Solid Phase Extraction of Organophosphorus Pesticides in Water with Agilent Bond Elut PPL

Application Note

Environmental

Authors

Marica Beggio and Michele Pozzebon
dtoLABS, via Fratta 25 31023,
Resana (TV), Italy

Joan Stevens and Francesco Spadola
Agilent Technologies, Inc.

Abstract

This application note describes an SPE method to prepare water samples for pesticides analysis by liquid chromatography/tandem mass spectrometry. Sixteen organophosphorus pesticides of different polarity were analyzed. The sample preparation was done using Agilent Bond Elut PPL versus Liquid-liquid extraction (LLE). Solid phase extraction (SPE) is a useful sample preparation technique since it yields extremely clean extracts and has a built-in concentration step. With SPE, many of the problems associated with LLE can be prevented, such as incomplete phase separations, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents. SPE is more efficient than LLE, yields quantitative extractions that are easy to perform, is rapid, and can be automated. Solvent use and lab time are reduced.

The analysis was completed by LC/MS/MS using dynamic multiple reaction monitoring (dMRM) mode. The limit of quantification (LOQ) for all pesticides was under 5 µg/L. At 5 µg/L, the recoveries ranged from 70 to 135%. The relative standard deviation associated with these recoveries was less than 10% in all cases.

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Introduction

When many organochlorine pesticides became banned in the 1970s, the agrochemical industry turned to the less persistent organophosphorus pesticides (OPs). OPs are widely used in agriculture and in veterinary medicine, in addition to home and work use worldwide. These pesticides have a short-term environmental persistence but high toxicity to humans and other mammals. Although they concentrate in fatty tissues, they are quickly metabolized, making analysis in the food chain very difficult [1]. Their extensive use and acute toxicity implies an environmental risk and an increasing social concern with respect to the presence of OPs in drinking, surface, and ground waters, fruits, vegetables, and other foodstuffs [2,3]. The presence of OPs in the environment is monitored by many agencies, including the United States Environmental Protection Agency (EPA).

The determination of OPs from water usually requires sample preparation to facilitate extraction and concentration of the pesticides for GC/MS or LC/MS analysis. Liquid-liquid extraction (LLE) is a common sample preparation approach but can be labor intensive, requiring large volumes of hazardous and expensive solvents. Solid phase extraction (SPE) significantly reduces solvent consumption. Its built-in concentration step is especially useful for large sample volumes, and SPE is less labor-intensive, and can be automated.

Agilent Bond Elut PPL can be effective for extracting OPs. Its larger particles are effective with particulate-laden samples. The styrene-divinylbenzene (SDVB) particle, with a unique, proprietary, polar functionalization, offers both nonpolar and electrostatic/polar/pi-pi retention opportunities for nonpolar analytes as well as OPs and other polar, predominately water-soluble compounds.

The goal of this work was to develop a rapid and efficient method for the analysis of OPs from various water sources. LC/MS/MS was used to provide high sensitivity for quantification in dynamic multiple reaction monitoring mode (dMRM). The LC/MS/MS run time was 15 minutes. Polymeric BE-PPL was used as the SPE sorbent to enrich the 16 OPs, resulting in high extraction efficiencies.

Experimental

All reagents and chemicals were LC/MS grade. Acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), and isopropanol, glacial acetic acid, and ammonium formate were from Sigma-Aldrich, Corp. (St. Louis, MO, USA). Hydrochloric acid 37% w/v was from Fluka (Sleinheim, Germany). Buffer solution at pH 4.01, 7.00 and 10.01 were from Agilent Technologies (Agilent Technologies Inc., Wilmington, DE, USA). Milli-Q ultrapure water came from Sartorius (Sartorius AG, Goettingen, Germany). Formic acid (FA) was obtained from Millipore (EDM Millipore Corporation, MA, USA). The custom pesticides mix standard solution was supplied by Ultra Scientific (North Kingstown, RI, USA).

Solutions and standards

A 1:1 conditioning solution of ethanol/acetonitrile was made by combining 50 mL ethanol with 50 mL acetonitrile. The 5 mM ammonium formate 0.01% formic acid in mobile phase A was made by dissolving 0.157 g of ammonium formate and 50 µL of formic acid in 500 mL of Milli-Q water. The 5 mM ammonium formate 0.01% formic acid in mobile phase B was made by dissolving 0.157 g of ammonium formate and 50 µL of formic acid in 500 mL of methanol.

