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Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Pesticides in Water by Graphitized Carbon-Based Solid-Phase Extraction and High-Performance Liquid Chromatography/Mass Spectrometry

Water-Resources Investigations Report 01–4134

U.S. Department of the Interior
U.S. Geological Survey

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CONVERSION FACTORS AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
centimeter (cm)	3.94×10^{-1}	inch
gram (g)	3.53×10^{-2}	ounce, avoirdupois
kilopascal (kPa)	1.45×10^{-1}	pounds per square inch
liter (L)	3.38×10^{-1}	ounce
meter (m)	3.28×10^{0}	foot
microgram (µg)	3.53×10^{-8}	ounce, avoirdupois
microliter (µL)	3.38×10^{-5}	ounce
micrometer (µm)	3.94×10^{-5}	inch
milligram (mg)	3.53×10^{-5}	ounce, avoirdupois
milliliter (mL)	3.38×10^{-2}	ounce
millimeter (mm)	3.94×10^{-2}	inch
nanogram (ng)	3.53×10^{-11}	ounce, avoirdupois

Degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) using the following equation:

$$°F = 9/5\ (°C) + 32.$$

ABBREVIATED WATER-QUALITY UNITS

g/L	gram per liter
g/mL	gram per milliliter
kg/m^2	kilogram per square meter
µg/L	microgram per liter
µg/µL	microgram per microliter
µg/mL	microgram per milliliter
µL/L	microliter per liter
µL/mL	microliter per milliliter
mg/L	milligram per liter
mg/mL	milligram per milliliter
mL/L	milliliter per liter
mL/min	milliliter per minute
ng/L	nanogram per liter
ng/µg	nanogram per microgram
ng/µL	nanogram per microliter
ng/mL	nanogram per milliliter

ABBREVIATIONS AND ACRONYMS

amu	atomic mass unit
BDMC	4-bromo-3,5-dimethyl phenyl-n-methylcarbamate
cat. no.	catalog number
CCB	continuing calibration blank
CCV	continuing calibration verification
DAD	photodiode-array detection
“E”	second elution fraction
FEB	field equipment blank
FMS	field matrix spike
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
HPLC	high-performance liquid chromatography
HPLC/MS	high-performance liquid chromatography/mass spectrometry
kV	kilovolt
LOQ	limit of quantitation
LRB	laboratory reagent blank
LRL	laboratory reporting level
LRS	laboratory reagent spike
“M”	first (methanol) elution fraction
MDL	method detection limit
MHz	megahertz
MS	mass spectrometry
m/z	mass-to-charge ratio
NAWQA	National Water-Quality Assessment Program
NWIS	National Water Information System
NWQL	National Water Quality Laboratory
ODS	octadecylsilane
POC	polar organic compound
QA/QC	quality assurance/quality control
QC	quality control
RF	response factor
SPE	solid-phase extraction
TFA	trifluoroacetic acid
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
≡	identical with, congruent
≅	equals approximately
±	plus or minus

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By Edward T. Furlong, Bruce D. Anderson, Stephen L. Werner, Paul P. Soliven, Laura J. Coffey, and Mark R. Burkhardt

Abstract

In 1996, the U.S. Geological Survey National Water Quality Laboratory (NWQL) developed and implemented a graphitized carbon-based solid-phase extraction and high-performance liquid chromatographic analytical method to determine polar pesticide concentrations in surface- and ground-water samples. Subsequently, the NWQL developed a complementary analysis that uses high-performance liquid chromatography/ mass spectrometry to detect, identify, and quantify polar pesticides and pesticide metabolites in filtered water at concentrations as low as 10 nanograms per liter. This new method was designed to improve sensitivity and selectivity over the prior method, and to reduce known interferences from natural organic matter.

In this new method, pesticides are extracted from filtered water samples by using a 0.5-gram graphitized carbon-based solid-phase extraction cartridge, eluted from the cartridge, and concentrations determined by using high-performance liquid chromatography with electrospray ionization–mass spectrometry. The upper concentration limit is 1.000 microgram per liter (µg/L) for most compounds. Single-operator method detection limits in organic-free water samples fortified with pesticides at a concentration of 0.025 µg/L ranged from 0.0019 to 0.022 µg/L for all compounds in the method. The grand mean (mean of mean recoveries for individual compounds) recoveries in organic-free water samples ranged from 72 to 89 percent, fortified with pesticides at three concentrations between 0.025 and 0.5 µg/L. Grand mean recoveries in ground- and surface-water samples ranged from 46 to 119 percent, also fortified with pesticides at three concentrations between 0.025 and 0.5 µg/L. Long-term recoveries from reagent water spikes were used to demonstrate that 38 of 65 compounds can be reported without qualification of the quantitative result across the analytical range of the method. The remaining 27 are reported with qualified estimates of concentration because of greater variability of recovery.

INTRODUCTION

In 1996, the National Water Quality Laboratory (NWQL) developed and implemented a graphitized carbon-based solid-phase extraction and high-performance liquid chromatographic analytical method to determine pesticide concentrations in surface- and ground-water samples (Werner and others, 1996). The National Water-Quality Assessment Program (NAWQA) uses this method for determining concentrations of 41 polar pesticides and pesticide metabolites. NAWQA used the methods of Werner and others (1996) and Zaugg and others (1995) to develop a detailed and extensive data set for nationwide assessment of pesticide presence, concentrations, and distribution (Larson and others, 1999).

The method by Werner and others (1996) initially was developed because several pesticide classes that might be found in ground-water and surface-water samples are not readily amenable to analysis by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS). Analysis by GC/MS is a long-established method for unequivocal identification and quantitation of organic compounds, but these compound classes could not be analyzed without derivitization or other sample-modifying steps. Examples of these pesticide classes include phenylurea herbicides, chlorophenoxyacetic acid herbicides, methylcarbamate insecticides, sulfonylurea, sulfonamide, and imidazolinone herbicides, and uracil-derivative pesticides. Because these pesticides are used on a national scale in large quantities, a broad-spectrum, sensitive analytical method for monitoring selected pesticides in these classes was needed. The method of Werner and others (1996) initially fulfilled this requirement, but in routine use, shortcomings were identified that were inherent to the available analytical technologies used in the method. Several important polar pesticides could not be adequately resolved and identified by high-performance liquid chromatography (HPLC) with photodiode-array detection. In addition, detection, identification, and quantitation of polar pesticides in the presence of co-isolated and ubiquitous dissolved organic matter required a more specific means of detection and identification to routinely achieve the detection limits needed for ambient environmental concentrations.

Concurrent with development of the method by Werner and others (1996), improvements were made in high-performance liquid chromatography/mass spectrometry (HPLC/MS) interfaces so that sensitive and selective detection of polar pesticides and pesticide metabolites can be made routinely in extracts from 1-L water samples (Ferrer and Barcelo, 1998). These newer atmospheric pressure ionization interfaces allow unattended and nearly continuous operation of HPLC/MS systems, with detection limits (expressed as a filtered-water concentration in a nominal 1-L water sample) as low as the tens of nanograms per liter (Crescenzi and others, 1997).

The use of HPLC/MS as a routine analytical tool under production conditions was tested at the NWQL by Furlong and others (2000) for determination of sulfonylurea, imidazolinone, and sulfonamide herbicides. This study demonstrated that with sufficient laboratory and field quality control, hundreds of samples could be successfully analyzed by HPLC/MS, at concentrations in the nanogram-per-liter range. The problems of analyte coelution and interference from dissolved organic carbon are substantially reduced by combining the instrumental approach of Furlong and others (2000) with the solid-phase extraction procedure by Werner and others (1996). As noted by Werner and others (1996), the advantages of HPLC coupled with solid-phase extraction over other methods include use of less solvent, rapid extraction, field-extraction capabilities, lower solvent exposure to technicians, and the ability to automate the extraction procedure and determine thermally sensitive compounds. Although the addition of mass spectrometry increases the analytical cost, this cost is outweighed by improvements in specificity and detectability. Collectively, slightly increased analytical costs are outweighed by improvements in data quality.

The method described herein was developed by the U.S. Geological Survey (USGS) for use at the NWQL. It uses graphitized carbon-based solid-phase extraction coupled with HPLC/MS. The method complements the method by Werner and others (1996) by increasing the number of compounds identified and quantified from 39 to 65. It provides similar method detection limits and enhanced detection of low

concentrations of compounds in sample matrices with high dissolved organic carbon concentrations and other matrix interferences. It also supplements other methods of the USGS for determination of organic substances in water that are described by Wershaw and others (1987) and by Fishman (1993).

This report provides a detailed description of all aspects of the method, including equipment, reagents, sample extraction and elution techniques, sampling protocol, tabulated quality-control data, calculations, and reporting of results. Bias and variability data and method detection limits for 65 pesticides are presented.

The scope of the report includes determination of method performance in organic-free water samples and in two natural-water types—a ground-water sample and a surface-water sample from the Denver, Colorado, region. Method performance was determined at three pesticide concentrations—0.025, 0.10, and 0.50 µg/L—in each water type, with the exception of surface water, where the lowest concentration was 0.05 µg/L. Method detection limits (MDLs) were determined in organic-free water at a concentration of 0.025 µg/L by using the method outlined by the U.S. Environmental Protection Agency (1997).

The method described in this report was approved in June 1999 as a custom analytical method and implemented for routine sample analysis as provisional laboratory analytical schedule LC9060. It was intended primarily for the analysis of samples associated with the NAWQA program. The method has remained in use until the present (2001) with only minor modifications.

ANALYTICAL METHOD

Organic Compounds and Parameter Codes: Pesticides, Dissolved, Graphitized Carbon-Based Extraction, High-Performance Liquid Chromatography/Mass Spectrometry, O-2060-01

1. Application

This method is suitable for use with filtered water samples for the determination of the pesticides and related degradation products that are specified in table 1. Many parent compounds, such as 2,4-D, were included because of their relative importance in agriculture in the United States, as indicated by the amount applied nationally (Gianessi and Anderson, 1996). Other compounds, such as aldicarb, have or can have substantial impact on human or ecosystem health (Gustafson, 1993). Some compounds, such as caffeine, are ubiquitously detected and might indicate human wastewater contamination (Gulyas, 1997). Several compounds, such as 2-hydroxyatrazine, are important degradation products of commonly used pesticides (Kolpin and others, 1998). The method is applicable for determining pesticides and pesticide metabolites that are (1) efficiently isolated from the sample matrix and adsorbed onto a graphitized carbon black sorbent-filled cartridge, and (2) chromatographically resolved and identified using an HPLC coupled by an electrospray ionization source to a quadrupole mass spectrometer.

The method has been tested and validated for filtered surface water and ground water. Although not specifically tested for other aqueous matrices, it could be applied to precipitation, wastewater, tile drain discharge, and other sample types as long as

Table 1. Compound names, uses, pesticide class, Chemical Abstract Service registry numbers, and codes

[conc., concentration; Y/N, yes/no; CAS, Chemical Abstract Service; NWIS, National Water Information System; Use codes in alphabetical order; F, Fungicide; H, Herbicide; H-Degr., Herbicide degradant; I, Insecticide; I-Degr., Insecticide degradant. Class codes in alphabetical order: AA, Acylalanine; ACA, Acetamide; B., Biological agent; BI, Benzimidazole; BN, Benzonitrile; BTZ, Benzothiadiazole; BZ, Benzoic acid; C, Carbamate; CN, Chloro-Nicotinyl; CP, Chlorophenoxy Acid; DNA, Dinitroaniline; DNP, Dinitrophenol; DPE, Diphenyl ether; IMI, Imidazolinone; PH, Phthalimide; PU, Phenylurea; PY, Pyridine; PYA, Pyridyloxyacetic acid; PYD, Pyridazine; SA, Sulfonamide; SU, Sulfonylurea; T, Triazine; TC, Thiocarbamate; TDZ, Thiadiazole; TR, Triazole; U, Uracil; n/a, not applicable]

Compound name	Alternative or common name	Reported as an estimated conc.[1] (Y/N)	Use	Class	CAS registry number	NWIS parameter code	Ionization mode for identification and quantitation
2,4,5-T (Negative Ion Surrogate)	Trichlorophenoxyacetic acid	N	H	CP	93-76-5	99958	Negative Ion
2,4-D	Weedone-2,4-DP	N	H	CP	94-75-7	39732	Negative Ion
2,4-D methyl ester	2,4-Dichlorophenoxyacetic methyl ester	N	H	CP	1928-38-7	50470	Positive Ion
2,4-DB	4-(2,4-dichlorophenoxy) butanoic acid	Y	H	CP	94-82-6	38746	Negative Ion
2,4-Dichlorophenylacetic acid (DCAA; negative ion internal standard)		n/a			19719-28-9		Negative Ion
2-Hydroxyatrazine	4-(ethylamino)-6-[(1-methylethyl)amino]-1,3,5-Triazin-2(1H)-one	Y	H-Degr.	T	2163-68-0	50355	Positive Ion
3(4-chlorophenyl)-1-methyl urea	N'-(4-chlorophenyl)-N-methylurea	N	H-Degr.	PU	5352-88-5	61692	Positive Ion
3-Hydroxycarbofuran	2,3-dihydro-2,2-dimethyl-3,7-Benzofurandiol, 7-(methylcarbamate)	N	I-Degr.	C	16655-82-6	49308	Positive Ion
3-Ketocarbofuran	3-oxocarbofuran	Y	I-Degr.	C	16709-30-1	50295	Positive Ion
Acifluorfen	Tackle; Blazer	N	H	DPE	50594-66-6	49315	Negative Ion
Aldicarb	Temik	Y	I	C	116-06-3	49312	Positive Ion
Aldicarb sulfone	Aldoxycarb	Y	I-Degr.	C	1646-88-4	49313	Positive Ion
Aldicarb sulfoxide	Temik Sulfoxide	Y	I-Degr.	C	1646-87-3	49314	Positive Ion
Atrazine	Atrex; Atratol	N	H	T	1912-24-9	39632	Positive Ion

Table 1. Compound names, uses, pesticide class, Chemical Abstract Service registry numbers, and codes—Continued

Compound name	Alternative or common name	Reported as an estimated conc.[1] (Y/N)	Use	Class	CAS registry number	NWIS parameter code	Ionization mode for identification and quantitation
Barban (positive ion surrogate)	Carbyne; Neoban; Caryne	N	H	C	101-27-9	90640	Positive Ion
Bendiocarb	Ficam	N	I	C	22781-23-3	50299	Positive Ion
Benomyl	Benlate	N	F	BI	17804-35-2	50300	Positive Ion
Bensulfuron-methyl	Londax	N	H	SU	83055-99-6	61693	Positive Ion
Bentazon	Adagio; Galaxy; Storm	Y	H	BTZ	25057-89-0	38711	Negative Ion
Bromacil	Bromax; Hyvar; Uragon	Y	H	U	314-40-9	04029	Positive Ion
Bromoxynil	Bromanil; Buctril; Torch	Y	H	BN	1689-84-5	49311	Negative Ion
Caffeine	1,3,7-Trimethylxanthine	N			58-08-2	50305	Positive Ion
Caffeine-C13 (positive ion surrogate)		N				99959	Positive Ion
Carbaryl	Sevin	N	H	C	63-25-2	49310	Positive Ion
Carbofuran	Crisfuran; Furadan	N	H	C	1563-66-2	49309	Positive Ion
Chloramben methyl ester	Amiben, methyl ester	Y	H	CP	7286-84-2	61188	Positive Ion
Chlorimuron-ethyl	Classic	N	H	SU	90982-32-4	50306	Positive Ion
Chlorothalonil	Bravo; Forturf	Y	H	PH	1897-45-6	49306	Negative Ion
Clopyralid	Stinger; Lontrel	N	H	PY	1702-17-6	49305	Negative Ion
Cycloate	Ro-Neet; Marathon	Y	H	TC	1134-23-2	04031	Positive Ion
Dacthal monoacid	DCPA Monoacid; Monomethyl tetrachloroterephtalate	N	H-Degr.	CP	887-54-7	49304	Negative Ion
Deethylatrazine	2-Amino-4-isopropylamino-6-chloro-s-triazine	Y	H-Degr.	T	6190-65-4	04040	Positive Ion
Deethyldeisopropylatrazine	6-chloro-1,3,5-triazine-2,4-diamine	Y	H-Degr.	T	3397-62-4	04039	Positive Ion
Deisopropylatrazine	Amino-2-chloro-6-ethylamino-s-triazine	Y	H-Degr.	T	1007-28-9	04038	Positive Ion
Dicamba	Banval; Marksman; Clarity	N	H	BZ	1918-00-9	38442	Negative Ion
Dichlorprop	2,4-DP,Weedone DP	N	H	CP	120-36-5	49302	Negative Ion
Dinoseb	DNPB; Caldon: Dynamite	N	H	DNP	88-85-7	49301	Negative Ion

Table 1. Compound names, uses, pesticide class, Chemical Abstract Service registry numbers, and codes—Continued

Compound name	Alternative or common name	Reported as an estimated conc.[1] (Y/N)	Use	Class	CAS registry number	NWIS parameter code	Ionization mode for identification and quantitation
Diphenamid	Dymid; Enide; Rideon; Dyfen	N	H	ACA	957-51-7	04033	Positive Ion
Diuron	DCMU; Diurex; Aguron	N	H	PU	330-54-1	49300	Positive Ion
Fenuron	Beet-Klean	N	H	PU	101-42-8	49297	Positive Ion
Flumetsulam	DE 498; XRD 498	Y	H	SA	98967-40-9	61694	Positive Ion
Fluometuron	Cortoran; Lanex; Cottonex	N	H	PU	2164-17-2	38811	Positive Ion
Imazaquin	Image 1.5LC; Scepter 1.5L	Y	H	IMI	81335-37-7	50356	Positive Ion
Imazethapyr	Pursuit; Pursuit DG	Y	H	IMI	81335-77-5	50407	Positive Ion
Imidacloprid	Admire; Gaucho; Merit	N	I	CN	138261-41-3	61695	Positive Ion
Linuron	Lorox; Afalon; Linurex; Linex	N	H	PU	330-55-2	38478	Positive Ion
MCPA	Bordermaster; Metaxon	N	H	CP	94-74-6	38482	Negative Ion
MCPB	Tropotox; Can-Trol; PDQ	Y	H	CP	94-81-5	38487	Negative Ion
Metalaxyl	Apron; Subdue; Ridomil	N	F	AA	57837-19-1	50359	Positive Ion
Methiocarb	Mercaptodimethur; Draza; Mesurol	Y	I	C	2032-65-7	38501	Positive Ion
Methomyl	Nudrin; Lannate; Lanox	Y	I	C	16752-77-5	49296	Positive Ion
Methomyl oxime	1-(methylthio)-acetaldehyde oxime	Y	I-Degr.	C	13749-94-5	61696	Positive Ion
Metsulfuron-methyl	Escort;Gropper; Ally	Y	H	SU	74223-64-6	61697	Positive Ion
Monuron (positive ion internal standard)	Telvar	n/a	H	PU	150-68-5		Positive Ion
Neburon	Granurex; Herbalt; Kloben	N	H	PU	555-37-3	49294	Positive Ion
Nicosulfuron	Accent; Accent DF	N	H	SU	111991-09-4	50364	Positive Ion
Norflurazon	Zorial; Evital; Solicam; Telok	Y	H	PYD	27314-13-2	49293	Positive Ion
Oryzalin	Ryzelan; Surflan; Dirimal	N	H	DNA	19044-88-3	49292	Positive Ion
Oxamyl	Vydate: Thioxamyl	N	I	C	23135-22-0	38866	Positive Ion

Table 1. Compound names, uses, pesticide class, Chemical Abstract Service registry numbers, and codes—Continued

Compound name	Alternative or common name	Reported as an estimated conc.[1] (Y/N)	Use	Class	CAS registry number	NWIS parameter code	Ionization mode for identification and quantitation
Oxamyl oxime	2-(Hydroxyimino)-N,N-dimethyl-2-(methylmercapto)acetamide	Y	I-Degr.	C	30558-43-1	50410	Positive Ion
Picloram	Tordon; Amdon; Grazon	N	H	PY	1918-02-1	49291	Negative Ion
Propham	IPC; Chem-Hoe; Premalox	N	H	C	122-42-9	49236	Positive Ion
Propiconazole	Tilt; Orbit; Banner: Proconazole; Wocosin	N	F	TR	60207-90-1	50471	Positive Ion
Propoxur	Baygon; PHC; Suncide; Unden	N	I	C	114-26-1	38538	Positive Ion
Siduron	Tupersan; Trey	N	H	PH	1982-49-6	38548	Positive Ion
Sulfometuron-methyl	Oust; DPX-T5648	N	H	SU	74222-97-2	50337	Positive Ion
Tebuthiuron	Graslan; Spike; Perflan	N	H	TDZ	34014-18-1	82670	Positive Ion
Terbacil	Sinbar; DPX-D732; Geonter	Y	H	U	5902-51-2	04032	Positive Ion
Tribenuron-methyl	Express; tribenuron methyl ester; DPX-L5300	Y	H	SU	101200-48-0	61159	Positive Ion
Triclopyr	Garlon; Curtail; Redeem; Remedy	N	H	PYA	55335-06-3	49235	Negative Ion

[1]The term "estimated concentration" is used in this electronic file. It was changed from "qualified estimate," which was used in the original published report. Tables 13–24 and tables 26–31 also reflect this change. (Modified: February 4, 2002, etf)

(1) the samples have been filtered using the method of Shelton (1994), and (2) the user recognizes that performance characteristics of new matrices are not tested and any results would be provisional.

Two classes of determinations are reported for samples analyzed by this method. Compounds for which the results of the determination are reported without qualification make up the first class. Compounds for which the results of the determination are reported as qualified estimates, and the concentration qualified with an "E", are in the second class. The classification for each compound in the method is listed in table 1.

These classes, and the criteria used to establish them, are discussed in detail in section 11.6. Compounds that are reported without qualification are reproducibly well-recovered using this method, as defined by median recoveries of long-term laboratory reagent spikes (LRS) between 60 and 120 percent and with variation (as indicated by the nonparametric statistic F-pseudosigma) less than ± 25 percent. Compounds that are reported as qualified estimates do not meet these long-term method performance criteria, but are retained in the method because they are used in substantial quantity and have important environmental or toxicological effects. Although quantitative method performance for this class lies outside the criteria described above, the criteria for qualitative identification of the detected compounds are the same as that used for compounds reported without quantitative qualifications. Greater certainty exists in the quantitative determination of concentration for compounds reported without qualification—this is the only difference between the reported concentrations of the two data classes.

It is important to note, however, that the long-term performance criteria for the compounds in the method span a continuum of performance, rather than corresponding to categories. As a consequence, the criteria used to discriminate between the two classes of data, although consistent with common analytical practice, are inherently arbitrary. The qualitative identification of the compounds in both classes is equally reliable. Thus, data users should consider the estimated concentration as a categorical warning to pay extra attention to potential use of numerical concentrations, but not as a distinct boundary between good and poor data.

2. Summary of Method

This method is designed for the determination of 65 pesticides and pesticide degradation products and caffeine (table 1) in filtered natural-water samples. The method is applicable to pesticides that are (1) efficiently partitioned from the water onto a graphitized carbon-based solid-phase extraction (SPE) material, (2) can be quantitatively eluted from the SPE material, and (3) can be efficiently ionized by HPLC/MS with electrospray interface.

Pesticides are extracted from prefiltered water samples by using disposable polypropylene syringe cartridges that contain 0.5 g of a graphitized carbon sorbent. A prefiltered water sample of 1 L is pumped through the SPE cartridge at a flow rate of 20 mL/min. After extraction, the adsorbed compounds are eluted from the SPE cartridge using two sequential elutions of

(1) 1.5 mL methanol, followed by

(2) 13 mL of an 80-percent methylene chloride and 20-percent methanol mixture that has been acidified with trifluoroacetic acid anhydride (0.2 percent).

The two fractions are reduced under nitrogen to near dryness and then combined. The final volume for the extract is 1,000 µL. Analytes are chromatographically separated by HPLC using a reverse-phase octadecylsilane HPLC column, which is coupled to an electrospray ionization interface and quadrupole mass spectrometer for detection, identification, and quantitation. Each extract is analyzed twice using separate ionization modes. The first analysis is for those compounds that preferentially form positive ions under electrospray conditions, and the second is for those compounds that preferentially form negative ions.

The terms "extraction" and "elution" are used to define specific actions during sample processing. Extraction is the transfer of the selected compounds from the sample onto the SPE cartridge. Elution is the removal of the selected compounds from the SPE cartridge.

3. Interferences

Interferences might be caused by compounds recovered from a sample matrix that contains one or more of the same ions as the selected compound and that cannot be chromatographically resolved from the selected compounds. Compared to optical detection methods typically used with HPLC, mass spectrometry is less affected by interferences, but the potential for interferences remains and requires special attention to the ratios of the characteristic ions of interest to avoid false positive detections. In addition, for the sulfonylurea and imidazolinone compound classes, sample matrix components have been shown empirically to result in an apparent increase in compound concentration (Furlong and others, 2000).

4. Apparatus and Instrumentation

NOTE: During the development and implementation of this method, Hewlett-Packard Corporation, the manufacturer of the HPLC/MS instrumentation used in determining method performance, formed a new company, Agilent Technologies, from the original Hewlett-Packard Chemical Analysis Division and other company components. The HPLC/MS systems used in this study were originally made by Hewlett-Packard, and after formation of the new company, by Agilent Technologies. The phrase “Hewlett-Packard/Agilent Technologies” is used in this report to describe this identical instrumentation.

