Determination of Drug Residues in Surface and Municipal Wastewaters by SPE and LVI-LC/MS/MS

Application Note

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Introduction

Pharmaceutical residues in the environment are an issue of growing attention. Due to detection of diverse drug residues in aquatic environments (surface and groundwater), as well as in influents and effluents of sewage treatment plants, the German Environment Ministers' Conference held in 1999 saw a need to establish a program for monitoring the occurrence of pharmaceuticals in aquatic systems. Over the past few years, the amount of research on the potential environmental effects and risks of pharmaceuticals has greatly increased. It is therefore of general interest to regularly monitor the aquatic environment. The first components to be controlled are drugs that have been found most often and/or in the highest concentrations and which are prioritized in various papers as the most important representatives[1]. The following drugs, which are part of the prioritization lists, are included in this application: carbamazepine, bezafibrate, clofibric acid, sulfamethoxazole, ibuprofen, diclofenac, fenofibric acid, iopromide and iopamidole.

The amounts of the different pharmaceutical compounds detected in water show a very large scatter[2]. Our goal was to develop a fast and sensitive analysis method to allow detection down to 0.05 µg/L in surface water, and in addition using dilution with an adjusted limit of quantification for highly polluted samples such as effluents of sewage treatment plants. The method is based on Solid Phase Extraction (SPE), Large Volume Injection (LVI) with HPLC analysis and MS/MS detection.

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Instrumentation and Materials

Autosampler:	Agilent ProStar 410
Column Oven:	Agilent ProStar 510
HPLC:	Agilent ProStar 210
Mass Spectrometer:	Agilent 1200L triple quadrupole with ESI source
SPE Cartridge:	Bond Elut PPL 200 mg/3.0 mL (part number 12105005)
HPLC Column:	Pentafluorophenyl 3 µm, 250 x 2.0 mm
Guard Cartridge:	C18, 4 x 2.0 mm

Method

Our method development focused on reliability, sensitivity and fast analysis time. To achieve high sensitivity and shorten the time for sample preparation, we chose a LVI technique and injected 200 µL into the LC/MS/MS.

Using LVI, the total sample volume required was smaller than in current analytical methods that need to concentrate the analytes from a typical sample volume ranging from 50-250 mL[3]. We diluted 2 mL surface water with 48 mL LC/MS grade water and cleaned-up with a fast SPE step on a Bond Elut PPL cartridge. Bond Elut PPL was chosen because the high surface area of the functionalized styrene-divinylbenzene polymer is optimal for the extraction of polar and mid-polar species.

Large volume injections and separations were performed on a Agilent ProStar HPLC system equipped with a binary pump and a ProStar autosampler. The LC was coupled to a Varian 1200L triple quadruple mass spectrometer system fitted with an ESI source and operated in both positive and negative ion mode, respectively. The ionization mode selected for each compound is shown in Table 1.

Table 1. Ionization mode and SRM selected for each compound

Analyte	SRM	Capillary Voltage (V)	Collision Energy (V)
Carbamazepine	(+) 237>194	56	20.5
Sulfamethoxazole	(+) 254>156	48	12
Ibuprofen	(-) 205>161	-30	7
Ibuprofen D3	(-) 208>164	-60	5
Clofibric acid	(-) 213>85	-30	10.5
Clofibric acid	(-) 213>127	-30	15
Diclofenac	(-) 294>214	-40	12
Diclofenac	(-) 294>250	-40	16
Fenofibric acid	(-) 317>231	-32	11.5
Bezafibrate	(-) 360>154	-44	34.5
Bezafibrate	(-) 360>274	-44	18
Iopamidole	(-) 776>127	-48	16
Iopromide	(-) 790>127	-32	16

Sample Preparation and SPE Method
Add 2 mL sample to 48 mL LC/MS grade water.
Add 0.35 g (7 g/L) NaCl.
Condition Bond Elut PPL cartridge:
3 mL methanol, 6 mL LC/MS grade water
Apply sample:
pre-treated sample with 1-1.5 mL/min
Wash: 6 mL LC/MS grade water
Dry: 5 min
Elute:
1.5 mL methanol, soak for 5 min, repeat one time
Evaporate to dryness, re-dilute in 250 µL methanol, and fill up to 4 mL with LC/MS grade water

Results and Discussion

LC Conditions
Mobile Phase:

Solvent A:	LC/MS grade water, 10% methanol, 0.003% formic acid (pH4)
Solvent B:	methanol
Flow Rate:	0.2 mL/min
Oven Temperature:	40 °C

Table 2. LC Program

Time (min)	%A	%B
0	100	0
7	100	0
8	25	75
25	0	100
30	0	100
31	100	0
41	100	0

MS Parameters

Ionization Mode:	ESI (positive and negative)
Drying Gas:	29 psi at 290 °C
Nebulizing Gas:	41 psi
Needle:	6000 V
Collision Gas:	1.3 mTorr Argon

