APPLICATION NOTE

Quantitative Comparison of Hormones in Drinking Water Between MS/MS and Orbitrap Technology

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Key Words

Contaminants of emerging concern, CEC, endocrine disrupting compound, EDC, micropollutants, EPA Method 539, Q Exactive

Goal

To demonstrate a liquid chromatography – high-resolution, accurate mass (LC-HRAM) methodology using Orbitrap™ technology as a sensitive, accurate, and reliable alternative to the use of triple quadrupoles mass spectrometers in the quantification of hormones in drinking water according to EPA guidelines.

Introduction

Increasingly, contaminants of emerging concern (CEC) including pharmaceuticals and personal care products, such as the contraceptive pill and antibiotics, are being detected at low levels in surface water. Many of these CEC are endocrine disrupting compounds (EDCs), which can alter the normal functions of hormones and cause a variety of health effects.[1,2] As a result, the United States Environmental Protection Agency (EPA) has developed EPA Method 539[3] for the Unregulated Contaminant Monitoring Rule 3 (UCMR 3) program, which collects data for contaminants suspected to be present in drinking water but that do not have health-based standards set under the Safe Drinking Water Act (SDWA).[4]

The identification and quantification of micropollutants at low concentrations requires both sensitivity and selectivity against complex matrices. Selected reaction monitoring (SRM) of precursor-product ion transitions, which makes use of a triple quadrupole mass analyzer, has been the method of choice.[5] However, other screening strategies employing full scan mode and other advanced MS/MS scan modes can potentially offer a valuable alternative to SRM based methodology due to the development of more rugged, sensitive, and selective instrumentation.

The quantitative performance of the latest generation of high-resolution instruments is comparable to that of a triple quadrupole MS, even though different scanning modes are used. Higher-resolution instrumentation also allows flexibility concerning compound identification because the experiment can be set up for targeted quantitation, screening, or both. In an Orbitrap-based instrument, the parallel reaction monitoring (PRM) mode performs most closely to a triple quadrupole mass analyzer using SRM mode. This study compares the quantitation performance between a triple quadrupole (MS/MS) to that of an Orbitrap-based detector using EPA Method 539: *Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization and Tandem Mass Spectrometry (LC-ESI-MS/MS).* All other aspects of the method including sample preservation, storage, preparation, and chromatographic separation were kept the same. The only difference was the MS detector.

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Experimental

Sample Preparation

The sample preparation is based on EPA Method 539. Any modifications and text are highlighted for clarity and discussion purposes. Five hundred milliliters of a dechlorinated sample with Omadine™ biocide was extracted through solid phase extraction (SPE) using an octadecyl (C-18) stationary phase after adding surrogates. The eluent from SPE was concentrated to dryness and then diluted to 1 mL with 50:50 methanol/water. An aliquot was injected into the LC-MS/MS after adding internal standards and quantified against the internal standard (IS).

LC-MS Conditions

Under the EPA Method, flexibility is allowed for columns, eluents, and MS conditions in general. Table 1 shows the conditions optimized and used in the analysis.

Table 1. LC-MS conditions optimized and used for the experiments described.

Mass Analyzer	Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer
Mass Resolving Power	70,000 (FWHM) at *m/z* 200
Scan Mode	PRM
AGC	2e5
IT	200 ms
Isolation Window	1.0 *(m/z)*
HPLC	Thermo Scientific™ UltiMate™ 3000 RS UHPLC, binary pump, autosampler, and column heater with 100 µL sample loop
Column	Thermo Scientific™ Acclaim™ PolarAdvantage II (2.1 x 150 mm, 3 µm, 120 Å, P/N 063187)
Eluents	A) 1 mM ammonium fluoride in water B) 50:50 (v/v) acetonitrile/methanol Gradient flow at 0.3 mL/min with a 21.4 min run
Injection Volume	50 µL

EPA Method 539 uses a triple quadruple method using an SRM scan mode (also known as MRM). According to EPA Method 539, section 3.16, "MRM... a mass spectrometric technique in which a precursor ion is first isolated, then subsequently fragmented into a product ion(s). Quantitation is accomplished by monitoring a specific production." In this study, a similar set of conditions was used.

