Detection of Estrogens in Aqueous and Solid Environmental Matrices by Direct Injection LC-MS/MS

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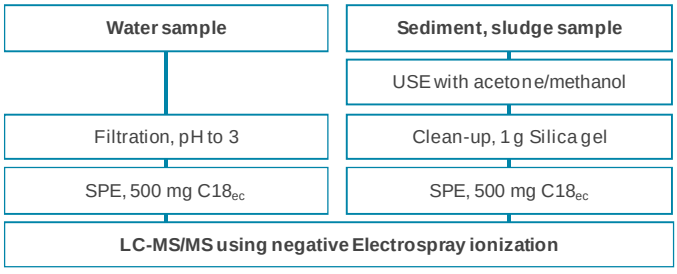
Introduction

Various analyses of water have shown ubiquitary presence of pharmaceutical residues in the aqueous environment.[1] Due to their ecotoxic effects native and synthetic estrogens, Estrone (E1), 17β-Estradiol (E2), Estriol (E3) and 17α-Ethinylestradiol (EE2), are of special relevance even at very low concentrations. A significant feminization could be observed at a concentration of approximately 1 pg/mL reflecting the strong endocrine potential of these compounds.[2] As a result of these very low concentrations a powerful analytical set-up is essential for their reliable detection and quantification. Residues of estrogens in aqueous and solid environmental samples are commonly analyzed by GC-MS^n, however the necessary derivatization steps are time consuming and laborious. This study investigates the power of LC-MS/MS for the analysis of estrogens, and compares a traditional Solid Phase Extraction (SPE) approach to direct injections of filtered wastewater, sediment and sludge samples.

Experimental

Sample Preparation

Direct injections of environmental samples were compared to samples prepared using the following procedure. Wastewater (250-500 mL), sediment (5 g), and sludge samples (0.5 g) were prepared according to the following scheme:



Liquid Chromatography

- Clean-up column: Phenomenex MercuryMS Luna C18(2) 20x2 mm, 3 µm
- Analytical column: Phenomenex Gemini 50x2 mm, 5 µm
- Eluent A: water, eluent B: acetonitrile
- Eluent C (post column): water + 2.5% NH_3

- Injection volume: 20 µL of extracts and 100 µL of wastewater without clean-up

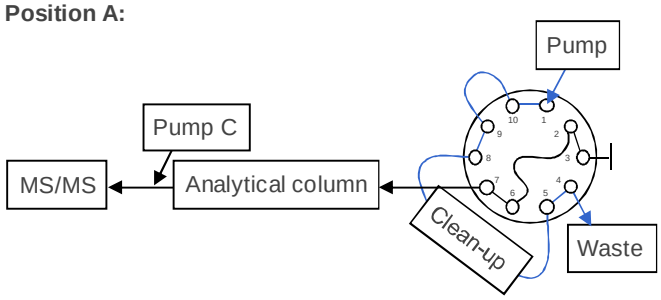
Table 1. LC Flow and Gradient

Time (min)	Flow (µl/min)	A/B	C Flow (µl/min)
0.0	1000	90/10	0
4.0	1000	90/10	0
4.5	250	90/10	10
15.0	250	34/66	10
16.0	250	0/100	10
20.0	250	0/100	10
4.0	Re-equilibration to 1000 µL/min and 90/10		

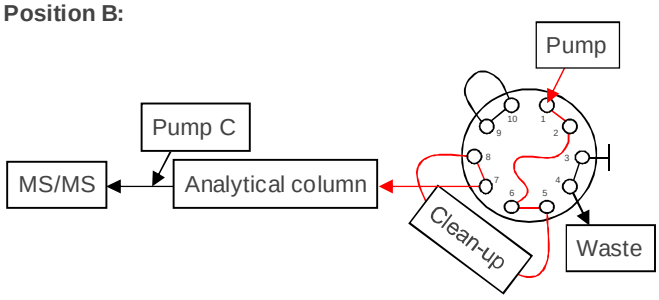
Switching Valve

Switching valve: 0.0 min position A, 4.6 min position B, 25.0 min position A For valve connections see the following schematics:

Position A:



Position B:



Mass Spectrometric Detection

An AB SCIEX API 5000™ LC/MS/MS system with Turbo V™ source with Electrospray Ionization (ESI) probe in negative polarity was used. Gas and source parameters:

CUR: 20 psi; GS1: 45 psi; GS2: 65 psi; TEM: 360°C (optimized for Ethinylestradiol); and CAD value: 7; IonSpray voltage (IS) was set to 0 V between 0.0-4.7 min and 20.0-21.0 min, while IS was set to -4500 V between 4.7- 20.0 min, respectively.

