Application Note

A Confirmatory Method for the Determination of Synthetic Pyrethroids in Wastewater

Peter Hancock, David Douce, Pat Cummings

Waters Corporation, ALcontrol Laboratories

Abstract

In this application note, a method is described for the determination and confirmation of seven synthetic pyrethroids in wastewater using the Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer.

Benefits

The Quattro micro GC provides high selectivity to reduce any matrix interferences, high sensitivity to reach the reporting levels required by European Union legislation and quantitative and confirmatory data in a single injection

Introduction

Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the resin extract of dried chrysanthemum flowers. They were introduced to provide a less hazardous and less persistent alternative to organochlorine and organophosphate insecticides. They are among the safest insecticides available on the basis of their low acute toxicity to mammals. Pyrethroid formulations are used in agriculture, animal husbandry, horticulture and in the home. They are extensively used for mothproofing woollen materials in the textile industry and as the active ingredient in commercial sheep dips.

In the UK, an estimated 200 million litersof sheep dip are disposed of each year. Dips from some facilities are discharged directly into water courses, while others use the soakaway method of disposal where chemical is allowed to soak into the ground near to the dip facility.[1] Pyrethroids are designed to breakdown more slowly than the naturally occurring pyrethrins; however, they will break down after a few days of application in direct sunlight. Although pyrethroids have a low acute toxicity to mammals, they are extremely toxic to aquatic organisms, including fish such as the bluegill and lake trout, with LC50 values less than 1 ppb.[2] These levels are similar to those for mosquito, black fly and tsetse fly larvae, often the actual target of the pyrethroid application.

Two of the synthetic pyrethroids are listed in the Dangerous Substances Directive (76/464/EEC). Substances are either listed in List 1 or 2. List 1 concerns substances having the potential to cause the most harm to aquatic life due to their persistence, toxicity or bioaccumulation. The Directive requires the elimination of discharges of these substances to the environment in order to reduce pollution.

List 2 concerns substances that are thought to be harmful, but not to the same degree as List 1 substances. The Directive requires the reduction of discharges of these substances to the environment in order to reduce pollution.

Cyfluthrin and Permethrin are the two pyrethroids in List 2. The discharge of these two compounds into the aquatic environment should be reduced; consequently, there is a need for environmental monitoring. Under the directive, the environmental quality standards (EQSs) for Cyfluthrin is 0.001 μg/L and Permethrin is 0.01 μg/L. To put these values into context, without a concentration step, Cyfluthrin would need to be detected with 1 fg on column to reach the EQS. Normally, samples are concentrated by a factor of 100 or 1000 to reach the required reporting levels. Bifenthrin, λ-Cyhalothrin, Cypermethrin, Fenvalerate and Deltamethrin are all examples of othersynthetic pyrethroids that are not regulated by an EQS, but these compounds are extremely toxic to aquatic organisms, and need to be monitored.

In this application note, a method is described for the determination and confirmation of seven synthetic pyrethroids in wastewater using the Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer, Figure 1.

Figure 1. Quattro micro GC tandem quadrupole mass spectrometer.

Experimental

Extraction Method

Sample extraction was based around liquid-liquid extraction with an solid-phase extraction (SPE) cleanup procedure. 10 mL hexane was added to 1 liter of wastewater and shaken for 20 minutes. In this example, 1 liter of sample was extracted but sample volumes as low as 50 mL can be extracted from heavily polluted effluents, including textile dye baths. The hexane fraction was then passed through an amino (NH_2) SPE cartridge. The cartridge was washed with pentane to remove non-polar matrix interferences. The pyrethroids were eluted with 10 mL hexane/isopropyl alcohol (50:50). The concentration factor of this extraction method was 100x.

GC Method

To achieve the EQS for Cyfluthrin, 10 µL was injected with a CTC CombiPal autosampler. The injection system was cyro-cooled programmable temperature vaporization (PTV) in solvent vent mode at a vent pressure of 1.3 psi for 1.5 min. The carrier gas was helium at a constant flow rate of 1.0 mL/min delivered from an Agilent 6890 GC. The capillary column was a J & W Scientific DB-5MS 30 m x 0.25 mm i.d., 0.25 µm. The following oven temperature ramp rate was used: 100 °C (2 min.) to 200 °C at 25 °C/min., to 300 °C at 10 °C/min. The total run time was 20 min. The temperature of the interface was held at 260ºC during the chromatographic run.

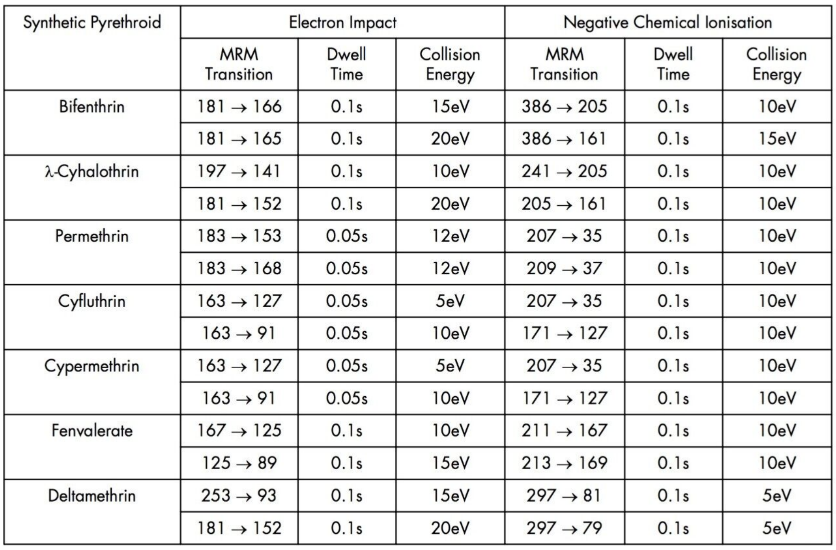
MS Method

The Waters Micromass Quattro micro GC tandem mass spectrometer was used.

In electron impact (EI) mode, the ion source was operated at 180 °C with an electron energy of 70 eV and a trap current of 200 µA. The mode of acquisition was Multiple Reaction Monitoring (MRM) at an argon collision gas pressure of 3.0×10^{-3} mBar.

