Targeted Forensic Screening and Semi-Quantitation of Drugs in Urine Using an HRAM Mass Spectrometer

Kristine Van Natta, Jingshu Guo, Tanis Correa, Bradley Hart, Sarvesh Iyer, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA, USA 95134

ABSTRACT

Purpose: Demonstrate performance of an established UHPLC-HRAM MS screening method on a next-generation orbitrap-based HRAM mass spectrometer. Identified compounds are screened against an extensive HRAM MS/MS spectral library and a database containing molecular formula, exact mass, retention times, and fragment ions.

Methods: Urine samples were spiked with analytes, separated chromatographically and detected on a next-generation orbitrap-based HRAM mass spectrometer in FS-ddMS2 mode.

Results: HRAM MS2 spectra collected on the new system successfully matched a library generated on an older model of mass spectrometer.

INTRODUCTION

Toxicology laboratories face many challenges, including extremely high volumes of samples in complex matrices and the proliferation of designer drugs. Laboratories must screen and quantify quickly and at low cost. While these challenges can be addressed individually, it is far more difficult to address all of them in a single analytical approach. Here, we present a novel workstream that combines liquid chromatography and high-resolution accurate-mass (HRAM) mass spectrometry to screen and quantify large panels in a single run while retaining the ability to retrospectively interrogate the analytical data for novel or unexpected compounds. Further, we demonstrate that the method, developed on one model of mass spectrometer, can be run successfully on a newer instrument model.

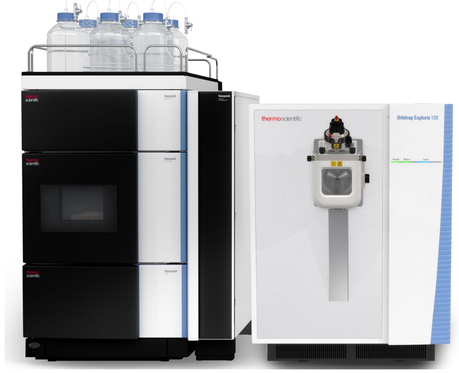
MATERIALS AND METHODS

Sample Preparation

Over 1500 standard solutions were analyzed on a first-generation HPLC/quadrupole-Orbitrap system using consistent HPLC and HRAM MS conditions. The resulting retention times and HRAM MS/MS spectra were used to generate a spectral library and compound database.

From the original list, 101 compounds covering a wide range of compound classes, hydrophobicities, and polarities were selected for proof-of-concept analyses. The compound classes represented include: opiates, amphetamines, benzodiazepines, synthetic cannabinoids, cathinones, barbiturates, and over-the-counter medications. Mixes of the selected compounds, with 8 internal standards (cotinine-d3, amphetamine-d5, naloxone-d5, 6-MAM-d3, benzoylecgonine-d8, 7-amino-flunitrazepam, imipramine-d3, diazepam-d5), were spiked into urine in a range of 0.1–2000 ng/mL and then diluted 20x with water to mimic a standard urine sample processing scheme.

Figure 2. Thermo Scientific™ Orbitrap Exploris™ 120 coupled to a Thermo Scientific™ Vanquish™ Flex UHPLC.



Chromatography

Gradient elution was performed using a Thermo Fisher™ Vanquish™ Flex ultra-high performance liquid chromatography (UHPLC) system equipped with a Thermo Scientific™ Accucore™ phenyl hexyl, 100 x 2.1 mm, 2.6 µm column. Mobile phases were 2 mM ammonium formate with 0.1% formic acid in (A) water and (B) methanol:acetonitrile (1:1), respectively and run with the gradient in Figure 1. All samples were injected in triplicate.

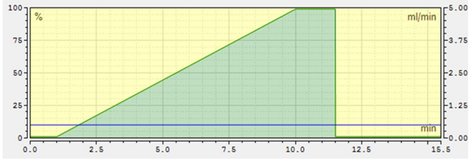
Mass Spectrometry

The Thermo scientific™ Orbitrap Exploris™ 120 HRAM mass spectrometer (Figure 2) was used for the targeted screening and quantitation analysis. Full scan and targeted data-dependent MS/MS scanning were used with an inclusion list for the targeted compounds. The inclusion list contains the exact mass of the compound, polarity, and retention time. Resolution of 60,000 was used for the full scan and 15,000 for the MS^2 scan. The isolation window was m/z 1.5 and stepped collision energies (18.75, 37.5, 56.25) were used to generate MS/MS spectrum. Polarity switching was performed, allows the acquisition of both positively and negatively ionized analytes during a single analysis.

