On-line SPE Enrichment of Trace Organic Contaminants in Water and Juices

Application Note

Environment and Food

Authors

E. Michael Thurman, Imma Ferrer
Center for Environmental Mass Spectrometry
Department of Environmental Engineering
University of Colorado
Boulder, CO
USA

Jerry A. Zweigenbaum
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

Abstract

An on-line SPE method for the identification and quantitation of trace levels of organic contaminants (pharmaceuticals and pesticides) in environmental waters and juices was developed with the Agilent Flex Cube solution followed by detection with triple quadrupole mass spectrometry. A small volume (900 µL) was preconcentrated on polymeric cartridges. Analytical performance data of the on-line SPE system for linearity, recovery, and detection limits in real samples is shown and discussed. Recoveries varied from 41 to 115%. Method detection limits ranged from 1 to 500 ng/L.

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Introduction

Automation of a manual SPE method can provide many benefits, such as health and safety of the operator, improved results, and cost savings. Automating a manual SPE method with on-line SPE removes the analyst from extended contact with environmental water samples containing hazardous substances, as well as contact with organic elution solvents. Operator burnout from repeated motions of SPE, which often leads to errors in manual methods, is also eliminated. Furthermore, overnight runs are possible, maximizing the use of sample preparation time. The most important benefit of on-line SPE is the ability to maximize the recovery of analytes due to sample losses in manual SPE and solvent handling.

This application note shows how to automate SPE methods for environmental water samples containing pesticides, and pharmaceuticals and juice samples containing pesticides. The importance of SPE in water samples has been known since the early 1980s, because of the need to improve the detected concentration from µg/L levels to ng/L by modern GC/MS and LC/MS instrumentation. Typically, this has been done by manual SPE methods using a vacuum manifold approach. With this system, the water sample is manually passed through a conditioned cartridge and then eluted into a test tube for evaporation under nitrogen. Finally, the extract is transferred to a vial for LC/MS analysis. The multiple steps in sample preparation result in losses of approximately 10% of the sample, even with the most care possible. In addition, there may be losses due to partial elution from the SPE cartridge. Both of these problems are overcome by on-line SPE because the sample is in line with the LC/MS system and the elution solvent is the same solvent used to run the chromatogram. This means that large volumes pass the on-line SPE column and result in a very effective elution of the cartridge. With respect to juice analysis, the small volumes needed for juice analysis, for example, 1 mL, are easily accommodated by automated SPE, but are difficult to do with manual methods because of the small volumes that must be handled. For these reasons, on-line SPE is the method of choice for these two applications.

Experimental

Standards and reagents

Calibration standards were obtained from Cerilliant and AccuStandards at the highest available purity. Calibration standard solutions were prepared in the range of 1– 1,000 ng/L. Labeled surrogate internal standard, carbamazepine d-10 was purchased from Cambridge Isotopes. All solvents used were of highest purity available. Pesticide-grade water, methanol and acetonitrile were obtained from Burdick & Jackson.

Instrumentation

Agilent 1290 Infinity Series on-line solution system comprising:

- Agilent 1290 Infinity Binary Pump with internal degasser, (G4220A)
- Agilent 1260 Infinity Standard Autosampler with 900-µL loop (G1329B #020) and thermostat (G1330B)
- Agilent 1290 Infinity Flexible Cube (G4227A) with 2-position/10-port valve (G4232C)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)

The MS detection was carried out with the Agilent 6460 Triple Quadrupole LC/MS with Agilent Jet Stream technology.

Figure 1 shows the schematic of the different modules. Two cartridges were used in this system; while one column is loading, the second column is eluted, thus minimizing the total analysis time.