In negative chemical ionisation (NCI) mode, the ion source was operated at 100 ºC with an electron energy of 70 eV and an emission current of 200 µA. Methane was employed as the reagent gas at a source pressure of approx. 1.8×10^{-4} mBar. The mode of acquisition was MRM at an argon collision gas pressure of 3.0×10^{-3} mBar.

The Quattro micro GC was tuned so that the precursor and product ions were resolved with a peak width at half height of less than 0.7 Da. The MRM transitions, along with the collision energies and dwell times for the method in both EI and NCI ionisation modes are listed in Table 1. Twelve MRM transitions were monitored with a quantification and confirmation transition for each of the seven pyrethroids (Cyfluthrin and Cypermethrin have the same MRM transitions). The dwell times were optimized so that approximately 15 data points described each chromatographic peak.



Synthetic Pyrethroid	Electron Impact			Negative Chemical Ionisation		
	MRM Transition	Dwell Time	Collision Energy	MRM Transition	Dwell Time	Collision Energy
Bifenthrin	181 → 166	0.1s	15eV	386 → 205	0.1s	10eV
	181 → 165	0.1s	20eV	386 → 161	0.1s	15eV
λ-Cyhalothrin	197 → 141	0.1s	10eV	241 → 205	0.1s	10eV
	181 → 152	0.1s	20eV	205 → 161	0.1s	10eV
Permethrin	183 → 153	0.05s	12eV	207 → 35	0.1s	10eV
	183 → 168	0.05s	12eV	209 → 37	0.1s	10eV
Cyfluthrin	163 → 127	0.05s	5eV	207 → 35	0.1s	10eV
	163 → 91	0.05s	10eV	171 → 127	0.1s	10eV
Cypermethrin	163 → 127	0.05s	5eV	207 → 35	0.1s	10eV
	163 → 91	0.05s	10eV	171 → 127	0.1s	10eV
Fenvalerate	167 → 125	0.1s	10eV	211 → 167	0.1s	10eV
	125 → 89	0.1s	15eV	213 → 169	0.1s	10eV
Deltamethrin	253 → 93	0.1s	15eV	297 → 81	0.1s	5eV
	181 → 152	0.1s	20eV	297 → 79	0.1s	5eV

Table 1. MRM method parameters for EI and NCI modes.

Results and Discussion

A typical total ion chromatogram (TIC) for the seven synthetic pyrethroids in EI mode is illustrated in Figure 2. Multiple peaks can be observed for some of the pyrethroids; Permethrin is split into cis-and trans-isomers; Cyfluthrin and Cypermethrin have four isomers each; and Fenvalerate has two isomers. For quantification purposes, it was chosen to report the isomers of Cyfluthrin and Cypermethrin as a combined group, while Permethrin and Fenvalerate were reported as single isomers.

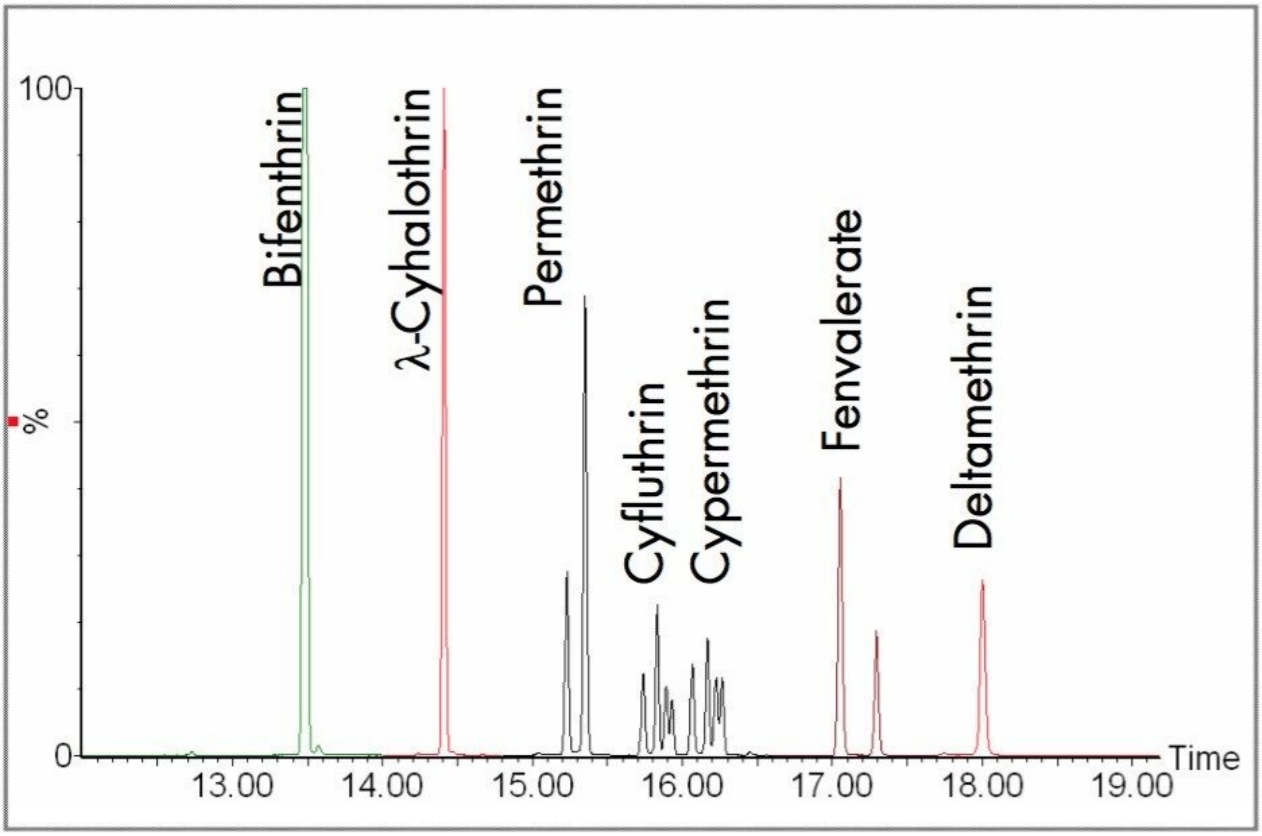


Figure 2. TIC of the seven pyrethroids in EI mode.

