High-Resolution, Accurate-Mass Forensic Toxicology Screening in Blood Samples Using a Q Exactive Mass Spectrometer

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Overview

Purpose: To evaluate the Thermo Scientific™ Q Exactive™ High-Resolution Mass Spectrometer in Forensic Toxicology Screening for whole blood analysis and make a comparison with Targeted Screening on a Triple Quadrupole MS using the SRM (Selected Reaction Monitoring) mode and also UPLC/Diode Array Detection (DAD).

Methods: Blood samples were spiked with internal standards and extracted with TOXI-TUBES™ A (Agilent Technologies, Santa Clara, CA). LC separation was performed with a 30 minute gradient. Mass spectrometry data were acquired in Full Scan and MS^2 mode using the Q Exactive MS.

Results: Data collected show benefits of high-resolution screening over both the triple quadrupole approach and DAD detection.

Introduction

Forensic scientists and forensic toxicologists need to identify an unlimited number of compounds in complex matrixes with the capability of retrospective data analysis for quick and confident analysis. The major challenge is to separate the analytes of interest from the matrix and accurately identify them. Here we evaluated the Q Exactive MS, a bench-top quadrupole-Orbitrap™ ultra-high resolution mass spectrometer routinely capable of better than 5 ppm mass accuracy and 140,000FWHM resolution, with Thermo Scientific™ Exact Finder™ data processing software, for forensic toxicology screening in blood samples. We will also compare the results with those obtained by forensic targeted screening using an SRM approach and DAD detection.

Method

Sample Preparation

500 µl of each blood sample was spiked with 20 µl of an internal standard solution (Flurazepam at 1 mg/L) and extracted with TOXI-TUBES A™ (Agilent Technologies). The organic layers were transferred, evaporated to dryness, reconstituted in 2.5 ml of a mixture containing 70% of mobile phase A and 30% of mobile phase B, and injected onto the Q Exactive MS. For triple quadrupole analysis and DAD detection, the sample was reconstituted in 500 µl and 100 µl, respectively, of the mixture described above.

Liquid Chromatography

The U-HPLC comprises Thermo Scientific™ Accela™ 1250 pumps with an Accela Autosampler. Mobile phases are 10 mM Ammonium formate and 0.1% Formic acid in water (A) and 0.1% Formic acid in Acetonitrile (B). The LC separation was performed on a Thermo Scientific™ Hypersil™ GOLD PFP column 150 x 2.1 mm 3µm.

Start (min)	Flow (mL/min)	%A	%B
0.00	0.2	95	5
5	0.2	55	45
18	0.2	30	70
20	0.2	5	95
27	0.2	5	95
27.1	0.2	95	5
32	0.2	95	5

Figure 1. HPLC Gradient Method

Mass Spectrometry

Compounds are detected on a Q Exactive mass spectrometer equipped with an Orbitrap mass analyzer. A schematic diagram of the Q Exactive MS is illustrated in Figure 2. A Heated Electrospray Source Ionization (HESI) probe was used as an ion source. The instrument was operating in alternating positive and negative full scan mode. Each Full Scan was followed by 8 high-resolution MS^2 scans in positive mode and 3 high-resolution MS^2 scans in negative mode. Precursor selection was done in the data-dependent operation mode where the most intense ion of the previous scan was selected for fragmentation. Resolution was set to 70,000 FWHM for each full scan mode and 17,500 FWHM for MS^2 scan acquisition. MS^2 spectra were acquired with a Normalized Collision Energy (NCE) of 70. Relevant scan and source parameters are shown in Figures 3 and 4.

DAD Detection

Data have been acquired on a UPLC-Acquity™ (Waters Corporation, Milford, MA) equipped with a DAD detector. The library contains 612 molecules. Acquisition is performed using a 15 minute LC gradient.

Triple Quadrupole Detection

Six different targeted LC-MS/MS methods have been used to acquire data in SRM (Selected Reaction Monitoring) mode. This method includes 97 molecules.

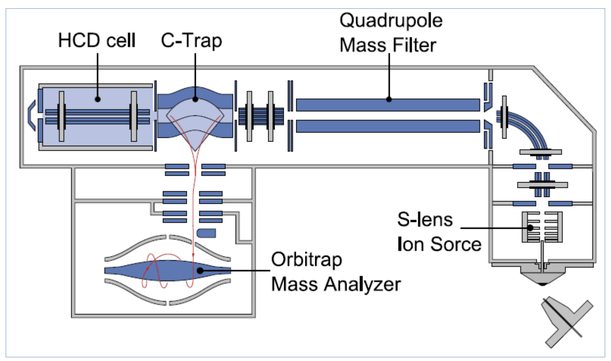

Figure 2. Schematic diagram of the Q Exactive High-Resolution, Accurate-Mass Instrument.

FIGURE 3. Scan Parameters for Q Exactive Mass Spectrometer.

