Analyze Pesticides in Water with Stir Bar Sorptive Extraction and Thermal Separation

Application Note

Environmental

Authors

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Abstract

Multiple pesticides in water samples were successfully analyzed using an Agilent 5975T LTM GC/MSD equipped with an Agilent J&W DB-5ms Ultra Inert low thermal mass column module, with stir bar sorptive extraction and an Agilent Thermal Separation Probe for sample preparation. The method was simple, rapid, effective, and showed good linearity (R^2 >0.9914) and high sensitivity for most of the target pesticides.

Introduction

An effective sample preparation and analysis method is required to monitor trace pesticide residues in environmental samples. For aqueous samples, conventional extractions require 1 to 2 L of water to obtain sufficient pesticide residue for analysis. Extraction and enrichment steps in these traditional sample preparation techniques are tedious, time consuming, labor intensive, and use large amounts of organic solvents.

Stir bar sorptive extraction (SBSE) was introduced in 1999 as a solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrixes [1]. The method is based on sorptive extraction, where the solutes are extracted into a polymer coating on a magnetic stirring rod. The extraction is controlled by the partitioning coefficient of the solutes between the polymer coating and the sample matrix, and by the phase ratio between the polymer coating and the sample volume. For a polydimethylsiloxane coating and aqueous samples, this partitioning coefficient resembles the octanol-water partitioning coefficient. The technique has been applied successfully to trace analysis in environmental, biomedical, and food applications [2,3,4].

The Agilent Thermal Separation Probe (TSP) for GC injection is a technology that minimizes sample preparation and can provide a rugged analytical approach for complex matrixes.

The use of SBSE and TSP together, for sample preparation, was found to be rapid, effective and solvent-free. These techniques were, therefore, used in developing a method for the determination of pesticides in water on the Agilent 5975T LTM GC/MSD, according to the Agilent Japanese positive list pesticide solution [5].

Materials and Methods

All reagents and solvents were HPLC grade. The pesticide standards and triphenyl phosphate internal standard (TPP) were provided by internal customers. Analyses were performed on an Agilent 5975T LTM GC/MSD system using a split/splitless inlet. The TSP was connected to the GC with split/splitless inlet.

GC conditions

Analytical column:	Agilent J&W DB-5ms Ultra Inert LTM, 30 m × 0.25 mm, 0.25 µm (p/n G3900-63005)
Guard column:	0.5 m column with same phase as analytical column, connected to the injector
Carrier gas:	Helium, constant flow mode, 1.0 mL/min
LTM oven temperature:	50 °C (1 min), 25 °C/min to 125 °C, 10 °C/min to 300 °C (10 min)
Inlet:	250 °C, split 10:1
Injection:	1 µL
Retention time locking:	Chlorpyrifos-methyl locked at 13.443 min

MSD conditions

Ion source temperature:	230 °C
Quadrupole temperature:	150 °C
Ionization:	EI mode, 70 eV
Acquisition mode:	Scan/SIM, full scan, *m/z* 45 to 550
Transfer line temperature:	280 °C
Solvent delay:	3 min
Probe:	Agilent Thermal Sample Probe (p/n G4381A)
Liner:	Low pressure drop, Ultra Inert liner with glass wool (p/n 5190-2295)
Stir bar coated with 24 µL PDMS:	Twister, GERSTEL GmbH, film thickness: 0.5 mm; length: 10 mm

Sample preparation

Forty milliliters of water sample and 60 µL internal standard spiking solution were transferred into a 50 mL centrifuge tube. A stir bar (Twister) was added and the centrifuge tube was sealed with a screw cap. SBSE was performed at room temperature for 35 minutes while stirring at 1,500 rpm. After extraction, the stir bar was removed with forceps, dipped briefly in Milli-Q purified water, dried with a lint-free tissue, and placed in the TSP micro-vial. Spiked samples were treated in the same way.

Results and Discussion

The pesticides studied in this work included organochlorines, organophosphates, and pyrethroids (Table 1). Figure 1 shows the total ion chromatogram (TIC) of the matrix blank and a 25 µg/L fortified water sample. No peaks in the TIC of matrix blank interfered with the target analytes, and all target pesticides were well separated using the DB-5ms Ultra Inert LTM column module.

