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Quantification of Pyrethroids in Environmental Samples Using NCI-GC-MS with Stable Isotope Analogue Standards

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ABSTRACT: Stable isotope internal standards are useful in correcting for matrix effects and instrumental variability when environmental samples such as wastewaters and biosolids are analyzed by mass spectral methods. This paper reports the use of deuterium-labeled analogues of eight pyrethroid insecticides to improve accuracy for the analysis of environmental samples by negative chemical ionization gas chromatography with mass spectrometric detection (NCI-GC-MS). Data for the analysis of effluent water from wastewater treatment facilities are presented which demonstrate that the method is rugged and capable of achieving limits of quantification (LOQs) as low as 0.5 ng/L (ppt), with individual recoveries within the range of 81−94% for those compounds with minimal control background concentrations. In addition, an alternate use of the deuterium-labeled standards is proposed for the determination of method recoveries at low levels that would normally have been precluded due to background pyrethroid levels present in environmental samples being used for control fortifications.

KEYWORDS: *biosolids, chromatography, method, mass spectrometry, pyrethroid, stable isotope, water*

INTRODUCTION

Because of their insecticidal effectiveness and low mammalian toxicity, synthetic pyrethroids are widely used for home and lawn applications as well as for crop protection purposes. Ever-increasing scrutiny, focused upon environmental effects of synthetic pyrethroids at trace levels,[1] is driving the need for analytical methods that are rugged, reliable, and extremely sensitive. For pyrethroids, methods are ideally tailored to account for challenges associated with this chemical class, including extremely low water solubility and compound stability following sampling. Multianalyte methods for this compound class are commonly based upon gas chromatography.[2−10] The vulnerability of gas chromatographic methods to signal enhancement due to matrix coextractives is well-known.[11,12] Additionally, detector sensitivity can drift over time, due to either matrix contamination or other factors. The nature of complex environmental samples (such as biosolids and wastewaters) often precludes the use of matrix-matched standards as a corrective for matrix effects because of the difficulties in obtaining controls that are analyte-free while at the same time mimicking the unknown sample compositions. (Our experience with analyzing control sample candidates from several wastewater treatment facilities on both the East and West Coasts of the United States has demonstrated that samples without measurable amounts of at least some of the pyrethroids being monitored are not available.) Although the addition of analyte protectants[12] can prevent peak tailing and in some cases minimize specific analyte effects due to coextractives, this does not correct for instrumental drift over time. We have investigated the use of stable deuterium-labeled isotopic analogues of eight pyrethroid insecticides to improve method accuracy and performance when applied to the analysis of environmental samples, such as surface waters, wastewaters, and biosolids by negative chemical ionization gas chromatography with mass spectrometric detection (NCI-GC-MS), and we report here methodology for the analysis specifically of effluent water samples collected from publicly owned treatment works (POTWs). The use of hexane as a partitioning solvent limits the coextractables and provides for a clear chromatographic background. Although the use of stable isotope standards for mass spectral methods has become a standard technique,[10,13,14] there are some nuances due to the GC separation of stereoisomers that have been investigated and are reported herein. In addition, an alternate, and unique, use of the deuterated (d_6) standards is proposed for the determination of method recoveries at low levels that would normally have been precluded due to background pyrethroid levels present in environmental samples being used for control fortifications.

MATERIALS AND METHODS

Safety. Flammable solvents were stored in fire-resistant solvent cabinets when not in use, and solvent waste was collected and disposed of as required by local, state, and federal regulations.

Chemicals and Reagents. Solvents used were acetone, acetonitrile, cyclohexane, diethyl ether, hexane, and methanol (Fisher Optima grade or equivalent, Fisher Scientific, Fair Lawn, NJ, USA). Formic acid was 88%, GR ACS (EMD Chemicals, Gibbstown, NJ, USA). Peanut oil was cooking grade (Planters Co., East Hanover, NJ, USA). Sodium sulfate was analytical reagent grade, anhydrous granular (Fisher Scientific). Sodium chloride was 99% GR ACS (EMD Chemicals). Solid phase extraction cartridges were Agilent Bond

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Elut LRC-Si, 500 mg (Agilent Technologies, Walnut Creek, CA, USA).

Laboratory Supplies and Equipment. Evaporation tubes used were Zymark, glass, 200 mL, and were used with a Zymark Turbo-vap II concentrator (Zymark Corp., Hopkinton, MA, USA). The final evaporation step was accomplished with an N-Evap Laboratory Sample Evaporator model 115 attached to a nitrogen source (Organomation Associates, South Berlin, MA, USA).

Analytical Standards. Analytical reference standards of bifenthrin, cyfluthrin (or β-cyfluthrin, depending upon the intended application of the method), cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, λ-cyhalothrin, and permethrin were obtained from ChemService (West Chester, PA, USA) or directly from the individual pesticide registrants (cyfluthrin, β-cyfluthrin, and deltamethrin from Bayer CropScience, Research Triangle Park, NC, USA; esfenvalerate from DuPont Crop Protection, Newark, DE, USA; bifenthrin, cypermethrin, and permethrin from FMC, Ewing, NJ, USA; λ-cyhalothrin from Syngenta Crop Protection, Greensboro, NC, USA; and fenpropathrin from Valent USA Corp., Walnut Creek, CA, USA). Stable isotope (d_6) analogues of bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, λ-cyhalothrin, and permethrin were custom synthesized by Kalexsyn, Inc. (Kalamazoo, MI, USA).

Standard Solutions. All standard solutions, prepared as described below, were stored frozen (−8 to −22 °C) in amber glass bottles, when not in use. For the analytical reference standards, 25.0 mg (corrected for purity) of bifenthrin, cypermethrin, cyfluthrin/β-cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, λ-cyhalothrin, and permethrin were accurately weighed and quantitatively transferred to separate 25 mL volumetric flasks and brought to volume with acetonitrile. The resulting stock solution concentrations were 1000 μg/mL. For the d_6 internal standards, 5.0 mg (corrected for purity) of each internal standard was accurately weighed directly into separate 25 mL volumetric flasks and brought to volume with acetonitrile. The resulting concentration, corrected for purity and actual amount weighed for each stock internal standard, was approximately 200 μg/mL.

