

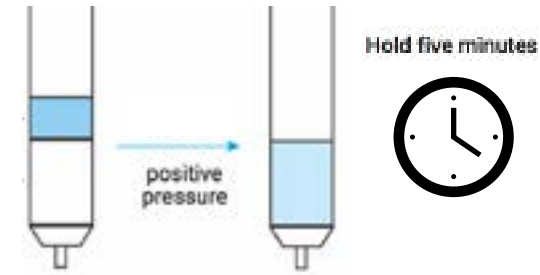


Quantitative Determination of a Panel of Endogenous Steroids in Human Serum by LC/MS/MS using an Agilent Supported Liquid Extraction (SLE) Chem Elut S Plate

- Use Chem Elut S supported liquid extraction plates or cartridges for more rugged, reliable, and reproducible methods
- Chem Elut S (synthetic based SLE) has better well-to-well reproducibility and calibration curve linearity than diatomaceous earth-based SLE

1. Aliquot 200 μ L of serum samples into a 1 mL collection plate. Add 10 μ L of IS spiking solution to each sample. Cover the plate and vortex for 30 seconds.

2. Transfer the entire sample mixture to an Agilent Chem Elut S plate (200 mg) with a 2 mL collection plate beneath. Apply 2 to 3 PSI pressure to load the aqueous sample into the SLE sorbent bed.

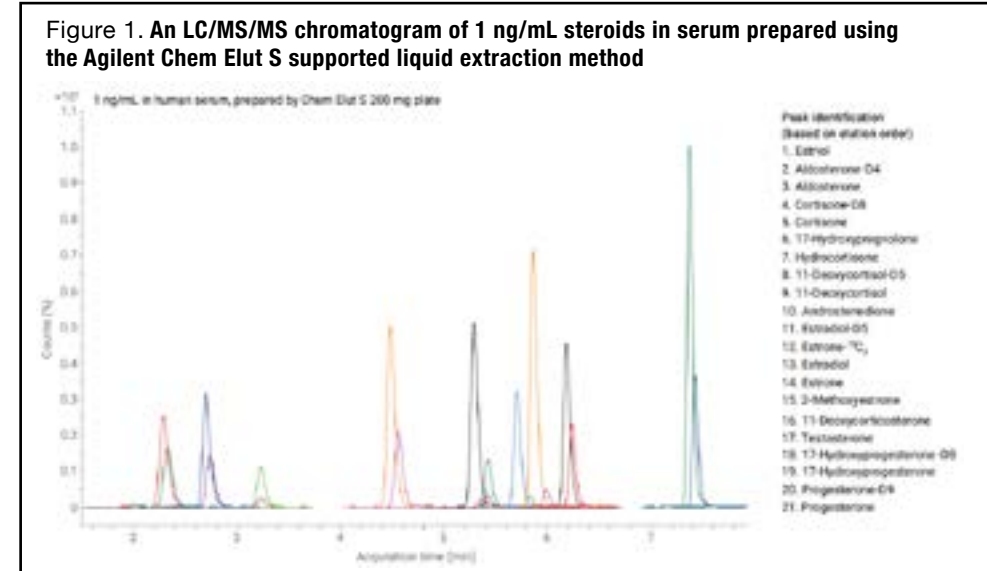
3. Add 400 μ L of 1:1 MTBE/EtOAc into each well, and allow gravity elution. When there is no visible liquid left in wells, add another 400 μ L of 1:1 MTBE/EtOAc for gravity elution. Repeat the above step. In total, use 400 μ L \times three for gravity elution. When there is no visible liquid left in all of the wells, apply \sim 6 PSI to dry the cartridge.

Add solvent

4. Remove the collection plate, and dry the entire eluent with N₂ flow at 40 $^{\circ}$ C. Reconstitute with 100 μ L of 1:1 MeOH/H₂O; cover the plate with a square plate mat. Vortex the plate for two minutes, sonicate for three minutes, then centrifuge for two minutes. Samples are then ready for LC/MS/MS analysis.

Get reproducible organic phase separation—every time.

Elute Gravity, positive pressure to dry



HPLC Conditions

Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 μ m (959758-902)

Guard: Agilent ZORBAX RRHD C18 guard, 2.1 x 5 mm, 1.8 μ m (821725-901)

Flow rate: 0.4 mL/min

Column temp: 40 $^{\circ}$ C

Inj. volume: 20 μ L

Mobile phase: A) 0.2 mM ammonium fluoride in water
B) 0.2 mM ammonium fluoride in MeOH

Needle wash: 1:1:1 ACN/MeOH/IPA/H₂O w/ 0.2% FA

Gradient: 0:00 50%B 0.4 mL/min
3:00 60%B 0.4 mL/min
8:00 90%B 0.4 mL/min
8:50 100%B 0.4 mL/min

