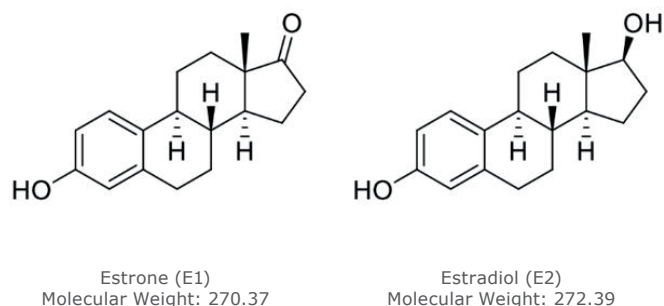


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# Extraction of Estrone and Estradiol from Human Serum Using ISOLUTE® SLE+ Prior to HPLC-MS/MS (Without Derivatisation or Fluorine Adduct Stabilization)



**Figure 1.** Structures of Estrone (E1) and Estradiol (E2).

## Introduction

Estrone (E1) and estradiol (E2) are female steroid hormones typically monitored in peri- and postmenopausal human serum samples to assess a number of conditions.

This application note describes a simple supported liquid extraction method for extraction of estrone and estradiol from human serum. An analytical HPLC-MS/MS method that avoids the use of derivatization or fluoride adduct stabilization (two approaches often used to enhance method sensitivity) is used. High, reproducible analyte recovery and low detection limits are achieved.

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

## Analytes

Estrone (E1) and Estradiol (E2)

## Sample Preparation Procedure

### Format:

ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate, Part Number 820-0400-P01

### Sample Pre-treatment

Add 250 µL of stripped serum to 100 µL of 25% IPA (aq) followed by 12.5 µL of 1000 ng/mL 1 stock of E1 and E2 in methanol and equilibrate for 10 minutes to simulate a 50 ng/mL sample. Dilute subsequent standards (0.001, 0.002, 0.005 and 5 ng/mL) from a 0.1 µg/mL stock in MeOH using H<sub>2</sub>O/ACN/MeOH (2:1:1, v/v).

### Sample Loading

Load 350 µL of pre-treated sample into each well using a Biotage® PRESSURE+ 96 Positive Pressure Manifold (PPM-96) and allow to absorb for 5 minutes.

### Analyte Extraction

Apply 600 µL of dichloromethane (DCM) and wait for 5 minutes. Apply a second aliquot of 600 µL of DCM and elute under gravity. Repeat with a third aliquot of DCM (600 µL). Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

### Post Elution and Reconstitution

Dry the extract to dryness in a stream of air or nitrogen using a Biotage® SPE Dry® 96 (40 °C at 80 L/min).

Reconstitute with 250 µL of H<sub>2</sub>O/ACN/MeOH (2:1:1, v/v).

## HPLC Conditions

### Instrument

Shimadzu Nexera X2 Series UHPLC (Tokyo, Japan)

### Column

Restek Raptor Biphenyl, 100 mm x 2.1 mm, 2.7 µm

### Column Temperature

30 °C

### Sample Temperature

25 °C

### Mobile Phase

A: 0.2% NH<sub>4</sub>OH and 1% IPA in Water

B: 0.2% NH<sub>4</sub>OH and 1% IPA in Methanol

### Wash Solvent

Water/Methanol (50:50, v/v) with 0.1% formic acid

### Flow Rate

0.3 mL min<sup>-1</sup>

### Injection Volume

50 µL

**Table 1.** HPLC Gradient Conditions.

Step	Time (min)	Flow Rate (µL/min)	% A	% B	PCI (µL/min)
1	0.01	300	30	70	6.0
2	1.0	300	20	80	6.0
3	1.1	300	5	95	6.0
4	3.0	300	5	95	6.0
5	3.1	300	30	70	6.0
6	5.0	300	30	70	6.0

**Table 3.** Multiple reaction monitoring transitions for target analytes.

	MRM Transition (m/z)	Declustering Potential (V)	Entrance Potential (V)	Collision Energy (V)	Collision Cell Exit Potential (V)	Dwell Time
<b>E1 Quant</b>	269.2 → 145.1	-100	-10	-60	-18	300
<b>E1 Qual</b>	269.2 → 183.1	-100	-10	-60	-18	300
<b>E2 Quant</b>	271.2 → 145.4	-100	-10	-60	-18	300
<b>E2 Qual</b>	271.2 → 183.4	-100	-10	-60	-18	300



## MS Conditions

### Instrument

Sciex 5500 triple quadrupole mass spectrometer (Sciex, Foster City, CA.) equipped with a Turbo Ionspray® interface for mass analysis.

