Application of a novel screening workflow for the detection of illicit and medicinal drugs in human hair

Emily G Armitage[1]; Thomas Brema[2]; Christopher Bowen[3]; Alan Barnes[1]; Rohan Steel[4]; Neil J Loftus[1]

[1]Shimadzu Corporation, Manchester, United Kingdom; [2]Shimadzu Deutschland GmbH, Duisburg, Germany; [3]Shimadzu Scientific Instruments, Melbourne, Australia; [4]Biological Research Unit, Racing Analytical Services Limited, Flemington, Australia

Overview

- A second-generation component detection algorithm integrated into Insight Discovery software[1] was applied to the detection of illicit and medicinal drugs in human hair using a high resolution QTOF LC-MS/MS platform.
- The non-targeted screening (NTS) application identified single and polydrug use with high reporting confidence following high resolution MS/MS library matching.

1. Introduction

Hair analysis is involved in several applications in forensic toxicology, clinical toxicology, occupational medicine, and doping control given its longer detection window enabling drugs to be detected weeks or even months after consumption, depending on the length of the hair. The detection window for hair analysis provides a long-term drug history as opposed to blood or urine analysis which provides short term information related to drug use.

In this study, hair extracts have been analysed using an untargeted high-resolution LC-DIA-MS/MS method with a novel screening software application for the identification of illicit and medicinal drugs.

2. Materials and Methods

Hair extracts were analysed using an untargeted high resolution accurate mass method.

- **Reverse phase LC Separation.**
  - Shim-pack Velox™ Biphenyl (2.1x100mm 2.7µm); 40ºC, flow rate 0.3 mL/min
  - Binary gradient; water + 2mM ammonium formate + 0.002% formic acid, and methanol + 2mM ammonium formate + 0.002% formic acid
  - Cycle time 17 minutes.
- **LC-MS/MS Mass Spectrometry Detection**. High resolution QTOF LC-MS/MS (LCMS-9030, Shimadzu Corporation, Japan) was applied to hair samples to screen for drugs of abuse, medications and dietary drugs using a non-targeted screening (NTS) workflow.
  - TOF MS mass scan m/z 100-1000; 100 msecs
  - 17 DIA MS/MS scans (m/z 40-500) with a precursor isolation width of 25 Da for precursors with m/z<500, scan time 40 msecs; 12 DIA MS/MS scans (m/z 40-900) with a precursor isolation width of 35 Da for precursors with m/z>500, scan time 25 msecs; collision energy spread 5-55V. Scan cycle time 1.095 seconds (30 mass scans in total).
  - ESI positive ion mode data was acquired using external mass calibration.
- **Data processing**. Insight Discovery as a research application was used in batch data processing (LabSolutions Insight™, Shimadzu Corporation). Insight Discovery automates untargeted component detection using a second-generation peak detection algorithm, formula prediction, suspect screening workflows and library matching. The high-resolution MS/MS library repositories include:
  - Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology (1245 compounds registered from authentic standards including retention time)
  - HighResNPS library (an open-source repository for new psychoactive compounds) https://highresnps.com/
  - Wiley Registry of Tandem Mass Spectral Data: MS for ID; 12048 entries
  - Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites; 5006 entries
  - .msp spectral libraries https://systemsomicslab.github.io/compms/msdial/main.html#MSP

3. Results

3.1 Insight Discovery Workflow

There are several processing workflows that are supported by Insight Discovery, the core engine driving untargeted screening is the Analyze component detection algorithm. Analyze is a precursor component detection algorithm that first creates a series of overlapping spectral bins; the same ion signals which are found in neighboring spectral data windows and behave as a chromatographic peak are registered as a component. To negate the impact of false positive or idiosyncratic ion behavior, quality factors such as jaggedness, peak shape and intensity thresholding are applied.

1 Untargeted **Component Detection**

- **Precursor peak detection to generate a candidate list of components.** To simplify peak detection in an untargeted screening workflow, a user defined threshold for a response setting can be saved in the settings file ('low' is used for trace level analysis).
- All other parameters are set to default.
- The algorithm will detect missing data points in a peak and reject the component. It also applies quality factors such as isomer detection, jaggedness and peak shape quality.
- The candidate component list includes all ion signals that behave as a chromatographic peak. In step 2 a search list is applied to find all matched precursor ions and in step 3 MS/MS provides further reporting confidence using a dot-product score on each detected target.

Batch driven processing
Storing peak detection and search parameters in a single processing file for routine processing and reporting

- **Stored user defined parameters for routine screening.** Once the parameters are configured (component detection thresholds, screening search list, library search options and formula prediction), the user simply clicks on the **'Calculate'** icon to run untargeted data processing on a batch file.
- **Batch reporting**. The batch process can also include a printed report based on 4 templates designed specifically for screening.

2 Precursor **Screening**

- **Screening list used to match targets with the candidate list of components.** The screening list includes the compound name, m/z, formula, ion and Rt. The search list is fully editable and can be scaled to meet the needs of the toxicology screen.
- In this work, the screening list included all compounds registered in the Shimadzu Forensic Toxicology Library Database (over 1280 compounds).

