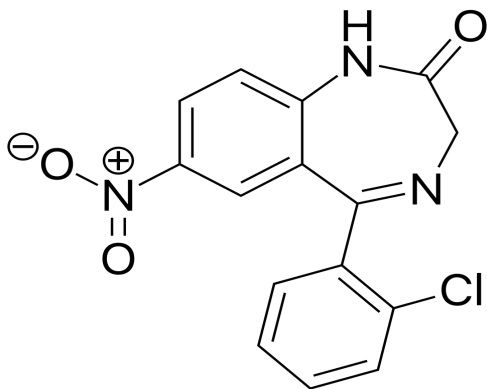


Cocaine and Benzoylecgonine In Serum, Plasma, Or Whole Blood For HPLC Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

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

Or

ZCDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
With CLEAN-THRU® Tips

Procedure:

1. Prepare Sample: β-Glucuronidase Hydrolysis

- To 2 mL of urine, add internal standard(s)* and 1 mL of β-Glucuronidase solution.
- β-Glucuronidase solution contains 5,000 F units/mL *Patella vulgata* in 100 mM acetate buffer (pH 5.0).
- Mix/vortex.
- Hydrolyze for 3 hours at 65 °C.
- Cool before proceeding.

2. Condition Clean Screen® Extraction Column

- 1 x 3 mL CH₃OH.
 - 1 x 3 mL D.I. H₂O.
 - 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 to 2 mL/minute.

4. Wash Column

- 1 x 2 mL D.I. H₂O.
- 1 x 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0).
- Dry column (5 minutes at > 10 inches Hg).
- 1 x 2 mL hexane.

5. Elute Clonazepam / 7-Aminoclonazepam

- 1 x 3 mL ethyl acetate with 2% NH₄OH: Collect eluate at 1 to 2 mL/minute.
- Note:** Prepare fresh daily.

6. Dry Eluate

- Evaporate to dryness at < 40 °C.

7. Derivatize

- Add 50 µL ethyl acetate and 50 µL MTBSTFA (with 1% TBDMCS)****.
 - Mix/vortex.
 - React 20 minutes at 90 °C.
 - Remove from heat source to cool.
- Note:** Do not evaporate MTBSTFA solution.

8. Analysis

- Inject 1 to 2 µL sample.
- For MSD monitor the following ions:



Compound	Primary Ion**	Secondary	Tertiary	Cerilliant #
Clonazepam-TBDMS	372	374	326	C-907
7-Aminoclonazepam-TBDMS	342	344	399	A-915
Clonazepam-D4-TBDMS	376	378	377	C-905
7-Aminoclonazepam-D4-TBDMS	346	348	403	A-917

*Suggested internal standard for GC/MS: Clonazepam-D₄, 7-aminoclonazepam-D₄.

**Quantitation ion

***Part # SMTBSTFA-1-1,10, 25, 100

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