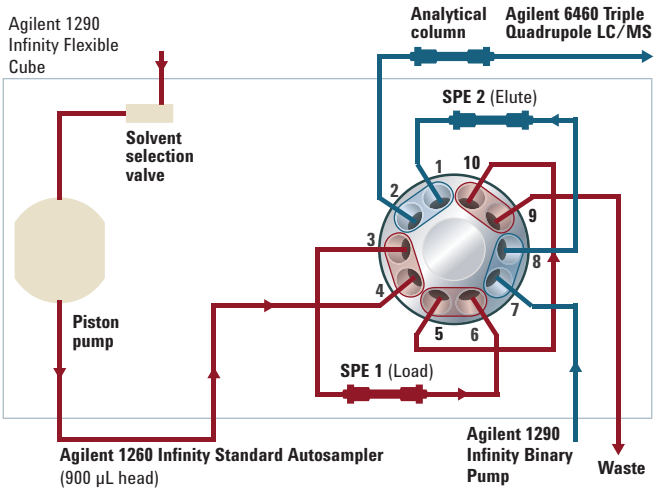


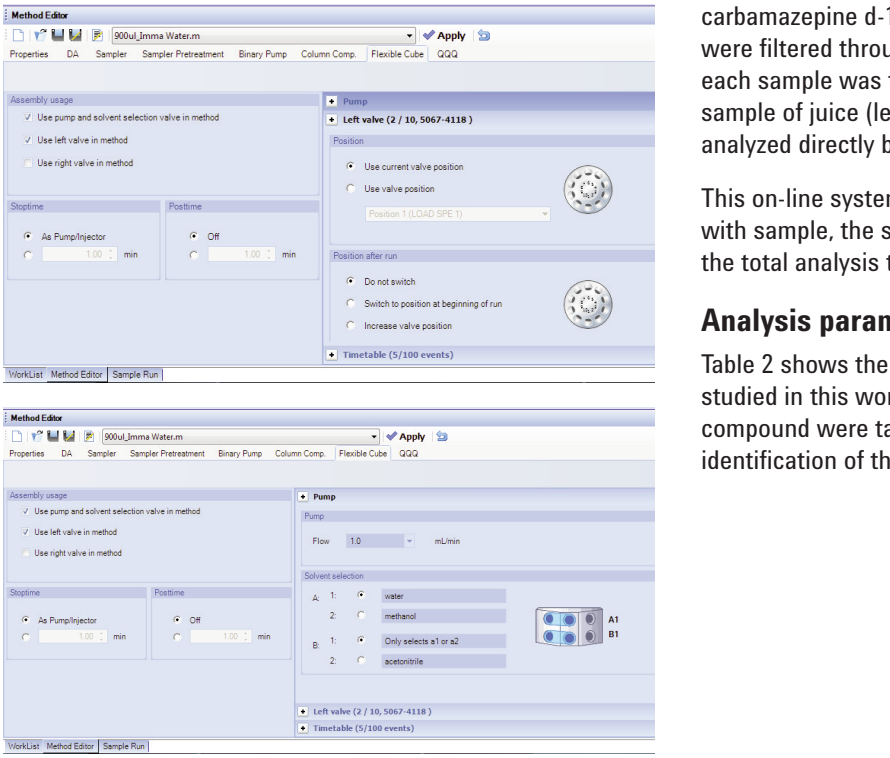
Figure 1. Schematic of the on-line process.

Table 1 shows the analytical operating conditions for both the 1290 Infinity Flexible Cube and the LC/MS systems.

Table 1. On-line SPE Conditions

Agilent 1290 Infinity Flexible Cube	
Online SPE cartridges	PLRP-S, 4.6 × 12.5 mm (p/n 5982-1270)
Temperature	25 °C
Volume	900 µL
Injection draw speed	1,000 µL/min
Eject speed	1,000 µL/min
Draw position	0.5 mm
Flow rate	1 mL/min
Solvents	A1) Water A2) MeOH B2) ACN
Cartridge conditioning	2 mL MeOH 4 mL Water
Cartridge wash	2 mL ACN
HPLC conditions	
Analytical column	Agilent ZORBAX Eclipse XDB C8 150 × 4.6 mm, 3.5 µm (p/n 963967-906)
Temperature	25 °C
Mobile phase	A) Water (0.1% acetic acid) B) Acetonitrile
Flow rate	0.6 mL/min
Gradient for elution from SPE column	Time (min) A B 0 90% 10% 2 90% 10% 10 0% 100% 12 0% 100%
MS conditions	
Acquisition parameters	Dynamic MRM mode
Sheath gas temperature	350 °C
Sheath gas flow rate	11 L/min
Drying gas temperature	250 °C
Drying gas flow rate	10 L/min
Nebulizer pressure	45 psig
Nozzle voltage	0 V positive; 1,500 negative
Vcap	3,500 V
EMV	200 V positive; 400 V negative

Figure 2 shows a series of snapshots for the positions and timetables of the SPE valve.



