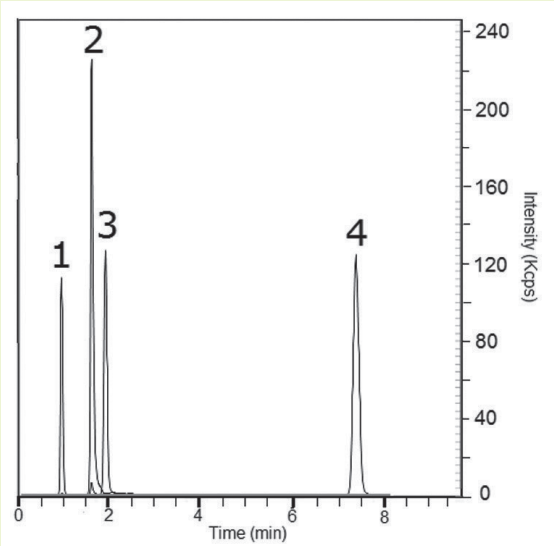
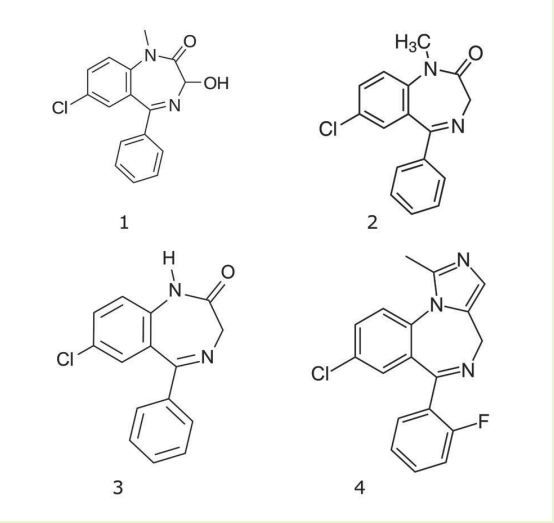
1,4-Benzodiazepines in Urine

LC-MS method with SPE





Note: Benzodiazepines have very important clinical applications for the therapy of anxiety, sleep disorders and convulsive attacks. Diazepam is metabolized in the body to temazepam. In medical practice, there is a need for analytical control of the concentration of 1,4-benzodiazepines in body fluids due to their narrow therapeutic range (toxic effects at low doses of the drugs). On the other hand, in forensic toxicology, benzodiazepines are often found in fatal cases of drug intoxication.

Method Conditions

Column: **Cogent Diamond Hydride 2.ō™**, 2.2µm, 120Å

Catalog No.: 70200-05P-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:

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

Post time: 3 min

Injection vol.: 1 µL

Flow rate: 0.4 mL/min

Detection: ESI – POS – Perkin Elmer 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 10ppm spiked sample was filtered through a 0.45 µm 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 $[M+H]^+$

t_0: 0.9 min

Discussion

The Cogent Diamond Hydride 2.ō column was successfully used in analysis of 1,4-benzodiazepines in urine samples after SPE extraction. Four available compounds were well retained and separated. The procedure could be used for determination of this class of compounds in urine samples and other body fluids.

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