4.1 *High-performance liquid chromatograph*—Hewlett-Packard Model 1090M Series II or Hewlett-Packard/Agilent Technologies Model 1100 high-performance liquid chromatographic system equipped as follows: a binary (Model 1100) or direct-ratio (DR5; Model 1090M) ternary-solvent delivery system, a photodiode-array ultraviolet-absorbance detector (optional), a 250-µL automatic syringe sampler, a 100-position random-access autosampler equipped with a cooling module, and thermostated column heating. An IBM-compatible computer workstation also is required, with a minimum configuration of an Intel Pentium II Processor operating at a minimum of 266 MHz, 96 megabytes of Random Access Memory, and a minimum 20-gigabyte hard disk. Hewlett-Packard/Agilent Technologies LC/MSD ChemStation Revision A.06.03 software, and Thru-Put Systems Target Version 3.4 Software, or equivalent, are also required.

4.2 *Mass spectrometer*—Hewlett-Packard/Agilent Technologies Series 1100 LC/MSD mass spectrometer, or equivalent, equipped with an electrospray ionization source and capable of operating in positive and negative ionization mode.

4.3 *Analytical columns—Chromatographic columns,* MetaChem Technologies, Inc. Inertsil octadecylsilane (ODS-3), 5 µm; 2.1-mm inside diameter by 15-cm stainless-steel column or equivalent. A guard column (MetaChem Technologies, Inc., MetaGuard 2.0-mm Inertsil octadecylsilane (ODS-3) direct-connect, 3 µm, or equivalent, also is required.

4.4 Manual sample extraction apparatus

4.4.1. *Solid-phase extraction manifold*—Supelco, Inc., Visiprep Solid-Phase Extraction Vacuum Manifold or equivalent.

4.4.2 *Ceramic-piston valveless sample pumps*—Capable of pumping 0 to 25 mL/min, Fluid Metering Inc. Model QSY-2 CKC or equivalent.

4.4.3 *Sample flow path*—All Teflon-perfluoralkoxy (PFA) 1/8-inch tubing (3.18 mm) or equivalent.

4.4.4 Tefzel-tetrafluoroethylene Luer connectors or equivalent.

4.4.5 *Luer stopcock—flow control on-off valves*—Burdick & Jackson Inert PTFE flow-control valves or equivalent.

4.4.6 *Vacuum pump*—Must be able to draw a vacuum equivalent to at least 102 kPa.

4.5 Automated sample extraction apparatus

4.5.1 *Zymark AutoTrace 6-place SPE workstation or equivalent*—Equipped for processing 6-mL SPE tubes.

4.5.2 *Laboratory-auto trace barrel adapters*—Made from 6-mL polypropylene syringe tubes (structurally identical to the SPE cartridge without the SPE packing), cut to one-half the original length (various manufacturers).

4.5.3 *SPE cartridge connectors*—Polyethylene (Restek #26007 or equivalent).

4.5.4 *Evaporative concentrator*—Temperature controlled to 34°C and nitrogen gas pressure of 69 kPa Zymark Turbo-Vap or equivalent.

4.6 Liquid-handling apparatus

4.6.1 *Syringes*—Hamilton Gastight 1750RN, 500 µL (cat. no. 81131); Gastight 1001LTN, 1,000 µL (cat. no. 81317); and Hamilton Microliter 701, 10 µL (cat. no. 80366) or equivalent.

4.6.2 *Micropipets*—Van Waters and Rogers (VWR) 10- to 100-µL variable volume digital microdispenser (cat. no. 53506201), VWR 100-µL fixed-volume microdispenser (cat. no. 53506675), and VWR 100-µL replacement tubes (cat. no. 53508499) or equivalent.

4.6.3 *Autosampler vials*—National Scientific Company, 2-mL, graduated amber glass for use with screw-top caps (cat. no. C4000-2W) or equivalent.

4.6.4 *Vial caps and septa*—National Scientific Company, screw-top caps with 11-mm dual Teflon-faced silicone septa (cat. no. C4000-53B) or equivalent.

4.6.5 *20-mL solution storage vials*—Eagle-Picher Technologies, LLC, 20 mL, amber with Teflon-faced silicone rubber-lined screw caps (cat. no. 139-20A/CT) or equivalent.

4.7 Consumables

4.7.1 *Amber-glass bottles*—1,000 mL, baked at 450°C for 2 hours, fitted with Teflon-lined screw caps or equivalent.

4.7.2 *Solid-phase extraction cartridges*—Supelco ENVIRO-Carb Graphitized Carbon Black, graphitized nonporous carbon, 500 mg, 120/400 mesh, in 6-mL syringe barrel or equivalent.

4.7.3 *Nitrogen gas* for sample extract concentration, ultrapure.

4.7.4 *Test tubes*, graduated 14-mL polypropylene round-bottom, 17- by 100-mm style or equivalent.

4.7.5 *Disposable Pasteur pipets* cleaned by ashing at 440°C for 2 hours.

5. Reagents and Solutions

NOTE: Material Safety Data Sheets for all materials described herein need to be read prior to using any of these materials to ensure safe handling and proper disposal. Unless otherwise specified, solutions should be stored at room temperature and should be used for no longer than 6 months.

5.1 Neat reagents

5.1.1 *Acetonitrile*—Burdick and Jackson, ultraviolet (UV) grade or equivalent.

5.1.2 *L–(+)–Ascorbic acid*—J.T. Baker, reagent grade or equivalent.

5.1.3 *Formic acid solution*—88 percent, Mallinckroft AR or equivalent.

5.1.4 *Liquinox, liquid detergent*—Alconox Inc. or equivalent.

5.1.5 *Methanol*—Burdick and Jackson, HPLC grade or equivalent.

5.1.6 *Methylene chloride*—Burdick and Jackson, pesticide grade or equivalent.

5.1.7 *Trifluoroacetic acid anhydride (TFA)*—Pierce Chemical, Inc., reagent grade or equivalent.

5.1.8 *Water, organic-free*—Deionized and distilled water that is free from interfering organic compounds and chlorine.

5.1.9 *Ammonium acetate*—J.T. Baker, reagent grade or equivalent.

5.1.10 *Sodium chloride*—EM Science, reagent grade or equivalent.

5.1.11 *Sodium hydroxide pellets*—Reagent grade or equivalent.

5.1.12 *Isopropyl alcohol*—Burdick and Jackson, HPLC grade or equivalent.

5.2 Solutions

5.2.1 SPE cartridge conditioning solutions

5.2.1.1 *Solution 1*—80-percent methylene chloride/20-percent methanol. Mix 400 mL of methylene chloride with 100 mL of methanol. Store in a calibrated adjustable dispenser. Replace solution at least weekly.

5.2.1.2 *Solution 2*—Ascorbic acid solution. Dissolve 10 g of ascorbic acid (5.1.2) in 1 L organic-free water and mix. Refrigerate at 4°C and replace solution at least weekly.

5.2.2 *SPE cartridge elution solution*—80-percent methylene chloride/20-percent methanol/0.2-percent trifluoroacetic acid anhydride (TFA). Mix 400 mL of methylene chloride, 100 mL methanol, and

1,000 µL of TFA (5.1.7). Prepare solution daily and store it in AutoTrace reservoir.

5.2.3 *Eluent neutralization solution*—10 percent w/v sodium hydroxide solution. Weigh 10 g of sodium hydroxide pellets (5.1.11). Dissolve sodium hydroxide pellets in 100 mL organic-free water and mix.

5.2.4 *AutoTrace workstation cleaning solution* (*Liquinox detergent solution)*—Dilute four drops of Liquinox (5.1.4) with 4 L of organic-free water and mix.

5.2.5 *Concentrated ammonium acetate in organic-free water solution*— Weigh 20 g ammonium acetate (crystalline) (5.1.9) into a 1-L flask. Dissolve the ammonium acetate with organic-free water (5.1.8) and dilute to volume in the flask. Filter the solution through a 47-mm, 0.2-µm nylon filter membrane into a clean, burned filter flask. Transfer solution into a clean and burned 1-L bottle. The final concentration is 20 g/L (0.26 molar). Store at room temperature.

5.2.6 *Concentrated ammonium acetate in methanol and acetonitrile solution*—Weigh 20 g ammonium acetate (crystalline) (5.1.9) into a tared 1-L flask. Dissolve the ammonium acetate in the flask with 500 mL of methanol, then add 500 mL of acetonitrile. Do not premix the methanol and acetonitrile because ammonium acetate does not dissolve easily in the presence of acetonitrile. Use a stirring magnet and mixer with no heat to fully dissolve all ammonium acetate. Once it is dissolved, the ammonium acetate will stay in solution. The final concentration is 20 g/L (0.26 molar). Store at room temperature.

5.3 HPLC mobile phase preparation

NOTE: The concentrations of formic acid in the mobile phase are varied from instrument-to-instrument and column-to-column to optimize chromatographic separation. The following concentrations are for reference.

5.3.1 *Organic solvent eluent (acetonitrile modified with ammonium acetate solution)*—Use a 7-mL class A pipet to measure 7 mL of the ammonium acetate in acetonitrile:methanol solution (5.2.6), and transfer to a 500-mL volumetric flask. Dilute the solution in the flask to 500 mL with HPLC-grade acetonitrile (5.1.1) and mix. Filter the solution through a 47-mm, 0.2-µm nylon filter membrane into a clean, burned filter flask. Transfer to HPLC eluent reservoir B for use. The final concentration is 0.28 g/L (3.63 millimolar).

5.3.2 *Aqueous eluent* (*organic-free water modified with ammonium acetate solution and formic acid)*—Use a 7-mL class A pipet to measure 14 mL (in two aliquots) of the ammonium acetate in organic-free water solution (5.2.5), and place in a 1-L volumetric flask. Use a graduated cylinder to add 40 mL of acetonitrile to the 1-L volumetric flask. Use a 500-µL syringe to add 200 µL of formic acid (5.1.3) to the solution in the flask. Dilute the solution in the flask to 1 L with organic-free water (5.1.8) and mix. Filter the solution through a 47-mm, 0.2-µm nylon filter membrane into a clean, burned filter flask. Transfer to HPLC eluent reservoir A for use. The final concentration of ammonium acetate in the solution is 0.28 g/L (3.63 millimolar). The final concentration of formic acid in the solution is 0.24 g/L (5.22 millimolar).

5.4 Calibration and quality-control standard solutions

In this method, each sample is analyzed twice, once for pesticides that preferentially form negative ions and once for pesticides that preferentially form positive ions. Although the chromatographic conditions are identical for both analyses, it is important that the stock solutions used for calibration and quality-control standards be prepared separately rather than in combination. There is a substantial possibility that interferences from co-eluting compounds or cross-reactivity among compounds will affect the identification and quantitation of method analytes if solutions are combined. This problem also might be exacerbated by the high concentrations of the stock solutions. All calibration and quality control (QC) standard solutions are derived from three common mixed standards, mixed from the high-concentration, single-component solutions (see 5.4.1 and 5.4.2 below). These solutions are referred to as positive mix 1, positive mix 2, and negative mix. The purpose of using these solutions for all subsequent calibration and QC standard solutions is to reduce potential discrepancies in interpreting QC results.

The overall approach is to prepare three 20,000-µg/L multicomponent solutions that together include all compounds in the method except for surrogates and internal standards. From these solutions, working standards are made that contain the appropriate positive and negative surrogate compounds. The calibration solutions are then derived from the working standards. The specifications for the solutions are contained in the following sections and should be used if an external (commercial) source of standard solutions is used for this method. Individual single-component stock solutions (at about 1 to 10 mg/mL) are prepared from neat material or purchased at a known purity from a commercial source. After formulation, all solutions, except the calibration standards made prior to analysis, are stored in a freezer in labeled, amber 20-mL glass vials with Teflon-faced, silicone rubber-lined screw caps.

5.4.1 *Calibration solutions for compounds determined under negative ionization conditions*—For compound mixtures determined under negative ionization conditions, single compound stock solutions and multicomponent stock and calibration solutions are prepared with HPLC-grade methanol (5.1.5). Prepare individual stock solutions of 10 mg/mL by dissolving 50 mg of the selected pesticides in a 5-mL amber-glass volumetric flask and dilute to volume using methanol to dissolve each compound. For negative ionization analysis, prepare a multicomponent stock solution for each listed compound by calculating the aliquot of each individual stock solution necessary to produce a final concentration of 20.0 ng/µL, calculated as follows:

$$V_{ss} = C_f \left[\frac{V_f}{C_{ss}} \right] \qquad (1)$$

where V_{ss} = the stock solution volume used, in microliters, typically ≅200 µL;

C_f = the final solution concentration (for this solution 20.0 ng/µL≡20,000 µg/L);

V_f = the final solution volume (for this solution, 100 mL ≡100,000 µL); and

C_{ss} = the stock solution concentration (for this solution, 10 mg/mL ≡10,000 ng/µL).

Use a variable-volume microdispenser (see 4.6.2) to add the calculated aliquot of each compound to a 100-mL amber-glass volumetric flask. Dilute the combined compounds to volume with methanol. Prepare a new primary fortified standard solution every 6 months.

Store all solutions in a refrigerator in cleaned and burned amber glass vials or bottles with Teflon-lined screw caps. For all compounds except triclopyr and chlorothalonil, single component stock solutions are usable for no more than 12 months. For triclopyr and chlorothalonil, single component stock solutions are usable for no more than 3 months. The components of the negative ion solution are listed in table 2.

5.4.2 *Preparation of multicomponent standard solutions for compounds determined under positive ionization conditions*—Calibration, calibration verification, lab reagent spike fortification, and field matrix spike fortification solutions are prepared by diluting high-concentration (20,000 µg/L), multicomponent standard solutions. These solutions are prepared from individual compound stock solutions, made up in one or more solvents, and are listed for each single compound in table 3. Prepare individual stock solutions of 10 mg/mL by dissolving 50 mg of the selected pesticides in a 5-mL amber-glass volumetric flask and dilute to volume by using methanol or appropriate solvent to dissolve the compound (listed in table 3).

Two multicomponent calibration solutions are prepared for compounds determined under positive ionization conditions. The first solution contains all the positive ionization compounds except for deethyldeisopropylatrazine and 2-hydroxy-atrazine. The second solution contains only deethyldeisopropylatrazine and 2-hydroxy-atrazine. Two separate solutions are prepared because deethyldeisopropylatrazine and 2-hydroxyatrazine require acid to dissolve the compounds in the stock solutions, and, if combined into the primary solution, would have potential for degradation of other compounds in the primary solution. There also is potential for cross-reactivity with other compounds in the primary mixed standard solution. For positive ionization analysis, prepare two multicomponent stock solutions by calculating the aliquot of each individual stock solution necessary to produce a final concentration of 20.0 ng/µL, calculated as indicated in equation (1) and repeated below:

Table 2 Calibration solution composition for compounds determined under negative ionization conditions

[CAS, Chemical Abstract Services]

Compound name	CAS number	Stock solution solvent
2,4-D	94-75-7	Methanol
2,4-DB	94-82-6	Methanol
Acifluorfen	50594-66-6	Methanol
Bentazon	25057-89-0	Methanol
Bromoxynil	1689-84-5	Methanol
Chlorothalonil	1897-45-6	Methanol
Clopyralid	1702-17-6	Methanol
Dacthal monoacid (Monomethyl tetrachloroterephthalate)	887-54-7	Methanol
Dicamba	1918-00-9	Methanol
Dichloprop	120-36-5	Methanol
Dinoseb	88-85-7	Methanol
MCPA (4-Chloro-*o*-tolyloxyacetic acid)	94-74-6	Methanol
MCPB	94-81-5	Methanol
Picloram	1918-02-1	Methanol
Triclopyr	55335-06-3	Methanol

Table 3 Calibration solution composition for compounds determined under positive ionization conditions

[CAS, Chemical Abstract Services; %, percent]

Compound name	CAS number	Stock solution solvent
Positive Solution Mixture 1		
2,4-D methyl ester	1928-38-7	Methanol
3(4-chlorophenyl)-1-methyl urea	1897-46-6	Methanol
3-Hydroxycarbofuran	16655-82-6	Methanol
3-Ketocarbofuran	16709-30-1	Acetone
Aldicarb	116-06-3	Methanol
Aldicarb sulfone	1646-88-4	Methanol
Aldicarb sulfoxide	1646-87-3	Acetonitrile
Atrazine	1912-24-9	Methanol
Bendiocarb	22781-23-3	Methanol
Benomyl	17804-35-2	Acetone/Methanol (75:25)
Bensulfuron-methyl	83055-99-6	Dichloromethane
Bromacil	314-40-9	Methanol
Caffeine	58-08-2	Methanol
Carbaryl	63-25-2	Methanol
Carbofuran	1563-66-2	Methanol
Chloramben methyl ester	7286-84-2	Methanol
Chlorimuron-ethyl	90982-32-4	Acetonitrile
Cycloate	1134-23-2	Methanol
Deethylatrazine	6190-65-4	Methanol
Deisopropylatrazine	1007-28-9	Dichloromethane
Diphenamid	957-51-7	Methanol
Diuron	330-54-1	Methanol
Fenuron	101-42-8	Methanol
Flumetsulam	98967-40-9	Methanol/Acetone (50:50)
Fluometuron	2164-17-2	Methanol
Imazaquin	81335-37-7	Dichloromethane/Methanol (60:40)
Imazethapyr (Pursuit)	81335-77-5	Acetone
Imidacloprid	138261-41-3	Methanol
Linuron	330-55-2	Methanol
Metalaxyl	57837-19-1	Methanol
Methiocarb	2032-65-7	Methanol
Methomyl	16752-77-5	Methanol
Methomyl oxime	13749-94-5	Methanol
Metsulfuron-methyl	74223-64-6	Methanol
Neburon	555-37-3	Methanol
Nicosulfuron (Accent)	111991-09-4	Acetonitrile/Methanol/Acetone (50:25:25)
Norflurazon	27314-13-2	Methanol
Oryzalin	19044-88-3	Methanol
Oxamyl	23135-22-0	Methanol
Oxamyl oxime	30558-43-1	Dichloromethane/Methanol (60:40)
Propham	122-42-9	Methanol
Propiconazole (Tilt)	60207-90-1	Methanol
Propoxur (Baygon)	114-26-1	Methanol
Siduron	1982-49-6	Methanol
Sulfometuron-methyl	74222-97-2	Acetonitrile/Dichloromethane (50:50)
Tebuthiuron	34014-18-1	Acetone
Terbacil	5902-51-2	Methanol
Tribenuron-methyl	101200-48-0	Acetonitrile
Positive Solution Mixture 2		
2-Hydroxyatrazine	2163-68-0	Methanol/Acetone / Hydrochloric Acid (aq) [36%] (49.9:49.9:0.2)
Deethyldeisopropylatrazine	3397-62-4	Methanol/Acetone / Hydrochloric Acid (aq) [36%] (49.9:49.9:0.2)

$$V_{ss} = C_f \left[\frac{V_f}{C_{ss}} \right] \qquad (2)$$

where V_{ss} = the stock solution volume used, in microliters, typically ≅200 µL;

C_f = the final solution concentration (for this solution, 20.0 ng/µL);

V_f = the final solution volume (for this solution, 100 mL ≡100,000 µL); and

C_{ss} = the stock solution concentration (for this solution, 10 mg/mL ≡10,000 ng/µL).

Prepare each positive and negative multicomponent standard solution separately by using a variable-volume microdispenser (4.6.2) to add the calculated aliquot of each compound to a 100-mL amber-glass volumetric flask. Dilute the combined compounds to volume with methanol. Prepare new primary fortified standard solution for each mixture every 6 months. Store the solutions in a cleaned and burned amber-glass bottle with Teflon-lined screw caps. For all compounds except 3-hydroxy- carbofuran, single component stock solutions are usable for no more than 12 months. For 3-hydroxycarbofuran, single component stock solutions are usable for no more than 3 months. The components of the two multicomponent positive solutions and the appropriate solvents for the single component stock solutions are listed in table 3.

5.4.3 *Surrogate solution*—A single surrogate solution is used for both ionization modes. The surrogates used for each analysis are listed in table 4. Individual surrogate stock solutions are made up in HPLC-grade methanol to a concentration of 10 mg/mL. The multicomponent surrogate solution is made up in HPLC-grade methanol at a final concentration of 20 ng/µL of each listed compound. All solutions are transferred to and stored in a cleaned and burned amber-glass bottle with Teflon-lined screw caps. Stock solutions for the individual surrogate compounds are usable for no more than 12 months.

5.4.4 *Laboratory reagent spike fortification and field matrix spike fortification solutions*—The same solutions are used for laboratory reagent and matrix spikes. Prepare three separate laboratory reagent spike fortification solutions (excluding positive and negative ionization surrogates) at a final concentration of 2.5 ng/µL each. These solutions are prepared with a 100-mL volumetric flask, with methanol as the dilution solvent. The aliquot of each individual compound to be used is calculated as follows:

$$V_{ss} = C_t \left[\frac{V_t}{C_{ss}} \right] \qquad (3)$$

where V_{ss} = the aliquot volume required, in microliters;

C_t = the target concentration (in this solution, 2.5 ng/µL);

V_t = the volume of target solution required, in milliliters (100 mL for this solution); and

C_{ss} = the compound standard solution concentration, in milligram/milliliter (1,000 ng/µL for this solution).

Table 4. Surrogate solution composition for compounds determined under positive and negative ionization conditions

[CAS, Chemical Abstract Services; %, percent]

Compound name	CAS number	Stock solution solvent
2,4,5-T (negative ionization analysis surrogate)	93-76-5	Methanol
Barban (positive ionization analysis surrogate)	101-27-9	Methanol
^{13}C-Caffeine (Caffeine (2-^{13}C,99%:1,3-$^{15}N_2$,98%+); positive ionization analysis surrogate)		Methanol

Use a variable-volume micro-dispenser (see 4.6.2) to add each compound to the volumetric flask. Bring the final solution to volume with methanol. Make three solutions, two for compounds determined under positive ionization conditions and one for compounds determined under negative ionization conditions (5.4.1). Addition of 100 µL of each of these solutions to 1.000 L of organic-free water will result in a concentration of 0.25 µg/L of each compound in the laboratory fortification sample.

5.4.5 *Internal standard solution*—Prepare an internal standard solution of Monuron and dichloroacetic acid at concentrations of 0.1 and 1.0 mg/mL, respectively, according to the following formula:

$$V_{ss} = C_t\left[\frac{V_t}{C_{ss}}\right] \qquad (4)$$

where V_{ss} = the aliquot volume required, in microliters;

C_t = target concentration, typically 100 ng/µL;

V_t = volume of target solution required, in milliliters (100 mL for this solution); and

C_{ss} = the compound standard solution concentration, in milligram/milliliter (1,000 ng/µL for this solution).

5.4.6 C*alibration solutions*—A set of quantitative calibration solutions is analyzed and a calibration curve is generated at the beginning of analysis to determine the concentrations of qualitatively identified compounds. To do this, prepare a series of working standard solutions, ranging in concentration from 100 to 100,000 ng/L, from the stock solutions prepared in sections 5.4.1 and 5.4.2. At least five concentrations of calibration standards and a compound-free blank solution (system or instrument blank) are analyzed. Each concentration is prepared as needed by addition of a specified volume of the stock multicomponent standards to produce standards with concentrations equivalent to 0.010, 0.050, 0.10, 0.20, 0.50, and 1.00 µg/L in water, assuming an injection volume of 50 µL and a 1.00-L water sample. The preparation of these standard solutions is described in detail in the following sections.

5.4.6.1 *Working standard solutions for calibration standard formulation*. Mix 25 mL of surrogate solution (5.4.3) with 25 mL of positive mix 1 and negative mix (5.4.1) in separate 50-mL volumetric flasks. The concentration of this intermediate solution is 10,000 µg/L. Prepare the working standards for positive mix 1 and negative mix by using the 10,000-µg/L intermediate solutions and the dilutions listed in table 5(A). Prepare working standards for positive mix 2 using the 10,000-µg/L intermediate solution and the dilutions listed in table 5(B).

5.4.6.2 *Calibration standards.* Calibration standards are prepared just prior to instrumental analysis. Measure 900 µL of organic-free water (5.1.8) into the number of vials required for the desired number of calibration standards. Add 100 µL of a working standard solution to each vial. Add 5 µL of quantitation internal standard solution (5.4.5), cap, and mix. Note that for ease of sample concentration determination, the calibration standard concentrations are expressed by an equivalent concentration in a 1,000-mL water sample (sample equivalence). The concentration of the calibration standards is calculated by using the following formulae, in which the dilution of a 5,000-µg/L working standard to a 0.5-µg/L (sample equivalence) calibration standard is listed:

(A) 100 µL of working standard (5,000 µg/L) is added to 900 µL of organic-free water:

$$5{,}000\ \mu g/L \times 100\ \mu L \times L/10^6\ \mu L = 0.5\ \mu g$$
$$0.5\ \mu g/mL \times 1{,}000\ mL/L = 500\ \mu g/L \text{ in vial}$$

(B) On-column mass of standard made in (A), when using a 50-µL injection:

$$500\ \mu g/L \times 50\ \mu L \times L/10^6\ \mu L = 0.025\ \mu g \text{ on column}$$
$$0.025\ \mu g \text{ on column} \times 1{,}000\ ng/\mu g = 25\ ng \text{ on column}$$

(C) Concentration of calibration standard (sample equivalence):

$$25\ ng \times 1{,}000\text{-}\mu L \text{ vial}/50\ \mu L \text{ injected} \times 1/1{,}000\ mL = 0.5\ ng/mL$$
$$0.5\ ng/mL \times 1{,}000\ mL/L \times 1\ \mu g/1{,}000\ ng = 0.5\ \mu g/L$$
(calibration standard's concentration equivalence in a 1-L water sample)

Table 5. Volume dilutions for method working standards

[mL, milliliter; µg/L, microgram per liter; µL, microliter]

Volumetric flask used (mL)	Volume of 10,000 µg/L standard used (µL)	Final concentration of working standard (µg/L)
(A) Working standards for positive mix 1 and negative mix using the 10,000-µg/L solution		
10	5,000	5,000
25	5,000	2,000
50	5,000	1,000
100	5,000	500
200	2,000	100
(B) Working standard dilutions for positive mix 2 using the 20,000-µg/L solution		
10	5,000	10,000
10	2,500	5,000
25	2,500	2,000
100	2,500	500

5.4.6.3 *Concentration of calibration standards.* Working standard concentrations and their sample equivalence are listed in table 6.

