



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

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 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

SLPFPF100ID21-5UM - SELECTRA® PFPF 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<sub>3</sub>OH.  
1 x 3 mL D.I. H<sub>2</sub>O.  
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:**

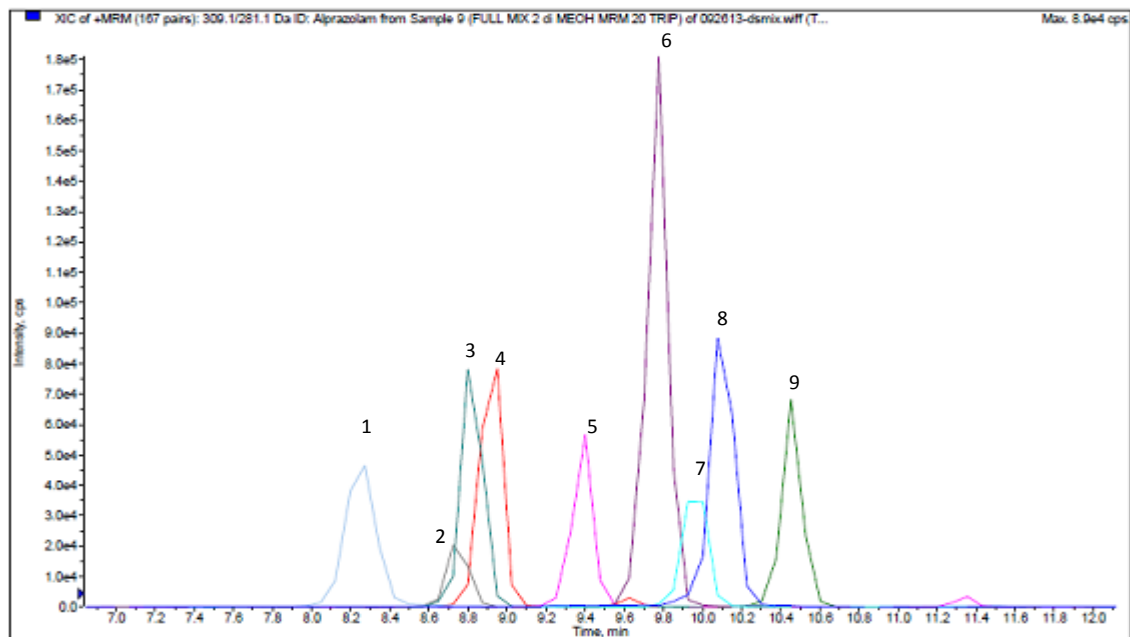
1 x 3 mL D.I. H<sub>2</sub>O.  
1 x 3 mL 5% Acetonitrile in 100 mM phosphate buffer (pH 6.0).  
Dry column (5 minutes at full vacuum or pressure).  
1 x 2 mL Hexane.
- 5. ELUTE BENZODIAZEPINES:**

1 x 3 mL Ethyl Acetate containing 2% NH<sub>4</sub>OH  
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<sub>2</sub> 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<sub>2</sub>O

