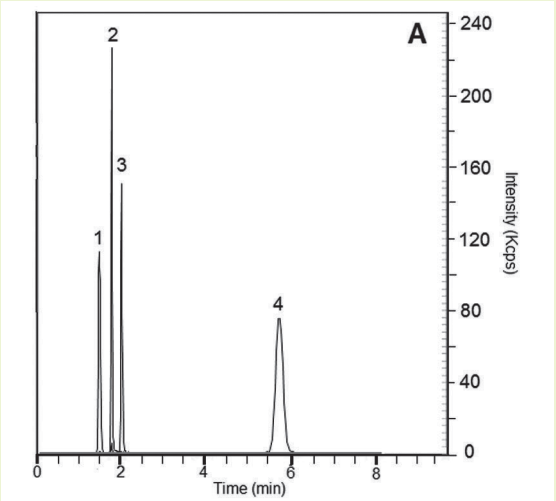
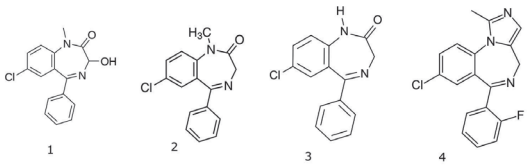
1,4-Benzodiazepines in Urine

LC-MS method with SPE





Note: Benzodiazepines are prescribed for conditions such as anxiety disorders, insomnia, seizures, and alcohol withdrawal. However, they also have potential for abuse as recreational drugs.

Method Conditions

Column: **Cogent Diol™**, 4.4µm, 100Å

Catalog No.: 40060-05P-2

Dimensions: 2.1 x 50 mm

Mobile Phase: A: DI H_2O / 0.1% formic acid (v/v)
B: Acetonitrile / 0.1% formic acid (v/v)

Gradient:

(Fig. A)		(Fig. B)	
time (min.)	%B	time (min.)	%B
0	85	0	10
6	70	6	10
7	20	7	50
9	20	9	50
10	85	10	10

Post Time: 3 min

Injection vol.: 1 µL

Flow rate: 0.4mL/min

Detection: ESI – POS - PerkinElmer AxiON 2 TOF mass spectrometer

Sample: Extraction method: Spiked urine sample was loaded into SPE cartridge I (Clean Screen Xcel™ purchased from UCT Bristol, PA, USA) and eluted with 0.78 mL of acetonitrile, 200 microL of 2-propanol, 20 microL of ammonia. After the elution, the sample was dried under N_2 gas and dissolved in 100 microL of 50% methanol / 50% DI H_2O / 0.1% formic acid. Before injection, the sample was filtered through a 0.45µm nylon syringe filter (MicroSolv Tech Corp.).

Peaks: 1. Temazepam 301.0739 m/z $[M+H]^+$
2. Diazepam 285.0790 $[M+H]^+$
3. Nordiazepam 271.0633 $[M+H]^+$
4. Midazolam 326.0855 $[M+H]^+$

Discussion

The Cogent Diol column was used in analysis of 1,4-benzodiazepines in urine samples after SPE extraction using two types of retention modes. Four available compounds were well retained and separated in both modes; however, the retention order was changed depending on the gradient used. It is worth noticing that the peak intensities were three times higher when using Gradient A (ANP mode) compared to Gradient B (RP mode).

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