

# On-line SPE-LC/MS/MS to Detect Organonitrogen and Triazine Pesticides at 10 ng/L in Drinking Water

**Monitoring water for pesticides requires sensitive and robust methods suitable for a broad range of compounds, and current UK legislation limits permissible levels of pesticides in drinking water to 100 ng/L. This study investigated the feasibility of on-line solid phase extraction (SPE) coupled to liquid chromatography with tandem mass spectrometry (LC/MS/MS) to detect organonitrogen and triazine pesticides at concentrations of 10 ng/L in drinking water. Sensitivity, reproducibility, linearity and recovery in tap water samples and water samples spiked with humic acid were investigated to evaluate sample preparation efficiency and possible matrix effects.**

## Introduction

Pesticides in water can be derived from agricultural use as well as non-agricultural use, such as weed control on paved areas. Current legislation for drinking waters in the UK requires a lower limit of quantitation (LOQ) for pesticides of 100 ng/L (ppt) with a coefficient of variation (CV) below 12.5 per cent and a limit of detection (LOD) of 20 ng/L [1]. In order to achieve these criteria, traditional methods usually use different sample pre-treatments for each pesticide class, with

different procedures, eg. SPE, to concentrate the analytes and remove matrix components. After extraction and specific chemical derivatisation processes, several different analytical techniques are often needed to perform the actual detection and determination of pesticides at these low levels. Gas chromatographic (GC) techniques are usually used to analyse thermally stable pesticides, and thermally labile compounds are usually determined by liquid chromatography (LC).

This article describes an automated SPE sample clean-up and pre-concentration step using large volume injection (5 mL of untreated water samples) prior to LC/MS/MS analysis. With this approach, all investigated 26 pesticides are first trapped on an SPE cartridge and then back-flushed onto a reverse phase LC column. The pesticides are separated on the analytical column using a binary LC gradient and are determined with a LC/MS/MS system (API 3200™ System, Applied Biosystems) in multiple reaction monitoring (MRM) mode.

Trapping the analytes on an on-line SPE cartridge has three major advantages over methods that employ direct column injection:

1. analytes can be rapidly enriched on the SPE column, allowing detection at lower LOD;
2. matrix components, such as humic acid or inorganic salts, are washed away prior to the analytical column separation, extending the longevity of the column life while maximising assay robustness;
3. the reproducibility of the analysis is improved by automation, which minimises human errors during sample preparation.

## Experimental procedures

### HPLC

Tap water (5 mL) was loaded onto a Phenomenex Strata C18 on-line SPE cartridge using a CTC HTC PAL autosampler (fitted with a 5 mL syringe, a 5 mL loop and a 32 x 10 mL sample rack). The trap cartridge was fitted into load position A of a high pressure, Valco 10 port switching valve. An Agilent 1200 isocratic pump was used to load the sample at 3 mL/min onto the trapping cartridge. After 2.5 minutes the valve was switched to position B where an Agilent 1200 Binary pump was used to provide the gradient elution of pesticides. For pesticide separation, an Agilent Zorbax 1.8 µm 4.6 x 50 mm column with a flow rate of 600 µL/min at ambient temperature was used, where mobile phase A was 2 mM ammonium formate with 0.1 per cent formic acid in water, and mobile phase B was 2 mM ammonium formate with 0.1 per cent formic acid in methanol. After 14.5 minutes the switching valve was switched back to the loading position to analyse the next sample.

Local tap water without any pre-treatment was spiked with pesticides and injected directly onto the SPE-LC/MS/MS system. To study matrix effects,

commercially available humic acid (Sigma-Aldrich) was dissolved in 0.2 per cent formic acid at a level of 10 ppm and pesticides were spiked into this matrix.

