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APPLICATION NOTE 65383

Rapid determination of ethinylestradiol (17α-EE2) to 15 pg/L using EQuan MAX online SPE coupled to a Q Exactive Focus Orbitrap LC/MS/MS system

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Keywords

Environmental analysis, water analysis, wastewater, ethinylestradiol, 17α-EE2, 17β-EE2, Q Exactive Focus MS, EQuan MAX Plus system, Orbitrap MS

Application benefits

- Excellent quantitation and confirmation performance using the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer with 15 pg/L LOD and 29 pg/L LOQ
- Analysis in under 30 minutes using the Thermo Scientific™ EQuan MAX Plus™ online sample preparation system coupled to the Q Exactive Focus mass spectrometer
- Productivity: 30× faster than traditional offline methods
- Potential chromatographic resolution of 17α-EE2 and 17β-EE2

Goal

To demonstrate the feasibility of using a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer with the EQuan MAX Plus LC/MS online solid phase extraction system to achieve the EU Water Frame Framework limit of detection for 17α-ethinylestradiol (35 pg/L) and provide confirmation.

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Introduction

The presence of endocrine disrupting compounds (EDCs), particularly mixtures, and their effects on aquatic environments are significant concerns.[1] Of the many EDCs, 17α-ethinylestradiol (17α-EE2) is recognized as possessing the greatest estrogenic potency and risk to freshwater ecosystems and drinking water resources.[2] Due to its environmental significance, 17α-EE2 was incorporated into the EU Water Framework Directive (WFD), with a stipulated limit of detection (LoD) of 35 pg/L, which presents a significant analytical challenge.

Current methods generally involve large-volume solid phase extraction (SPE), normal phase SPE clean up, and size exclusion fractionation, which involve considerable time, expense, and sampling logistics[3] (Figure 1). This work assessed the feasibility and performance of using online SPE with 5 mL sample volume and a Q Exactive Focus mass spectrometer for the determination of 17α-EE2 at the WFD LOD of 35 pg/L.[4]