Mixed intermediate/fortification standard solutions were prepared, as described, with the first listed concentration referring to bifenthrin, cyfluthrin/β-cyfluthrin, cypermethrin, esfenvalerate, fenpropathrin, and λ-cyhalothrin, the second to deltamethrin, and the third to permethrin. All intermediate/fortification standards were brought to volume with acetonitrile and mixed well. For 5.0/10/50 μg/mL, 125 μL each of the bifenthrin, cypermethrin, cyfluthrin/β-cyfluthrin, esfenvalerate, fenpropathrin, and λ-cyhalothrin stock standard solutions, 250 μL of the deltamethrin stock standard solution, and 1.25 mL of the permethrin stock standard solution were added to a 25 mL volumetric flask. Serial dilutions were then made to produce intermediate/fortification standards at concentrations of 0.50/1.0/5.0, 0.050/0.10/0.50, and 0.0050/0.010/0.050 μg/mL. Intermediate calibration standard solutions were prepared as mixtures as follows. For 0.50/1.0/5.0 μg/mL, 2.5 mL of the intermediate 5.0/10/50 μg/mL mixed standard solution was transferred to a 25 mL volumetric flask and brought to volume with acetone/cyclohexane (50:50, v/v). For 0.050/0.10/0.50 μg/mL, 5.0 mL of this 0.50/1.0/5.0 μg/mL mixed standard solution in (approximately) acetone/cyclohexane (50:50, v/v) was added to a 50 mL volumetric flask and brought to volume in cyclohexane and mixed well.

Mixed internal standard solutions were prepared, as described, with the first listed concentration referring to bifenthrin-d_6, cyfluthrin-d_6, cypermethrin-d_6, esfenvalerate-d_6, fenpropathrin-d_6, and λ-cyhalothrin-d_6, the second to deltamethrin-d_6, and the third to permethrin-d_6. An internal standard mixture at concentrations of 1.0, 2.0, and 10 μg/mL was prepared by transferring 125 μL, 250 μL, and 1.25 mL, respectively, of the 200 μg/mL internal standard stock solutions to a 25 mL volumetric flask, bringing to volume with acetone/cyclohexane (50:50, v/v), and mixing well. A 10/20/100 ng/mL mixed standard was prepared by transferring 2.5 mL of the 1.0/2.0/10 μg/mL internal standard solution to a 250 mL volumetric flask, bringing to volume with 0.1% peanut oil in cyclohexane, and mixing well.

GC-MSD calibration standards were prepared in 0.1% peanut oil in cyclohexane solution, with the peanut oil additive used to minimize possible peak tailing due to adsorption.[2] All calibration curve standards prepared as described below were brought to volume with the 0.1% peanut oil in cyclohexane and mixed well. The mixed internal standard components were prepared to remain constant (at 10/20/100 ng/mL) at each point of the native analyte curve range. For 10/20/100 ng/mL + 10/20/100 ng/mL IS, 5.0 mL of the 0.050/0.10/0.50 μg/mL mixed standard solution in cyclohexane and 250 μL of the 1.0/2.0/10 μg/mL mixed internal standard solution in acetone/cyclohexane (50:50, v/v) were transferred to a 25 mL volumetric flask. Four additional calibration standards, ranging from 5.0/10/50 ng/mL + 10/20/100 ng/mL IS to 0.25/0.50/2.5 ng/mL + 10/20/100 ng/mL IS, were similarly prepared, using appropriate volumes of the 0.050/0.10/0.50 μg/mL mixed standard and 250 μL of the 1.0/2.0/10 μg/mL mixed internal standard in 25 mL volumetric flasks.

Water Sample Preservation. Effluent water is the end product of the water treatment processes that are performed by POTWs on the aqueous portion of incoming raw sewage, prior to discharge into surface water. Effluent water used for method development was obtained from a local POTW and stored under refrigeration (2−8 °C).

Effluent water samples should be collected in glass containers (500 mL volume is recommended). Unless the samples will be analyzed within a week of collection, formic acid should be added (to achieve a pH 5−6) at the time of collection, followed by the addition of methanol (at a volume ca. 10% of the water volume, with shaking) as soon as feasible. Regardless of the anticipated holding time, 25 mL of hexane should be added upon sample receipt (unless the samples are to be immediately extracted with hexane as described below). Samples should be stored refrigerated pending analysis.

Sample Extraction and Cleanup. Unless added previously, 50 mL of methanol and 50 g of sodium chloride were added to each 500 mL sample. Hexane (50 mL) was added to each sample (75 mL if the sample has not already had 25 mL of hexane added). The glass sample jars were capped tightly, placed horizontally on their sides, and shaken for 20 min on a platform shaker. After removal from the shaker, the jars were allowed to sit for ca. 10 min to allow time for phase separation. Using a 25 mL disposable glass pipet, the upper hexane layer was removed and drained through a glass funnel with a glass wool plug and ca. 20 g of sodium sulfate into a 200 mL Zymark tube, rinsing the sodium sulfate with ca. 10 mL of hexane into the 200 mL Zymark tube. Another 75 mL of hexane was added to the aqueous samples, and the above procedure was repeated. The remaining aqueous/methanol samples were discarded. Any emulsions that were present after the second partition step were centrifuged to facilitate separation; any remaining emulsions were transferred to the sodium sulfate and rinsed with additional hexane. The combined hexane extracts were concentrated to ca. 1.0 mL using a Turbo-Vap evaporator set to ca. 40 °C. Each sample extract was quantitatively transferred with hexane to a test tube calibrated at 2.0 mL. Volumes were adjusted to 2.0 mL, and the samples were mixed by vortexing. SPE cleanup of the hexane extracts was accomplished using an Agilent Bond Elut LRC-Si solid phase extraction cartridge (500 mg), using a vacuum manifold. After rinsing the SPEs with hexane (3 mL under vacuum at ca. 2 mL/min, draining the hexane only to the top frit), 2.0 mL sample aliquots were passed through the cartridges under gravity or low vacuum, discarding the eluate. After each SPE had been rinsed with 15 mL of hexane (discarded), again under gravity or low vacuum, collection tubes (e.g., 13 × 100 mm) were placed in the manifold rack. Analytes were eluted from each SPE with 6 mL of hexane/diethyl ether solution (9:1, v/v), drawing through under gravity or low vacuum at a rate of approximately 2 mL/min, and collecting the eluates in the tubes. Each eluate was evaporated to dryness under a stream of nitrogen using an N-Evap evaporator set to ∼40 °C. Each dried extract was redissolved in 0.5 mL of 10/20/100 ng/mL internal standard solution, using ultrasonication. Any necessary dilutions to bring the response of any analyte(s) within the demonstrated linear curve range were prepared using the internal standard solution as the diluent, thus maintaining a constant internal standard concentration.

