



BENZODIAZEPINES IN HAIR BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

CSDAU206 – CLEAN SCREEN® DAU 200 mg, 6 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:

Add 10 -50 mg of decontaminated hair into a clean glass sample tube

Add 3 mL of 0.1 M phosphate buffer and internal standards and mix

Sonicate for 12 hours at room temperature

Centrifuge for 10 minutes at 3000 rpm

Transfer clear liquid to a clean glass sample tube

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

1 x 3 mL 5% Acetonitrile in 100 mM phosphate buffer (pH 6.0.0).

Dry column (5 minutes at full vacuum or pressure).

5. ELUTE BENZODIAZEPINES:

1 x 3 mL Ethyl Acetate containing 2% NH₄OH

collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

6. DRY ELUATE:

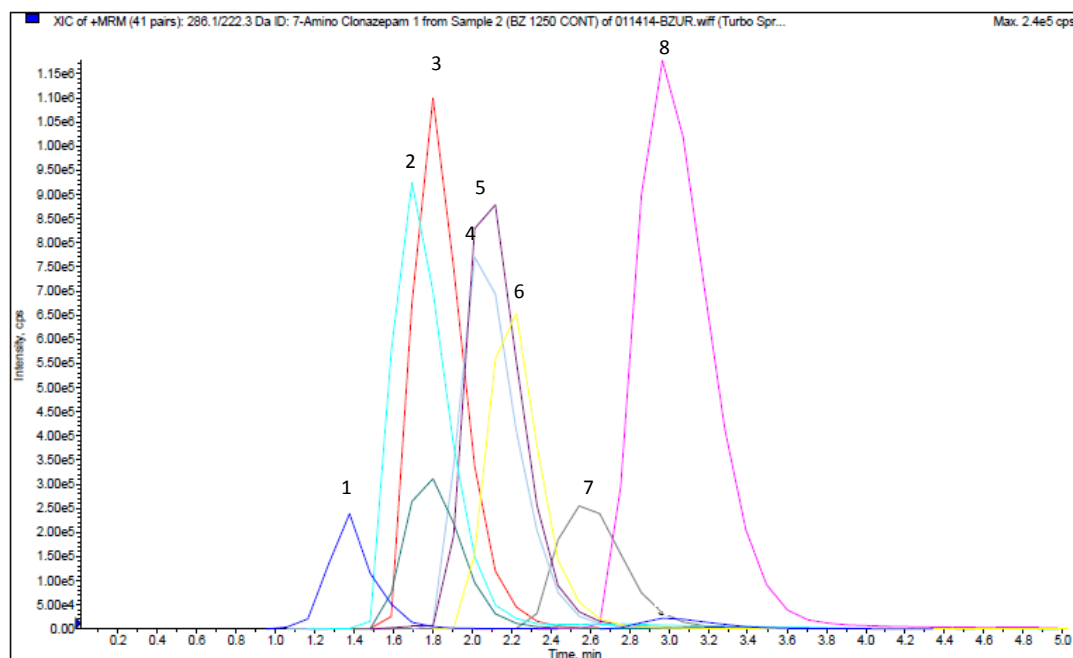
Evaporate to dryness at < 40 °C.

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 10 µL.
- **GC-MS:** Dissolve residue in 50 µL of Acetonitrile and
50 µL MTBSTFA w/ 1% TBDMCS
Overlay with N₂ 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. 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: 0.1% Formic Acid in D.I. H₂O

