Introduction

Estradiol, a female sex hormone, not only plays critical role in human reproductive functioning, but also impacts bone health, cardiovascular system, and affects mode of behavior. It may also fuel certain diseases, such as uterine fibroids and breast cancer. Therefore, it is important to measure estradiol in human plasma for diagnostic and clinical research. Since estradiol is usually in a pg/mL concentration level in human plasma, the development of a sensitive quantitation method is critical. The purpose of this study is to develop and validate a sensitive and rapid UPLC-MS/MS method for the determination of estradiol in human plasma.

Methods

Sample Preparation:
Due to endogenous presence of estradiol in human plasma, bovine serum albumin (BSA) in phosphate buffered saline (PBS) was used for the preparation of calibration standards, LLOQ, low QC and mid QC samples. The high QC samples were prepared in authentic human plasma. Estradiol and the added internal standard were extracted from 200 μL of human plasma or surrogate matrix by liquid–liquid extraction using MTBE (then derivatized with dansyl chloride). After vortexing and centrifugation, the supernatant was transferred into a 96-well plate for LC-MS/MS analysis.

Liquid Chromatography:

- **Pump:** Shimadzu UPLC-TSA
- **Autosampler:** Shimadzu SIL–10AC
- **System Controller:** Shimadzu CRM–20A
- **Analytical Column:** Kinetex 1.7μm, 100 x 2.0 mm
- **Gradient:** Analyte was eluted using a gradient with MPB (0.1% FA in methanol) and MPh (0.1% FA in water)
- **Flow Rate:** 0.5 mL/min
- **Injection Volume:** 5 μL

Mass Spectrometry:

- **MS System:** AB Sciex Triple Quad 5500
- **Condition:** (a) APCI-ABMS
- **Estradiol:** 506.2 → 171.1; Estradiol-d$_4$: 511.2 → 171.1

Figure 1. Typical Chromatograms of Estradiol

Figure 2. Typical Calibration Curve of Estradiol in Human Plasma (0 – 10,000 pg/mL)

### Results and Discussion

#### Table 1: Calibration Curve and Other Parameters

<table>
<thead>
<tr>
<th>Concentration (pg/mL)</th>
<th>LLOQ</th>
<th>LQC</th>
<th>MQC</th>
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<tbody>
<tr>
<td></td>
<td>1.00</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

• This assay was validated within a nominal range of 10 to 10,000 pg/mL for human plasma with the correlation coefficients (r$^2$) ≥ 0.9944. The LLOQ, LQC and MQC were prepared in surrogate matrix while HQC was prepared in R_EDETA plasma. The intra-day and inter-day precision and accuracy were within the acceptance criteria for both surrogate and plasma matrix QCs as shown in Table 1, indicating using BSA in PBS as surrogate matrix is suitable for the quantitation of estradiol in human plasma. No obvious interference peak was observed in the surrogate matrix.

• The stability of estradiol in surrogate matrix and human plasma has been evaluated at room temperature for 6 hours, through 3 cycles of freeze-thaw and after storage at approximately -20°C and -70°C for 47 days. It was found that estradiol was stable under those conditions.

Conclusions

After derivatization using dansyl chloride, a sensitive and specific UPLC-MS/MS assay for the quantitation of estradiol in human plasma has been developed and validated. BSA in PBS was used as surrogate matrix is suitable for the quantitation of estradiol in human plasma. The assay has been used for analysis of estradiol in human plasma in support of clinical studies.

A Sensitive and Rapid UPLC-MS/MS Quantitation of Estradiol in Human Plasma

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