HPLC Application

ID No.: 15712



Protein Precipatation vs. Ion Suppression of Medium Polar Drugs on strata Impact Square Well Plate

Gemini® 3 µm C18 110 Å, LC Column 150 x 3 mm, Ea

Dimensions: 150 x 3 mm ID Order No: 00F-4439-Y0 **Elution Type:** Gradient

Eluent A: 0.1% Formic acid/Water **Eluent B:** 0.08% Formic acid/Acetonitrile

Gradient	Step No.	Time (min)	Pct A	Pct B
Profile:	1	0	90	10
	2	5	50	50
	3	7	50	50

0.5 mL/min Flow Rate: Col. Temp.: ambient

Detection: Refractive Index @ 0.000000000 (ambient)

Protein Precipitation Protocol: **Analyst Note:**

Phase: Strata Impact Square Well Plate, 2 mL (CEO-7565)

- 1. Dispense 300 uL acetonitrile into each well using an automatic pipettor.
- 2. Place the protein precipitation plate onto a suitable 96-well vacuum manifold . Make sure that a 96-well collection plate is positioned inside the manifold to collect the filtrate.
 3. Dispense 100 uL of Porcine plasma into each well (acetonitrile:plasma = 3:1). Let it stand for 2 mins (no vortex /mixing required).
- 4. Apply 5-10" of mercury for 30-40 secs.
- 5. Collect the filtrate and blow down to dryness under slow stream of nitrogen @ 40 deg. C.
- 6. Reconstitute with 100 uL of mobile phase containing 10.0 ng of analye.

Note: For ion suppression or enhancement estimation, a set of 4 blank (100 uL of water instead of plasma) was run in parallel.

Observation:

Filtrate looked very clean and clear

Results:

Analyte	logP	m/z	% Variation	Effect
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1. Pindolol 1.75 249 15% Suppression 2. Metoprolol 1.88268 13% Suppression

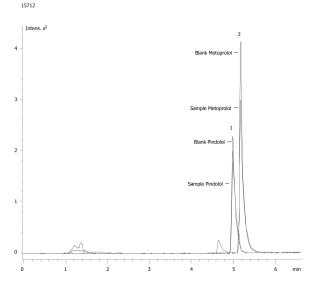


Products used in this application:



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ANALYTES:

- Pindolol 1
- Metoprolol