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Note d'application

Measuring Estrogens at Low Levels in Plasma

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For research use only. Not for use in diagnostic procedures.

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates a selective and analytically sensitive method for the measurement of estrone and estradiol in human plasma samples for endocrine research.

Benefits

Measuring estrogens in plasma with LC-MS

Introduction

The role of the estrogenic steroids, estrone (E1) and estradiol (E2), is well established in the development of secondary sex characteristics and fertility in females. In men, pre-pubescent children, post-menopausal women, and women taking aromatase inhibitors to treat cancers, however, their role is less well understood and is a field of active research. E1 and E2 are most commonly measured by immunoassays and these assays are, unfortunately, plagued by a high degree of cross-reactivity with structurally similar steroids and metabolites and conjugates of E1 and E2. This lack of selectivity confounds research studies and diminishes their conclusions. A

more analytically sensitive and selective method is needed for measuring E1 and E2 and this technical brief outlines one such method that uses simple sample pretreatment and online solid phase extraction coupled to LC-tandem mass spectrometry.

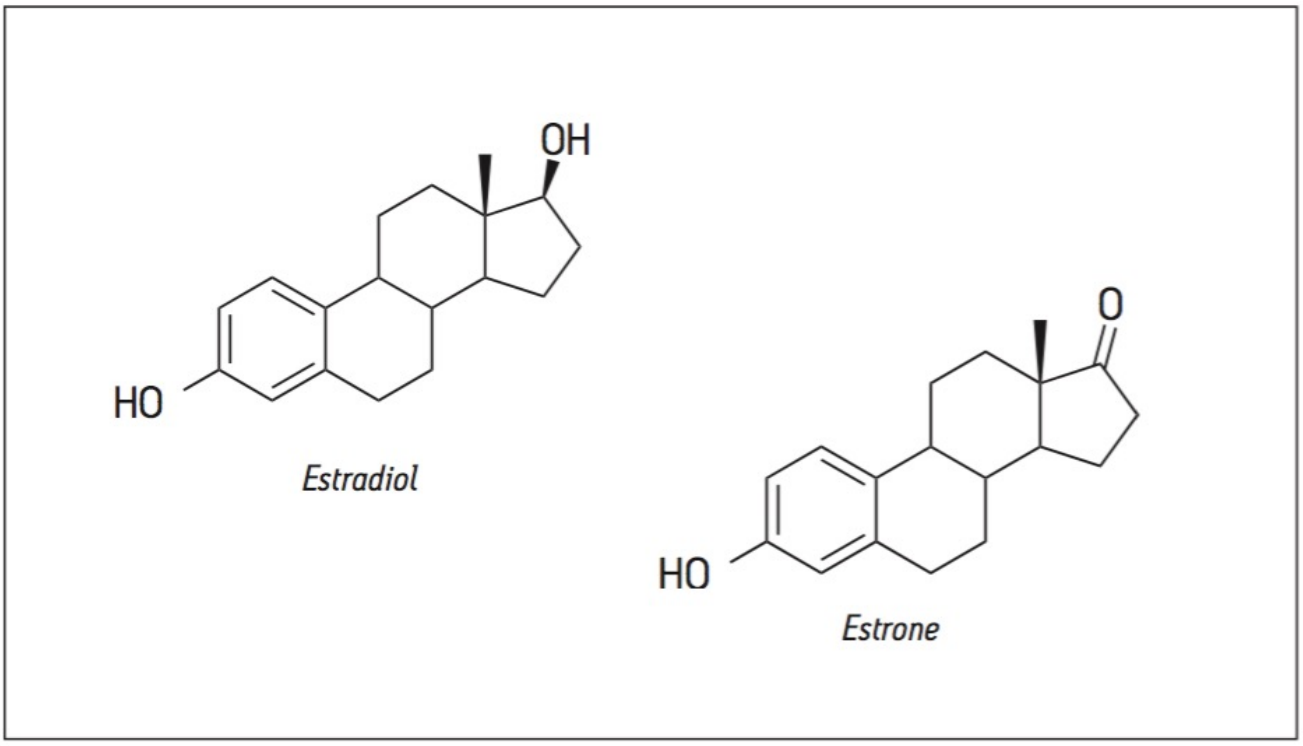

Figure 1: Structures of estradiol and estrone.

Experimental

Method Details

LC System:	ACQUITY UPLC
Mass Spectrometer:	Xevo TQ-S, negative ion mode
Column:	ACQUITY UPLC C_{18} SB, 1.8 µm, 2.1 x 30 mm
Sample Preparation:	ACQUITY UPLC Online SPE

	Manager (OSM)
SPE:	MassTrak C_{18} OSM Cartridge

Sample Preparation

250 µL of plasma were pretreated by extraction with an SPE plate with 900 µL MTBE. Samples were then reconstituted with 100 µL of 40% methanol. OSM cartridges were preconditioned with 0.5 mL methanol and equilibrated with 0.5 mL water. 75 µL of sample with 0.5 mL water were loaded onto the OSM cartridge and subsequently washed with 0.5 mL 30% methanol. After washing, samples were eluted directly onto the analytical column by the OSM for separation.

