Application Note: 530

Fast and Sensitive LC-APCI-MS/MS Quantitative Analysis of Estrone and Estradiol in Serum without Chemical Derivatization

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Key Words

- TSQ Vantage
- Accela UHPLC
- Clinical Research

Introduction

In the clinical research setting, quantitative measurements of estrone (E1) and estradiol (E2) in serum typically have been done with immunoassay or liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS is preferred over immunoassay and other analytical techniques because of its high sensitivity.

E1 and E2 are usually chemically derivatized before they are detected by mass spectrometry for enhanced sensitivity. The derivatization step extends the sample preparation procedure and usually involves chemicals/reagents that might compromise the performance of the mass spectrometer in the long term.

Goal

To develop and validate a simple, fast and sensitive analytical method for measuring E1 and E2 in serum or plasma by LC-APCI-MS/MS.

Methods

Sample Preparation

Serum was spiked with internal standard (IS, deuterated E2) and underwent liquid-liquid extraction (LLE) with methyl tert-butyl ether (MTBE). After extraction, the MTBE layer was dried under nitrogen and re-suspended with 60% methanol. The reconstituted sample was centrifuged to remove particulates and the supernatant was injected for LC-MS/MS analysis.

LC-MS/MS Conditions

LC-MS/MS analysis was performed on a Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer coupled with a Thermo Scientific Accela UHPLC system. UHPLC was carried out on a Thermo Scientific Hypersil GOLD column (150 × 2.1 mm, 3 µm) at room temperature using water and methanol as mobile phases. The total LC run time was 6 minutes. The mass spectrometer was operated with an atmospheric pressure chemical ionization (APCI) source in negative ion mode. Data was acquired in selected reaction monitoring (SRM) mode.

Validation

The validation procedure included tests for 1) recovery of sample preparation; 2) calibration range; 3) lower limit of quantitation (LLOQ), dynamic range, accuracy; 4) precision; 5) ion suppression; and 6) carryover.

Results and Discussion

Sample Preparation

LLE was used to extract E1 and E2 from serum/plasma and was found to be efficient. MTBE was selected as the extraction solvent for its excellent recovery and ease of handling.

Validation

1. Recovery for LLE Sample Preparation

The absolute recovery of E1, E2 and their internal standard from liquid-liquid extraction ranged from 70% – 115% (n=4).

2. Calibration Range

Calibration curves (Figures 1 and 2) using calibrators in charcoal stripped serum (CSS) showed excellent linearity (R^2 > 0.998) between 5 and 1000 pg/mL.

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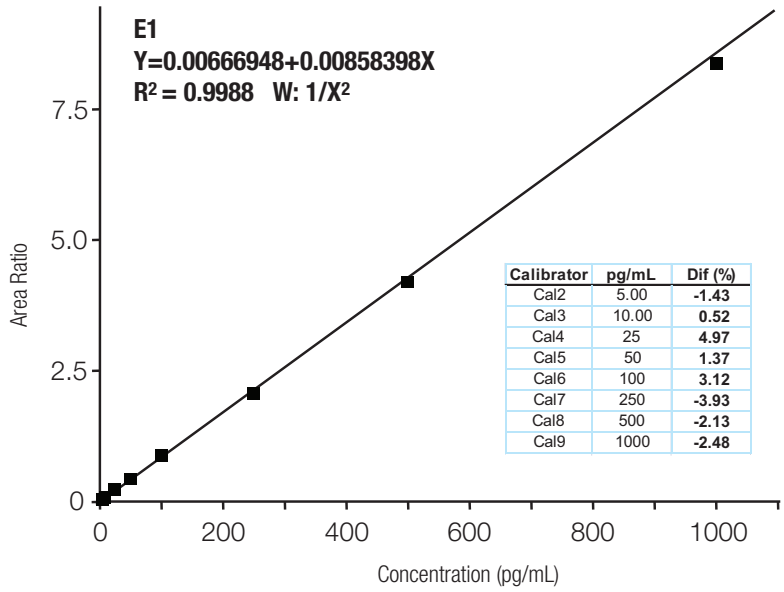