Table 6. Working standard concentrations and equivalent sample concentrations for this method

[µg/L, microgram per liter]

Working standard concentration (µg/L)	Equivalent sample concentration (µg/L)
10,000	1.00
5,000	.50
2,000	.20
1,000	.10
500	.05
100	.01
50	.005

5.4.7 *Third-party check solution*—An independently verified or "third-party" standard solution, containing the same or a subset of the compounds in the calibration standard solutions dissolved in methanol, can be used to determine acceptable performance of the instrument calibration. This solution should not contain the method internal standards or surrogates. If available, obtain this solution from a vendor that has prepared a solution and has independently validated it by instrumental analysis. Store this solution in a freezer at −15°C, being sure to use the solution prior to the manufacturer's expiration date. Select a concentration near the midpoint of the method calibration range (0.1 to 0.5 µg/L). Use this concentration to determine the volume of solution required to fortify a 1-L water sample to the chosen concentration. For example, the concentration of the components in third-party check solution used in this study was 17.5 µg/L. Add 10 µL of the third-party standard to 990 µL of water in a 1.5-mL amber-glass screw-cap autosampler vial and 5 µL of the internal standard solution to produce a per-component solution concentration of 0.175 µg/L. Then seal the vial with a Teflon-lined septum cap, and add to the sequence of sample, calibration, and quality-control samples. The concentration of the third-party standard solution should be sufficiently high so that the solvent in the aliquot that is diluted will not affect chromatographic separation.

5.4.8 *Instrument quality-control standards*—The two instrument quality-control standards that monitor instrument performance over the course of analysis are continuing calibration verification standards (CCVs) and continuing calibration blanks (CCBs). The CCVs are interspersed within a set of samples and are used to verify that the instrument has stayed within analytical calibration over the course of analysis. The CCBs are monitored to determine if sample or standard injections caused cross-contamination.

5.4.8.1 *Continuing calibration verification (CCV) standards*. Prepare a 0.2-µg/L midcalibration-level-check sample by adding an appropriate amount of the calibration standard for the ionization mode to be analyzed into an appropriate amount of organic-free water in an autosampler vial. Add 5 µL of the internal standard (5.4.5), and seal with a Teflon-lined septum cap. Concentrations other than 0.2 µg/L may be used for CCVs if desired, as long as the concentration is near the midpoint of the calibration range.

5.4.8.2 *Continuing calibration blank (CCB) standards*. Place 1,000 µL of organic-free water in an autosampler vial.

NOTE: Do not add internal standard solution to the CCB. Seal with a Teflon-lined septum cap.

6. Safety Precautions

6.1 Use a well-vented fume hood for all steps involving organic solvents and acids.

6.2 Wear eye protection and the appropriate type of gloves when using any reagents.

6.3 Ensure that the electrospray waste exhaust tube and the vacuum pump exhaust tube of the mass spectrometer are vented out of the ambient laboratory atmosphere through ventilation ducting expressly specified for that purpose.

7. Procedure

7.1 Sample filtration

This method is applicable only to filtered water samples. All samples should be filtered in the field, preferably at the time of collection. Filtration will reduce the likelihood of compound degradation by removing particulate-associated bacteria. Removal of particulates also will prevent clogging of the retaining frit and stationary phase of the solid-phase extraction cartridge, thus improving operation and extraction efficiency. Sandstrom (1995) describes a USGS-approved filtration method appropriate for samples analyzed by this method. Occasionally, samples are not filtered on site or become cloudy (particulate formation caused by chemical reactions or nanobacterial growth) during transit to the laboratory. Filter these cloudy samples at the laboratory according to the procedure outlined by Sandstrom (1995) by using a 14.2-cm filter holder and positive pressure pump. Use a 0.7-µm pore size, 14.2-cm diameter, glass-fiber filter, ashed at 440°C for 2 hours. Flush the filtration apparatus with 100 mL of Liquinox solution (5.2.4), 100 mL of organic-free water (5.1.8), 50 mL of methanol (5.1.5), and again with 100 mL of organic-free water. Repeat this cleaning procedure between samples. Use a separate filter for each sample to prevent sample cross-contamination.

7.2 Solid-phase extraction cartridge cleaning and conditioning

NOTE: The extraction and elution procedure used in this method was designed to perform equally well by manual operation or by automated SPE workstations. The same cleaning and conditioning procedure is used for both.

7.2.1 Prepare, as needed, the 80-percent methylene chloride/20-percent methanol (v/v) and 10-g/L aqueous ascorbic acid solutions for conditioning the SPE cartridges (5.2.1.1 and 5.2.1.2). These SPE cartridge conditioning solutions are prepared once a week because evaporation might alter the solvent composition. Store the ascorbic acid solution in the refrigerator at all times to prevent premature degradation of the acid.

7.2.2 The performance of Supelco Graphitized carbon-based SPE (6-mL) cartridges may vary from lot to lot. Be sure to use cartridges from a single lot for any set of environmental and QC samples. Install the graphitized carbon-based SPE cartridges on the vacuum extraction manifold. The vacuum pump is used to pull the first two conditioning solutions through the cartridge. Do not exceed 20 mm Hg vacuum pressure, or the extraction chamber will implode.

7.2.3 Cartridges are conditioned by sequentially eluting the cartridge with two 5-mL aliquots of 80-percent methylene chloride/20-percent methanol (v/v) (5.2.1.1) with the vacuum pump to facilitate elution. This step is followed by elution with 5 mL of methanol (5.1.5) through each cartridge using the vacuum pump. Eluting three 5-mL aliquots of the ascorbic acid solution (5.2.1.2) under gravity flow completes conditioning. The ascorbic acid conditioning step should take place slowly to ensure activation of binding sites in the cartridge bed. Keep the flow rate of ascorbic acid solution at no more than 3 mL/min. Cover conditioned cartridges with foil and set aside until ready for use. Cartridges can be used up to 8 hours after conditioning. Cartridges that have not been used within 8 hours need to be reconditioned. Collect the

conditioning solvents in the vacuum manifold; place these solvents in a chlorinated waste container for proper disposal.

NOTE: To avoid deactivation of the SPE sorbent surface, cartridges should never be allowed to dry after conditioning.

7.3 Solid-phase extraction

NOTE: The following description is for the automated SPE workstation method. This method can be carried out manually through the elution step by using the same conditions outlined in the following procedure.

7.3.1 Prior to extraction, the approximate pH of the sample is determined by removing a small (0.05 mL) aliquot with a disposable Pasteur pipet (4.7.5) and applying the volume to pH paper with a range of 0 to 14, and recording the pH. Do not adjust the sample pH. Record the combined sample and bottle weight. Note any unusual appearance of the sample and record it. Add 100 µL of surrogate solution (5.4.3) and 1 g of sodium chloride (NaCl) to each sample. Shake samples well to dissolve the NaCl and uniformly disperse and mix the surrogate.

7.3.2 Prepare laboratory reagent blank (LRB) and laboratory reagent spike (LRS) samples. Obtain two cleaned and burned 1-L amber bottles. Fill them with 1,000 mL organic-free water and add 1 g of NaCl to each bottle. Shake well to dissolve the NaCl. In preparing the fortification sample, add 100 µL each of the three multicomponent matrix fortification solutions to one bottle containing 1,000 mL of reagent water. Record the solution code and bottle preparation date of the fortification solutions (section 5.4.4). Add 100 µL of surrogate solution to each bottle (5.4.3). This step will result in a final concentration in the set fortification of 0.25 µg/L. Shake the fortification and blank vigorously to mix the surrogate and NaCl in the water. Laboratory reagent blank and reagent spike samples must be prepared with each set of environmental samples. A set of samples in this procedure consists of the reagent spike and reagent blank samples and up to 10 environmental samples. The environmental sample total may include duplicate field samples or field samples that are to be fortified in the laboratory (laboratory matrix spike samples). Most analytes are stable under refrigeration for up to 4 days prior to extraction on the basis of results from on-going holding-time studies.

7.3.3 Clean the AutoTrace pumps and tubing prior to use by flushing each AutoTrace position with sequential aliquots of 50 mL of Liquinox detergent solution (5.2.4), 50 mL of water (5.1.8), and 50 mL of methanol (5.1.5). Pass nitrogen through the lines at about 103.4 kPa (15 pounds per square inch) for about 5 minutes to ensure that all traces of methanol are removed.

7.3.4 Install six conditioned, 6-mL graphitized carbon-based SPE cartridges (4.7.2) on the AutoTrace SPE workstation. Attach one SPE cartridge connector and one of the 6-mL polypropylene syringe barrel adapters to each cartridge. Lower the plunger/cartridge clamp into the adapter, thus ensuring that the entire assembly fits snugly in the apparatus. It is critical that the plunger is lowered fully and snugly into the adapter because this snug fit, and the plunger O-ring, are the primary means for sealing the SPE cartridge into the flow stream for SPE loading and elution. Leaks caused by poor sealing will adversely and irreproducibly affect method performance.

7.3.5 Pump water samples through the conditioned cartridges by using a flow rate of 20 mL/min. Approximate extraction time for 1 L of sample is 50 minutes. The AutoTrace workstation will emit an audible signal and suspend operation when extraction is complete.

7.3.6 Upon completion of extraction, the SPE cartridges require drying. Two methods have been used with equal success. The first is to pass nitrogen gas through the cartridge, followed by chemical drying of the cartridge with methanol. After some experimentation, it was found that the nitrogen gas step could be omitted. This optional nitrogen gas-drying step is described in the following section. The methanol drying procedure is explained in section 7.3.8.

7.3.6.1 *Optional nitrogen gas drying.* Place empty plastic test tubes in the AutoTrace eluent collection rack. Continue AutoTrace operation, and the programmed step for nitrogen flow will begin. Nitrogen will flow at a pressure of 103 kPa for 1 minute. Remove the empty polyethylene test tubes and dispose of the residual water. Remove cartridges from the AutoTrace and attach to nitrogen manifold. Dry for at least 15 minutes at 550 to 620 kPa. Return to appropriate positions on the AutoTrace.

7.3.7 Weigh the empty sample bottle and record the weight. The difference between this weight and the initial bottle weight (7.3.1) provides the sample mass in grams, which is assumed to be equal to the sample volume in milliliters. Note that this procedure assumes that the volumetric density of a typical freshwater sample is 1 g/mL. For samples collected from saline environments, a salinity or density determination should be made and a volume correction applied.

Occasionally a cartridge will clog, even if a sample has been filtered. This is likely the result of adsorption of coextracted natural organic matter onto the cartridge bed. In this event, the entire sample mass may not have been extracted. Weigh the bottle and remaining sample, discard the remaining sample and re-weigh the empty bottle. Record these results and this condition. This information is required to accurately determine sample concentration.

7.3.8 SPE cartridge elution

7.3.8.1 After sample loading and the optional nitrogen gas drying step, cartridges retain a small amount (≅ 0.1 mL) of residual water. Chemically dry the cartridge by eluting the analytes with 1.5 mL of methanol. Some compounds are eluted in this fraction. This 1.5-mL fraction is collected separately in unused polypropylene test tubes by placing six tubes positioned in the AutoTrace rack and labeled with the appropriate sample identifications. In addition, this label should also include the letter "M" to indicate that this is the methanol fraction. Start the first step of the AutoTrace elution program to begin collection of the "M" fraction for all six tubes. When the AutoTrace has completed this first part of the elution, it will emit an audible signal. The last drops of methanol in the cartridge bed are removed by passing nitrogen gas through the cartridge at 103 kPa for 30 seconds. When the AutoTrace has completed this methanol-drying step, it will emit an audible signal. Remove the "M" fractions from the AutoTrace rack and bring the fraction to a volume of 1 mL with organic-free water. Refrigerate the extract until completion of elution.

7.3.8.2 Most of the analytes are collected in the second elution fraction of the SPE cartridge. Place unused polypropylene test tubes in the elution rack of the AutoTrace workstation to collect the second elution fraction. Label these tubes with the appropriate sample identifications. Indicate that these tubes hold the second elution fraction by labeling each tube with an "E". Make sure the tubes are arranged in the correct order so that they will receive the eluent from appropriate SPE cartridges. Elute each SPE cartridge with three aliquots of 80-percent methylene chloride/20-percent methanol (v/v) with 0.2 percent trifluoroacetic acid anhydride (TFA) solution (5.2.2).

The three aliquot volumes are, in sequence, 5, 5, and 3 mL. This elution sequence is programmed for automated operation by the AutoTrace workstation. Eluting with three aliquots improves analyte recoveries compared to continuous elution with a single 13-mL aliquot. Start the "E" elution portion of the AutoTrace sequence. Note: If performing this method manually, do not let the cartridge beds dry. When the elutions of the three aliquots for each sample are completed, the AutoTrace will emit an audible signal. Remove the "E" extract tubes from the AutoTrace rack, cover with foil, and set aside in a refrigerator until it is time for the concentration step.

7.3.8.3 The fluid-flow paths of the AutoTrace workstation consist of polytetrafluoroethylene tubing, which may adsorb nonpolar analytes. A postextraction cleaning of the AutoTrace workstation (or manual SPE extraction apparatus) is therefore required and should be performed immediately after elution. Discard the SPE sample cartridges, and seal the empty adapter cartridges into the elution stations. Wash each of the six AutoTrace concentration and elution stations with 50 mL of Liquinox detergent solution, 50 mL of water, and 50 mL of methanol, at a flow rate of 20 mL/min.

7.3.9 Concentration

7.3.9.1 Before concentration, add 10 µL of a 10-percent w/v sodium hydroxide solution (5.2.3) to the "E" fractions of the samples. This is added to neutralize the TFA in the eluent and reduce potential analyte degradation during volume reduction.

7.3.9.2 The two fractions are reduced in volume under a nitrogen gas vortex stream. To reduce volume, place the "M" and "E" fraction tubes into the TurboVap sample concentration apparatus. Samples are concentrated under a nitrogen gas stream of 69 kPa (10 lb/in^2) while kept at 34°C in a water bath. The volume of the "E" fraction is about 13 mL, which nearly fills the 15-mL volume of the test tube. The nitrogen gas pressure is therefore increased slowly from 0 to 69 kPa, while monitoring the behavior of the fractions in the TurboVap. This prevents ejecting a portion of the "E" extract from the tube into the TurboVap water bath or into other samples, which might cause cross-contamination. When tubes are initially placed in the TurboVap, they are spaced apart as much as possible to minimize potential cross-contamination. TurboVap nozzles should be cleaned between samples. Note that the settings for bath temperature and nitrogen pressure are optimized for analyte recovery within a reasonable total time for extract volume reduction. Exceeding the specified temperature or pressure settings will adversely affect compound recoveries.

7.3.9.3 The "M" fraction will take 30 minutes to concentrate to about 400 µL because of the volume of water in the fraction. Carefully monitor the fraction reduction and do not allow the extracts to dry, or analyte recoveries will be adversely affected. Take the "M" fraction out of the TurboVap after 30 minutes of concentration time and cover with foil. Set the "M" fraction aside in the refrigerator until the vialing process is to begin.

7.3.9.4 The "E" fraction will normally take 45 to 60 minutes to concentrate. Allow the "E" fraction to concentrate for 30 minutes along with the "M" extract. After the "M" extract has been concentrated and removed from the TurboVap, continue to concentrate the "E" fraction for an additional 15 minutes. Concentrate the sample to about 400 µL. Carefully monitor the fraction reduction and do not allow extract volume to decrease to less than 400 µL, or analyte recoveries will be adversely affected. If the extract has been concentrated for 60 minutes and the extracts

are greater than 400 µL, however, there may be residual methylene chloride in the "E" extract. If this occurs, go to step 7.3.9.5. If there is no residual methylene chloride in the "E" extract, then the concentrated sample is ready to be combined with the "M" extract and to be placed in a sample vial for instrumental analysis.

7.3.9.5 If the "E" fraction contains methylene chloride, it must be removed to avoid altering the chromatographic separation of analytes and decreased analyte recoveries. Typically, methylene chloride is present as an immiscible layer underneath the aqueous phase or as small bubbles at the bottom of the test tube, or as a cloudy or "milky" appearance. As this description indicates, the presence of methylene chloride in a sample may be barely perceptible, so each sample must be carefully examined for the presence of methylene chloride. If a test tube is determined to contain methylene chloride, evaporate the volume in the test tube to about 400 µL. Then add about 200 to 300 µL of organic-free water and mix with a vortexing mixer. Evaporate the liquid in the test tube down to 400 µL again. Repeat this process until there is no more methylene chloride in the test tube. Note for each sample where treatment to remove residual methylene chloride is required.

7.3.10 Sample transfer into vials and preparation prior to analysis

7.3.10.1 Use clean and pre-ashed 1.5-mL amber screw-top autosampler vials.

7.3.10.2 Label each vial with appropriate lab identification and set number. This information is important for evaluating individual sample results by comparison to set quality-control samples (duplicates, set blanks, set reagent fortifications).

7.3.10.3 Pair the "M" and "E" extracts for each sample. Briefly swirl the "M" fractions with a vortexing mixer to wash the test tube walls. Transfer the contents of the "M" extract tube into the "E" extract tube by using a silicone-rubber bulb and a separate cleaned and burned disposable Pasteur pipet (4.7.5) for each sample. If the sample appears "cloudy," methylene chloride may be present and the sample will need further concentration to remove it (section 7.3.9.5). Mix the combined "M" and "E" fractions with a vortexing mixer and transfer the extract into the appropriate sample vial by using the same pipet that was used for combining the two fractions. Repeat this procedure for the entire sample set.

7.3.10.4 Ensure that the final combined extract volume is about 1,000 µL by comparison to a vial with a known volume of liquid. An exact volume is not required because quantitation is by internal standard. If the final sample volume is less than 900 µL, dilute to volume with organic-free water. Seal vials with screw cap by ensuring a tight seal but not so tight that the Teflon-lined septum is puckered or wrinkled, which can result in evaporation of the sample. Place the vials in a vial tray, organized by set. Store sample extracts in a freezer at –14ºC until analyzed.

7.3.10.5 Just prior to analysis, inject 5 µL of the internal standard solution (5.4.5) into each vial through the septa, using a dedicated 10-µL syringe (4.6.1). The internal standard is added to all samples, including the LRS and the LRB. The internal standard solution contains the internal standards for both positive and negative analysis, and is only added once. The same volume of internal standard is added to the instrument calibration solutions and continuing calibration verification solutions analyzed with the environmental samples. These samples and the sequence they are analyzed in are discussed in section 7.4.2.5.2.

7.4 Instrumental analysis

7.4.1 Instrumental analysis overview—The analytes contained in sample extracts are separated by HPLC with a reverse-phase octadecylsilane column and water:acetonitrile gradient elution. The separated components are transported in a flowing stream to the electrospray ionization interface. In the interface, compounds, solvent, and any coeluting components of the sample matrix are nebulized into small droplets and desolvated. During desolvation, compounds are ionized by charge adduction, ion evaporation, or a combination of protonating processes (Kebarle and Ho, 1997). The ionized compounds are separated from the neutral compounds by means of a ≅3 kV potential difference between the nebulizing electrospray needle and the aperture leading to the mass analyzer. The nebulized neutral compounds do not enter the mass spectrometer, but rather they condense with the solvent and drain to a waste reservoir.

The ions transit through the capillary aperture, and, as they exit the capillary to a reduced (1 to 2 millitorr) pressure region, are subject to an accelerating voltage (called a fragmentor voltage or capillary exit voltage by some manufacturers). This accelerating voltage provides the ions with sufficient momentum to induce fragmentation by collision with neutral nitrogen gas molecules. The ionized fragments are swept from this low-pressure region to the mass spectrometer by momentum from a pressure difference between the low-pressure region and the analyzer region. Electrostatic lenses collimate the ions, and a quadrupole mass analyzer is used selectively to transport ions of a specific mass-to-charge ratio to a continuous electron multiplier. The current induced by the impact of the ion on the multiplier surface is amplified and transmitted as a voltage signal, with the number of ions proportional to the total voltage of the signal. Time-programmed, selected-ion monitoring is used to maximize detectability of specific ions and reduce chemical noise from coeluting interferences.

Coordinated, automated computerized programming is used to control most aspects of chromatographic separation, ionization, fragmentation, ion focusing, mass analysis, detection, and data handling. A typical separation of a standard mixture of the compounds determined under positive ionization conditions is shown in figure 1. Note that coeluting peaks are not distinguished because this is a reconstructed ion chromatogram of selected-ion monitoring results. These coeluting peaks would be separated and identified by using Target Software or equivalent automated graphic data-handling software. A similar chromatogram can be produced for compounds eluting under negative ionization conditions.

7.4.2 High-performance liquid chromatographic separation

7.4.2.1 Sample vials are placed in the autosampler of either a Hewlett-Packard 1090 Series II HPLC or a Hewlett-Packard/Agilent Technologies Series 1100 HPLC. The autosampler temperature is kept at 4ºC either by recirculating fluid chiller (for the HP 1090 Series II HPLC; Neslab Coolflow CFT-33 or equivalent) or by a Peltier cooling unit (Hewlett-Packard/Agilent Technologies 1100 Series HPLC autosampler). A 50-µL aliquot of the sample extract is injected into the HPLC eluent stream to start separation.

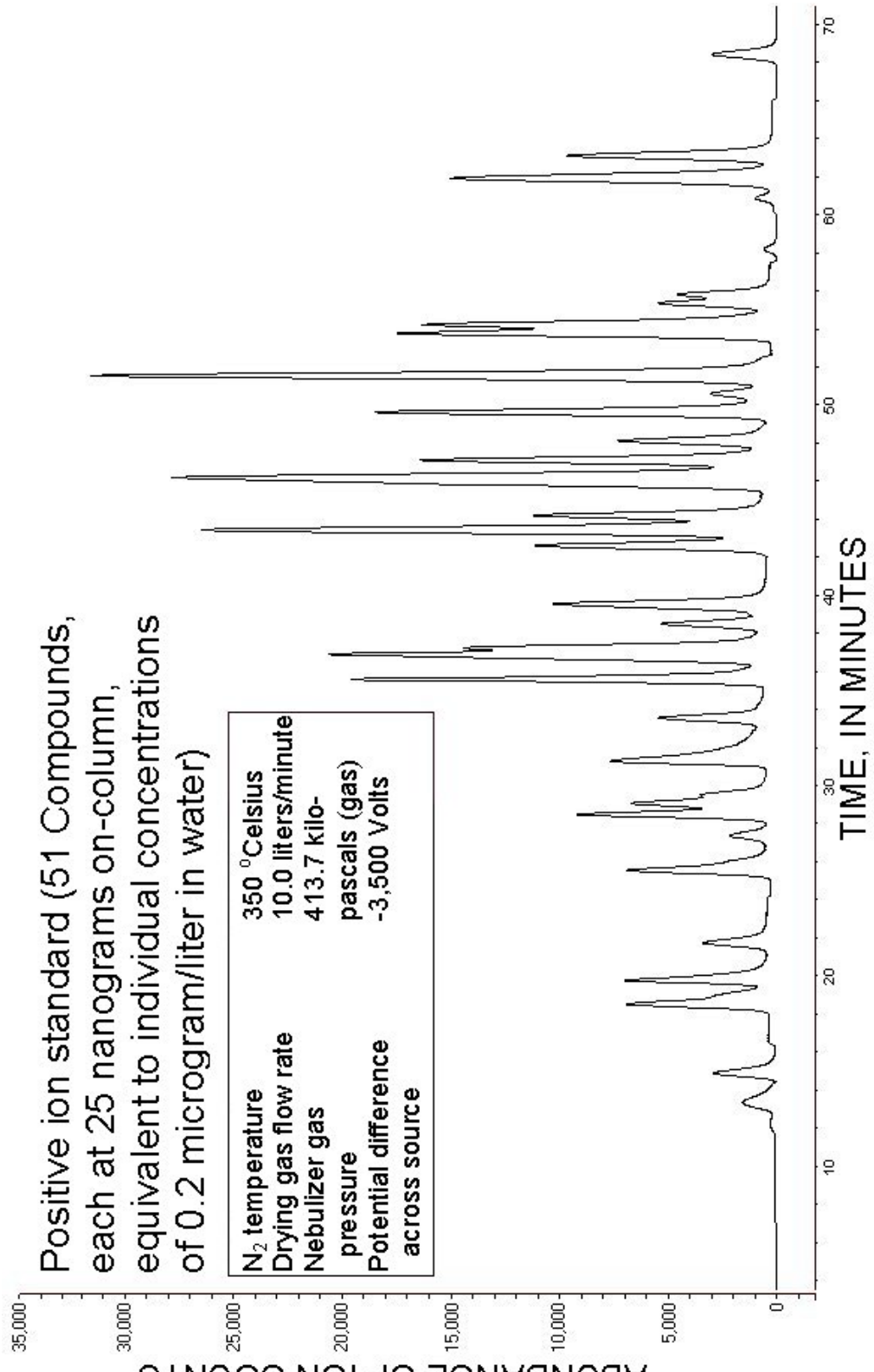

Figure 1. Chromatogram of a positive ion standard used in this method.

7.4.2.2 The analytical separation for the method is carried out by using a reverse-phase octadecylsilane column (MetaChem Technologies Inertsil ODS-3, particle size of 5 µm, column dimensions of 2 mm inside diameter by 150 mm long or equivalent). An integral guard column (MetaChem Technologies Metaguard Inertsil ODS-3, particle size of 5 µm, 2 mm inside diameter or equivalent) also is used.

