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應用手冊

UPLC with On-line-SPE Technology for the Analysis of Pesticides and Pharmaceuticals in Drinking Water

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Abstract

This application note details the analysis of pesticide residues and pharmaceuticals of concern in drinking water samples present at trace levels (sub ppb).

Benefits

Waters UPLC with On-line SPE Technology provides a number of benefits compared to traditional off-line sample prep:

- The same on-column concentration can be achieved with a much smaller sample volume
- A reduction in manual sample handling resulting in excellent precision
- Unattended operation frees up analyst for other tasks
- A reduction in cost per test due to reduced labor costs
- A reduction in turnaround time resulting in increased throughput

Introduction

Target contaminants in water are usually present at trace levels (sub ppb). In order to bring the final concentration of analytes into the detectable range for a chosen detector (e.g. UV, ELSD, MS), a pre-concentration stage is required. An enrichment factor is calculated from the original sample volume and the final extract volume. For example, an initial sample volume of 500 mL is concentrated to a final volume of 1 mL for a 500:1 enrichment. It is common to have methods starting with higher volumes, such as 1 L, and a final extract as low as 500 µl. These methods require extensive manual labor with loading, evaporation, and reconstitution steps that may take several hours.

This application note details the analysis of pesticide residues and pharmaceuticals of concern in drinking water samples present at trace levels (sub ppb). Sample volumes of less than 20 mL were used to achieve the same trace level results as 1 L samples using the off-line approach. Samples can be collected in the field directly into 20 mL vials.

The UPLC with On-line SPE Technology used for these analyses provides a number of advantages:

- Eliminates tedious evaporation and reconstitution steps
- Manages several methods without operator intervention
- Manages methods requiring different conditions

- Reduces the required sample volume
- Minimizes sample transfer and handling

Figure 1. UPLC with On-line SPE Technology.

Experimental

SPE conditions

Column:	Oasis HLB Direct Connect HP 20 µm, 2.1 x 30mm Column
Load/wash:	2% NH_4OH (both for pesticides and pharmaceuticals)
Recondition:	2% NH_4OH in methanol

LC conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C^{18} 2.1 x 50 µm, 1.7 µm
Mobile phases for pharmaceutical method:	A: 20 mM NH_4HCO_2, pH 3.2 B: 20 mM NH_4HCO_2 in 50:50 methanol/acetone, pH 3.2
Mobile phases for pesticide method:	A: 0.5% HCO_2H B: 0.5% HCO_2H in 80:20 methanol/acetonitril
Gradient:	5% B to 95% B in 5 min; 95% B held for 3 min before returning to initial conditions
Total run time:	13 min

MS conditions

MS system:	ACQUITY TQD
Capillary voltage:	3.5 kV
Source temp:	140 °C
Desolvation temp:	350 °C
Desolvation gas:	550 L/hr

MRM conditions: Pharmaceuticals listed in Table 1 Pesticides listed in Table 2

Pharmaceuticals	Precursor	Product	Cone Voltage	Collision
Carbamezapine	237.1	194.0	30	20
Cimetidine	253.1	95.0	25	25
Diphenhydramine	256.1	167.0	15	0
Atenolol	267.1	74.0	30	25
Metoprolol	268.1	72.0	30	20
Chlorpheniramine	275.1	230.0	20	15
Tripolidine	279.1	208.0	20	15
Trimethoprime	291.1	123.0	40	25
Terbinafine	292.1	141.0	25	25
Codeine	300.1	165.0	40	30
Cocaine	304.1	182.0	30	20
Clotrimazole	345.1	277.0	20	10
Miconazole	417.0	158.9	40	30
Erythromycin	734.5	158.0	25	25
Azithromycin	749.5	591.2	15	30

Table 1: Pharmaceuticals MRM conditions.

Pesticides	Precursor	Product	Cone Voltage	Collision
Aldicarb	191.0	88.9	10	10
Simazine	202.0	95.9	30	25
Propuxur	210.0	110.9	15	15
Propachlor	212.0	169.0	20	15
Simetryn	214.1	96.0	30	25
Atrazine	216.1	174.0	25	25
Carbofuran	222.1	165.0	20	10
Methiocarb	226.0	169.0	20	10
Propazine	230.0	145.9	40	25
Terbuthylazine	230.0	173.9	30	20
Cyanazine	241.0	214.0	30	20
Prometryn	242.1	158.0	20	25
Metolachlor	284.1	251.9	20	15
Tebuconazole	308.1	69.9	25	25
Propiconazole	342.0	68.9	30	20

Table 2: Pesticides MRM conditions.

