High Sensitivity Detection of Pesticides in Water Using Online SPE Enrichment

Application Note

Environmental

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Abstract

An online solid phase extraction (SPE) method for analyzing 17 chlorinated phenoxy acid herbicides and pentachlorophenol has been developed on an Agilent 6410 Triple Quadrupole LC/MS system. This method meets the performance requirements set by the UK Drinking Water Inspectorate for standard deviation, bias, recovery and total error, and it is accredited by the United Kingdom Accreditation Service (UKAS).

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Introduction

Chlorinated phenoxy acid herbicides and their derivatives are widely used for control of broadleaf weeds in crops and brush along roads. While runoff from treated areas can contaminate surface and groundwater, some chlorinated phenoxy acid herbicides have been directly applied to waterways and reservoirs for the control of aquatic weeds and algae.

Traditionally, these pesticides were monitored in water by extracting 1 L of sample using liquid-liquid or solid phase extraction followed by methylation using diazomethane. The derivatized sample was then analyzed by gas chromatography/mass spectrometry (GC/MS). However, liquid chromatography/mass spectrometry (LC/MS) methods eliminate the need for derivatization and offer the ability to significantly reduce the amount of sample required, depending on the sensitivity of the mass spectrometer used. An LC/MS method using off-line solid phase extraction was previously developed at South East Water in the United Kingdom using 50 mL of sample.

This application note describes the development and validation of a new method for 17 chlorinated phenoxy acid herbicides and pentachlorophenol (PCP) using online solid phase extraction, which enables sensitive detection with only 1.5 mL of water. The required amount of sample has been reduced by almost three orders of magnitude over the original GC/MS method. The method uses a polymeric solid phase extraction cartridge that is attached online to an Agilent 1200 Quaternary LC pump, which in turn is coupled to an Agilent 6410A LC/MS Triple Quadrupole system upgraded with a hotbox. The method meets the performance requirements set by the UK Drinking Water Inspectorate for standard deviation, bias, recovery, and total error and is accredited by the UKAS.

Experimental

Reagents and Standards

Reagents were obtained as follows: formic acid 98%, LC/MS grade from Fluka; glacial acetic acid, HPLC grade, Fisher Scientific; acetonitrile, HPLC gradient grade, JT Baker; acetone and methanol, JT Baker. The PLRP-S SPE cartridge was obtained from Agilent (p/n 5062-8547). All pesticide standards and internal standards were obtained as solids with certified purity from QMX Laboratories.

Stock standards were prepared for each pesticide using 50 mg of solid standard weighed into a 50-mL volumetric flask and made to volume with acetone, for a final concentration of 1,000 mg/L. Mixed Intermediate standards were prepared by adding 10 µL of each individual stock standard to a 100-mL volumetric flask and made to volume with methanol, for a final concentration of 100 µg/L of each pesticide standard.

Stock internal standard was prepared using 10 mg of dichlorophenyl acetic acid (DCPAA) weighed into a 100-mL volumetric flask and made to volume with acetone, to a final concentration of 100 mg/L. The working internal standard was prepared by adding 250 µL of stock internal standard added to a 50-mL volumetric flask and brought to volume with methanol for a final concentration of 500 µg/L.

Calibration standards were prepared by first acidifying 1 L of ultrapure water (from Milli-Q system) by adding 5 mL of formic acid. A 50-mL amount of acidified water was then added to each of five 60-mL amber bottles, and 50 µL of internal standard were added to each bottle. The calibration standards were then prepared in the five 60-mL bottles per the following matrix:

Bottle number	Volume of mixed intermediate standard added (µL)	Final concentration of standard (µg/L)
1	100	0.20
2	50	0.10
3	20	0.04
4	10	0.02
5	0	0.00

All bottles were shaken well, and 2 mL was removed from each bottle and placed in a 2-mL labeled vial for analysis. The calibration range was 0.0–0.20 µg/L, and spiking of Analytical Quality Control samples was done at 0.10 µg/L.

