Screening of 300 Drugs in Blood Utilizing Second Generation Exactive Plus High-Resolution, Accurate Mass Spectrometer and ExactFinder Software

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Overview

Purpose: Evaluate Thermo Scientific Exactive Plus high performance bench-top mass spectrometer for drug screening of whole blood for forensic toxicology purposes.

Methods: Whole blood samples were processed by precipitation with $ZnSO_4$/ methanol. Samples were injected onto an HPLC under gradient conditions and detected on an Exactive™ Plus mass spectrometer. Results were analyzed with Thermo Scientific ExactFinder software.

Results: Over 400 drugs were detected at LODs ranging from 5-100 ng/mL in whole blood.

Introduction

Forensic scientists and toxicologists need to search for many different compounds in samples of human blood. Endogenous matrix components and the wide variety of possible compounds make the task daunting. The second generation Exactive Plus high-resolution accurate mass spectrometer with fast polarity switching enables identification of compounds in a wide chemical space with minimum interference from endogenous compounds. Additionally, full scan data allows for retrospective analysis of data for previously unknown compounds.

Methods

Sample Preparation

Mix 50 µL of blood with 5 µL of internal standard solution containing reserpine and tolbutamide-d9. Add 150 µL of 40mM $ZnSO_4$ in 66% methanol and vortex immediately. Place samples in a –20°C freezer for 20-30 minutes. Centrifuge at 13,000 rpm for 10 minutes. Transfer supernatant to and autosampler vial, cap, and inject 20 µL onto HPLC system.

Liquid Chromatography

The HPLC used is a Thermo Scientific Accela 600 pump with Accela™ open autosampler. Mobile phases are 10 mM ammonium acetate in water (A), 0.1% formic acid in acetonitrile (B), and acetonitrile:1-propanol:acetone (45:45:10) (C). The HPLC column used is a Thermo Hypersil GOLD, 5 µm, 100 x 3 mm run under the gradient shown in Figure 1.

FIGURE 1. HPLC gradient method

Start (min)	Sec	Flow (mL/min)	%A	%B	%C
0.00	60	0.50	95	5	
1.0	660	0.50		100	
12.0	60	0.75		100	
13.0	30	1.00			100
13.5	60	2.00	95	5	
14.5	30	0.50	95	5	

Mass Spectrometry

Compounds are detected on a Exactive Plus high performance bench-top mass spectrometer equipped with an Orbitrap™ mass analyzer. A schematic diagram of the Exactive Plus instrument is illustrated in Figure 2.

An atmospheric pressure chemical ionization (APCI) probe was used as an ion source. The instrument was operating in alternating positive and negative full-scan and all-ion fragmentation (AIF) mode with fast polarity switching at a resolution of 70,000 to 35,000 (FWHM) at *m/z* 200.

The Exactive Plus has an S-lens for improved ion transmission. The performance and robustness of the Automatic Gain Control (AGC) is improved using a C-Trap charge detector (CTCD). The higher-energy collisional dissociation (HCD) cell collects fragment-rich spectra similar to those generated in the collision cell of a triple stage quadrupole instrument. Relevant scan and source parameters are shown in Figure 3 and 4.

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

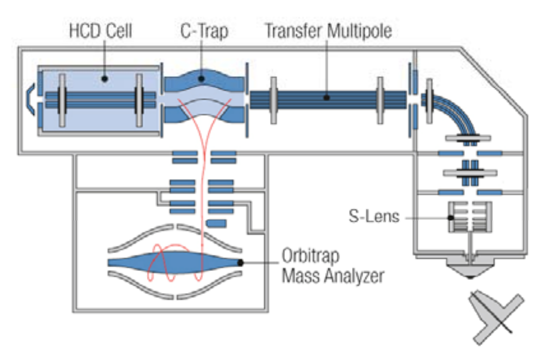


FIGURE 3. Scan Parameters for Exactive Plus Mass Spectrometer

Parameter	Value
Full MS	
Micro scans	1
Resolution	70,000
AGC Target	3e6
Maximum IT	200 msec
Scan Range	*m/z* 120-1200
AIF	
Micro scans	1
Resolution	35,000
AGC Target	3e6
Maximum IT	100 msec
NCE	35.0
Scan Range	*m/z* 80-1200
Parameters are the same for positive and negative mode	

FIGURE 4. Source Parameters for APCI Probe.