7.4.2.3 The separation is carried out using a binary eluent system of (1) organic-free water modified with ammonium acetate and formic acid (3.6 millimolar ammonium acetate; 5.22 millimolar formic acid; Mobile Phase A; see section 5.3.2 for preparation), and (2) acetonitrile modified with ammonium acetate (3.6 millimolar; Mobile Phase B; see section 5.3.1 for preparation). Initial HPLC conditions follow: Autosampler, 4°C; column oven, 40°C; binary mobile phases (Mobile Phases A and B; sections 5.3.1 and 5.3.2). The combined mobile-phase flow rate is constant at 0.20 mL/min. The mobile-phase gradient used for both positive and negative ionization analyses is listed in table 7. Each HPLC analysis requires 85 minutes to complete, including a post-analysis column re-equilibration period of 11 minutes. HPLC separation and mass spectrometric (MS) analysis are synchronized by computer control at the start of each analysis. For each HPLC/MS system, specific elution compositions and times are tested iteratively to achieve optimal separation, so the specific times and mobile-phase compositions listed in table 7 should be used as a starting point for developing an acceptable separation.

Table 7. High-performance liquid chromatograph mobile-phase gradient for this method

Time, in minutes	Percentage of mobile phase A	Percentage of mobile phase B
0.00	96.0	4.0
0.50	96.0	4.0
20.50	79.0	21.0
63.50	32.4	67.6
68.00	0.0	100.0
72.00	0.0	100.0
74.00	96.0	4.0

7.4.2.4 Mass spectral analysis parameters

7.4.2.4.1 *Ionization source operating conditions*—The mass spectrometer ionization source conditions for both positive and negative ionization analyses are listed in table 8. These conditions are held constant during the analysis.

7.4.2.4.2 *Programmable conditions during positive ion analysis*—The computer-controlled aspects of mass spectrometer operation during positive ion analysis are listed in table 9a. These time-programming conditions are synchronized with the HPLC programming at the start of each analysis.

Table 8. Mass spectrometer operating conditions during positive and negative ionization analyses used in this method

Nitrogen dry gas temperature	350 degrees Celsius
Drying gas flow rate	10.0 liters per minute
Nebulizer gas pressure	212 to 414 kilopascals [30 to 60 pounds per square inch (gas); optimized for each instrument]
Potential difference between nebulizer and capillary	-3,500 volts (polarity of voltage reverses for negative ions)

Table 9a. Mass spectrometer time-programmed operating conditions for individual compounds determined under positive ionization conditions

[m/z, mass-to-charge ratio]

Time, in minutes	Selected-ion group[1]	Selected-ion monitoring ion mass, in m/z	Typical electrometer gain	Fragmentor voltage, in volts	Individual ion dwell time, in milliseconds
1.00	1	142	2	70	400
7.00	2	106, 108, 132, 133 163, 164, 185,207	2	50	48
16.11	3	106, 163, 174, 175 176, 195, 196, 198 199, 220, 223, 237 238, 240	2	40	27
22.70	4	146, 160, 161, 163 165, 166, 175, 181 188, 190, 192, 198 199, 209, 220, 256 312, 313, 326, 348	2	80	18
33.71	5	100, 116, 128, 151 167, 168, 172, 179 185, 187, 205, 213 229, 230, 236, 290 291, 312, 313, 411 412, 413	2	70	16
38.80	6	144, 161, 163, 199 201, 205, 207, 290 291, 312	2	90	43
41.92	7	111, 145, 146, 153 165, 167, 168, 202 216, 217, 218, 220 222, 223, 224, 233 234, 248, 280, 290 291, 365, 366	2	60	15
49.79	8	120, 134, 138, 149 155, 182, 220, 222 240, 241, 290, 291 304, 305, 306, 396 411	2	80	22
53.63	9	121, 122, 137, 160 169, 186, 213, 233 234, 249, 251, 415	2	75	31
57.79	10	160, 249, 251, 252 253, 254	2	35	65
60.56	11	114, 143, 160, 178, 249, 251, 275 277, 305, 342, 343 344, 347	2	85	29
67.03	12	134, 154, 216	2	80	132

[1]Selected-ion group number refers to specific-mass charge ratios monitored in a time interval.

7.4.2.4.3 *Programmable conditions during negative ion analysis*—The computer-controlled aspects of mass spectrometer operation during negative ion analysis are listed in table 9b. These time-programming conditions are synchronized with the HPLC programming at the start of each analysis.

7.4.2.5 Mass spectrometer tuning and calibration

7.4.2.5.1 *Mass spectrometer autotuning*—Prior to any analysis, the mass spectrometer is brought to temperature and gas pressure equilibrium, then tuned to ensure accurate mass assignment and a minimum detector response. An automated tuning (autotuning) procedure is used, with proprietary tuning solutions (provided by the instrument manufacturer) for positive and negative ion analysis. The autotune procedure uses a proprietary algorithm that combines the gain and voltage applied to the instrument electron multiplier to produce the minimal acceptable ion current across the mass range of the mass spectrometer.

The ions that must be present are listed in table 10, and the target peak width for acceptable tuning in positive and negative ion modes is listed in table 11. Note that for both positive and negative ion modes, the autotune mass axis calibration must be within 0.13 atomic mass units (amu). In addition, note that although a typical electrometer gain is listed in tables 9a and 9b, the appropriate electrometer gain varies with the condition and age of the electron multiplier in the mass spectrometer, and the electrometer gain listed should be used as a starting point.

Table 9b. Mass spectrometer time-programmed operating conditions for individual compounds determined under negative ionization conditions

[m/z, mass-to-charge ratio]

Time, in minutes	Selected-ion group[1]	Selected-ion monitoring ion mass, in m/z	Typical electrometer gain	Fragmentor voltage, in volts	Individual ion dwell time, in milliseconds
7.00	1	146, 190, 192	2	45	192
13.00	2	195, 239, 241	2	45	192
18.00	3	175, 177	2	45	289
30.00	4	239, 240, 241	2	45	192
37.00	5	141, 161, 195, 196 198, 199, 201, 203 218, 219, 221, 233 235, 253, 255, 271 273, 274, 275, 276 278	2	45	26
49.00	6	141, 143, 161, 163 217, 219, 227, 239 240 245, 247, 316, 318 360	2	45	43

[1]Selected-ion group number refers to specific-mass charge ratios monitored in a time interval.

Table 10. Quantitation and confirmation ions used for the compounds determined in this method

[Note that the absence of an ion indicates that fewer than three ions are used for quantitation and confirmation.]

Compound	Retention time, in minutes	Quantitation ion	Primary confirmation ion	Secondary confirmation ion
Compounds analyzed under positive ion conditions				
2,4-D methyl ester	57.47	252	254	
2-Hydroxyatrazine	20.89	198	199	
3(4-chlorophenyl)-1-methyl urea	35.85	185	128	187
3-Hydroxycarbofuran	27.88	163	181	220
3-Ketocarbofuran	36.66	179	236	151
Aldicarb	35.51	116	213	
Aldicarb sulfone	17.35	240	223	
Aldicarb sulfoxide	13.71	132	207	
Atrazine	45.67	216	218	217
Barban (Internal Std)	60.09	178	143	
Bendiocarb	42.82	167	224	
Benomyl	24.57	192	160	161
Bensulfuron-methly	50.75	411	149	182
Bromacil	37.92	205	207	
Caffeine	18.67	195	196	
Caffeine C13 (internal standard)	18.66	198		
Carbaryl	45.14	145	202	146
Carbofuran	42.80	222	165	223
Chloramben methyl ester	50.19	220	222	
Chlorimuron-ethyl	55.05	415	186	
Cycloate	67.94	216	134	154
Deethylatrazine	28.43	188	190	146
Deethyldeisopropylatrazine	5.11	142		
Deisopropylatrazine	20.79	174	176	175
Diphenamid	50.94	240	241	134
Diuron	47.35	233	234	
Fenuron	26.59	165	166	
Flumetsulam	32.67	326	348	
Fluometuron	45.56	233	234	
Imazaquin	35.02	312	313	
Imazethapyr	31.96	290	291	
Imidicloprid	28.65	256	175	209
Linuron	54.59	249	251	160
Metalaxyl	46.62	280	248	220
Methiocarb	53.54	169	121	122
Methomyl	18.94	163	106	
Methomyl oxime	10.51	106	108	
Metsulfuron-methyl	38.83	167	168	
Monuron (surrogate)	38.68	199	201	
Neburon	62.47	275	277	114
Nicosulfuron	36.61	411	213	
Norflurazon	48.98	304	306	305
Oryzalin	60.83	347	305	
Oxamyl	17.97	237	220	238
Oxamyl oxime	12.06	163	185	164

Table 10. Quantitation and confirmation ions used for the compounds determined in this method—Continued

Compound	Retention time, in minutes	Quantitation ion	Primary confirmation ion	Secondary confirmation ion
Compounds analyzed under positive ion conditions—Continued				
Propham	49.88	138	120	
Propiconazole	61.33	342	344	343
Propoxur	42.10	168	111	153
Siduron	53.06	233	234	137
Sulfometuron-methyl	43.45	365	366	
Tebuthiuron	36.47	229	172	230
Terbacil	38.91	161	163	144
Tribenuron-methyl	50.53	155	396	
Compounds analyzed under negative ion conditions				
2,4,5-T	47.38	253	255	195
2,4-D	42.07	219	221	161
2,4-DB	55.53	161	163	
Acifluorfen	56.97	316	360	318
Bentazon	37.02	239	240	241
Bromoxynil	45.95	276	278	274
Chlorothalonil	62.65	245	247	
Clopyralid	13.66	190	146	192
Dacthal, monoacid	42.08	273	271	275
DCAA	60.70	217	219	
Dicamba	28.59	175	177	
Dichlorprop	45.89	233	235	161
Dinoseb	62.17	239	240	
MCPA	42.50	199	141	201
MCPB	55.89	141	143	
Picloram	19.73	241	239	195
Triclopyr	44.13	196	198	218

Table 11. Autotune parameters for acceptable tuning criteria used in this method

[m/z, mass-to-charge ratio; amu, atomic mass unit]

Tune ion mass, m/z	Target peak width, amu
(A) Tuning paramaters for positive ion mode	
118.08	0.65
622.03	.65
922.05	.65
1,521.95	.65
2,121.95	.71
(B) Tuning parameters for negative ion mode	
112.99	0.65
601.98	.65
1,033.99	.65
1,633.95	.65
2,233.91	.74

The signal intensity of the system is further optimized by using the ion at a mass-to-charge ratio of 622.03 as part of the autotune process. A compensated gain-voltage calibration curve is produced, and the appropriate gain and electron multiplier voltages produce a manufacturer-defined minimum ion abundance of 5×10^4 to 1×10^6 (manufacturer's arbitrary units). This process is automatic and is written to the autotune file. The autotune file also contains all the pertinent instrument settings for mass axis calibration and peak width. For other manufacturers' HPLC/MS systems, the

appropriate manual or automated tuning procedure is used to meet instrument specifications for calibration and minimal ion signal. Regardless of the HPLC/MS system used, electronic and paper copies of tuning conditions should be stored chronologically to monitor long-term HPLC/MS system performance, assist in determining if maintenance or repairs are required, and allow comparison of samples analyzed over extended (greater than 6 months) periods.

The mass spectrometer performance needs to meet manufacturers' specifications for peak width, mass axis, calibration, and minimum acceptable ion signal intensity. If it does, proceed to calibrate quantitatively. If the performance is not acceptable, however, then diagnostic, preventative or corrective procedures might be required. Manufacturer-supplied diagnostic procedures are used to identify and correct any autotune-identified problems. Following any corrections, repeat the autotune procedure to verify that the corrections result in acceptable instrument performance. When acceptable mass spectrometer mass axis and signal intensity have been achieved, as indicated by a successful autotune, the instrument can be calibrated for quantitative analysis.

7.4.2.5.2 *Quantitative calibration*—A multiple concentration calibration for quantitative analysis is carried out for all compounds after acceptable mass spectrometer tuning is completed. The seven concentrations for this calibration are listed in table 6 of section 5.4.6.3 and are the same for positive and negative ion analysis. The seven calibration concentrations are analyzed sequentially, and calibration curves are produced. A minimum of four quantitation levels must be used to determine the calibration curve, and the curve should have a correlation coefficient (r^2) greater than 0.995. Most compounds will exhibit linear calibration curves with acceptable r^2 values, but some analytes (typically atrazine, deethyl-atrazine, deisopropylatrazine, deethyl-deisopropylatrazine, and 2-hydroxyatrazine) require quadratic curve fitting to achieve acceptable r^2 values. If one or two of the calibration curve standard levels are not used, there must be a legitimate reason for dropping the point from the curve, such as an incomplete or inaccurate injection or some evidence that standard solution quality has fallen below acceptable levels. Corrective actions, such as preparing new standards, also would be required. Note that two analytes, 2-hydroxyatrazine and deethyldsisopropyl-atrazine, require separate calibration curves. A limited set of three to four calibration points is used because both compounds are always reported as estimates. This second calibration is calculated for positive ion analysis only.

If the initial calibration is acceptable, the set(s) of environmental samples, set quality-control (QC) samples, and instrument QC samples are combined into a batch and analyzed. A batch typically consists of up to six environmental sample sets of 10 samples each, the associated set QC samples, and instrumental QC samples to monitor performance. Note that analysis of the entire analytical batch can require up to a week or more of continuous instrument operation. As a result, QC data need to be reviewed during the analysis of the batch to ensure acceptable instrument operation throughout the analysis.

Instrumental QC samples are interspersed between environmental and set QC samples. The presence of interspersed instrumental QC samples allows use of part of the data collected in the batch analysis in case of calibration problems or instrument failure during the batch sequence. The first instrument QC sample type used to monitor batch performance is the continuing calibration verification (CCV) sample. The CCVs ensure ongoing acceptable calibration performance during analysis of the batch.

When CCV results vary by more than 30 percent, data are reported as estimates; if the CCV results vary by more than 40 percent for five analytes, a new calibration is required. A continuing calibration blank (CCB) follows each CCV, and is used to monitor possible cross-contamination between injections as a result of incomplete injection or insufficient injection-needle washing. Typical batch analytical sequences for positive and negative ion analyses are listed in table 12. Note that this sequence includes analyses for producing a calibration curve. The sequence would be adjusted if a calibration curve was not required. Note also that the position of the reagent blank in the preparation set is varied to monitor for position-specific contamination. As a result, the position of the set reagent blank can vary within the instrument analytical sequence.

7.4.2.6 Evaluation of instrument analysis results

7.4.2.6.1 The QC and environmental sample data are reviewed to ensure that they meet acceptance criteria upon completion or partial completion of the analysis of a batch (partial completion in the case of a CCV or CCB failure). These data are reviewed qualitatively and quantitatively to ensure that (1) detected compounds are correctly identified, and (2) the quantified concentrations are correctly calculated.

7.4.3 *Qualitative determination*—Several criteria are used to determine that a compound identification is qualitatively correct. Correct identification is based on (1) the time at which the apex of the chromatographic peak elutes from the HPLC into the MS, (2) the presence of up to three compound-specific ions in the selected-ion monitoring mass spectrum (table 10), and (3) the relative abundances of these ions in the selected-ion monitoring signal and mass spectra. These criteria are determined from analysis of authentic standards, and are verified by analysis of standards in each batch, to compensate for long-term changes in the HPLC/MS system. Compounds are qualitatively detected when the following criteria are met:

- **Retention time**—The intensities of the characteristic ions of a compound are at a maximum that should coincide within ± 0.1 minute of the retention time of the selected compound. In addition, the maxima of the primary (quantification) ion and secondary and tertiary (qualification) ions should be within 0.05 minute of each other. Matrix effects and sample-to-sample pH variations can have a substantial influence on liquid chromatographic retention times, thereby resulting in substantial variations of absolute retention time reproducibility, which also can be compound-dependent. An absolute retention-time criterion is therefore evaluated in comparison to previously analyzed standards and samples, and with cognizance of known problems that can result in retention-time variations.

- **Spectra**—The identity of each compound is verified by comparing the selected-ion monitoring spectrum of the suspected compound with a reference selected-ion monitoring spectrum obtained from a standard for that compound analyzed in the same batch. Two criteria apply. First, the same two or three ions must be present in the reference and sample spectra, although there may be additional ions present if ions for additional compounds are collected

Table 12. Typical minimum sample analysis sequence for high-performance liquid chromatography/mass spectrometry analysis in this method

[*, indicates quality-control samples analyzed in positive ion analysis only; µg/L, microgram per liter]

Injection number	Quality-control or environmental-sample type
1	Continuing calibration blank (ultrapure solvent)
2	0.005 µg/L concentration standard
3	0.01 µg/L concentration standard
4	0.05 µg/L concentration standard
5	0.10 µg/L concentration standard
6	0.20 µg/L concentration standard
7	0.50 µg/L concentration standard
8	1.00 µg/L concentration standard
9	0.05 µg/L concentration atrazine-degradate standard*
10	0.20 µg/L concentration atrazine-degradate standard*
11	0.50 µg/L concentration atrazine-degradate standard*
12	1.00 µg/L concentration atrazine-degradate standard*
13	Continuing calibration blank (ultrapure solvent)
14	0.175 µg/L concentration third-party check standard
15	Environmental sample- #1
16	Environmental sample- # 2
17	Environmental sample- #3
18	Environmental sample- #4
19	Environmental sample- # 5
20	Environmental sample- #6
21	Environmental sample- #7
22	Environmental sample- #8
23	Environmental sample- #9
24	Environmental sample- #10
25	Set quality control sample- #11 (typically set reagent blank, but can vary in sequence position)
26	Set quality control sample- #12 (typically set reagent spike)
27	0.20 µg/L continuing calibration verification standard
28–39	Twelve sequence entries for environmental/quality control samples (#13–#24)
40	0.20 µg/L continuing calibration verification standard
41	Continuing calibration blank (ultrapure solvent)
42–53	Twelve sequence entries for environmental/quality control samples (#25–#36)
54	0.20 µg/L continuing calibration verification standard
55–66	Twelve sequence entries for environmental/quality control samples (#37–#48)
67	0.20 µg/L continuing calibration verification standard
68	Continuing calibration blank (ultrapure solvent)
69-80	Twelve sequence entries for environmental/quality control samples (#49–#60)
81	0.20 µg/L continuing calibration verification standard
82–93	Twelve sequence entries for environmental/quality control samples (#61–#72)
94	0.20 µg/L continuing calibration verification standard
95	0.20 µg/L concentration atrazine-degradate standard*
96	0.05 µg/L limit of quantitation standard
97	0.05 µg/L limit of quantitation atrazine-degradate standard*
98	Continuing calibration blank (ultrapure solvent)

concurrently. Second, the areas of these ions, determined from integrated peak areas of mass chromatograms, must be within 20 percent of the absolute ratios obtained on injection of a standard solution generated using the conditions of this method. Meeting these criteria for qualitative identification requires careful consideration. One must determine whether the abundances in the selected-ion profiles are appropriate and if the profiles have relative intensities that are consistent with the reference mass spectrum, or if there are contributions to the relative abundances resulting from interference. Experience and training are necessary to recognize the salient features of individual mass spectra and potential interferences. Exercise careful judgment in making a qualitative identification, given the variability inherent in identifying compounds at low concentrations in environmental samples.

7.4.4 *Quantitative determinations*—When a compound is qualitatively determined to be present, a quantitative determination of the compound concentration can then be made. The concentration of that compound will be based on the integrated area from the primary quantitation ion of that compound, the regression line fitted to the initial calibration curve, the area of the internal standard in the sample, and the internal standard response factors relative to the internal standard response factor from the calibration standards. This method typically uses linear-fitted curves. For atrazine and the atrazine degradates measured in this method, a quadratic fitted curve is used for more accurate concentration determination across the method calibration range. In practice, quadratic and linear curves provide equally acceptable results, and a quadratic curve can be used for all compounds if found to be more practical.

7.4.5 *Analysis of dilutions*—Samples must be analyzed within the range of the calibration curve. In environmental samples, compound responses that exceed the response of the highest standard in the calibration curve, 1.0 µg/L, should be brought within the range of the calibration curve by diluting the extract, using the initial calculated concentration as a guide for determining the appropriate dilution volume, and then reanalyzing. For example, an undiluted sample with an initial calculated concentration of 5.0 µg/L could be diluted to 10 percent of its original concentration, so a predicted concentration of the diluted extract should be within the calibration range of the method.

8. Calculations

In this method, the calculation of a final concentration of a polar organic compound (POC) in a filtered water sample requires multiple calculations, as follows.

8.1 Calculate the relative response factors for each POC from the calibration analyses conducted in 7.4.2.5.2 by using a best-fit linear regression or quadratic fit model. Rearrange the equation of the linear form $y=mx + b$ to $m = (y-b)/x$ as follows:

$$RRF_c = \left[\frac{\left(\frac{area_c}{area_{is}} \right) - b}{\left(\frac{amt_c}{amt_{is}} \right)} \right] \qquad (5)$$

where RRF_c = the relative response factor for the polar organic compound (POC) of interest;

$area_c$ = the integrated peak area of the POC of interest;

$area_{is}$ = the integrated peak area of the positive or negative ionization internal standard used for the POC of interest;

amt_c = the mass of the POC of interest, in nanograms;

amt_{is} = the mass in nanograms of the POC internal standard (see section 5.4.5) used for the POC of interest; and

b = the y-intercept of the best-fit linear regression line.

NOTE: A similar calculation can be made for fitted quadratic curve calibrations by rearranging the equation $y=ax^2+bx+c$, where a, b, and c are experimental constants determined from the fitted curve by iterative mathematical extraction with curve-fitting software.

8.2 Calculate the volume of water extracted, in liters (V_S):

$$V_S = (V_i - V_f)/1{,}000 \qquad (6)$$

where V_i = initial weight of sample and sample bottle, in grams (≡mL; 7.3.7);

V_f = final weight of sample and sample bottle, in grams (≡mL; 7.3.7); and

1,000 = conversion factor for milliliters to liters.

NOTE: This procedure assumes that the volumetric density of a typical freshwater sample is 1 g/mL. For samples collected from saline environments, a salinity or density determination should be made and a volume correction applied (see 7.3.7).

8.3 Calculate sample polar organic compound concentrations

If the compound of interest has met the qualitative identification criteria listed in 7.4.3, calculate the compound concentration in the sample as follows:

$$C = \left(\frac{amt_{is} \times A_c}{RRF_c \times A_{is} \times V_s} \right) \qquad (7)$$

where C = the concentration of the compound of interest in the sample, in micrograms per liter;

amt_{IS} = the mass of internal standard added to the sample, in micrograms;

A_C = the area of the quantitation ion for the compound of interest;

RRF_C = the relative response factor for the compound of interest, calculated above in 8.1;

A_{IS} = the area of the quantitation ion for the internal standard; and

V_S = the volume of sample extracted, in liters, calculated in 8.2 (equation 6).

8.4 Calculate the percentage recovery of the surrogate compounds in each sample by using

$$R_a = \left[\frac{C_s}{(C_a \times V_a)/V_s} \right] \times 100 \qquad (8)$$

where R_a = recovery of surrogate in sample, in percent;

C_s = concentration of surrogate in sample, in micrograms per liter, calculated by using equation 7;

C_a = concentration of compound in the surrogate solution added to the sample, in micrograms per microliter (5.4.3);

V_a = volume of POC surrogate solution added to the sample, typically 100 µL (7.3.1); and

V_s = volume of sample, in liters (calculated in 8.2).

8.5 Calculate the percentage recovery of compounds in set reagent spike sample by using

$$R_b = \left[\frac{C_s}{(C_b \times V_b)/V_s} \right] \times 100 \qquad (9)$$

where R_b = recovery of fortified compound in the set POC fortification sample, in percent;

C_s = concentration of compound in set reagent spike sample, in micrograms per liter, calculated using equation 7;

C_b = concentration of compound in reagent spike fortification solution added to sample, in micrograms per microliter (5.4.4);

V_b = volume of reagent spike fortification solution added to the sample, typically 100 µL (7.3.2); and

V_s = Set reagent spike sample volume, in liters (calculated in 8.2).

9. Reporting of Results

9.1 *Reporting units*—Report compound concentrations for field samples in micrograms per liter. Report compound concentrations for field samples to 4 decimal places, but no more than 3 significant figures. Report data for compounds reported as qualified estimates to 4 decimal places, but no more than 2 significant figures. Report surrogate data for each sample type as percent recovered, and report to 1 decimal place (tenths of a percent), but no more than 3 significant figures. Report data for the set fortification sample as percent recovered, and report to 1 decimal place (tenths of a percent), but no more than 3 significant figures. Compounds quantified in the set blank sample are reported in micrograms per liter, and are reported to 4 decimal places, but no more than 3 significant figures.

9.2 *Reporting limits and levels*—Method detection limits (MDLs) that use the procedures outlined by the U.S. Environmental Protection Agency (1997) have been calculated for this method and are discussed further in section 11.8. The interim laboratory reporting level (LRL) for each compound determined using this method is calculated according to Childress and others (1999) and is twice the method detection limit. Report qualitatively identified compound concentrations (those POCs that are identified from relative retention time and MS spectral fit) that are less than the MDL or less than the lowest calibration standard as estimated concentrations. Compounds that are not detected are reported as less than the interim LRL.

10. Quality Assurance/Quality Control

Laboratory extraction samples are formed into sets, each consisting of 10 environmental samples, a set reagent spike and set reagent blank, for a total of 12

samples. Field equipment blanks and laboratory matrix spikes, whose frequency is determined by the method user, provide additional quality assurance/quality control (QA/QC). The frequency of analysis of these QA/QC samples and the aspects of the analytical process they monitor are described.

10.1 *Surrogate*. Surrogates are organic compounds that are placed into all filtered water samples prior to extraction on the SPE cartridge. Surrogates are expected to behave similarly to selected compounds for SPE recovery and are not expected to be present in the environment. Three surrogates are used in this method, two (^{13}C-caffeine and Barban) for compounds determined under positive ionization conditions, and one (2,4,5-T), for compounds determined under negative ionization conditions. The herbicide 2,4,5-T had been previously used as a pesticide, although registrations for all uses in the United States have been canceled since 2 January 1985 (U.S. Environmental Protection Agency, 2000). In 12,064 surface- and ground-water samples, 2,4,5-T was detected 68 times using a similar analytical method (Werner and others, 1996) Given the limited number of appropriate negative ionization surrogates, 2,4,5-T was used as a surrogate because the frequency of environmental presence was very low, and the frequency of detections in the future is likely to decrease further.

