Expanded Analysis of Human Hormones in Drinking Water Using Solid Phase Extraction and Liquid Chromatography Tandem Mass Spectrometry

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ABSTRACT

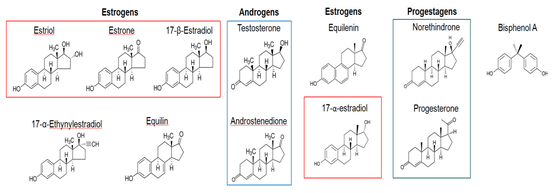
The presence of hormones in drinking water is a human health concern. As a result, several of these hormones are routinely monitored as part of the U.S. Environmental Protection Agency (EPA) Unregulated Contaminant Monitoring Rule 3 (UCMR3). To monitor the levels of the seven most common hormones in drinking water, EPA Method 539 was developed. The work presented here updates this method to include five additional hormones and describes the use of an automated solid phase extraction (SPE) system containing high-surface-area, reversed-phase (HRPHS) cartridges, followed by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) with timed selected-reaction monitoring (TSRM) mode for detection and quantification. The results of chromatographic separation, SPE recoveries, and Lowest Concentration Minimum Reporting Level (LCMRL) are presented.

INTRODUCTION

Hormones in drinking water are a human health concern, although safe exposure limits have yet to be established due to the need for further studies to determine the impact of long-term and synergistic exposure.[1] Hormone pharmaceuticals often end up in the sewage system as a result of excretion and disposal of unwanted quantities. These may not be effectively removed during wastewater treatment, and as a result, significant amounts of these hormones may be present in drinking water sources. Estriol, estrone, estradiol, ethynylestradiol, equilin, androstenedione, and testosterone (Figure 1) are routinely monitored as part of the U.S. Environmental Protection Agency (EPA) Unregulated Contaminant Monitoring program.[2]

Figure 1. Human Hormones Monitored in EPA Method 539.

Figure 2. Additional Hormones Monitored in Revised Method.



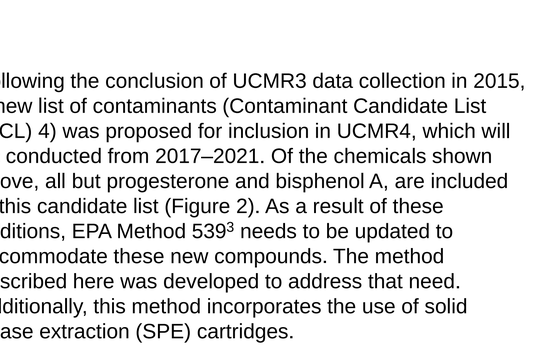
Following the conclusion of UCMR3 data collection in 2015, a new list of contaminants (Contaminant Candidate List (CCL) 4) was proposed for inclusion in UCMR4, which will be conducted from 2017–2021. Of the chemicals shown above, all but progesterone and bisphenol A, are included in this candidate list (Figure 2). As a result of these additions, EPA Method 539[3] needs to be updated to accommodate these new compounds. The method described here was developed to address that need. Additionally, this method incorporates the use of solid phase extraction (SPE) cartridges.

MATERIALS AND METHODS

Instrumentation

- Thermo Scientific™ Dionex™ AutoTrace™ 280 Solid Phase Extraction instrument
- Thermo Scientific™ Dionex™ SolEx™ Hydrophobic Reversed Phase High-Surface area (HRPHS) Resin SPE cartridges
- Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
- Thermo Scientific™ Acclaim™ Rapid Separation LC (RSLC) Polar Advantage II column (2.1 × 150 mm)
- Thermo Scientific™ TSQ Endura™ Triple Quadrupole Mass Spectrometer

Figure 3. Solid Phase Extraction.



Water samples were collected in bottles containing preservatives. Hormone extraction was automated using the Dionex AutoTrace 280 SPE instrument, extracts dried, and the resultant resuspended in 50% methanol (Figure 3). The Dionex SolEx HRPHS cartridges contain a neutral resin comprised of high-surface-area, divinylbenzene-based particles that have both hydrophilic and reversed-phase properties.

Figure 4. HPLC and MS Systems.

Injection volume	50 µL
Column temperature	30 °C
Mobile phase A	1 mM Ammonium fluoride in water
Mobile phase B	1 mM Ammonium fluoride in methanol
Flow rate	200 µL/min

Gradient:

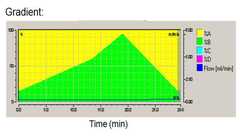
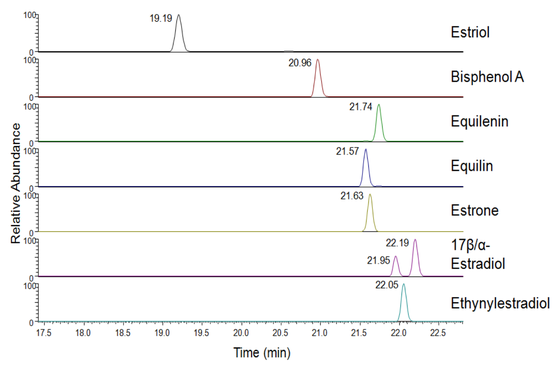


Table 2. MS Conditions.

