Rapid Determination of Ethynylestradiol (17αEE2) to 15 pg/L in wastewater using Thermo Scientific EQuan MAX Plus LC-MS on-line SPE and Thermo Scientific Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer

Neville Llewellyn[1]; Gary Woffendin[1]; Olaf Scheibner[2]; Jonathan Beck[3]; Thermo Fisher Scientific, London, UK; [2]Thermo Fisher Scientific, Dreieich, Germany[3]Thermo Fisher Scientific, San Jose, CA, USA.

ABSTRACT

Purpose: To demonstrate the feasibility of using a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitap™ mass spectrometer with the Thermo Scientific™ EQuan MAX Plus™ LC-MS on-line solid phase extraction system to achieve the EU Water Frame Framework LoD for 17αEthynylestradiol (35 pg/L) and provide confirmation.

Methods: EQuan MAX Plus LC-MS on-line SPE High Resolution Accurate Mass using Parallel Reaction Monitoring

Results:

- Excellent quantitation and confirmation performance
- LoD - 15 pg/L
- LoQ - 29 pg/L
- Analysis in under 30 minutes - approximately 30 x faster than current methods
- Potential chromatographic resolution of 17αEE2 and 17β-EE2

INTRODUCTION

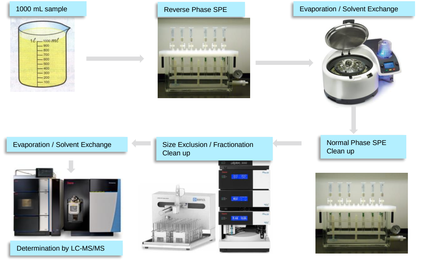
The occurrence and effects of endocrine disrupting compounds (EDCs), particularly mixtures, in aquatic environments is a significant concern[1] . Of the many EDCs, 17αEE2 is recognised as possessing the greatest estrogenic potency and risk to freshwater ecosystems and drinking water resources[2].

Due to its environmental significance, 17αEE2 was incorporated into the EU Water Framework Directive, with a stipulated Limit of Detection of 35 pg/L, which presents a significant analytical challenge.

Current methods generally involve large-volume SPE; normal phase SPE clean up and size exclusion fractionation, which take considerable time, expense, and sampling logistics[3] (Figure 1).

The aim of this work is to assess the feasibility and performance of using a 5 mL sample on-line solid phase extraction and a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer for the determination of 17αEE2 at the WFD LoD of 35 pg/L[4].

Figure 1. Typical workflow for steroid estrogen analysis.



MATERIALS AND METHODS

Liquid Chromatography

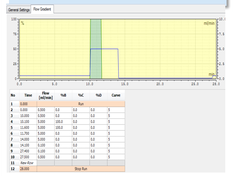
Liquid chromatography separations were carried out on the EQuan™ UHPLC system including binary analytical pump, CTC autosampler, quaternary loading pump and column compartment; see Figure 2.

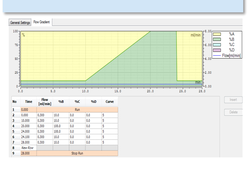
Loading Conditions:
Column: Thermo Scientific™ Accucore™ aQ 12 µm 5x2.1
Injection volume: 5 mL
Loading rate: 0.5 mL/min
Loading solvent: HPLC water
Column Wash Solvent: MeOH

Syringe and valve Cleaning Solvents:
1 - 50% MeOH & 50% ACN
2 - 50% HPLC Water

Analytical Conditions:
Column: Thermo Scientific™ Acclaim™ VANQUISH™ Polar Advantage 150x2.1, 2.2 µm
Injection volume: na mL
Flow rate: 0.3 mL/min
Needle Wash Solvent: 50%MeOH & 50% ACN

Mobile phase:
A - 0.3 mM Ammonium Fluoride
B - 0.3 mM Ammonium Fluoride in MeOH





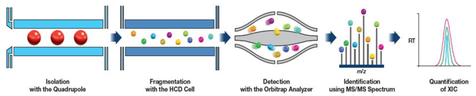
Mass Spectrometry

The MS analysis was performed on a Thermo Scientific Q Exactive Focus hybrid quadrupole-Orbitrap bench-top high resolution mass spectrometer using heated electrospray ionization (HESI). Acquisition and quantitation was performed using Parallel Reaction Monitoring (PRM) where MS/MS data were collected at a resolving power of 70,000 (FWHM m/z 200) in negative polarity; see Figure 3.