Examination of surrogate recovery for individual samples provides insight into overall method performance for that particular sample. Control limits, determined by using statistical process control techniques and an extended sequence of laboratory reagent spike and reagent blank surrogate recoveries, are used to evaluate surrogate recoveries of individual samples.

10.2 *Laboratory reagent spike (LRS).* A 1-L organic-free water sample is fortified at 0.25 µg/L for all compounds determined in this method. This sample then is included with each sample set and is carried through the entire extraction, elution, and analytical procedure. The LRS recoveries reflect method performance in the absence of any environmental sample matrix. These results are used to determine if overall set recoveries are acceptable, or if there was a gross change in method performance in the set. Acceptability is defined from analysis of a series of LRS samples, typically 30 or more, processed by multiple operators, who used different instruments. Statistical process control analysis is used with these data to develop acceptance criteria.

10.3 *Laboratory reagent blank (LRB).* A 1-L organic-free water sample is fortified with method surrogates only. One LRB is included with each sample set and is carried through the entire extraction, elution, and analysis procedure. The LRB is used to monitor for impurities and contamination, and, because it follows the LRS in the sample instrumental analysis sequence, it also monitors for carryover between sample injections.

10.4 *Continuing calibration verification (CCV).* For each analysis type (positive ionization or negative ionization), a 0.25-µg/L calibration standard that contains all of the selected compounds, including surrogates and internal standards, is inserted in an autosampler vial and placed between every 12 environmental and set QA/QC samples throughout the HPLC/MS analysis. These CCV samples are used to ensure that the calibration of the HPLC/MS system is within acceptable limits, typically ±30 percent. If the control limits are exceeded, environmental samples that follow the last acceptable CCV are reanalyzed. Control limits for the CCV rarely were exceeded during the course of this study.

10.5 *Continuing calibration blank (CCB)*. A sample of organic-free water is

placed in an autosampler vial behind a CCV, with a typical frequency of one every third set of samples. The CCB follows the CCV, and thus monitors for potential injection-to-injection carryover, as well as instrumental contamination.

10.6 *Limit-of-quantitation (LOQ) standard*. The LOQ standard is an aliquot of the lowest concentration calibration solution used to develop the calibration curve (typically 0.005 µg/L; see section 5.4.6.3). The LOQ is analyzed at the end of a sample analytical sequence to verify that sufficient instrument sensitivity has been maintained throughout the sequence.

10.7 *Field equipment blank (FEB)*. A volume of organic-free water is processed exactly as environmental samples by using all appropriate on-site sampling equipment and techniques. This process includes bottles, compositing, splitting, and filtering. The FEB is processed at the start of sampling and then about every 15 to 20 samples. The FEB monitors for contamination or carryover, or both, resulting from field sampling and equipment cleaning techniques that could cause equipment contamination of environmental samples.

10.8 *Field matrix spike (FMS)*. The FMS is a duplicate environmental sample that is fortified at 0.25 µg/L for all compounds determined in this method. The unfortified duplicate is used to determine naturally present concentrations of any compounds measured in the sample. If concentrations of method compounds are determined, they are subtracted from the measured concentrations in the fortified sample. The corrected recoveries of method compounds are determined from the background concentration-subtracted results. The FMS measures the effects of the sample matrix on the recovery of method compounds. Several effects are possible, including matrix-enhanced compound degradation, matrix-introduced coeluting interferences, and matrix enhancement of compound concentration. The frequency of FMS analyses is determined by data-quality objectives.

11. Method Performance

This method was originally developed as a custom analytical method by the USGS and was put into routine use in June 1999 at the NWQL. As part of the initial custom method testing, compound recovery was determined in June 1999 in three water types at a fortified concentration of 0.100 µg/L. The three matrices were laboratory-produced, pesticide-free, organic-free water; a ground-water sample from a private domestic well; and a surface-water sample collected from the South Platte River in Denver, Colorado. The surface- and ground-water samples were collected following the precautions suggested in Shelton (1994) for avoiding sample contamination.

Upon acceptance of these results, the method was applied to surface- and ground-water samples collected across the United States from March 1999 to July 2000. Additional method validation samples were analyzed in February 2000 using the same water types and fortified at nominal concentrations of 0.025 and 0.50 µg/L during this period when the method was being used routinely for custom analysis. One compound, dichlorprop, which was not present in the initial 0.100 fortifications, was added to the spiking solutions after a suitable standard was obtained. The combined performance studies at fortifications of 0.025, 0.100, and 0.50 µg/L are used to evaluate method performance. Provisional MDLs were determined using the initial organic-free water data, fortified at 0.100 µg/L. Later MDL determinations were made using the results from organic-free water fortified at 0.025 µg/L for organic-free water and ground water, and at 0.050 µg/L for surface water.

As of July 2000, about 3,300 surface- and ground-water samples were analyzed with this method. The results for set quality-control samples (197 laboratory reagent blanks and 285 laboratory reagent spikes) were analyzed concurrently with these environmental samples, aggregated, and the data evaluated for long-term (multiple instrument, multiple operator) method performance.

11.1 Recoveries from fortified water samples

11.1.1 The performance of this method for the extraction and analysis of POCs was evaluated by adding aliquots of standard solutions to a minimum of six water samples and processing the fortified samples through the entire method. Two unfortified water samples were processed with each set of fortified samples to determine the concentrations of any POCs present in the water prior to spiking. Reagent spikes and unfortified laboratory reagent blank samples also were processed with each set. Three water types were used for evaluating method performance and are described below.

11.1.2 The ground-water sample was collected from a single-family domestic supply well near Evergreen, Colorado. Water was collected from the well after a sustained period of domestic use to minimize contributions of water that had been stored in a lined pressurization tank. The well penetrates 85 m into a fractured rock aquifer with minimal overlying soil. This well was part of a cooperative U.S. Geological Survey-Jefferson County, Colorado, ground-water monitoring program near Evergreen, Colorado (Schwartz, 1997). Water was collected into a precleaned, 40-L stainless-steel container and filtered in the laboratory using the procedure described in Sandstrom (1995). Samples were collected sequentially into individual, pre-ashed 1-L amber bottles for analysis. Water was collected several times from this site over the course of the study. Dissolved organic carbon (DOC) was not measured each time a water sample was collected at this site; instead, DOC concentrations were measured as part of the ground-water monitoring program described by Schwartz (1997).

11.1.3 The surface-water samples were collected from the South Platte River as it passes through metropolitan Denver, Colorado. The water quality of the South Platte River has been extensively studied; Litke and Kimbrough (1998) provide an overview. Grab samples of South Platte River water were collected in stainless-steel containers of either 10- or 40-L capacity that had been washed with soap and water and sequentially rinsed with water and solvent. The water samples were filtered using the procedure described in Sandstrom (1995), and split into individual 1-L aliquots in pre-ashed 1-L amber bottles for analysis. Over the course of the study, water was collected several times from two sites, one upstream and one downstream of Denver. The DOC concentrations were not measured on the individual samples used in this study. Median DOC concentrations for the South Platte River from 1993–1995 were 5.2 mg/L at Denver and 7.0 mg/L at Henderson, Colorado, on the basis of measurements made as part of the Survey's National Water-Quality Assessment (NAWQA) program. These data are summarized in Litke and Kimbrough (1998).

11.1.4 The pesticide-free, organic-free water was produced at the NWQL using a Solution 2000 water purification system (Model 2002AL, Solution Consultants, Inc., Jasper, Ga.). The following method was used. Laboratory-distilled water was introduced into a 1-µm activated carbon prefilter, passed through a series of ion exchange resin beds to remove dissolved inorganic constituents, followed by high-intensity UV radiation

oxidation to remove dissolved organic carbon, and then filtered through a 0.22-µm sterile filter. The organic-free water was dispensed into pre-ashed 1-L amber bottles for analysis. This water also was used for extraction set QC samples (laboratory reagent spikes and blanks). In these water samples, DOC was not determined, but routine monitoring of DOC in the water produced by this system did not detect DOC concentrations at the long-term method detection level of 0.016 mg/L.

11.2 Organic-free water fortification recovery results

11.2.1 Sets of 10 samples of organic-free water were fortified with a laboratory reagent spike solution (5.4.4) at concentrations of 0.025, 0.100, and 0.500 µg/L. The results are listed in tables 13 to 18. The mean, median, and the standard deviation of recovery for all compounds in the tables were calculated from the data for mean recoveries of individual compounds. No method compounds were detected in unspiked reagent water.

11.2.2 The mean positive ionization recovery results for compounds reported without qualification (tables 13, 15, and 17) were 103.5 ± 11.5, 92 ± 10.4, and 93.1 ± 9.0 percent at fortifications of 0.025, 0.100, and 0.500 µg/L, respectively. The median recoveries were similar to the means, 99.5, 88.0, and 93.0 percent, respectively. The mean positive ionization recovery results for compounds reported as qualified estimates were 87.9 ± 31.6, 89.3 ± 42.3, and 82.0 ± 26.1 percent at fortifications of 0.025, 0.100, and 0.500 µg/L, respectively. The median recoveries for qualified estimates also were similar, at 94.0, 88.0, and 88.5 percent, respectively.

11.2.3 Mean recoveries for compounds determined by negative ionization and reported without qualification (tables 14, 16, and 18) averaged slightly less than the positive ionization results, and were 76.9 ± 5.4, 84.7 ± 6.4, and 75.8 ± 17.4 percent, at fortifications of 0.025, 0.100, and 0.500 µg/L, respectively. The corresponding median recoveries were similar to the means, at 78.0, 85.0, and 78.0 percent, respectively. For negative ionization compounds reported as qualified estimates, mean recoveries decreased and were more variable, at 64.6 ± 10.7, 59.2 ± 27.5, and 64.4 ± 39.8 percent, respectively, with corresponding median recoveries of 65.0, 69.0, and 62 percent.

11.2.4 Average recoveries of all compounds reported without qualification at all three fortification levels varied about ±10.3 percent for compounds determined by positive ionization and ±9.7 percent for compounds determined by negative ionization. This result, however, does not reflect the analytical precision, which is better reflected by the relative standard deviation of individual compound recoveries in organic-free water, particularly those that are reported without quantitation qualification. Under positive ionization conditions, the mean relative standard deviations for compounds reported without qualification in organic-free water were 8.9 ± 4.8, 11 ± 3.4, and 6.3 ± 3.6 percent for 0.025-, 0.100-, and 0.500-µg/L fortifications, respectively. The corresponding relative standard deviations for negative ionization results reported without qualification were 10.4 ± 3.7, 13.1 ± 3.6, and 13.5 ± 10.5 percent for 0.025-, 0.100-, and 0.500-µg/L fortifications, respectively.

11.3 Ground-water fortification recovery results

11.3.1 Sets of 10 individual ground-water samples were fortified at concentrations of 0.025, 0.100, and 0.500 µg/L (tables 19 through 24). The mean, median, and the standard deviation of recovery for all compounds in the tables were calculated

Table 13. Accuracy and precision data from 10 determinations of the method compounds fortified at 0.025 microgram per liter in organic-free water, under positive ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)	Method detection limit (µg/L)
2,4-D methyl ester	N	0.0279	0.00136	5	111	0.0043
2-Hydroxyatrazine	Y	.0241	.00126	5	96	.0040
3(4-chlorophenyl)-1-methyl urea	N	.0283	.00382	14	113	.0121
3-Hydroxycarbofuran	N	.0236	.00092	4	94	.0029
3-Ketocarbofuran	Y	.0156	.00224	14	62	.0071
Aldicarb	Y	.0233	.00625	27	93	.0198
Aldicarb sulfone	Y	.0227	.00309	14	91	.0098
Aldicarb sulfoxide	Y	.0231	.00130	6	92	.0041
Atrazine	N	.0243	.00143	6	97	.0045
Bendiocarb	N	.0235	.00398	17	94	.0126
Benomyl	N	.0240	.00060	2	96	.0019
Bensulfuron-methyl	N	.0324	.00251	8	130	.0079
Bromacil	Y	.0265	.00514	19	106	.0163
Caffeine	N	.0236	.00150	6	95	.0048
Carbaryl	N	.0274	.00447	16	110	.0142
Carbofuran	N	.0235	.00090	4	94	.0028
Chloramben methyl ester	Y	.0130	.00282	22	52	.0089
Chlorimuron-ethyl	N	.0293	.00153	5	117	.0048
Cycloate	Y	.0220	.00206	9	88	.0065
Deethylatrazine	Y	.0288	.00444	15	115	.0141
Deethyldeisopropylatrazine	Y	.0436	.00702	16	175	.0222
Deisopropylatrazine	Y	.0241	.00164	7	96	.0052
Diphenamid	N	.0220	.00418	19	88	.0132
Diuron	N	.0253	.00235	9	101	.0075
Fenuron	N	.0275	.00498	18	110	.0158
Flumetsulam	Y	.0242	.00180	7	97	.0057
Fluometuron	N	.0282	.00488	17	113	.0155
Imazaquin	Y	.0236	.00247	10	94	.0078
Imazethapyr	Y	.0244	.00265	11	98	.0084
Imidicloprid	N	.0242	.00107	4	97	.0034
Linuron	N	.0277	.00228	8	111	.0072
Metalaxyl	N	.0241	.00315	13	96	.0100
Methiocarb	Y	.0242	.00127	5	97	.0040
Methomyl	Y	.0234	.00071	3	94	.0022
Methomyl oxime	Y	.0088	.00166	19	35	.0053
Metsulfuron-methyl	Y	.0237	.00387	16	95	.0123
Neburon	N	.0251	.00189	8	101	.0060
Nicosulfuron	N	.0244	.00205	8	98	.0065
Norflurazon	Y	.0263	.00259	10	105	.0082
Oryzalin	N	.0299	.00277	9	120	.0088
Oxamyl	N	.0222	.00194	9	89	.0061
Oxamyl oxime	Y	.0070	.00211	30	28	.0067
Propham	N	.0260	.00153	6	104	.0048
Propiconazole	N	.0318	.00332	10	127	.0105
Propoxur	N	.0223	.00127	6	89	.0040
Siduron	N	.0283	.00266	9	113	.0084
Sulfometuron-methyl	N	.0233	.00140	6	93	.0044
Tebuthiuron	N	.0241	.00098	4	97	.0031
Terbacil	Y	.0231	.00156	7	92	.0049
Tribenuron-methyl	Y	.0079	.00140	18	32	.0044

Table 14. Accuracy and precision data from 10 determinations of the method compounds fortified at 0.025 microgram per liter in organic-free water, under negative ionization

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)	Method detection limit (µg/L)
2,4-D	N	0.0239	0.00334	14	96	0.0109
2,4-DB	Y	.0207	.00245	12	83	.0080
Acifluorfen	N	.0232	.00101	4	93	.0033
Bentazon	Y	.0153	.00170	11	61	.0055
Bromoxynil	Y	.0151	.00260	17	60	.0085
Chlorothalonil	Y	.0147	.00531	36	59	.0173
Clopyralid	N	.0258	.00213	8	103	.0069
Dacthal monoacid	N	.0257	.00179	7	103	.0058
Dicamba	N	.0244	.00197	8	97	.0064
Dichlorprop	N	.0258	.00213	8	103	.0069
Dinoseb	N	.0064	.00184	29	26	.0060
MCPA	N	.0230	.00250	11	92	.0081
MCPB	Y	.0194	.00236	12	78	.0077
Picloram	N	.0230	.00303	13	92	.0099
Triclopyr	N	.0249	.00344	14	100	.0112

Table 15. Accuracy and precision data from seven determinations of the method compounds fortified at 0.10 microgram per liter in organic-free water, under positive ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D methyl ester	N	0.0800	0.01376	17	80
2-Hydroxyatrazine	Y	.1131	.03065	27	113
3(4-chlorophenyl)-1-methyl urea	N	.1003	.01455	15	100
3-Hydroxycarbofuran	N	.0924	.00991	11	92
3-Ketocarbofuran	Y	.0822	.01150	14	82
Aldicarb	Y	.0619	.01297	21	62
Aldicarb sulfone	Y	.0672	.02544	38	67
Aldicarb sulfoxide	Y	.0765	.00431	6	77
Atrazine	N	.0940	.01172	12	94
Bendiocarb	N	.0860	.00973	11	86
Benomyl	N	.0820	.00349	4	82
Bensulfuron-methyl	N	.0939	.00767	8	94
Bromacil	Y	.0944	.01283	14	94
Caffeine	N	.1094	.01280	12	109
Carbaryl	N	.0885	.00999	11	89
Carbofuran	N	.0850	.00901	11	85
Chloramben methyl ester	Y	.0939	.01812	19	94
Chlorimuron-ethyl	N	.0864	.00584	7	86
Cycloate	Y	.0720	.00864	12	72
Deethylatrazine	Y	.1089	.01381	13	109
Deethyldeiopropylatrazine	Y	.0882	.00953	11	88
Deisopropylatrazine	Y	.1063	.01173	11	106
Diphenamid	N	.0847	.00925	11	85
Diuron	N	.0918	.01262	14	92
Fenuron	N	.0863	.01169	14	86
Flumetsulam	Y	.1341	.01377	10	134

Table 15. Accuracy and precision data from seven determinations of the method compounds fortified at 0.10 microgram per liter in organic-free water, under positive ionization conditions—Continued

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
Fluometuron	N	0.0873	0.00982	11	87
Imazaquin	Y	.1664	.01639	10	166
Imazethapyr	Y	.2007	.01399	7	201
Imidicloprid	N	.0987	.01688	17	99
Linuron	N	.0872	.01105	13	87
Metalaxyl	N	.0840	.00909	11	84
Methiocarb	Y	.0884	.01265	14	88
Methomyl	Y	.0810	.01222	15	81
Methomyl oxime	Y	.0121	.00162	13	12
Metsulfuron-methyl	Y	.0759	.01810	24	76
Neburon	N	.0901	.01188	13	90
Nicosulfuron	N	.1295	.01039	8	130
Norflurazon	Y	.0925	.01232	13	93
Oryzalin	N	.0874	.01131	13	87
Oxamyl	N	.0836	.00255	3	84
Oxamyl oxime	Y	.0259	.01025	40	26
Propham	N	.0843	.01141	14	84
Propiconazole	N	.1000	.01024	10	100
Propoxur	N	.0854	.00945	11	85
Siduron	N	.0960	.01484	15	96
Sulfometuron-methyl	N	.0982	.00618	6	98
Tebuthiuron	N	.1062	.01220	11	106
Terbacil	Y	.0966	.01517	16	97
Tribenuron-methyl	Y	.0268	.01078	40	27

Table 16. Accuracy and precision data from seven determinations of the method compounds fortified at 0.10 microgram per liter in organic-free water, under negative ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter; nd, not determined]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D	N	0.0722	0.01231	17	72
2,4-DB	Y	.0705	.00856	12	71
Acifluorfen	N	.0847	.00990	12	85
Bentazon	Y	.0612	.00307	5	61
Bromoxynil	Y	.0827	.00909	11	83
Chlorothalonil	Y	.0123	.01182	96	12
Clopyralid	N	.0862	.00654	8	86
Dacthal monoacid	N	.0851	.01148	13	85
Dicamba	N	.0918	.01527	17	92
Dichlorprop	N	nd	nd	nd	nd
Dinoseb	N	.0823	.00683	8	82
MCPA	N	.0786	.00931	12	79
MCPB	Y	.0690	.00994	14	69
Picloram	N	.0904	.01133	13	90
Triclopyr	N	.0907	.01604	18	91

Table 17. Accuracy and precision data from nine determinations of the method compounds fortified at 0.50 microgram per liter in organic-free water, under positive ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D methyl ester	N	0.6060	0.04205	7	121
2-Hydroxyatrazine	Y	.5415	.09270	17	108
3(4-chlorophenyl)-1-methyl urea	N	.5098	.02956	6	102
3-Hydroxycarbofuran	N	.4725	.04037	9	95
3-Ketocarbofuran	Y	.2774	.1582	57	55
Aldicarb	Y	.3883	.05049	13	78
Aldicarb sulfone	Y	.3783	.04853	13	76
Aldicarb sulfoxide	Y	.3747	.04017	11	75
Atrazine	N	.5250	.01725	3	105
Bendiocarb	N	.4330	.02770	6	87
Benomyl	N	.4329	.04982	12	87
Bensulfuron-methyl	N	.4756	.02557	5	95
Bromacil	Y	.4304	.05635	13	86
Caffeine	N	.4857	.04800	10	97
Carbaryl	N	.4523	.05996	13	90
Carbofuran	N	.4954	.02407	5	99
Chloramben methyl ester	Y	.4370	.05847	13	87
Chlorimuron-ethyl	N	.4794	.04320	9	96
Cycloate	Y	.3198	.05180	16	64
Deethylatrazine	Y	.4836	.02359	5	97
Deethyldeiopropylatrazine	Y	.4199	.03419	8	84
Deisopropylatrazine	Y	.4416	.05683	13	88
Diphenamid	N	.4554	.02355	5	91
Diuron	N	.4639	.00897	2	93
Fenuron	N	.4364	.05450	12	87
Flumetsulam	Y	.4383	.02062	5	88
Fluometuron	N	.4581	.01034	2	92
Imazaquin	Y	.5825	.02403	4	117
Imazethapyr	Y	.5589	.02199	4	112
Imidicloprid	N	.3844	.05725	15	77
Linuron	N	.4911	.01474	3	98
Metalaxyl	N	.4808	.01269	3	96
Methiocarb	Y	.4505	.03728	8	90
Methomyl	Y	.3890	.03807	10	78
Methomyl oxime	Y	.0757	.02830	37	15
Metsulfuron-methyl	Y	.6061	.08198	14	121
Neburon	N	.4703	.01215	3	94
Nicosulfuron	N	.4886	.01786	4	98
Norflurazon	Y	.4721	.02388	5	94
Oryzalin	N	.4175	.01890	5	84
Oxamyl	N	.3890	.03985	10	78
Oxamyl oxime	Y	.1049	.05437	52	21
Propham	N	.4630	.01057	2	93
Propiconazole	N	.4655	.03045	7	93
Propoxur	N	.4466	.02265	5	89
Siduron	N	.4446	.02779	6	89
Sulfometuron-methyl	N	.3896	.01961	5	78
Tebuthiuron	N	.5213	.01444	3	104
Terbacil	Y	.4191	.05142	12	84
Tribenuron-methyl	Y	.4235	.07477	18	85

Table 18. Accuracy and precision data from nine determinations of the method compounds fortified at 0.50 microgram per liter in organic-free water, under negative ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D	N	0.4431	0.05134	12	89
2,4-DB	Y	.3078	.02720	9	62
Acifluorfen	N	.3918	.03392	9	78
Bentazon	Y	.2891	.04762	16	58
Bromoxynil	Y	.3734	.03328	9	75
Chlorothalonil	Y	.0384	.02494	65	8
Clopyralid	N	.3147	.04925	16	63
Dacthal monoacid	N	.1600	.06604	41	32
Dicamba	N	.3894	.02944	8	78
Dichlorprop	N	.4239	.02782	7	85
Dinoseb	N	.3922	.04564	12	78
MCPA	N	.4662	.03263	7	93
MCPB	Y	.5971	.05247	9	119
Picloram	N	.3828	.07625	20	77
Triclopyr	N	.4256	.03132	7	85

Table 19. Accuracy and precision data from 10 determinations of the method compounds fortified at 0.025 microgram per liter in ground-water samples, under positive ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)	Method detection limit (µg/L)
2,4-D methyl ester	N	0.0228	0.00153	7	91	0.0049
2-Hydroxyatrazine	Y	.0230	.00142	6	92	.0045
3(4-chlorophenyl)-1-methyl urea	N	.0226	.00106	5	90	.0034
3-Hydroxycarbofuran	N	.0294	.00233	8	118	.0074
3-Ketocarbofuran	Y	.0099	.00035	4	40	.0011
Aldicarb	Y	.0218	.00112	5	87	.0036
Aldicarb sulfone	Y	.0229	.00088	4	92	.0028
Aldicarb sulfoxide	Y	.0232	.00072	3	93	.0023
Atrazine	N	.0245	.00088	4	98	.0028
Bendiocarb	N	.0238	.00111	5	95	.0035
Benomyl	N	.0231	.00149	6	93	.0047
Bensulfuron-methyl	N	.0386	.00340	9	154	.0108
Bromacil	Y	.0225	.00105	5	90	.0033
Caffeine	N	.0238	.00061	3	95	.0019
Carbaryl	N	.0237	.00062	3	95	.0020
Carbofuran	N	.0238	.00080	3	95	.0025
Chloramben methyl ester	Y	.0210	.00101	5	84	.0032
Chlorimuron-ethyl	N	.0239	.00150	6	95	.0048
Cycloate	Y	.0240	.00307	13	96	.0097
Deethylatrazine	Y	.0229	.00092	4	92	.0029
Deethyldeiopropylatrazine	Y	.0243	.00061	3	97	.0019
Deisopropylatrazine	Y	.0229	.00091	4	92	.0029
Diphenamid	N	.0255	.00132	5	102	.0042
Diuron	N	.0244	.00056	2	98	.0018

Table 19. Accuracy and precision data from 10 determinations of the method compounds fortified at 0.025 microgram per liter in ground-water samples, under positive ionization conditions—Continued