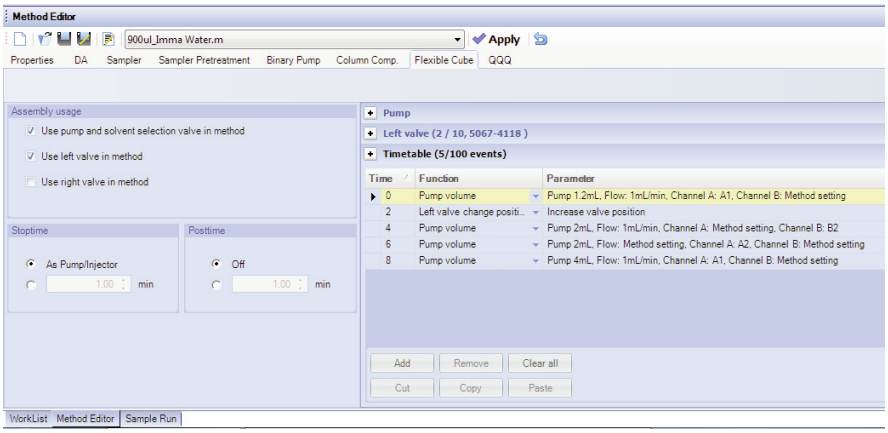


Figure 2. Screenshots of the SPE valve functions.

Sample preparation

Surface water samples were taken downstream of wastewater treatment plants. Samples were fortified with carbamazepine d-10 surrogate standard at 80 ng/L. Samples were filtered through 0.2-µm syringe filters. A 6-mL aliquot of each sample was transferred to 6-mL vials. Again, a 6-mL sample of juice (lemon juice and fruit juice) was filtered and analyzed directly by the on-line SPE system.

This on-line system shows that while one column was loaded with sample, the second column was eluted, thus minimizing the total analysis time to less than 12 minutes per sample.

Analysis parameters

Table 2 shows the MRM transitions for all the analytes studied in this work. Two transitions (when available) for each compound were taken into account for the correct identification of the contaminants.

Table 2. MRM ESI Analysis Parameters for All Analytes Studied

Compound name	Precursor ion	Product ion	Ret time (min)	Fragmentor	Collision energy	Polarity
2,4-D	219	161	10.22	70	10	Negative
2,4-D	219	125		70	25	Negative
Atenolol	267	190	5.47	110	15	Positive
Atenolol	267	145		110	20	Positive
Atrazine	216	174	9.57	120	15	Positive
Atrazine	216	146		120	20	Positive
Caffeine	195	138	6.33	110	15	Positive
Caffeine	195	110		110	25	Positive
Carbamazepine	237	194	8.56	120	15	Positive
Carbamazepine	237	179		120	35	Positive
Carbamazepine-d10	247	204	8.5	120	15	Positive
Carbendazim	192	160	6.3	80	15	Positive
Carbendazim	192	132		80	20	Positive
Clarithromycin	748.5	590	7.22	110	15	Positive
Clarithromycin	748.5	158		110	25	Positive
Cotinine	177	98	5.41	90	25	Positive
Cotinine	177	80		90	25	Positive
DEET	192	119	9.62	110	15	Positive
DEET	192	91		110	30	Positive
Diazinon	305	169	11.72	90	15	Positive
Diazinon	305	153		90	20	Positive
Diltiazem	415	178	7.02	130	25	Positive
Diltiazem	415	150		130	25	Positive
Diphenhydramine	256	167	7.01	70	15	Positive
Diphenhydramine	256	152		70	35	Positive
Diuron	235	72	9.53	90	20	Positive
Diuron	233	72		90	20	Positive
Fluridone	330	310	9.98	110	30	Positive
Fluridone	330	294		110	50	Positive
Gemfibrozil	249	121	11.19	70	5	Negative
Imazalil	297	159	7.4	120	20	Positive
Imazalil	297	255		120	20	Positive
Imidacloprid	256	209	7.7	80	10	Positive
Imidacloprid	256	175		80	10	Positive
Lamotrigine	258	213	6.34	120	25	Positive
Lamotrigine	256	211		120	25	Positive
Metoprolol	268	116	6.31	110	15	Positive
Metoprolol	268	56		110	30	Positive
Propranolol	260	116	6.83	110	15	Positive
Propranolol	260	56		110	30	Positive
Sucralose	419	239	6.59	110	15	Positive
Sucralose	419	221		110	15	Positive
Sulfamethoxazole	254	156	8.02	80	10	Positive
Sulfamethoxazole	254	92		80	30	Positive
Thiabendazole	202	175	6.5	120	30	Positive
Thiabendazole	202	131		120	30	Positive
Triclopyr	256	198	10.19	50	5	Negative
Triclopyr	254	196		50	5	Negative
Trimethoprim	291	261	5.98	110	25	Positive
Trimethoprim	291	230		110	20	Positive