In PRM mode, a list of targeted precursor ions, retention times, and collision energies can be included in the method. When detecting a targeted ion, the system isolates that precursor ion in the quadrupole and triggers MS/MS experiments, generating MS/MS spectra that can be used for both quantitation and identification. Both the quantitation and identification are performed taking into account product ions generated after the isolation of a specific precursor ion. This operating mode is similar to an SRM (or MRM) experiment using a triple quadrupole instrument. In PRM mode, the third quadrupole is substituted with an HRAM (high-resolution, accurate mass) mass analyzer, enabling the parallel detection of all target product ions (Figure 1).

The number of scans across the chromatographic peak is dependent on the cycle time of the instrument and, therefore, on the set of conditions used (e.g., resolving power). These conditions can and should be optimized depending on the objectives of the experiment. In this case, accurate quantitation as well as unambiguous identification has been targeted. Optimized conditions can be found in Table 1.

Requirements

The EPA has strict requirements that should be met before the analysis of any sample, referred to as the Initial Demonstration of Capability (IDC). These requirements include the demonstration of low background noise, precision by analyzing four to seven extracted laboratory fortified reagent water blanks (LFB) at mid-level, the demonstration of accuracy and, finally, the demonstration of capability necessary to meet the minimum reporting limit (MRL). The percent relative standard deviation (%RSD) of the results of the replicate analyses must be ≤ 20%. The average percent recovery for each analyte must be within ± 30% of the true value.

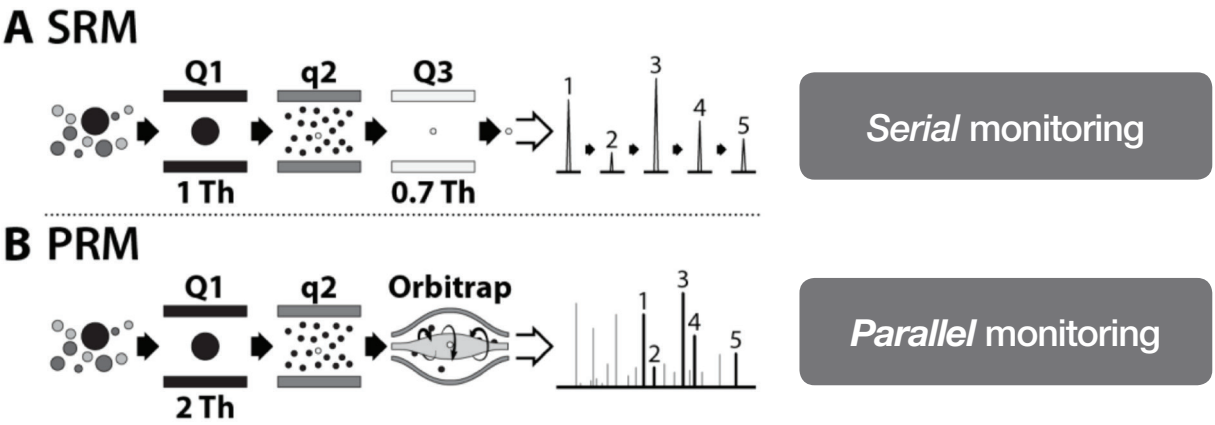

Figure 1. Schematic representation of selective reaction monitoring (SRM) mode and parallel reaction monitoring (PRM) mode.

Results and Discussion

Excellent linearity has been demonstrated from a range starting at one-fourth of the MRL (Figure 2). Table 2 compares the MRL and LCMRL obtained when using both SRM and PRM modes. Tables 3, 4, and 5 summarize precision and accuracy of the method after the LC-HRAM analysis of different types of samples—reagent water spiked at different levels and UCMR3 water samples.

