

# Analysis of a Complex Mixture of Orphines Using HPLC-PDA Detection

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A mixture of thirteen piperidinylbenzimidazolone opioid standards (more commonly known as 'orphines') was prepared at 100 µg/mL each and analyzed using HPLC-PDA. All compounds are well resolved ( $R_s > 1.2$ ).

## Introduction

Brorphine was first reported to the UNODC Early Warning Advisory on NPS in 2019 and was controlled in 2022. Subsequent analogs continued to be developed and were detected in 2024.<sup>1,2</sup> As more orphine analogs emerge, this indicates a need for the detection of multiple orphine compounds. This work develops a method for the analysis of 13 such compounds in a single run.

## Methods

Each of the 13 compounds was made into individual stock solutions in either methanol or methanol containing 1-2% formic acid. Solubility varied from 2 to 20 mg/mL.

After the stock solutions were made, an appropriate amount of each compound was added to a vial and diluted with methanol to make a 100 µg/mL mixed standard solution. Individual 100 µg/mL standard solutions of each compound were made for retention time comparison purposes.

## Materials

Analysis was performed by injecting 1 µL into a Shimadzu Nexera LC-40D XR HPLC, using a 100 x 2.1 mm, 1.7 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 0.1% formic acid in methanol. The 0.4 mL/min gradient was 25% B from 0 to 1 min, 25-35% B from 1 to 3 min, 35-40% B from 3 to 8 min, 40-55% B from 8 to 12 min, 55-72% B from 12 to 17 min, 72-95% B from 17 to 18 min, 95% B from 18 to 19.5 min, then 25% B for a 3 minute equilibration. The chromatogram was captured using a photodiode array (PDA) detector at a wavelength of 220 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

In response to the need for orphine opioid analysis, a method has been generated that can resolve 13 different compounds. It may be possible to add more compounds to this method as they become available.

## References

1. Iula, D.M., St. Germaine, D.M., and Layle, N.K. NPS Snapshot: Orphines. *Cayman Chemical* (September 2025).
2. UNODC Lab and Scientific Service Portals, *Emerging analogues of brorphine*. 30 MAY 2025.