Figure 1: Calibration curve of E1 in CSS

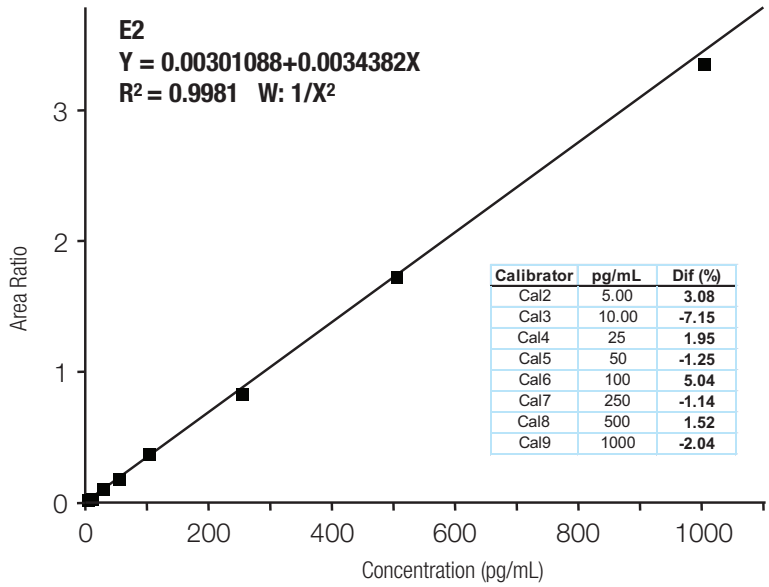

Figure 2. Calibration curve of E2 in CSS

3. Determination of LLOQ, Linearity and Accuracy

CSS was first evaluated by comparing it to human plasma to determine if it was suitable. During this stage of the validation, CSS samples with progressively lower concentrations of E1 and E2 were prepared in triplicate along with one set of CSS calibrators.

The method was linear between 3.5 and 1019.3 pg/mL with accuracy (n=3) from 85.8% to 107.0% for E1, and between 4.4 and 1032.5 pg/mL with accuracy (n=3) from 92.9% to 112.8% for E2 (Table 1 and Figure 3). The LLOQ for E1 and E2 are 3.5 and 4.4 pg/mL, respectively (Table 1 and Figure 4).

Table 1. LLOQ, dynamic range and accuracy

		E1			E2		
Dilution factor	**Expected (pg/mL)**	**Measured (mean, pg/mL)**	**CV of Triplicates (%)**	**Accuracy (%)**	**Measured (mean, pg/mL)**	**CV of Triplicates (%)**	**Accuracy (%)**
256	3.91	3.5	18.8	90.5	4.4	7.1	112.8
128	7.81	8.4	4.5	107.0	8.0	9.0	102.2
64	15.63	15.8	9.4	101.2	18.0	5.1	115.2
32	31.25	28.7	0.6	92.0	31.0	8.8	99.1
16	62.50	56.7	4.8	90.7	60.8	6.7	97.2
8	125.00	107.2	3.9	85.8	116.1	6.8	92.9
4	250.00	224.2	7.4	89.7	242.2	4.4	96.9
2	500.00	484.2	3.5	96.8	492.2	2.4	98.4
1	1000.00	1019.3	8.9	101.9	1032.5	9.1	103.2
Mean				**95.1**			**102.0**

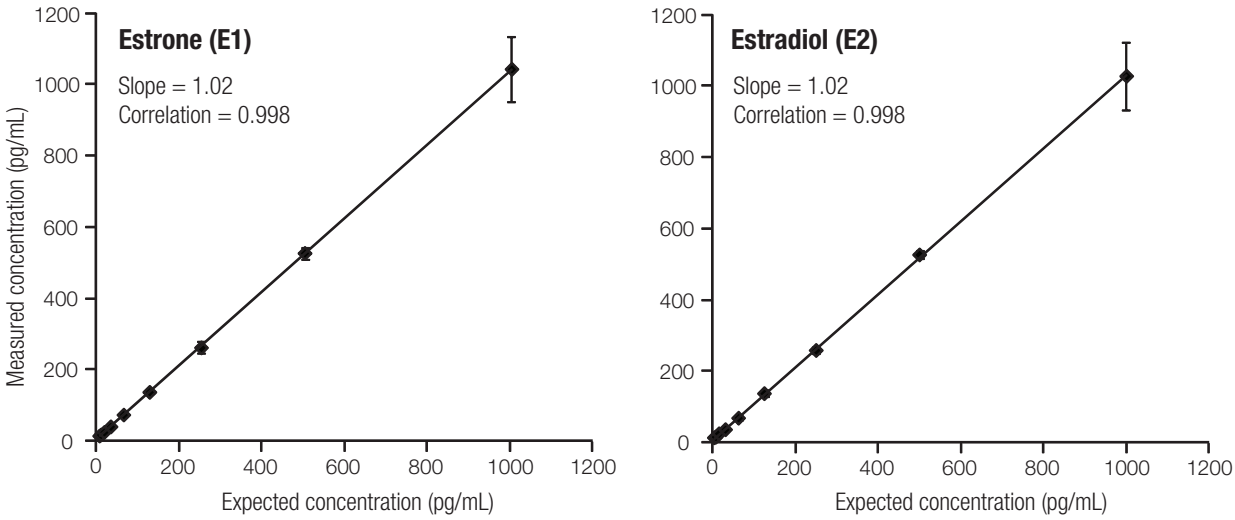

Figure 3: Linearity (Deming regression)