NCI-GC-MS. Quantification of pyrethroid concentrations, with instrumental conditions that were based upon the work of Robinson,[2] was accomplished using an Agilent 6890 gas chromatograph equipped with an Agilent 5973N mass selective detector operated in negative chemical ionization mode, an Agilent 7683 autosampler, and Agilent ChemStation software, G1701CA version C.00.00 and G1701EA version E.02.02. The analytical capillary column used to effect separation of the analytes was a 30 m × 0.25 mm i.d. × 0.25 μm film thickness, Varian CP-SIL 8CB-MS (Varian Corp., now Agilent, Walnut Creek, CA, USA). Table 1 summarizes the operating conditions, Table 2 shows the ions monitored, and Table 3 lists, in the order of analyte elution, approximate retention times for each component of each analyte, including the d_6 analogues. It was noted experimentally that there were slight retention time differences between the native analyte and corresponding d_6 analogue peaks, with the d_6 stable isotopes for some compounds (bifenthrin, fenpropathrin, λ-cyhalothrin, permethrin, cyfluthrin, and cypermethrin) eluting slightly earlier, with deltamethrin exhibiting slightly later retention times for the d_6 analogue peaks.

Table 1. NCI-GC-MS Operating Conditions

inlet liner	4 mm i.d. gooseneck splitless liner packed with Carbo Frit
injection volume	4 μL
injection mode	pulsed splitless, 15 psi for 1 min, purge flow to split vent 50 mL/min at 2 min
carrier gas	helium
column flow	0.9 mL/min, constant flow
detector reagent gas	methane at 30%
dwell time	50 ms
tuning	prior to analysis, the instrument is autotuned for ions m/z 185, 351, and 449
temperatures	injector, 275 °C
	GC-MSD transfer line, 300 °C
	column
	initial, 80 °C, hold 1.00 min
	rate 1, 40 °C/min to 180 °C
	rate 2, 5 °C/min to 285 °C
	rate 3, 30 °C/min
	final, 305 °C hold 5.00 min

Table 2. Ions Monitored by NCI-GC-MS

	target ion	qualifier 1	qualifier 2
bifenthrin	m/z 386	m/z 387	m/z 241
cyfluthrin	m/z 207	m/z 209	m/z 171
cypermethrin	m/z 207	m/z 209	m/z 171
deltamethrin	m/z 299	m/z 295	m/z 297
esfenvalerate	m/z 211	m/z 213	
fenpropathrin	m/z 141		
λ-cyhalothrin	m/z 205	m/z 241	m/z 243
permethrin	m/z 207	m/z 209	
bifenthrin-d_6	m/z 392	m/z 393	m/z 247
cyfluthrin-d_6	m/z 213	m/z 215	m/z 177
cypermethrin-d_6	m/z 213	m/z 215	m/z 177
deltamethrin-d_6	m/z 305	m/z 301	m/z 303
esfenvalerate-d_6	m/z 217	m/z 219	
fenpropathrin-d_6	m/z 147		
λ-cyhalothrin-d_6	m/z 211	m/z 247	m/z 249
permethrin-d_6	m/z 213	m/z 215	

Table 3. NCI-GC-MS Retention Times

compound[a]	peak(s)	approximate retention time (min)
bifenthrin-d_6	1	18.0
bifenthrin	1	18.1
fenpropathrin-d_6	1	18.4
fenpropathrin	1	18.5
λ-cyhalothrin-d_6	1	19.5
	2	19.9
λ-cyhalothrin	1	19.6
	2	20.0
permethrin-d_6	1	21.4
	2	21.7
permethrin	1	21.5
	2	21.8
cyfluthrin-d_6	2	22.7
	4	22.9
cyfluthrin	1	22.6
	2	22.8
	3	22.9
	4	23.0
cypermethrin-d_6	1	23.1
	2	23.3
	3	23.4
	4	23.5
cypermethrin	1	23.2
	2	23.4
	3	23.5
	4	23.6
esfenvalerate-d_6	1	24.5
	2	24.7
esfenvalerate	1	24.5
	2	24.7
deltamethrin	1	25.1
	2	25.3
deltamethrin-d_6	1	25.2
	2	25.4

[a]The analytes are listed in order of retention time.

Calculations. Peak areas (calculated by the ChemStation software) were measured for quantification. For analytes with multiple peaks (isomers), the total area under all peaks for that analyte was used in the calculations. The sum of selected peak areas for each corresponding d_6 internal standard was used to calculate an internal standard (IS) response ratio of native analyte response versus d_6 internal standard response for each pyrethroid.

Linear regression was used to calculate a best-fit line (from a set of standard concentrations in ng/L versus IS ratio, typically injected at the end of each analytical run) to demonstrate the linearity of response for the GC-MSD detector system upon completion of the sample injections. On the basis of this verified linear response, the average response factor of each analyte for the bracketing (injected before and after the sample extracts) standards was used to determine sample residue concentrations, where

Table 4. Summary of Percent Recoveries for Pyrethroids in Effluent Wastewater[a]

	bifenthrin				cyfluthrin				cypermethrin			
added, ng/L	measured, ng/L	background, ng/L	% rec	% av (RSD)	measured, ng/L	background, ng/L	% rec	% av (RSD)	measured, ng/L	background, ng/L	% rec	% av (RSD)
0.5	1.65	1.64	2		0.779	<0.5 (0.428)	70		2.84	2.64	40	
0.5	0.707	<0.5 (0.353)	71	72	0.493	<0.5 (0.0324)	92	79	0.649	<0.5 (0.227)	84	91
0.5	1.91	1.57	68	(67)	0.842	<0.5 (0.395)	89	(13)	3.02	2.45	114	(34)
0.5	0.552	<0.5 (0.146)	81	(*N* = 5)	0.474	<0.5 (0.111)	73	(*N* = 5)	0.517	nd	103	(*N* = 5)
0.5	2.26	1.58	136		0.842	<0.5 (0.477)	73		4.17	3.59	116	
2.5	2.38	<0.5 (0.156)	89		2.26	nd	90		2.38	<0.5 (0.101)	91	
2.5	3.57	1.51	82		2.70	<0.5 (0.471)	89		5.26	3.15	84	
2.5	2.28	<0.5 (0.226)	82		2.20	nd	88		2.28	nd	91	
2.5	3.18	1.23	78	83	2.55	<0.5 (0.341)	88	92	4.67	2.56	84	90
2.5	2.30	<0.5 (0.334)	79	(5.9)	2.42	nd	97	(5.1)	2.35	<0.5 (0.168)	87	(4.8)
2.5	3.69	1.68	80	(*N* = 9)	2.59	<0.5 (0.402)	88	(*N* = 9)	5.42	3.01	96	(*N* = 9)
2.5	2.67	<0.5 (0.457)	89		2.38	nd	95		2.66	<0.5 (0.309)	94	
2.5	2.34	<0.5 (0.127)	89		2.66	<0.5 (0.140)	101		2.64	<0.5 (0.278)	94	
2.5	2.33	<0.5 (0.405)	77		2.24	nd	90		2.26	nd	90	
12.5	11.1	<0.5 (0.0955)	88		11.7	nd	94		11.7	nd	94	
12.5	11.6	0.548	88		11.8	<0.5 (0.0461)	94		12.0	<0.5 (0.382)	93	
12.5	11.9	<0.5 (0.430)	92		11.5	<0.5 (0.109)	91		11.8	<0.5 (0.266)	92	
12.5	11.4	<0.5 (0.193)	90	91	11.5	nd	92	93	11.6	<0.5 (0.161)	92	94
12.5	11.9	0.704	90	(3.5)	11.4	<0.5 (0.359)	88	(4.5)	12.3	0.610	94	(3.0)
12.5	11.7	<0.5 (0.284)	91	(*N* = 8)	11.3	nd	90	(*N* = 8)	11.7	nd	94	(*N* = 8)
12.5	12.3	<0.5 (0.0943)	98		12.7	nd	102		12.6	<0.5 (0.107)	100	
12.5	12.1	0.672	91		11.6	<0.5 (0.169)	91		12.2	0.831	91	