Results and Discussion

Reproducibility

Since the drinking water samples were free from suspended particulates, they were injected directly into the system. To calculate reproducibility, six replicates of seven different concentrations of each analyte (ranging from 10 ppt to 5,000 ppt) were injected. Overall, the calculated concentrations for 2 orders of magnitude showed excellent reproducibility with CV's (coefficient of variation) between 0.6 and 14.1%. A quadratic linear regression (0.99) with 1/x2 weight was used for the study. Tables 3 and 4 show the calculated average concentration for six replicates with the corresponding CV values in parenthesis at a 100 ppt spike in drinking water samples.

Pesticides	100 ppt	1000 ppt
Simazine	103.3 (4.7)	1011.7 (4.6)
Aldicarb	100.5 (8.5)	1028.7 (4.4)
Propoxure	98.5 (3.9)	941.9 (7.8)
Propachlor	102.6 (7.2)	1014.4 (1.7)
Simetryn	104.2 (8.5)	975.1 (4.7)
Atrazine	101.5 (4.7)	1027.9 (4.7)
Carbofuran	98.7 (6.9)	918.6 (5.7)
Methiocarb	99.1 (5.1)	955.1 (7.6)
Propazine	95.1 (8.10	980.1 (3.1)
Terbuthylazine	96.2 (11.5)	1046.6 (4.9)
Cyanazine	95.3 (6.9)	988.6 (5.1)
Prometryn	104.0 (5.4)	986.6 (4.1)
Metolachlor	99.1 (3.9)	974.2 (6.8)
Tebuconazole	105.8 (6.9)	1011.5 (5.3)
Propiconazole	96.6 (12.5)	981.3 (5.8)

Pharmaceuticals	100 ppt	1000 ppt
Carbamezepine	106.2 (4.1)	979.9 (4.2)
Cimetidine	98.8 (7.4)	957.7 (6.9)
Diphenhydramine	95.2 (11.2)	987.4 (9.1)
Atenolol	97.8 (10.2)	1018.7 (8.8)
Metoprolol	101.4 (7.1)	958.7 (9.1)
Chlorpheniramine	92.8 (10.6)	968.4 (11.1)
Tripolidine	99.0 (5.5)	921.5 (8.5)
Trimethoprime	97.3 (9.1)	928.3 (5.6)
Terbinafine	97.1 (11.3)	980.2 (9.4)
Codeine	100.8 (2.9)	941.2 (5.2)
Cocaine	95.1 (11.5)	952.0 (13.3)
Clotrimazole	100.6 (9.0)	986.8 (5.1)
Miconazole	99.8 (10.7)	1018.1 (6.4)
Erythromycine	93.8 (8.7)	1052.4 (5.7)
Azithromycine	99.3 (7.4)	960.5 (8.3)

Pesticides and Pharmaceutical reproducibility at 100 and 1000 ppt spike in drinking water (n=6).

Chromatograms

Figure 2 shows extracted chromatograms of two pesticides (atrazine and carbofuran) in drinking water spiked at 10 ppt. Overall, the peaks show a well-defined Gaussian shape indicating they were refocused well on the analytical column following elution off the SPE extraction column.

