Screening for Xenobiotics in Postmortem Biological Samples using High Resolution Liquid Chromatography Mass Spectrometry

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1. Overview

- High resolution LCMS for more selective, more sensitive sample screening in forensic toxicology laboratories
- Method package complete with instrument parameters and optional spectral library containing >900 compounds
- Easy spectral library creation and expansion capabilities
- Faster data processing using Insight Explore software

2. Introduction

- The ability to screen biological samples quickly and accurately is of utmost importance in a Medical Examiner's laboratory.
- To keep up with the workflow, a sensitive and robust analytical technique combined with spectral library matching and user-friendly software is required
- With the constant discovery of novel drugs, forensic laboratories need the ability to retrospectively search through a data file to find a substance not yet added to their library

3. Methods

- Shimadzu Nexera LC40 UHPLC coupled with 9030 QToF Mass Spectrometer (**Figure 1**)
- Chromatographic separation using Shimadzu Forensic Toxicology Database® parameters
- The acquisition method design was set-up with one MS scan and several DIA-MS/MS events. HRAM data acquired using sequential mass scans with a cycle timeless than 1 second (MS and subsequent DIA-MS/MS mass scans with an isolation width of *m/z* 20 Da). In each MS/MS event, a collision energy (CE) spread (5-55V) was applied to generate precursor MS and merged product ion MS/MS spectra.

Figure 1

4. Results/Workflow

4.1 Library Creation

- An in-house library containing 425 entries was created using certified reference material and the analytical conditions listed in Section 3. Each library entry included compound metadata information and retention time. (**Figure 2**)

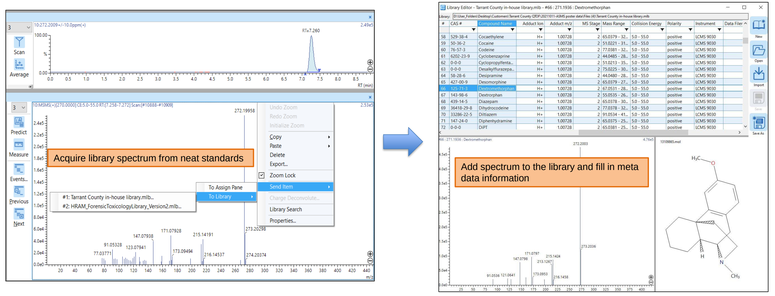


Figure 2

4.2 Sample Extraction

- Drugs from Postmortem blood and tissue samples were extracted using a protein precipitation method, centrifuged, dried down, and reconstituted in mobile phase prior to injection.
- End results were compared with the laboratory's previous methodology.
- This dry down technique requires a much lower sample volume, a quicker sample extraction, and 5x lower injection volume.

4.3 Data Analysis and Reporting

- Data was analyzed using Shimadzu Insight Explore Screening software
- An analyte was deemed a match based on the following criteria:
  - Spectral match >60% similarity index score
  - Mass error < 10 ppm of the calculated mass
  - Retention time +/- 0.2 min from the target
- A 1-point calibrator was included as a pass/fail concentration threshold
- Insight Flagging criteria used to accelerate data review process by allowing review by exception **(Figure 3)**

Data Review

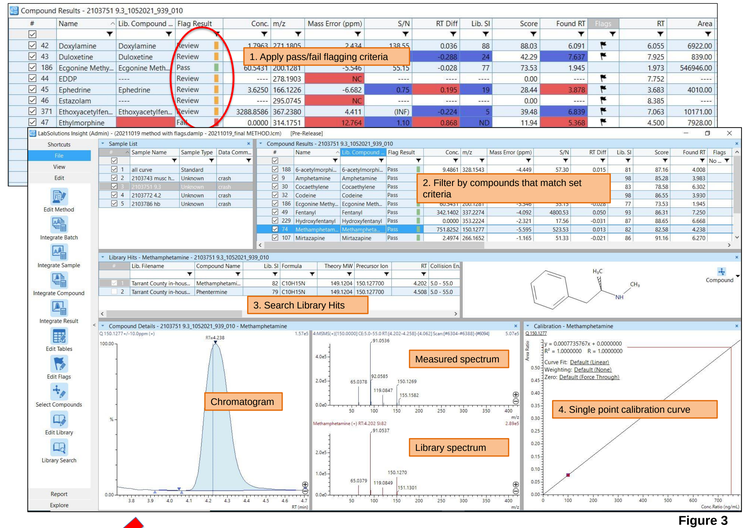
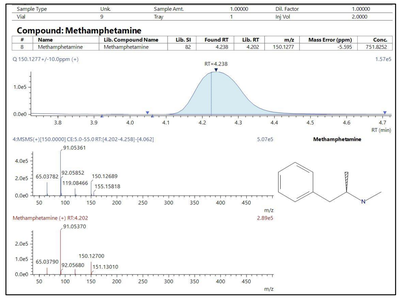


Figure 3

Report



5. Conclusion

- Faster, more-encompassing screening methodology for forensic casework
- Less sample volume, lower injection volume required
- Easily expandable spectral library for novel compounds
- Data-independent acquisition methodology provides retrospective data analysis capability

Acknowledgements

Shimadzu Scientific Instruments would like to thank Tarrant County Medical Examiner's Office for their collaborative efforts in making this poster.