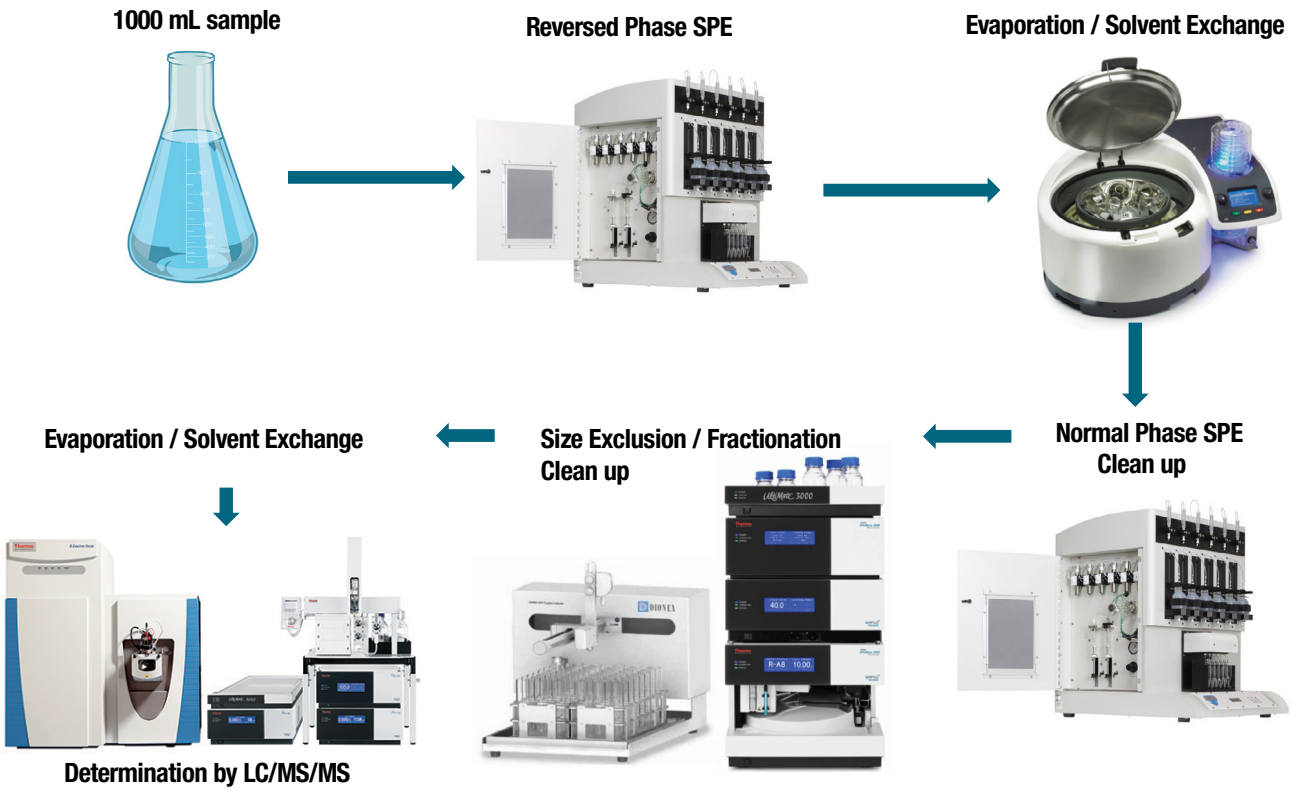


Figure 1. Typical workflow for steroid estrogen analysis

Experimental

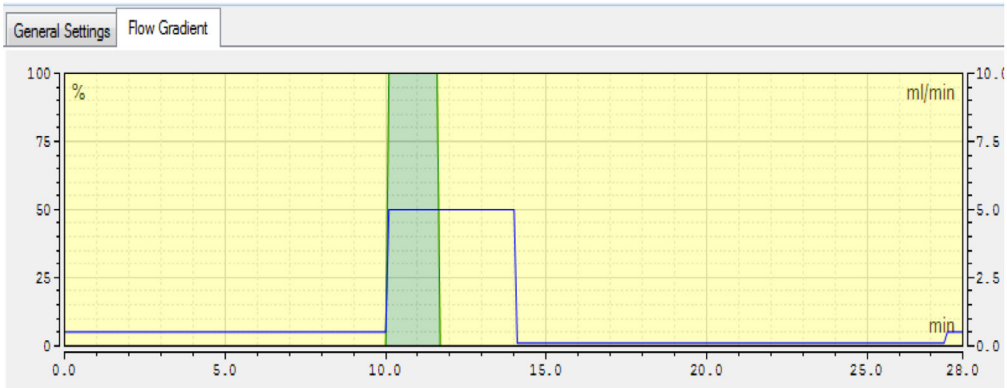
Liquid chromatography

Liquid chromatography separations were carried out on the EQuan MAX Plus LC/MS system, which included a binary analytical pump, CTC Analytics autosampler, quaternary loading pump, and column compartment (Figure 2). The LC conditions are listed in Table 1.

Figure 2. Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer with EQuan MAX Plus LC/MS online SPE system

