HPLC Application

ID No.: **15712**

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

Column: 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 Profile:

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

Flow Rate: 0.5 mL/min
Col. Temp.: ambient
Detection: Mass Spectrometer (MS) @ 249 amu (ambient)

Products used in this application:

Analyst Note:

Protein Precipitation Protocol:

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
1. Pindolol	1.75	249	15%	Suppression
2. Metoprolol	1.88	268	13%	Suppression

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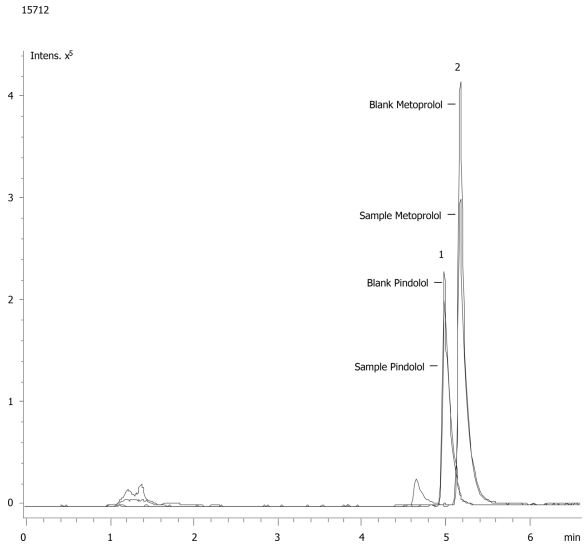

ANALYTES:

1 Pindolol

2 Metoprolol