Direct Determination of Trace Hormones in Drinking Water by Large Volume Injection at Sub ng/L Levels Using LC-MS/MS

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1. Introduction

Endocrine disrupting compounds enter the aquatic environment primarily through the discharge of treated and raw sewage and are detrimental to aquatic organisms even at sub nanogram per litre levels. In the majority of North American and European cities wastewater treatment plant effluent is indirectly re-used, through discharge into rivers which are also a source of drinking water. Consequently, there is the possibility that trace amounts may enter into drinking water even after special treatment processes. Several hormones are routinely monitored by the US EPA in drinking water as part of the Unregulated Contaminant Monitoring program (UCMR3). In this study, the LCMS-8050 triple quadrupole mass spectrometer was used for the highly selective and sensitive detection of hormones in water to meet the requirements of UCMR3. This direct high volume injection method of analysis avoids the disadvantages associated with extracting samples using SPE as is commonly performed. Ammonium fluoride as an aqueous mobile phase additive was found to significantly improve response for all studied hormones in comparison to ammonium hydroxide. The excellent sensitivity of the final method provided detection limits ranging from 0.005 ng/L (testosterone) to 0.330 ng/L (17-α-ethynylestradiol).

2. Materials and Methods

Liquid chromatography	
UHPLC	Nexera LC system
Analytical column	Shim-pack XR-ODS III column (150 x 2 mm, 2.2 µm)
Column temperature	45°C
Column fitted between the mixer and autosampler	Kinetex XB-C18 column (50 x 2.1, 1.7 µm particle size)
Injection cycle	3 x 400 µL injections (500 µL loop fitted) Total injection volume 1200 µL
Flow rate	0.3mL/minute
Solvent A	0.15mM ammonium fluoride
Solvent B	Methanol
Binary Gradient	10% B (0min) →10% B (0.3min) → 45% B (1min) → 100%B (15min) → 100% B (17min) → 10% B (17.1min) → 10% B (22min)
Mass Spectromtery	
LC-MS/MS	LCMS-8050
Dwell times	10 – 100 ms
Polarity switching time	5ms
Interface/Heating block temp.	400°C / 400°C
Heating/Drying/Nebulising gas	10 / 5 / 2.8 L/min

3. Results and Discussion

3.1. Method Development

Previously published methods for the analysis of endocrine disruptors have typically used ammonium hydroxide as the mobile modifier and it is the currently recommended approach in EPA method 539. In this study ammonium fluoride was tested at different concentrations (0.1, 0.2, 0.3 and 0.5mM) in the aqueous phase, with methanol used as the organic phase. Improved response was observed for all hormones using ammonium fluoride, in comparison to ammonium hydroxide, as is shown in Figure 1. Ammonium fluoride (approx. pH 6) offers further benefits in comparison to ammonium hydroxide (approx. pH 9.5) as the lower pH means that analytical columns, other than those stable at high pH, can be employed.

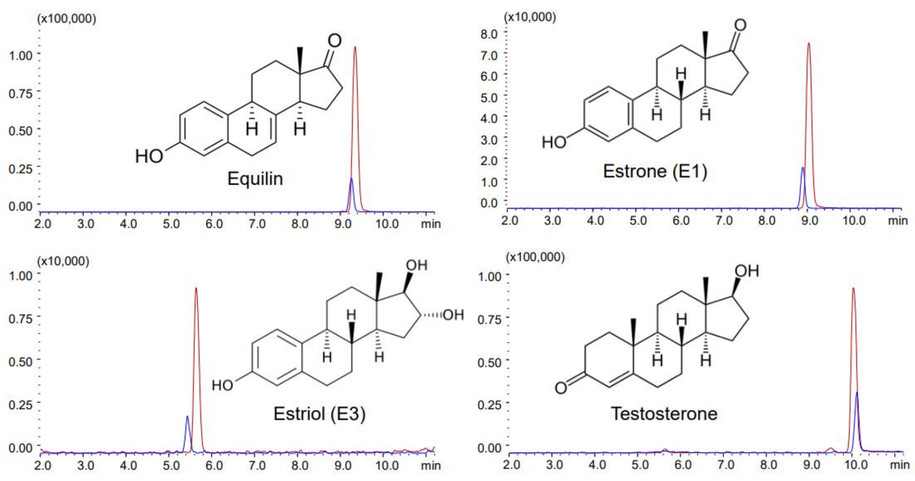

Figure 1: Comparison between the response generated using ammonium hydroxide (blue trace) and ammonium fluoride (red trace). Ammonium fluoride delivers an increased S/N for all compounds (for example, equilin x4.0, estrone x4.8, estriol x4.5 and testosterone x2.8