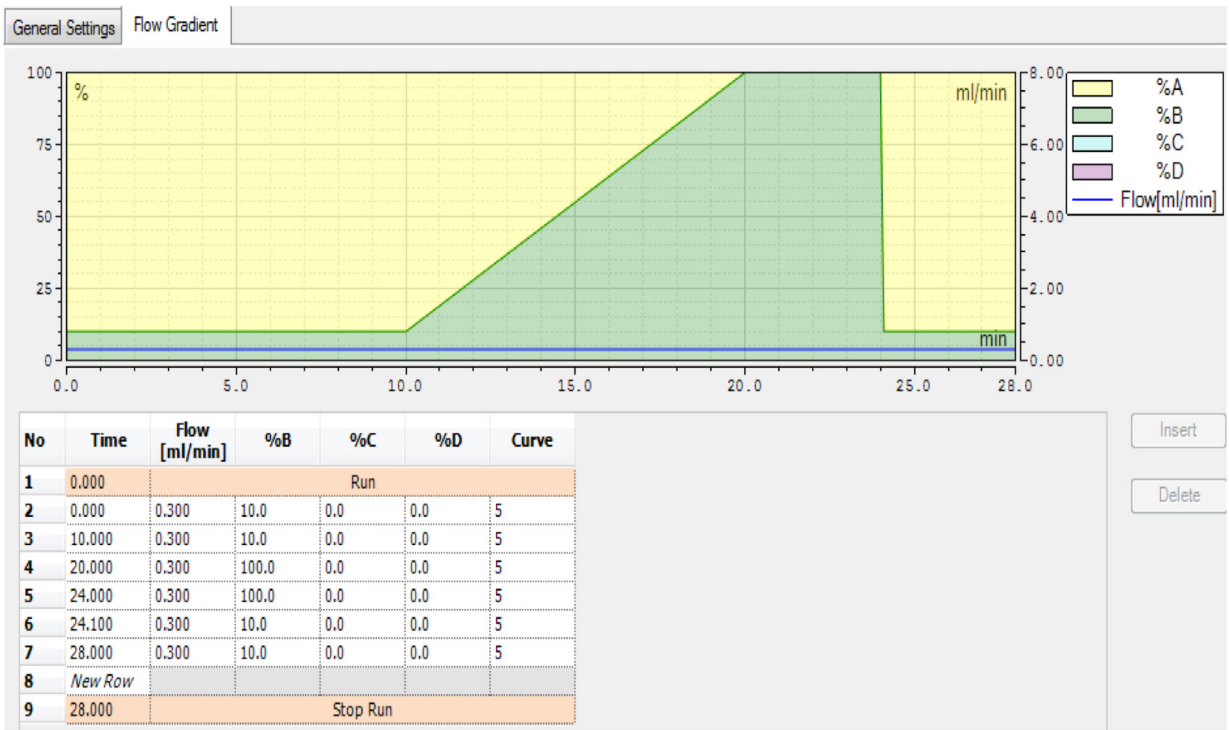
Table 1. LC conditions

	Loading conditions	Analytical conditions
Column	Thermo Scientific™ Hypersil GOLD™ aQ 20 × 2.1 mm, 12 µm	Thermo Scientific™ Acclaim™ VANQUISH™ Polar Advantage 150 x × 2.1 mm, 2.2 µm
Column temperature	Ambient	40 ˚C
Injection volume	5 mL	n/a
Loading rate	0.5 mL/min	n/a
Flow rate	See Figure 3	0.3 mL/min
Mobile phase	LC/MS grade water	A. 0.3 mM Ammonium fluoride B. 0.3 mM Ammonium fluoride in methanol
Column wash solvent	Methanol	n/a
Syringe and valve cleaning solvents	1. 90% Methanol, 10% water 2. 90% Water, 10% methanol	n/a
Gradient	See Figure 3	See Figure 4



No	Time	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	Run				
2	0.000	0.500	0.0	0.0	0.0	5
3	10.000	0.500	0.0	0.0	0.0	5
4	10.100	5.000	100.0	0.0	0.0	5
5	11.600	5.000	100.0	0.0	0.0	5
6	11.700	5.000	0.0	0.0	0.0	5
7	14.000	5.000	0.0	0.0	0.0	5
8	14.100	0.100	0.0	0.0	0.0	5
9	27.400	0.100	0.0	0.0	0.0	5
10	27.500	0.500	0.0	0.0	0.0	5
11	*New Row*					
12	28.000	Stop Run				

Figure 3. Loading gradient



No	Time	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	Run				
2	0.000	0.300	10.0	0.0	0.0	5
3	10.000	0.300	10.0	0.0	0.0	5
4	20.000	0.300	100.0	0.0	0.0	5
5	24.000	0.300	100.0	0.0	0.0	5
6	24.100	0.300	10.0	0.0	0.0	5
7	28.000	0.300	10.0	0.0	0.0	5
8	*New Row*					
9	28.000	Stop Run				

Figure 4. Analytical gradient

MS

The MS analysis was performed on a Q Exactive Focus hybrid quadrupole-Orbitrap benchtop high-resolution mass spectrometer using heated electrospray ionization (HESI-II). Acquisition and quantitation were performed using Parallel Reaction Monitoring (PRM) where MS/MS data were collected at a resolving power of 70,000 (FWHM) at *m/z* 200 in negative polarity mode (Figure 5). MS conditions are listed in Table 2.

