High Resolution Mass Spectrometry Screening in Impaired Driving Investigations

Jessica Lynn Ayala*, M.S., D-ABFT-FT and Sarah Kerrigan, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

Jessica Lynn Ayala, M.S., D-ABFT-FT
Jessica.Ayala@shsu.edu

INTRODUCTION

Impaired Driving investigations have become increasingly more challenging with the influx of new psychoactive substances (NPS) into the drug market. According to the Drug Enforcement Administration (DEA), seventeen new substances were discovered in 2020, equivalent to one new substance every three weeks. NPS become more prevalent as drug users pursue "legal highs." However, as these compounds are subject to regulation, new structural analogues are produced to meet the demand. As a result, traditional immunoassay-based drug screening cannot keep pace with NPS proliferation. Furthermore, standards for scope and sensitivity of toxicology testing in impaired driving investigations were recently published **[1]** following long-standing recommendations or best practices **[2]**. Immunoassays are not available for every drug, and they take a relatively long time to develop. Although simple and amenable to various biological matrices, they are limited in scope and specificity **[3]**. As a result, forensic toxicology laboratories are beginning to transition from traditional immunoassay-based technologies to high resolution mass spectrometry (HRMS)-based toxicological screening.

OBJECTIVE AND MATERIALS

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

Blood samples were prepared for analysis by supported liquid extraction. Sample analysis was conducted using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) in All Ions mode. This method, which is capable of detecting > 200 common drugs of abuse and NPS, was validated in accordance with ANSI/ASB 036 **[4]**.

All solvents 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. Extracts were analyzed using an Agilent 6530 LC-QTOF-MS operated in both positive and negative electrospray ionization (ESI) modes.

METHODS

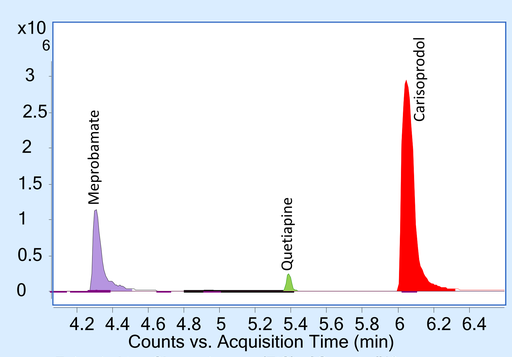
OPTIMIZED SLE (BIOTAGE ISOLUTE SLE+ 1 mL COLUMNS) PROTOCOL

- Add internal standard to 600 µL of blood
- Add 300 µL of 0.1 M acetic acid
- Centrifuge samples 4000 rpm for 10 mins
- Load supernatant on 1 mL 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
- Evaporate under nitrogen at 40°C
- Reconstitute in 20 µL 60:40 (MPA:MPB)
  - MPA: 5mM ammonium formate; 0.01% formic acid (FA) in DIW
  - MPB: 0.01% FA in acetonitrile
- Centrifuge extracts 4000 rpm for 10 mins and transfer to autosampler vials

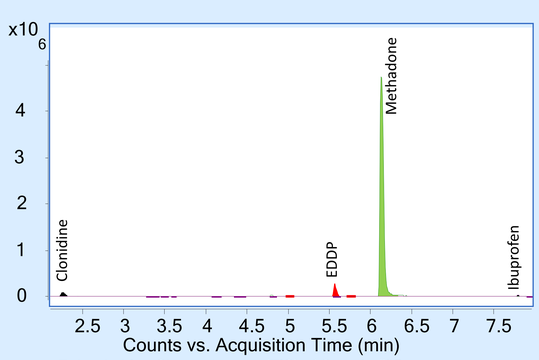
RESULTS

Selected data below highlights the number of potentially impairing substances that were not identified using immunoassay-based screening. The case samples shown below were reported as negative.

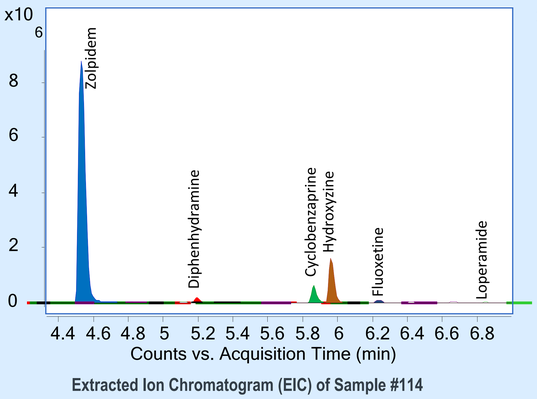
- **Sample #10**
  - Carisoprodol
  - Meprobamate
  - Quetiapine
- **Sample #35**
  - Clonidine
  - EDDP
  - Ibuprofen
  - Methadone
- **Sample #114**
  - Cyclobenzaprine
  - Diphenhydramine
  - Fluoxetine
  - Hydroxyzine
  - Loperamide
  - Zolpidem
- **Sample #115**
  - Acetaminophen
  - Butalbital
  - Diphenhydramine
- **Sample #135**
  - Citalopram
  - Dextromethorphan
- **Sample #165**
  - Citalopram
  - Carbamazepine
  - Doxylamine
  - Phenobarbital