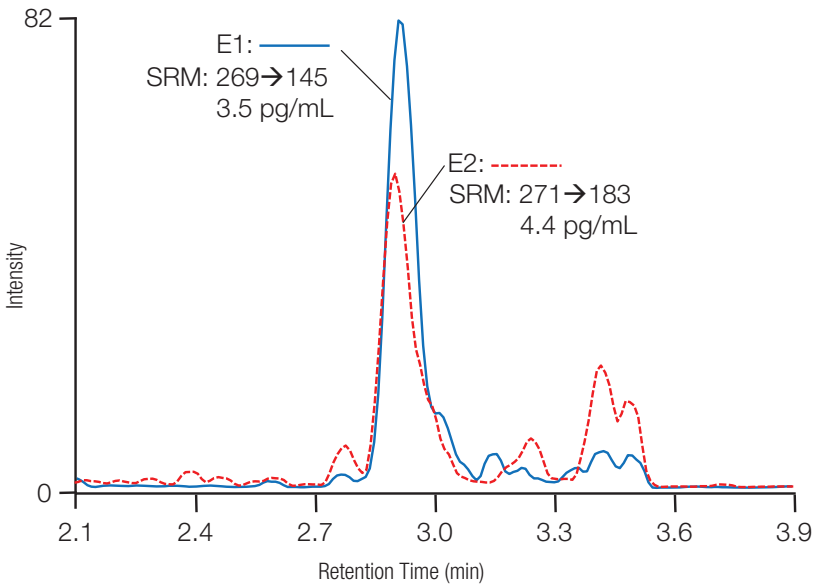

Figure 4. SRM chromatograms of E1 and E2 at their LLOQ in spiked CSS

4. Precision

A) CSS samples: Precision was first assessed with spiked CSS at two concentration levels (12 and 300 pg/mL). Inter- (n=15) and intra-batch (n=5) CV values ranged between 1.6% to 12.5% (Table 2).

B) Pooled human plasma samples: Precision was also assessed with a spiked human plasma pool (35.4 pg/mL of E1 and 18.1 pg/mL of E2, n=5) and the determined intra-batch CV was 2.2% and 3.6% for E1 and E2, respectively.

Table 2. Precision data

		E1		E2	
	Charcoal Stripped Serum	**Low (12 pg/mL)**	**High (300 pg/mL)**	**Low (12 pg/mL)**	**High (300 pg/mL)**
Batch 1	Intra-assay Precision (n=5, %)	7.1	6.9	9.4	6.7
Batch 2	Intra-assay Precision (n=5, %)	5.5	1.6	12.5	3.0
Batch 3	Intra-assay Precision (n=5, %)	7.2	4.9	8.0	3.1
Batch 1-3	Inter-assay Precision (n=15, %)	7.3	4.7	10.9	4.4

	Spiked Pooled Plasma	E1 (35.4 pg/mL)	E2 (18.1 pg/mL)
	Precision (n=5, %)	2.2	3.6

5. Ion Suppression

Results from the post-column infusion experiments are shown in Figure 5. Compared to solvent blank (60% methanol), no obvious ion suppression was detected in the SRM chromatograph of IS using a processed human plasma sample without IS. The red arrow indicates where E1 and E2 elute during the LC gradient.