Data Analysis

Data was acquired and processed with Thermo Scientific™ TraceFinder ™ software, version 5.1. TraceFinder software utilizes its Compound Database which stores information including molecular formula, exact mass, retention time and fragment ions for all compounds of interest.

Figure 1. UHPLC Chromatographic gradient used for data acquisition.



RESULTS

Targeted Screening

Five parameters were set to ensure positive confirmation during screening: exact mass of the precursor ion, retention time of UHPLC chromatography, isotopic pattern match, fragment ion match and match with the *m*zVault library. Additional details on the criteria for each of these parameters can be found in Table 1.

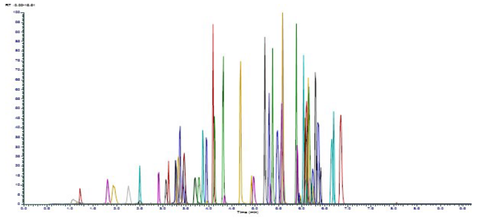
An overlay of extracted ion chromatograms (XIC) of all the compounds is shown in Figure 3. Figure 4 highlights the mass accuracy over a chromatographic peak for both an early and late eluting compound.

An example of the screening data review is found in Figure 5, where the software quickly indicates which compound was positive in the sample. Morphine was found in Mix 5 for the entire calibration curve. The screening criteria are depicted in the bottom portion of the figure: extracted chromatogram, isotope matching, fragments, and library matching. The exact mass of 286.1436 m/z is an isomer for four drugs of abuse compounds. Two out of the four were positive for library matching. However, with the Tox Explorer Collection, chromatography of these isomers is resolved with different retention times.

Table 1. Criteria assigned in TraceFinder software for targeted drugs of abuse screening.

Parameter	Criteria
Precursor Ion	m/z < 5 ppm mass deviation
Retention Time	30 second window
Isotopic Pattern	< 10 ppm mass deviation, < 20% intensity deviation, fit > 70%
Fragment Ion	At least 2 fragments with < 10 ppm mass deviation in MS^2 spectra
m/zVault HRAM Library	Reverse search with > 70% match of $ddMS^2$ spectra

Figure 3. Combined chromatogram showing all compounds extracted from the Full Scan data with 5 ppm mass accuracy.



RESULTS (cont.)

Quantitation

Over hundred analytes of different classes, retention times, and polarities were evaluated for limit of detection (LOD), LOQs, and limit of identification (LOI). Specific criteria for quantitation that were used can be found in Table 2.

An example of the calibration curve for 7-aminoflunitrazepam is shown in Figure 6. Figure 7 is representative XICs of 0.1 ng/mL for different analytes in each of the five mixes tested. Figure 8 reports the % of compounds satisfying the quantitation criteria.

Figure 4. From full scan spectrum, mass accuracy at different points under the chromatography peak of a) early eluter, nicotine and b) later eluter, THC-COOH.

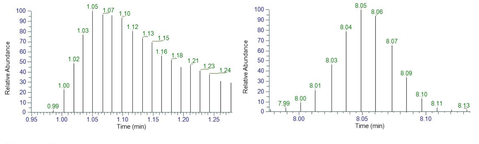


Figure 5. Data review for screening of the analyte, morphine demonstrating retention time, isotope matching, fragment ions and library match.

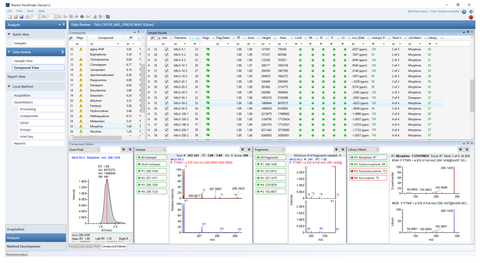


Table 2. Criteria assigned in TraceFinder software for quantitation.

