Application Note 611

LC-MS/MS Analysis of Estradiol with Dansyl Derivatization

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Key Words

Estradiol, dansyl chloride, TSQ Quantiva, derivatization

Goal

To develop a high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the clinical research analysis of estradiol in human plasma with a limit of quantitation of 1 pg/mL.

Introduction

Estradiol is an endogenous steroid in the human body. The current goal of researchers is to determine estradiol concentrations at 1 pg/mL in plasma. Here a method for the analysis of estradiol in human plasma was evaluated for clinical research based on these requirements.

Experimental Methods

Sample Preparation

Samples were processed by liquid-liquid extraction (LLE) and subsequently derivatized. Charcoal stripped serum (CSS) was used as the matrix for the calibration curve. The calibration curve was prepared by spiking the CSS with known amounts of estradiol. A 500 µL aliquot of CSS was fortified with internal standard (estradiol-d_5) and extracted with 6 mL of methyl tert-butyl ether (MTBE). The samples were vortexed, centrifuged, and frozen. The resulting organic layer was decanted into a clean test tube and evaporated to dryness. The residue was reconstituted and derivatized with dansyl chloride dissolved in acetone and carbonate buffer. An aliquot was then injected into the HPLC-MS/MS.

Liquid Chromatography

Chromatographic separations were performed under gradient conditions using a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system and UltiMate™ 3000 RS autosampler. The analytical column was a Thermo Scientific™ Hypersil GOLD™ column (50 x 2.1 mm, 1.9 µm particle size). The column was heated to 50 °C. The injection volume was 50 µL. Mobile phases A and B consisted of 0.1% formic acid in water and methanol, Fisher Chemical™ Optima™ grade solvents, respectively. The total run time was 9 minutes.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer equipped with a Thermo Scientific™ Ion Max NG source and heated electrospray ionization (HESI-III) probe. Two selected-reaction monitoring (SRM) transitions were monitored for estradiol and its deuterated internal standard to provide ion ratio confirmations (IRC). Mass spectrometer and SRM parameters are shown in Tables 1 and 2, respectively.

Table 1. Mass spectrometer parameters for estradiol

Parameter	Value
Spray Voltage	4500 V
Sheath Gas	30 Arb
Aux Gas	10 Arb
Sweep Gas	1 Arb
Ion Transfer Tube	380 °C
Vaporizer	400 °C
CID Gas	2.5 mTorr
Cycle Time	0.3 s
Divert Valve	5.0–7.5 min

Table 2. SRM parameters

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE	Lens
Estradiol	506.2	156.2	35	120
		171.2	35	120
Estradiol-d_5	511.2	170.2	35	120
		171.2	35	120

Data was acquired and processed with Thermo Scientific™ TraceFinder™ software.

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Method Performance

Precision and accuracy were determined by analyzing triplicate calibration curves. Method ruggedness and matrix effects were determined by performing a mixing test using a plasma sample analyzed at 1-fold, 2-fold, and 4-fold dilution with water.

Results and Discussion

Estradiol was linear in the range of 1 to 1000 pg/mL. Figure 1 shows a representative calibration curve for estradiol in human plasma. Table 3 shows the excellent precision and accuracies of the calibration curve using this method. In addition to the precision shown, accuracies for all calibrators across the method were within 14.3%. Figures 2 and 3 show chromatograms of the quantifier and confirming ions for estradiol at the lowest calibrator level of 1 pg/mL and 5 pg/mL, respectively. The method proved to be rugged with no matrix effects observed in the mixing test. All diluted plasma samples showed good recovery when compared to an undiluted sample (Table 4). Figure 4 shows a chromatogram of a donor plasma sample.