The following parameters were used:
Ionization mode: Negative HESI; Scan Mode (PRM): 195.1705 m/z; Ion source: HESI-II;
Spray voltage (KV): -3.0; Heated capillary temp (ºC): 275; S-lens RF level: 50.0; Heater temp (ºC): 400

Figure 2. Q Exactive Focus hybrid quadrupole-Orbitrap Mass Spectrometer, showing EQuan MAX Plus LC-MS On-Line SPE System

Figure 3. Parallel Reaction Monitoring with the Q Exactive Focus Mass Spectrometer.



LCMS Analysis

Calibration and method performance

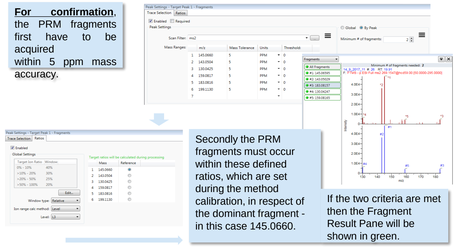
17αEE2 calibration standards were prepared in LCMS grade water with 5% LCMS grade methanol; 5 mL volumes were used for analysis and the calibration was carried out using external standardisation. Calibrants were prepared at 25, 100, 200, 400, 800, and 1600 pg/L

To assess the limit of detection (LoD) and limit of quantitation.(LoQ) , the 100 pg/L standard was run six times and the standard deviation used to derive the performance data.

Acquisition, Processing and Confirmation

The data were acquired, process and confirmed using TraceFinder 4.1 software. Data were confirmed using the accurate mass of the MS2 fragment ions, see Figure 4.

Figure 4. PRM Confirmation Workflow



Method Application

To assess the performance of the method on real-world samples, a sample of wastewater effluent from a treatment works in Glasgow (UK) was analysed; prior to analysis the sample was diluted with 5% LCMS grade methanol to match the composition of the calibration standards.

Results - Calibration and method performance

A typical calibration plot for EE2 is shown in Figure 5 showing excellent linearity with an R^2 value of 0.9998; also shown is the chromatogram for the 25 pg/L standard.

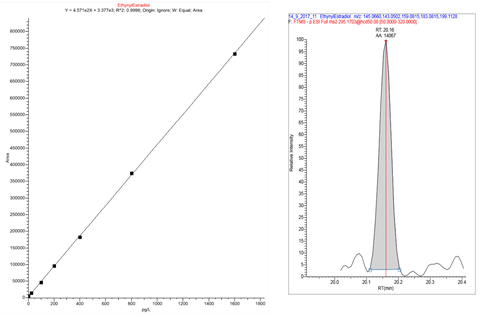
Figure 6 shows the raw calibration data along with the excellent MS2 mass error of < 1 ppm; also shown is the percentage difference between specified and observed calibrant concentrations, none of which are above 10 %.

Figure 6. Mass error for the most abundant PRM MS2 ion (m/z 145.0660) and the % difference between actual and observed calibration levels.

Sample Results

	Filename	Area	RT	Comments	Calculated Amt	m/z (Delta)	%Diff	m/z (Apex)	Fl
1	14_9_2017_10	390113	20.17	Effluent	846.012	.0000 (ppm)	N/A	145.0660	
2	14_9_2017_11	14067	20.17	Cal1 EE2 25 pg/L	23.386	.7363 (ppm)	-6.46	145.0661	
3	14_9_2017_12	45870	20.17	Cal2 EE2 100 pg/L	92.957	.3156 (ppm)	-7.04	145.0661	
4	14_9_2017_13	96477	20.17	Cal3 EE2 200 pg/L	203.663	.1052 (ppm)	1.83	145.0660	
5	15_9_2017_14	182066	20.17	Cal4 EE2 400 pg/L	390.895	.5259 (ppm)	-2.28	145.0661	
6	15_9_2017_15	374865	20.17	Cal5 EE2 800 pg/L	812.657	.0000 (ppm)	1.58	145.0660	
7	15_9_2017_16	732933	20.17	Cal6 EE2 1600 pg/L	1595.955	.0000 (ppm)	-0.25	145.0660	
8	15_9_2017_18	5885	20.17	BLANK	5.486	-.4207 (ppm)	548606667.21	145.0660	

Figure 5. External calibration plot for 17α Ethynylestradiol and chromatogram for the 25 pg/L standard



Results - Limit of Detection and Quantitation

Run 1	98
Run2	94
Run 3	96
Run 4	89
Run 5	96
Run 6	97
Mean	95
RSD	**3.4%**
LOD	**15**
LOQ	**29**

The standard deviation (SD) of six replicates of the 100 pg/L standard was used to calculate LoD and LoQ.