The pesticide mix standard stock solution (100 µg/mL, 16 pesticides in methanol) was stored at 4 °C. A 1,000 µg/L pesticide solution was prepared daily by diluting 10 µL of pesticide mix standard stock solution to 1,000 µL in acetonitrile.

Solutions to build calibration curves were made by diluting 1, 2, 5, 10, 20, 50, and 100 µL of 1,000 µg/L pesticide solution to 1,000 µL in acetonitrile. The final concentration of every calibration point was 1, 2, 5, 10, 20, 50, and 100 µg/L, respectively. Three QCs (quality controls) were made at 5 µg/L by diluting the 1,000 µg/L pesticide solution in acetonitrile.

For the recovery test, 500 mL of Milli-Q water were spiked with 20 µL of the 1,000 µg/L pesticide solution and acidified to pH 2 with hydrochloric acid before the SPE procedure.

For the recovery test on a real sample, 500 mL of underground water was spiked with 20 µL of the 1,000 µg/L pesticide solution and acidified to pH 2 with hydrochloric acid before SPE.

Instrumentation

- Agilent 1290 Infinity LC System
- Agilent 6460 Triple Quadrupole LC/MS with Agilent Jet Stream source
- Agilent 3200P pH meter
- Agilent Vac Elut vacuum manifold, 30 bar max pressure (p/n 12234103)
- Agilent Bond Elut adapters (p/n 12131001)
- Agilent Bond Elut PPL SPE cartridge 200 mg, 3 mL (p/n 12105005)
- Sartorius Arium Pro Milli-Q water producer.
- Agilent autosampler 2 mL glass vials and caps (p/n 5190-2279)

Conditions

Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm, 1,200 bar (p/n 959758-902)	
Mobile phase:	A) water 5 mM ammonium formate, 0.01% formic acid; B) methanol 5 mM ammonium formate, 0.01% formic acid	
Flow rate:	0.3 mL/min	
Column temperature:	45 °C	
Injection volume:	2 µL	
Needle wash:	80:15:5 Isopropanol:water:glacial acetic acid, 30 s	
Gradient:	Time	% B
	0.0	5
	0.8	40
	3.5	40
	5.0	60
	6.0	60
	12.0	65
	17.0	95
Post run:	3.0 min	
Total cycle time:	20.0 min	

MS conditions

Parameter	Value (+)	Value (–)
Gas temperature:	325 °C	325 °C
Gas flow:	9 L/min	9 L/min
Nebulizer pressure:	40 psi	40 psi
Sheath gas temperature:	400 °C	400 °C
Sheath gas flow:	11 L/min	11 L/min
Capillary:	3,500 V	3,500 V
Nozzle voltage:	400	0

Instrument acquisition data for the analysis of the organophosphate pesticides is shown in Table 1.

Table 1. Instrument acquisition data for the analysis of 16 organophosphate pesticides by LC/MS/MS.

Organophosphorus pesticide	**MRM transitions (m/z)**	**Fragmentor (V)**	**CE (V)**	**Retention time (min)**
Azinphos-ethyl	346.0 → 137.1 346.0 → 97.0 346.0 → 77.1 346.0 → 160.0 346.0 → 132.0	80	20 32 45 10 10	10.60
Azinphos-methyl	318.1 → 261.0 318.1 → 132.0	80	14 8	7.71
Chlorfenvinphos	359.1 → 155.1 359.1 → 99.0	120	10 10	14.35
Coumaphos	363.0 → 306.9 363.0 → 227.0	120	15 20	13.82
Demeton-S-methyl	231.0 → 89.0 231.0 → 61.0	80	10 35	4.60
Dichlorvos	223.0 → 109.0 221.0 → 145.0 221.0 → 127.0 221.0 → 109.0	120	10 15 20 15	4.50
Dimethoate	230.0 → 199.0 230.0 → 125.0	80	8 20	3.17
Fenitrothion	278.0 → 125.0 278.0 → 109.0	121	16 12	9.63
Fenthion	279.1 → 246.8 279.1 → 168.9 279.0 → 109.1 279.0 → 105.1	115	4 12 24 20	13.01
Malathion	331.0 → 285.0 331.0 → 127.0	80	5 5	9.41
Mevinphos	225.1 → 193.0 225.1 → 127.0	100	15 15	3.55
Omethoate	214.0 → 183.0 214.0 → 125.0	80	5 20	1.95
Parathion	292.0 → 264.0 292.0 → 236.0 292.0 → 97.0 292.0 → 94.1	100	5 10 32 45	12.21
Parathion-methyl	264.0 → 125.0 264.0 → 79.0	120	20 40	8.19
Triazophos	314.1 → 162.1 314.1 → 119.0	120	20 20	10.13
Trichlorfon	256.9 → 221.0 256.9 → 109.0	120	5 15	3.24

