



Determination and Separation of Iohexol in Human Serum by LC-MS/MS using a Kinetex 2.6 μm Polar C18 Core-Shell Column

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Iohexol is an iodinated medical imaging dye that is typically used to add contrast to body parts before X-ray or CT scans. Alongside Iohexol intake, a patient may be required to take other therapeutics such as furosemide, metoprolol, atenolol or acetaminophen, which may interfere with the chromatographic analysis of Iohexol. In this application note, we selected acetaminophen, atenolol and metoprolol to display separation of Iohexol from possible interference drugs due to the nature of those analytes. From this study we developed two simplified extraction methods from human serum alongside analysis by LC-MS/MS using a Kinetex 2.6 μm Polar C18 core-shell HPLC/UHPLC column

Sample Extraction Procedures

Extraction Option 1

Acidified Protein Precipitation

1. Aliquot 50 μL of human serum to 1.8 mL amber microcentrifuge tube, add 1 mL DI water, mix
2. Add 30 μL of 70 % HClO₄ to sample and mix well, centrifuge at 13,500 rpm for 10 min
3. Take 200 μL supernatant into amber autosampler vial
4. Inject 5 μL on the system

Extraction Option 2

Phree™ 30 mg/well, 96-well plate
Part No.: 8E-S133-TGB

1. Add 1 mL Acetonitrile to Phree 96-well plate vial, then aliquot 50 μL of human serum sample into 96-well plate
2. Using pipette/or automation liquid handler pipette tip aspirate sample 4-5 times for mixing, then apply vacuum (10 - 20 inches Hg) to allow all organic solvent going through and into a 96-well collection plate for up to 5 minutes
3. Dry down the collection plate under N₂ at 45 °C
4. Reconstitute the collection 96-well plate with 300 μL 10 mM ammonium acetate (pH unadjusted), cap and mix.
5. Inject 5 μL on the system

LC-MS/MS Conditions

Column: Kinetex 2.6 μm Polar C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4759-AN
Mobile Phase: A: 10 mM Ammonium Acetate (unadjusted pH)
B: 10 mM Ammonium Acetate in Methanol

Gradient	Time (min)	(%) B
	0.01	0
	0.3	0
	2.5	65
	3.0	65
	3.1	95
	4.1	95
	4.5	0
	7.0	0

Injection: 5 μL
Flow Rate: 0.3 mL/min
Temperature: 40 °C
Pressure: 110 Bar
Detector: SCIEX 4000 QTRAP® (ESI+)
Sample: 1. Iohexol (10 μg/mL)
2. Ioversol (20 μg/mL, IS for Iohexol)
3. Acetaminophen (0.4 μg/mL)
4. Metoprolol (0.4 μg/mL)
5. Atenolol (0.8 μg/mL)

ESI Ionization Source Parameters:

CUR	10
CAD:	High
TEM:	600
GS1:	50
GS2:	50
IS:	5000
EP:	10
CXP:	13

Mass Transitions:

ID	Q1	Q3	Dwell (msec)	CE	DP
Iohexol 1	821.9	803.9	50	30	60
Iohexol 2	821.9	603.2	50	30	60
Ioversol 1	807.9	588.9	50	30	60
Ioversol 2	807.9	717	50	30	60
Metoprolol 1	268.1	116	25	30	60
Metoprolol 2	268.1	159.1	25	30	60
Atenolol 1	267.1	145.1	25	30	60
Atenolol 2	267.1	189.9	25	30	60
Acetaminophen 1	152	92.9	25	25	60
Acetaminophen 2	152	81.9	25	28	60



APPLICATIONS

Figure 1.
Representative chromatogram of serum sample after HClO₄ Protein Precipitation

