Measurement of oestrone and oestradiol by liquid-chromatography tandem mass spectrometry

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Introduction

The two major metabolically active oestrogens in the non-pregnant population are oestrone (E1) and oestradiol (E2). Accurate measurement of E2 at low concentrations is important in a variety of clinical situations including inborn errors of metabolism, disorders of puberty, post-menopausal women, monitoring aromatase inhibitor treatment, and in men. Measuring oestrogens at very low concentrations remains an analytical challenge. Many published methods involve complex and time-consuming sample preparations, large sample volumes and chemical derivatisation. Here we present a simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure serum E1 and E2 in the low pmol/L range.

Sample preparation

250 µL of serum was spiked with diluent containing deuterated internal standards (d4-E1 and d5-E2), loaded onto a Biotage ISOLUTE® supported liquid extraction (SLE) 96-well plate and eluted using 1 mL dichloromethane. This process was automated using the Biotage® Extrahera™. Samples were evaporated to dryness and reconstituted in 50 µL of 50% methanol.

UPLC conditions

System	ACQUITY UPLC™	
Analytical column	Waters® ACQUITY UPLC™ BEH phenyl column 1.7 µm, 2.1 x 50 mm	
Mobile phase A	Aqueous 0.02 mM ammonium fluoride	
Mobile phase B	Methanol	
Gradient	Time (min)	%B
	0	57
	1.5	57
	1.6	100
	2.5	100
	2.6	57
	3.5	57
Flow rate	0.45 mL/min	
Column temp	50°C	
Injection volume	30 µL	

Table 1: Analytical conditions.

MS conditions

Detection was performed using the Waters Xevo™ TQ-S instrument in negative ion mode with transitions 269>145 (quantifier) and 269>159 (qualifier) for E1 and 271>183 (quantifier) and 271>145 (qualifier) for E2.

Results

Baseline resolution of E1 and E2 is shown in Figure 1.

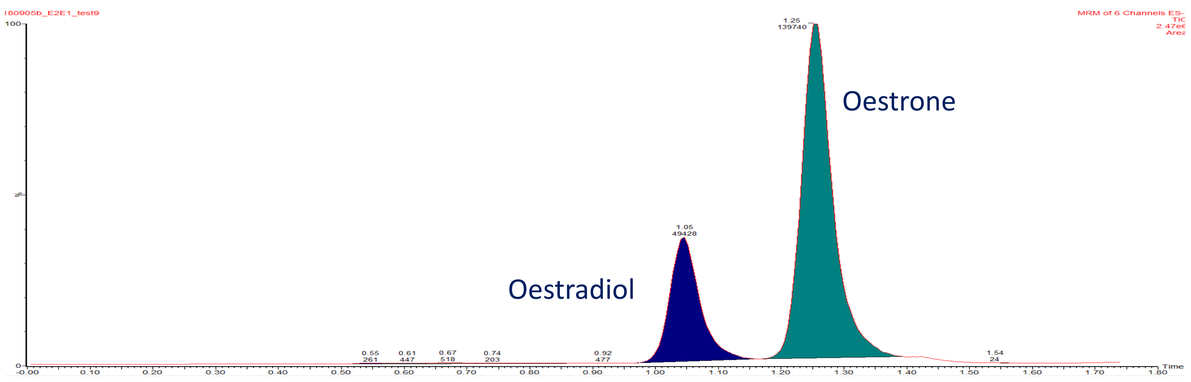

Figure 1: Chromatogram of E1 and E2 measured at 441 pmol/L and 367 pmol/L, respectively.

The functional limit of detection was 3.7 pmol/L for E1 and 18.5 pmol/L for E2 (Figure 2) and the calibration curve was linear up to 2570 pmol/L for both with a coefficient of determination (r^2) >0.99 (Figure 3).