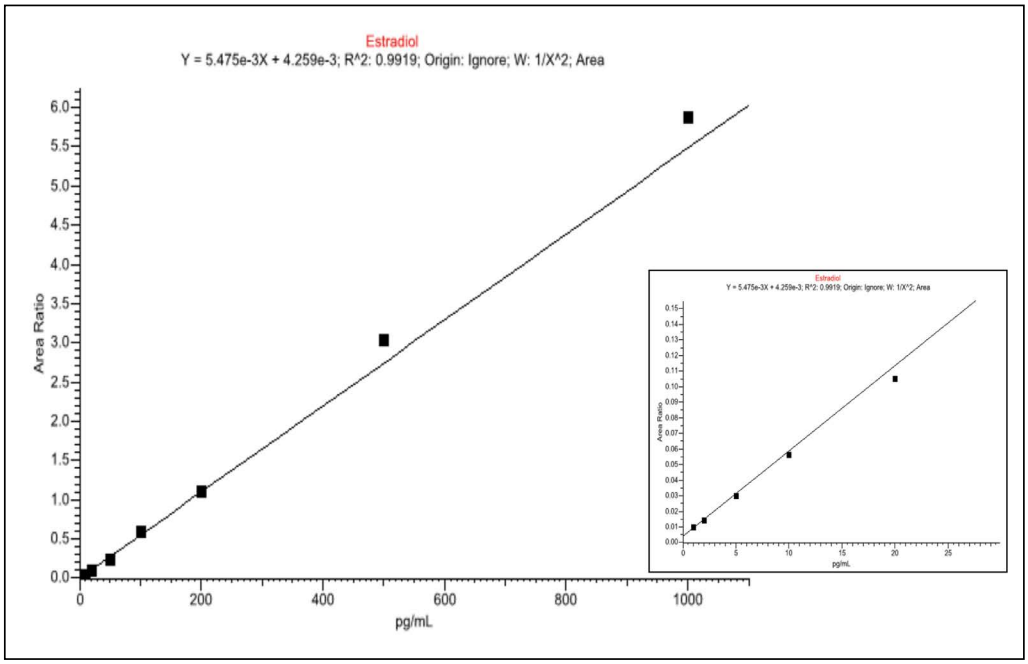

Figure 1. Representative calibration curves for estradiol in human CSS

Table 3. Precision and accuracies of replicate injections of calibrators

Concentration	% RSD (n = 3)	% Diff
1 pg/mL	6.86	6.70
2 pg/mL	8.09	-9.55
5 pg/mL	1.74	-5.48
10 pg/mL	2.06	-4.50
20 pg/mL	1.96	-7.62
50 pg/mL	1.51	-8.96
100 pg/mL	0.122	9.45
200 pg/mL	1.76	1.38
500 pg/mL	0.886	11.0
1000 pg/mL	0.807	7.52

