NICOTINE, COTININE, AND ANABASINE IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

SLPFPP100ID21-3UM – Selectra® PFPP HPLC Column, 100 x 2.1 mm, 3 µm

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
   Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
   Mix/vortex and let stand for 5 minutes
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   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. H_2O.
   1 x 2 mL 200 mM HCl
   Dry column (5 minutes at full vacuum or pressure).
   1 x 3 mL Methanol
   Dry column (5 minutes at full vacuum or pressure).

5. **ELUTE NICOTINE, COTININE, ANABASINE:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

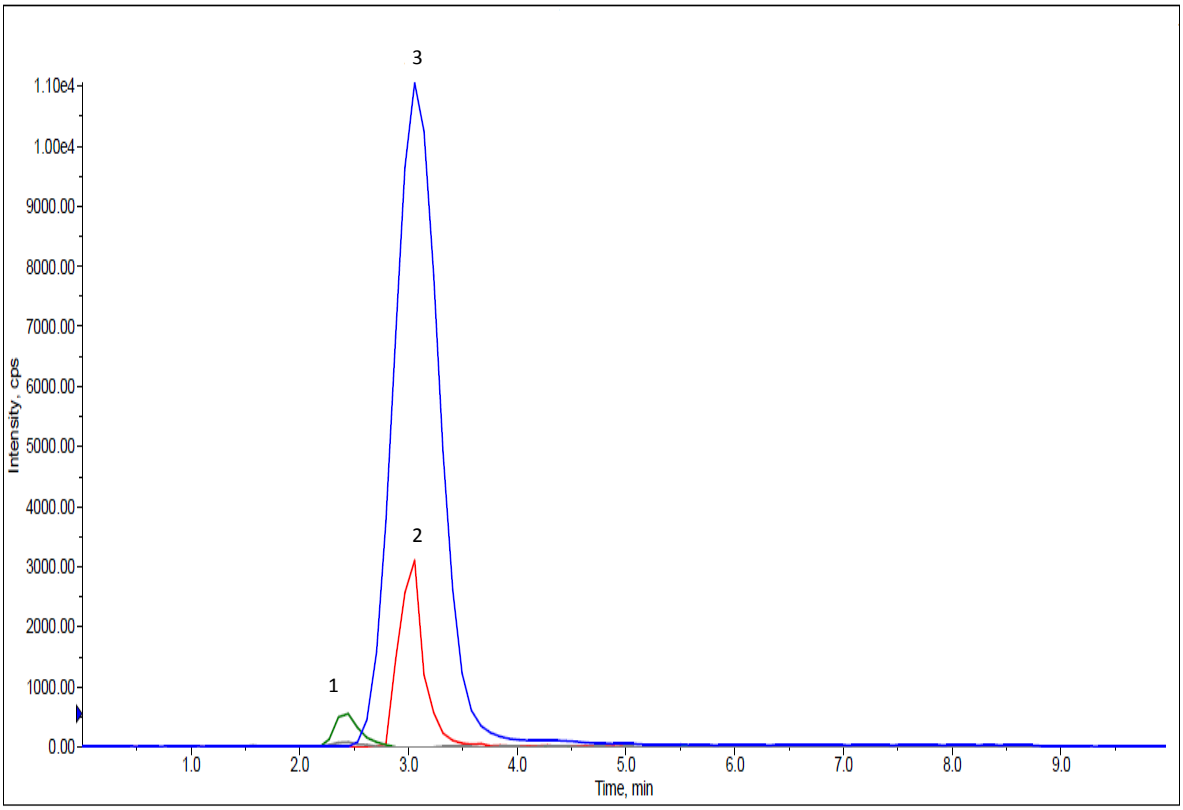
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

7. **RECONSTITUTE / DERIVATIZE**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Cotinine	117.2	80.1	2.42
Cotinine D_3	180.2	101.2	-
2. Nicotine D_4	167.2	136.1	3.03
3. Nicotine	163.2	132.2	3.06

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.3 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 3 µm

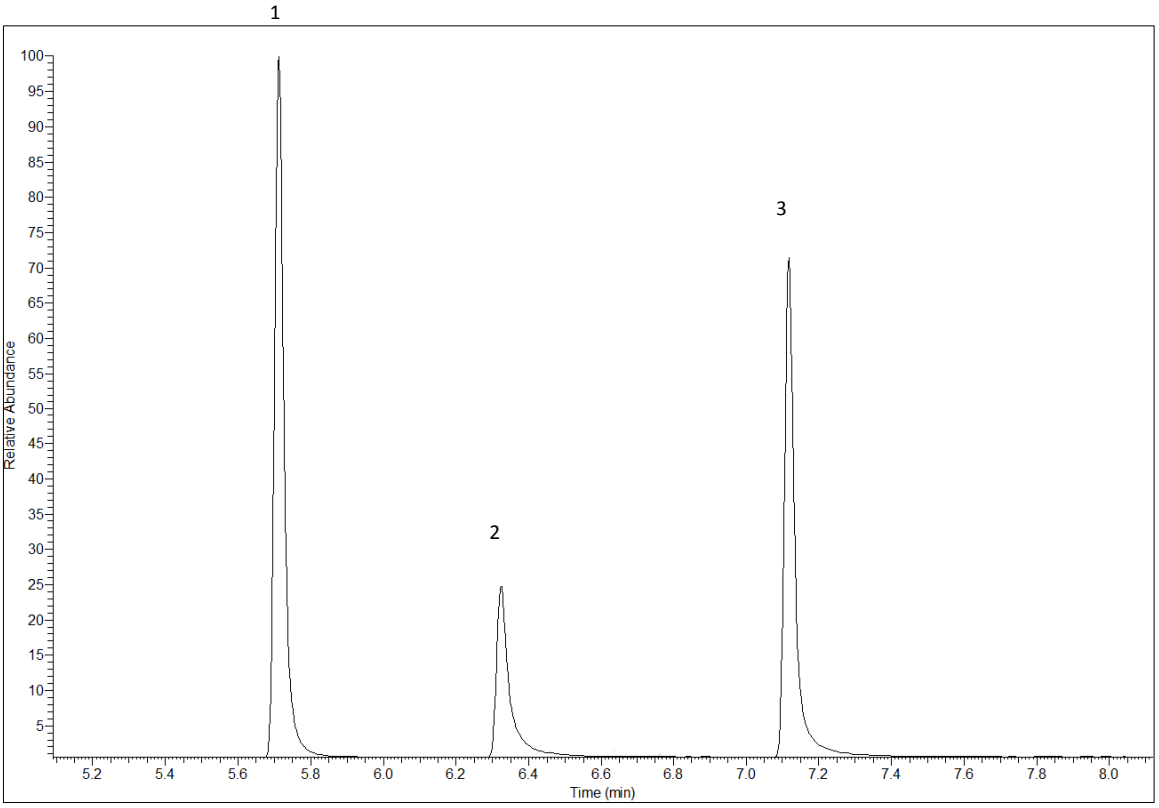
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	80	20
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Nicotine	84	133	162	5.71
Nicotine D_4	88	137	166	-
2. Anabasine	84	105	133	6.32
3. Cotinine	98	119	176	7.12
Cotinine D_4	101	122	179	-

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

GC capillary column: 30 m x 0.25 mm (0.25 µm) TG-1MS

Injector: 1 µL Splitless, 250 °C

Oven temperature program: 50 °C (0.5) to 320 °C (30 °C/ minute): hold (5 minutes)

Carrier gas: Helium (1.2 mL/ minute)

MSD condition: Aux temperature: 280 ºC, MS Source: 300 ºC, MS Quad: 150 ºC