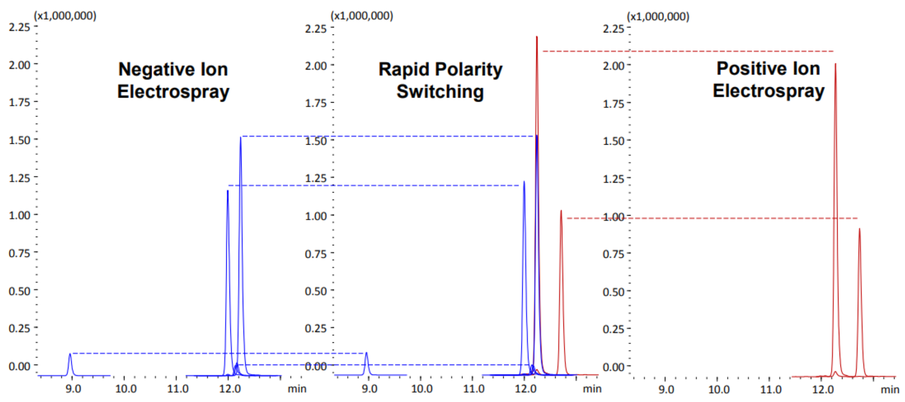

Figure 2: Rapid positive/negative switching using a 5ms switching time results in the highest data quality for all target hormone compounds in a single analysis

3.2. Final Method Performance

In order to test the performance of the developed method, limits of detection, linearity, repeatability (low and high concentrations), and longer term robustness were assessed. Linearity was assessed from 0.5 x the required reporting level to 100 x times the reporting level. The concentration for each compound in spiked drinking water is listed in Table 1. All seven hormones achieved excellent correlation coefficients R2>0.999 (Linear, 1/C, zero not forced). Calibration curves for equilin, estradiol, androstenedione and testosterone are shown in Figure 2. Peak area reproducibility (n=8) was assessed at the reporting level corresponding to 'low concentration' (level 2) and a 'high concentration' (level 5). At the low concentration repeatability was < 4.3 %RSD, with the exception of 17-α-ethynylestradiol (12.2 %RSD). At the high concentration repeatability was < 3.9 %RSD for all compounds.

Compound	Level 1 (ng/L)	Level 2 (ng/L)	Level 3 (ng/L)	Level 4 (ng/L)	Level 5 (ng/L)	Level 6 (ng/L)	Level 7 (ng/L)	Level 8 (ng/L)
Equilin	2	4	8	20	40	80	200	400
Estrone	1	2	4	10	20	40	100	200
17-α-Ethynylestradiol	0.45	0.9	1.8	4.5	9	18	45	90
Estriol	0.4	0.8	1.6	4	8	16	40	80
17-β-Estradiol	0.2	0.4	0.8	2	4	8	20	40
Androstenedione	0.15	0.3	0.6	1.5	3	6	15	30
Testosterone	0.05	0.1	0.2	0.5	1	2	5	10

Table 1: Concentration of each compound in the calibration series in drinking water

