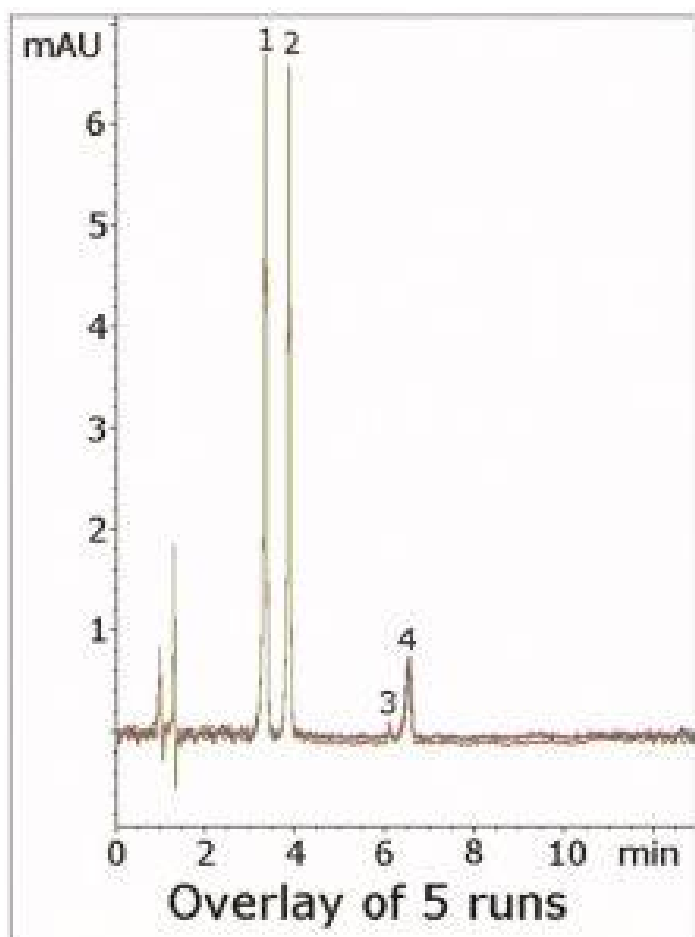


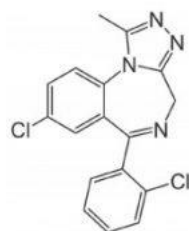
## Alprazolam Analyzed with HPLC - AppNote

### Robust Separation of API from USP Internal Standard

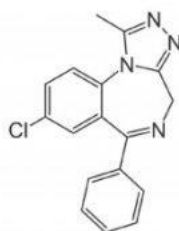
The USP Assay Method for Alprazolam uses a bare Silica Column and a complex Mobile Phase consisting of Acetonitrile, Chloroform, Butyl Alcohol, and Acetic Acid. In this Method, a simple LCMS compatible Mobile Phase is used and produces excellent Peak shapes for both the API and its USP internal standard.

Furthermore, a resolution of 4.3 was obtained between the two Peaks, which meets the USP system suitability of  $R_s \geq 2.0$ . 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.





Peak 1



Peak 2

**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<sub>0</sub>: 0.9 minutes

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



**Attachment**

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