Forensic Screening for Drugs in Urine Using High-Resolution MS/MS Spectra and Simplified High-Performance Screening Software

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Application Note 616

Key Words
Q Exactive Focus, ToxFinder, forensic toxicology, screening, drugs of abuse

Goal

To evaluate the performance of the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap mass spectrometer as an LC-MS/MS screening platform for forensic detection and quantitation of very large numbers of drugs in human urine.

Introduction

Forensic toxicologists need an economical solution to screen for a virtually unlimited number of compounds in urine. Here we present a method for screening using a dilute-and-shoot approach with the Q Exactive Focus mass spectrometer and Thermo Scientific™ ToxFinder™ software.

Experimental Methods

Sample Preparation

Samples were processed by simple dilution. Briefly, an aliquot of centrifuged urine was spiked with stable-isotope-labeled internal standard and diluted 30-fold before an aliquot was analyzed by gradient HPLC on a Q Exactive Focus mass spectrometer. No hydrolysis was performed. The internal standard used was tolbutamide-d_9. This compound was used for its versatility because it ionizes in both positive and negative mode. Limits of detection were determined by spiking compounds of interest into pooled blank urine in the range of 1 to 500 ng/mL.

Liquid Chromatography

Gradient elution was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC with OAS autosampler (Figure 1). Mobile phases consisted of 10 mM ammonium formate in water and methanol (Fisher Chemical brand) for mobile phases A and B, respectively. The column used was a Thermo Scientific™ Accucore™ PFP column, 2.6 µm, 100 x 2.1 mm fused core (PN 17426-102130). The gradient was run at a flow rate of 0.45 mL/min from 5 to 100% mobile phase B over 6 minutes followed by a column wash and re-equilibration to starting conditions. The total run time was 10 minutes.

Figure 1. Q Exactive Focus MS with UltiMate 3000 RSLC pump and UltiMate 3000 OAS autosampler.

Mass Spectrometry

Compounds were detected on a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer equipped with a Thermo Scientific™ Ion Max source and heated electrospray ionization (HESI II) sprayer. Data were acquired in full-scan data-dependent MS^2 ($ddMS^2$) mode. In this mode, both positive and negative high-resolution, full-scan data at resolution of 70k were collected, then MS^2 spectra at a resolution of 17.5k were triggered for compounds entered in the inclusion list (Figure 2). Figure 3 shows an example of the data acquired with this method.

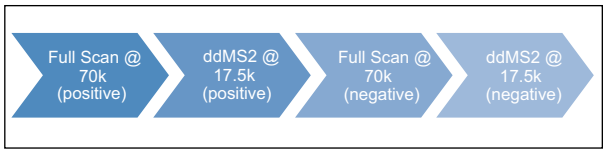

Figure 2. Diagram of data-dependent MS^2 method for detection and quantitation of drugs in urine.

