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

Robert J. Seward, Bonnie Marmor, and Andrew Tyler

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

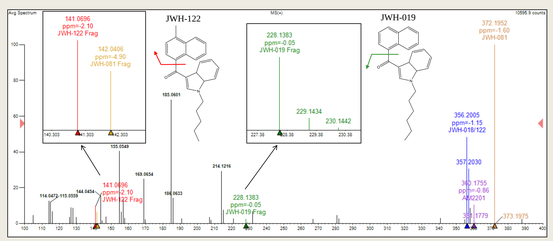


Figure 2. Example analysis of synthetic cannabinoid mixture containing an isobaric pair: JWH-019 and JWH-122 which were distinguished by unique fragment ions. Identified ions are highlighted in color.

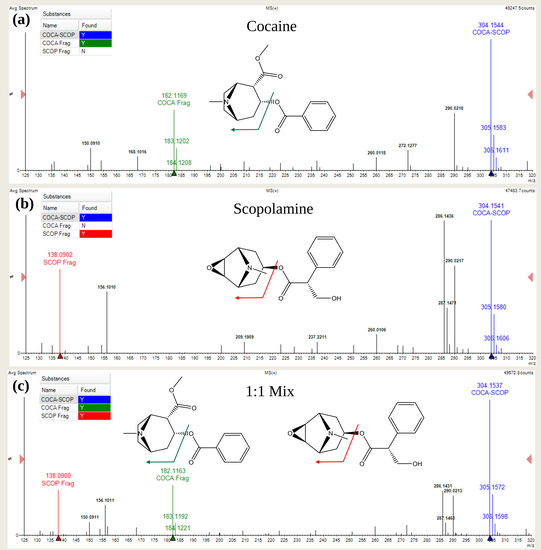


Figure 3. Analysis of the isobaric pair cocaine and scopolamine. (a) Cocaine only. (b) Scopolamine only. (c) 1:1 cocaine and scopolamine mix. Identified ions are highlighted in color.

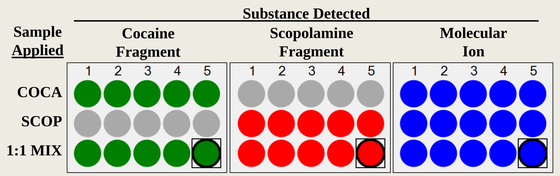


Figure 4. AxION Solo analysis of cocaine and scopolamine. Numbers 1-5 are replicate samples. Colored circles indicate positive identification of a fragment or molecular ion. Top row, cocaine only samples. Middle row, scopolamine only samples. Bottom row, 1:1 mixture of cocaine and scopolamine samples.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

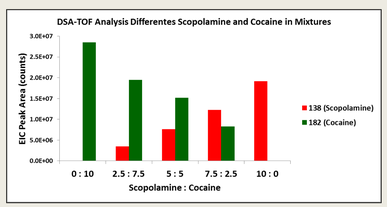


Figure 5. Analysis of cocaine and scopolamine mixed in varied proportions. Cocaine and scopolamine were mixed in the ratios indicated on the x-axis. Extracted ion chromatograms for cocaine (green) and scopolamine (red) were generated and peak areas were quantitated (y-axis). These results show that the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

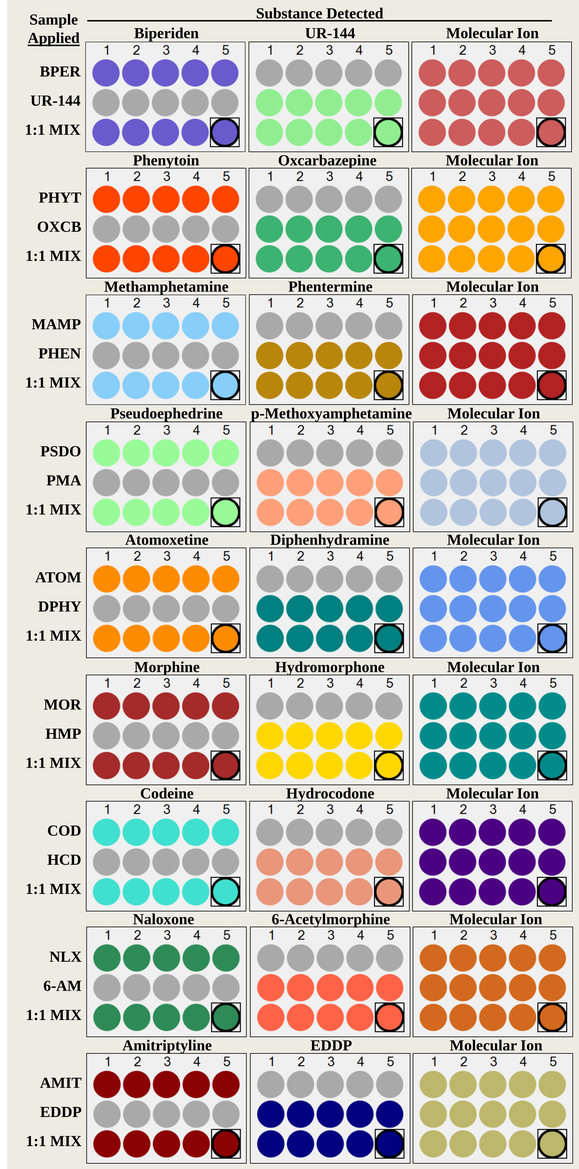
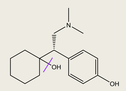
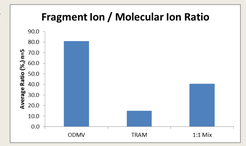


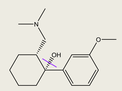
Figure 6. Summary analysis of 9 isobaric pairs . Cutoff (%) of strong signal was set to 1% for all analyte pairs except for the following: phenytoin / oxcarbazepine (2%), methamphetamine / phentermine (2%), and Naloxone / 6-Acetylmorphine (4%).

O-Desmethylvenlafaxine





Tramadol



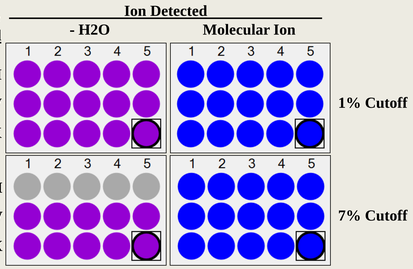


Figure 7. Analysis of the isobaric pair O-Desmethylvenlafaxine and Tramadol which did not produce isomer-specific fragment ions. Top panel, the ratio of the water loss ion to the molecular ion is greater with ODMV than for TRAM. This ratio is affected when a mixture of analytes is present. Bottom panels,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

PerkinElmer, Inc., 940 Winter Street, Waltham, MA USA (800) 762-4000 or (+1) 203 925-4602
www.perkinelmer.com