Table 2. MS conditions

Ion source	HESI-II
Ionization mode	Negative HESI
Scan mode (PRM)	195.1705 *m/z*
Spray voltage	-3.0 kV
Capillary temperature	275 °C
S-lens RF level	50.0
Heater temperature	400 °C
Isolation width	1 *m/z*
HCD collision energy	50 ev
AGC target	$2e^5$
Resolution (@ 200 *m/z*)	70,000

LC/MS analysis

Calibration and method performance

17α-EE2 calibration standards were prepared in LC/MS grade water with 5% LC/MS grade methanol; 5 mL volumes were used for analysis and the calibration was carried out using external standardization. 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 was used to derive the performance data.

Acquisition, processing, and confirmation

The data were acquired, processed, and confirmed using Thermo Scientific™ TraceFinder™ software version 4.1, using a 2 ppm mass tolerance.

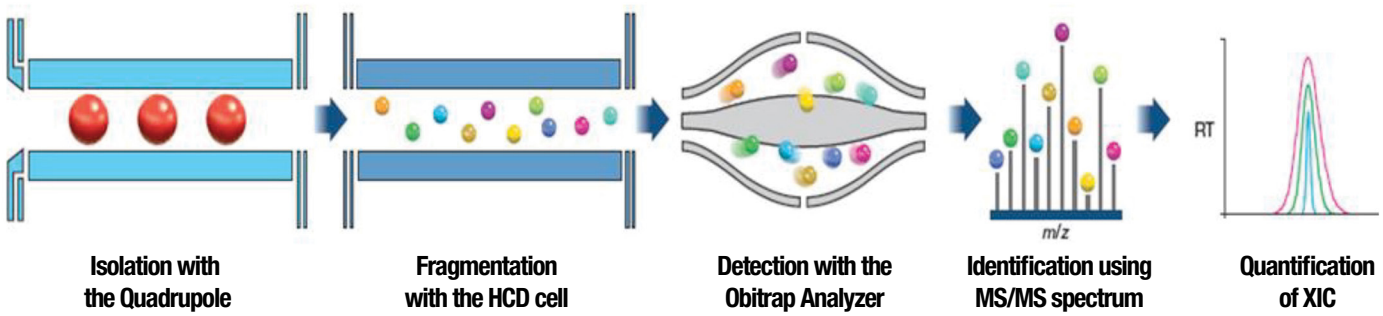


Figure 5. PRM with the Q Exactive Focus mass spectrometer

The ions used for quantitation are shown in Figure 6; fragment ion 143.0502 *m/z* was used as a confirming ion.

17α-EE2 was confirmed with the ratio of ion 143.0502 *m/z*, with respect to the ions used for quantitation, using an absolute window of ±20% (Figure 6); 17α-EE2 was further confirmed using the accurate mass and ratios of the fragments shown in Figure 7.

Method application

To assess the performance of the method on real-world samples, wastewater effluent from a treatment plant in Glasgow, Scotland (UK) was analyzed. Prior to analysis the sample was diluted with 5% LC/MS grade methanol to match the composition of the calibration standards.