Figure 1 shows the six-point calibration curve of diclofenac in the concentration range from 0.05 µg/L up to 2.5 µg/L. The chromatogram (Figure 2) obtained from an actual sample under LVI-LC/MS/MS conditions indicated good separation and detection of all analytes. The injection of a large volume (200 µL) onto a guard column filled with C18 material and the separation on a pentafluorophenylpropyl analytical column gave narrow and symmetrical peaks and did not show peak broadening. The limit of detection for surface water samples is 0.05 µg/L. Highly-polluted samples, such as influents and effluents of sewage treatment plants, can be diluted and detected with an adjusted limit of quantification.

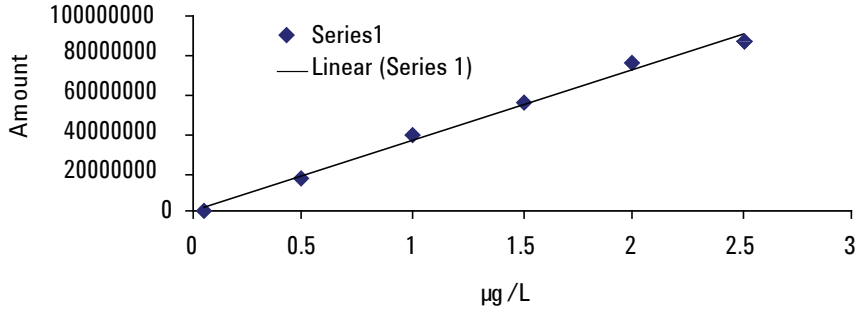

Figure 1. Calibration curve of diclofenac

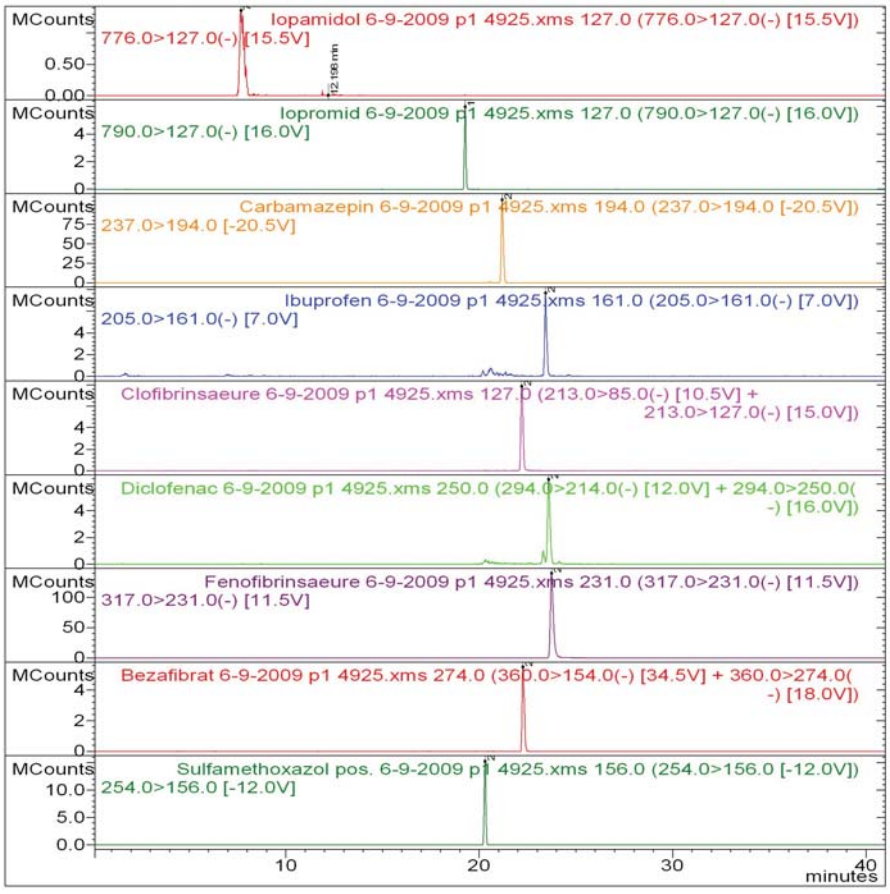

Figure 2. MRM chromatograms of drug residues in surface water after SPE and LVI

Conclusions

The method incorporated pharmaceuticals from a wide variety of classes. The optimization of a single SPE extraction procedure for the neutral, basic and acidic compounds in combination with a large volume injection streamlined the method and is a significant improvement in sample throughput.

References

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