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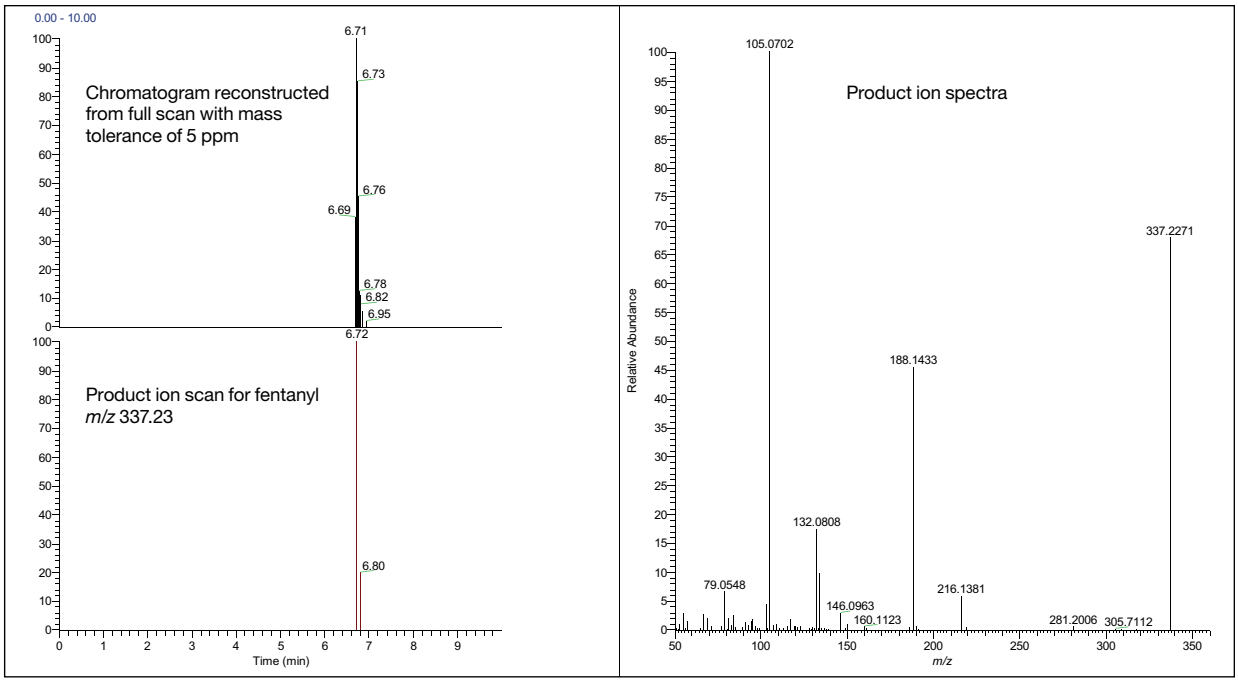

Figure 3. Fentanyl data, showing full scan at 5 ppm, product ion scan, and product ion spectra.

Method Evaluation

Three hundred compounds, both positively and negatively ionizing, from different classes including therapeutic drugs, drugs of abuse, and environmental toxins, were selected for evaluation. Spiking solutions of 8–10 compounds each were prepared and used to make test mixes at concentration of 500, 100, 50, 10, 5, and 1 ng/mL in pooled donor urine.

Test mixes were processed using the previously described sample preparation procedure and then analyzed. Additionally, positive donor samples from a collaborator laboratory were processed and analyzed.

Data Analysis

Data was processed using ToxFinder software. ToxFinder software uses a database that contains compound-related information and tolerances for identification. It also utilizes proprietary spectral libraries including forensic toxicology libraries containing drugs of abuse, therapeutic drugs, and environmental toxins, and food safety and environmental libraries containing pesticides, mycotoxins, veterinary drugs, and PFCs. Other important features include semi-quantitation, relative retention time calculation, a custom reporting package, and easy output for importation into LIMS systems.

The ToxFinder software database and libraries are combined into a processing method (Figure 4). After a method is created, the analyst imports a sample list and processes the data. Results can be printed immediately or reviewed before printing (Figure 5).

In this note, the primary method identified compounds based on retention time, accurate *m/z*, and spectral library matching. The LOD/cut-off for each compound was determined to be the lowest spiked concentration in which peaks were identified by the ToxFinder software. If even greater identification confidence is required, isotopic pattern matching can also be added to the method parameters. Table 1 shows the parameters used in each method.