Results and Discussion

On-line SPE performance

Table 3 shows the recoveries obtained for the analytes studied in surface water samples. For the majority of analytes, a satisfactory recovery was obtained, with the exception of gemfibrozil and sucralose. Gemfibrozil is a rather hydrophobic compound and might not completely elute from the polymeric cartridge. Conversely, sucralose is polar in nature and probably breaks through the sorbent. Other phases such as C-18 or ionic phases might be better choices for these two compounds. All other compounds were recovered at high percentages, thus making this method amenable to analysis of surface waters that are wastewater impacted.

Table 3. Recoveries Obtained After the Preconcentration of 900 µL onto PLRP-S Cartridges

Compound	% Recovery	LODs (ng/L)
2,4-D	88	10
Atenolol	102	5
Atrazine	98	5
Caffeine	102	5
Carbamazepine	88	5
Clarithromycin	107	5
Cotinine	114	5
DEET	82	10
Diazinon	96	1
Diltiazem	115	5
Diphenhydramine	114	2
Diuron	79	10
Fluridone	96	5
Gemfibrozil	35	50
Lamotrigine	96	10
Metoprolol	110	5
Propranolol	109	5
Sucralose	41	500
Sulfamethoxazole	83	10
Triclopyr	79	10
Trimethoprim	104	5

Table 3 shows the limits of detection (LODs) obtained for all compounds studied in surface water samples. These LODs were calculated taking into account the confirmatory transition as well. The LODs varied depending on the compound studied. Some analytes were as sensitive as 1 ng/L. The majority of compounds can be detected at or lower than 10 ng/L.

Calibration curves were built from 1 ng/L to 1,000 ng/L showing good linearity range. Figure 3 shows an example of carbamazepine, one of the most commonly detected pharmaceuticals in water. Figure 3 also shows the detection of both transitions for a concentration level of 5 ng/L.

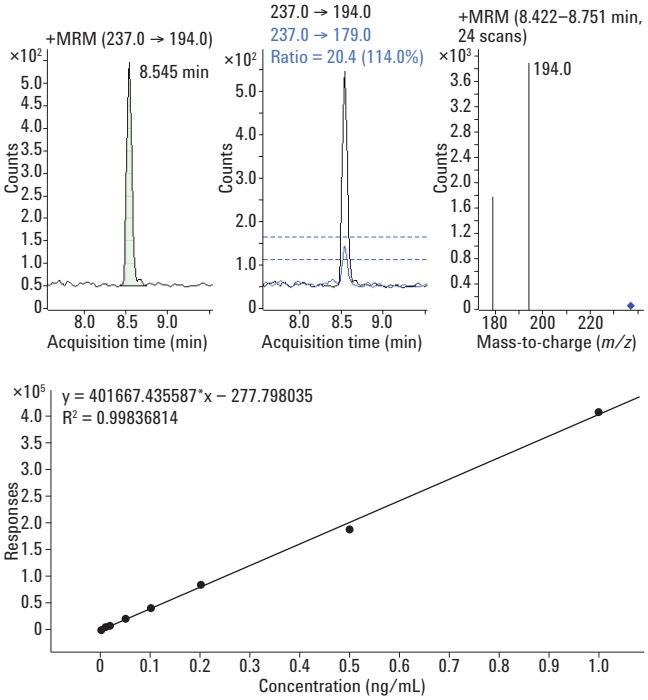


Figure 3. Quantitation and linear curve of carbamazepine in surface water samples.

Sample analysis

Figure 4 shows a chromatogram corresponding to a surface water sample impacted by wastewater. A total of 12 analytes were detected in this sample at low concentration levels.

Two juice samples (lemon juice and fruit punch) were also analyzed by this methodology. A positive detection for carbendazim and imazalil was found in the lemon juice sample, as shown in Figure 5. This allowed for a rapid detection method of pesticides in beverages with minimal sample preparation. However, this matrix tended to foul the on-line SPE cartridge and, for routine use, the samples may need to be diluted. Optimization of the analysis of fruit juices was not pursued for this study.