Stop time: 8.5 min

Post time: 2.5 min

MS Conditions

Gas temp: 180 $^{\circ}$ C

Gas flow: 11 L/min

Nebulizer: 20 PSI

Sheath gas heater: 400 $^{\circ}$ C

Sheath gas flow: 10 L/min

Capillary: 3,500 V (pos) 3,000 V (neg)

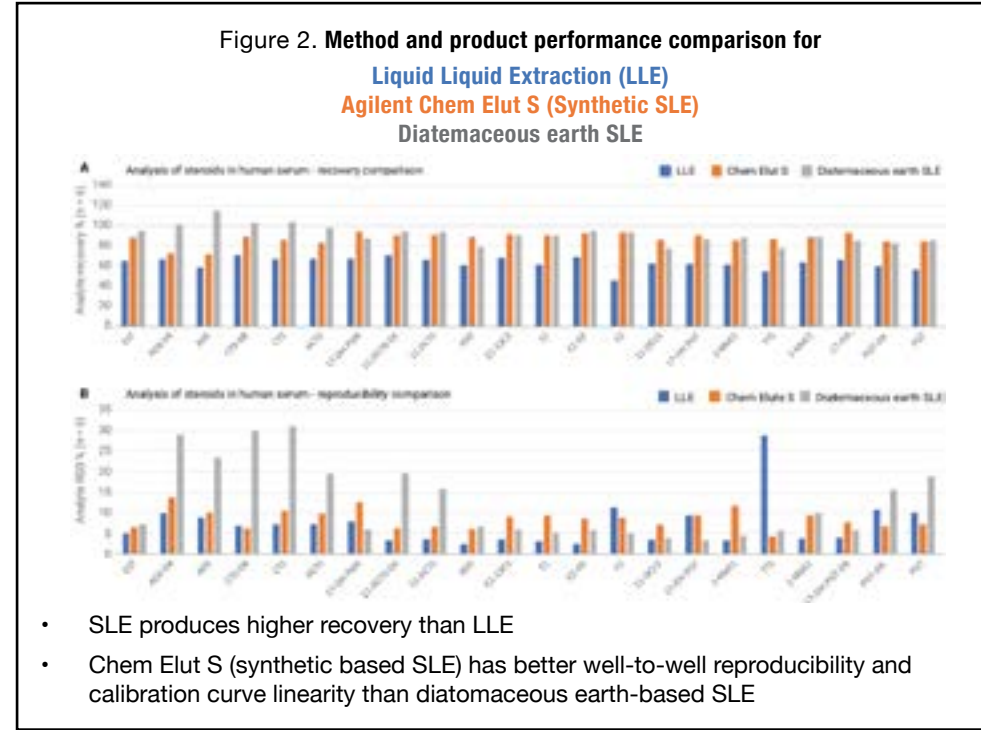
iFunnel: High-pressure RF: 150 V (pos & neg)

Parameters: Low-pressure RF: 100 V (pos & neg)

Data acquisition: dMRM

TECH TIP

The use of ammonium fluoride buffer not only increased ionization of these difficult steroid compounds in negative mode, but also increased the ionization of other compounds in positive mode




AGILENT CHEM ELUT S & ACCESSORIES

PART NO	DESCRIPTION	QTY
5610-2003	Chem Elut S, 2 mL 96 well plate, 200 μ L sample volume	ea
5610-2004	Chem Elut S, 2 mL 96 well plate, 400 μ L sample volume	ea
959758-902	Agilent ZORBAX RRHD Eclipse Plus C18, 1.8 μ m, 2.1 x 100 mm	ea
821725-901	Agilent ZORBAX RRHD C18 guard, 1.8 μ m, 2.1 x 5 mm	ea
96-6001NR	96 Round-Well plate, 1 mL, no rim, polypropylene	50/cs
96-6009	96 Square-Well, V bottom plate, 2 mL, polypropylene	50/cs
96-0662	96 Square pierceable cap mat for 2 mL square	50/pk
BST-9790	2 mil PTFE sealing tape	100/pk

AGILENT CHEM ELUT S ORDERING INFORMATION

PART NO	DESCRIPTION	QTY
5610-2003	Chem Elut S, 2 mL 96 well plate, 200 μ L sample volume	ea
5610-2004	Chem Elut S, 2 mL 96 well plate, 400 μ L sample volume	ea
5610-2005	Chem Elut S, 1 mL tube, 200 μ L sample volume	100/pk
5610-2006	Chem Elut S, 3 mL tube, 400 μ L sample volume	100/pk
5610-2007	Chem Elut S, 6 mL tube, 1 mL sample volume	100/pk



TECH TIP

Steroid compounds are very sensitive to glass surfaces, especially at low concentrations. The use of glass vials could result in significant variations and loss of steroid compounds, especially in highly aqueous samples. As a result, glass vials and tubes should be avoided during standard and sample preparation and handling.



www.chromtech.com/customcontent/CT-5994-0949.pdf

Quantitative Determination of a Panel of Endogenous Steroids in Human Serum by LC/MS/MS