	esfenvalerate				λ-cyhalothrin			
added, ng/L	measured, ng/L	background, ng/L	% rec	% av (RSD)	measured, ng/L	background, ng/L	% rec	% av (RSD)
0.5	0.525	<0.5 (0.141)	77		0.931	0.532	80	
0.5	0.444	<0.5 (0.0364)	82	81	0.524	<0.5 (0.0661)	92	94
0.5	0.536	<0.5 (0.152)	77	(4.4)	1.15	0.538	122	(17)
0.5	0.415	nd	83	(*N* = 5)	0.425	nd	85	(*N* = 5)
0.5	0.622	<0.5 (0.197)	85		1.17	0.718	90	
2.5	2.31	<0.5 (0.0189)	92		2.28	<0.5 (0.0434)	89	
2.5	2.20	<0.5 (0.155)	82		2.56	0.612	78	
2.5	2.23	nd	89		2.18	<0.5 (0.0360)	86	
2.5	2.58	<0.5 (0.143)	97	90	2.53	<0.5 (0.409)	85	90
2.5	2.29	nd	92	(5.3)	2.32	<0.5 (0.0605)	90	(12)
2.5	2.59	<0.5 (0.184)	96	(*N* = 9)	2.88	0.612	91	(*N* = 9)
2.5	2.29	<0.5 (0.0313)	90		2.39	<0.5 (0.0937)	92	
2.5	2.29	<0.5 (0.0276)	90		2.98	<0.5 (0.0715)	116	
2.5	2.15	<0.5 (0.0224)	85		2.23	<0.5 (0.127)	84	
12.5	11.3	<0.5 (0.0130)	90		11.4	<0.5 (0.0202)	91	
12.5	11.8	<0.5 (0.0557)	94		11.1	<0.5 (0.141)	88	
12.5	12.6	<0.5 (0.0389)	100		11.5	<0.5 (0.0607)	92	
12.5	11.7	<0.5 (0.0163)	93	94	11.3	<0.5 (0.0385)	90	93
12.5	12.0	<0.5 (0.0683)	95	(3.6)	11.9	<0.5 (0.258)	93	(4.7)
12.5	11.6	<0.5 (0.0133)	93	(*N* = 8)	11.6	<0.5 (0.0720)	92	(*N* = 8)
12.5	12.2	<0.5 (0.0125)	98		12.5	<0.5 (0.0287)	100	
12.5	11.5	<0.5 (0.0630)	91		11.9	<0.5 (0.229)	100	

permethrin			
added, ng/L	measured, ng/L	background, ng/L	% rec	% av (RSD)
5.0	18.5	16.0	50	
5.0	5.41	<5.0 (0.975)	89	78
5.0	20.2	16.3	78	(21)
5.0	4.28	nd	86	(*N* = 5)
5.0	25.3	21.0	86	

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Table 4. continued

permethrin				
added, ng/L	measured, ng/L	background, ng/L	% rec	% av (RSD)
25	23.1	nd	92	
25	39.0	17.5	86	
25	22.4	<5.0 (0.699)	87	
25	35.2	12.0	93	90
25	23.4	<5.0 (0.920)	90	(2.7)
25	38.0	15.2	91	(N = 9)
25	24.2	<5.0 (1.65)	90	
25	25.5	<5.0 (2.36)	93	
25	22.6	nd	90	
125	117	nd	94	
125	117	<5.0 (1.80)	92	
125	111	<5.0 (1.51)	88	
125	115	<5.0 (0.872)	91	92
125	115	<5.0 (1.84)	91	(2.5)
125	118	<5.0 (2.65)	92	(N = 8)
125	122	<5.0 (2.49)	96	
125	118	<5.0 (2.58)	92	

[a]RSD, relative standard deviation; nd, nondetected. Background values in parentheses indicate measured values that are less than the method LOQ, but that were subtracted from the measured value of the fortified samples prior to calculated percent recovery. Background values were determined from the analysis of a separate (unfortified) aliquot of the sample used for the recovery experiment.

$$\text{std response factor} = \frac{\text{IS ratio of std}}{\text{std concn of analyte}}$$

and

$$\text{av response factor} = \frac{\text{sum of std response factors of bracketing standards}}{2}$$

The concentration of analyte (in ng/L) found in the sample is then calculated according to the equation

$$\text{ng/L} = \frac{\text{IS ratio sample}}{\text{av response factor}} \times \frac{\text{FV (mL)}}{\text{sample vol (mL)}} \times \frac{\text{solvent vol (mL)}}{\text{aliquot 1 (mL)}} \times \frac{\text{reconst vol (mL)}}{\text{aliquot 2 (mL)}} \times (1000\ \text{mL/L}) \times \text{GC dil factor}$$

where FV (mL) is the reconstitution volume of extract (typically 0.5 mL), sample vol (mL) is the volume of sample taken through the extraction procedure (typically 500 mL), solvent vol (mL) is the volume of extraction solvent added (typically 150 mL), aliquot 1 (mL) is the volume of extraction solvent taken through the method (typically 150 mL), reconst vol (mL) is the volume of reconstitution solvent (typically 2.0 mL), aliquot 2 (mL) is the aliquot of sample extract taken for SPE (typically 2.0 mL), and GC dil factor reflects the factor for any dilution of sample extract, as required to produce analyte responses bracketed by standards (if no dilution, the value is 1).

