

## DETERMINATION OF ENDOCRINE DISRUPTING COMPOUNDS IN RIVER WATER BY ACQUITY UPLC TANDEM QUADRUPOLE MS

Peter Hancock and James Morphet  
Waters Corporation, Manchester, UK

### INTRODUCTION

Endocrine disrupting compounds (EDCs) have become important emerging contaminants, due to their presence in environmental waters (following incomplete removal in wastewater treatment or point-source contaminations). EDCs pose a threat to drinking water sources, and there are additional concerns about possible estrogenic and other effects, both to wildlife and humans<sup>1</sup>.

Compounds identified as EDCs include pesticides, polychlorinated biphenyls (PCBs), dioxins, furans, alkylphenols, and steroid hormones (natural and synthetic). The steroid hormones are of special concern due to their potency.

Natural (estrone and 17-estradiol) and synthetic (17-ethynylestradiol) estrogens found in contraceptive birth control pills and in hormone replacement therapies, contribute to a large extent to the estrogenicity of wastewater treatment plant effluents<sup>2</sup>.

Other EDCs, such as the alkylphenols (bisphenol A) are derived from industrial and domestic activities and can also occur in environmental waters. Bisphenol A is used in the production of epoxy resins and polycarbonate plastics, which are utilized extensively in the manufacture of food and beverage packaging materials.

These chemicals have been shown to provoke endocrine disruption at ng/L levels so analyte enrichment may be required to achieve the required detection limits. SPE is considered to be the most appropriate technique for the extraction of EDCs from aqueous samples<sup>3</sup>.

The aim of this work was to develop a simple, sensitive and reliable method for the determination of natural and synthetic estrogens and alkylphenols in surface water at ng/L levels using LC/MS/MS. The developed method is based on Waters® Oasis® HLB solid phase extraction (SPE), Waters ACQUITY UPLC® and tandem quadrupole MS/MS with electrospray ionization in negative ion mode.

### EXPERIMENTAL

#### Extraction

The SPE method used for this application note has been described previously<sup>4</sup>. The method is based on Oasis HLB glass cartridges designed specifically for low level estrogens in river water.

The extraction method was changed slightly to accommodate the early eluting compound; estriol. Bisphenol A is the earliest eluting compound described in the extraction method. The first wash step was changed from 40% methanol in water to 10% methanol in water resulting in the following procedure.

Cartridge:	Waters Oasis HLB glass 5 cc/200 mg
Condition:	3 mL methyl <i>t</i> -butyl ether (MTBE) 3 mL methanol 3 mL water
Load:	200 mL sample adjusted to pH 3.0
Wash 1:	3 mL 10% methanol in water
Re-equilibrate:	3 mL water
Wash 2:	3 mL 10% methanol/2% NH <sub>4</sub> OH in water
Elute:	2 x 3 mL 10% methanol in MTBE
Final:	Dry under N <sub>2</sub> and reconstituted in 10% methanol in water



Figure 1. ACQUITY UPLC and Quattro Premier XE tandem quadrupole mass spectrometer.

**LC conditions**

LC system:	Waters ACQUITY UPLC System		
Column:	ACQUITY UPLC BEH C <sub>8</sub> Column 2.1 x 50 mm, 1.7 μm		
Column temp:	40 °C		
Flow rate:	450 μL/min		
Mobile phase A:	Water + 0.1% NH <sub>4</sub> OH		
Mobile phase B:	Methanol + 0.1% NH <sub>4</sub> OH		
Gradient:	Time	0.00 min	90% A
	Time	0.50 min	90% A
	Time	4.00 min	5% A
	Time	5.00 min	5% A
	Time	5.10 min	90% A
Total run time:	8.00 min		
Injection volume:	20 μL full loop		

**MS conditions**

MS system:	Waters Quattro Premier™ XE Mass Spectrometer
Ionization mode:	ESI negative
Capillary voltage:	2000 V
Desolvation gas:	Nitrogen, 800 L/Hr at 400 °C
Cone Gas:	Nitrogen, 20 L/Hr
Source Temp:	120 °C
Collision Gas:	Argon, 3.5 x 10 <sup>-3</sup> mBar
Acquisition Mode:	Multiple Reaction Monitoring (MRM)

**Acquisition and Processing**

The data were acquired using Waters MassLynx™ Software V. 4.1 and processed using the TargetLynx™ Application Manager.

The Quattro Premier™ XE was tuned so that the precursor and product ions were resolved with a peak width at half height of less than 0.7 Da. The list of endocrine disrupting compounds, and the MRM transitions, along with the retention times, dwell times, cone voltages, and collision energies for the method are listed in Table 1. The dwell times were optimized so that ten to fifteen data points were acquired across each chromatographic peak.

