Screening for unknowns in assessing emerging threats and new developments in the use of illicit drugs

Emily G Armitage[1]; Christopher Bowen[2]; Alan Barnes[1]; Chloe Hutton[1]; Simon Ashton[1]; Neil J Loftus[1]; John Fitzgerald[3]

[1]Shimadzu Corporation, Manchester, UK; [2]Shimadzu Scientific Instruments, Melbourne, Australia; [3]School of Social and Political Sciences, The University of Melbourne, Victoria, Australia

Overview

- Application of an innovative screening software tool to rapidly identify illicit drugs in discarded drug paraphernalia analyzed by high-resolution LC-DIA-MS/MS.
- The analysis of 32 user submitted syringes positively identified 6-MAM, acetylcodeine, cocaine, codeine, heroin, methamphetamine, morphine and noscapine reflecting poly-drug use using a primary list of targeted drugs verified by authentic standards.
- As part of the non-targeted screening (NTS) workflow, testosterone propionate was positively identified in a subset of the samples.

1. Introduction

Changes to the illicit drug market are typically revealed through monitoring population level trends associated with known drug substances via wastewater analysis and conducting surveys of people who use drugs. This can lead to a lack of empirical data relating to dynamic alterations in drug availability, purity, adulteration, poly-drug use, and the introduction of novel drug substances at the “street-level”, that are directly proximal to the point of consumption. Here we present a pilot study which has led to the implementation of the RAPID early warning system at The University of Melbourne. In this work, samples from discarded drug paraphernalia were analyzed using a non-targeted high-resolution LC-DIA-MS/MS method with an innovative screening software application for the identification of illicit drugs and the potential detection of novel substances.

2. Materials and Methods

In a pilot study, the drug contents from 32 syringes collected from a Melbourne community syringe disposal bin were analyzed by high resolution LC-MS/MS (LCMS-9050, Shimadzu Corporation). The method was previously optimized for targeted LC-MS/MS analysis and non-targeted toxicology screening to detect and identify a range of compounds including illicit drugs, adulterants, unregulated supplements, and prescription medications. Reserpine was added to all samples to help monitor assess the quality of analysis.

Reversed 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.
- Sample cycle time 17 minutes.

LC-MS/MS Mass Spectrometry Detection.

- Positive ion mode TOF MS survey scan (m/z 80-1000; 100 msecs)
- 17 DIA-MS/MS mass scans (precursor isolation 25 Da m/z <500)
- 12 DIA MS/MS scans (precursor 35 Da m/z >500)
- Collision energy spread 5-55V; External mass calibration. Total cycle time <1 sec.

Data Processing.

- LabSolutions Insight Discovery software was used to screen for suspected illicit drug content.
- Identifications were putatively confirmed by library verification considering data repositories including the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology, HighResNPS, NIST, MassBank and HMDB.

3. Results

3.1 Insight Discovery batch analysis for NTS

To enhance unknown screening strategies and workflows LabSolutions Insight Discovery software was applied to increase the effectiveness of peak detection and support compound identification. There are several processing workflows supported by the software. Figure 1 highlights a workflow of component detection, MS/MS library searching and formula prediction to help identify drugs in the samples using the NTS approach.

1 Untargeted **Component Detection**
- **User defined threshold set to medium**
- With this threshold applied, 529 components were detected (ion signals that behaved as recognizable chromatographic peaks)

2 MS/MS **Library Searching**
- **Library identification with mass and RT tolerance set**
- 6 targets confirmed with high dot product library scores

3 Support for **Formula Prediction**
- **Formula prediction applied to library verified targets**
- Experimental target formula in agreement with theoretical precursor ion distribution for 6 compounds

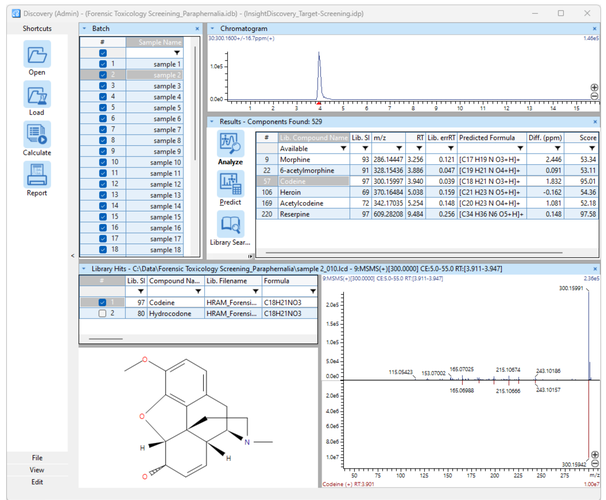

Figure 1. Insight Discovery was applied to the analysis of a batch of discarded drug paraphernalia samples following data acquisition using a TOF MS mass scan and DIA-MS/MS mass scans with a collision energy spread of 5-55 V. In this sample, 5 drugs were detected including morphine, 6-acetylmorphine codeine, heroin and acetylcodeine; reserpine was also detected as it was added to each sample as a marker to assess system performance. The data analysis workflow consisted of 3 steps including untargeted component detection, MS/MS library searching within a RT range of +/- 0.3 min and formula prediction.

