Comparison of Forensic Tandem Mass Spectral Data Obtained on Portable Instrumentation to an Established Reference Library

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INTRODUCTION

The variety of chemical evidence present at crime scenes is often quite extensive. While several analytical techniques have proven to be advantageous in combating the wide-ranging complexity of criminal evidence, limitations in sample throughput have caused forensic laboratories to become plagued by significant backlogs. The implementation of portable mass spectrometric instrumentation would permit on-site evidentiary analysis, expediting criminal investigations and reducing the burden on off-site laboratories, but any platform employed would need to be robust toward potential non-technical use. To this end, implementing a standard reference library for chemical identification is appealing. In this work, tandem mass spectral data collected on forensically-relevant analytes is compared to that of a commercially-available, spectral reference library.

EXPERIMENTAL

For these studies, a Griffin AI-MS 1.2 cylindrical ion trap (CIT) mass spectrometer (FLIR Systems, West Lafayette, IN, USA) was utilized. All AC/DC voltages needed for instrument operation and ESI/DESI ionization are built in, as well as an on-board syringe pump for DESI and ESI solvent delivery and a cartridge-based helium supply for the CID damping gas. DESI nebulizing gas (N_2) and an external PSI source are not incorporated. A spray solvent of 1:1 methanol:water with 0.1% formic acid was utilized for all DESI-MS and PSI-MS studies, and the following parameters were used for DESI: solvent flow rate of 3 μL/min, spray voltage of 4 kV, and nebulizing gas pressure (N_2) of 100 psi.

SAMPLE PREPARATION

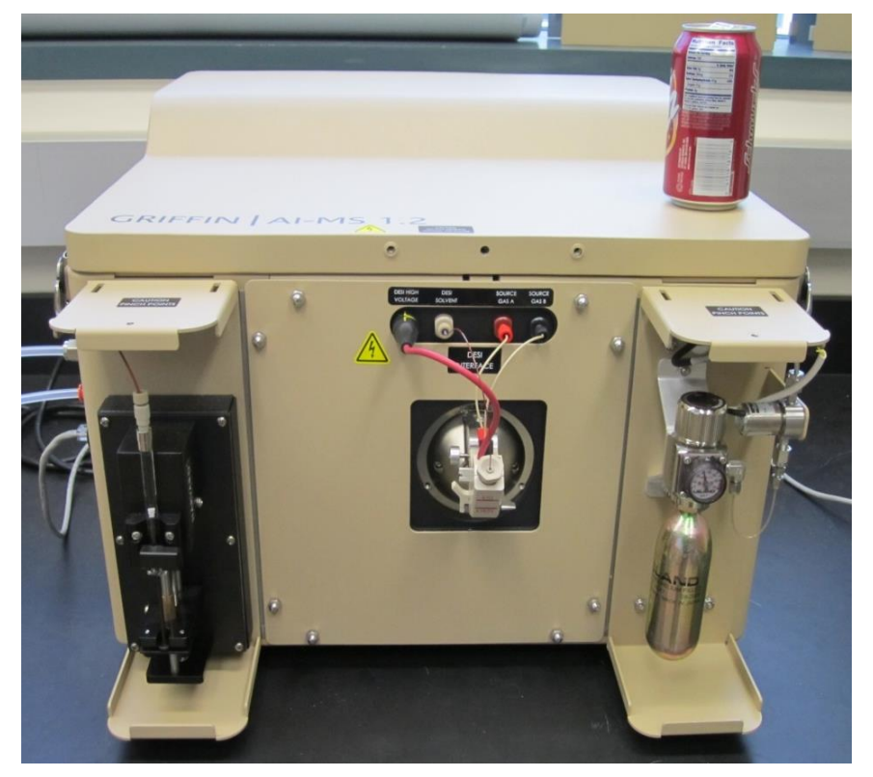
Liquid-Based Samples

- Analytical standards of target analytes were purchased from Cerilliant Corp. (Round Rock, TX, USA), and stock solutions of the target analytes were prepared *via* serial dilution in methanol.
- These known composition solutions were then analyzed as-is via ESI-MS or spotted as 1 μL aliquots onto porous Teflon well slides (Prosolia, Inc., Indianapolis IN, USA), dried, and subsequently analyzed via DESI-MS.