RT min	Compounds	Precursor Ion	Product Ion	Cone Voltage (V)	Collision Energy (eV)
2.35	Estriol	287.0	171.0	60	38
		287.0	145.0		44
2.81	Bisphenol A	227.0	212.1	40	20
		227.0	133.0		25
3.03	Estrone	269.0	145.0	60	41
		269.2	143.0		53
3.05	17β-estradiol	271.1	145.0	60	42
		271.1	183.0		42
3.09	17α-ethinyl estradiol	295.0	145.0	55	40
		295.0	143.0		55
3.05	17α-estradiol	271.1	145.0	60	42
		271.1	183.0		42

Table 1. Acquisition parameters for the six endocrine disrupting compounds.

Two MRM transitions were acquired for each residue so that quantification and confirmation could be performed with a single injection, assuming that the ion ratio between the two transitions is consistent for standards and samples.

## RESULTS AND DISCUSSION

The separation of the endocrine disrupting compounds was optimized for UPLC<sup>®</sup>, to reduce the total run time, while maintaining sensitivity and chromatographic resolution between the structural isomers 17 $\alpha$ - and 17 $\beta$ -estradiol.

The total run-time was reduced to eight minutes from 25 minutes, as reported in the previous method<sup>5</sup>. The baseline resolution of 17 $\alpha$ - and 17 $\beta$ -estradiol and the peak shape are illustrated in Figure 2. Figure 2 also demonstrates the good sensitivity of the Quattro Premier XE in negative ion electrospray mode using the MRM chromatograms from a 20  $\mu$ L injection of a 1 ng/mL solvent standard. This sensitivity resulted in the estimated instrumental LODs being less than 0.1 ng/mL for all compounds.

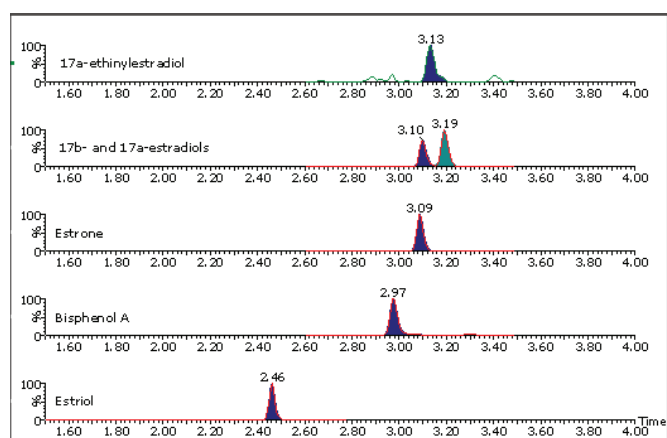


Figure 2. Sensitivity of the Quattro Premier XE for 1 ng/mL estrogens and bisphenol A in solvent standards.

To test the extraction method described, five recovery experiments were performed from 200 mL river water spiked at two different levels, 10 and 100 ng/L. These concentration levels equate to 2 and 20 ng/mL on the calibration curve. Calibration curves were constructed by injecting matrix-matched standards at concentration

levels of 0.1, 0.2, 0.5, 1, 5, 10, 20, and 50 ng/mL. The mean recovery and relative standard deviation (%RSD) in parenthesis of each analyte are listed in Table 2. The extraction method gives good recovery for the six analytes with satisfactory reproducibility even at the low concentration level of 10 ng/L.

Compounds	Mean Recovery (%RSD)	
	10 ng/L	100 ng/L
Estriol	109 (8)	103 (2)
Bisphenol A	122 (29)	109 (6)
Estrone	120 (14)	109 (4)
17 $\beta$ -estradiol	107 (8)	97 (3)
17 $\alpha$ -ethinyl estradiol	132 (17)	99 (11)
17 $\alpha$ -estradiol	129 (8)	108 (7)

Table 2. Mean recovery and %RSD of the estrogens and bisphenol A from river water spiked at 10 and 100 ng/L.

The excellent sensitivity and selectivity of the Quattro Premier XE in negative ion electrospray mode is illustrated in Figure 3 with the MRM chromatograms from a 20  $\mu$ L injection of the 10 ng/mL matrix-matched standard (equivalent to 50 ng/L from a 200 mL sample).