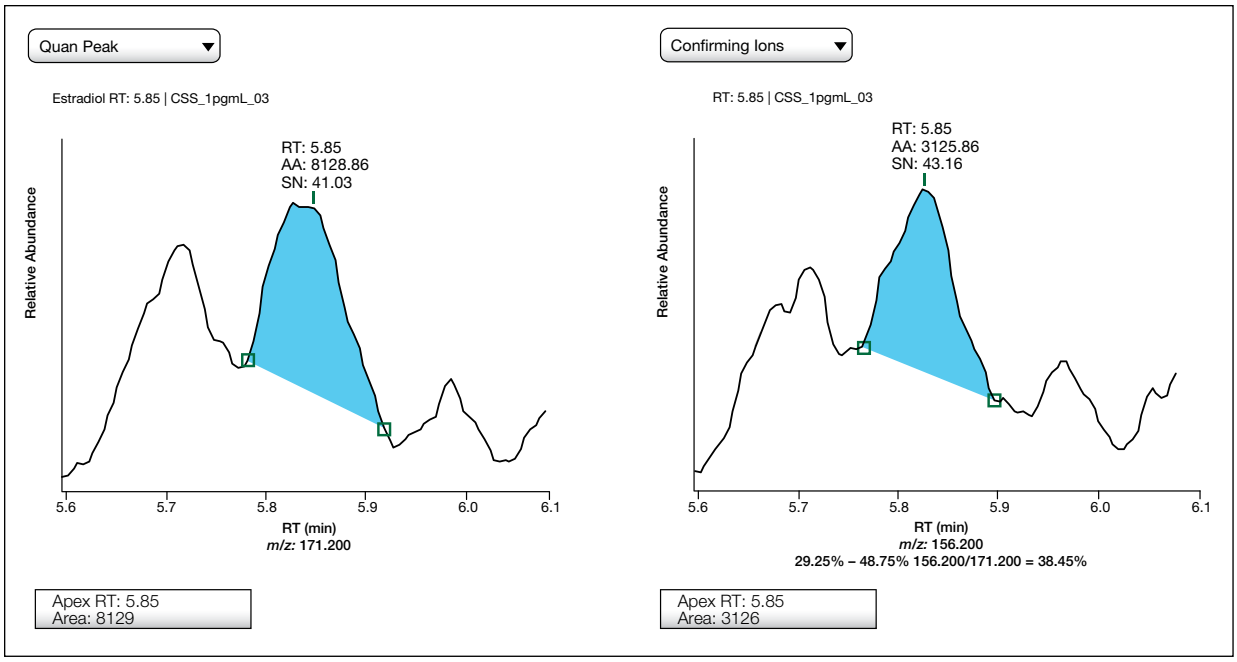

Figure 2. Chromatogram of 1 pg/mL calibrator for estradiol in CSS showing quantifier and confirming ion with passing ion ratio

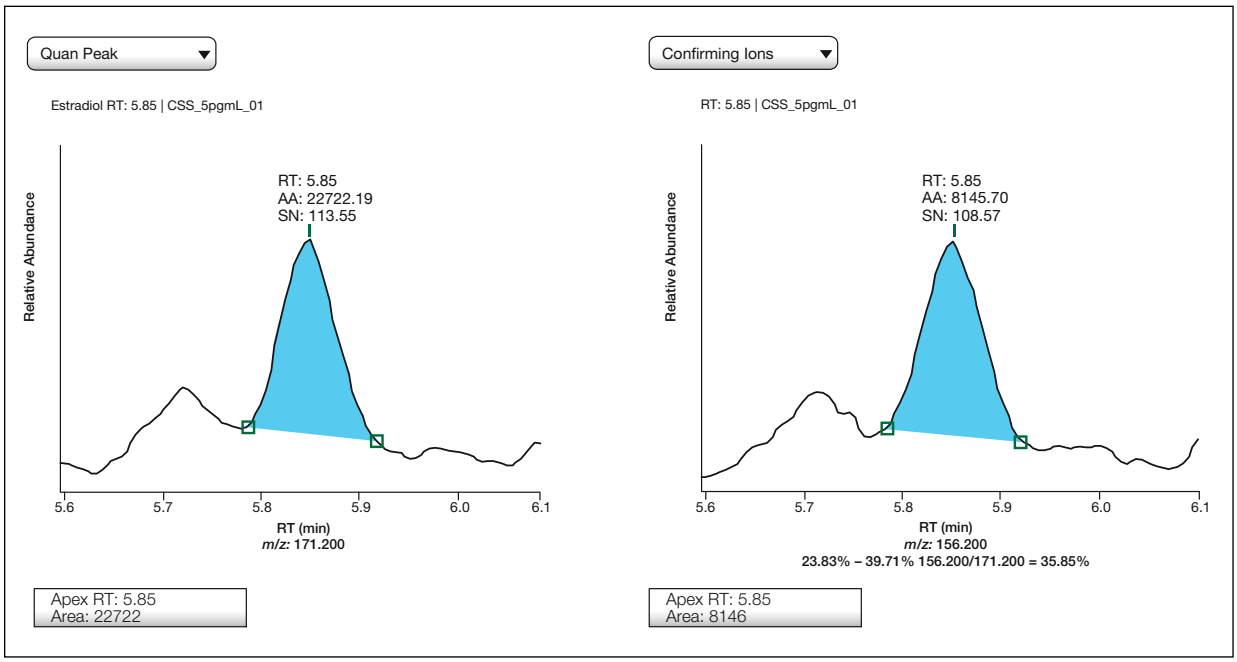

Figure 3. Chromatogram of 5 pg/mL calibrator for estradiol in CSS showing quantifier and confirming ion with passing ion ratio