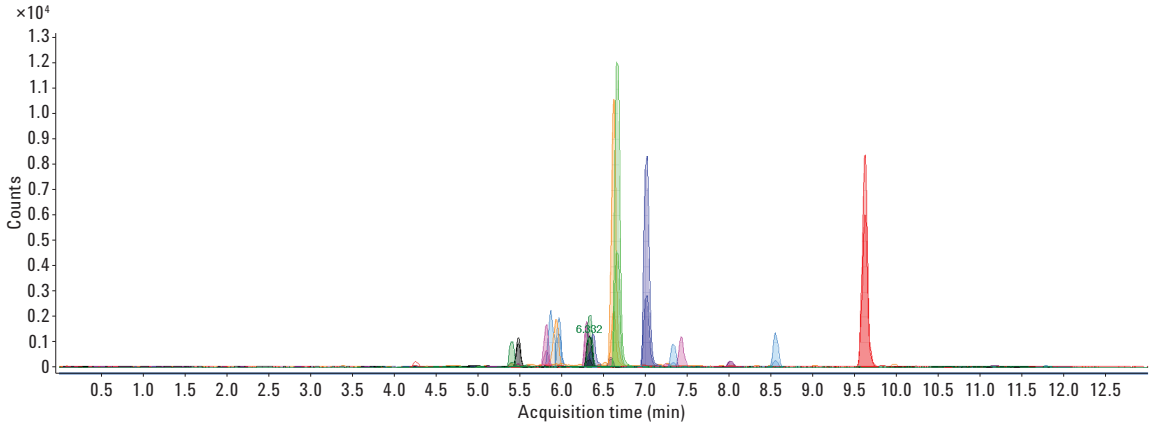


Figure 4. LC/MS/MS of a surface water sample collected in Colorado.

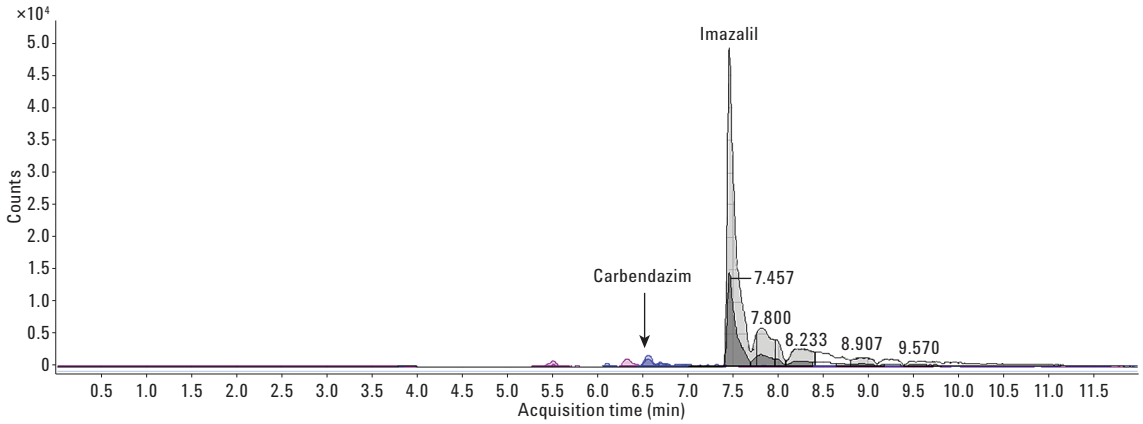


Figure 5. LC/MS/MS chromatogram of a lemon juice sample.

Conclusions

This application note shows that an automated on-line SPE is a valuable tool for water sample analysis for trace levels of pesticides and pharmaceuticals in environmental water samples. Several juice samples are easily analyzed with small volumes, less than 1 mL, by on-line SPE LC/MS analysis. However, additional sample conditioning would be required to make the on-line method routine for fruit juices. The combination of the Agilent 1290 Infinity Flexible Cube and the Agilent 1260 Infinity Standard Autosampler allow the analyst to do on-line SPE with chromatography while the second cartridge is being loaded. Thus, no time is lost in sample preparation. Proper cleaning and elution techniques allow the same SPE cartridge to be used in multiple applications before replacement.

For More Information

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