The Importance of Autosampler Vial Selection in the GC-MS Analysis of Pyrethroid Pesticides at Low Concentration

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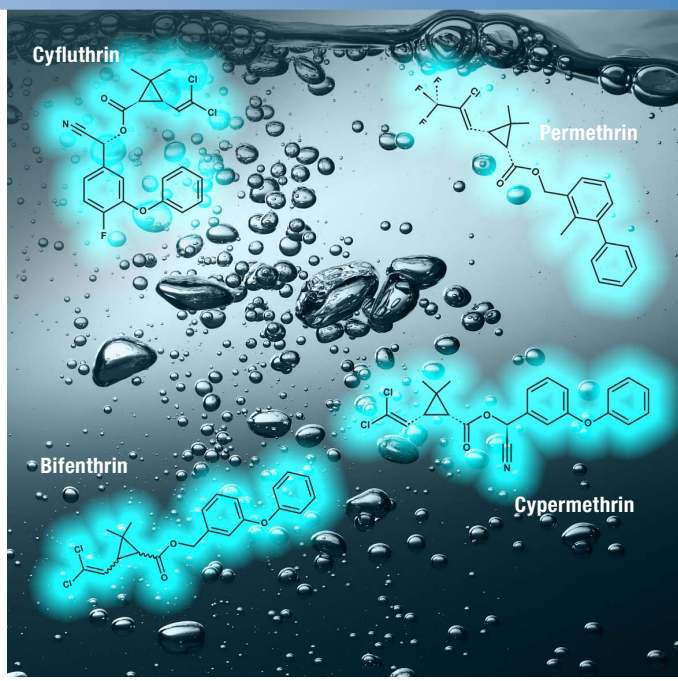
Key Words

Pyrethroid pesticides, adsorption, 33 expansion high purity clear neutral borosilicate glass vial

Abstract

The selection of the correct autosampler vial type is vital to the success of analyzing pyrethroid pesticides at low levels. A method for the determination of pyrethroids at 0.10 ng/mL was developed using solid phase extraction (SPE) for pre-concentration with subsequent analysis by GC with PTV simulated on-column injection. Careful selection of the autosampler vial type was needed to minimize adsorption effects.



Introduction

Pyrethroids are a class of synthetically produced insecticides that are mainly used for domestic purposes to control insects such as house flies and mosquitoes. They behave very similarly to natural pyrethrins, which are derived from chrysanthemum flowers, and are extremely toxic to fish and aquatic organisms but have low toxicity towards humans. However, repeated exposure to pyrethroids increases the risk of anaphylaxis and allergic reaction to very low concentrations and, therefore, pyrethroid levels should be monitored.

Analyzing pyrethroids at low concentration levels can be challenging due to their adsorption onto glass surfaces, such as sample bottles, GC inlet liners and vials. To reduce adsorption of pyrethroids onto the surface of glass, a comparison with plastic and high purity clear neutral borosilicate glass vials was carried out with a GC method utilizing a Programmable Temperature Vaporizer (PTV) simulated on-column injection.

The separation of the pyrethroid extracts was carried out using a Thermo Scientific™ TraceGOLD™ TG-5SilMS column with a Thermo Scientific GuardGOLD™ pre-column. This column is based on silarylene chemistry, which provides more stability and lower bleed than standard 5% phenyl dimethylpolylsiloxane phase GC columns. This in turn gives rise to better sensitivity due to reduced background signal. This phase can also partially resolve complex mixtures of cyfluthrin and cypermethrin isomers.

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Experimental Details

Consumables		Part Number
Columns:	TraceGOLD TG-5SilMS, 30 m x 0.25 mm x 0.25 µm GuardGOLD 2 m x 0.53 mm ID Press-Fit	26096-1420 26050-0253 64000-001
Injection port septum:	BTO, 12.7 mm	313032280
Liner:	PTV Silcosteel liner for PTV simulated on-column, 1 x 2.75 x 120 mm	45322052
Column ferrules:	100% graphite ferrules for Thermo Scientific TRACE™ injector, 0.53 mm ID	29053486
Colum ferrules:	Graphite/Vespel® for transfer line, 0.25 mm ID	29033496
Injection syringe:	85 mm 26s Gauge, 10 µL fixed needle syringe for a Thermo Scientific TriPlus™ RSH Autosampler	365D0321
Sample vials:	Thermo Scientific National™ 9 mm Target DP Polypropylene Vial, 300 µL	C4000-11
	Thermo Scientific National 9 mm Target DP™ Vial, Total Recovery with 10 µL Reservoir	C4000-9TR
	Thermo Scientific Chromacol™ 9 mm screw caps with High Purity Silicone/PTFE septa	9-SC(B)-ST101
	Fisher Scientific™ LC-MS grade water	W/0112/17

