

Benzodiazepines from urine using Strata-X-Drug N on a Kinetex C18 2.6µm, 50x2.1mm

Column: Kinetex® 2.6 µm C18 100 Å, LC Column 50 x 2.1 mm, Ea

Dimensions: 50 x 2.1 mm ID

Order No: 00B-4462-AN

Elution Type: Gradient

Eluent A: 0.1% Formic acid

Eluent B: 0.1% Formic acid in methanol

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	65	35
	2	4	5	95
	3	4.01	65	35
	4	7	65	35

Flow Rate: 300 µL/min

Col. Temp.: ambient

Detection: Mass Spectrometer (MS) @ amu (ambient)

Detector Info: <a target="_blank"

href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<

Source: Cur-11, CAD-12, IS-4500, Tem-450, NEB-12

Dwell: 25ms

Polarity: Positive

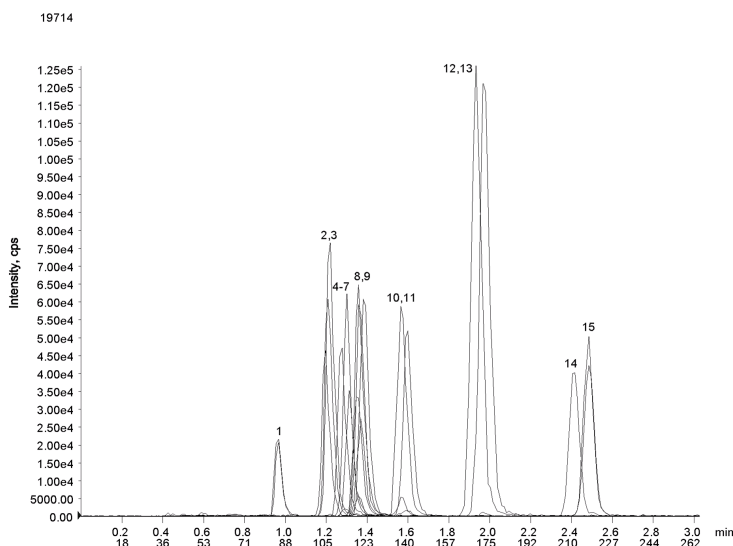
Analyst Note: SecurityGuard™ ULTRA Guard Cartridge System extends column lifetime.

- SecurityGuard ULTRA Cartridges, UHPLC C18 for 2.1mm ID Columns, 3/Pk Part No.: AJ0-8782

- Holder Part No.: AJ0-9000



Products used in this application:



Benzodiazepines from urine using Strata-X-Drug N on a Kinetex C18 2.6 μ m, 50x2.1mm

ANALYTES:

- 1** alpha-Hydroxyalprazolam (325.4>279.2, 325.4>204.9)
- 2** Oxazepam-d5 (292.3>246.3)
- 3** Oxazepam (287.1>241.1, 287.1>268.9)
- 4** Alprazolam-d5 (314.2>210.2)
- 5** Alprazolam (309.1>205.1, 309.1>281.1)
- 6** Nordiazepam-d5 (276.3>140.1)
- 7** Nordiazepam (271.4>140.3, 271.3>164.9)
- 8** Lorazepam-d4 (325.1>279.2,)
- 9** Lorazepam (321.3>275.3, 321.3>239.1)
- 10** Clonazepam-d4 (320.1>274.1,)
- 11** Clonazepam (316.3>270.3, 316.3>214)
- 12** Temazepam-d5 (306.3>260.2)
- 13** Temazepam (301.3>255.2, 301.3>180.1)
- 14** Diazepam-d5 (Valium) 290.2>154.2
- 15** Diazepam (Valium) 285.3>193.2, 285.3>154.2



Sample Preparation Details

for **UHPLC** Application ID No.: **19714**

Benzodiazepines from urine using Strata-X-Drug N on a Kinetex C18 2.6µm, 50x2.1mm

PRODUCT DESCRIPTION:

Strata™-X-Drug N 100 µm Polymeric Reversed Phase, 100 mg / 6 mL, Tubes , 30/Pk

Order No.: 8B-S129-ECH

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 100 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Pre-Treatment: To 2mL urine, add 1mL beta-glucuronidase (contains 5,000 F units/mL Patella vulgata in 100mM acetate buffer(pH 5.0)).

Mix/vortex.

Hydrolyze for 3 hours at 60C.

Let cool and add 1mL 100mM Phosphate buffer (pH 6.0).

Centrifuge for 5 minutes at 5000 rpm and discard pellet. Load directly onto SPE cartridge - NO CONDITIONING REQUIRED (Extraction automated using a Gilson GX-274 ASPEC liquid handler)

Wash:

Dry:

10 minutes @ full vacuum

Elute:

Final Prep and Analysis:

Evaporate eluant under a stream of N2 gas at 50°C. Reconstitute with 1mL of 35% methanol

Inject: 5 µL on HPLC Mass Spectrometer (MS) @ amu (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 alpha-Hydroxyalprazolam	300			92.4	10.6
2 Oxazepam-d5	300			100	0.01
3 Oxazepam	300			105.3	5.6
4 Alprazolam-d5	300			100	0.01
5 Alprazolam	300			98.4	2.1
6 Nordiazepam-d5	300			100	0.01
7 Nordiazepam	300			103.7	3.7
8 Lorazepam-d4	300			100	0.01
9 Lorazepam	300			101.1	1
10 Clonazepam-d4	300			100	0.01
11 Clonazepam	300			99.6	0.5
12 Temazepam-d5	300			100	0.01
13 Temazepam	300			104.6	3.9
14 Diazepam-d5	300			100	0.01
15 Diazepam	300			101.1	1

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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