Application Note

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Extraction and Analysis of Pesticides from Water by Solid Phase Extraction and GC/MS

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Abstract

A cartridge-based solid phase extraction method using an Agilent SPE cartridge as per EPA 525.2 was used for extraction of pesticides in trace quantities and analyzed by Agilent 8890-5977 GC/MS. Water samples were spiked with a mixture of pesticides at 0.005–0.080 ng/mL and extracted, followed by 1,000-fold concentration. Matrix-matched linearity ranging from 5–80 ng/mL was generated by spiking the required amount of pesticides in blank matrix. Excellent linearity with R^2 > 0.995 was obtained for all the pesticides considered for the study. Recoveries were obtained in the range of 80–120% with associated RSDs < 15%.

Introduction

Drinking water is defined as water intended for human consumption obtained from different sources. Several guidelines/regulations exist for ensuring that undesirable substances such as pesticides are not present in excessive amounts that may be harmful to human health. These regulations ensure that water intended for human consumption can be consumed safely. In India, the Bureau of Indian Standards (BIS) has set regulatory limits for residual pesticides in drinking water (IS 10500)[1], packaged drinking water (IS 14543)[2], and natural mineral water (IS 13428).[3] A total of 26 pesticides are regulated by BIS standards, of which four compounds (monocrotophos, phorate, 2,4-D, and isoproturon) are more suitable for analysis by liquid chromatography.

EPA Method 525.2 is a general-purpose method for determination of organic compounds in finished drinking water or water from any source or treatment stage.[4] This method is applicable to a range of organic compounds that are partitioned from the water sample onto a C18 organic phase packed in a disk or cartridge. The organic compounds are then eluted using a suitable eluent, concentrated, and analyzed by GC/MS.

This application note describes the use of EPA Method 525.2 for extraction of pesticide residues at trace levels in water using solid-phase extraction (Agilent Bond Elut C18: Straight barrel cartridges, p/n 12102118) and analysis using Agilent 8890-5977 GC/MS.

Excellent linearity with $R^2 > 0.995$ was obtained for all pesticides considered for the study. Recoveries were obtained in the range of 80–120% with associated RSDs < 15% at 0.005–0.080 ng/mL level of fortification.

Experimental

Reagent and other supplies

- Solid phase extraction cartridges: Bond Elut C18 cartridge, 1 g, 3 mL (p/n 12102118)
- Methylene chloride, ethyl acetate, acetone, toluene, and methanol (high purity pesticide quality or equivalent)
- Reagent water: Water in which an interference is not observed at the method detection limit of the compound of interest
- Hydrochloric acid: 6N

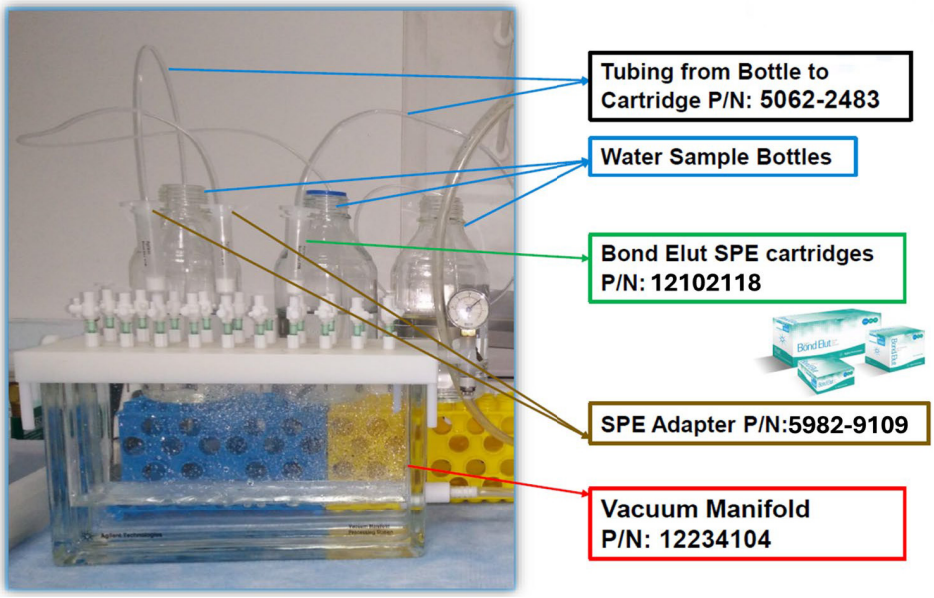

Figure 1. Setup of the SPE apparatus for large volume samples.

