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Offline Automated Extraction of Non-Polar Pesticides in Water with Analysis by GC/MS

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First Published: 2015

Introduction

Non polar pesticides are a group of chemicals which are present in the environment at low concentrations. Pesticides are used on farmland and through leaching, can enter the river water and therefore the water treatment processes.

Due to their non-polar nature they are insoluble in water but may be found at low concentrations in environmental waters. These pesticides have relatively low predicted no effect concentration (PNEC, the highest predicted concentration in solution whereby the chemical has no toxic effect to the environment) as to the high K_{ow} (the octanol – water partitioning coefficient) values which means the chemicals will favour partitioning from water to the lipids of plants and animals through natural processes. This causes bio-concentration which can cause levels with animals to be at a level which maybe toxic. As well as the environment, it is important to have a low concentration of pesticides in drinking water. The level of these pesticides must be controlled and strict measurement and control of concentrations in our water systems is required.

This application note is designed to show the results of three days of work in collaboration with ALS (Wakefield) to determine an offline proof of principle method for an automated method for the extraction of these compounds from aqueous environmental samples. A suite of 45 pesticides were extracted using the method shown which was based on liquid-liquid extraction automated using the GERSTEL MPS2. Analysis of the prepared extracts was performed using an Agilent 7890 gas chromatograph with triple quadrupole mass spectrometer as a detector (GC/QQQ) with a large volume injection.

The use of small scale automated liquid-liquid extraction saves solvent, time and man hours. It also improves the repeatability of the experiments. This application note shows how a set of spiked water samples were extracted, analysed and quantified against a set of matrix matched standards.

Instrumentation

GERSTEL MPS 2 XL-*xt*
GERSTEL Agitator
Maestro software (version 1.4.18.25/3.5)
Agilent 7890 GC with a 7000B Triple quadrupole mass spectrometer

Method

Two sets of aqueous spiked standards were prepared by spiking volumes of 200 mL of water with an appropriate level of a spiking solution containing the suite of pesticides to be analyzed. Set A were determined as the standards and Set B were used as quality control (QC) samples. The concentration levels were 20, 50, 80, 120 and 150 ng/L in solution for each set.

Triplicate aliquots of 15 mL of the aqueous solutions were placed into separate 20 mL headspace vials and placed on the MPS2. The MPS2 added ethyl acetate to each sample as a co-solvent. The extraction solvent was hexane and 1 mL was added to the same vial and the samples were agitated at 500 rpm for 10 minutes and an aliquot of the upper layer was transferred to a GC vial for analysis by the GC/QQQ. The samples were transferred to the GC/QQQ for analysis and a 25 µL injection was made. Each non-polar pesticide was monitored using individual MS/MS MultiReaction Monitoring (MRM) transitions to give specificity and increase signal to noise. A suite of internal standards were used to give better reproducibility, linear response and reduce the matrix effects.

Results

An extracted MRM chromatogram is shown in Figure 1 of the top standard which contained 150 ng/L of each pesticide.

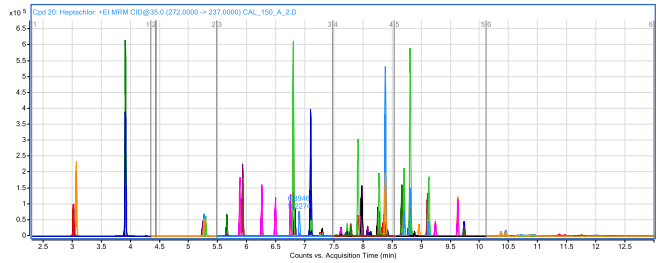

Figure 1: Extracted MRM transitions of all pesticides at 150 ng/L

Figure 2 shows a typical overlay of a single pesticide at the 5 calibration levels. This shows clearly that the lowest level tested in this work 20 ng/L shows enough sensitivity that, when the signal to noise is used to calculate an approximate detection level, the method could be used at levels below 1 ng/L however this was not tested in this work.

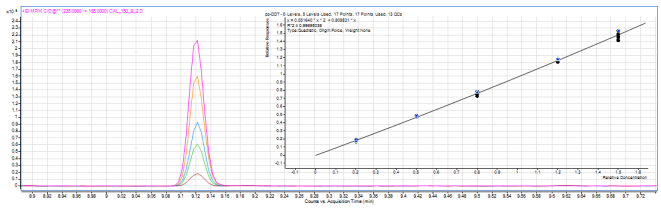

Figure 2: Overlaid chromatogram of pp-DDT at 20, 50, 80, 120 and 150 ng/L in solution

The results indicated that a linear or quadratic fit gave a r^2 values of 0.99 or greater for each of the 45 compounds analysed (Table 1). The accuracy of measurement at 20 ng/L was within the range of 100 ± 20 % for 35 and all were within 30 % of nominal.

