# Rapid Differentiation of Isobaric Drugs Using a Novel Direct Sample Analysis Source, CID, and Accurate Mass TOF Mass Spectrometry

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## 1 Introduction

Identification of isobaric drugs is an increasing challenge for analytical research laboratories. These isomers cannot be distinguished by molecular weight alone. Liquid or gas chromatographic methods can often separate structural isomers, but sample throughput may be reduced. Collisionally induced dissociation (CID) in the mass spectrometer has the potential to distinguish structural isomers without the need for chromatography. Here, we examine the potential to differentiate isobaric drugs using direct sample ionization combined with accurate mass TOF MS analysis.

## 2 Experimental



Figure 1. AxION 2 TOF MS fitted with a Direct Sample Analysis (DSA) Source.

- Analysis was performed on a PerkinElmer AxION 2 TOF MS system fitted with a Direct Sample Analysis (DSA) source operated in positive mode.
- Samples were acquired using AxION DSA Controller and data was processed with AxION Solo software using a strong signal setting of 5000 counts and a cutoff of 1% strong signal except where noted. Isotope search window ±0.003 Da. Monoisotopic weight 7.
- Analytes were obtained from Cerilliant and Sigma-Aldrich.
- Analytes were prepared in methanol to 1 µg/mL and 5 µL was spotted on the stainless steel sample mesh.
- 5 replicates were acquired per sample, 1 second acquisition per sample, 10 spectra per second acquisition rate.

**Table 1. Isobaric Pairs, Collisional Energy, and Fragments Ions**

| Molecular Ion | | CE (V) | Isobar 1 Fragment | | | Isobar 2 Fragment | | |
|---|---|---|---|---|---|---|---|---|
| Formula | [M+H]+ | | Analyte | Formula | m/z | Analyte | Formula | m/z |
| C10H15N | 150.1277 | 120 | Methamphetamine | C9H11 | 119.0855 | Phentermine | C10H13 | 133.1012 |
| C10H15NO | 166.1226 | 120 | Pseudoephedrine | C10H14N | 148.1121 | p-Methoxyamphetamine | C10H13O | 149.0961 |
| C15H12N2O2 | 253.0972 | 150 | Oxcarbazepine | C15H10NO2 | 236.0706 | Phenytoin | C14H12N2O | 224.0944 |
| C17H21NO | 256.1696 | 120 | Atomoxetine | C10H14N | 148.1121 | Diphenhydramine | C13H11 | 167.0855 |
| C16H25NO2 | 264.1958 | 150 | Tramadol | C16H23NO | 246.1852 | O-Desmethylvenlafaxine | C16H23NO | 246.1852 |
| C20H23N | 278.1903 | 160 | Amitriptyline | C15H11 | 191.0855 | EDDP | C18H19N | 249.1512 |
| C17H19NO3 | 286.1438 | 150 | Morphine | C17H18NO2 | 268.1332 | Hydromorphone | C15H15O3 | 243.1016 |
| C18H21NO3 | 300.1594 | 150 | Codeine | C18H20NO2 | 282.1489 | Hydrocodone | C16H17O3 | 257.1172 |
| C17H21NO4 | 304.1543 | 150 | Cocaine | C10H16NO2 | 182.1176 | Scopolamine | C8H12NO | 138.0913 |
| C21H29NO | 312.2322 | 180 | Biperiden | C6H12N | 98.0964 | UR-144 | C14H16NO | 214.1226 |
| C19H21NO4 | 328.1543 | 150 | Naloxone | C19H20NO3 | 310.1438 | 6-Acetylmorphine | C17H18NO2 | 268.1332 |
| C25H25NO | 356.2009 | 220 | JWH-019 | C15H18NO | 228.1383 | JWH-122 | C11H9 | 141.0699 |

## 3 Results

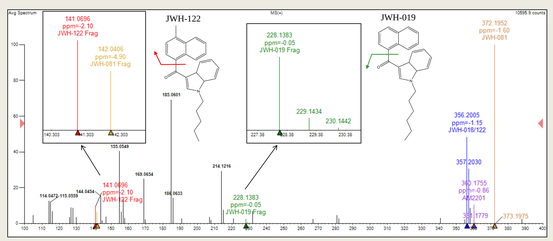



Figure 2. Example analysis of synthetic cannabinoid mixture containing an isobaric pair: JWH-019 and JWH-122 which were distinguished by unique fragment ions. Identified ions are highlighted in color.

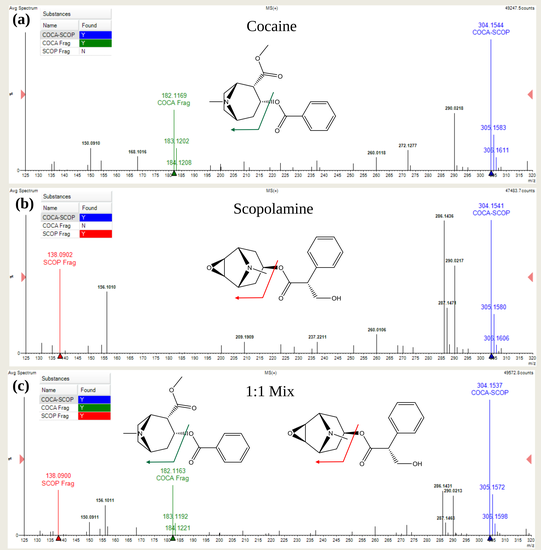



Figure 3. Analysis of the isobaric pair cocaine and scopolamine. (a) Cocaine only. (b) Scopolamine only. (c) 1:1 cocaine and scopolamine mix. Identified ions are highlighted in color.

