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Forensic and toxicology screening in human blood using a QTOF mass spectrometer and DIA

Evelyn H. Wang[1]; Sarah R. Olive[1]; Neil J Loftus[2]; Jennifer Davis[1]; Rachel Lieberman[1]; Priyanka Chitranshi[1]; Samantha Olendorff[1]; Liz Barnabe[1]; Christopher T Gilles[1]

[1]Shimadzu Scientific Instruments, Columbia, MD; [2]Shimadzu MS/BU, Manchester, United Kingdom

Overview

- Applying a high-resolution mass spectrometer LC-MS (QTOF) to forensic toxicology screening.
- Compounds were identified using accurate mass, isotopic distribution and retention time data from the TOF survey scan data and accurate mass fragment spectrum using data independent acquisition (DIA-MS/MS).
- In house libraries and external data repositories were used for compound identification.

1. Introduction

High resolution mass spectrometry (HRMS) is being increasingly applied to analytical toxicology, particularly in clinical and forensic toxicology. The capability to deliver accurate mass data and product ion spectral matching for compound identification has resulted in HRMS LC-MS being used for a considerable range of applications including targeted or non-targeted (also named untargeted) screening, quantification, forensic chemistry, doping control. In this paper a validated routine LC-MS/MS triple quadrupole method using in routine screening was transferred to a LC-MS/MS QTOF platform with a highly curated forensic toxicology library developed in house for compound identification.

2. Methods

- **Sample Preparation**. Blood samples were spiked with a panel of drugs of abuse (benzoylecgonine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, methamphetamine, morphine, and temazepam) ranging from 5-5000 ng/mL and extracted by solid phase extraction before analysis.
- **LC Separation**.
  - Shim-pack Velox column (2.1 x 100 mm; 2.7um)
  - Flow rate 0.3 mL/min
  - Binary gradient; water and methanol with 2 mM ammonium formate and 0.002% formic acid
  - Cycle time 17 minutes
- **Mass Spectrometry Detection**. QTOF LCMS-9030 using external mass calibration for positive and negative mode ESI;
  - MS mass scan m/z 40-900; 100 msecs
  - DIA-MS/MS mass scans m/z 40-500; 40 msecs; isolation width 25 Da; collision energy spread 5-55V; 17 mass scan events
  - DIA-MS/MS mass scans m/z 40-900; 25 msecs; isolation width 35 Da; collision energy spread 5-55V; 12 mass scan events
  - Cycle time 1.08 second (30 mass scan in total)
- **Data processing**. The targeted method included 900 compounds (all compounds were registered in the Shimadzu Forensic Toxicology DataBase; a curated MS/MS library developed using authentic standards).

3. Results

For routine clinical and forensic toxicology screening a high-resolution LC-MS/MS method was developed to target a panel of ~1000 compounds to report compounds with high confidence. The criteria for reporting compounds included accurate mass error (nominally within ± 5 ppm), isotopic pattern matching score, retention time (if available) and product ion spectral library verification using in house developed MS/MS libraries or external MS/MS data repositories.

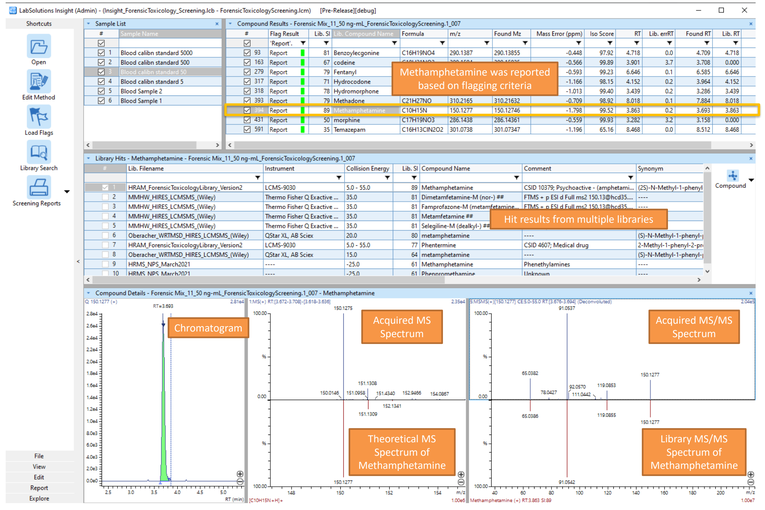


Figure 1 LabSolutions Insight data review application shows the results of the sample analysis, 9 drugs of abuse compounds have been positively identified (within the criteria for reporting a compound with high confidence) highlighting the detection of methamphetamine in a blood extract.