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)	Method detection limit (µg/L)
Fenuron	N	0.0223	0.00101	5	89	0.0032
Flumetsulam	Y	.0383	.00304	8	153	.0096
Fluometuron	N	.0244	.00065	3	97	.0021
Imazaquin	Y	.0527	.00965	18	211	.0306
Imazethapyr	Y	.0350	.00191	5	140	.0060
Imidicloprid	N	.0382	.00320	8	153	.0101
Linuron	N	.0240	.00106	4	96	.0034
Metalaxyl	N	.0239	.00093	4	95	.0029
Methiocarb	Y	.0239	.00097	4	96	.0031
Methomyl	Y	.0235	.00113	5	94	.0036
Methomyl oxime	Y	.0091	.00097	11	37	.0031
Metsulfuron-methyl	Y	.0396	.00543	14	159	.0172
Neburon	N	.0237	.00100	4	95	.0032
Nicosulfuron	N	.0429	.00327	8	171	.0104
Norflurazon	Y	.0242	.00065	3	97	.0021
Oryzalin	N	.0225	.00172	8	90	.0054
Oxamyl	N	.0238	.00139	6	95	.0044
Oxamyl oxime	Y	.0071	.00498	70	29	.0158
Propham	N	.0238	.00065	3	95	.0021
Propiconazole	N	.0221	.00158	7	88	.0050
Propoxur	N	.0237	.00072	3	95	.0023
Siduron	N	.0236	.00090	4	94	.0029
Sulfometuron-methyl	N	.0388	.00292	8	155	.0092
Tebuthiuron	N	.0268	.00272	10	107	.0086
Terbacil	Y	.0229	.00062	3	92	.0020
Tribenuron-methyl	Y	.0157	.00203	13	63	.0064

Table 20. Accuracy and precision data from nine determinations of the method compounds fortified at 0.025 microgram per liter in ground-water samples, under negative ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)	Method detection limit (µg/L)
2,4-D	N	0.0239	0.00175	7	96	0.0057
2,4-DB	Y	.0227	.00169	7	91	.0055
Acifluorfen	N	.0236	.00132	6	94	.0043
Bentazon	Y	.0207	.00099	5	83	.0032
Bromoxynil	Y	.0163	.00185	11	65	.0060
Chlorothalonil	Y	.0148	.00272	18	59	.0088
Clopyralid	N	.0233	.00104	4	93	.0034
Dacthal monoacid	N	.0238	.00090	4	95	.0029
Dicamba	N	.0232	.00082	4	93	.0027
Dichlorprop	N	.0238	.00159	7	95	.0052
Dinoseb	N	.0062	.00085	14	25	.0028
MCPA	N	.0236	.00098	4	94	.0032
MCPB	Y	.0205	.00099	5	82	.0032
Picloram	N	.0234	.00102	4	94	.0033
Triclopyr	N	.0242	.00115	5	97	.0038

Table 21. Accuracy and precision data from six determinations of the method compounds fortified at 0.10 microgram per liter in ground-water samples, under positive ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D methyl ester	N	0.0826	0.01318	16	83
2-Hydroxyatrazine	Y	.1226	.02965	24	123
3(4-chlorophenyl)-1-methyl urea	N	.0918	.01323	14	92
3-Hydroxycarbofuran	N	.0822	.00729	9	82
3-Ketocarbofuran	Y	.0554	.01110	20	55
Aldicarb	Y	.0585	.01442	25	59
Aldicarb sulfone	Y	.0473	.02192	46	47
Aldicarb sulfoxide	Y	.0735	.00649	9	73
Atrazine	N	.0827	.01322	16	83
Bendiocarb	N	.0653	.00282	4	65
Benomyl	N	.0748	.00470	6	75
Bensulfuron-methyl	N	.1260	.00945	8	126
Bromacil	Y	.0768	.01486	19	77
Caffeine	N	.0991	.00840	8	99
Carbaryl	N	.0845	.01063	13	84
Carbofuran	N	.0844	.00944	11	84
Chloramben methyl ester	Y	.0811	.00521	6	81
Chlorimuron-ethyl	N	.0982	.01716	17	98
Cycloate	Y	.0724	.00824	11	72
Deethylatrazine	Y	.0702	.01419	20	70
Deethyldeiopropylatrazine	Y	.0825	.00639	8	82
Deisopropylatrazine	Y	.0651	.01304	20	65
Diphenamid	N	.0842	.01009	12	84
Diuron	N	.0900	.01335	15	90
Fenuron	N	.0796	.01081	14	80
Flumetsulam	Y	.1725	.00761	4	172
Fluometuron	N	.0863	.01027	12	86
Imazaquin	Y	.1455	.01904	13	145
Imazethapyr	Y	.1807	.05831	32	181
Imidicloprid	N	.1453	.01455	10	145
Linuron	N	.0887	.01014	11	89
Metalaxyl	N	.0872	.00980	11	87
Methiocarb	Y	.0891	.01143	13	89
Methomyl	Y	.0785	.01676	21	78
Methomyl oxime	Y	.0111	.00100	9	11
Metsulfuron-methyl	Y	.1436	.03049	21	144
Neburon	N	.0860	.01542	18	86
Nicosulfuron	N	.1740	.01222	7	174
Norflurazon	Y	.0930	.01094	12	93
Oryzalin	N	.0905	.01251	14	90
Oxamyl	N	.0700	.00724	10	70
Oxamyl oxime	Y	.0238	.01125	47	24
Propham	N	.0803	.01164	14	80
Propiconazole	N	.0972	.00808	8	97
Propoxur	N	.0807	.00926	11	81
Siduron	N	.0996	.01481	15	100
Sulfometuron-methyl	N	.1546	.01200	8	155
Tebuthiuron	N	.0961	.01555	16	96
Terbacil	Y	.0795	.01433	18	80
Tribenuron-methyl	Y	.0434	.02271	52	43

Table 22. Accuracy and precision data from six determinations of the method compounds fortified at 0.10 microgram per liter in ground-water samples, under negative ionization conditions

[conc., concentration; Y/N; yes/no; µg/L, microgram per liter; nd, not determined]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D	Y	0.0646	0.00468	7	65
2,4-DB	N	.0748	.00654	9	75
Acifluorfen	N	.0780	.01180	15	78
Bentazon	N	.0600	.00282	5	60
Bromoxynil	Y	.0738	.00531	7	74
Chlorothalonil	Y	.0486	.01359	28	49
Clopyralid	N	.0755	.00975	13	75
Dacthal monoacid	N	.0777	.00770	10	78
Dicamba	Y	.0786	.00959	12	79
Dichlorprop	Y	nd	nd	nd	nd
Dinoseb	N	.0792	.00390	5	79
MCPA	Y	.0725	.00556	8	73
MCPB	N	.0651	.00614	9	65
Picloram	N	.0821	.00625	8	82
Triclopyr	Y	.0835	.01317	16	83

Table 23. Accuracy and precision data from 11 determinations of the method compounds fortified at 0.50 microgram per liter in ground-water samples, under positive ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D methyl ester	N	0.6056	0.03970	7	121
2-Hydroxyatrazine	Y	.5503	.09067	16	110
3(4-chlorophenyl)-1-methyl urea	N	.5286	.07316	14	106
3-Hydroxycarbofuran	N	.4740	.03803	8	95
3-Ketocarbofuran	Y	.2994	.03088	10	60
Aldicarb	Y	.2440	.15406	63	49
Aldicarb sulfone	Y	.2038	.16250	80	41
Aldicarb sulfoxide	Y	.2546	.11533	45	51
Atrazine	N	.5031	.07281	14	101
Bendiocarb	N	.6663	.16411	25	133
Benomyl	N	.4042	.10879	27	81
Bensulfuron-methyl	N	.4340	.13865	32	87
Bromacil	Y	.3884	.09041	23	78
Caffeine	N	.3404	.21684	64	68
Carbaryl	N	.4448	.10781	24	89
Carbofuran	N	.4703	.09763	21	94
Chloramben methyl ester	Y	.3528	.14311	41	71
Chlorimuron-ethyl	N	.4784	.03925	8	96

Table 23. Accuracy and precision data from 11 determinations of the method compounds fortified at 0.50 microgram per liter in ground-water samples, under positive ionization conditions—Continued

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
Cycloate	Y	0.2102	0.13361	64	42
Deethylatrazine	Y	.4838	.02207	5	97
Deethyldeiopropylatrazine	Y	.4176	.18583	44	84
Deisopropylatrazine	Y	.3387	.19134	56	68
Diphenamid	N	.4130	.13814	33	83
Diuron	N	.4515	.04772	11	90
Fenuron	N	.4367	.05099	12	87
Flumetsulam	Y	.4407	.02060	5	88
Fluometuron	N	.4563	.01552	3	91
Imazaquin	Y	.5570	.09995	18	111
Imazethapyr	Y	.5188	.08684	17	104
Imidicloprid	N	.3771	.06654	18	75
Linuron	N	.4922	.01486	3	98
Metalaxyl	N	.4376	.14267	33	88
Methiocarb	Y	.2805	.19175	68	56
Methomyl	Y	.2756	.14205	52	55
Methomyl oxime	Y	.0641	.03562	56	13
Metsulfuron-methyl	Y	.6685	.16345	24	134
Neburon	N	.4716	.01157	2	94
Nicosulfuron	N	.4627	.08009	17	93
Norflurazon	Y	.3877	.12897	33	78
Oryzalin	N	.3836	.11295	29	77
Oxamyl	N	.3199	.09500	30	64
Oxamyl oxime	Y	.0887	.04992	56	18
Propham	N	.4312	.12601	29	86
Propiconazole	N	.4241	.13628	32	85
Propoxur	N	.4251	.07483	18	85
Siduron	N	.4064	.10948	27	81
Sulfometuron-methyl	N	.3587	.10319	29	72
Tebuthiuron	N	.5225	.01363	3	105
Terbacil	Y	.4115	.05046	12	82
Tribenuron-methyl	Y	.3196	.11716	37	64

from the data for mean recoveries of individual compounds. No method compounds were detected in the ground-water samples, so no background correction was required.

11.3.2 The mean positive ionization recoveries for compounds from the ground-water samples reported without qualification (tables 19, 21, and 23) were 104.8 ± 23.1, 95.0 ± 25.1, and 90.2 ± 14.6 percent at fortifications of 0.025, 0.100, and 0.500 µg/L, respectively. The median recoveries were similar to the means, at 95.0, 86.5, and 88.5 percent, respectively. Mean recoveries of compounds reported as qualified estimates were more variable, at 96.6 ± 40.8, 84.7 ± 43.9, and 70.6 ± 30.1 percent, with corresponding medians of 92.0, 77.5, and 69.5 percent, respectively.

11.3.3 Mean recoveries determined by negative ionization and reported without qualification in the ground-water samples (tables 20, 22, and 24)

Table 24. Accuracy and precision data from 10 determinations of the method compounds fortified at 0.50 microgram per liter in ground-water samples, under negative ionization conditions

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter; nd, not determined]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D	N	0.0722	0.01231	17	72
2,4-DB	N	.0705	.00856	12	71
Acifluorfen	Y	.0847	.00990	12	85
Bentazon	N	.0612	.00307	5	61
Bromoxynil	N	.0827	.00909	11	83
Chlorothalonil	N	.0123	.01182	96	12
Clopyralid	N	.0862	.00654	8	86
Dacthal monoacid	N	.0851	.01148	13	85
Dicamba	N	.0918	.01527	17	92
Dichlorprop	Y	nd	nd	nd	nd
Dinoseb	Y	.0823	.00683	8	82
MCPA	N	.0786	.00931	12	79
MCPB	Y	.0690	.00994	14	69
Picloram	N	.0904	.01133	13	90
Triclopyr	Y	.0907	.01604	18	91

averaged slightly lower, at 87.6 ± 22.0, 74.0 ± 7.6 and 73.1 ± 23.5 percent, at fortifications of 0.025, 0.100, and 0.500 µg/L, respectively. The median recoveries were similar to the means, at 94.0, 76.5, and 81.0 percent, respectively. In this matrix, mean recoveries of compounds reported as qualified estimates were similar to compounds reported without qualification, at 76.0 ± 13.4, 70.5 ± 12.2, and 81.8 ± 9.3 percent, with corresponding medians of 92.0, 77.5, and 69.5 percent, respectively.

11.3.4 In the ground-water samples, average recoveries of all compounds reported without qualification at all three fortification levels varied about ±20.9 percent for compounds determined by positive ionization and ±17.7 percent for compounds determined by negative ionization. This result, however, does not reflect the analytical precision, which is better reflected by the relative standard deviation of individual compound recoveries in the ground-water samples, particularly those that are reported without quantitation qualification. Under positive ionization conditions, the mean relative standard deviations for compounds reported without qualification in the ground-water samples were 5.4 ± 2.2, 11.7 ± 3.6, and 20.5 ± 13.5 percent for 0.025-, 0.100-, and 0.500-µg/L fortifications, respectively. The corresponding relative standard deviations for negative ionization results reported without qualification in the ground-water samples were 5.9 ± 3.1, 9.2 ± 3.5, and 20.4 ± 26.8 percent for 0.025-, 0.100-, and 0.500-µg/L fortifications, respectively. The greater variability in the relative standard deviation for compounds determined by negative ionization in the 0.500-µg/L fortification was the result of particularly variable recovery of chlorothalonil. If this result is excluded, then the mean relative standard deviation was 12 ± 3.8 percent, similar to the mean relative standard deviations reported for the other fortifications.

11.4 Surface-water fortification recovery results

11.4.1 In contrast to the other matrices tested, one set of 10 surface-water samples was fortified at 0.050 µg/L as the lowest fortification concentration rather than 0.025 µg/L, as was the case with the reagent-water and ground-water fortification experiments. This change was made because of nonspecific matrix interferences. Concentrations of dissolved organic carbon, the likely source of these interferences in the South Platte River, typically ranged between 3 and 30 mg/L (Litke and Kimbrough, 1998), although median concentrations were 5.2 and 7.0 mg/L in the South Platte River at Denver and Henderson, Colorado, respectively. The remaining two sets of samples were fortified at 0.100 and 0.500 µg/L, identical to the organic-free and ground-water fortifications. The surface-water fortification experiments at 0.100 µg/L were conducted 8 months prior to the 0.05 and 0.5 fortifications. All results are listed in tables 25 through 31. The mean, median, and the standard deviation of recovery for all compounds in the tables were calculated from the data for mean recoveries of individual compounds. In comparison to the reagent-water and ground-water samples, measurable concentrations of a few method analytes were detected. Caffeine, 2-hydroxy-atrazine, atrazine, diuron, 2,4-D, and MCPA were detected in at least two of three replicate unspiked South Platte surface-water samples. The mean concentrations are listed in table 25. Spiked South Platte River surface-water results were corrected for ambient concentrations prior to calculation of recoveries.

11.4.2 Compound recoveries from the surface-water samples were higher, often substantially greater than 100 percent at the 0.05- and 0.5-µg/L fortifications, and more variable between compounds than in either the organic-free water or the ground-water samples. The mean positive ionization recovery results for compounds reported without qualification in the surface-water samples were 131 ± 66.3, 90.8 ± 10.0, and 125 ± 93.5 percent at fortifications of 0.050, 0.100, and 0.500 µg/L, respectively. The median recoveries were similar to the means at 119.0, 87.0, and 108.0 percent, respectively. For those compounds determined under positive ion conditions and

Table 25. Ambient pesticide concentrations measured in South Platte River samples during this study [µg/L, microgram per liter]

Compound	Mean detected concentrations, in µg/L	Standard deviation of mean detected concentrations, in µg/L	Number of detections/number of determinations
2,4-D	0.1925	0.1025	2/2
2-Hydroxyatrazine	.0645	.0021	2/3
Atrazine	.0065	.0007	3/3
Caffeine	.1033	.0112	3/3
Diuron	.0380	.0066	3/3
MCPA	.0250	.0156	2/2

Table 26. Accuracy and precision data from nine determinations of the method compounds fortified at 0.05 microgram per liter in surface-water samples, under positive ionization conditions. Recoveries corrected for ambient compound concentrations listed in table 25

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)	Method detection limit (µg/L)
2,4-D methyl ester	N	0.0173	0.00543	31	35	0.0177
2-Hydroxyatrazine	Y	.0362	.00449	12	72	.0146
3(4-chlorophenyl)-1-methyl urea	N	.0442	.00380	9	88	.0124
3-Hydroxycarbofuran	N	.0501	.00525	10	100	.0171
3-Ketocarbofuran	Y	.0032	.00045	14	6	.0015
Aldicarb	Y	.0439	.00668	15	88	.0217
Aldicarb sulfone	Y	.0363	.00700	19	73	.0227
Aldicarb sulfoxide	Y	.0247	.00173	7	49	.0056
Atrazine	N	.0536	.00416	8	107	.0135
Bendiocarb	N	.0124	.00453	36	25	.0147
Benomyl	N	.0459	.00417	9	92	.0135
Bensulfuron-methyl	N	.1446	.00757	5	289	.0246
Bromacil	Y	.0289	.00314	11	58	.0102
Caffeine	N	.0541	.01065	20	108	.0346
Carbaryl	N	.0412	.00474	11	82	.0154
Carbofuran	N	.0484	.00317	7	97	.0103
Chloramben methyl ester	Y	.0737	.00433	6	147	.0141
Chlorimuron-ethyl	N	.1143	.00953	8	229	.0320
Cycloate	Y	.0236	.00609	26	47	.0198
Deethylatrazine	Y	.0466	.00305	7	93	.0099
Deethyldeiopropylatrazine	Y	.0256	.00277	11	51	.0093
Deisopropylatrazine	Y	.0386	.00309	8	77	.0100
Diphenamid	N	.0676	.00388	6	135	.0126
Diuron	N	.0606	.00604	10	121	.0196
Fenuron	N	.0519	.00688	13	104	.0224
Flumetsulam	Y	.1003	.00873	9	201	.0284
Fluometuron	N	.0623	.00346	6	125	.0113
Imazaquin	Y	.1069	.00643	6	214	.0209
Imazethapyr	Y	.2210	.01377	6	442	.0447
Imidicloprid	N	.0729	.01179	16	146	.0383
Linuron	N	.0683	.00632	9	137	.0206
Metalaxyl	N	.0663	.00374	6	133	.0122
Methiocarb	Y	.0396	.00515	13	79	.0167
Methomyl	Y	.0654	.00543	8	131	.0177
Methomyl oxime	Y	.0038	.00158	42	8	.0053
Metsulfuron-methyl	Y	.0124	.00450	36	25	.0146
Neburon	N	.0583	.00450	8	117	.0146
Nicosulfuron	N	.1563	.01168	7	313	.0380
Norflurazon	Y	.0671	.00369	5	134	.0120
Oryzalin	N	.0606	.01500	25	121	.0488
Oxamyl	N	.0487	.01150	24	97	.0374
Oxamyl oxime	Y	.0241	.00326	14	48	.0106
Propham	N	.0457	.00566	12	91	.0184
Propiconazole	N	.0637	.00502	8	127	.0163
Propoxur	N	.0516	.00240	5	103	.0078
Siduron	N	.0744	.00439	6	149	.0143
Sulfometuron-methyl	N	.1291	.00645	5	258	.0210
Tebuthiuron	N	.0638	.00432	7	128	.0141
Terbacil	Y	.0236	.00334	14	47	.0112
Tribenuron-methyl	Y	.0974	.00665	7	195	.0216

Table 27. Accuracy and precision data from nine determinations of the method compounds fortified at 0.05 microgram per liter in surface-water samples, under negative ionization conditions. Recoveries corrected for ambient compound concentrations listed in table 25

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter; nd, not determined]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)	Method detection limit (µg/L)
2,4-D	N	0.0226	0.00477	21	45	0.0155
2,4-DB	Y	.0252	.00295	12	50	.0096
Acifluorfen	N	.0261	.00112	4	52	.0036
Bentazon	Y	.0249	.00289	12	50	.0094
Bromoxynil	Y	.0260	.00132	5	52	.0043
Chlorothalonil	Y	.0258	.00311	12	52	.0101
Clopyralid	N	.3057	.01425	5	611	.0463
Dacthal monoacid	N	.0159	.00401	25	32	.0130
Dicamba	N	.0251	.00280	11	50	.0091
Dichlorprop	N	.0134	.00837	62	27	.0272
Dinoseb	N	.0591	.00337	6	118	.0110
MCPA	N	nd	nd	nd	nd	nd
MCPB	Y	.0263	.00187	7	53	.0061
Picloram	N	.0261	.00302	12	52	.0098
Triclopyr	N	.0234	.00113	5	47	.0037

Table 28. Accuracy and precision data from six determinations of the method compounds fortified at 0.10 microgram per liter in surface-water samples, under positive ionization conditions. Recoveries corrected for ambient compound concentrations listed in table 25

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter; nd, not determined; na, not available]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D methyl ester	N	0.0780	0.01388	18	78
2-Hydroxyatrazine	Y	.1086	.03100	29	109
3(4-chlorophenyl)-1-methyl urea	N	.0997	.01584	16	100
3-Hydroxycarbofuran	N	.0916	.01059	12	92
3-Ketocarbofuran	Y	.0822	.01260	15	82
Aldicarb	Y	.0622	.01417	23	62
Aldicarb sulfone	Y	.0649	.02707	42	65
Aldicarb sulfoxide	Y	.0758	.00418	6	76
Atrazine	N	.0925	.01202	13	92
Bendiocarb	N	.0852	.01039	12	85
Benomyl	N	.0809	.00212	3	81
Bensulfuron-methyl	N	.0921	.00657	7	92
Bromacil	Y	.0928	.01322	14	93
Caffeine	N	.1085	.01380	13	109
Carbaryl	N	.0877	.01070	12	88
Carbofuran	N	.0846	.00981	12	85
Chloramben methyl ester	Y	.0928	.01962	21	93
Chlorimuron-ethyl	N	.0848	.00448	5	85
Cycloate	Y	.0705	.00838	12	71
Deethylatrazine	Y	.1080	.01490	14	108
Deethyldeiopropylatrazine	Y	.0877	.01034	12	88
Deisopropylatrazine	Y	.1055	.01265	12	105
Diphenamid	N	.0834	.00939	11	83
Diuron	N	.0899	.01267	14	90

Table 28. Accuracy and precision data from six determinations of the method compounds fortified at 0.10 microgram per liter in surface-water samples, under positive ionization conditions. Recoveries corrected for ambient compound concentrations listed in table 25—Continued

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
Fenuron	N	0.0856	0.01263	15	86
Flumetsulam	Y	.1302	.01003	8	130
Fluometuron	N	.0865	.01046	12	86
Imazaquin	Y	.1620	.01256	8	162
Imazethapyr	Y	nd	nd	nd	nd
Imidicloprid	N	.0956	.01620	17	96
Linuron	N	.0864	.01184	14	86
Metalaxyl	N	.0835	.00985	12	83
Methiocarb	Y	.0870	.01324	15	87
Methomyl	Y	.0796	.01282	16	80
Methomyl oxime	Y	.0129	.00008	1	13
Metsulfuron-methyl	Y	.0746	.01945	26	75
Neburon	N	.0886	.01225	14	89
Nicosulfuron	N	.1261	.00532	4	126
Norflurazon	Y	.0918	.01335	15	92
Oryzalin	N	.0860	.01175	na	86
Oxamyl	N	.0841	.00244	3	84
Oxamyl oxime	Y	.0256	.01120	44	26
Propham	N	.0824	.01114	14	82
Propiconazole	N	.0978	.00927	9	98
Propoxur	N	.0846	.01011	12	85
Siduron	N	.0939	.01512	16	94
Sulfometuron-methyl	N	.0962	.00355	4	96
Tebuthiuron	N	.1052	.01302	12	105
Terbacil	Y	.0952	.01614	17	95
Tribenuron-methyl	Y	.0278	.01141	41	28

Table 29. Accuracy and precision data from six determinations of the method compounds fortified at 0.10 microgram per liter in surface-water samples, under negative ionization conditions. Recoveries corrected for ambient compound concentrations listed in table 25

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter; nd, not determined]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D	N	0.0692	0.00858	12	69
2,4-DB	N	.0672	.00855	13	67
Acifluorfen	Y	.0732	.00775	11	73
Bentazon	N	.0573	.00425	7	57
Bromoxynil	N	.0649	.00613	9	65
Chlorothalonil	N	.0764	.01028	13	76
Clopyralid	N	.0501	.01623	32	50
Dacthal monoacid	N	.0744	.00804	11	74
Dicamba	N	.0733	.00890	12	73
Dichlorprop	Y	nd	nd	nd	nd
Dinoseb	Y	.0743	.00440	6	74
MCPA	N	.0751	.00535	7	75
MCPB	Y	.0651	.00921	14	65
Picloram	N	.0871	.00765	9	87
Triclopyr	Y	.0789	.00898	11	79

Table 30. Accuracy and precision data from eight determinations of the method compounds fortified at 0.50 microgram per liter in surface-water samples, under positive ionization conditions. Recoveries corrected for ambient compound concentrations listed in table 25

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D methyl ester	N	0.0123	0.01210	98	2
2-Hydroxyatrazine	Y	.4449	.16531	37	89
3(4-chlorophenyl)-1-methyl urea	N	.4648	.04682	10	93
3-Hydroxycarbofuran	N	.1589	.10825	68	32
3-Ketocarbofuran	Y	.0058	.00096	17	1
Aldicarb	Y	.3174	.07261	23	63
Aldicarb sulfone	Y	.2719	.07615	28	54
Aldicarb sulfoxide	Y	.5699	.15729	28	114
Atrazine	N	.5258	.07168	14	105
Bendiocarb	N	.0069	.00544	79	1
Benomyl	N	.4231	.04948	12	85
Bensulfuron-methyl	N	1.9925	.30680	15	399
Bromacil	Y	.2469	.01911	8	49
Caffeine	N	.6533	.06538	10	131
Carbaryl	N	.0839	.07925	94	17
Carbofuran	N	.2638	.14014	53	53
Chloramben methyl ester	Y	.8634	.07006	8	173
Chlorimuron-ethyl	N	1.4456	.10097	7	289
Cycloate	Y	.1804	.08792	49	36
Deethylatrazine	Y	.4493	.06076	14	90
Deethyldeiopropylatrazine	Y	.0443	.01743	39	9
Deisopropylatrazine	Y	.3958	.05684	14	79
Diphenamid	N	.6759	.07794	12	135
Diuron	N	.6533	.06118	9	131
Fenuron	N	.5284	.04383	8	106
Flumetsulam	Y	1.0458	.09840	9	209
Fluometuron	N	.6446	.06166	10	129
Imazaquin	Y	1.3143	.14009	11	263
Imazethapyr	Y	1.5173	.04774	3	303
Imidicloprid	N	.8706	.08315	10	174
Linuron	N	.5155	.07320	14	103
Metalaxyl	N	.7384	.08215	11	148
Methiocarb	Y	.1597	.02914	18	32
Methomyl	Y	.6341	.12760	20	127
Methomyl oxime	Y	.0399	.04037	101	8
Metsulfuron-methyl	Y	.0071	.00569	80	1
Neburon	N	.5493	.12373	23	110
Nicosulfuron	N	1.5246	.11032	7	305
Norflurazon	Y	.7425	.06540	9	149
Oryzalin	N	.4705	.06710	14	94
Oxamyl	N	.0676	.06188	92	14
Oxamyl oxime	Y	.4521	.06752	15	90
Propham	N	.4011	.10534	26	80
Propiconazole	N	.8118	.16649	21	162
Propoxur	N	.3371	.13514	40	67
Siduron	N	.7349	.06842	9	147
Sulfometuron methyl	N	1.2940	.10436	8	259
Tebuthiuron	N	.6379	.05940	9	128
Terbacil	Y	.3790	.04253	11	76
Tribenuron-methyl	Y	1.4160	.00000	0	283

Table 31. Accuracy and precision data from eight determinations of the method compounds fortified at 0.50 microgram per liter in surface-water samples, under negative ionization conditions. Recoveries corrected for ambient compound concentrations listed in table 25

[conc., concentration; Y/N, yes/no; µg/L, microgram per liter; nd, not determined]

Compound	Reported as an estimated conc. (Y/N)	Mean observed conc. (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true conc.)
2,4-D	N	0.4874	0.32028	66	97
2,4-DB	N	.2254	.15642	69	45
Acifluorfen	Y	.2168	.16552	76	43
Bentazon	N	.2014	.12745	63	40
Bromoxynil	N	.2520	.17770	71	50
Chlorothalonil	N	nd	nd	nd	nd
Clopyralid	N	.1158	.08788	76	23
Dacthal monoacid	N	.1428	.11184	78	29
Dicamba	N	.1913	.13510	71	38
Dichlorprop	Y	.2376	.16962	71	48
Dinoseb	Y	.3043	.21623	71	61
MCPA	N	.2393	.16637	70	48
MCPB	Y	.2320	.16347	70	46
Picloram	N	.1385	.08582	62	28
Triclopyr	Y	.2076	.14622	70	42

reported as qualified estimates, the corresponding mean recoveries were 103.9 ± 96.1, 82.9 ± 33.8, and 104.5 ± 91.0 percent at fortifications of 0.050, 0.100, and 0.500 µg/L, respectively. The corresponding medians were 75.0, 87.0, and 84.0 percent.