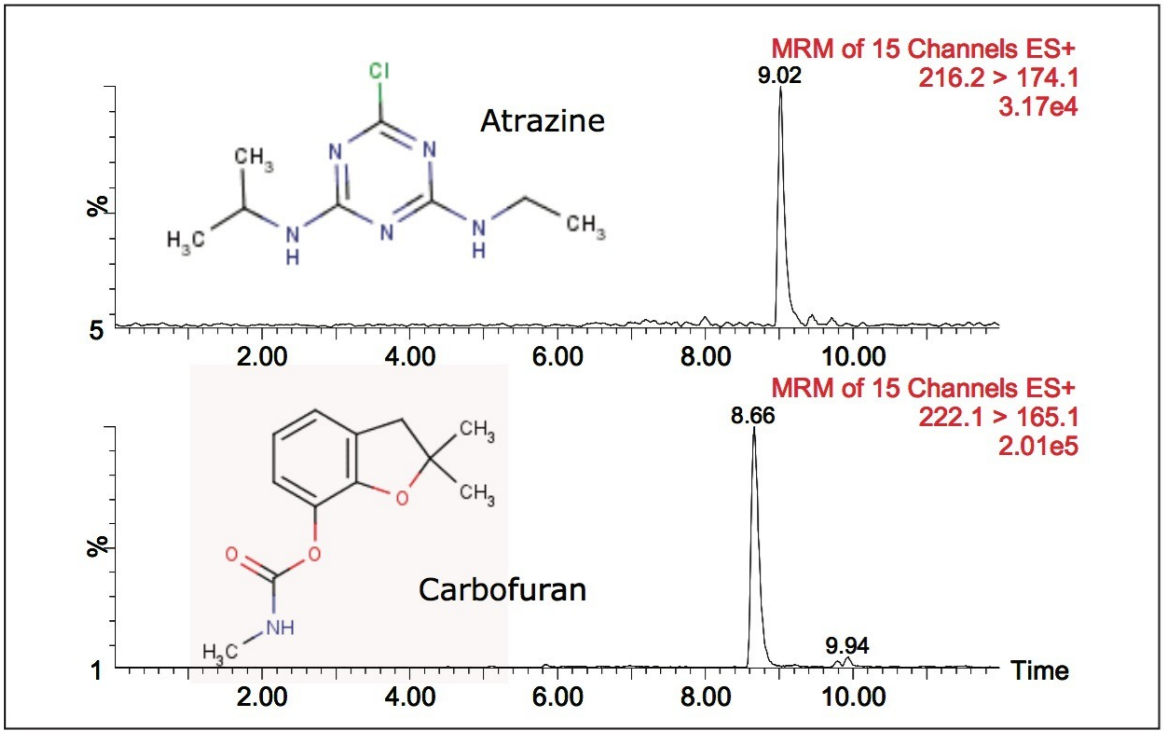

Figure 2. Extracted chromatograms of atrazine and carbofuran at 10 ppt in drinking water (15 mL).

The pesticide carbofuran (from the carbamate class of compounds) gave an average RSD (area) of less than 15% across a 3 order of magnitude calibration curve. The baseline signal was flat demonstrating that the 20% methanol wash was effective for removing interferences. Figure 3 shows extracted chromatograms for clotrimazole and tripolidine in drinking water spiked at 10 ppt. Again, the peaks show an excellent Gaussian shape, ensuring a high level of confidence in the results.

