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POSTER NOTE 65050

Targeted Forensic Screening and Semi-Quantitation of Drugs in Plasma Using High-Resolution Accurate-Mass Detection and On-line Sample Preparation

ABSTRACT

Purpose: The aim of this work was to generate a large forensic screening panel in a short chromatographic run. Then, the method was tested to combine the screening capabilities of a Thermo Scientific™ Q Exactive™ Focus mass spectrometer to the quantitation of 41 drugs in plasma matrix for a partial analytical validation of the screening method.

Methods: Two different analytical methods were used, one based on HPLC with a run time of 15.5 minutes, and the other based on on-line extraction using Thermo Scientific™ TurboFlow™ technology prior to HPLC separation, with a runtime of 16.75 minutes. For the generation of the spectral library and compound database, 1513 standard solution were injected with the both methods to obtain retention times and MS/MS spectra. The limit of detection (LOD), the limit of quantification (LOQ) and the limit of identification (LOI) were determined for 41 compounds in spiked plasma with the on-line extraction approach.

Results: A compound database and a spectral library for the screening of 1513 compounds were implemented on a Thermo Scientific™ Transcend™ II TLX-1 system coupled to a Q Exactive Focus Orbitrap high-resolution, accurate-mass spectrometer. A partial analytical method validation was performed in plasma. The compounds can be used as a basis for the method validation since they cover different drug classes, retention times and polarities.

INTRODUCTION

In forensic toxicology, it is of high importance to be able to screen a large panel of compounds on a single injection of sample for further confirmation by more specific methods. Methods developed for this purpose need to use a low volume of sample and to include the capability of monitoring a very large panel of compounds; it is also desirable to reduce the runtime of these methods to increase throughput. The development of a spectral library and compound database for the screening and semi-quantitation of more than 1500 compounds in plasma samples, but which is applicable to other biological matrices, is reported. For each compound, the database includes the exact mass, chemical formula, retention time, and exact masses of main fragments.

Figure 1. System configuration used for this work consisting on a Transcend II TLX1 system coupled to a Q Exactive Focus mass spectrometer.

For a quantitation method, analytical validation is generally based on the evaluation of the LOQ and the intra-day and inter-day accuracy and precision. The approach is difficult to apply in this case considering the large number of compounds in the panel. This would suggest the preparation, injection, acquisition, and processing of data for more than 1400 compounds. Moreover, there are no official guidelines regarding the analytical validation of a screening method. A possible solution consists of selecting some compounds that are representative of different drug groups that appear in the complete retention time window of the chromatographic run, and that can present different polarities.

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MATERIALS AND METHODS

Sample Preparation

Standard solutions for library generation were prepared in groups of 20 compounds at a concentration of 0.1 µg/mL in methanol/water 30:70 v/v solution.
Calibrators were prepared by spiking the compounds into blank plasma matrix from Innovative Research (Le Perray-en-Yvelines, France). Sample preparation previous to injection consisted of the precipitation of proteins as follows: 25 µL of a solution containing isotopically labeled internal standards (2 mg/L amphetamine-d5, 1 mg/L THC-COOH-d3, 5 mg/L haloperidol-d4, prazepam-d5 and morphine-d3, and 0.2 mg/L trimipramine-d3 in methanol) and 100 µL of acetonitrile were added to 100 µL of calibrator. After vortex mixing, the calibrators were centrifuged and the supernatant was transferred to a vial for sample injection.

Liquid Chromatography

The system used for this method was a Transcend II TLX1 system. This system is presented in Figure 1. The system used allows for the use of either an HPLC-only method or an HPLC method combined with on-line extraction of the sample. Both methods are reported in table 1 and Table 2 accordingly.

Table 1. Gradient conditions for the HPLC screening method

Step	Time (min)	Duration (s)	Loading pump					Tee	Loop	Eluting pump			
			Flow	Grad	%A	%B	%C			Flow	Grad	%A	%B
1	0	60	0	Step	100	-	-	-	Out	0.5	Step	99	1
2	1	540	0	Step	100	-	-	-	Out	0.5	Ramp	1	99
3	10	90	0	Step	100	-	-	-	Out	0.5	Step	1	99
4	11.5	240	0	Step	100	-	-	-	Out	0.5	Step	99	1

Table 2. Gradient conditions for the TurboFlow extraction coupled to HPLC separation screening method

Step	Time (min)	Duration (s)	Loading pump					Tee	Loop	Eluting pump			
			Flow	Grad	%A	%B	%C			Flow	Grad	%A	%B
1	0	20	2	Step	100	-	-	-	Out	0.5	Step	99	1
2	0.3	5	0.5	Step	100	-	-	-	In	0.5	Step	99	1
3	0.4	60	0.5	Step	99	1	-	T	In	0.05	Step	99	1
4	1.4	540	0.5	Ramp	1	99	-	T	In	0.05	Ramp	1	99
5	10.4	90	0.5	Step	1	99	-	T	In	0.05	Step	1	99
6	11.9	10	1	Step	-	-	100	-	In	0.5	Step	-	100
7	12.1	10	1	Step	100	-	-	-	In	0.5	Step	-	100
8	12.2	10	1	Step	-	-	100	-	In	0.5	Step	-	100
9	12.4	10	1	Step	100	-	-	-	In	0.5	Step	-	100
10	12.6	10	1	Step	-	-	100	-	In	0.5	Step	-	100
11	12.8	60	0.3	Step	100	-	-	T	In	0.05	Step	99	1
12	13.8	180	1	Step	100	-	-	-	In	0.5	Step	99	1