Furlong and others (2000) have previously noted apparent matrix enhancement of concentration observed in the 0.05- and 0.5-µg/L fortifications in this study. The sample used for these fortifications was collected about 12 months after the 0.1-µg/L fortification. The apparent matrix enhancement observed in the surface-water samples also appears to be compound class-specific and is further discussed in a subsequent section (11.5.3) of this report. The fact that the South Platte River water used for these fortifications was collected at a different time and location may have a substantial role in determining the type and extent of matrix effects.

11.4.3 Mean recoveries for compounds reported without qualification and determined by negative ionization in the surface-water samples were lower than in positive ionization, at 115 ± 188, 69.3 ± 10.4, and 44.2 ± 21.9 percent, at fortifications of 0.05, 0.100, and 0.500 µg/L, respectively. The corresponding median recoveries were 50.0, 71.0, and 40.0 percent, and reflect the higher variablity observed among compounds in negative ionization analysis. The mean recoveries of compounds reported as qualified estimates and determined by negative ionization in surface water were lower but less variable, at 51.4 ± 1.3, 72.8 ± 5.8, and 48.0 ± 7.6 percent at fortifications of 0.05, 0.100, and 0.500 µg/L, respectively. The corresponding median recoveries were 52.0, 73.5, and 46.0 percent. The determination of whether a compound was reported as a qualified estimate was a function of long-term recoveries in reagent water (11.6.2). In this case, matrix effects may have resulted in lower but less variable recoveries.

11.4.4 Average surface-water recoveries of all compounds at all three fortification levels varied about ±65 percent

for compounds determined by positive ionization and ±59 percent for compounds determined by negative ionization. This variation indicates that the accuracy of the method in the surface-water samples is compound dependent, and might reflect compound-specific bias resulting from the surface-water matrix. Understanding analytical precision is equally important to using any analytical method, however, and analytical precision is better reflected by the relative standard deviation of individual compound recoveries from the surface-water samples. Under positive ionization conditions, the mean relative standard deviations for all compounds in the surface-water samples were 12 ± 9, 15 ± 9, and 27 ± 27 percent for 0.050-, 0.100-, and 0.500-µg/L fortifications, respectively. The corresponding relative standard deviations for negative ionization results were 14 ± 15, 12 ± 6, and 70 ± 5 percent for 0.050-, 0.100-, and 0.500-µg/L fortifications, respectively. Surface-water matrix effects, particularly the higher DOC concentration in the South Platte River, might be responsible for the substantially higher variability observed in the 0.500-µg/L fortifications for both positive and negative ionization compounds than in the ground-water samples and the organic-free-water samples (11.2 and 11.3).

11.5 Compound-specific recovery results

11.5.1 The method validation results by class for all three tested matrices are shown in figures 2, 3, 4, and 5. In figures 2, 3, and 4, each figure contains the analytical results under positive ionization for one matrix at three concentrations, subdivided by compound class. The four compound class groupings were as follows: (A) carbamate insecticides; (B) triazine and phenylurea herbicides; (C) sulfonylurea, sulfonamide, and imidazolinone herbicides; and (D) miscellaneous herbicides and other compounds. Pesticides determined by negative ionization HPLC/MS are grouped by matrix in figure 5.

11.5.2 A review of the positive ionization results for each matrix (figs. 2 through 4) reveals consistent similarities across matrices. Two carbamate degradation products, oxamyl oxime and methomyl oxime, have poor recoveries (averaged only 23, 22, and 29 percent in organic-free water, ground-water and surface-water samples, respectively) compared to the remaining carbamate insecticides in which recoveries averaged 87, 82, and 66 percent, respectively. In the other groupings, tribenuron was lower than the average recoveries for other miscellaneous compounds in the organic-free water and in the ground-water samples. Tribenuron in the organic-free water and in the ground-water samples averaged 48 and 57 percent, respectively, but in contrast, recovery was apparently enhanced in the surface-water samples, at 178 percent, although recovery in surface water varied between fortification levels, with the greatest variation measured at the highest level (fig. 4D).

11.5.3 When compared with the organic-free water and the ground-water samples (figs. 2 and 3), fortified surface-water recoveries were more variable, which indicated that several compound classes experienced apparent enhancement of recovery. As noted above, this finding applied to tribenuron, but also was observed for the sulfonylurea, sulfonamide, and imidazolinone herbicides. This result is consistent with apparent matrix enhancement of recovery for sulfonylurea, sulfonamide, and imidazolinone herbicides observed in a range of natural-water samples and South Platte River water (Furlong and others, 2000). The results shown in figure 4 suggest that enhancement varies by compound class. The carbamate insecticides (fig. 4A) appear to experience the least enhancement and, for 3-ketocarbofuran, perhaps some suppression of recovery. The recoveries of the triazine and phenylurea herbicides (fig. 4B) are consistent with recoveries in the organic-free water and in the ground-water samples (figs. 2B and 3B),

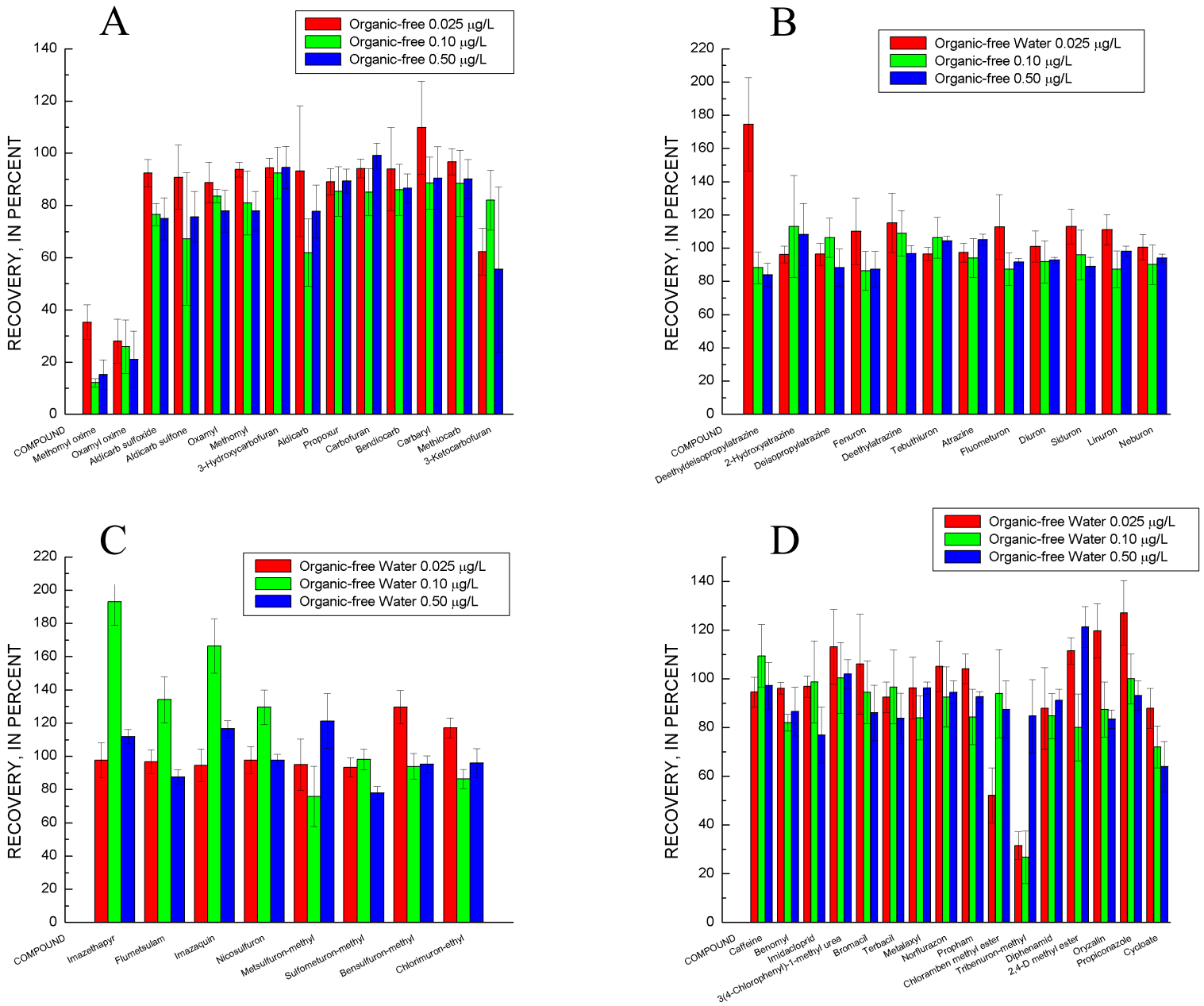

Figure 2. Recoveries, in percent, for compounds fortified at three levels in organic-free water and determined under positive ionization conditions. (A) N-methylcarbamate and related compounds; (B) phenylurea and triazine compounds; (C) sulfonylurea, imidazolinone and related compounds; and (D) other classes of compounds. Error bars indicate ±1 standard deviation.

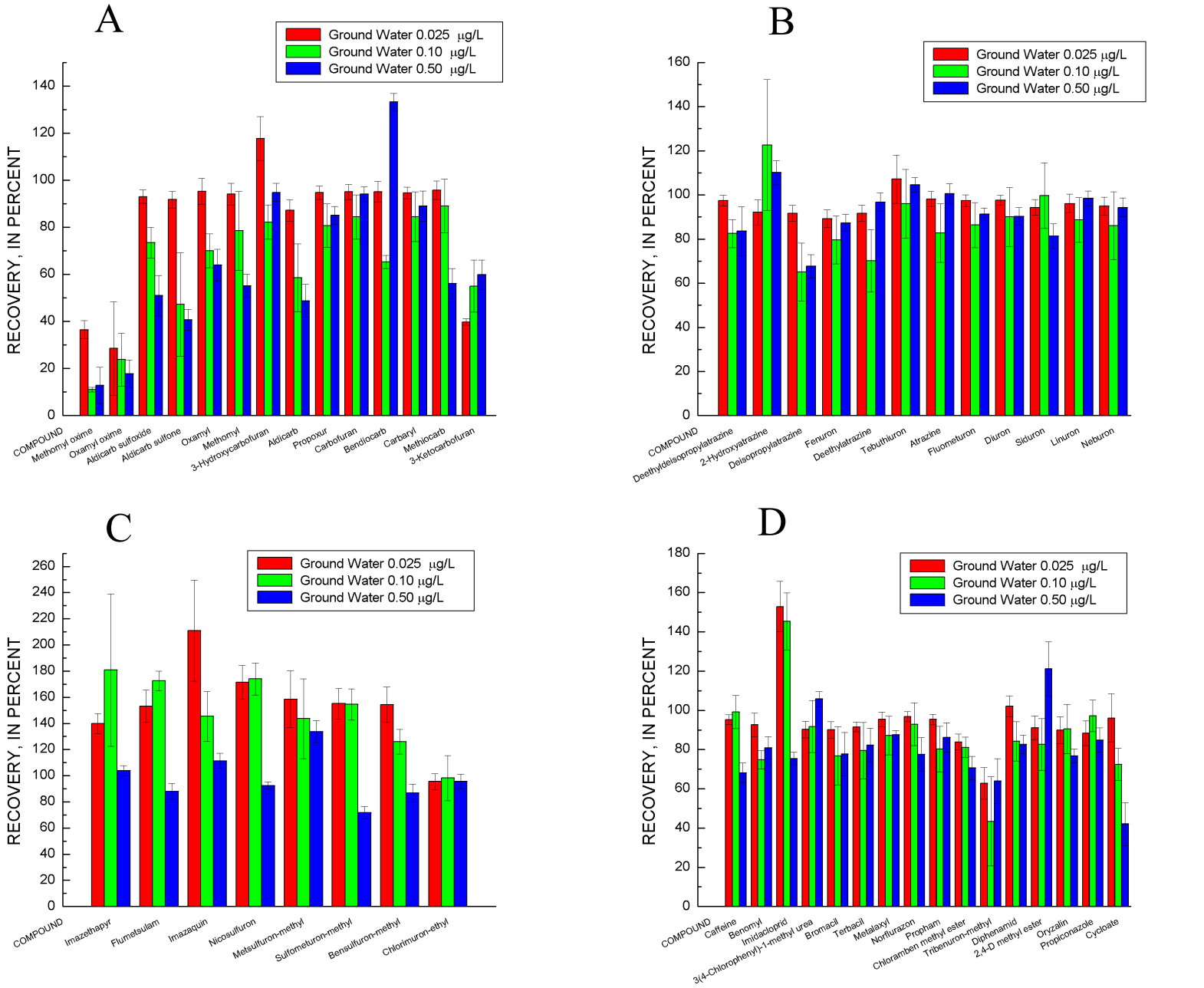

Figure 3. Recoveries, in percent, for compounds fortified at three levels in ground-water samples and determined under positive ionization conditions. (A) N-methylcarbamate and related compounds; (B) phenylurea and triazine compounds; (C) sulfonylurea, imidazolinone and related compounds; and (D) other classes of compounds. Error bars indicate ±1 standard deviation.

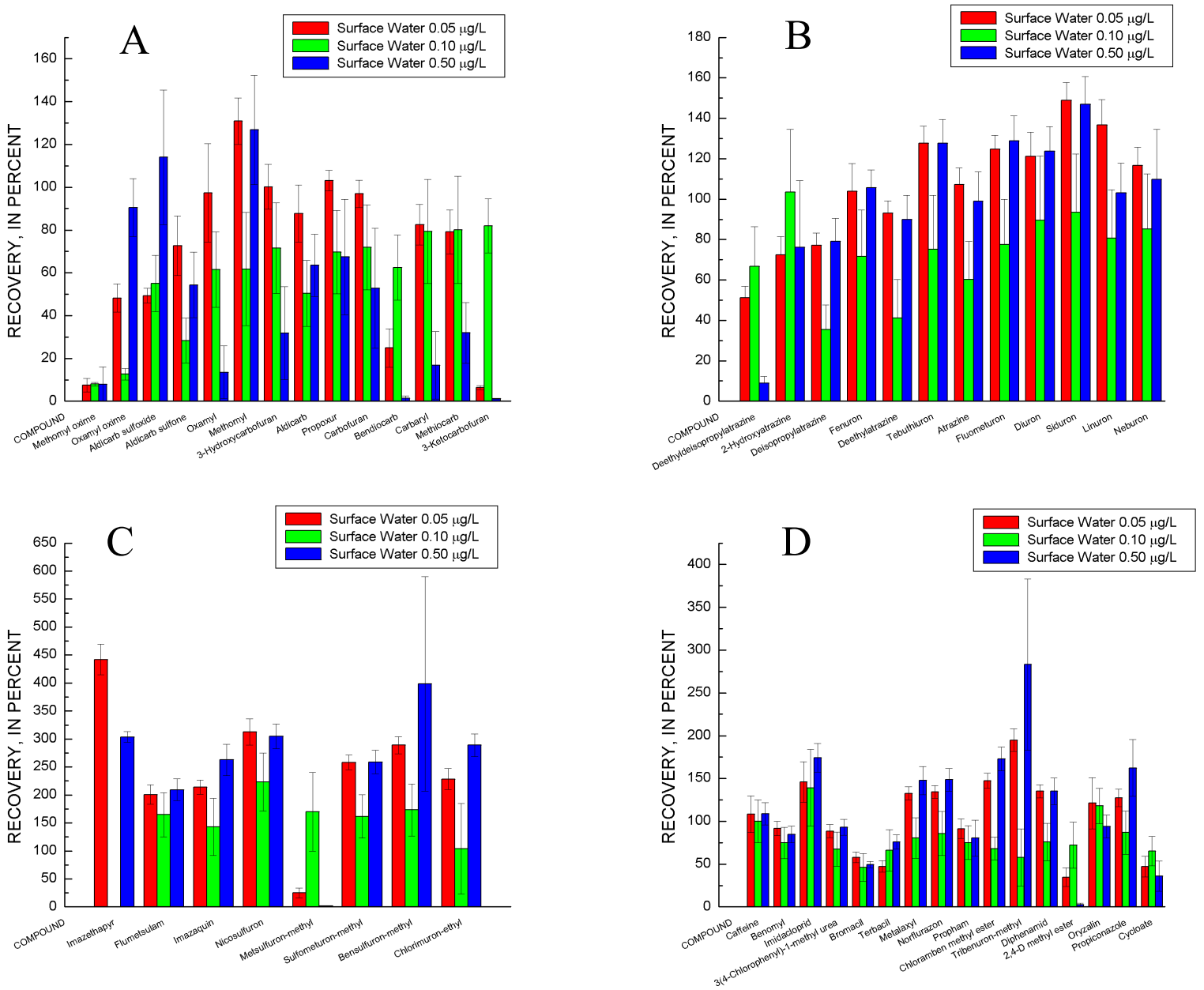

Figure 4. Recoveries, in percent, for compounds fortified at three levels in surface-water samples and determined under positive ionization conditions. (A) N-methylcarbamate and related compounds; (B) phenylurea and triazine compounds; (C) sulfonylurea, imidazolinone and related compounds; and (D) other classes of compounds. Error bars indicate ±1 standard deviation.

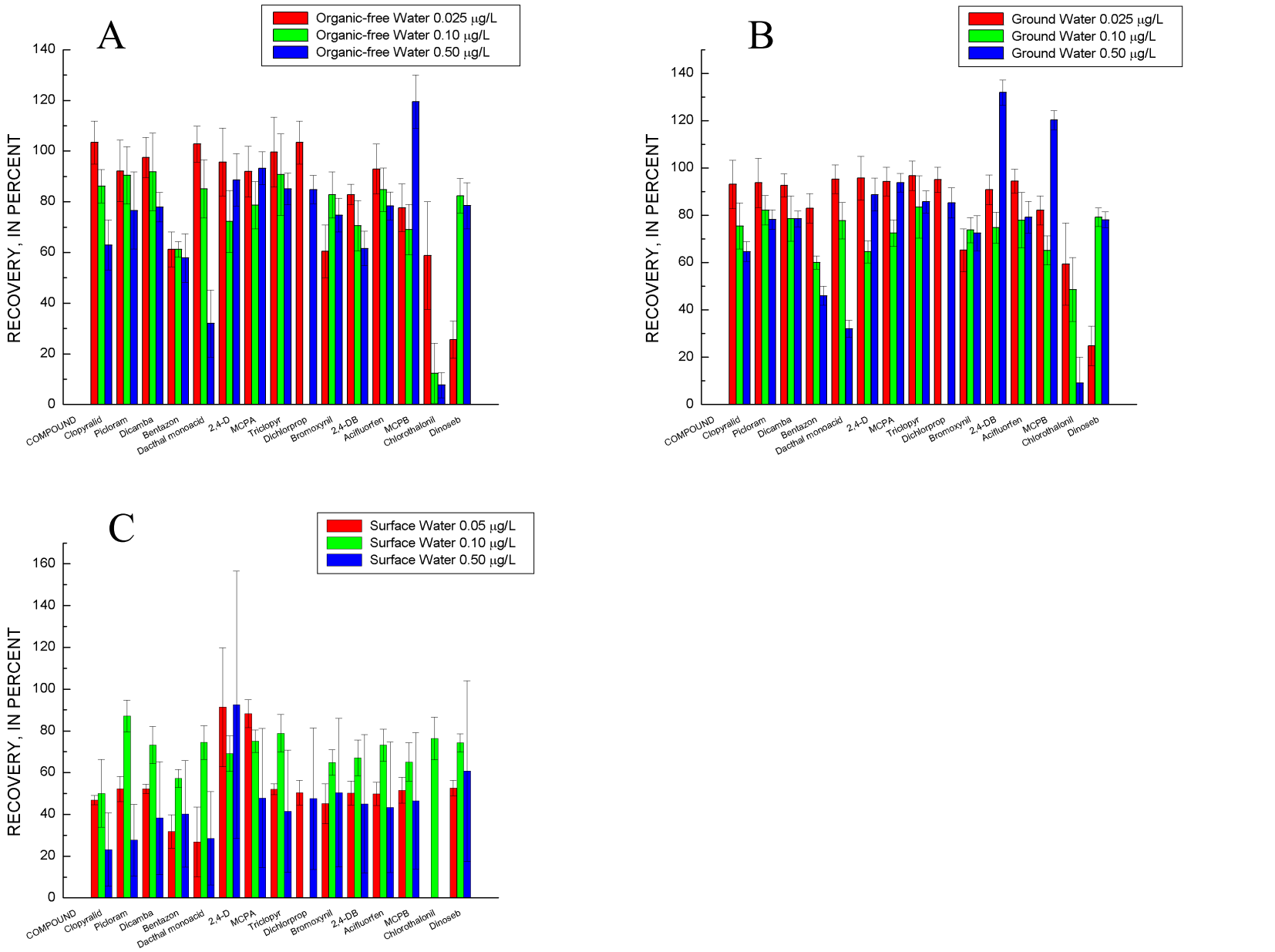

Figure 5. Recoveries, in percent, for compounds determined under negative ionization conditions. (A) Compounds fortified at three levels in organic-free water; (B) compounds fortified at three levels in ground-water samples; and (C) compounds fortified at three levels in surface-water samples. Error bars indicate ±1 standard deviation.

although they are somewhat more variable in the surface-water samples. Recoveries of the sulfonylurea, sulfonamide, and imidazolinone herbicides (fig 4C) are the most enhanced of all the compound classes in the surface-water samples and averaged 214 ± 35 percent compared to 108 ± 9 and 134 ± 14 percent in the organic-free and in the ground-water samples, respectively. The miscellaneous compounds are variable, with the most enhancement measured in the recoveries of tribenuron, but with significant enhancement in the recoveries of imadacloprid, metalaxyl, norflurazon, and propiconazole (fig. 4D).

Concentrations of compounds determined under negative ionization conditions (fig. 5) suggest no matrix enhancement. Recoveries were typically less than 100 percent. Recoveries for all compounds at all fortifications averaged 75.2 ± 9.3, 80.0 ± 6.5, and 45.8 ± 18.8 percent in samples of organic-free water, ground water, and surface water, respectively. Chlorothalonil was variably and poorly recovered in all matrices and was a substantial influence on the observed recoveries and standard deviations in all matrices (fig. 5). Recoveries also were lower and more variable in the surface-water samples and may reflect either matrix interference or suppression of recovery.

11.6 Long-term laboratory reagent spike results

11.6.1 As noted earlier, fortified organic-free water or laboratory reagent spike (LRS) samples were analyzed as part of the quality control for each environmental sample set. These LRS samples were made up from the same pesticide-free water used for reagent-water fortification recovery experiments and were at concentrations of 0.250 µg/L for each compound. The LRS provides set-specific quality-control information for interpretation of individual samples in the set. When aggregated (table 32), LRS data also provide long-term, multiple instrument, and multiple operator performance data. For the purposes of determining long-term method performance, results from fiscal year 2000 were aggregated and summary statistics were produced. The aggregation was made for fiscal year 2000 so that the data reflect the performance of the method after implementation and training of the chemists who perform the analyses. A total of 285 reagent spikes was determined in fiscal year 2000.