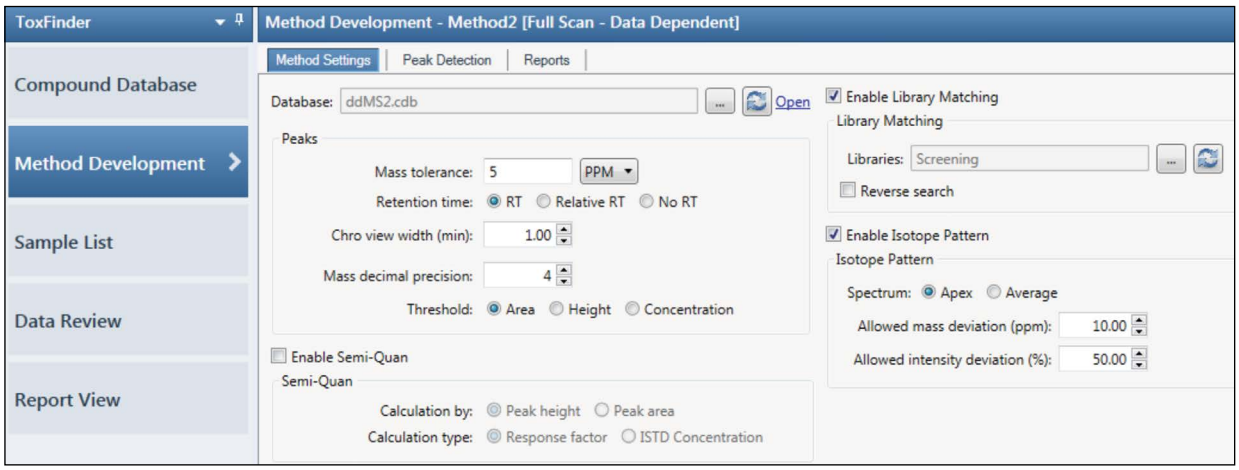

Figure 4. Example of a ToxFinder method using library matching (Method #2).

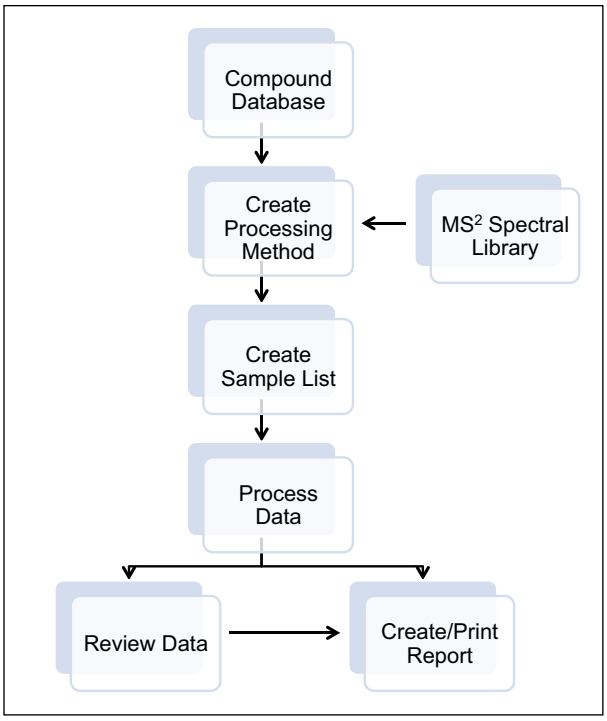

Figure 5. ToxFinder software workflow.

Table 1. ToxFinder method parameters used for compound identification.

Method 1	Method 2
Accurate *m/z*	Accurate *m/z*
Retention time	Retention time
Library search	Library search
—	Isotopic pattern

Results

Using the primary data processing method, the vast majority of compounds analyzed had detection limits at or below 10 ng/mL. Figure 6 shows the number of compounds detected at each concentration, and Table 2 shows the limits of detection for all compounds. When the additional requirement of isotopic pattern matching was employed, the limits of detection were slightly higher. This is to be expected because of the naturally lower abundance of isotopic ions. Figure 7 shows the ToxFinder software Data Review page with precursor scan, library search results matching, and isotopic pattern comparison results for oxycodone. Complete results for this method are again shown in Figure 6 and Table 2.

Figure 8 shows reconstructed chromatograms for compounds detected in a donor urine sample. Figure 9 shows the ToxFinder results for cyclobenzaprine from the same samples.

