



Sensitive Detection of Pyrethroids in Surface Water and Sediment

Application Note

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Abstract

A method has been developed on the Agilent 7000 series Triple Quadrupole GC/MS using NCI GC/MS/MS with backflushing. It delivers estimated method detection limits (EMDLs) for pyrethroids as low as 0.05 parts per trillion (ppt) in water samples and 0.02 parts per billion (ppb) for sediment, enabling efficient monitoring of water sources for toxic pyrethroid levels.

Introduction

Pyrethroids are synthetic derivatives of pyrethrins, which are naturally occurring insecticides produced by the flowers of pyrethrum plants (*Chrysanthemum cinerariaefolium* and *C. coccineum*). Pyrethroids were introduced 30 years ago to replace highly toxic organophosphates and DDT. Natural pyrethrins rapidly knock down flying insects, have low mammalian toxicity, and low persistence in the environment. However, they have poor efficacy in the field due to rapid degradation when exposed to light and air.

The synthetic pyrethroids are substantially more resistant to degradation than pyrethrins and are now widely used in commercial pesticides, including more than 3500 EPA-registered products. However, they have significantly higher mammalian toxicities and are highly toxic to fish and other aquatic organisms at extremely low concentrations (parts per billion). Pyrethroids have been found at acutely toxic levels in sediments and waterways in California, with residential water run-off being a major source of contamination due to structural and landscape pest control [1]. Monitoring for pyrethroids in water and sediments in urban and suburban water sources is therefore a high priority, and requires sensitive detection methods.



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Initially, pyrethroid analysis was done using gas chromatography and an electron capture detector (GC/ECD). The use of gas chromatography/mass spectrometry (GC/MS) in electron ionization (EI) mode was later adopted as the method of choice, but it does not have sufficient sensitivity to meet all of the pyrethroids' monitoring requirements. Negative Chemical Ionization (NCI) provides a much more sensitive and highly selective method for detecting pyrethroids, exploiting the electrophilic nature of halogenated compounds in the same way as electron capture detectors.

This application note describes a method for high-sensitivity detection of pyrethroids in water and sediment using an efficient extraction procedure, GC backflushing with Agilent Capillary Flow Technology, and an Agilent 7000 series Triple Quadrupole GC/MS in (NCI) mode [2]. Multiple Reaction Monitoring (MRM) MS/MS analysis delivers detection of pyrethroids at levels as low as 0.01 ppb, with a dynamic range of 0.01 to 10 ppb.

Experimental

Standards and Reagents

All certified pyrethroid standards were obtained from Chem Service, Inc. (West Chester, PA). Second source standards of each analyte were obtained from AccuStandard (New Haven, CT) and Ultra Scientific (North Kingstown, RI) for verification of calibration standards. Individual stock solutions of each pyrethroid analyte were made in acetone from neat and combined, resulting in a stock mixture containing the six compounds: bifenthrin, es/fenvalerate, lambda-cyhalothrin, cyfluthrin, permethrin and permethrin. From this mixture, a matrix spiking solution used to fortify samples was made in acetone. The pyrethroid stock mixture was also used to make an instrument calibration mixture in isoctane, ranging from 0.01–10 ppb. Results are reported as total concentration for each analyte rather than as individual stereoisomer concentrations. External standard calibration was used to quantify the pyrethroids.

The surrogate (used to monitor extraction and sample preparation) for water samples was dibromooctafluorobiphenyl (DBOB) from Restek Corp (Bellefonte, PA). For sediment samples the surrogate was dibutylchlorodate (DBCE), obtained from AccuStandard. GPC Calibration Standard Solution was obtained from Ultra Scientific and Florisil, 60/100 mesh, PR grade was obtained from Floridin Corp (Berkeley Spring, WV). OI Analytical gel permeation chromatography (GPC) columns (J2 Scientific, Columbia, MO) were used to clean up extracts. The PFE cellulose filters were obtained from Dionex (Salt Lake City, UT).

Instruments

The experiments were performed on an Agilent 7890A gas chromatograph equipped with a multimode inlet (MMI) and coupled to an Agilent 7000B Triple Quadrupole GC/MS in negative chemical ionization (NCI) mode. Gas chromatography was performed using backflushing with a Purged Ultimate Union (Figure 1). The instrument conditions are listed in Table 1.