**Mobile Phase B:** 0.1% Formic Acid in Methanol

**Flow Rate:** 0.5 mL/minute

**Polarity:** Positive

**Reconstitute:** 100 µL

**Injection Volume:** 20 µL

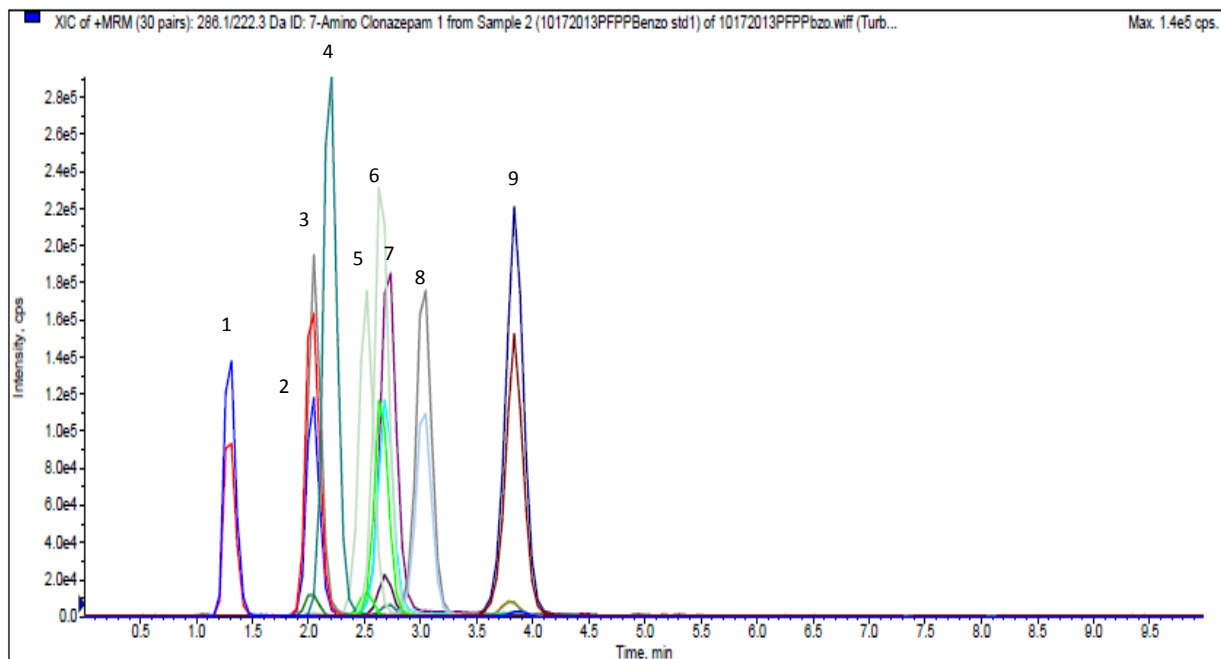
**LC Column:** Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

**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<sub>2</sub>O

**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

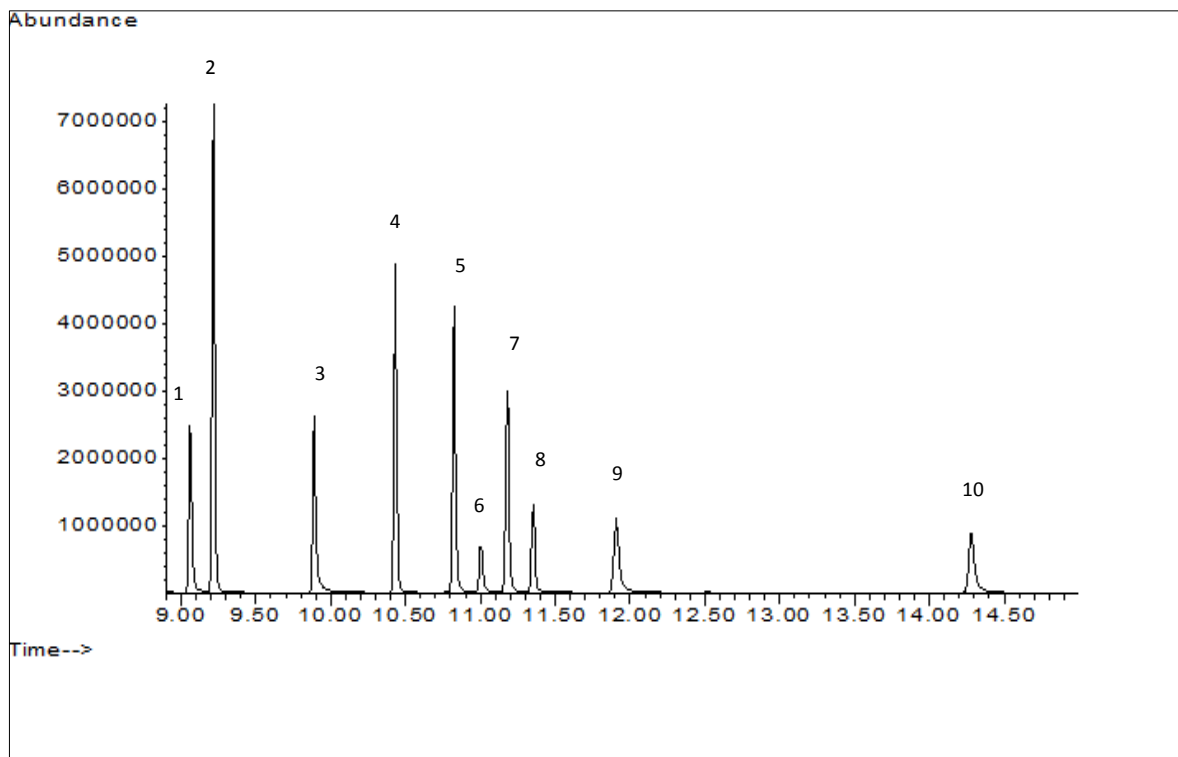
**Instrument:** API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

### 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. Midazolam	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 30m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

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

Carrier gas: Helium

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