BENZODIAZEPINES IN BLOOD, PLASMA/SERUM, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® BNZ EXTRACTION COLUMN

Part #
ZSBNZ030 – CLEAN SCREEN® BNZ 300 mg, 10 mL Tube
SMTBSTFA-1-1 – SELECTRA- SIL® MTBSTFA w/ 1% TBDMCS
SLDA50ID21-5UM – SELECTRA® DA HPLC Column, 50 x 2.1 mm, 5 µm
or
SLPFPP100ID21-5UM – SELECTRA® PFPP HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
   Add 1-2 mL of blood, plasma/ serum, or 1 g (1:4) tissue homogenate.
   Mix/vortex and let stand for 5 minutes
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 3 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O.
   1 x 3 mL 5% Acetonitrile in 100 mM phosphate buffer (pH 6.0).
   Dry column (5 minutes at full vacuum or pressure).
   1 x 3 mL Hexane.

5. **ELUTE BENZODIAZEPINES:**
   2 x 3 mL Ethyl Acetate containing 2% NH_4OH
   Collect eluate at 1 to 2 mL/minute.

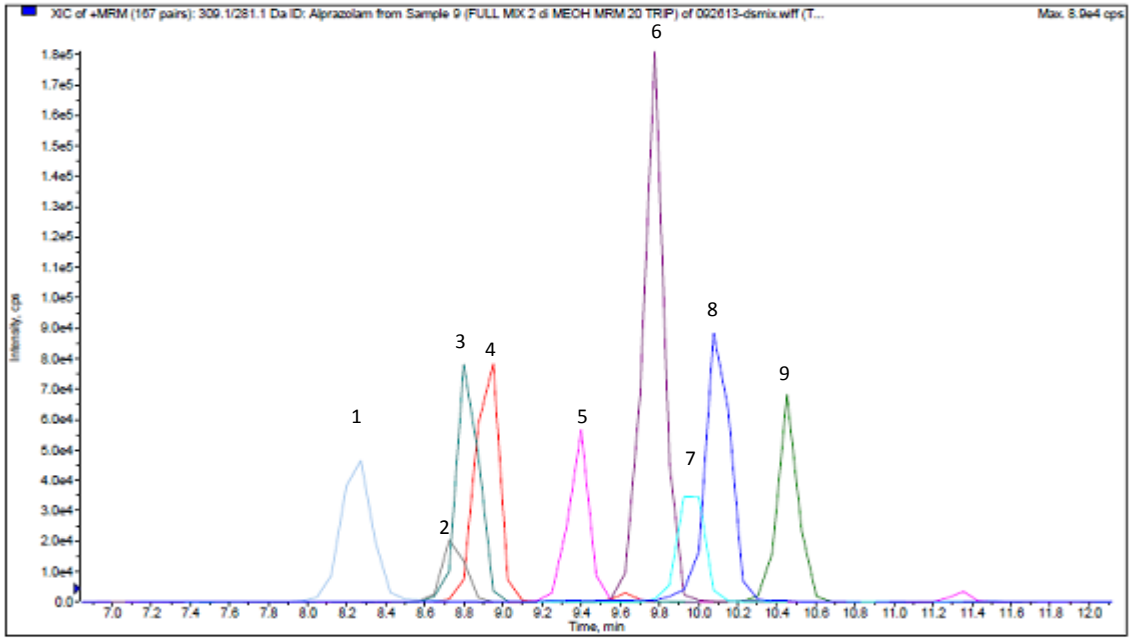
   NOTE: Prepare elution solvent daily.

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

7. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10-20 µL.
   - **GC-MS**: Dissolve residue in 50 µL of ACN and 50 µL MTBSTFA w/ 1% TBDMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70° C; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Midazolam	326.1	291.1	8.25
2. Lorazepam	321.0	229.1	8.70
3. Oxazepam	287.1	241.1	8.80
4. Clonazepam	316.1	270.1	8.95
5. Nordiazepam	271.1	140.1	9.40
6. Temazepam	301.1	255.1	9.75
7. Triazolam	343.0	239.0	10.0
8. Alprazolam	309.1	281.1	10.1
9. Diazepam	285.1	193.1	10.5