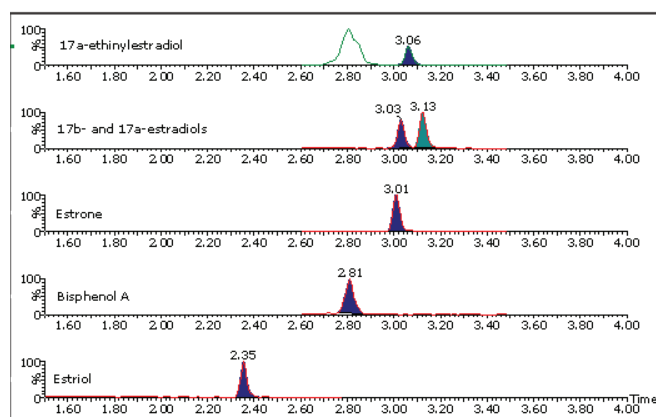


Figure 3. Sensitivity and selectivity of the Quattro Premier XE for 50 ng/L estrogens and bisphenol A in matrix-matched river water.

This sensitivity resulted in the estimated method LODs that are listed in Table 3. The method LODs shown here are sufficient to enable monitoring of real surface water samples but optimization of the sample preparation method, such as increasing the volume of water extracted, could improve LODs if required.

Compounds	Method LOD ng/L 200 mL sample
Estriol	4
Bisphenol A	2
Estrone	0.5
17 $\beta$ -estradiol	1
17 $\alpha$ -ethinyl estradiol	1.5
17 $\alpha$ -estradiol	1

Table 3. Estimated method LODs for estrogens and bisphenol A in matrix-matched standards.

Matrix interference can be observed in the 17 $\alpha$ -ethinylestradiol MRM chromatogram, however, this is chromatographically resolved from the peak and does not present any problems for the automatic integration algorithm in TargetLynx.

The ion ratio between the two acquired MRMs is fundamental when confirming the identity of compounds. Figure 4 illustrates the reproducibility of the ion ratio for three of the endocrine disrupting compounds at various concentrations in river water.

The twenty-five injections illustrated were taken from a fifty-five injection series containing matrix blanks and recovery samples. No ion ratio difference was greater than 20% for ion ratios > 0.5 (estriol) and no ion ratio difference was greater than 25% for ion ratios between 0.2 and 0.5 (bisphenol A and 17 $\alpha$ -estradiol). No specific legislation exists for endocrine disrupting compounds in surface water, however, these ion ratio differences meet the accepted legislation for pesticides<sup>6</sup> and veterinary drugs<sup>7</sup>.

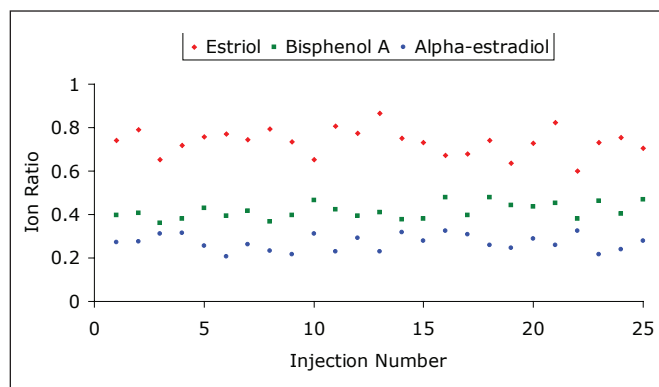


Figure 4. Reproducibility of the ion ratio between the two acquired MRMs for estriol, bisphenol A and 17 $\alpha$ -estradiol.

## CONCLUSION

A method has been successfully developed for the determination of six endocrine disrupting compounds in surface water sufficient to enable monitoring of real surface water samples.

ACQUITY UPLC conditions resulted in a 68% reduction in total run time compared to HPLC based methods, from 25 to eight minutes. This was achieved while maintaining sensitivity and chromatographic resolution between the structural isomers 17 $\alpha$ - and 17 $\beta$ -estradiol.

The sensitivity of the Quattro Premier XE for the six endocrine disruptors in solvent standards was very good with LODs of less than 0.1 ng/mL for all compounds.

The solid-phase extraction method gives good recovery for the six endocrine disrupting compounds with satisfactory reproducibility even at a concentration level of 10 ng/L.

In matrix-matched standards, the sensitivity was again fit for purpose with LODs of less than 5 ng/L for all compounds from a 200 mL sample volume of river water.

Confirmation of identity was achieved by acquiring two MRMs per compound. The reproducibility of the ion ratio was consistent across a sample batch of fifty-five injections meeting the closest available legislation that exists for pesticides and veterinary drugs.

The benefits of UPLC for a revenue conscious laboratory are shown with increased speed, along with reduced solvent usage, and the associated costs of solvents and solvent disposal.

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**Waters Corporation**  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)