Sample preparation

Five hundred milliliters of water was measured in a graduated cylinder and put into an 800 mL glass bottle. The pH was adjusted to pH 2 using a calibrated pH meter with 37% hydrochloric acid. A PTFE tube 1/8 in od was placed into the sample bottle at the bottom and to one side. The other end of the tube was attached to an adapter cap that was secured onto the top of a Bond Elut PPL SPE cartridge mounted on the quick-release valve of an Agilent Vac Elut vacuum manifold. The sample can be drawn directly from the sample container and loaded onto the preconditioned SPE cartridge by vacuum (Figure 1).

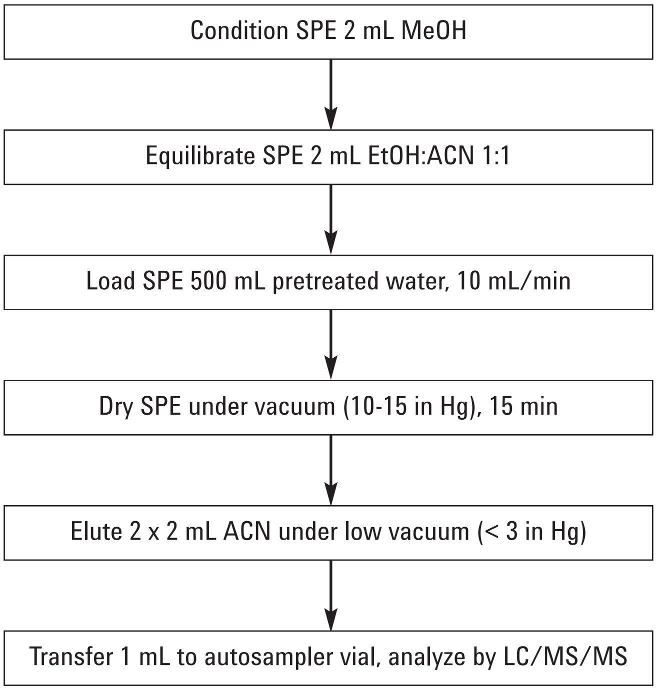

Figure 1. Procedure for the solid phase extraction of organophosphorus pesticides in water.

Results and Discussion

Chromatographic separation

The 16 OPs were separated using the optimized LC/MS/MS conditions. A typical chromatogram is shown in Figure 2. The identification of OPs was based on standard solution retention time and mass spectra transitions.