Parameter	Criteria
Limit of Detection (LOD)	Presence of peak at correct retention time (see Table 1)
Limit of Quantitation (LOQ)	Back-calculated concentration on calibration curve within 30%.
Limit of Identification (LOI)	IP = passing isotopic pattern score (70) FI = presence of diagnostic fragment ions LS = passing library score (70)

Figure 6. Data review for quantitation of 7-amino-flunitrazepam demonstrating peak area, percent different and percent RSDs for calibration curve of 0.1 to 1000 ng/mL

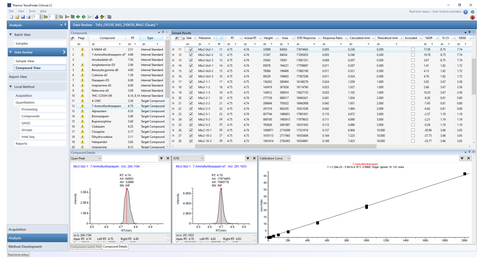
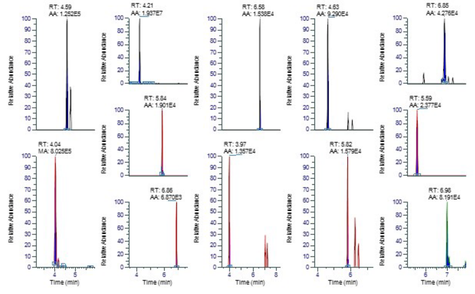


Figure 7. Analyte's XIC at the concentration 0.1 ng/mL for the five mixes run for quantitation using Tox Explorer method.



Robustness

Robustness of the analytical method instills confidence in the data. This robustness can be demonstrated by showing high mass accuracy and mass stability across multiple injections. To demonstrate mass accuracy, internal standards 6-MAM-d3, amphetamine-d5, diazepam-d5, and imipramine-d3 were monitored over 2 days (48 hrs). The masses were within 5 ppm with %RSD <0.001% over 2 days (48 hrs) without recalibration (Figure 9). The %CV for peak abundance for the internal standards were below 10% (imipramine-d3 was 13% in Mix 4), and retention time for the compounds was ±0.01 min, demonstrating the reproducibility and robustness of this method.

Figure 8. Summary of percentage of compounds analyzed for this poster meeting LOD, LOQ and LOI parameters.

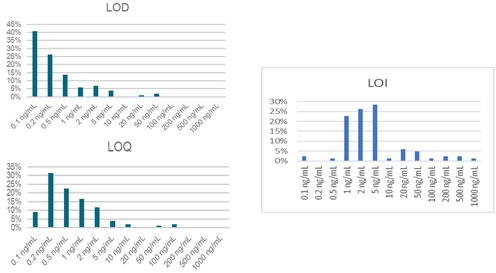
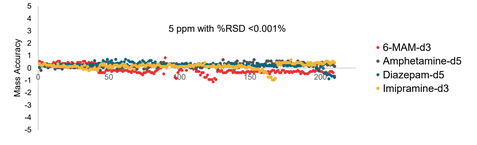


Figure 9. Robustness of method – Mass accuracy of four internal standards for continuous injections spanning over two days.



CONCLUSIONS

- A screening method was used to detect, identify, and quantitate 101 drugs of abuse by Orbitrap Exploris 120.
- Identifications were made by comparing MS2 spectra collected on a previous generation orbitrap mass spectrometer to Orbitrap Exploris 120.
- The Orbitrap Exploris 120 can both screen and quantitate compounds in one platform and run.
- While this study reports data from a dilute-and-shoot method, limits may be improved with analyte-class specific sample processing methods.
- TraceFinder software with its integrated compound database enables data acquisition, processing for both screening and quantitation and reporting in one platform.

REFERENCES

1. Technical Note 73771: LC-MS/MS Toxicology Platform and Method for High-resolution, Accurate Mass (HRAM) Detection, Screening, and Quantitation of Drugs

TRADEMARKS/LICENSING

PO66158-EN0422S