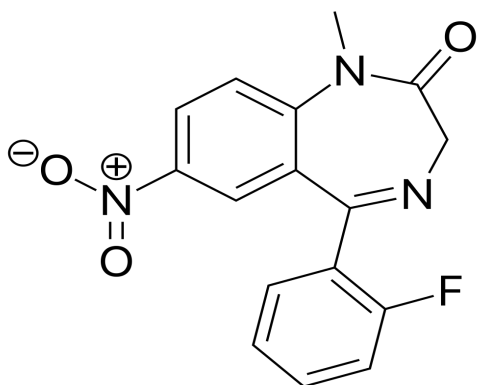


Flunitrazepam and Metabolites In Urine For GC OR GC/MS Confirmations Using: 200 mg Clean Screen® Extraction Column



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.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.

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 mL/minute.

4. Wash Column

- 1 x 3 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 Flunitrazepam, 7-Aminoflunitrazepam, and Desmethyflunitrazepam

- 1 x 3 mL ethyl acetate with 2% NH₄OH.
- Collect eluate at 1 to 2 mL/minute.
- 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)***.
- Overlay with N₂ and cap. Mix/vortex.
- React 20 minutes at 70°C. Remove from heat source to cool.

Note: Do not evaporate MTBSTFA solution.

8. Quantitate

- Inject 1 to 2 µL onto gas chromatograph.
- For MSD monitor the following ions:



Compound	Primary Ion****	Secondary	Tertiary	Cerilliant #
Flunitrazepam-TBDMS	312	286	266	F-907
7-Aminoflunitrazepam-TBDMS	283	255	254	A-911
Desmethyflunitrazepam-TBDMS	356	357	310	D-918
*Oxazepam-D5 -TBDMS	462	464	463	O-901

* Suggested internal standard for GC/MS: D5-Oxazepam

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

**** Quantitation ion

References:

[1] UCT Internal Publication

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255

www.unitedchem.com Email: methods@unitedchem.com

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