



AM2201 METABOLITES IN URINE BY LC-MS/MS OR GC-MS CLEAN SCREEN[®] THC EXTRACTION COLUMN

Part #

CSTHC206 – CLEAN SCREEN THC 200mg 5mL Tube

BETA-GLUC-10 - Slectrazyme[®] Beta-glucuronidase

SMSTFA-1-1 – SELECTRA-SIL[®] MSTFA w/ 1% TMCS

SLC-1850ID21-3UM - Selectra[®] C18 HPLC Column, 50 x 2.1 mm, 3µm

1. PREPARE SAMPLE:

Urine: PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:

To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Slectrazyme[®] β-glucuronidase. Optionally, add 1 mL of 1M acetate buffer and 25-50 µL of concentrated β-glucuronidase.

Vortex and heat for 1-2 hours at 65°C.

Allow sample to cool

Do not adjust pH~ sample is ready to be added to the extraction column.

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

1 x 3 mL 100 mM phosphate buffer (pH 6.0).

NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

4. WASH COLUMN:

1 x 3 mL D.I. H₂O

1 x 3 mL of 100 mM phosphate buffer containing 20% Acetonitrile

NOTE: Aspirate at full vacuum or pressure

5. ELUTE AM2201 ANALYTES:

2 x 3 mL Ethyl Acetate containing 10 % CH₃OH

Collect eluate at 1-2 mL /minute

6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

7. . RECONSTITUTE / DERIVATIZE:

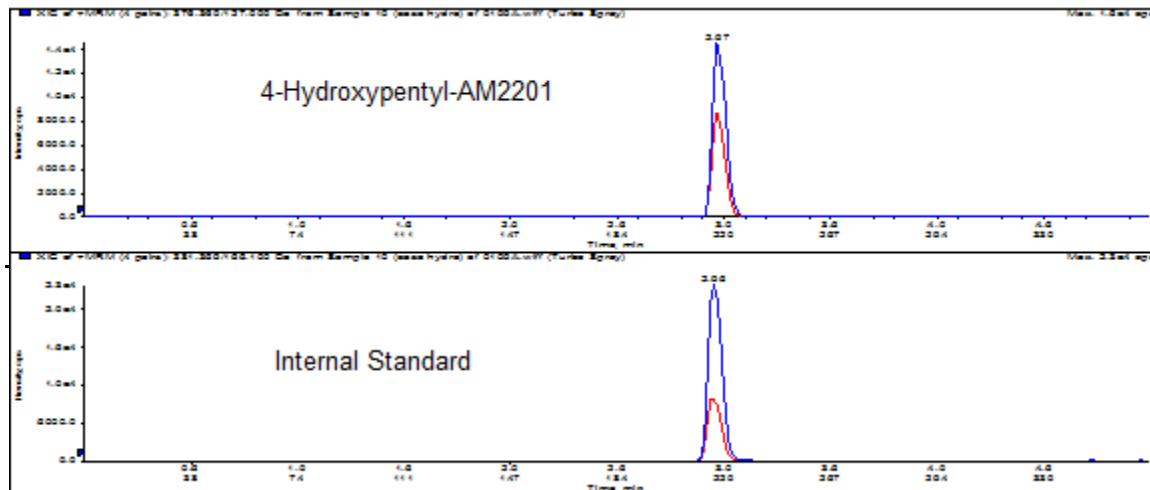
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 10 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA w/
1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 mL

NOTES:

This method is used for the extraction of AM2201 and metabolites in urine. LC/MS/MS instrumentation is used for analysis with a linear range of 1 ng/mL to 100 ng/mL with average recoveries found to be greater than 90%.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



PARAMETERS

Analyte	MRM Transitions		
	Precursor Ion	Product Ion 1	Product Ion 2
AM-2201	360.2	155.1	127.1
AM 2201 2-OH INDOLE	376.3	127.1	154.9
AM 2201 4-OH INDOLE 1	376.3	127.1	154.9
AM 2201 4-OH INDOLE D ₅	381.3	126.1	155.1
AM 2201 5-OH INDOLE	377.1	154.9	56.9
AM 2201 N4 OH PENTYL	377.1	155.0	57.0

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 µL

LC Column: Selectra[®] C18 HPLC Column 50 x 2.1 mm 3 µm

Instrument: API 3200 QTrap MS/MS Compound MRM Transition

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Polarity: Positive

Injection Volume: 10 µL

Gradient:

Time	%A	%B
0	65	35
0.5	65	35
3.5	10	10
4.0	65	35
5.0	65	35