Preparation of Calibration Standards

A stock standard solution of 1 mg/mL of bifenthrin, permethrin, cyfluthrin, and cypermethrin was prepared in ethyl acetate. Calibration standard solutions were then prepared in ethyl acetate at the following concentrations: 50, 100, 200, 500, 1000, and 2000 ng/mL. A 100 µL aliquot of each calibration standard was then placed into an autosampler vial followed by the addition of 10 µL of 10 µg/mL of internal standard to each vial.

Sample Preparation: SPE Extraction Protocol		Part Number
SPE cartridge:	Thermo Scientific HyperSep™ C18 SPE column, 2000 mg/15 mL	60108-701
Compound:	(i) Bifenthrin, (ii) *cis/trans* permethrin, (iii) cyfluthrin, and (iv) cypermethrin 1 L each at 0.10 ng/mL in water	
Matrix:	LC/MS water	
Conditioning stage:	10 mL ethyl acetate, 10 mL acetone, 2 x 10 mL aliquots water applied sequentially to the SPE cartridge and then pulled through under vacuum at 4-5 mL/min	
Application stage:	1 L of sample was applied to the SPE cartridge under vacuum at 4-5 mL/min	
Washing stage:	10 mL of water was added to a 1 L vessel, swirled, and placed onto the SPE cartridge. Then the cartridge was dried for 20 min under vacuum.	
Elution stage:	10 mL ethyl acetate was added to the sample vessel, swirled, and then placed onto the SPE cartridge. Then an additional 10 mL ethyl acetate was applied directly onto the cartridge.	
Additional stages:	Solvent was evaporated to dryness at 40 °C and the residue reconstituted in 100 µL of ethyl acetate to give the final concentration of pyrethroids at 1000 ng/mL. Then 10 µL of 10 µg/mL of internal standard was added to the vial.	

Separation Conditions	
Instrumentation:	Thermo Scientific TRACE GC Ultra™
Carrier gas:	Helium
Split flow:	50 mL/min
Column flow:	1.2 mL/min, Constant flow
Oven temperature:	80 °C (0.5 min), 30 °C/min, 220 °C (4 min), 10 °C/min, 320 °C (10 min)
Injector type:	PTV simulated on-column
Injector mode:	Splitless (10 min) 30 mL/min flow rate, constant septum purge
Injector temperature phases:	40 °C (0.10 min), transfer 12 °C/sec, 330 °C (10 min) min
Detector type:	Thermo Scientific ISQ™ mass spectrometer
Transfer line temperature:	260 °C
Source temperature:	220 °C
Ionization conditions:	EI
Electron energy: 70	eV
Emission current:	25 µA
SIM scan parameters:	Table 1

Scan Window Start Time (min)	Compound Name	Mass List (Quan), Qual ions	Total Scan Time (s)
5.50	1,2,3,4-tetrachloro-naphthalene (IS)	(264), 268, 266,	0.222
9.50	Bifenthrin	(181), 165, 166, 182	0.216
14.00	*cis/trans* permethrin	(183), 184, 163, 165	0.216
15.40	Cyfluthrin/cypermethrin	(226), (181), 182, 163, 165, 166	0.234

Table 1: SIM Scan parameters

Injection Conditions	
Instrumentation:	Thermo Scientific TriPlus RSH Autosampler
Injection Volume:	2 µL
Injection depth:	70 mm
Penetration speed:	10 mm/s
Injection speed:	50 µL/s

Results

Due to the possible breakdown of pyrethroids if exposed to light during sample preparation, standard solutions were initially prepared in amber glass vials. Investigation showed that the choice of a non-silanized 51A amber (type 1, class B) glass autosampler vial was impacting the recovery of the compounds from the vials. The results showed that the overall recovery of the pyrethroids was poor and the calibration of both bifenthrin and permethrin showed non-linear response (Figure 1). The higher levels of iron oxide present in the amber vials used as a coloring agent leaches out when in contact with water. The glass surface then becomes more active and interacts with pyrethroids.