Instruments

The system was built using Agilent 1200 Infinity Series LC modules coupled to a 6410A Triple Quadrupole LC/MS with 'hotbox' upgrade. The hotbox upgrade kit (G2573A) comprised an additional MS turbo-pump with controller and replacement entrance and exit lenses for the collision cell. The online enrichment used the 1200 Quaternary LC pump, an Agilent Autosampler with 900 µL metering device and multidraw capability, and a programmable 6-position selection valve. A second programmable 6-port/2-position valve is used to select between loading sample on a re-useable solid phase extraction cartridge or elution on to the analytical column. The instrument system configuration is shown in Figure 1, and the instrument operating conditions are shown in Tables 1 and 2. The quantitation and qualifier ions, fragmentation voltages, and collision energies for each compound were optimised using Mass Optimizer.

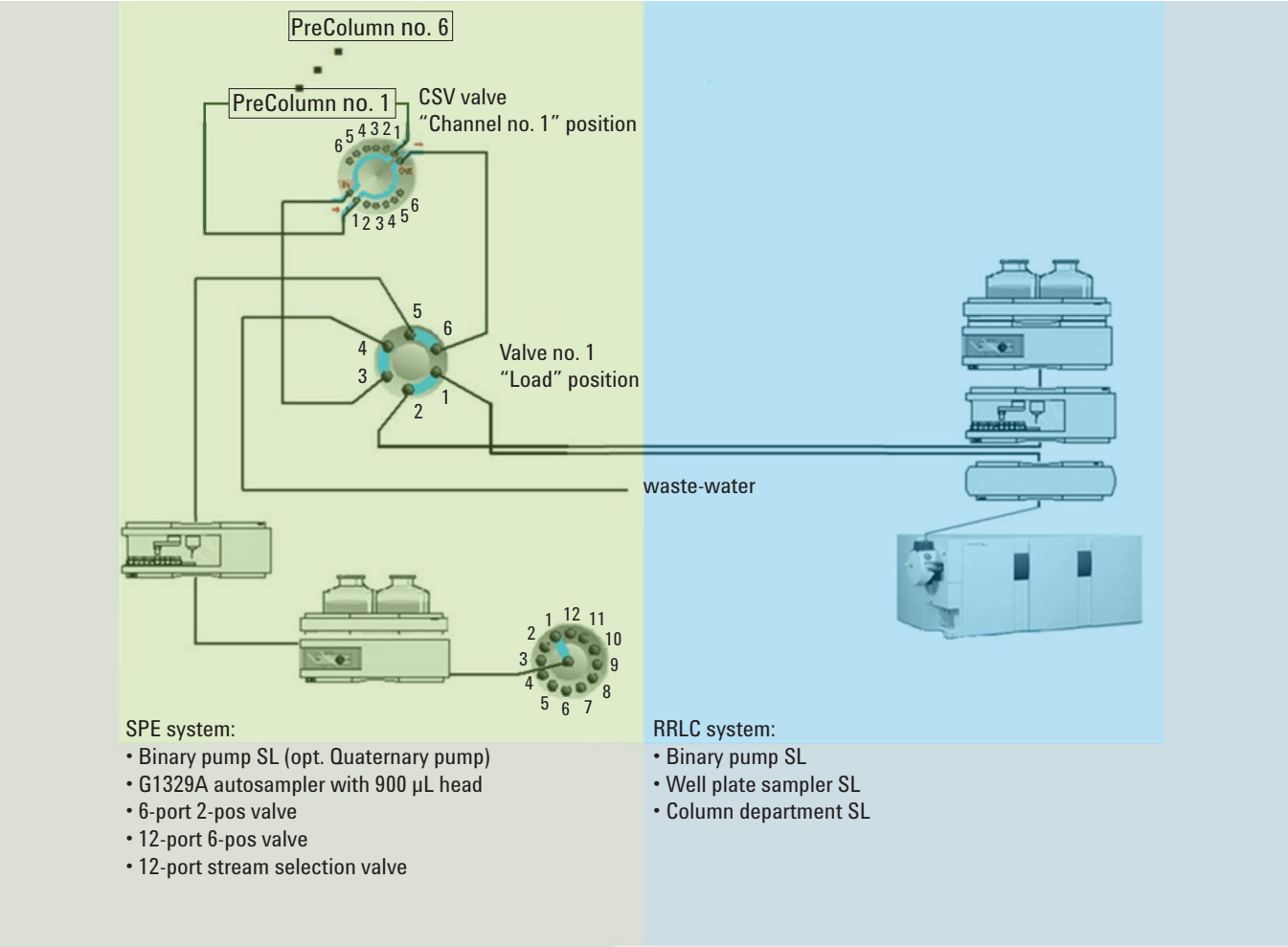

Figure 1. Online SPE LC/MS system configuration.

