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"DETERMINACIÓN DE HIDROCARBUROS LIGEROS EN SEDIMENTOS Y SUELOS DEL RÍO CHILLÓN POR CROMATOGRAFÍA DE GASES"

Tesis para optar el Título profesional de Licenciado en Química

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DEDICATORIA

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ABREVIATURAS

BTEX: Acrónimo que define la mezcla de Benceno, Tolueno, Metilbenceno y los tres isómeros del Xileno (orto, meta y para)

BFB: Acrónimo que se le asigna al estándar de surrogado (1-Bromo-4-Fluorobenzene)

BLK: Muestra prepara con suelo libre de inferencia, llamado blanco de método

CROMATOGRAMA: es un gráfico de la respuesta del detector en función del tiempo

CON-SURR: Muestra sintética que sirve para verificar la curva de surrogado, Control de Surrogado

CON-LCS: Muestra preparada con material libre de interferencia, no ayuda como muestra control de Laboratorio

CON-VER: muestra sintética prepara con otro lote diferente al de calibración, que nos ayuda a verificar la curva.

DETECTOR: Dispositivo que responde a cierta característica del sistema que está sujeto a observaciones y convierte esa respuesta en una señal susceptible de medirse.

FD: Factor de dilución

FID: (Detector Ionizacin de llama)
GRO: Rango Orgánico de Gasolina-hidrocarburo de petróleo comprendido entre carbono C6-C10

GC/FID: Cromatografía de gases con detector por ionización de llama HS:

Espacio de cabeza

IM: Estándar de verificación

LDD: Acrónimo que define Limite de Detección, Cantidad más pequeña de analito en una muestra que puede ser detectada por una única medición, con un nivel de confianza determinada, pero no necesariamente cuantificada con un valor exacto.

RESUMEN

El objetivo de la investigación fue determinar la concentración de Hidrocarburos Ligeros en suelos y sedimentos, por Cromatografía de Gases en el Rio Chillón, para ello, fueron muestreados suelos y sedimentos en los meses de octubre del 2016 a enero 2017. El procedimiento abarco cuarenta pruebas, estableciendo diez réplicas en cada mes. La determinación analítica fue realizada mediante los métodos normalizados de la Agencia de Protección Ambiental de los Estado Unidos, EPA 8015 y 5021.Se utilizó un Cromatógrafo de gases del modelo 7890A y un Headspace Sampler 7697A.

Esta Técnica Instrumental es la más adecuada para la determinación de Hidrocarburos Ligeros en muestras de suelos y sedimentos debido a que no existe otra técnica para la determinación de estos.

En las réplicas de suelos tomadas en el mes de enero identificadas como C-suelo-3 y C-suelo-9 se encontraron concentraciones de 4.99 mg/Kg Ms y 5.72 mg/Kg Ms de Hidrocarburos Ligeros respectivamente, mientras que en las muestra de sedimentos se encontraron concentraciones mayores de 22.91 mg/Kg Ms y 21.20 mg/Kg Ms identificadas como C-sed-6 y C-sed-7, las cuales son mayores según la norma publicada en el Diario Oficial El Peruano (2013). 002-2013-MINAM.Publicada el 25 de marzo 2013.Concluyéndose de esta manera que las descargas de los diferentes sectores industriales están siendo vertidas al Rio Chillón contaminando de esta anera el medio ambiente.

Palabras claves: suelos, sedimentos, Cromatografía de Gases, Río Chillón, Hidrocarburos Ligeros, medioambiente.

ABSTRACT

The objective of the research was to determine the concentration of light hydrocarbons in soils and sediments, by Gas Chromatography in the Chillón River. For this, soils and sediments were sampled in the months of October 2016 to January 2017. The procedure covered forty tests, establishing ten replications in each month. The analytical determination was made through the standardized methods of the Environmental Protection Agency of the United States, EPA 8015 and 5021. A gas chromatograph of model 7890A of the brand Technologies Agilent and a Headspace Sampler 7697A of the brand Technologies Agilent were used.

This Instrumental Technique is the most suitable for the determination of Aliphatic Hydrocarbons in soil and sediment samples because there is no other technique for the determination of these.
In soil replicas taken in January identified as C-soil-3 and C-soil-9 concentrations of4.99 mg / Kg Ms and 5.72 mg / Kg Ms of Aliphatic Hydrocarbons respectively were found, while in the samples of sediments were found higher concentrations of 22.91 mg / kg Ms and 21.20 mg / kg Ms identified as C-sed-6 and C-sed-7, being higher than the permissible regulatory values; It is concluded that the discharges from the different industrial sectors are being discharged into the Chillón River thus contaminating the environment.

Keywords: soils, sediments, Gas Chromatography, Chillón River,Light Hydrocarbons

I. INTRODUCCIÓN

En el Perú, se presentaron derrames de petróleo con mayor frecuencia, en el 2016 debido a fugas de hidrocarburos originados en la Región Amazónica, y según el Organismo de Evaluación y Fiscalización Ambiental (OEFA), dio a conocer que este es uno de los desastres más importantes ocurridos en los últimos años.

La necesidad para realizar el estudio de hidrocarburos seria dar a conocer las concentraciones de Hidrocarburos Ligeros que contienen los efluentes vertidos por las diferentes industrias; cuya finalidad seria demostrar si los vertidos arrojados sobrepasan los límites máximos permisibles establecidos en los Estándares de Calidad Ambiental. Debido a que dentro de los contaminantes encontramos Benceno, Tolueno, Etilbenceno, Xilenos; además de residuos de gasolina que son tóxicos y nocivos para los diferentes ecosistemas.

El estudio fue desarrollado durante el 2016 entre los meses de octubre, noviembre, diciembre y enero del 2017 respectivamente.

El objetivo del presente trabajo fue determinar la concentración de Hidrocarburos Ligeros en la fracción de carbono seis al carbono diez, por Cromatografía de Gases en el Rio Chillón.

II. MARCO TEÓRICO

2.1 ANTECEDENTES.

Riser (1998) afirma. “que en cierto sentido uno de los problemas de la contaminación ambiental a nivel mundial es el vertido de hidrocarburos derivados del petróleo en ecosistemas acuáticos y terrestres” (p.500).

En publicaciones realizadas por Castellanos, Isaza y Torres (2015) aseguran que existen evidencias de que la contaminación del suelo por Hidrocarburos Totales de Petróleo produciendo cambios en las propiedades físicas y químicas, alterando así de esta manera su condición natural; principalmente ocurre en las capas expuestas directamente al vertimiento.

Siguiendo con los estudios de hidrocarburos. Campillay (2006).afirma “en su tesis sobre el origen de hidrocarburos en las puede incluir compuestos petrogénicos como biogénicos y dentro del primero se incluye principalmente al petróleo como Hidrocarburo Ligeros, gasolinas, Hidrocarburos Medianos, kerosén y diésel e Hidrocarburos Pesados, aceites”(p.150).

Las fuentes biogénicas, pueden incluir por ejemplo: algas, animales, plantas vasculares o bacterias. En síntesis toda la información de estos índices se puede demostrar que la contaminación por Hidrocarburos Totales de Petróleo es de origen petrogénica.

La tesis doctoral de (Rosales, 2013, p.180).Realiza un muestreo y el análisis cualitativo de compuestos orgánicos volátiles como el Benceno, Tolueno, Etilbenceno, m, p y o Xilenos además del Rango Orgánico de Gasolina (GRO), la cual debe realizarse de la forma más hermética posible, para evitar la pérdida de compuestos debido a su alta volatilidad; es decir, hacer lo más rápido posible tras el conocimiento de fugas o derrames en el subsuelo.

Los investigadores. Pinto y Bonert (2005) Afirman.” que es posible que nos explican sobre la Técnica de Cromatografía de Gases que es la más tratada para el análisis de Hidrocarburos Alifáticos y compuestos orgánicos volátiles en los suelos, debido a que estos compuestos son muy ligeros y necesitan un acoplamiento de headspace al Cromatógrafo de Gases para la determinación de los mismos”(p.250), así mismo Esteve-Turrillas,Armenta,Garrigues y Pastor (2007) afirman que los hidrocarburoas alifaticos son compuestos volatiles.

En los artículos de investigación de Méndez ,Rennola y Peña (2011). Nos afirma que para la determinación de Hidrocarburos de Petróleo se realiza con un Cromatógrafo de Gases con Detector de Ionización de Llama (GC-FID).

Rincón (2015) refiere. “que para la determinación de CO2 y H2S se realiza por cromatografía de gases en gas natural” (p.350)

2.2 GENERALIDADES

2.2.1. Hidrocarburos alifáticos

Los Hidrocarburos Alifáticos son compuestos orgánicos no derivados del benceno, que están formados por átomos de carbono e hidrógeno, formando cadenas las cuales pueden ser abiertas o cerradas.

Los Alcanos o Hidrocarburos Saturados Alifáticos responden a la formula general C_nH_{2n+2} y no poseen grupo funcional propiamente dicho, y están constituidos exclusivamente por enlaces simples –C-C y C-H, donde los cuatro orbitales híbridos del carbono son equivalentes y están orientados hacia los cuatro vértices de un tetraedro regular.

En la figura 1, 2 se muestran las estructuras de n-hexano, n-octano respectivamente.

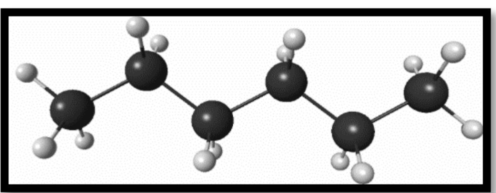

Figura 1. Estructura del n-hexano

Fuente: (García et al. ,2011)

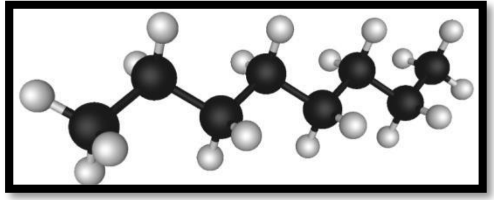

Figura 2. Estructura del n-octano

Fuente: (García et al. ,2011)

2.2.1.2. Propiedad Física de los Hidrocarburos Alifáticos (Ligeros)

a) Punto de Ebullición

Los Hidrocarburos Alifáticos son compuestos formados por carbono e hidrógeno, y pueden ser moléculas ramificadas o lineales de cadena abierta, saturada o insaturada. Las moléculas más pequeñas son gases a temperatura ambiente (C1 a C4) que al aumentar de tamaño su complejidad estructural de dicha molécula se hace líquida y su viscosidad aumenta con el número de carbonos (C5 a C16).

Los hidrocarburos de alto peso molecular son sólidos a temperatura ambiente (mayores de C16). Los Hidrocarburos Alifáticos de uso industrial derivan principalmente del petróleo, que es una mezcla compleja de hidrocarburos (Wade, 2007, p.95).

b) Punto de Fusión

Los Alcanos son apolares, por lo que se disuelven en disolventes orgánicos apolares o débilmente polares. Los alcanos tienen densidades aproximadas de 0,7 g/ml, comparadas con la densidad del agua 1.0 g/ml, Los puntos de ebullición de los alcanos aumentan ligeramente a medida que aumenta el número de átomos de carbono, y por tanto sus masas moleculares.
Las moléculas más grandes tienen mayores áreas superficiales, por lo que aumenta la atracción intermolecular de Van Der Waals. Al ser mayor esta fuerza, mayor es el punto de ebullición.

En la Tabla 1 se muestran el punto de ebullición y fusión de los hidrocarburos alifáticos del carbono 5 al carbono 12 respectivamente.

Tabla 1: Punto de ebullición y fusión de los hidrocarburos alifáticos

Hidrocarburos alifáticos	Formula	Punto de ebullición (°C)	Punto de fusión
Pentano	$CH_3CH_2CH_2CH_2CH_3$	36	-130
Hexano	$CH_3CH_2CH_2CH_2CH_2CH_3$	69	-95
Heptano	$CH_3CH_2CH_2CH_2CH_2CH_2CH_3$	98	-91
Octano	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$	125.5	-57
Nonano	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$	151	-51
Decano	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$	174	-30
Undecano	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$	196	-26
Dodecano	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$	216	-10

2.2.2. Hidrocarburos aromáticos

Los Hidrocarburos Aromáticos forman una gran familia de compuestos, que tienen un núcleo común que es, el núcleo bencénico. El benceno contiene 92.3 % de carbono y 7.7 % de hidrógeno con la fórmula química C6H6, la molécula de Benceno se representa mediante un hexágono formado por los seis conjuntos de átomos de carbono e hidrógeno unidos con alternancia de enlaces simples y doble hace referencia.García,Herrero,Pérez y Moreno (2011).

Estos compuestos químicos que tienen una alta presión de vapor en condiciones normales, para evaporarse significativamente al entrar en la atmósfera. Los Hidrocarburos Aromáticos como el Benceno, Tolueno, Etilbenceno y los Xilenos son algunos de estos compuestos los cuales se obtienen del carbón y del petróleo. (Wade, 2007, p.150).

En las figuras 3, 4,5 y 6 se muestran las moléculas de Benceno, Tolueno, Etilbenceno y xilenos respectivamente.

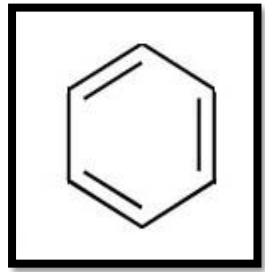

Figura 3. Estructura Benceno
Fuente: (García et al. ,2011)

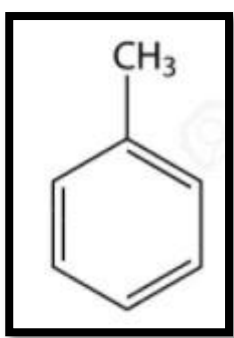

Figura 4. Estructura Benceno
Fuente: (García et al. ,2011)

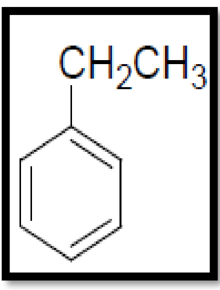

Figura 5 Estructura Benceno
Fuente: (García et al. ,2011)

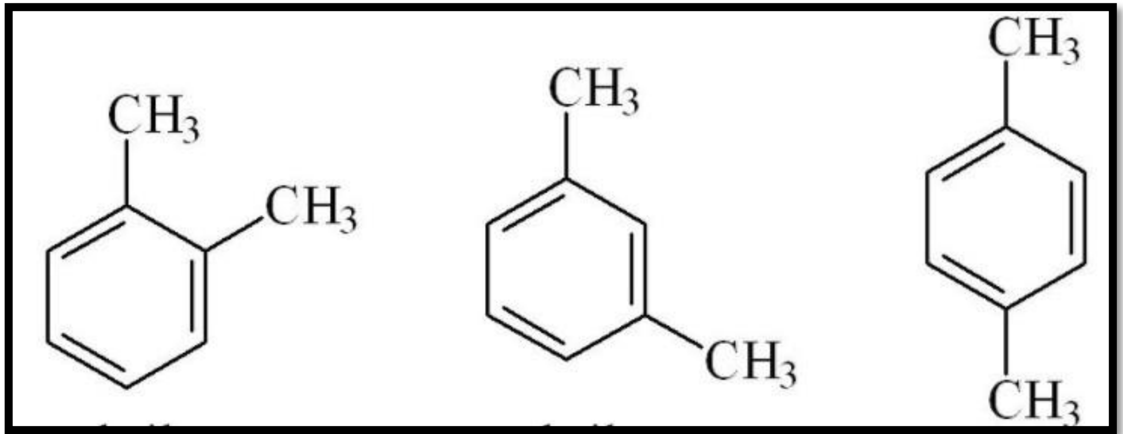

Figura 6 Estructura del Xileno (orto, meta y para)

Fuente: (García et al. ,2011)

2.2.3. Características Fisicoquímicas para BTEX y Gasolina comercial

Tabla 2. Características para Hidrocarburos Fracción Ligera

Contaminante	Peso molecular (g/mol)	Solubilidad en agua a 25ºC (mg/L)
Benceno	78	1780
Tolueno	92	490-627
Etilbenceno	106	152-208
Xilenos	106	162-200
Gasolina	107-114	

Fuente: (Davis, 1997 y Heath, 1993)

2.2.4. Gasolina

La gasolina es una mezcla de Hidrocarburos Alifáticos, obtenida del petróleo por destilación fraccionada, que se utiliza como combustible en motores de combustión interna por encendido de una chispa convencional o por compresión, así como en estufas, lámparas, limpieza con solventes y otras aplicaciones.

En general se obtiene a partir de la gasolina de destilación directa, que es la fracción líquida más ligera del petróleo (exceptuando los gases).

La nafta también se obtiene a partir de la conversión de fracciones pesadas del petróleo (gasoil de vacío) en unidades de proceso. Cabrera (2014, p.106).

Normalmente se considera nafta a la fracción del petróleo cuyo punto de ebullición se encuentra aproximadamente entre 28°C y 177 °C (umbral que varía en función de las necesidades comerciales de la refinería). A su vez, este subproducto se subdivide en: nafta ligera (hasta unos 100 °C) y Nafta Pesada, por encima de 100ºC. La Nafta Ligera es uno de los componentes de la gasolina, con unos números de octano en torno a 70, y la Nafta pesada no tiene la calidad suficiente como para ser utilizada para ese fin, y su destino es la transformación mediante reformado catalítica, proceso químico por el cual se obtiene también hidrógeno, a la vez que se aumenta el octanaje de dicha nafta.

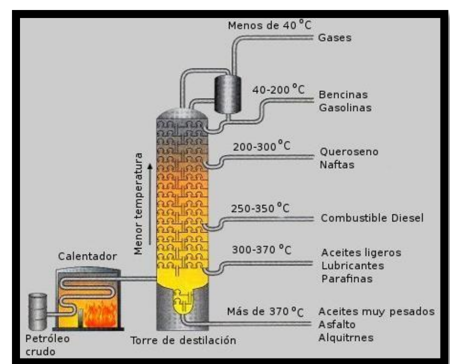

Figura7 Esquema de destilación del petróleo

Fuente: (García et al. ,2011)

2.2.5. Componentes de la gasolina

Los hidrocarburos que componen la gasolina están comprendidos entre los que poseen 4 átomos de carbono y los que tienen átomos de carbono C10-C11. Cabrera (2014, p.116), así como se muestra en el Tabla 3

Tabla 3. Estructuras de los hidrocarburos del C6-C10

Nombres	# C	Estructuras
n-hexano	6	H_3C CH_3
Ciclo hexano	6	
Benceno	6	
n-Heptano	7	H_3C CH_3
Tolueno	7	CH_3
n-octano	8	H_3C CH_2 CH_2 CH_2 CH_2 CH_2 CH_2 CH_3
Etilbenceno	8	CH_3
xilenos	8	CH_3 CH_3 CH_3 CH_3 CH_3 CH_3
n-nonano	9	H_3C CH_3
n-decano	10	H_3C CH_3

Fuente: Elaboración propia

2.2.6. Estándares de calidad ambiental

Los productos asociados a los derrames de hidrocarburos y fracciones para los límites máximos permisibles de contaminación en suelos describen en el Tabla 4.

Tabla 4. Estándares de Calidad Ambiental (ECA)

Compuestos orgánicos	Usos del suelos (mg/kg MS)		
	Suelo agrícola	Suelo residencial	Suelo industrial
Benceno	0.03	0.03	0.03
Tolueno	0.37	0.37	0.37
Etilbenceno	0.082	0.082	0.082
Xilenos	11	11	11
Naftaleno	0.1	0.6	22
Fracción de hidrocarburos C6-C10	200	200	500

Fuente: Elaboración propia

2.2.7. Contaminación de los suelos por hidrocarburos

Un suelo se puede degradar al acumularse en ellas sustancias como Hidrocarburos Totales de Petróleo (TPH), a unos niveles tales que repercuten negativamente en el comportamiento de los suelos.

Los derrames de estos hidrocarburos a niveles de concentración se vuelven tóxicas para los organismos del suelo, se trata de una degradación química que provoca la pérdida parcial o total de la productividad del suelo.

La contaminación es básicamente un cambio indeseable en las características físicas, químicas y biológicas del medio natural, producido sobre todo para la actividad humana (incluida la contaminación de las aguas superficiales y freáticas, del suelo y del aire) según (Sharapin, 2000, p.189)

La variedad y cantidad de productos contaminantes de un suelo es prácticamente inabarcable, pero los de mayor relevancia son:

- Metales pesados
- Sustancias Orgánicas
- Sustancias Inorgánicas

Los efectos de los Hidrocarburos en muestras de suelo dependen de la cantidad y composición del producto derivado del petróleo, así como también de la frecuencia de tiempo de exposición, estado físico del derrame, características del área afectada además de variables medioambientales como: temperatura, humedad, oxígeno y sensibilidad de la biota, especifica del ecosistema impactado por el derrame.

La presencia de contaminantes en un suelo, supone la existencia de efectos nocivos para el hombre, fauna y la vegetación, estas características dependerán de los contaminantes que se encuentren, además de la concentración en un determinado tiempo.

En la siguiente Tabla mediremos el grado de Toxicidad, Persistencia e Inflamabilidad dándoles valores como: 3: alto, 2: medio ,1: bajo y 0: sin riesgo.

Tabla 5: Características de algunos Hidrocarburos ligeros

Compuestos	Características	Toxicidad	Persistencia	Inflamable	Reactividad
Benceno		3	1	3	0
Etilbenceno	CH_3	2	1	3	0
Petroleo,Kerosene		3	1	2	0
Tolueno	CH_3	2	1	3	0
Xilenos	CH_3 CH_3 CH_3 CH_3 CH_3 CH_3	2	1	3	0

Fuente: Elaboración propia

Los compuestos de Benceno, Tolueno, Etilbenceno y Xilenos son denominados BTEX, estos compuestos son los más peligrosos y comunes en la fuga de gasolina. El benceno es el más peligroso de estos compuestos por sus características carcinógenas y toxicas. Para este compuesto la Agencia Protección Ambiental de los Estados Unidos (US EPA), ha establecido un límite de cuantificación de 5ppb en el suelo. (US EPA, 2011; ASTDR, 2011)

En España la legislación Real Decreto (R.D. 9/2005), indica los límites de concentración de los BTEX (nivel genérico de referencia, NGR) para la protección de la salud humana que se muestra en el Tabla 6 y ecosistemas en función del uso del suelo que se muestra en los Tabla 7.

Listado de Contaminantes y Niveles Genéricos de Referencia para Protección de la Salud en Función del uso del Suelo

Tabla 6: Listado de contaminantes de referencia para protección de la salud

Analitos	Número de CAS	Uso Industrial	Uso Urbano	Otros usos
		mg/kg masa seca de suelo		
	Protección de la salud humana			
Benceno	71-43-2	10	1	0.1
Etilbenceno	100-41-4	100	20	2
Tolueno	108-88-3	100	30	3
Xilenos	1330-20-7	100	100	35

Fuente: Elaboración propia

Listado de Contaminantes y Niveles Genéricos de Referencia para Protección de los ecosistemas

Tabla 7. Listado de contaminantes de referencia para protección del ecosistema

Analitos	Número de CAS	Organismos del suelo	Organismos acuáticos	Vertebrados terrestres
		mg/kg masa seca de suelo		
	Protección de los ecosistemas			
Benceno	71-43-2	1	0.2	0.11
Etilbenceno	100-41-4	-	0.08	4.6
Tolueno	108-88-3	0.3	0.24	13.5
Xilenos	1330-20-7	-	0.07	-

Fuente: Elaboración propia

2.2.8. Efectos de los hidrocarburos de petróleo a la salud humana y medio ambiente.

a) Efectos a la Salud Humana

Es conocido que algunos Hidrocarburos tienden a acumularse en los organismos a través de las cadenas alimenticias con creciente riesgo de cáncer y de mutagénesis, por ello los organismos más expuestos son los humanos, ya que estos contaminantes incluyen el benceno, los fenoles, y a los Hidrocarburos Policíclicos que son muy peligrosos por sus efectos cancerígenos.

Los efectos en la salud humana dependen de muchos factores, estos incluyen:

- ✓ Tipo de hidrocarburo al que se encuentra expuesto
- ✓ Tiempo de exposición
- ✓ Cantidad de la sustancia química con la que se está en contacto

La Agencia Internacional para la Investigación del Cáncer (IARC) ha determinado que el Benceno es cancerígeno en los seres humanos, produce Leucemia, y se considera que otros hidrocarburos, por ejemplo: Benzo(a) pireno y la gasolina también producen cáncer en los seres humanos.

b) Efectos al Medio Ambiente

- Los hidrocarburos interfieren en el intercambio de gases entre el aire y el agua, esto elimina el abastecimiento de oxígeno para los animales de respiración branquial y obstruye, en muchos casos la posibilidad de respiración pulmonar de otros animales acuáticos.
- Los hidrocarburos se adhieren a sedimentos, allí forman depósitos que se van liberando al ambiente lentamente durante mucho tiempo, y actúan como fuentes de contaminación por un largo período.

En la Tabla 8, se observan los efectos que ocasionan los hidrocarburos ligeros de petróleo como es el caso de Benceno, n-hexano y Gasolina.

Tabla 8. Efecto de asimilación de los hidrocarburos

Compuesto	Concentración (mg/L)	Tiempo (horas)	Síntomas	Observaciones
Benceno	100	>3	-Fatiga -dolor de cabeza -náuseas -adormecimiento	-Cuando la exposición cesa, los síntomas desaparecen.
n-Hexano H_3C … CH_3	500-2500	>24	Afecta al sistema nervioso central ,característico por pérdida de la sensación en los pies y piernas	-En caso graves produce parálisis -Exposición al compuesto en el aire
Gasolina			-Irritación a la garganta y estómago. -Depresión del sistema nervioso. -Dificultad al respirar	-Debido a la ingestión

Fuente: Elaboración propia

2.2.9. Cromatografía de gases

La Cromatografía de Gases permite separar los componentes de una muestra vaporizada, en virtud de que éstos se distribuyen entre una fase móvil la cual es un gas y una fase estacionaria, en donde esta podría ser líquida o sólida contenida en una columna.

Los analitos que se van analizar se lleva a la fase gaseosa y se inyecta en una de las cabezas de la columna cromatográfica; es decir, en el puerto de inyección.

La elusión de los componentes se realiza mediante el flujo de una fase móvil, en donde esta es inerte y no interactúa con las moléculas de las especies de la muestra; sólo las transporta a través de la columna.

Existen dos tipos de cromatografía de gases: la de gas-líquido y la de gas-sólido. La primera, es la que tiene más aplicaciones en todos los campos de las ciencias y se le conoce comúnmente como cromatografía de gases, mientras que la segunda nos habla del gas-sólido, el cual tiene menos aplicaciones debido a que muchas moléculas reactivas o polares poseen tiempos de retención muy largos y las colas de los picos de elución no son aceptables. (Skoog, 2001, p.162).

La Cromatografía es un método de separación basado en las diferencias de afinidad de los diferentes compuestos (analitos) entre una fase móvil y una fase estacionaria, dado que cada analito tiene una afinidad especifica en relación a estas fases, la migración entre las fases es diferente para cada uno dando origen a la separación a lo largo del desarrollo de la cromatografía.

La distintiva movilidad permite la separación de los componentes en bandas o zonas discretas (Skoog, 1992,p.250).

Los componentes que son fuertemente retenidos por la fase estacionaria se mueven lentamente con el flujo de la fase móvil, por el contrario, los componentes que se unen débilmente a la fase estacionaria se mueven con rapidez.

La clasificación más fundamental de los métodos se basa en el tipo de fase móvil y estacionaria, y en la clase de equilibrios implicados en la transferencia de analítos entre fases.

La Cromatografía Gaseosa, es una técnica de análisis que ofrece resoluciones excelentes con sensibilidad del orden de miligramos a picogramos, sus resultados son cuantitativos y se obtienen en un espacio de tiempo relativamente corto. Los componentes de las muestras deben ser estables a la temperatura de operación, las muestras tienen que ser volátiles.

La fase estacionaria, es un líquido poco volátil que recubre un soporte sólido o las paredes de la columna, y el mecanismo de separación se produce mediante la partición de las moléculas de la muestra entre la fase estacionaria y la fase móvil (líquida y gaseosa).
Esta modalidad se utiliza en más del 95% de las aplicaciones de la cromatografía líquida, el cual debe solubilizar selectivamente las sustancias de la muestra que debe ser termoestable, es decir presentar una baja volatilidad a la temperatura en la cual se realiza el análisis, y ser químicamente inerte en relación con los componentes de la muestra. (Sharapin, 2000, p.250).

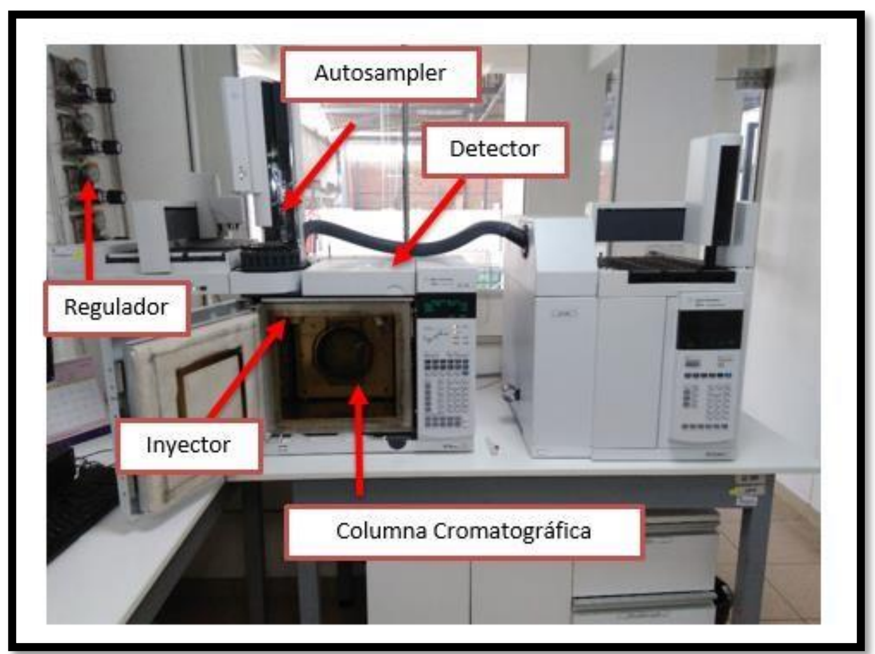

Figura8 Cromatógrafo de gases

2.2.10. Componentes de un cromatógrafo de gases

2.2.10.1 Fase móvil

La fase móvil gaseosa proporciona un rápido equilibrio con la fase estacionaria, el cual se utiliza con mayor eficiencia en la obtención de los análisis. Los gases más utilizados son: nitrógeno, helio, hidrógeno y argón.

La fase móvil no debe interactuar con la fase estacionaria ni con la muestra, ya que debe tener un bajo costo y ser compatible con el detector al tener alta pureza.

Para dar una mayor reproducibilidad al análisis, la saturación del gas debe ser constante y debe ser controlada a través de válvulas de aguja (Skoog, 2001, p120).

2.2.10.2 Sistema de inyección

La inyección se realiza generalmente con micro jeringas, que contienen la muestra donde el volumen inyectado no debe superar la capacidad de la columna, y entre más pequeño sea el volumen usado de la muestra mayor será la eficiencia y la reproducibilidad del análisis. La temperatura aplicada debe ser suficiente para la volatilización de la muestra (Skoog, 2001, p125).

2.2.10.3 Columna

La columna consiste en un tubo largo que contiene la fase estacionaria, y los materiales más usados son: el cobre, acero, aluminio, vidrio y teflón. El material de la columna no debe interaccionar con la fase estacionaria ni con la muestra. (Skoog, 2001, p.148).en la figura 9 se muestra una columna capilar.

Fig. 9. Columna Capilar

2.2.10.4 Sistema de detección

Uno de los elementos más importantes en el Cromatògrafo de Gases es el detector. El cual es un dispositivo que indica y mide los solutos en la corriente del gas portador, convirtiendo una señal no medible directamente en una señal elaborable, de una propiedad física.
"Esta señal es elaborada por una comparación entre el gas portador puro y el mismo gas, llevando cada uno de los componentes previamente separados en la columna, y este es traducido en una señal eléctrica que es amplificada y registrada al momento de salir de la columna" (Olguín y Rodríguez ,2004).

"Las sustancias presentes en la muestra pasan a través de la columna, en donde son separadas y llegan al sistema de detección. Con relación a la selectividad, los detectores pueden ser clasificados en universales y selectivos o específicos nos hace referencia" (Hernández, 2016, p.251)

Los detectores universales miden la variación de una propiedad del gas de arrastre que sale de la columna, mientras que los detectores específicos miden una propiedad característica de una determinada clase de sustancias. Con respecto a la sensibilidad, los detectores cuya respuesta varía poco por cambios en la velocidad de flujo y de la fase móvil, los cuales son llamados detectores sensibles de flujo de masa.

En estos detectores la sensibilidad se define como la relación del área de pico a la masa inyectada.

En los detectores sensibles a la concentración, la respuesta varía en función del flujo de la fase móvil y su sensibilidad, que son definidas como el producto del área del pico por el flujo de la fase móvil, dividida por la masa inyectada. La respuesta del detector debe ser lineal dentro de un amplio intervalo de concentraciones y este intervalo de concentraciones se conoce como intervalo lineal dinámico, que corresponde a la diferencia entre la concentración máxima del intervalo lineal dinámico y la mínima concentración del mismo, que debe distinguirse de la señal de fondo o ruido del detector.

El análisis cuantitativo se puede llevar a cabo gracias a la relación directa que existe entre la respuesta del detector y la concentración de la muestra, siempre y cuando la respuesta obtenida para la muestra se encuentre dentro de ese intervalo (Skoog,2001,p.126).

2.2.10.5 Detector de Ionización de Llama (FID)

El detector de ionización de llama es el que más se utiliza, y por lo general es uno de los que más se aplican en cromatografía de gases. El efluente de la columna se dirige a una pequeña llama de hidrogeno y aire, y la mayoría de los compuestos orgánicos producen iones y electrones cuando se pirolizan a la temperatura de una llama de hidrogeno-aire.

La detección implica controlar la corriente producida al recolectar estos portadores de carga, y cuando se aplica una diferencia de potencial de unos pocos cientos de volts entre el extremo del quemador y un electrodo colector situado por encima de la llama, los iones y los electrones se dirigen hacia el colector. (Skoog, 2008,p.159).

"La llama de hidrogeno-aire genera un medio ionizante de gran eficacia para las sustancias orgánicas, y por lo tanto, es un sensor electrónico adecuado, constituyendo un transductor de elevada sensibilidad se refiere" (Davis, 1997,p.15).

El Detector de Ionización por Llama, funciona mediante la pirólisis del material que eluye de la columna en una llama de hidrógeno/aire con exceso de oxígeno, y cuando pasan por el detector los compuestos separados por la columna, estos reaccionan en la llama produciendo cationes. "Los iones producidos son conducidos mediante un campo eléctrico hacia el colector, donde la corriente generada se amplifica para producir una respuesta" (Sogorb y Vilanova, 2004,).

Dicho detector manifiesta una elevada sensibilidad (10-13 g/s), en un gran intervalo de respuesta lineal y una bajo ruido. La desventaja de este detector es la destrucción de la muestra durante el paso de la combustión y la necesidad de gases adicionales y controladores. (Skoog, 2008, p.109).

El FID es un detector de muy alta sensibilidad, apto para hidrocarburos ya que permite detectar los iones de carbono que se forman durante la combustión a alta temperatura, sumado a la alta sensibilidad, este detector presenta la característica de poseer un factor de respuesta (en masa) casi idéntico para todos los hidrocarburos: "La misma masa de distintos componentes produce la misma intensidad de señal en el detector, esta característica transforma al FID en un detector de mucha utilidad, puesto que no es necesario conocer la fórmula de un componente para conocer su aporte a la masa total del sistema"(Montoya y Páez, 2012).

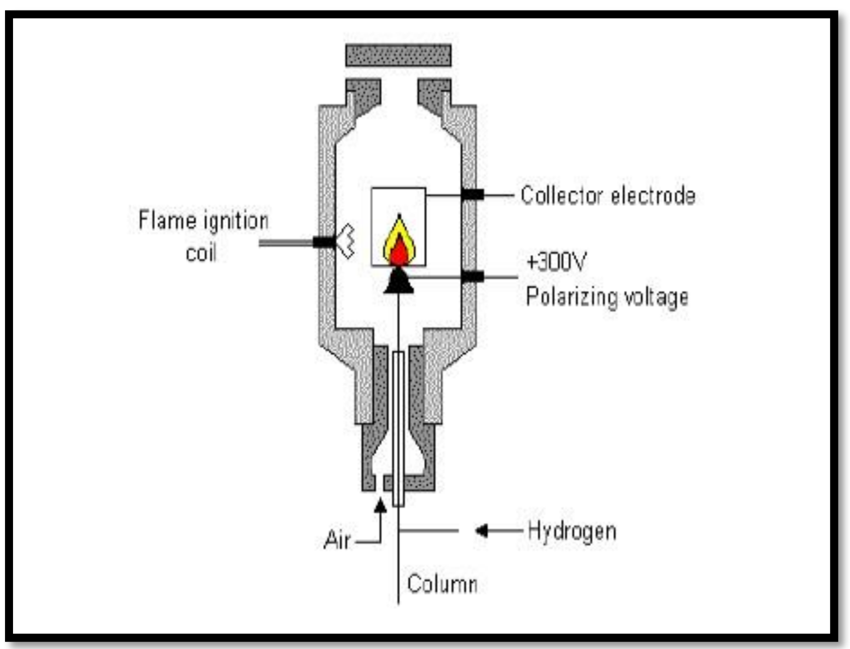

Fig. 10. Detector de ionización de llama

Debido a que este detector posee una alta sensibilidad especialmente para los hidrocarburos, también es el que se usa con mayor frecuencia en el análisis de estos, como es el caso de un trabajo realizado en la región Vasco-Cantábrica en España, en donde se reporta un análisis de Hidrocarburos Alifáticos en muestras correspondientes a diferentes ambientes sedimentarios y diagenéticos.

En dicho procedimiento, la fracción de hidrocarburos saturados se inyectó en un Cromatógrafo de Gases equipado con un Detector de Ionización de Llama, usando un sistema de inyección splitless (FID) (Agirrezabal et.al ,2009).

2.2.11. Técnicas de extracción: Automuestreador headspace

Esta técnica es usada para la concentración y análisis de componentes orgánicos Volátiles. “Es una técnica relativamente simple y de buena sensibilidad, la popularidad de la técnica Headspace ha crecido mundialmente para diferentes tipos de análisis donde se desee observar compuestos volátiles como los hidrocarburos”.Pavón, Martin, Pinto y cordero (2009)
Se conocen en el mercado dos técnicas de Headspace, una estática y otra dinámica. En la primera de estas se introduce la muestra en un vial llenándolo hasta la mitad, este se sella y coloca en un termostato para dirigir los componentes volátiles como hidrocarburos hacia la fase de vapor (espacio superior del vial conocido como Headspace), con la ayuda de una jeringa o un dispositivo similar se toma una alícuota de dicha fase, que posteriormente será inyectada al Cromatógrafo de Gases. La técnica implica que la muestra fue tomada de una fase en equilibrio

Para incrementar la sensibilidad se desarrolló del headspace dinámico, donde la fase de equilibrio está siendo desplazada continuamente, dirigiendo el Head Space fuera del vial con ayuda de un gas inerte hacia un sistema de purga y trampa, donde los analitos serán atrapados por un adsorbente y posteriormente desorbidos por calor para introducirse en el Cromatógrafo de Gases. “El aditamento de Espacio de Cabeza dinámico requiere de una inversión inicial alta, por lo que no resulta un método de fácil acceso” (Peña, 2010, p.256).

Fig. 11. Automuestreador Headspace

En la figura 12, se muestra las etapas de inyección en el Headspace; en donde la etapa 1, nos muestra cuando la muestra alcanza el equilibrio, en la etapa 2 nos muestra la presurización y la etapa 3 es la extracción e inyección de la muestra a la columna Cromatográfíca.

Mientras que en la figura 13, nos muestra el proceso de agitación que ocurren en el Headspace

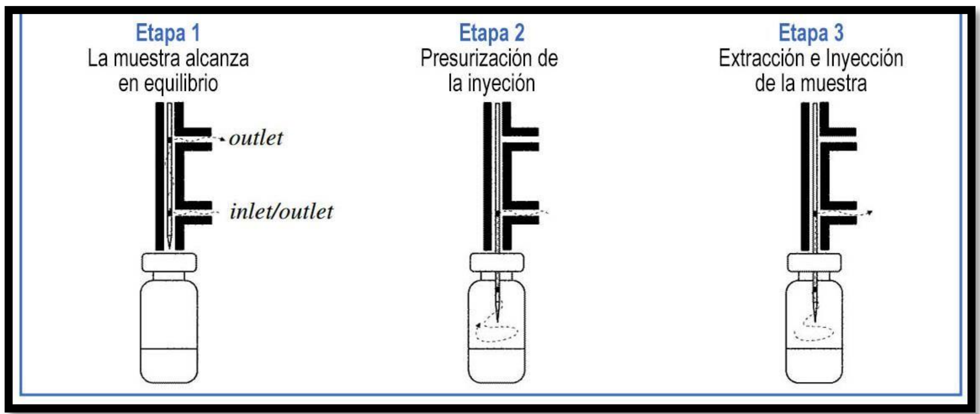

Fig. 12. Etapas del sistema de inyección del headspace

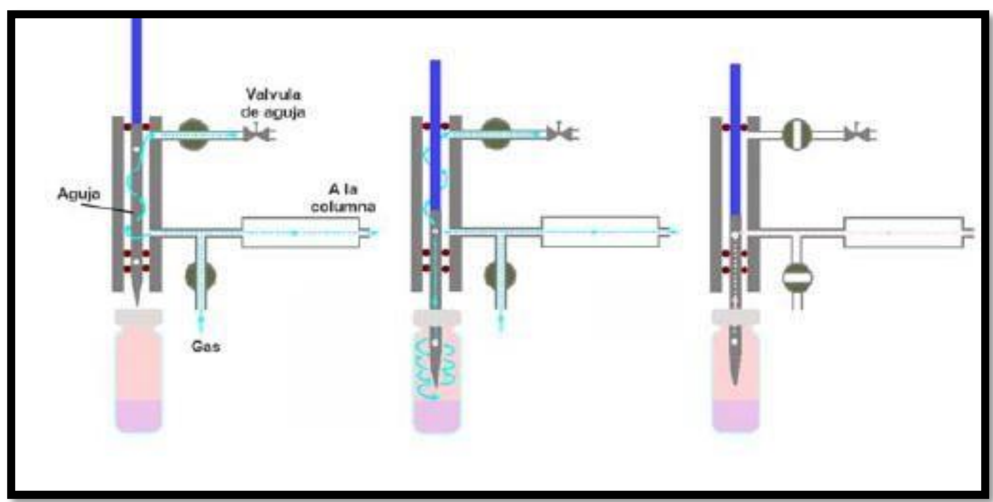

Fig. 13. Proceso de agitación del vial headspace

III. MATERIALES Y MÉTODOS

3.1 Lugar de muestreo de suelo y sedimentos

Las muestras fueron recogidas del Rio Chillón ubicado en el distrito de Puente Piedra, dicho río se origina en la laguna de Chonta a 4,800 msnm, alimentándose con las precipitaciones que caen en las partes de su cuenca colectora y con los deshielos de la cordillera de la viuda.

La cuenca del Rio Chillón se halla comprendida entre las coordenadas geográficas 76º 20' y 77º 10' de longitud Oeste, 11º 20' y 12º 00'de latitud sur; ubicada en el departamento de Lima, Perú.

Este lugar de muestreo se escogió debido que el Rio Chillón es depositario de una variedad de efluentes domésticos, eléctricos, mineros etc.; en tal sentido es posible que en las matrices de sedimentos y suelos haya una alta concentración de hidrocarburos ligeros C6-C10.

Para determinar las muestras previamente se realizó un muestreo en octubre del 2016, a cual se le realizó un análisis cualitativo y cuantitativo, a fin de detectar Hidrocarburos Ligeros C6-C10; él cual nos evidencio una concentración de hidrocarburos ligeros por encima de los límites máximos permisibles.
Luego se realizó 4 muestreos de 10 puntos cada uno, que abarcaron desde el mes de octubre del 2016 hasta enero del 2017; tomando como referencia las coordenadas 18 L 274149.02 m E8681689.39 m elevación 112m.

3.2 Determinación de rango orgánico de gasolina (GRO)

La mezcla de Hidrocarburos que contengan entre cinco y diez átomos de carbono (C5 a C10), son llamados Rango Orgánico de Gasolina dentro de los cuales se encuentran en mezclas de productos desconocidos derivados del petróleo, petróleo crudo, gasavión, gasolvente, gasolinas, gas nafta. Para lo cual se siguió el procedimiento siguiente:

3.2.1 Extracción de hidrocarburos de suelos mediante headspace

Para la extracción de Hidrocarburos de suelos contaminados se realizó por Headspace, que toma como referencia el método de la Agencia de Protección Ambiental de los Estados Unidos (US EPA) 5021A

3.3 Equipos

- Cromatográfo de Gases FID, Agilent Technologies 7890A, con detector FID, Columna Cromatográfíca Agilent J&W DB-VRX 20m x 0.18mm x 1.0 m
- Equipo Muestreador Headspace, Agilent Technologies 7890A
- Fase mòvil:

-Gas de Nitrógeno Praxair, con una pureza de 99.999%

-Gas de Aire, Praxair con una pureza de 99.999%

-Gas de Hidrógeno, Praxair con una pureza de 99.999%

3.4 Materiales

- Balanza analítica digital. Precisión ± 0.0001g modelo Mettler Toledo
- Fiolas Volumétricas ambar, clase A de 10,25 y 50 ml con tapa de vidrio esmerilado
- Micro jeringas de 250ul, 1000ul
- Viales Head space 22 ml, con tapa de aluminio y septa de silicona
- Vial Crimping Tool 20 mm
- Botella de Reactivo de 1000 ml con tapa azul
- Estufa

3.5 Reactivos

- Metanol (CH_3OH) para cromatografía al 99.8% de pureza
- Cloruro de Sodio (Nacl) o Cloruro de potasio (Kcl) para análisis grado de pureza
- Agua reactivo: agua ultrapura de grado tipo I, libre de compuestos orgánicos
- Aliphatic Mix (C5-C12) 2000 ug/mL
- 1-Bromo-4-Fluorobenzene 10000 mg/L
- Combustible Gasolina de fuente comercial
- a, a, a-Triofluorotolueno Standard 10000 mg/mL

3.6 Preparación de soluciones

3.6.1 Estándar de calibración

Se pesa 0.25g de gasolina comercial en una fiola de 25ml y se enraza con metanol obteniendo una solución de 10000 mg/L.

Tabla 9. Preparación de gasolina

Estándar de Calibración de Gasolina (mg/L)	Alícuota (ul)	Volumen (ml)	Intermedio de Calibración(mg/L)
10000	250	25	100

Fuente: Elaboración propia

3.6.2 Estándar de surrogado

A partir del estándar de 1-Bromo-4-Fluorobenzene de 10000 mg/L se realizó un estándar intermedio de 100mg/L.

Tabla 10. Preparación del 4-Bromofluorobenceno

Estándar de BFB (mg/L)	Alícuota (ul)	Volumen (ml)	Intermedio de Surrogado BFB (mg/L)
10000	250	25	100

Fuente: Elaboración propia

3.6.3 Preparación de solución modificadora de matriz (SMM)

Se adiciono 180g de cloruro de sodio y llevarlo a foro a 500 ml de agua libre de compuestos orgánicos. Se verifica que la solución es libre de contaminantes mediante la lectura de solución como blanco de método.

3.6. Curva de calibración de rango orgánico de gasolina (GRO)

A partir de las soluciones intermedias de calibración de gasolina de 100mg/L, se prepararon 9 soluciones de calibración de diferentes concentraciones como indica en el Tabla11

Tabla 11. Curva de calibración de rango orgánico de gasolina

Concentración (mg/L)	Intermedio de Calibración Gasolina(ul)	Volumen Final (ml)
	100mg/L	
0.5	50	**10**
1.0	100	**10**
2.0	200	**10**
3.0	300	**10**
4.0	400	**10**
5.0	500	**10**
7.0	700	**10**
8.0	800	**10**
10.0	**1000**	**10**

Fuente: Elaboración propia

3.7 Curva de surrogado del 1-bromo-4-fluorobenzene(BFB)

A partir de las soluciones intermedios de surrogado 1-Bromo-4-Fluorobenzene (BFB) de 100mg/L. se preparan 9 soluciones de calibración de diferentes concentraciones, procediendo como se indica en la Tabla 12.

