



BENZODIAZEPINES IN URINE FOR GC OR GC/MS CONFIRMATIONS USING:

200 mg CLEAN SCREEN[®] EXTRACTION COLUMN

Part # ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU[®] Tips

February 3, 2009

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 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 BENZODIAZEPINES

1 x 5 mL ethyl acetate containing 4% ammonium hydroxide
collect eluate at 1 to 2 mL/minute.

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 μ L ethyl acetate and 50 μ L BSTFA* (with 1% TMCS)*.

Overlay with Nitrogen and cap. Mix/vortex.

React 20 minutes at 70°C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

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

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

**** Quantitation ion

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

8. QUANTITATE

Inject 1 to 2 μ L onto gas chromatograph.

For MSD monitor the following ions:

<u>Generic Name</u>	<u>Trade Name</u>	<u>Primary Ion****</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerilliant#</u>
Alprazolam	Xanax®	308	279	204	A- 907
α-Hydroxyalprazolam-TMS		381	396	383	A-903
Chlordiazepoxide	Librium®	282	283	284	C-022
Clonazepam	Clonopin®	387	352	306	C-907
Diazepam	Valium®	256	283	221	A-907
Desalkylflurazepam-TMS		359	341	245	D-915
Hydroxyethylflurazepam-TMS		288	360	389	F-902
Lorazepam-TMS	Ativan®	429	430	347	L-901
Nordiazepam-TMS		341	342	343	N-905
Oxazepam-TMS	Serax®	429	430	313	O-902
Prazepam*		269	241	324	P-906
Temazepam-TMS	Restoril®	343	283	257	T-907
Triazolam	Halcion®	313	314	342	T-910
α-Hydroxytriazolam-TMS		415	417	430	T-911

NOTE: Flurazepam does not extract under these conditions; However metabolites such as desalkylflurazepam and hydroxyethylflurazepam will extract with high recovery.

ALTERNATE DERIVATIZATION

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL MTBSTFA***** (with 1% MTBDMCS).

Overlay with Nitrogen 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:

<u>Generic Name</u>	<u>Trade Name</u>	<u>Primary Ion****</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerilliant#</u>
Nordiazepam -D5-TBDMS		332	334	333	N-903
Nordiazepam-TBDMS		327	328	329	N-902
Oxazepam-D5-TBDMS		462	519	462	O-901
Oxazepam-TBDMS	Serax®	457	513	459	O-902
Temazepam-D5-TBDMS		362	390	288	T-903
Temazepam-TBDMS	Restoril®	357	359	385	T-903
Lorazepam-TBDMS	Ativan®	491	513	493	L-901
Clonazepam	Clonopin®	372	374	326	C-907
7-Aminoclonazepam -TBMS		456	458	513	A-915
Diazepam	Valium®	256	283	221	A-907
Desalkylflurazepam-TBDMS		345	347	402	D-915
Prazepam*		269	241	324	P-906
α-Hydroxymidazolam-TBDMS	Versid®	398	400	440	H-902
Desmethylflunitrazepam-TBDMS		357	310	356	D-919
7-Aminoflunitrazepam-TBDMS		397	324	398	D-912
Alprazolam	Xanax®	308	279	204	A-907
α-Hydroxyalprazolam-D5-TBDMS		386	388	387	A-904
α-Hydroxyalprazolam-TBDMS		383	384	381	A-903
Triazolam	Halcion®	313	314	342	T-910
α-Hydroxytriazolam-TBDMS		415	417	190	T-9111

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