The following Multiple Reaction Monitoring (MRM) transitions were detected with a dwell time of 80 ms:

E1: 269/145, E2: 271/145, E3: 287/171, EE2: 295/145, Internal Standards E1-D4: 273/147, E2-13C2: 273/147and EE2-D2: 297/145.

Results and Discussion

Ecotoxic effects down to sub-ng/L levels in combination with the limited sensitivity of older LC-MS/MS systems are the reasons to use time consuming sample preparation steps. A typical sample preparation step is Solid Phase Extraction (SPE) of wastewater or Ultrasonic Extraction (USE) followed by SPE of sediment and sludge samples.[3-4] However, simultaneously matrix components are enriched as well, leading to an increased background which in the worst case leads to false positive results.

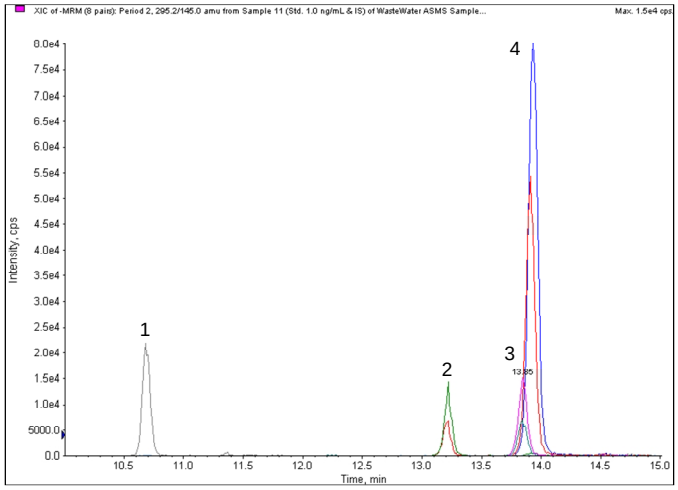


Figure 1. Chromatogram of E3 (1), E2 (2), EE3 (3), E1 (4) and their internal standards E1-D_4 and E2-$^{13}C_2$