Powder-Based Samples

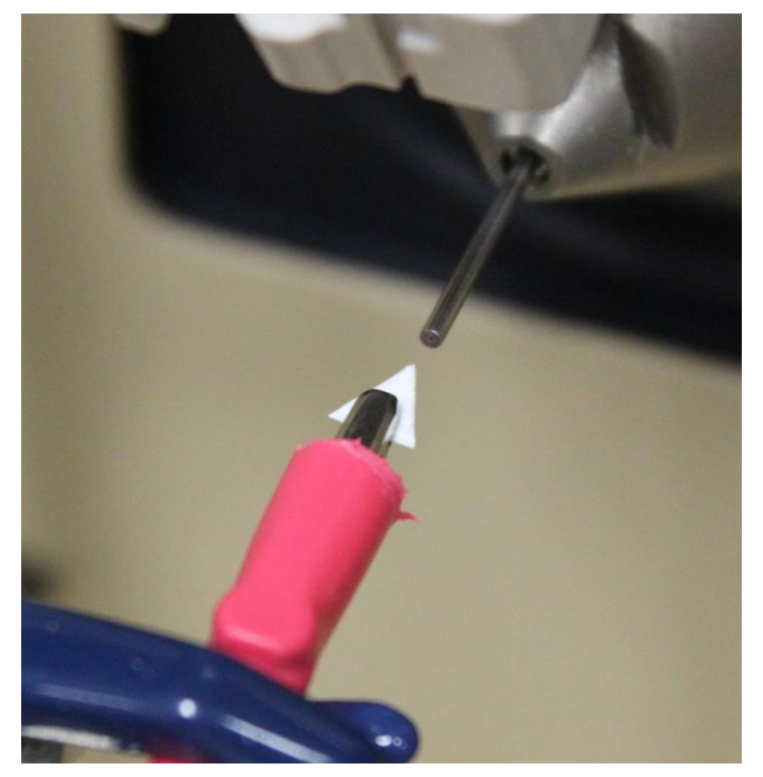
- **For DESI-MS**, a small amount of the powder (~1 mg) was deposited onto Scotch® double-sided tape adhered to a glass microscope slide using a spatula.
  - The deposited powder was then pressed into the adhesive, and a low flow of compressed air was used to remove excess loose powder.
- **For PSI-MS**, ~1 mg of powder was sampled via spatula from the bulk evidence and dissolved in a 1 mL aliquot of methanol.
  - The resulting solutions were spotted as 2 μL aliquots onto MQuant paper-based testing strips (EMD Millipore Corp., Billerica, MA, USA) and allowed to dry.

PORTABLE MASS SPECTROMETER



Griffin AI-MS 1.2

DESI/ESI Source



PSI Source

- Cylindrical ion trap (CIT)
- Size: 24" x 20" x 15", L x W x H
- Weight: ~98 lbs