The sensitivity of MRM mode for the two regulated compounds as solvent standards is illustrated in Figure 3. The amount injected was equivalent to the EQS after extraction using the described method. Good signal to noise (S/N) ratios were obtained for the isomers of Permethrin and Cyfluthrin; therefore, the EQS can be met for these two compounds using EI. The extra peak in the Cyfluthrin chromatogram is actually the first isomer of Cypermethrin as they have the same MRM transition.

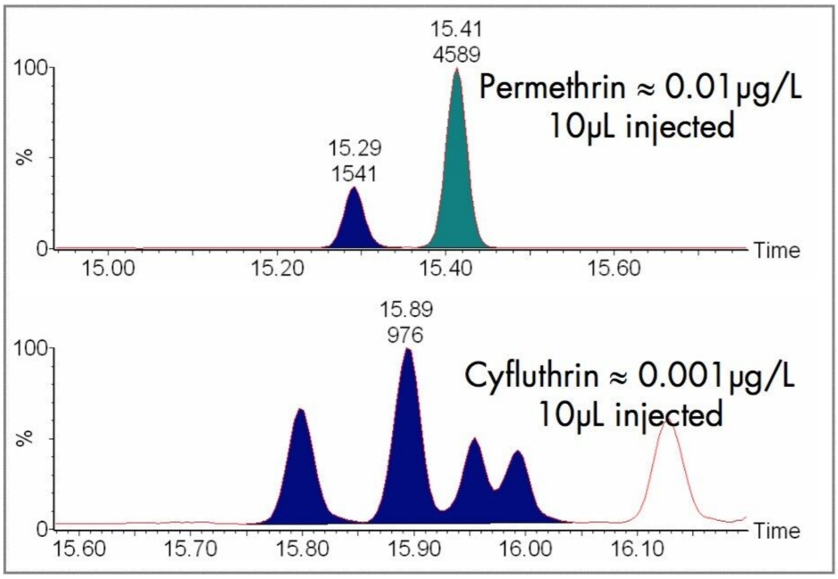
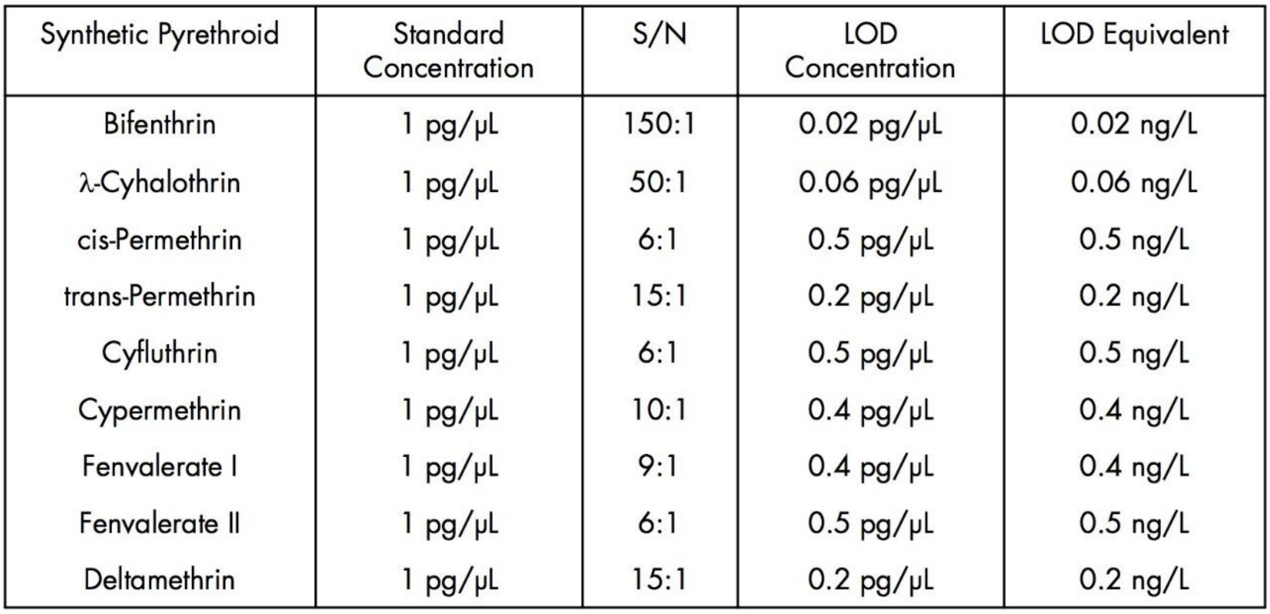


Figure 3. Sensitivity of MRM in EI mode for Permethrin and Cyfluthrin at a concentration equivalent to the EQS.

The S/N ratio for the lowest calibration standard in EI mode was calculated for each pyrethroid using the confirmation transition and these are listed in Table 2. The LOD concentration was then estimated on the basis that the minimum S/N ratio was ≥3:1.

