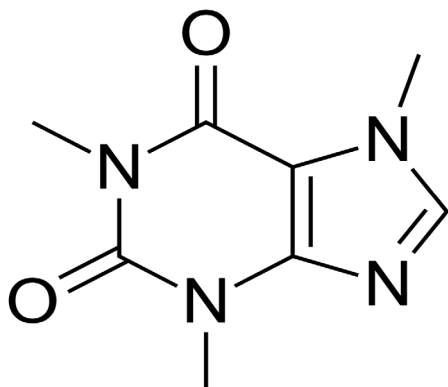


Caffeine, Theophylline, and Theobromine In Blood, Plasma/Serum, and Urine Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

ZSDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
Without Tips

Procedure:

1. Prepare Sample

- To 1 mL of 100 mM acetic acid add internal standard.*
- Add 1 mL Blood, Serum/ Plasma, or Urine.
Add 2 mL of 100 mM acetic acid.
- Vortex and centrifuge as appropriate.

2. Condition Clean Screen® Column

- 1 x 3 mL CH₃OH.
 - 1 x 3 mL D.I. H₂O.
 - 1 x 1 mL 100 mM acetic acid.
- Note:** Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

- Load at 1 to 2 mL/minute.

4. Wash Column

- 1 x 3 mL D.I. H₂O.
- 1 x 3 mL 100 mM acetic acid.
- Dry column (5 minutes at > 10 inches Hg)

5. Elute Caffeine/Theobromine/Theophylline

- 1 x 3 mL ethyl acetate : methanol (90:10)
- Collect eluate at 1-2 mL/minute.

6. Evaporation

- Combine eluates
- Evaporate eluates under a gentle stream of nitrogen < 40°C

7. Reconstitute sample in 1000 µL of

0.1% Formic Acid (aq)

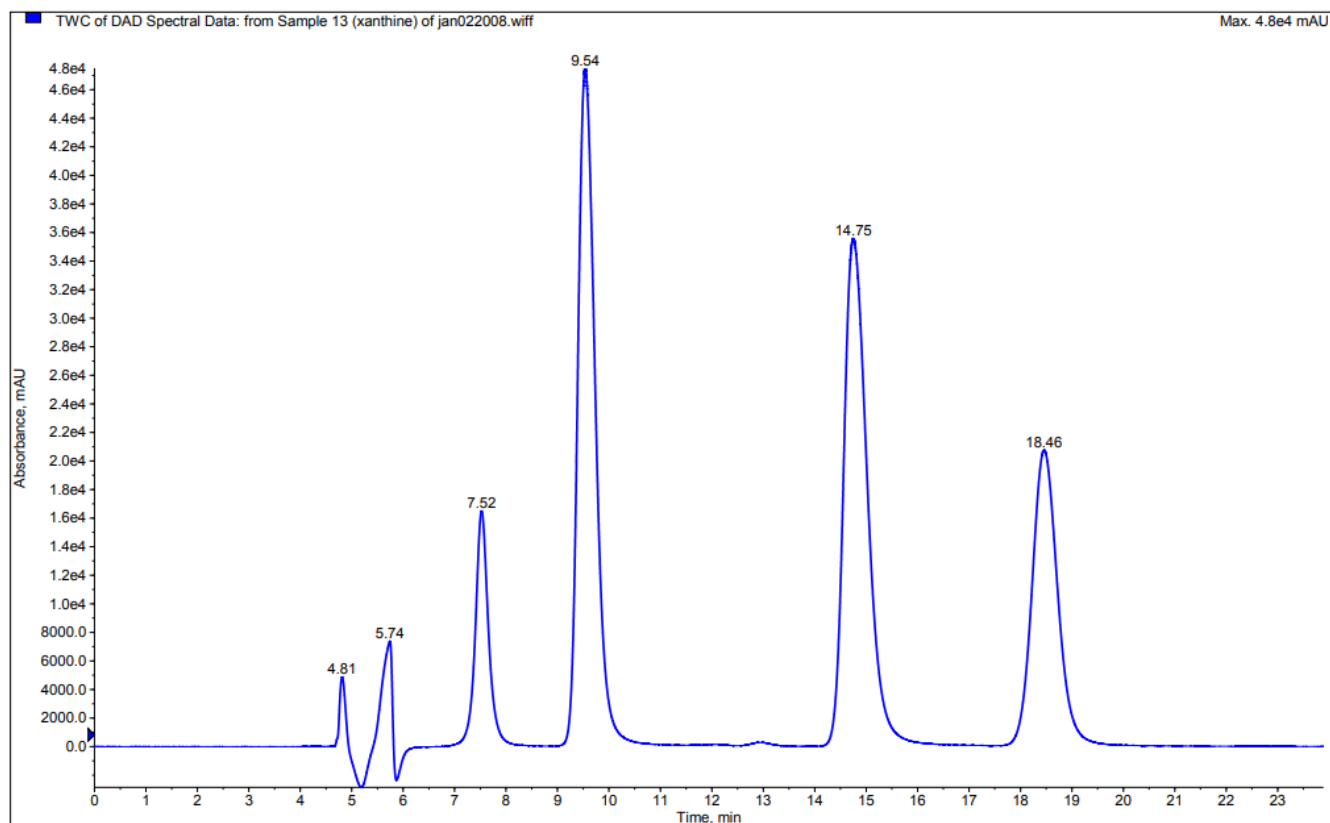
- Inject 20 µL.

Instrument Conditions

Column	150 x 2.1 mm (3 µm) Gold C ₁₈ (ThermoFisher)
Mobile Phase	Acetonitrile: 0.1% Formic acid aqueous (10:90)
Flowrate	0.1 mL/ minute
Column Temperature	ambient
Detector	Diode Array (200-350 nm)



Compound	Time	Cerilliant #
Theobromine	7.5 minutes	T--013
Theophylline	9.5 minutes	IMPC-051-01
Caffeine	14.5 minutes	C-0151
*8-Chlorotheophylline	18.0 minutes	



Recovery: > 90% (N=10)

LOD: 1 µg/mL

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