Results and Discussion

A new method was developed for clinical research that utilizes a novel, fully integrated online SPE-LC/MS system for the measurement of E1 and E2 in human plasma at low concentrations (pmole/L). The system provides sample preparation and good chromatography while minimizing matrix effects. The analysis resulted in quantitation of low levels of these steroid hormones in a short analytical run time.

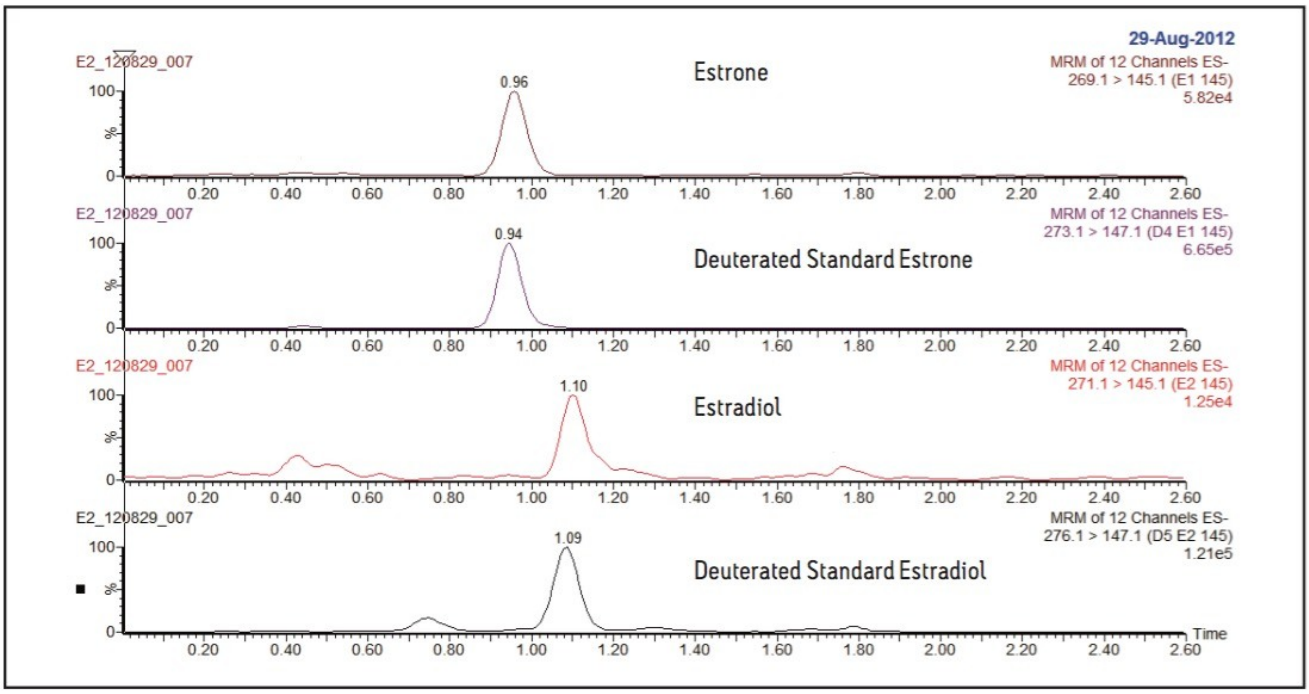

Figure 2: LC-MS analysis of estrone (E1) and estradiol (E2) after sample preparation.