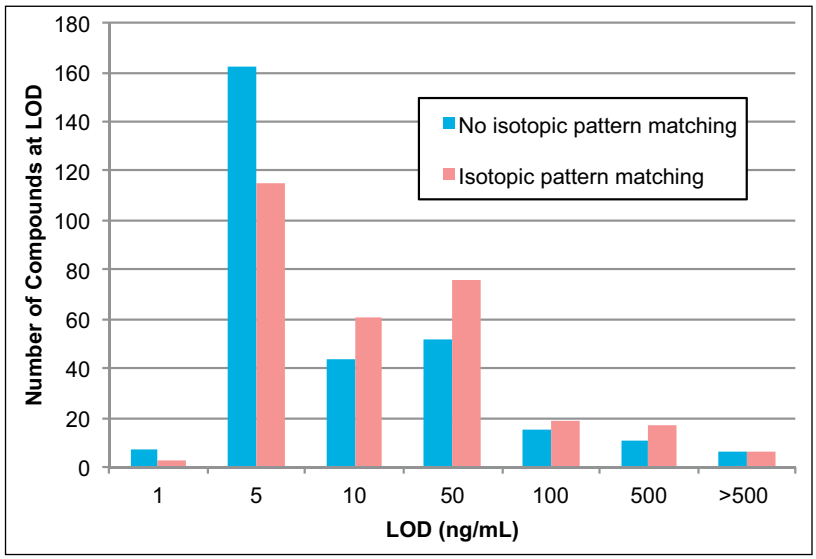

Figure 6. Number of compounds at each limit of detection using ToxFinder methods with and without isotopic pattern matching.

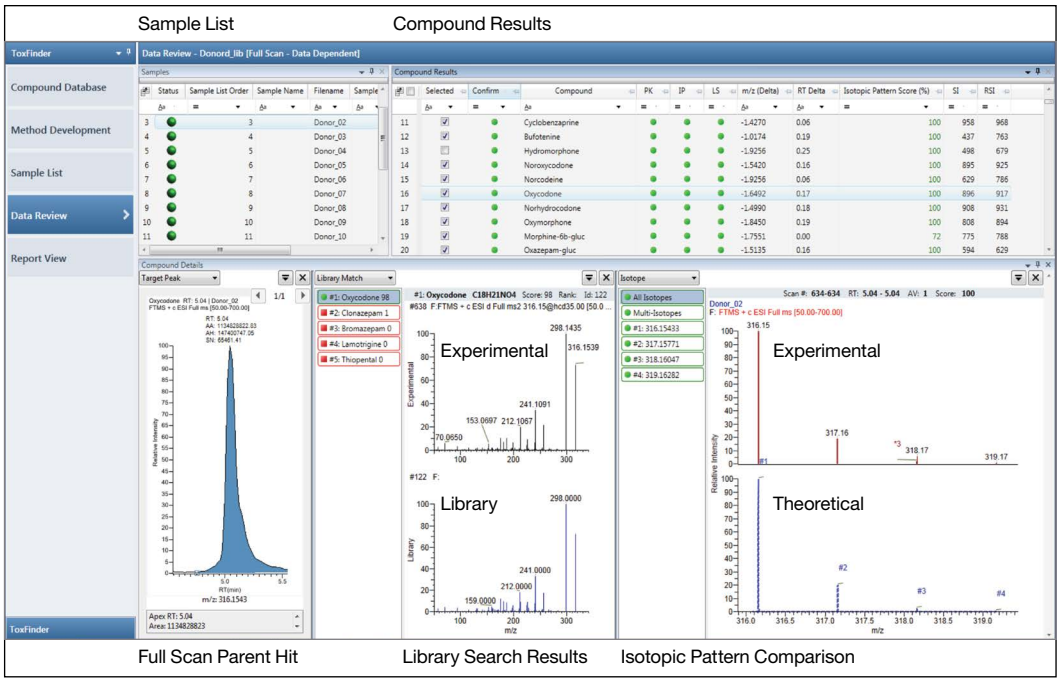

Figure 7. ToxFinder software data review page.

Table 2. Limits of detection for compound, with and without isotopic pattern matching.

