Determination of α-Estradiol in Human Plasma by a LC/MS/MS Method

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Introduction

In biological samples estradiol can be detected utilizing HPLC for separation followed by electrochemical or mass spectrometric techniques. The purpose of this study was to develop a method that provides a simple, rapid and specific route for the determination of the alpha isomer of estradiol in human plasma. The assay parameters and validation results of this method are presented.

Analytical Method Summary

- **HPLC solvent system:**
  - Gradient: Initially 35% B, ramp to 80% B at 3 min, held until 4 min, ramp to 35% B at 4.5 min, hold until 7 min.
  - Solvent B: 0.1% ammonium hydroxide in acetonitrile
- **Column:** Ace C18, 100 X 2.1 mm, 5 µm, analytical column (Advanced Chromatography Technologies, Aberdeen, Scotland)
- **Instrumentation:** Agilent 1100 HPLC coupled to a Micromass Quattro Ultima tandem quadrupole mass spectrometer
- **Calibration Equation:** Power
- **Sample Extraction Method:** Liquid/Liquid with MTBE/hexane
- **Sample Extraction Volume:** 1.00 mL
- **Retention Times:** α-Estradiol: ~3.7 min; β-Estradiol: ~4.0 min
- **Mass Transitions:** α-Estradiol: 276.25 > 147.15; β-Estradiol: 271.25 > 145.14
- **Mass Accuracy:** ≤5%

Representative Calibration Curve For α-Estradiol in Human Plasma

![Representative Calibration Curve](image)

Analytical performance data included the extraction of α-Estradiol and the internal standard d5-estradiol from a high QC sample with 1.0 mL of MTBE/hexane. Calibration curve construction was performed with quintuplicate injections of the analyte and I.S. standards from 50.0 to 20,000.0 pg/mL. The linearity of the calibration curve was assessed over the concentration range of 50.0 to 20,000.0 pg/mL using a Power Calibration Equation. The limit of quantitation (LOQ) was established at 50.0 pg/mL based on the signal to noise ratio of 10.8 and a value greater than 1.00 indicates enhancement from the matrix.

<table>
<thead>
<tr>
<th>Concentration (pg/mL)</th>
<th>Mean (pg/mL)</th>
<th>SD (pg/mL)</th>
<th>%CV</th>
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<th>SD (pg/mL)</th>
<th>%CV</th>
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<tr>
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<td>53.1</td>
<td>6.0</td>
<td>11.4</td>
<td>46.6</td>
<td>5.0</td>
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<td>444.0</td>
<td>2.8</td>
<td>15751.5</td>
<td>438.6</td>
<td>2.8</td>
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</table>

Sensitivity (LLOQ)

- **LOQ:** 50.0 pg/mL
- **LOQ:** 27.5 pg/mL
- **LOQ:** 11.9 pg/mL
- **LOQ:** 4.8 pg/mL
- **LOQ:** 3.7 pg/mL

Stability

- **Reagent Blank High Std:**
  - Mean: 122.8 pg/mL
  - SD: 8.0 pg/mL

- **Plasma Blank High QC:**
  - Mean: 40000.0 pg/mL
  - SD: 114.8 pg/mL

Partial Volume Analysis

The partial volume analysis was performed by diluting the high and low QC samples 10-fold with plasma. The absolute recoveries of α-Estradiol and the internal standard were determined by analyzing the unextracted recovery standards spiked into extracted reagent blanks and extracted plasma blanks.

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Conclusions

The method is suitable to quantify human plasma in pharmacokinetic studies.

References