Ion source	HESI III
Spray voltage (Polarity Switching)	3000 V (-) 3250 V (+)
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	15 arbitrary units
Sweep gas pressure	1 arbitrary units
Ion transfer capillary temperature	300 °C
Vaporizer temperature	350 °C
Scan type	SRM

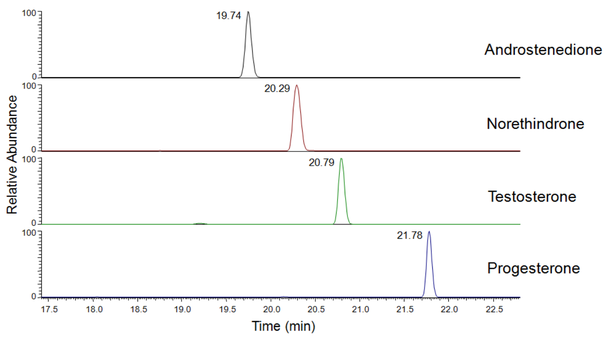
RESULTS

Figure 5. MS Chromatograms – Negative Ionization Mode.



The chromatographic conditions were optimized to ensure separation of the isobaric compounds 17-β- and 17-α-estradiol. All of the analytes were well resolved (Figures 5 and 6), with the exception of equilin and estrone, but because these have different mass/charge (*m/z*), baseline resolution was not critical for this pair.

Figure 6. MS Chromatograms – Positive Ionization Mode.



The method accuracy and precision were determined from five replicates of samples fortified with a mid - calibration concentration of hormones (Figures 7 and 8). The recoveries for reagent (deionized) water ranged from 86–129% with RSDs from 6–19. For drinking water, recovery was 64–130% with RSDs from 3–17. The generally acceptable range of % recovery (70–130) is highlighted in green.

Figure 7. Accuracy and Precision of Hormones in Reagent Water.

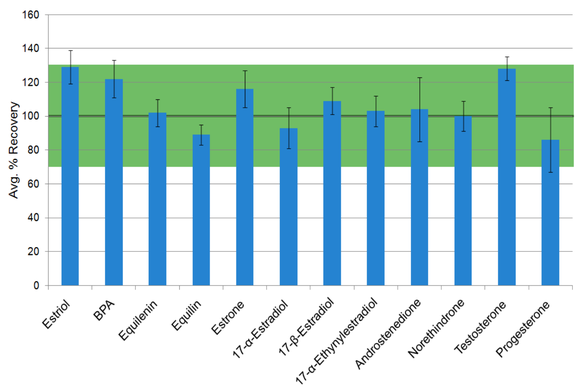


Figure 8. Accuracy and Precision of Hormones in Drinking Water.

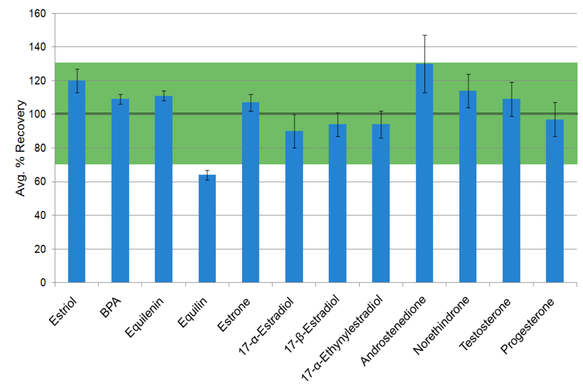


Table 3. Lowest Concentration Minimum Reporting Levels.

Hormone	Draft Method Data (ng/L)	Calculated (ng/L)*
Estriol	4.3	63
Bisphenol A	111	80
Equilenin	2.7	6.7
Equilin	5.2	0.15
Estrone	2.4	3.3
17-α-Estradiol	3.5	1.8
17-β-Estradiol	6	0.67
17-α-Ethynylestradiol	23	9.2
Androstenedione	0.17	0.16
Norethindrone	0.36	0.71
Testosterone	0.031	0.021
Progesterone	0.072	0.069

*Four replicates of seven concentrations

The Lowest Concentration Minimum Reporting Level (LCMRL) for each hormone was determined by entering the data from four replicates of seven different concentrations of hormone into the LCMRL calculator application that was developed by the U.S. EPA. Most of the LCMRLs were comparable if not better (green) than the sample data from the draft method (Table 3). The exception was estriol, which was about 10-fold higher than expected. When data was corrected for surrogate recovery, the value obtained was closer to that expected (7.3 ng/L).

CONCLUSIONS

- EPA Method 539 has been revised to include automated extraction of hormones in drinking water using the Dionex AutoTrace 280 Solid Phase Extraction instrument with Dionex SolEx HRPHS SPE cartridges
- Chromatographic conditions using the Dionex UltiMate 3000 RSLC system with an Acclaim Rapid Separation LC Polar Advantage II column separated the isobaric hormones, 17-β- and 17-α-estradiol, with baseline resolution
- Data collected using polar switching on a TSQ Endura Mass Spectrometer produced LCMRLs that were comparable to those included in a draft revision of EPA method 539
- Accuracy and precision were within the range of 64–130% and 3–19, respectively

REFERENCES

1. World Health Organization (WHO), United Nations Environment Programme, Inter-Organization Programme for the Sound Management of Chemicals. State of the Science of Endocrine Disrupting Chemicals 2012: Summary for Decision-Makers. Bergman, A.; Heindel, J.J.; Jobling, S.; Kidd, K.A.; Zoeller, R.T. WHO Document Number WHO/HSE/PHE/IHE/2013.1.

2. U.S. Environmental Protection Agency (EPA). Basic Information about the Unregulated Contaminant Monitoring Rule 3 (UCMR3). http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/basicinformation.cfm#four

3. Smith, G.A.; Zaffiro, A.D.; Zimmerman, M.L.; Munch, D.J. Method 539: Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS). Document No. 815-B-10-001. U.S. Environmental Protection Agency, Cincinnati, OH, 2010.

TRADEMARKS/LICENSING