Method Performance Data. This method, using a single hexane extraction (rather than the two extractions described herein), has been applied to the analyses of six of the pyrethroids in effluent water. (The double extraction is now being utilized for current analyses, but these data are not included here.) Along with each set of samples analyzed, concurrent fortifications of effluent water were prepared and analyzed. Each analyte was fortified at the targeted method LOQ, 5 times the LOQ, and 25 times the LOQ. Five of the analytes with similar responses were assigned a targeted LOQ of 0.50 ng/mL, whereas the LOQ for the less-responsive analyte permethrin was assigned an LOQ of 5.0 ng/L. Method limits of detection (LODs) were estimated to be approximately one-third of the LOQ. Results of the analysis of these recovery samples are presented in Table 4. Measured concentrations of fortified samples were corrected (by subtraction) for any background concentration amount measured in the analysis of a separate (unfortified) aliquot of the sample used for each recovery experiment. These corrected values were used for all recovery calculations. Deionized water control samples analyzed by this method (not reported herein) typically show no analyte responses above their respective LODs.

Use of the internal standards to normalize for instrumental variability when sets of samples are injected has proven to be successful. As an example, from a set of water analyses, a linear regression curve was calculated using four concentration levels of cypermethrin, plus three bracketing standard injections at a fifth level (a total of seven data points). Using the cypermethrin native analyte peak responses, an r value of 0.992 was obtained, but this improved to 0.998 when the internal standard ratios were used instead.

RESULTS AND DISCUSSION

Selection of the appropriate stable isotopes for the eight targeted analytes took several factors into consideration. For optimal use as internal standards for the normalization of instrumental response, the labeled location for each molecule was required to be in the primary target ion for each compound, the isotope labels needed to be chemically stable, and the difference in molecular weight needed to be such that the stable isotope target ion response was at an m/z value for which the native analyte has minimal response. Seven of the eight targeted compounds contain a dimethylated cyclopropyl

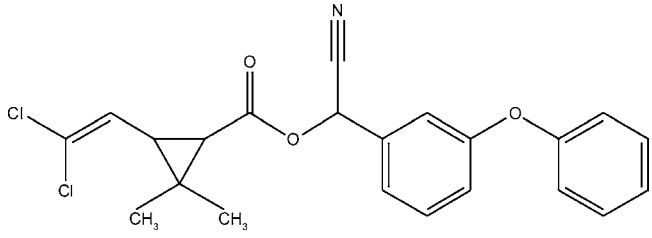

Figure 1. Cypermethrin structure.

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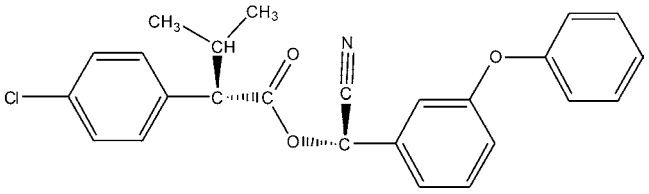

Figure 2. Esfenvalerate structure.

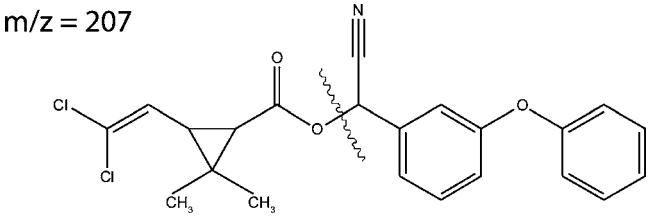

Figure 3. Cypermethrin target ion fragmentation.

moiety (and all eight contain a phenoxyphenyl substituent), for example, cypermethrin (Figure 1). Esfenvalerate is structurally unique among the eight analytes by the absence of a cyclopropyl group (but it does still contain two methyl groups) (Figure 2).

Commercially available ^{13}C-labeled (all six carbons on the phenoxyphenyl substituent) standards were not suitable for the existing method; after fragmentation, that portion of the molecule was not part of the primary and secondary ions being used for quantification.

Kalexsyn, Inc., Kalamazoo, MI, USA, was contracted to synthesize deuterium-labeled analogues of the eight compounds, via substitution of the six hydrogens on the dimethyl substituents, producing d_6 analogues. The deuterium atoms, due to their location on isolated methyl groups on the molecules, are expected to be chemically stable and not subject to proton exchange under the conditions of their use. This has been demonstrated empirically by the consistency of the instrumental response for the selected ions. The dimethyl component of all eight compounds was part of the primary target ion being used for quantification. The +6 MW differential ensured minimal native analyte contribution to the +6 target ion from the internal standard. As an example, for cypermethrin (Figure 3) the primary ion of m/z 207, which is monitored as part of the method, represents a distribution of two ^{35}Cl atoms, with the qualifier ion of 209 representing one ^{35}Cl and one ^{37}Cl atom. Although not one of the monitored ions, there is a response at 211 when two ^{37}Cl atoms are present. Mass spectra of cypermethrin and d_6 cypermethrin are presented in Figures 4 and 5. As can be seen, cypermethrin native analyte shows no

