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

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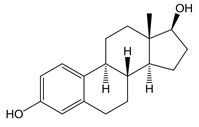
Overview

To support clinical studies, an LC-MS/MS bioanalytical method was validated for the quantitation of estradiol in human plasma using surrogate matrix. The method utilized a liquid-liquid extraction procedure followed by derivatization using dansyl chloride prior to LC-MS/MS analysis. Estradiol-d_5 was used as the internal standard (IS). Method validation parameters including selectivity, sensitivity, precision, bias, and stability were evaluated and are presented in this report.

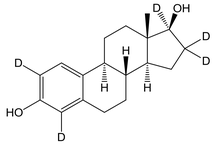
Introduction

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.

Structure



Estradiol



Estradiol-d5, IS

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-30AD
Autosampler:	Shimadzu SIL-30AC
System Controller:	Shimadzu CBM-20A
Analytical Column:	Kinetex PFP 2.6µm, 100 x 2.0 mm
Gradient:	Analyte was eluted using a gradient with MPA (0.1% FA in water) and MPB (0.1% FA in methanol)
Flow Rate:	0.5 mL/min
Injection Volume:	5 µL

Mass Spectrometry:

MS System:	AB Sciex Triple Quad 5500
Condition:	(+) APCI-MRM,

Estradiol: 506.2 → 171.1 ; Estradiol-d_5: 511.2 → 171.1

Results and Discussion

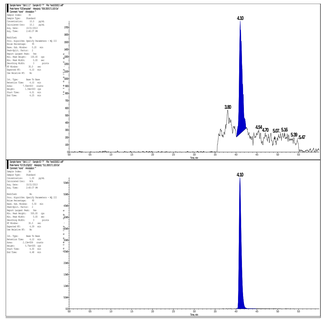


Figure 1. Typical Chromatograms of Estradiol LLOQ Sample (Upper: Estradiol, Lower: Estradiol-d_5 (IS)

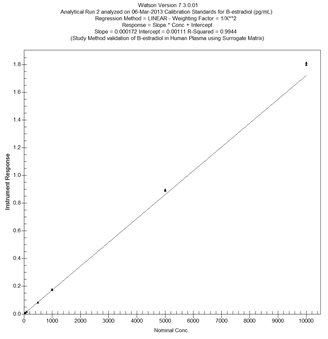


Figure 2. Typical Calibration Curve of Estradiol in Human Plasma (10 – 10,000 pg/mL)

Table 1 Estradiol Intra-Day and Inter-Day Precision and Bias

Run Number	Estradiol Nominal Concentrations (pg/mL) LLOQ (10)	Low (25)	Mid (400)	High (8000)
	Estradiol Measured Concentrations (pg/mL)			
1	14.8	21.0	403	7930
	9.94	26.3	406	8150
	11.9	26.3	390	8300
	9.80	21.5	365	7870
	11.0	21.8	390	7450
	8.80	--	404	7660
Mean	11	23.4	393	7890
S.D.	2.13	2.68	15.4	311
%CV	19.4	11.5	3.9	3.9
%Bias	10.0	-6.4	-1.8	-1.4
n	6	5	6	6
2	11.9	26.3	407	8690
	11.9	27.9	384	7980
	12.3	26.5	435	8270
	11.7	22.0	439	8910
	12.1	26.2	415	8700
	8.40	26.5	432	8270
Mean	11.4	25.9	419	8470
S.D.	1.48	2.01	21.0	351
%CV	13.0	7.8	5.0	4.1
%Bias	14	3.6	4.8	5.9
n	6	6	6	6
4	11.7	22.6	419	8470
	9.71	19.2	419	8640
	9.12	24.1	436	9080
	12.0	23.1	402	9470
	10.9	23.1	408	9030
	8.66	21.9	423	8230
Mean	10.3	22.3	418	8820
S.D.	1.39	1.70	11.9	455
%CV	13.5	7.6	2.9	5.2
%Bias	3	-10.8	4.5	10.3
n	6	6	6	6
Overall Mean	10.9	23.9	410	8390
Overall S.D.	1.65	2.54	19.8	529
Overall %CV	15.1	10.6	4.8	6.3
Overall %Bias	9	-4.4	2.5	4.9
n	18	17	18	18

•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 K_2EDTA 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 -20ºC and -70°C for 47 days. It was found that estradiol was stable under those conditions.

Figure 2 Recovery of Estradiol Extracted from Surrogate Matrix and Human Plasma

	Area	
	Extracted	Neat Standard
Replicate	LQC	
1	4530	5060
2	4690	4650
3	4780	4780
4	3710	4180
5	4480	4170
6	4720	4060
n	6	6
Mean	4485	4483
SD	397	404
CV (%)	8.9	9
Recovery (%)	100.0	—
Replicate	MQC	
1	79000	64200
2	77300	75700
3	60200	85200
4	68900	79100
5	73700	76000
6	70000	75800
n	6	6
Mean	71517	76000
SD	6803	6835
CV (%)	9.5	9
Recovery (%)	94.1	—
Replicate	HQC (plasma)	
1	1630000	1890000
2	1690000	1660000
3	1680000	1870000
4	1740000	1660000
5	1600000	1650000
6	1700000	1780000
n	6	6
Mean	1673333	1751667
SD	50465	110529
CV (%)	3.0	6.3
Recovery (%)	95.5	—

The recovery from surrogate matrix (LQC and MQC) was from 94.1% to 100% , while from human plasma (HQC) was 95.5%..

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.