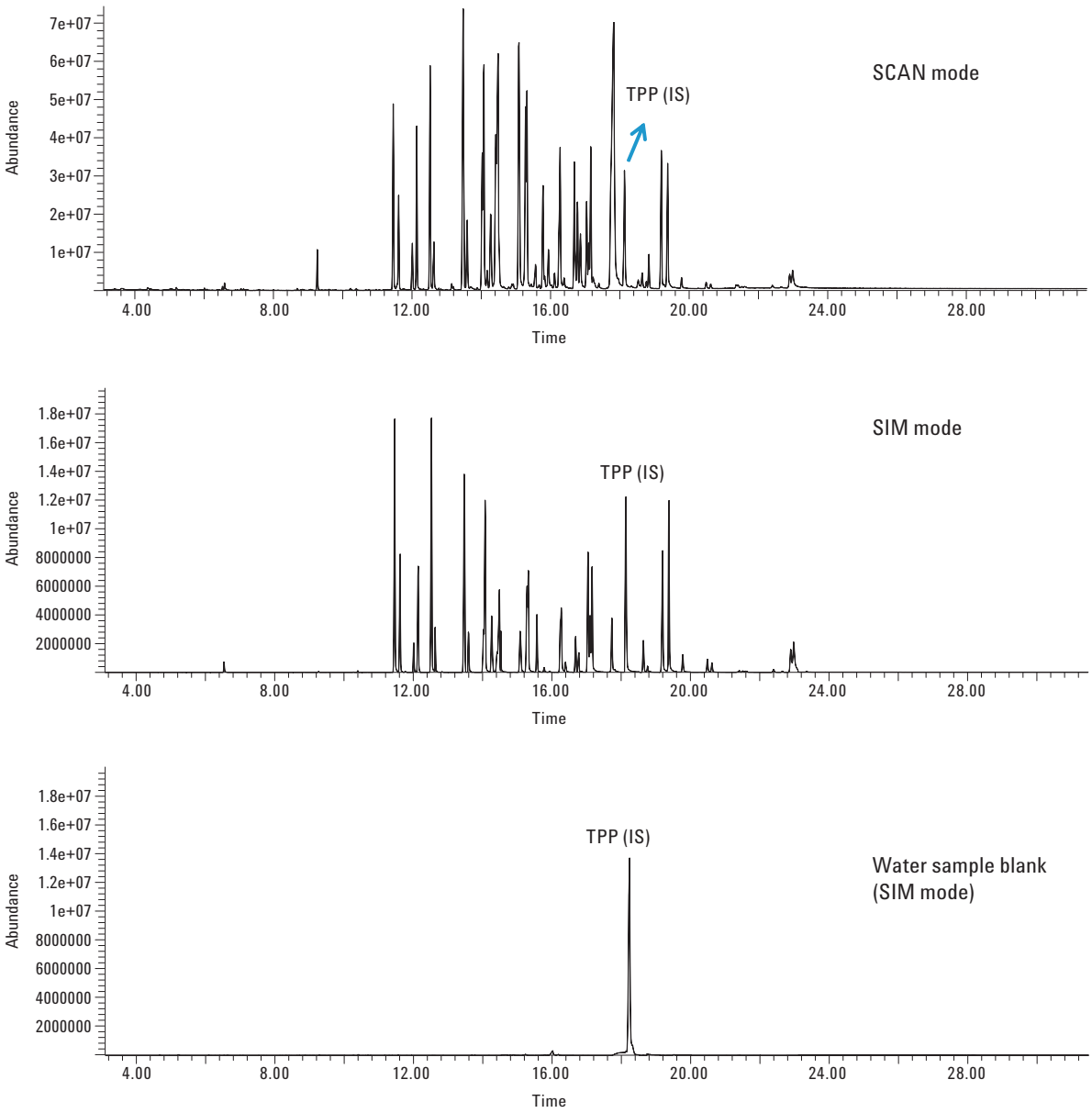

Figure 1. TIC of water sample blank and spiked water sample (25 µg/L pesticides).