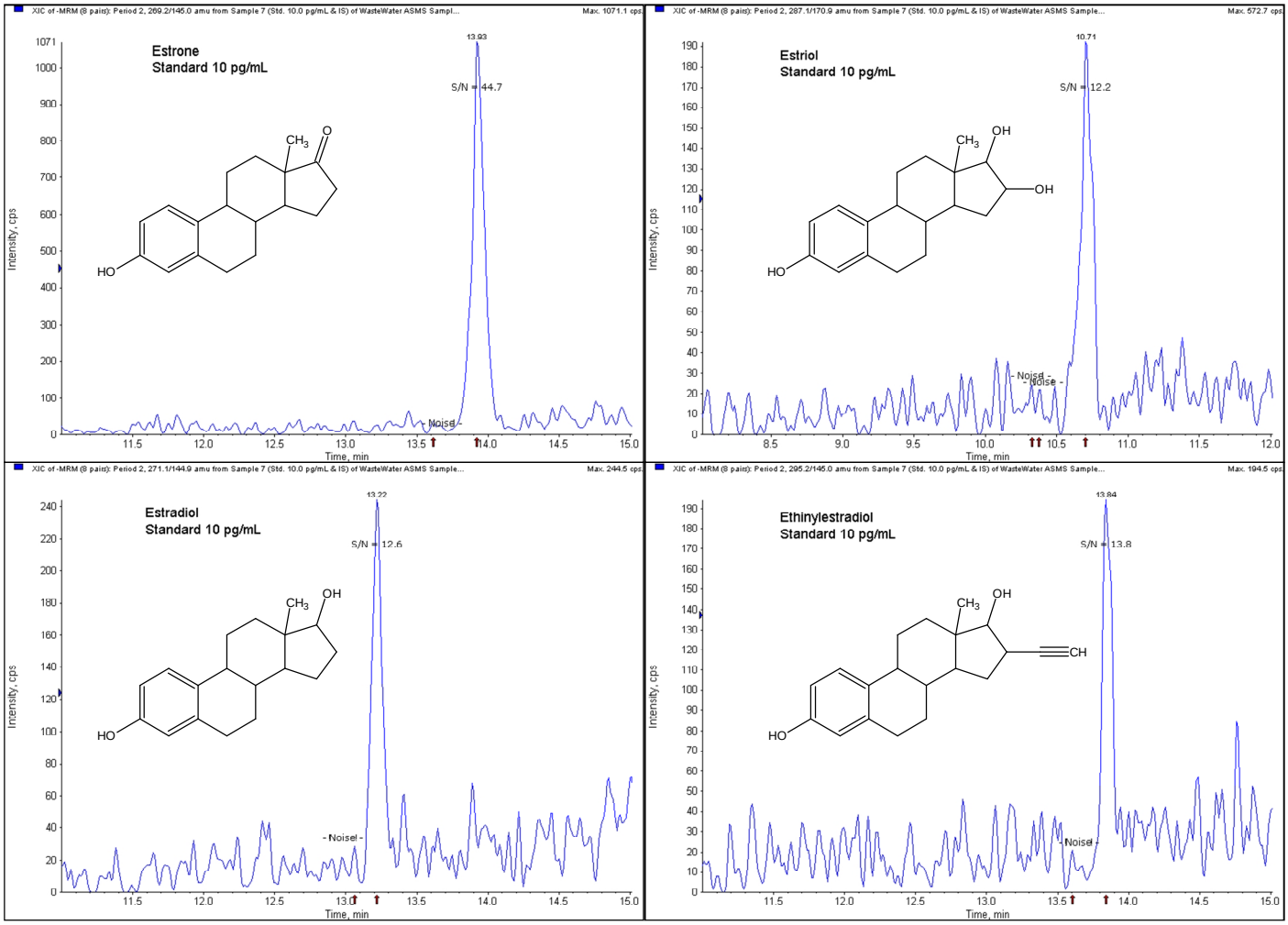


Figure 2. Injection of a 10 pg/mL standard of Estrone, Estradiol, Estriol, and Ethinylestradiol

An example chromatogram of all analytes and internal standards is presented in Figure 1. The ionization conditions were optimized for Ethinylestradiol, the least sensitive analyte. The use of APCI or APPI sources results in thermal degradation of Ethinylestradiol to Estradiol. Electrospray Ionization (ESI) in negative polarity with post column infusion of ammonia shows highest intensity. The sensitivity of these conditions using the API 5000™ LC/MS/MS system is presented in Figure 2. All investigated estrogens have a Signal-to-Noise ratio (S/N) higher than 10 at a concentration of 10 pg/mL.

It is known that Estriol can be entirely eliminated during clean-up. However, the direct injection of 100 µL of a filtered wastewater sample results in an Estriol response of 3.4e4 counts per second (Figure 3).

Extraction of sediment and sludge samples followed by a traditional SPE clean-up leads to high background as well as many interfering signals. Figure 4 shows chromatograms of Estrone, Estradiol, and Ethinylestradiol after a traditional clean-up procedure and non- SPE treated sediment samples which were diluted by a factor of 100. The analysis of non-SPE treated 100 times diluted samples delivers results with comparable S/N ratios to those samples analyzed after complete clean-up procedure.

Estriol could not be detected in sludge samples.

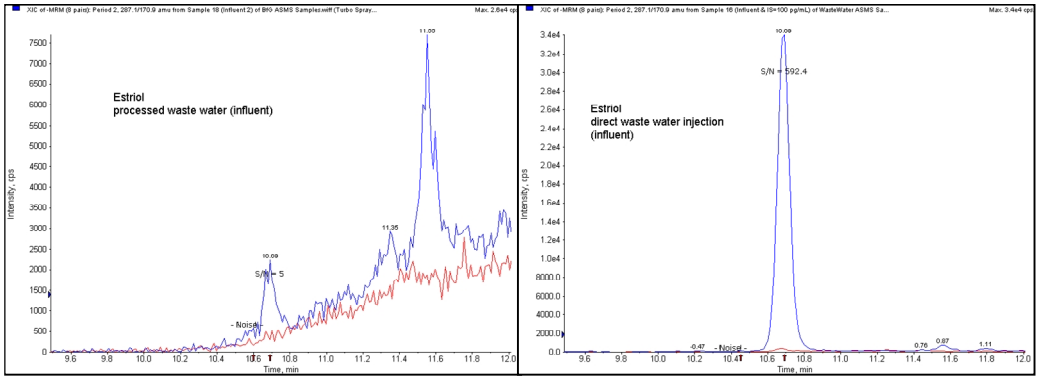


Figure 3. Comparison of Signal-to-Noise ratios of Estriol in extracted (left) and directly injected wastewater (right), red trace: internal standard

