Evaluation of Ultra High Resolution Mass Spectrometer in Targeted Forensic Screening Method for Urine Analysis in Comparison to Immunoassay and GC-MS Techniques

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Overview

Purpose: To evaluate Exactive [TM] ultra high resolution mass spectrometer in targeted forensic screening applications for urine analysis.

Methods: Urine samples were diluted 20 times with DI water and analyzed with 15-minute gradient LC method. Full scan data followed by all ion fragmentation spectra were collected in polarity switching experiment.

Results: Data collected with Exactive screening method correlates very well with immunoassay, Remedy and GC-MS data. More compounds and metabolites were detected with LC-MS method when compared to the other two analytical techniques.

Introduction

Fast screening methods allowing for quick and confident identification of unlimited number of compounds in urine samples with capability of retrospective data analysis are required in forensic toxicology . Ultra high resolution mass spectrometers are the only mass spectrometry platforms meeting these expectations. Among those instruments, the Exactive with Orbitrap™ mass analyzer stands out with up to 100K resolution for data specificity and high quality of all ions fragmentation (AIF) spectra collected in higher-energy collisional dissociation (HCD) cell.

Sample preparation procedure is a critical step in screening applications. Sample processing methods in which urine is simply diluted can detect a greater number of compounds and metabolites than those methods using SPE or LLE extractions.

Possibility of eliminating glucuronides hydrolysis by detection of conjugated metabolites allows faster data delivery.

Method

Sample Preparation

Mix 50 µL of urine with 50 µL of internal standard solution and 900 µL of DI water. Vortex and transfer samples to an autosampler vial. Inject 20 µL onto HPLC system.

Internal standard spiking solution containing 200 ng/mL of Hydromorphone-D6, 200 ng/mL of Methamphetamine-D5 and 500 ng/mL of Phenobarbital-D5 was prepared in 50% MeOH.

Hydromorphone-D6 and Methamphetamine-D5 were used as internal standards for positively ionized compounds, and Phenobarbital-D5 was used as internal standard for negatively ionized compounds.

LC method

The HPLC used is a Thermo Scientific Accela 600 pump with Accela™ open autosampler. Mobile phases are 10 mM ammonium formate in water (A) and methanol (B), and acetonitrile:1-propanol:acetone (45:45:10) (C). The HPLC column used is a Thermo Hypersil GOLD PFP, 5 µm, 100 x 2.1 mm run under the gradient shown in Figure 1. Divert valve is set to waste from 0 to 1.8 minutes and to mass spectrometer from 1.8 minutes to the end of the run.

FIGURE 1. HPLC gradient method

Start (min)	Sec	Flow (mL/min)	%A	%B	%C
0.00	60	0.50	98	2	
1.0	660	0.50		100	
12.0	60	0.75		100	
13.0	30	1.00			100
13.5	60	2.00	98	2	
14.5	30	0.50	98	2	

Mass Spectrometry

Compounds are detected on an Exactive high performance bench-top mass spectrometer equipped with an Orbitrap mass analyzer. A schematic diagram of the new Exactive Plus instrument is illustrated in Figure 2. A HESI probe was used as an ion source.

The instrument was operating in alternating positive and negative full-scan and all-ion fragmentation mode. Relevant scan and source parameters are shown in Figures 3 and 4.

FIGURE 2. Schematic diagram of the new Exactive Plus high resolution accurate mass benchtop mass spectrometer.

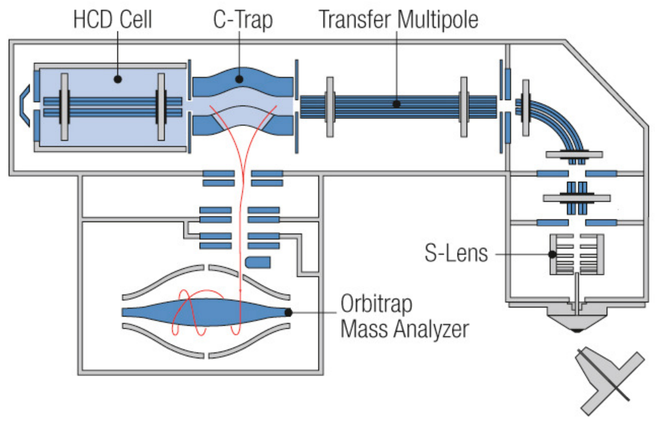


FIGURE 3. Scan Parameters for Exactive Mass Spectrometer

Parameter	Value
Full MS	
Microscans	1
Resolution	50,000
AGC Target	1e6
Maximum IT	100 msec
Scan Range-pos	135-2000 m/z
Scan Range-neg	100-2000 m/z
AIF	
Microscans	1
Resolution	25,000
AGC Target	1e6
Maximum IT	100 msec
NCE	35.0
Scan Range	50-1000 m/z

FIGURE 4. Source Parameters for HESI Probe.