Compound	Retention Time	S/N of 10ng/L In Tap Water	Standard Deviation of 10ng/L (n=6)	%CV of 100ng/L (n=6)	R Value for Calibration Line (10-500ng/L)
Ametryn	10.1	51	1.01	2.53	0.9997
Atraton	8.6	46	0.52	2.93	0.9998
Atrazine	10.3	65	0.95	1.76	0.9988
Desmetryn	9.3	47	0.65	2.59	0.9992
Epoxiconazole	11.6	88	0.51	1.05	0.9987
EPTC	11.9	9	1.99	4.47	0.9993
Fenpropidin	10.4	178	0.58	1.70	0.9996
Fenpropimorph	12.9	56	0.66	6.72	0.9979
Flusilazole	11.6	169	0.44	1.69	0.9987
Flutriafol	10.2	12	1.13	1.92	0.9994
Metazachlor	10.3	117	0.63	1.82	0.9997
Pendimethalin	11.1	64	0.93	3.70	0.9946
Prometon	9.6	55	0.75	7.54	0.9998
Prometryn	10.9	128	0.43	1.75	0.9991
Propachlor	10.2	28	0.65	1.95	0.9994
Propazine	11.3	75	1.39	2.82	0.9994
Propiconazole	11.9	55	0.51	2.37	0.9998
Propyzamide	11.3	160	0.46	3.00	0.9994
Simazine	9.5	39	0.98	3.05	0.9991
Simeetryn	9.3	43	0.51	3.00	0.9994
Tebuconazole	11.9	116	0.64	2.18	0.9996
Terbutylazine	10.5	171	0.28	2.12	0.9995
Terbutryn	10.9	498	0.39	3.13	0.9991
Triadimefon	11.2	42	0.62	2.02	0.9998
Tri-allate	12.9	22	1.21	4.09	0.9985
Trietazine	11.7	13	1.76	2.02	0.9993

Table 1. Retention times, signal-to-noise, reproducibility and linearity data for each detected pesticide in tap water without the use of internal standards.

### Mass spectrometry

An API 3200 LC/MS/MS System with a Turbo V™ source and electrospray ionisation probe was used for all experiments, with curtain gas 25 psi; gas 1 70 psi; gas 2 30 psi; CAD gas 4; temperature 700 °C; ionspray voltage 5500 V. Detected MRM transitions with compound-dependent parameters were optimised using Analyst® software quantitative optimisation for each pesticide (data not shown).

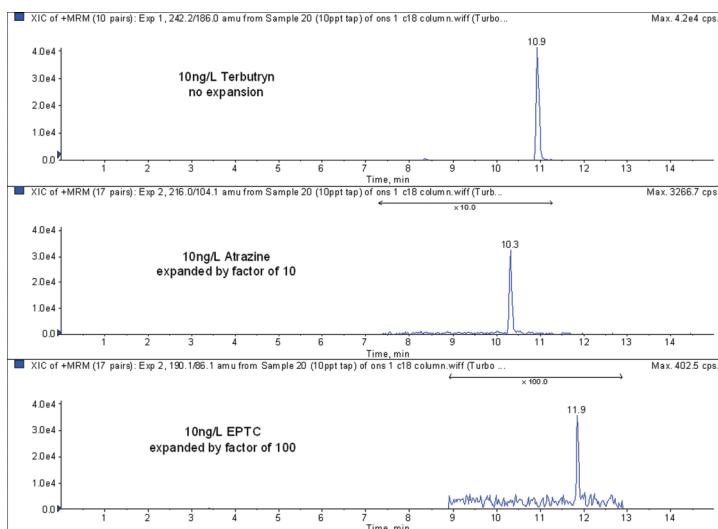


Figure 1. MRM transitions illustrating differences in the relative sensitivities of 10 ng/L terbutryn (S/N=498), atrazine (S/N=65) and for EPTC (S/N=9) calculated with peak-to-peak algorithm.

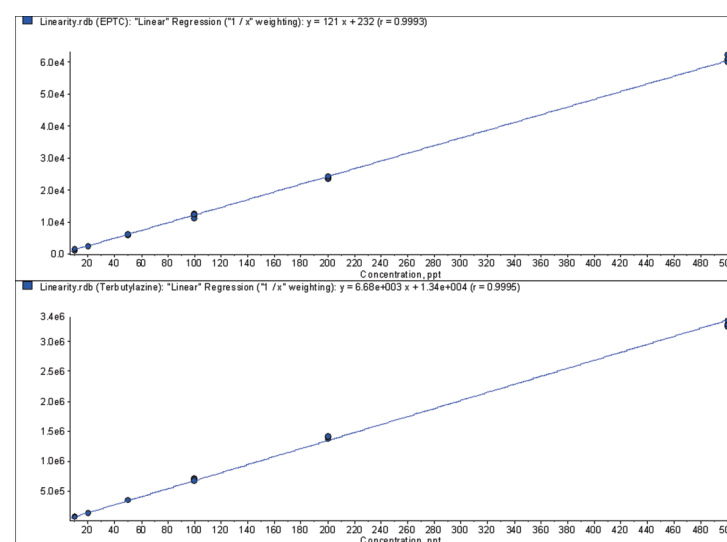


Figure 2. Carryover assessment using an injection of a 500 ng/L standard of fenpropidin and EPTC followed by an injection of blank water directly afterwards (corresponding MRM transitions for the same compounds are shown for the two injections).