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
1-(3-Chlorophenyl)-Piperizine	5	5
1,1-Dimethylbiguanide	50	50
2-Bromo-Alpha-Ergocryptine	10	50
4bromo-2,5dimethoxyphenethylamine	5	10
6-Acetylcodeine	5	5
6-Acetylmorphine	50	5
7-Amino-Flunitrazepam	10	10
Acebutolol	5	5
Acetaminophen	5	5
Albuterol	5	10
Allobarbital	100	500
Alprazolam	5	5
Alprenolol	50	50
Aminorex	5	5
Amitriptyline	1	5
Amobarbital	100	500
Amoxapine	5	10
Amphetamine	5	10
AnhydroecgonineMethylEster	1	5
Antipyrine	5	5
Apomorphine	500	500
Aprobarbital	100	500
Astemizole	50	500
Atenolol	5	5
Atropine	5	5
Barbital	>500	>500
BDB	5	5
Benzocaine	10	10

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
Benzoylecgonine	5	5
Benzylpiperazine	5	10
Betamethasone	50	50
Betaxolol	5	10
Bisoprolol	10	10
Bromazepam	50	50
Brompheniramine	5	10
Bufotenine	10	50
Bupivocaine	5	5
Buprenorphine	5	10
Buprenorphine-glucuronide	>500	>500
Buspirone	1	1
Butabarbital	100	500
Butorphanol	5	5
Caffeine	5	5
Carbamazepine	5	5
Carbinoxamine	5	5
Chloroquine	500	500
Chlorothiazide	50	50
Chlorpromazine	10	50
Chlorprothixene	10	50
Cimetidine	10	100
Cinnarizine	100	100
Ciprofloxacin	100	100
Cisapride	5	50
Citalopram	5	5
Clozapine	5	10
Clenbuterol	10	50

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
Clobazam	5	5
Clomipramine	5	10
Clonazepam	10	10
Clozapine N-Oxide	50	50
Cocaethylene	5	5
Cocaine	5	5
Codeine	5	5
Codeine-glucuronide	>500	>500
Coumetetrayl	5	5
Cyclobenzaprine	5	5
Desalkylflurazepam	10	10
Desipramine	5	5
Desmethyl-citalopram	5	5
Desmethyl-clomipramine	5	5
Desmethyldoxepin	5	5
Desmethyl-flunitrazepam	10	50
Desmethyl-selegiline	5	5
Dexamethasone	50	50
Dextromethorphan	5	5
Diazepam	5	10
Diclofenac	10	50
Dihydrocodeine	5	5
Disopyramide	1	5
Dothiepin	10	50
Doxepin	5	5
Doxylamine	5	5
EcgonineMethylEster	5	5
EDDP	5	5
EMDP	10	10
Enalapril	10	10
Ephedrine	10	10
Estazolam	5	5
Ethylamphetamine	5	5
Etomidate	5	5
Fendiline	10	10
Fenoprofen	500	500
Fentanyl	1	1
Flecainide	5	5
Flumethasone	50	50
Flunitrazepam	5	5
Flunixin	5	5
Fluoxetine	10	10
Fluphenazine	50	50
Flurazepam	5	5
Flurbiprofen	>500	>500
Fluvoxamine	5	10
Furosemide	100	100
Gabapentin	10	50
Glafenine	10	10
Gliclazide	5	50

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
Glimepiride	50	100
Glipizide	50	100
Glutethimide	50	50
Glyburide	50	100
Haloperidol	5	50
Heroin	500	500
Hexobarbital	500	500
HMMA	50	50
Hydrocodone	10	10
Hydromorphone	5	10
Hydroxy-Benzoylecgonine	5	5
Hydroxy-Ethyl-Flurazepam	50	50
Hydroxy-Midazolam	50	50
Hydroxy-Nordiazepam	5	5
Hydroxy-Triazolam	50	50
Hydroxyzine	50	50
Ibogaine	5	5
Imipramine	5	5
Indomethacin	1	5
Isocaffeine	5	5
Isoproterenol	500	500
Ketamine	5	10
Ketoconazole	10	50
Ketoprofen	10	10
Ketorolac	5	50
Labetalol	50	50
Lamotrigine	5	5
Levotiracetam	50	50
Lidocaine	5	5
Loratadine	50	50
Lorazepam	10	50
Lorazepam-glucuronide	>500	>500
Lormetazepam	5	50
LSD	5	5
Malathion	50	100
Maprotiline	5	5
MBDB	5	5
MDA	5	5
MDEA	5	5
MDMA	5	5
MeclofenamicAcid	500	500
Meperidine	5	5
Mepivacaine	5	5
Meprobamate	50	50
Mescaline	10	50
Mesoridazine	5	50
Metaproterenol	10	50
Methadone	5	5
Methamphetamine	5	10
Methaqualone	5	5