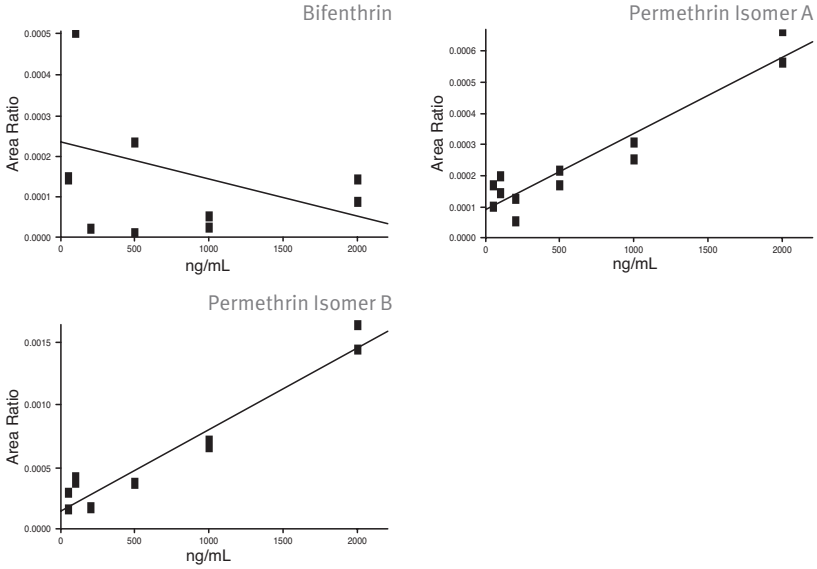

Figure 1: Calibration curves for bifenthrin and permethrin isomers (50–2000 ng/mL) using non-silanized amber glass vial

To determine if the glass vial was responsible for adsorption of pyrethroids, it was decided to substitute the non-silanized amber vials with an alternative plastic vial. The results obtained showed improved recovery and linearity of sample response (Figure 2, Table 2). However, the plastic vials, composed of polypropylene, would also be likely to introduce leachable organic species when exposed to the organic solvent for any extended period of time. The contact time between the solvent and plastic vials had to be kept to a minimum to avoid introduction of these polypropylene extractables into the mass spectrometer.

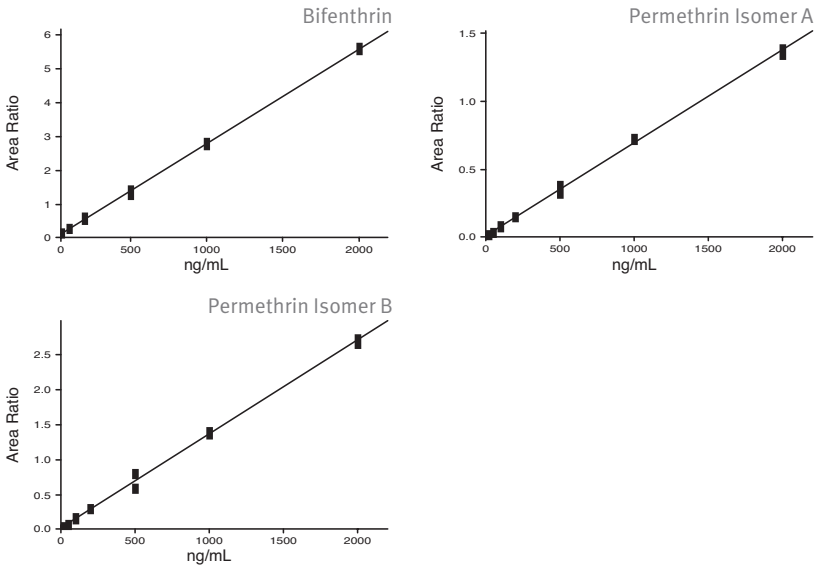

Figure 2: Calibration curves for bifenthrin and permethrin isomers (50–2000 ng/mL) using a plastic vial

To determine if the type of glass could have an effect on adsorption of pyrethroids, a high purity clear neutral borosilicate glass vial was evaluated. Thermo Scientific National 9 mm Target DP Total Recovery Vials, 33 expansion borosilicate clear (Type 1, Class A) were used. This gave improved linearity and extraction recoveries for all pyrethroids (Figure 3, Table 2). In this case, the contact time was not found to be a limiting factor, and the pyrethroid samples could be safely stored in the vial, if shielded from direct sunlight.

