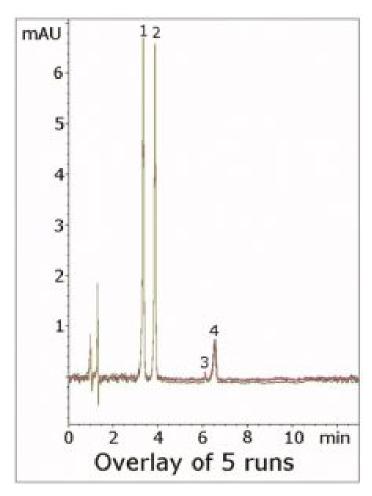


## Alprazolam Analyzed with HPLC - AppNote

## **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.



1. Triazolam (internal standard)



2. Alprazolam (API)

3, 4. Impurities

## **Method Conditions**

**Column:** Cogent Diamond Hydride<sup>™</sup>, 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<sub>0</sub>: 0.9 minutes

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



Attachment

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