11.6.2 Long-term LRS recoveries averaged 73.8 percent for all compounds, with an average standard deviation of 24.2 percent. The average median recovery for all compounds was similar to the mean, at 73.4 percent, and the average *f*-pseudosigma of recovery was 21.4 percent. All compounds in the method ranked according to increasing mean recoveries are shown in figure 6.

Two statistics were used to determine if the reported concentration of any compound required quantitation qualification. Median recoveries calculated from long-term LRS data are used to estimate the accuracy of concentration. A nonparametric statistic, *f*-pseudosigma (Hoaglin, 1983) is calculated to determine the variation of LRS recoveries. These statistics are used instead of the mean and standard deviation because they minimize the effects of outlier values, and are thus more representative of long-term method performance under multiple operators and instruments. Median recoveries had to fall within 60 and 120 percent for a compound to be considered reportable without qualification. In addition, the *f*-pseudosigma statistic had to be less than 25 percent for a compound to be reported without qualification.

Median recoveries and the *f*-pseudosigma statistics for those recoveries were calculated for set LRS data determined between October 1, 1999, and September 31, 2000. This data period was used because it was considered representative of method performance after the

Table 32. Long-term accuracy and precision calculated from multiple determinations of the method compounds fortified in sample-set spikes with the compounds added to organic-free water at 0.25 microgram per liter

[N, number of determinations]

Compound	N	Number of missing values	Mean recovery (percent)	Standard deviation of recovery (percent)	Median recovery (percent)	*F*-pseudosigma of recovery (percent)	Minimum recovery (percent)	Maximum recovery (percent)
Compounds reported without qualification (Median Recovery between 60 and 120 percent and *f*-pseudosigma less than 25 percent)								
2,4-D	281	4	101.8	43.6	102.0	16.3	0.1	754
2,4-D methyl ester	282	3	83.5	19.3	84.5	15.4	0.1	150
3(4-chlorophenyl)-1-methyl urea	282	3	95.5	14.8	96.0	9.5	0.1	155
3-Hydroxycarbofuran	282	3	91.7	18.7	92.0	12.6	0.1	178
Acifluorfen	280	5	84.0	20.2	86.0	18.5	0.0	134
Atrazine	282	3	74.4	19.3	77.0	16.3	0.1	135
Bendiocarb	282	3	75.8	21.1	79.0	12.6	0.1	154
Benomyl	280	5	89.0	22.9	90.0	13.5	0.0	169
Bensulfuron-methyl	282	3	97.2	22.0	95.0	13.3	0.0	177
Caffeine	282	3	101.2	23.5	103.5	20.6	0.1	172
Carbaryl	282	3	87.7	17.1	89.0	9.5	0.0	173
Carbofuran	282	3	90.7	16.1	90.0	9.6	0.1	166
Chlorimuron-ethyl	282	3	102.6	26.3	101.5	19.1	0.0	179
Clopyralid	281	4	78.8	17.9	80.0	14.8	0.0	117
Dacthal monoacid	279	6	82.1	14.3	83.0	14.1	0.1	115
Dicamba	281	4	88.8	19.6	89.0	17.8	0.0	144
Dichlorprop	280	5	75.5	15.0	78.0	14.8	0.1	106
Dinoseb	281	4	80.0	21.6	79.0	21.5	0.0	139
Diphenamid	282	3	83.4	16.2	84.0	11.7	0.1	158
Diuron	282	3	89.8	15.5	90.0	9.6	0.1	160
Fenuron	282	3	73.6	19.5	75.0	21.3	0.0	145
Fluometuron	282	3	85.7	14.9	86.0	11.1	0.1	155
Imidacloprid	282	3	106.2	22.7	106.5	19.3	0.1	178
Linuron	282	3	89.5	16.7	91.0	11.1	0.1	154
MCPA	281	4	70.5	18.6	72.0	14.8	0.1	156
Metalaxyl	282	3	85.8	39.5	84.0	9.6	0.1	701
Neburon	281	4	89.8	18.2	92.0	9.6	0.1	160
Nicosulfuron	282	3	103.9	27.7	103.5	22.2	0.1	205
Oryzalin	282	3	80.7	17.9	81.0	14.1	0.1	150
Oxamyl	282	3	64.1	19.4	66.0	16.9	0.0	144
Picloram	281	4	88.7	17.4	89.0	15.6	0.0	130
Propham	282	3	90.9	16.3	90.0	13.3	0.1	161
Propiconazole	281	4	102.4	20.2	105.0	14.1	0.1	157
Propoxur	282	3	83.6	14.2	85.0	11.1	0.1	126
Siduron	282	3	80.3	21.0	83.0	23.0	0.1	148
Sulfometuron-methyl	282	3	85.5	21.6	88.0	15.6	0.0	148
Tebuthiuron	282	3	100.4	16.1	101.0	11.1	0.1	172
Triclopyr	281	4	83.2	16.7	85.0	14.1	0.1	136

Table 32. Long-term accuracy and precision calculated from multiple determinations of the method compounds fortified in sample-set spikes with the compounds added to organic-free water at 0.25 microgram per liter—Continued

Compound	N	Number of missing values	Mean recovery (percent)	Standard deviation of recovery (percent)	Median recovery (percent)	*F*-pseudosigma of recovery (percent)	Minimum recovery (percent)	Maximum recovery (percent)
Compounds qualified with an E-code because bias was outside acceptable range (median recovery less than 60 percent or greater than 120 percent)								
2,4-DB	281	4	53.1	15.0	55.0	12.6	0.1	99
3-Ketocarbofuran	282	3	44.7	28.5	41.5	32.6	0.0	112
Aldicarb	281	4	11.4	15.5	4.0	13.3	0.0	66
Aldicarb sulfone	279	6	55.2	18.9	59.0	17.0	0.0	109
Aldicarb sulfoxide	271	14	30.5	27.8	26.0	37.8	0.0	113
Bentazon	278	7	38.6	35.4	25.0	43.6	0.0	121
Bromoxynil	281	4	60.3	61.4	52.0	51.9	0.1	890
Chloramben methyl ester	275	10	56.2	30.7	57.0	37.1	0.0	157
Chlorothalonil	281	4	20.1	21.2	17.0	25.2	0.0	80
Cycloate	277	8	38.2	30.7	31.0	40.8	0.0	123
Flumetsulam	282	3	130.0	34.8	128.5	34.8	0.1	212
Imazaquin	277	8	123.0	36.9	124.0	26.7	0.1	259
MCPB	281	4	55.9	42.8	56.0	13.3	0.1	725
Methiocarb	282	3	41.6	36.6	30.0	53.9	0.0	159
Methomyl	282	3	43.2	32.9	39.0	46.0	0.0	152
Methomyl oxime	281	4	3.3	4.0	3.0	4.4	0.0	24
Metsulfuron-methyl	282	3	25.3	30.7	11.0	31.9	0.0	105
Oxamyl oxime	271	14	10.3	9.9	10.0	11.9	0.0	65
Tribenuron-methyl	278	7	6.1	12.2	0.0	5.9	0.0	117
Compounds qualified with an E-code because variation was outside acceptable range (*f*-pseudosigma greater than 25 percent)								
2-Hydroxyatrazine	282	3	102.6	33.1	100.0	28.2	0.2	213
Bromacil	282	3	67.7	47.5	68.0	36.1	0.1	691
Deethylatrazine	282	3	69.2	27.7	69.0	30.9	0.1	132
Deethyldeisopropylatrazine	282	3	64.7	26.5	62.5	31.1	0.1	179
Deisopropylatrazine	282	3	67.3	29.2	67.0	30.4	0.1	149
Imazethapyr	229	56	113.5	54.3	115.0	35.6	0.0	336
Norflurazon	282	3	69.9	24.4	73.0	28.9	0.1	154
Terbacil	282	3	65.7	31.7	70.5	40.8	0.1	141

method had been implemented and the operators of the method experienced in its use. The results of these calculations are listed in table 32. Median recoveries for all compounds are plotted in figure 6 to provide a reference set of typically acceptable performance. Median recovery and *f*-pseudosigma limits are used as a guide, thereby resulting in 27 compounds reported as qualified concentration estimates.

Of these 27 compounds, nine are pesticide degradates. Two compounds, flumetsulam and imazaquin, had median recoveries greater than 120 percent. These two compounds are known to experience matrix enhancement of concentration when determined with a completely different SPE isolation method and by positive ion electrospray HPLC/MS (Furlong and others, 2000). Although there should be no matrix present in these set fortification samples, it is possible that matrix carryover, or minor amounts of matrix contamination that result from leaching from the Carbopack SPE phase, might result in matrix enhancement.

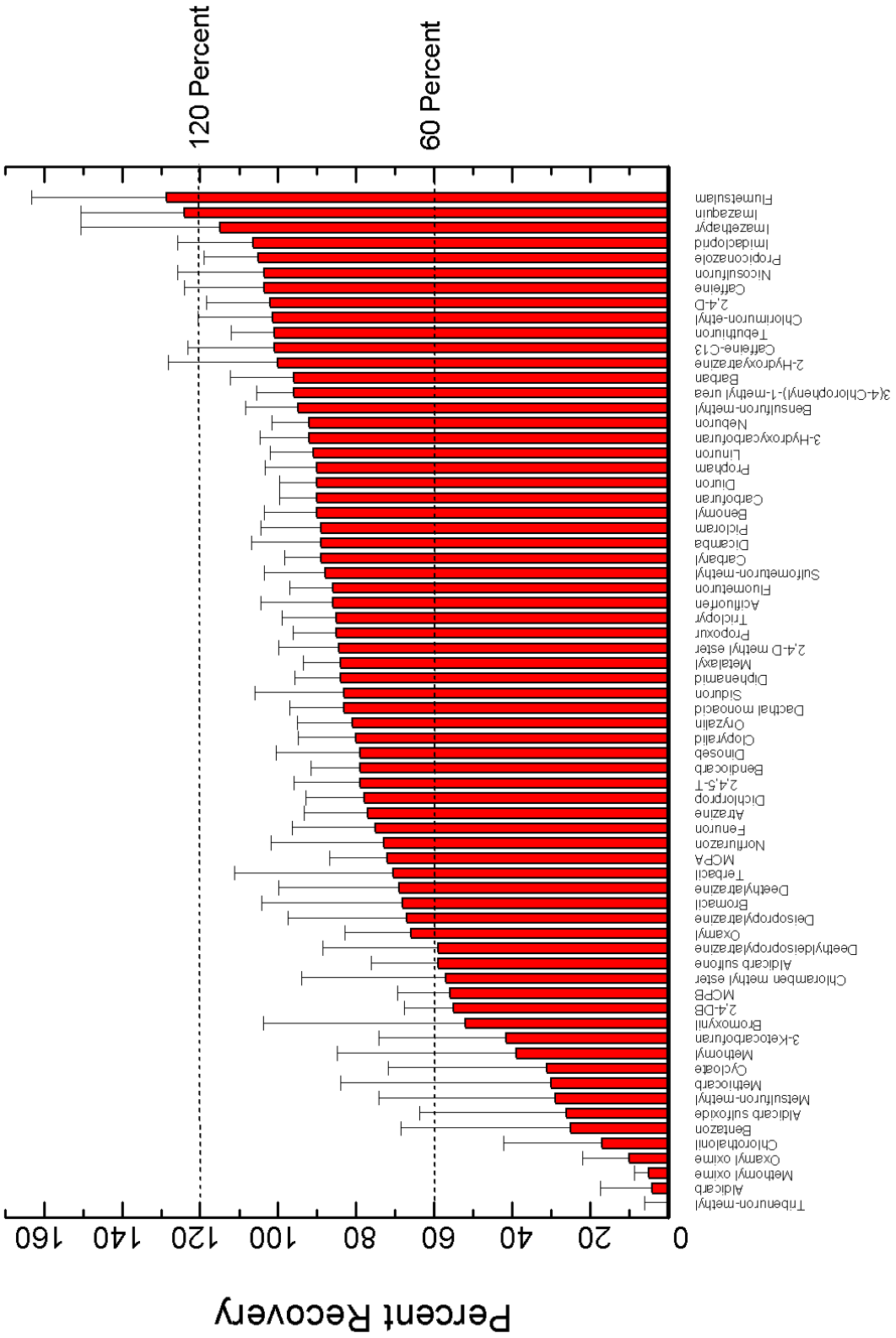

Figure 6. Median recoveries of method compounds from 285 laboratory reagent spikes determined from October 1, 1999 to September 30, 2000. Error bars are from the *f*-pseudosigma statistic.

For these 27 compounds, recoveries averaged 55.5 percent for all compounds, with an average standard deviation of 29.7 percent. The average median recovery for all compounds was similar to the mean, at 53.5 percent, and the average *f*-pseudosigma of recovery was 30.4 percent.

The remaining 38 compounds met recovery performance criteria and were reported without qualification. For these compounds, recoveries averaged 87.3 percent for all compounds, with an average standard deviation of 20.1 percent. The average median recovery for all compounds was similar to the mean, at 88.2 percent, and the average *f*-pseudosigma of recovery was 14.7 percent.

11.7 Laboratory reagent blanks

A total of 498 LRBs was analyzed while this method was used as a provisional custom method. A subset of 197 LRBs was reviewed in detail to determine the frequencies and concentrations of possible blank contamination. These results are listed in table 33. Twenty-eight of 65 compounds measured in this method were detected in the set blanks. Except for

Table 33. Detections of compounds in 197 set blanks analyzed with this method

[µg/L, microgram per liter]

Compound	Number of detections	Mean concentration, in µg/L	Median concentration, in µg/L
2,4-D methyl ester	1	0.0166	0.0166
2-Hydroxyatrazine	1	.041	.0041
3(4-chlorophenyl)-1-methyl urea	1	.0018	.0018
3-Hydroxycarbofuran	2	.0014	.0014
Atrazine	4	.0016	.0014
Bendiocarb	1	.0004	.0004
Benomyl	1	.0018	.0018
Bromacil	1	.0089	.0089
Caffeine	3	.0059	.0057
Carbaryl	2	.0023	.0023
Cycloate	4	.0109	.0106
Deethylatrazine	2	.0011	.0011
Deethyldeisopropylatrazine	1	.0015	.0015
Dinoseb	5	.0011	.0011
Diphenamid	1	.0007	.0007
Diuron	6	.0109	.0047
Fenuron	38	.0116	.0059
Fluometuron	4	.0018	.0020
Imazaquin	7	.0054	.0073
Imazethapyr	3	.0295	.0298
Metalaxyl	2	.0006	.0006
Methiocarb	2	.0275	.0275
Neburon	3	.0013	.0011
Norflurazon	5	.0018	.0014
Propiconazole	12	.0011	.0011
Siduron	1	.0014	.0014
Sulfometuron-methyl	5	.0036	.0041
Tebuthiuron	6	.0016	.0015

fenuron, the frequency of detections ranged from 1 to 12 in 197 blanks. The median frequency of detection in all blanks, except for fenuron, was 2.5 detections. Detections were clustered in 38 blank samples. In total, 93 detections of all compounds were measured, a rate of 0.73 percent, calculated on the basis of 12,805 individual compound determinations made for 65 compounds in 197 blank samples.

One compound, imazethapyr, was detected one time at a concentration greater than its median method laboratory reporting level (LRL is calculated as twice the method detection limit; see section 11.8). Only 11 of the method analytes detected were at concentrations greater than 0.0025 µg/L. For these 11 compounds (sulfometuron-methyl, norflurazon, methiocarb, imazethapyr, imazaquin, diuron, cycloate, caffeine, bromoxynil, 2-hydroxyatrazine, 2,4-D), the median detected concentration was 0.0018 µg/L.

Fenuron was determined in 38 of 197 blanks, a rate of 19 percent, and was the only compound identified frequently enough to be considered a chronic contaminant for the method. It is unclear whether this chronic contamination was a result of actual fenuron contamination or the result of a coeluting interference that contributed ions that were identical to those used for the selected-ion monitoring determination of fenuron. For chronic contamination of this type, the statistics of the distribution should be calculated, the 95th percentile of the distribution determined, and the result used to qualify detections in field samples.

11.8 Method detection limits

11.8.1 Method detection limits (MDLs) were determined for this method using the procedures of the U.S. Environmental Protection Agency (1997). Data for the MDL determination were for the lowest concentration of fortification used to determine method recoveries (0.025 µg/L for the organic-free samples and the ground-water samples, and 0.05 µg/L for the surface-water samples). The calculated MDLs are listed in table 34. The MDLs calculated for the predecessor method of Werner and others (1996) are also listed in table 34. The extraction part of this method is based on that report. There are 30 compounds in the present method that are not included in the method by Werner and others; the latter contains six compounds not in the present method. These exceptions are listed separately at the bottom of table 34.

11.8.2 On average, the MDLs for both methods were comparable. The mean MDLs for all compounds in the present method were 0.019 ± 0.090, 0.007 ± 0.012, and 0.017 ± 0.010 µg/L for the organic-free water, ground-water, and surface-water samples, respectively. The mean MDLs for all compounds in the method by Werner and others (1996) were 0.014 ± 0.006, 0.018 ± 0.009, and 0.029 ± 0.023 µg/L for organic-free water, ground-water, and surface-water samples, respectively. The median MDLs for the present method were 0.007, 0.003, and 0.014 µg/L for the organic-free water, ground-water, and surface-water samples, respectively. These MDLs compare favorably with median MDLs of 0.007, 0.009, and 0.026 µg/L for organic-free water, ground-water, and surface-water samples, respectively, in the method by Werner and others (1996). The mean MDL for all analytes in the present method, regardless of long-term performance, was 0.033 µg/L.

11.8.3 If the comparison of MDLs is limited to the 35 compounds common to both methods, the data suggest that this new method offers improved detection at lower concentrations. In the new method, the mean MDLs for the 35 compounds were 0.008 ± 0.004, 0.004 ± 0.002, and 0.016 ± 0.011 µg/L for organic-free water, ground-water, and surface-water samples, respectively.

Table 34. Method detection limits for the method described in this study and the predecessor method of Werner and others (1996)

[Concentrations in microgram per liter (µg/L). MDL, method detection limit; nd, not determined]

Compound	This method, MDL in organic-free water	Werner method, MDL in organic-free water[1]	This method, MDL in ground-water samples	Werner method, MDL in ground-water samples[1]	This method, MDL in surface-water samples	Werner method, MDL in surface-water samples[1]
2,4-D	0.0109	0.013	0.0057	0.023	0.0463	0.021
2,4-DB	.0080	.013	.0055	.015	.0091	.012
3-Hydroxycarbofuran	.0029	.014	.0074	.015	.0171	.026
Acifluorfen	.0033	.008	.0043	.021	.0094	.022
Aldicarb	.0198	.016	.0036	.014	.0217	.020
Aldicarb sulfone	.0098	.021	.0028	.016	.0227	.037
Aldicarb sulfoxide	.0041	.016	.0023	.019	.0056	.043
Bentazon	.0055	.014	.0032	.022	.0130	.029
Bromacil	.0163	.011	.0033	.040	.0102	.050
Bromoxynil	.0085	.012	.0060	.011	.0155	.011
Carbaryl	.0142	.008	.0020	.018	.0154	.016
Carbofuran	.0028	.028	.0025	.028	.0103	.044
Chloramben methyl ester	.0089	.011	.0032	.011	.0141	nd
Chlorothalonil	.0173	.007	.0088	.012	nd	.021
Clopyralid	.0069	.018	.0034	.007	.0037	.023
Dacthal monoacid	.0058	.017	.0029	.035	.0272	.024
Dicamba	.0064	.011	.0027	.025	.0036	.021
Dichlorprop	.0069	.032	.0052	.018	.0096	.043
Dinoseb	.0060	.010	.0028	.032	.0061	.013
Diuron	.0075	.012	.0018	.012	.0196	.040
Fenuron	.0158	.013	.0032	.017	.0224	.067
Fluometuron	.0155	.010	.0021	.010	.0113	.038
Linuron	.0072	.006	.0034	.016	.0206	.024
MCPA	.0081	.014	.0032	.009	.0110	.016
MCPB	.0077	.010	.0032	.009	.0101	.038
Methiocarb	.0040	.026	.0031	.015	.0167	.058
Methomyl	.0022	.017	.0036	.008	.0177	.047
Neburon	.0060	.015	.0032	.015	.0146	.044
Norflurazon	.0082	.024	.0021	.014	.0120	.024
Oryzalin	.0088	.019	.0054	.008	.0488	.042
Oxamyl	.0061	.018	.0044	.008	.0374	.042
Picloram	.0099	.004	.0033	.011	.0098	.022
Propham	.0048	.011	.0021	.011	.0184	.021
Propoxur	.0040	.008	.0023	.033	.0078	.027
Triclopyr	.0112	.010	.0038	.007	.0043	.021

Table 34. Method detection limits for the method described in this study and the predecessor method of Werner and others (1996)—Continued

Compound	This method, MDL in organic-free water	Werner method, MDL in organic-free water[1]	This method, MDL in ground-water samples	Werner method, MDL in ground-water samples[1]	This method, MDL in surface-water samples	Werner method, MDL in surface-water samples[1]
Compounds analyzed in the present method only						
2,4-D methyl ester	0.0043		0.0049		0.0177	
2-Hydroxyatrazine	.0040		.0045		.0146	
3(4-chlorophenyl)-1-methyl urea	.0121		.0034		.0124	
3-Ketocarbofuran	.0071		.0964		.0015	
Atrazine	.0045		.0028		.0135	
Bendiocarb	.0126		.0035		.0147	
Benomyl	.0019		.0047		.0135	
Bensulfuron-methyl	.0079		.0108		.0246	
Caffeine	.0048		.0019		.0346	
Chlorimuron-ethyl	.0048		.0048		.0320	
Cycloate	.0065		.0097		.0198	
Deethylatrazine	.0141		.0029		.0099	
Deethyldeisopropylatrazine	.0222		.0019		.0093	
Deisopropylatrazine	.0052		.0029		.0100	
Diphenamid	.0132		.0042		.0126	
Flumetsulam	.0057		.0096		.0284	
Imazaquin	.0078		.0306		.0209	
Imazethapyr	.0084		.0060		.0447	
Imidicloprid	.0034		.0101		.0383	
Metalaxyl	.0100		.0029		.0122	
Methomyl oxime	.0053		.0031		.0053	
Metsulfuron-methyl	.0123		.0172		.0146	
Nicosulfuron	.0065		.0104		.0380	
Oxamyl oxime	.0067		.0158		.0106	
Propiconazole	.0105		.0050		.0163	
Siduron	.0084		.0029		.0143	
Sulfometuron-methyl	.0044		.0092		.0210	
Tebuthiuron	.0031		.0086		.0141	
Terbacil	.0049		.0020		.0112	
Tribenuron-methyl	.0044		.0064		.0216	
Compounds analyzed in the method of Werner and others (1996) but not determined in the present method						
1-Naphthol		0.007		0.023		0.027
2,4,5-T		.010		.037		.027
Dichlobenil		.012		.12		.16
DNOC		.006		.015		.011
Esfenvalerate		.019		.033		.039
Silvex		.021		.021		.015

[1]Werner, S.L., Burkhardt, M.R., and DeRusseau, S.N., 1996, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of pesticides in water by Carbopak-B solid-phase extraction and high-performance liquid chromatography: U.S. Geological Survey Open-File Report 96-216, 42 p.

The mean MDLs for the same compounds in the method by Werner and others (1996) were 0.014 ± 0.006, 0.017 ± 0.009, and 0.031 ± 0.014 µg/L for organic-free water, ground-water, and surface-water samples, respectively. The median MDLs of the common compounds for the present method were 0.008, 0.003, and 0.014 µg/L for the organic-free water, ground-water, and surface-water samples, respectively. These compare favorably with median MDLs of 0.006, 0.009, and 0.026 µg/L for organic-free water, ground-water, and surface-water samples, respectively, in the method by Werner and others (1996).

The difference between the mean and standard deviations for all compounds in relation to mean and standard deviations for the compounds common to both methods is likely the result of the additional compounds in the present method being more difficult to isolate, more subject to degradation during sample preparation, or more difficult to resolve chromatographically. These effects would likely introduce additional variation to the concentrations measured. The MDLs for these difficult compounds would be greater than for the compounds common to both methods because the MDL determination is a function of standard deviation. The method documented in this report is likely to result in more frequent detections at comparable or lower concentrations than the method of Werner and others (1996), given that HPLC/MS offers more specific detection in the presence of matrix interferences than does HPLC with ultraviolet detection.

CONCLUSIONS

Solid-phase extraction coupled with high-performance liquid chromatography/ mass spectrometry (HPLC/MS) analysis can be used to determine and quantify 65 polar pesticides and pesticide degradates in surface- and ground-water samples, at concentrations as low as 0.002 µg/L (microgram per liter) in environmental water samples. This method has comparable or better method detection limits than the existing complementary method of Werner and others (1996), with the additional advantage that 65 compounds from 25 pesticide classes are measured compared to 40 compounds in 15 pesticide classes in the Werner method. The single-operator mean standard deviation at 0.025 µg/L in organic-free water samples is 11 percent. Recoveries in organic-free water samples ranged from 28 to175 percent, with elevated recoveries reflecting apparent matrix enhancement, likely from elevated dissolved organic carbon concentrations. The multiple operator (five), multiple instrument (five) mean relative standard deviation is 24.2 percent for all compounds in 285 organic-free water samples fortified at 0.25 µg/L and analyzed for 1 year from October 1999 through September 2000. Mean recoveries in these same samples were 73.8 percent for all compounds. The mean long-term set fortification recovery of the 38 compounds in the method reported without qualification ranged between 60 and 120 percent. An interference corresponding to the chromatographic and mass spectrometric characteristics of fenuron was identified as a chronic contaminant and required qualified reporting of this compound. Eleven compounds were detected sporadically in blanks at concentrations greater than 0.025 µg/L. The single operator, single instrument validation data and the long-term quality-control data reported here provide strong evidence for the application of HPLC/MS to large-scale, routine monitoring programs for pesticides in surface and ground water at concentrations as low as 10 parts per trillion (0.010 µg/L or 10 ng/L).

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