Tabla 12. Curva de calibración de 4-fluorobenzene (BFB)

Concentración (mg/L)	Intermedio de surrogado BFB (ul)	Volumen Final (ml)
	100mg/L	
0.3	30	**10**
0.5	50	**10**
0.7	70	**10**
1.0	100	**10**
1.5	150	**10**
2.0	200	**10**
2.5	250	**10**
3.0	300	**10**
3.5	**350**	**10**

Fuente: Elaboración propia

3.8 Lugar de muestreo

Las muestras fueron recogidas del Rio Chillón ubicado en el distrito de Puente Piedra, a la altura del kilómetro 23 de la panamericana norte cruce con Gerardo Unger.

La recolección y preservación de muestras sólidas y sedimentos se realizó tomando de referencia los procedimientos de muestreo estipulados en el CHAPTER FOUR,la cual nos indica que para muestras volátiles o fracciones ligeras deben ser recolectadas en frasco de vidrio y ámbar con tapa teflonada teniendo la preservación a una temperatura de 0-6°C, hasta al llegada al laboratorio para su respectivo análisis.

3.9 Procedimiento de preparación de muestra

1) Se pesa aproximadamente 5± 1 g de muestra en luna de reloj y se agrega a los viales Headspace de 22ml, en la balanza Mettler Toledo.

Fig. 14. Pesado de muestra

2) Se Agrega 10 ml de solución modificadora de matriz (SMM), en el vial Headspace de 22ml.

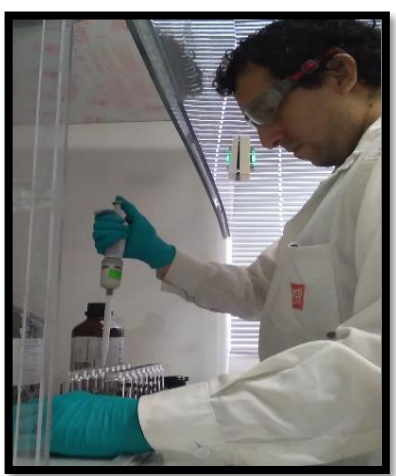

Fig. 15. Adiciones de solución modificadora

3) Se Adiciona 250 ul de solución estándar de surrogado (BFB),100mg/L.

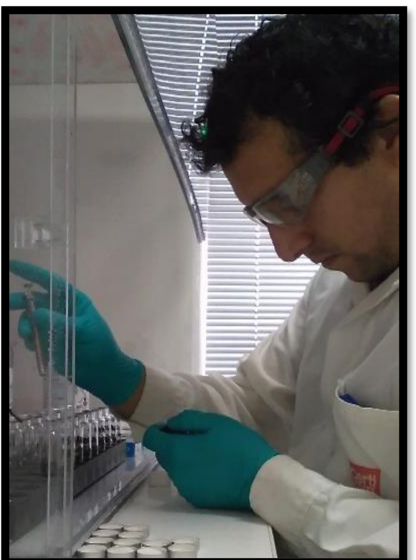

Fig. 16. Adiciones de estándar surrogado

4) Se tapa el vial Headspace con el Tool Crimping.

Fig. 17. Adiciones de estándar surrogado

5) Se llevará al automuestreador Headspace para su respectiva extracción.

Fig. 18. Lectura de GC-HS

6) Posteriormente el auto inyector contiene los vapores que ingresara a la columna Cromatográfica para su respectiva separación y llegando al detector de ionización de llama, donde la muestra se destruye originado un perfil Cromatográfico (adquisición de datos), tal como se muestra a continuación.

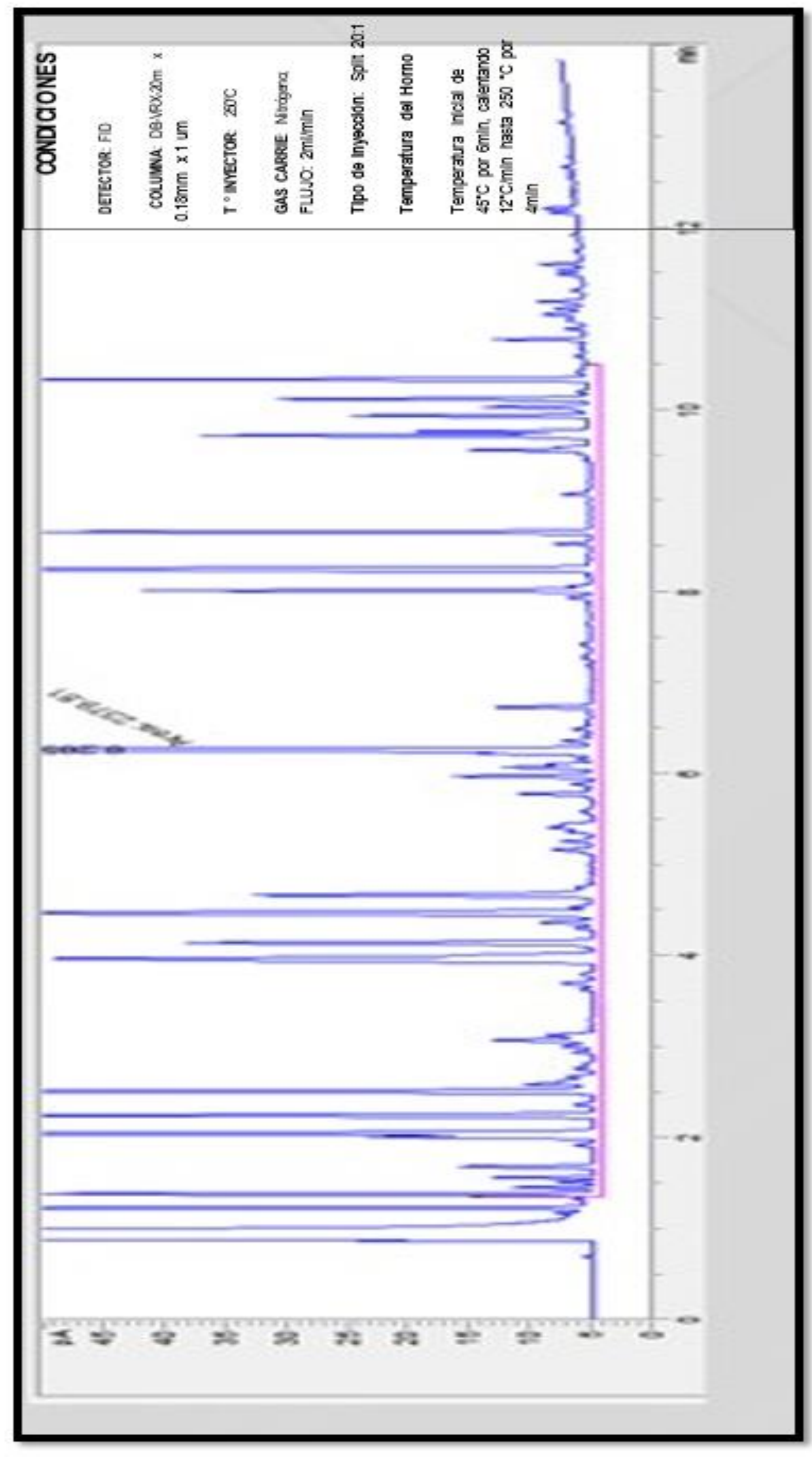

Fig. 19. Perfil Cromatográfíca de una muestra de suelo (t: 1.3min -10.5 min), donde está presente los hidrocarburos ligeros

3.10 Procedimiento del análisis instrumental

1) Las muestras son analizadas en un batch llamado secuencia. La secuencia comienza con la verificación de la calibración, seguido por el análisis de las muestras ya adicionalmente se analiza un estándar de verificación (CON-VER) cada 12 horas.

2) Las muestras son analizadas con el mismo método instrumental que se usó durante la calibración.

3) Las muestras se diluyen para que la concentración de analitos esté dentro del rango de aceptación de la curva de calibración.

4) La medición del área por encima de la línea de base se integra.

3.11 Calibración del cromatógrafo de gases con detector de ionización de Llama

Para el proceso de calibración del equipo GC/FID, se debe correr un IM (estándar para definir los tiempo de retención).tal como se muestra en la tabla 13 y el cromatográma en la figura 20.

Tabla 13: Tiempo de retención para definir los intervalos de integración de Rango Orgánico de Gasolina

Compuesto	Tiempo de retención (tr)
C6-C11	2.4min – 10.15min

Fuente: Elaboración propia

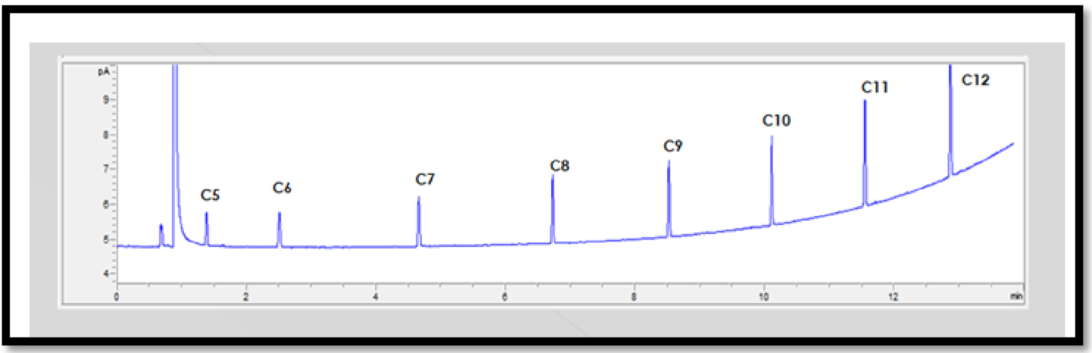

Fig. 20. Tiempo de elución del estándar alifático

A continuación, en la Tabla 14, se muestra los puntos de calibración en mg/L del estándar de gasolina con las áreas correspondientes y en la Fig. 20 se muestra la ecuación lineal y el factor de correlación R2 = 0.99801

Tabla 14: Punto de calibración

Calibration Table

Enter | Delete | Insert... | Print | OK | Help

#	RT	Signal	Compound	Lvl	Amt[mg/L]	Area	Rsp.Factor	Ref	ISTD	#
1	6.400	FID2 B	F1	1	0.500	206.420	2.4222e-3	No	No	
				2	1.000	326.510	3.0627e-3			
				3	2.000	528.610	3.7835e-3			
				4	3.000	776.270	3.8646e-3			
				5	4.000	889.020	4.4993e-3			
				6	5.000	1219.200	4.1012e-3			
				7	7.000	1513.500	4.6251e-3			
				8	8.000	1827.200	4.3782e-3			
				9	10.000	2181.900	4.5832e-3			

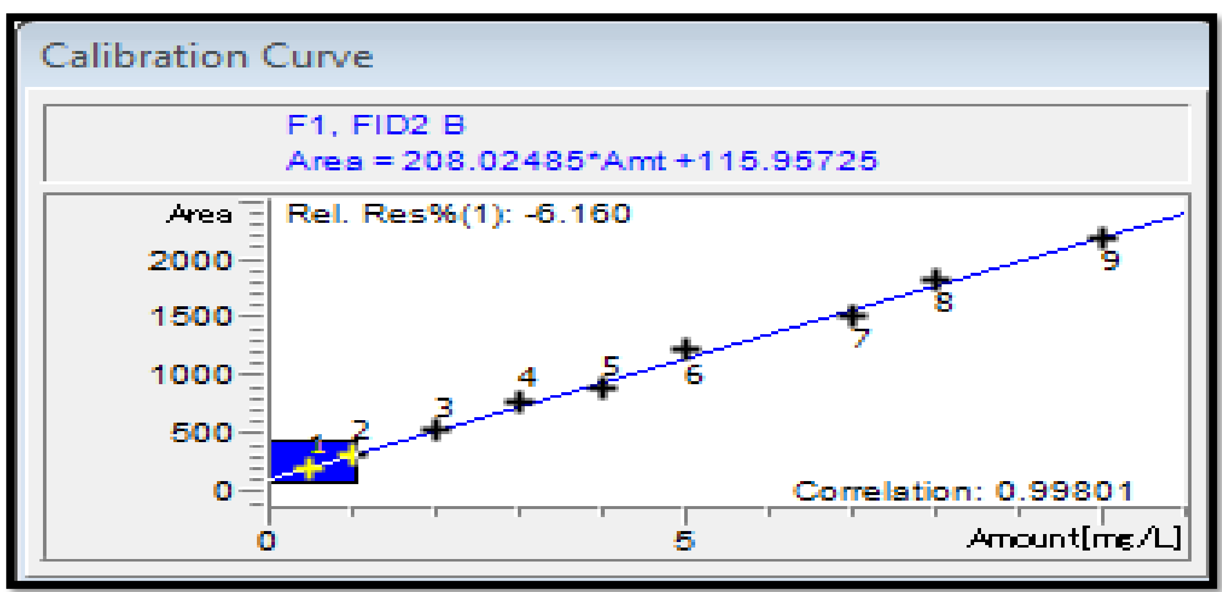

Fig. 21 Ecuación del área vs concentración (mg/L)

3.12 Lectura mediante cromatografo de gases con detector de ionización de Llama

Para la lectura de la fracción liviana de hidrocarburos, el equipo toma una alícuota de 1ul para las muestras colocadas en el rack.

✓ Abrir el software y cargar el método. Crear la secuencia de muestras para un buen análisis; seguir la siguiente secuencia.

Tabla 15: secuencia de lectura

Vial	Nombre de la muestra	Nombre del método	Inyección del vial
1	Rinse	TPH-F1-GRO-RUN	1
2	IM	TPH-F1-GRO-RUN	1
3	Rinse	TPH-F1-GRO-RUN	1
4	CON-CCB	TPH-F1-GRO-RUN	1
5	CON-CCV	TPH-F1-GRO-RUN	1
6	CON-LIM	TPH-F1-GRO-RUN	1
7	CON-BLK	TPH-F1-GRO-RUN	1
8	CON-LCS	TPH-F1-GRO-RUN	1
9	Rinse	TPH-F1-GRO-RUN	1
10	SMP	TPH-F1-GRO-RUN	1
11	CON-DUP	TPH-F1-GRO-RUN	1
12	CON-ADI	TPH-F1-GRO-RUN	1
13	Rinse	TPH-F1-GRO-RUN	1
14	R-CH-1	TPH-F1-GRO-RUN	1
15	R-CH-2	TPH-F1-GRO-RUN	1
16	R-CH-3	TPH-F1-GRO-RUN	1
17	R-CH-4	TPH-F1-GRO-RUN	1
18	R-CH-5	TPH-F1-GRO-RUN	1
19	R-CH-6	TPH-F1-GRO-RUN	1
20	R-CH-7	TPH-F1-GRO-RUN	1
21	R-CH-8	TPH-F1-GRO-RUN	1
22	R-CH-9	TPH-F1-GRO-RUN	1
23	R-CH-10	TPH-F1-GRO-RUN	1

Fuente: Elaboración propia

3.13 Verificación de calibración

La curva de calibración y los tiempos de retención se verifican cada 12 horas, esta verificación es complementada con la medición de 1 o más estándares (concentración de CON-LCS), que contiene a los analitos de interés y surrogado.

Para los analitos y surrogado o n-alcanos en el análisis de verificación de la calibración deben ser previamente establecidas las ventanas de tiempo de retención de la n-alcano de C6-C10. (EPA 8000C ,2003)

3.14 Cálculos de concentraciones

Los resultados del equipo CG/FID tras procesar las muestras y definir el rango de calibración para la fracción C6-C10 (GRO).

La curva o rectas de calibrado para la fracción ligera se relaciona el área de integración versus la concentración en mg/L., dando una ecuación lineal la cual es Área(A)= 208.02485*Conc+115.95725, la cual aparece en la Fig. 21.
Para expresar estos resultados obtenidos en mg/kg de suelo en base seca, se utiliza la siguiente ecuación de conversión para los hidrocarburos.

$$\frac{mg}{Kg}MS = (\frac{C \ x \ V_L \ x \ F_d}{W \ x \ F_s})$$

Dónde:

C (mg/L): concentración dada por equipo GC/FID V_L: Volumen (mL)

F_d: Factor de dilución

W: peso en gramo (g)

F_s: Factor seco

IV. RESULTADOS

4.1 Condiciones de Análisis

Las condiciones para la identificación y cuantificación de Hidrocarburos Ligeros C6- C10 en un Cromatógrafo de Gases con Detector de Ionización de Llama (GC/FID) acoplada a un Automuestreador Headspace, en la Tabla 16 y 17 muestran los parámetros con sus condiciones del cromatógrafo y Headspace respectivamente.

4.1.1 Cromatografo de gases

A continuación, se mencionan el parámetro y condiciones del Cromatógrafo de Gases

Tabla 16: Condiciones del cromatografo de gases para el análisis de hidrocarburos ligeros

Parámetro	Condiciones			
Detector	FID/250°C			
Inyector	250°C			
Columna	Columna cromatografía Agilent J&W DB-VRX 20m x 0.18mm x 1.0 m			
Rampa del Horno	Rate °C/ min	Value °C	Hold Time min	Run Time min
Inicial	-	45	6	6
Rampa 1	12	250	4	27.083

Fuente: Elaboración propia

4.1.2 Headspace

A continuación se mencionan el parámetro y condiciones del Headspace.

Tabla 17: Condiciones del automuestreador Headspace en el Análisis Hidrocarburos

Parámetro	**Condiciones**
Temperatura	Oven: 80°C
	Loop: 90°C
	Transfer Line
Tiempo	Tiempo de Equilibrio :15 min
	Tiempo de Inyección : 1min
	Ciclos del CG :35 min
Vial and Loop	Vial size: 20ml Frecuencia: 36shakes/min Aceleración:125 cm/s2 Presión : 15 psi

Fuente: Elaboración propia

4.2 Resultados de las muestras de suelos

En la Tabla 18, se muestras las concentraciones en mg/Kg MS de los 10 puntos de suelos tomados en los meses de octubre 2106 a enero 2017.En donde las concentraciones más altas están en los puntos 3 y 9 respectivamente.

Tabla 18: Resultados de hidrocarburos livianos en suelo

	Muestra	Tipo de muestra	Código	Concentración de Hidrocarburos ligeros: mg/kg MS de suelo			
				octubre	noviembre	diciembre	enero
Rio Chillón	1	suelo	C-Suelo-1	1.32	1.69	1.52	1.05
	2	suelo	C-Suelo-2	1.75	1.73	1.50	1.05
	3	suelo	C-Suelo-3	1.06	<LC	<LC	**4.99**
	4	suelo	C-Suelo-4	<LC	<LC	<LC	<LC
	5	suelo	C-Suelo-5	1.48	1.54	1.08	1.15
	6	suelo	C-Suelo-6	1.24	1.20	1.50	1.09
	7	suelo	C-Suelo-7	<LC	1.15	<LC	1.95
	8	suelo	C-Suelo-8	<LC	<LC	<LC	<LC
	9	suelo	C-Suelo-9	1.48	<LC	<LC	**5.72**
	10	suelo	C-Suelo-10	1.69	1.75	1.45	2.30

Fuente: Elaboración propia

4.3 Resultados de las muestras de sedimentos

En la Tabla 19 se muestras las concentraciones en mg/Kg MS de los 10 puntos de sedimentos tomados en los meses de octubre 2106 a enero 2017.en donde las concentraciones más altas están en los puntos 6 y 7 respectivamente.

Tabla 19: Resultados de hidrocarburos livianos en sedimentos

	Muestra	Tipo de muestra	Código	Concentración de Hidrocarburos Ligeros: mg/kg MS de Sedimento			
				octubre	noviembre	diciembre	enero
Rio Chillón	1	sedimento	C-Sed-1	2.92	2.09	2.10	3.00
	2	sedimento	C-Sed-2	2.35	<LC	2.50	2.05
	3	sedimento	C-Sed-3	3.06	3.2	3.12	1.05
	4	sedimento	C-Sed-4	<LC	<LC	<LC	<LC
	5	sedimento	C-Sed-5	2.81	2.40	2.18	2.55
	6	sedimento	C-Sed-6	2.54	2.50	2.80	**22.91**
	7	sedimento	C-Sed-7	2.52	<LC	<LC	**21.20**
	8	sedimento	C-Sed-8	<LC	<LC	<LC	<LC
	9	sedimento	C-Sed-9	5.48	3.89	3.45	1.50
	10	sedimento	C-Sed-10	2.95	2.75	2.52	3.30

Fuente: Elaboración propia

V. DISCUSIONES

Se evaluaron las muestras de suelos y sedimentos del Rio Chillón, en donde se encontraron concentraciones de hidrocarburos ligeros en los meses de octubre, noviembre, diciembre del 2016 a enero del 2017. Así mismo estos resultados se comparan con los resultados de Hidrocarburos ligeros según la tesis de (Rosales, 2013,p.205); donde T1: Primero Sondeo o muestreo, T2: Segundo sondeo o muestreo y T3: Tercer sondeo o muestreo tal como se muestra en la tabla a continuación.

Tabla 20: Resultados de Rosales sobre hidrocarburos ligeros en suelos mg/kg MS

	GROs(media), mg/kg Ms de suelo		
Profundidad (m)	T1	T2	T3
1	0.0140	0.02092	**3.18574**
2	0.00602	0.01741	0.01763
3	0.03771	0.01809	0.0653
4	0.0899	0.01011	0.0649
5	0.0579	0.0509	
6	0.03704	0.0422	
7	0.0633	0.0825	
8	0.01340	0.0283	
9	0.0340	0.0324	
10	0.0811	0.0218	

Fuente: Rosales, 2013

Tabla 21: Resultados de hidrocarburos livianos en suelos mg/kg MS

	Muestra	Tipo de muestra	Código	Concentración de Hidrocarburos ligeros: mg/kg MS de suelo			
				octubre	noviembre	diciembre	enero
Rio Chillón	1	suelo	C-Suelo-1	1.32	1.69	1.52	1.05
	2	suelo	C-Suelo-2	1.75	1.73	1.50	1.05
	3	suelo	C-Suelo-3	1.06	<LC	<LC	**4.99**
	4	suelo	C-Suelo-4	<LC	<LC	<LC	<LC
	5	suelo	C-Suelo-5	1.48	1.54	1.08	1.15
	6	suelo	C-Suelo-6	1.24	1.20	1.50	1.09
	7	suelo	C-Suelo-7	<LC	10.15	<LC	1.95
	8	suelo	C-Suelo-8	<LC	<LC	<LC	<LC
	9	suelo	C-Suelo-9	1.48	<LC	<LC	**5.72**
	10	suelo	C-Suelo-10	1.69	1.75	1.45	2.30

Fuente: Elaboración propia

En los resultados de Rosales se encontró un valor máximo de GROs que se encuentra situado en los 30 cm del sondeo T3, con un valor de 3.18574 mg/kg MS de suelo, mientras en la determinación de hidrocarburos de GRO se encuentra valores de 4.99 y 5.72 mg/kg MS en las muestras 3, 9 de suelos respectivamente. También se aprecian ciertas discrepancias en tipo de suelos, condiciones y otros factores climáticos; esto puede deberse a la infiltración de derrames ocasionados por el llenado y fugas de tanques de gasolina.

Los resultados en comparación fueron aplicados por las mismas metodologías, cromatografía de gases con automuestreador Headspace (EPA 8015, EPA 5021).

-Los datos de Hidrocarburos fracción Ligera de las muestras de suelos tomadas en Rio Chillón en la estación C-Suelo-3 y C-Suelo-9, muestran promedios de hidrocarburos ligeros similares 4.99 mg/Kg Ms y 5.72 mg/Kg Ms respectivamente, en cambio en las otras estaciones se observan concentraciones muy bajas.

- Los datos de Hidrocarburos fracción Ligera de las muestras de sedimentos tomadas en Rio Chillón en la estación C-Sed-6 y C-Sed-7, muestran promedios de hidrocarburos ligeros similares 22.91 mg/Kg Ms y 21.20 mg/Kg Ms respectivamente, en cambio en las otras estaciones se observan concentraciones muy bajas.

-los niveles encontrados en las muestras recolectadas, son significativos. Los máximos niveles encontrados se produjeron en los dos muestreos del mes de enero tanto para suelos y sedimentos (C-Suelo-3; C-Suelo-9; C-Sed-6, C-Sed-7). Esto indicaría una acumulación de hidrocarburos fracción ligera en las muestras tomadas del Rio Chillón, la cual dependería por los contaminantes vertidos al rio por las empresas que están alrededor.

-“Para explicar esta tendencia de variación en la concentración de Hidrocarburos fracción ligera en los diferentes muestreos, hay que tener en cuenta que los hidrocarburos se introducen al ambiente marino en forma de solución y suspensión coloidal y gotas, lo cuales pueden ser absorbido por el plancton, partículas suspendidas, sedimentos” (Stegeman y Teal, 1973).

-La Técnica cromatografía de gases acoplada a un equipo Headspace, es muy fácil de usar y requiere poco solvente, el tiempo de lectura por muestra dura aproximadamente 40 minutos para la determinación de suelos y sedimentos.

-el tiempo de elución de los hidrocarburos fracción volátiles, se dan en los primeros 15 minutos como, por ejemplo: Benceno., Tolueno, Etilbenceno y los xilenos orto, meta y para en el resto de tiempo salen los otros hidrocarburos con mayor peso molecular.

VI. CONCLUSIONES

- Las muestras de suelos que se tomaron en el mes de enero en el Rio Chillón identificadas con C-Suelo-3 y C-Suelo-9, presentan concentraciones de Hidrocarburos ligeros de 4.99 mg/kg MS y 5.72 mg/kg MS por encima de los límites máximos permisibles establecidos por los Estándares de Calidad Ambiental de Suelos (2013), esto puede ser debido, a que el lugar en el que se encuentra es zona de vertedero de desechos de la actividad industrial eléctrica entre otras.

- Las concentraciones de hidrocarburos Ligeros fueron mayores en sedimentos que en suelos, lo que refleja que los vertimientos de hidrocarburos en cuerpos de agua que van acumulando en el tiempo, tal como se observa en la Tabla 17 y 18 resultados de Hidrocarburos Ligeros en sedimentos.

- En las muestras de suelos tomadas en los meses de octubre a diciembre presenta concentraciones de Hidrocarburos Alifáticos Ligeros, alcanzando los límites máximos permisibles establecidos por los estándares de calidad ambiental (ECA) para suelos.

- El Análisis por Cromatografía de Gases acoplado con un automuestreador Headspace (EPA 8015, EPA 5021) es una técnica muy específica para la identificación y Cuantificación de Hidrocarburos Ligeros C6-C10 porque hace posible que analice los hidrocarburos fracción liviana sin la posibilidad de perderse en el análisis.

- Los valores de límites máximos permisibles (LMP) establecido en los Estándares de Calidad Ambiental son Benceno 0.03 mg/kg Ms, Tolueno 0.37 mg/kg Ms, Etilbenceno 0.082 mg/kg Ms , o-xileno 3.6mg/kg Ms, y naftaleno 0.1 mg/kg Ms; una suma total de 4.18 mg/kg Ms de Hidrocarburos Ligeros.

VII. RECOMENDACIONES

- Se recomienda utilizar frascos estériles y tapas de teflón, para evitar las contaminaciones o interferentes de matriz.
- Tratar de preparar las muestras de inmediato una vez llegada al laboratorio y evitar la pérdida de hidrocarburos ligeros C6-C10.
- En el proceso de análisis, para disminuir alguna interferencia trabajar con agua ultra pura o agua libre de compuestos orgánicos.
- En el proceso de preparación de soluciones, utilizar metanol grado purga y trampa o metanol grado GC.
- Se debe utilizar una solución modificadora preparada con 180 g de Cloruro de Sodio en 500 ml de agua libre de orgánico, para evitar la pérdida de analitos con alto contenido de materia orgánica (EPA 5021A, 2014).

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IX. INDICE DE ANEXOS

ANEXO 1
FIGURAS DE PUNTO DE MUESTREO Y EQUIPOS

Fig. 22 Punto de muestreo parte lateral

Fig. 23 Vista satelital del punto de muestreo en el Rio Chillón.

Fig. 24 Muestreo de suelos del Rio Chillón

Fig. 25 Zona de descargas del sector industrial

Fig. 26 Muestreo de muestras de sedimentos

Fig. 27 Muestreo de muestras de suelos

Fig. 28 Secado de sedimentos para el factor de humedad

Fig. 29 Secado de suelos para el factor de humedad

Fig. 30Cromatógrafo de gases

Fig. 31 Automuestreador   headspace

Fig. 32 Cromatografo de gases acoplado al automuestreador headspace

ANEXO 2

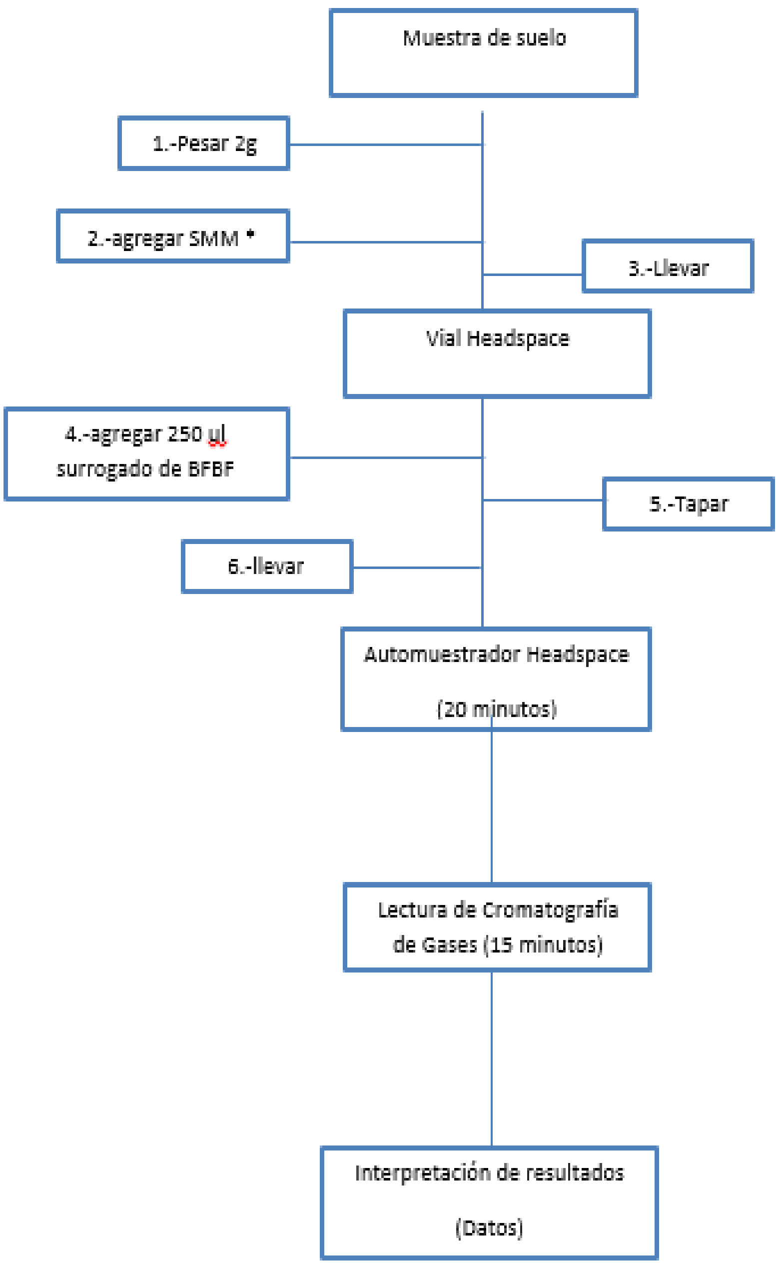
Métodos y parámetros para la determinación de hidrocarburos en suelos y sedimentos

Acrónimo	Compuestos	Definición	Técnica de preparación	Técnica de determinación	comentarios
BTEX	Benceno,Tolueno,Etilbenceno ,xilenos(isómeros)	BTEX	EPA 5021,static Headspace	EPA 8260B :GC/MS	Viales de muestra por punto de muestreo para la determinación de volátiles y de contenido en humedad
GRO	GROS: según Wisconsin DNR (1995):metil-t- butileter.1,2,4-trimetilbenceno.1,3,5-trimetilbenceno	GRO:Rango orgánico de gasolina (Algunos autores incluyen BTEX como GRO). EPA Y API: rango de C6-C10 con puntos de ebullición de 60-170°C) Según Wisconsin DNR (1995) el rango de ebullición desde 55.2°C-220°C incluyendo el naftaleno 218°C	EPA 5035:Purga y trampa	EPA 8021:GC/PID	Wisconsin DNR (1995), define GRO como todas las respuestas cromatograficos entre comienzo del pico del metil-t-butileter y la conclusión en el pico del naftaleno. La cuantificación está basada en la comparación directa del rango total en este rango respecto a un estándar de componentes de una gasolina, que contiene la mezcla de D componentes mas típicos
	GRO:n-hexano,ciclohexano,benceno,n-heptano,metilciclohexano,tolueno,n-octano,etilbenceno,p-xileno,n-nonano,n-decano y naftaleno		EPA5032	EPA 8015B:GC/FID.	

Fuente: Rosales (2013)

ANEXO 3

Flujo del proceso de análisis para la determinación de hidrocarburos ligeros fracción C6-C10



Fuente Elaboración propia

ANEXO 4

Límites máximos permisibles de hidrocarburos según el tipo de suelo

Fracción de hidrocarburos	Uso de suelo predominante (mg/kg base seca)[1]		
	Agrícola[2]	Residencial[3]	Industrial
Ligera	200	200	500
Media	1200	1200	1200
Pesada	3000	3000	6000

Fuente: NOM-138-SEMARNAT/SS-2003

1. Para usos de suelo mixto, deberá aplicarse la especificación al menor valor de los usos de los suelos involucrados

2. Agrícola incluye suelo forestal, recreativo y de conservación

3. Incluye comercial

ANEXO 5

Copias de certificados de análisis de patrones de estándares

RESTEK CERTIFIED REFERENCE MATERIAL

110 Benner Circle
Bellefonte, PA 16823-8812
Tel: (800)356-1688
Fax: (814)353-1309
www.restek.com

Certificate of Analysis

ACCREDITED

ilac-MRA

ACCREDITED

FOR LABORATORY USE ONLY-READ SDS PRIOR TO USE.
This Reference Material is intended for Laboratory Use Only as a standard for the qualitative and/or quantitative determination of the analyte(s) listed.

Catalog No. : 30082 **Lot No.:** A0108738

Description : 4-Bromofluorobenzene Standard

4-Bromofluorobenzene 10,000µg/mL, P&T Methanol, 1mL/ampul

Container Size : 2 mL **Pkg Amt:** > 1 mL

Expiration Date : April 30, 2020 **Storage:** 0°C or colder

CERTIFIED VALUES

Elution Order	Compound	Grav. Conc. (weight/volume)	Expanded Uncertainty (95% C.L.; K=2)
1	1-Bromo-4-fluorobenzene (BFB) CAS # 460-00-4 (Lot 20401KOV) Purity 99%	10,004.0 µg/mL	+/- 58.5756 µg/mL Gravimetric +/- 113.0222 µg/mL Unstressed +/- 129.9960 µg/mL Stressed

Solvent: P&T Methanol
CAS # 67-56-1
Purity 99%

Fig. 33 Certificado del surrogado 4-bromofluorobenceno

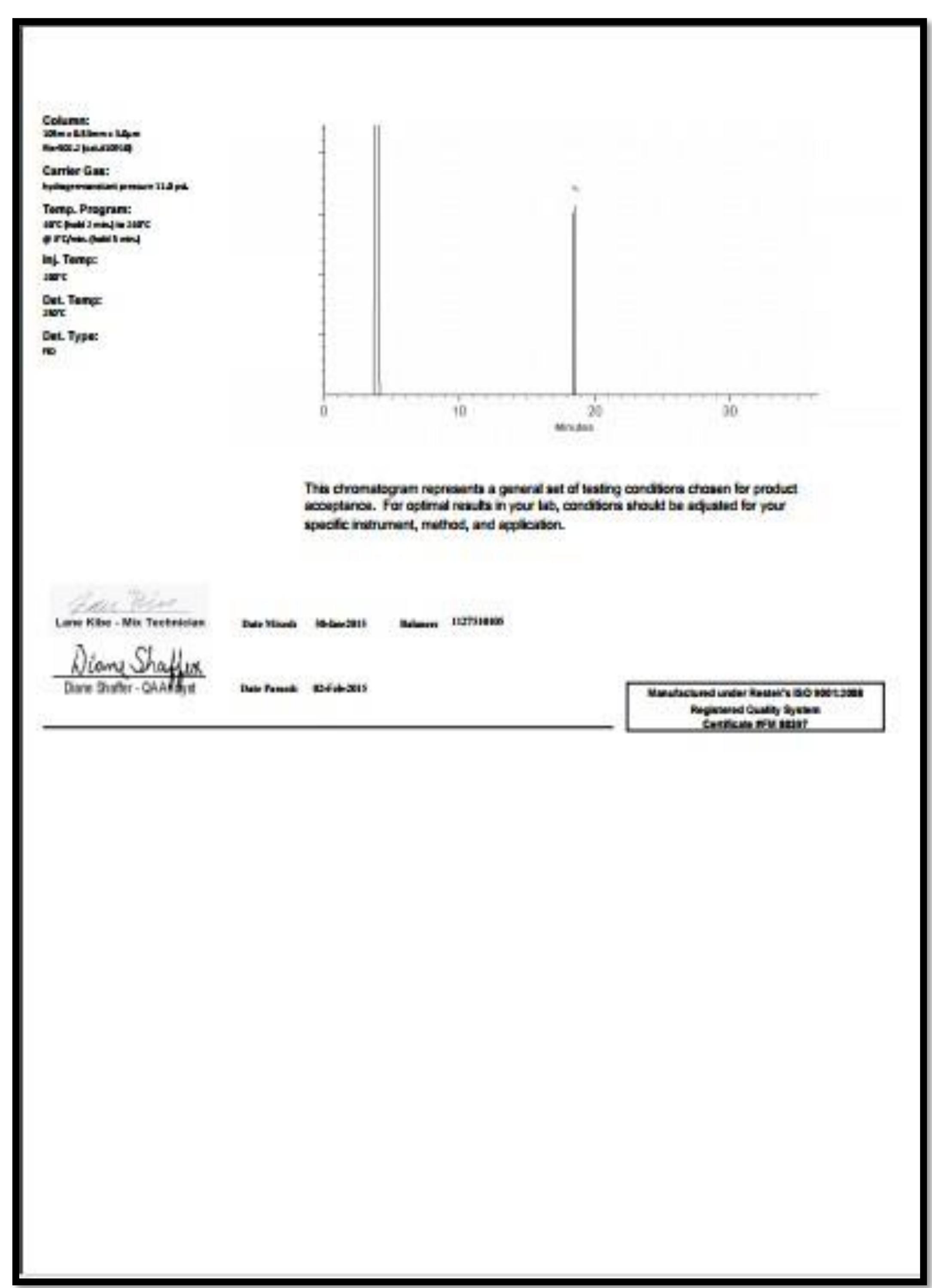
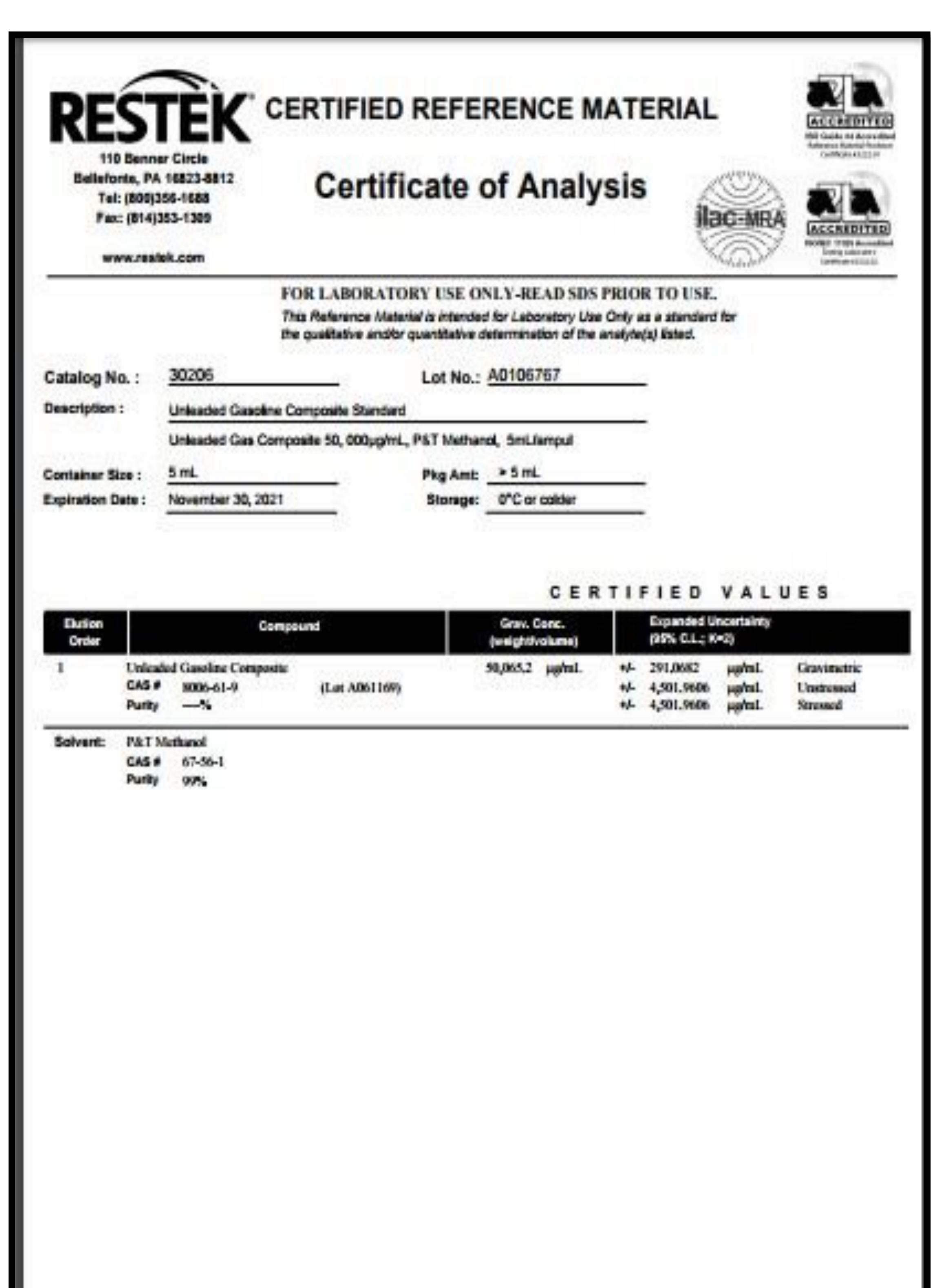


Fig. 34 Perfil del surrogado 4-bromofluorobenceno



RESTEK CERTIFIED REFERENCE MATERIAL

110 Benner Circle
Bellefonte, PA 16823-8812
Tel: (800)356-1688
Fax: (814)353-1309
www.restek.com

Certificate of Analysis

FOR LABORATORY USE ONLY-READ SDS PRIOR TO USE.

This Reference Material is intended for Laboratory Use Only as a standard for the qualitative and/or quantitative determination of the analyte(s) listed.

Catalog No. : 30206 Lot No.: A0106767

Description : Unleaded Gasoline Composite Standard

Unleaded Gas Composite 50, 000µg/mL, P&T Methanol, 5mL/ampul

Container Size : 5 mL Pkg Amt: > 5 mL

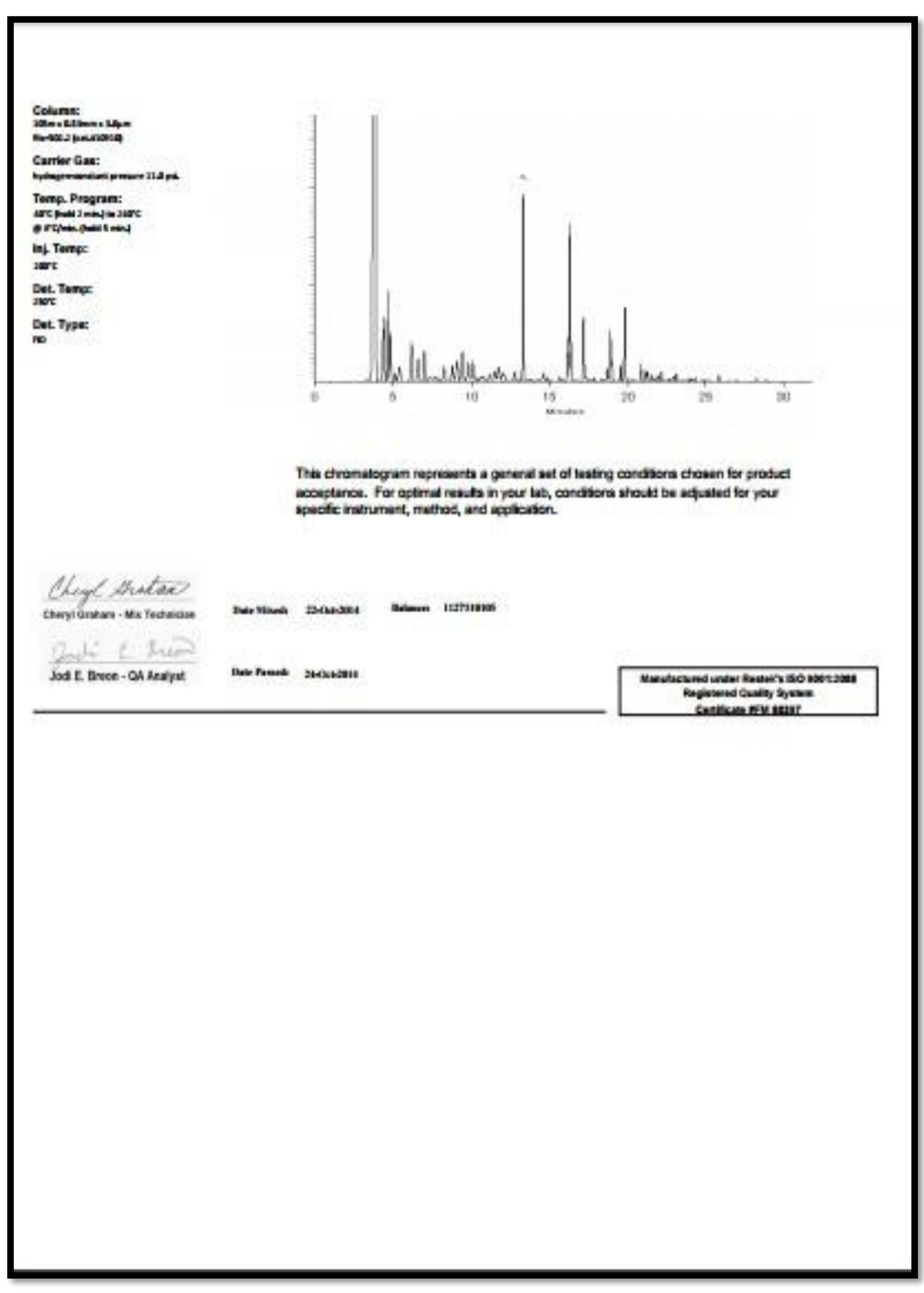
Expiration Date : November 30, 2021 Storage: 0°C or colder

CERTIFIED VALUES

Elution Order	Compound			Grav. Conc. (weight/volume)	Expanded Uncertainty (95% C.L.; K=2)			
1	Unleaded Gasoline Composite			50,065.2 µg/mL	+/-	291.0682	µg/mL	Gravimetric
	CAS #	8006-61-9	(Lot A061169)		+/-	4,501.9606	µg/mL	Unstressed
	Purity	—%			+/-	4,501.9606	µg/mL	Stressed

Solvent: P&T Methanol
CAS # 67-56-1
Purity 99%

Fig. 35 Certificado del estándar de gasolina


Column:

[illegible]

Carrier Gas:

[illegible]

Temp. Program:

[illegible]

Inj. Temp:

[illegible]

Det. Temp:

[illegible]

Det. Type:

[illegible]

This chromatogram represents a general set of testing conditions chosen for product acceptance. For optimal results in your lab, conditions should be adjusted for your specific instrument, method, and application.

