High Resolution Mass Spectrometry Screening in Impaired Driving Investigations

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1. Introduction

Impaired Driving investigations have become increasingly more challenging with the influx of new psychoactive substances (NPS) into the drug market. In 2020, seventeen new substances were newly discovered which equals one new substance encountered approximately every three weeks (DEA, 2020). NPS become more prevalent as drug users pursue "legal highs." However, as these compounds gradually become controlled substances, new structural analogues emerge. As a result, traditional immunoassay-based drug screening is unable to keep pace with the emergence of new and emerging drug trends (Lee, 2009). Furthermore, published standards and best practices **[1, 2]** for the scope and sensitivity for toxicology testing in impaired driving investigations place increasing demands on operational laboratories.

Immunoassays are not available for all drugs or drug classes, and due to their reliance on antibody-based reagents, they are expensive and time consuming to develop. When used alone, they have insufficient scope and sensitivity **[3]**. As a result, forensic toxicology laboratories are exploring high resolution mass spectrometry (HRMS)-based technologies for toxicological drug screening.

2. Objective and Materials

The purpose of this study was to re-analyze adjudicated blood specimens and compare HRMS-based drug screening to reported immunoassay results. Blood specimens were initially screened for six common drug classes including opiates, methamphetamine, benzodiazepines, cocaine metabolite, phencyclidine and cannabinoids.

Drugs were isolated from blood using supported liquid extraction (SLE). Sample analysis was conducted using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) in All Ions mode in positive (P) and negative (N) electrospray ionization (ESI). This method was developed for common drugs of abuse and NPS. It has been validated according to the ANSI/ASB standard 036 **[4]**.

All solvents used were HPLC grade or equivalent. Reference standards were purchased from Cerilliant, Corp., Lipomed, and Cayman Chemical. An Agilent LC Infinity II was used with a poroshell 120 EC C-18 column (2.1 X 100 mm; 2.7 µm) and guard column. Chromatographic separations were analyzed by an Agilent 6530 LC-QTOF-MS.

3. Methods

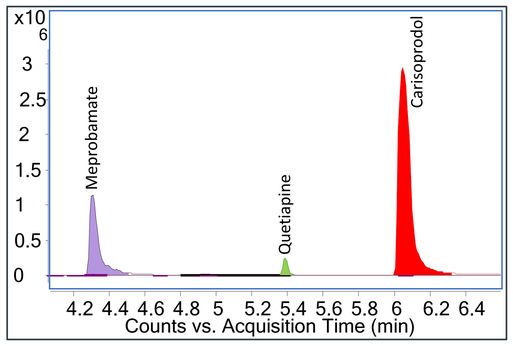
OPTIMIZED SLE (BIOTAGE ISOLUTE SLE+ 1mL COLUMNS) PROTOCOL

- Add 300 µL of 0.1M acetic acid to 600 µL blood specimen
- Centrifuge samples 4000 rpm for 10 mins.
- Load supernatant on 1mL SLE column and wait 5 mins.
- Add 3 mL of 70:23:7 (v/v) Hexane:Ethyl Acetate:Isopropanol
- Apply vacuum for 30 secs.
- Add 3 mL of 70:23:7 (v/v) Hexane:Ethyl Acetate:Isopropanol
- Apply vacuum for 5 mins.
- Add 30 µL of acidic methanol (1% conc. HCl in methanol)
- Evaporate under nitrogen at 40°C
- Reconstitute in 20 µL 60:40 Mobile Phase A/B
  - Mobile Phase A: 5mM ammonium formate; 0.01% FA in DIW
  - Mobile Phase B: 0.01% FA in acetonitrile
- Centrifuge extracts 4000 rpm for 10 mins and transfer to autosampler vials
- Inject 2 µL in P-ESI and 5 µL in N-ESI

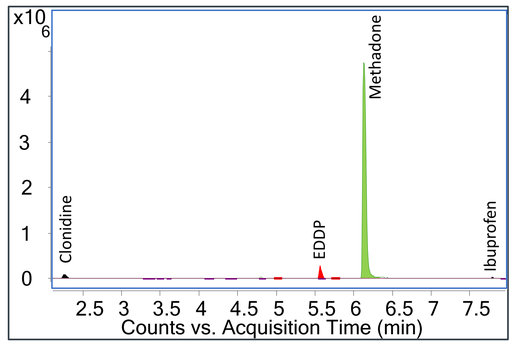
4. Results

Specific case samples are used to highlight the limitations of immunoassay-based screening for impaired driving investigations. The specimens below were all negative (no drugs detected) using a routine immunoassay-based screening. However, several potentially impairing drugs were presumptively identified using HRMS.