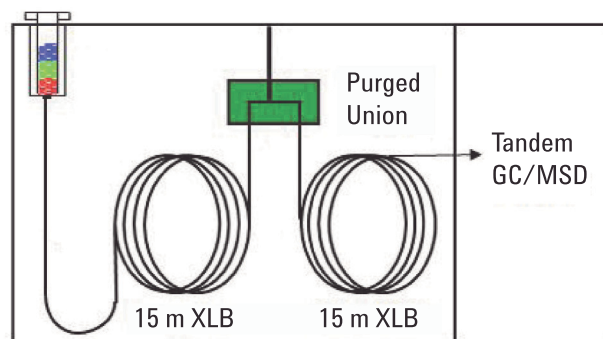


Figure 1. Tandem GC/MS system configuration with mid-column back flushing. Backflushing removes higher boiling substances from the column prior to each subsequent run by flushing late eluting peaks out of the inlet split flow vent instead of driving them through the entire column and into the Agilent Triple Quadrupole GC/MS. Agilent uses a unique approach to backflushing employing Capillary Flow Technology that minimizes flow addition and subsequent decrease in sensitivity due to sub-optimal carrier gas flows into the mass spectrometer. Please refer to technical overview 5990-5484EN which describes mid-column backflushing in detail.

Table 1. Agilent 7890N/7000B Gas Chromatograph and Triple Quadrupole Mass Spectrometer Conditions

GC run conditions

Pre-column	DB-XLB 15 m × 0.25 mm, 0.25 µm J&W 122-1212
Analytical columns	DB-XLB 15 m × 0.25 mm, 0.25 µm J&W 122-1212
Injection volume	2 µL
Inlet temperature	240 °C
Injection mode	Pulsed splitless
Oven program	150 °C; 0 min hold 30 °C/min to 220 °C; 1 minute hold 5 °C/min to 300 °C; 2 minute hold
Post-run oven program	Time: 0.5 minutes Pre-column flow: -19.7 mL/min Analytical column flow: 20 mL/min
Carrier gas	Helium
Transfer line temp	300 °C

MS conditions

Tune	NCI Ammonia Autotune
EMV gain	25
Acquisition parameters	NCI mode with multiple reaction monitoring (MRM)
Reagent gas	Ammonia, 35% flow
Collision gas	Nitrogen, constant flow, 0.75 mL/min
Quench gas	Helium, constant flow, 2.25 mL/min
Solvent delay	10 minutes
MS temperatures	Source 150 °C; Quadrupole 150 °C

Sample Preparation

Water and sediment samples were prepared as previously described [3], with the procedure summarized in Figure 2. One liter water samples were collected in amber glass bottles, refrigerated at 4 °C and extracted as soon as possible. All water samples were fortified with pyrethroid pesticide spiking solution and DBOB surrogate directly into the sample bottles. The fortified water samples were manually agitated before extraction twice with 120 mL of dichloromethane (DCM). The extracts were dried using sodium sulfate and evaporated to approximately 2–5 mL using K-D glassware. The samples were then solvent exchanged by adding petroleum ether (PE) (~50 mL three times). The extracts were evaporated to 5 mL and further concentrated to less than 1 mL and brought to a final volume of 2 mL with isoctane.

Sediment samples were homogenized and then extracted by automated Pressurized Fluid Extraction (PFE) with an ASE 200 Accelerated Solvent Extractor from Dionex (Salt Lake City, UT) equipped with 33 mL extraction cells and cellulose filters. Cleanup of extracts was accomplished using automated gel permeation chromatography. The mobile phase was dichloromethane (DCM) at a flow rate of 5 mL/min. The GPC program was as follows: dump for 19 minutes, collect for 24 minutes, and then rinse for 10 minutes. The cleaned extracts were solvent exchanged from DCM to petroleum ether, then fractionated using 25 g of Florisil (60/100 mesh) in an 11 mm × 300 mm column. The appropriate fractions were then concentrated and reconstituted with isoctane to a final volume of 2 mL.

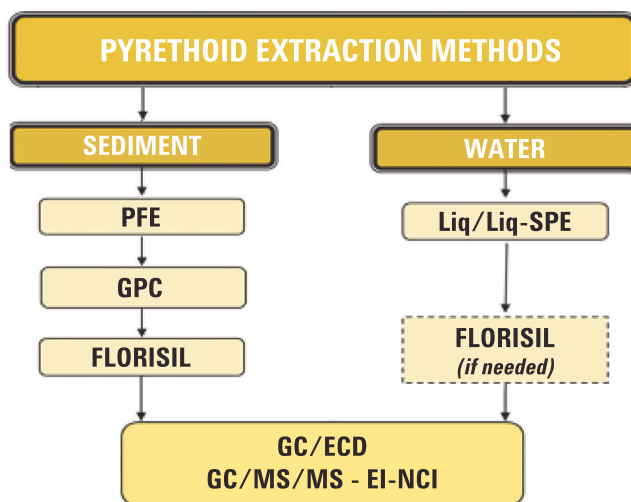


Figure 2. A flow diagram of the sample preparative procedure shows the differences between the water and sediment procedures. PFE is an abbreviation for Pressurized Fluid Extraction and GPC is an abbreviation for Gel Permeation Chromatography.