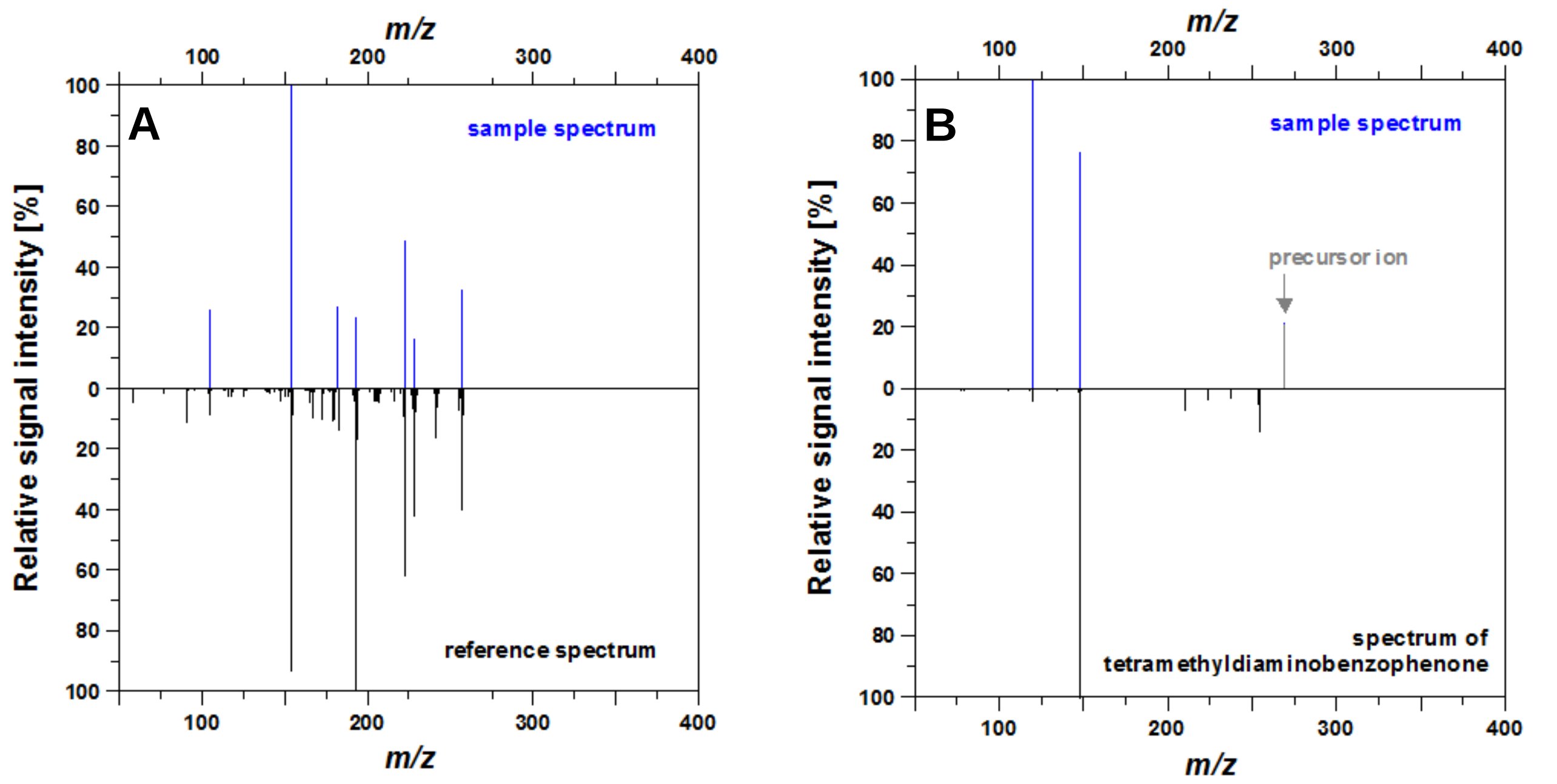
REFERENCE LIBRARY

- The Wiley Registry of Tandem Mass Spectral Data, "MSforID" was used as the reference library.
- The library was developed on a QqTOF instrument using electrospray ionization in positive and negative ion mode.
- For each reference compound, product-ion spectra were typically acquired at ten different collision energy values.
- At the current stage of development the library contains 12,122 spectra of 1,208 compounds.

INTER-LIBRARY SEARCHING

- Library searching focused on the m/z of the precursor ion as well as the m/z of the fragment ions and the corresponding relative signal intensities.
- Each collected spectrum was compared with that stored in the reference library, and the spectral similarity was determined.
- The spectral information retrieved was used to calculate a 'reference spectrum-specific match probability' (mp).
- All reference compound-specific mp-values were combined and normalized to yield the compound-specific 'relative average match probability' (ramp).
- A high compound-specific ramp-value indicated high similarity between the unknown and the reference compound.

