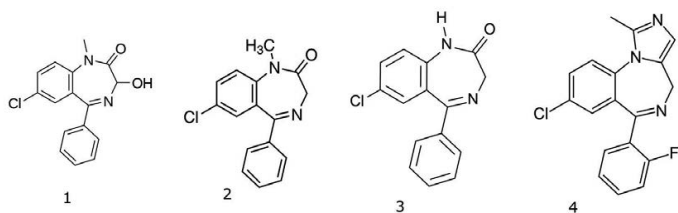
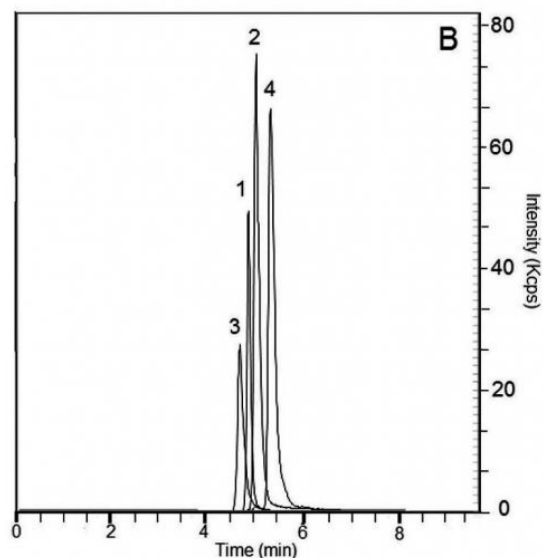
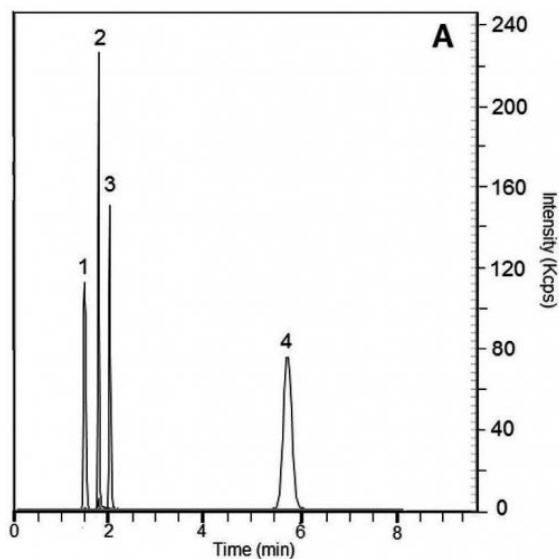


## Benzodiazepines in Urine Analyzed using RP and ANP – AppNote

### 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:

1. Temazepam 301.0739 m/z [M+H]<sup>+</sup>

2. Diazepam 285.0790 [M+H]<sup>+</sup>
3. Nordiazepam 271.0633 [M+H]<sup>+</sup>
4. Midazolam 326.0855 [M+H]<sup>+</sup>

## 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.



## Attachments

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Date: 05-07-2024