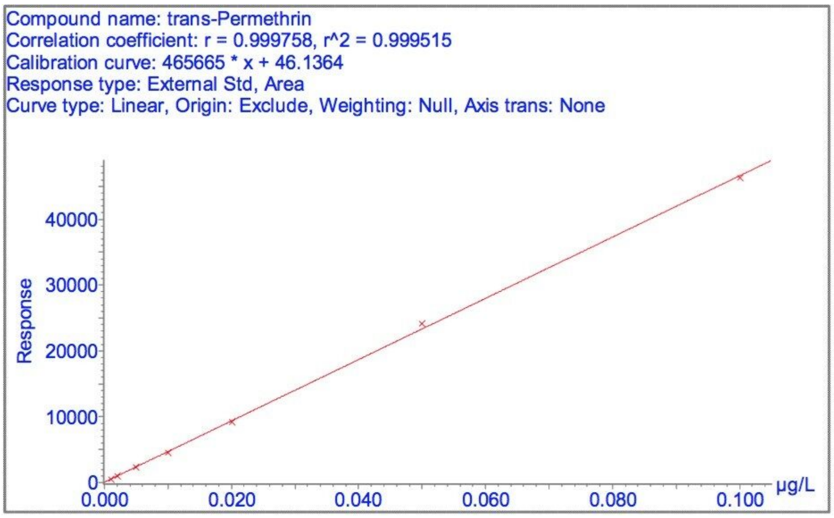


Synthetic Pyrethroid	Standard Concentration	S/N	LOD Concentration	LOD Equivalent
Bifenthrin	1 pg/µL	150:1	0.02 pg/µL	0.02 ng/L
λ-Cyhalothrin	1 pg/µL	50:1	0.06 pg/µL	0.06 ng/L
cis-Permethrin	1 pg/µL	6:1	0.5 pg/µL	0.5 ng/L
trans-Permethrin	1 pg/µL	15:1	0.2 pg/µL	0.2 ng/L
Cyfluthrin	1 pg/µL	6:1	0.5 pg/µL	0.5 ng/L
Cypermethrin	1 pg/µL	10:1	0.4 pg/µL	0.4 ng/L
Fenvalerate I	1 pg/µL	9:1	0.4 pg/µL	0.4 ng/L
Fenvalerate II	1 pg/µL	6:1	0.5 pg/µL	0.5 ng/L
Deltamethrin	1 pg/µL	15:1	0.2 pg/µL	0.2 ng/L

Table 2. S/N ratios, LOD concentrations and estimated LOD equivalent concentrations in EI mode.

The LOD equivalent was then calculated, accounting for the concentration factor in the extraction method and the injection volume. The LOD equivalents listed are all less than the EQS, indicating the EU regulations can be met with this ionization technique.

Solvent standards were generated at the 1, 2, 5, 10, 20, 50, and 100 pg/μL concentrations levels. The standards were injected in a typical batch analysis. The data was then processed using Waters TargetLynx Application Manager for MassLynx Software. A representative curve for trans-Permethrin with a correlation coefficient of r^2= 0.9995 is illustrated in Figure 4.



Compound name: trans-Permethrin
Correlation coefficient: r = 0.999758, r^2 = 0.999515
Calibration curve: 465665 * x + 46.1364
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

Figure 4. Typical calibration curve for trans-Permethrin in EI mode, 1 –100 pg/μL.

The sensitivity and selectivity of the technique from a real wastewater extract is illustrated in Figure 5. Permethrin is at a concentration of approximately 0.03 μg/L, only 3 times greater than the EQS. The cis-and trans-isomers can be easily observed. There is little or no matrix interference making peak detection and automatic integration routine.

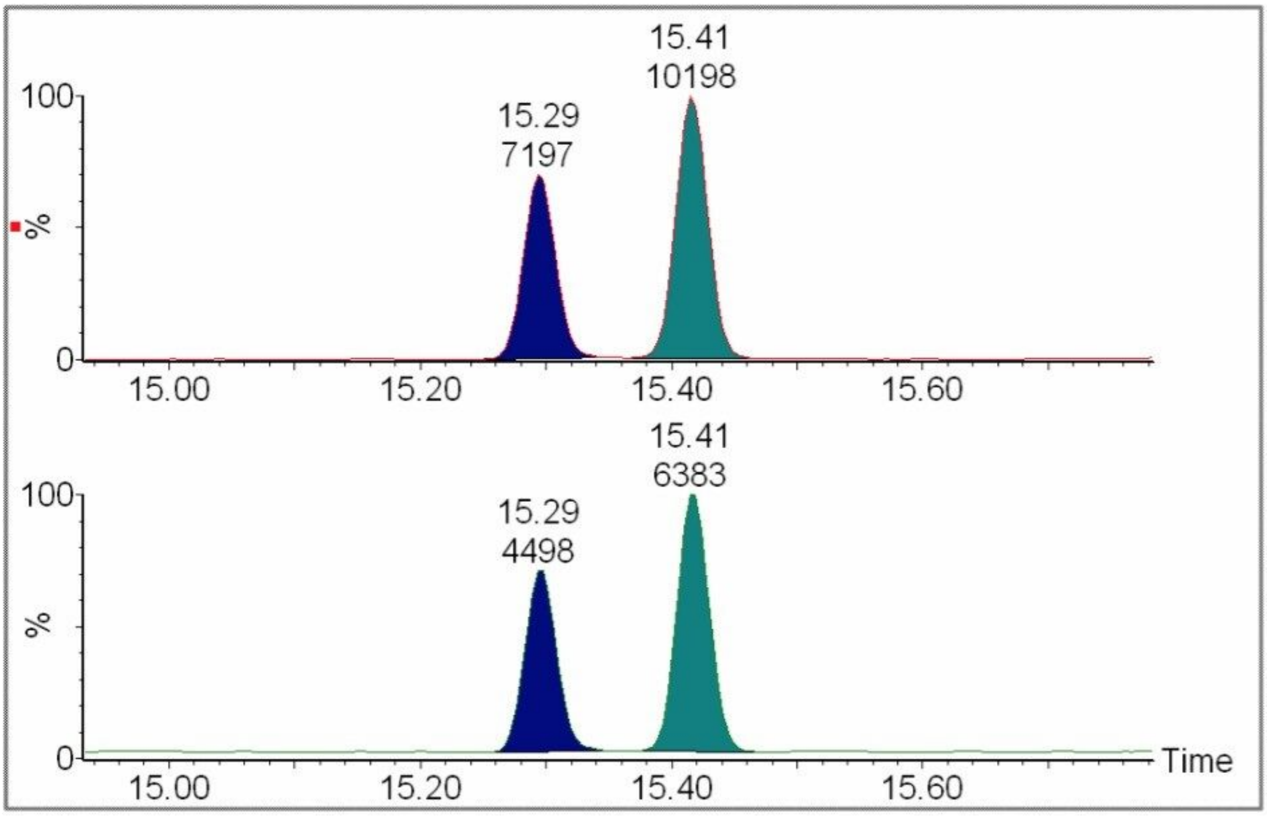


Figure 5. Sensitivity and selectivity in EI mode of Permethrin in a wastewater extract at ~0.03 µg/L.

