

# APPLICATIONS

## Separation of Low-level Estrogen Analytes from Plasma by LC-MS/MS Analysis in Conjunction with Novum™ PRO MAX Simplified Liquid Extraction (SLE)

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### Overview

In this study estrogen analytes estrone, beta-estradiol, and estriol were derivatized from plasma using Novum PRO MAX 96-Well Plates SLE sample preparation. These samples were then injected onto LC-MS/MS for analysis. Analytes were successfully separated giving excellent recovery and lowest level of quantitation. Novum PRO utilized an optimized manufacturing process to provide less background noise which leads to cleaner samples post sample preparation.

### Sample Preparation

<b>Pretreatment:</b> 200 µL spiked plasma + 200 µL 50 mM Sodium phosphate buffer, pH unadjusted
<b>Load:</b> Novum PRO MAX 96-Well Plate, wait 5 minutes
<b>Part No.:</b> <a href="#">8E-S539-5GA</a>
<b>Elution:</b> 1:3 Hexane:Ethyl Acetate
<b>Dry:</b> Under Nitrogen
<b>Add:</b> 50 µL Dansyl chloride/Sodium bicarbonate (50:50)
<b>Incubate:</b> 60 °C for 10 minutes
<b>Inject:</b> Inject 10 µL onto LC-MS/MS

### LC-MS/MS Conditions

**Column:** Kinetex® 1.7 µm Biphenyl 100 Å  
**Dimension:** 50 x 2.1 mm  
**Part No.:** [00B-4628-AN](#)  
**Mobile Phase:** A: 0.1 % Formic acid in Water  
 B: 0.1 % Formic acid in Methanol  
**Gradient:**

Time (min)	%B
0	80
1	80
2	90
3	95
6	95
6.1	80
8	80

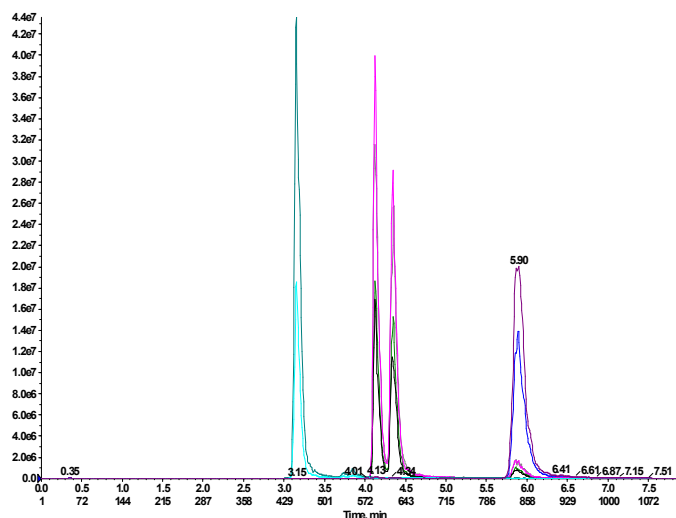
**Flow Rate:** 0.4 mL/min  
**Temperature:** Ambient  
**Detection:** SCIEX® 6500 MS/MS

### MS/MS Conditions

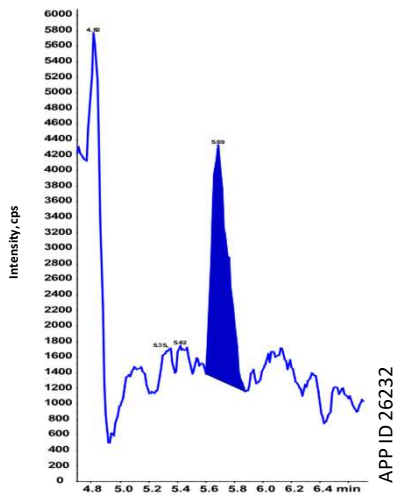
**Ion Source:** Positive  
**CUR:** 20  
**CAD:** High  
**GS1:** 30  
**GS2:** 10  
**IS:** 4000  
**Source Temp:** 450°C

Q1	Q3	Time	ID	DP	EP	CE	CXP
504.2	156.1	50	(E1)Estrone-1	50	10	80	14
504.2	171.1	50	(E1)Estrone-2	40	10	50	18
522.2	171.1	50	(E3)Estriol-1	100	10	50	8
522.2	156.1	50	(E3)Estriol-2	120	10	60	8
524.2	156.1	50	(E3)Estriol-D2-1	100	10	50	8
524.2	171.1	50	(E3)Estriol-D2-2	120	10	60	8
511.2	156.1	50	(E2)Estradiol-D5-1	40	10	60	8
511.2	171.1	50	(E2)Estradiol-D5-2	30	10	50	8
506.201	156.1	50	(E2)Estradiol-1	40	10	60	8
506.201	171.1	50	(E2)Estradiol-2	30	10	50	8
506.211	156.1	50	(E1)Estrone-D2-1	60	10	60	8
506.211	171.1	50	(E1)Estrone-D2-2	100	10	50	8