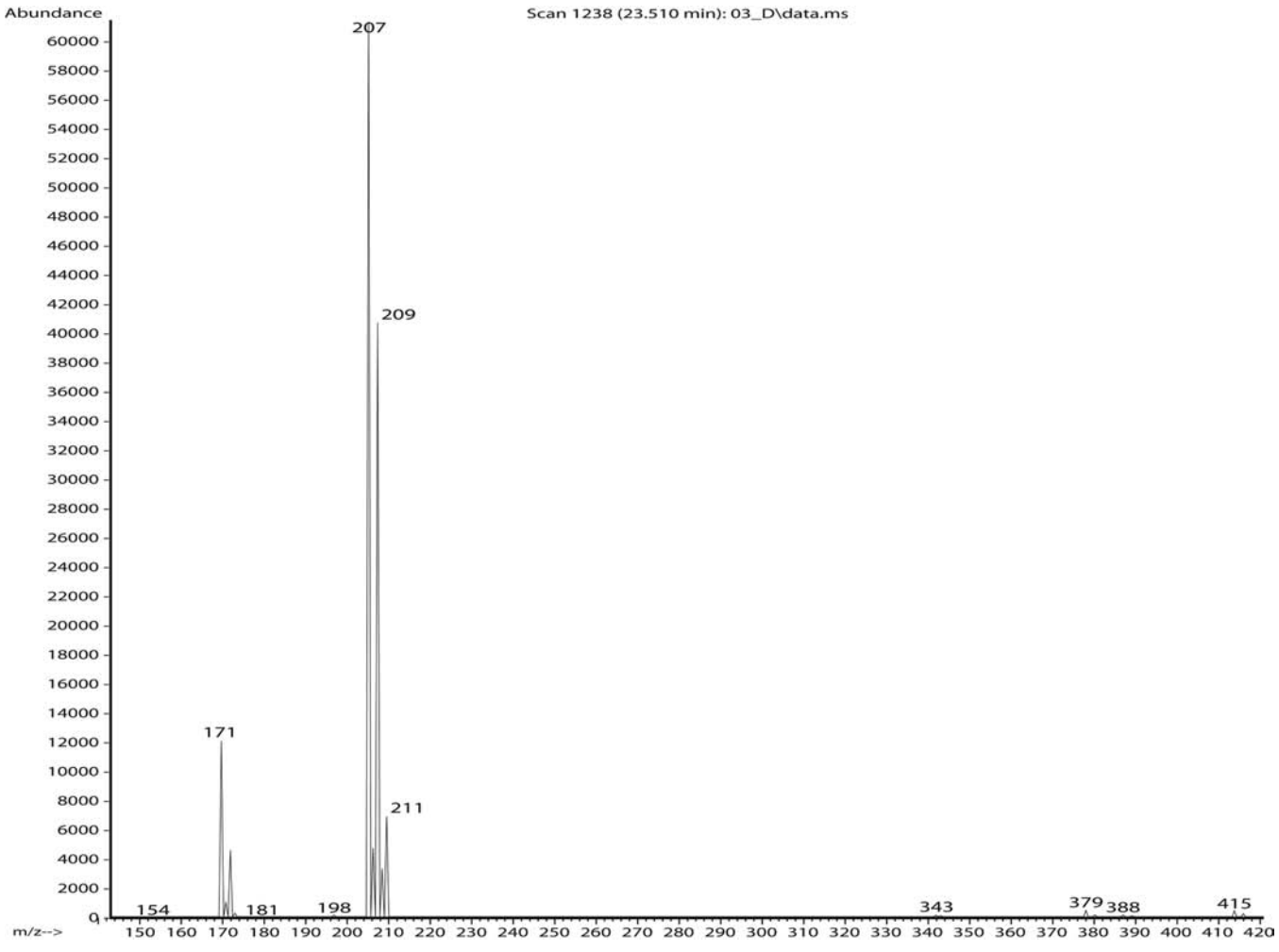

Figure 4. Mass spectrum of cypermethrin.

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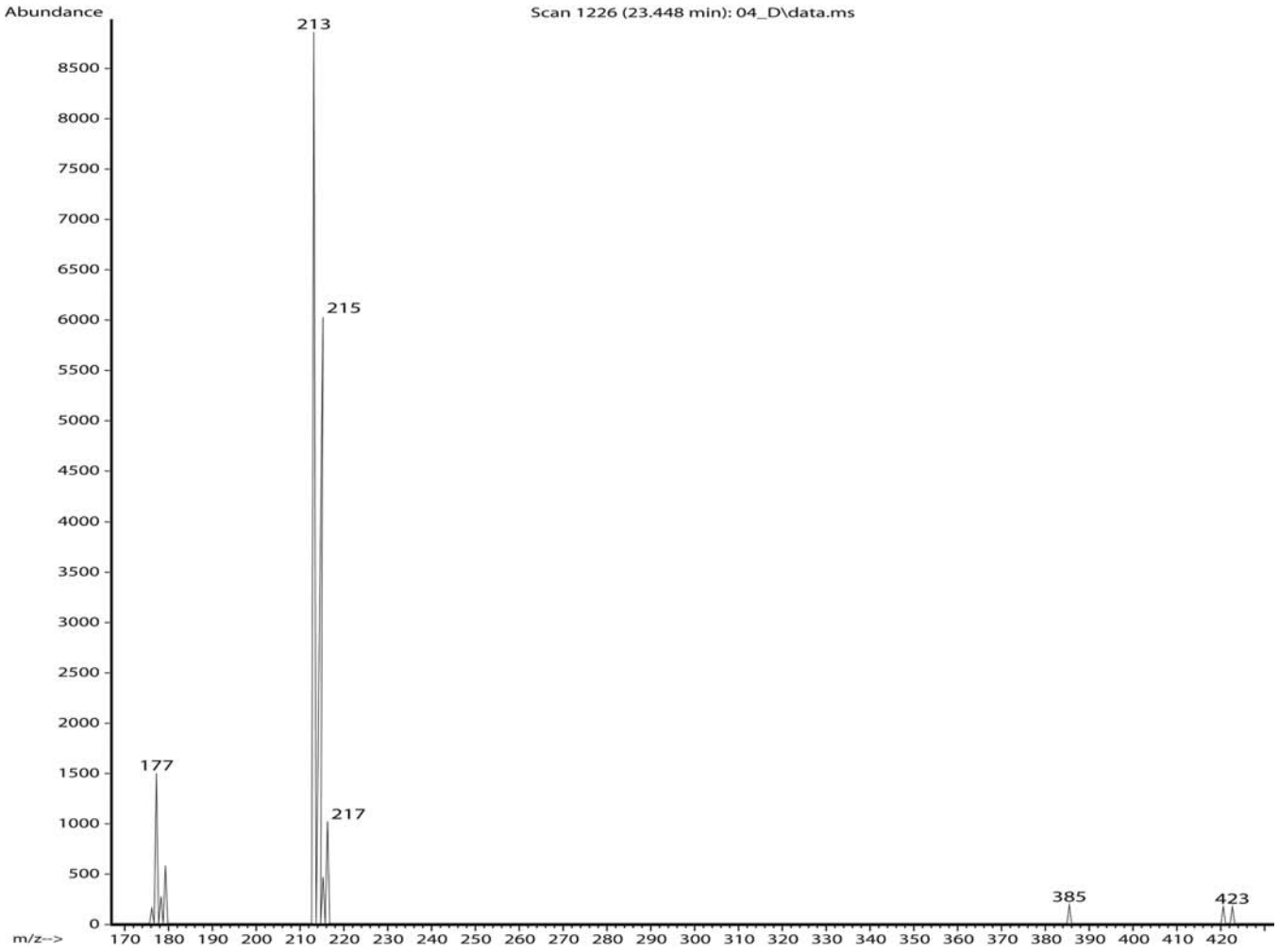

Figure 5. Mass spectrum of cypermethrin-d_6.

response at m/z 213, which is the primary ion monitored for the d_6 cypermethrin internal standard.