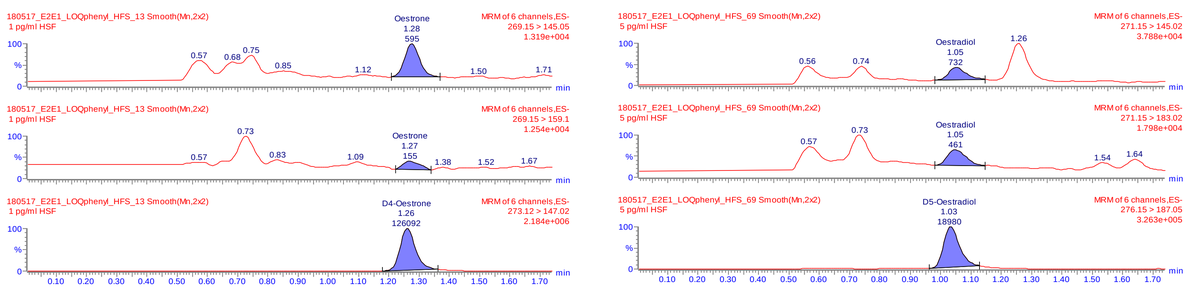

Figure 2: Typical chromatograms of low levels samples for E1 (3.7 pmol/L) and E2 (18.5 pmol/L).

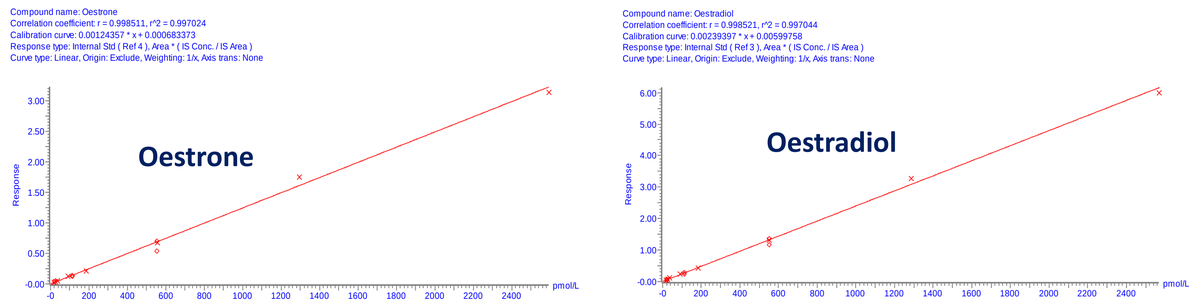

Figure 3: Representative calibration curves for E1 and E2.

The intra-assay and inter-assay precision, expressed as percentage CV, was <15% at 22 pmol/L, 111 pmol/L and 554 pmol/L for E1 and E2.

	Oestrone (E1) %CV	Oestradiol (E2) %CV
QC 22 pmol/L (*n*=16)	14.4	14.2
QC 111 pmol/L (*n*=16)	7.3	5.8
QC 554 pmol/L (*n*=16)	7.7	7.6

Table 2: Precision performance summary for E1 and E2.

Accuracy was evaluated using BCR®-576 certified reference material (CRM) with a bias of 3.8% for E2. The average recovery for E2 was 105%. The LC-MS/MS E2 assay was compared with an Abbott Architect i2000SR chemiluminescent immunoassay (*n*=70) with r^2 of 0.91.

Conclusions

We have developed and validated a fit for purpose LC-MS/MS assay for the simultaneous measurement of serum E1 and E2 in men, children and menopausal women. The concentration of ammonium fluoride in the aqueous mobile phase was critical in achieving an improved functional limit of detection[1], and avoidance of an interfering peak in the E2 quantifier transition (Figure 4). It has high throughput owing to the readily automatable sample extraction and uses a relatively small sample volume with no requirement for chemical derivatisation. This assay offers significant advantages in terms of sensitivity when compared to our current commercial immunoassay. We hope to further improve the functional limit of detection, as recommended in The Endocrine Society position statement[2], following the purchase of a Waters Xevo™ TQ-XS instrument.