Table 1. Pesticides studied and corresponding octanol-water partitioning coefficients (log K_{OW}), selected ions for determination, theoretical recovery, real recovery, linearity, and method detection limits (MDL).

Rt (min)	Compound	Log K_{ow}	*m/z*	Theoretical recovery (%)	Recovery (%)	R^2	MDL (µg/L)
6.536	Dichlorvos	1.90	109	4.55	4.18	0.9953	0.03
11.457	BHC-*alpha*	3.82	181	79.86	48.15	0.9994	0.05
11.609	Hexachlorobenzene	3.93	284	83.62	42.36	0.9934	0.06
11.723	Atraton	2.69	211	22.71	38.91	0.9950	1.28
11.829	Simazine	2.30	201	10.69	12.75	0.9999	1.30
11.946	Atrazine	2.70	200	23.12	23.87	0.9964	0.85
12.010	BHC-*beta*	4.26	181	75.90	67.47	0.9959	0.16
12.042	Propazine	3.95	214	84.25	69.45	0.9975	1.06
12.131	BHC-*gamma*	3.68	181	74.17	54.67	0.9990	0.20
12.257	Terbutylazine	3.40	214	60.11	38.60	0.9988	0.95
12.513	Diazinon	3.69	179	74.61	69.98	0.9986	0.21
12.638	BHC-*delta*	3.68	181	74.17	47.97	0.9972	0.20
12.651	Secbumeton	3.64	196	72.37	48.79	0.9936	1.01
13.442	Vinclozolin	3.02	212	38.59	53.53	0.9961	0.26
13.447	Parathion-methyl	3.00	263	37.50	44.42	0.9914	0.16
13.449	Chlorpyrifos-methyl	4.00	286	85.71	67.90	0.9980	0.23
13.517	Simetryn	2.80	213	27.46	17.54	0.9998	1.15
13.587	Heptachlor	5.44	272	99.40	79.59	0.9941	0.09
13.617	Ametryn	2.63	227	20.38	15.13	0.9976	1.50
13.701	Prometryn	3.34	241	56.76	33.25	0.9994	0.69
13.946	Terbutryn	3.66	226	73.28	54.90	0.9997	0.92
14.010	Fenitrothion	3.32	260	55.63	64.58	0.9976	0.13
14.051	Pirimiphos-methyl	3.90	290	82.66	68.61	0.9989	0.47
14.232	Malathion	2.75	173	25.23	37.66	0.9992	0.37
14.272	Aldrin	6.50	263	99.95	89.64	0.9930	0.11
14.385	Fenthion	4.84	279	97.65	83.18	0.9968	0.42
14.430	Chlorpyrifos	4.70	197	96.78	90.44	0.9958	0.22
14.450	Parathion	3.83	291	80.22	72.48	0.9984	0.28
15.027	Pendimethalin	5.20	162	98.96	82.94	0.9972	0.10
15.057	Heptachlor exo-epoxide	4.24	81	91.25	92.74	0.9993	0.28
15.070	Chlordane-oxy	5.48	185	99.45	98.24	0.9989	0.15
15.246	Isofenphos	4.04	213	86.81	73.57	0.9994	0.25
15.288	Quinalphos	4.44	146	94.29	82.68	0.9993	0.32
15.555	Methidathion	2.57	145	18.23	27.66	0.9995	0.18
16.238	DDE-p,p'	6.00	246	99.83	29.11	0.9994	0.25
16.263	Dieldrin	3.70	79	75.04	79.79	0.9988	0.20
16.390	Myclobutanil	2.89	179	31.78	44.00	0.9961	0.51
16.677	Endrin	3.20	263	48.74	51.78	0.9993	0.23
16.754	Chlorfenapyr	4.83	59	97.59	105.34	0.9956	0.15
17.031	DDD-p,p'	5.87	235	99.78	75.32	0.9951	0.17
17.093	DDT-p,p'	6.91	235	99.98	74.75	0.9959	0.23
17.146	Ethion	5.07	231	98.60	89.33	0.9982	0.06
17.733	DDT-o,p'	6.70	235	99.97	75.54	0.9928	0.28