Parameter	Value
Full MS	
Microscans	1
Resolution (FWHM)	70,000
AGC Target	1e6
Maximum IT	250 msec
Scan Range	150-800 m/z
MS^2 Experiments	
Microscans	1
Resolution	17,500
AGC Target	1e5
Maximum IT	250 msec
NCE	70.0

FIGURE 4. Source Parameters for HESI Probe.

Parameter	Value
Sheath Gas	30
Aux gas	15
Spray voltage (V)	3500
Capillary temp (°C)	320
Vaporizer Temp (°C)	350

* Parameters are the same for positive and negative modes

Data Analysis

All MS data have been processed using ExactFinder 2.0 software. Identification of the analytes is performed using the exact mass of the precursor, the retention time, the isotopic distribution and the fragment exact masses.

Results

Data Processing

Chromatograms were reconstructed with a 5 ppm mass accuracy. The method was set to identify compounds based on the exact mass of the parent and the retention time. Confirmation was performed using the isotopic pattern and up to 5 fragment ions obtained from each precursor. A database containing up to 650 analytes was selected for processing. Figure 5 shows an example of the results page showing the XIC (extracted ion chromatogram) for Nordiazepam reconstructed with 5 ppm mass accuracy (a), isotopic pattern (b) and fragment ion confirmation (c).

Results are reported using flags of different colors :

- (green circle): When the sample/compound/peak combination is identified and fully confirmed.
- (yellow triangle): When the sample/compound/peak combination is identified but not fully confirmed.
- (red square): When the sample/compound/peak combination is not identified.

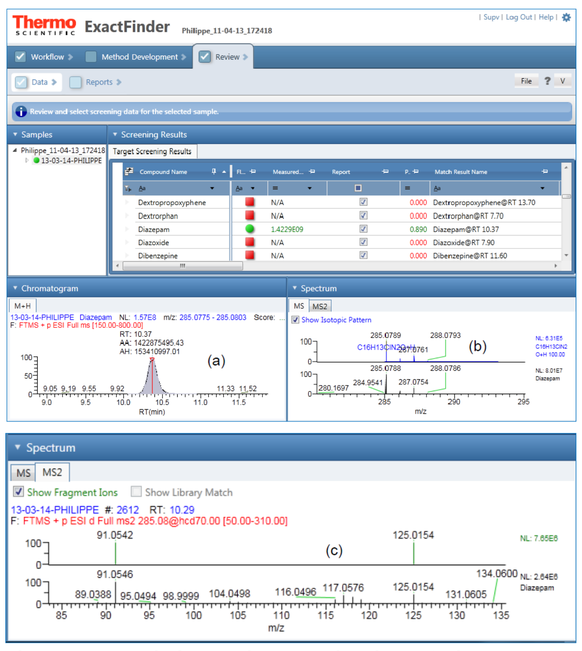

Figure 5. ExactFinder results page showing XIC chromatogram for Diazepam reconstructed with 5 ppm mass window (a), isotopic pattern (b) and fragment ion confirmation (c).

Metabolite Identification

In addition to compound identification, it is possible to confirm the results by identifying potential metabolites present in the sample. The approach is simple. As the acquisition is performed in Full Scan mode, identification of metabolites can be realized with the same HR-MS analysis by only extracting theoretical m/z values for predicted biotransformations. Figure 6 shows an example of metabolites identified from a single sample. The main compound identified is methadone and we have also been able to identify two major metabolites: EDDP and EMDP.

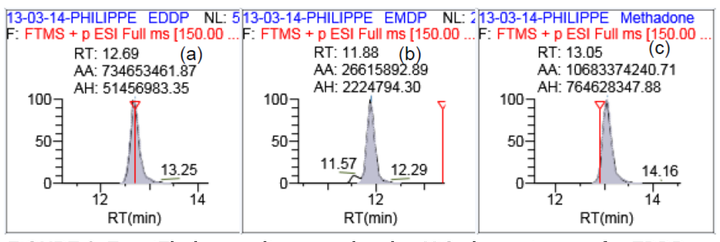

FIGURE 6. ExactFinder results page showing XIC chromatogram for EDDP (a), EMDP (b) and Methadone (c) reconstructed with 5 ppm mass window.

Comparison between the different approaches: DAD detection, targeted screening using a triple quadrupole, HRAM screening using the Orbitrap technology

We've analyzed and compared 39 samples using the 3 different technologies. Overall, the HRAM approach allowed identification of a higher number of analytes than the other approaches. We have been able to identify 143 compounds with the HRAM approach, 121 with the six targeted forensic screening methods performed on the triple quadrupole MS and 69 compounds using the DAD. Some of the results are reported in Figure 7 where we compare for 40 analytes (among the 77 identified) the number of positive hits obtained for each approach.