Parameter	Value
Sheath Gas	30
Aux gas	15
Spray voltage	4
Capillary temp	320
Vaporizer Temp	350
Parameters are the same for positive and negative modes	

Study Design

40 urine samples were analyzed with LC-MS, GC-MS, Immunoassay and Remedy methods. Results were compared.

GC-MS method

Urine samples were hydrolyzed, processed with SPE procedure and derivatized with BSTFA. The analysis was performed with EI-GC-MS Thermo CSQ II. Compounds were separated on TR-5 MS capillary column and compound identities were established against standard libraries.

Immunoassay

Immunoassays for drugs of abuse were performed by EMIT technique on Siemens DB RxL instrument.

LC-MS data analysis

LC-MS data were analyzed with ExactFinder[TM] software. Chromatograms were reconstructed with mass accuracy of 5 ppm. ExactFinder processing method was set to identify compounds based on exact mass and retention time. Compounds were confirmed by fragments and isotopic pattern. Database containing 220 compounds with information required for compounds identification was created (Figure 5).

FIGURE 5. ExactFinder processing method and database.

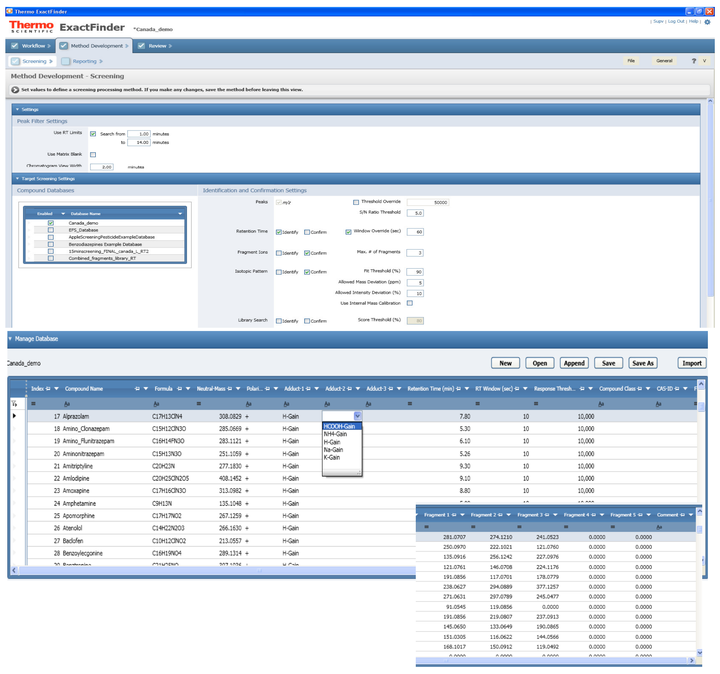
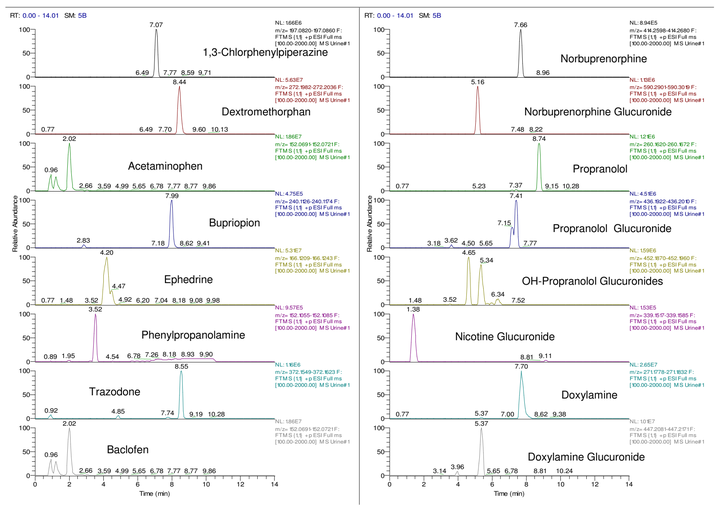


FIGURE 5. Chromatograms of some of the compounds detected in urine sample #1 reconstructed with mass accuracy of 5 ppm



Results

ExactFinder uses novel algorithms for background noise subtraction, isotopic pattern comparison and fragment matching. It also uses parameter less peak detection and integration algorithm. These features insure high confidence in reported results.