Cheryl Graham - Mix Technician

Jodi E. Breon - QA Analyst

Manufactured under Restek's ISO 9001:[illegible]
Registered Quality System
Certificate [illegible]

Fig. 36 Perfil del estándar de gasolina

ANEXO 6

Perfiles cromatográficos en el cromatógrafo de gases

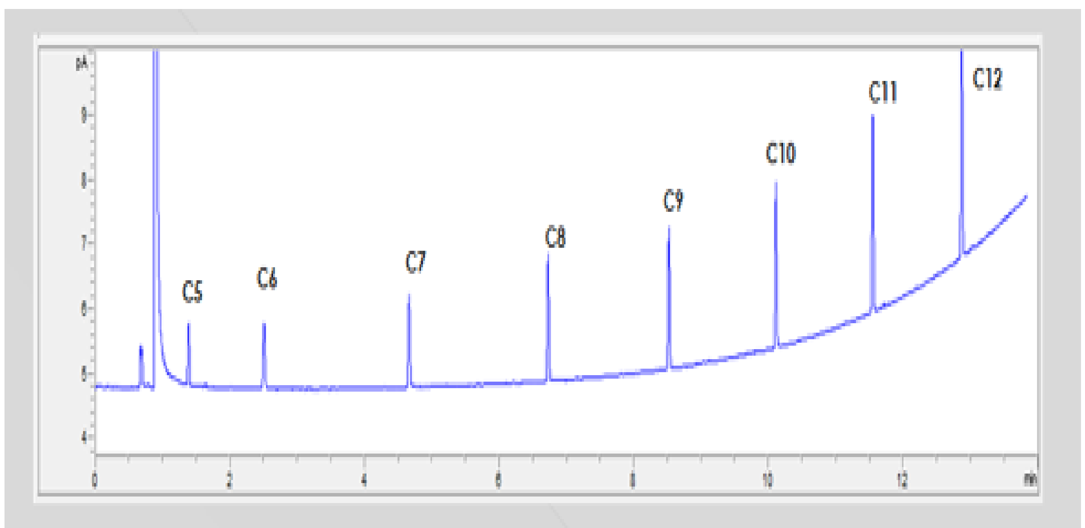


Fig. 37 Perfil Cromatográfíco de un estándar alifático para obtener el tiempo de retención

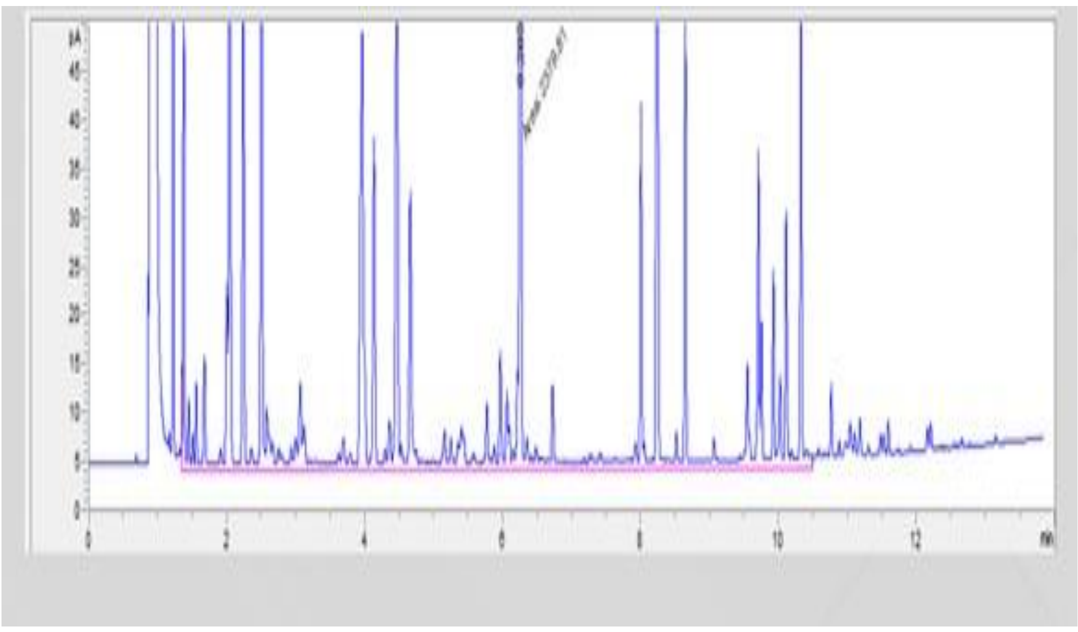


Fig. 38 Perfil Cromatográfíco de un estándar control del laboratorio de hidrocarburos ligeros

ANEXO 7

Método de la norma de la Agencia de Protección Ambiental (EPA 8015C)

METHOD 8015C

NONHALOGENATED ORGANICS BY GAS CHROMATOGRAPHY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed Standard Operating Procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 This method may be used to determine the concentrations of various nonhalogenated volatile organic compounds and semivolatile organic compounds by gas chromatography. The following RCRA compounds were quantitatively determined by this method, using the preparative techniques indicated.

Compound	CAS No.[a]	Appropriate Technique				
		Purge-and-Trap[b]	Head-space[c]	Direct Aqueous Injection	Azeo. Dist.[c]	Vacuum Dist.[d]
Acetone	67-64-1	pp / ht	x	x	x	x
Acetonitrile	75-05-8	pp	ne	x	x	ne
Acrolein	107-02-8	pp	ne	x	x	x
Acrylonitrile	107-13-1	pp	ne	x	x	x
Allyl alcohol	107-18-6	ht	ne	x	x	ne
t-Amyl alcohol (TAA)	75-85-4	ht	x	ne	ne	x
t-Amyl ethyl ether (TAEE)	919-94-8	x/ ht	x	ne	ne	x
t-Amyl methyl ether (TAME)	994-05-8	x/ ht	x	ne	ne	x
Benzene	71-43-2	x	x	ne	ne	x
t-Butyl alcohol (TBA)	75-65-0	ht	x	x	x	x
Crotonaldehyde	123-73-9	pp	ne	x	x	ne
Diethyl ether	60-29-7	x	ne	x	ne	ne
Diisopropyl ether (DIPE)	108-20-3	x/ ht	x	ne	ne	x
Ethanol	64-17-5	I	x	x	x	x
Ethyl acetate	141-78-6	I	x	x	x	ne
Ethyl benzene	100-41-4	x	x	ne	ne	x
Ethylene oxide	75-21-8	I	ne	x	x	ne
Ethyl tert-butyl ether (ETBE)	637-92-3	x/ ht	x	ne	ne	x
Isopropyl alcohol (2-Propanol)	67-63-0	pp	x	x	x	ne

Compound	CAS No.[a]	Appropriate Technique				
		Purge-and-Trap[b]	Head-space[e]	Direct Aqueous Injection	Azeo. Dist.[c]	Vacuum Dist.[d]
Methanol	67-56-1	I	x	x	x	ne
Methyl ethyl ketone (MEK, 2-Butanone)	78-93-3	pp	x	x	x	x
Methyl *tert*-butyl ether (MTBE)	1634-04-4	x/ ht	x	x	ne	x
N-Nitroso-di-*n*-butylamine	924-16-3	pp	ne	x	x	ne
Paraldehyde	123-63-7	pp	ne	x	x	ne
2-Pentanone	107-87-9	pp	x	x	x	ne
2-Picoline	109-06-8	pp	ne	x	x	ne
1-Propanol (*n*-Propyl alcohol)	71-23-8	pp	x	x	x	ne
Propionitrile (Ethyl cyanide)	107-12-0	ht	ne	x	x	ne
Pyridine	110-86-1	I	ne	x	x	ne
Toluene	108-88-3	x	x	ne	ne	x
o-Toluidine	95-53-4	I	ne	x	x	ne
o-Xylene	95-47-6	x	x	ne	ne	x
m-Xylene	108-38-3	x	x	ne	ne	x
p-Xylene	106-42-3	x	x	ne	ne	x

a Chemical Abstract Service Registry Number
b Purge-and-Trap (Methods 5030 or 5035)
c Azeotropic distillation (Method 5031)
d Vacuum distillation (Method 5032)
e Headspace (Method 5021)
x Adequate response using this technique
ht Method analyte only when purged at 80 °C (high temperature purge)
I Inappropriate technique for this analyte
ne Not evaluated
pp Poor purging efficiency, resulting in higher limits of quantitation. Use of an alternative sample preparative method is strongly recommended. May be amenable to purging at elevated temperature.

1.2 This method may be applicable to the analysis of other analytes, including triethylamine and petroleum hydrocarbons. The petroleum hydrocarbons include gasoline range organics (GRO) and diesel range organics (DRO). The sample preparation techniques are shown in the table below.

Compound	CAS No.[a]	Appropriate Technique			
		Purge-and-Trap	Head-space	Direct Aqueous Injection	Solvent Extraction
Triethylamine	121-44-8	I	ne	x	I
Gasoline range organics (GRO)	--	x	x	x	I
Diesel range organics (DRO)	--	I	x	I	x

a Chemical Abstract Service Registry Number
x: Adequate response using this technique; I: Inappropriate technique for this analyte;
ne: Not evaluated

1.2.1 This method was applied to the analysis of triethylamine in water samples by direct aqueous injection onto a different GC column than is used for any other analytes. Descriptions of the GC column, temperature program, and performance data for triethylamine are provided in this method (see Secs. 6.2.5 and 11.2.6, and Table 6).

1.2.2 GRO corresponds to the range of alkanes from C_6 to C_{10} and a boiling point range of approximately 60 °C - 170 °C (Reference 6). DRO corresponds to the range of alkanes from C_{10} to C_{28} and a boiling point range of approximately 170 °C - 430 °C (Reference 6). The quantitative analyses of these fuel types are based on the procedures described in Sec. 11.11. The identification of specific fuel types may be complicated by environmental processes such as evaporation, biodegradation, or when more than one fuel type is present. Methods from other sources may be more appropriate for GRO and DRO, since these hydrocarbons are not regulated under RCRA. Consult State and local regulatory authorities for any specific regulatory requirements.

1.2.3 This method may be applicable to classes of analytes and to fuel types and petroleum hydrocarbons other than those listed in Secs. 1.1 and 1.2. However, in order to be used for additional analytes, fuel types, or petroleum hydrocarbons, the analyst must demonstrate that the gas chromatographic conditions, including the GC column, are appropriate for the analytes of interest. The analyst must also perform the initial demonstration of proficiency described in Sec. 9.4 and Method 8000. Expansion of this method to other fuel types or petroleum hydrocarbons will also necessitate careful defining of the boiling point range or carbon number range of the material and modification of the quantitation approach to match such ranges. Analysts are advised to consult authoritative sources, such as the American Petroleum Institute (API), for relevant definitions of other fuel types or petroleum fractions.

NOTE: Mention of the analyses of other fuel types and petroleum fractions does *not* imply a regulatory requirement for such analyses, using this or any other method.

1.3 This method can also be used as a screening tool (for both volatile and semivolatile organics) to obtain semiquantitative data to prevent overloading the GC/MS system during quantitative analysis. This may be accomplished using a purge-and-trap method (e.g., Method 5030), an automated headspace method (e.g., Method 5021), direct aqueous injection, or by direct injection, if a solvent extraction method has been utilized for sample preparation. Single-point calibration is acceptable in this situation. Performance data are not provided for screening.

1.4 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing

requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.5 This method is restricted for use by, or under the supervision of, analysts appropriately experienced and trained in the use of a gas chromatograph and skilled in the interpretation of gas chromatograms. In addition, if this method is to be used for the analysis of petroleum hydrocarbons, its use then should be limited to analysts experienced in the interpretation of hydrocarbon data. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 This method provides gas chromatographic conditions for the detection of certain nonhalogenated volatile and semivolatile organic compounds.

2.2 Depending on the analytes of interest, samples may be introduced into the GC by a variety of techniques, including:

- Purge-and-trap (Methods 5030 or 5035)
- Equilibrium headspace (Method 5021)
- Direct injection of aqueous samples
- Injection of the concentrate from azeotropic distillation (Method 5031)
- Vacuum distillation (Method 5032)
- Following solvent extraction (Methods 3510, 3520, 3535, 3540, 3541, 3545, 3546, 3550, 3560, or other appropriate technique)

2.3 Groundwater or surface water samples generally need to be analyzed in conjunction with Methods 5021, 5030, 5031, 5032, 3510, 3520, or other appropriate preparatory methods to obtain the necessary lower limits of quantitation. Method 3535 (solid-phase extraction) may also be applicable to some of the target analytes, however, this method has not been validated by EPA in conjunction with this determinative method.

2.4 Samples to be analyzed for diesel range organics may be prepared by an appropriate solvent extraction method.

2.5 Gasoline range organics may be introduced into the GC/FID by purge-and-trap (Methods 5030 and 5035), automated headspace (Method 5021), vacuum distillation (Method 5032), or other appropriate technique.

2.6 Triethylamine may be analyzed by direct injection of aqueous samples. This compound has not been found to be amenable to purge-and-trap techniques.

2.7 An appropriate column and temperature program are used in the gas chromatograph to separate the organic compounds. Detection is achieved by a flame ionization detector (FID).

2.8 This method allows the use of capillary or packed columns for the analysis and confirmation of the non-halogenated individual analytes. The GC columns and conditions listed have been demonstrated to provide separation of those target analytes. Other columns and conditions may be employed, provided that the analyst demonstrates adequate performance for the intended application.

2.9 The quantitative analyses of GRO and DRO are based on the definitions provided in Sec. 1.2.2 and the procedures described in Sec. 11.11.

2.10 Given the large number of components to be separated, fused-silica capillary columns are necessary for the analysis of petroleum hydrocarbons, including GRO and DRO, and are recommended for all other analytes. A capillary column is also necessary for the analysis of triethylamine.

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four for general guidance on the cleaning of glassware.

4.2 When analyzing for volatile organics, samples can be contaminated by diffusion of volatile organics (particularly chlorofluorocarbons and methylene chloride) through the sample container septum during shipment and storage. A trip blank prepared from organic-free reagent water and carried through sampling and subsequent storage and handling will serve as a check on such contamination.

4.3 Contamination by carryover can occur whenever high-concentration and low-concentration samples are analyzed in sequence. To reduce the potential for carryover, the sample syringe or purging device needs to be rinsed out between samples with an appropriate solvent. Whenever an unusually concentrated sample is encountered, it should be followed by injection of a solvent blank to check for cross contamination.

4.3.1 Clean purging vessels with a detergent solution, rinse with distilled water, and then dry in a 105 °C oven between analyses. Clean syringes or autosamplers by flushing all surfaces that contact samples using appropriate solvents.

4.3.2 All glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water. Drain the glassware and dry it in an oven at 130 °C for several hours or rinse it with methanol and drain. Store dry glassware in a clean environment.

4.4 The flame ionization detector (FID) is a non-selective detector. There is a potential for many non-target compounds present in samples to interfere with this analysis. There is also the potential for analytes to be resolved poorly, especially in samples that contain many analytes. The data user should consider this and may wish to alter the target analyte list accordingly.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list common laboratory glassware (e.g., beakers and flasks).

6.1 Gas chromatograph -- Analytical system equipped with gas chromatograph suitable for solvent injections, direct aqueous injection, headspace, vacuum distillation sample introduction, or purge-and-trap sample introduction, and equipped with all necessary accessories, including detectors, column supplies, recorder, gases, and syringes. A data system for measuring peak heights and/or peak areas is recommended.

6.2 Recommended GC columns

The choice of GC column will depend on the analytes of interest, the expected concentrations, and the intended use of the results. The packed columns listed below are generally used for screening analyses. The capillary columns are necessary for petroleum hydrocarbon analyses and for triethylamine analyses and are recommended for all other analyses.

The columns listed in this section were the columns used to develop the method. The listing of these columns in this method is not intended to exclude the use of other columns that are available or that may be developed. The laboratory may use either the columns listed in this

method or other columns and columns of other dimensions, provided that the laboratory documents method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) that are appropriate for the intended application.

6.2.1 Column 1 -- 8-ft x 0.1-in. ID stainless steel or glass column, packed with 1% SP-1000 on Carbopak-B 60/80 mesh or equivalent.

6.2.2 Column 2 -- 6-ft x 0.1-in. ID stainless steel or glass column, packed with *n*-octane on Porasil-C 100/120 mesh (Durapak) or equivalent.

6.2.3 Column 3 -- 30-m x 0.53-mm ID fused-silica capillary column bonded with DB-Wax (or equivalent), 1-µm film thickness.

6.2.4 Column 4 -- 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 5% methyl silicone (DB-5, SPB-5, RTx, or equivalent), 1.5-µm film thickness.

6.2.5 Column 5 -- 30-m x 0.53-mm ID fused-silica capillary column bonded with HP Basic Wax (or equivalent), 1-µm film thickness. This column is used for triethylamine.

6.2.6 Wide-bore columns should be installed in 1/4-inch injectors, equipped with deactivated liners designed specifically for use with these columns.

6.3 Detector -- Flame ionization (FID)

6.4 Sample introduction and preparation apparatus

6.4.1 Refer to the 5000 series sample preparation methods for the appropriate apparatus for purge-and-trap, headspace, azeotropic distillation, and vacuum distillation analyses.

6.4.2 Samples may also be introduced into the GC via injection of solvent extracts or direct injection of aqueous samples.

6.5 Syringes

6.5.1 5-mL Luer-Lok glass hypodermic and 5-mL gas-tight syringe equipped with a shutoff valve, for volatile analytes.

6.5.2 Microsyringes -- 10- and 25-µL equipped with a 0.006-in. ID needle (Hamilton 702N or equivalent) and 100-µL.

6.6 Volumetric flasks, Class A -- Appropriate sizes equipped with ground-glass stoppers.

6.7 Analytical balance -- 160-g capacity, capable of measuring to 0.0001 g.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Organic-free reagent water -- All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

7.3 Methanol, CH_3OH, pesticide quality or equivalent -- Store away from other solvents.

7.4 Fuels, e.g., gasoline or diesel -- Purchase from a commercial source. Low-boiling components in fuel evaporate quickly. As applicable and available, obtain the fuel from the leaking tank on site.

7.5 Alkane standard -- A standard containing a homologous series of *n*-alkanes for establishing retention times (e.g., C_{10}-C_{28} for diesel).

7.6 Standard solutions

The following sections describe the preparation of stock, intermediate, and working standards for the compounds of interest. This discussion is provided as an example, and other approaches and concentrations of the target compounds may be used, as appropriate for the intended application. See Method 8000 for additional information on the preparation of calibration standards.

7.7 Stock standards -- Stock solutions may be prepared from pure standard materials or purchased as certified solutions. When methanol is a target analyte or when using azeotropic distillation for sample preparation, standards should not be prepared in methanol. Standards must be replaced after 6 months or sooner, if comparison with check standards indicates a problem.

7.8 Secondary dilution standards -- Using stock standard solutions, prepare secondary dilution standards, as needed, that contain the compounds of interest, either singly or mixed together. The secondary dilution standards should be prepared at concentrations such that the aqueous calibration standards prepared in Sec. 7.9 will bracket the working range of the analytical system. Secondary dilution standards should be stored with minimal headspace for volatiles and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.9 Calibration standards -- Prepare calibration standards at a minimum of five different concentrations in organic-free reagent water (for purge-and-trap, direct aqueous injection, azeotropic distillation, or vacuum distillation) or in methylene chloride (for solvent injection) from the secondary dilution of the stock standards. For headspace, prepare the standards as directed in Method 5021. One of the standards should be at or below the concentration equivalent to the appropriate lower limit of quantitation for the project. The

remaining concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC. Each standard should contain each analyte to be determined by this method (e.g., some or all of the compounds listed in Sec. 1.1 may be included). In order to prepare accurate aqueous standard solutions, the following precautions need to be observed:

7.9.1 Do not inject more than 20 µL of methanolic standards into 100 mL of water.

7.9.2 Use a 25-µL Hamilton 702N microsyringe or equivalent (variations in needle geometry will adversely affect the ability to deliver reproducible volumes of methanolic standards into water).

7.9.3 Rapidly inject the primary standard into the filled volumetric flask. Remove the needle as fast as possible after injection.

7.9.4 Mix diluted standards by inverting the flask three times only.

7.9.5 Fill the sample syringe from the standard solution contained in the expanded area of the flask (do not use any solution contained in the neck of the flask).

7.9.6 The negative pressure generated by pipettes makes them inappropriate for routine use in the transfer of spiked solutions. As such, use of pipettes to dilute or transfer aqueous standards should be avoided. When sample transfer is absolutely necessary, (such as in the performance of headspace sample preparation for water samples) only high quality, automatic pipettes should be used, and then with extreme care.

7.9.7 Aqueous standards used for purge-and-trap analyses (Method 5030) are not stable and should be discarded after 1 hr, unless held in sealed vials with zero headspace. If so stored, they may be held for up to 24 hrs. Aqueous standards used for azeotropic distillation (Method 5031) may be stored for up to a month in polytetrafluoroethylene (PTFE)-sealed screw-cap bottles with minimal headspace, at ≤6 °C, and protected from light.

7.9.8 Standards for direct aqueous injection of triethylamine are prepared by dissolving an appropriate weight of neat triethylamine in organic-free reagent water and diluting to volume in a volumetric flask.

7.10 Internal standards (if internal standard calibration is used) -- To use this approach, the analyst needs to select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst needs to further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples. The following internal standards are recommended when preparing samples by azeotropic distillation (Method 5031): 2-chloroacrylonitrile, hexafluoro-2-propanol, and hexafluoro-2-methyl-2-propanol.

7.11 Surrogate standards -- Whenever possible, the analyst should monitor both the performance of the analytical system and the effectiveness of the method in dealing with each

sample matrix, by spiking each sample, standard, and blank with one or two surrogate compounds which are not affected by method interferences.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to Chapter Four, "Organic Analytes," and Method 5035.

8.2 If the headspace technique is used, also see Method 5021.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Methods 3500 and 5000 for QC procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques. If an extract cleanup procedure is performed, refer to Method 3600 for the appropriate QC procedures. Any more specific QC procedures provided in this method will supersede those noted in Methods 8000, 3500, 5000, or 3600.

9.3 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Methods 5000 and 8000 for information on how to accomplish a demonstration of proficiency.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If a peak is observed within the retention time window of any analyte that would prevent the determination of that analyte, determine the source and eliminate it, if possible, before processing the samples. The blanks should be carried through all stages of sample preparation and analysis. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample when surrogates are used. Any method blanks, matrix spike samples, and replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.6.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair. Consult Method 8000 for information on developing acceptance criteria for the MS/MSD.

9.6.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike, when appropriate. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. Consult Method 8000 for information on developing acceptance criteria for the LCS.

9.6.3 Also see Method 8000 for details on carrying out sample quality control procedures for preparation and analysis. In-house method performance criteria for evaluating method performance should be developed using the guidance found in Method 8000.

9.7 Surrogate recoveries

If surrogates are used, the laboratory should evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate limits. Procedures for evaluating the recoveries of multiple surrogates and the associated corrective actions should be defined in an approved project plan.

9.8. It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Secs. 11.3 through 11.5 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Introduction/preparation methods

Various techniques are available for sample introduction. All internal standards, surrogates, and matrix spikes (when applicable) need to be added to samples before introduction into the GC/FID system. Consult the applicable sample introduction method regarding when to add standards.

Other sample introduction techniques may be appropriate for specific applications and the techniques described here also may be appropriate for other matrices and analytes. Whatever technique is employed, *including* those specifically listed below, the analyst *must* demonstrate adequate performance for the analytes of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Sec. 9.6, using a clean reference matrix. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

11.1.1 Direct aqueous injection - This technique involves direct syringe injection of an aliquot of an aqueous sample into the GC injection port. This technique is applicable to the following groups of analytes in this method.

11.1.1.1 Volatile organics (includes GRO)

This technique may involve injection of an aqueous sample containing a very high concentration of analytes. Direct injection of aqueous samples has very limited application in the analysis of volatile organics. It is only appropriate for the determination of volatiles at the toxicity characteristic (TC) regulatory limits or at concentrations in excess of 10,000 µg/L. It may also be used in conjunction with

the test for ignitability in aqueous samples (along with Methods 1010 and 1020) to determine if alcohol is present at > 24%.

11.1.1.2 Triethylamine in aqueous samples

Triethylamine may be determined by injecting a portion of an aqueous sample directly into the GC injection port. This technique has been demonstrated to be appropriate for samples containing low µg/L (ppb) concentrations of triethylamine.

11.1.2 Purge-and-trap of volatile organics (includes GRO)

This includes purge-and-trap for aqueous samples (Method 5030) and purge-and-trap for solid samples (Method 5035). Method 5035 also provides techniques for extraction of solid and oily waste samples by methanol (and other water-miscible solvents) with subsequent purge-and-trap from an aqueous matrix using Method 5030. Normally, purge-and-trap for aqueous samples is performed at ambient temperatures, while soil/solid samples utilize a 40 °C purge to improve extraction efficiency. Heated purge may also be used to improve the purging of compounds with high solubilities in water, particularly alcohols associated with fuel oxygenates such as TBA and TAA. Occasionally, there may be a need to perform a heated purge for aqueous samples to lower detection limits; however, using a 25-mL sample will often provide the sensitivity needed in most situations.

11.1.3 Azeotropic distillation

This technique exploits the ability of selected water-soluble organic compounds to form binary azeotropes with water during distillation. The organic compounds are removed from the bulk water sample and concentrated in a distillate, as described in Method 5031. An aliquot of the distillate is then injected into the GC/FID.

11.1.4 Vacuum distillation of volatile organics

This technique employs a vacuum distillation apparatus to introduce volatile organics from aqueous, solid, or tissue samples into the GC/FID system, as described in Method 5032.

11.1.5 Automated static headspace

This technique employs a device that collects the volatile organics from the headspace over a sample contained in a sealed vial and introduces them into the GC/FID system, as described in Method 5021.

11.1.6 Solvent injection

This technique involves the syringe injection of solvent extracts of aqueous samples prepared by Methods 3510, 3520, 3535, or other appropriate technique, or extracts of soil/solids prepared by Methods 3540, 3541, 3545, 3546, 3550, 3560, or other appropriate technique. It is applicable to many semivolatile organics, including DRO.

CAUTION: Ultrasonic extraction (Method 3550) may not be as rigorous a method as the other extraction methods for soil/solids. This means that it is critical that the method be followed explicitly to achieve an extraction efficiency which approaches that of Soxhlet extraction. Consult Method 3550 for information on the critical aspects of this extraction procedure.

11.2 Suggested chromatographic conditions

Establish the GC operating conditions appropriate for the GC column being utilized and the target analytes specified in the project plan. Optimize the instrumental conditions for resolution of the target analytes and sensitivity. Suggested operating conditions are given below for the columns recommended in Sec. 6.2. The laboratory may use either the columns listed in this method or other columns and columns of other dimensions, provided that the laboratory documents method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) that are appropriate for the intended application.

11.2.1 Column 1

Carrier gas (helium) flow rate: 40 mL/min
Temperature program:
Initial temperature: 45 °C, hold for 3 min
Program: 45 °C to 220 °C, at 8 °C/min
Final temperature: 220 °C, hold for 15 min.

11.2.2 Column 2

Carrier gas (helium) flow rate: 40 mL/min
Temperature program:
Initial temperature: 50 °C, hold for 3 min
Program: 50 °C to 170 °C, at 6 °C/min
Final temperature: 170 °C, hold for 4 min.

11.2.3 Column 3

Carrier gas (helium) flow rate: 15 mL/min
Temperature program:
Initial temperature: 45 °C, hold for 4 min
Program: 45 °C to 220 °C, at 12 °C/min
Final temperature: 220 °C, hold for 3 min.

11.2.4 Column 4 (DRO)

Carrier gas (helium) flow rate: 5-7 mL/min
Makeup gas (helium) flow rate: 30 mL/min
Injector temperature: 200 °C
Detector temperature: 340 °C
Temperature program:
Initial temperature: 45 °C, hold for 3 min

Program:	45 °C to 275 °C, at 12 °C/min
Final temperature:	275 °C, hold for 12 min.

11.2.5 Column 4 (GRO)

Carrier gas (helium) flow rate:	5-7 mL/min
Makeup gas (helium) flow rate:	30 mL/min
Injector temperature:	200 °C
Detector temperature:	340 °C
Temperature program:	
Initial temperature:	45 °C, hold for 1 min
1st Ramp:	45 °C to 100 °C at 5 °C/min
2nd Ramp:	100 °C to 275 °C, at 8 °C/min
Final temperature:	275 °C, hold for 5 min.

11.2.6 Column 5 (triethylamine only)

Carrier gas (helium) flow rate:	5 mL/min
Makeup gas (helium) flow rate:	30 mL/min
Injector temperature:	200 °C
Detector temperature:	250 °C
Temperature program:	
Initial temperature:	110 °C
Program:	110 °C to 175 °C, at 10 °C/min
Final temperature:	175 °C, hold for 3 min.

11.3 Initial calibration

11.3.1 Set up the sample introduction system as outlined in the method of choice (see Sec. 11.1). A separate calibration is necessary for each sample introduction mode because of the differences in conditions and equipment. Establish chromatographic operating parameters that provide instrument performance appropriate for the intended application. Prepare calibration standards using the procedures described above (see Sec. 7.9). The external standard technique is described below. Analysts wishing to use the internal standard technique should refer to Sec. 7.10 and Method 8000.

11.3.2 External standard calibration procedure for single-component analytes

11.3.2.1 For each analyte and surrogate of interest, prepare calibration standards at a minimum of five different concentrations. For headspace analysis, the standards should be prepared in methanol or organic-free reagent water and then spiked into the organic-free water in the headspace vial. The spiking solutions should be at concentrations which will dilute to the desired standard concentrations when added into the organic-free water in the headspace vials. Otherwise, standards should be made by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with an appropriate solvent. One of the standards should be at a concentration at or below the lower limit of quantitation necessary for the project (based on the concentration in the final volume described in the preparation method, with no dilutions). The

concentrations of the other standards should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

11.3.2.2 Introduce each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph. Tabulate peak height or area responses against the mass injected. Calculate the calibration factor (CF) for each single-component analyte as described in Method 8000.

11.3.3 External standard calibration procedure for DRO and GRO

The calibration of DRO and GRO is markedly different from that for single-component analytes. In particular, the response used for calibration needs to represent the entire area of the chromatogram within the retention time range for the fuel type (DRO or GRO), including the unresolved complex mixture that lies below the individual peaks. See Sec. 11.11 for information on calculating this area.

11.3.3.1 For each fuel type, prepare calibration standards at a minimum of five different concentrations by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with an appropriate solvent (for headspace analysis, follow the instructions in Sec. 11.3.2.1, above). One of the standards should be at a concentration at or below the lower limit of quantitation necessary for the project (based on the concentration in the final volume described in the preparation method, with no dilutions). The concentrations of the other standards should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

NOTE: Whenever possible, the calibration should be performed using the specific fuel that is contaminating the site (e.g., a sample of the fuel remaining in the tank suspected of leaking). Where such samples are not available or not known, use recently purchased commercially-available fuel. A qualitative screening injection and GC run may be performed to identify unknown fuels.

11.3.3.2 Introduce each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph. Determine the area of the response as described in Sec. 11.10. Calculate the calibration factor (CF) for each fuel type as shown below:

$$\text{Calibration Factor} = \frac{\text{Total Area within Retention Time Range}}{\text{Mass injected (nanograms)}}$$

11.3.4 Calibration linearity

The linearity of the calibration must be assessed. This applies to both the single-component analytes and the fuel types.

11.3.4.1 If the percent relative standard deviation (%RSD) of the calibration factors is less than 20% over the working range, then linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve.

11.3.4.2 If the RSD is more than 20% over the working range, linearity through the origin cannot be assumed. See Method 8000 for other calibration options that may be employed, which may include a linear calibration not through the origin or a non-linear calibration model (e.g., a polynomial equation).

11.4 Retention time windows

Single-component target analytes (see Sec. 1.1) are identified on the basis of retention time windows. GRO and DRO are distinguished on the basis of the ranges of retention times for characteristic components in each type of fuel.

11.4.1 Before establishing retention time windows, make sure that the chromatographic system is functioning reliably and that the operating parameters have been optimized for the target analytes and surrogates in the sample matrix to be analyzed. Establish the retention time windows for single component target analytes using the procedure described in Method 8000.

11.4.2 The retention time range for GRO is defined during initial calibration. Two specific gasoline components are used to establish the range, 2-methylpentane and 1,2,4-trimethylbenzene. Use the procedure described in Method 8000 to establish the retention time windows for these two components. The retention time range is then calculated based on the lower limit of the RT window for the first eluting component and the upper limit of the RT window for the last eluting component.

11.4.3 The retention time range for DRO is defined during initial calibration. The range is established from the retention times of the C_{10} and C_{28} alkanes. Use the procedure described in Method 8000 to establish the retention time windows for these two components. The retention time range is then calculated based on the lower limit of the RT window for the first eluting component and the upper limit of the RT window for the last eluting component.

11.4.4 If this method is expanded to address other fuel types or petroleum fractions, then the analyst needs to establish appropriate retention time ranges for the boiling point range or carbon number range used to define each additional fuel type or petroleum fraction. Use the procedure described in Method 8000 to establish the retention time windows.

11.5 Calibration verification

11.5.1 The initial calibration and retention times need to be verified at the beginning of each 12-hr work shift, at a minimum. When individual target analytes are being analyzed, verification is accomplished by the analysis of one or more calibration standards (normally mid-concentration, but a concentration at or near the action level may be more appropriate) that contain all of the target analytes and surrogates. When petroleum hydrocarbons are being analyzed, verification is accomplished by the measurement of the fuel standard and the hydrocarbon retention time standard. Additional analyses of the verification standard(s) throughout a 12-hr shift are strongly

recommended, especially for samples that contain visible concentrations of oily material. See Method 8000 for more detailed information on calibration verification.

11.5.2 Calculate the % difference as detailed in Method 8000. If the response for any analyte is within ±20% of the response obtained during the initial calibration, then the initial calibration is considered still valid, and the analyst may continue to use the mean CF or RF values from the initial calibration to quantitate sample results. If the response for any analyte varies from the predicted response by more than ±20%, corrective action must be taken to restore the system or a new calibration curve must be prepared for that compound.

11.5.3 All target analytes, surrogates, and/or *n*-alkanes in the calibration verification analyses need to fall within previously established retention time windows. If the retention time of any analyte does not fall within the established window, then corrective action must be taken to restore the system or a new calibration curve must be prepared for that compound.

11.5.4 Solvent blanks and any method blanks should be run with calibration verification analyses to confirm that laboratory contamination does not cause false positive results.

11.6 Gas chromatographic analysis

11.6.1 Samples are analyzed in a set referred to as an analytical sequence. The sequence begins with calibration verification followed by sample extract analyses. Additional analyses of the verification standard(s) throughout a 12-hr shift are strongly recommended, especially for samples that contain visible concentrations of oily material. A verification standard is also necessary at the end of a set (unless internal standard calibration is used). The sequence ends when the set of samples has been injected or when retention time and/or % difference QC criteria are exceeded.

If the criteria are exceeded, inspect the gas chromatographic system to determine the cause and perform whatever maintenance is necessary before recalibrating and proceeding with sample analysis. All sample analyses performed using external standard calibration need to be bracketed with acceptable data quality analyses (e.g., calibration and retention time criteria). Therefore, all samples that fall between the standard that failed to meet the acceptance criteria and the preceding standard that met the acceptance criteria need to be reanalyzed. Samples analyzed using internal standard calibration need not be bracketed (see Method 8000).

11.6.2 Samples are analyzed with the same instrument configuration as is used during calibration. When using Method 5030 for sample introduction, analysts are cautioned that opening a sample vial or drawing an aliquot from a sealed vial (thus creating headspace) will compromise samples analyzed for volatiles. Therefore, it is recommended that analysts prepare two aliquots for purge-and-trap analysis. The second aliquot can be stored for 24 hrs to ensure that an uncompromised sample is available for analysis or dilution, if the analysis of the first aliquot is unsuccessful or if results exceed the calibration range of the instrument. Distillates from Method 5031 may be split into two aliquots and held at 4 °C prior to analysis. It is recommended that the distillate be analyzed within 24 hrs of distillation. Distillates should be analyzed within 7 days of distillation.

11.6.3 Sample concentrations are calculated by comparing the sample response with the response from the initial calibration of the system (see Sec. 11.3). Therefore, if the sample response exceeds the limits of the initial calibration range, a dilution of the sample or sample extract needs to be analyzed. For volatile organic analyses of aqueous samples, the dilution needs to be performed on a second aliquot of the sample which has been properly sealed and stored prior to use and reanalysis. Samples and/or sample extracts should be diluted so that all peaks are on scale, as overlapping peaks are not always evident when peaks are off scale. Computer reproduction of chromatograms, manipulated to ensure that all peaks are on scale over a 100-fold range, is acceptable as long as calibration limits are not exceeded. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

11.6.4 Tentative identification of a single-component analyte occurs when a peak from a sample extract falls within the daily retention time window. Confirmation may be necessary on a second column or by GC/MS. Since the flame ionization detector is non-specific, it is highly recommended that GC/MS confirmation be performed on single-component analytes unless historical data are available to support the identification(s). See Method 8000 for additional information on confirmation.

11.6.5 Second-column confirmation is generally not necessary for petroleum hydrocarbon analysis. However, if analytical interferences are indicated, analysis using the second GC column may be necessary. Also, the analyst needs to ensure that the sample hydrocarbons fall within the retention time range established during the initial calibration.

NOTE: The identification of fuels, especially gasoline, is complicated by their inherent volatility. The early eluting compounds in fuels are obviously the most volatile and the most likely to have weathered unless the samples were taken immediately following a spill. The most highly volatile fraction of gasoline constitutes 50% of the total peak area of a gasoline chromatogram. This fraction is the least likely to be present in an environmental sample or may be present at only very low concentration in relation to the remainder of a gasoline chromatogram.

11.6.6 The performance of the entire analytical system should be checked every 12 hrs, using data gathered from analyses of blanks, standards, and samples. Significant peak tailing needs to be corrected. Tailing problems are generally traceable to active sites on the column, cold spots in a GC, the detector operation, or leaks in the system. See Sec. 11.8 for GC/FID system maintenance. Follow manufacturer's instructions for maintenance of the introduction device.

11.7 Screening

11.7.1 This method can be used with a single-point calibration for screening samples prior to GC/MS analyses (e.g., Methods 8260 and 8270). Such screening can reduce GC/MS down-time when highly-contaminated samples are analyzed.

11.7.2 When this method is used for screening, it is recommended that the same sample introduction device (e.g., purge-and-trap versus direct injection) that is used for the subsequent GC/MS analyses also be used for the screening analysis. This will improve the correlation between the results and make the screening results more useful in predicting those samples that may overload the GC/MS system. However, other sample introduction techniques may be employed as well.

11.7.3 Establish that the system response and chromatographic retention times are stable. Analyze the high-point GC/MS calibration standard.

11.7.4 Analyze samples or sample extracts. Compare peak heights in the sample chromatograms with the high-point standard to establish that no compound with the same retention time as a target analyte exceeds the calibration range of the GC/MS system.

<u>CAUTION</u>: The FID is much less sensitive to halogenated compounds than the MS detector. As a result, a simple peak height comparison for such compounds in the GC/MS standard may underestimate the actual concentration of halogenated compounds. When using this method as a screening tool, such an underestimate could lead to GC/MS results over the calibration range or result in contamination of the GC/MS system. Therefore, the analyst should exercise caution when screening samples that also contain halogenated compounds.

11.7.5 There are no formal QC requirements applied to screening analyses using this method. However, it is recommended that the high-point standard be run at least once every 12 hrs to confirm the stability of the instrument response and chromatographic retention times. The analyst should consider the costs associated with making the wrong decision from the screening results (e.g., GC/MS instrument down-time and maintenance) and use appropriate judgment.

11.8 Instrument maintenance

11.8.1 Injection of sample extracts from waste sites often leaves a high boiling residue in the injection port area, splitters (when used), and the injection port end of the chromatographic column. This residue affects chromatography in many ways (i.e., peak tailing, retention time shifts, analyte degradation, etc.) and, therefore, instrument maintenance is very important. Residue buildup in a splitter may limit flow through one leg and therefore change the split ratios. If this occurs during an analytical run, the quantitative data may be incorrect. Proper cleanup techniques will minimize the problem and instrument QC will indicate when instrument maintenance is necessary.

11.8.2 Recommended chromatograph maintenance

Corrective measures may need any one or more of the following remedial actions. Also see Method 8000 for additional guidance on corrective action for capillary columns and the injection port.

11.8.2.1 Splitter connections -- For dual columns which are connected using a press-fit Y-shaped glass splitter or a Y-shaped fused-silica connector, clean and deactivate the splitter or replace with a cleaned and deactivated splitter. Break off the first few inches (up to one foot) of the injection port side of the column. Remove the columns and solvent backflush according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the columns.

11.8.2.2 Column rinsing -- Rinse the column with several column volumes of an appropriate solvent. Both polar and nonpolar solvents are recommended. Depending on the nature of the sample residues expected, the first rinse might be water, followed by methanol and acetone; methylene chloride is a

satisfactory final rinse and in some cases may be the only solvent necessary. The column should then be filled with methylene chloride and allowed to remain flooded overnight to allow materials within the stationary phase to migrate into the solvent. The column is then flushed with fresh methylene chloride, drained, and dried at room temperature with a stream of ultrapure nitrogen passing through the column.

11.9 Calculations and data handling

Results need to be reported in units commensurate with their intended use and all dilutions need to be taken into account when computing final results.

11.10 The concentration of each analyte in the sample may be determined by calculating the amount of standard purged or injected, from the peak response, using the mean CF or RF from the initial calibration, or another appropriate calibration model (see Method 8000).

11.11 While both diesel fuel and gasoline contain a large number of compounds that will produce well-resolved peaks in a GC/FID chromatogram, both fuels contain many other components that are not chromatographically resolved. This unresolved complex mixture results in the "hump" in the chromatogram that is characteristic of these fuels. In addition, although the resolved peaks are important for the identification of the specific fuel type, the area of the unresolved complex mixture contributes a significant portion of the area of the total response.

11.11.1 For the analysis of DRO, sum the area of all peaks eluting between C_{10} and C_{28}. This area is generated by projecting a horizontal baseline between the retention times of C_{10} and C_{28}.

11.11.2 Because the chromatographic conditions employed for DRO analysis can result in significant column bleed and a resulting rise in the baseline, it is appropriate to perform a subtraction of the column bleed from the area of the DRO chromatogram. In order to accomplish this subtraction, analyze a methylene chloride blank during each 12-hr analytical shift during which samples are analyzed for DRO. Measure the area of this chromatogram in the same fashion as is used for samples (see Sec. 11.11.1), by projecting a horizontal baseline across the retention time range for DRO. Then subtract this area from the area measured for the sample and use the difference in areas to calculate the DRO concentration, using the equations in Method 8000.

11.11.3 For the analysis of GRO, sum the areas of all peaks eluting between 2-methylpentane and 1,2,4-trimethylbenzene. This area is used to calculate the GRO concentration, using the equations in Method 8000. Column bleed subtraction is not generally necessary for GRO analysis.

11.12 Refer to Method 8000 for the calculation formulae. The formulae cover external and internal standard calibration, aqueous and non-aqueous samples, and linear and non-linear calibrations.

12.0 DATA ANALYSIS AND CALCULATIONS

See Sec. 11.9 for information on data analysis and calculation.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. <u>These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.</u>

13.2 Example method performance data for non-purgeable volatiles prepared using the azeotropic microdistillation technique from Method 5031 are included in Table 1 and 2 for aqueous matrices and in Table 3 for solid matrices. Typical chromatograms are included in Figs. 4 and 5. These data are for guidance purposes only.

13.3 Example method performance information are provided in Tables 4 and 5 for diesel fuel spiked into soil as are chromatograms of GRO and DRO standards in Figures 1 to 3. These data are for guidance purposes only.

13.4 Table 6 contains example precision and bias data for the analysis of triethylamine. Reagent water was spiked with triethylamine at 1.0 µg/L and analyzed by direct aqueous injection in a GC/FID equipped with an HP Basic Wax column (30-m x 0.53-mm ID). These data are for guidance purposes only.

13.5 Table 7 contains example single-laboratory data on the pressurized fluid extraction of diesel range organics (DRO) from three types of soil (sand, loam, and clay). The soils were spiked at two levels of DRO, approximately 5 mg/kg each and approximately 2000 mg/kg. Seven replicates of each level and soil type were extracted using pressurized fluid extraction (Method 3545), using a mixture of methylene chloride and acetone (1:1). The data are taken from Reference 8 and are for guidance purposes only. This extraction technique may be applicable to other analyte classes, fuel types, or petroleum fractions (see Sec. 1.2.3).

13.6 This method was the determinative technique used by one of the three laboratories participating in the study of headspace analysis of oxygenated gasoline contaminated groundwater samples. Refer to the new version of Method 5021, which can be found at http://www.epa.gov/epaoswer/hazwaste/test/pdfs/5021a_r1.pdf, for a discussion and the results of that study. These data are provided for guidance purposes only.

13.7 This method was used in combination with Method 5021 to analyze a standard with several gasoline components, including MTBE and 2-methylpentane. As can be seen from the chromatogram in Figure 6, the two analytes were resolved quite well (column: Restek 502.2 105m x 0.53 um, 3 df). See http://www.epa.gov/epaoswer/hazwaste/test/pdfs/5021a_r1.pdf for more information. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention

techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

1. T. A. Bellar, and J. J. Lichtenberg, "Determining Volatile Organics at Microgram-per-Liter Levels by Gas Chromatography," *J. Amer. Water Works Assoc.*, 66(12), pp. 739-744 (1974).

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3. "Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters: Category 11 - Purgeables and Category 12 - Acrolein, Acrylonitrile, and Dichlorodifluoromethane," Report for EPA Contract 68-03-2635.

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5. S. F. Tsang, N. Chau, P. J. Marsden, and K. R. Carter, "Evaluation of the EnSys PETRO RISc kit for TPH," Report for Ensys, Inc., Research Triangle Park, NC, 27709, 1992.

6. "Interlaboratory Study of Three Methods for Analyzing Petroleum Hydrocarbons in Soils," API Publication Number 4599, American Petroleum Institute, March 1994.

7. Science Applications International Corporation, "Carbamates Method Evaluation Report," Report for EPA Contract No. 68-W6-0068, August 1998.

8. B. E. Richter, "Single Laboratory Method Validation Report. Extraction of Diesel Range Organics (DRO), Waste Oil Organics (WOO), and Total Petroleum Hydrocarbons (TPH) using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC," Document 118357, Dionex Corporation, June 21, 1999.

9. R. J. Pirkle, and P. W. McLoughlin, "The Analysis of Selected Components of Reformulated Gasoline in Environmental Samples" *from* MTBE Handbook, *ed.* Kostecki, P. and Moyer, E. Amherst Scientific Publishers, 2002.

10. USEPA OUST, *Environmental Fact Sheet: Analytical Methods for Fuel Oxygenates*, EPA 510-F-03-001, April 2003, http://www.epa.gov/oust/mtbe/omethods.pdf

11. H. White, B. Lesnik, and J. T. Wilson, "Analytical Methods for Fuel Oxygenates," *LUSTLine* (Bulletin #42), October, 2002, http://www.epa.gov/oust/mtbe/LL42Analytical.pdf

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

TABLE 1

EXAMPLE METHOD PERFORMANCE DATA FOR NON-PURGEABLE VOLATILES IN GROUND WATER BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

Analyte	Low Concentration[a]		Med. Concentration[b]		High Concentration[c]	
	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD
Acetone[e]	126	17	N/A	--	N/A	--
Acetonitrile	147	5	105	8	92	9
Acrolein	146	13	120	27	80	20
Acrylonitrile	179	7	143	28	94	21
1-Butanol	127	8	86	8	90	9
t-Butyl alcohol	122	7	N/A	--	N/A	--
1,4-Dioxane	124	16	96	10	99	8
Ethanol	152	10	N/A	--	N/A	--
Ethyl acetate	142	7	135	33	92	25
Ethylene oxide	114	10	N/A	--	N/A	--
Isobutyl alcohol	122	8	87	13	89	13
Isopropyl alcohol	167	13	N/A	--	N/A	--
Methanol	166	14	94	9	95	7
Methyl ethyl ketone	105	6	N/A	--	N/A	--
Methyl isobutyl ketone	66	4	N/A	--	N/A	--
2-Pentanone	94	3	N/A	--	N/A	--
1-Propanol	N/A	--	91	7	91	7
Propionitrile	135	5	102	14	90	14
Pyridine	92	12	N/A	--	N/A	--

[a] 25 µg/L spikes, using internal standard calibration.
[b] 100 µg/L spikes, using internal standard calibration.
[c] 750 µg/L spikes, using internal standard calibration.
[d] Mean of 7 replicates.
[e] Problematic, due to transient laboratory contamination.

N/A = Data not available
These data are provided for guidance purposes only.