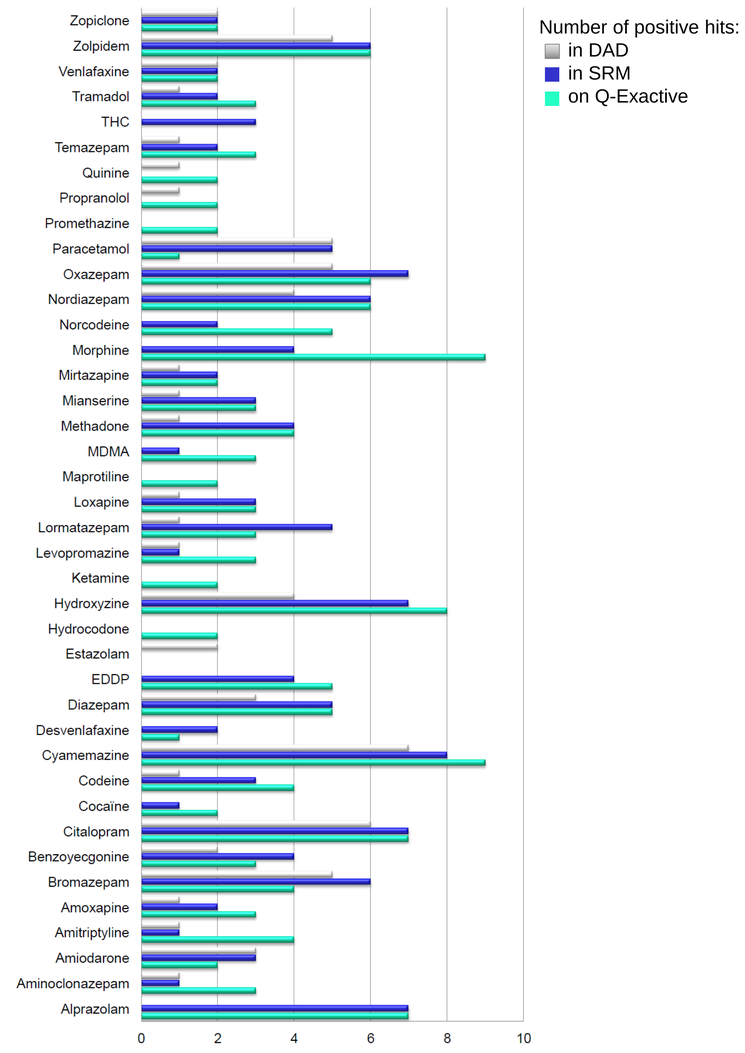

Figure 7. List of analytes that have been identified among 39 samples and confirmed using the 3 approaches: targeted screening in SRM, DAD and Q Exactive screening.

DAD Approach

Fewer analytes have been identified using this approach despite the size of the library (612 analytes). Sensitivity is certainly the main concern with this technique. Moreover, DAD may provide in some cases some false positive results. For example estazolam has been identified in DAD but not confirmed using the MS technologies. This approach is well known for its poor sensitivity in benzodiazepines analysis. As reported in Figure 7, alprazolam is not detected with DAD but is confirmed using the other two approaches.

Triple Quadrupole Approach Using the Six Targeted SRM Methods. This approach gives good results in terms of positive hits identified. THC was identified using this approach as the sample preparation was done in acidic conditions unlike the other approaches where basic conditions were used. There are still some limitations. The identification is confirmed using six different SRM methods which means that we may have to inject the same sample several times. Moreover these six methods contain only 97 analytes. The run is performed in SRM mode and for this reason there is no capability for retrospective analysis and potential metabolite identification.

HRAM Approach Using the Q Exactive MS

This approach is able to identify the largest number of analytes with the 650 analytes library. But there are still some limitations to overcome. Precursor selection was done in the data-dependent operation mode where the most intense ion of the previous scan was selected for fragmentation. So we may, in some cases, have to add the compounds in the inclusion list in order to not miss the MS^2 acquisition. Some of the analytes listed are isomers (eg: maprotiline, paroxetine and EDDP). As they have exactly the same exact mass, we have to make sure they present different fragment ions in MS^2 or elute at different retention times. All data have been processed though ExactFinder 2.0 software with a 5 ppm mass accuracy. In this version of the software, the mass accuracy is set and can't be adjusted. For this reason, low mass fragments like the one we have with paracetamol at m/z 110.0595 are in some cases not properly identified with an accuracy of 5 ppm. This limitation is nevertheless going to be overcome with the launch of Thermo Scientific™ TraceFinder™ 3.0 where the mass accuracy is set by the user and can be expressed in ppm or milli-amu.

Conclusion

- The Q Exactive MS provides high confidence with high-resolution capabilities (up to 140,000 FWHM) for forensic screening.
- Data processing is performed using ExactFinder 2.0 software. Compounds are identified and confirmed using the exact mass of the precursor, the isotopic distribution, the retention time and the exact mass of up to 5 fragment ions.
- HRAM LC-MSMS method identified more compounds for forensic toxicology than Diode Array Detection and Triple Quadrupole Targeted SRMs methods.
- Additional information such as metabolites identification can be easily obtained by extracting the theoretical m/z values for predicted biotransformations
- This HRAM method also allows for retrospective data analysis.
- A new HRAM database (https://www.mzcloud.org/) will soon be available to perform targeted and also unknown identification.

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