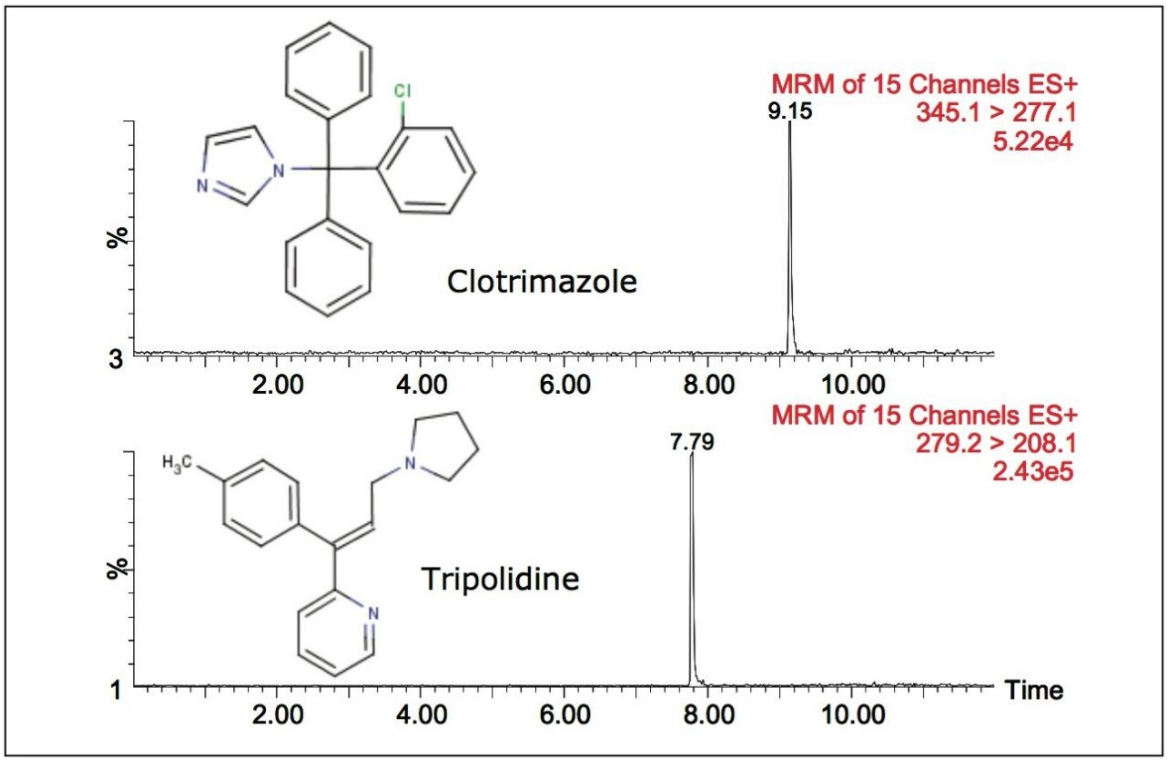

Figure 3. Extracted chromatograms of clotrimazole and tripolidine at 10 ppt in drinking water (15 mL).

Lifetime

The extraction column gave good results after 500 injections of drinking water samples. Figures 4 and 5 show extracted chromatograms at the first, 250th and 500th injections for metolachlor and diphenhydramine with excellent reproducibility on the retention time (CV less than 2%) and a coefficient of variation (CV's) of less than 15% for signal areas.

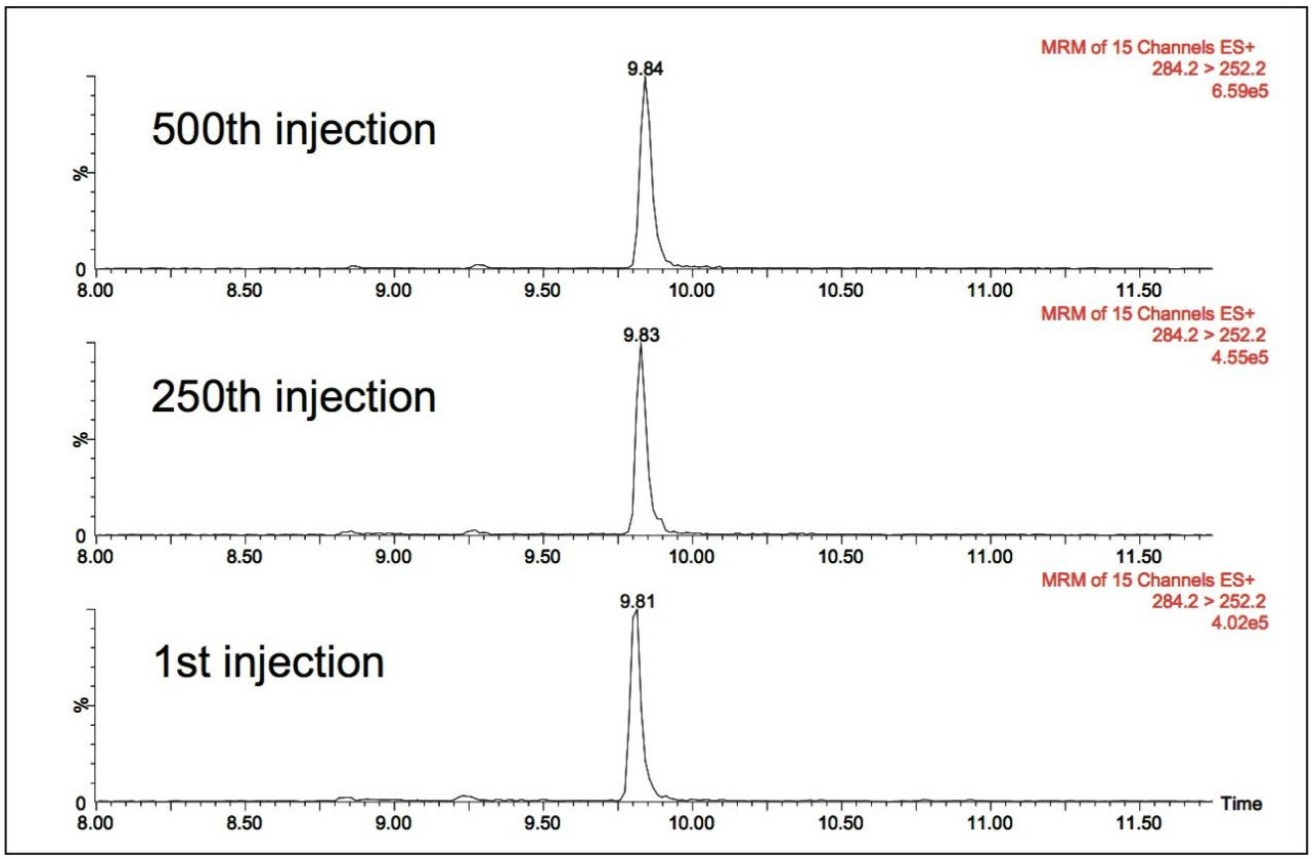

Figure 4. Lifetime chromatograms of metolachlor at 100 ppt in drinking water (15 mL).