Parameter	Value
Sheath Gas	35
Aux gas	15
Sweep gas	1
Discharge current	4
Capillary temp	320
S-Lens RF Level	60
Vaporizer Temp	350

Study Design

To determine LOD for each compound, aliquots of whole blood were spiked with sets of compound at 5, 10, 20, 50, and 100 ng/mL. Matrix effects were determined by processing a sample spiked in water in place of blood.

Data Analysis

Chromatograms are reconstructed with a mass tolerance of 5 ppm. Compounds are identified based on exact mass and retention time; confirmation is with fragments and isotopic pattern using ExactFinder™ software. Figure 5 shows the ExactFinder method used in this study along with the compound database.

Results

Over 400 different compounds were tested in this study: 487 were detected at 5 ng/mL, 39 at 10 ng/mL, 10 at 20 ng/mL, 18 at 50 ng/mL, and 7 at 100 ng/mL. Figure 6 shows a representative chromatogram. Fast polarity switching enabled identification of both positively and negatively charged species in one analytical run. Average mass accuracy was <3 ppm. Figure 7 shows the limits of detection of compounds in this study.

FIGURE 5. ExactFinder processing method and database.

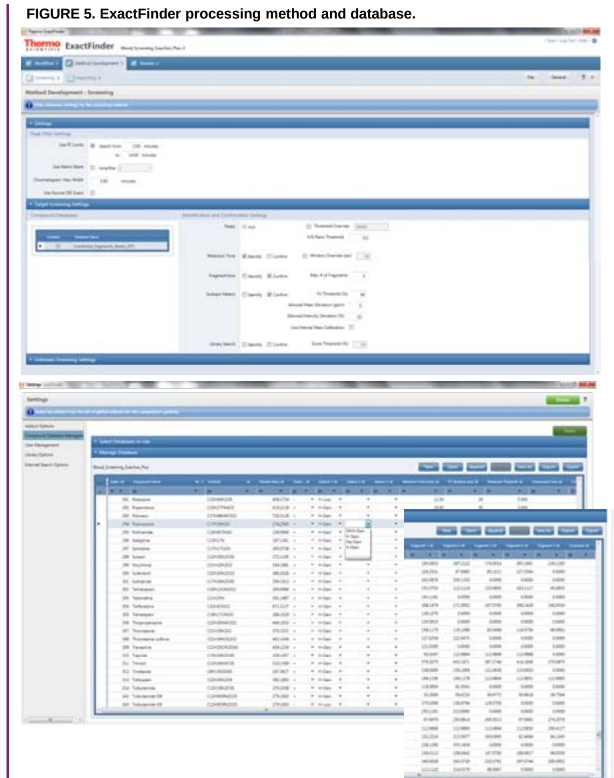


FIGURE 6. Chromatograms reconstructed with a 5 ppm mass window at 5 ng/mL.