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
Methohexital	500	500
Methotrexate	50	50
Methoxyverapamil	5	5
Methylphenydate	5	5
Methyprylon	5	5
Metoclopramide	5	5
Metronidazole	5	50
Mexiletine	5	5
Mianserin	5	5
Miconazole	500	500
Midazolam	5	5
Mirtazapine	5	5
Molsidomine	5	10
Morphine	1	1
Morphine-3-beta-glucuronide	100	500
Morphine-6-beta-glucuronide	>500	>500
Nabumetone	100	100
N-Acetylprocainamide	5	10
Nalbuphine	5	5
Nalorphine	5	10
Naloxone	5	5
Naltrexol	5	5
Naltrexone	5	5
Naproxen	10	50
N-desmethyl-cis-tramadol	50	50
N-Desmethylselegiline	10	10
N-Desmethyltrimipramine	5	5
Nicardipine	50	50
Nifedipine	50	50
Nimodipine	50	50
Nitrazepam	5	5
Nitrendipine	50	50
Nizatidine	50	50
Norbenzoylecgonine	5	10
Norbuprenorphine	10	50
Norcocaethylene	10	10
Norcocaine	5	5
Norcodeine	10	10
Nordiazepam	5	5
Nordoxepin	5	5
Norfentanyl	5	5
Norfluoxetine	50	50
Norhydrocodone	10	10
Norketamine	5	10
Nor-LSD	50	50
Normeperidine	5	50
Normorphine	10	50
Noroxycodone	5	10
Noroxymorphone	50	50
Norpropoxyphene	50	50
Nortriptyline	5	5

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
Noscapine	10	10
O-demethyl-cis-tramadol	10	10
O-desmethyl-venlafaxine	50	50
Ondansetron	5	5
Opipramol	5	50
Oxazepam	5	5
Oxazepam-glucuronide	>500	>500
Oxcarbazepine	50	50
Oxycodone	5	10
Oxymorphone	5	10
Papaverine	5	5
Paraxanthine	5	10
Paroxetine	10	10
Pentazocine	5	10
Pentobarbital	50	100
Perphenazine	50	100
Phenobarbital	50	100
Phenolphthalein	50	50
Phentermine	10	10
Phenylpropanolamine	10	50
Phenyltoloxamine	5	5
Phenytoin	500	500
Physostigmine	5	5
Pindolol	5	5
Piroxicam	100	100
PMA	5	50
PMMA	5	5
Prazosin	5	5
Prilocaine	5	5
Primidone	50	50
Procainamide	5	5
Procaine	5	5
Promazine	5	10
Promethazine	50	50
Prometryn	100	100
Propafenone	5	10
Propoxyphene	10	10
Propranolol	5	5
Protriptyline	5	5
Pseudoephedrine	5	5
Pyrilamine	5	5
Quetiapine	5	50
Quinidine	5	5
Quinine	1	1
Ranitidine	50	50
Risperidone	5	5
Ritalinic Acid	5	10
Scopolamine	5	10
Secobarbital	100	500
Selegiline	50	50
Sertraline	10	50

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
Sotalol	5	5
Spironolactone	50	50
Strychnine	10	10
Sufentanil	50	50
Sulindac	10	50
Sulpiride	5	5
Tamoxifen	100	100
Tapentadol	5	5
Telmisartan	5	10
Temazepam	50	50
Tenoxicam	5	50
Terbutaline	5	10
Terfenadine	50	10050
Tetracaine	50	5
Theophylline	5	100
Thiopental	100	100
Thioridazine	100	50
Thiothixene	5	100
Tiagabine	5	5
Tiapride	5	5