Table 1. Online SPE Conditions

Mobile phase	A: 1% Formic acid B: Acetonitrile		
SPE cartridge	PLRP-S 10 × 2 mm, 15–25 µm		
Temperature	Ambient		
Flow (load)	1 mL/min		
Gradient program	Time	Gradient (%B)	Flow rate (mL/min)
	0.0	0	1.0
	3.0	0	1.0
	3.5	0	0.5
	4.5	100	0.5
	18.0	100	0.5
	18.2	100	1.0
	25.0	100	1.0
	25.5	0	1.0
Injector program	Command: DRAW def. amount from sample 750 µL speed 800 VALVE mainpass WAIT 1.5 minutes EJECT def. amount into seat, max. speed DRAW def. amount from sample 750 µL speed 800 VALVE mainpass WAIT 1.5 minutes REMOTE start pulse EJECT def. amount into seat, max. speed		

Table 2. LC and MS Instrument Conditions

LC conditions		
Analytical column	Agilent ZORBAX C-18 Eclipse Plus, 2.1 mm × 150 mm, 3.5 µm (p/n 959763-902)	
Column temperature	60 °C	
Injection volume	Injection program, 2 × 750 µL, for a total of 1.5 mL	
Mobile phase	A = 0.1% Acetic acid B = Acetonitrile	
Run time	29.0 minutes	
Flow rate	0.25 mL/min	
Gradient program	Time (min)	Gradient (%B)
	0	15
	1.00	15
	1.01	25
	2.00	25
	17.00	70
	18.00	100
	20.00	100
	21.00	15
MS conditions		
Acquisition parameters	ESI mode, pos/neg ionization; MRM (7 time segments)	
Gas temperature	250 °C	
Drying gas	8 L/min Nitrogen	
Nebulizer pressure	40 psig	
Vcap voltage	3,000 V	

Sample Preparation

From each sample source, 50 mL were added to a 60-mL amber bottle. If the samples contained particulates, they were filtered through Whatman GF filter paper before measuring 50 mL. To each sample were added 250 µL of formic acid and 50 µL working internal standard solution. The bottles were shaken and 2 mL removed and placed in a 2-mL labeled vial for analysis.

Analysis Parameters

The Triple Quadrupole LC/MS multiple reaction monitoring (MRM) acquisition parameters are shown in Table 3.

Table 3. Agilent 6410 Triple Quadrupole LC/MS MRM Acquisition Parameters

Retention time (min)	Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell (msec)	Fragmentor voltage	Collision energy (V)	Polarity
5.387	Clopyralid	194	148	100	65	19	Pos
		192	146	100	65	19	Pos
6.103	Picloram	243	197	100	75	18	Pos
		241	195	100	75	18	Pos
6.576	Imazapyr	262.2	234.3	50	130	14	Pos
			217.2	50	130	17	Pos
8.933	Dicamba	221	177	100	60	0	Neg
		219	175	100	60	0	Neg
9.455	Benazolin	242	198	70	100	0	Neg
			170	70	100	8	Neg
9.782	Fluoroxypyr	255	197	75	100	8	Neg
		253	195	75	100	8	Neg
11.361	Bentazone	239	197	25	130	20	Neg
			132	25	130	25	Neg
12.379	2, 4-D	221	163	35	80	15	Neg
		219	161	35	80	15	Neg
12.426	Bromoxynil	276	81	75	110	35	Neg
		274	79	75	110	35	Neg
12.613	MCPA	201	143	35	100	15	Neg
		199	141	35	100	15	Neg
12.63	DCPAA (internal standard)	205	161	35	50	0	Neg
		202.9	159	35	50	0	Neg
13.476	Triclopyr	256	198	200	60	5	Neg
		254	196	200	60	5	Neg
14.114	Ioxynil	369.8	214.9	50	120	30	Neg
			126.9	50	120	35	Neg
14.223	Dichlorprop	235	163	50	80	10	Neg
		233	161	50	80	10	Neg
14.359	2,4,5-T	254.9	196.9	50	80	10	Neg
		252.9	194.9	50	80	10	Neg
14.371	MCPP	215	143	50	80	20	Neg
		213	141	50	80	20	Neg
15.319	2,4-DB	249	163	75	80	10	Neg
		247	161	75	80	10	Neg
15.466	MCPB	229	143	75	105	2	Neg
		227	141	75	105	2	Neg
19.636	PCP	266.9	266.9	50	126	0	Neg
		264.9	264.9	50	126	0	Neg
		262.9	262.9	50	126	0	Neg

