TN-1408

Derivatization-Free LC–MS/MS Analysis of Estrogens with Streamlined Sample Cleanup

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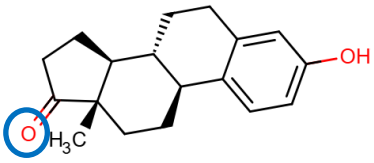
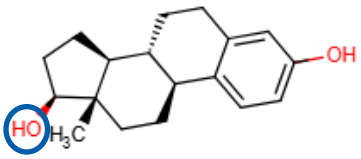
Introduction

Precise measurement of estrone (E1) and estradiol (E2) is essential for assessing reproductive and endocrine function in clinical research. Due to their low circulating levels, poor ionization efficiency, and structural similarity, direct LC-MS quantification without derivatization is analytically challenging. Although recent improvements in sample preparation and mass spectrometry sensitivity have enhanced assay performance, derivatization remains a common approach to achieve the sensitivity required for reliable detection of E1 and E2 in serum. Here, we present a sensitive method for LC-MS/MS quantitation of estrone and estradiol without derivatization using supported liquid extraction (SLE).



Estrone (E1)
LogP: 4.3
pKa : 10.3



Estradiol (E2)
LogP: 3.74
pKa : 10.3

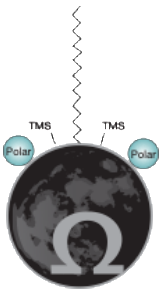
Reagents and Chemicals

Analytical reference standards and human serum were purchased from Cerilliant® (Round Rock, TX, USA) and Golden West Diagnostics, LLC® (Temecula, USA), respectively. Ultrapure D.I. water was obtained via Sartorius® Arium® Comfort II from Sartorius Corporation (Bohemia, NY, USA). All other chemicals were obtained from Sigma-Aldrich® Company (St. Louis, MO, USA).

Column Screening Methods

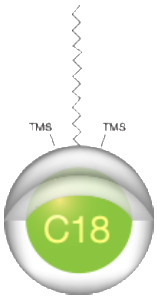
Four columns were screened to determine the better separation for E1 and E2: Luna Omega Polar C18 (P/N:00B-4760-Y0), Kinetex C18 (P/N: 00B-4462-Y0), Kinetex F5 (P/N: 00B-4723-Y0) and Kinetex Biphenyl (P/N: 00B-4622-Y0O).

Luna Polar C18



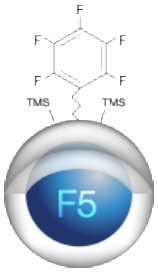
Fully porous silica bonded with C18 with a polar modified surface

Kinetex C18



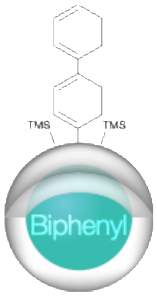
Hydrophobic compounds (separation based on LogP)

Kinetex F5



Closely related compounds and aromatic hydrocarbons

Kinetex Biphenyl



Closely related compounds and aromatic hydrocarbons

Column Screening LC Conditions

LC Column: Four Columns were screened
Dimensions: 50 x 3.0 mm

Mobile Phase: A: 0.5 mM Ammonium Fluoride in Water
B: Methanol

Gradient:

Time (min)	%B
0	40
3.0	95
3.5	95
3.51	40
5.0	40
6.5	40
8	40

Flow Rate: 0.8 L/min
Injection Volume: 5 µL
Temperature: 30 °C
LC System: Agilent® 1290 Infinity
Detection: MS/MS
Detector: SCIEX® 7500 Triple Quad™

MS/MS Conditions

Ion Source: ESI
Polarity: Positive or Negative
Source Temperature: 700 °C
GS1: 60 psi
GS2: 60 psi
CUR: 40 psi
CAD: 10
IS: -3000 V
EP: -10 V

Optimized Method for E1 and E2

Table 1. Strata SE Sample Preparation Method

Step	Description
Sample Pretreatment:	Combine 200 µL human serum sample (Mass Spec Gold, Cat # 4000 with verified ultra-low hormone levels, spiked with 20 µL of 400 pg/mL isotopically labeled internal standard) with 200 µL 25 mM ammonium acetate buffer, pH 6.9 (unadjusted).
Load:	400 µL pretreated sample onto the SLE Plate
Apply:	Vacuum briefly. Wait 5 minutes before eluting.
Elute 1:	900 µL Ethyl Acetate/Hexane (9:1) Briefly apply vacuum. Briefly apply vacuum.
Elute 2:	900 µL Ethyl Acetate/Hexane (9:1) Briefly apply vacuum. Briefly apply vacuum.
Dry:	Under a gentle stream of Nitrogen at 45 °C
Reconstitute:	200 µL of 0.5 mM Ammonium Fluoride (aq)/Methanol (60:40, v/v)