Mass Spectrometry

Data were acquired on a Q Exactive Focus Orbitrap mass spectrometer. The detection was performed by Full Scan acquisition in data dependent acquisition with an inclusion list. Full Scan data were acquired in both positive and negative mode with a resolution of 35,000 FWHM at *m/z* 200, and the MS² spectra for confirmation were acquired with a resolution of 17,500 FWHM at *m/z* 200. The experiment schematics are presented in Figure 2.

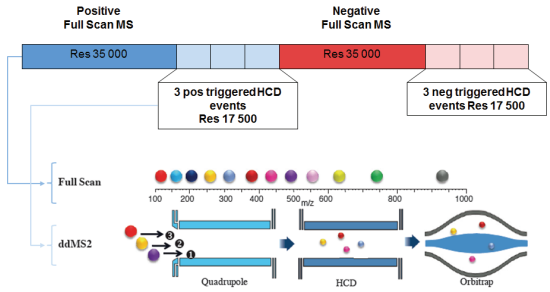

Figure 2. Full Scan data dependent acquisition schematics

Data Analysis

Data were acquired and analysed with Thermo Scientific™ TraceFinder™ 4.1 software. TraceFinder software uses a database that contains compound-related information for identification and confirmation. It also uses proprietary MS² spectral libraries containing the spectra of the 1513 compounds tested. The spectra generated for this application were imported into a Thermo Scientific™ mzVault™ library. mzVault library is a new library search algorithm from mzCloud for improved library matching. mzCloud is a high resolution accurate masses fragmentation library available through the site: www.mzCloud.org. It contains spectral information on multi-energy, multi-level and multi-fragment techniques.

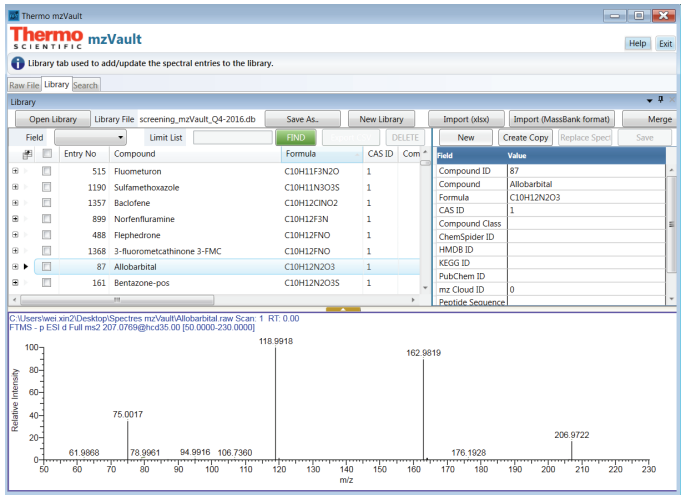

Figure 3. View of the screening mzVault spectral library

RESULTS

A database containing compound related information was created for both methods, one using HPLC-only and one using TurboFlow technology on-line extraction on a Transcend II TLX1 system. For the development of the database, concentrated solutions were used. 1433 out of 1513 injected solutions were detected in both approaches. The somewhat lower number for the TurboFlow approach is due to poor retention of some of the analytes in the extraction columns. An example of the review of the data oriented to a screening approach in TraceFinder 4.1 software is presented in Figure 4.

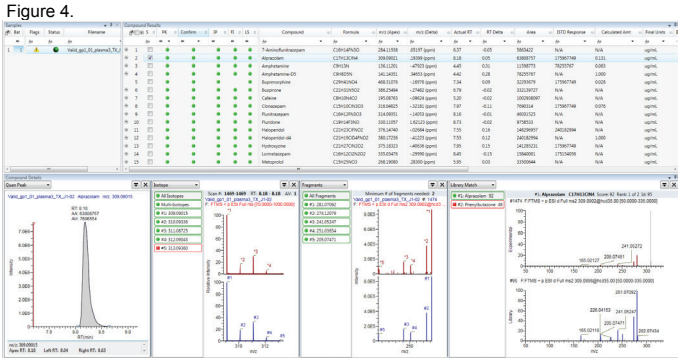

Figure 4. View of the screening data review in TraceFinder 4.1 software

The analytical method was then partially validated. To this end, 41 compounds were selected from the panel, covering different compound classes, retention times and polarities. The 41 compounds used for this stage were divided in three groups for the preparation of the calibrators according to the levels of concentration of the analytes to be assessed in plasma samples. Calibrators had concentrations going from 0.1 ng/mL to 250ng/mL for compounds on group A, and from 10ng/mL to 5000ng/mL for groups B and C. TraceFinder 4.1 software has the possibility to perform in the same batch a screening workflow with identification and confirmation of compounds, and to obtain a quantitative result based calibration curves. The quantitation data review is presented in Figure 5.