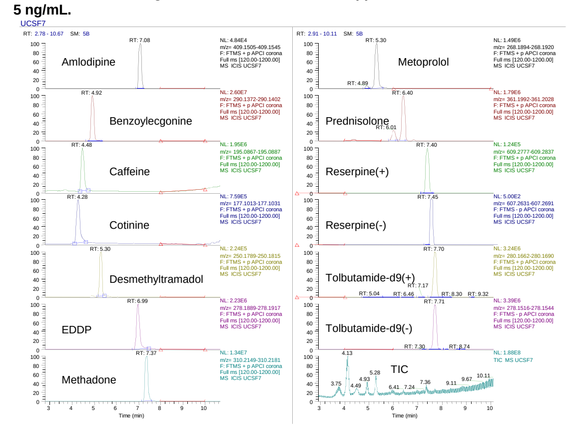


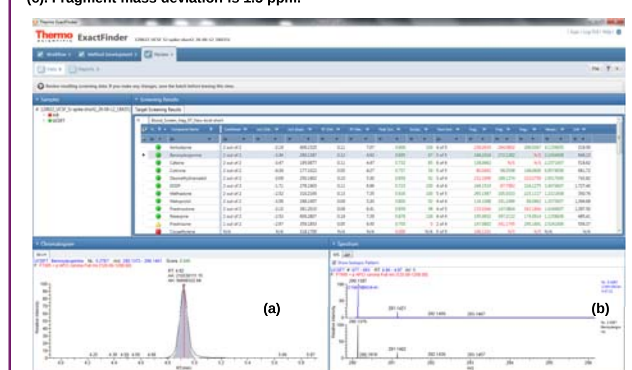
FIGURE 7. LODs for around 490 compounds

Compound	LOD	Compound	LOD	Compound	LOD	Compound	LOD
1-(3-Chlorophenyl)piperazine	5	Deacetyl Diltiazem	5	Lormetazepam	5	Perimetazine	5
10-hydroxycarbazepine	5	Demexiptiline	5	Loxapine	5	Phenacetin	5
5-(p-Methylphenyl)-5-phenylhydantoin	10	Des(2-hydroxyethyl)opipramol	5	LSD	5	Phenazone	5
6-Acetylcodeine	100	Desalkylflurazepam	5	Maprotiline	5	Pheniramine	5
6-Methylthiopurine	5	Desipramine	5	Maraviroc	5	Phenobarbital	5
6-Monoacetylmorphine	5	Desmethylcitalopram	5	MBDB	5	Phenylbutazone	5
7(2,3dihydroxypropyl)Theophylline	5	Desmethylclozapine	50	MDEA	5	Phenytoin	10
7-Aminoclonazepam	5	Desmethyldoxepin	5	MDMA	10	Pholcodine	5
7-Aminoflunitrazepam	5	Desmethylmirtazapine	5	Meclofenamic Acid	10	Pimozide	5
9-hydroxy-risperidone	5	Desmethylnefopam	5	Medazepam	5	Pindolol	5
Acebutolol	5	Desmethyltramadol	5	Medifoxamine	5	Pipamperone	5
Acenocoumarol	5	Dexamethasone	5	Mefloquine	5	Pipotiazine	5
Acepromazine	5	Dextromethorphan	5	Melatonin	5	Piroxicam	5
Aceprometazine	5	Dextropropoxyphene	5	Meperidine	5	Pizotifen	5
Acetazolamide	5	Dextrorphan	10	Mephenesin	10	Posaconazole	50
Ajmaline	5	Diazepam	5	Mepivacaine	5	Pramipexole	5
Albuterol	20	Diazoxide	5	Meprobamate	5	Prazepam	5
Alfentanyl	5	Dibenzepine	5	Mescaline	10	Prazepam-d5	5
Alfuzosine	5	Dihydrocodeine	5	Mesoridazine	5	Prednisolone	5
Alimemazine	5	Diltiazem	5	Metapramine fumarate	50	Prednisone	5
Allobarbital	10	Dimetotiazine	5	Metenolone	20	Primaquine	5
Alpha-hydroxy-midazolam	5	Diphenhydramine	5	Methadone	5	Primidone	10
Alprazolam	5	Dipyridamole	5	Methamphetamine	5	Procainamide	50
Amantadine	5	Disopyramide	5	Methandienone	100	Prochlorperazine	5
Amidopyrine	5	Dobutamine	100	Methaqualone	5	Procyclidine	5
Amiloride	5	Domperidone	5	Methcathinone	10	Proguanil	5
Amineptine	5	Dosulepin	5	Methohexital	10	Promazine	10
Amiodarone	5	Doxepine	5	Methoxsalen	5	Promethazine	5
Amisulpride	5	Doxorubicin	5	Methoxyamphetamine	50	Prometryn	5
Amitriptyline	5	Droperidol	5	Methoxymethamphetamine	5	Propafenone	5
Amlodipine	5	Dropopizine	5	Methylene Blue	10	Propericiazine	5