Two MRM transitions were chosen so that quantification and confirmation could be performed in a single run, assuming that the ion ratio between the transitions was consistent for standards and samples. The confirmation criteria chosen are listed in Table 3. They were dependent on the relative abundance of the confirmation transition to the quantification transition. This ratio was used to confirm or reject pyrethroids in the extracts.

Relative Abundance Ratio	Confirmation Criteria
> 0.5	± 10%
> 0.2 but < 0.5	± 15%
> 0.1 but < 0.2	± 20%

Table 3. Confirmation criteria used for the basis of confirmation and rejection of pyrethroids in extracts.

The expected ion ratio of each pyrethroid was calculated from the mean of the seven calibration standards. For Permethrin, the expected ion ratio was 0.613. The presence of Permethrin was considered confirmed if the observed ion ratio from any extract does not deviate by more than 10% from this expected value. The ion ratio between the quantification transition (7197) and the confirmation transition (4498) for cis-Permethrin in Figure 5 is 0.625. The confirmation criteria has been passed with a percentage difference of 2.0% between the expected and measured ratios. This is significantly less than the acceptable limits of ±10%.

In general, synthetic pyrethroids fragment around the ester oxygen single bond. The phenoxybenzyl portion is normally detected in positive ion mode while the cyclopropane portion is normally detected in negative ion mode (Figure 6). This specific fragmentation allows the possibility for negative chemical ionization (NCI) mode to be used.

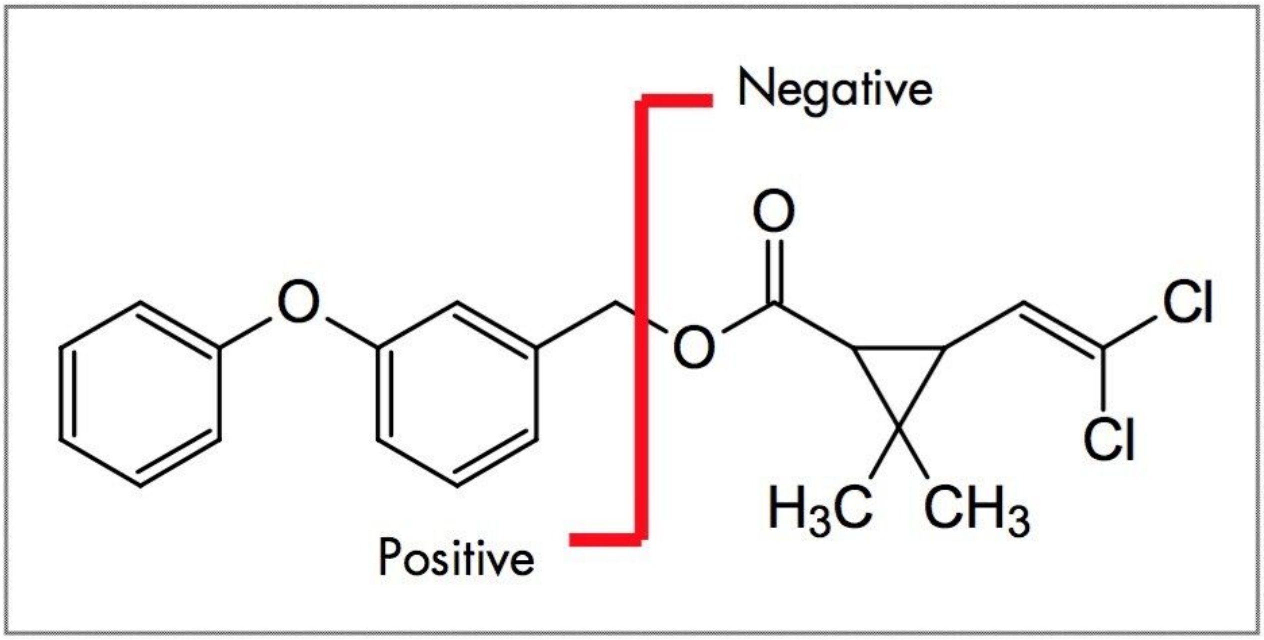


Figure 6. Normal fragmentation of synthetic pyrethroids, Permethrin is illustrated.

As the pyrethroids have a positive electron affinity they ionize well in NCI mode. The selectivity of NCI is greater than EI for the pyrethroids as less compounds are ionized in this manner. Therefore, the matrix interference/background noise is greatly reduced. This gain in selectivity should lead to lower LODs being achieved.

The difference in sensitivity between EI and NCI modes is illustrated in Figure 7. Fenvalerate has been injected (10 pg on column) in both modes and the S/N ratio calculated. Fenvalerate has an increase in sensitivity of 30 times by changing ionization modes from EI to NCI. The mean sensitivity difference across all seven synthetic pyrethroids is approximately 20 times. This difference in sensitivity will undoubtedly allow lower LODs to be reached in the wastewater extracts.