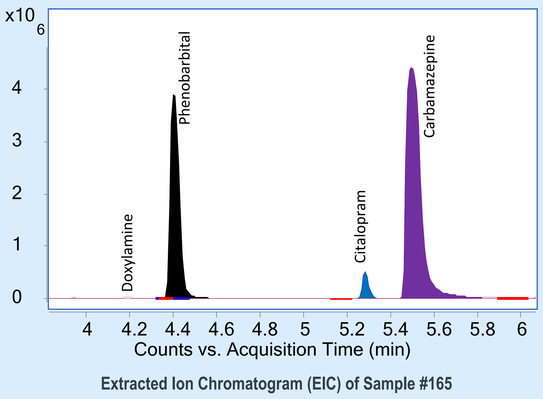
Extracted Ion Chromatogram (EIC) of Sample #10



Extracted Ion Chromatogram (EIC) of Sample #35



Extracted Ion Chromatogram (EIC) of Sample #114



Extracted Ion Chromatogram (EIC) of Sample #165

DISCUSSION

Advantages of HRMS-based Drug Screening

Although traditional immunoassay-based drug screening has adequate sensitivity, the approach has limited scope and specificity. The technique has limited ability to identify specific compounds within a class of drugs. In contrast, HRMS-based drug screening techniques (e.g. LC-QTOF-MS) have much greater discriminating power, and new compounds can be added to the scope of testing with relative ease. Specificity is improved 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

Most routine screening does not target common NPS, potentially resulting in false negative results. As the demands for scope and sensitivity of testing increase, an increasing number of laboratories (68%) are outsourcing their work. According to the most recent BJS census, forensic toxicology is now the most outsourced forensic discipline, surpassing forensic biology **[5]**.

NPS are often encountered months after their initial emergence on the drug market. As a result, forensic toxicology laboratories must constantly adapt. The transitory nature of the drug market and geographical trends place an enormous analytical burden upon toxicology laboratories. Retrospective data analysis is extremely valuable because it allows the laboratory to identify compounds and make data-driven decisions regarding the need to offer additional testing.

Advantages of All Ions Data Analysis

Data acquisition using All Ions mode improves analytical detection and allows for retrospective data analysis. All Ions mode ionizes and fragments all ions that enter the ionization source. Unlike targeted MS/MS detection, it does not require abundance thresholds or expected ion transitions. Therefore, non-targeted drugs of abuse, even at low abundance, are not overlooked. This is a critical advantage of All Ions data acquisition because NPS are often found at low concentrations. In addition, distinctive fragmentation patterns provide 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, targeted MS-based methods, or data-dependent acquisition.

CONCLUSIONS

The speed with which the illicit drug market changes places a significant analytical burden on forensic toxicology laboratories. To maintain relevance, instrumental approaches must have adequate scope and high sensitivity. From an analytical detection standpoint, HRMS-based drug screening has many advantages compared with traditional immunoassay-based methods.

Immunoassays have limited specificity (which can result in false positive results) and limited scope, potentially increasing the likelihood that drugs are not identified (false negative results). LC-QTOF-MS with All Ions detection is an invaluable tool for comprehensive toxicological drug screening. Major disadvantages of this approach include the cost of instrumentation, and level of training/experience for operation.

REFERENCES

1. ANSI/ASB STD 120, Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing of Blood in Impaired Driving Investigations, First Edition (2021).
2. Logan, B.K., D'Orazio, A.L., Mohr, A.L.A., Limoges, J.F., Miles, A.K.; Scarneo, C.E., Kerrigan, S., Liddicoat, L.J., Scott, K.S., and Huestis, M.A. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities. J Anal Toxicol 42, 63-68 (2018).
3. Saitman, A., Park, H.D., and Fitzgerald, R.L. False-positive Interferences of Common Urine Drug Screen Immunoassays: A Review. J Anal Toxicol, 38, 387-396 (2014).
4. ANSI/ASB STD 036, Standard Practices for Method Validation in Forensic Toxicology, First Edition (2019).
5. Bureau of Justice Statistics. Census of Publicly Funded Forensic Crime Laboratories Series: Publicly Funded Forensic Crime Laboratories: Resources and Services, 2014. United States Department of Justice, Office of Justice Programs (2016).

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