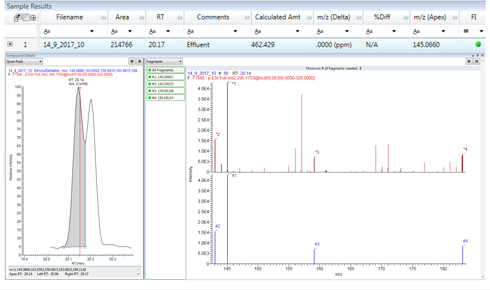
The RSD for the six replicates was 3.4 %. The LoD was derived using 4.65 x SD and the LoQ as 9 x SD.

Limit of Detection	15 pg/L
Limit of Quantitation	29 pg/L

Results - Method Application

Figure 7 shows a confirmed peak for 17αEE2 in wastewater effluent at a concentration of 462 pg/L, which is typical of the range reported in the scientific literature[2].

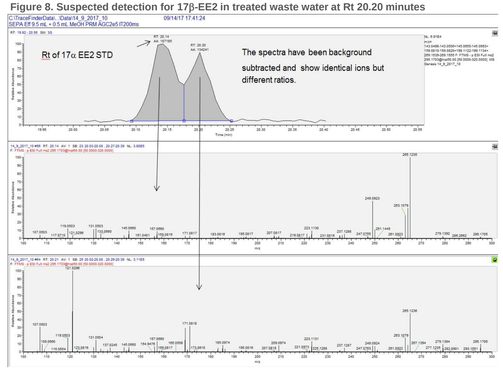
Figure 7. Confirmed detection for 17αEE2 in treated waste water at 462 pg/L



Suspected detection of 17β-EE2

Closer examination of the MS2 spectra for the second peak in the effluent chromatogram shows that the ion masses for the second peak are identical to those of 17αEE2, though in slightly different ratios, see Figure 8.

Figure 8. Suspected detection for 17β-EE2 in treated waste water at Rt 20.20 minutes



DISCUSSION/CONCLUSIONS

Using external calibration and PRM acquisition, the initial results have shown excellent method performance both in terms of quantitation and confirmation using MS2 fragment ions, and in speed of analysis compared to traditional approaches.

With external calibration the limit of detection and quantitation was determined at 15 pg/L and 29 pg/L respectively. It is planned to further improve the robustness of the method using deuterated internal standards and to extend the scope of the method to cover other steroid estrogens cited in the EU Water Framework Directive: estrone and 17β-estadiol.

If the duration of current methods (see Figure1) is assumed to be in the order of 10 hours, then the method described is approximately 30 times faster, which has implications in terms of sampling logistics, capital expense and maintenance, as well as expense of consumables.

Lastly, though yet to be confirmed , the chromatographic resolution of the methods appears to be able to differentiate between 17αEE2 and 17β-EE2 . If this is indeed the case and present methods do not differentiate between the two isomers then current analysis programmes could be potential be over estimating the concentration of 17αEE2 by approximately 100%.

REFERENCES

1. Thrupp, T.J.; Runnalls, T.J.; Scholze, M.; Kugathas, S.; Kortenkamp, A.; Sumpter; J.P. (2018). The consequences of exposure to mixtures of chemicals: Something from 'nothing' and 'a lot from little when fish are exposed to steroid hormones. Sci. Tot. Env. 6119-620.
2. Runnalls, T.J.; Margiotta-Casaluci L., Kugathas S.; Sumpter J.P. (2010). Pharmaceuticals in the aquatic environment: steroids and anti-steroids as high priorities for research. Hum. Ecol. Risk. Assess., 19 pp. 1318-1338
3. The determination of steroid oestrogens in waters using chromatography and mass spectrometry (2008); Standing Committee of Analysts; https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/316788/steroids220_2107605.pdf
4. EU Water Frame Work Directive. http://ec.europa.eu/environment/water/water-framework/index_en.html

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