FIGURE 7. ExactFinder results page showing XIC chromatogram for Baclofen reconstructed with 5 ppm mass window (a), isotopic pattern (b) and fragment ion confirmation (c).

A list of compounds detected in selected urine samples using Immunoassay and Remedy, GC-MS and LC-MS methods are presented in Figure 8.

Based on the data collected for 40 urine samples we observe:

1.All compounds detected with Immunoassay and Remedy and GC-MS methods were also detected with LC-MS method.
2.GC-MS and LC-MS methods did not detect THC or THC metabolites in sample #6
3.Neither Remedy and Immunoassay together nor GC-MS methods can detect all compounds present in urine samples.
4.LC-MS method detected more compounds and more metabolites then the other analytical techniques

Some of targeted compounds (3-methoxytyramine, levamisole, ziprasidone) were not initially present in EaxctFinder database and were not detected by processing method.
Missing compounds were added to database, data was reprocessed and compounds were reported.

Conclusion

- LC-MS method identifies more compounds for forensic toxicologists then Remedy combined with Immunoassay and GC-MS techniques with the exception of low-level THC and THC metabolites.
- Immunoassay is more sensitive method for THC and THC metabolites detection than current LC-MS method which analyzes 20-fold diluted urine .
- LC-MS method implemented on Exactive ultra high resolution mass spectrometer allows for retrospective data analysis.
- LC-MS method uses simple urine dilution as sample preparation method which allows for faster data turn-around time when compared to GC-MS method
- Hydrolysis of urine samples is not required in LC-MS method since compound glucuronides (including very polar like nicotine and morphine glucuronides) can be identified.

FIGURE 8. Compounds detected with different techniques –data for selected samples

Sample	Remedy and Immunoassay	GC-MS	LC-MS
#1	•Dextrometorphan •Doxylamine •Bupriopion •Ephedrione	•Dextromethorphan •Doxylamine •Bupriopion •Ephedrine •Phenylpropanolamine •Propranolol •Nor-buprenorphine •Trazodone	•Dextromethorphan •Doxylamine •Bupriopion •Ephedrine •Phenylpropanolamine •Propranolol •Nor-buprenorphine •Trazodone •1,3-Chlorphenylpiperazine •Baclofen •Acetaminophen •Nicotine Glucuronide •Ketamine
#2	•Ketamine	•Ketamine •Nor-ketamine	•Ketamine •Nor-ketamine •Cotinine •Nicotine •Nicotine Glucuronide •Benzoylecgonine •Caffeine
#3	•Ritalinic acid •Trazodone •Hydroxy risperidone •EDDP •Methadone •Benzodiazepines	•Ritalinic acid •Trazodone •Hydroxy risperidone •EDDP •Methadone	•Ritalini acid •Trazodone •Hydroxy risperidone •EDDP •Methadone •7-Amino Clonazepam •Norfluoxetine •Acetaminophen •1,3-Chlorphenylpiperazine •Diphenhydrazine •Methylphenidate •Cotinine •Nicotine •Caffeine
#4	•Codeine •Tramadol •Propoxyphene	•Codeine •Tramadol •Naproxen	•Codeine •Tramadol •Naproxen •Desipramine •Caffeine •N-desmethyl-cis-tramadol •Tramadol Glucuronide
#6	•Methamphetamine •MDMA •Diphenhydramine •Cocaine •Methadone •EDDP •THC	•Methamphetamine •MDMA •Diphenhydramine •Cocaine •Methadone •EDDP •Temazepam •Benzoylecgonine	•Methamphetamine •MDMA •Diphenhydramine •Cocaine •Methadone •EDDP •Temazepam •Benzoylecgonine •Aminoclonazepam •MDA
#15	•Trazodone •Clonidine •Benzodiazepines	•Trazodone	•Trazodone •Clonidine •Oxazepan •Hydroxy nordiazepam •Naproxen
#16	•Fentanyl	•Fentanyl •Codeine •Nor codeine	•Fentanyl •Codeine •Codeine Glucuronide •Nor codeine •Nor codeine Glucuronide •Nor fentanyl •Acetaminophen •Nicotine •Nicotine Glucuronide •Cotinine
#22	•Ethopropazine •Zuclopenthixol •Benzodiazepines	•Ethopropazine •Zuclopenthixol •Promethazine	•Ethopropazine •Zuclopenthixol-OH •Promethazine •Lorazepam Glucuronide •Oxazepam Glucuronide
#23	•Psilocin	•Psilocin	•Psilocin •Acetaminophen •Cotinine •Nicotine Glucuronide

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