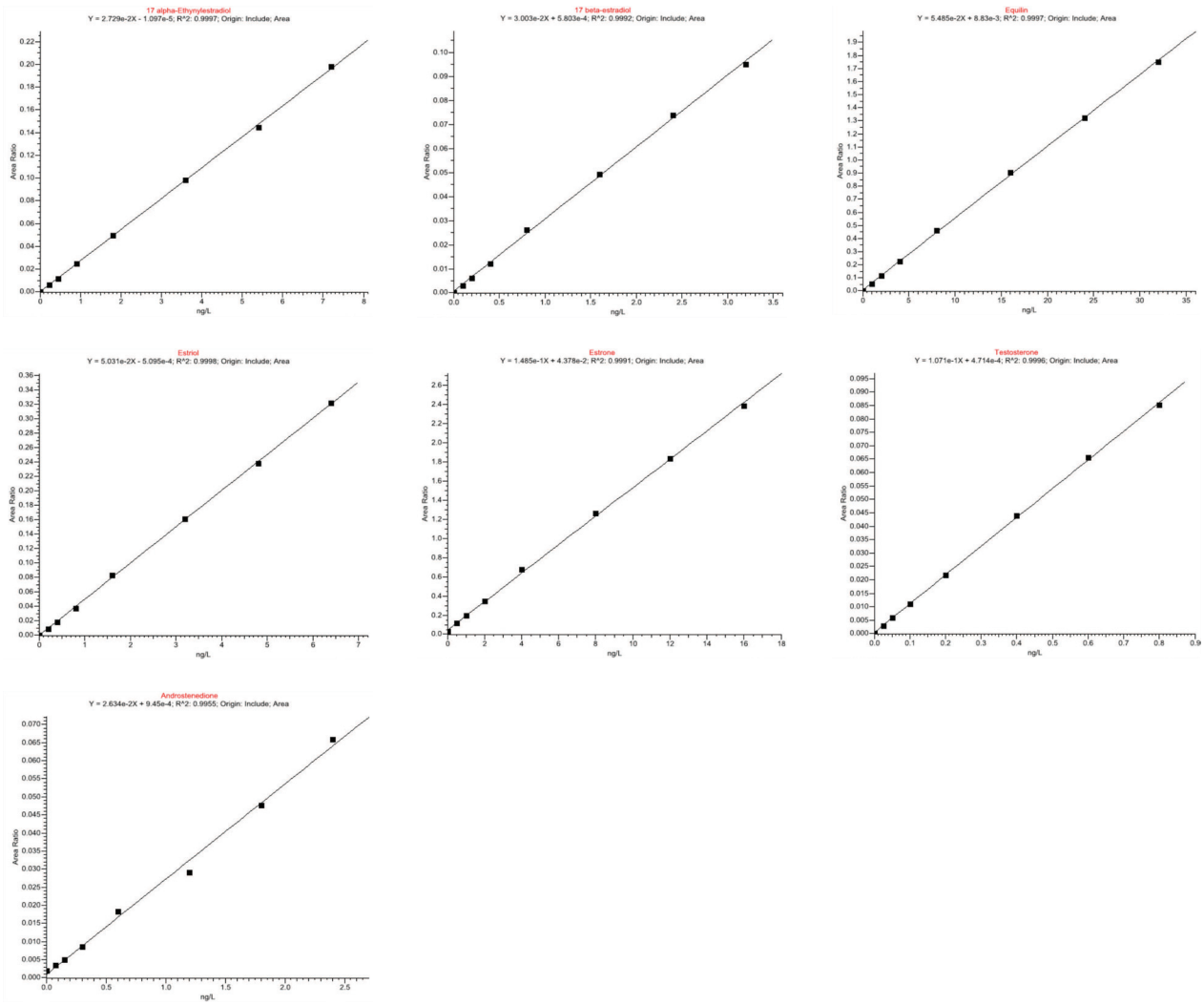

Figure 2. Calibration curves for all EPA Method 539 analytes.

Table 2. MRL and LCMRL comparison when using triple quadrupole and Orbitrap mass analyzers in reagent water preserved according to EPA Method 539.

