

Determination of Benzodiazepines in Hair Samples Using Solid Phase Extraction and LC-MS/MS



UCT Part Numbers

SLDAGDC21-5UM
Selectra® DA Guard Column
10 mm x 2.1 mm, 5 µm

SLDA100ID21-5UM
Selectra® DA Column
100 x 2.1 mm, 5 µm

CSDAU206
Clean Screen® DAU
200 mg, 6 mL

SPHPHO6001-5
Select pH Buffer Pouch
100 mM Phosphate Buffer
pH 6.0

Procedure:

1. Sample Preparation

- Add 10 -50 mg of decontaminated hair into a clean glass sample tube
- Add 3 mL of 0.1 M phosphate buffer 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
- Add internal standards* and mix

2. Condition Clean Screen® DAU Extraction Column

- 1 x 3 mL CH₃OH
- 1 x 3 mL D.I. H₂O
- 1 x 1 mL 0.1 M phosphate buffer (pH 6.0)

Note: Aspirate at < 3 inches Hg to prevent sorbent drying

3. Apply Sample

- Load sample at 1 to 2 mL/minute

4. Wash Column

- 1 x 3 mL DI H₂O
- 1 x 3 mL 0.1 M phosphate buffer (pH 6.0) containing 5% acetonitrile
- Dry column (5 minutes at > 10 inches Hg)

5. Elute Benzodiazepines

- 1 x 3 mL of ethyl acetate containing 2% ammonium hydroxide
- Collect eluate at 1 to 2 mL/minute

6. Dry Eluate

- Evaporate to dryness under nitrogen < 40°C
- Reconstitute in 100 µL of mobile phase



Instrument Conditions	
Column	Selectra® DA Column 100 x 2.1 mm, 5 µm (PN: SLDA100ID21-5UM)
Column Temperature	40 °C
Mobile phase	CH ₃ OH w/0.1% Formic acid: DI H ₂ O w/ 0.1% Formic acid (70:30)
Flowrate	0.4 mL/ minute
Injection Volume	10 µL
Detector	API 4000 MS/MS

MS Parameters	
Polarity	ESI+
Spray voltage V	4500 V
Source Temperature	650 °C
Curtain Gas	10
Gas 1	40
Gas 2	40
CAD Gas	Medium
Dwell Time	150 ms

SRM Transitions									
Compound	RT	Precursor Ion	Product Ion 1	CE 1	Product Ion 2	CE 2	DP	EP	CXP
7-aminoclonazepam	1.75	286.09	222.3	31	250.2	27	56	9	4
*7-aminoclonazepam-D4	1.73	290.12	93.98	30	121.02	30	56	9	4
Alpha-hydroxyalprazolam	3.64	325.18	297.1	33	216.3	33	56	9	4
Alpha-hydroxyalprazolam	3.62	330.18	302.2	33	284.2	53	56	10	4
Alprazolam	3.17	309.16	281.2	33	205.3	33	66	8	4
*Alprazolam-D5	3.15	314.15	286.5	43	279.3	33	56	7	4
Clonazepam	3.17	316.13	270.2	39	241.2	37	56	8	4
*Clonazepam-D4	3.14	320.1	270.4	37	244.2	37	56	8	4
Diazepam	5.99	285.11	193.1	43	154.1	37	56	10	4
*Diazepam-D5	5.95	290.16	198.3	29	227.3	21	36	7	4
Lorazepam	2.82	321.06	275	29	303.3	21	46	6.5	4
*Lorazepam-D5	2.79	325.11	279.2	37	307	35	51	5.5	4
Nordiazepam	3.91	271.09	140.1	35	165.2	37	56	9	4
*Nordiazepam-D5	3.86	276.08	213.4	29	165.1	51	41	8	4
Oxazepam	3.01	287.09	241.3	31	104.1	21	46	4.5	4
*Oxazepam-D5	2.95	292.12	246.2	29	274.3	19	41	4.5	4
Temazepam	4.27	301.12	255.2	50	177.2	50	60	4.5	4
*Temazepam-D5	4.23	306.17	260.8	27	288.8	19	41	3.5	4

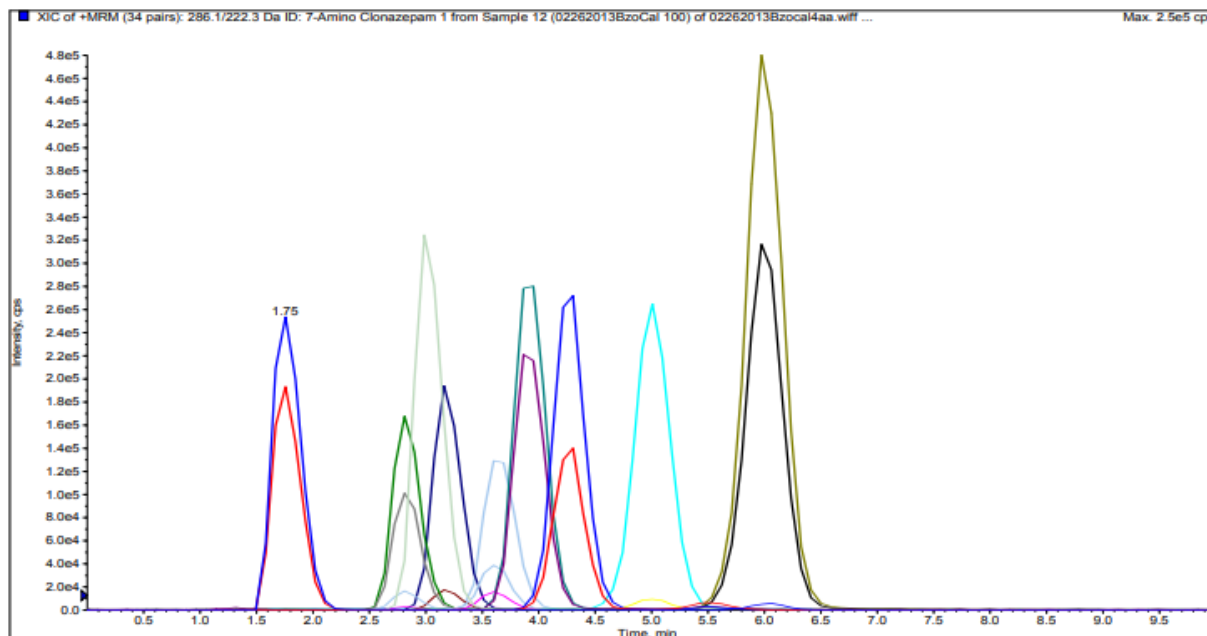
Note: DP= Declustering Potential; EP= Entrance Potential; CEP= Collision Entrance Potential;

CE= Collision Energy; CXP= Collision Exit Potential

*Internal Standard



Chromatogram of Benzodiazepines Extracted from Decontaminated Hair



Elution order: 7-aminoclonazepam, clonazepam/lorazepam, oxazepam, alpha-hydroxyalprazolam, nordiazepam, alprazolam, temazepam, and diazepam

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