During any chromatographic measurement, small drifts in retention time will be inevitably caused by gradual column degradation or small changes in temperature and mobile phase composition in between samples. When incorporating time windows (periods or segments) into the chromatogram, a small change in retention may result in pesticides falling outside the window and therefore going undetected during the MS/MS acquisition. This phenomenon becomes more profound as the number of compounds being monitored and/or windows within a given experiment



increases. Therefore, the ion path of the API 3200 System was set to constantly monitor all pesticides throughout the entire chromatogram using a linear accelerator (LINAC®) collision cell, making it possible to subsequently add more pesticides to the suite of screened compounds without the prior need to investigate the elution profile of the added analytes.

### Results and discussion

The analyte sensitivity varied markedly between pesticides, and the corresponding S/N (peak-to-peak) ratio for the pesticides varied from 498:1 (terbutryn) down to 9:1 (EPTC; see Fig 1 and Table 2). These findings qualify the need to use 5 mL sample for SPE to include weakly ionising compounds, such as EPTC, flutriafol and trietazine within the same sample injection.

Reproducibility was assessed by analysing tap water samples that had been spiked at two levels, 10 ng/L and 100 ng/L (the LOQ required for monitoring drinking water). Table 2 shows excellent standard deviations and coefficients of variation (% CV) for all pesticides.

Linearity was studied over a concentration range from 10 to 500 ng/L. Correlation coefficients were found to be greater than 0.994 for all calibration lines when a linear fit with 1/x weighting was applied (Table 2). No internal standards were used when reproducibility and linearity were investigated.

To further test the robustness of the method, 70 injections were made of the same 100 ng/L standard of terbutryn over a 20 hour period, which gave a CV of 6.6 per cent. A 500 ng/L standard was injected followed by a blank water sample to assess possible carryover using the developed method. Figure 2 shows a typical result for 2 pesticides and indicates that carryover is under 0.2 per cent.

Figure 3 shows examples of total ion chromatograms (TIC) obtained from the direct injection of different types of spiked water. In these examples it can be seen that there is a very low effect on the intensity of the response observed when tap water and water containing a high level of humic acid are compared to distilled water. The recoveries of all pesticides in tap water ranged from 77 to 115 per cent. When pesticides were spiked into water containing 10 ppm humic acid, there was a decrease in sensitivity but recoveries compared to distilled water were still over 67 per cent. The effect of humic acid was to decrease recovery in comparison to tap water samples, typically by under 15 per cent (the only exception being tri-allate at 20 per cent).

### Conclusion

The method described provides a simple, robust and cost-effective approach for the routine analysis of organonitrogen and triazine pesticides in potable waters by on-line SPE-LC/MS/MS. The use of on-line SPE eliminates the additional cost and time required for traditional SPE methods and offers improved robustness and reproducibility.

Limits of detection (LOD) have been calculated using both peak-to-peak measurements and also by calculating the LOD as 4.65 times the standard deviation of a 10 ng/L spike. Both calculations show that all the tested pesticides can be detected below 10 ng/L with good CV (below 8 per cent at 100 ng/L) and excellent linearity from 10 to 500 ng/L.

The sensitivity of the method was only slightly affected by the type of water sample analysed and has been shown to be sensitive enough for all the pesticides in potable waters. In water samples with a high total organic carbon, such as humic acid at 10 ppm, some ion suppression