Analyte	UCMR3 MRL (ng/L)	EPA 539 published LCMRL (ng/L)	LC-HRAM[a] LCMRL (ng/L)	LC-HRAM[a] LCMRL Calc -DL (ng/L)
17α-ethynylestradiol	0.9	1.3	Critical level 0.05[b]	0.1
17β-estradiol	0.4	0.32	0.17	0.047
equilin	4	0.28	Critical level 0.23[b]	0.48
estriol	0.8	3	0.27	0.2
estrone	2	4	0.84	0.48
testosterone	0.1	0.062	0.033	0.027
4-androstene-3,17-dione	0.3	0.37	0.19	0.08

[a]The detection limits reported in EPA Methos 539 reflect the MS/MS, Ion Trap, and Hybrid MS technology used at the time of method validation. They are shown here for reference purposes. Detection limits for newer MS/MS instruments can either be lower or higher depending on many variables including operator performance, instrumentation, sample preparation, and other factors. Thus, the lower DL for Orbitrap technology shown here demonstrate that quantitatively the results are comparable with the reported method.

[b]The critical level calculation can't find the MRL as the lowest standard wasn't low enough for exact determination. Thus a lower level spiking concentration is required to determine the LCMRL for these compounds.

As shown in Table 2, the LCMRL and DL were much lower when using LC-HRAM than the detection limits reported in EPA Method 539. This demonstrates the greater sensitivity using Orbitrap HRAM compared to the MS/MS and hybrid instruments used during method validation. In order to demonstrate method robustness, the EPA requires the demonstration of performance using a fortified matrix in blanks, reagent water, and real samples. Results are summarized in Tables 3, 4, and 5.

Table 3. LC-HRAM method: Precision and accuracy in fortified reagent water spiked at 10 x MRL.

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17α-ethynylestradiol	7.2	82%	4
17β-estradiol	3.2	84%	3
equilin	32.0	81%	3
estriol	6.4	100%	4
estrone	16.0	83%	4
testosterone	0.8	87%	5
4-androstene-3,17-dione	2.4	85%	8

n=4

Table 4. LC-HRAM method: Precision and accuracy in fortified matrix (UCMR3 water sample 1) spiked at MRL.

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17α-ethynylestradiol	0.72	95%	2
17β-estradiol	0.32	87%	1
equilin	3.20	92%	8
estriol	0.64	101%	4
estrone	1.60	95%	3
testosterone	0.08	99%	0.1
4-androstene-3,17-dione	0.24	118%	0.1

n=4

Table 5. LC-HRAM method: Precision and accuracy in fortified matrix (UCMR3 water sample 2) spiked at 10 × MRL.

Analyte	Fortified Concentration (ng/L)	Avg. %Recovery	%RSD
17α-ethynylestradiol	7.2	98%	3
17β-estradiol	3.2	113%	0.8
equilin	32.0	102%	0.7
estriol	6.4	103%	2.4
estrone	16.0	110%	1.7
testosterone	0.8	103%	0.3
4-androstene-3,17-dione	2.4	104%	1.4

n=4

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Conclusion

The LC-HRAM methodology proved to be sensitive, accurate, reproducible, and a reliable alternative to the use of triple quadrupoles in the quantification of hormones in drinking water according to the EPA guidelines. By the use of different scanning modes within the Q Exactive MS, quantitation on precursor ions and identification of fragments ions are possible. These scanning modes are consistent with the requirements in many regulated methods and can possibly be used for compliance monitoring in place of a triple quadrupole MS. The latest LC-HRAM technology assures sensitivity and selectivity in the quantitation of known contaminants in drinking water, while potentially enabling the combination of targeted and non-targeted analysis in the same run, which cannot be accomplished using MS/MS alone.

References

1. https://www.epa.gov/wqc/contaminants-emerging-concern-including-pharmaceuticals-and-personal-care-products (last accessed May 5, 2016).

2. Winslow, S.D.; Pepich, B.V.; Martin, J. J.; Hallberg, G.R.; Munch D.J.; Frebis, C.P.; Hedrick, E.J.; Krop, R.A. Statistical Procedures for Determination and Verification of Minimum Reporting Levels for Drinking Water Methods. *Environ. Sci. Technol.* **2006**, *40*, 281-288.

3. EPA 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)' version 1.0 (2010).

4. Unregulated Contaminant Monitoring Rule 3 (UCMR3) http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/ (last accessed May 5, 2016).

5. Richardson, S.D. and Ternes, T. A. Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem.* **2014**, *86,* 2813-2848.

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