TABLE 2

EXAMPLE METHOD PERFORMANCE DATA FOR NON-PURGEABLE VOLATILES IN TCLP LEACHATE BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

Analyte	Low Concentration[a]		Med. Concentration[b]		High Concentration[c]	
	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD
Acetone[e]	99	91	N/A	--	N/A	--
Acetonitrile	107	17	111	10	95	11
Acrolein	88	10	109	29	87	41
Acrylonitrile	133	13	123	29	103	38
1-Butanol	119	7	89	12	86	8
t-Butyl alcohol	70	31	N/A	--	N/A	--
1,4-Dioxane	103	20	103	16	102	7
Ethanol	122	13	N/A	--	N/A	--
Ethyl Acetate	164	12	119	29	107	41
Ethylene oxide	111	12	N/A	--	N/A	--
Isobutyl alcohol	115	4	86	13	82	13
Isopropyl alcohol	114	8	N/A	--	N/A	--
Methanol	107	10	102	6	N/A	--
Methyl ethyl ketone	87	13	N/A	--	N/A	--
Methyl isobutyl ketone	78	13	N/A	--	N/A	--
2-Pentanone	101	8	N/A	--	N/A	--
1-Propanol	N/A	--	98	10	89	7
Propionitrile	100	16	100	11	90	17
Pyridine	46	59	N/A	--	N/A	--

[a] 25 µg/L spikes, using internal standard calibration.
[b] 100 µg/L spikes, using internal standard calibration.
[c] 750 µg/L spikes, using internal standard calibration.
[d] Mean of 7 replicates.
[e] Problematic, due to transient laboratory contamination.

N/A = Data not available
These data are provided for guidance purposes only.

TABLE 3

EXAMPLE METHOD PERFORMANCE DATA FOR NON-PURGEABLE VOLATILE COMPOUNDS IN SOLID MATRICES BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

	Incinerator Ash				Kaolin			
	Low Conc.[a]		High Conc.[b]		Low Conc.[a]		High Conc.[b]	
	Mean Rec[c]	%RSD	Mean Rec[c]	%RSD	Mean Rec[c]	%RSD	Mean Rec[c]	%RSD
Acrylonitrile	50	53	10	31	102	6	12	52
1-Butanol	105	14	61	12	108	5	58	25
t-Butyl alcohol	101	21	60	13	97	9	59	23
1,4-Dioxane	106	19	48	18	105	10	48	25
Ethanol	117	25	52	20	108	11	48	24
Ethyl acetate	62	19	39	12	90	5	41	25
Isopropyl alcohol	119	21	61	15	108	11	58	24
Methanol	55	53	33	28	117	17	37	22
Methyl ethyl ketone	81	21	40	12	91	8	42	20
Methyl isobutyl ketone	68	11	57	14	71	5	55	23
2-Pentanone	79	13	54	10	91	5	54	19
Pyridine	52	24	44	20	50	10	49	31

[a] 0.5 mg/kg spikes, using internal standard calibration.
[b] 25 mg/kg spikes, using internal standard calibration.
[c] Mean of seven replicates.

These data are provided for guidance purposes only.

TABLE 4

EXAMPLE RESULTS FROM ANALYSIS OF LOW AROMATIC DIESEL BY GC/FID
(5 replicates per test)

Spike Concentration	Analysis Results
12.5 ppm	ND
75 ppm	54 ± 7 ppm
105 ppm	90 ± 15 ppm
150 ppm	125 ± 12 ppm
1000 ppm	960 ± 105 ppm

ND = Not detected

Samples were prepared using 2-g aliquots of sandy loam soil spiked with known amounts of low aromatic diesel. Low aromatic diesel is sold in California. It was purchased for this study at a gas station in San Diego, California. Extractions were accomplished using methylene chloride as a solvent (Method 3550, high concentration option).
These data are provided for guidance purposes only.

TABLE 5

EXAMPLE RESULTS FROM ANALYSIS OF DIESEL BY GC/FID
(5 replicates per test)

Spike Concentration	Analysis Results
25 ppm	51 ± 6 ppm
75 ppm	76 ± 8 ppm
125 ppm	99 ± 5 ppm
150 ppm	160 ± 10 ppm

Samples were prepared using 10-g aliquots of sandy loam soil spiked with known amounts of regular #2 diesel purchased at a gas station in Northern Virginia. Extractions were accomplished using methylene chloride as a solvent (Method 3550).
These data are provided for guidance purposes only.

TABLE 6

EXAMPLE TRIETHYLAMINE PERFORMANCE DATA FOR SPIKED REAGENT WATER
(Analyses by Direct Aqueous Injection)

				Triethylamine Concentration (µg/L)				
Spike Conc.	**Rep. 1**	**Rep. 2**	**Rep. 3**	**Rep. 4**	**Rep. 5**	**Rep. 6**	**Rep. 7**	**Mean**
1.00	1.12	1.17	1.14	1.20	1.19	1.18	1.18	1.169
Mean Recovery 117%		S.D. 0.0288 µg/L						

The estimated lower limit of quantitation was derived from the analyses of seven aliquots of water spiked at 1.00 µg/L, using external standard calibration, on a 30-m, 0.53-mm ID, HP Basic Wax GC column. A 1-µL injection volume was used. **Lower limits of quantitation provided in SW-846 are for illustrative purposes and may not always be achievable.** Laboratories should establish their own in-house lower limits of quantitation, if necessary to document method performance.

Data are taken from Reference 7.
These data are provided for guidance purposes only.

TABLE 7

EXAMPLE SINGLE-LABORATORY DATA FOR PRESSURIZED FLUID EXTRACTION (METHOD 3545)
OF DIESEL RANGE ORGANICS FROM THREE SOIL MATRICES

Matrix and Spiking Level	DRO Concentration in mg/kg								Mean Recovery (%)	%RSD
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6	Rep. 7	Mean		
Low Sand	3.2	8.2	5.9	6.3	7.0	7.7	6.4	6.4	127	25.4
Low Loam	6.5	6.0	7.9	5.1	6.9	9.5	6.4	6.9	138	20.7
Low Clay	4.3	5.8	5.7	8.6	5.4	7.5	7.6	6.4	128	23.6
High Sand	1850	1970	2030	2390	2210	2400	2430	2183	108	10.8
High Loam	1790	1870	1860	1970	1790	1990	1990	1894	94	4.7
High Clay	1910	1890	1990	2860	2880	2150	2040	2246	112	19.4

Low level samples were spiked with approximately 5 mg/kg of DRO.

High level samples were spiked with approximately 2000 mg/kg of DRO.

Seven replicates of each sample were extracted and analyzed by GC/FID.

Data are taken from Reference 8.
These data are provided for guidance purposes only.

FIGURE 2

EXAMPLE CHROMATOGRAM OF A 30 PPM DIESEL STANDARD

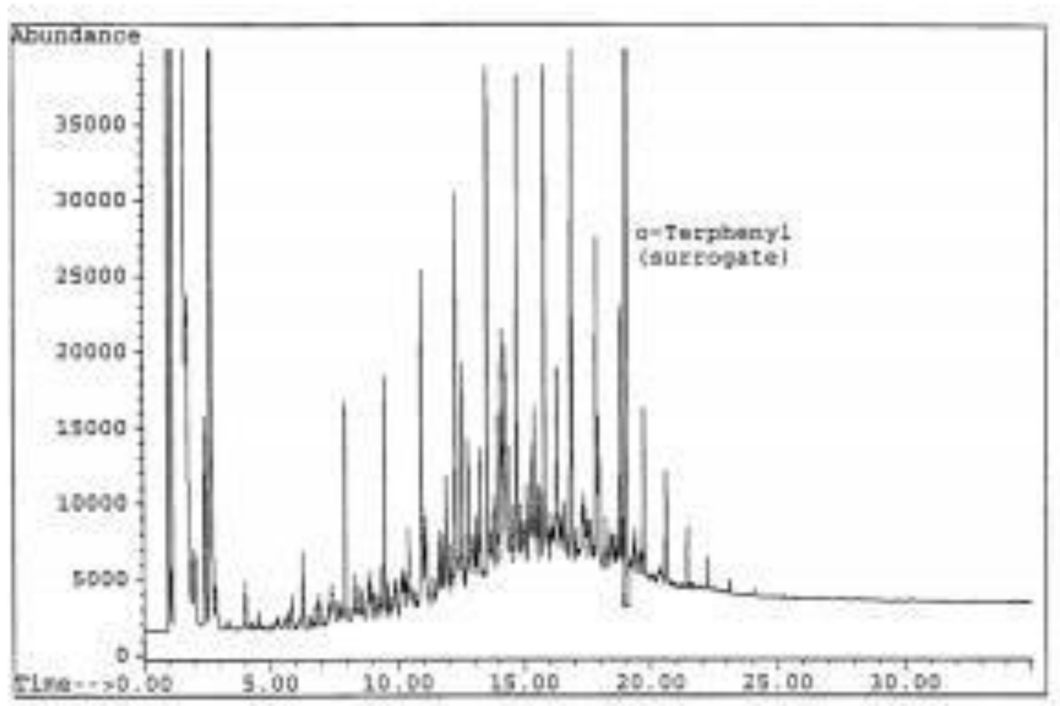


FIGURE 3

EXAMPLE CHROMATOGRAM OF A 30 PPM DIESEL STANDARD WITH THE BASELINE PROJECTED BETWEEN C_{10} AND C_{28}

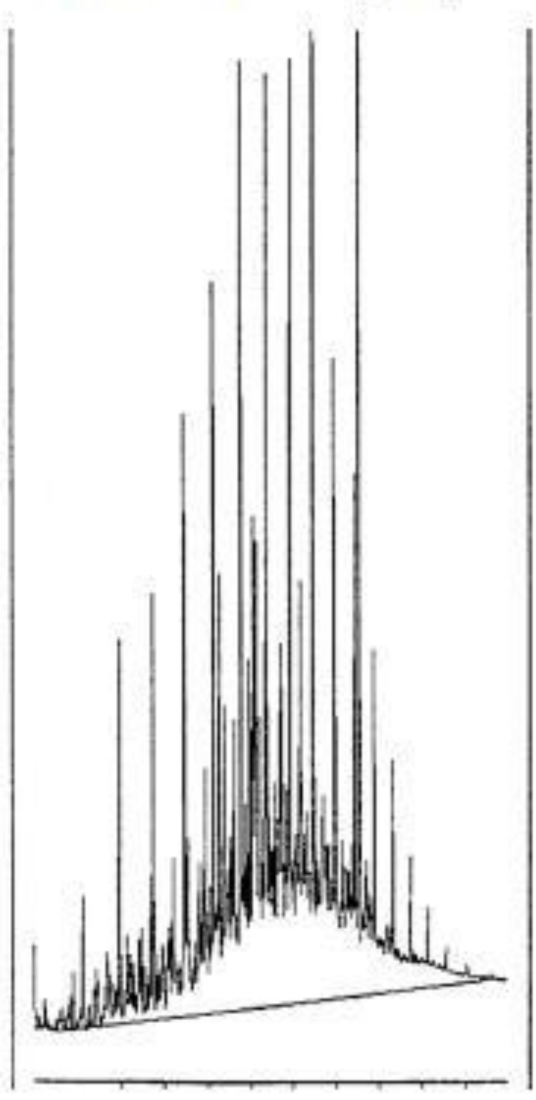
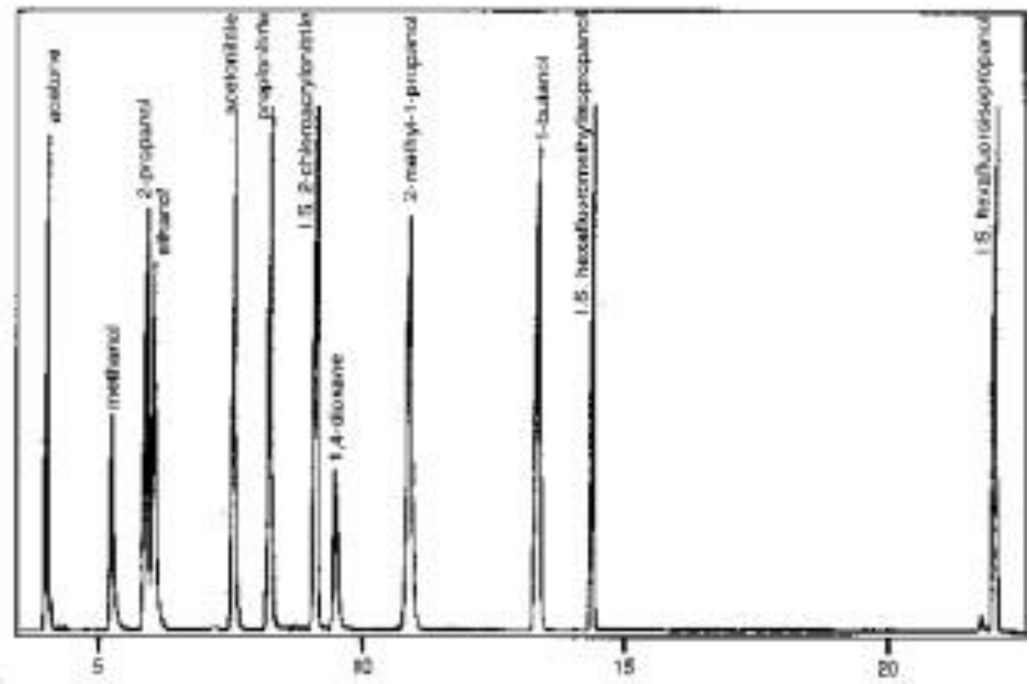


FIGURE 4

EXAMPLE CHROMATOGRAM OF SEVERAL NONPURGEABLE VOLATILE COMPOUNDS IN SPIKED REAGENT WATER USING AZEOTROPIC MICRODISTILLATION (METHOD 5031)



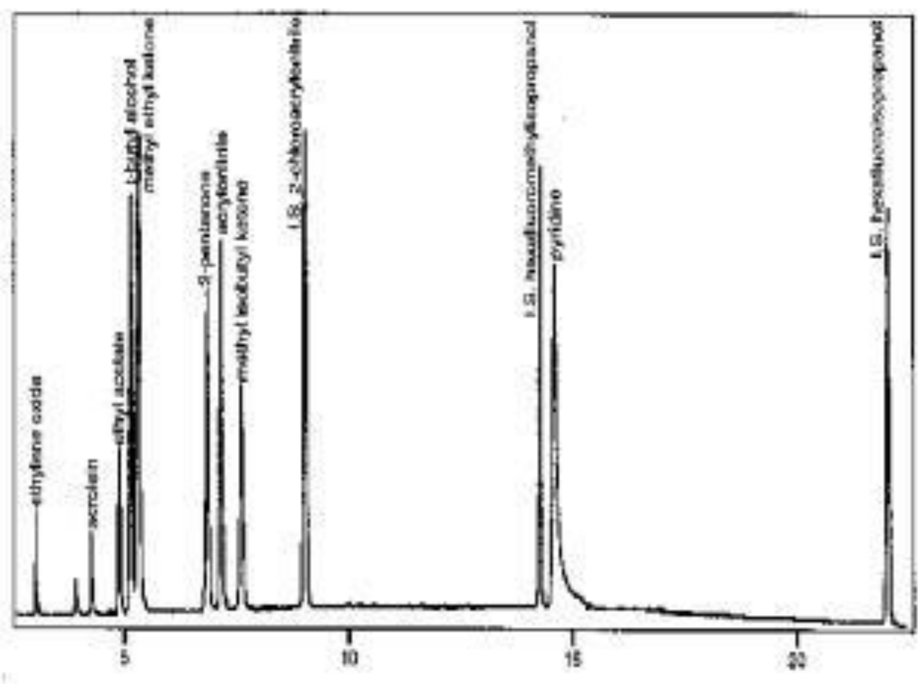
Mix 1: Analytes distilled at 0.25 mg/L, internal standards at 2.5 mg/L

GC Column: J&W DB-Wax column with 0.53-mm ID

Temperature program: 30 °C for 2 min.
3 °C/min. to 100 °C and held for 0 min.
25 °C/min. to 200 °C and held for 4 min.

FIGURE 5

EXAMPLE CHROMATOGRAM OF SEVERAL NONPURGEABLE VOLATILE COMPOUNDS IN SPIKED REAGENT WATER USING AZEOTROPIC MICRODISTILLATION (METHOD 5031)



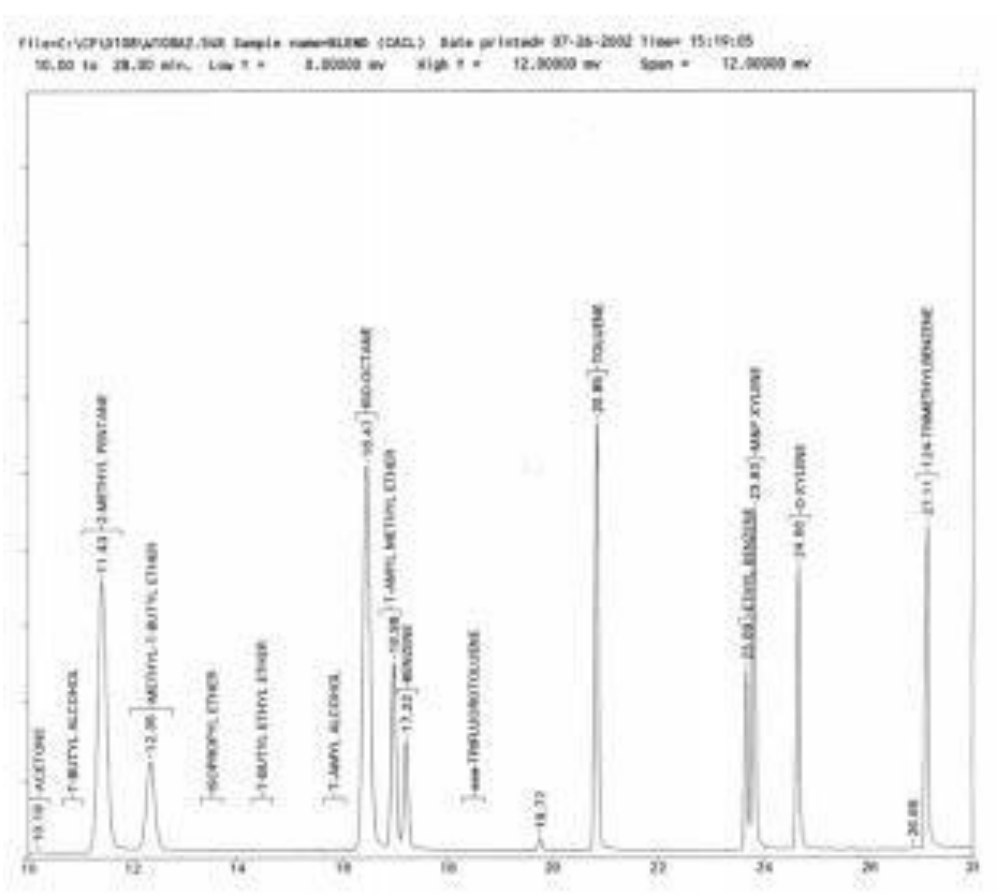
Mix 2: Analytes distilled at 0.25 mg/L, internal standards at 2.5 mg/L

GC Column: J&W DB-Wax column with 0.53-mm ID

Temperature program:
30 °C for 2 min.
3 °C/min. to 100 °C and held for 0 min.
25 °C/min. to 200 °C and held for 4 min.

FIGURE 6

EXAMPLE CHROMATOGRAM OF MULTI-COMPONENT MIXTURE



ANEXO 8

Método de la norma de la Agencia de Protección Ambiental (EPA 5021A)

METHOD 5021A

VOLATILE ORGANIC COMPOUNDS IN VARIOUS SAMPLE MATRICES USING EQUILIBRIUM HEADSPACE ANALYSIS

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria or for the purpose of laboratory accreditation.

1.0 SCOPE AND APPLICATION

Please see Appendix A at the end of this document for a summary of changes from the previous version.

1.1 This method describes equilibrium-based static headspace preparation of volatile organic compounds (VOCs) in soil/sediment, solid waste, aqueous and water-miscible liquid samples for determination by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS). This method is applicable to a wide range of organic compounds that have sufficiently high volatility to be effectively removed from samples using the described conditions. While the method is designed for use on samples containing low levels of VOCs or aqueous dilutions thereof to be analyzed by direct vapor partitioning, a solvent extraction and extract introduction procedure is also described for solid samples containing high concentrations of VOCs or for oily materials that may not be appropriate for the low level technique. This preparation method is intended to be combined with a determinative method such as Methods 8015, 8021 or 8260. This preparation method is appropriate for the compounds listed below, and it may also be appropriate for other VOCs included in the determinative method (e.g., Sec. 1.1 of 8260), provided method performance is demonstrated to be acceptable for the intended use of the data.

Compound	CAS No.[a]	Response	Stability
Acetone	67-64-1	ws	hs
t-Amyl alcohol (TAA)	75-85-4	nd	hs
t-Amyl ethyl ether (TAEE)	919-94-8	nd	nd
t-Amyl methyl ether (TAME)	994-05-8	nd	hs
Benzene	71-43-2	c	hs
Bromochloromethane	74-97-5	p	hs
Bromodichloromethane	75-27-4	c	ms
Bromoform	75-25-2	p	hs
Bromomethane	74-83-9	c	hvs

Compound	CAS No.[a]	Response	Stability
t-Butyl alcohol (TBA)	75-65-0	ws	nd
Carbon tetrachloride	56-23-5	c	hvs
Chlorobenzene	108-90-7	c	hvs
Chloroethane	75-00-3	c	ms
Chloroform	67-66-3	c	hs
Chloromethane	74-87-3	c	hvs
Dibromochloromethane	124-48-1	p	nd
1,2-Dibromo-3-chloropropane	96-12-8	p	ms
1,2-Dibromoethane	106-93-4	p	hs
Dibromomethane	74-95-3	p	hs
1,2-Dichlorobenzene	95-50-1	c	hs
1,3-Dichlorobenzene	541-73-1	c	ms
1,4-Dichlorobenzene	106-46-7	c	ms
Dichlorodifluoromethane	75-71-8	c	hs
1,1-Dichloroethane	75-34-3	c	hs
1,2-Dichloroethane	107-06-2	p	hs
1,1-Dichloroethene	75-35-4	c	hvs
trans-1,2-Dichloroethene	156-60-5	c	ms
1,2-Dichloropropane	78-87-5	c	hs
Diisopropyl ether (DIPE)	108-20-3	c	hs
Ethanol	64-17-5	ws	nd
Ethylbenzene	100-41-4	c	hvs
Ethyl *tert*-butyl ether (ETBE)	637-92-3	c	hs
Hexachlorobutadiene	87-68-3	c	ms
Isopropanol	67-63-0	ws	nd
Methyl *tert*-butyl ether (MTBE)	1634-04-4	c	hs
Methylene chloride	75-09-2	c	hs
Naphthalene	91-20-3	p	ms
Styrene	100-42-5	c	hvs
1,1,1,2-Tetrachloroethane	630-20-6	c	hs
1,1,2,2-Tetrachloroethane	79-34-5	p	nd
Tetrachloroethene	127-18-4	c	ms
Toluene	108-88-3	c	hs
1,2,4-Trichlorobenzene	120-82-1	c	hs
1,1,1-Trichloroethane	71-55-6	c	ms
1,1,2-Trichloroethane	79-00-5	p	hs
Trichloroethene	79-01-6	c	ms
Trichlorofluoromethane	75-69-4	c	ls
1,2,3-Trichloropropane	96-18-4	p	ls
Vinyl chloride	75-01-4	c	hvs
o-Xylene	95-47-6	c	hvs
m-Xylene	108-38-3	c	hvs
p-Xylene	106-42-3	c	hvs
Gasoline range organics			

[a] Chemical Abstracts Service Registry Number

c = Response in reagent water is acceptable; similar response expected in matrix modifier solution (< 50% improvement).
p = Response in matrix modifier solution expected to improve >50% compared to reagent water; Use of matrix modifier is recommended.
ws = Highly water soluble analyte. Method sensitivity expected to be poorer than for other analytes due to poor partitioning into headspace; matrix modifier expected to be critical for acceptable method performance.
nd = Not determined
hs = High stability in preserved water samples (> 60 days). Longer holding times may be appropriate, see Method 5035, Appendix A, Table A.1 footnote and Ref. 47 for additional information
ms = Medium stability in preserved water samples (15 - 60 days). Longer holding times may be appropriate, see Method 5035, Appendix A, Table A.1 footnote and Ref. 47 for additional information
ls = Low stability in preserved water samples (< 14 days), analyses should be performed as soon as possible. May be degraded if acid preserved.
hvs = Highly variable stability depending on the sample matrix. Longer holding times may be appropriate, see Method 5035, Appendix A, Table A.1 footnote and Ref. 47 for additional information.

1.2 The following compounds may also be analyzed by this procedure or may be used as surrogates:

Compound	CAS No.[a]	Response	Stability
Bromobenzene	108-86-1	c	nd
n-Butylbenzene	104-51-8	c	nd
sec-Butylbenzene	135-98-8	c	nd
tert-Butylbenzene	98-06-6	c	nd
2-Chlorotoluene	95-49-8	c	nd
4-Chlorotoluene	106-43-4	c	nd
cis-1,2-Dichloroethene	156-59-4	c	hs
1,3-Dichloropropane	142-28-9	c	nd
2,2-Dichloropropane	590-20-7	c	nd
1,1-Dichloropropene	563-58-6	c	nd
Isopropylbenzene	98-82-8	c	nd
4-Isopropyltoluene	99-87-6	c	nd
n-Propylbenzene	103-65-1	c	nd
1,2,3-Trichlorobenzene	87-61-6	c	nd
α,α,α-Trifluorotoluene	98-08-8	nd	nd
1,2,4-Trimethylbenzene	95-63-6	c	nd
1,3,5-Trimethylbenzene	108-67-8	c	nd

[a] Chemical Abstracts Service Registry Number

1.3 In order to produce quantitative data with this technique, all of the quality control criteria described in the determinative method and/or Method 8000 should be met. Alternatively, this method may be utilized as a screening protocol. If used for screening, semi-quantitative or estimated sample results may be obtained with minimal calibration and quality control, such as a reagent blank and a single calibration standard.

As with any preparative method for volatiles, screening samples prior to low level analysis may help minimize problems associated with carryover contamination from samples that contain very high concentrations of volatiles above the calibration range of the determinative method. In addition, because removing a sample aliquot from a container may compromise the integrity of the sample, multiple sample aliquots should be collected to allow for screening and re-analysis.

1.4 In order to accommodate analysis of a variety of sample matrices and VOCs, a matrix modifier (Sec. 7.7) is generally recommended to be used with this method. The matrix modifier is a water soluble salt solution that is added to each sample and standard vial prior to analysis. The matrix modifier solution acts to increase the VOCs mass transfer into the headspace of the vial. The principal benefits of using the matrix modifier are:

1) better response and reproducibility of the VOCs that do not otherwise partition efficiently into the headspace of the vial from the aqueous phase (identified with 'p' or 'ws' in the response column in the table in Sec. 1.1); and

2) less potential for measurement bias resulting from aqueous activity differences between standards and samples.

Measurement bias results from VOCs partitioning into the vial headspace differently in a sample than in the calibration standards. Some potential sources of measurement bias and the anticipated effects of the matrix modifier on these sources of bias are described below.

1.4.1 Aqueous field samples containing high dissolved solute concentrations:

At higher solute concentrations substantially larger fractions of some VOCs partition into the headspace leading to high bias in the determined concentration. The VOCs most prone to high bias measurement at higher dissolved solute concentrations are also the VOCs whose responses are most substantially improved in the matrix modifier solution relative to reagent water (identified with 'p' or 'ws' in the response column in the tables in Sec. 1.1). The VOCs identified with 'c' in the response column in the analytes table in Secs. 1.1 and 1.2 are not as subject to this source of measurement bias. The matrix modifier is used to normalize the solute concentration between samples and calibration standards, thereby minimizing this source of bias.

1.4.2 Aqueous field samples containing water miscible organic component:

The presence of a water miscible organic component (e.g., cosolvent or surfactant) may result in low bias measurement of VOCs with high octanol-water partitioning coefficients (e.g., C3 and C4 alkylbenzenes, trichlorobenzenes and naphthalene), while recovery of the lighter and more highly water soluble VOCs is unlikely to be strongly affected unless the proportion of the water miscible organic component in the sample is high. The matrix modifier helps improve the recovery of VOCs whose partitioning into the headspace is most strongly affected by this source of measurement bias.

1.4.3 Field samples containing a water immiscible component:

For samples with a separate water immiscible phase, partitioning of VOCs into the headspace competes with the water immiscible phase. While addition of the matrix modifier has a favorable effect on partitioning of VOCs into the headspace from the aqueous phase, it may also increase partitioning into the water immiscible phase(s) (e.g., soils with >1% organic matter, oily materials), potentially exaggerating matrix effects relative to the calibration standards. This matrix effect is more pronounced for VOCs with higher octanol-water partitioning coefficients when the matrix modifier is used for the analysis. Recovery of the lighter and more water soluble VOCs is expected to be less affected.

For complex samples, more than one of these types of matrix effects may be relevant, and a compromise may have to be made for data quality of some analytes in order to obtain reliable data for the analytes deemed most critical for the project. For simple sample matrices and VOCs

not expected to subject to measurement bias (*e.g.*, analysis of BTEX and other alkylbenzenes in surface water samples) the matrix modifier solution may be omitted.

1.5 This method, in conjunction with determinative Method 8015 (GC/FID), may be used for analysis of the aliphatic hydrocarbon fraction in the light ends of petroleum hydrocarbons, *e.g.*, gasoline. For the aromatic fraction (BTEX), use this method and Method 8021 (GC/PID). A total determinative analysis of gasoline and other volatile petroleum hydrocarbon fractions may be obtained using Method 8021 in series with Method 8015. If MS detection is desired for these target analytes, Method 8260 (Volatile Organic Chemicals by GC/MS) may be used.

1.6 Measurements of VOCs using this method may be subject to bias from several sources, including differences in partitioning of VOCs between the aqueous phase and headspace in samples relative to standards, differences in headspace volume in samples relative to standards, and adsorption of VOCs to surfaces or absorption into compatible phases (*e.g.*, soil organic matter). Measurement bias is monitored through internal standard, surrogate, and matrix spike recovery when appropriate for the project and determinative method. Use of the matrix modifier (Sec. 7.7) will help minimize measurement bias resulting from differences in partitioning behavior of VOCs in samples relative to standards. Measurement bias resulting from adding solid material to the vial, which changes the headspace volume in the sample relative to the calibration standards, is expected to be negligible as long as the volume of material is small relative to the headspace volume. The magnitude of this bias may be reduced by adding a similar volume of solid organic-free control material to calibration standards as the volume of the bulk material being tested. Measurement bias related to sorption of VOCs to solid samples with fine particle size distributions and/or significant organic content may be substantial. The magnitude of this bias may be reduced by analyzing a smaller amount of material or by solvent extraction (Sec. 11.4).

1.7 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (*e.g.*, Methods 3500, 3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.8 This method is restricted to use by, or under supervision of, appropriately experienced and trained analysts for volatile organic analysis in general and specifically the use of equilibrium headspace devices interfaced to the determinative method selected by the analyst. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Sample collection and headspace vial preparation

2.1.1 Water samples – A 40-mL volatile organic analyte (VOA) vial is filled to capacity and capped so no headspace remains in the vial. The water sample may be preserved at the time of sampling by addition of a chemical preservative (*e.g.*, hydrochloric acid solution, solid sodium bisulfate or solid trisodium phosphate) to the vial. At the laboratory, that vial is sub-sampled into a headspace vial, and internal standards and surrogates are added, if used. The matrix modifying solution (Sec. 7.7) should be added to the headspace vial during subsampling if used for the analysis.

2.1.2 Low concentration soil samples – Approximately 2 g of soil is collected with an appropriately sized coring tool and placed in a pre-weighed crimp seal or screw top glass headspace vial, and then the vial is sealed. Depending on the analytes of interest, the soil sample may be preserved by addition of a pH modifying chemical preservative (*e.g.*, sodium bisulfate, trisodium phosphate) prior to sealing the sample vial, and the matrix modifying solution (Sec. 7.7) should also be added prior to capping the vial if used for the analysis. Sec. 8.3.3 also describes the use of a sealable coring device as an alternative sampling technique, which may simplify collection and handling of soils in the field.

Surrogates and internal standards may be added to the vials during sampling or at the laboratory. If the matrix modifying solution is used for the analysis and was not added to sample vials in the field, it should be added when any surrogates and internal standards are added at the laboratory. Adding the matrix modifying solution or reagent water to a vial after adding the sample may cause loss of gas phase VOCs from the container due to displacement of a portion of the vial headspace. Adding the matrix modifying solution (Sec. 7.7) to the vial prior to adding the sample and sealing quickly will help to limit loss of VOCs from the sample container and maintain sample representativeness.

NOTE: The choice of chemical preservative(s) will depend on the VOCs that will be measured in the samples and to some extent on the sample matrix. The matrix modifying solution acts as a chemical preservative, but it does not otherwise alter the sample pH and may not protect against degradation of some classes of VOCs, including hydrolysis of ethers or dehydrohalogenation of chlorinated aliphatics (Sec. 4.7). Sodium bisulfate has also been identified as inappropriate for use as a preservative for calcareous soils, which may off-gas CO_2 when exposed to acid due to chemical reaction with any carbonate salts, which may cause loss of VOCs from the container or build up pressure once the container is sealed, potentially leading to rupture.

2.1.3 High concentration soils or other solid materials – A representative portion of soil is collected with an appropriately sized coring tool and placed in a pre-weighed glass VOA vial, and then the vial is sealed. The soil sample may be preserved by addition of extraction solvent (*e.g.*, methanol) at the time of sampling or upon receipt by the laboratory. At the laboratory, the methanol extract is then diluted with the matrix used for the calibration standards (organic-free reagent water or the matrix modifying solution) and analyzed as an aqueous sample. Sec. 8.3.3 also describes the use of an air-tight sealable coring device as an alternative sample collection technique that may be useful, and Sec. A.6 of SW-846 method 5035A provides additional information pertaining to methanol extraction of soils.

NOTE: Surrogate compounds may either be spiked into the solvent at the time of extraction or into reagent water containing an aliquot of the extract prior to analysis. Since the surrogate recovery data from these two options provides assurances of either extraction or analytical efficiencies, the decision as to

when the surrogates are added depends on what questions need to be answered for a given sample matrix and the intended uses of the data.

2.2 For soil samples, additional aliquot(s) are collected in VOA vials for dry weight determination.

2.3 In the laboratory, the vials are rotated to allow for diffusion of internal standards and surrogates throughout the matrix. The vials are placed in the autosampler carousel of the headspace analyzer and maintained at room temperature. Approximately 1 hr prior to analysis, the individual vials are moved to a heated zone and mechanically agitated while the elevated temperature is maintained, allowing the VOCs to equilibrate between the headspace, liquid and any solid phases in the vial.

2.4 The autosampler then pressurizes the vial with helium and forces a portion of the headspace gas mixture into the gas chromatograph through a heated transfer line, either passing through the GC inlet or directly connected to the analytical column via an inert, low dead volume connector.

2.5 Determinative analysis is performed using the appropriate GC or GC/MS method. Any chemical preservative and matrix modifier added to the field samples should also be added to the calibration standards and other QC samples.

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four for general guidance on the cleaning of glassware. Also refer to the determinative methods to be used for information regarding potential interferences.

4.2 Volatile organic analyses are subject to major interference problems because of the prevalence of volatile organics in a laboratory. See Method 5000 for common problems and precautions to be followed.

4.3 Samples can be contaminated by diffusion of volatile organics (particularly methylene chloride and fluorocarbons) through the septum seal of the sample vial during shipment and storage. A trip blank, prepared from an appropriate organic-free matrix and sample container and carried through sampling and handling protocols, serves as a check on such contamination.

4.4 The sample matrix itself can cause severe interferences by one of several processes or a combination of these processes. These include, but are not necessarily limited to, the absorption potential of the soil, the biological activity of the soil, and the actual composition of the soil. Soils high in organic matter or oily material and organic sludge wastes inhibit the

partitioning of the volatile target analytes into the headspace. Therefore, analyte recovery by direct vapor partitioning may be low and will depend on the properties of the particular chemical. This matrix effect can be difficult, if not impossible, to overcome. It is recommended that surrogates or additional deuterated compounds (for GC/MS methods) be added to a matrix and analyzed to determine the percent recovery of these compounds. The calculated percent recovery can give some indication of the degree of the matrix effect, but not necessarily correct for it. Alternatively, the use of the high-concentration procedure in this method should minimize the problem with oily waste and other organic sludge wastes.

4.5 Contamination by carryover can occur whenever high concentration and low concentration samples are analyzed sequentially. Where practical, samples with unusually high concentrations of analytes should be followed by an analysis of one or more method blanks or instrument blanks to check for cross-contamination. If the target compounds present in an unusually concentrated sample are also found to be present in subsequent samples, the analyst must demonstrate that the compounds are not affected by carryover contamination. Conversely, if those target compounds are not present in the subsequent sample, then the analysis of a blank is not necessary.

4.6 The laboratory where volatiles analysis is performed should be free of any solvents that may interfere with the analysis. Special precautions must be taken when analyzing for methylene chloride. The analytical and sample storage areas should be isolated from all atmospheric sources of methylene chloride. Otherwise, random background levels can result. Since methylene chloride can permeate through polytetrafluoroethylene (PTFE) tubing, all GC carrier gas lines and purge gas plumbing should be constructed of stainless steel or copper tubing. Laboratory workers' clothing previously exposed to methylene chloride fumes during common liquid/liquid extraction procedures can contribute to sample contamination. The presence of other organic solvents in the laboratory where volatile organics are analyzed can also lead to random background levels, and the same precautions must be taken.

4.7 Ethers in acidic samples (*i.e.*, samples with a pH < 7) will hydrolyze at the higher temperatures used in this method. As such, basic preservatives should be used if the target analytes are ethers or the alcohols that those ethers would form if hydrolyzed. Strong bases may catalyze substitution and elimination reactions that can occur if halogenated compounds are present. Halogenated aliphatic VOCs are particularly susceptible to dehydrohalogenation reactions in neutral to basic conditions at elevated temperature such as with a heated sample preparation procedure as is described here. Accordingly, acidic preservatives may be necessary to prevent dehydrohalogenation if halogenated aliphatic VOCs are analytes of interest or their presence is suspected and their transformation products are of interest. Acetone has also been observed to form in high organic content soils preserved with sodium bisulfate (Sec. A.8 in the Appendix of method 5035A provides more information). The chemical reactivity introduced by the preservative should be monitored by analyzing a matrix spike of a field sample with each batch. The spiking solution should contain all analytes which the client intends to monitor.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals included in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list all common laboratory glassware (e.g., beakers and flasks) that might be used.

6.1 Headspace containers - Clear glass, 22-mL vials equipped with PTFE-lined septa that are compatible with the analytical system. Vials of other sizes may be employed, provided that they can be hermetically sealed and equipped with suitable septa. Ideally, the vials and septa should have a uniform tare weight. The septa should be unpunctured, as piercing the PTFE face may allow target analytes to diffuse into and adsorb to the silicone backing material. New, disposable vials may be used without pretreatment provided they are demonstrated to be clean through method blank analysis. Store the vials in an area free of organic solvents. If vials are suspected of being a source of contamination, first wash the vials in a detergent solution, then thoroughly rinse with tap water followed by distilled water, and finally dry the vials in an oven at 105 °C for 1 hour. Allow vials to cool prior to use.

6.2 Headspace system - The operating conditions listed in Sec. 11.0 are those selected for the equipment used in developing this method. See Reference #1 in Sec. 16 for more detail. Other equipment and conditions may be employed, provided that the laboratory demonstrates performance for the analytes of interest using the determinative method appropriate for the intended application. The system used must meet the following specifications:

6.2.1 The system must be capable of holding samples at elevated temperatures and establishing a reproducible equilibrium between a wide variety of sample types and the headspace.

6.2.2 The system must be capable of accurately transferring a representative portion of the headspace into a gas chromatograph fitted with a capillary column without adversely affecting the chromatography or the detector.

6.3 Field sampling equipment

6.3.1 Water samples - Clear or amber 40 mL volatile organic analysis (VOA) vials with screw-cap PTFE lined vials.

6.3.2 Soil samples

6.3.2.1 A soil sampler which delivers at least 2 g of soil is necessary, e.g., Purge and Trap Soil Sampler Model 3780SPT (Associated Design and Manufacturing Company, 814 North Henry Street, Alexandria, VA 22314), or equivalent.

6.3.2.2 An automatic syringe or bottle top dispenser calibrated to deliver a 10.0 mL liquid volume.

6.3.2.3 Crimping tool for headspace vials - If using screw-top vials, this is not needed.

6.3.2.4 VOA vials (22, 40 or 60 mL) with PTFE faced septa and crimp-seal caps or screw-top caps. These vials will be used for sample screening, high concentration analysis (if needed) and dry weight determination.

6.3.2.5 Sealable, air-tight coring device – A soil coring device with an internal volume appropriate for approximately 2 g of sample for direct vapor partitioning analysis, or other size as appropriate for high level analysis, equipped with an o-ring seal or equivalent air-tight sealing mechanism, constructed of materials that will not absorb or react with the target chemicals of interest and with a cross-sectional diameter appropriate for a VOA vial compatible with the headspace analyzer or for use with methanol extraction.

6.4 Miscellaneous equipment

6.4.1 For the preparation of blanks, standards and water samples, it is necessary to have the crimping tool addressed in 6.3.2.3 available in the laboratory.

6.4.2 Graduated microsyringes for standard preparation and for addition of internal standard and surrogate spiking solutions.

6.4.3 5-mL glass hypodermic syringes with Luer-Lok™ tip (other sizes are acceptable depending on sample volume used).

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Organic-free reagent water - Reagent water must be interference free. All references to water in this method refer to organic-free reagent water, unless otherwise specified.

7.3 Methanol - Pesticide quality or equivalent. Store away from other solvents. Purchase in small quantities (1 Liter size or less) to minimize shelf life to reduce potential for contamination.

7.4 See the determinative method and Method 5000 for guidance on the preparation of stock standards and a secondary standard for internal standards, calibration standards, and surrogates.

7.4.1 Calibration spiking solutions - Prepare five or more spiking solutions in methanol or water that contain all the target analytes. The concentrations of the calibration solutions should be such that the addition of 1.0 µL of each to the headspace vials will bracket the analytical range of the detector. Alternatively, calibration standards may be prepared by adding different volumes of one or more stock solutions provided that the

linearity of the calibration is not affected by the methanol content. For analysis of methanol extracts, it may be appropriate to calibrate surrogates at multiple concentration levels as well to demonstrate calibration linearity at the surrogate level measured in diluted extracts.

7.4.2 Internal and surrogate standards – Follow the recommendations of the determinative method for the selection of internal and surrogate standards. Selection and use of surrogates with physical properties similar to the classes of target analytes that are of interest for the project will provide more meaningful sample-specific quality assurance information. A concentration of 20 mg/L in methanol for both internal and surrogate standards may be used for spiking each sample. The concentration may vary depending on the relative sensitivity of the detector used in the determinative method. If determination is by GC, external standard calibration may be preferred and the internal standard omitted.

7.5 Blank preparation - Transfer 10.0 mL of matrix modifying solution (Sec. 7.7) or reagent water to a sample vial. Inject the necessary amounts of internal standards and surrogate compounds under the surface of the water in the headspace vial, and seal the vial. Place in the autosampler and analyze in the same manner as an unknown sample. Any chemical preservative and/or matrix modifier added to the field samples must also be included in the blank(s).

7.6 Preparation of calibration standards - Prepare calibration standards in the same manner as blanks (Sec. 7.5), adding the standard spiking solution(s) prepared in Sec. 7.4.1 in the same manner that internal standards and surrogates are added. Any chemical preservative and/or matrix modifier added to the field samples should also be included in the calibration standards.

7.7 Preparation of matrix-modifying solution - Add 180 g of ACS-grade sodium chloride (NaCl) to 500 mL of reagent water. Mix well until all components are dissolved. Other water soluble salts may be appropriate. The matrix modifier solution should not affect the pH of the sample to the extent that preservation or analyte stability is compromised. Analyze a 10.0-mL portion from each batch according to Sec. 7.5 to verify that the solution is free of contaminants. Store the prepared matrix-modifying solution in a sealed bottle in an area free of organic chemicals at ≤6 °C.

CAUTION: The matrix modifying solution may not be appropriate for analysis of some VOCs in soil samples having high organic matter content.

7.8 Preparation of chemical preservative for low level (vapor partitioning) analysis - The preservative should be chosen based on the analytes of interest and should be mixed with the sample at the time of sampling.

7.8.1 If a basic preservative is chosen, 100 mg of ACS-grade trisodium phosphate dodecahydrate (TSP; $Na_3PO_4 \cdot 12H_2O$) should be added to either a 22-mL headspace vial or a 40-mL water sample vial to raise the pH above 10.

7.8.2 If an acidic preservative is chosen, 2-3 drops of 6N hydrochloric acid (HCl) should be added to a 40-mL water sample vial. The HCl solution should be prepared by the 1:1 dilution of ACS-grade concentrated HCl. For acid preservation of a soil sample, 1 g of solid, ACS-grade sodium bisulfate ($NaHSO_4$) should be added to each 22-mL vial.

CAUTION: If samples containing MTBE, TAME, ETBE or other fuel ethers have been acid preserved with either sodium bisulfate or hydrochloric acid, these samples must be adjusted to pH >10 with trisodium phosphate

dodecahydrate (TSP) (Sec. 7.8.1) prior to initiation of the headspace analysis.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining an appropriate plan for sample collection, preservation and storage prior to sample collection and analysis.

8.1 Refer to Chapter Four and Method 5035A or general sample collection information. All samples should be stored in capped vials at ≤6 °C in an area free of solvent fumes If any evidence of leakage is found, the sample can be considered corrupted and should be discarded.

Pre-testing of a representative soil or aqueous sample, prior to collection, with acid or bisulfate may show effervescence if carbonaceous materials are present. If bubbling occurs during chemical preservation, an increased potential for loss of volatile constituents exists and samples should therefore be collected without preserving with acid or bisulfate.

8.2 Water samples - Fill the 40-mL vial and, according to the analyte list to be analyzed, chemically preserve the sample (Sec. 7.8) as necessary. Ensure that there is no headspace in the vial and seal it. At least two vials should be collected per sample and more may be necessary for duplicate and MS/MSD analyses, if desired. Transfer of the sample into a headspace vial and the addition of the matrix modifier and standards should be performed at the laboratory.

In general, liquid samples should be poured into the vial without introducing any air bubbles into the sample as the vial is being filled. Should bubbling occur as a result of violent pouring, the sample should be poured out and the vial refilled. The vials should be completely filled at the time of sampling, so that when the septum cap is fitted and sealed, and the vial inverted, no headspace is visible. The sample should be hermetically sealed in the vial at the time of sampling, and not opened prior to analysis to preserve its integrity.

Due to differing solubility and diffusion properties of gases in liquid matrices at different temperatures, it is possible for the sample to generate some headspace during storage. This headspace will appear in the form of micro bubbles and should not invalidate a sample for volatiles analysis. The diameter of any bubble caused by degassing upon cooling the sample should not exceed 5 - 6 mm. When a bubble is present, also inspect the cap and septum to ensure that a proper seal was made at the time of sampling. The presence of a macro bubble in a sample vial generally indicates either improper sampling technique or a source of gas evolution within the sample. The latter case is usually accompanied by a buildup of pressure within the vial, (e.g. carbonate-containing samples preserved with acid). Studies conducted by the USEPA (EMSL-Ci, unpublished data) indicate that "pea-sized" bubbles (i.e., bubbles not exceeding 1/4 inch or 6 mm in diameter) did not adversely affect volatiles data. These bubbles were generally encountered in wastewater samples, which are more susceptible to variations in gas solubility than are groundwater samples.

8.3 Soil samples - Three alternative procedures are presented below for collection of soil samples in headspace sample vials. Sec. A.7 in the appendix of method 5035A describes some additional alternatives that may also be appropriate. The choice between these alternatives should be based on knowledge of the field conditions, the organic carbon content of

the soil, the specific volatile analytes and concentration levels of interest, and the intended use of the analytical results. For low level analysis by direct vapor partitioning, 3 or 4 replicate samples should be collected from each sampling point to allow for reanalysis, while duplicate samples may be sufficient for high level analysis because the solvent extract can be diluted and reanalyzed. Additional sample replicates should also be collected for duplicate and MS/MSD analyses, as well as separate portions for dry weight determination. If samples will be analyzed by the low level method but have the potential to contain high levels of VOCs, samples may be collected for both low level and high level analysis. This is due to the difficulty of diluting samples prepared for low level analysis once they are sealed in the vials.