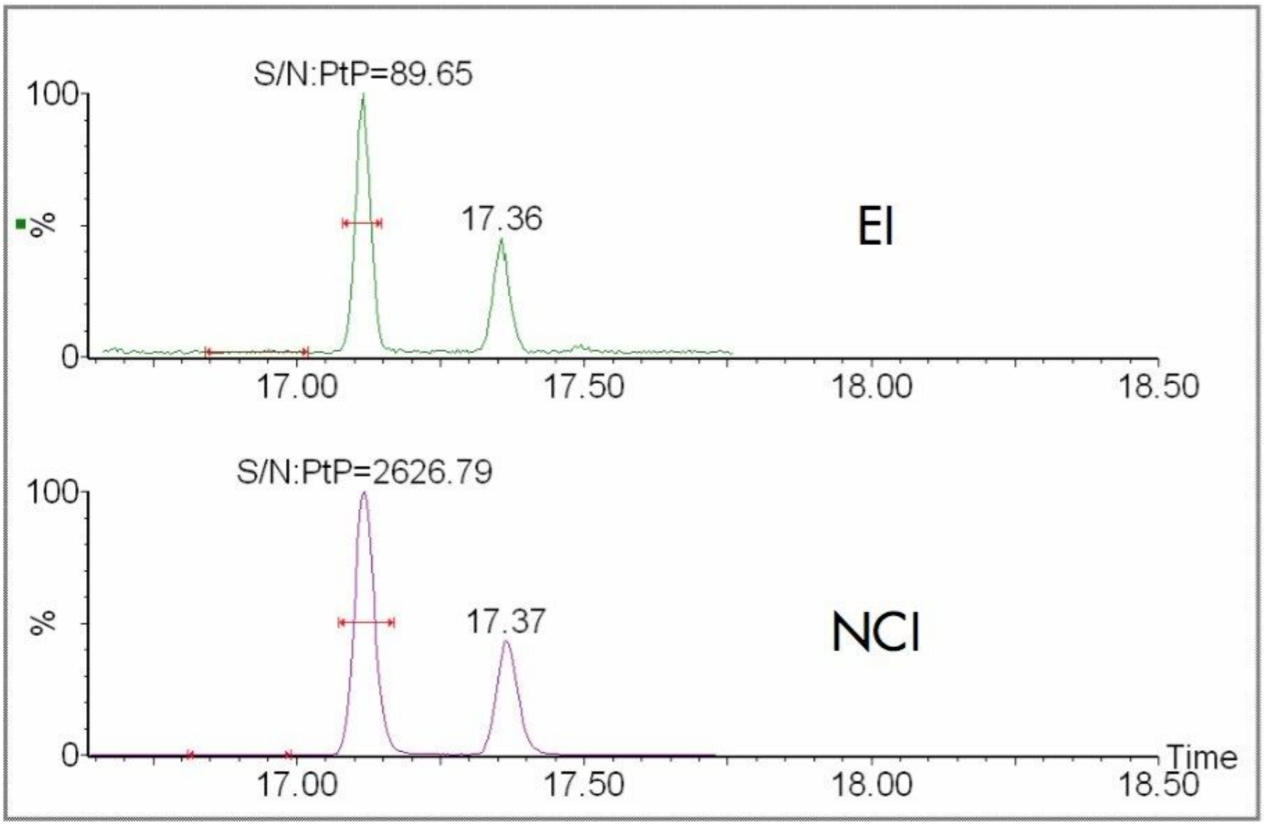


Figure 7. Sensitivity difference between EI and NCI modes for Fenvalerate.

The sensitivity of MRM mode for Permethrin and Cyfluthrin as solvent standards is illustrated in Figure 8. The amount injected was equivalent to the EQS after extraction using the described method. Excellent S/N ratios were obtained for the isomers of Permethrin and Cyfluthrin; therefore, the EQS can easily be met for these two compounds using NCI.

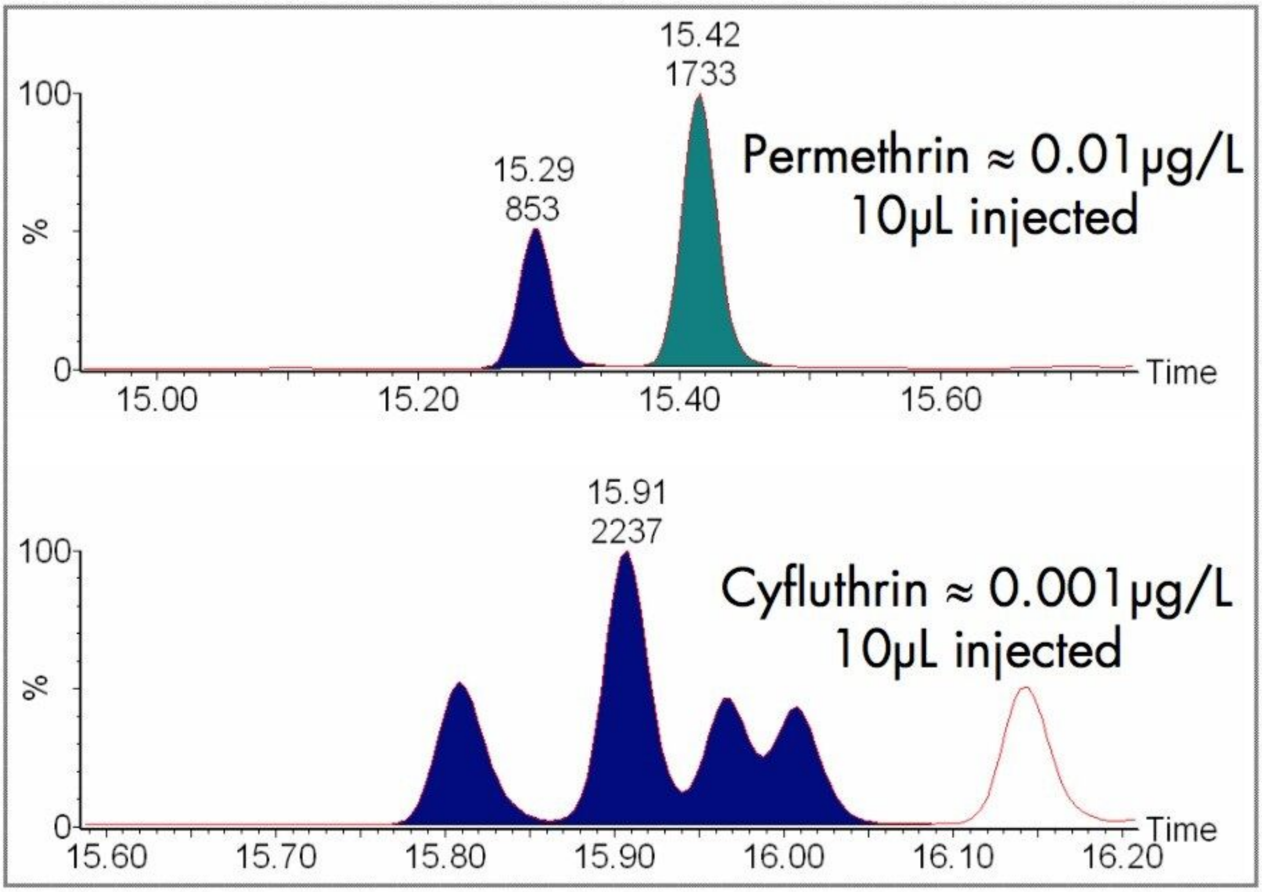
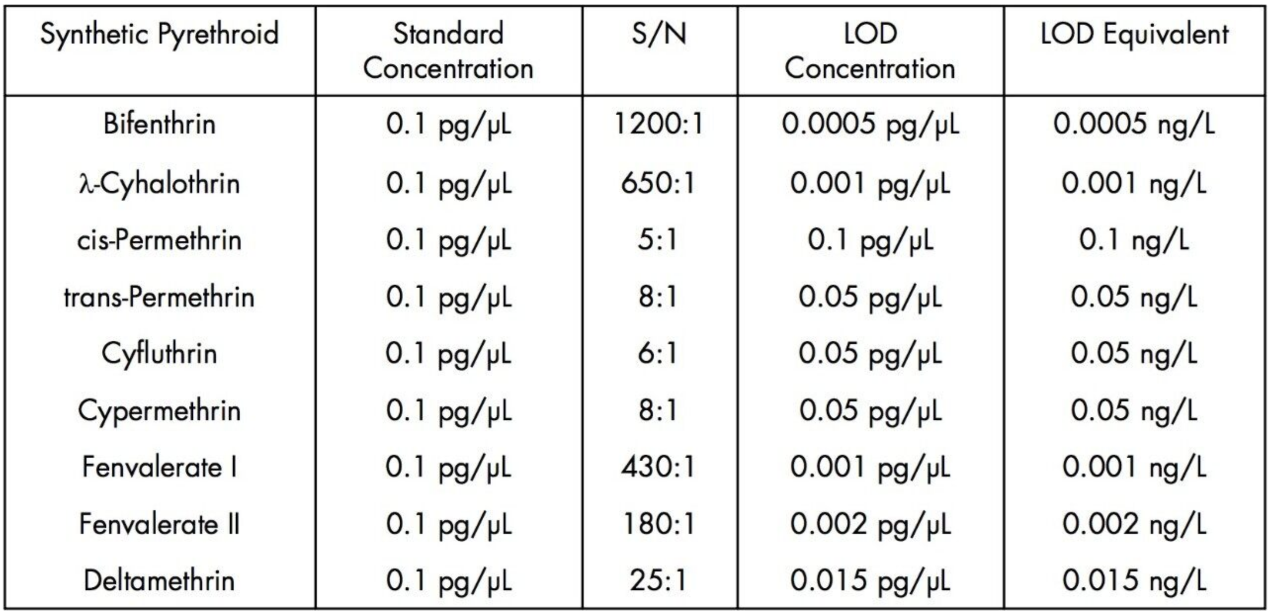