Figure 2. Specially designed adapter is required to route the waste water from the vacuum manifold to waste.

Sample preparation

Step 1. Conditioning the SPE cartridge

Elute each cartridge with a 5 mL aliquot of ethyl acetate. Let the cartridge drain dry.

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Elute each cartridge with a 5 mL aliquot of methylene chloride. Let the cartridge drain dry.

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Elute each cartridge with a 10 mL aliquot of methanol. Do not allow the methanol to elute below the top of the cartridge packing.

Step 2. Loading water samples (sample volume required: 1 L water sample, adjusted to ~pH 2)

Add 10 mL of reagent water (de-ionized water blank) to the cartridge. Add sample to the cartridge. Sample addition can be automated using the vacuum setup shown in Figure 1.

↓

Do not let the cartridge dry at any stage during sample loading. A 1 L sample should pass in approximately 2 hours. Vacuum should be ~13 cm of Hg.

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Draw air or nitrogen for 10 minutes after 1 L of sample has passed.

Step 3. Elution and collection

Place a collection tube below the cartridge, sufficient to hold at least 10 mL.

↓

Rinse the sample jar with 5 mL of ethyl acetate and elute the cartridge with this rinse into the collection tube.

↓

Rinse the sample jar with 5 mL of methylene chloride and elute the cartridge with this rinse into the collection tube.

Instrument parameters

Table 1. Agilent 8890-5977 GC/MS parameters.

Parameter	Value
GC	
Column	Agilent J&W HP-5ms UI 30 m × 0.25 mm, 0.25 µm
Carrier Gas	Helium, constant flow, 1.2 mL/min
Inlet	Agilent Multimode Inlet (MMI)
Inlet Temperature	280 °C
Injection Mode	Splitless, 1 µL
Oven Temperature Program	Rate (°C/min) Temperature Hold --- 60 1 40 170 0 10 310 3
Transfer Line Temperature	310 °C
MS	
Source Temperature	300 °C, Extractor Source with 3 mm lens
Quadrupole Temperature	150 °C
Acquisition Mode	SIM (parameters presented in Table 2)
Instrument Supplies	
Inlet Septa	Non-Stick Inlet Septa, bleed and temperature optimized (p/n 5183-4757)
Inlet Liner	Ultra Inert, Splitless, Single Taper with Glass Wool (p/n 5190-2293)
Syringes	Syringe, 10 µL Tapered, FN 23–26s/42/HP (p/n 5181-1267)
Inlet Column Nut	Column Nut, Collared, Self-Tightening (p/n G3440-81011)
MS Transfer Line Nut	Column Nut, Collared, Self-Tightening, MSD Transfer Line (p/n G3440-81013)
Ferrule (Inlet/Detector Connections)	Ferrule, 0.4 mm id, 15% Graphite/85% Vespel, 0.1 to 0.25 mm Column (p/n 5181-3323)

Small amounts of residual water from the sample container and the LSE cartridge may form an immiscible layer with the eluate. The eluate can be passed through 2–3 g of anhydrous sodium sulphate on a glass funnel plugged with glass wool at the neck. The sodium sulfate column/bed is washed with at least 2 mL methylene chloride and collected.

The extract is further concentrated in a low-volume concentrator under a gentle stream of nitrogen to 1 mL and transferred to GC vials.

Preparation of calibration standards and extracted calibration standards

Working standard solutions of 400 ng/mL were prepared by diluting the stock solutions in ethyl acetate.

Extracted calibration standards were prepared by spiking blank water samples (1 L) with the working standard solution, prior to extraction, to obtain concentrations of 5, 10, 20, 40, and 80 ng/L. After extraction and concentration (1 L to 1 mL, 1,000-fold), standards of 5, 10, 20, 40, and 80 ng/mL were obtained.

Results and discussion

TIC of the standards is presented in Figure 3. The reagent blank was free from any of the pesticides under study.

Calibration curve for each pesticide 5–80 ng/mL were plotted using peak area against concentration of pesticide in both extracted water and solvent. Correlation coefficients (R^2) > 0.995 were obtained for all pesticides as shown in Table 2.

Recovery experiments were carried out at 5–80 ng/L level of fortification indicated satisfactory recoveries within 80–120% with < 15% associated RSDs.