Analyte	LOD (ng/mL)	
	No Isotopic Pattern Matching	Isotopic Pattern Matching
Timolol	5	5
Tolmetin	5	10
Topiramate	5	5
Tramadol	5	10
Trazodone	10	10
Triazolam	5	10
Trifluoperazine	10	50
Trimethoprim	5	5
Trimipramine	5	5
Triprolidine	5	5
Venlafaxine	5	105
Verapamil	5	5
Vincamine	5	5
Warfarin	5	5
Zaleplon	5	5
Zimelidine	5	10
Zolpidem	5	5
Zolpidem phenyl-4-COOH	10	10
Zonisamide	5	10
Zopiclone	500	500

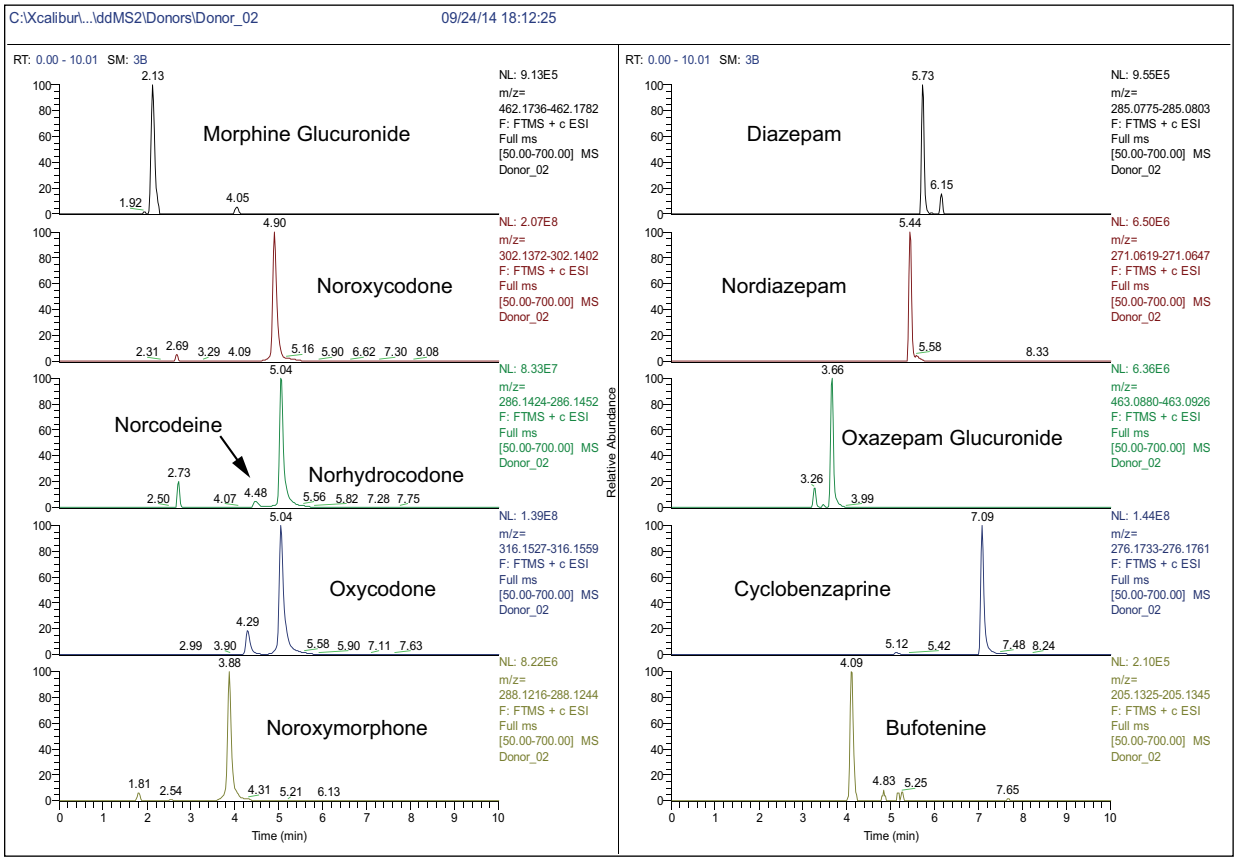

Figure 8. Donor #2 urine analysis results - identified compounds.