3.2 NTS workflow reveals poly-drug use

The most common drugs of abuse reported in this set of discarded drug paraphernalia samples included 6-MAM, acetylcodeine, cocaine, codeine, heroin, methamphetamine, morphine and noscapine. Figure 2 shows the identification of these compounds using the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology with over 2000 MS/MS spectra registered from authentic standards (including retention time). With the exception of cocaine, these compounds were positively identified in a single sample, reflecting poly-drug use.

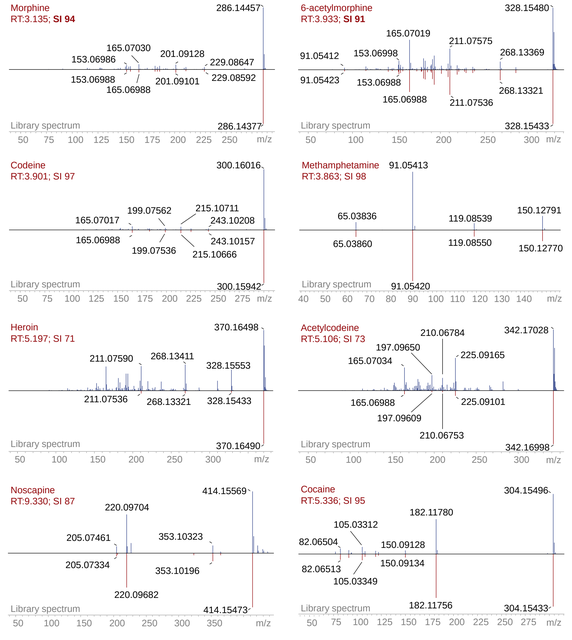

Figure 2. Positive identification of 6-MAM, acetylcodeine, codeine, heroin, methamphetamine, morphine and noscapine from a single sample and cocaine from another sample following the workflow described in Figure 1. Despite the complexity of poly-drug use, the methodology resulted in library searchable DIA-MS/MS spectra. Acquired DIA-MS/MS spectra are reflected against the curated MS/MS library spectra from the analysis of authentic standards and the similarity index (SI) is given for each.

3.3 NTS workflow reveals emerging threats

Library verification considered multiple data repositories including the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology, HighResNPS, NIST, MassBank and HMDB. The identifications with highest confidence were achieved using the Shimadzu library due to MS/MS spectra being acquired with the same analytical conditions (collision energy spread and retention time comparable to the measured spectra). However, the workflow is not limited to this library; the Insight Discovery NTS workflow supports library searching against up to five different libraries simultaneously, resulting in up to 25 hits for every component detected in every sample automatically.

As part of the NTS workflow, testosterone propionate was positively identified in a subset of the samples. This compound was identified by comparison to HMDB and NIST and represents a potential emerging threat in this sample set. Figure 3 shows the library hits pane from Insight Discovery highlighting the identification of testosterone propionate in one of the discarded drug paraphernalia samples in which the compound was detected.

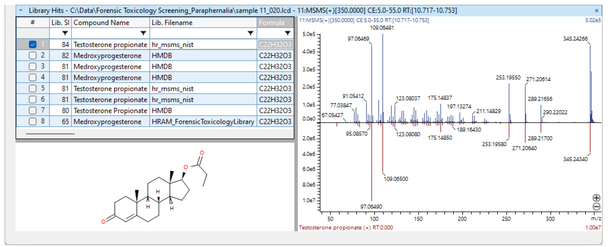

Figure 3. Insight Discovery was applied to the analysis of a batch of discarded drug paraphernalia samples following data acquisition using a TOF MS mass scan and DIA-MS/MS mass scans with a collision energy spread of 5-55 V. A component identified as testosterone propionate was identified in several samples through library matching to external repositories NIST and HMDB. This search consisted of 3 steps including untargeted component detection, MS/MS library searching and formula prediction.

4. Conclusions

- Insight Discovery automated a forensic toxicology NTS workflow for the batch analysis of discarded drug paraphernalia samples.
- There are several processing workflows that are supported by Insight Discovery. This study followed a 3-step workflow consisting of untargeted component detection, library identification (MS/MS) and formula prediction.
- The most frequently observed illicit compounds could be identified using the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology with over 2000 MS/MS spectra registered from authentic standards (including retention time). Additional compounds such as testosterone propionate were identified using NIST and HMDB.

Authors would like to acknowledge Mayumi Allinson, School of Social and Political Sciences, The University of Melbourne; James Ziogas, Department of Pharmacology and Biochemistry, The University of Melbourne; Paul Dietze, Burnet Institute, Melbourne; Andrea Fischer ,coHealth, Melbourne and Tom Lyons, Victorian Department of Health, Melbourne for their collaboration in this research. The work was financially supported by the Victorian Department of Health.