Figure 8. Sensitivity of MRM in NCI mode for Permethrin and Cyfluthrin at a concentration equivalent to the EQS.

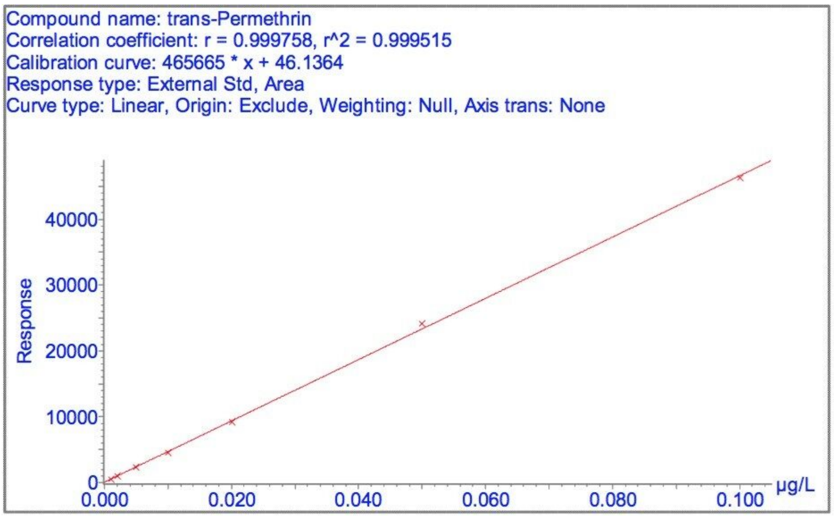
The S/N ratio for the lowest calibration standard in NCI mode was calculated for each pyrethroid using the confirmation transition and these are listed in Table 4. The LOD concentration was then estimated on the basis that the minimum S/N ratio was ≥3:1. The LOD equivalent was then calculated, accounting for the concentration factor in the extraction method and the injection volume. The LOD equivalents listed are all less than the EQS, indicating the EU regulations can easily be met with this ionization technique.



Synthetic Pyrethroid	Standard Concentration	S/N	LOD Concentration	LOD Equivalent
Bifenthrin	0.1 pg/μL	1200:1	0.0005 pg/μL	0.0005 ng/L
λ-Cyhalothrin	0.1 pg/μL	650:1	0.001 pg/μL	0.001 ng/L
cis-Permethrin	0.1 pg/μL	5:1	0.1 pg/μL	0.1 ng/L
trans-Permethrin	0.1 pg/μL	8:1	0.05 pg/μL	0.05 ng/L
Cyfluthrin	0.1 pg/μL	6:1	0.05 pg/μL	0.05 ng/L
Cypermethrin	0.1 pg/μL	8:1	0.05 pg/μL	0.05 ng/L
Fenvalerate I	0.1 pg/μL	430:1	0.001 pg/μL	0.001 ng/L
Fenvalerate II	0.1 pg/μL	180:1	0.002 pg/μL	0.002 ng/L
Deltamethrin	0.1 pg/μL	25:1	0.015 pg/μL	0.015 ng/L

Table 4. S/N ratios, LOD concentrations and estimated LOD equivalent concentrations in NCI mode.

