BENZODIAZEPINES IN URINE CLEAN SCREEN FASt® COLUMN

Part #

CSFAS203 – CLEAN SCREEN FASt ® 200 mg, 3 mL Tube

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

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

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. H_2O)

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:

Set up extraction manifold with FASt cartridges and auto-sampler collection vials.

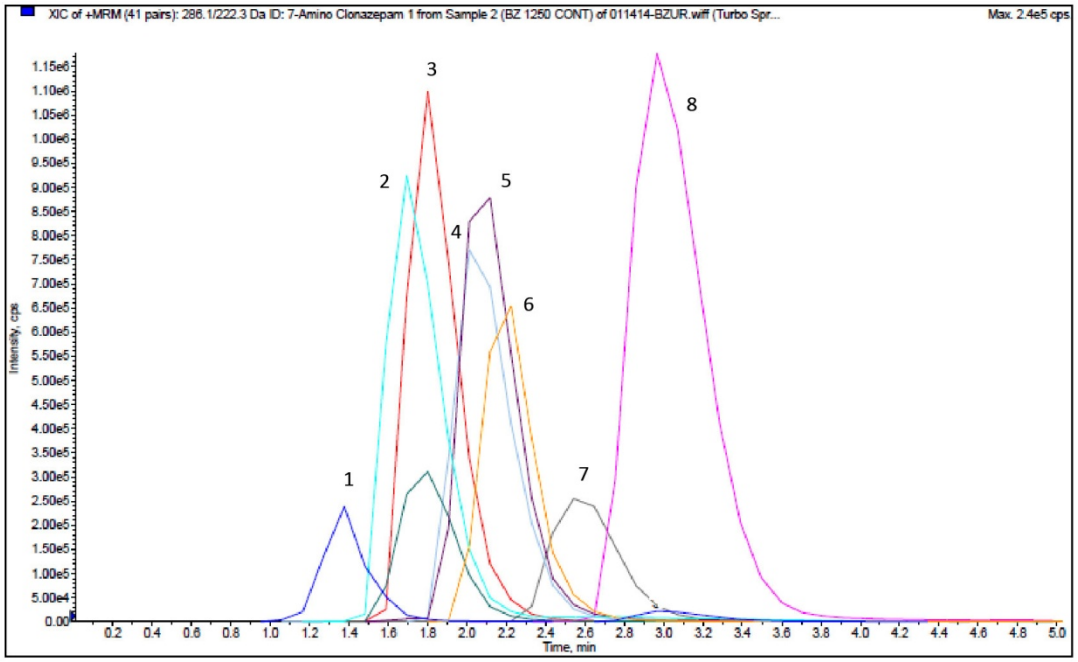
Pour sample into FASt cartridge and elute sample directly into auto-sampler vials.

4. ANALYSIS:

Cap vials and put directly onto LC/MS for analysis.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	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

PARAMETERS

Mobile Phase A: .02% Formic Acid in D.I. H_2O

Mobile Phase B: .02% Formic Acid in Methanol

Flow Rate: 0.1 mL/minute

Polarity: Positive

Injection Volume: 10 µL

LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

Instrument: API 3200 Qtrap MS/MS. with Shimadzu Prominence UFLC

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	STOP	