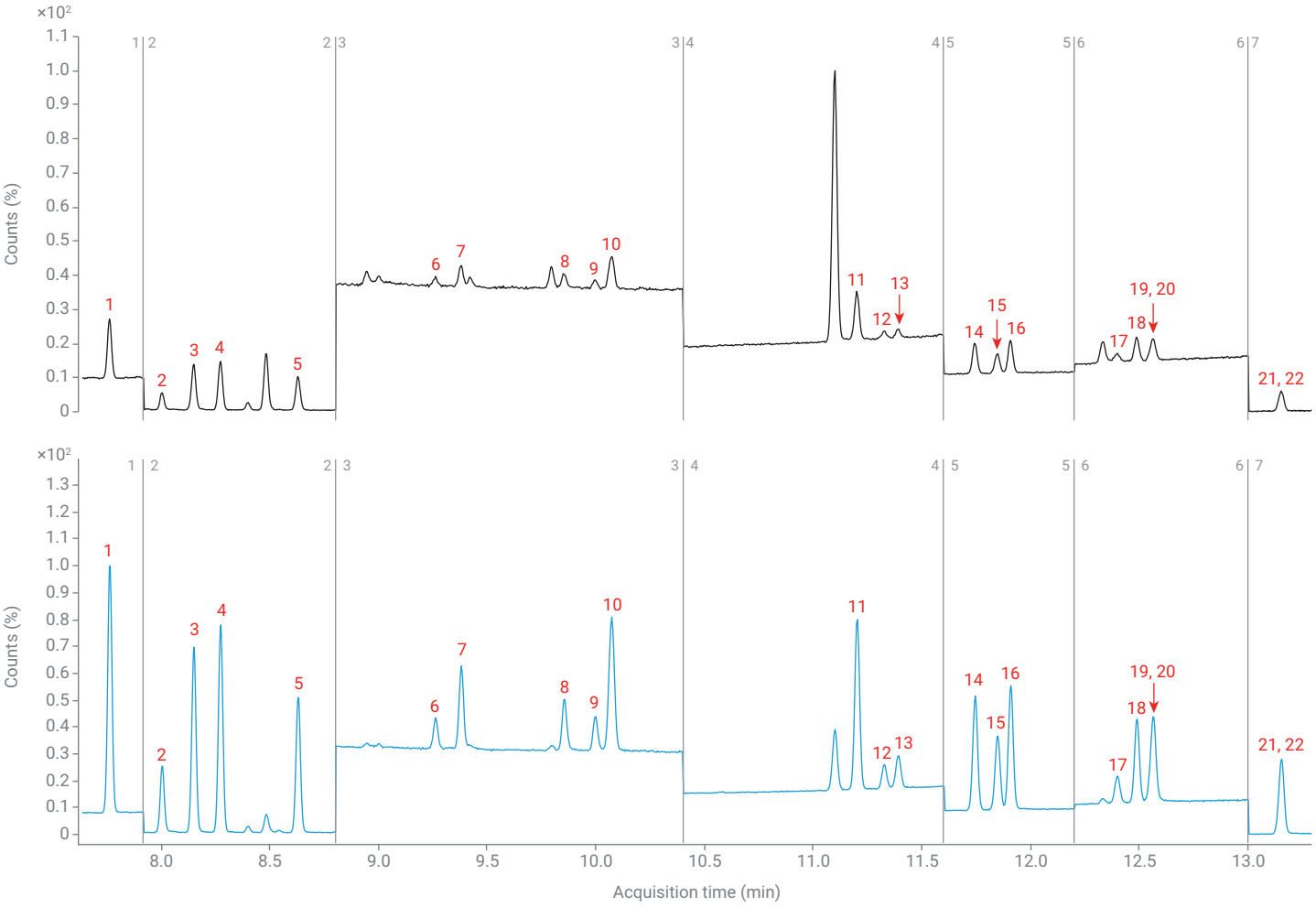

Figure 3. SIM chromatogram of 10 and 80 ppb pesticide standard.

Table 2. SIM parameters, retention time, and linearity.

