Expanding capabilities in routine clinical toxicology screening using HRAM QTOF

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Overview

- High resolution accurate mass (HRAM) spectrometry using QTOF technologies has a number of advantages in general unknown screening (GUS) workflows for both targeted compound quantitation and for untargeted compound identification given the ability to acquire high mass accuracy MS and MS/MS data, use library verification and retrospectively analyze components.
- In this study, a HRAM QTOF MS/MS system was used for targeted and untargeted workflows in a routine clinical pathology laboratory to detect panels of drugs of abuse in patient samples using MS/MS library verification.

1. Introduction

Low resolution mass spectrometry with triple quadrupole MS/MS systems deliver highly sensitive, robust, reproducible and proven technology platforms for targeted clinical toxicology screening and identifying unknown compounds in patient samples. However, high resolution accurate mass-spectrometers (HRAM) provide support for targeted and untargeted workflows in which no spectral data are lost and retrospective data analysis can be supported. To reduce false positive reporting a HRAM data-independent-acquisition MS and MS/MS method was used together with a HRAM MS/MS clinical toxicology library for routine screening assays.

2. Methods

The same sample preparation and LC analytical conditions developed for a validated triple quadrupole MRM analysis MS/MS were used for the untargeted HRAM MS and DIA-MS/MS data acquisition method.

- **Sample Preparation**. Plasma was spiked with target compounds and extracted with a QuEChERS method protocol (where possible deuterated internal standards were also included).
- **LC Separation**. Chromatographic conditions developed for diverse chemical space for clinical toxicology with a cycle time of 17minutes.
  - Nexera LC system (Shimadzu Corporation); flow rate 0.3 mL/min
  - Restek Raptor Biphenyl (2.7um 100 x 2.1mm) column
  - Binary gradient; Water/Methanol 2mM ammonium formate + 0.002% formic acid
- **Mass Spectrometry Detection**. QTOF LCMS-9030 (Shimadzu Corporation)
  - External mass calibration; TOF recalibration between 3-5 days
  - MS mass scan m/z 100-1000; 200 msecs
  - DIA-MS/MS mass scans m/z 40-1000; 20 msecs; isolation width 20 Da; Collision energy spread 5-55V; 45 mass scan events
  - Cycle time 1.1 seconds
- **High Resolution Accurate Mass (HRAM) Clinical Library**.
  - All MS/MS spectra were acquired using authentic reference standards
  - MS/MS spectra acquired with a collision energy spread of 5-55V