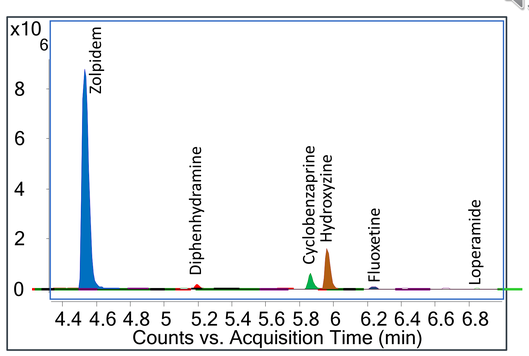
- **Specimen A**
  - Carisoprodol
  - Meprobamate
  - Quetiapine
- **Specimen B**
  - Clonidine
  - EDDP
  - Ibuprofen
  - Methadone
- **Specimen C**
  - Acetaminophen
  - Butalbital
  - Diphenhydramine
- **Specimen D**
  - Cyclobenzaprine
  - Diphenhydramine
  - Fluoxetine
  - Hydroxyzine
  - Loperamide
  - Zolpidem
- **Specimen E**
  - Citalopram
  - Dextromethorphan
- **Specimen F**
  - Citalopram
  - Dextromethorphan
  - Doxylamine
  - Fluoxetine
  - Paroxetine
  - Trazodone
- **Specimen G**
  - Citalopram
  - Carbamazepine
  - Doxylamine
  - Phenobarbital



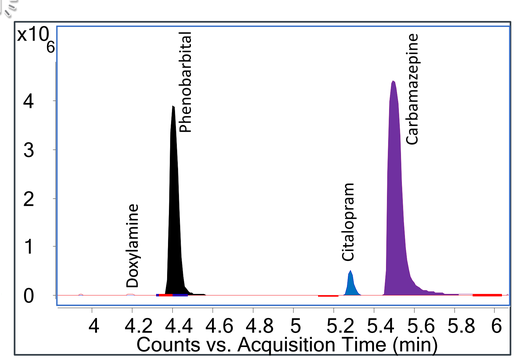
Extracted Ion Chromatogram (EIC) of Specimen A



Extracted Ion Chromatogram (EIC) of Specimen B



Extracted Ion Chromatogram (EIC) of Specimen D



Extracted Ion Chromatogram (EIC) of Specimen G

5. Discussion

Advantages of HRMS-based Drug Screening

Traditional immunoassay-based drug screening methods limit the scope and specificity of analyte detection. These techniques aim to identify common drug classes and not individualize specific analyte detection. In contrast, utilizing HRMS-based drug screening techniques such as LC-QTOF-MS enable the forensic toxicologist to target specific drugs of abuse and NPS. Sensitivity and specificity are improved by using high mass accuracy, isotopic patterns, and characteristic fragmentation for analyte detection. Furthermore, it broadens the scope of analytical testing by supporting retrospective data analysis.

Advantages of Retrospective Data Analysis

NPS complicate impaired driving forensic toxicology investigations because most compounds are not targeted or detectable by the testing laboratory. Therefore, toxicological analysis is often outsourced to other qualified laboratories. As a result, forensic toxicology has become the most outsourced forensic discipline surpassing forensic biology. The vast majority (68%) of toxicology laboratories are now outsourcing their work **[5]**.

Novel compounds are often encountered months after their initial emergence on the drug market. As a result, forensic toxicology laboratories are constantly challenged to maintain relevant drug screening procedures. The transitory nature of the drug market impresses this analytical burden upon toxicologists. Using HRMS-based drug screens, unknown compounds may be extracted from previously analyzed data files by retrospective data analysis. This process eliminates the need to re-sample and re-extract biological samples conserving case specimens.

Advantages of All Ions Data Analysis

Data acquisition using All Ions mode improves analytical detection and retrospective data analysis. All Ions mode ionizes and fragments all ions that enter the ionization source. It does not require abundance thresholds and expected ion transitions like targeted tandem mass spectrometry-based techniques. Therefore, relevant drugs of abuse encountered at low abundances are less likely to be overlooked. This is a critical advantage of All Ions data acquisition because NPS are often found at low concentrations. For example, synthetic cannabinoids in whole blood are often seen at ng/mL concentrations while designer amphetamines are in the µg/mL range **[6]**. In addition, distinctive fragmentation patterns employ another level of confidence in analyte detection and identification.

HRMS-based drug screening with All Ions data acquisition exhibits distinct advantages compared to conventional immunoassay drug screening techniques.

6. Potential for Impact

As NPS continue to evolve, immunoassay will become less practical for their detection. Immunoassay will remain useful for common drugs of abuse. However, transitioning to HRMS-based drug screening methods will benefit forensic toxicology laboratories in terms of increasing scope of testing and improving analyte sensitivity and specificity.

High resolution instruments have a high initial capital compared to immunoassay. However, this project emphasizes the benefits of utilizing HRMS-based drug screening such as LC-QTOF-MS. Future studies hope to show the increased benefits gained over the cost of employing HRMS instrumentation.

7. Conclusion

The scope of the drug market is constantly changing as NPS are consistently introduced. To maintain relevant drug screens, forensic toxicology laboratories require highly sensitive and specific analytical instrumentation. This presentation has highlighted some of the benefits of transitioning to high resolution drug screening. HRMS-based drug screening techniques such as LC-QTOF-MS have the potential to replace traditional immunoassay methods.

Immunoassay drug screening does not adequately respond to emerging drug threats and is time consuming in terms of commercial kit development. It also suffers from a high rate of false negative results. LC-QTOF-MS is a viable alternative for forensic toxicology drug screening because it can specifically detect more drugs of interest broadening the scope of analysis. Although instrument cost, training of personnel, data storage are considerable challenges, HRMS-based technologies offer unique possibilities for comprehensive toxicological drug screening.

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Acknowledgments

Disclaimer: This presentation was supported by Award No. 2018-75-CX-0040, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/ program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

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