3 MS/MS **Library Searching**

- **Library searching applied to the list of targets detected for compound identification.** Multiple MS/MS libraries can be used in identifying the detected components. In this processing settings file, 4 libraries have been selected as part of the search space.
  - Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology.
  - HighResNPS library.
  - Wiley Registry of Tandem Mass Spectral Data: MS for ID.
  - Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites.
  - Every MS/MS spectrum in the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology has been highly curated. Each fragment ion is theoretically mass assigned with a collision energy spread of 5-55 V and includes retention time data generated on a Shim-pack Velox™ Biphenyl column.

4 Support for **Formula Prediction**

- **Formula prediction can also be applied to provide further evidence for compound identification.** For each detected precursor, formula prediction software can be used to confirm identified components or to propose formulae for unknown components that are not present in either the screening list or spectral libraries.

Figure 1. Insight Discovery workflow for batch driven untargeted screening.

3.2 Insight Discovery Applied to Hair Analysis

Insight Discovery was applied to a batch of hair samples for forensic screening analysis following data acquisition using a high resolution TOF MS scan event and DIA-MS/MS mass scans. The example shown below highlights the data processing cascade.

1 Untargeted **Component Detection**

- **User defined threshold set to low for trace analysis screening.**
- 3384 components detected (ion signals that behaved as a recognizable chromatographic peak)

2 Precursor **Screening**

- **Forensic toxicology screening list applied with over 1200 compounds.**
- Precursor mass tolerance and retention time window matched 13 targets.

3 MS/MS **Library Searching**

- **Library identification**
- 11 targets confirmed with high dot product scores.

4 Support for **Formula Prediction**

- **Formula prediction was applied to library verified targets**
- Experimental target formula in agreement with theoretical precursor ion distribution for 11 compounds.

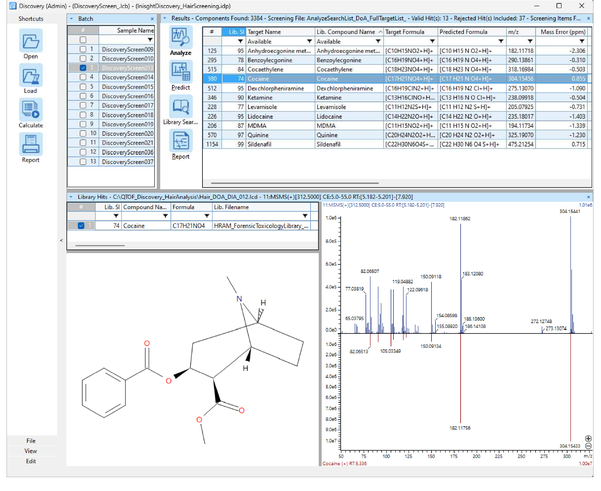

Figure 2. Insight Discovery was applied to the analysis of a batch of hair samples following data acquisition using a TOF MS mass scan and DIA-MS/MS mass scans with a collision energy spread of 5-55 V. In this sample, 11 target drugs were detected including benzoylecgonine, cocaethylene, cocaine, levamisole, MDMA, sildenafil and quinine.

Quinine has been reported as an adulterant in the illicit drug supply, including heroin, fentanyl, methamphetamine and cocaine, for many decades. It is generally added to increase total drug volume for distribution and to dilute the drug being cut.

3.3 Insight Discovery Batch Analysis

In a batch of 24 samples, four tested positive for cocaine and its primary metabolite benzoylecgonine. Caffeine, nicotine and DEET were also widely detected across samples. Codeine was detected at low levels in two samples.

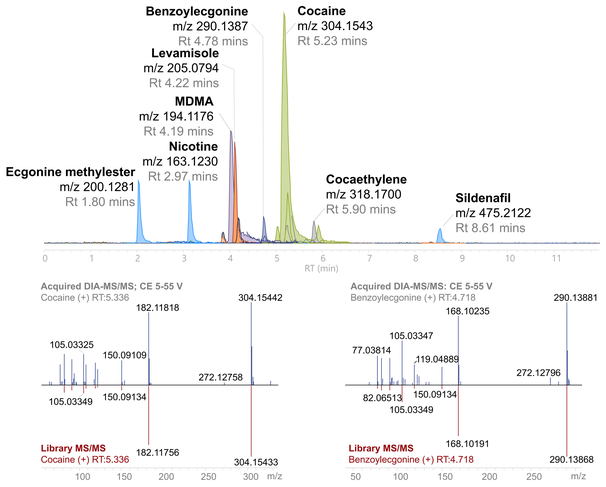

Figure 3. The most common drugs of abuse reported in this set of hair samples included benzoylecgonine, cocaethylene, cocaine, ecgonine methyl ester, levamisole, MDMA, nicotine and sildenafil (upper figure highlights overlapping mass chromatograms in a series of hair samples). Despite the complexity of polydrug use in some samples, the methodology resulted in library searchable DIA-MS/MS spectra (lower figure shows the acquired DIA-MS/MS mass spectra for cocaine and benzoylecgonine together with the curated MS/MS library spectra).

4. Conclusions

- Insight Discovery automated a forensic toxicology screening workflow for the batch analysis of hair samples with library identification.
- All target drugs were detected with high reporting confidence and verified following a manual workflow using authentic reference standards.
- The work provides evidence of both single and poly drug use.

[1]Insight Discovery is a research application within LabSolutions Insight™.