Optimized LC Conditions for E1 and E2

LC Column: Kinetex 2.6 mm Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4462-Y0
Mobile Phase: A: 0.5 mM Ammonium Fluoride in Water
B: Methanol

Gradient:

0.0	40
3.0	95
3.5	95
3.51	40
5.0	40

Flow Rate: 0.8 L/min
Injection Volume: 20 µL
Temperature: 30 °C
LC System: Agilent® 1290 Infinity
Detection: MS/MS (source parameters were the same as column screening)
Detector: SCIEX® 7500 Triple Quad™

Results

Estrone and estradiol co-eluted on the Luna Omega Polar C18 column. They were separated by 0.1 minutes on the Kinetex C18, and by 0.2 minutes on the Kinetex F5. Separation on the Kinetex Biphenyl was almost 1 minute (Figures 2-5). The gradient was optimized to shorten the run time and still maintain good separation of estrone and estradiol (Figure 1). Calibration curves are shown in Figure 6. Extracted ion chromatograms for E1 and E1 at low concentrations are in Figure 7.

Figure 1. Optimized E1 and E2 separation on Kinetex Biphenyl

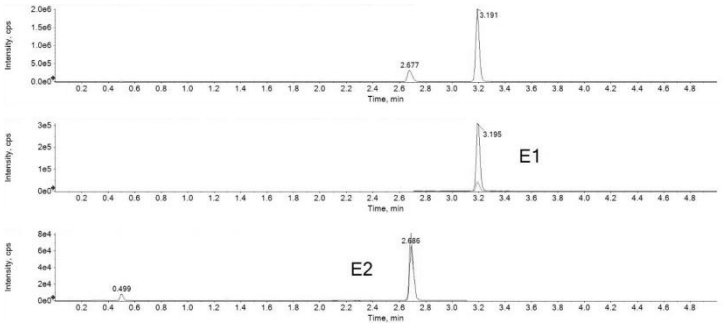


Figure 2. Estrogens separation Luna Omega Polar C18

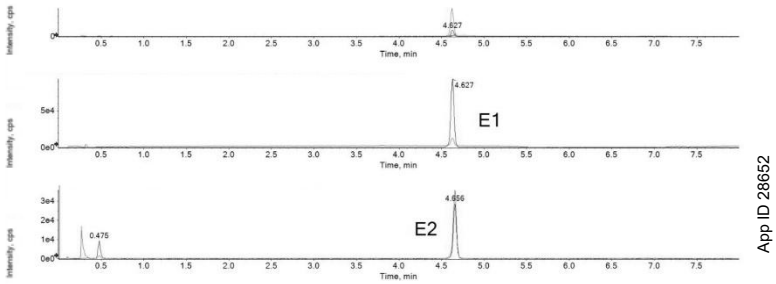


Figure 3. Estrogens separation Kinetex C18

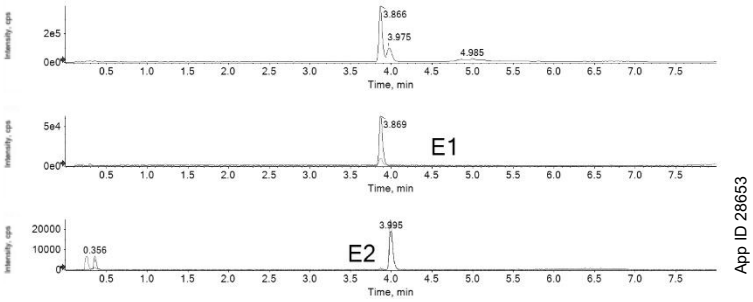


Figure 4. Estrogens separation Kinetex F5

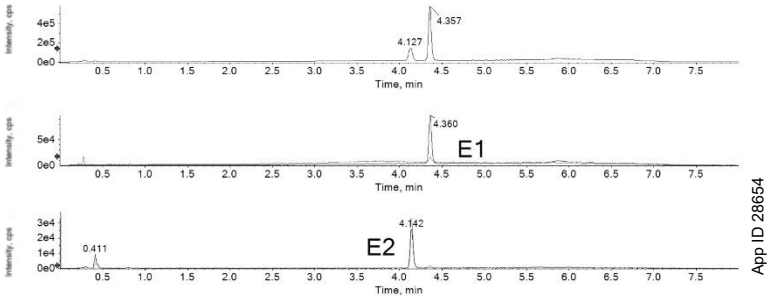
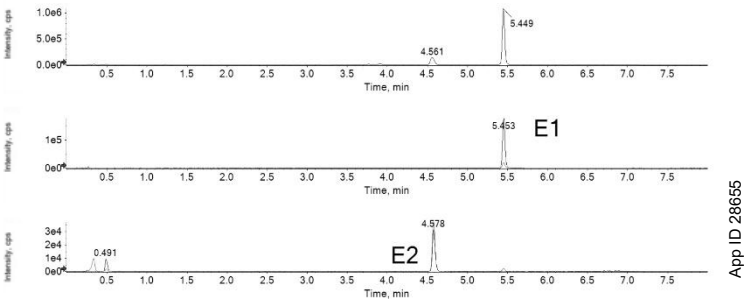