COMPARISON OF SPECTRAL DATA

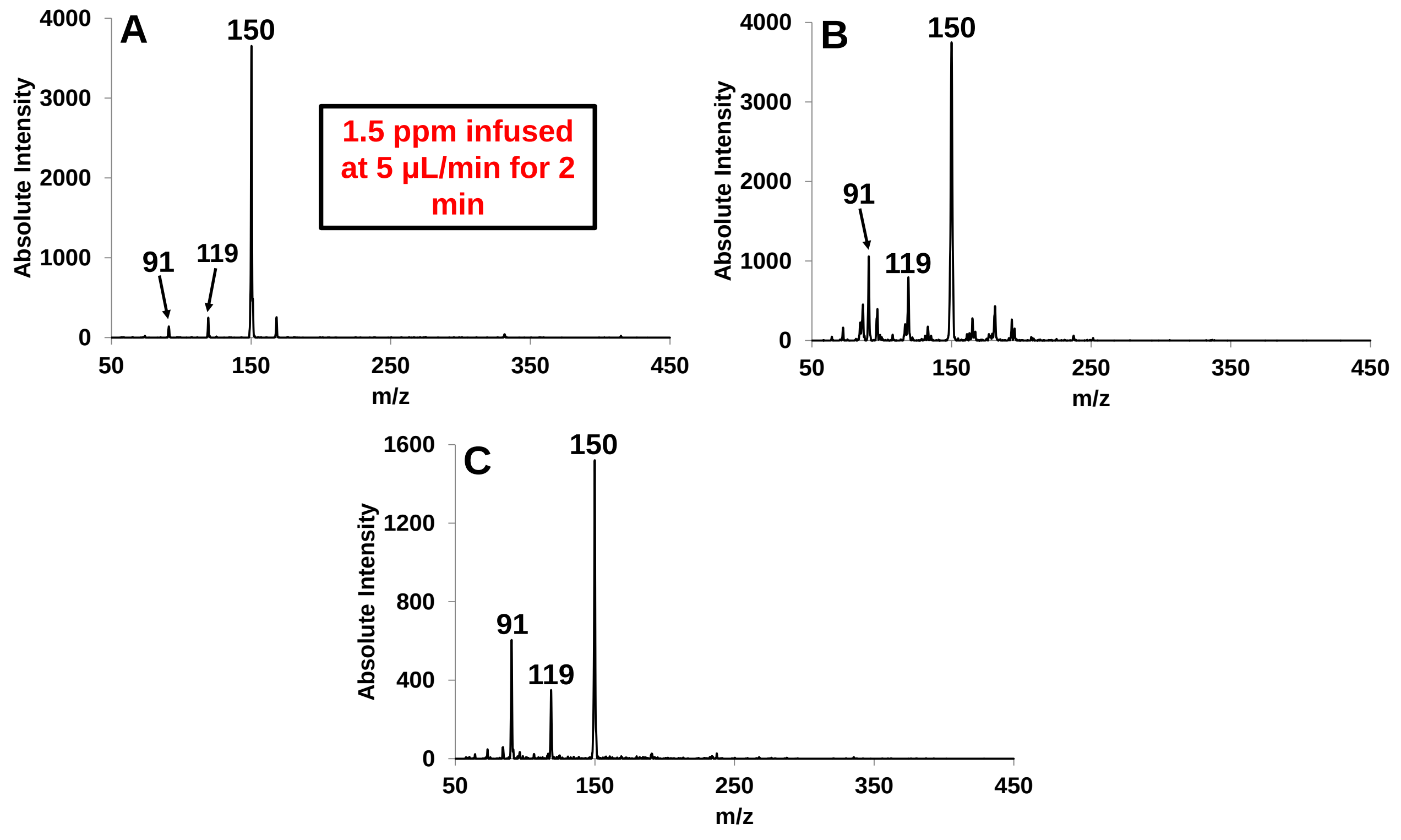


(A) a positive control sample (Diazepam) and (B) a false-positively identified negative control sample (Ethyl centralite).