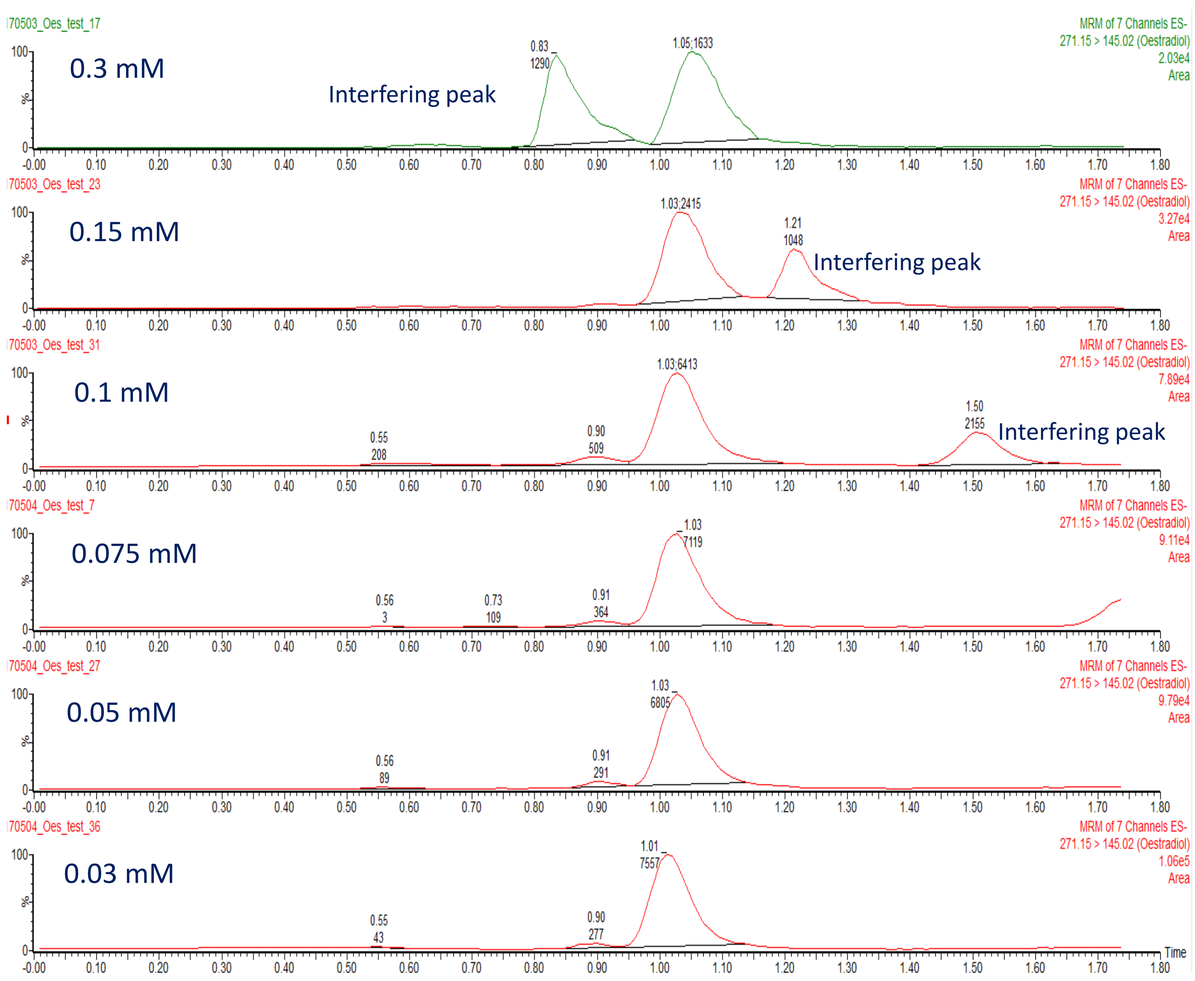

Figure 4: Interfering peak in the 271>145 E2 MRM with varying [ammonium fluoride].

Acknowledgements
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References

1. Keevil BG. Novel liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for measuring steroids. Best *Practice & Research Clinical Endocrinology & Metabolism* 2013 (27): 663-674.
2. Rosner W, Hankinson SE, Sluss PM, et al. Challenges to the measurement of estradiol: an endocrine society position statement. *J Clin Endocrinol Metab* 2013; 98(4): 1376-1387.