Compound	Peak Area of 100ng/L in Distilled Water	Peak Area of 100ng/L in TapWater	Peak Area of 100ng/L in Water with 10ppm Humic Acid	Recovery (%) from Tap Water	Recovery (%) from Water with 10ppm Humic Acid	Effect of Humic Acid on Recovery (%)
Ametryn	2.68E+05	2.08E+05	1.94E+05	77.8	72.3	-5
Atraton	2.68E+05	2.12E+05	2.04E+05	78.9	75.8	-3
Atrazine	1.53E+05	1.22E+05	1.02E+05	79.9	66.5	-13
Desmetryn	4.32E+05	3.37E+05	2.95E+05	77.9	68.4	-10
Epoxiconazole	3.87E+05	4.14E+05	3.58E+05	107.2	92.5	-15
EPTC	1.57E+04	1.65E+04	1.56E+04	105.2	99.1	-6
Fenpropidin	1.03E+06	8.51E+05	8.15E+05	82.8	79.2	-4
Fenpropimorph	4.59E+05	3.89E+05	3.80E+05	84.7	82.6	-2
Flusilazole	2.59E+05	2.88E+05	2.56E+05	111.2	98.7	-12
Flutriafol	6.93E+04	7.47E+04	6.88E+04	107.9	99.4	-8
Metazachlor	5.19E+05	4.41E+05	4.04E+05	84.9	77.8	-7
Pendimethalin	1.24E+05	1.41E+05	1.27E+05	113.7	102.6	-11
Prometon	6.82E+05	5.39E+05	5.18E+05	79.1	76	-3
Prometryn	8.61E+05	7.74E+05	7.00E+05	89.8	81.2	-9
Propachlor	2.59E+05	2.27E+05	2.05E+05	87.9	79.3	-9
Propazine	3.97E+05	3.51E+05	2.95E+05	88.3	74.4	-14
Propiconazole	2.18E+05	2.40E+05	2.17E+05	110.2	99.6	-11
Propyzamide	1.31E+05	1.22E+05	1.06E+05	93.1	81.4	-12
Simazine	1.52E+05	1.20E+05	1.16E+05	79.1	76.3	-3
Simetryn	4.87E+04	3.73E+05	3.50E+05	76.6	71.9	-5
Tebuconazole	2.09E+06	2.24E+05	2.15E+05	107.2	102.8	-4
Terbuthylazine	9.68E+05	8.86E+05	7.43E+05	91.5	76.7	-15
Terbutryn	2.45E+06	2.13E+06	1.93E+06	86.7	78.6	-8
Triadimefon	1.50E+05	1.71E+05	1.60E+05	114.5	107	-7
Tri-allate	4.13E+04	4.59E+04	3.76E+04	111.1	91.1	-20
Trietazine	2.74E+05	2.39E+05	2.08E+05	87.3	76	-11

Table 2. Recovery data at 100 ng/L for each detected pesticide in tap water and water spiked with 10 ppm humic acid

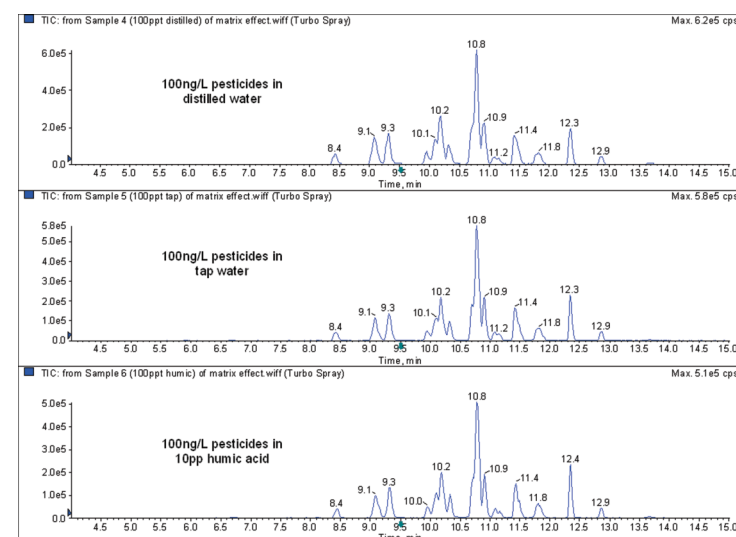


Figure 3. Total ion chromatograms of a 100 ng/mL spike of organonitrogen and triazine pesticides in distilled water (top), tap water (middle) and 10 ppm humic acid (bottom) to assess ion suppression effect.

effects were seen but they were under 20 per cent. The application of this technique to the determination of other pesticides such as acidic herbicides and organophosphorus pesticides is currently being investigated.

### REFERENCE

1. A manual of analytical quality control for the water industry (NS30), Drinking Water Inspectorate, UK.

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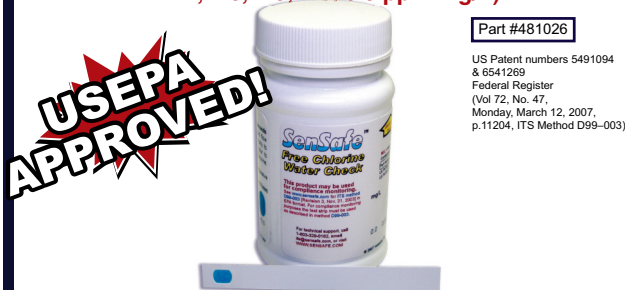


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