Number	Compound	RT (min)	Q1	Q2	Q3	R^2
1	BHC-alpha	7.76	219	181	111	0.9986
2	Atrazine	8.003	215	200	173	0.9999
3	BHC-beta	8.148	219	181	111	0.9997
4	BHC-gamma (lindane)	8.268	219	181	111	0.9999
5	BHC-delta	8.626	219	181	111	0.9998
6	Parathion-methyl	9.259	263	125	109	0.9999
7	Alachlor	9.373	188	160	45	0.9997
8	Malathion	9.85	173	127	125	0.9997
9	Aldrin	10.065	293	263	66	0.9991
10	Chlorpyrifos	10.083	314	199	197	0.9994
11	DDE-o,p'	11.196	318	248	246	0.9982
12	Butachlor	11.326	188	176	160	0.9968
13	Endosulfan isomer 1	11.394	339	241	195	0.9971
14	DDE-p,p'	11.739	318	248	246	0.9956
15	Dieldrin	11.844	263	81	79	0.9993
16	DDT-o,p'	11.903	237	235	165	0.9979
17	Endosulfan isomer 2	12.393	231	153	97	0.9994
18	DDT-p,p'	12.484	237	235	165	0.9979
19	Ethion	12.553	231	153	97	0.9994
20	DDD-o,p'	12.56	237	235	165	0.9978
21	Endosulfan sulfate	13.141	387	272	237	0.9998
22	DDD-p,p'	13.15	237	235	165	0.9978

Table 3. Recovery tables.

Compound	Day 1			Day 2			Average	RSD (%)
	R1	R2	R3	R1	R2	R3		
Recovery at 5 ppt								
BHC-alpha	119	95	106	104	95	118	106	10
Atrazine	117	102	105	104	98	106	105	6
BHC-beta	100	94	96	104	119	109	104	9
BHC-gamma (lindane)	97	89	91	117	116	98	101	12
BHC-delta	102	97	108	115	103	113	106	6
Parathion-methyl	115	101	102	100	111	110	106	6
Alachlor	117	100	99	102	116	108	107	7
Malathion	100	95	108	102	116	111	105	7
Aldrin	112	92	95	106	102	103	102	7
Chlorpyrifos	106	87	92	100	90	96	95	7
DDE-o,p'	113	93	106	111	114	110	108	7
Butachlor	99	84	100	104	97	104	98	8
Endosulfan isomer 1	102	100	107	113	114	113	108	6
DDE-p,p'	101	93	102	99	95	97	98	3
Dieldrin	108	105	108	100	89	91	100	8
DDT-o,p'	99	98	108	101	86	99	98	7
DDT-p,p'	94	98	108	100	103	101	101	5
Ethion	109	109	116	104	109	104	108	4
DDD-o,p'	98	93	104	107	100	102	101	5
Endosulfan isomer 2	112	87	95	114	102	103	102	10
Endosulfan sulfate	97	90	104	108	109	101	101	7
DDD-p,p'	115	97	89	102	112	102	103	9
Recovery at 10 ppt								
BHC-alpha	97	87	83	104	106	102	96	10
Atrazine	99	99	101	111	111	108	105	6
BHC-beta	98	96	94	102	110	104	101	6
BHC-gamma (lindane)	97	93	86	106	106	105	99	8
BHC-delta	99	94	87	107	116	106	101	10
Parathion-methyl	101	89	90	115	119	119	106	13
Alachlor	99	95	89	119	112	118	105	12
Malathion	97	92	86	111	113	103	100	11
Aldrin	89	89	88	93	95	90	91	3
Chlorpyrifos	95	84	83	103	110	95	95	11
DDE-o,p'	90	83	85	104	106	96	94	10
Butachlor	88	85	85	108	110	89	94	13
Endosulfan isomer 1	101	96	102	93	93	96	97	4
DDE-p,p'	90	83	84	106	106	94	94	11
Dieldrin	95	81	84	101	112	101	96	12
DDT-o,p'	88	83	81	106	110	96	94	12
DDT-p,p'	88	83	90	106	110	96	95	11
Ethion	93	84	92	111	112	92	97	12
DDD-o,p'	88	88	88	109	111	100	97	11
Endosulfan isomer 2	95	88	88	109	114	99	99	11
Endosulfan sulfate	90	81	81	103	108	100	94	12
DDD-p,p'	84	86	87	95	103	89	91	8

