



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

UCT Part Numbers:

CSDAU206: Clean Screen[®] DAU 200mg x 6mL

SLDA100ID21-5UM: Selectra[®] DA (100mm x 2.1mm, 5 µm) LC Column

SLDAGDC21-5UM: Selectra[®] DA (10mm x 2.1mm) Guard Column

SPHPHO6001-5: 0.1M Phosphate Buffer pH 6

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Procedure

1. Extraction

- a) Add 10 -50 mg of decontaminated hair into a clean glass sample tube
- b) Add 3 mL of 0.1 M phosphate buffer and mix
- c) Sonicate for 12 hours at room temperature
- d) Centrifuge for 10 minutes at 3000 rpm
- e) Transfer clear liquid to a clean glass sample tube
- f) Add internal standards* and mix

2. Condition Clean Screen[®] Extraction Column

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

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

3. Apply Sample

- a) Load at 1 to 2 mL/minute.

4. Wash Column

- a) 1 x 3 mL D.I. H₂O.
- b) 1 x 3 mL 0.1 M phosphate buffer (pH 6.0). containing 5% acetonitrile.
- c) Dry column (5 minutes at > 10 inches Hg).

5. Elute Benzodiazepines

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

6. Dry Eluate

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

Instrument Conditions

Column: 100 x 2.1 mm (5 µm) Selectra® DA

Column Temperature: 40°C

Mobile phase: CH₃OH w/0.1% Formic acid: DI H₂O 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	4500V
Source Temperature	650° C
Curtain Gas	10
Gas 1	40
Gas 2	40
CAD Gas	Medium
Dwell Time	150ms

SRM Transitions

Compound	RT	Precursor Ion	Product Ion 1	CE1	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-D5	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

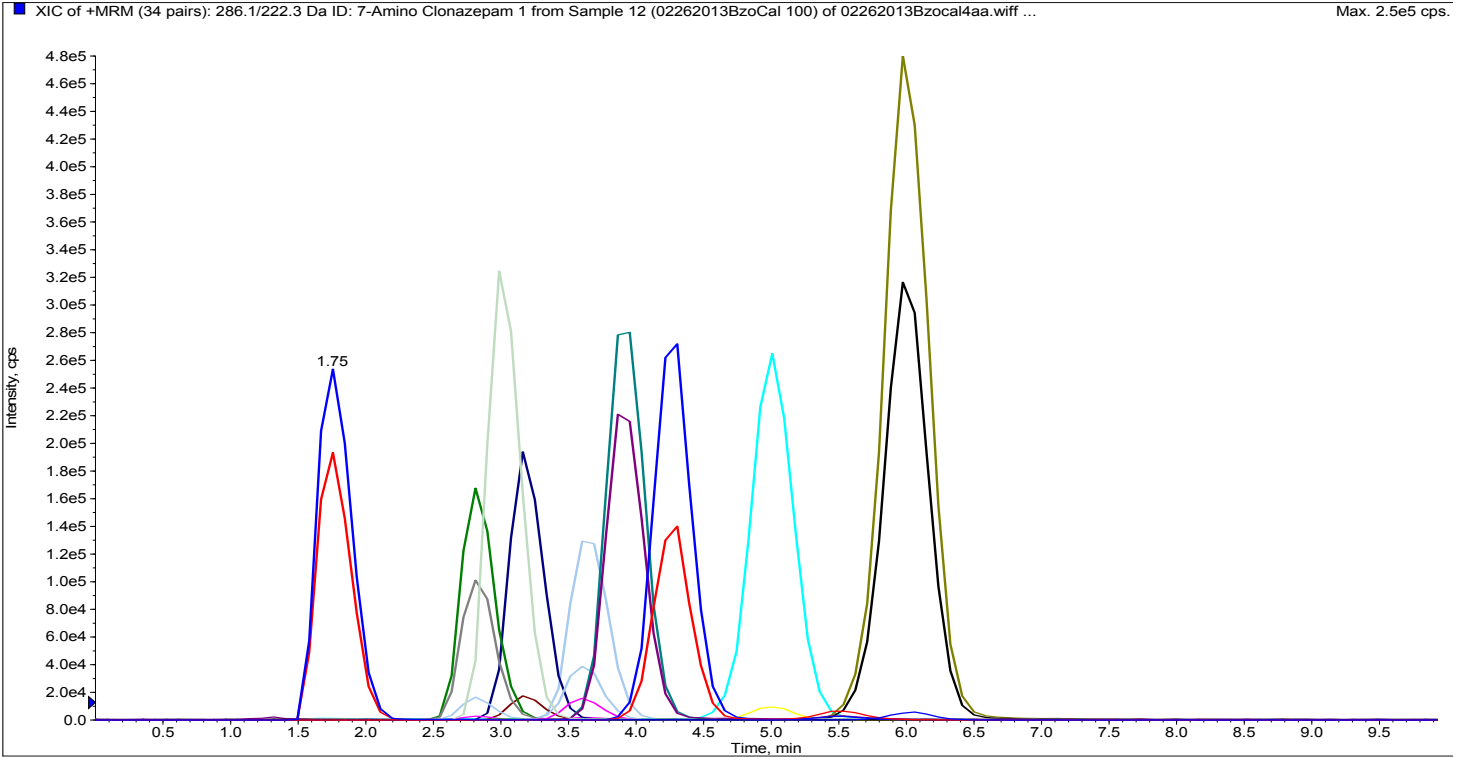
*Alprazolam-D5	3.15	314.15	286..5	43	279.3	33	56	7	4
Clonazepam	3.17	316.13	270.2	39	241.2	37	56	8	4
*Clonazepam-D4	3.14	320.1	270.4	37	244.2	37	56	8	4
Diazepam	5.99	285.11	193.1	43	154.1	37	56	10	4
*Diazepam-D5	5.95	290.16	198.3	29	227.3	21	36	7	4
Lorazepam	2.82	321.06	275	29	303.3	21	46	6.5	4
*Lorazepam-D5	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= Decustering 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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