Most of the targeted pyrethoids exhibit multiple peaks (due to the presence of different stereoisomers) when analyzed by GC; these peak areas are summed to generate a total response for calculation of concentration for each analyte. The stereoisometric compositions of some of the IS materials differ from the corresponding native analytes; in these cases the number of peaks summed for the IS response may differ from the native analyte summation. As shown in Figures 6 and 7, for β-cyfluthrin, the four isomer peaks characteristic of this compound are summed, whereas the custom-synthesized d_6 cyfluthrin has only two primary peaks. These two peaks, which correspond to the second and fourth (in order of GC elution) peaks in the native β-cyfluthrin chromatogram, are summed for the calculation of the IS ratio. This has not been a detriment to usage, as evidenced by the linear response and stability of the calibration standards when using the analyte/IS ratios. The assumption that each stereoisomer behaves similarly with regard to instrumental effects that modulate detector response is strengthened by the fact that acceptable method recoveries are obtained when using this approach. For λ-cyhalothrin, two peaks are measured under these GC conditions (Figure 8). However, for d_6 λ-cyhalothrin, the synthetic route used did not control the stereochemistry of the product, and six peaks were observed between 19.4 and 20.4 min (Figure 9). (The designation of this material as d_6 λ-cyhalothrin is thus, strictly speaking, inaccurate, but is retained to clarify its use as an internal standard for the λ-cyhalothrin native analyte.) For this internal standard, only the two "λ-cyhalothrin" peaks (which represent the third and fourth highest peaks in the chromatogram but match up acceptably well, in terms of retention times, with the two peaks being monitored for the native analyte) are summed to determined the IS response used to calculate the IS ratio. Again, the demonstrated linearity and stability of calibration response, along with the acceptable method recoveries, indicate that this has not been a detriment to the use of this d_6 material as an IS to normalize for variation in instrumental response.

Storage stability testing results for selected analytes from water samples preserved as described are summarized in Table 5 and indicate stability (based upon corrected recovery values of at least 90%) for the six analytes tested through at least 14 days.

The results from concurrent recovery experiments for effluent water, using the d_6 internal standards (as summarized in Table 4), show that in most cases the average recoveries for each analyte at each fortification level fell within the range of 81−94%, with relative standard deviations (RSDs) at or below 12%. The only exceptions were in cases when the measured background levels for some of the samples used for the fortification/recovery experiments were significant (ca. 50% or

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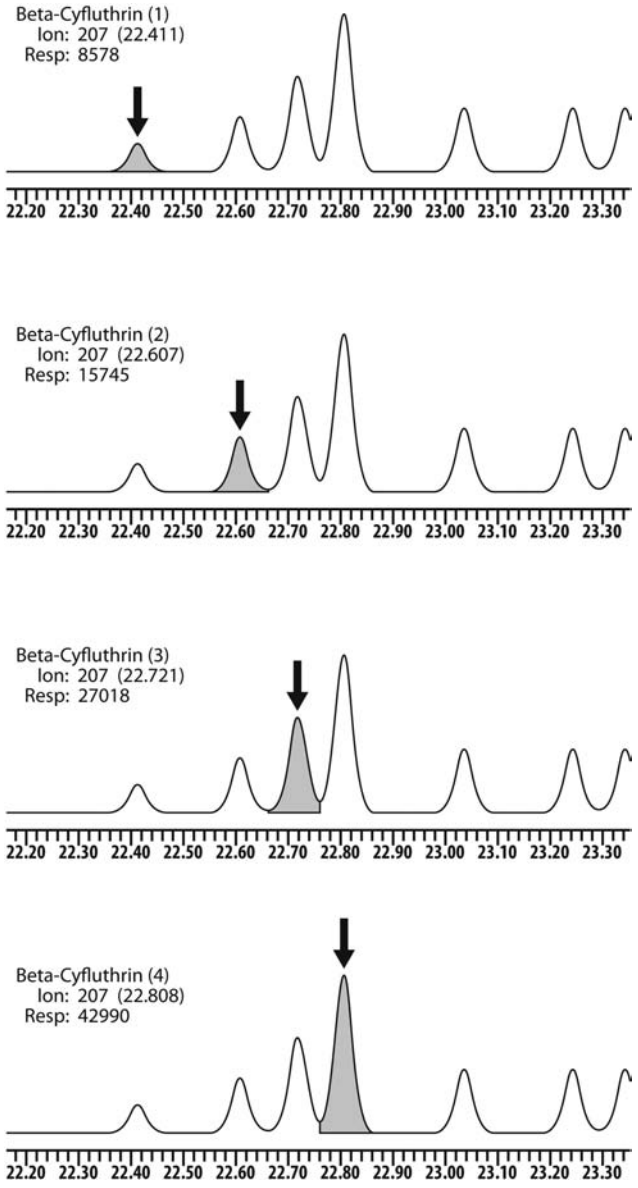

Figure 6. Chromatogram of β-cyfluthrin showing peaks summed for quantification.

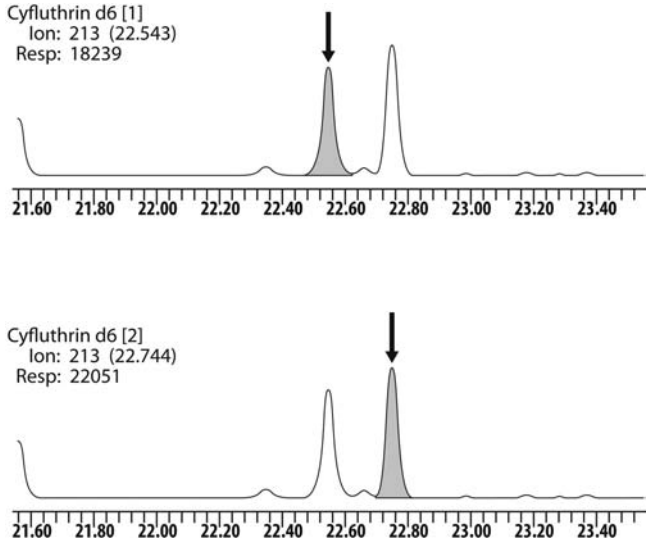

Figure 7. Chromatogram of cyfluthrin-d_6 showing peaks summed for quantification.