Results and Discussion

Analysis of Small Sample Volumes

Using online SPE sample preparation enables the use of a 1.5-mL sample volume in standard 2-mL autosampler vials, which, in turn, enables the use of the 100-position sample tray and ample throughput. Figure 2 shows a representative total ion chromatogram (TIC) for a 0.10 µg/L standard, using a 1.5-mL sample. Example EICs of a few of the herbicides and pentachlorophenol (PCP) at 0.10 µg/L are shown in Figure 3.

Sensitivity and Accurate Quantification

This method enables detection of these herbicides at concentrations at and below 0.010 µg/L, as shown in Figure 4. Calibration curves were constructed using four concentrations of standard from 0.02 to 0.20 µg/L. All coefficients of correlation (R^2) were greater than 0.999. Figure 5 shows three representative calibration curves.

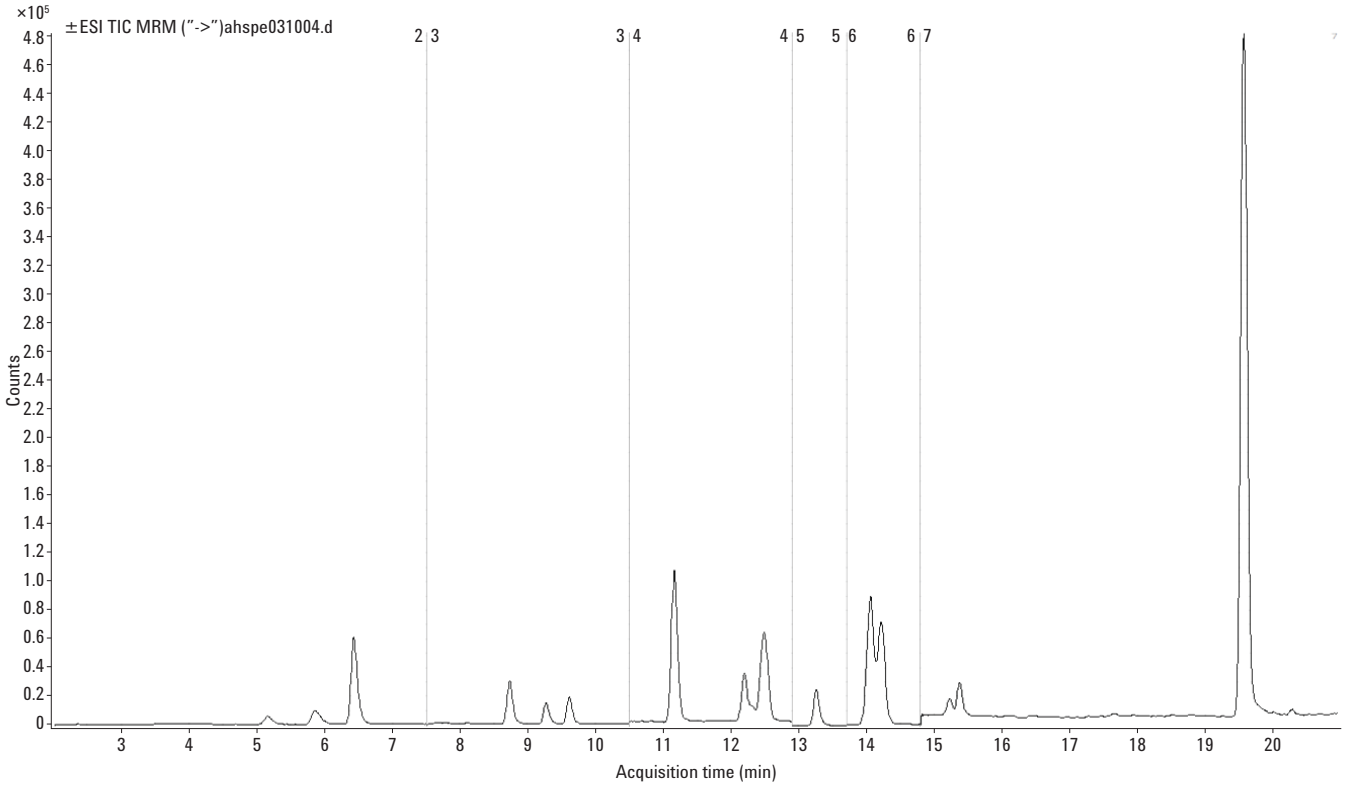

Figure 2. Total ion chromatogram for a 0.10 µg/L standard of 17 herbicides as well as the positions of the time segments.