LIBRARY SEARCH RESULTS

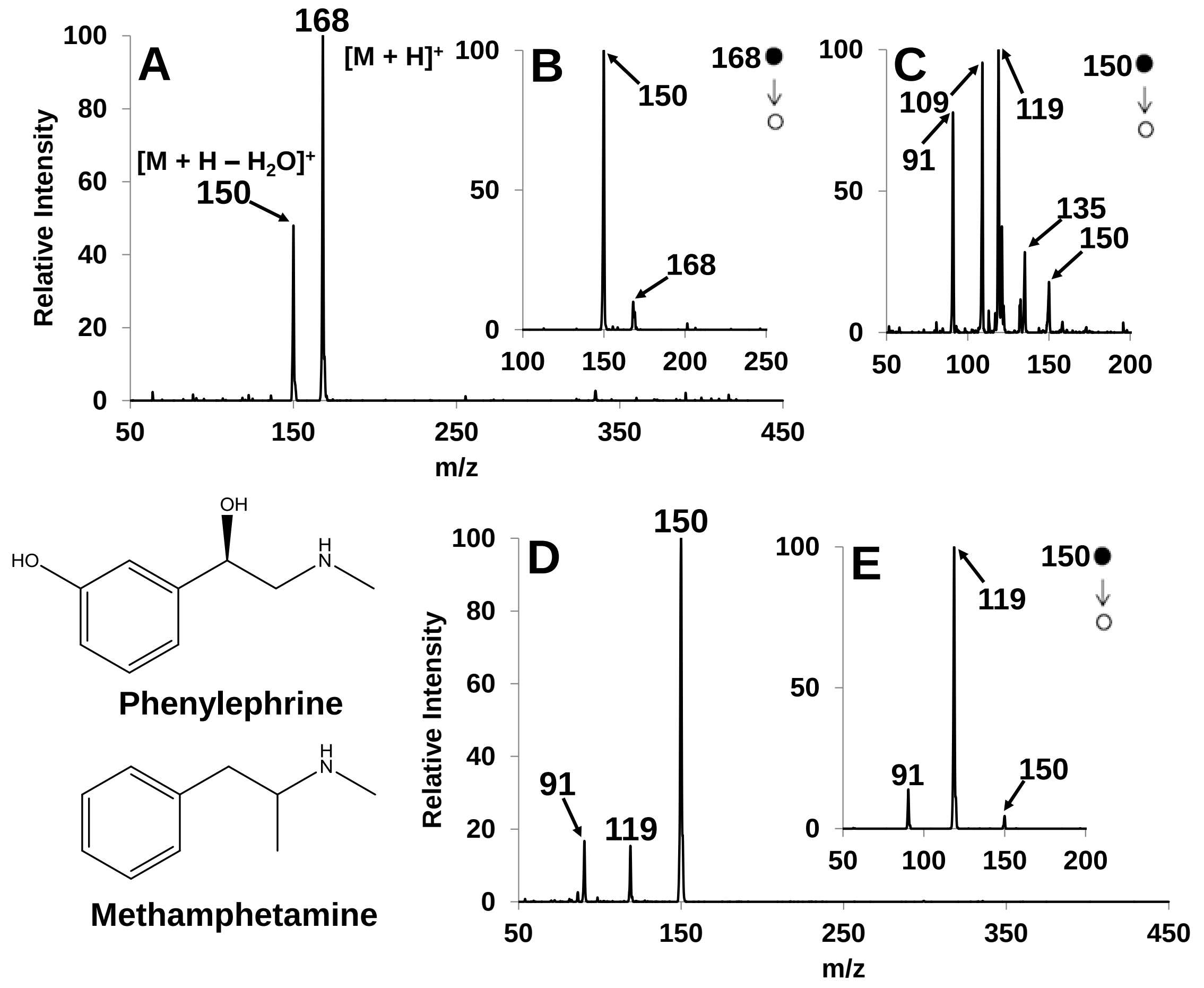
True Identity	Match	*ramp*
Positive Control Samples		
Cocaine	Cocaine	96.4, 96.3, 96.3
Methamphetamine	Methamphetamine	71.2, 73.3, 74.4
Fentanyl	Fentanyl	93.7, 93.7, 93.7
Lidocaine	Lidocaine	85.3, 85.3, 85.3
Benzocaine	Benzocaine	79.2, 89.3, 84.6
Cocaethylene	Cocaethylene	93.7, 92.9, 93.4
Codeine	Codeine	33.9, 43.4, 43.5
Dextromethorphan	Dextromethorphan	93.2, 92.8, 92.3
Diazepam	Diazepam	87.8, 89.0
Diphenylamine	Diphenylamine	48.6, 48.5
Hydrocodone	Hydrocodone	90.3, 89.1
Hydromorphone	Hydromorphone	53.2, 72.1, 71.7
Ketamine	Ketamine	96.0, 95.9
Mescaline	Mescaline	78.8, 78.8, 78.8
Methadone	Methadone	95.3, 95.3
Methylphenidate	Methylphenidate	85.0, 91.4, 85.0
Morphine	Morphine	65.7, 61.4, 62.4
Oxycodone	Oxycodone	94.7, 94.7, 94.7
Phenacetin	Phenacetin	53.1, 55.5, 55.3
Ephedrine	Ephedrine	87.9, 87.9, 87.9
Zolpidem	Zolpidem	97.2, 96.1, 96.1
Phencyclidine	Phencyclidine	49.1, 47.1, 47.9
Hydroxyzine	Hydroxyzine	90.3, 90.3, 89.4
Phenylephrine	Phenylephrine	67.2, 67.2, 67.2
Benzocaine	Benzocaine	79.2, 89.3, 84.6
Negative Control Samples		
Lysergic acid diethylamide	no match	N/A
Methyl centralite	no match	N/A
Ethyl centralite	Tetramethyldiaminobenzophenone	72.5, 80.5, 80.1
Dimethylphthalate	Temozolomide	49.0, 49.0, 49.0
Authentic Forensic Evidence Samples		
Cocaine	Cocaine	67.0
Methamphetamine	Methamphetamine	75.2
Pentedrone (NEG)	no match	N/A