Table 4. Results of mixing test showing recovery of estradiol in a sample of human plasma diluted 1-, 2-, and 4-fold

Sample	Mean Conc (pg/mL)	% RSD	Mean % Rec
Plasma	20.6	1.1	–
2-fold dilution	9.67	1.7	94.0
4-fold dilution	4.34	3.7	84.4

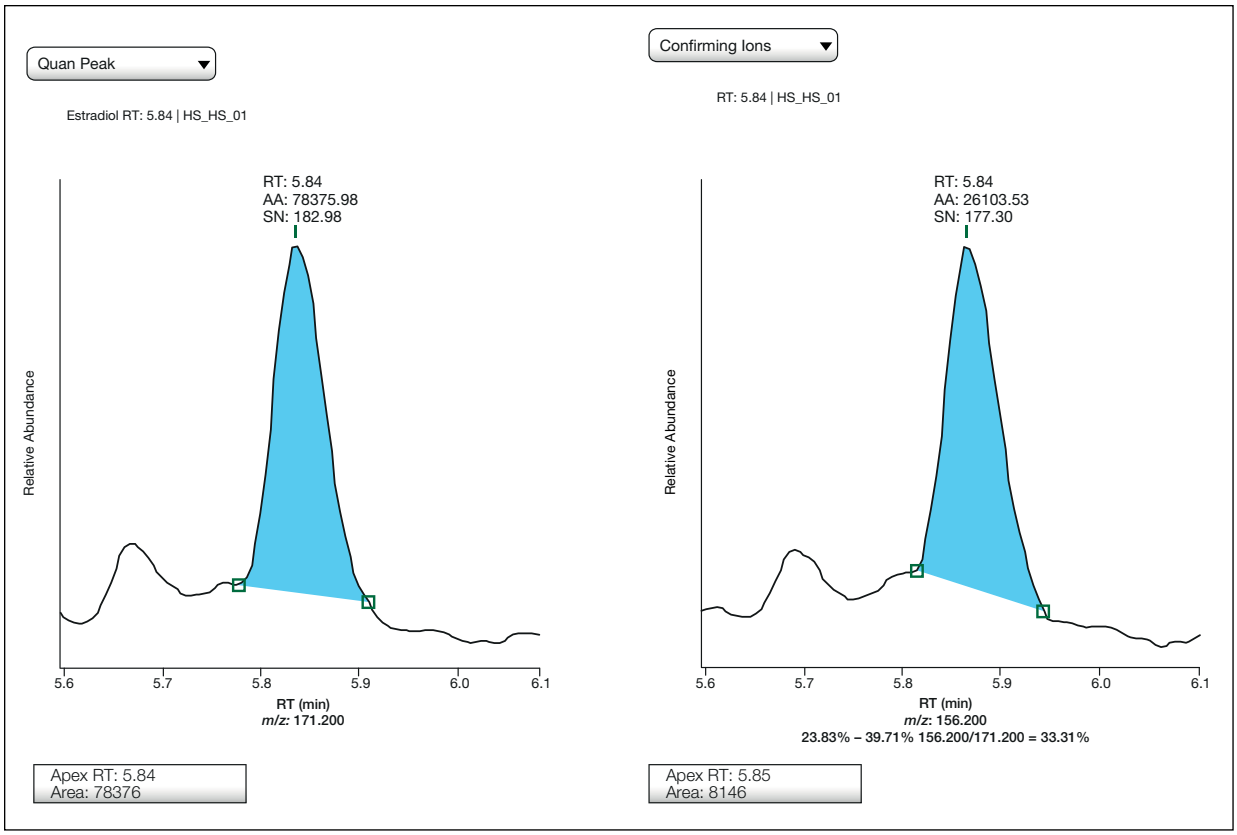

Figure 4. Chromatogram of estradiol in donor plasma at 22.6 pg/mL showing quantifier and confirming ion with passing ion ratio

Conclusion

- This clinical research method was able to reach the desired limit of quantitation of 1 pg/mL in human plasma.
- The method shows excellent precision, accuracy, and ion ratio confirmation over the entire calibration range of 1 to 1000 pg/mL.
- No matrix effects were observed.

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