Compound	Day 1			Day 2			Average	RSD (%)
	R1	R2	R3	R1	R2	R3		
Recovery at 20 ppt								
BHC-alpha	105	109	115	114	111	109	110	3
Atrazine	101	112	107	93	101	98	102	6
BHC-beta	102	110	105	115	118	115	111	6
BHC-gamma (lindane)	100	105	100	114	118	119	109	8
BHC-delta	100	116	107	118	115	115	112	6
Parathion-methyl	109	113	110	112	110	109	111	2
Alachlor	103	112	107	111	117	119	111	6
Malathion	102	118	108	96	104	98	104	8
Aldrin	102	116	109	112	107	109	109	4
Chlorpyrifos	101	116	109	117	114	116	112	5
DDE-o,p'	100	115	104	116	114	112	110	6
Butachlor	94	107	103	102	111	119	106	8
Endosulfan isomer 1	96	109	102	91	94	91	97	7
DDE-p,p'	91	101	101	99	107	105	101	6
Dieldrin	104	104	106	95	99	95	100	5
DDT-o,p'	96	114	101	99	104	99	102	6
DDT-p,p'	96	114	101	100	105	101	103	6
Ethion	100	117	104	91	100	97	101	8
DDD-o,p'	96	118	97	111	113	112	108	8
Endosulfan isomer 2	96	113	106	100	114	107	106	7
Endosulfan sulfate	95	117	98	108	106	101	104	7
DDD-p,p'	97	116	103	98	101	102	103	7
Recovery at 40 ppt								
BHC-alpha	99	105	100	114	102	97	103	6
Atrazine	102	115	107	108	113	112	109	4
BHC-beta	95	107	102	110	103	98	102	5
BHC-gamma (lindane)	99	106	105	115	102	101	105	5
BHC-delta	101	112	104	115	109	107	108	5
Parathion-methyl	102	112	109	117	119	120	113	6
Alachlor	100	116	107	111	111	109	109	5
Malathion	100	118	106	106	112	113	109	6
Aldrin	97	110	100	114	102	100	104	6
Chlorpyrifos	94	113	103	108	108	105	105	6
DDE-o,p'	96	111	99	119	105	103	106	8
Butachlor	95	105	102	109	110	109	105	5
Endosulfan isomer 1	89	91	91	115	102	103	98	10
DDE-p,p'	95	111	98	119	104	102	105	9
Dieldrin	98	112	99	117	107	107	107	7
DDT-o,p'	94	113	98	100	104	102	102	6
DDT-p,p'	94	113	98	104	104	102	102	6
Ethion	93	108	97	101	103	101	101	5
DDD-o,p'	94	114	98	106	105	105	104	7
Endosulfan isomer 2	96	119	100	108	108	106	106	7
Endosulfan sulfate	95	113	98	114	106	106	105	7
DDD-p,p'	95	115	99	113	107	110	106	8

Compound	Day 1			Day 2			Average	RSD (%)
	R1	R2	R3	R1	R2	R3		
Recovery at 80 ppt								
BHC-alpha	101	100	95	102	99	104	100	3
Atrazine	109	105	101	114	106	114	108	5
BHC-beta	101	99	98	106	99	111	102	5
BHC-gamma (lindane)	102	98	96	104	98	106	101	4
BHC-delta	107	99	98	110	103	110	105	5
Parathion-methyl	113	104	102	115	110	119	111	6
Alachlor	109	102	103	114	106	115	108	5
Malathion	110	102	100	113	107	118	108	6
Aldrin	105	98	94	104	99	106	101	5
Chlorpyrifos	110	101	100	109	101	117	106	6
DDE-o,p'	107	99	95	107	99	111	103	6
Butachlor	111	102	97	115	105	113	107	6
Endosulfan isomer 1	106	109	112	105	116	116	111	4
DDE-p,p'	107	99	96	109	101	115	104	7
Dieldrin	106	99	94	108	100	113	103	7
DDT-o,p'	106	99	95	109	101	115	104	7
DDT-p,p'	106	99	95	109	101	115	104	7
Ethion	106	99	93	106	97	111	102	7
DDD-o,p'	107	99	95	110	102	116	105	7
Endosulfan isomer 2	111	103	98	111	102	119	107	7
Endosulfan sulfate	106	99	95	111	103	116	105	8
DDD-p,p'	106	100	96	112	102	117	106	8

Conclusion

A simple, easy, and efficient method based on EPA 525.2 was implemented for the determination of 22 pesticides in water. The method demonstrated good sensitivity, precision, and accuracy, and allows for rapid analysis. The results demonstrate that pesticide residues can be detected below the current maximum residue levels (MRL) required by the BIS specifications. Acceptable recoveries and precision were obtained at concentrations as low as 5 ng/L for all pesticides. The method is ideally suited for use in a regulatory laboratory for the determination of pesticides in surface, drinking, and packaged drinking water.

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