

BENZODIAZEPINES IN URINE CLEAN SCREEN FAST[®] COLUMN



UCT Featured Products

CSFAS203
CLEAN SCREEN FAST[®]
200 mg, 3 mL Tube

SLDA50ID21-5UM
Selectra[®] DA HPLC Column,
50 x 2.1 mm, 5 μm

ASBETA-GLUC-10
Selectrazyme[®]
Beta-glucuronidase

Procedure:

1) Prepare Sample for Enzyme Hydrolysis of Glucuronides

To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme[®] β-glucuronidase.
Optionally, add 1 mL of acetate buffer and 25-50 μL of concentrated β-glucuronidase.
Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool.
Do not adjust pH~ sample is ready to be added to the extraction column.

2) Load Sample and Sample Dilute Ratio

Sample Dilution Ratio: Sample Volume* : Diluent** Volume
NOTE: *If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

Dilution Ratio	Urine	Diluent**
1:1	500 μL	500 μL
1:4	200 μL	800 μL
1:9	100 μL	900 μL

** Diluent is 50:50 (Acetonitrile: D.I. H₂O)

Sample and diluents are added in an appropriately labeled tube.

Add appropriate volume internal standard(s).
It is recommended to use an internal standard volume of no more than 200 μL

3) Extraction and Collection

Wash with 2 mL (84: 15: 1) D.I. H₂O: Acetonitrile: NH₄OH (made fresh daily). Dry column under full vacuum or pressure for 10-15 minutes.

4) Analysis

1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49: 49:2). Collect at 1-2 mL/ minute.



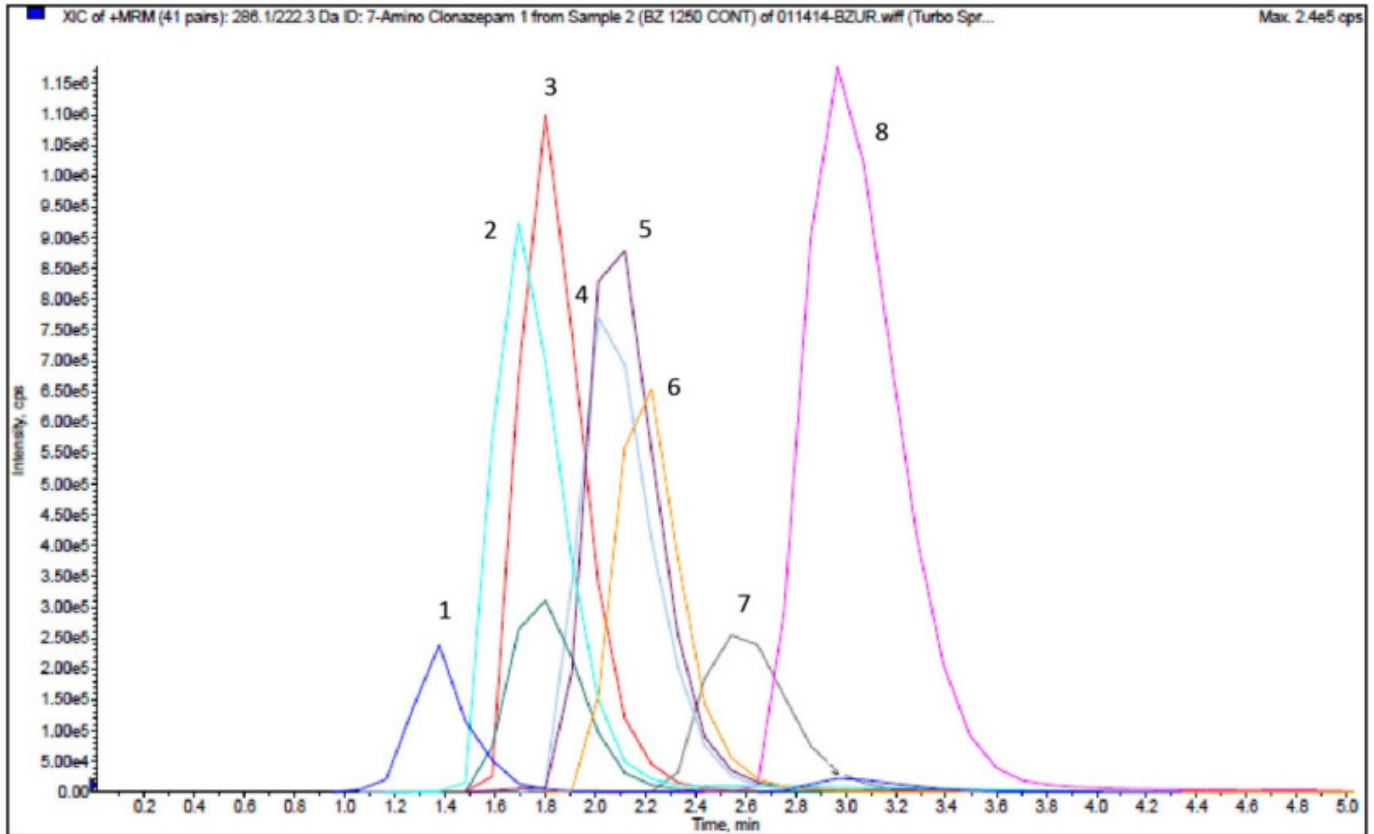
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INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. 7-Amino Clonazepam	286.09	222.3	1.40
2. Oxazepam	287.09	241.3	1.70
3. Alpha-Hydroxy-Alprazolam	325.18	297.1	1.80
4. Clonazepam	316.13	270.2	2.10
5. Nordiazepam	271.09	140.1	2.10
6. Temazepam	301.12	255.2	2.20
7. Alprazolam	309.16	205.3	2.60
8. Diazepam	285.1	193.1	3.00

LC-MS/MS Parameters	
Mobile Phase A	.02% Formic Acid in D.I. H ₂ O
Mobile Phase B	.02% Formic Acid in Methanol
Flow Rate	0.1 mL/minute
Injection Volume	10 µL
Instrument	API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC
LC Column	Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Polarity	Positive



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