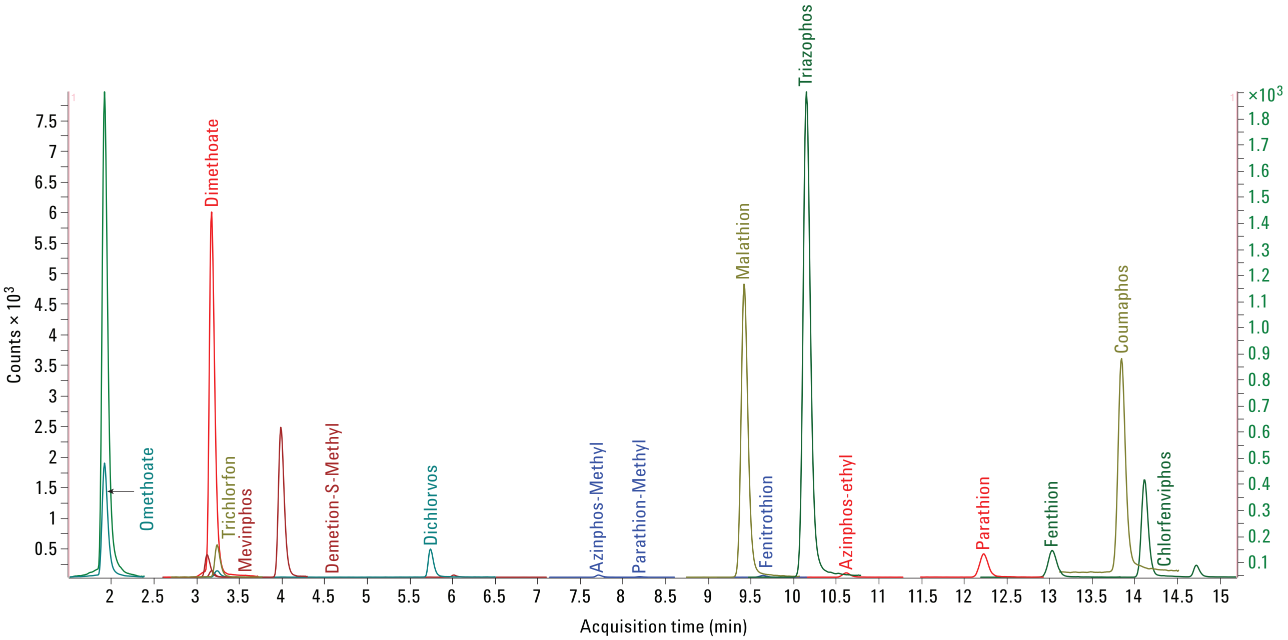

Figure 2. Chromatogram of 16 organophosphate pesticides from a 5 µg/L extracted water sample.

Recovery and reproducibility

Recovery and reproducibility was tested with MilliQ water, and with an underground water sample. The underground water was tested and determined to be free of pesticides. Both MilliQ and underground water were spiked with 20 µL of a 1,000 µg/L pesticide standard mix in acetonitrile, acidified at pH 2 with 37% hydrochloric acid, and then processed by the SPE cartridge, with an expected final concentration of 5 µg/L.

Recovery with MilliQ water was done in triplicate using three separate samples and three separate SPE cartridges. Recovery with underground water was done with five different samples and five different cartridges. All quantitation results are based on peak area. Every extract was injected three times.

Recoveries for the 5 µg/L OP spiked sample in MilliQ water and for the 5 µg/L OPs spiked in underground water, and relative standard deviations for underground water spiked with 16 OPs, are shown in Table 2. All compounds had linear calibration curves in the range of 1 to 100 µg/L.

The calibration curve was prepared by diluting a 1,000 µg/L pesticide mix solution in acetonitrile. The LOQ was calculated as 10 times the standard deviation of the concentration of N repeats, in agreement with officially recognized guidelines [4]. Calculation was done using Agilent MassHunter Quantitative Software, for five repeats, at 5 µg/L concentration (Table 2).

Table 2. Recovery from water blank and ground water at 5 µg/L for 16 organophosphate pesticides.

Pesticide	**Recovery water blank at 5 µg/L**	**Recovery ground water at 5 µg/L**	**% RSD (underground water)**	**LOQ (µg/mL)**	**Linearity (R^2)**
Omethoate	73.0	84.5	7.3	3.04	0.9996
Dimethoate	108.8	117.5	4.9	2.96	0.9999
Trichlorfon	135.0	142.4	4.3	3.65	0.9874
Mevinphos	109.9	110.3	4.0	2.47	0.9996
Demeton-S-methyl	98.6	99.2	6.0	1.55	0.9997
Dichlorvos	96.1	92.4	3.7	1.02	0.9986
Azinphos-methyl	108.3	111.9	4.6	2.01	0.9998
Parathion-methyl	109.3	110.2	6.7	4.83	0.9993
Malathion	108.3	106.7	3.4	2.12	0.9995
Fenitrothion	108.6	105.0	5.0	3.38	0.9995
Triazophos	107.9	105.8	3.2	1.88	0.9998
Azinphos-ethyl	108.4	105.8	4.5	2.24	0.9998
Parathion	108.4	105.0	3.7	2.08	0.9997
Fenthion	86.1	75.5	8.1	3.59	0.9998
Coumaphos	107.4	102.5	3.4	2.14	0.9992
Chlorfenvinphos	109.2	106.9	3.2	1.84	0.9998

Conclusions

This simple SPE procedure using Bond Elut PPL is an effective technique for preconcentration of 16 OPs in water samples at or close to monitored MRL levels in ground water. The technique is simple and economical, with excellent recovery and reproducibility. The workflow solution includes essential concentration of trace analytes by SPE, efficient separation on the chromatographic LC system, and superior sensitivity and selectivity provided by LC/MS/MS.

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