8.3.1 Sampling directly into prepared headspace vials for low level analysis:

Soil may be sampled by addition to a prepared vial that contains 10.0 mL of matrix modifier or reagent water, plus any necessary pH altering chemical preservative. The preservative and matrix modifier or water are added to the vial prior to sampling in order to prevent displacing a portion of the headspace from the vial, along with any associated VOCs . The matrix modifying solution has the additional benefits of reducing the biodegradation potential of the sample matrix and increasing partitioning of the VOCs into the vial headspace from water. Problems related to contamination of the aqueous solution in a field sampling situation and incorrect measurement and transfer into the sample vials can be minimized by adding it to the vials at the laboratory and sealing them prior to sending them to the field. Samples should be obtained and transferred to a vial rapidly after sampling (<10 seconds) to minimize volatilization losses. In order to estimate the sample mass added, the vial, cap and any added solutions should be tared and the masses recorded prior to and after adding a soil sample to the vial. If the vials were not prepared in the laboratory prior to sampling, the analyzing laboratory must be made aware of the identities and amounts of any reagents added to each vial in the field.

8.3.1.1 Use standard glass headspace vials with PTFE faced septa.

8.3.1.2 Using the soil sampler (Sec. 6.3.2.1), add 2-3 cm (approximately 2 g) of the soil sample to a tared headspace vial containing 10.0 mL of matrix modifier or reagent water and any pH modifying chemical preservative used. The samples should be introduced into the vials gently to reduce agitation which might drive off volatile compounds. Seal immediately with the PTFE side of the septum facing toward the sample.

8.3.2 Sampling directly into empty or prepared headspace vials for high level analysis:

If high concentrations of VOCs are expected (greater than 200 µg/kg), collection of the sample in an empty headspace vial or a vial containing methanol is appropriate for use with the high concentration procedure described in Sec. 11.4.

8.3.2.1 Use standard 22-mL crimp-cap or screw-top glass headspace vials with PTFE faced septa (other vials may be used, as described in Sec. 6.1).

8.3.2.2 Using the soil sampler (Sec. 6.3.2.1), add 2-3 cm (approximately 2 g) of the soil sample to a headspace vial and seal immediately with the PTFE side of the septum facing toward the sample. The samples should be obtained and transferred to a vial rapidly after sampling (<10 seconds) to minimize volatilization losses, and they should be introduced into the vials gently to reduce agitation which might drive off volatile compounds. If methanol is added to the vial

prior to the sample, the vial, cap and methanol should be tared and the masses recorded prior to and after adding a soil sample to the vial. The recorded mass should be checked by the analyzing laboratory to verify that solvent was not lost during shipping and/or storage.

8.3.3 Sampling with a sealable, air-tight coring device for low or high level analysis:

For cohesive soils, soil samples can be taken in appropriately sized air-tight sealable coring devices for refrigeration and shipping to the laboratory, where the samples are further preserved or immediately prepared for analysis.

8.3.3.1 Insert a clean coring device into a fresh surface for sample collection and ensure that no air is trapped between the coring tool and the sample. The volume of material collected should not cause excessive stress on the coring tool during intrusion into the material. Just before capping, a visual inspection of the lip and threads of the sample vessel should be made and any foreign debris should be wiped clean, allowing an airtight seal to form.

8.3.3.2 Upon laboratory receipt, the soil plug in each sealable coring device is extruded into individual tared headspace VOA vials containing the appropriate solution (either matrix modifier or reagent water for low level analysis, with pH modifying preservative as appropriate, or methanol for high level analysis). The coring device must fit into the mouth of the headspace vial or other VOA vial into which the sample is extruded, or losses of VOCs will result. In order to estimate the sample mass added, the vial, cap and any added solutions should be tared and the mass recorded prior to and after adding a soil sample to the vial.

8.4 Field blanks should be prepared, regardless of which alternative is employed for soil sample collection. If the matrix modifying solution is not added in the field, then the field blank(s) should be prepared by adding any reagents used in the field (e.g., 10.0 mL of organic-free reagent water, methanol, or matrix modifying solution, plus any other chemical preservatives) to a clean vial and immediately sealing the vial.

8.5 Sample storage

8.5.1 Samples should be stored at ≤6 °C until analysis in order to limit diffusion of the analytes out of the water, reduce the ability of the analytes to react with the glass walls of the sampling container and further hinder sample biodegradation. Water samples in VOA vials with no headspace should not be frozen, but subsamples added to prepared headspace vials may be frozen, provided the integrity of the container seal is maintained. Freezing of soil samples is also appropriate provided the storage temperature is not lower than the minimum temperature recommended by the manufacturer for maintaining integrity of the container seal. Freezing in this temperature range may be used to extend the holding time of soils in sealed air-tight coring devices and in sealed headspace vials with reagent water, even if no other chemical preservative is added. See Table A1 in the Appendix of method 5035A for more details. The sample storage area should be free of organic solvent vapors.

8.5.2 All samples should be analyzed within 14 days of collection or sooner if labile compounds are target analytes. See the cautionary notes in Table 4-1 of Chapter Four, Method 5035, Appendix A, Table A-1, and the list of analytes in Sec. 1.1 of this method pertaining to certain compound classes and applicable preservation options that

may affect target analyte stability and analytical holding times. Samples not analyzed within this period should be identified to the data user and the results considered minimum values unless it can be demonstrated that the reported VOC concentrations are not adversely affected by preservation, storage and analyses performed outside the recommended holding times.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. It should be noted that several methods (e.g., Method 8000) also contain general QC criteria and guidance that pertain to the individual methods referenced therein (e.g., Methods 8081, 8082, 8260 and 8270). Individual methods may also contain QC criteria specific only to that method. The QC criteria in the general methods take precedence over chapter QC criteria. Method-specific QC criteria take precedence over general method QC criteria.

Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Initial Demonstration of Proficiency (IDP)

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000D, Sec. 9.3 for information on how to accomplish a demonstration of proficiency.

9.3 Lower Limit of Quantitation (LLOQ) check standard

The laboratory shall establish the LLOQ as the lowest point of quantitation, which in most cases, is the lowest concentration in the calibration curve. LLOQ verification is recommended for each project application to validate quantitation capability at low analyte concentration levels. This verification may be accomplished with either clean control material (e.g., reagent water, solvent blank, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix, free of target compounds. Optimally, the LLOQ should be less than the desired regulatory action levels based on the stated DQOs.

In order to demonstrate the entire sample preparation and analysis process at the lower limit of quantitation (LLOQ), a LLOQ check standard (not part of an initial calibration) is prepared by spiking a clean control material with the analyte(s) of interest at the predicted LLOQ concentration level(s). Alternatively, a representative sample matrix may be spiked with the analytes of interest at the predicted LLOQ concentration levels. The LLOQ check is carried through the same preparation procedures as environmental samples and other QC samples.

Recovery of target analytes in the LLOQ check standard should be within established in-house limits, or other such project-specific acceptance limits, to demonstrate acceptable method performance at the LLOQ. Until the laboratory has sufficient data to determine

acceptance limits, the LCS criteria ± 20% may be used for the LLOQ acceptance criteria. This acknowledges the poorer overall response at the low end of the calibration curve. Historically-based LLOQ acceptance criteria should be determined as soon as practical once sufficient data points have been acquired. Additional information on LLOQ can be found in 8000D, Sec. 9.7.

9.4 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If a peak is observed within the retention time window of any analyte that would interfere with measurement of that analyte, determine the source and eliminate it, if possible, before analyzing the samples. The blanks should be carried through all stages of sample preparation and analysis. When new reagents or chemicals are received, the laboratory should monitor method and/or instrument blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks must be prepared for each set of reagents.

The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" may lead to negative sample results. If the method blank results do not meet the project-specific acceptance criteria and reanalysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory.

9.5 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, bias, method sensitivity). At a minimum, each batch of 20 or fewer field samples should include at least one method blank, a laboratory control sample (LCS), and either a matrix spike/matrix spike duplicate (MS/MSD) pair or a matrix spike and duplicate analysis of one field sample. When used, surrogates may be added to each field sample and QC sample and their recovery monitored to evaluate the effect of the sample matrix. Any method blanks, matrix spike samples, and duplicate QC samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

See Methods 5000 and 8000 for procedures to follow to demonstrate acceptable continuing performance on each set of samples to be analyzed.

9.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.7 The laboratory should have quality control procedures to make sure that sample integrity is not compromised during the sample collection and sample handling process, e.g., through analysis of trip blanks, method blanks, etc. In addition, it would be advisable for the laboratory to monitor the internal standard (IS) area counts for all samples; leaks attributed to a poor seal with the vial caps and septa will be evident by low IS area counts. Sample containers and data results for instances where low IS area counts are observed and leaks are suspected should be discarded. Low area counts of the less volatile internal standards may also be attributed to matrix effects and should not be confused with a leaking vial.

9.8 Heating the sample/chemical preservative/matrix modifier mixture can exacerbate chemical interferences such as those introduced by acid catalyzed hydrolysis or base catalyzed substitution and elimination reactions. This can only be monitored through a matrix spike of a sample from every project analytical batch. The spiking solution should be the same as that used to prepare the calibration standards in order to minimize sources of variability in evaluating spike recovery. The acceptance criteria shall be those recommended in the determinative method or specified by a properly executed systematic planning document. If these criteria cannot be met, the analyst may adjust the pH of the mixture through the addition of solid $NaHSO_4$ to excessively basic mixtures or solid $Na_3PO_4 \cdot 12H_2O$ to excessively acidic mixtures. After this is done, the matrix spike analysis should be repeated with an unanalyzed vial. If the results are acceptable, this pH adjustment should be made to all samples in the appropriate analytical batch. Even if the pH-adjusted matrix spike analysis is acceptable, the data user must be made aware that the initial matrix spike failed and the pH adjustment was necessary. The results from the pH adjusted samples should be reported, and the data user must be made aware that the results for the analytes for which the initial matrix spike failed are questionable.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization and refer to the appropriate determinative method for additional calibration and standardization procedures.

11.0 PROCEDURE

11.1 Sample preparation - Sample preparation in the laboratory will be necessary except when a soil sample is collected and used only for screening purposes. The procedure for sample preparation depends upon the matrix of the sample and the target analyte concentration range. To minimize loss of VOCs from the samples or exchange of the vial headspace with the room air, add spiking solutions quickly to cold sample vials soon after removing from refrigerated storage and either reseal or place a new cap on top of the vial and apply slight pressure in between preparation steps.

CAUTION: Adding standard solutions (e.g., internal standards) to a sealed vial by puncturing the PTFE septum face with a microsyringe exposes the gas phase contents of the vial to the silicone material backing the septum. This material may absorb some of the gas phase VOCs in the vial, causing problems with calibration, measurement in samples, spike recovery, *etc.*, as a function of exposure time. This problem is generally worse for the higher molecular weight VOCs with high octanol-water partition coefficients, and this practice should be avoided or the vial caps should be exchanged for caps with un-punctured septa soon after spiking if these VOCs are analytes of interest.

11.1.1 Water samples - The preparation of water samples inevitably involves some sample manipulation and exposure to the laboratory atmosphere. Extreme caution should be exercised to minimize any volatilization of analytes out of the sample contents and into the laboratory atmosphere. The first precaution is to prepare the water samples immediately after removal from cold storage. The decreased temperature reduces analyte volatility, and the benefits of this are substantially greater than the inaccuracies introduced by measuring sample volume at lower temperatures.

11.1.1.1 Add 5 mL of the matrix modifier solution to a headspace vial (Sec. 6.0), if used. Otherwise, add 5 mL reagent water. Set the septum and crimp top onto the vial and move the crimping tool to a readily available position.

11.1.1.2 Insert the tip of a 5-mL gas tight syringe through the septum of the vial to withdraw the sample. Fill the syringe, taking care to prevent air from leaking into the syringe while filling it, then remove the syringe from the sample and place it in the liquid phase in the headspace vial. Inject the entire aliquot into the headspace vial, then quickly add the internal standard and/or surrogate standard solution, if used, and immediately seal the vial. This process of taking an aliquot destroys the validity of the liquid sample for future analysis. Therefore, if there is only one VOA vial, the analyst should prepare a second sample in the same manner as the first at this time to protect against possible loss of sample integrity. This second sample is stored at <6°C until the analyst has determined that the first sample has been analyzed properly. If a second analysis is needed, it should be completed within 24 hr.

11.1.2 Soil samples - If the sample will be analyzed by direct vapor partitioning for low level analysis, follow the instructions in this section. If the sample will be extracted with solvent and the extract diluted for high level analysis, proceed to Sec.11.4.

11.1.2.1 If the soil sample was placed into a headspace vial with neither water nor matrix modifier and the sample mass was not recorded in the field, estimate the sample mass by weighing the vial plus soil and subtract the mass of an empty vial and cap. Then, unseal the vial, add 10.0 mL of matrix modifying solution, if used, or reagent water, along with any internal standard and/or surrogate standard used, and immediately reseal the vial. As noted in Sec. 8.0, VOC losses may occur as a result of opening the vial and displacing 10 mL of headspace.

CAUTION: Only open and prepare one vial at a time to minimize loss of volatile organics.

11.1.2.2 If the soil sample was placed into a headspace vial with reagent water or the matrix modifier solution at the time of sampling, first weigh the sealed vial and its contents to 0.01 g. If the matrix modifying solution was added at the time of sampling (Sec. 8.3.1), the tare weight does not include 10 mL of matrix modifying solution. Therefore, weigh the field blank associated with those samples and subtract from it the tare weight of the vial in which the field blank was prepared. Use the difference as the weight of the matrix modifying solution in the samples. (Although this approach may introduce some error into the sample results, that error should be much less than the changes that will occur in an unpreserved sample shipped to the laboratory without the modifier). If surrogates and/or internal standards were not added at the time of sampling, they should be added at this time.

11.2 The low-concentration method utilizing an equilibrium headspace technique is found in Sec. 11.3 and sample preparation for the high-concentration method is found in Sec. 11.4. The high-concentration method is recommended for samples that obviously contain oily material or organic sludge waste (see Sec. 4.4). See Method 8000 for guidance on the selection of a GC or GC/MS determinative method. For the analysis of gasoline, use Method 8021 with GC/PID (photoionization detector) for BTEX (benzene, toluene, ethylbenzene, and xylenes) in series with Method 8015 with the GC/FID (flame ionization detector) detector for other gasoline components. If GC/MS analysis is preferred, follow Method 8260. For the analysis of MTBE and

the other fuel oxygenates, use either Method 8015 with the GC/FID detector or Method 8260 using GC/MS.

11.3 Low-concentration (direct vapor partitioning) method for water, soil/sediment and solid waste amenable to the equilibrium headspace method.

11.3.1 Calibration

Prior to using this introduction technique for any GC or GC/MS method, the system must be calibrated. General calibration procedures are discussed in Method 8000, while the determinative methods and Method 5000 provide specific information on calibration and preparation of standards. Normally, external standard calibration is preferred for the GC methods because of possible interference problems with internal standards. If interferences are not a problem, based on historical data, internal standard calibration is acceptable. The GC/MS methods normally utilize internal standard calibration. The GC/MS methods require instrument tuning prior to proceeding with calibration.

11.3.1.1 GC/MS tuning

If a GC/MS determinative method is employed, prepare a headspace vial containing reagent water and the amount of 4-bromofluorobenzene (BFB) listed in the determinative method.

11.3.1.2 Initial calibration

Prepare a minimum of five headspace vials for calibration standards, as described in Sec. 7.6, and a reagent blank (Sec. 7.5), and proceed according to Sec. 11.3.2 and the determinative method selected. The mixing step is unnecessary, because no soil is present in the vial. See method 8000D for the minimum number of calibration standards recommended for each type of calibration model.

11.3.1.3 Calibration verification

Prepare a headspace vial, as described in Sec. 7.6, by spiking with the mid-concentration calibration standard. Proceed according to Sec. 11.3.2.1 (beginning by placing the vial into the autosampler) and the determinative method. If a GC/MS determinative method is employed, prepare a second headspace vial containing reagent water and the amount of BFB listed in the determinative method.

11.3.2 Headspace analyzer operating conditions

The conditions described throughout Sec. 11.3 were experimentally optimized using the equipment described in Reference #1 in Sec. 16 and employing Method 8260 as the determinative method. If other headspace systems and determinative methods are utilized, it is recommended that the manufacturer's headspace operating conditions be followed, provided that they are appropriate for the determinative method to be employed.

11.3.2.1 Mix the samples (on a rotator or shaker) for at least 2 min. For samples that contain water insoluble materials, care must be exercised during mixing to prevent this material from adhering to the inner surface of the vial seal; otherwise the sampling needle can become contaminated with this material upon puncturing the seal. Care must also be exercised to avoid over filling the vial to prevent contaminating the needle with aqueous sample.

Place the vials in the autosampler carrousel at room temperature. The individual vials are heated to 85 °C and allowed to equilibrate for 50 min. Each sample is mixed by mechanical agitation during this equilibrium period. Each vial is pressurized with helium carrier gas to a minimum pressure of 10 psi.

11.3.2.2 A representative and reproducible sample of the pressurized headspace is transferred to the GC column through a heated transfer line according to the manufacturer's instructions.

11.3.2.3 Proceed with the analysis as per the determinative method of choice.

<u>NOTE</u>: If maintaining a specified pH is critical to quality assured measurement of the analyte(s) of concern (Sec. 4.7), the pH of each sample should be verified. If basic preservation is necessary, the pH of the sample should be verified to be ≥10 (see Sec. 7.8.1). If acid preservation is necessary, the pH should be verified to be ≤ 2, (see Sec. 7.8.2). This check may be performed after analysis of the sample in order to avoid compromising sample integrity. Wide-range pH paper should provide sufficient information to verify efficacy of the preservative.

11.4 High-concentration soil method

11.4.1 If the sample was collected as described in Sec. 8.3.2 without the addition of methanol to the vial, then weigh the sample to the nearest 0.01 g. Add twice the volume of methanol as the nominal sample mass to a tared VOA vial and immediately reseal the vial. Open only one vial at a time to minimize loss of VOCs. If the sample was collected in a sealable coring device as described in Sec. 8.3.3, add the methanol to a vial first, weigh the vial with the methanol and the cap together to obtain the tare mass, and then add the soil plug, seal immediately, reweigh, and calculate the sample mass.

11.4.2 If the procedure in Sec. 8.3.1 was employed for sample collection and either the matrix modifying solution or organic-free reagent water was added to the sample vials, subsamples for high concentration analysis should be taken from the separate VOA vials collected without matrix modifying solution or reagent water as described in Sec. 8.3.2 or from the vials collected for dry solids determination. Transfer approximately 5 g of sample from the 40 or 60 mL VOA vial into a tared VOA vial containing 10.0 mL of methanol, seal the vial, and reweigh to estimate the mass of sample transferred. Open only one vial at a time to minimize the loss of volatile organics. Substantial VOC losses may occur as a result of transferring a subsample from one vial to another using this procedure. See Sec. A.5 in the Appendix of Method 5035A for more details.

11.4.3 Mix by shaking for 10 min at room temperature. Decant 2 mL of the methanol extract to a screw-top vial with PTFE-faced septa and seal. Withdraw 10 µLand inject into a headspace vial containing 10.0 mL of matrix modifying solution or organic free reagent water. A larger volume of methanol may be added provided the methanol content does not adversely affect the analyte responses (refer to Sec. 7.4.1). Add internal standards and/or surrogates as appropriate, and analyze by the headspace procedure by placing the vial into the autosampler and proceeding with Sec. 11.3.2.1.

12.0 DATA ANALYSIS AND CALCULATIONS

There are no data analysis and calculation steps directly associated with this procedure. Follow the directions given in the determinative method.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance goals should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method.

13.2 Water samples

This method was used to measure several VOCs in groundwater samples. The samples were collected from two sites: twenty-four samples were collected from the first site (site A) and twenty-three samples were collected from the second site (site B). Using a basic preservative to prevent the hydrolysis of ethers such as MTBE, multiple groundwater vials were collected at each sampling point. The samples were analyzed by three independent laboratories. All of the laboratories used this method for sample preparation, and each laboratory used a different determinative method. One laboratory used a GC/MS technique with a quadrupole mass spectrometer (Method 8260), another used a GC/MS technique with an ion-trap mass spectrometer (Method 8260), and the third used a GC/FID technique (Method 8015). The example results of the analyses are shown in Figures 1 through 6. Since all three laboratories followed the same project plan and the same data quality objectives, the data generated by the three laboratories is mutually comparable, even though they used different techniques. As recommended in Sec. 9.8, matrix spike studies were done at each site. The example percent recoveries from the site A studies are shown in Figure 7, while those from site B are shown in Figure 8. Figure 8 shows that one of the labs had poor recovery for MTBE. However, the recovery of the other ethers was acceptable, indicating that hydrolysis was unlikely to be the source of the problem. The effect was attributed to sample matrix interference.

13.3 Soil samples - Single-laboratory accuracy and precision data were obtained for the method analytes in two soil matrices, i.e., sand and garden soil. These data are found in Tables 26-28 of Method 8260C.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety,
http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the ACS publication listed in Sec. 14.2.

16.0 REFERENCES

1. P. Flores, and T. Bellar, "Determination of Volatile Organic Compounds in Soils using Equilibrium Headspace Analysis and Capillary Column Gas Chromatography/Mass Spectrometry," U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH, December, 1992.

2. B. V. Loffe, and A. G. Vitenberg, "Headspace Analysis and Related Methods in Gas Chromatography," John Wiley and Sons, 1984.

3. R. J. Pirkle, and R. P. McLoughlin, "The Analysis of Selected Components of Reformulated Gasoline in Environmental Samples" *from* MTBE Handbook, *ed.* Kostecki, P. and Moyer, E. Amherst Scientific Publishers, 2002.

4. USEPA OUST, *Environmental Fact Sheet: Analytical Methods for Fuel Oxygenates*, EPA 510-F-03-001, April, 2003.

5. H. White, B. Lesnik, and J. T. Wilson, "Analytical Methods for Fuel Oxygenates", *LUSTLine* (Bulletin #42), October, 2002, http://www.epa.gov/oust/mtbe/LL42Analytical.pdf.

6. RCRA Organic Methods Workgroup Meeting Minutes, March 20, 2012.

7. RCRA Organic Methods Workgroup Meeting Minutes, March 22, 2012.

8. RCRA Organic Methods Workgroup Meeting presentation describing changes to Method 5021, "Proposed Changes to SW-846 Method 5021A, VOCs by Static Headspace", March 1, 2012.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

FIGURE 1

EXAMPLE RESULTS FOR SITE A STUDY OF ETHYL *TERT*-BUTYL ETHER

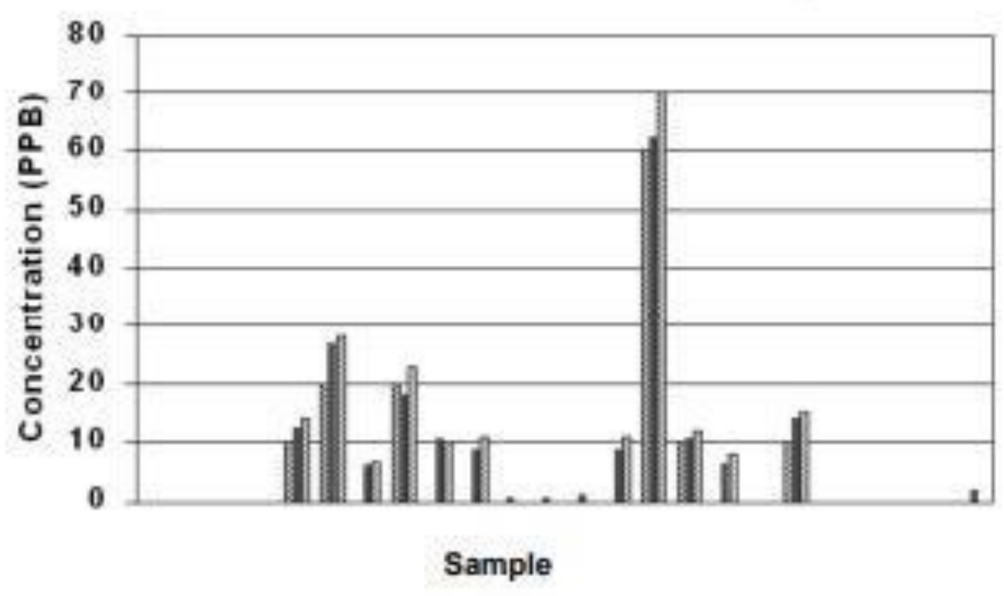


FIGURE 2

EXAMPLE RESULTS FROM SITE A STUDY FOR *TERT* AMYL METHYL ETHER

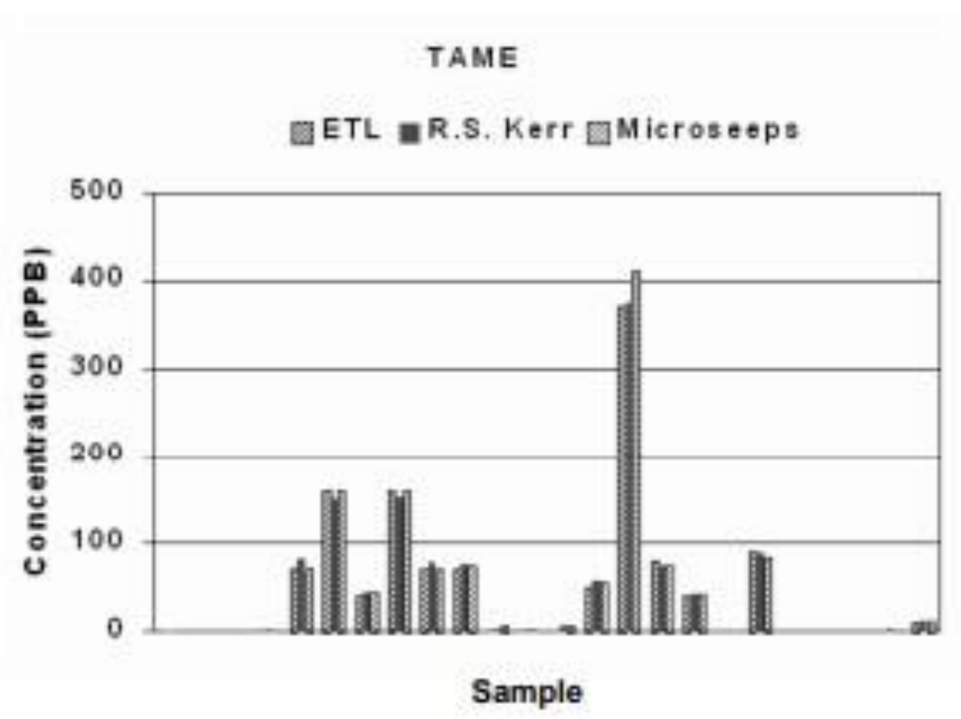


FIGURE 3

EXAMPLE RESULTS FROM SITE A STUDY FOR METHYL *TERT*-BUTYL ETHER

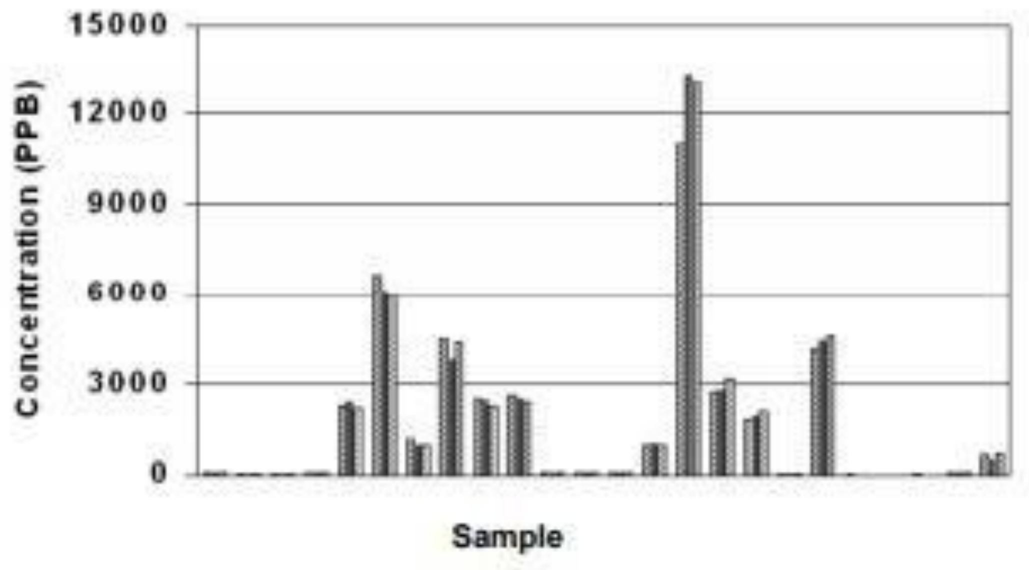
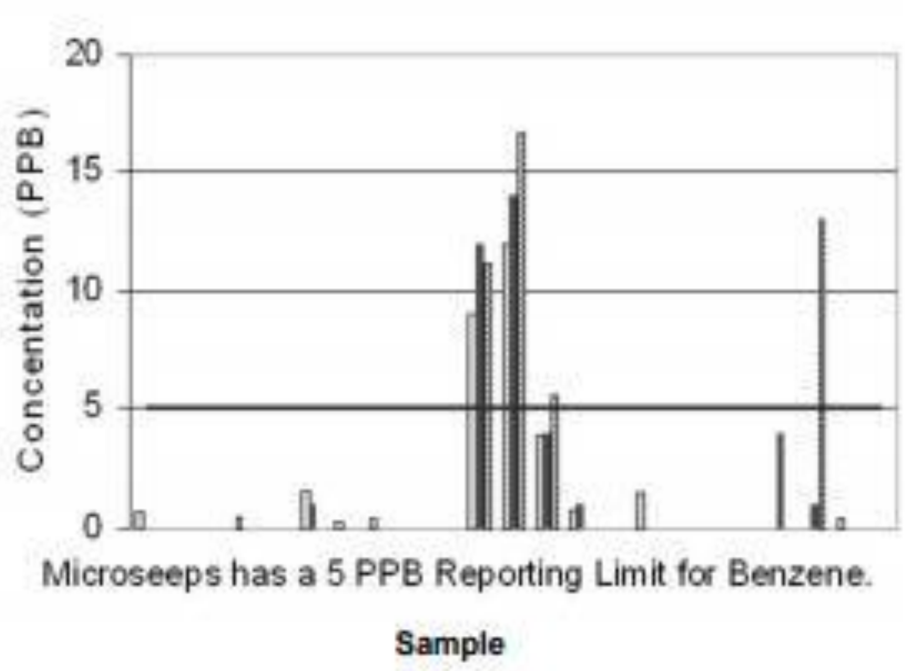


FIGURE 4

EXAMPLE RESULTS FROM SITE B STUDY FOR BENZENE



Sample

FIGURE 5

EXAMPLE RESULTS FROM SITE B STUDY FOR METHYL *TERT*-BUTYL ETHER

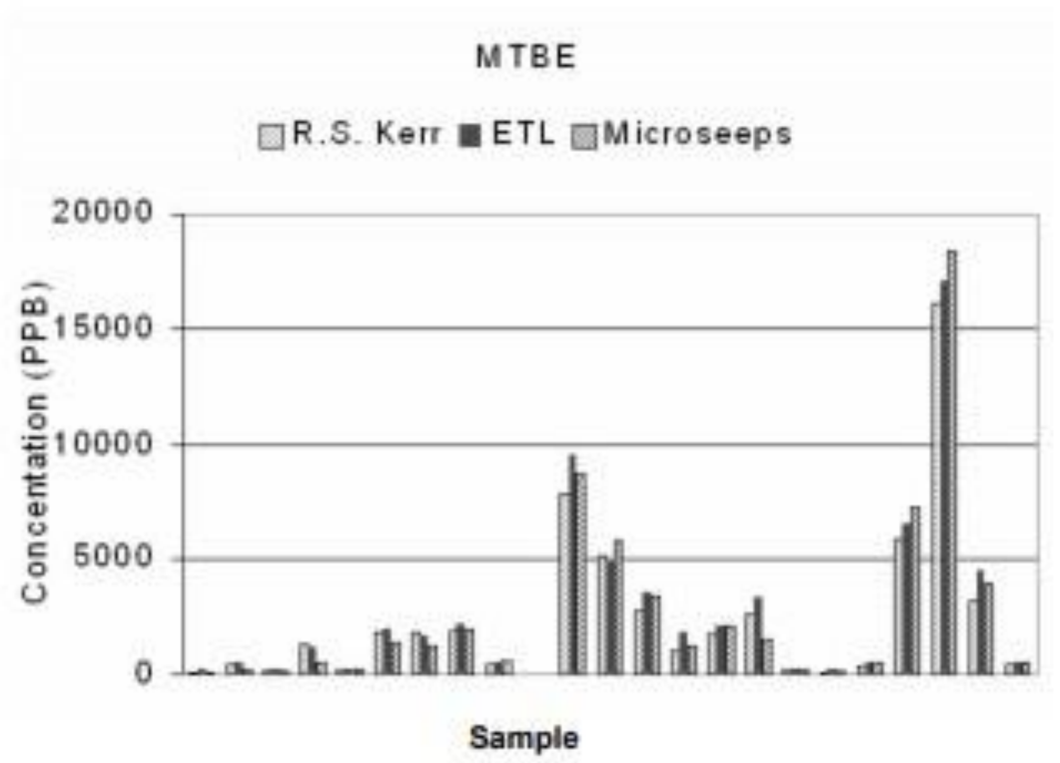


FIGURE 6

EXAMPLE RESULTS FROM SITE B STUDY FOR *TERT*-BUTYL ALCOHOL

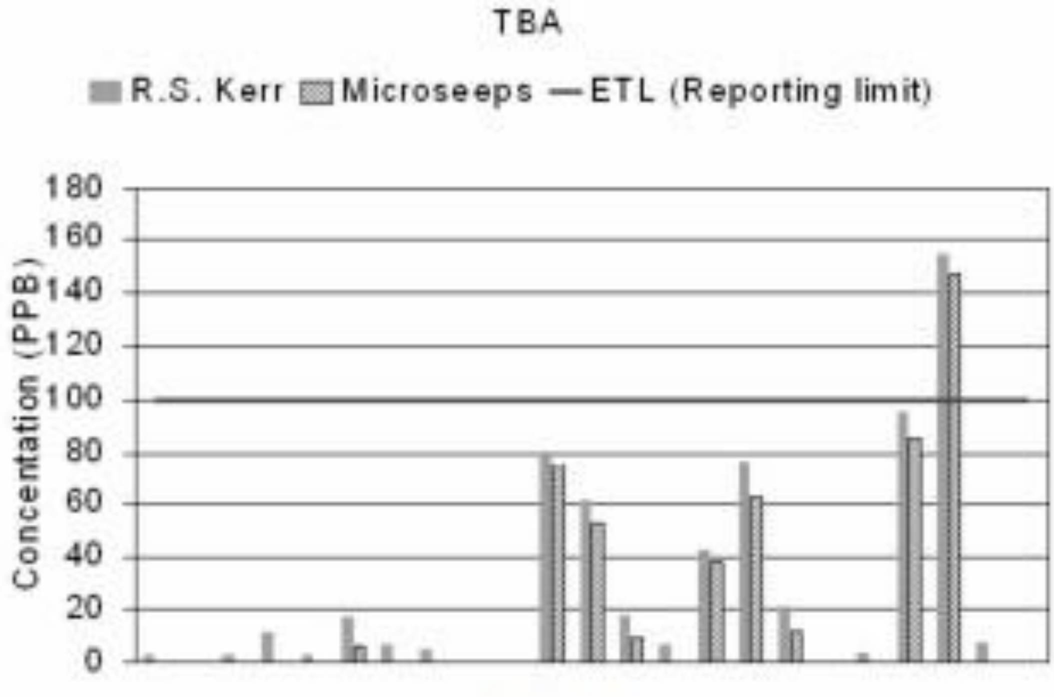


FIGURE 7

EXAMPLE PERCENT RECOVERIES FROM THE MATRIX SPIKE STUDIES OF SITE A

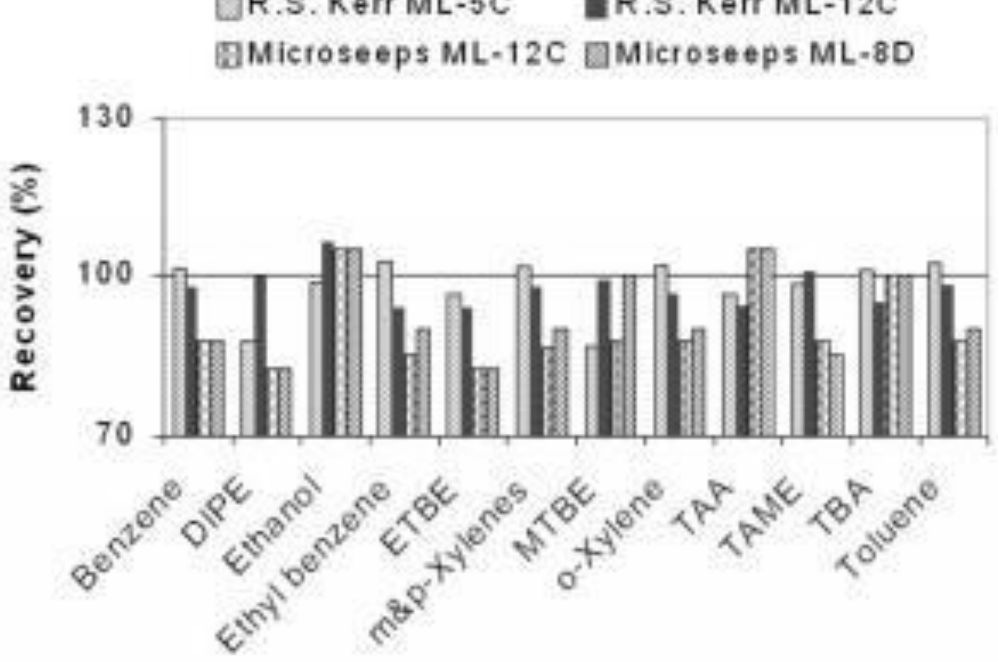
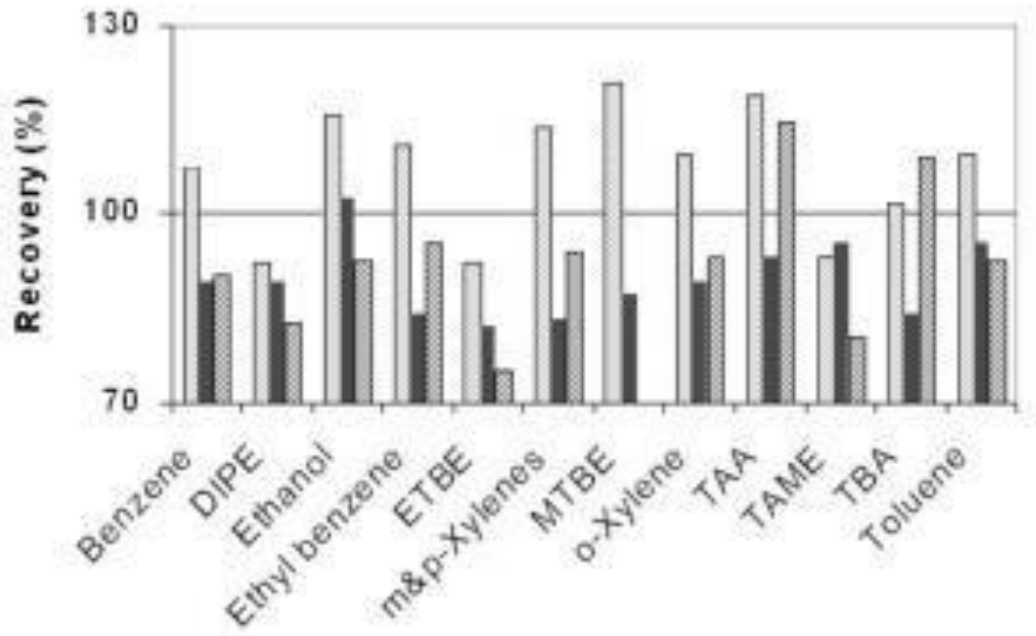


FIGURE 8

EXAMPLE PERCENT RECOVERIES FROM THE MATRIX SPIKE STUDIES OF SITE B



Appendix A:

Summary of Revisions to Method 5021A (as compared to previous Revision 1, June 2003)

1. Improved overall method formatting for consistency with new SW-846 methods style guidance. The format was updated to Microsoft Word .docx.
2. Minor editorial and technical revisions were made throughout to improve method clarity.
3. The revision number was changed to 2 and the date published was changed to July 2014.
4. This appendix was added showing changes from the previous revision.
5. Added updated IDP language and LLOQ verification standard language to Sections 9.2 and 9.3.
6. Included response column and a classification system for analytes in Secs. 1.1 and 1.2 to provide an indication of which VOC responses were improved by the matrix modifier.
7. Added a sealable, air-tight coring device as an alternative sample collection option for soils to Sec. 8.3.3.
8. Added an alternative for calibration standard preparation to Sec. 7.4.1 that allowed for multiple calibration levels prepared by adding different volumes of one or more stock solutions.
9. Clarified in Sec. 1.4 the major sources of measurement bias expected for sample analysis using this method, as well as which sources of measurement bias the matrix modifier may improve, which sources of measurement bias may be made worse by the matrix modifier, and under what other circumstances not adding the matrix modifier may be appropriate.
10. Added a caution after Sec. 11.1 regarding the expected effect of compromising the PTFE face of a vial seal on recovery of oil soluble target analytes.

ANEXO 9

Método de la norma de la Agencia de Protección Ambiental (EPA 8000D)

METHOD 8000D

DETERMINATIVE CHROMATOGRAPHIC SEPARATIONS

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods in this manual, with the exception of required use for the analysis of method-defined parameters, are intended to be guidance documents. They contain general information on how to perform an analytical procedure or technique, which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. Performance data included in this method are for guidance purposes only and must not be used as absolute quality control (QC) acceptance criteria for the purpose of laboratory QC or accreditation.

1.0 SCOPE AND APPLICATION

Please see Appendix A at the back of this document for a summary of revisions to Method 8000C (From Revision 3, March 2003).

1.1 Method 8000 is not a determinative method but instead provides guidance on analytical chromatography and describes calibration and QC requirements common to all SW-846 chromatographic methods. However, more specific QC requirements provided in the applicable determinative method will supersede those noted in Method 8000. Method 8000 should be applied in conjunction with all SW-846 determinative chromatographic methods. The methods include, but are not limited to, the following:

Method Number	Analytes	Chromatographic Technique (Sec. 1.5)	Detector
6850	Perchlorate	HPLC	MS, MS/MS
6860	Perchlorate	IC	MS, MS/MS
7580	White phosphorus (P_4)	GC	NICI/MS
8011	EDB, DBCP	GC, capillary column	ECD
8015	Non-halogenated volatiles	GC, packed & capillary column	FID
8021	Volatiles	GC, capillary column	PID, ELCD
8031	Acrylonitrile	GC, packed column	NPD
8032	Acrylamide	GC, packed column	ECD
8033	Acetonitrile	GC, capillary column	NPD
8041	Phenols	Underivatized or derivatized; GC, capillary column	FID, ECD
8061	Phthalates	GC, capillary column	ECD
8070	Nitrosamines	GC, packed column	NPD, ELCD, TED
8081	Organochlorine pesticides	GC, capillary column	ECD, ELCD

Method Number	Analytes	Chromatographic Technique (Sec. 1.5)	Detector
8082	Polychlorinated biphenyls	GC, capillary column	ECD, ELCD
8091	Nitroaromatics and cyclic	GC, capillary column	ECD
8100	Polynuclear aromatic hydrocarbons	GC, packed & capillary column	FID
8111	Haloethers	GC, capillary column	ECD
8121	Chlorinated hydrocarbons	GC, capillary column	ECD
8131	Aniline and selected	GC, capillary column	NPD
8141	Organophosphorus pesticides	GC, capillary column	FPD, NPD, ELCD
8151	Acid herbicides	Derivatized; GC, capillary column	ECD
8260	Volatiles	GC, capillary column	MS
8261	Volatiles	GC, capillary column	MS
8265	Volatiles	NA	DS/ITMS
8270	Semivolatiles	GC, capillary column	MS
8275	Semivolatiles	Thermal extraction/GC	MS
8276	Toxaphene and Congeners	GC, capillary column	NICI/MS
8280	Dioxins and Dibenzofurans	GC, capillary column	Low resolution MS
8290	Dioxins and Dibenzofurans	GC, capillary column	High resolution MS
8310	Polynuclear aromatic hydrocarbons	HPLC, reverse phase	UV, Fluorescence
8315	Carbonyl compounds	Derivatize; HPLC	UV
8316	Acrylamide, acrylonitrile, acrolein	HPLC, reverse phase	UV
8318	N-Methyl carbamates	Derivatize; HPLC	Fluorescence
8321	Extractable non-volatiles	PLC, reverse phase	TS/MS, UV
8323	Organotin compounds	HPLC, reverse phase	ES/ITMS
8325	Extractable non-volatiles	HPLC, reverse phase	PB/MS, UV
8330	Nitroaromatics and nitramines	HPLC, reverse phase	UV
8331	Tetrazene	HPLC, ion pair, reverse	UV
8332	Nitroglycerine	HPLC, reverse phase	UV
8410	Semivolatiles	GC, capillary column	FT-IR
8430	Bis(2-chloroethyl) ether hydrolysis products	GC, capillary column	FT-IR

DBCP = Dibromochloropropane
DS/ITMS = Direct sampling/ion trap mass spectrometry
ECD = Electron capture detector
EDB = Ethylene dibromide
ES/ITMS = Electrospray ionization/ion trap mass spectrometry
ELCD = Electrolytic conductivity detector
FID = Flame ionization detector
FPD = Flame photometric detector
FT-IR = Fourier transform-infrared
GC = Gas chromatography
HPLC = High performance liquid chromatography
MS = Mass spectrometry
MS/MS = Mass spectrometry/Mass spectrometry
NICI/MS = Negative Ion Chemical Ionization/Mass spectrometry
NPD = Nitrogen/phosphorous detector
NA = Not applicable
PAHs = Polynuclear aromatic hydrocarbons
PB/MS = Particle beam mass spectrometry
PID = Photoionization detector
TED = Thermionic emission detector
TS/MS = Thermospray mass spectrometry
UV = Ultraviolet

1.2 Analytical chromatography is used to separate target analytes from co-extracted interferences in samples. Chromatographic methods can be divided into two major categories: GC and HPLC.

1.2.1 GC is the separation technique of choice for organic compounds which can be volatilized without being decomposed or chemically rearranged.

1.2.2 HPLC is a separation technique useful for semivolatile and non-volatile chemicals or for analytes that decompose upon heating. Successful liquid chromatographic separation requires that the analyte(s) of interest be soluble in the solvent(s) selected for use as the mobile phase.

1.3 All chromatographic processes achieve separation by passing a mobile phase over a stationary phase. Constituents in a mixture are separated because they partition differently between the mobile and stationary phases and thus have different retention times. Compounds that interact strongly with the stationary phase elute slowly (i.e., longer retention times), while compounds that remain in the mobile phase elute quickly (i.e., shorter retention times).

1.3.1 The mobile phase for GC is an inert gas, usually hydrogen or helium, and the stationary phases are generally polymer bases.

1.3.2 In "normal phase" HPLC, the mobile phase is less polar than the stationary phase. In "reverse phase" HPLC, the converse is true. Reverse phase HPLC is the technique of choice for environmental and waste analyses of non-volatile organic target analytes.

1.3.3 Ion exchange chromatography is used to separate ionic species through competition with ions in the mobile phase for oppositely charged exchange sites on a stationary phase. Differential selectivities of the ionic species and the mobile phase ions for exchange sites are responsible for the chromatographic separation of the ions.

1.4 A number of specific GC and liquid chromatography (LC) techniques are used for environmental and waste analyses. Specific techniques are distinguished by the chromatographic hardware and chemical mechanisms used to achieve separations.

1.4.1 GC methods, including those in SW-846, can be categorized on the basis of the chromatographic columns employed.

1.4.2 HPLC methods in SW-846 are categorized on the basis of the mechanism of separation.

1.5 SW-846 methods describe columns and conditions that have been demonstrated to provide optimum separation of all or most target analytes listed in that specific procedure. Most often, those columns were the ones used by EPA during method development and testing. Analysts may change those columns and conditions, provided that they demonstrate performance for the analytes of interest that is appropriate for the intended application. This is especially true when limited groups of analytes are to be monitored (i.e., if only a subset of the list of target analytes in a method are needed, the chromatographic conditions and columns may be optimized for those analytes).