Figure 5. Estrogens separation Kinetex Biphenyl



Have questions or want more details on implementing this method? We would love to help!
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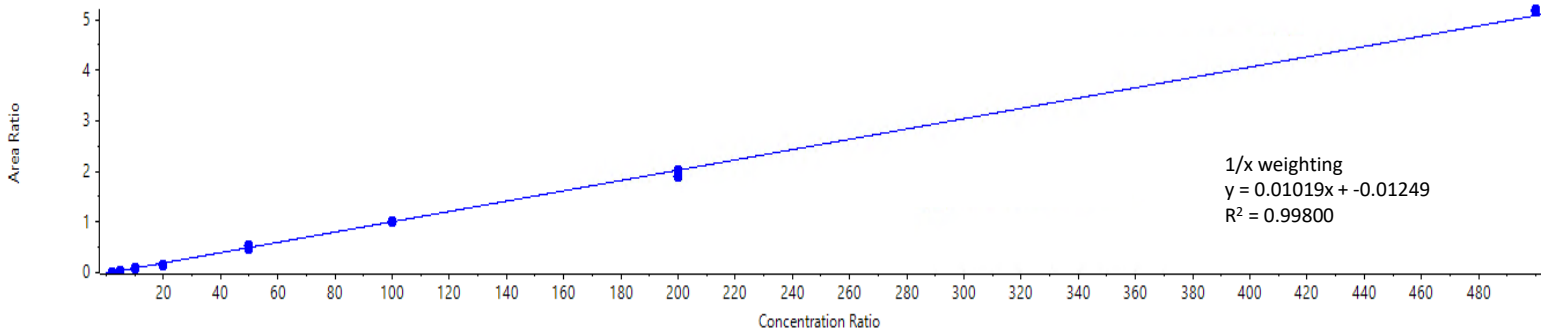
Figure 6. Calibration Curves

Estrone (E1) 1-500 pg/mL



Estradiol (E2) 5-500 pg/mL

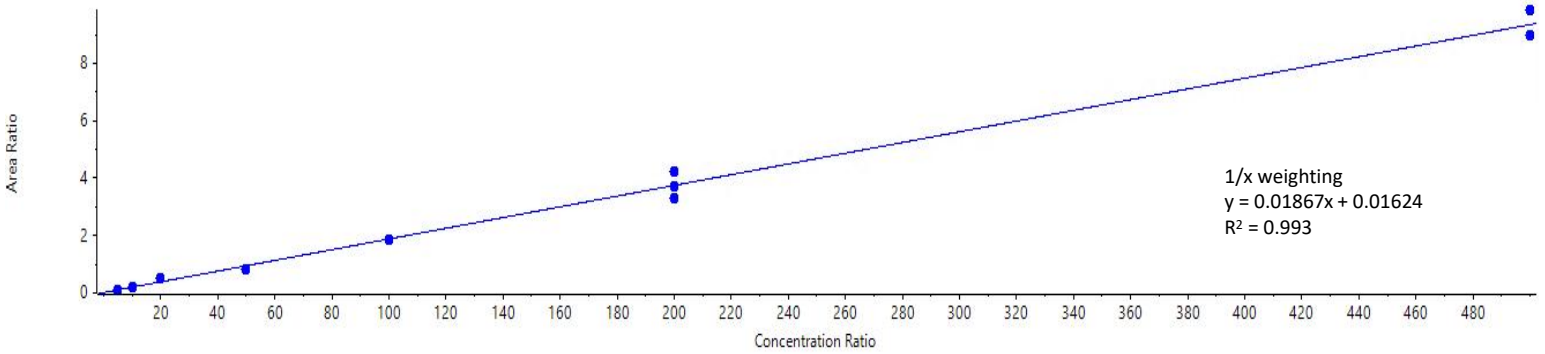
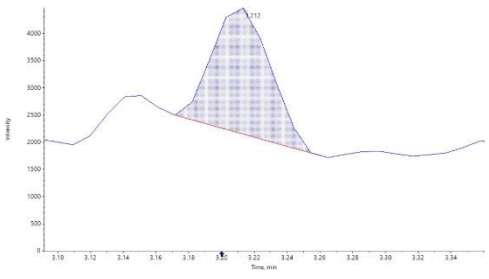
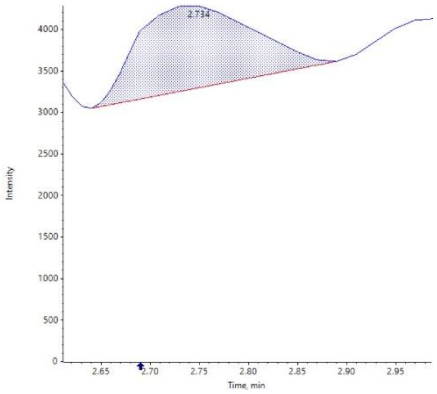