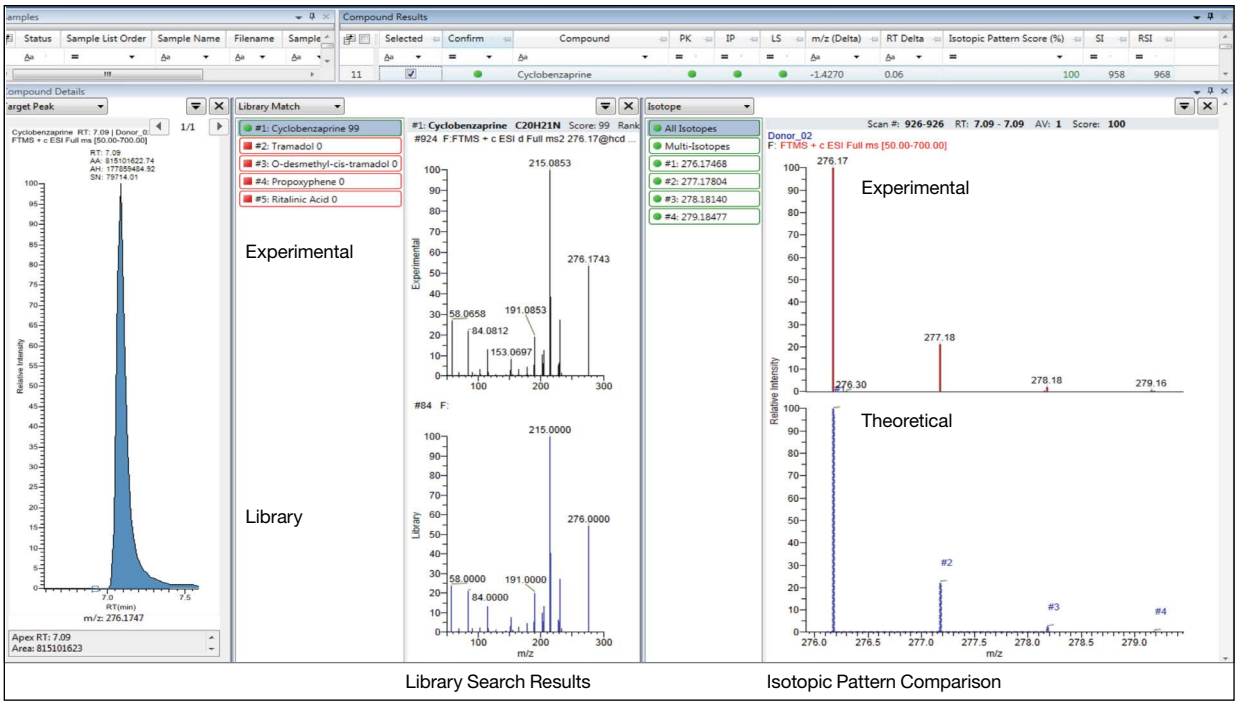

Figure 9. Cyclobenzaprine identified in donor sample.

Conclusion

- A urine screening method for about 300 compounds, both positively and negatively ionizing, including drugs of abuse and environmental toxins, was successfully evaluated.
- Collected data demonstrated good method sensitivity and specificity in diluted urine samples.
- ToxFinder software's simple user interface enabled quick method development and rapid data review.
- The Q Exactive Focus mass spectrometer and ToxFinder software together provided high confidence in data output by combining the power of an Orbitrap mass analyzer with the comprehensive identification software workflow.

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