1.5.1 Chromatographic performance is demonstrated by the resolution of standards and the ability to model the response of the detector during calibration, and by sensitivity, precision, bias, frequency of false positives, and frequency of false negatives

during analysis. The laboratory must demonstrate that any chromatographic procedure it uses provides performance satisfying the analytical requirements of the specific application for which it is being used. Such demonstrations should be performed using the procedures outlined in Secs. 9.2 to 9.8 of this method and appropriate sections in Chapter One.

1.5.2 Laboratories must also be cautious whenever the use of two dissimilar columns is included in a method for confirmation of identification and quantitation. For instance, a DB-5 column generally cannot be used for confirmation of results obtained using an SPB-5 column because the stationary phases are not sufficiently dissimilar and the changes in elution order (if any) will not provide adequate confirmation.

1.6 When GC conditions are changed, retention times and analytical separations are often affected. For example, increasing the oven temperature changes the rate of partitioning between the mobile and stationary phases, leading to shorter retention times. GC retention times can also be changed by selecting a column with a different length, stationary-phase loading (i.e., capillary column film thickness or percent loading for packed columns), or alternative liquid phase. As a result, two critical aspects of any SW-846 chromatographic method are the determination and/or verification of retention times and analyte separation.

1.7 HPLC retention times and analytical separations are also affected by changes in the mobile and stationary phases. The HPLC mobile phase is easily altered by adjusting the composition of the solvent mixture being pumped through the column. In reverse phase HPLC, increasing the ratio of water-miscible organic solvent to water generally shortens retention times. HPLC retention times can also be changed by selecting a column with a different length, alternative bonded phase, or dissimilar particle size (e.g., smaller particles and/or a longer column generally increase column resolution, while different bonded phases may resolve specific components differently). HPLC methods are also particularly sensitive to small changes in chromatographic conditions, including temperature. HPLC column temperature control ovens should be used to maintain constant retention times because ambient laboratory temperatures may fluctuate throughout the day. SW-846 methods provide conditions that have been demonstrated to provide good HPLC separations using specific instruments to analyze a limited number of samples. Analysts (particularly those using HPLC/MS) may need to tailor the chromatographic conditions listed in the method for their specific application and/or instrument.

1.8 Chromatographic methods can be used to produce data of appropriate quality for the analysis of environmental and waste samples. However, data quality can be greatly enhanced when the analyst understands both the intended use of the results and the limitations of the specific analytical procedures employed. Therefore, these methods are recommended for use only by, or under the close supervision of, experienced analysts. Many difficulties observed in the performance of SW-846 methods for the analysis of RCRA wastes can be attributed to the lack of skill and training of the analyst.

1.8.1 Methods using selective (e.g., PID, NPD, ELCD) or non-selective (e.g., FID) detectors may present serious difficulties when used for site investigations, including coelution of target analytes, false negatives due to retention time shifts, and false positives and quantitation errors due to coeluting non-target sample components.

1.8.2 In contrast, GC methods employing selective or non-selective detectors may be appropriate for remediation activities where the analytes of concern are known, of limited number, and of significantly greater concentration than potentially interfering materials.

1.8.3 If the site is not well characterized, and especially if large numbers of target analytes are of concern, analysis by GC/MS or HPLC/MS may be more appropriate.

1.9 Each chromatographic method includes a list of the compounds recommended for analysis given the procedures described therein. Lists in some methods are lengthy; it may not be practical or appropriate to determine all the analytes simultaneously. Such analyte lists do not imply a regulatory requirement for the analysis of any or all of the compounds, but rather indicate the method(s) applicable to those compounds.

1.10 Analysts should consult the disclaimer statement at the front of the manual and the information in Chapter Two for 1) guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies; and 2) the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrices of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly required in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance for the analyst and regulated community in making judgments necessary to generate results meeting the data quality requirements for the intended application.

1.11 All of the SW-846 determinative chromatographic methods that reference this method are restricted to use by, or under the supervision of, analysts experienced in the use of gas or high performance liquid chromatographs and skilled in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate an acceptable initial demonstration of proficiency (IDP) along with acceptable results according to method recommendations and stated project data quality objectives (DQOs). Method 8000 is intended to be a supplement to, but is <u>not</u> intended to be a substitute for, formal training in the basic principles of GC or HPLC.

2.0 SUMMARY OF METHOD

This method describes general considerations in achieving chromatographic separations and performing calibrations. It is to be used in conjunction with all SW-846 determinative chromatographic methods, including, but not limited to, each method listed in Sec. 1.1. Each of these chromatographic methods recommends appropriate procedures for sample preparation, extraction, cleanup, and/or derivatization. Consult the specific procedures for additional information on these crucial steps in the analytical process.

2.1 Sec. 4.2 of this method provides general guidance on minimizing contamination, including cross-contamination between samples. Sample screening procedures are strongly recommended, and discussed in Sec. 4.3.

2.2 Before any sample or blank is introduced into a chromatographic system, the appropriate resolution criteria and calibration procedure(s) described in Method 8000 must be satisfied.

2.3 Secs. 4.4 and 4.5 provide information on the effects of chromatographic interferences.

2.4 Sec 6.0 of this method contains generalized specifications for the components of both GC and HPLC systems used in SW-846 analyses.

2.5 Calibration of the analytical system is another critical step in the generation of quality data. Sec. 11.5 discusses specific procedures and calculations for both linear and non-linear calibration models. Continued use of any chromatographic procedure necessitates a verification of the calibration model, and procedures for such verifications are described in this method as well (Sec. 11.7).

2.6 Identification of target compounds by any chromatographic procedure is based, at least in part, on retention times. Sec. 11.6 provides procedures for the determination of retention times and retention time windows to be used with the specific methods listed in Sec. 1.1.

2.7 Calculations necessary to derive sample-specific concentrations from the instrument responses are common to most of the analytical methods listed in Sec. 1.1. Commonly used calculations are summarized in Sec. 11.10.

2.8 Preventive maintenance and corrective actions are essential to the generation of quality data in a routine laboratory setting. Suggestions for such procedures are found in Sec. 11.11.

2.9 Most of the methods listed in Sec. 1.1 employ a common approach to QC. While some of the overall procedures are described in Chapter One, Sec. 9.0 describes routinely used procedures for calibration verification, instrument performance checks, demonstrating acceptable performance, etc.

2.10 Before performing analyses of specific samples, analysts should work with data users to determine acceptable recovery ranges for all target analytes of interest in the type of matrices to be tested. Analysts must also be able to demonstrate that the sensitivity of the procedure employed is appropriate for the intended application. One approach to such a demonstration is to estimate the method sensitivity for the analytes of interest using the procedures in Chapter One or other appropriate procedures.

3.0 DEFINITIONS

Refer to Chapter One, the individual determinative methods, and the manufacturer's instructions for definitions that may be relevant.

4.0 INTERFERENCES/CHROMATOGRAPHIC PERFORMANCE

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences during sample analysis. All of these materials must be demonstrated to be free from interferences under conditions of analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on QC procedures and to Chapter Four for general guidance on the cleaning of glassware.

4.2 Contamination by carryover can occur whenever high- and low-concentration samples are analyzed in sequence. To reduce potential for carryover, the sample syringe or purging device must be thoroughly rinsed between samples with an appropriate solvent (including water). Purge-and-trap or headspace devices should be thoroughly baked out between samples.

Where practical, samples with unusually high concentrations of analytes should be followed by method blanks or instrument blanks or by analysis of organic-free reagent water to check for carryover contamination. If target compounds present in an unusually highly concentrated sample are also found to be present in subsequent samples, the analyst must demonstrate that the compounds are not affected by carryover contamination. Conversely, if those target compounds are not present in the subsequent sample(s), then they do not need to be reanalyzed.

Purging vessels may be cleaned by rinsing with methanol, followed by a distilled water rinse and drying in a 105 °C oven between analyses. Detergent solutions may also be used, but care must be taken to remove the detergent residue from the purging vessel. Other approaches to cleaning purging vessels, such as some modern autosamplers which rinse the vessel(s) between runs, may also be employed, provided that the laboratory can demonstrate that they are effective in removing contaminants.

4.3 In addition to carryover of compounds from one sample to the next, the analysis of high-concentration samples can lead to contamination of the analytical instrument itself. Eliminating this contamination can cost significant time and effort that cannot be spent analyzing samples. The most reliable procedure for ensuring minimum down time is to screen samples by a higher level technique. Samples to be analyzed for volatiles can be screened using an automated headspace sampler (Method 5021) connected to a GC/PID/ELCD detector (Method 8021) or by analyzing large (e.g., 100-fold) dilutions of the samples on the GC/MS. Samples to be analyzed for semivolatiles can be screened using GC/FID. Other screening methods are also acceptable. The analyst should use screening results to choose an appropriate dilution factor for the GC/MS analysis that will prevent system contamination yet still provide adequate sensitivity for the major constituents of the sample.

4.4 Elevated chromatographic baselines (e.g., baseline humps) should be minimized or eliminated during these analyses by application of appropriate sample clean-up (Method 3600), extract dilution, use of pre-columns and/or inserts, or employing a selective detector. Integration of "hump-o-grams" can result in significant quantitative errors. When elevated baselines are observed during analysis of blanks and standards, the chromatographic system should be considered contaminated. This contamination can result from impure carrier gas, inadequate gas conditioning, septum bleed, column oxidation, incomplete elution of non-target interferences, and/or pyrolysis products in the injector or column. Such contamination is unacceptable and must be addressed through a program of preventive maintenance and corrective action.

4.5 See Sec. 11.11 for suggested preventative maintenance activities that may prevent or ameliorate deterioration of chromatographic performance.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals and instrumentation included in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

6.1 Mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for

use. Products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list all common laboratory glassware (e.g., beakers and flasks) that might be used.

6.2 GC inlet systems

6.2.1 Volatile organics

Volatile organic analytes are introduced into a GC through a purge-and-trap system, by direct injection, or other technologies. The purge-and-trap apparatus is described in Method 5030 for water samples and in Method 5035 for soil and other solid samples. See Method 5000 for guidance on all forms of sample introduction of volatiles into the GC and GC/MS system.

6.2.2 Semivolatile organics

Sample extracts containing semivolatile organic compounds are introduced into a GC with a syringe that passes through a septum into an injection port. The injection port allows the sample extract to be vaporized prior to being flushed onto the GC column, hence the term "gas" chromatography. Correct setup and maintenance of the injector port is necessary to achieve acceptable performance with GC methods.

6.2.2.1 An injection port septum and liner should be installed in the GC inlet as appropriate for the system.

6.2.2.2 Packed columns and wide-bore capillary columns (> 0.32-mm ID) should be mounted in ¼-inch injectors.

6.2.2.3 Narrow-bore capillary columns (≤ 0.32 mm ID) should be mounted in split/splitless (Grob-type) injectors. Split/splitless injectors should have automated valve closures that direct most of the flow (and sample) onto the head of the analytical column. After a predetermined splitless introduction time the split valve is opened so that most of the flow is vented during analysis, thus eliminating the solvent tail while maintaining proper flow through the column. The initial oven temperature should be below the boiling point of the injection solvent if the solvent front interferes with early-eluting analytes or if the solvent effect is needed to resolve difficult to separate analytes.

6.2.2.4 Cool on-column injection and programmable temperature vaporizer inlets allow the analysis of labile compounds that degrade on packed columns and in split/splitless injectors.

6.3 GC flow control

Precise control of the gas mobile phase is necessary to achieve reproducible GC retention times. Flow controllers within any GC used for analyses described in SW-846 methods should deliver a precisely metered gas flow at a rate appropriate for the GC column mounted in the instrument.

6.3.1 Most GCs have restrictors built into electronic pressure controllers (EPCs) monitored using a digital readout. These restrictors are used to provide precise flow at the carrier gas flow rate listed in the method (e.g., use <20 mL/min restrictors for wide-bore capillary methods).

6.3.2 Analysts should ensure that cylinder pressures are regulated properly and manifold pressures are sufficiently large that a change in the head pressure of an individual instrument does not affect the flow through all instruments. Toggle valves that allow instruments to be isolated are recommended for all multi-instrument gas delivery systems.

6.3.3 Carrier gas should be of high purity, and conditioned between the cylinder and the GC with a scrubber to remove any residual water, oxygen and hydrocarbons as necessary. Gas regulators should contain stainless steel diaphragms. (Neoprene diaphragms are a potential source of gas contamination, and should not be used.)

6.4 GC columns

Each determinative method in SW-846 provides a description of a chromatographic column or columns with associated column specifications. Other GC columns may be substituted in SW-846 methods to improve performance if (1) the criteria described in Sec. 9.3 are satisfied, and (2) target analytes are sufficiently resolved from one another and from co-extracted interferences to provide data of appropriate quality for the intended application.

Use of capillary columns has become standard practice in environmental and waste analysis. Capillary columns have an inherently greater ability to separate analytes than packed columns. However, packed columns can provide adequate resolution of some analytes and are most appropriately employed when the list of analytes to be determined is relatively short.

6.4.1 Narrower columns are more efficient (i.e., can resolve more analytes) but have a lower capacity (i.e., can accept less sample without peak distortion).

6.4.2 Longer columns can resolve more analytes; resolution increases as a function of the square root of column length. Run-time is also increased.

6.4.3 Columns with greater film thickness (i.e., loading) increases column capacity and retention times.

6.5 GC detectors

Detectors are the transducers that respond to components eluting from a GC column and produce the electrical signal used for quantitative determinations. SW-846 analyses in this manual are conducted using the detectors listed in Sec. 1.1. Except where otherwise recommended by the instrument manufacturer, selective non-MS detectors should be maintained at least 20 °C above the highest oven temperature employed to prevent condensation and detector contamination. To prevent condensation between the GC and an MS detector, transfer lines should be maintained at a temperature above the highest temperature of the oven program, or as specified by the instrument manufacturer.

6.6 HPLC injectors

Liquids are essentially uncompressible, so a mechanical device is necessary that allows introduction of the sample into a high pressure flow without significant disruption in the flow rate and hydraulic pressure. Normally, a 6-port valve is used for this purpose. A sample loop is isolated from the flow of the mobile phase and filled with a sample extract. (Larger sample loops may be used to increase sensitivity; however, they may degrade chromatographic performance). The extract is then injected by the valve being turned so that the mobile phase flows through the loop. This procedure virtually eliminates dead volume in the injector and is fully compatible with automated operation.

6.7 HPLC pumps

The mobile phase used for HPLC should be accurately pressurized before it enters the injector. HPLC pumps are generally capable of delivering solvent at 5000 psi or above with excellent precision. Rate of delivery depends on the column used for the separation. Flow rates should be checked by collecting column effluent in a graduated cylinder for a designated time period.

Most pumping systems are capable of changing solvent concentration during an analysis (i.e., gradient elution). Gradients are generated by either high pressure mixing of two streams between the pump and the injector or by proportional mixing of the solvents before they are pumped. In either case, solvent mixing can cause changes in the solubility of dissolved gases, formation of bubbles in the mobile phase, or non-reproducible gradients.

6.8 HPLC Columns

HPLC columns are generally constructed of stainless steel tubing and are sealed with compression fittings. These columns should be constructed with minimum dead volume and a narrow particle size distribution. Manufacturers provide columns bonded with dissimilar functional groups (e.g., C_{18}, cyano, TMS) and have different percent carbon loading.

6.8.1 Use of high quality columns that are uniformly packed with the appropriate particle size and bonded phase will result in optimal chromatographic performance. For example, columns with silica-based particles with free silol groups show less tailing of polar materials (e.g., amines).

6.8.2 A smaller particle (and pore) size generally gives better resolution, higher back pressure, and smaller sample capacity.

6.8.3 Lifetime and performance of HPLC columns can be improved through proper maintenance. Analysts should filter sample extracts and use compatible guard columns.

6.9 HPLC column temperature control ovens

HPLC retention times are more reproducible if the column is held at a constant temperature. Temperature control ovens capable of maintaining the HPLC column at ± 0.1 °C should be utilized to provide consistent retention times throughout the course of an HPLC analysis. Normal oven operating temperature should be 3 – 5 °C above ambient laboratory temperature.

6.10 HPLC detectors

Detectors are the transducers that respond to components eluting from a HPLC column and produce the electrical signal used for qualitative and quantitative determinations. SW-846 analyses are conducted using selective detectors or mass spectrometers listed in Sec. 1.1. HPLC/MS involves the use of a sophisticated interface that separates target analytes from the aqueous mobile phase. Examples include the thermospray (TSP), electrospray (ESP), and the atmospheric pressure chemical ionization (APCI) interfaces.

6.11 Data systems

Raw chromatographic data have to be reduced in order to provide the quantitative information needed by analysts. Sophisticated data systems are strongly recommended for SW-846 chromatographic methods because the ability to store and re-plot chromatographic data is invaluable during data reduction and review. Organizations should select the system most suitable for their applications.

6.12 Supplies

Chromatographers use a variety of supplies. Specific items that should be stocked depend on laboratory instrumentation and the analyses performed. At a minimum, laboratories need PTFE tape, stainless steel regulators, acid-washed copper tubing, syringes, and replacement parts for instruments.

6.12.1 Laboratories performing GC analyses also need supplies such as high purity gases, scrubbers for gas conditioning, gas-tight fittings, capillary cutters, magnifying glasses, septa with proper temperature limits, appropriate ferrules, dichlorodimethylsilane (for deactivating surfaces), glass wool, spare columns and injection port liners.

6.12.2 Laboratories performing HPLC analyses need supplies such as high purity solvents, column packing material, frits, narrow inner diameter tubing, appropriate ferrules, solvent filtration apparatus, and solvent degassing equipment.

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent leaching of contaminants from plastic containers.

7.2 See specific extraction and determinative methods for the reagents and standards needed.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, they must be followed. In the absence of specific regulatory requirements, use the information in Chapter Four as guidance in determining sample collection, preservation, and storage

requirements. Additional information may be found in some of the individual sample extraction, preparation, and determinative methods.

9.0 QUALITY CONTROL

9.1 General Guidance

Refer to Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific and Chapter One criteria; technique-specific QC criteria take precedence over Chapter One criteria. Any effort involving collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results.

Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. Development of in-house QC limits for each method is encouraged, as described in Sec. 9.6. Use of instrument–specific QC limits is encouraged, provided such limits will generate data appropriate for use in the intended application. All data sheets and QC data should be maintained for reference or inspection.

9.2 Evaluating chromatographic performance

The analyst's expertise in performing chromatography is a critical element in the successful performance of chromatographic methods. Successful generation of data demands selection of suitable preparative and determinative methods and an experienced staff to use these methods.

9.2.1 For each 12-hour period during which analysis is performed, the performance of the instrument system should be checked. These checks should be part of a formal QC program that includes analysis of instrument blanks, calibration standards, and other QC as appropriate for that method. In addition to these instrument QC checks, performance of the entire analytical process (i.e., preparation, cleanup and analysis) should be monitored. These additional checks should include method blanks, matrix spikes/matrix spike duplicates (MS/MSD), laboratory control samples (LCS), replicate samples and other QC as appropriate for that method or project. It is generally advisable, although not required, that all method QC samples be run at the same time as the samples on the same instrument.

9.2.2 Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method and/or the measurement quality objectives (MQOs) established for intended application.

9.2.3 In addition to the quantitative measures of comparison described below and in the individual methods, analysts should evaluate chromatograms and instrument operation. Questions that should be asked include the following:

Do the peaks look normal (Gaussian)?
Is the response obtained comparable to the response from previous calibrations?
Are non-target peaks present in calibration analyses?
Are contaminants present in the blanks?
Is the injector leaking (e.g., does the GC injector septum need replacing)?

Do the column fittings need tightening?
Does the HPLC guard column need replacement?

9.2.4 Significant peak tailing, leaks, changes in detector response and laboratory contamination should be corrected. Tailing problems are generally traceable to active sites on the column, cold spots in a GC, improper choice of HPLC mobile phase, the detector inlet, or leaks in the system.

9.2.5 Recalibration of the instrument must take place when performance changes to the point that the calibration verification acceptance criteria (Sec. 11.7) cannot be achieved. Recalibration of the instrument should be performed as required per determinative methods.

9.2.6 Before processing any samples, the analyst should demonstrate that all parts of the equipment contacting the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. Each time samples are extracted, cleaned up, and analyzed, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. Consult the appropriate 3500 or 5000 series method for specifics of the preparation of method blanks. The following general guidelines apply to the interpretation of method blank results.

9.2.6.1 Method blanks should be prepared at a frequency of at least 5%: one method blank for each group of up to 20 samples prepared at the same time, by the same procedures. For volatile samples analyzed by purge-and-trap, preparation is equivalent to the analysis. Therefore, one purge-and-trap method blank must be analyzed with each group of up to 20 samples analyzed on the same instrument during the same analytical shift.

9.2.6.2. When samples that are extracted together are analyzed on separate instruments or in separate analytical shifts, the method blank associated with those samples (e.g., extracted with the samples) must be analyzed on at least one of those instruments. A solvent blank should be analyzed on all other instruments on which the set of samples was analyzed to demonstrate the instrument is not contributing contaminants to the samples.

9.2.6.3 Unless otherwise described in a determinative method, the method blank may be analyzed immediately after the calibration verification standard to ensure that there is no carryover from the standard or at another point in the analytical shift.

9.2.6.4 When sample extracts are subjected to cleanup procedures, the associated method blank must also be subjected to the same cleanup procedures.

9.2.6.5 Results of the method blank should be less than the lower limit of quantitation (LLOQ) (Sec. 9.7) for the analyte or less than the level of acceptable blank contamination specified in the approved QAPP or other appropriate systematic planning document.

9.2.6.6 If the method blank results do not meet the acceptance criteria above, the laboratory should take corrective action to locate and reduce the source of the contamination and re-extract and reanalyze any samples associated with the

contaminated method blank. If the method blank results still do not meet the acceptance criteria in 9.2.6.5 and re-analysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory. Qualification of the samples may be needed.

9.2.6.7 The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is inappropriate for the GC and HPLC methods addressed here and may lead to negative sample results.

9.2.6.8 Blanks – Before processing any samples, the analyst should demonstrate through the analysis of a method blank that equipment and reagents are free from contaminants and interferences. If a peak is found in the blank that would prevent the identification or bias the measurement of an analyte, the analyst should determine the source and eliminate it, if possible. As a continuing check, each time a batch of samples is extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank must be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. Method blanks, trip blanks, and other field blanks should be carried through all stages of sample preparation and analysis. At least one method blank or instrument blank must be analyzed on every instrument after calibration standard(s) and prior to the analysis of any samples.

9.2.6.9 Blanks are generally considered to be acceptable if target analyte concentrations are less than one-half the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e., targets are not present in samples or sample concentrations are ≥10X the blank). Other criteria may be used depending on the needs of the project.

9.2.6.10 If an analyte of interest is found in a sample in the batch near a concentration confirmed in the blank (refer to Sec. 9.5.2), the presence and or/concentration of that analyte should be considered suspect and may require qualification. Contaminants in the blank should meet most or all of the qualitative identifiers in Section 11.6 to be considered. Samples may require re-extraction and/or re-analysis if the blanks do not meet lab established or project specific criteria. Re-extraction and/or re-analysis is not necessary if the analyte concentration falls well below the action or regulatory limit or if the analyte is deemed not important for the project.

9.2.6.11 When new reagents or chemicals are received, the lab should monitor the blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.2.6.12 Method and/or solvent blanks may also be used to check for contamination by carryover from a high-concentration sample into subsequent samples (Sec. 4.2). When analysis of such blanks is not possible, such as when an unattended autosampler is employed, the analyst should carefully review the results for at least the next sample after the high-concentration sample. If analytes in the

high-concentration sample are not present in the subsequent sample, then lack of carryover has been demonstrated. If there is evidence that carryover may have occurred, then the affected samples should be reanalyzed.

9.3 Initial demonstration of proficiency (IDP)

Prior to implementation of a method, each laboratory must perform an IDP consisting of at least four replicate reference samples spiked into a clean matrix taken through the entire sample preparation and analysis. Whenever a significant change to instrumentation or procedure occurs, the laboratory must demonstrate that acceptable precision and bias can still be obtained by the changed conditions (Sec. 9.3.1). Whenever new staff members are trained, an analyst IDP must be performed. (Sec. 9.3.2).

9.3.1 Demonstration of proficiency for instrument or method changes

If a major change to the sample preparation procedure is made (e.g., a change in solvent), the IDP must be repeated for that preparation procedure by a minimum of four spiked reference samples. Alterations in instrumental procedures only, such as changing GC temperature programs or HPLC mobile phases or the detector interface, require a new calibration but not a new IDP because the preparation procedure is unchanged. Each laboratory must have policy for performance and documentation of IDP.

9.3.2 Demonstration of proficiency for new analysts

Each laboratory should have a training program which documents that a new analyst is capable of performing the method, or portion of the method, for which the analyst is responsible. This demonstration should document that the new analyst is capable of successfully following the SOP established by the laboratory.

For example, when analysts are trained for a subset of analytes for an 8000 series method, the new sample preparation analyst should prepare reference samples for a representative set of analytes (e.g., the primary analyte mix for Method 8270, or a mix of Aroclor 1016 and 1260 for Method 8082) for each preparation method the analyst will be performing. The instrument analyst being trained will need to analyze prepared samples (such as semi-volatile extracts).

9.3.3 Preparation of reference samples

9.3.3.1 Reference samples are prepared from a spiking solution containing each analyte of interest. The reference sample concentrate (spiking solution) may be prepared from pure standard materials or purchased as certified solutions. This reference standard should be made from the same source as the calibration standards to eliminate any additional variability due to differences between sources.

9.3.3.2 Preparation of the reference sample concentrate is dependent upon the method being evaluated. Guidance for certain methods is listed in Methods 3500 and 5000. In other cases, the determinative methods contain guidance on preparing the reference sample concentrate and the reference sample. If no guidance is provided, prepare a reference sample concentrate in methanol (or any water-miscible solvent) at a concentration such that the spike will provide a concentration in the clean matrix near the middle of the calibration range for each analyte in that matrix.

9.3.3.3 Concentrations of target analytes in the reference sample may be adjusted to reflect more accurately the concentrations to be analyzed by the laboratory. If the concentration of an analyte is being evaluated relative to a regulatory limit or action level, see Sec. 9.4.1 for information on selecting an appropriate spiking level.

9.3.4 Evaluation

9.3.4.1 To evaluate the performance of the total analytical process, reference samples must be handled in exactly the same manner as actual samples. Additional LCS or MS/MSD samples can be omitted. Use a clean matrix for spiking purposes (one without any target or interference compounds) such as organic-free reagent water for aqueous matrices and organic-free sand or soil for solid matrices.

9.3.4.2 Prepare and analyze at least four replicate aliquots of the well-mixed reference samples by the same procedures used to analyze actual samples (procedure section for each of the methods). This will include a combination of the sample preparation method (usually a 3500 series method for extractable organics or a 5000 series method for volatile organics) and the determinative method (an 8000 series method).

9.3.4.3 Calculate the mean recovery ($\bar{x}$) and the standard deviation of the recovery (s) for each analyte of interest using the four results.

9.3.4.4 Multiple-laboratory performance data are included in some determinative methods and may be used as guidance in evaluating performance in a single laboratory. However, comparison with single-laboratory performance data is much more indicative regarding expectations of how any individual laboratory will perform, than in comparison with multi-laboratory data. Compare s and $\bar{x}$ for each analyte with the corresponding performance data for precision and bias given in the performance table at the end of the determinative method. If s and ($\bar{x}$) for all analytes of interest meet the appropriate acceptance criteria, then the system performance is acceptable and analysis of actual samples can begin. If any individual s value exceeds the precision limit or any $\bar{x}$ value falls outside the range for bias, then the system performance may be unacceptable for that analyte. Once sufficient data points are available, each laboratory is strongly encouraged to develop in-house control limits.

NOTE: The large number of analytes in each of the methods presents a substantial probability that one or more analyte will fail at least one of the performance criteria when all analytes of a given method are determined.

9.3.4.5 Performance data in many of the methods are based on single-laboratory performance. As with multiple-laboratory data, the criteria in those methods may be used as guidance when evaluating laboratory performance. When comparing your laboratory data to performance data developed from single-laboratory data, certain analytes may be outside the limits; however, the majority should be within the acceptance limits.

9.3.4.6 When one or more of the analytes fail at least one of the performance criteria, the analyst should repeat the test only for those analytes that fail to meet criteria. Repeated failure, however, will confirm a general problem with

the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning at Sec. 9.3.4.

9.4 Matrix spike, laboratory control samples and method blanks

9.4.1 General Discussion

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision and bias). At a minimum, this check should include the analysis of at least one matrix spike and one duplicate unspiked sample or preferably, one MS/MSD pair with each preparation batch of up to 20 samples of the same matrix processed together (Chapter One). If samples are expected to contain target analytes of concern, laboratories may use one matrix spike and a duplicate of an unspiked field sample as an alternative to the MS/MSD pair (Sec. 9.4.3).

For samples requiring an extraction procedure separate from analysis (e.g., semivolatiles by Method 8270), the MS/MSD, or matrix spike and duplicate sample, should be extracted with the batch of samples but may be analyzed at any time. Conversely, if calibration standards and other analytical QC are processed identically to the field samples (e.g., volatiles by Method 8260), the MS/MSD, or matrix spike and duplicate sample, should be prepared and analyzed concurrently with the samples.

When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. In the case of very contaminated samples or when the lab does not receive enough samples to perform a single matrix spike, an LCS and LCS duplicate (LCSD) may be performed to document precision and bias. An LCS should be included with each preparation batch. The LCS is an aliquot of the same clean (control) matrix used for the method blank(s) and of a similar weight or volume as the method blank and field samples. The LCS is spiked with similar analytes at the same concentrations as in the matrix spike and is processed identically to the samples.

In the case of samples that need an extraction procedure separate from analysis (e.g., semivolatiles by Method 8270), the LCS should be extracted with the batch of samples but may be analyzed at any time. However, if calibration standards and other analytical QC are processed identically to the field samples (e.g., volatiles by Method 8260), the LCS should be prepared and analyzed concurrently with the samples and may also serve as the continuing calibration verification (CCV) standard.

<u>NOTE</u>: If an LCS also serves as a CCV, acceptance criteria of the CCV should be used.

9.4.2 Spiking procedure for the MS/MSD and LCS

The solution used to fortify a sample and/or an LCS should contain all of the target analytes and their concentration levels should be determined as described in Secs. 9.4.1 and 9.4.2. For those methods that apply to a large list of analytes or that contain compounds that may interfere with an accurate assessment (i.e., coeluting or multi-peak analytes), a smaller subset of analytes may be used (see the specifics directions in the appropriate individual method).

9.4.2.1 If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit or

action level, the spike should be at or below the limit, or 1 - 5 times the background concentration (if historical data are available), whichever concentration is higher.

If historical data are not available, a background sample of the same matrix from the site may be submitted for matrix spiking purposes to ensure that high concentrations of target analytes and/or interferences will not prevent calculation of recoveries.

NOTE: If the background sample concentration is very low or non-detect, a spike of greater than 5 times the background concentration is still acceptable. To assess data precision with duplicate analyses, it is preferable to use a low concentration field sample to prepare a MS/MSD for organic analyses.

9.4.2.2 If the concentration of a specific analyte in a sample is not being checked against a limit specific to that analyte, then the analyst may spike the matrix spike or MS/MSD sample(s) at the same concentration as the reference sample (Sec. 9.3.3) at 20 times the estimated LLOQ in the matrix of interest, or at a concentration near the middle of the calibration range. It is suggested that a background sample of the same matrix from the site be submitted as a sample for matrix spiking purposes.

NOTE: Preparing the spiking solution from the same source as the calibration standards helps minimize additional variability due to differences between sources.

9.4.2.3 To develop precision and bias data for the spiked compounds, the analyst has two choices: analyze the original sample, and an MS/MSD pair; or analyze the original sample, a duplicate sample, and one spiked sample. If samples are not expected to contain the target analytes of concern, then the laboratory may use a MS/MSD pair. If samples are expected to contain the target analytes of concern, then the laboratory may use one matrix spike and a duplicate analysis of an unspiked field sample as an alternative to the MS/MSD pair.

9.4.2.4 Begin by analyzing one sample aliquot to determine the background concentration of each analyte. Prepare a matrix spike concentrate according to one of the options described in Sec. 9.4.2.1 or 9.4.2.2.

9.4.2.5 Prepare a matrix spike sample by adding the appropriate volume of the matrix spike concentrate to another aliquot of the sample to yield the desired concentration (Secs. 9.4.2.1 and 9.4.2.2). If MS/MSD analysis will be performed, prepare a matrix spike duplicate sample from a third aliquot of the sample.

9.4.2.6 Analyze the MS/MSD samples using the same procedures employed for the original sample, and calculate the concentration of each analyte in the matrix spike and matrix spike duplicate. Likewise, analyze the LCS samples using the same procedures employed for the original sample, and calculate the concentration of each analyte in the LCS.

9.4.3 MS/MSD, Duplicate and LCS calculations

9.4.3.1 Calculation of % recovery (bias)

Bias is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of analytes in the LCS. Calculate the recovery of each spiked analyte in the matrix spike, matrix spike duplicate (if performed) and LCS according to the following formula.

$$Recovery = \%R = \frac{(C_s - C_u)}{C_n} x\ 100$$

where:

C_s = Measured concentration of spiked sample aliquot
C_u = Measured concentration of unspiked sample aliquot (use 0 for LCS)
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).

MS/MSD recoveries may not be meaningful if the amount of analyte in the sample is large relative to the amount spiked.

9.4.3.2 Calculation of relative percent difference (RPD)

Precision is estimated from the RPD of the concentrations (<u>not</u> the recoveries) measured for MS/MSD pairs, or for duplicate analyses of unspiked samples. Calculate RPD according to the formula below.

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} X\ 100$$

where:
C_1 = Measured concentration of first sample aliquot
C_2 = Measured concentration of second sample aliquot.

<u>NOTE</u>: A difference in the amount of sample used for the MS/MSD results in an artificially high RPD when based on concentration. Using approximately the same sample size or scaling the spike amount to the sample size for the MS/MSD will minimize bias in the RPD calculation for MS/MSD.

9.4.4 Recommended QC acceptance criteria for matrix spike samples and LCS

The laboratory should develop performance data for precision and bias in the matrices of interest (Sec. 9.6). In addition, laboratories should monitor method performance in each matrix, through the use of control charts and other techniques.

Many methods may not contain recommended acceptance criteria for LCS results. The laboratory should use 70 - 130% as interim acceptance criteria for recoveries of spiked analytes, until in-house LCS limits are developed (Sec. 9.6). Where in-house limits have been developed for matrix spike percent recoveries, the LCS results should be similar to or tighter than those limits, as the LCS is prepared in a clean matrix.

Ideally, the acceptance criteria for MS/MSD recovery and/or duplicate relative % difference will be established for the field samples through the DQOs contained in a written QAPP. These criteria should be established with consideration given to performance data provided in the reference method and/or by the laboratory in order to avoid overly conservative expectations. In the absence of site- or project-specific acceptance criteria for matrix spike and duplicate QC samples, these criteria should be based on in-house performance data generated by the laboratory or on the performance data in the reference method.

Even when the project QAPP or determinative methods provide performance criteria for matrix spikes and LCS, laboratories must develop in-house performance criteria based on their historical data for use in project planning and for comparison to any relevant performance criteria in the reference methods. Development of in-house performance criteria is discussed in Sec. 9.6. Where methods do contain performance data for the matrix of interest, use Secs. 9.4.4.1 - 9.4.4.3 below as guidance in evaluating data generated by the laboratory.

9.4.4.1 When multi-laboratory performance data for the matrix of interest are provided in the determinative method, compare the recovery for each analyte with the method performance data of the same matrix. Given that such method performance criteria were developed from multi-laboratory data, they should be met by almost all laboratories. See Sec. 9.6.10 for more information on comparisons between limits. Performance data include an allowance for error in measurement of both the background and spike concentrations. If spiking was performed at a concentration substantially lower than the level used to generate the recovery data in the reference method, the recovery data in the method may not be appropriate for assessing the quality of the sample results, and criteria generated from in-house data may be more relevant.

9.4.4.2 When a method is initially established in a laboratory, the LCS limits may be applied to the matrix spikes until the laboratory has sufficient data (a minimum of 20 or more MS/MSD samples of the same matrix) to generate their own statistical limits. These data should be used as the basis for determining MS/MSD precision and bias limits. Alternatively, acceptance criteria based on historical LCS data may continue to be used for evaluating bias in matrix spike recovery and may be more sensitive to matrix effects than acceptance limits based on MS/MSD data. It is generally preferable to use statistically calculated MS/MSD, rather than LCS recovery limits once sufficient data points have been collected (i.e., ≥ 20 MS/MSD samples). See Secs. 9.6.1 - 9.6.3 for calculating in-house performance criteria for LCS, MS/MSD and surrogate recoveries.

9.4.5 Also, compare the recovery data from the matrix spike with the LCS data (use the average recovery if an MS/MSD were analyzed). If any individual %R in the MS/MSD falls outside the designated range for recovery, the laboratory should determine if there is a matrix effect or a laboratory performance problem. A matrix effect is indicated if the LCS data are within limits but the MS/MSD data exceed the limits. Surrogate recovery data (Sec. 9.5) should also be used to evaluate the data. Recoveries of both matrix spike

compounds and surrogates outside of the acceptance limits suggest more pervasive analytical problems in the batch and/or instrument than problems with the recoveries of either matrix spikes or surrogates alone.

9.5 Surrogate recoveries

9.5.1 It is necessary that the laboratory evaluate surrogate recovery data from individual samples versus in-house surrogate recovery limits. General considerations for developing in-house acceptance criteria for surrogate recoveries are described in Sec. 9.6.

9.5.2 Surrogate recovery is calculated below.

$$Recovery\ (\%) = \left(\frac{Concentration\ (or\ amount)\ found}{Concentration\ (or\ amount)\ added}\right) \times 100$$

If recovery is not within in-house surrogate recovery limits, the following procedures are necessary.

9.5.2.1 Check for errors in the calculations, surrogate solutions or internal standards. If errors are found, recalculate the data accordingly.

9.5.2.2 Examine chromatograms for interfering peaks and proper peak integration.

9.5.2.3 Check instrument performance. If an instrument problem is identified, correct the problem and reanalyze the sample or extract.

9.5.2.4 If no instrument problem is found, the sample should be re-extracted and reanalyzed (or another vial reanalyzed for volatiles).

9.5.2.5 If upon re-analysis (in either 9.6.2.2 or 9.6.2.4), the recovery is again not within limits, report the data as an "estimated concentration." If the recovery is within the limits in the reanalysis, report the reanalysis data for the samples. If the method holding time for the sample has expired prior to re-extraction and/or reanalysis, qualify the data accordingly.

9.5.2.6 Some samples may need dilution to bring one or more target analytes within the calibration range or to overcome significant interferences. This may result in dilution of the surrogate responses to the point that recoveries cannot be measured. If surrogate recoveries are available from a less-diluted (or undiluted) aliquot of the sample or sample extract, those recoveries may be used to demonstrate that the surrogates are within the QC limits and no further action is needed.

9.6 Generation of performance criteria for MS/MSD, duplicates, surrogates, LCS, and IDP

Usefulness of developing in-house performance criteria and control charting or similar procedures to track laboratory performance cannot be overemphasized. Many data systems and commercially available software packages support the use of control charts.

Procedures for calculating in-house performance criteria for MS/MSD, LCS and surrogate recoveries are provided below.

9.6.1 Once sufficient data have been acquired and the recovery and RPD calculated as in Secs. 9.4.3 and 9.5 for a given sample matrix, the following statistics should be used to calculate acceptance criteria.

9.6.1.1 Mean percent recovery ($\bar{x}$) and standard deviation (s) for:

1) Each added target compound in the MS/MSD samples;
2) Each added target compound in the LCS samples; and
3) Each added surrogate in the field samples.

9.6.1.2 Mean RPD and standard deviation for MS/MSD or duplicate QC samples.

A minimum of 20 data points should be used to generate meaningful criteria. Inclusion of additional data should result in more robust criteria that better describe variance in method performance and result in fewer outliers. If the lower limit of the acceptance range is calculated to be <10%, it should be set to 10%. However, an alternative lower acceptance limit may be established by the laboratory or at the project level through the DQOs in a QAPP.

9.6.2 Calculate the upper and lower control limits for % recovery of each target or surrogate compound in LCS, MS/MSD and field samples using the respective $\bar{x}$ and s values calculated in Sec. 9.6.1.

Acceptance range = $\bar{x}$ (mean percent recovery) ± $3s$ (standard deviation)

Upper control limit = $\bar{x} + 3s$
Lower control limit = $\bar{x} - 3s$

9.6.3 Calculate the upper control limit for the RPD for the MS/MSD using the mean RPD value + $3s$ of the RPDs of historical MS/MSD pairs. RPD should be calculated based on the concentration or amount, not the spike recovery.

NOTE: The RPD limit only has a maximum value, because perfect agreement between C1 and C2 would result in a RPD of 0. Refer to Sec. 9.4.3.2 for the calculation.

9.6.4 Any matrix spike or surrogate recovery outside of control limits necessitates evaluation by the laboratory such as comparison with the LCS recovery.

9.6.4.1 If recoveries of analytes in the LCS are outside of the control limits, then the problem may lie with application of the extraction and/or cleanup procedures applied to the sample matrix, or with analysis. Once the problem has been identified and addressed, corrective action may include reanalysis of samples, or extraction and analysis of new sample aliquots, including new matrix spike samples and LCS. However, when there are a large number of analytes in the LCS or matrix spike, the statistical probability of a few analytes outside of control limits

becomes high. Therefore, a number of analytes should be allowed to marginally fail the limits without requirement for corrective action. Laboratories should have a documented procedure to assess and qualify marginal exceedance limits.

9.6.4.2 When LCS results are within control limits but matrix spike results are not, the problem may either be related to the specific sample matrix or to an inappropriate choice of extraction, cleanup, and/or determinative method. If results are to be used for regulatory compliance monitoring, the analyst must take steps to demonstrate that the analytes of concern can be determined in the sample matrix at the levels of interest.

9.6.5 Once established, control limits should be reviewed regularly and updated on a routine basis as established by the laboratory's quality management plan. Reviewing appropriateness of these criteria with respect to generated data is especially important for newly implemented procedures or those not in continual use. The laboratory should monitor trends in both analyte recovery performance and also in the control limits. Control limits used to evaluate sample results should be those in place at the time of sample analysis. Once control limits are updated, they should apply to all subsequent analyses of new samples.

9.6.6 For analytes, methods, and matrices with very limited data (e.g., unusual analytes or matrices not analyzed often), interim limits should be established using available data or by analogy to similar methods or matrices.

9.6.7 Results used to develop acceptance criteria should meet all other QC criteria associated with the determinative method. For example, matrix spike recoveries from a GC/MS procedure should be generated from samples analyzed after a valid tune and initial calibration that includes the matrix spike compounds. Analytes in GC or HPLC methods should fall within the established retention time windows in order to be used to develop acceptance criteria.

9.6.8 Laboratories are advised to consider effects of spiking concentration on matrix spike performance criteria. Acceptance criteria for matrix spike recovery and precision are often a function of the spike concentration used. Therefore, use caution when pooling data in establishing acceptance criteria. Not only should results all be from roughly the same type matrix but spiking levels should also be similar.

9.6.9 Similarly, acceptance criteria for matrix spike, LCS and surrogate results should all be generated using the same combination of extraction, cleanup, and analysis techniques. For example, do not mix results from solid samples extracted by ultrasonic extraction with those extracted by Soxhlet.

9.6.10 Another common error in developing acceptance criteria is discarding data that do not meet a preconceived notion of acceptable performance (i.e., while professional judgment is important in evaluating data used to develop acceptance criteria, do not discard specific results simply because they do not meet one's expectations). This practice results in a censored data set, which when used to develop acceptance criteria, will lead to unrealistically narrow criteria. Rather, employ a statistical test for outlier values, or at least calculate the acceptance limits both with and without the results considered suspect. Then, observe the effect of deleting suspect data.

9.6.11 In-house QC limits must be examined for reasonableness; it is not EPA's intent to legitimize poor recoveries due to incorrect choice of methods or spiking levels.

In-house limits should also be compared with the DQOs of specific analyses. For example, recovery limits (for surrogates, MS/MSD, LCS, etc.) that include allowance for a relatively high positive bias (e.g., 70 - 170%) may be appropriate for determining that an analyte is not present in a sample. However, they would be less appropriate for analysis of samples near but below a regulatory limit because of the potential for high bias.

9.6.12 It may be useful to compare QC limits generated in the laboratory with performance data listed in specific determinative methods. However, the analyst must be aware that performance data generated from multi-laboratory studies tend to be significantly wider than those generated from a single laboratory.

9.7 Lower Limit of Quantitation (LLOQ)

The LLOQ is the lowest concentration at which the laboratory has demonstrated target analytes can be reliably measured and reported with a certain degree of confidence, which must be ≥ the lowest point in the calibration curve. The laboratory shall establish the LLOQ at concentrations where both quantitative and qualitative requirements can consistently be met (see Sections 9.7.3 and 11.6). The laboratory shall verify the LLOQ at least annually, and whenever significant changes are made to the preparation and/or analytical procedure, to demonstrate quantitation capability at lower analyte concentration levels. The verification is performed by the extraction and/or analysis of an LCS (or matrix spike) at 0.5-2 times the established LLOQ. Additional LLOQ verifications may be useful on a project-specific basis if a matrix is expected to contain significant interferences at the LLOQ. The verification may be accomplished with either clean control material (e.g., reagent water, solvent blank, Ottawa sand, diatomaceous earth) or a representative sample matrix, free of target compounds. Optimally, the LLOQ should be less than the desired decision level or regulatory action level based on the stated DQOs.

9.7.1 LLOQ Verification – The verification of LLOQs using spiked clean control material represents a best-case scenario because it does not evaluate the potential matrix effects of real-world samples. For the application of LLOQs on a project-specific basis, with established DQOs, a representative matrix-specific LLOQ verification may provide a more reliable estimate of the lower quantitation limit capabilities.

9.7.2 The LLOQ verification (to be performed after the initial calibration) is prepared by spiking a clean control material with the analyte(s) of interest at 0.5-2 times the LLOQ concentration level(s). Alternatively, a representative sample matrix free of targets may be spiked with the analytes of interest at 0.5-2 times the LLOQ concentration levels. The LLOQ check is carried through the same preparation and analytical procedures as environmental samples and other QC samples. It is recommended to analyze the LLOQ verification on every instrument where data is reported; however, at a minimum, the lab should rotate the verification among similar analytical instruments such that all are included within 3 years. Frequently performed analyses, such as Methods 8260C and 8270D, should have an LLOQ check standard be verified, at minimum, once a year.

9.7.3 Recovery of target analytes in the LLOQ verification should be within established in-house limits or within other such project-specific acceptance limits to demonstrate acceptable method performance at the LLOQ. Until the laboratory has sufficient data to determine acceptance limits, the LCS criteria ± 20% (i.e., lower limit minus 20% and upper limit plus 20%) may be used for the LLOQ acceptance criteria. This practice acknowledges the potential for greater uncertainty at the low end of the calibration curve. Where practical, historically based LLOQ acceptance criteria should be determined once sufficient data points have been acquired.

9.7.4 Reporting concentrations below LLOQ - Concentrations that are below the established LLOQ may still be reported; however, these analytes must be qualified as estimated. The procedure for reporting analytes below the LLOQ should be documented in the laboratory's SOP or in a project-specific plan. Analytes below the LLOQ that are reported should meet most or all of the qualitative identification requirements in Sec. 11.6.

9.8 It is recommended that the laboratory adopt additional QA practices for use with 8000-series methods. Specific practices that are most productive depend upon the needs of the laboratory, nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess precision of the environmental measurements. When doubt exists over identification of a peak on the chromatogram, confirmatory techniques such as GC with a dissimilar column, element-specific detector, or mass spectrometer (selected ion monitoring or full scan) must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

Refer to the appropriate determinative method for detailed calibration and standardization procedures and the general guidance as noted in Sec. 11.0.

11.0 PROCEDURE

Extraction and cleanup are critical for successful analysis of environmental samples and wastes. Analysts should pay particular attention to selection of sample preparation procedures to obtain reliable measurements.

11.1 Extraction

Individual determinative methods for organic analytes in SW-846 often recommend appropriate sample extraction procedures. General guidance on semivolatile extraction procedures can be found in Method 3500. Guidance on volatile procedures can be found in Method 5000.

11.2 Cleanup and separation

Individual determinative methods for organic analytes in SW-846 often recommend appropriate cleanup procedures. General guidance on cleanup procedures can be found in Method 3600. While some relatively clean matrices (e.g., groundwater samples) may not need extensive cleanups, the analyst should carefully balance time savings gained by skipping cleanups against potential increases in instrument downtime and loss of data quality that can occur as a result.