Aldrin	Endrin	(E)-Permethrin
Carbophenothion	Fenitrothion	(Z)-Permethrin
α-Chlordane	Fenvalerate	Phorate
Chlorothalonil	Fluazifop-Butyl	PCB 28
Cyfluthrin	Heptachlor	PCB 52
Cypermethrin	(Z)-Heptachlor Epoxide	PCB 101
Deltamethrin	(E)-Heptachlor Epoxide	PCB 118
Dichlobenil	Hexachlorobenzene	PCB 138
Dieldrin	Hexachlorobutadiene	PCB 153
op-DDE	α-HCH	PCB 180
pp-DDE	β-HCH	Tecnazene
op-DDT	ɣ-HCH	op-TDE
pp-DDT	δ-HCH	pp-TDE
α-Endosulphan	Isodrin	124-Trichlorobenzene
β-Endosulphan	Methoxychlor	Trifluralin

Table 1: Compounds included in the analyses

Discussion

The method gave satisfactory results for 35 of 45 non-polar pesticides over the calibration range in terms of response and accuracy but further development would be required to give a complete method.

By automating the extraction process, using large volume injection and the specificity of the QQQ mass spectrometer, this method has been shown to have good reproducibility, accuracy and linearity between 20 and 150 ng/L.. This method could be improved further by increasing the sample to solvent ratio and by injecting a larger amount of sample for example 100 µL utilizing the GERSTEL CIS injector but this was not investigated in this application note. By using the larger injection size it was possible to cut out a concentration step between extraction and analysis.

The advantage of short preparation steps is that utilizing the Maestro software 'prepahead' function is that the extracts were made just prior to the analysis. This means that the preparation time is minimized. Although in this experimental work the preparation was done offline it would be possible to fully integrate the preparation and GC analysis to give great time savings overall.
Figure 3 (below) shows how the different stages of the extraction overlap to ensure quick results. Full automation of this method and further optimization will be investigated in further application notes.

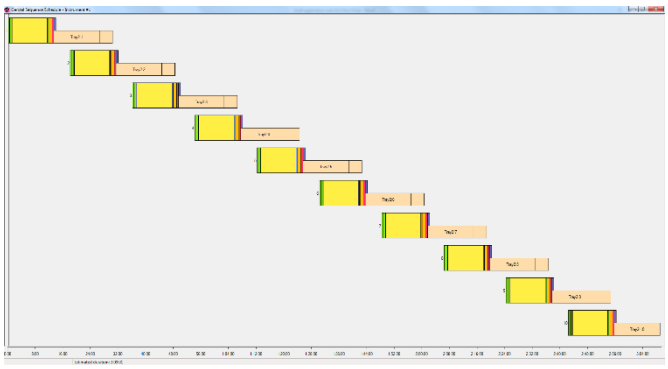

Figure 3: By preparing the samples whilst the previous sample is being analysed

Another advantage of the smaller scale automation is that the volume of solvent is reduced significantly whereas with most liquid-liquid extractions the volumes of solvent can be 10's if not 100's of millilitres, this method used 1 mL of the extraction solvent which means that solvent costs and exposures are reduced in orders of magnitude.

Further Work

The work covered in this application note was a preliminary study into the feasibility of simple automated extraction of nonpolar pesticides. This study should be repeated using the suggested modifications below;

1. Full automation of the method.
2. Use of an Agilent 7010 QQQ triple quadrupole mass spectrometer to improve sensitivity.
3. Larger volume injection would further increase the detection limits of the methodology.
4. Investigation of sample to extraction solvent ratio and the time for equilibration would lead to optimized extraction conditions.

By using the above method improvements a fully automated method which can analyse the non-polar pesticides to the required level of quantitation (0.1 ng/L)

Once the parameters have been optimized a full laboratory testing program to assess the limits of detection, quantification, linearity of response and recovery in more complex aqueous samples would be needed to assess the robustness of the method.

Conclusion

The work in this application note shows that it is possible to automate an extraction method for the analysis of non-polar pesticides. Following the further work suggested, this method would save analysts time, solvent and money compared to the current manual method. This would enable the analysts to be free to process the larger amount of data produced from the higher throughput which this automated process would provide. The reduction of solvent has two benefits which are reduction in costs of solvent overall and a reduction of exposure to the analyst, hence making the technique safer. Generaly, in all analytical work and especially the highly competitive contract laboratory sector, increased sensitivity would either

allow for smaller sample / injection volumes or lower detection limits for all analytes which would give a competitive edge. Further development and testing is required for this method to be used in a regulatory or analytical testing laboratory currently but this work indicates this would be possible.

Acknowledgements

Pat Cummings, Katy Buck and Dave Evans at ALS (Wakefield) for providing their knowledge, laboratory facilities and additional assistance in the laboratory.