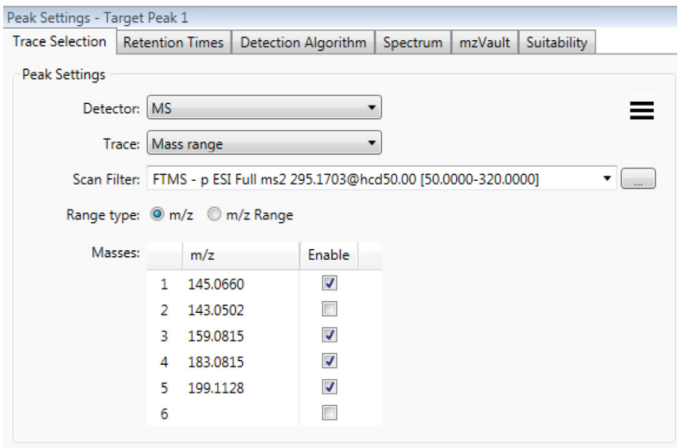


Figure 6. PRM quantitation ions

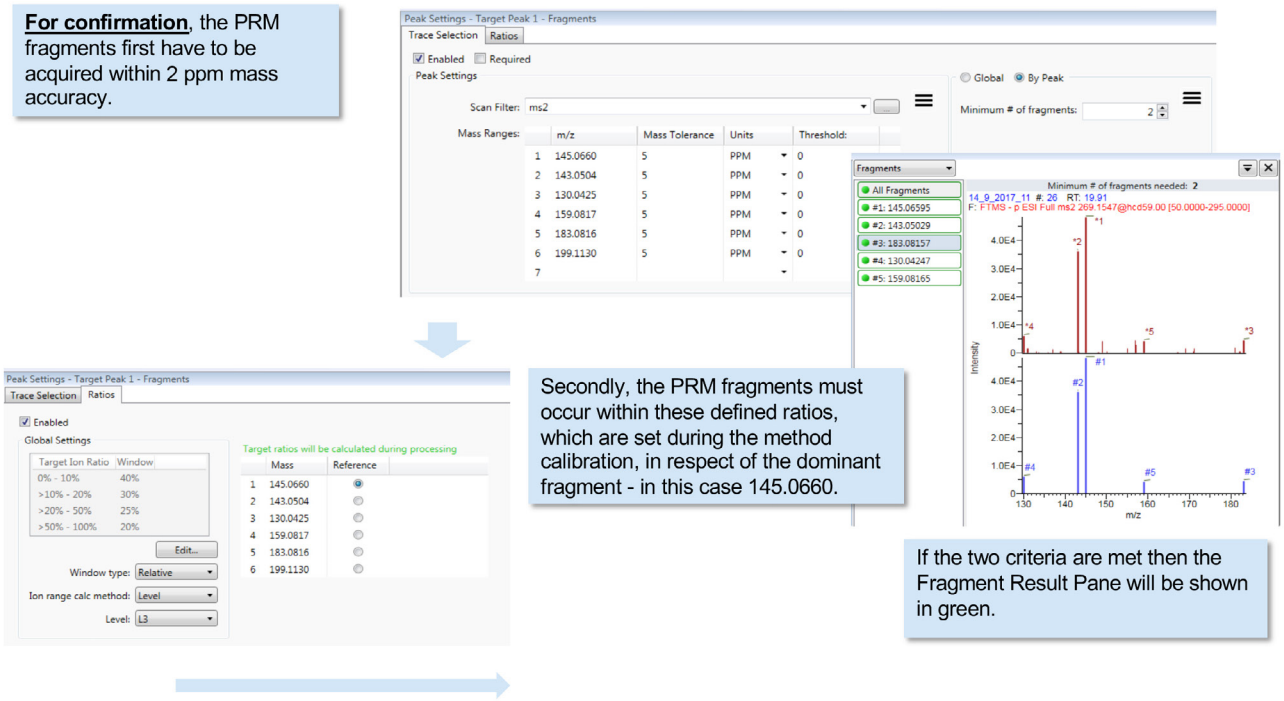


Figure 7. PRM fragment ion confirmation workflow

Results and discussion

Calibration and method performance

Figure 8 shows the TraceFinder Sample Result view showing a typical calibration plot for 17α-EE2 demonstrating excellent linearity with an R^2 value of 0.9998. Also shown are the chromatogram for the 25 pg/L standard and the calibration and sewage sample data, which are confirmed with correct fragment ion data (FI) and confirming ion ratio (IR), as well as the excellent MS^2 fragment ion (145.0660 *m/z*) mass errors of <1 ppm. The percentage differences between specified and observed calibrant concentrations are also displayed, all of which are below 10%.