11.3 Chromatographic Performance

Recommended chromatographic columns and instrument conditions are described in each determinative method. As noted earlier, these columns and conditions are typically those used during development and testing of the method. However, other chromatographic systems may have somewhat different characteristics as analytical instrumentation continues to evolve. Therefore, SW-846 methods allow analysts flexibility to change conditions as long as adequate performance is demonstrated.

Chromatographic performance is demonstrated by resolution of standards and ability to model the response of the detector during calibration; also important are sensitivity, bias, precision, frequency of false positives, and frequency of false negatives during analysis. For any chromatographic procedures or conditions used, the laboratory must demonstrate that the performance satisfies the analytical requirements of the specific application for which the chromatographic procedure is being used. Such demonstrations should be performed using procedures outlined in Secs. 9.2 - 9.5 of this method and in Chapter One.

11.4 Initial Calibration

Calibration of an analytical instrument involves delineation of the relationship between response of the instrument and amount or concentration of an introduced analyte. The graphical depiction of this relationship is often referred to as the calibration curve. To perform quantitative measurements, this relationship, termed initial calibration, must be established before the analyses of any samples.

Historically, many analytical methods have relied on linear models of the calibration relationship, where the instrument response is directly proportional to the amount of a target compound. The linear model has many advantages including simplicity and ease of use. However, given the advent of new detection techniques and because many methods cannot be optimized for all the analytes to which they may be applied, the analyst is increasingly likely to encounter situations where the linear model neither applies nor is appropriate.

Initial calibration for SW-846 chromatographic methods involves analysis of standards containing the target compounds at a minimum of five different concentrations within the working range of the instrument. In order to produce acceptable sample results, instrument response must be within the range established by the initial calibration.

Extrapolation of the calibration to concentrations above or below those of the actual calibration standards is not appropriate and may lead to significant quantitative errors, regardless of the calibration model chosen. It may be necessary to prepare calibration standards that cover concentration ranges appropriate for specific projects or types of analyses. For instance, the analyst should not necessarily expect to perform a calibration appropriate for sub-ppb level analyses and use the same calibration data for high-ppb or ppm-level samples. Preparation of calibration standards is described in general terms in Sec. 11.4.1.

SW-846 methods in this manual for quantitative chromatographic analysis rely on one of three commonly used calibration approaches:

- External standard calibration
- Internal standard calibration
- Isotope dilution calibration

These approaches are described in general terms in Secs. 11.4.2 - 11.4.4.

General calibration criteria are provided in Sec. 11.5 for GC and HPLC procedures using non-MS detection. Calibration procedures for GC/MS (e.g., Methods 8260, 8270, 8276, 8280, and 8290), HPLC/MS (e.g., Methods 8321 and 8325), and GC/FT-IR (e.g., Method 8410) are described in those methods. Some determinative methods may provide specific guidance on calibration such as Method 8085, GC/AED with compound-independent calibration.

11.4.1.4 For each analyte, at least one of the calibration standards MUST correspond to a sample concentration at or below the quantitation levels needed for the project; this may include establishing compliance with a regulatory or action limit. Given that different limits may be associated with different analytes, the same standard should not be expected to fulfill this requirement for all analytes.

11.4.1.5 Given the large number of target compounds addressed by some of the methods listed in Sec. 1.1, it may be necessary to prepare several sets of calibration standards, each set consisting of different analytes. Initial calibration will then involve analysis of each of these sets of standards.

11.4.2 External standard calibration

External standard calibration is one of the most common approaches to calibrations. It involves a simple comparison of instrument responses from the sample to the target compound responses in the calibration standards. Sample peak responses are compared with calibration standard peak responses. The ratio of the detector response to the amount (mass) of analyte in the calibration standard is defined as the calibration factor (CF).

$$CF = \frac{peak\ response\ of\ the\ standard\ compound}{mass\ of\ the\ compound\ (nanograms)}$$

Advantages of external standard calibration are that it is simple and can be applied to a wide variety of specific chromatographic methods. The primary disadvantage is that it is greatly affected by stability of the chromatographic detector system and presence of chromatographic interferences in a sample or sample extract.

The CF may also be calculated using the standard concentration rather than mass in the denominator of the equation above. However, use of concentration in calculating the CF will necessitate changes to the equations used to calculate sample concentration (Sec. 11.10.3).

For multi-component analytes (e.g., PCBs and toxaphene), see the appropriate determinative method for information on which peaks to employ for CF calculation.

11.4.3 Internal standard calibration

Internal standard calibration involves comparison of instrument responses from the target compounds in the sample to responses of other standards added to the sample or extract before injection. Response of the target compound is normalized to the response of the other standard. This other standard is called an internal standard because it is contained within the aliquot of the sample or sample extract injected into the instrumentation.

A constant amount of the internal standard is added to all samples or extracts. That same amount of the internal standard is also included in each of the calibration standards. In the sample or sample extract, the peak response ratio of the target compound to the internal standard is compared with a similar ratio derived for each calibration standard. This ratio is termed the response factor (RF) or relative response factor (RRF), indicating that the target compound response is calculated relative to that of the internal standard.

Advantages of internal standard calibration include that it can account for routine change in response of the chromatographic system as well as variation in the volume of the introduced sample or sample extract. In addition to normalizing the peak response of the target compound to the response of the internal standard in the sample or extract for that injection, the internal standard may be used to calculate the relative retention time (RRT) of the target compound. RRT is expressed as a unitless quantity.

$$RRT = \frac{Retention\ time\ of\ the\ analyte}{Retention\ time\ of\ the\ internal\ standard}$$

If RRT is used by the laboratory to establish peak identity, the RRT of each target analyte in each calibration standard should agree within established limits set by the laboratory in their SOP. These limits should be appropriate for reliable identification of the target analyte. If this criterion is not met and there are no other indicators of an analyte's identification (such as a very unique and high probability mass spectral match), that analyte may not be considered as identified by RRT.

The RRT of the analyte in the sample should be within the RRT limits of the analyte in the standards. If this criterion is not met and there are no other indicators of a component's identification (such as a very unique and high probability mass spectral match), that component may not be considered as identified by RRT.

RRT evaluation allows the analyst to compensate for modest shifts in the chromatographic conditions that can occur due to interferences and day-to-day instrument variability. Many methods that employ internal standard calibration use more than one internal standard; target compounds are related to the internal standards based on similarity of their respective chromatographic retention times or physical and chemical properties.

Principal disadvantages of internal standard calibration are that internal standards must be compounds not found in the samples to be analyzed and they must produce an unambiguous response on the chromatographic detector system. Many SW-846 methods recommend brominated or fluorinated compounds and/or stable isotopically labeled analogs of target compounds (e.g., a compound containing a deuterium atom instead of hydrogen, or a ^{13}C atom instead of a ^{12}C atom) as internal standards. Isotopically labeled compounds are most often employed in MS detection methods because the detector can differentiate between the target compound and the labeled internal standard based on its added mass even when the two compounds elute at the same retention time. In general, internal standard calibration is not as useful for GC and HPLC methods with non-MS detectors because of the inability to chromatographically resolve many internal standards from the target compounds.

Internal standards recommended in many SW-846 methods were used during development of the method. Analysts may employ other internal standards in place of, or in addition to, those recommended. If internal standards are not recommended in the method, the analyst should select one or more compounds similar in analytical behavior to the analytes of interest and not expected to be found in the samples. Whichever internal standards are employed, the analyst should demonstrate (as detailed in the determinative method) that measurement of the internal standard is not affected by target analytes, surrogates, or matrix interferences.

When preparing calibration standards, add the same amount of the internal standard solution to each calibration standard. Therefore, the internal standard concentration is the same in each calibration standard, whereas concentrations of the target analytes will vary.

The internal standard solution may contain more than one internal standard, and their relative concentrations may differ within the spiking solution. However, the mass of each internal standard added to the samples or sample extracts immediately before injection must be the same as in each calibration standard. The volume of the internal standard solution spiked into a sample extract should cause minimal dilution of the extract.

An ideal internal standard concentration would yield a response factor (RF) of 1 for each analyte. However, this is unlikely to be the case when dealing with more than a few target analytes. Therefore, as a general rule, the internal standard should produce an instrument response ≤ 100 times that produced by the least responsive target analyte associated with the internal standard. This should result in a minimum RF of approximately 0.01 for the least responsive target compound.

For each of the initial calibration standards, calculate the RF values for each target analyte relative to one of the internal standards as follows.

$$RF = \frac{A_x \ x \ C_{is}}{A_{is} \ x \ C_x}$$

where:

A_x = Peak response of the analyte or surrogate

A_{is} = Peak response of the internal standard

C_x = Mass of the analyte or surrogate in the sample aliquot

C_{is} = Mass of the internal standard in the sample aliquot

Response factors for GC/MS methods may also be calculated using sums of the areas of two ions (expressed as mass over charge, *m/z*) for each target analyte and internal standard.

Note that in the equation above, RF is unitless. Therefore, units such as ng or µg may be used for amounts of the analyte, surrogate, and internal standard, provided that they are uniform.

Because internal standards are used to compensate for routine variations in the chromatographic separation of target compounds, there is a significant advantage to using more than one internal standard when dealing with a large number of target compounds or when those compounds elute over a long timeframe. When multiple internal standards are employed, target compounds are associated with the internal standards on the basis of their respective retention times. Therefore, the internal standards should be chosen to cover the expected retention time range of the target compounds. Accordingly, internal standards can compensate for small retention time shifts or response changes in the portion of the chromatographic run in which they occur. Ideally, the analyst will employ enough internal standards to result in a RRT for each target compound in the range 0.80 - 1.20, though other RRT ranges may be appropriate as well.

Many methods that utilize internal standard calibration include acceptance limits for responses of the internal standards in the calibration standards, samples, or both. Those limits are typically expressed in terms of peak areas because the concentration of the internal standard cannot be measured directly (e.g., one has to assume that the entire mass injected into the sample or sample extract is present during analysis). Common consensus

limits are 50 - 200% of the area of the internal standard in the most recent calibration standard. Representing a factor of two, these limits are used as a gross diagnostic check on addition of the internal standards to the samples or extracts and injection of the sample aliquot into the instrument.

11.4.4 Isotope dilution calibration

Isotope dilution calibration is a special case of internal standard calibration. In isotope dilution, the internal standards are stable isotopically labeled analogs of the target analytes and are added to the sample prior to extraction or other sample preparation steps such as pH adjustment, drying, or extraction solvent addition. Physical and chemical properties of each labeled compound are virtually the same as its unlabeled "native" analog. Thus, any losses of the target compound that may occur during sample preparation or determinative steps will be mirrored by a similar loss of the labeled standard. Similarities between labeled compounds and unlabeled analogs mean that RFs and RRTs for the unlabeled compounds are very close to 1.0.

Labeled compounds are spiked into samples and standards at a constant amount. RFs developed from the calibration standards assume that all of the labeled compounds added to the sample reach the instrument. This assumption, termed recovery correction, allows for correction to observed concentrations of the target compound relative to its labeled counterpart.

The degree to which the labeled compounds meet this assumption is monitored by use of traditional internal standards added to the sample extract immediately prior to injection. Separate RFs relate the concentrations of the labeled compounds to the traditional internal standards. Most isotope dilution methods include some limits on the apparent recovery of the labeled compounds. However, those limits are often consensus limits that may be overly conservative. As long as responses for both native and labeled compounds can be distinguished from the background instrumental noise, isotope dilution calibration can provide excellent results, even when the apparent spike recovery of the labeled compound is low. Labeled compound recoveries >100% are allowed as well. Such recoveries can occur as a result of the inherent variability in calibration of the labeled compounds, and are not indicative of contamination or other problems.

Built-in recovery correction is one of the principal advantages of isotope dilution calibration. Isotope dilution requires an MS detection system and isotopically labeled analogs of target analytes and generally produces more precise data with lower bias. The added cost of isotopically labeled compounds is a disadvantage, but can be offset by higher quality data, as well as eliminating some routine QC analyses, such as surrogates and MS/MSDs used with internal standard calibration. However, whether or not to add surrogate or prepare MS/MSD aliquots should be described in a QAPP and not left to the analyst's professional judgment.

Isotope dilution calibration is often used in conjunction with selected ion monitoring (SIM) GC/MS procedures, such as those for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. These procedures, along with a relatively small list of target compounds, allow the instrument to be operated in a mode that detects only those ions (*m/z*) corresponding to the target compounds and their labeled analogs; this application significantly increases the sensitivity of the method and reduces interferences. Because isotope dilution methods have acceptance limits for recovery of the labeled analogs added prior to extraction or other sample preparation steps, these methods typically do not contain limits on responses of the traditional internal standards used to monitor those recoveries.

A RF is calculated for each target compound relative to its labeled analog and for each labeled analog relative to the traditional internal standard added immediately prior to injection. These calculations may involve areas of more than one ion (*m/z*) for each compound (e.g., Methods 8280 and 8290).

11.4.5 Extracted internal standards

Extracted internal standard calibration is a hybrid of internal standard calibration and isotope dilution calibration. In this approach, traditional internal standards are added to the samples before extraction instead of just prior to analysis. Results for the target compounds can be corrected for recovery of the internal standards using the same assumptions made for isotope dilution.

This approach is most helpful when the compounds used as internal standards are very closely related to the target compounds. It is similar to the internal standard procedure used for volatile organic analyses by purge-and-trap or headspace extraction.

11.5 Calibration models and acceptance criteria

SW-846 chromatographic methods allow the use of three different calibration models: average calibration factor or response factor (Sec. 11.5.1), linear regression (Sec. 11.5.2), and non-linear regression (Sec. 11.5.3). Any of these models can be applied to either external or internal standard calibration data. This section also provides suggested criteria for calibration models; however, method- or project-specific criteria will always supersede general guidance.

Choice of calibration model may begin with the simplest approach, the average calibration factor or response factor model, and then progress through linear and then non-linear regression until the calibration acceptance criteria are met. Another appropriate approach is to choose a calibration model based on previous experience, knowledge of the physics of the detector, or specific manufacturer's recommendations. For the calibration model to be usable, it must be continuous and monotonic throughout the calibration range. More calibration points are required for more complex models. The chromatographic methods in SW-846 employ a minimum of five standards for average response factor or linear (first-order) calibration models, six standards for a quadratic (second-order) model, and seven standards for a cubic (third-order) model.

NOTE: The option of using non-linear calibration may be necessary to address specific instrumental techniques. However, it is not EPA's intent to allow non-linear calibration to compensate for detector saturation or avoid proper instrument maintenance. Regardless of the calibration model chosen, an X value of zero should not be included as a calibration point.

The following sections describe various options for initial calibration evaluation and provide the calibration acceptance criteria used to evaluate each option. In addition to this suggested acceptance criteria, two general methods for assessing the accuracy of the calibration curve for all allowed curve models are presented in 11.5.4. It is further recommended that each calibration model be inspected to ensure that the data are representative of the model chosen as described in Sec. 11.5.6. Whichever calibration model is selected, samples with concentrations that exceed the calibration range must be diluted to fall within the range. Criteria listed in these sections are designed for quantitation of trace-level concentrations of the analytes of interest. If data of lower quality will satisfy project-specific data needs, less stringent criteria may be employed provided they are documented and approved in a QAPP.

11.5.1 Linear calibration using average calibration or response factor

As calculated in Sec 11.4, each CF or RF represents the slope of the line between the origin and the given standard response. If the relative standard deviation (RSD) of variation in the factors is ≤ 20%, the linear model is generally representative over the range of calibration standards. Representativeness beyond the range of calibration standards is not to be inferred. At least five calibration levels should be used to construct the average CF or RF model (Sec. 11.5).

To evaluate linearity of the initial calibration, calculate the mean CF (external standard calibration) or RF (internal standard calibration), the standard deviation (SD), and the RSD (also called coefficient of variance, CV) as follows:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(CF_i - \overline{CF})^2}{n-1}} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n}(RF_i - \overline{RF})^2}{n-1}}$$

$$mean\ CF = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n} \qquad mean\ RF = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n}$$

$$RSD = \frac{SD}{\overline{CF}}\ x\ 100 \qquad RSD = \frac{SD}{\overline{RF}}\ x\ 100$$

Where *n* is the number of calibration standards and RSD is expressed as a percentage (%).

11.5.1.1 If the RSD is ≤20% over the calibration range, the slopes of the lines for each standard are sufficiently close to one another that the use of the linear model is generally appropriate <u>over the range of standards that are analyzed</u>; $\overline{CF}$ or $\overline{RF}$ may be used to determine sample concentrations. Alternatively, either of the two methods described in 11.5.4 may be used to determine calibration function acceptability.

<u>NOTE</u>: The RSD approach is equivalent to a $\frac{1}{x^2}$ weighted linear least square regression line that is forced through the origin.

11.5.1.2 Given the potentially large numbers of analytes that may be analyzed in some methods, it is likely that some analytes may exceed the acceptance limit for the RSD for a given calibration. In those instances, it is recommended, but not required, that corrective actions as described in Sec. 11.5.6.1 be followed. Sec. 11.5.6.1 also provides alternative uses for initial calibrations that do not meet their criteria of acceptability.

11.5.1.3 Calculation of sample amounts

If all the conditions in Secs. 11.5.1.1 and 11.5.1.2 are met, the $\overline{CF}$ or $\overline{RF}$ may be used to determine sample concentrations, as described in Sec. 11.10. It is recommended that the curve generated by the $\overline{CF}$ or $\overline{RF}$ be examined for

acceptability using the refitting check described in Sec. 11.5.4.1. The calculated amount introduced into the instrument, x_s, is:

$$X_s = \frac{A_s}{\overline{CF}} \qquad and \qquad X_s = \frac{A_s}{\overline{RF}} \, x \, \frac{C_{is}}{A_{is}}$$

where:

X_s = Calculated mass of the analyte or surrogate in the sample aliquot introduced into the instrument (in ng)

A_s = Peak response of the analyte or surrogate in the sample

A_{is} = Peak response of the internal standard in the sample

C_{is} = Mass of the internal standard in the sample aliquot introduced into the instrument (in ng)

$\overline{CF}$ = Average calibration factor from the most recent initial calibration

$\overline{RF}$ = Average RF from the most recent initial calibration.

Units for the mass of analyte should be the same units used to calculate the CFs or RFs. If different units are used for amount (e.g., µg/L), these calculations and those found in Sec. 11.10 should be adjusted accordingly.

11.5.2 Linear calibration using a least squares regression

A linear calibration model based on a least squares regression may be employed based on past experience or a priori knowledge of the instrument response. Based on the professional judgment of the analyst, this approach also may be used for analytes that do meet the RSD criteria in Sec. 11.5.1 This is most easily achieved by performing a linear least squares regression of the instrument response versus the mass of the chromatographed analyte. Treat the instrument response as the dependent variable (*y*) and the amount as the independent variable (*x*). This is a statistical requirement and is not simply a graphical convention. At least five calibration levels should be used to construct the linear regression model (Sec. 11.5).

For external standard calibration, *x* is the mass of the analyte in the sample aliquot introduced into the instrument and *y* is the instrument response.

$$x = C_S \qquad\qquad y = A_S$$

For an internal standard calibration, x and y can be assigned in various ways where x is the amount of the analyte introduced into the instrument and y is the instrument response to that analyte. Two options are provided here using the mass introduced into the instrument. If other assignments for x and y are used, e.g., concentration, subsequent equations used for calculating mass of the analyte introduced into the instrument must be changed accordingly.

Option 1: X_s is the mass of the analyte in the calibration standard aliquot introduced into the instrument and Y_s is the ratio of response of the analyte to the response of

internal standard times the mass of the internal standard in the calibration standard aliquot introduced into the instrument.

$$X_s = C_s \qquad and \qquad Y_s = A_s \times \frac{C_{is}}{A_{is}}$$

Option 2: x is the ratio of the analyte mass in the calibration standard aliquot introduced into the instrument to the internal standard mass in the calibration standard aliquot introduced into the instrument and y is the ratio of response of the analyte to the response of internal standard.

$$x = \frac{C_s}{C_{is}} \qquad\qquad y = \frac{A_s}{A_{is}}$$

where:

C_s = Mass of analyte in the volume of calibration standard introduced into the instrument.

C_{is} = Mass of internal standard in the volume of calibration standard injected into the instrument.

A_s = Peak response of analyte.

A_{is} = Peak response of internal standard.

A linear least squares regression attempts to construct a linear equation of the form,

$$y = ax + b$$

by minimizing the sum of squared differences between the observed response (y_i, the instrument response) and the predicted response (y'_i, the response calculated from the constructed equation) at each calibration level.

Weighting the sum of the squares of the differences may significantly improve the ability of the least squares regression to fit the linear model to the data, especially at the low end of the calibration curve. The general form of the sum of the squares of the differences containing the weighting factor is:

$$\sum_{i=1}^{n} W_i (y_i - y'_i)^2$$

where:

W_i = Weighting factor for the ith calibration standard (w=1 for unweighted least squares regression, or 1/x or 1/x^2 for weighted least squares regression)

y_i = Observed instrument response for the ith calibration standard.

y'_i = Predicted (or calculated) response for the ith calibration standard.

n = Total number of calibration standards.

Mathematics used in least squares regression favors numbers of larger value over numbers of smaller value. Thus, unweighted regression curves will tend to fit points that are at upper calibration levels better than those points at lower calibration levels. If concentrations of concern are at lower calibration levels, an unweighted regression curve tends to give less accurate results. A weighting factor which reduces this tendency can be used as compensation.

11.5.2.1 Do not include an X-value of zero as a calibration point. However, most data systems and many commercial software packages will allow the analyst to "force" the regression through the origin. Forcing the curve through the origin is not the same as including the origin as a fictitious point in the calibration. In essence, if the curve is forced through the origin, the intercept is set to 0 *before* the regression is calculated, thereby setting the bias to favor the low end of the calibration range by "pivoting" the function around the origin to find the best fit and resulting in one less degree of freedom. It may be appropriate to force an unweighted regression though the origin for some calibrations, but not when the regression is weighted.

However, forcing the regression through the origin may NOT be used as a rationale for reporting results below the calibration range demonstrated by the analysis of the standards. Results should not be reported at a concentration below the LLOQ unless qualified as estimated.

11.5.2.2 In the specific case of an unweighted linear least squares regression (i.e., a regression that varies both a and b), the correlation coefficient (r) can be used to measure the "goodness of fit."

$$r = \frac{n\sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{\left(\sqrt{n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2}\right)\left(\sqrt{n\sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2}\right)}$$

The value of r is such that $-1 \leq r \leq +1$.

The instrument data system will typically calculate r. An r-value of +1.00 indicates a positive perfect correlation; an r-value of -1.00 indicates a negative perfect correlation; an r-value of 0 indicates no correlation.

However, if the regression line is forced through the origin or the weighting factor is variable, then the coefficient of determination, more often termed r^2, should be used to measure the "goodness of fit", such that $0 \leq r^2 \leq 1$. This shows the strength of the association between x and y. The r^2 value allows the analyst to determine the percent of the data closest to the line of best fit. For consistency, it is acceptable to use r^2 for linear unweighted curves as well. An r^2 value of 1.00 indicates that all variability in response is due to variation in concentration.

In order for the linear regression model to be used for quantitative

purposes, r or r^2 should be ≥ 0.995 or 0.99, respectively. Alternatively, either of the two methods described in Sec. 11.5.4 may be used to determine whether the calibration function meets acceptance criteria. It is recommended that the resulting calibration curve be inspected by the analyst as described in Sec. 11.5.4.1.

11.5.2.3 To calculate the mass (x) of the analyte in the sample aliquot introduced into the instrument, the regression equation is rearranged.

$$x = \frac{(y - b)}{a}$$

External standard calibration allows the mass of the analyte in the sample aliquot introduced into the instrument to be calculated.

$$x_S = \frac{(A_S - b)}{a}$$

For the internal standard method, the calculation will depend on which of the two options described in Sec. 11.5.2 is chosen.

$$Option\ 1 \qquad X_s = \frac{\left(\frac{(A_s \times C_{is})}{A_{is}}\right) - b}{a}$$

$$Option\ 2 \qquad X_s = \frac{\left(\left(\frac{A_s}{A_{is}}\right) - b\right) \times C_{is}}{a}$$

where:

X_s = Calculated mass of the analyte or surrogate in the sample aliquot introduced into the instrument (in ng)
A_s = Peak response of the analyte or surrogate in the sample
A_{is} = Peak response of the internal standard in the sample
C_{is} = Mass of the internal standard in the sample aliquot introduced into the instrument (in ng).

Units for analyte mass should be the same as those used to determine the regression equation. If alternative units such as concentrations are used, calculations for the final sample concentrations found in Sec. 11.10 should be adjusted accordingly.

11.5.3 Non-linear calibration

In situations where the analyst knows the instrument response does not follow a linear model over a sufficiently wide calibration range, or when other approaches described here have not met acceptance criteria, a non-linear calibration model may be employed. At least six calibration levels are recommended to construct a quadratic (second-order) calibration curve, and at least seven levels should be used for a cubic (third-order) curve (Sec. 11.5).

NOTE: It is not EPA's intent to allow non-linear calibration to compensate for detector saturation or to avoid proper instrument maintenance.

When a calibration model for quantitation is used, the curve must be continuous: continuously differentiable and monotonic over the calibration range. The model chosen should have no more than four parameters, as in this equation:

$$y = f(a, b, c, d, x)$$

where *f* indicates a function with up to four parameters, *a* – *d*, and *x* is the independent variable. If the model is polynomial, it may be no more than third-order, as in the equation:

$$y = ax^3 + bx^2 + cx + d$$

When the linear regression model is used to estimate model parameters for the calibration data, the instrumental response (*y*) must be treated as the dependent variable, and the amount of the calibration standard (*x*) must be the independent variable. An *x*-value of zero should not be included as a calibration point, although the curve may either be weighted or forced through the origin as long as calibration criteria are met.

Model estimates from the regression must be used as calculated, and no term (i.e., *a, b, c, or d*) calculated as a result of the least squares regression can be modified. Weighting in a calibration model or forcing through the origin may significantly improve the ability of the least squares regression to fit the data at lower concentration levels. However, forcing the regression through the origin may NOT be used as a rationale for reporting results below the calibration range demonstrated by the analysis of the standards.

11.5.3.1 Linear and non-linear least squares regressions are mathematical methods that minimize differences (the residuals) between observed instrument response, y_i, and calculated response, y_i', by adjusting coefficients of the polynomial (*a, b, c, and d*) to obtain the polynomial best fitting the data.

The coefficient of determination (r^2)may be used as a measure of goodness of fit. See Sec. 11.5.2.2 for the definition of r^2.

11.5.3.2 Under ideal conditions (i.e., a "perfect" fit of the model to the data), the r^2 will equal 1.00. In order to be an acceptable non-linear calibration, the r^2 must be ≥ 0.99. Alternatively, either of the two methods described in 11.5.4 may be used to determine calibration function acceptability. It is recommended that the resulting calibration curve be inspected by the analyst, as described in Sec. 11.5.4.1.

As noted in Sec. 11.5, whichever of these options is employed, an analyte or surrogate concentration must fall within the calibration range. Analysts are also advised to check both second- and third-order calibration models to ensure that all tangents to the curve within the calibration range are of the same sign and no tangent is zero. Samples with concentrations that exceed the calibration range must be diluted to fall within the range.

11.5.4 Acceptance criteria independent of calibration model

Either of the two procedures described in Secs. 11.5.4.1 and 11.5.4.2 may be used to determine calibration function acceptability for linear and non-linear curves. These include refitting the calibration data back to the model. Both % Error and Relative Standard Error (RSE) evaluate the difference between the measured and the true amounts or concentrations used to create the model.

11.5.4.1 Calculation of the % Error

$$\% \, Error = \frac{x_i - x'_i}{x_i} \; x \, 100$$

where:

x'_i = Measured amount of analyte at calibration level *i*, in mass or concentration units

x_i = True amount of analyte at calibration level *i*, in mass or concentration units.

Percent error between the calculated and expected amounts of an analyte should be ≤ 30% for all standards. For some data uses, ≤50% may be acceptable for the lowest calibration point.

11.5.4.2 Calculation of Relative Standard Error (RSE - expressed as %)

$$RSE = 100 \times \sqrt{\sum_{i=1}^{n} \left[\frac{x'_i - x_i}{x_i}\right]^2 \Big/ (n-p)}$$

where:

x_i = True amount of analyte in calibration level *i*, in mass or concentration units

x'_i = Measured amount of analyte in calibration level *i*, in mass or concentration units

p = Number of terms in the fitting equation

(average = 1, linear = 2, quadratic = 3, cubic = 4)

n = Number of calibration points.

The RSE acceptance limit criterion for the calibration model is the same as the RSD limit for $\overline{CF}$ or $\overline{RF}$ in the determinative method. If the RSD limit is not defined in the determinative method, the limit should be set at ≤20% for good performing compounds and ≤30% for poor performing compounds. A list of known poorly performing compounds can be found in Sec. 16 of this document.

11.5.5 Data transformations

An understanding of the fundamental behavior of the detector may be used to choose a data transformation that will then allow for a simple calibration model. For example, the response of a flame photometric detector in the sulfur mode is known to be proportional to the square of the sulfur concentration. Therefore, using the data system to take the square root of the instrument response or peak height allows for a calibration factor approach rather than a polynomial calibration curve. Instrument response may be transformed prior to any calculations (including integration) subject to the following constraints:

11.5.5.1 Any parameters used in the transformation must be fixed for the calibration and all subsequent analyses and verifications until the next calibration.

11.5.5.2 The transformation model chosen must be consistent with the behavior of the instrument and detector. All data transformations must be clearly defined and documented by the analyst and related back to the fundamental behavior of the detector. In other words, this approach may not be used in the absence of specific knowledge about the behavior of the detector.

11.5.5.3 No transformations should be performed on areas or other results (e.g., the transformation must be applied to the instrument response itself).

11.5.5.4 When the transformed data are used to develop calibration factors, those factors should meet the acceptance criteria described in Sec. 11.5.1, and it is recommended that the resulting calibration "curve" be inspected by the analyst as described in Sec. 11.5.4.

11.5.6 Inspecting the calibration model and recommended corrective actions

Given the potentially large numbers of analytes that may be analyzed in some methods, it is likely that some analytes may exceed acceptance limits for a given calibration. If the criteria is not met by a target analyte, the acceptability of the initial calibration for other analytes that have met their criteria is not invalidated. Information obtained from the initial calibration of targeted analytes not meeting the acceptability criteria may have other uses such as for screening and for estimation of quantitation (see Sec 11.5.6.1), but those uses should still fit the needs of the project objectives.

Whichever calibration model is selected, it is recommended that the model be subjected to an additional check to establish the representativeness of the data that were used to produce the model. This check is the refitting of the calibration data back to the model or the comparison of the calculated amount of each of the standards against the expected amount, as described in Sec. 11.5.4. Criteria for acceptability based upon the additional check would have a similar impact upon the usability of a calibration for quantitation as is discussed in the above paragraph.

11.5.6.1 Corrective action may be needed if the calibration criteria (RSD/r^2 and %Error/RSE) are not met. If any analyte for any calibration standard has a percent error > ±30% as described in Section11.5.4.1, corrective action may be needed. Some recommended courses of action and additional options for modifying the calibration ranges follow. More specific corrective actions that are provided in the applicable determinative methods will supersede those noted in Method 8000. Generally, the calibration should not be used for quantitative analyses of that analyte when the calibration criteria (RSD/r^2 and % Error/RSE) are not met.

11.5.6.2 For all calibration models the following options are allowed. However, if none result in an acceptable calibration, a new initial calibration must be performed.

11.5.6.3 Generally, the first option is to check the instrument operating conditions. The suggested maintenance procedures in Sec. 11.11 may be useful in guiding such adjustments. This option will apply in those instances where a linear instrument response is expected. It may involve some trade-offs to optimize performance across all target analytes. For instance, changes to the operating conditions necessary to achieve linearity for problem compounds may cause the RSD for other compounds to increase, but as long as all analytes meet the RSD limits for linearity, the calibration is acceptable.

If the initial calibration for any analyte does not meet the acceptance criteria (e.g., RSD/RSE > 20% or $r^2 < 0.99$), the analyst may wish to review the results (proper identification, area counts, calibration or RFs, and RSD/RSE) for those analytes to ensure that the problem is not associated with just one of the initial calibration standards.

11.5.6.4 As a second option, if the problem appears to be associated with a single standard, that one standard may be reanalyzed, to rule out problems due to random error, and the calibration function may be recalculated and reevaluated against the acceptance criteria. Replacing the standard may be necessary in some cases. If the criteria still cannot be met, the entire initial calibration should be performed again.

NOTE: An initial calibration should be considered a single event process and a reanalysis of a calibration standard should be performed immediately to ensure that the reanalysis is still part of the original initial calibration event, and before any samples are analyzed.

11.5.6.5 A third option is to narrow the calibration range by replacing one or more of the calibration standards with standards that cover a narrower range. If linearity can be achieved using a narrower calibration range, document the calibration linearity, and proceed with analyses. Changes to the upper end of the calibration range will affect the need to dilute samples above the range, while changes to the lower end may increase the LLOQ. Consider the regulatory limits or action levels associated with the target analytes when adjusting the lower end of the range. Replacing one or more of the standards is not to be confused with discarding results from a given standard. The minimum number of standards described in Sec. 11.5.3.1 should still be used for calibration.

11.5.6.6 A fourth option is to narrow the calibration range by removing data points from either extreme end of the range and recalculating the calibration function. It is prohibited to remove data points from within a calibration range while still retaining the extreme ends of the calibration range. The minimum number of calibration levels described in Sec. 11.5 should still be met for the model.

NOTE: As noted in Sec. 11.4.1.2, the LLOQ is established by the concentration of the lowest standard analyzed during the initial calibration. Hence, narrowing the calibration range by changing the concentration of the lowest standard will, by definition, change the LLOQ. When the purpose of the analysis is to demonstrate compliance with a specific regulatory limit or action level, the analyst should ensure that the LLOQ is at least one calibration point below the regulatory limit or action level.

If criteria for RSD/RSE/r^2 has been met for the calibration model but the % error of one or more of the individual calibration points at the extreme ends of the calibration range exceeds the criteria described in Sec. 11.5.4.1, the usable range of the calibration may be narrowed to the standards that meet the % error criteria, but the calibration points used to generate the initial curve are retained. The LLOQ becomes the lowest end of the adjusted calibration range. The calibration model should meet the RSD/RSE/r^2 criteria (Secs. 11.5.1 – 11.5.3) and the minimum number of data points (Sec. 11.5.3.1) before this option can be used.

NOTE: This guidance allows the use of a calibration model constructed using all of the data points (with the exception of the highest or lowest point, which may be dropped, but will change linear range) but limits the range for usefulness to only those data points that refit the model within the criteria set in Sec. 11.5.5.1 (i.e., < 30% difference).

11.5.6.7 A fifth alternative is available for target analytes that do not meet the acceptance criteria for the initial calibration. Without reanalysis of standards or manipulations of the model, the initial calibration can be used to estimate quantitation and information from the calibration can be used to verify the identification of target analytes when used to screen samples.

If the initial calibration does not meet the acceptance criteria, it may not be used for quantitative analyses; however, estimates of the quantitation can be made. Estimates of quantitation can be useful when screening for the level of contamination and determining the degree of dilutions that may be necessary when high levels of contamination are encountered. If quantitation estimates for a positively identified analyte are not within the scope of the project DQOs, then an acceptable initial calibration should be prepared for that analyte.

If information from the initial calibration will be used to verify the identification of a targeted analyte for screening purposes, there should be sufficient sensitivity at the screening level to verify identification. Reasonable responses found at the lowest level of the calibration standards may be used as a verification of identity at that level of concentration.

11.6 Retention time windows

Retention time windows are crucial to the identification of target compounds. Absolute retention times are used for compound identification in all GC and HPLC methods that do *not*

employ internal standard calibration. Retention time windows are established to compensate for minor shifts in absolute retention times as a result of sample loadings and normal chromatographic variability. Width of the retention time window should be carefully established to minimize the occurrence of both false positive and false negative results. Tight retention time windows may result in false negatives and/or may cause unnecessary reanalysis of samples when surrogates or spiked compounds are erroneously not identified. Overly wide retention time windows may result in false positive results that may not be confirmed.

The following subsections describe one approach that may be used to establish retention time windows for GC and HPLC methods. Other approaches may be employed, provided the analyst can demonstrate performance appropriate for the intended application.

11.6.1 Before establishing retention time windows, make sure that the chromatographic system is operating reliably and that the system conditions are optimized for the target analytes and surrogates in the sample matrix to be analyzed. Make three injections of all single component standard mixtures and multi-component analytes (such as PCBs) over the course of a 72-hour period. Serial injections or injections over a period of less than 72 hours may result in retention time windows that are too tight.

11.6.2 Record the retention time (in minutes) for each single component analyte and surrogate to three decimal places. Calculate the mean and standard deviation of the three absolute retention times for each single component analyte and surrogate. For multi-component analytes, choose three to five major peaks (see the determinative methods for more details) and calculate the mean and standard deviation of those peaks.

11.6.3 If the standard deviation of the retention times for a target compound is 0.000 (i.e., no difference between the absolute retention times), then the laboratory may either collect data from additional injections of standards or use a default standard deviation of 0.01 minutes. (Recording retention times to three decimal places rather than only two should minimize the instances in which the standard deviation is calculated as 0.000).

11.6.4 Width of the retention time window for each analyte, surrogate, and major constituent in multi-component analytes is defined as ±3 times the standard deviation of the mean absolute retention time established during the 72-hour period or 0.03 minutes, whichever is greater.

11.6.5 Establish the center of the retention time window for each analyte and surrogate by using the absolute retention time for each analyte and surrogate from the calibration verification standard at the beginning of the analytical shift. For samples run during the same shift as an initial calibration, the retention time of the midpoint standard of the initial calibration should be used.

11.6.6 If the instrument data system is not capable of employing compound-specific retention time windows, then the analyst may choose the widest window and apply it to all compounds. As noted above, other approaches may also be employed, but must be documented by the analyst.

11.6.7 Surrogates are added to each sample, blank, and QC sample and are also contained in each calibration standard. Although the surrogates may be diluted out of certain sample extracts, their retention times in the calibration standards may be useful in tracking retention time shifts. Whenever the observed retention time of a surrogate is outside of the established retention time window, the analyst is advised to determine the cause and correct the problem before continuing analyses.

11.7 Calibration verification

The calibration relationship established during the initial calibration (Sec. 11.5) must be verified at periodic intervals. The process of calibration verification applies to both external standard and internal standard calibration techniques, as well as to linear and non-linear calibration models.

As a general rule, the initial calibration in a SW-846 method must be verified at the beginning of each 12-hour analytical shift during which samples are analyzed using a calibration verification standard prepared at the appropriate level of concern. (Some methods may specify more frequent verifications and recommended standard concentrations). The 12-hour analytical shift begins with the injection of the calibration verification standard (or the MS tuning standard in MS methods). The shift ends after the completion of the analysis of the last sample or standard that can be injected within 12 hours of the beginning of the shift.

If the % Difference (when using average RF calibration) or % Drift (for all other types of calibration) of an analyte is within ±20% of the expected concentration or amount based on the initial calibration, then the initial calibration is considered still valid, and the analyst may continue to use the calibration curve to quantitate sample results. The ±20% criterion may be superseded in certain determinative methods.

Except where the determinative method contains alternative calibration verification criteria, if the % Drift or % Difference is >± 20%, the initial calibration relationship may no longer be valid.

NOTE: The process of calibration verification is fundamentally different from the approach called "continuing calibration" in some methods from other sources. As described in those methods, the calibration factors or RFs calculated during continuing calibration are used to update the calibration factors or RFs used for sample quantitation. This approach, while employed in other EPA programs, is equivalent to a daily single-point calibration, and is neither appropriate nor permitted in SW-846 chromatographic procedures for trace environmental analyses.

If the calibration does not meet the acceptance criteria, perform any necessary instrument maintenance, and inject another aliquot of the calibration verification standard. If the response for the analyte is still not ± 20%, then a new initial calibration may be necessary.

11.7.1 Calibration verification criteria

Use the equations below to calculate % Drift or % Difference, depending on the procedure described in the determinative method.

$$\% \text{ Drift} = \frac{\text{Measured Amount}}{\text{True Amount}} \text{ x } 100$$

where:

Measured amount = mass or concentration determined by the calibration model

True amount = prepared mass or concentration of the analyte in the standard.

$$\% \text{ Difference} = \frac{CF_v - \overline{CF}}{\overline{CF}} \times 100\% \quad = \frac{RF_v - \overline{RF}}{\overline{RF}} \times 100$$

where:

CF_v = calibration factor calculated for the calibration verification standard
RF_v = response factor calculated for the calibration verification standard
$\overline{CF}$ = mean calibration factor from the initial calibration
$\overline{RF}$ = mean response factor from the initial calibration

If >10% of the analytes in a multi-analyte method exceed the calibration verification criteria, and instrument maintenance does not correct the problem, then a new initial calibration is necessary. If ≤10% of the analytes exceed the calibration verification criteria, then the initial calibration may still be used, but any detected analytes exceeding the limit must be reported as estimated. Non-detected analytes may be reported if the calibration verification for that specific analyte exceeds the upper acceptance criteria (e.g., >+20%). In order to report non-detected analytes that exceeds the lower acceptance criteria (e.g., <-20%), a sensitivity verification standard at or below the LLOQ should be analyzed in the analytical batch. The analyte should be detected in the LLOQ standard and meet all of the qualitative identification criteria that the laboratory routinely uses (for example, qualifier ions of columns, signal to noise, etc). In any event, the limitation to no more than 10% of analytes exceeding the calibration verification criteria applies to both detected and non-detected analytes.

11.7.2 Verification of non-linear calibration

Calibration verification of a non-linear calibration is performed using the % Drift calculation and criteria described in Sec. 11.7.1, above.

It may also be appropriate to employ two standards at different concentrations to verify the calibration. One standard should be near the quantitation limit or action limit. Choice of specific standards and concentrations is generally a method- or project-specific consideration.

11.7.3 Calibration verification may be performed using both high and low concentration standards from time to time. This is particularly true when the ECD or ELCD is used. These detectors drift and are not as stable as FID or FPD, and periodic use of the high and low concentration standards serves as a further check on the initial calibration. Concentrations of these standards should generally reflect those observed in samples.

11.7.4 Additional analyses of the midpoint calibration verification standard during a 12-hour analytical shift are strongly recommended for methods involving external standard calibration. The same evaluation criteria described in Sec. 11.7.1 should be used.

Frequency of verification necessary to ensure accurate measurement is dependent on the detector and the sample matrix. Very sensitive detectors that operate in the sub-nanogram range are generally more susceptible to changes in response caused by column contamination and changes in ambient conditions. Therefore, more frequent verification of calibration (i.e., after every 10 samples) may be necessary for some types of

detectors (i.e., electron capture, electrochemical conductivity, photoionization, fluorescence detectors).

Sec. 11.8.2 states that samples analyzed using external standards must be bracketed by periodic analyses of standards that meet the QC acceptance criteria (e.g., calibration and retention time). Therefore, more frequent analyses of standards will minimize the number of sample extracts to be reinjected if the QC limits are violated for the standard analysis. Results from these bracketing standards should meet the calibration verification criteria in Secs. 11.7.1 and 11.7.2 and the retention time criteria in Sec. 11.6. However, if the standard analyzed after a group of samples exhibits a response for an analyte that is above the acceptance limit (i.e., >20%), and the analyte was not detected in any of the previous samples during the analytical shift, then the sample extracts do not need to be reanalyzed, as the verification standard has demonstrated that the analyte would have been detected were it present.

11.7.5 Any method blanks described in the preparative methods (Methods 3500 and 3600) may be run immediately after the calibration verification analyses to confirm that laboratory contamination does not cause false positive results, or at any other time during the analytical shift. If the method blank indicates contamination, then it may be appropriate to analyze a solvent blank to demonstrate that the contamination is not a result of carryover from standards or samples.

11.8 Chromatographic analysis of samples

11.8.1 Introduction of samples or sample extracts into the GC or HPLC varies, depending on the physical and chemical properties of the compound and the solvent matrix. Volatile organics are primarily introduced by purge-and-trap techniques (Method 5030, water and Method 5035, soils). Other techniques include azeotropic distillation (Method 5031), vacuum distillation (Method 5032), headspace (Method 5021), or direct aqueous injection. Use of Method 5021 or another headspace technique may be advisable for screening volatiles in some sample matrices to prevent overloading and contamination of the purge-and-trap system. Semivolatile and non-volatile analytes are introduced by direct or split/splitless injection.

11.8.1.1 Manual injection (GC)

Inject a small volume (i.e., 0.5 - 5 µL) of the sample extract. However, other injection volumes may be used if the analyst can demonstrate appropriate performance for the intended application. Use of the solvent flush technique is necessary for packed columns.

11.8.1.2 Automated injection (GC)

Automated injectors can provide volumes both larger and smaller than 1 - 2 µL. The analyst should ensure that the appropriate injector design is used for the volume to be injected and that the injection volume is reproducible. Other injection volumes may be used if the analyst can demonstrate appropriate performance for the intended application.

Large Volume Injection (LVI) is the injection of large volumes (greater than 5 µL) into cooled inlets that allow the solvent to be vented while retaining less volatile analytes. LVI is used to increase the sensitivity of the analysis, either to decrease LLOQs or to decrease the amount of sample extracted, or extraction solvent used.

This procedure is typically performed with inlets made specifically for this technique. The analyst should ensure that all of the QC requirements of both the preparation and determinative methods are met.

11.8.1.3 Purge-and-trap

Refer to Methods 5000, 5030, or 5035 for details.

11.8.1.4 Manual injection (HPLC)

Inject 10 - 100 µL. This is generally accomplished by overfilling the injection loop of a zero dead-volume injector. Larger volumes may be injected if better sensitivity is needed; however, chromatographic performance may be affected.

11.8.1.5 Automated injection (HPLC)

Inject 10 - 100 µL. Laboratories should demonstrate that the injection volume is reproducible. Larger volumes may be injected if greater sensitivity is needed; however, the solvent of the standards and samples should be matched to the initial mobile phase to avoid chromatographic performance degradation.

11.8.2 All analyses, including field samples, duplicates, MS/MSDs, LCS, method blanks, and any other QC samples are performed during an analysis sequence. The sequence begins with instrument calibration, which is followed by the analysis of sample extracts. Verification of calibration and retention times is necessary no less than once every 12-hour analytical shift. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded. As noted in Secs. 11.5 and 11.7, when employing external standard calibration, run a calibration verification standard at the end of the sequence to bracket the sample analyses. Acceptance criteria for the initial calibration and calibration verification are described in Secs. 11.5 - 11.7.

Analysis of calibration verification standards between every set of 10 samples is strongly recommended, especially for highly sensitive GC and HPLC detectors at sub-nanogram concentrations. Frequent analysis of calibration verification standards helps ensure that chromatographic systems are performing acceptably and that false positives, false negatives and poor quantitation are minimized. Samples analyzed using external standard calibration should be bracketed by the analyses of calibration standards that meet the QC limits for verification of calibration and retention times. If criteria are exceeded, corrective action should be taken (Sec. 11.11) to restore the system and/or a new calibration curve prepared for that compound and the samples reanalyzed.

Certain methods may also include QC checks on column resolution, analyte degradation, mass calibration, etc., at the beginning of a 12-hour analytical shift.

11.8.3 Sample concentrations are calculated by comparing sample responses with the initial calibration of the system (Sec. 11.5). If sample response exceeds the limits of the initial calibration range, dilute the extract (or sample) and reanalyze. Extracts should be diluted so that all peaks are on scale, as overlapping peaks are not always evident when peaks are off scale. When overlapping peaks cause errors in peak area integration, the use of peak height measurements is suggested.

11.8.4 If chromatographic peaks are masked by the presence of interferences, further sample cleanup or dilution may be necessary. See Method 3600 for guidance.

11.9 Compound Identification

Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Confirmation is necessary when the composition of samples is not well characterized. Confirmation techniques include further analysis using a second column with dissimilar stationary phase, GC/MS (full scan or SIM) or HPLC/MS (if concentration permits), GC or HPLC with two different types of detectors, or by other recognized confirmation techniques. For HPLC/UV methods, analyte confirmations at two different UV wavelengths with a UV or UV diode array detector is not recommended because of the broadband nature of UV absorption spectra of many organic compounds. Positive identification of a target analyte using an HPLC/UV method may be confirmed with a different type of detector such as a mass-selective detector or a fluorescence detector at different excitation and emission wavelengths.

When confirmation is made on a second column, that analysis should meet all of the QC criteria described above for calibration, retention times, etc. Confirmation is not needed with GC/MS and HPLC/MS methods.

Confirmation may not be necessary if the composition of the sample matrix is well established by prior analyses as when a pesticide known to be produced or used in a facility is found in a sample from that facility.