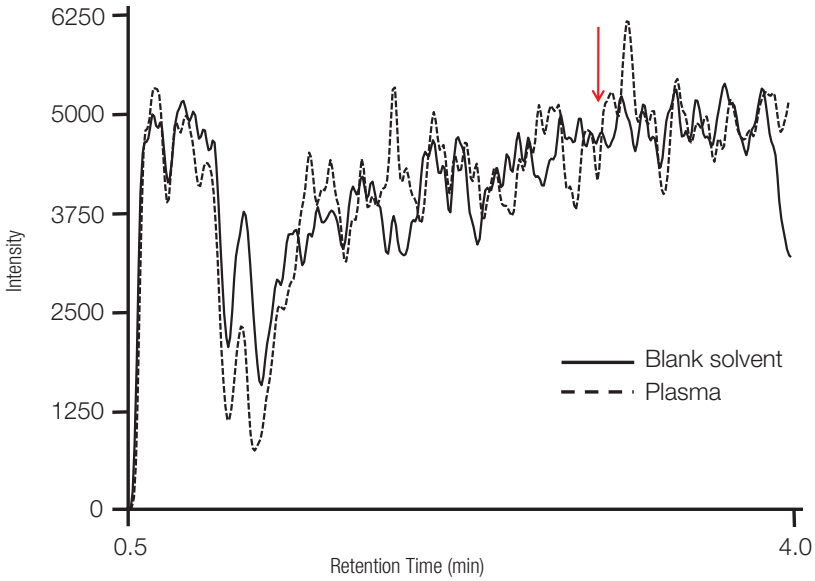

Figure 5: Ion suppression test

6. Carryover

No carryover was observed in the solvent blank injection that was right after a processed spiked CSS sample with E1 and E2 concentration at 300 pg/mL.

Figures 6 and 7 show the SRM chromatograms of E1 and E2 in two individual plasma samples.

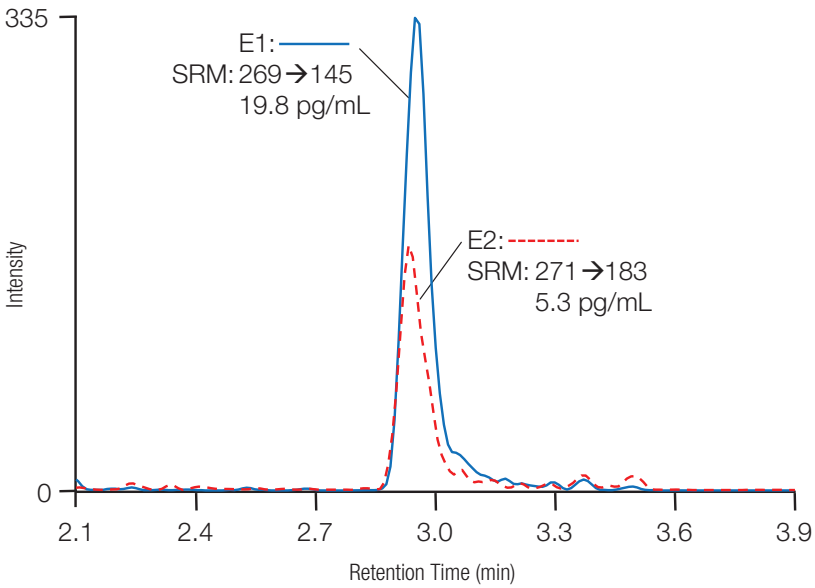

Figure 6: SRM chromatograms of E1 and E2 in human plasma sample 1 (male)

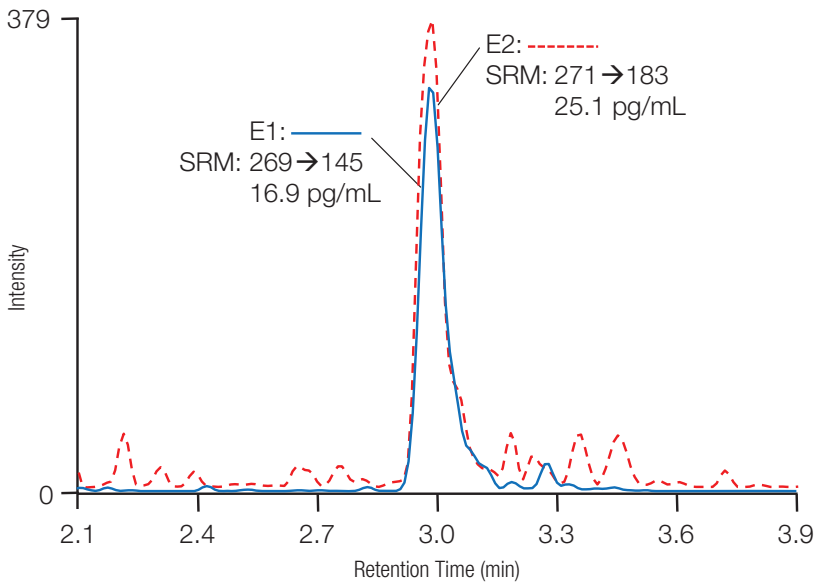

Figure 7: SRM chromatograms of E1 and E2 in human plasma sample 2 (female)

Conclusion

We have developed and fully validated a simple, fast and sensitive LC-APCI-MS/MS method for measurement of E1 and E2 in serum/plasma without derivatization. The LLOQ for E1 and E2 are 3.5 and 4.4 pg/mL, respectively. The method was linear between 3.5 and 1019.3 pg/mL for E1, and 4.4 and 1032.5 pg/mL for E2. No ion suppression or carryover was observed. In addition, for clinical research laboratories, this method offers high precision and recovery.

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