Continued

Rt (min)	Compound	Log K_{ow}	*m/z*	Theoretical recovery (%)	Recovery (%)	R^2	MDL (µg/L)
18.641	Phosmet	2.96	160	35.37	46.42	0.9973	0.30
18.769	Bifenthrin	6.60	181	99.96	80.53	0.9962	0.42
19.181	Tetradifon	4.61	159	96.07	78.12	0.9991	0.30
19.371	Phosalone	4.01	182	85.99	71.71	0.9997	0.07
19.788	Cyhalothrin	6.80	181	99.97	108.57	0.9988	0.62
20.625	Permethrin	6.10	183	99.87	80.88	0.9982	0.42
22.421	Fenvalerate	5.01	125	98.40	88.82	0.9994	0.35
22.916	Difenoconazole I	4.36	265	93.22	84.29	0.9972	0.47
23.364	Deltamethrin	4.60	181	95.98	81.27	0.9973	0.56

Triphenyl phosphate (internal standard)

Extraction time

The extraction time is a critical parameter in the SBSE sampling process. The spiked samples treated according to the procedure were analyzed to evaluate extraction effectiveness. Figure 2 shows the relationship between extraction time and response peak areas. The large increase in peak areas can be found from 20 to 60 minutes. This means the time factor is very critical until equilibrium between the stir bar and the sample is reached. However, when extraction time is more than 60 minutes, the equilibrium for most of target compounds will have been reached and so small changes in extraction time after that have no critical influence on the quantitative results. Normally, extraction time can be selected as 60 minutes, but, in this application, extraction time was 35 minutes to achieve faster analysis and higher throughput, which, because of sensitivity, was sufficient.

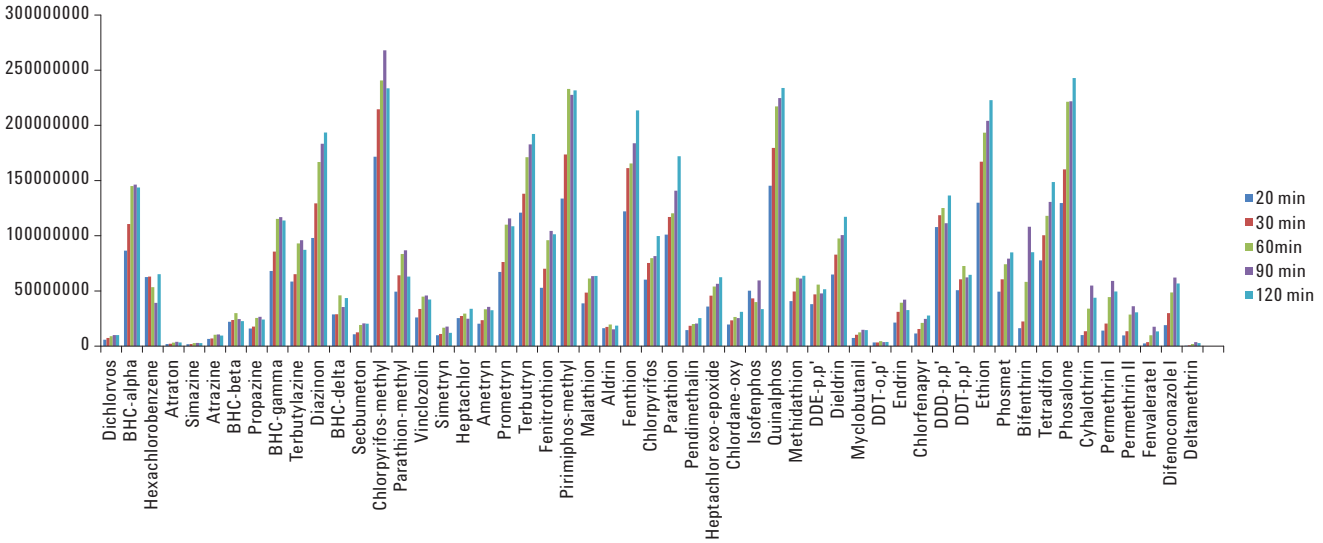

Figure 2. Relationship of extraction time and response peak area.