Flow Rate: 0.1 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

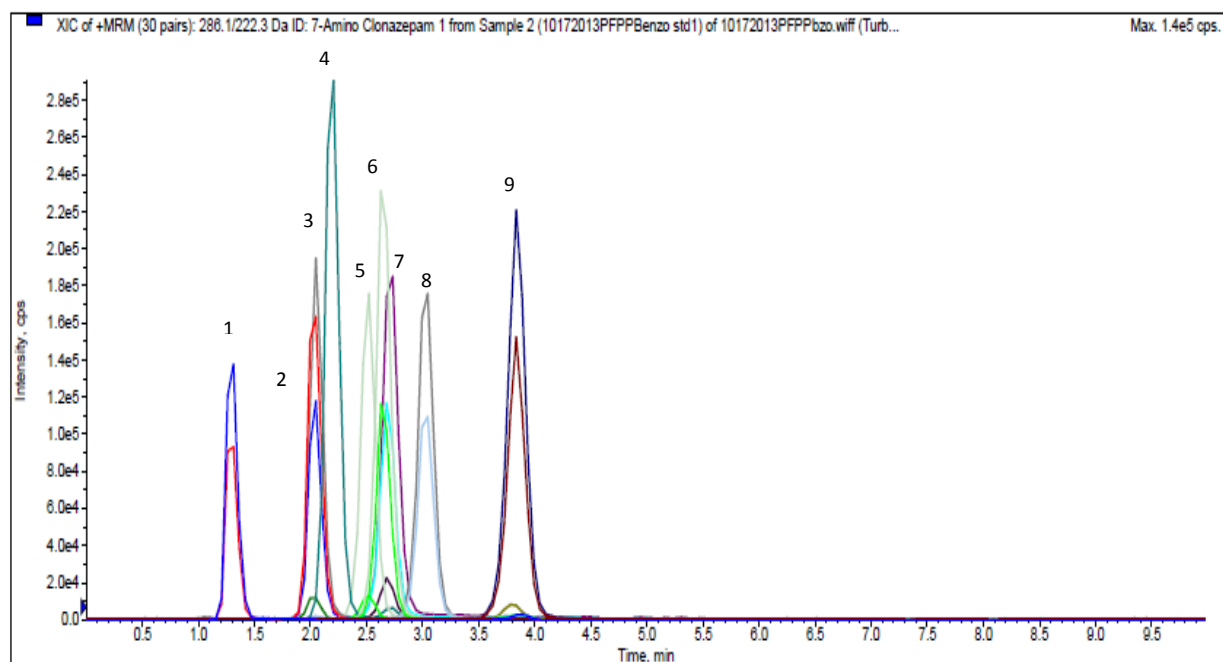
Polarity: Positive

Injection Volume: 10 µL

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	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. H₂O

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

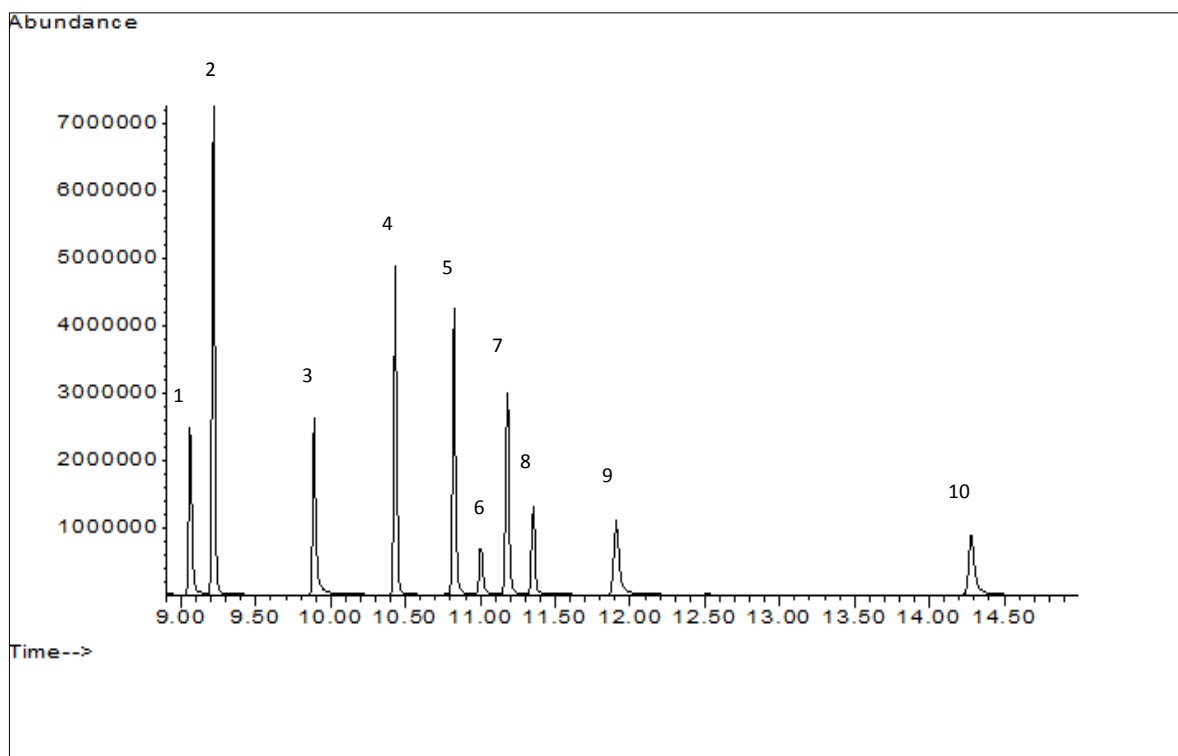
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 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 °C, MS Source: 250 °C, MS Quad: 150 °C