SPECTRAL INTENSITY COMPARISON BETWEEN IONIZATION SOURCES



(A) ESI, (B) PSI, and (C) DESI mass spectra of 15 ng of methamphetamine.

POTENTIAL FALSE POSITIVES



(A) ESI-MS spectrum of phenylephrine, with the protonated molecule present at m/z 168, as well as an in-source fragment at m/z 150. (B) ESI-MS/MS of protonated phenylephrine. (C) ESI-MS/MS of the m/z 150 in-source fragment. Several products are present, with m/z 119 and m/z 91 being similar to MS/MS analysis of methamphetamine, potentially resulting in a false positive. (D) ESI-MS of methamphetamine, showing the protonated molecule at m/z 150 and characteristic in-source fragments at m/z 119 and 91. (E) ESI-MS/MS of protonated methamphetamine.

CONCLUSIONS

- All 28 positive control samples were correctly identified with the MSforID search algorithm.
- Of the 5 negative control samples, only three were misidentified and later rebutted by comparison of the mass spectra.
- Mass spectra obtained from authentic forensic evidence were successfully matched and correctly identified using the MSforID search algorithm.
- The results generated from the comparison of mass spectral data collected on the Griffin AI-MS 1.2 to the Wiley Registry of Tandem Mass Spectral Database support its implementation as a method of on-site forensic analysis and evidentiary identification.

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