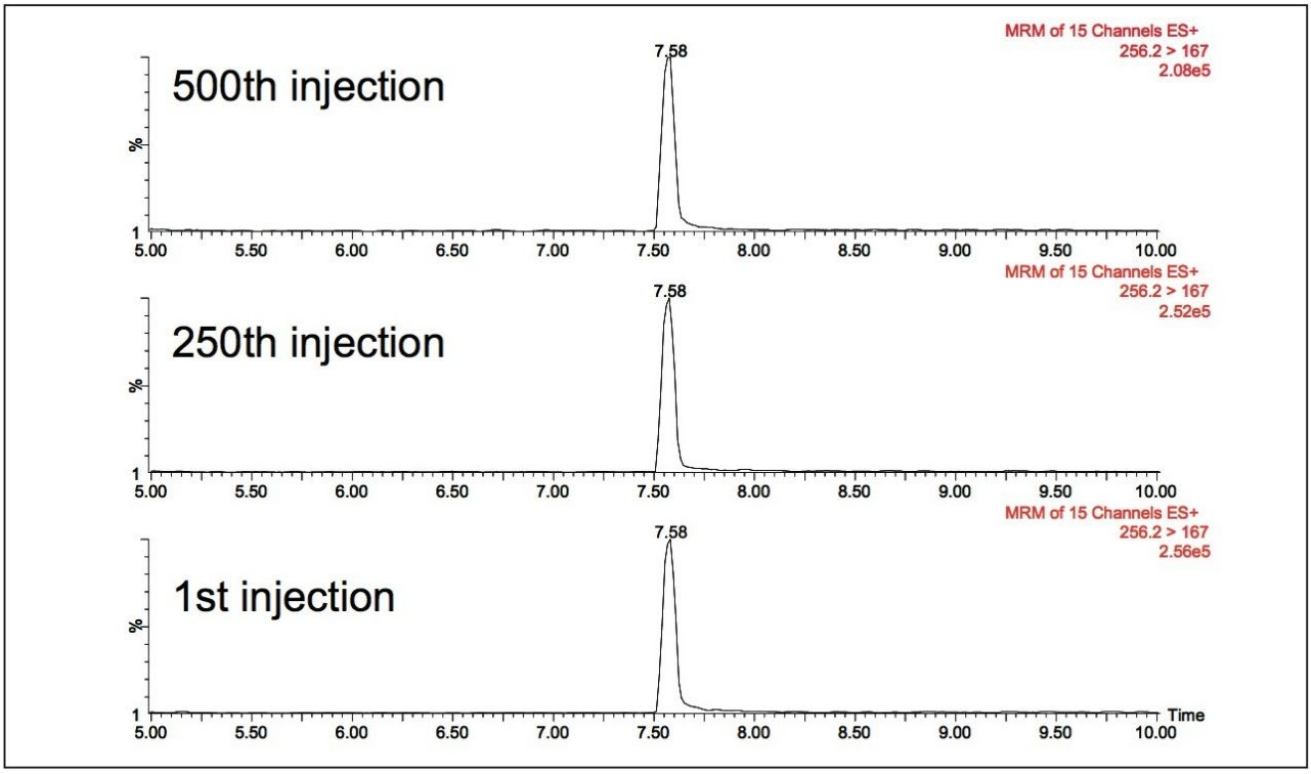

Figure 5. Lifetime chromatograms of diphenhydramine at 100 ppt in drinking water (15 mL).

Conclusion

The UPLC with on-line SPE Technology using ACQUITY TQD for detection gave an average quantification limit of 10 ppt for pesticides and pharmaceuticals in drinking water samples. The overall reproducibility on the area was less than 15% across 3 orders of magnitude. The extraction column gave excellent results even after 500 injections of drinking water samples.

The UPLC with on-line SPE Technology provides multiple benefits:

- The same on-column concentration can be achieved with a much smaller sample volume
- A reduction in manual sample handling resulting in excellent precision
- Unattended operation frees up analyst for other tasks
- A reduction in cost per test due to reduced labor costs
- A reduction in turnaround time resulting in increased throughput

The UPLC with On-line SPE System streamlines both the extraction and analysis into a turnkey total solution

platform.

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- ACQUITY UPLC System <https://www.waters.com/514207>

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