Solvent standards were generated at the 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 pg/μL concentrations levels. The standards were injected in a typical batch analysis. The data was then processed using TargetLynx. A representative curve for Fenvalerate I with a correlation coefficient of r^2= 0.9997 is illustrated in Figure 9.



Compound name: trans-Permethrin
Correlation coefficient: r = 0.999758, r^2 = 0.999515
Calibration curve: 465665 * x + 46.1364
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

Figure 9. Typical calibration curve for Fenvalerate I in NCI mode, 0.1 –100 pg/μL.

The sensitivity and selectivity of the technique from a real wastewater extract is illustrated in Figure 10.

Deltamethrin is at a concentration of approximately 0.007 µg/L. The peak immediately before Deltamethrin is an isomer that was not present in the standard solution used. There is little or no matrix interference making peak detection and automatic integration routine.

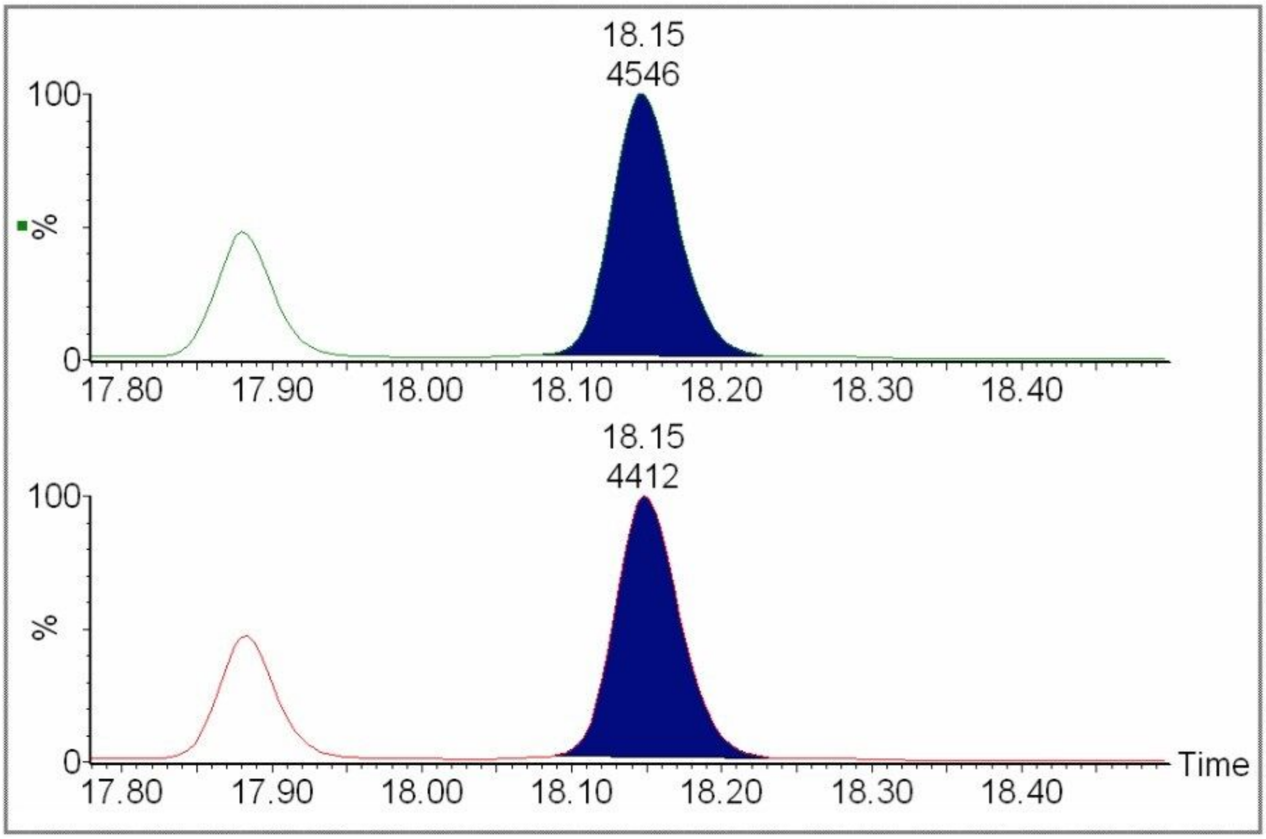


Figure 10. Sensitivity and selectivity in NCI mode of Deltamethrin in a wastewater extract at ~0.007 µg/L.

Once again, two MRM transitions were chosen so that quantification and confirmation could be performed in a single run. The confirmation criteria chosen were the same as for EI. This ratio was used to confirm or reject pyrethroids in the extracts.

The expected ion ratio of each pyrethroid was calculated from the mean of the ten calibration standards. For Deltamethrin, the expected ion ratio was 0.987. The presence of Deltamethrin was considered confirmed if the observed ion ratio from any extract does not deviate by more than 10% from this expected value. The ion ratio between the quantification transition (4546) and the confirmation transition (4412) for Deltamethrin in Figure 10 is 0.971. The confirmation criteria has been passed with a percentage difference of 1.6% between the expected and measured ratios. This is significantly less than the acceptable limits of ±10%.

Conclusion

A method has been described for the determination and confirmation of seven synthetic pyrethroids in wastewater using the Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer.

The Quattro micro GC provides high selectivity to reduce any matrix interferences, high sensitivity to reach the reporting levels required by European Union legislation and quantitative and confirmatory data in a single injection.

The programmable temperature vaporization (PTV) injector in solvent vent mode provides the ability to inject larger volumes on the GC column.

The TargetLynx Application Manager for MassLynx Software provides advanced quantification with a range of automatic quality control checks.

For synthetic pyrethroids, negative chemical ionization (NCI) provides even greater selectivity and sensitivity than electron impact (EI) mode, allowing even lower limits of detection (LODs) to be reached.

References

1. http://www.pan-uk.org/pestnews/Pn36/ pn36p11.htm.
2. http://www.beyondpesticides.org/pesticides/ factsheets/Synthetic%20Pyrethroids.pdf.

Featured Products

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

720001093, February 2005