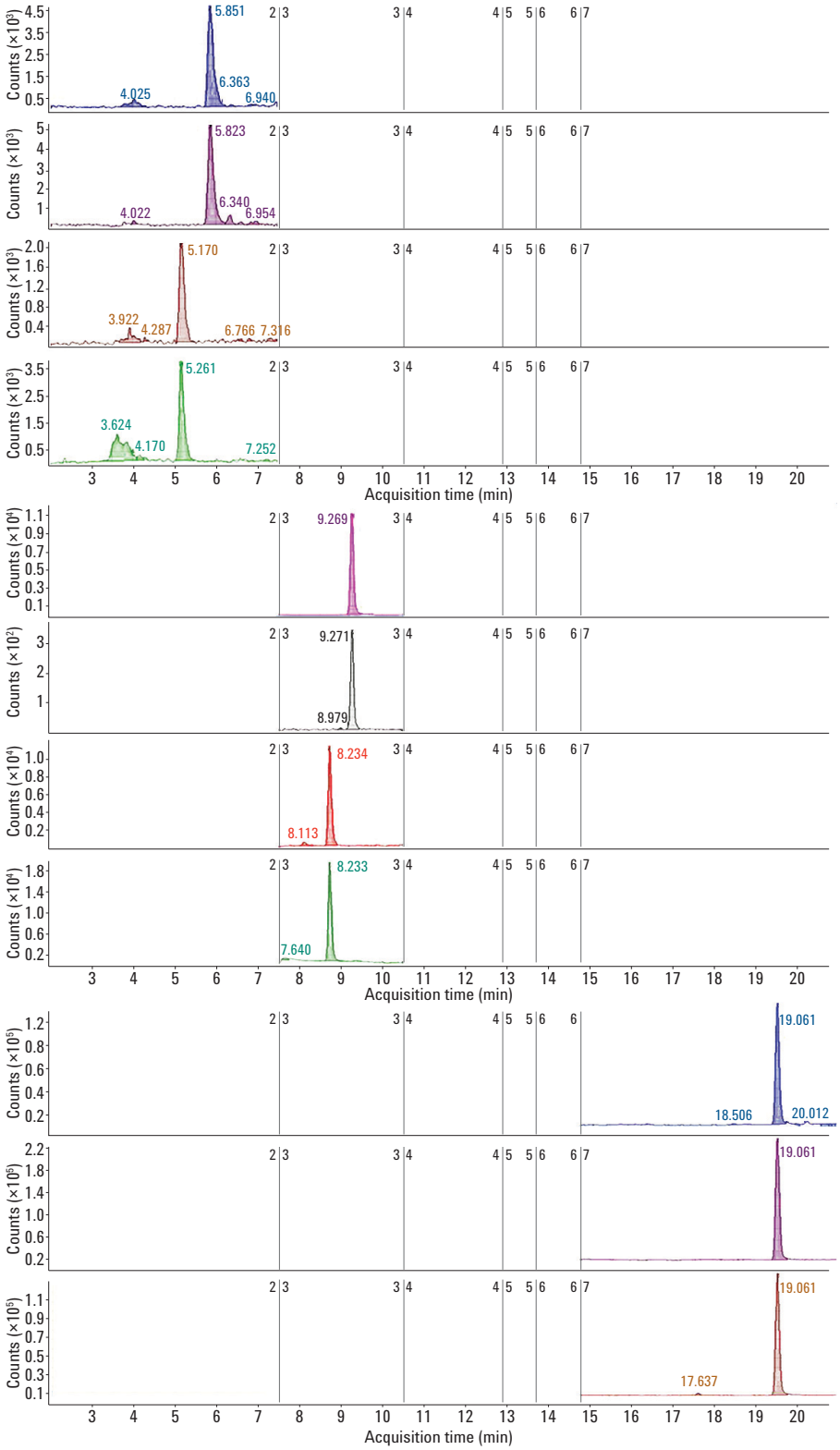

Figure 3. EICs of the transitions for 0.10 µg/L standards of picloram (top two traces in top panel) and clopyralid (bottom two traces in top panel); benazolin (top two traces in middle panel) and dicamba (bottom two traces in middle panel); pentachlorophenol (bottom panel, all three traces).

