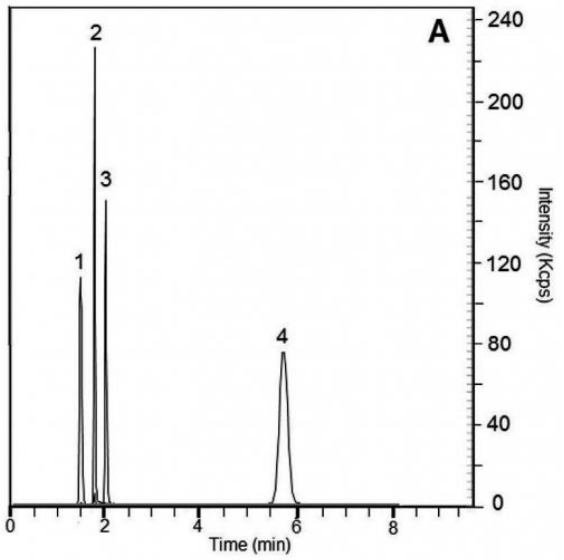
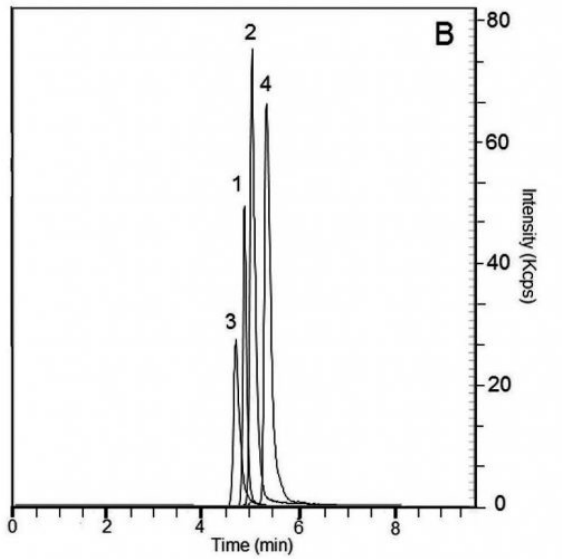
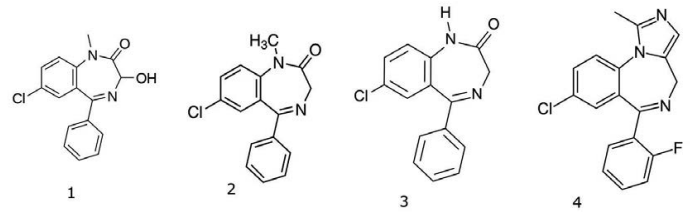
Benzodiazepines in Urine Analyzed using RP and ANP – AppNote

Two Types of Retention Modes are Described in this Application Note

Four Compounds were well retained and separated in both HPLC Modes shown in Figures A and B; 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).







Peaks:

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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]+

Method Conditions

Column: Cogent Diol™, 4.4µm, 100Å

Catalog No.: 40060-05P-2

Dimensions: 2.1 x 50mm

Mobile Phase:

A: DI Water with 0 .1% Formic Acid (v/v)

B: Acetonitrile with 0.1% Formic Acid (v/v)

Gradient:

Figure A		Figure B	
Time *(minutes)*	%B	Time *(minutes)*	%B
0	85	0	10
6	70	6	10
7	20	7	50
9	20	9	50
10	85	10	10

Post time: 3 minutes

Injection vol.: 1µL

Flow rate: 0.4ml / minute

Detection: ESI – POS – Perkin Elmer AxION 2 TOF Mass Spectrometer

Flow rate: 0.4ml / minute

Sample Preparation: Extraction Method: Spiked urine sample was loaded into SPE cartridge I *(Clean Screen Xcel™ purchased from UCT Bristol, PA, USA)* and eluted with 0.78mL of Acetonitrile, 200µL of 2-Propanol, 20µL of Ammonia. After the elution, the Sample was dried under Nitrogen gas and dissolved in 100µL of 50:50 Methanol / DI Water / 0.1% Formic Acid. Before injection, the 10ppm spiked Sample was filtered through a 0.45µm Nylon Syringe Filter (MicroSolv Tech Corp).

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

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Attachments

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