Vials	Plastic Vial			Target DP 33 Expansion High Purity Clear Neutral Borosilicate Glass Vial			Non-Silanized Amber Glass Vial		
Compound	Linearity R^2	% Recovery	% RSD (n=3)	Linearity R^2	% Recovery	% RSD (n=3)	Linearity R^2	% Recovery	% RSD (n=3)
Bifenthrin	0.9995	81.8	7.2	0.9988	101.8	4.0	0.1426	49.5	–
Permethrin Isomer a	0.9984	85.7	12.1	0.9975	117.1	4.4	0.9016	46.33	–
Permethethrin Isomer b	0.9979	85.6	5.7	0.9978	112.2	3.9	0.9296	8.08	–
Cyfluthrin Total Isomers	0.9953	101.6	6.0	0.9977	117.1	3.6	0.9967	62.61	–
Cypermethrin Total Isomers	0.9957	84.7	4.5	0.9963	113.9	4.2	0.9744	62.61	–

Table 2: Comparison data of linearity (50–2000 ng/mL) and extraction recovery for 0.10 ng/mL spiked pyrethroids in water using plastic, 33 expansion high purity clear neutral borosilicate glass, and non-silanized amber vials.

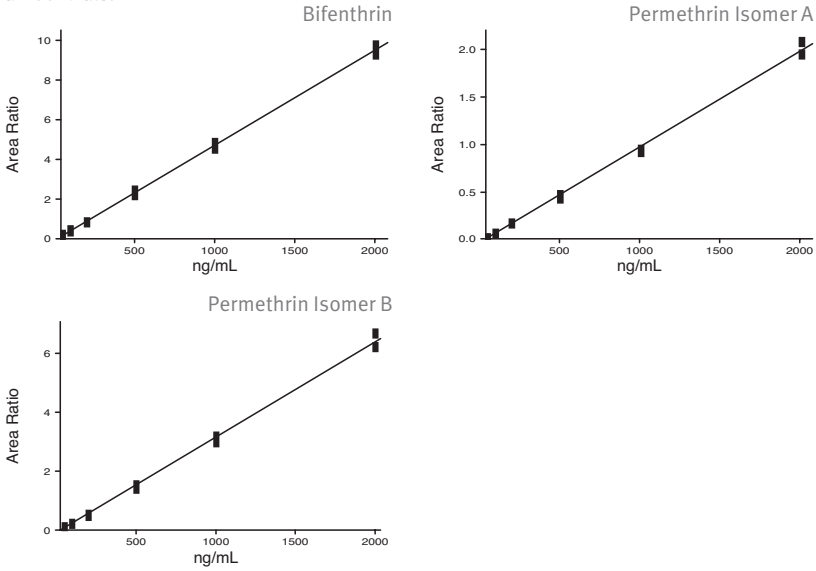

Figure 3: Calibration curves for bifenthrin and permethrin isomers (50–2000 ng/mL) using a 33 expansion high purity clear neutral borosilicate glass vial

A calibration curve (50-2000 ng/mL) was constructed for each compound using 1,2,3,4-tetrachloronaphthalene as the internal standard (IS). The coefficients of determination (R^2) between area ratio of sample and internal standard for all pyrethroids were greater than 0.99 for plastic and Target DP Total Recovery Vials, 33 expansion borosilicate clear (Type 1, Class A) (Table 2), demonstrating good method linearity. The analysis was performed in SIM mode. Figure 4 shows the TIC chromatogram of spiked pyrethroids in water at 0.10 ng/mL after the pre-concentration step.