Analysis Parameters

The Triple Quadrupole GC/MS parameters used are shown in Table 2.

Table 2. Agilent 7000B Triple Quadrupole GC/MS Analysis Parameters

Pyrethroid	RT (min)	NCI Mode			
		MRM	Dwell time (ms)	Collision energy (eV)	Gain
Bifenthrin	11.30	386→205	100	4	25
		386→161	100	10	
Cyhalothrin	13.15	241→205	100	5	25
		241→35	100	5	
Cyfluthrin and Cypermethrin	16.34	209→37	75	5	75
		207→35	75	5	
		171→127	75	5	
Deltamethrin	19.57	297→81	100	4	25
		297→79	100	4	
Fenvalerate and Es-fenvalerate	18.60	213→169	75	4	25
		211→35	75	10	
Permethrin	14.58	209→37	100	5	75
		207→35	100	5	

Results and Discussion

Setting Minimum Detection Limits

Due to the high toxicity of pyrethroids to aquatic life when present in water or sediments (<1 part per billion in some cases), very low detection limits are required of the methods used to test for these compounds. One meaningful way to set detection limits is to use the LC₅₀ as a guide. The LC₅₀ is the median lethal dose, which is defined as the concentration at which a toxic substance will kill half the members of a tested population. Pyrethroids are sequestered in soil sediments and break down in water, but water samples taken from aquatic sources contain dissolved solids which may be contaminated with pyrethroids. Therefore, the minimum detection limits (MDLs) can be set at the LC₅₀ in water, and 10% of the LC₅₀ in sediment. These limits are shown in Table 3.

Table 3. Minimum Detection Limits Required for Pyrethroids in Water and Sediment

Synthetic pyrethroid	Water ng/L (ppt)		Sediment ng/g (ppb)	
	LC ₅₀ Daphnia magna	LC ₅₀ Mysidopsis bahia	LC ₅₀ Hyalella	MDL goal: 10% LC ₅₀
Bifenthrin	1600	4	5	0.5
Cyfluthrin	–	–	11	1.1
Lambda Cyhalothrin	–	–	4.5	0.5
Cypermethrin	1000	5	3.8	0.4
Es-fenvalerate	150	–	15	1.5
Permethrin	320	46	108	11

MDL: Method detection limit

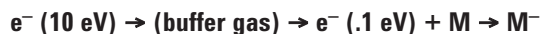
Sensitive Detection Using NCI GC/MS/MS

Initially, pyrethroid analysis was done using gas chromatography and an electron capture detector (GC/ECD). However, the high rate of false positives with GC/ECD led to the adoption of mass spectrometry in electron ionization (EI) mode as the method of choice. While GC/MS/MS-EI is much more selective than GC/ECD, it is not quite sensitive enough to meet all of the MDL requirements.

Negative Chemical Ionization (NCI) provides a less energetic and highly selective method for detecting and quantifying electrophilic compounds in complex matrices than electron ionization mass spectrometry. It exploits the electrophilic nature of halogenated compounds in the same way that electron capture detectors have in the past (Figure 3). The more critical type II pyrethroids such as cyfluthrin and permethrin are electronegative for NCI. However, there are at least two type I pyrethroids (resmethrin and fenprothrin) that are not detected by traditional NCI techniques. Ammonia is preferred as the reagent gas instead of methane, because it has approximately seven times the thermalizing power of methane [4].

The advantage of using a tandem quadrupole mass spectrometer is that a very selective precursor to product ion transition data is generated, minimizing interferences and maximizing signal-to-noise. In addition, using MS/MS in NCI mode can significantly enhance sensitivity over the EI mode, while maintaining selectivity.

Negative Chemical Ionization



NCI Attributes

- Only electrophilic molecules are capable of capturing thermal electrons.
- Electron capture is extremely efficient for halogenated compounds, leading to high sensitivity.
- Matrix interferences typically do not capture electrons, and detection limits are generally very low due to lack of response by contaminants or matrix.