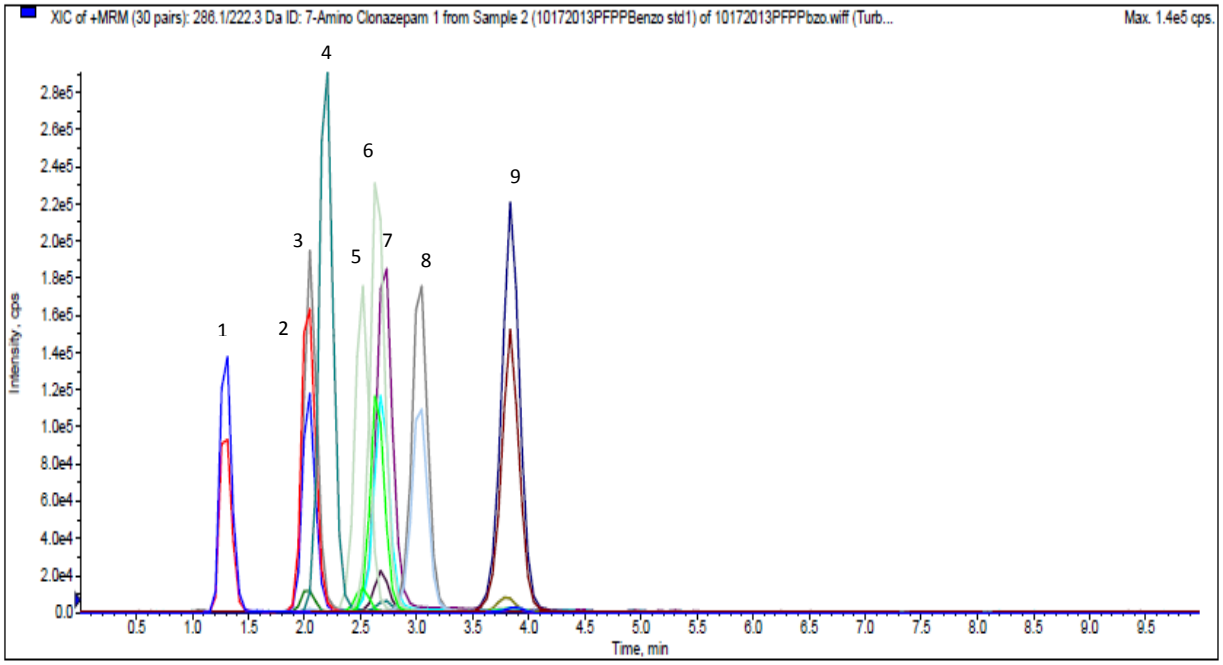
PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Reconstitute: 100 µL
Injection Volume: 20 µL
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. 7-Amino Clonazepam	286.09	222.3	1.30
2. Lorazepam	321.06	303.3	2.04
3. Alpha- Hydroxy- Alprazolam	325.18	297.1	2.05
4. Oxazepam	287.09	241.3	2.19
5. Clonazepam	316.13	270.2	2.51
6. Temazepam	301.12	255.2	2.65
7. Alprazolam	309.16	205.3	2.71
8. Nordiazepam	271.09	140.1	3.03
9. Diazepam	285.1	193.1	3.84

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

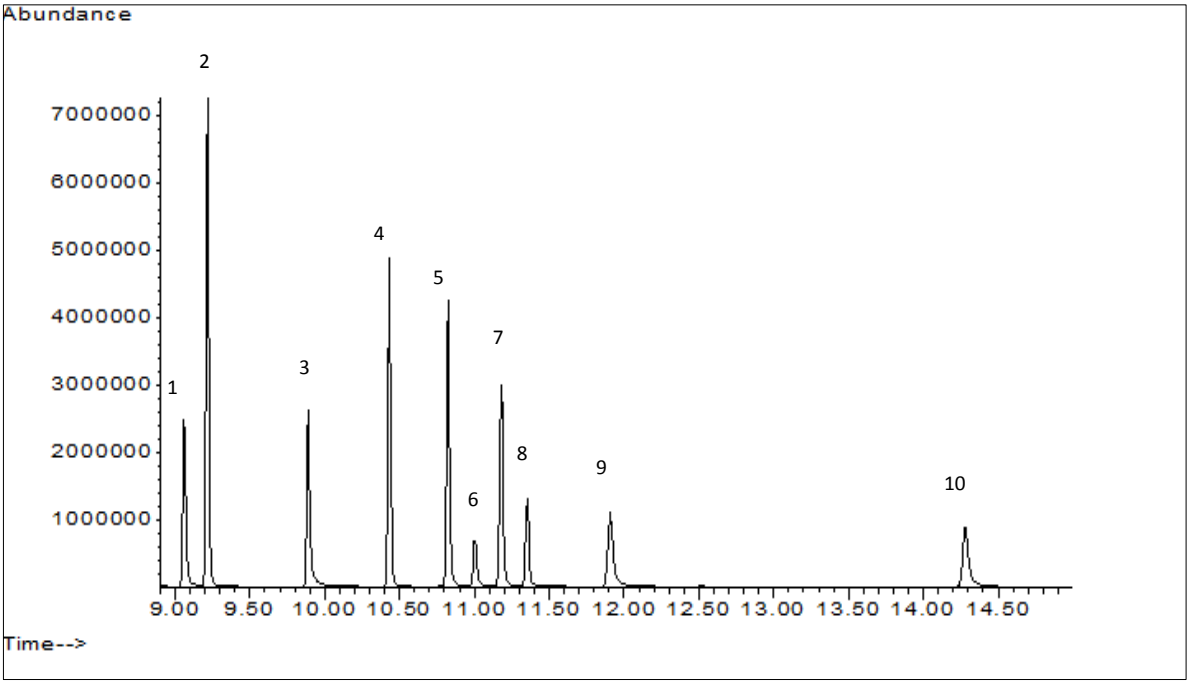
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic Flow:

Time	%A	%B
0.00	40	60
10.0	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Diazepam	256.0	283.0	221.0	9.06
2. Nordiazepam TBDMS	327.0	383.1	369.0	9.22
3. Midiazolam	310.0	325.0	297.0	9.89
4. Oxazepam 2TBDMS	457.1	513.2	383.1	10.43
5. Temazepam TBDMS	357.0	283.0	385.1	10.82
6. 7-Amino Clonazepam TBDMS	342.0	399.1	328.0	11.00
7. Lorazepam 2TBDMS	491.1	513.2	533.1	11.18
8. Clonazepam TBDMS	372.0	326.0	429.0	11.36
9. Alprazolam	279.0	204.0	308.0	11.91
10. Alpha-Hydroxy Alprazolam TBDMS	381.0	423.1	346.0	14.28

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30 m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160 °C for 0.5 min; 15 °C/min to 310 °C for 4.50 minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 ºC, MS Source: 250 ºC, MS Quad: 150 ºC