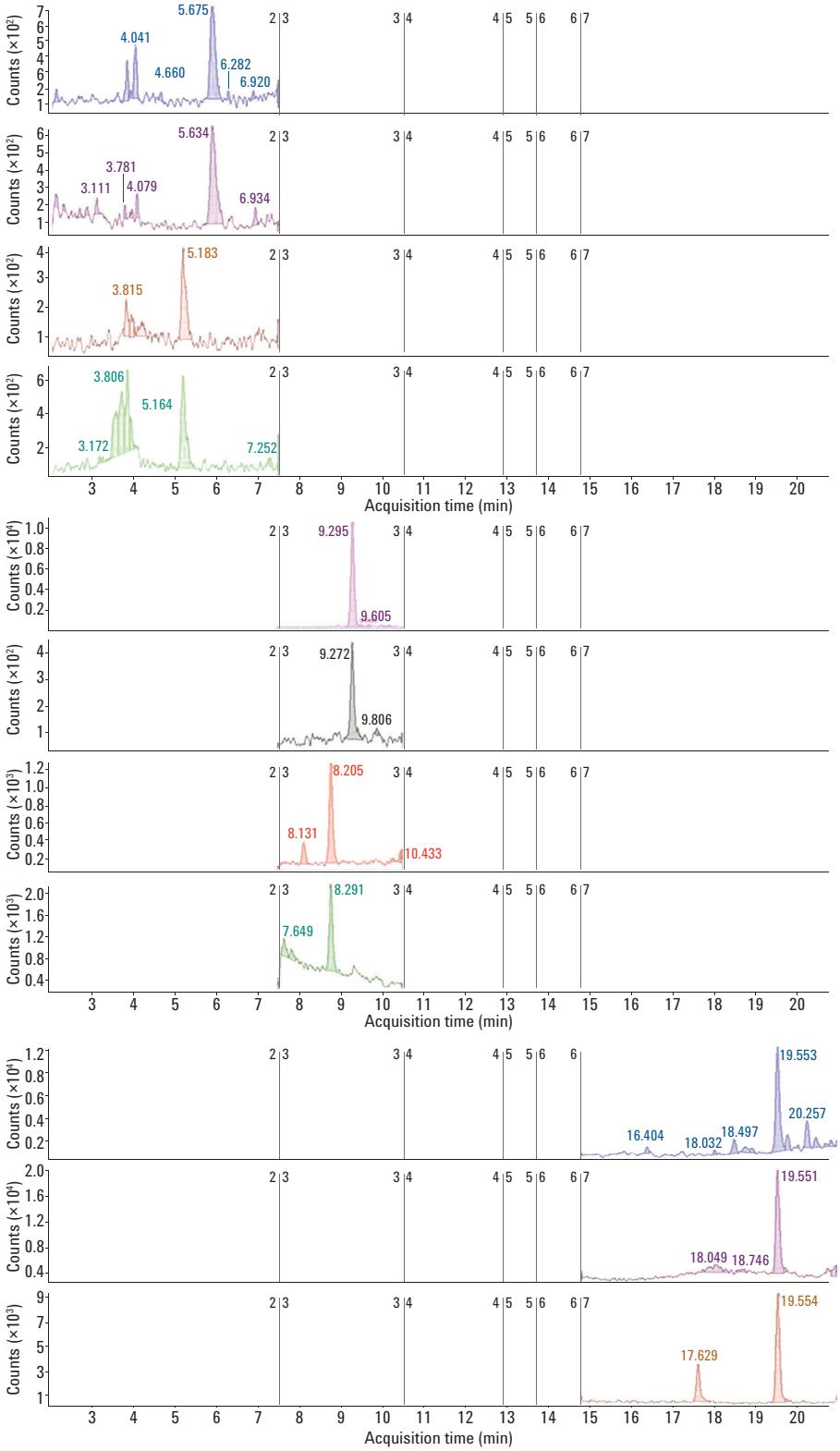

Figure 4. EICs of the transitions for 0.010 µg/L standards of picloram (top two traces in top panel) and clopyralid (bottom two traces in top panel); benazolin (top two traces in middle panel) and dicamba (bottom two traces in middle panel); pentachlorophenol (bottom panel, all three traces).

