



# NICOTINE AND COTININE IN URINE OR SERUM FOR GC OR GC/MS CONFIRMATIONS USING: 200 mg CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU<sup>®</sup> Tips

## 1. PREPARE SAMPLE

To 2 mL of 100 mM phosphate buffer (pH =6.0) add internal standards\*. Add 2 mL of urine or serum..  
Sample pH should be 6.0 ± 0.5.  
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphateMix/vortex.  
Centrifuge as appropriate.

## 2. CONDITION CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN

1 x 3 mL CH<sub>3</sub>OH.  
1 x 3 mL D.I. H<sub>2</sub>O.  
1 x 1 mL 100 mM phosphate buffer (pH 6.0).  
**NOTE:** Aspirate at < 3 inches Hg to prevent sorbent drying.

## 3. APPLY SAMPLE

Load at 1 mL/minute.

## 4. WASH COLUMN

1 x 3 mL D.I. H<sub>2</sub>O.  
1 x 2 mL 200 mM HCl.  
Dry column (5 minutes at > 10 inches Hg).  
1 x 2 mL Hexane.

## 5. WASH COLUMN

Remove rack of collection tubes to rewash columns.  
1 x 3 mL CH<sub>3</sub>OH.  
Dry column, (5 minutes at > 10 inches Hg).

## 6. ELUTE COTININE AND NICOTINE

Replace rack of collection tubes.  
1 x 3 mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2); Collect eluate at 1 mL/minute.  
**NOTE:** Prepare elution solvent daily. Add IPA/NH<sub>4</sub>OH, mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12).

## 7. CONCENTRATE

Evaporate to dryness at < 40 °C.  
Take care not to over-heat or over evaporate.  
Reconstitute with 100 µL ethyl acetate.

## 8. QUANTITATE

Inject 1 to 2 µL onto chromatograph.  
Monitor the following ions (GC/MS):

<u>Compound</u>	<u>Primary ion**</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerilliant#</u>
Nicotine	84	133	162	N-008
*Nicotine-D4	88	137	166	N-048
Cotinine	98	119	176	C-016
*Cotine-D3	101	122	179	C-017

\*\* Quantitation Ion

SOURCE - UCT Internal Publication