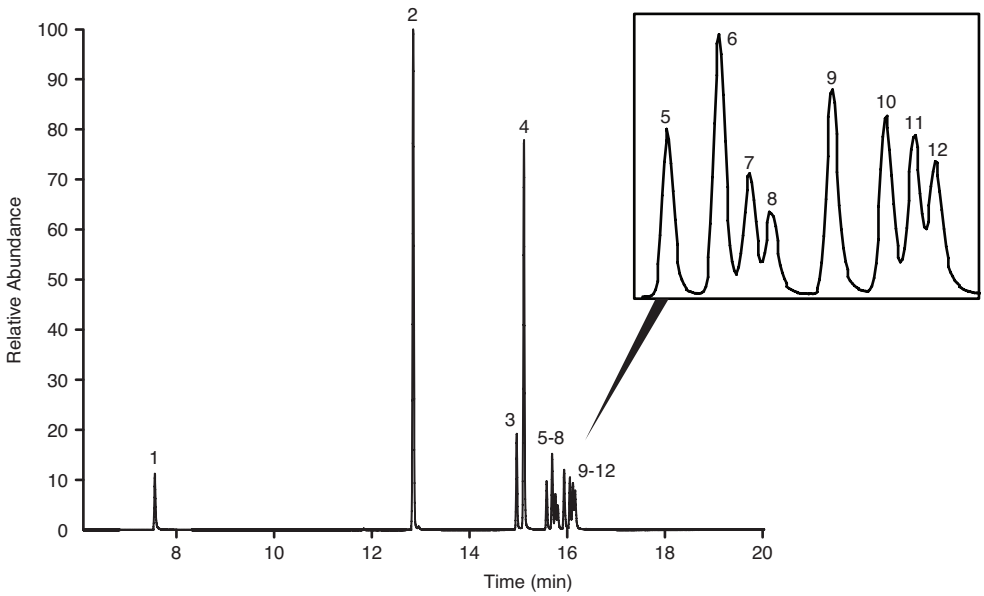

Figure 4: SIM Chromatogram of 0.10 ng/mL of pyrethroid pesticides separated on TG-5SilMS column after a pre-concentration step using a HyperSep C18 SPE cartridge

Peak Number	Compound	t_R (min)
1	1,2,3,4-Tetra Chloronaphthalene (IS)	7.56
2	Bifenthrin	12.84
3	Permethrin isomer a	14.97
4	Permethrin isomer b	15.11
5	Cyfluthrin isomer a	15.58
6	Cyfluthrin isomer b	15.69
7	Cyfluthrin isomer c	15.76
8	Cyfluthrin isomer d	15.81
9	Cypermethrin isomer a	15.94
10	Cypermethrin isomer b	16.06
11	Cypermethrin isomer c	16.12
12	Cypermethrin isomer d	16.16

Table 3: Peak indentification

Three replicate extractions of pyrethroids spiked at 0.10 ng/mL in water were carried out using a HyperSep C18 SPE cartridge. The extraction recoveries from plastic, non-silanized amber vials, and Target DP Total Recovery Vials, 33 expansion borosilicate clear (Type 1, Class A), were compared. The pyrethroids recoveries were measured to be 81%–117%, with relative standard deviations (RSD) of 3%–12% (see Table 2 for individual pyrethroids measured at each concentration). The extraction recovery was enhanced when using the high purity 33 expansion glass vials as shown in Figure 5.

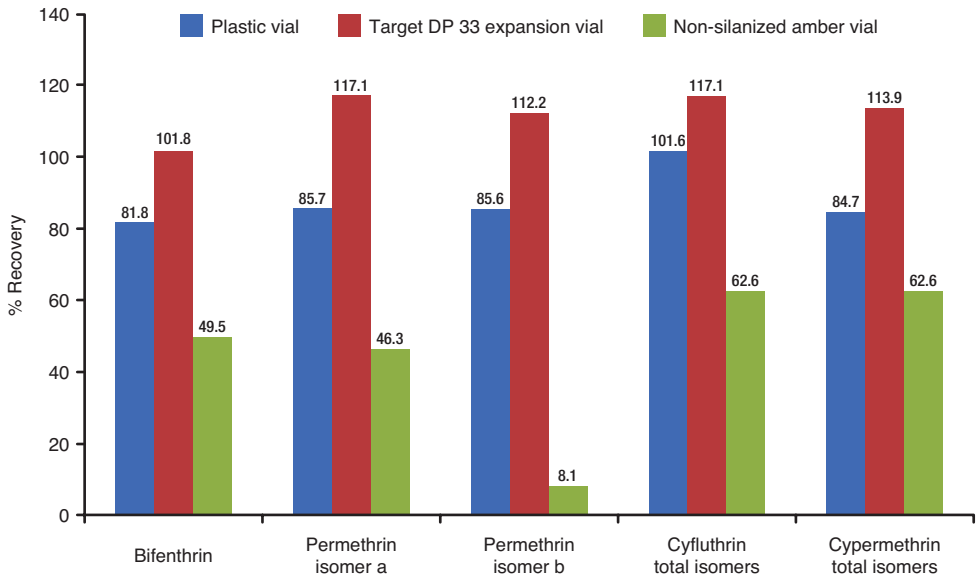

Figure 5: Comparison of extraction recoveries of pyrethroids using three different vial types

Conclusion

- Studies showed plastic and high purity clear neutral borosilicate glass vials reduce adsorption of pyrethroids at lower concentrations.
- The linearity and recovery of the pyrethroids was improved by the use of polypropylene vials but at the risk of absorbed material being introduced into the GC.
- The best results were obtained using a high purity 33 expansion clear glass vial with low surface activity, which gave higher sample recovery compared to the polypropylene vial.
- The SPE-GC/MS method demonstrated high recovery for 0.10 ng/mL of pyrethroids in water.
- The GC/MS method was found to be linear over the range of 50 to 2000 ng/mL.
- The HyperSep C18 silica SPE cartridge allowed the extraction and preconcentration of pyrethroids in water for quantification.

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