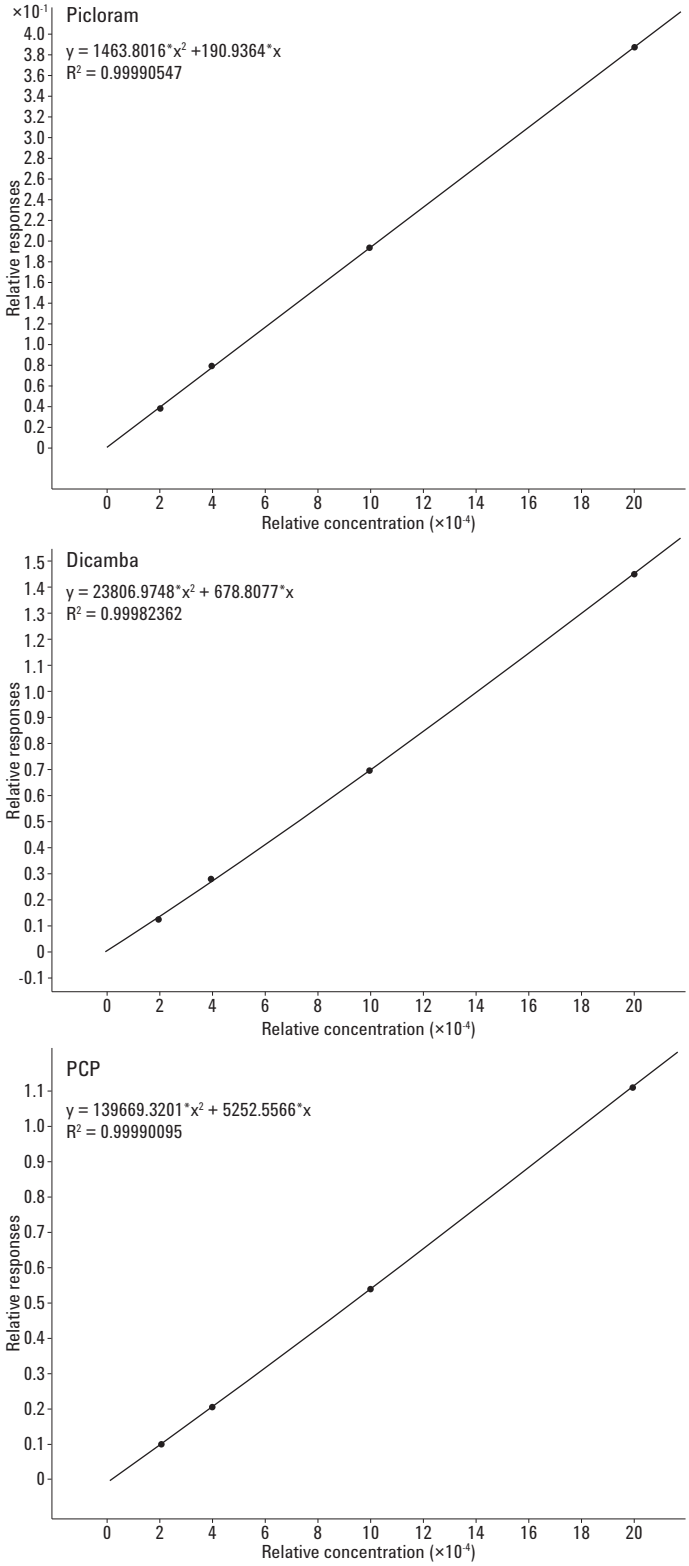

Figure 5. Representative calibration curves from 0.02 to 0.20 µg/L for picloram (top), dicamba (middle) and pentachlorophenol (bottom). All R^2 values were >0.999.

Method Validation

Validation of the method was carried out on 11 sets of spiked duplicates, blanks, and AQC samples using surface, borehole, and treated water sources spiked at 0.10 µg/L with the standard mix of the 17 pesticides plus PCP. Most method limits of detection (LODs) were well below 0.01 µg/L. All of the recoveries were between 86 and 125%, with the majority falling between 95 and 104% (Table 4). The Aquacheck Test is a proficiency testing scheme performed by LGC Standards, a UKAS accredited international provider of proficiency testing (PT) services. It showed excellent correlation with the assigned values, which must have a Z score within ±2 to pass the test (Table 5). In addition, the method meets the performance requirements set by the UK Drinking Water Inspectorate for standard deviation, bias, recovery, and total error (data not shown).

Conclusions

An online SPE method accredited by the UKAS has been developed on the 6410 Triple Quadrupole LC/MS for the analysis of 17 chlorinated phenoxy acid herbicides and pentachlorophenol that reduces the required sample size by a factor of almost one thousand versus the previous GC/MS method. Even so, it delivers ≤12 part per trillion (ppt) LODs, as well as recoveries >95% for most pesticides. In addition to a reduction in sample volume, this method provides faster results at lower cost. The solid phase extraction cartridges are reuseable, and less solvent is used for extraction. Finally, the results are more reproducible because the system is fully automated and less prone to extractor error.