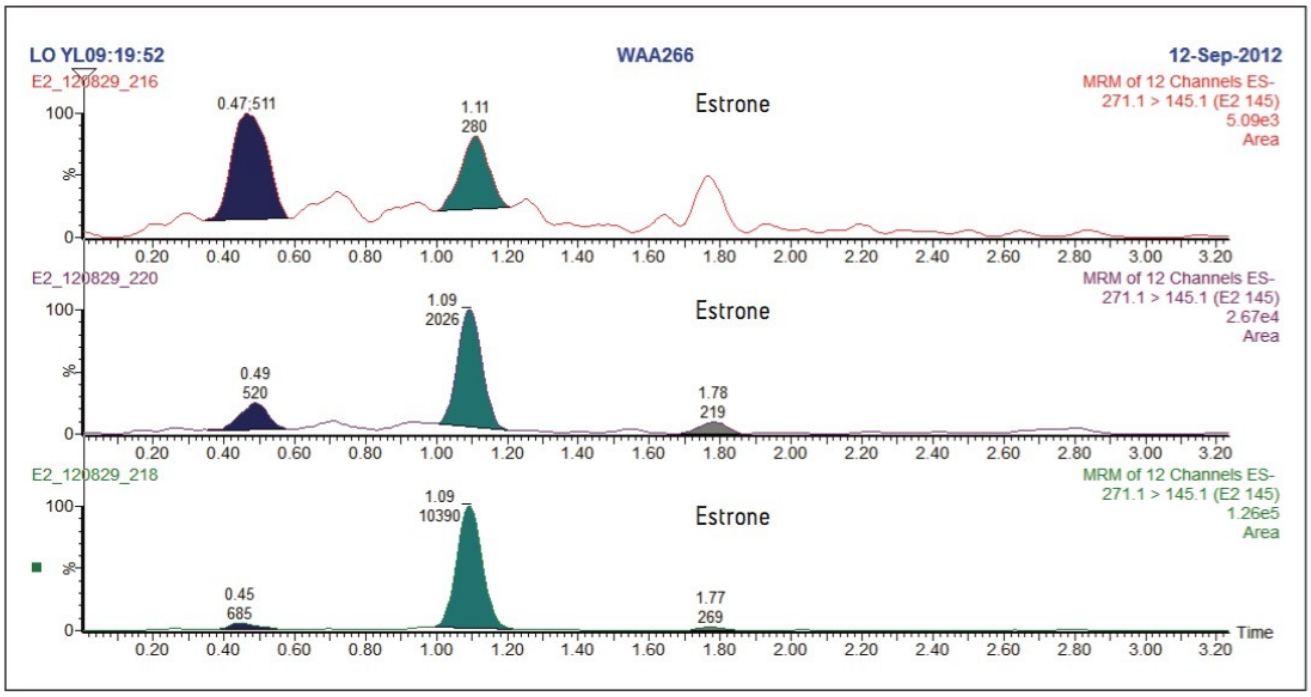

Figure 3: Quantitation of various levels of estradiol by LC-MS (Top: 6 pmol/L; Middle: 59 pmol/L; Bottom: 290 pmol/L).

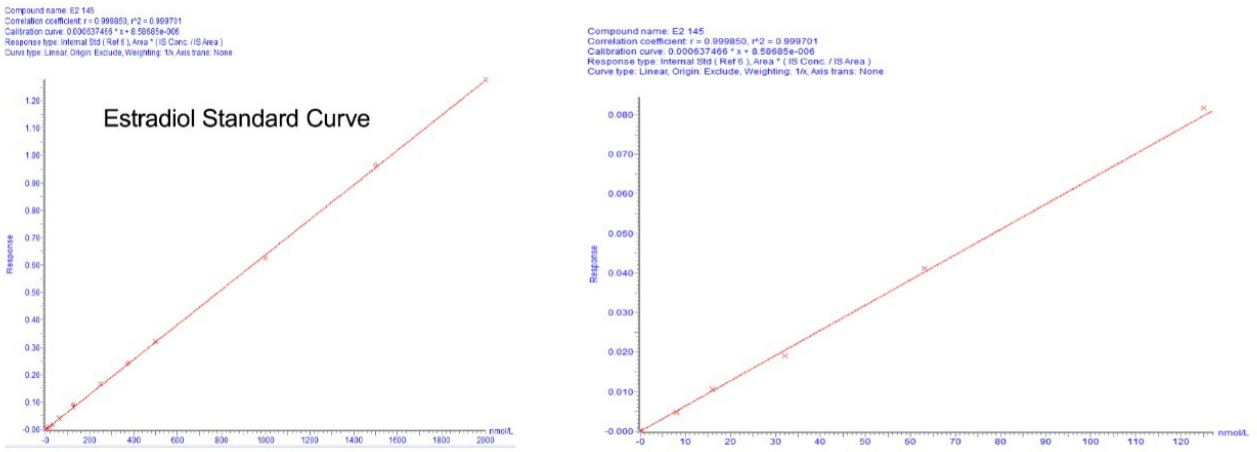

Figure 4: Standard curve of estradiol up to 2000 nmol/L. LLOQ 10 pmol/L for estradiol, 6 pmol/L for estrone.

Conclusion

Estrone and estradiol, and a host of other structurally similar steroid hormones, are often measured in research laboratories studying the role of endocrine function. These sex steroids are present in a wide range of concentrations (10 pmol/L – 2000 nmol/L) in different populations (e.g. males vs. females, pre-menopausal vs. post-menopausal women) and disease states (e.g. polycystic ovarian disease, breast cancer and osteoporosis). Due to the challenge of a wide analytical measurement range and the difficulty of developing selective immunoassay methods, liquid chromatography - tandem mass spectrometry methods are being rapidly adopted for endocrine research.

Liquid chromatography-tandem mass spectrometry methods are optimized when non-relevant interferences in the sample under analysis are removed. In this Technical Brief, the focus of the measurement procedure was on sample pretreatment and high analytical sensitivity mass spectrometry. The online solid-phase extraction approach described herein reduces the likelihood of measurement artifacts such as matrix effects and ion suppression. Rapid, quantitation of the estrogenic hormones estrone and estradiol at low concentrations has been demonstrated.

The method developed here provides:

- Simultaneous analysis of estradiol and estrone in a single assay
- Simple sample preparation and detection without derivatization
- Fast analysis time of < 5 min/sample

- Very good sensitivity LLOQs for all analytes in the low (6-10 picomolar) ranges
- Highly efficient SPE sample preparation integrated with LC-MS

Featured Products

- ACQUITY UPLC System <https://www.waters.com/514207>
- Xevo TQ-S <https://www.waters.com/10160596>

720004616, January 2014

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