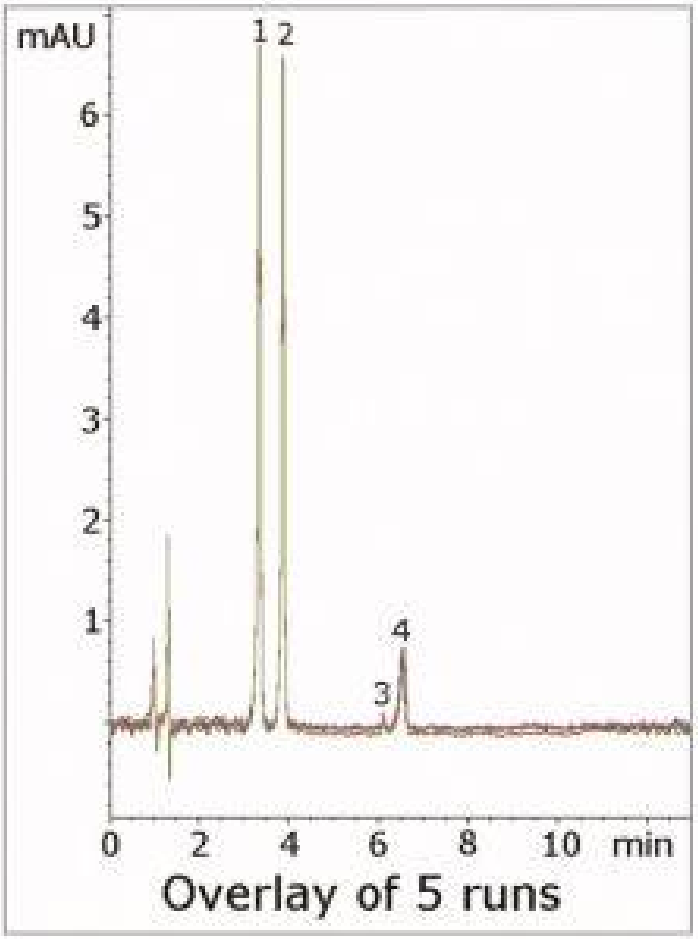
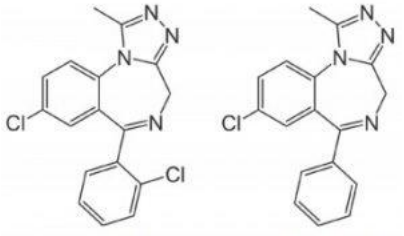
Alprazolam Analyzed with HPLC - AppNote

Robust Separation of API from USP Internal Standard

The USP Assay Method for Alprazolam uses a bare Silica Column and a complex Mobile Phase consisting of Acetonitrile, Chloroform, Butyl Alcohol, and Acetic Acid. In this Method, a simple LCMS compatible Mobile Phase is used and produces excellent Peak shapes for both the API and its USP internal standard.

Furthermore, a resolution of 4.3 was obtained between the two Peaks, which meets the USP system suitability of Rs≥2.0. Two impurity Peaks are also observed, which further illustrates the resolution capabilities of the analysis. This Method illustrates how the MS-compatible HPLC-UV Methods described in various application notes can be successfully adapted for LCMS.





Peaks:

1. Triazolam (internal standard)
2. Alprazolam (API)
3, 4. Impurities

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å
Catalog No.: 70000-7.5P
Dimensions: 4.6 x 75mm
Mobile Phase:
A: DI Water / 0.1% Formic Acid (v/v)
B: Acetonitrile / 0.1% Formic Acid (v/v)
Gradient:

Time *(minutes)*	%B
0	95
1	95
6	50
7	95

Post Time: 3 minutes
Injection vol.: 1µL
Flow rate: 1.0mL / minute
Detection: UV @ 254nm
Sample Preparation:
Tablet: A 0.25mg strength generic Xanax® tablet was ground and added to a 10mL volumetric flask. After diluting with 50% Solvent A / 50% Solvent B, it was sonicated for 10 minutes. A portion was filtered with a 0.45µm Nylon Syringe Filter (MICROSOLV Tech Corp.).
Internal Standard: 1mg / mL Triazolam in Methanol diluent.
Working Solutions: 20µL of the internal standard and 980µL of the tablet extract were mixed. Peak identities were confirmed by individual solutions of the tablet extract and the internal standard.
t_0: 0.9 minutes

Note: *Alprazolam is a member of the benzo-diazepine class of compounds, prescribed to treat a variety of anxiety-related conditions.*

COGENT HPLC Columns™

Attachment

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