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Table 4. Validation Data for the Online SPE Method

	% Recovery			
Compound	**Surface water**	**Borehole**	**Treated water**	**LOD* (µg/L)**
Clorpyralid	86.29	97.34	86.62	0.012
Picloram	99.58	96.74	93.33	0.009
Imazapyr	124.87	96.92	96.72	0.009
Dicamba	98.94	95.36	93.64	0.003
Benazolin	92.54	96.71	96.13	0.003
Fluroxypyr	92.50	97.26	96.96	0.003
Bentazone	97.59	97.58	96.50	0.003
2,4-D	98.48	96.67	97.47	0.003
Bromoxynil	95.84	95.00	96.75	0.003
MCPA	98.88	95.97	96.47	0.003
Triclopyr	98.22	95.78	96.15	0.003
Ioxynil	100.03	98.65	99.87	0.003
Dichlorprop	100.08	97.01	97.79	0.003
2,4,5-T	102.60	99.07	99.42	0.003
MCPP	101.86	98.29	98.45	0.003
2,4-DB	98.21	97.73	97.30	0.003
MCPB	99.45	98.18	98.83	0.003
PCP	103.68	98.22	98.08	0.006

*LOD = Limit of Detection (three times the standard deviation of the low standard for each pesticide)

Table 5. Aquacheck Test Data for the Online SPE Method

Compound	**Aquacheck assigned concentration (ng/L)**	**Online SPE method result (ng/L)**	**Z score***
Dicamba	115.6	116.7	0.09
Bentazone	85.9	81.85	-0.47
2,4-D	84.4	88.7	0.51
Bromoxynil	118.3	115.15	-0.27
MCPA	83.8	80.95	-0.34
Triclopyr	44.4	41.2	-0.64
Ioxynil	103	94.05	-0.87
Dichlorprop	54.1	54.95	0.17
MCPP	53	54.45	0.27
2,4-DB	32.8	28.35	-0.89
MCPB	42.6	39.55	-0.61

*Z Score is a measure of correlation with the assigned values, and must value within ±2 to pass the proficiency test.

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