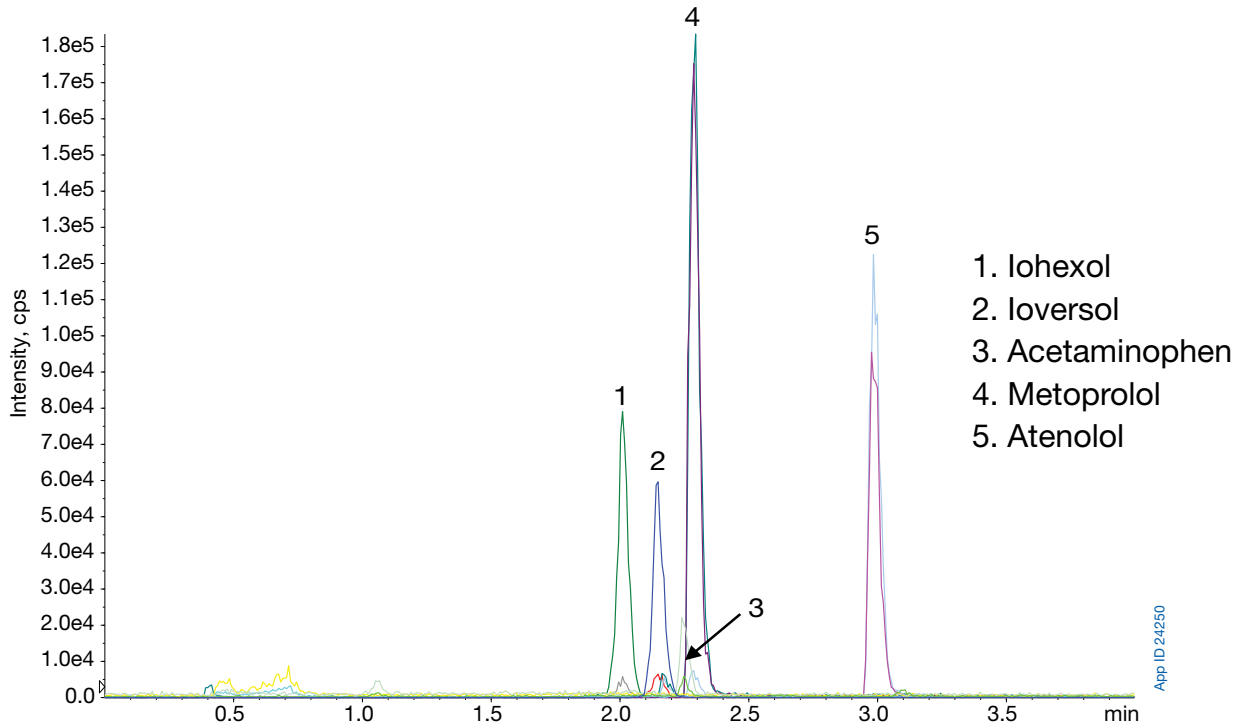
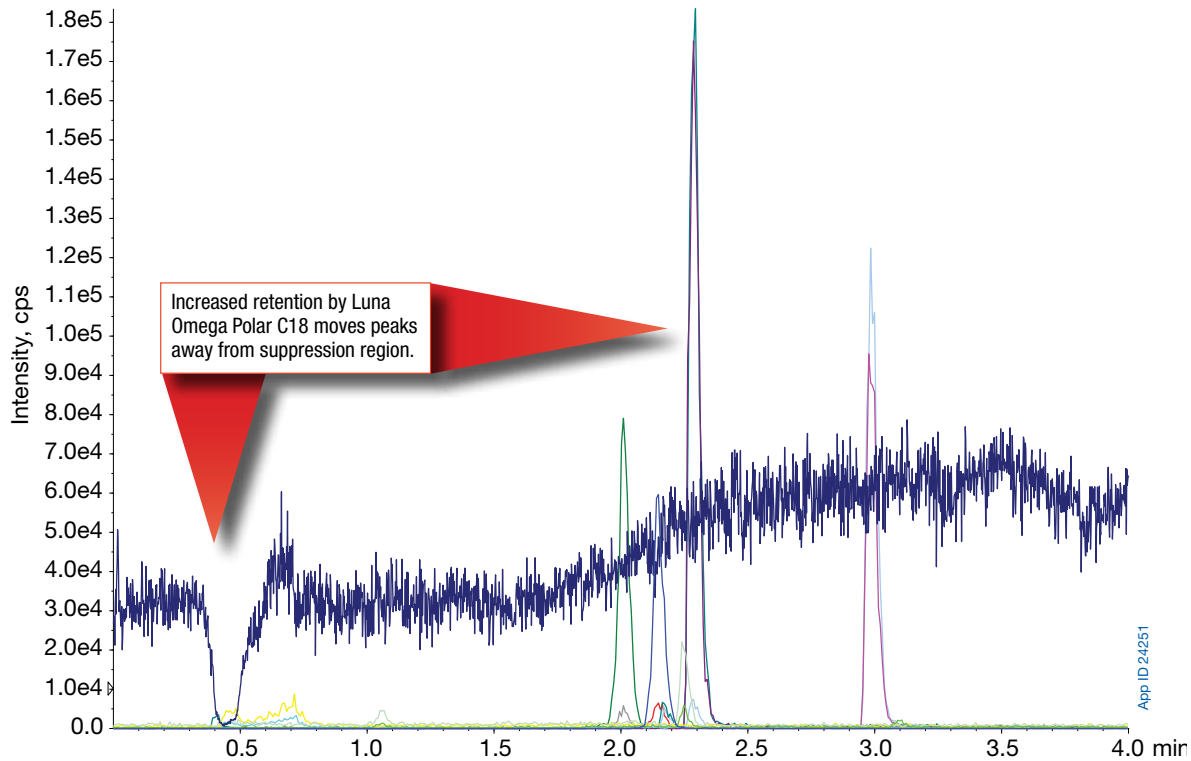
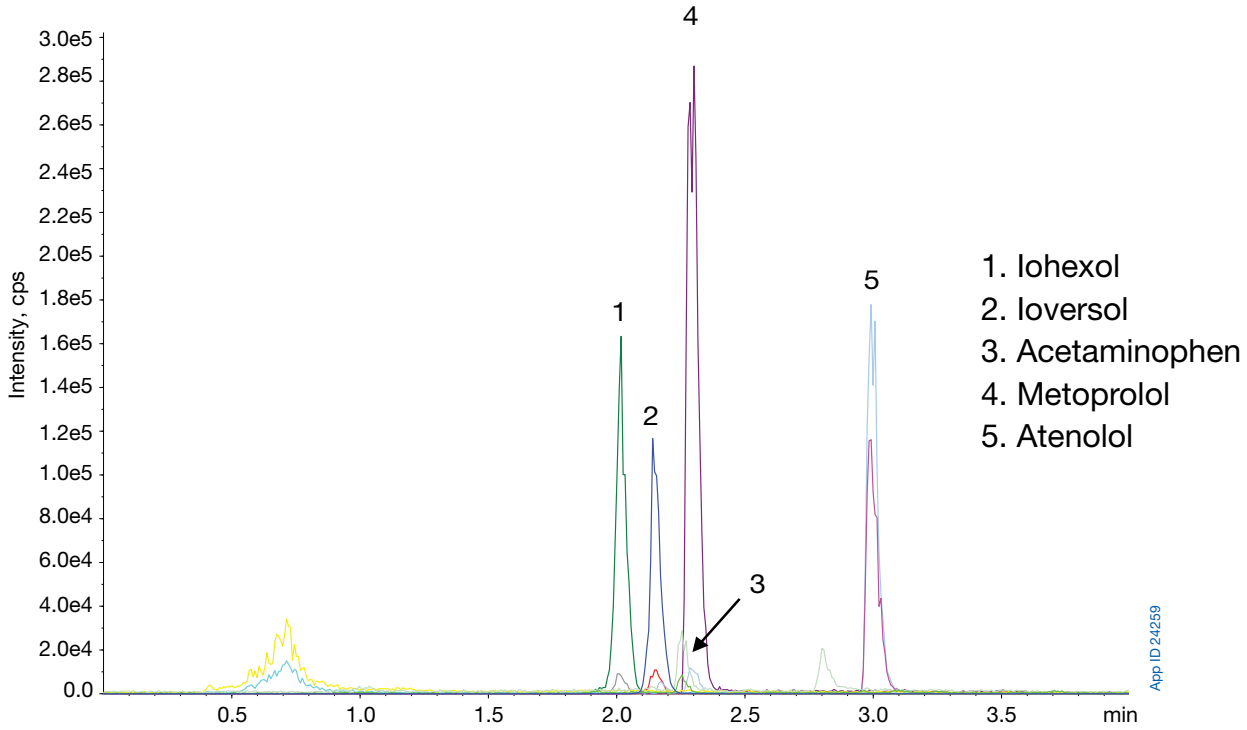


Figure 2.
Post column infusion after HClO₄ Protein Precipitation



APPLICATIONS

Figure 3.
Representative chromatogram of serum sample after Phree™ Extraction



APPLICATIONS

Kinetex Ordering Information

2.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges [†]
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00A-4759-AN	00B-4759-AN	00D-4759-AN	00F-4759-AN	AJ0-9532 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)					SecurityGuard™ ULTRA Cartridges [†]
Phases	30 x 3.0	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Polar C18	—	00B-4759-Y0	00D-4759-Y0	00F-4759-Y0	AJ0-9531 for 3.0 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges [†]
Phases	30 x 4.6	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Polar C18	—	00B-4759-E0	00D-4759-E0	00F-4759-E0	AJ0-9530 for 4.6 mm ID

[†] SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000.

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