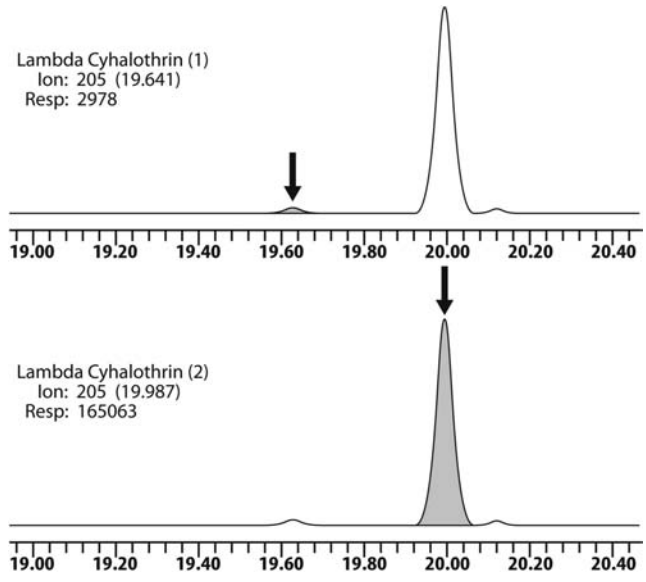

Figure 8. Chromatogram of λ-cyhalothrin showing peaks summed for quantification.

more of the amount added) relative to the fortification level. In cases when the background levels were equal to or greater than the amount added for fortification, the standard deviation of the percent recoveries, unsurprisingly, could be quite high (67% in one case, with the individual recoveries ranging from 2 to 136%). Other than these cases when the background was significant relative to the fortification level, the data were all well within the U.S. EPA environmental chemistry method acceptance criteria of mean recoveries at each fortification level in the 70−120% range, with RSD <20%.[15]

Difficulty in obtaining analyte-free control material for recovery experiments is not an uncommon problem. The availability of the d_6 stable isotopes provides an opportunity to address this challenge and extends their utility beyond their demonstrated use as internal standards to normalize instrumental response. Table 6 illustrates a hypothetical example, with a targeted method LOQ for effluent water for compound A = 0.5 ng/L (ppt), and the measured concentration of the unfortified control sample = 1.0 ppt compound A. This situation can cause accurate quantification of compound A (fortified at 0.5 ppt) by difference to be impractical. As shown in this illustration, one could (conservatively) assume that the variance of the control matrix compound A measurement to ±20% of the "true" value, and measurement of the amount fortified could vary ±10% (or more). Thus, the range of compound A recoveries, after background subtraction, might be expected to be as shown (Table 6) when the method is performed and the d_6 materials are used as instrumental internal standards. This recovery range demonstrates the variability that is inherent in measuring recoveries when the fortification level is similar to (or lower than) the background level in the cleanest available control, even when conservative assumptions are made (as well as ignoring the error in obtaining the "true" value by prior analyses). Even higher background levels can generate even wider recovery ranges. It is proposed that the d_6 standards could be used for fortifications to demonstrate the capability of

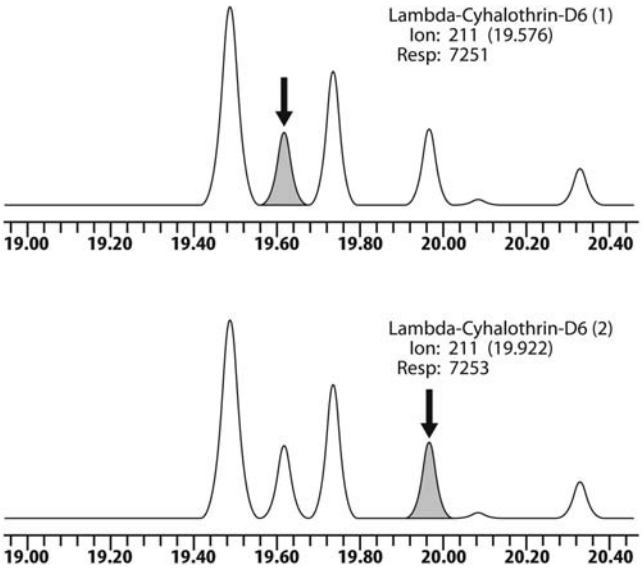

Figure 9. Chromatogram of λ-cyhalothrin-d_6 showing peaks summed for quantification.

Table 5. Water Stability Data for Selected Analytes

	% av stability recovery, corrected for av fresh recovery						
compound	3 days	7 days	14 days	37 days	68 days	90 days	180 days
bifenthrin	96	98	98	89	92	94	91
λ-cyhalothrin	97	94	94	89	81	78	74
permethrin	98	96	96	87	87	83	80
β-cyfluthrin	96	93	92	79	79	76	66
cypermethrin	96	92	92	73	84	82	74
deltamethrin	96	90	96	78	81	79	62

the method to achieve the typical U.S. EPA acceptability range of 70−120% for trace residue analysis for regulatory studies. No contribution of the d_6 from the control matrix would be expected, although this approach would necessitate the use of an alternate internal standard to normalize the response of the compound A-d_6 (although the remaining d_6 materials could continue to be used as internal standards). One option would be to use another of the d_6 pyrethroid compounds with NCI-GC-MS behavior similar to that of the internal standard for compound A-d_6. A second option would be to use the native compound A, at a concentration much greater than that in the sample, as the internal standard. As an example, the current method approach adds the d_6 internal standards, at a concentration of 10 ng/mL for most of the analytes, to all calibration curve standards and sample extracts. For the purpose of using the native analyte as the internal standard, the concentration could be increased to 50 ng/mL. On the basis of the earlier example, a 500 mL influent water sample with an endogenous level of compound A of 1.0 ppt, taken through the method and reconstituted in 0.5 mL, would calculate to a control contribution of 1.0 ng/mL to the concentration of the final extract. The bias introduced by the "extra" compound A in the samples, as compared to the standards, would then be a tolerable −2% (1 ng/mL relative to 50 ng/mL).

In conclusion, a method has been described for the analysis of eight pyrethroid insecticides in wastewater effluent, using NCI-GC-MS and stable isotope (d_6) internal standards. Although the small differences in retention time between the native analyte and stable isotope peaks being measured allow for the possibility of differential matrix effects occurring in the detector, this has not been observed for the matrices tested. The method is shown to be rugged and capable of achieving LOQs as low as 0.5 ng/L (ppt) for most of the analytes and uses instrumentation that is available to many analytical laboratories. This analytical approach is applicable to other environmental matrices, such as surface waters and sediments and POTW influent waters and biosolids. The alternate use of the d_6 stable isotope standards, for determining recoveries in cases when control sample availability is limited to those with significant analyte levels present as background, provides a new analytical tool for evaluating method recoveries at low targeted method LOQs.

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Notes

The authors declare no competing financial interest.

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Table 6. Theoretical Recovery Variance with Relatively High Background in Control Samples

compound A	background, ppb	fortified, ppb	total measured, ppb	fortified ppb, corrected for "true" value	corrected recovery, %
"true" value	1.00	0.50	1.50	0.50	100
measured low end	0.80 (± 20%)	0.45 (± 10%)	1.25	0.25	50
measured high end	1.20 (± 20%)	0.55 (± 10%)	1.75	0.75	150

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