

Analysis of a Complex Mixture of 13 Benzodiazepines Using HPLC-PDA Detection

Marc Gregerson, Ph.D., Cayman Chemical

Benzodiazepines remain common in forensic casework. Thirteen benzodiazepines mentioned in the CFSRE Q1 2026 Scope Recommendations were analyzed using HPLC-PDA. All compounds are well resolved ($R_s > 1.2$) using this newly developed method.

Introduction

The CFSRE & SOFT NPS Committee's Q1 2026 Scope Recommendations included 13 NPS benzodiazepines.¹ Some of these compounds are emerging, while others are well established and seen frequently in forensic/toxicological testing. This technical brief highlights the breadth of NPS benzodiazepines available at Cayman Chemical and provides a method for analyzing this complex mixture.

Materials

Each compound was dissolved in either methanol or acetonitrile at concentrations ranging from 2.5 to 5 mg/mL. These stock solutions were then used to prepare a mixture containing each of the 13 benzodiazepines at 200 µg/mL per compound in methanol. A 200 µg/mL standard of each compound was also made for retention time comparison purposes. Cayman standards (item numbers in parentheses) used in this study: 4'-chloro deschloroalprazolam (36057), pyrazolam (14901), desalkylflurazepam (18484), ethylflualprazolam (44429), etizolam (12063), flualprazolam (24481), flubromazepam (15157), clonazolam (18173), 8-aminoclonazolam (27247), ethylbromazolam (42723), desalkylgidazepam (36608), phenazolam (26700), and bromazolam (22665).

Methods

Analysis was performed by injecting 1 µL into a Shimadzu Nexera LC-40D XR HPLC, using a 150 x 4.6 mm, 2.6 µm Kinetex Biphenyl HPLC column (Phenomenex, Inc.). The column temperature was 50°C. Mobile phase A was 20 mM ammonium formate + 0.1% formic acid in water, mobile phase B was acetonitrile. The 1.2 mL/min flow rate was held at 29% B from 0 to 11 min, a 29-36% B gradient was applied from 11 to 17.5 min, held at 36% B from 17.5 to 18.5 min, and finally, 29% B for 3.5 min to equilibrate. The chromatogram was captured using a photodiode array (PDA) detector at a wavelength of 230 nm.

Results

The chromatogram is shown below in **Figure 1** with the 13 compound peaks identified. A resolution of >1.2 was achieved for all compounds.

Conclusions

Benzodiazepines continue to be detected in forensic and clinical toxicology laboratories. This method provides a way to separate all 13 of these substances and help labs develop internal methods.

Reference

1. Krotulski, *et al.* (2026) Recommended Scope for NPS Testing in the United States Q1 2026. In: Center for Forensic Science Research and Education [Internet]. United States. Available from: <https://www.cfsre.org/nps-discovery/scope-recommendations>

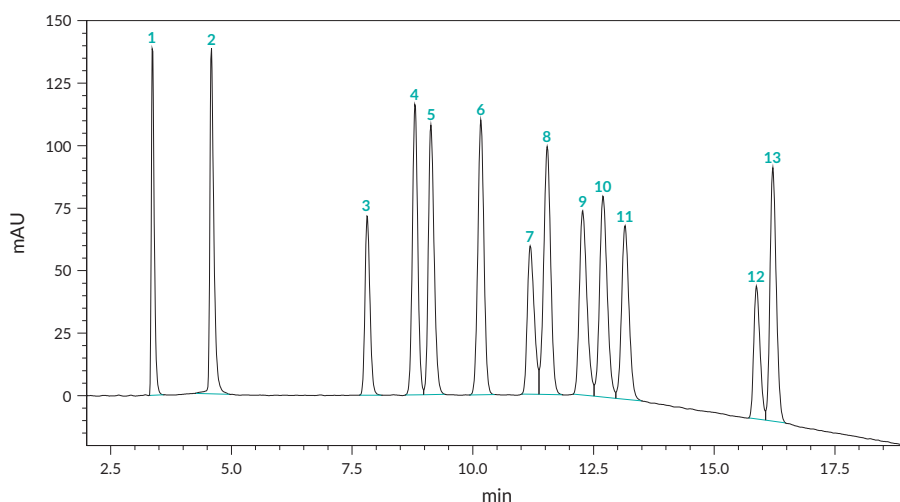


Figure 1 - Chromatogram at 230 nm showing separation of the 13 analytes:

8-Aminoclonazolam (1), Pyrazolam (2), Clonazolam (3), Desalkylflurazepam (4), Flualprazolam (5), Flubromazepam (6), 4'-chloro Deschloroalprazolam (7), Desalkylgidazepam (8), Bromazolam (9), Phenazolam (10), Ethylflualprazolam (11), Etizolam (12), and Ethylbromazolam (13).