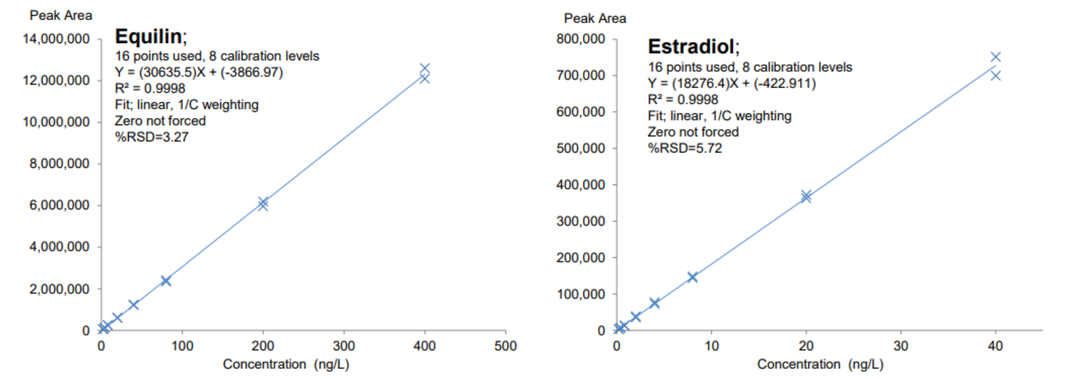

Figure 3. Calibration curves for equilin (2-400ng/L), and estradiol (0.2-40ng/L), spiked into drinking water

Hormone limits of detection were calculated based on the method described by the EPA Method 539. Using the developed method on the LCMS-8050 detection limits ranged from 0.0058 ng/L for testosterone to 0.33 ng/L for 17-α-ethynylestradiol.

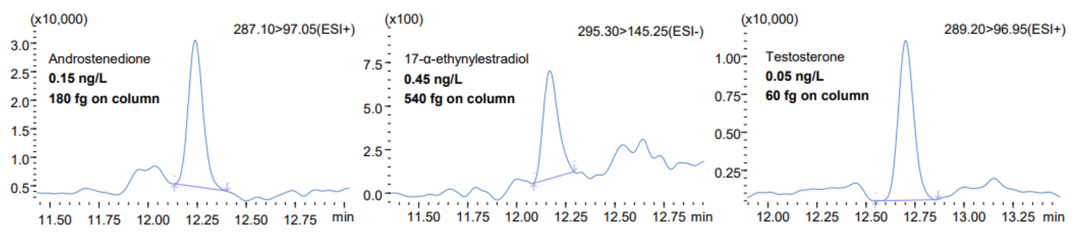

Figure 4. MRM chromatograms of target hormones at the lowest calibration standard (level 1) using an injection volume of 1200uL.

To assess the robustness of system, repeat injections were performed over a 62 hour period using drinking water spiked at level 5. Figure 5 displays the results for estriol, estradiol and EE. These three hormones were selected as they had the lowest S/N at the required reporting level.

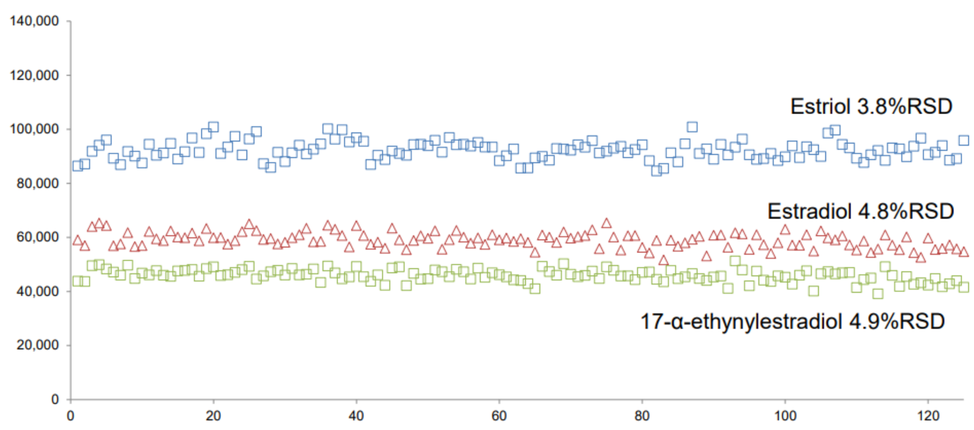

Figure 5. Peak area response for three hormones over 62 hours. The legend displays the %RSD for each compound. Y-axis displays the injection number

4. Conclusion

A fast, selective and highly sensitive method has been developed for the measurement of hormones in drinking water. By integrating a direct high volume injection cycle with a fully optimised LC/MS/MS method, the LCMS-8050 delivers precise and accurate detection limits regulated by EPA method 539 and is in accordance with UCMR3.