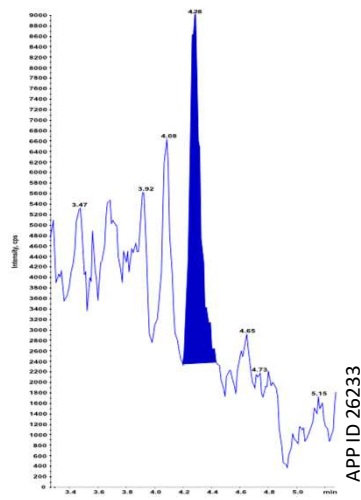
### Representative Chromatogram of Estrogens



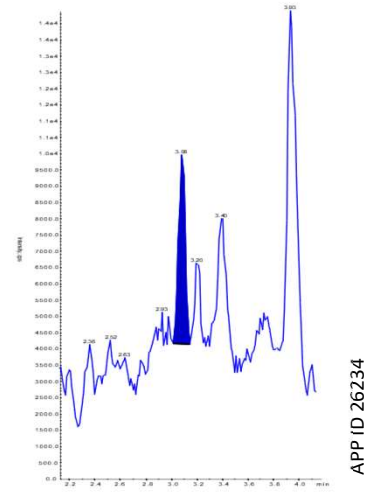
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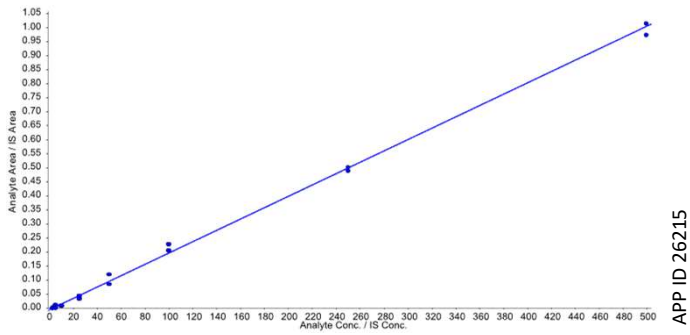
10 pg/mL E1 (LLOQ)



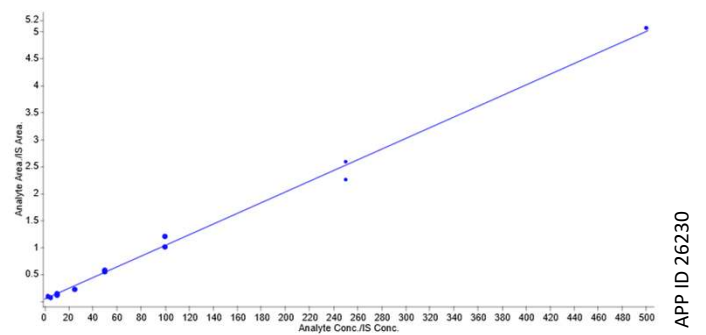
10 pg/mL E2 (LLOQ)



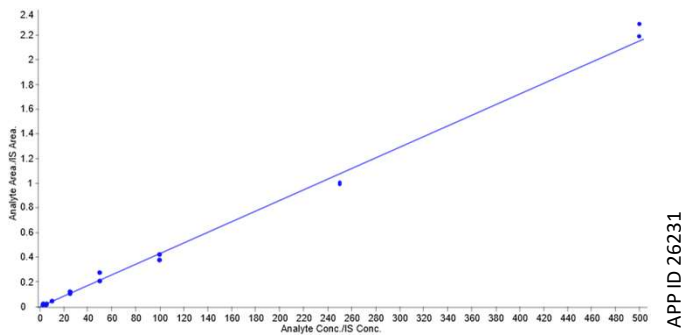
5 pg/mL E3 (LLOQ)



E1 Calibration Curve



E2 Calibration Curve



E3 Calibration Curve

Analyte	Retention Time (min)	% Recovery	% CV	LLOQ
E1	5.90	111	7	10 pg/ml
E2	4.35	92	7	10 pg/ml
E3	3.15	99	1	5 pg/ml

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Novum is patent pending.

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