Figure 3. Description of the Negative Chemical Ionization Process

EMDLs, IDLs and Quantification

The GC/MS/MS-NCI method can deliver estimated method detection limits (EMDLs) well below most of the MLDs set for pyrethroids (Table 4), using a 500 fold concentration of water samples. This method provides detection of deltamethrin down to 0.01 ng/L, or 0.01 parts per trillion (ppt) in water samples. The instrument detection limits (IDLs) of standards in isoctane are also listed in Table 4. Detection of deltamethrin on the instrument has a dynamic range up to 10 ppb and an excellent R^2 value (.9974) for the calibration (Figure 4). Cypermethrin is detected down to 0.1 ppt in water samples. Detection on the instrument has a dynamic range up to 10 ppb and an even higher R^2 value (.9996) for the calibration (Figure 5).

Table 4. Ammonia MS/MS-NCI Instrument Detection Limit (IDL) and Estimated Method Detection Limit (EMDL)*

Synthetic pyrethroid	IDL $\mu\text{g/L}$	Estimated Method Detection Limit (EMDL)	
		Water ng/L	Sediment ng/g
Bifenthrin	0.01	0.05	0.01
Cyfluthrin	0.05	0.1	0.2
Lambda Cyhalothrin	0.02	0.05	0.02
Cypermethrin	0.05	0.1	0.18
Deltamethrin	0.005	0.01	0.03
Es-fenvalerate	0.001	0.05	0.05
Permethrin	0.05	0.1	1.5

The estimated EMDL values are based on 500-fold concentration of original water samples and 10g sediment sample to 1mL, with 50% moisture. The EPA defines the method detection limit as, "the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte."

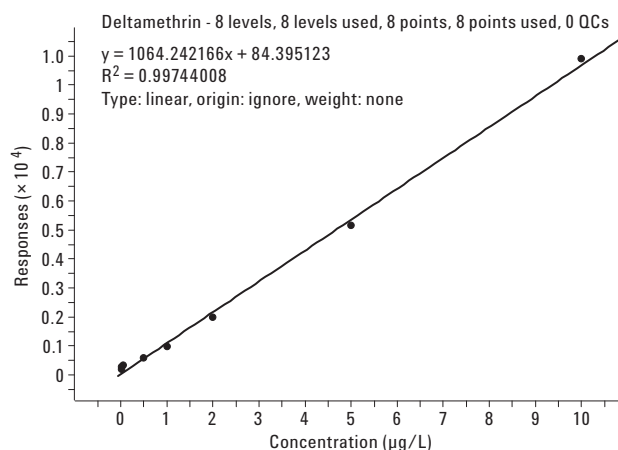


Figure 4. Calibration curve for deltamethrin at concentrations ranging from 0.01 to 10 $\mu\text{g/L}$, using ammonia as reagent gas in NCI GC/MS/MS.

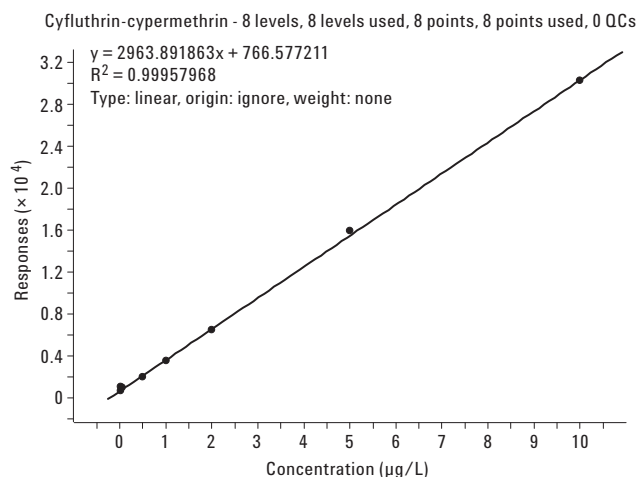


Figure 5. Calibration curve for cypermethrin at concentrations ranging from 0.050 to 10 $\mu\text{g/L}$, using ammonia as collision gas in NCI GC/MS/MS.

Conclusion

Ammonia NCI GC/MS/MS analysis of water and sediment sample extracts for pyrethroids provides increased sensitivity and selectivity over existing GC/ECD and GC/MS/MS-EI methods. EMDLs using NCI GC/MS/MS have been established for water and sediment that are well below the toxicity of type II pyrethroids such as cyfluthrin and permethrin. Analysis of pyrethroids by this technique yields the sub-ppb detection limits that are required to ensure the safety of aquatic organisms.

References

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