3. Results

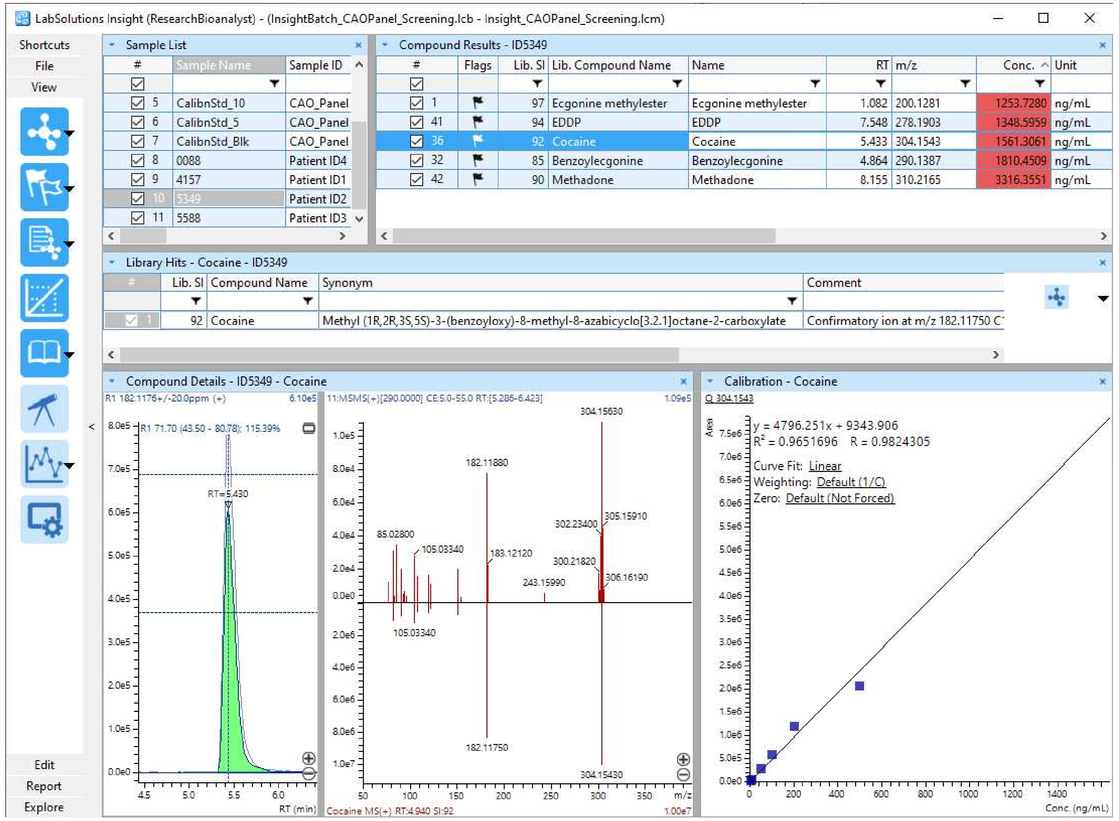

Figure 1 Insight data processing. Ecgonine methylester, EDDP, cocaine, benzoylecgonine and methadone were detected in this clinical sample submitted for toxicological screening.

- **Reducing false negative or false positive reporting**. To reduce or negate false negative or false positive reporting the user interface highlights ion ratio confirmation, library verification, mass accuracy tolerance and quantitative results. In the patient sample shown each reported drug was within a predefined RT, mass error, ion ratio tolerance and above the library similarity score threshold (similarity index score; SI).
- **Review by exception.** Flagging and filtering tools to support streamlined data processing allow for quicker data review with a higher reporting confidence (for example, only view compounds above a concentration threshold for review; check the library score, library MS/MS and ion ratio correspond to the true target; check Rt and mass error are within the expected tolerance)

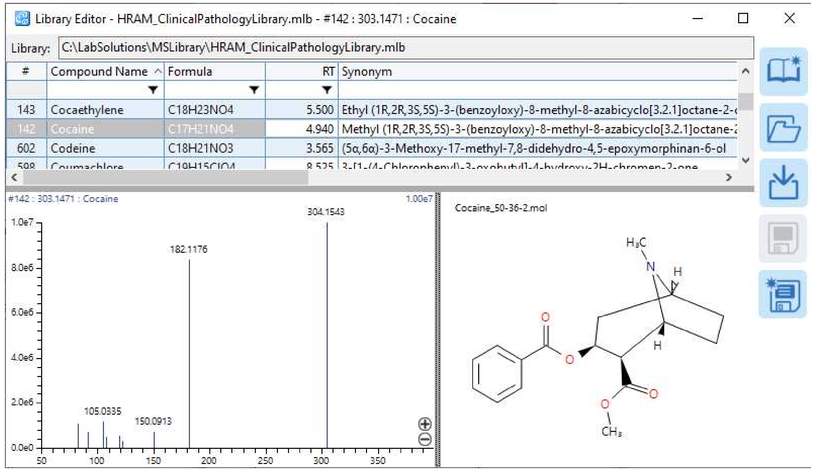

Figure 2 The HRAM MS/MS library editor supports DDA and DIA-MS/MS spectra, import of meta information from external data bases and also import from external MS/MS data repositories. The HRAM Clinical Library has over 650 clinically relevant compounds registered in the research application.

- **Theoretical mass fragment ions**. All fragment ions in the HRAM Clinical Library were corrected to a theoretical mass value using the Assign fragment annotation tool (Shimadzu, LabSolutions Insight software). All fragment ions were structurally verified using Assign.
- **Creating new libraries.** The Library Editor also enables third party MS/MS spectrum to be imported. This support is helpful in screening emerging compounds such as new psychoactive substances. All meta information can be simply copied/imported from spreadsheets to accelerate new library creation.
- **Collision Energy Spread**. A collision energy spread of 5-55V was used in acquiring the HRAM Clinical Library to include a range of product ions.

4. Conclusions

- High resolution accurate mass (HRAM) spectrometry method using untargeted and unbiased MS and DIA-MS/MS data acquisition was used to screen urine samples in clinical toxicology. The unbiased nature of the method design resulted in acquiring all tandem mass spectrometry data in a sample which can be useful in retrospective data analysis and discovering compounds outside the initial scope of the target panel.
- To reduce or negate false defect reporting a HRAM MS/MS Clinical Library was used to provide library searching and compound verification in addition to RT, ion ratio and mass error tolerances.
- The HRAM MS/MS Clinical Library can be expanded simply to meet changing clinical needs by all users.