Figure 7. Underivatized Estrone and Estradiol at Low Concentrations

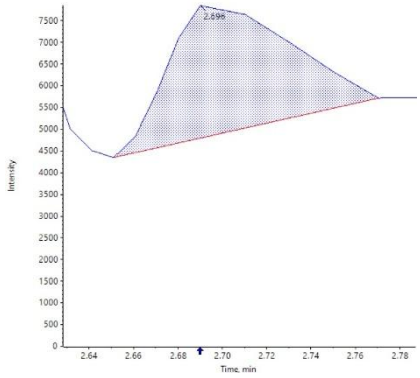
Estrone (E1) 5 pg/mL
S/N = 12



Estradiol (E2) 10 pg/mL
S/N = 8



Estradiol (E2) 20 pg/mL
S/N = 19



Conclusions

Concentration ranges for 95% of estrone and estradiol in a population of all males and females[1]: is 40-200 pg/mL for estrone and 40-350 pg/mL for estradiol. This method using Strata SE SLE and Kinetex Biphenyl on a SCIEX 7500 MS provides a solution for the majority of samples for clinical research applications without derivatization. The Kinetex Biphenyl column provided the best separation for E1 and E2 from the of columns evaluated. The optimized LC separation had excellent resolution of the target analytes with a short injection to injection time of 5 minutes. Strategies to attain higher sensitivity include increasing serum volume used, reducing the reconstitution volume, increasing the injection volume and derivatization. Strata SE SLE produced clean extracts with fewer steps than solid phase extraction (SPE).

Sample Preparation Steps Using Strata SE SLE

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

Ordering Information

Strata SE SLE

Interference FREE

Part No.	Description	Unit
8E-S542-5GB	MAX, 96 Well plate	2/pk
8E-S542-FGB	MINI, 96 Well plate	2/pk

Accessories

96-Well Plate Manifold**		
Part No.	**Description**	**Unit**
AH0-8950	96-Well Plate Manifold, Universal w/vacuum gauge	ea

Replacement Parts		
Part No.	**Description**	**Unit**
AH0-7285	96-Well Plate Manifold Replacement Gasket, Flat (to fit between acrylic chamber and 96-well plate), black	ea
AH0-7198	96-Well Plate Manifold Replacement Gasket, Profile, (to fit between acrylic chamber and manifold base), white	ea

**Manifold, compatible with 2 mL Impact plate, Novum SLE 96-well plate, Phree Phospholipid Removal Plate, Strata, and Strata-X 96-well plate formats.

Accessories

Collection Plates*		
Part No.	**Description**	**Unit**
AH0-7192	350 µL/well 96-Square Well Conical V-Bottom Collection Plate	50/pk
AH0-7193	1 mL/well 96-Square Well Conical V-Bottom Collection Plate	50/pk
AH0-7194	2 mL/well 96-Square Well Conical V-Bottom Collection Plate	50/pk
AH0-8636	2 mL/well 96-Round Well Round Bottom, 8 mm Collection Plate	50/pk
AH0-9332	1.2 mL/well 96-Round Well Round Bottom Collection Plate	50/pk
AH0-9333	0.5 mL/well 96-Round Well V-Bottom, 7 mm Collection Plate, Sterile	50/pk
AH0-9341	0.5 mL/well 96-Round Well Conical Bottom, 7 mm Collection Plate	50/pk

Sealing Mats*		
Part No.	**Description**	**Unit**
AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633**	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634**	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-8199	Sealing Mats, Pierceable, 96-Square Well, Santoprene™	100/pk
AH0-7195	Sealing Mats, Pieceable, 96-Square Well, Ethylene Vinyl Acetate (EVA)	50/pk
AH0-7362	Sealing Tape Pad	10/pk

*Square well sealing mats compatible with 2 mL Impact Plates, Novum SLE 96-well plate, Phree Phospholipid Removal plate, Strata and Strata-X 96-well plates, and 96-square collection plates.
**8 mm round-well sealing mats compatible with 2 mL round-well 8 mm collection plates (AH0-8636).

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

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