Limits of detection and quantitation

The standard deviation (SD) of six replicates of the 100 pg/L standard was used to calculate the LoD and LoQ. The RSD for the six replicates was 3.4%. The LoD was derived using 4.65 × SD and the LoQ using 9 × SD (Table 3).

Table 3. Determination of LoD and LoQ

Run 1	98 pg/L
Run 2	94 pg/L
Run 3	96 pg/L
Run 4	89 pg/L
Run 5	96 pg/L
Run 6	97 pg/L
Mean	**95 pg/L**
RSD	**3.4%**
LoD	15 pg/L
LoQ	29 pg/L

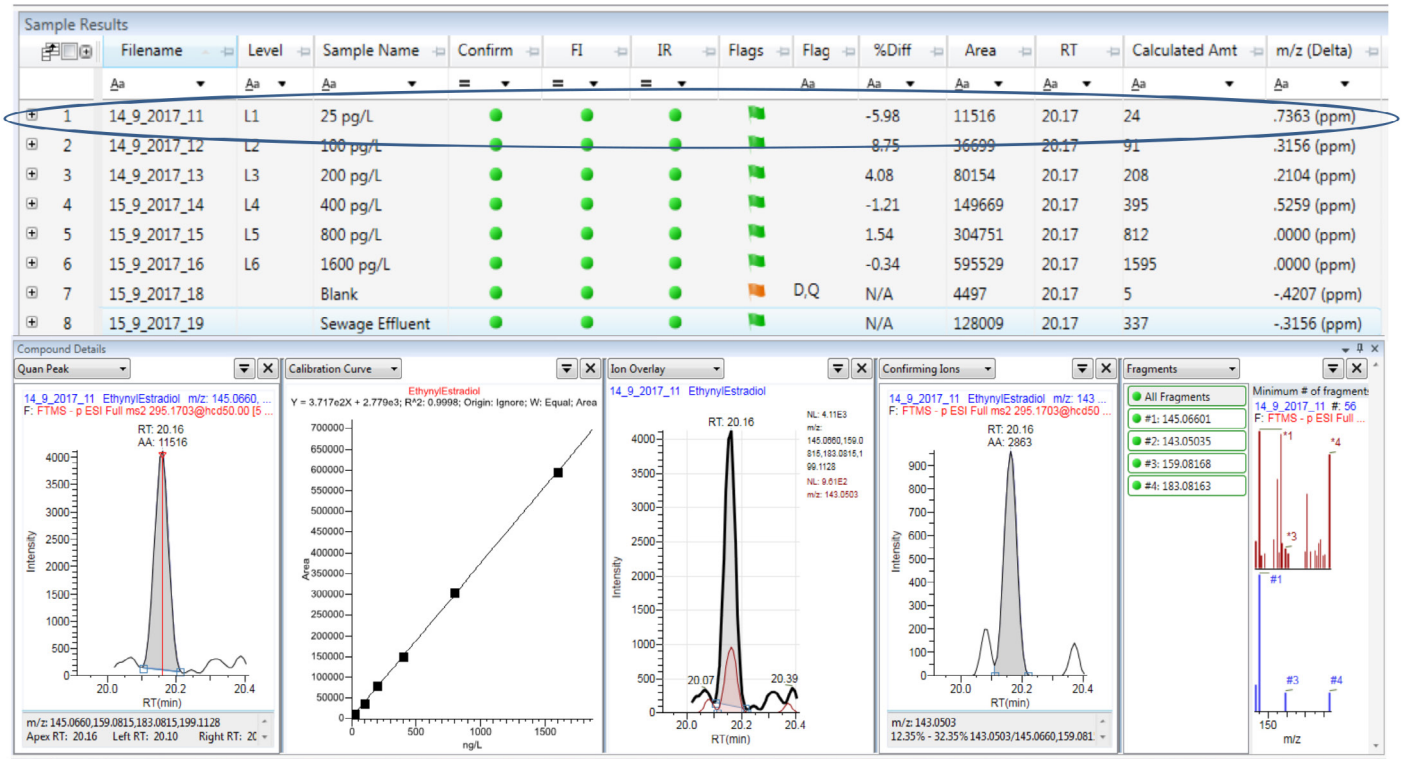


Figure 8. The TraceFinder Data Review view displaying Sample Results and Compound Details panes. Shown are the 17α-EE2 confirmed calibration and sample data, as well as performance data, along with the external calibration plot and the chromatogram for the 25 pg/L standard.

Method application

Figure 9 shows a confirmed peak for 17α-EE2 in wastewater effluent at a concentration of 337 pg/L, which is typical of the range reported in the scientific literature.[2]

Suspected detection of 17β-EE2

Closer examination of the MS^2 spectra for the second peak in the effluent chromatogram shows that the ion masses for the second peak are identical to those of 17α-EE2, though with slightly differing ratios (Figure 10).