Many chromatographic interferences result from coelution of one or more compounds with the analyte of interest, or may be the result of the presence of a non-analyte peak in the retention time window of an analyte. Such coelution problems affect quantitation as well as identification, and may result in poor agreement between the quantitative results from two dissimilar columns. Therefore, even when the identification has been confirmed on a dissimilar column, the analyst should evaluate the agreement of the quantitative results on both columns, as described in Sec. 11.10.4.

11.10 Calculations

Calculation of sample results depends on the type of calibration (external or internal standard) and the calibration model employed (linear or non-linear). Calculations of the mass of the analyte in the sample aliquot introduced into the instrument can be found in Secs. 11.5.1.3, 11.5.2.3, and 11.5.3. The following sections describe the calculations necessary to obtain the concentrations of analytes in the original sample, based on its volume or weight.

These calculations are provided for illustrative purposes only. Various dilution schemes and conventions for defining final volumes and injection volumes exist and they all cannot be addressed here. The analyst must clearly document and verify all of the calculations that are employed. Specific determinative methods may also contain additional information on how to perform these calculations.

11.10.1 Sample concentration by volume (µg/L), for aqueous samples

$$Concentration\ in\ \frac{\mu g}{L} = \frac{(X_s)(V_t)(D)}{(V_i)(V_s)}$$

where:

X_s = Calculated mass of analyte (in ng) in sample aliquot introduced into instrument. Type of calibration model used determines derivation of x_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3.

V_t = Total volume of concentrated extract (in µL). For purge-and-trap analysis, V_t is the purge volume and will be equal to V_i. Thus, units other than µL may be used for purge-and-trap analyses.

D = Dilution factor, if sample or extract was diluted prior to analysis. If no dilution, D=1. Always dimensionless.

V_i = Volume of extract injected (in µL). The nominal injection volume for samples and calibration must be the same. For aqueous purge-and-trap analysis or direct injection of a liquid sample into a GC or HPLC, V_i will be equal to V_t.

V_s = Volume of aqueous sample extracted or purged (in mL). If units of liters (L) are used for this term, multiply results by 1000 mL/L.

Using the units listed here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L.

11.10.2 Sample concentration by weight (µg/kg), for solid samples and non-aqueous liquids

$$Concentration\ in\ \frac{\mu g}{kg} = \frac{(X_s)(V_t)(D)}{(V_i)(W_s)}$$

where:

X_s = Calculated mass of analyte (in ng) in sample aliquot introduced into instrument. Type of calibration model used determines derivation of x_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3.

V_t = Total volume of concentrated extract (in µL). For purge-and-trap analysis where an aliquot of solvent (methanol, water, etc.) extract is added to reagent water and purged, V_t is total volume of solvent extract. Also includes any contribution from water present in samples prior to solvent extraction (Sec. 11.10.5).

D = Dilution factor, if sample or extract was diluted prior to analysis. If no dilution, D=1. This value is always dimensionless.

V_i = Volume of extract injected (in µL). The nominal injection volume for samples and calibration standards must be the same. For purge-and-trap analysis where an aliquot of solvent (methanol, water, etc) extract is added to reagent water and purged, V_i is the volume of solvent extract added to reagent water just prior to purging. Dilutions made to the initial volume of solvent extract are accounted for in *D*.

W_s = Weight of sample extracted or purged (in grams). If kg units are used for this term, multiply results by 1000 g/kg.

Using the units listed here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg. See Sec. 11.10.5 for situations in which calculated concentrations may need to be corrected based on the solvent/water dilution effect for extracted volatile organics.

11.10.3 Sample concentration when X_s is expressed as concentration during calibration

As noted in Sec. 11.4, the analyst may develop the calibration using the concentration of analyte and internal standard instead of mass. Using such an approach usually involves expressing concentrations as mass of the analyte or internal standard in the volume injected into the instrument (i.e., ng/µL). Thus, calculations for the final concentration of an analyte in a sample in Secs. 11.10.1 and 11.10.2 must be modified to include the injection volume, V_i, into the term X_s. Therefore, the equation for sample concentration by volume becomes:

$$Concentration\ in\ \frac{\mu g}{L} = \frac{(X_s)(V_t)(D)}{(V_s)}$$

And the equation for sample concentration by weight becomes:

$$Concentration\ in\ \frac{\mu g}{g} = \frac{(X_s)(V_t)(D)}{(W_s)}$$

where V_t, D, V_s, and W_s are the same as in Secs. 11.10.1 and 11.10.2 and

X_s = Calculated concentration of analyte (ng/µL) in the sample. Type of calibration model used determines derivation of X_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3.

Using the units listed here for these terms will result in concentrations in ng/mL, which is equivalent to µg/L, or in ng/g, which is equivalent to µg/kg. See Sec. 11.10.5 for situations in which calculated concentrations may need to be corrected based on the solvent/water dilution effect for extracted volatile organics.

11.10.4 Comparison between results from different columns or detectors

When sample results are confirmed using two dissimilar columns or with two dissimilar detectors, the agreement between the quantitative results should be evaluated after the identification has been confirmed. Large differences in the numerical results from the two analyses may be indicative of positive interferences with the higher of the results, which could result from poor separation of target analytes, or the presence of a non-target compound. However, they may also result from other causes. Thus, in order to ensure that the results reported are appropriate for the intended application, the analyst should make a formal comparison, as described below.

Calculate the RPD between the two concentrations using the formula below.

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} X\ 100$$

where C_1 and C_2 are concentrations on the two columns and the vertical bars in the numerator indicate the absolute value of the difference. Therefore, RPD is always a positive value.

11.10.4.1 If one result is significantly higher (e.g., >40%), check the chromatograms to see if an obviously overlapping peak is causing an erroneously high result. If no overlapping peaks are noted, examine the baseline parameters established by the instrument data system (or operator) during peak integration. A rising baseline may cause the incorrect integration of the peak for the lower result.

11.10.4.2 If no anomalies are noted, review the chromatographic conditions. If there is no evidence of chromatographic problems, it may be appropriate to report the lower result.

Regardless of the presence or absence of chromatographic problems, the data user must be advised of the disparity between the two results, because the user, not the laboratory, is responsible for ensuring that the most appropriate result is reported or utilized. Under some circumstances, including those involved in monitoring compliance with an action level or regulatory limit, further cleanup of the sample or additional analyses may be needed when the two values in question span the action level or regulatory limit.

11.10.5 Moisture-corrected reporting

Results for solid samples may be reported on the basis of wet weight (as received) or dry weight (moisture-corrected) sample concentration. There are merits to either approach; however, some regulatory limits associated with solid wastes and solid samples are based on the form of the waste as generated, which rarely involves oven-dry solids. As a result, there is no default preference for one form or the other.

The choice of "as received" or moisture-corrected reporting is always a project-specific decision that must be based on knowledge of intended use of the data.

When moisture-corrected reporting is required, concentration results for solid samples calculated in Secs. 11.10.2 and 11.10.3 may be converted to moisture-corrected results as follows:

$$Moisture\ corrected\ concentration = \frac{(\text{"}As\ received\text{"}\ concentration)}{(100 - \%\ moisture)} X\ 100$$

where % moisture is determined as described in the specific sample preparation or determinative method, typically by drying an aliquot of the sample at 105 °C overnight. Percent moisture is calculated as follows:

$$\% \, Moisture = \frac{(g \, of \, \text{"as received"} \, sample) - (g \, of \, dry \, sample)}{g \, of \, sample} \, X \, 100$$

The % moisture determination may also be called % solids in some methods. In this case, percent solids should be subtracted from 100, in order to attain % moisture as noted in the above moisture-corrected calculation. Units for the final results will be the same, regardless of the % moisture calculation.

Except when the sample is completely dry (i.e., the % moisture equals 0), moisture-corrected results will always be higher than "as received" results. In the absence of project-specific requirements, it may be most appropriate to report results on the "as received" basis of the sample and provide the % moisture for each sample. This will allow the data user to convert the results from one form to another, as needed. The approach used must be clearly described for the data user.

In volatile organic analysis, solid samples with significant moisture content (>10%) that are extracted prior to analysis in a water-miscible solvent such as methanol, are diluted by the total volume of the solvent/water mixture. The total mixture volume can only be calculated based on the sample moisture present as determined by the % moisture determination. This total volume is then expressed as V_t in the sample concentration calculations provided in Secs. 11.10.2 and 11.10.3. Therefore, in order to report results for volatile analysis of samples containing significant moisture content on either "as received" or "moisture-corrected" basis, the calculated concentration must be corrected using the total solvent/water mixture volume represented as V_t. This total solvent/water volume is calculated as follows:

$$\frac{\mu L \, solvent}{water V_t} = \left((mL \, of \, solvent) + \frac{(\% \, Moisture \, x \, g \, of \, sample)}{100} \right) x \, 1000 \, \mu L/mL$$

When the sample moisture content is >10%, it is recommended that the calculated concentrations of volatile samples that are extracted in a water-miscible solvent such as methanol be corrected for the solvent/water dilution effect. Potential underreporting of volatile concentrations is more pronounced as % moisture increases.

11.11 Suggested chromatographic system maintenance

Following is a list of corrective measures that may be employed to prevent or ameliorate the deterioration of chromatographic performance. This list is by no means comprehensive, and analysts should develop expertise in troubleshooting their specific instruments and analytical procedures. Manufacturers of chromatographic instruments, detectors, columns, and accessories generally provide detailed information regarding the proper operation and limiting factors associated with their products. Reading and reviewing this information cannot be overemphasized.

11.11.1 GC preventive maintenance and corrective action

To prevent or ameliorate deterioration of chromatographic performance, analysts should perform routine maintenance activities on the GC inlet, column, and gas delivery system.

11.11.1.1 Inlet maintenance

Appropriate injector liners should be installed and replaced as necessary to maintain chromatographic performance. Injection port septa should also be changed frequently enough to prevent retention time shifts of target analytes and peak tailing. Over-tightening the septum nut can cause the inlet to leak. The schedule for changing inlet liners and septa is dependent on the operation of the injection system, the nature of samples and parameters tested, and acceptance criteria in the reference method.

If chromatographic performance or ghost peaks are still a problem after performing these inlet maintenance steps, replacing the seal in the bottom of the inlet or cleaning and deactivating the metallic surfaces of the injection port itself may be necessary. Deactivation of the injection port necessitates the use of toxic reagents and should only be performed by knowledgeable personnel according to the instrument manufacturer's instructions.

11.11.1.2 Column maintenance

Capillary columns are reliable and easy to use, but overheating and exposure to oxygen can cause damage. Install and condition the column as recommended by the manufacturer, and flush the column with carrier gas before conditioning. Avoid contact between the capillary column and the metal surfaces in the GC oven or heating above the maximum column temperature.

Poor chromatographic performance may also be observed when the head of the GC column is contaminated with high-boiling material. Removing as much as 0.5 - 1 m from the injector side of the capillary column may restore chromatographic performance. If clipping the head of the column does not restore performance, replacement of the column may be necessary. Using a guard column may extend column life.

11.11.1.3 Gas delivery system maintenance

Analysts should periodically ensure that proper flow control is maintained. A search for leaks using an electronic leak detector or by isolating and pressure testing various parts of the delivery system may be conducted; static pressure tests may also be performed, or other appropriate measures taken. Electronic pressure controller flow rates should also be checked regularly (with both the injector and the oven heated) using a bubble meter or other appropriate device. A leak in the gas delivery system and/or change in delivery pressure can lead to retention time shifts of the target analytes in the GC chromatograms. If this is observed, corrective action should be taken. Monitoring retention times in standards over time can help to ensure that

11.11.2 HPLC preventive maintenance and corrective action

Band broadening occurs whenever there is a dead volume between the injector and detector. Therefore, plumbing connections should be of minimum length and diameter, and ferrules should be properly positioned on the tubing to minimize dead volume.

11.11.2.1 Injection port maintenance

Filtration of extracts and injection of solvent mixtures miscible with the mobile phase can help minimize solvent-related problems. Otherwise, contamination of subsequent injections may occur when the extract contains material that is not soluble in the mobile phase. Injectors also need maintenance, as the surfaces that turn past each other tend to wear down over time, potentially causing leaks. Injection loops are easily changed, but analysts must ensure that the compression fittings are properly installed to prevent leaks.

11.11.2.2 Column maintenance

Use of high quality columns that are uniformly packed with the appropriate particle size and bonded phase will result in optimal chromatographic performance. Column temperatures may be regulated by the use of temperature control ovens to ensure reproducibility of retention times.

Lifetime and performance of HPLC columns can be improved through proper maintenance. Sample extracts should be filtered prior to analysis, and care should be taken to ensure that storage conditions (e.g., freezing) do not cause subsequent precipitation of solids in the extracts prior to analysis. Guard columns should be used when dirty samples are analyzed because HPLC columns can become contaminated with particulates or insoluble materials. If degradation of resolution or changes in back pressure are observed, the replacing the guard column if one is installed may restore performance. Columns should not be stored dry or containing strong buffers, and they should be replaced when performance degrades (e.g., significant band broadening, peak splitting, or loss of chromatographic resolution occurs).

11.11.2.3 Mobile phase and pump maintenance

Pumping systems should deliver reproducible gradients at a uniform flow rate. Pumping flow rates can be checked by collecting solvent into a graduated cylinder for a designated time period.

Air bubbles tend to cause an erratic baseline and, in the case of low-pressure mixing, bubbles can cause the pump to cavitate. Therefore, HPLC solvents should be degassed prior to use.

Non-reproducible gradients can result from deterioration in pump performance and can cause unacceptable variation in retention times from run to run. Mobile phase solvents should be filtered to remove particles that cause pump piston wear. Seals in the HPLC pumps should be replaced regularly. Use of strong buffers or solvents such as tetrahydrofuran can significantly shorten the lifetime of pump seals and should be avoided where practical.

Small changes in the composition or pH of the mobile phase can have a significant effect on retention times. Buffering the mobile phase may help make the pH more reproducible from preparation to preparation, as long as it doesn't conflict with the method or cause other problems with the analysis. Precise measurement of reagents and care in mixing the mobile phase may help ensure consistency from one preparation to the next. A solvent mixer may be the best way to ensure reproducibility of the mobile phase over time.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 See Sec. 11.0 and the appropriate determinative method for information regarding data analysis and calculations.

12.2 Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. Data do not represent required performance goals for users of the methods. Instead, performance goals should be developed on a project-specific basis and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

13.2 Refer to individual determinative methods for performance data examples and guidance.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the EPA recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety, http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Laboratories are urged to protect air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations and complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2 above.

16.0 REFERENCES

16.1 For further information regarding these methods, review Methods 3500, 3600, 5000, the individual sample preparative, cleanup and determinative methods, and Chapter One.

16.2 Three references for poorly performing compounds in general are shown below.

Department of Defense:
Appendix G of DoD "*Quality Systems Manual for Environmental Laboratories*", Version 4.2
http://www.denix.osd.mil/edqw/upload/QSM-V4-2-Final-102510.pdf.

"*Quality Systems Manual for Environmental Laboratories*", Version 5.0
http://www.denix.osd.mil/edqw/upload/QSM-Version-5-0-FINAL.pdf.

EPA:
USEPA Contract Laboratory Program SOM01.2
http://www.epa.gov/superfund/programs/clp/som1.htm.

16.3 A list of quantitation limits for poorly performing volatile compounds can be found in Table App D XIII-1 at: http://www.epa.gov/region1/oeme/appendc.pdf.

16.4 A list of quantitation limits for poorly performing semivolatile compounds can be found in Table App E XIII-1 at: http://www.epa.gov/region1/oeme/appendd.pdf.

16.5 Department of Defense:
"Environmental Data Quality Workgroup (EDQW) Laboratory Control Sample Control Limits Study", July 2013 at:
http://www.denix.osd.mil/edqw/upload/Final-LCS-Study-July-2013.pdf.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

There are no tables or figures associated with this method.

Appendix A:

Summary of Revisions to Method 8000C (From Revision 3 March 2003)

1. In Sec. 9.3.3, additional text to indicate that the reference samples used for initial demonstration of proficiency (IDP) analyses should be prepared from the same source as the calibration standards was added. This change is now consistent with Methods 8260 and 8270. The IDP section was also expanded to include separate language for preparation and extraction chemists, as well as instrument chemists. This only requires analysts to perform an IDP on the portion of the procedure that they actually do.

2. References to the method quantitation limit (MQL) have been replaced with the lower limit of quantitation (LLOQ).

3. Inclusion of relative standard error (RSE) in Sec. 11.5.4.2.

4. Improved overall method formatting for consistency with new SW-846 methods style guidance. The format was updated to Microsoft Word.docx.

5. Many minor editorial and technical revisions were made throughout to improve method clarity.

6. The revision number was changed to 4 and the date published was changed to July 2014.

7. This appendix was added showing changes from the previous revision.

8. Items regarding instrument maintenance in Secs. 4 and 6 were condensed and moved to Sec. 11.11.

9. Update language discussing method blank acceptance criteria and LLOQ standards was added in Section 9.

ANEXO 10

Método CHAPTER FOUR

CHAPTER FOUR
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CHAPTER FOUR

ORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this chapter is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the data quality objectives (DQOs) or needs for the intended use of the data.

4.1 SAMPLING CONSIDERATIONS

4.1.1 Introduction

Following the initial and critical step of designing a sampling plan (Chapter Nine) is the implementation of that plan such that a representative sample of the solid waste (or other material) is collected. Once the sample has been collected it must be stored and preserved to maintain the chemical and physical properties that it possessed at the time of collection. The sample matrix, type of containers and their preparation, analytes of interest, preservation techniques, and sample holding times must be thoroughly examined in order to maintain the integrity of the samples. This section highlights practices relevant to maintaining sample integrity and representativeness from the time of sampling until analysis is complete. This section is, however, applicable primarily to trace analyses. Some of these considerations may be less relevant for source level samples.

4.1.2 Sample Handling and Preservation: General Considerations

This following sections deal separately with volatile organic chemicals (VOCs) and semivolatile organic chemicals (SVOCs). Refer to Chapter Two and Table 4-1 of this section for recommended sample containers, sample preservation, and holding time information. The guidelines in Table 4-1 are intended to improve chemical stability in the sample matrix between the time of sample collection and laboratory preparation/analysis by minimizing loss of the analytes of interest from the sample container and limiting biological and/or chemical degradation (e.g., hydrolysis) (Sec. 4.6 Refs 1, 3-6). Sample preservation recommendations for analysis of organic chemicals almost always include refrigeration or freezing and may also include chemical preservation (e.g., addition of pH modifier). Improper handling, preservation, and storage of samples can negatively impact the representativeness of the field sample data.

The preservation and holding time information presented in Table 4-1 does not represent EPA requirements, but rather is intended solely as guidance. Selection of preservation techniques and applicable holding times should be based on all available information, including the properties of the analytes of interest for the project, their anticipated concentration levels, the composition of the sample matrix itself, and the stated project-specific DQOs. A shorter holding time may be appropriate if the analytes of interest are reactive (e.g., 2-chloroethyl vinyl ether, acrylamide) or the sample matrix is complex (e.g., wastewater). Conversely, a longer holding time may be appropriate if it can be demonstrated that the analytes of interest are not adversely affected from preservation, storage and analyses performed outside the recommended holding times. Prior to collecting samples for analysis, the project team may consider existing information and data regarding analyte stability or conduct field screening for the samples to be collected in

order to determine how best to preserve sample integrity for the analytes of interest. The use of site-specific performance evaluation material is a high confidence mechanism to ensure reliability of project data. The references in Sec. 4.6 provide examples of study designs that may be useful for this purpose.

4.1.3 Sample Handling and Preservation for Volatile Organics

4.1.3.1 VOC Sample Containers

The containers used for collecting VOC samples are frequently volatile organics analysis (VOA) vials that are directly compatible with the equipment used for sample preparation and analysis in the laboratory. Use of these containers for sampling helps minimize loss of VOCs resulting from opening sample containers and/or transferring materials from one container to another. Certified pre-cleaned VOA vials are commonly used as sample containers for VOCs and are commercially available from a number of vendors. The vials should be absent of burrs around the caps that might prevent the vial from sealing, and septa should be lined with a polytetrafluoroethylene (PTFE) layer of sufficient thickness to limit diffusion of VOCs out of the vials during storage. PTFE thicknesses of 0.13 to 0.25 mm have been shown to be effective. See reference # 18 in Sec. 4.6 below and Sec. A.8 in Method 5035A for more detail. If they are suspected of being a source of interferences, VOA vials and unpunctured septa should be washed with soap and water and rinsed with distilled de-ionized water. After thoroughly cleaning the vials and septa, they should be placed in an oven and dried at 100 °C for approximately one hour.

NOTE: Heating the septa for extended periods of time (i.e., more than one hour) or at higher temperatures should be avoided, because the silicone begins to slowly degrade at 105 °C). Also, punctured silicone-backed PTFE-lined septa should generally not be reused, because some VOCs have high affinity for the silicone material, and puncturing the PTFE septum face exposes the gas phase vial contents to the silicone backing material, causing loss of certain VOCs depending on length of exposure time and vial temperature.

Air-tight, sealable coring devices (e.g., En Core™, Core N' One™ or equivalent) may also be useful for collection and storage of cohesive soil samples for VOC analysis. These devices are designed to limit loss of VOCs from samples during cold storage and shipping over a limited time frame and for quantitative transfer of solids and associated VOCs into VOA vials for immediate analysis or further preservation. Their use during field sampling of solids helps reduce or eliminate the need to handle solvents or chemical preservatives in the field and eliminates some shipping restrictions on field samples that may otherwise contain flammable solvents (e.g., methanol). Additional information regarding stability studies of VOCs in solid materials stored in sealable coring devices is contained in the Sec. A.7 of the appendix of Method 5035A and is described in more detail in the sources referenced therein. An American Society for Testing and Materials (ASTM) standard practice for use of the En Core™ type samplers is also included in the references in Sec. 4.6 below.

4.1.3.2 VOC Sample Collection:

When transferring samples into vials, liquids and solids should be introduced gently to minimize agitation which might drive off volatile compounds.

At least two replicate VOA vials should be collected and labeled immediately for each collected field sample. They should not be filled near a running motor or any type of exhaust system because discharged fumes and vapors may contaminate the samples. Replicate vials from a single sampling point may be sealed together in a single plastic bag, but different samples should be segregated into separate plastic bags to prevent contamination of samples with little to no VOCs from those with high concentrations. Sample containers may also become contaminated by diffusion of VOCs into the vials through the septa from the surrounding environment during shipment and storage. To monitor for this potential source of contamination, a trip blank prepared from organic-free reagent water (as defined in Chapter One) should be maintained with the samples throughout sampling, shipping, and storage. Including activated carbon in the bags containing the sample vials may help reduce concerns related to these potential sources of sample contamination.

Improper vial sealing (e.g., due to solids retained on the vial threads) and improper tightening of caps or closing of sealable coring devices are primary factors in the loss of volatiles due to sample collection activities. Sealing surfaces and any closure threads should be inspected to ensure they are free of debris prior to container closure.

Procedures should also be established for selection and appropriate use of sample collection devices (i.e., bailer, coring tool, etc.) including appropriate decontamination measures. If the sample comes in contact with the sampling device, organic free reagent water may be run through the device and tested as a field blank.

In general, liquid samples should be poured into vials without introducing any air bubbles into the samples as vials are filled. Should bubbling occur as a result of violent pouring, the sample should be poured out and the vial refilled. The vials should be completely filled at the time of sampling, so that when the septum cap is fitted and sealed and the vial is inverted, no headspace is visible. The sample should be hermetically sealed in the vial at the time of sampling, and not opened prior to analysis to preserve its integrity.

4.1.3.3 VOC Sample Preservation and Holding Times:

Samples containing analytes that can be subject to biological degradation need to be preserved as soon as possible (preferably in the field) to avoid the loss of target analytes. Refrigeration or freezing is a primary means of sample preservation, because rates of biotic and abiotic degradation decrease with decreasing temperature, and VOCs are also less volatile at lower temperature. Samples containing analytes that are most subject to biological degradation (e.g., aromatic hydrocarbons) also should be chemically preserved (e.g., by addition of acid), unless they are analyzed immediately. Chemical preservation may be inappropriate for highly reactive compounds (e.g., 2-chloroethyl vinyl ether, acrylamide, etc.), since it may accelerate loss by rapid chemical reaction. Aqueous samples containing free chlorine should also be preserved with a dechlorinating agent in order to minimize formation of trihalomethanes and other possible chemical reactions.

Although VOC samples may be held for up to 7 days unpreserved or 14 days or longer preserved, it is generally not recommended as good laboratory

practice to hold them that long. VOC samples should be run as soon as possible after receipt by the laboratory. Samples in which highly reactive compounds (e.g., 2-chloroethyl vinyl ether, acrylamide, etc.) are analytes of interest should be analyzed as soon as they are received in the laboratory.

4.1.4 Sample Handling and Preservation for Semivolatile Organics, Including Pesticides, PCBs and Herbicides

4.1.4.1 Sample Containers for Analysis of Semivolatile Organics

The containers specified for samples intended for analysis of SVOCs are typically constructed of glass with PTFE-lined threaded caps. In situations where PTFE liners are not available, solvent-rinsed aluminum foil may be used as a liner. However, acidic or basic samples may react with the aluminum foil, causing eventual contamination of the sample. Use of new, disposable pre-cleaned and certified containers reduces concerns about contamination from reusing sample containers. Plastic containers or plastic lids without PTFE liners should not be used for storage of samples due to potential contamination by phthalate esters and other hydrocarbons within the plastic or absorption of any chemicals of concern in the native sample into the container material. If sample containers are suspected of being a source of interferences, particularly for low-level analysis, they should be soap and water washed followed by rinsing with solvent(s) appropriate for the analytes of interest. (See Sec. 4.1.6 for specific instructions on glassware cleaning.). Caps may be cleaned by solvent rinsing or replaced with new ones. Monitoring for contamination introduced from sample containers should be accomplished through preparation and analysis of a method blank.

4.1.4.2 Sample Collection for SVOCs

Sample containers should be filled with care so as to prevent any portion of the collected samples from coming in contact with the sampler's gloves, potentially leading to sample contamination. Samples should not be collected or stored in the presence of exhaust fumes. If the sample comes in contact with the sampling device, run organic-free reagent water through the sampling device and test this water as a field blank.

4.1.4.3 Sample Preservation and Holding Times for SVOCs

Field samples to be analyzed for SVOCs are typically preserved by refrigeration or freezing. In order to minimize opportunities for the most labile SVOCs to degrade, these samples are typically recommended to be solvent extracted shortly after being taken, within 7-14 days for many classes of chemicals. However, some classes of SVOCs, like polychlorinated biphenyls and polychlorinated dibenzodioxins and dibenzofurans are very recalcitrant and do not readily degrade during refrigerated storage. Sample matrices to be analyzed for these SVOCs have no maximum recommended holding time. Depending on the composition of the sample matrix and the levels of concern for the target analytes, other classes of SVOCs (e.g., polycyclic aromatic hydrocarbons [PAHs]) may also be stable in refrigerated or frozen storage for longer than the maximum holding time recommended in Table 4-1 (see Reference #12 in Sec. 4.6 below). However, the composition of the sample matrix can be an important determinant of chemical stability, and minimizing the holding time between sampling and solvent extraction is generally a good practice to obtain representative data.

Solvent extracts of samples should be carefully maintained. Solvent extraction generally stabilizes SVOCs, because the chemicals are typically physically removed from the sample matrix, and some loss mechanisms are eliminated (i.e., biological degradation). Holding times of 40 days are recommended for solvent extracts for most classes of SVOCs. Many analytes of interest may be stable in solvent for a longer time period even in extracts of complex matrices, but problems maintaining small volumes of very volatile solvent extracts preclude storage of extracts indefinitely, and some SVOCs may still chemically degrade or may be slightly volatile in certain solvents.

Freezing solvent extracts particularly of complex sample matrices may cause precipitation of solids resulting from interaction of some co-extracted sample matrix components. Storing extracts at 0 to 6 °C may limit problems resulting from analyzing extracts containing precipitated solids, like contaminating or clogging the injector syringe or introducing insoluble components into the flow pathway of the mobile phase. One way to remove precipitated solids from a solvent extract is by filtration with a sub-micron particle size filter made of inert material (e.g., PTFE). As with other preparation steps, batch quality control (QC) samples should be subjected to the same filtration procedure as the field samples in order to assess the cumulative impact of all sample preparation steps on analyte recovery and evaluate the potential for contamination resulting from all reagents, and other materials that come into contact with the samples.

4.1.5 Safety

The methods listed in this chapter do not address all safety issues associated with their use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals used in these methods. A reference file of material safety data sheets (MSDSs) and/or safety data sheets (SDSs) should be available to all personnel involved in these analyses.

Safety should always be the primary consideration in the collection and analysis of samples. A thorough understanding of the waste production process, as well as all of the potential hazards of the waste itself, should be investigated whenever possible. The site should be evaluated just prior to sampling to determine whether any additional safety measures are necessary. Minimum protection of gloves and safety glasses should be worn to prevent sample contact with the skin and eyes. A respirator should be worn even when working outdoors if organic vapors are present. More hazardous sampling missions may require the use of supplied air and special clothing.

4.1.6 Cleaning of Reusable Glassware

In order to successfully analyze samples containing components in the parts per billion or lower concentration range, the preparation of scrupulously clean glassware is necessary. Failure to do so can lead to a myriad of problems interpreting data due to the presence of interferences resulting from contamination. Particular care must be taken with glassware such as Soxhlet extractors, Kuderna-Danish evaporative concentrators, sampling-train components, or any other glassware that comes into contact with an extract, particularly if the extract will be evaporated to a smaller volume. The process of concentrating the compounds of interest in this operation may similarly concentrate the contaminating substance(s), which may distort the results and complicate data interpretation.

The basic cleaning steps are:

1. Removal of surface residuals immediately after use

2. Hot soak to loosen and float most particulate material

3. Hot water rinse to flush away floated particulates

4. Soak with an oxidizing agent to destroy traces of organic compounds

5. Hot water rinse to flush away materials loosened by the deep penetrant soak

6. Distilled water rinse to remove metallic deposits from the tap water
7. Alcohol (e.g., isopropanol or methanol) rinse to flush off any final traces of organic materials and remove the water

8. Flushing the item immediately before use with some of the same solvent that will be used in the analysis

Comments regarding each of the eight fundamental steps are discussed here in the order in which they appeared above:

Step 1: As soon as analysis is complete, the glassware (e.g., beakers, pipettes, flasks, or bottles) that came into contact with samples or standards should be flushed with water and then alcohol or other appropriate solvent before it is placed in the hot detergent soak. Otherwise, the soak bath may serve to contaminate all other glassware placed therein.

Step 2: The hot soak consists of a bath of a suitable detergent in water at 50 °C or higher. The detergent, powder or liquid, should be entirely synthetic and not a fatty acid base. There are very few areas of the country where the water hardness is sufficiently low to avoid formation of some hard-water scum resulting from the reaction between calcium and magnesium salts with a fatty acid soap. This hard-water scum or curd would have an affinity particularly for many chlorinated compounds and, being almost wholly water-insoluble, would deposit on all glassware in the bath in a thin film.

There are many suitable detergents on the wholesale and retail market. Most of the common liquid dishwashing detergents sold at retail are satisfactory but are more expensive than other comparable products sold industrially. Alconox, in powder or tablet form, is manufactured by Alconox, Inc., New York, and is marketed by a number of laboratory supply firms. Sparkleen, another powdered product, is distributed by Fisher Scientific Company.

Step 3: No comments

Step 4: **Chromic acid should not be used to clean glassware.** Commercial, non-chromate products (e.g., Nochromix) may be used in place of chromic acid, if adequate cleaning is documented by an analytical quality assurance (QA) program. Chromic acid should also not be used with plastic bottles.

The potential hazards of using chromic-sulfuric acid mixture are great and have been well publicized. There are now commercially available substitutes that possess the advantage of safety in handling. These are biodegradable concentrates with a claimed cleaning strength equal to the chromic acid solution. They are alkaline, equivalent to roughly 0.1 N NaOH upon dilution, and are claimed to remove dried blood, silicone greases, distillation residues, insoluble organic residues, etc. They are further claimed to remove radioactive traces and will not attack glass or exert a corrosive effect on skin or clothing. One such product is "Chem Solv 2157," manufactured by Mallinckrodt and available through laboratory supply firms. Another comparable product is "Detex," a product of Borer-Chemie, Solothurn, Switzerland. Other similarly effective products are Nochromix (Godax Laboratories) and Contrad 70 (Decon Labs).

Steps 5, 6, and 7: No comments

Step 8: There is always a possibility that between the time of washing and the next use, the glassware could pick up some contamination from either the air or direct contact. To prevent this, it is good practice to flush the item immediately before use with some of the same solvent that will be used in the analysis.

The drying and storage of the cleaned glassware is of critical importance to realize the benefit of scrupulous cleaning. Pegboard drying is not recommended. It is recommended that laboratory glassware and equipment be dried at 100 °C. Under no circumstances should such small items be left in the open without protective covering. Otherwise, dust and soot in a laboratory environment can re-contaminate the clean glassware.

As an alternative to solvent rinsing, glassware may be heated to a minimum of 300 °C for sufficient time to vaporize any residual organic chemicals. Glassware should be allowed to cool fully before use. This high temperature treatment should not be used on volumetric glassware, glassware with ground glass joints, or sintered glassware.

4.1.7 High concentration samples

Cross contamination of trace concentration samples may occur when prepared in the same laboratory with high concentration samples. Ideally, if both type samples are being handled, a laboratory and glassware dedicated solely to the preparation of high concentration samples would be available for this purpose. If this is not feasible, at a minimum, disposable glassware or glassware dedicated solely to the preparation of high concentration samples should be used. Avoid cleaning glassware used for both trace and high concentration samples in the same area.

TABLE 4-1

RECOMMENDED SAMPLE CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES[a]

(Note: Footnotes are located on the last page of the table.)

VOLATILE ORGANICS

Sample Matrix	Container[1]	Preservative[2]	Holding Time[3]
Concentrated waste samples	Method 5035: See the method. Method 5021: See the method. Methods 5031 and 5032: See the methods. Use PTFE-lined lids for all procedures.	Cool to 0 - 6 °C.	14 days
Aqueous samples with no residual chlorine present	Methods 5021, 5030, 5031, and 5032: 3 x 40-mL vials with PTFE-lined septum caps	Cool to 0 - 6°C and adjust pH to less than 2 with H_2SO_4, HCl, or solid $NaHSO_4$	14 days
		If carbonaceous materials are present, or if MTBE and other fuel oxygenate ethers are present and a high temperature sample preparative method is to be used, do not acid preserve the samples.	7 days
		If compounds that readily degrade in acidified water (e.g., 2-chloroethyl vinyl ether[b]) are analytes of interest, collect a second set of samples without acid preservatives and analyze as soon as possible.	7 days

TABLE 4-1 (continued)
RECOMMENDED SAMPLE CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES[a]

VOLATILE ORGANICS (continued)			
Sample Matrix	Container[1]	Preservative[2]	Holding Time[3]
Aqueous samples WITH residual chlorine present	Methods 5021, 5030, 5031, and 5032: 3 x 40-mL vials with PTFE-lined septum caps	Collect sample in a 125-mL container which has been pre-preserved with 4 drops of 10% sodium thiosulfate solution. Gently swirl to mix sample and transfer to a 40-mL VOA vial. Cool to 0 - 6 °C and adjust pH to less than 2 with H_2SO_4, HCl, or solid $NaHSO_4$.	14 days
		If carbonaceous materials are present, or if MTBE and other fuel oxygenate ethers are present and a high temperature sample preparative method is to be used, do not acid preserve the samples.	7 days
		If compounds that readily degrade in acidified water (e.g., 2-chloroethyl vinyl ether[b]) are analytes of interest, collect a second set of samples without acid preservatives and analyze as soon as possible.	7 days
Acrolein and Acrylonitrile Aqueous samples	Methods 5021, 5030, 5031, and 5032: 3 x 40-ml vials with PTFE-lined septum caps	Adjust to pH 4 - 5. Cool to 0 - 6 °C. These compounds are highly reactive and should be analyzed as soon as possible.	7 days
Solid samples (e.g., soils, sediments, sludges, ash)	Method 5035: See the method. Method 5021: See the method. Methods 5031 and 5032: See the methods.	See the individual methods.	14 days
		If compounds that may be reactive in acidified soils (e.g., vinyl chloride, styrene, 2-chloroethyl vinyl ether) are analytes of interest, collect a second set of samples without acid preservatives and analyze as soon as possible.	7 days

TABLE 4-1 (continued)
RECOMMENDED SAMPLE CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES[a]

SEMIVOLATILE ORGANICS/ORGANOCHLORINE PESTICIDES AND HERBICIDES

Sample Matrix	Container[1]	Preservative[2]	Holding Time[3]
Concentrated waste samples	125-mL wide-mouth glass with PTFE-lined lid	Cool to 0 - 6 °C.	Samples extracted within 14 days and extracts analyzed within 40 days following extraction.
Aqueous samples with no residual chlorine present	4 x 1-L amber glass container with PTFE-lined lid, or other size, as appropriate, to allow use of entire sample for analysis.	Cool to 0 - 6 °C.	Samples extracted within 7 days and extracts analyzed within 40 days following extraction.
Aqueous samples WITH residual chlorine present	4 x 1-L amber glass container with PTFE-lined lid, or other size, as appropriate, to allow use of entire sample for analysis.	Add 3 mL 10% sodium thiosulfate solution per gallon (or 0.008%). Addition of sodium thiosulfate solution to sample container may be performed in the laboratory prior to field use. Cool to 0 - 6 °C.	Samples extracted within 7 days and extracts analyzed within 40 days following extraction.
Solid samples (e.g., soils, sediments, sludges, ash)	250-mL wide-mouth glass container with PTFE-lined lid	Cool to 0 - 6 °C.	Samples extracted within 14 days and extracts analyzed within 40 days following extraction.

TABLE 4-1 (continued)
RECOMMENDED SAMPLE CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES[a]

POLYCHLORINATED BIPHENYLS, POLYCHLORINATED DIBENZO-p-DIOXINS, AND POLYCHLORINATED DIBENZOFURANS

Sample Matrix	Container[1]	Preservative[2]	Holding Time[3]
Concentrated waste samples	125-mL wide-mouth glass with PTFE-lined lid	None	None
Aqueous samples with no residual chlorine present	4 x 1-L amber glass container with PTFE-lined lid, or other size, as appropriate, to allow use of entire sample for analysis.	Cool to 0 - 6 °C.	None
Aqueous samples WITH residual chlorine present	4 x 1-L amber glass container with PTFE-lined lid, or other size, as appropriate, to allow use of entire sample for analysis.	Add 3 mL 10% sodium thiosulfate solution per gallon (or 0.008%). Addition of sodium thiosulfate solution to sample container may be performed in the laboratory prior to field use. Cool to 0 - 6 °C	None
Solid samples (e.g., soils, sediments, sludges, ash)	250-mL wide-mouth glass container with PTFE-lined lid.	Cool to 0 - 6 °C.	None

[a] The information presented in this table does not represent EPA requirements, but rather it is intended solely as guidance. Selection of containers, preservation techniques and applicable holding times should be based on the stated project-specific DQOs.
[b] See References 1-10 for the preservation and holding times studies for volatile organics. It is the intention of the Agency that separate unpreserved vials be collected when 2-chloroethylvinyl ether is an analyte of interest.
[1] PTFE lined caps are acceptable for all recommended container types. Additional replicate sample containers should also be collected to perform all necessary laboratory QC (e.g., duplicate, matrix spike / matrix spike duplicate QC samples).
[2] The exact sample, extract, and standard storage temperature should be based on project-specific requirements and/or manufacturer's recommendations for commercially available standards. Furthermore, alternative storage temperatures may be appropriate based on demonstrated analyte stability in a given matrix, provided the stated DQOs for a project-specific application are still attainable.
[3] A longer holding time may be appropriate if it can be demonstrated that the reported analyte concentrations are not adversely affected from preservation, storage and analyses performed outside the recommended holding times.

4.2 SAMPLE PREPARATION METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

4.2.1 Extractions and preparations

The following methods are included in this section:

Method 3500C:	Organic Extraction and Sample Preparation
Method 3510C:	Separatory Funnel Liquid-Liquid Extraction
Method 3511:	Organic Compounds in Water by Microextraction
Method 3520C:	Continuous Liquid-Liquid Extraction
Method 3535A:	Solid-Phase Extraction (SPE)
Method 3540C:	Soxhlet Extraction
Method 3541:	Automated Soxhlet Extraction
Method 3542:	Extraction of Semivolatile Analytes Collected Using Method 0010 (Modified Method 5 Sampling Train)
Method 3545A:	Pressurized Fluid Extraction (PFE)
Method 3546:	Microwave Extraction
Method 3550C:	Ultrasonic Extraction
Method 3560:	Supercritical Fluid Extraction of Total Recoverable Petroleum Hydrocarbons
Method 3561:	Supercritical Fluid Extraction of Polynuclear Aromatic Hydrocarbons
Method 3562:	Supercritical Fluid Extraction of Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides
Method 3570:	Microscale Solvent Extraction (MSE)
Method 3571:	Extraction of Solid and Aqueous Samples for Chemical Agents
Method 3572:	Extraction of Wipe Samples for Chemical Agents
Method 3580A:	Waste Dilution
Method 3585:	Waste Dilution for Volatile Organics
Method 5000:	Sample Preparation for Volatile Organic Compounds
Method 5021A:	Volatile Organic Compounds in Soils and Other Solid Matrices Using Equilibrium Headspace Analysis
Method 5030B:	Purge-and-Trap for Aqueous Samples
Method 5031:	Volatile, Non-purgeable, Water-Soluble Compounds by Azeotropic Distillation
Method 5032:	Volatile Organic Compounds by Vacuum Distillation
Method 5035:	Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples
Method 5041A:	Analysis for Desorption of Sorbent Cartridges from Volatile Organic Sampling Train (VOST)

4.2.2 Cleanup

The following methods are included in this section:

Method 3600C: Cleanup
Method 3610B: Alumina Cleanup
Method 3611B: Alumina Column Cleanup and Separation of Petroleum Wastes
Method 3620C: Florisil Cleanup
Method 3630C: Silica Gel Cleanup
Method 3640A: Gel-Permeation Cleanup
Method 3650B: Acid-Base Partition Cleanup
Method 3660B: Sulfur Cleanup
Method 3665A: Sulfuric Acid/Permanganate Cleanup

4.3 DETERMINATION OF ORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

4.3.1 Gas chromatographic methods

The following methods are included in this section:

Method 8000D: Determinative Chromatographic Separations
Method 8011: 1,2-Dibromoethane and 1,2-Dibromo-3-chloropropane by Microextraction and Gas Chromatography
Method 8015C: Non-halogenated Organics by Gas Chromatography
Method 8021B: Aromatic and Halogenated Volatiles by Gas Chromatography Using Photoionization and/or Electrolytic Conductivity Detectors
Method 8031: Acrylonitrile by Gas Chromatography
Method 8032A: Acrylamide by Gas Chromatography
Method 8033: Acetonitrile by Gas Chromatography with Nitrogen-Phosphorus Detection
Method 8041A: Phenols by Gas Chromatography
Method 8061A: Phthalate Esters by Gas Chromatography with Electron Capture Detection (GC/ECD)
Method 8070A: Nitrosamines by Gas Chromatography
Method 8081B: Organochlorine Pesticides by Gas Chromatography
Method 8082A: Polychlorinated Biphenyls (PCBs) by Gas Chromatography

Method 8085:	Compound-independent Elemental Quantitation of Pesticides by Gas Chromatography with Atomic Emission Detection (GC/AED)
Method 8091:	Nitroaromatics and Cyclic Ketones by Gas Chromatography
Method 8095:	Explosives by Gas Chromatography
Method 8100:	Polynuclear Aromatic Hydrocarbons
Method 8111:	Haloethers by Gas Chromatography
Method 8121:	Chlorinated Hydrocarbons by Gas Chromatography: Capillary Column Technique
Method 8131:	Aniline and Selected Derivatives by Gas Chromatography
Method 8141B:	Organophosphorus Compounds by Gas Chromatography
Method 8151A:	Chlorinated Herbicides by GC Using Methylation or Pentafluorobenzylation Derivatization

4.3.2 Gas chromatographic/mass spectrometric methods

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

The following methods are included in this section:

Method 8260B:	Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)
Method 8261:	Volatile Organic Compounds by Vacuum Distillation in Combination with Gas Chromatography/Mass Spectrometry (VD/GC/MS)
Method 8270D:	Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)
Method 8275A:	Semivolatile Organic Compounds (PAHs and PCBs) in Soils/Sludges and Solid Wastes Using Thermal Extraction/Gas Chromatography/Mass Spectrometry (TE/GC/MS)
Method 8276:	Toxaphene and Toxaphene Congeners by Gas Chromatography/Negative Ion Chemical Ionization Mass Spectrometery (GC-NICI/MS)
Method 8280B:	Polychlorinated Dibenzo-*p*-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/Low Resolution Mass Spectrometry (HRGC/LRMS)
Method 8290A:	Polychlorinated Dibenzo-*p*-dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS)
	Appendix A: Procedures for the Collection, Handling,

Analysis and Reporting of Wipe Tests Performed within the Laboratory

4.3.3 High performance liquid chromatographic methods

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

The following methods are included in this section:

Method 8310: Polynuclear Aromatic Hydrocarbons
Method 8315A: Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)
Appendix A: Re-crystallization of 2,4-Dinitrophenylhydrazine (DNPH)
Method 8316: Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)
Method 8318A: *N*-Methylcarbamates by High Performance Liquid Chromatography (HPLC)
Method 8321B: Solvent-Extractable Nonvolatile Compounds by High-Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection
Method 8325: Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry (HPLC/PB/MS)
Method 8330A: Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)
Method 8331: Tetrazene by Reverse Phase High Performance Liquid Chromatography (HPLC)
Method 8332: Nitroglycerine by High Performance Liquid Chromatography

4.3.4 Infrared methods

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

The following methods are included in this section:

Method 8410: Gas Chromatography/Fourier Transform Infrared (GC/FT-IR) Spectrometry for Semivolatile Organics: Capillary Column
Method 8430: Analysis of Bis(2-chloroethyl) Ether and Hydrolysis Products by Direct Aqueous Injection GC/FT-IR
Method 8440: Total Recoverable Petroleum Hydrocarbons by Infrared Spectrophotometry

4.3.5 Miscellaneous spectrometric methods

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

The following method is included in this section:

Method 8520: Continuous Measurement of Formaldehyde in Ambient Air

4.4 IMMUNOASSAY METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

The following methods are included in this section:

Method 4000: Immunoassay
Method 4010A: Screening for Pentachlorophenol by Immunoassay
Method 4015: Screening for 2,4-Dichlorophenoxyacetic Acid by Immunoassay
Method 4020: Screening for Polychlorinated Biphenyls by Immunoassay
Method 4025: Screening for Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans (PCDD/Fs) by Immunoassay
Method 4030: Soil Screening for Petroleum Hydrocarbons by Immunoassay
Method 4035: Soil Screening for Polynuclear Aromatic Hydrocarbons by Immunoassay
Method 4040: Soil Screening for Toxaphene by Immunoassay
Method 4041: Soil Screening for Chlordane by Immunoassay
Method 4042: Soil Screening for DDT by Immunoassay
Method 4050: TNT Explosives in Soil by Immunoassay
Method 4051: Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Soil by

Immunoassay

Method 4425: Screening Extracts of Environmental Samples for Planar Organic Compounds (PAHs, PCBs, PCDDs/PCDFs) by a Reporter Gene on a Human Cell Line

Method 4430: Screening For Polychlorinated Dibenzo-p-Dioxins And Furans (PCDD/Fs) By Aryl Hydrocarbon-Receptor PCR Assay

Method 4435: Method For Toxic Equivalents (TEQS) Determinations For Dioxin-Like Chemical Activity with the CALUX® Bioassay

Method 4670: Triazine Herbicides as Atrazine in Water by Quantitative Immunoassay

4.5 MISCELLANEOUS SCREENING METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the DQOs or needs for the intended use of the data.

The following methods are included in this section:

Method 3815: Screening Solid Samples for Volatile Organics

Method 3820: Hexadecane Extraction and Screening of Purgeable Organics

Method 8510: Colorimetric Screening Procedure for RDX and HMX in Soil

Method 8515: Colorimetric Screening Method for Trinitrotoluene (TNT) in Soil

Method 8535: Screening Procedure for Total Volatile Organic Halides in Water

Method 8540: Pentachlorophenol by UV-Induced Colorimetry

Method 9074: Turbidimetric Screening Method for Total Recoverable Petroleum Hydrocarbons in Soil

Method 9078: Screening Test Method for Polychlorinated Biphenyls in Soil

Method 9079: Screening Test Method for Polychlorinated Biphenyls in Transformer Oil

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