Compound identification for all targets was confirmed by using accurate mass MS/MS libraries including

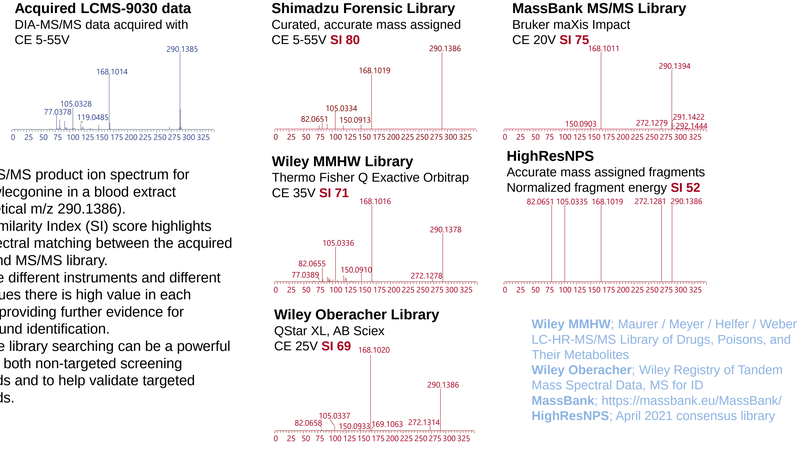
- Shimadzu Forensic Toxicology Data base (over 1000 compounds, MS/MS data acquired using authentic standards with a fragmentation energy of 5-55V, all product ion spectra corrected to theoretical accurate mass values using Insight Assign application, MS/MS spectra acquired using targeted MS/MS and a precursor ion isolation width of 1 Da, retention time mapped to a Shim-pack Velox Biphenyl LC separation).
- Wiley (Wiley Registry of Tandem Mass Spectral Data, MS for ID and LC-HR-MS/MS Library of Drugs, Poisons and Their Metabolites) and HighResNPS (an open-source library; https://highresnps.forensic.ku.dk/ with normalized MS/MS fragment energies).

The results show the library matching score (similarity score, SI) using several MS/MS data repositories. The dot product score was set to mass accuracy weighting 0.5, intensity weighting 0.75, with a minimum of 3 centroids to match. The forward search was used. In this example. methamphetamine was identified using a deconvoluted MS/MS spectra and matched with Shimadzu Toxicology DataBase, Wiley libraries (MMHW: LC-HR-MS/MS Library of Drugs, Poisons and Their Metabolites and Oberacher: MS for ID) and HighResNPS library.

3.1 Library verification in forensic toxicology

Clinical and forensic toxicology analysis is highly challenging; the nature of the biological sample is complex, a drug landscape which is changing with more than 800 novel psychoactive substances emerging over the past 10 years, and a diverse chemical space to search for over 10,000 potential poisons. Analytical strategies may need to adapt to the need dependent on whether it is a targeted screen to help clinical management of intoxication or a non-targeted approach to screen for a large compound panels. With such a need high resolution LC-MS/MS libraries have become indispensable tools for systematic toxicological analysis.

- **Working with multiple data repositories**. Figure 2 highlights the high value in using LC-MS/MS product ion spectral matching from multiple libraries despite data acquired on different LC-MS/MS platforms. Although the spectral matching value may differ between libraries it provides further confidence in compound identification.



- DIA-MS/MS product ion spectrum for benzoylecgonine in a blood extract (theoretical m/z 290.1386).
- The Similarity Index (SI) score highlights the spectral matching between the acquired data and MS/MS library.
- Despite different instruments and different CE values there is high value in each library providing further evidence for compound identification.
- Multiple library searching can be a powerful tool for both non-targeted screening methods and to help validate targeted methods.

Figure 1 Library verification and compound for benzoylecgonine detected in the spiked blood sample B using multiple libraries (100 ng/mL spike level). Although the data repositories use different LC-MS/MS instruments and different fragment ion intensities (CE spread 5-55V, fixed CE and normalized fragment ion intensity) each MS/MS library can provide positive evidence for compound identification.

4. Conclusions

- To meet the challenges in analytical toxicology, a workflow has been developed using TOF survey scan and DIA-MS/MS mass scans. To increase the probability of a positive compound identification a highly curated in-house forensic toxicology library has been developed using authentic standards.
- Library searching capability has been enhanced to work with multiple MS/MS data repositories to further increase compound identification and to enable a wider compound search particularly for NPS compounds.
- The method can be applied to targeted and non-targeted workflows.

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