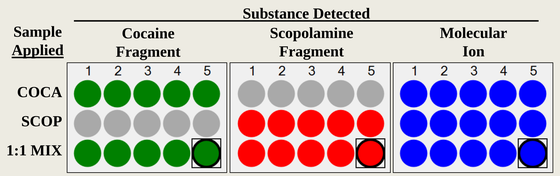



Figure 4. AxION Solo analysis of cocaine and scopolamine. Numbers 1-5 are replicate samples. Colored circles indicate positive identification of a fragment or molecular ion. Top row, cocaine only samples. Middle row, scopolamine only samples. Bottom row, 1:1 mixture of cocaine and scopolamine samples. Left panel, cocaine-specific fragment ion. Middle panel, scopolamine-specific fragment ion. Right panel, shared molecular ion for cocaine and scopolamine.

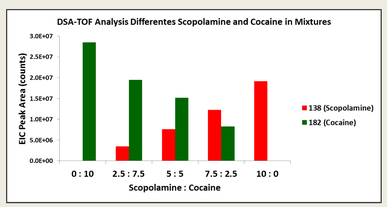



Figure 5. Analysis of cocaine and scopolamine mixed in varied proportions. Cocaine and scopolamine were mixed in the ratios indicated on the x-axis. Extracted ion chromatograms for cocaine (green) and scopolamine (red) were generated and peak areas were quantitated (y-axis). These results show that the relative proportion of each analyte can be determined using specific fragment ion intensities.

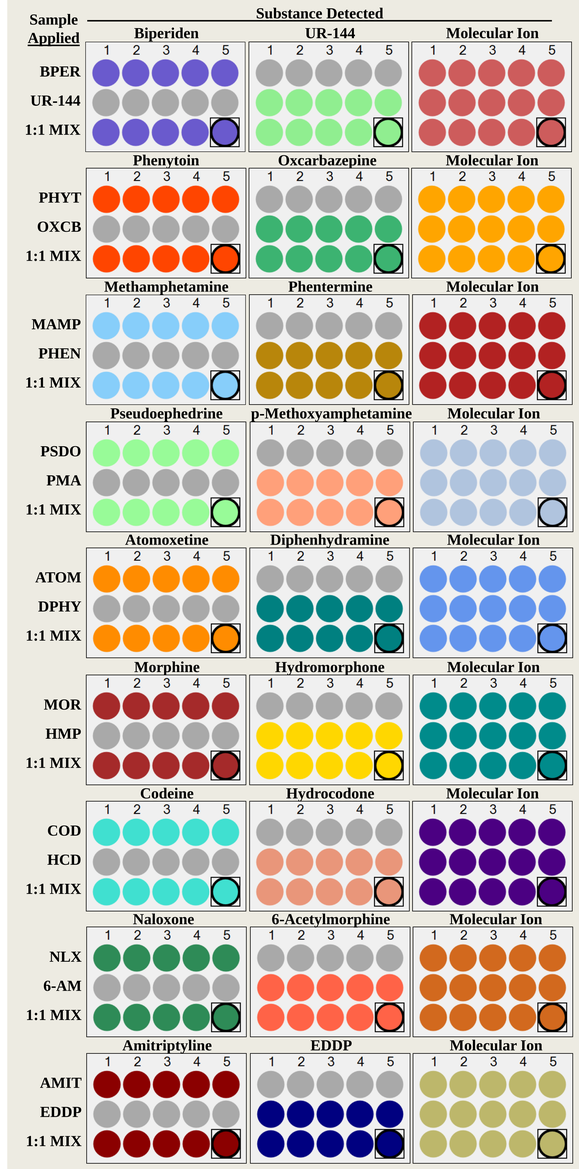
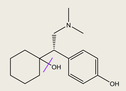
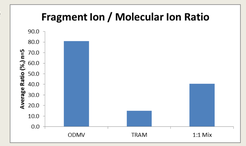


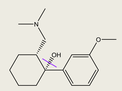

Figure 6. Summary analysis of 9 isobaric pairs . Cutoff (%) of strong signal was set to 1% for all analyte pairs except for the following: phenytoin / oxcarbazepine (2%), methamphetamine / phentermine (2%), and Naloxone / 6-Acetylmorphine (4%).

O-Desmethylvenlafaxine






Tramadol



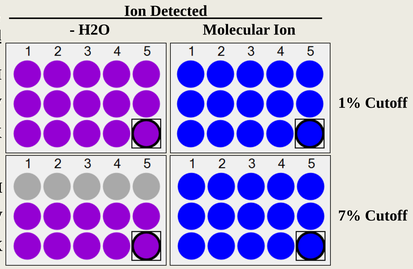



Figure 7. Analysis of the isobaric pair O-Desmethylvenlafaxine and Tramadol which did not produce isomer-specific fragment ions. Top panel, the ratio of the water loss ion to the molecular ion is greater with ODMV than for TRAM. This ratio is affected when a mixture of analytes is present. Bottom panels, adjusting the score cutoff threshold allows the differentiation of ODMV and TRAM.

## 4 Summary

- DSA TOF analysis allows for specific detection of many isobaric drugs
- Chromatographic separation is not required which greatly improves sample throughput
- We demonstrate that the relative proportion of isobaric components in mixtures can be determined although further work will be required for absolute quantitation.
- AxION Solo software allows for clear visualization of sample identifications and can rapidly generate sample reports.
- The DSA system can analyze samples in either liquid or powder form.

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