**Table 2.** Sciex electrospray ionization source parameters for target analytes.

ESI Interface Conditions	Operating Parameter(s)
Curtain Gas (psi)	50
Collision Gas(psi)	8
Ion Spray Voltage (kV)	-4.5
Source Temp (°C)	650
Nebulizing Gas GS1 (psi)	80
Ion Source Gas GS2 (psi)	70

Negative ions were acquired in the multiple reaction monitoring (MRM) mode, conditions are shown in table 3 below:

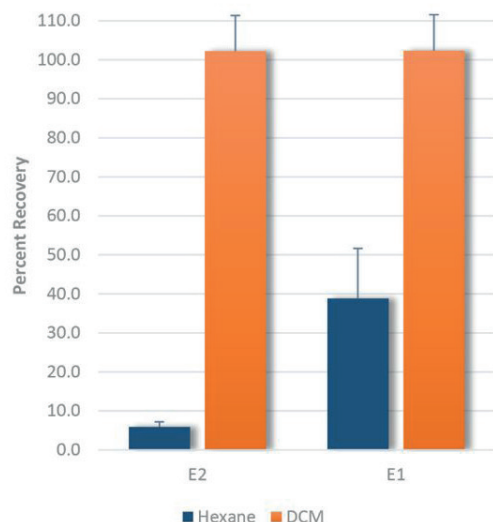
## Results

### HPLC-MS/MS Optimization

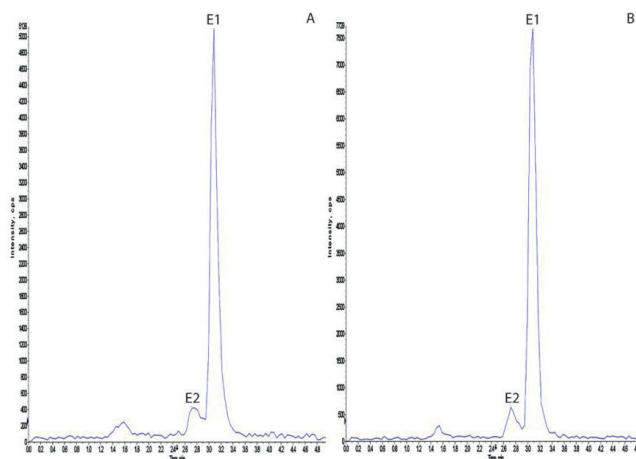
Infusion experiments focused on optimization of mobile phase additives to achieve low detection limits without the use of derivatization or fluorine additives in the mobile phase.

### Analyte Recovery

Recovery studies using hexane showed low recovery of both estradiol and estrone. However, the use of dichloromethane as extraction solvent produced excellent recoveries at or above 100% (Figure 2).



**Figure 2.** Percent recovery of E2 and E1 in stripped serum at 50 ng mL<sup>-1</sup> using 1.2 mL hexane or 1.8 mL DCM. n=8 for each analysis, error bars represent standard deviation.



**Figure 3.** Representative chromatograms of E1 and E2 extracted in serum with DCM analyzed on a Sciex 5500 mass spectrometer with 0.2% NH<sub>4</sub>OH and 1.0% IPA mobile phase additives. **(A)** Extracted serum at 0.001 ng mL<sup>-1</sup>. **(B)** Extracted serum at 0.002 ng mL<sup>-1</sup>.

Using optimum extraction and analytical conditions (as described) an LOD and LOQ of 0.001 and 0.002 ng/mL (respectively) were achieved from extracted serum samples (Figure 3).

## Conclusion

This application note describes a sensitive, robust method for extraction and analysis of estrone and estradiol in serum.

The use of ammonium hydroxide and isopropanol in the HPLC mobile phase provides a unique alternative to ammonium fluoride allowing low levels of detection of estrogens when extracted with DCM using ISOLUTE® SLE+ for sample preparation.

## Ordering Information

Part Number	Description	Quantity
<b>820-0400-P01</b>	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate	1
<b>SD-9600-DHS-EU</b>	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
<b>SD-9600-DHS-NA</b>	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
<b>PPM-96</b>	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1

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