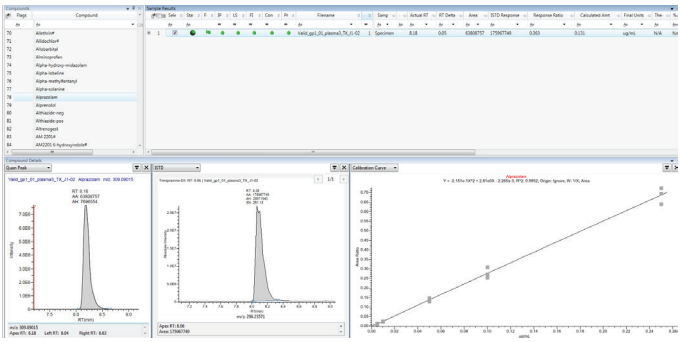

Figure 5. View of the quantitative data review in TraceFinder 4.1 software

For the TurboFlow approach, the limit of quantitation (LOQ), the limit of detection (LOD) and the limit of identification (LOI) were determined for spiked plasma samples. The LOD was obtained as the lowest concentration for which a peak is still observed for 3 different plasma matrices tested. The LOQ was obtained as the lowest concentration for which a quantitation has an accuracy with a bias inferior to 20% and a %RSD inferior as well to 20% for 3 repeated injections in three different plasma matrices. The bias determination was based on the calibration curves generated from 0.1 to 250ng/mL for group A compounds, and from 10ng/mL to 5000ng/mL for groups B and C. Finally, the LOI was determined as the lowest concentration for which a compound can be identified based on the following conditions: m/z of the parent (< 5 ppm), isotopic pattern match, fragment ion presence, and MS^2 spectra matching. The corresponding results are presented in Table 3.

Table 3. LOD, LOQ and LOI obtained for 41 compounds with the TurboFlow method

Group	Compound	LOD (ng/mL)	LOQ (ng/mL)	LOI (ng/mL)
A	Alprazolam	5	50	50
	Amphetamine	50	50	100
	Buprenorphine	5	5	50
	Buspirone	10	10	10
	Clonazepam	10	50	100
	Flunitrazepam	5	50	50
	Haloperidol	1	1	50
	Hydroxyzine	1	5	10
	Lormetazepam	10	10	100
	Mianserine	0.5	0.5	5
	Morphine	50	100	250
	Olanzapine	5	50	50
	Prazepam	5	5	50
	Zopiclone	50	50	100
B	Amoxapine	50	100	100
	Chlordiazepoxide	50	100	100
	Chlorpromazine	50	500	500
	Doxepine	50	50	50
	EDDP	50	100	100
	Estazolam	50	100	100
	Fluoxetine	50	1000	1000
	Norclobazam	100	1000	1000
	Nordiazepam	50	100	100
	Nortriptyline	50	100	100
	Temazepam	50	500	500
	Amitriptyline	10	50	50
C	Bisoprolol	10	50	50
	Clobazam	10	10	50
	Clomipramine	10	50	50
	Clozapine	10	10	50
	Codeine	10	10	50
	Cyamemazine	10	10	50
	Desipramine	10	10	10
	Doxylamine	10	50	50
	Fluvoxamine	50	50	50
	Imipramine	10	50	50
	Levomepromazine	10	50	50
	Metformin	50	250	500
	Methadone	10	50	50
	Tramadol	10	50	50
	Trimipramine	10	50	50

CONCLUSIONS

- A compound database and a spectral library for the **forensic** screening of 1513 compounds were implemented on a Transcend II TLX-1 system coupled to a Q Exactive Focus Orbitrap high resolution accurate mass spectrometer.
- The panel includes compounds **of interest in forensic toxicology** both positively and negatively ionized such as drugs of abuse and metabolites, antidepressants, beta-blockers, antibiotics, pesticides and other classes.
- This opens possibilities to increase even more the screening panel to new substances.
- The drug screening method presented in this work covers a large panel of compounds with a short run time of 15.5 minutes and an option for an on-line extraction approach of only 16.75 minutes.
- Analytical validation for the TurboFlow method was performed on 41 compounds spiked in plasma matrix.
- The screening and quantitation workflows can be used both at the same time within an acquired batch in TraceFinder 4.1 software

REFERENCES

1. Orbitrap technology for comprehensive metabolite-based liquid chromatographic-high resolution-tandem mass spectrometric urine drug screening - exemplified for cardiovascular drugs - Helfer A.G., Michely J.A., Weber A.A., Meyer M.R., Maurer H.H. – Anal Chim Acta, 891 (2015), 221-233.
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