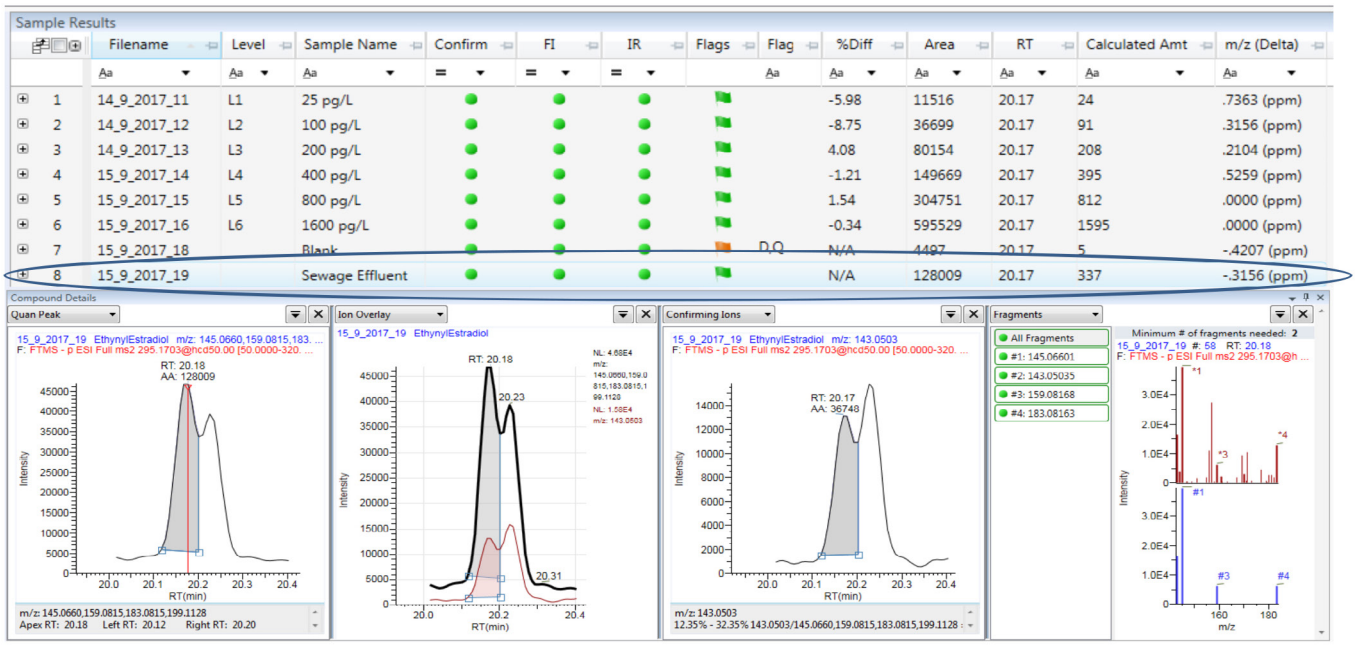


Figure 9. Confirmed detection for 17α-EE2 in treated wastewater at 337 pg/L

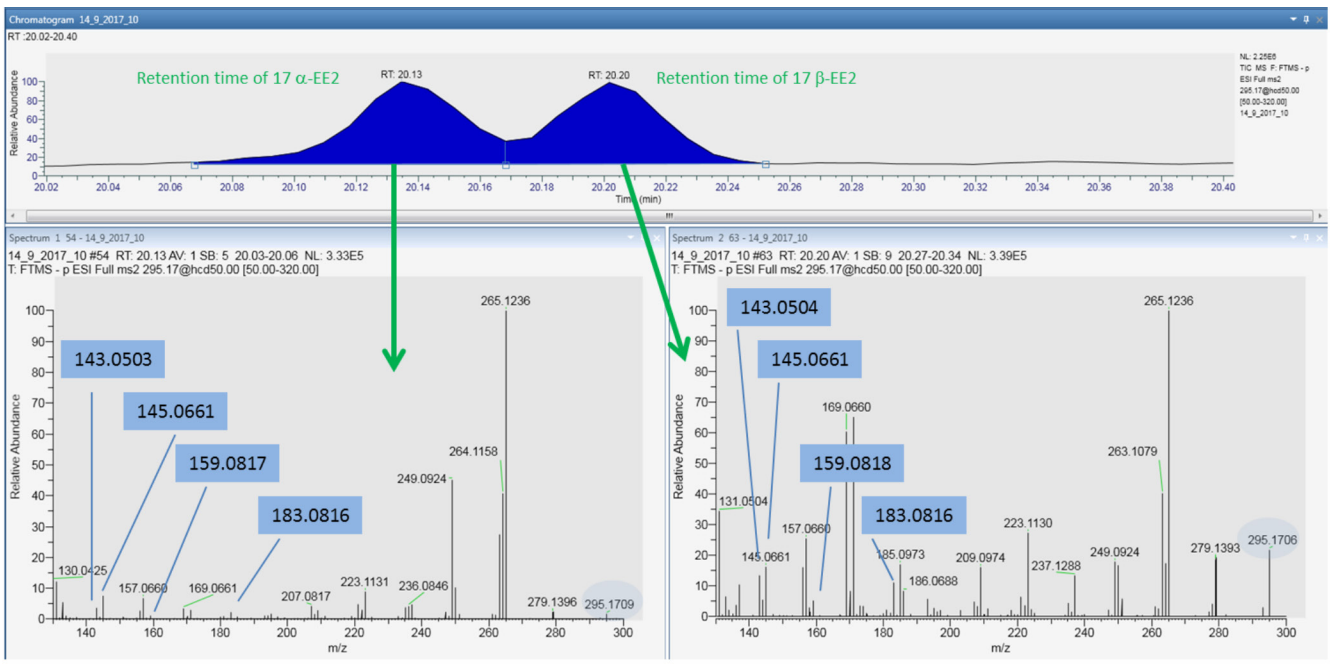


Figure 10. Suspected detection for 17β-EE2 in treated wastewater at RT 20.20 minutes

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Conclusions

- Using external calibration and PRM acquisition, this method for the determination of 17α-EE2 in wastewater has shown excellent performance in terms of quantitation and confirmation using MS^2 confirming and fragment ions and in the speed of analysis compared to traditional approaches.
- With external calibration, the limit of detection and limit of quantitation were determined at 15 pg/L and 29 pg/L, respectively.
- The method described here is nearly 30 times faster when compared to existing methods that require approximately ten hours to complete, representing considerable potential benefits in terms of sampling logistics, capital expense and maintenance, and expense of consumables.
- While subject to confirmation, initial results indicate that this method offers the ability to chromatographically resolve peaks corresponding to the 17α-EE2 and 17β-EE2 isomers, thereby avoiding the overestimation of the concentration of 17α-EE2 that may occur when using existing methods that are unable to distinguish between the two isomers.

References

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