Partitioning efficiency and recovery

Since SBSE is by nature an equilibrium technique, the extraction of solutes from the aqueous phase into the PDMS phase is controlled by the partitioning coefficients. Recent studies have correlated this partitioning coefficient with the octanol-water distribution constant (K_{OW}) [6]. Estimation of recoveries listed in Table 1 were calculated according to Equation 1. In general, the obtained recovery was lower than the theoretical value. Hydrophobic compounds with a high K_{OW} had high recovery; by contrast, hydrophilic compounds with a low K_{OW}, for example, polar pesticides, had low recovery. Therefore, most recoveries can be predicted based on the known analyte octanol-water partition coefficient. This prediction can offer a reference before embarking on an experiment.

$$\frac{m_{SBSE}}{m_0} = \frac{\left(\frac{K_{OW}}{\beta}\right)}{1 + \left(\frac{K_{OW}}{\beta}\right)}$$

m_{SBSE}= amount on PDMS
m_0 = initial amount
K_{ow} = octanol-water partitioning
β = phase ratio = sample volume/PD MS volume

Equation 1. Calculation of theoretical recovery.

Salt addition

It has been reported that recoveries for solutes with log K_{OW} of less than 4.0 dramatically increased with salt addition (SBSE with 30% NaCl) [7]. The recoveries of SBSE with NaCl from 10 to 40% were, therefore, evaluated. Since PDMS is a non-polar phase, the solutes with log K_{OW} less than 1 could not be extracted well by SBSE, with or without salt addition. Examples include methamidophos (log K_{OW} 0.79) and acephate (log K_{OW} 0.85). However, the recoveries for some solutes increased greatly, such as dichlorvos (log K_{OW} 1.90) and myclobutanil (log K_{OW} 2.89), when recoveries increased fourfold and threefold, respectively. With log K_{OW} close to or greater than 4.0, recoveries for these compounds dramatically decreased compared to SBSE without salt addition. Although sequential SBSE can increase recovery for some hydrophilic solutes, another 30 to 60 minutes is needed for extraction. Rapid methods are required by 5975T LTM GC/MSD users, and so salt addition was not used for extraction because log K_{OW} was close to or greater than 4.0 for most of the target compounds in this work.

Linearity

The linearity calibration range for organochlorine and organophosphate pesticides was 0.8 to 25 µg/L. For triazines, the range was 1 to 50 µg/L, and for pyrethroid pesticides the range was 5 to 50 µg/L. Calibration curves spiked in matrix blanks were made at six levels and TPP was used as an internal standard at 15 µg/L. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) against the relative concentration of analytes (concentration of analyte/concentration of IS). Good linearity was achieved with correlation coefficients (R^2) for all of the compounds between 0.9914 and 0.9999.

Method detection limit (MDL)

The method detection limit (MDL) is defined as "the minimum concentration that can be determined with 99% confidence that the true concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte." The MDL is calculated according to Equation 2.

$$MDL = t_{(n-1,1-\alpha = 0.99)} \times S$$

where:

MDL = the method detection limit

$t_{(n-1,1-\alpha = 0.99)}$ = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom

S = standard deviation of the replicate analyses

Equation 2. Calculation of the method detection limit.

Detection was by MSD in scan mode. MDL was calculated as 3.36 times the standard deviation obtained for six replicate analyses of the lowest concentration of the calibration curve for the different pesticides. Low MDLs in the range of 0.03 to 1.50 µg/L were obtained, as shown in Table 1.

Conclusions

This application note demonstrates a rapid and low-cost method for analysis of pesticides in water samples using the Agilent Thermal Sample Probe and stir bar sorptive extraction with the Agilent 5975T LTM GC/MSD. Agilent TSP in combination with SBSE provides a simple, fast, and effective method for extraction of representative pesticides in water samples. The Agilent low thermal mass GC technology also reduces the run time by heating and cooling the column very efficiently, for significantly shorter analytical cycle times. Recovery can be predicted based on the known analyte octanol-water partition coefficient, and the method shows good linearity and low MDL for pesticide analysis.

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