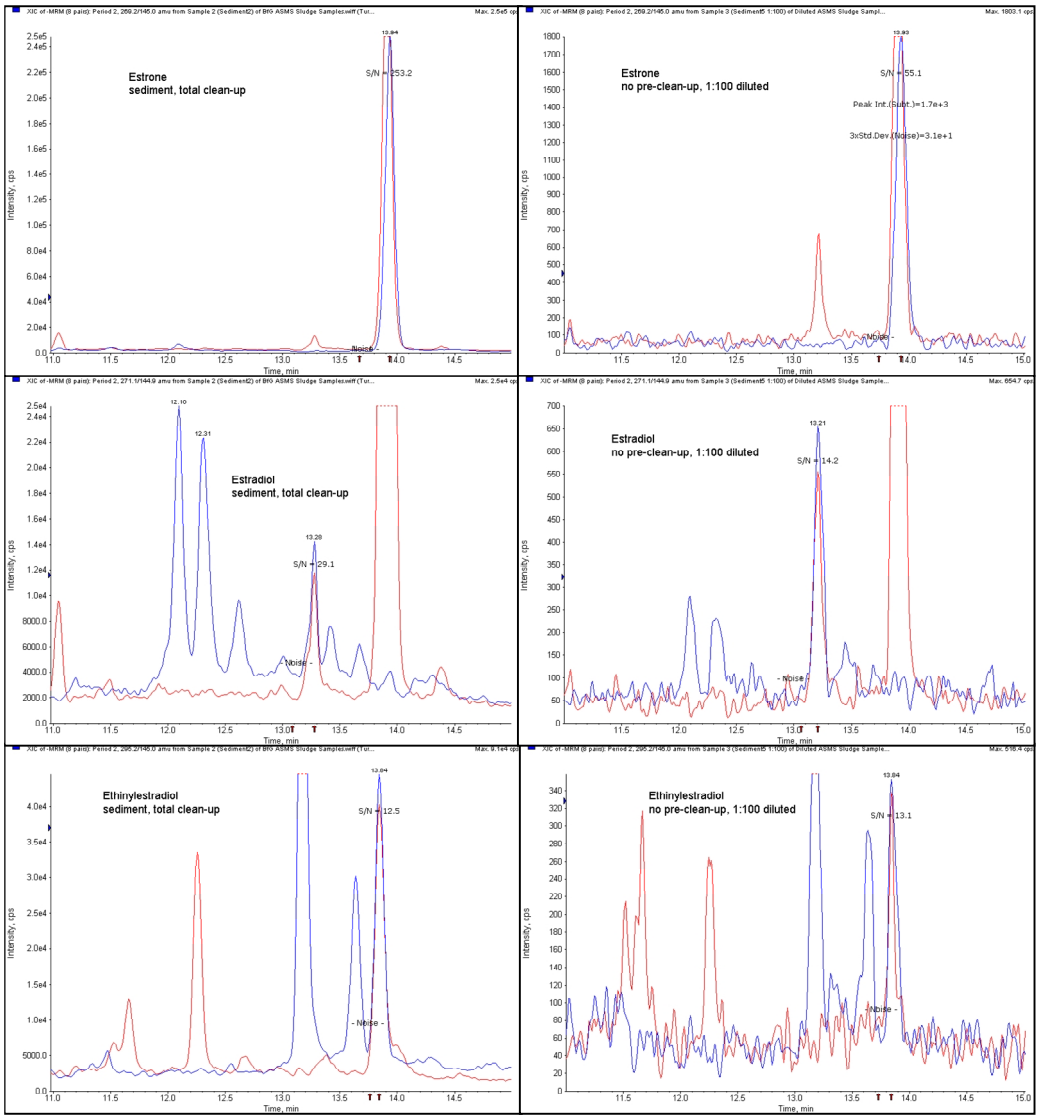


Figure 4. Comparison of Signal-to-Noise ratios of Estrone, Estradiol, and Ethinylestradiol in extracted sediments (left) and directly injected wastewater (right), red trace: internal standard

Summary

Limits of quantitation of all detected estrogens in a mix including Estrone, 17β-Estradiol, Estriol and 17α-Ethinylestradiol were found below 10 pg/mL. In this study highest sensitivity was achieved using Turbo V™ source in negative polarity on an API 5000™ LC/MS/MS with post column infusion of ammonia.

The developed direct injection method provides enough sensitivity to analyze estrogens in filtered wastewater samples with minimum sample preparation. This approach reduces time consuming sample preparation and avoids disturbing matrix signals. Furthermore it eliminates the loss of Estriol during traditional Solid Phase Extraction.

The improvement in sensitivity allows similar Signal-to-Noise ratios analyzing sediment and sludge samples to those prepared with traditional clean-up or a simplified procedure. Dilution of crude samples reduces the background noise and the presence of interfering signals while reducing time of sample treatment significantly.

References

1 Baronti et al.: Environ. Sci. & Technol. Vol. 34 (2000) 5059-50662

2 Routledgeet al.: Environ. Sci. & Technol. Vol. 32 (1998) 1559-1565

3 Ternes: Trends Anal. Chem. 20 (2001) 419-434

4 Ternes, et al.: Anal. Chem. 74 (2002) 3498-3504

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