Amobarbital	5	Duloxetine	5	Methylparaben	10	Propizepine	5
Amoxapine	10	EDDP	5	Methylphenidate	5	Propranolol	5
Ampicillin	20	Efavirenz	5	Methylprednisolone	10	Protriptyline	5
Amprenavir	5	EMDP	5	Metobromuron	5	Pseudoephedrine	10
Antipyrine	5	Enalaprilat	5	Metoclopramide	5	Pyrilamine	5
Apocodeine	5	Ephedrine	20	Metoprolol	5	Quinidine	5
Aprindine	5	Escitalopram	5	Metronidazole	5	Quinine	5
Aprobarbital	5	Esmolol	5	Mexiletine	5	Quinupramine	5
Astemizole	5	Estazolam	5	Mianserine	5	Raltegravir	5
Atazanavir	5	Ethyl Loflazepate	5	Miconazole	20	Ramipril	5
Atenolol	5	Ethylamphetamine	10	Midazolam	5	Ranitidine	5
Atropine	5	Ethylmorphine	5	Milnacipram	5	Repaglinide	5
AZT	50	Etifoxine	5	Mirtazapine	5	Riboflavin	5
Baclofen	5	Etodolac	5	Molsidomine	5	Rifabutin	5
Bendroflumethiazide	5	Etomidate	5	Morphine	5	Risperidone	5
Benperidol	5	Etoposide	5	Mycophenolic acid	5	Ritonavir	5
Benzoylecgonine	5	Felbamate	5	Nadolol	5	Ropivacaine	5
Bepridil	5	Felodipine	5	Nalbuphine	5	Roxithromycin	5
Betamethasone	5	Fenbufen	20	Nalorphine	5	Rufinamide	5
Betaxolol	5	Fenfluramine	5	Naloxone	5	Salicylamide	50
Bezafibrate	5	Fenofibrate	5	Naltrexone	5	Saquinavir	5
Biperiden	5	Fenoterol	5	N-DemethylTrimipramine	5	Scopolamine	5
Bisoprolol	5	Fenspiride	5	N-Desmethyl Clomipramine	5	Secobarbital	5
Brallobarbital	5	Finasteride	5	N-Desmethyl Mephenytoin	5	Selegiline	5
Bromazepam	10	Flecainide	5	N-Desmethylclobazam	5	Sertraline	5
Brompheniramine	5	Fluconazole	5	N-Desmethylflunitrazepam	5	Sotalol	5
Buflomedil	10	Flumethasone	5	N-Desmethyltrimipramine	5	Stanozolol	10
Bupivacaine	5	Flunarizine	20	N-Desmethylvenlafaxine	5	Strychnine	5
Bupropion	5	Flunitrazepam	5	Nebivolol	5	Sufentanil	5
Buspirone	5	Flunixin	5	Nefazodone	5	Sulfamethoxazole	5
Butabarbital	5	Fluoxetine	5	Nelfinavir	5	Sulindac	5
Butabital	5	Fluoxymesterone	5	Nelfinavir M8	5	Sultopride	5
Butalbital	5	Flupentixol	5	Netilmicin	5	Tacrine	5
Butorphanol	5	Fluphenazine	5	Nevirapine	5	Telmisartan	5
Cafeine	5	Flurazepam	5	Nialamide	50	Temazepam	5
Carbamazepine	5	Fluvoxamine	5	Niaprazine	5	Terbinafine	5
Carbamazepine epoxyde	5	Furosemide	50	Nicardipine	5	Terfenadine	5
Carbinoxamine	5	Galantamine	5	Nifedipine	5	Tetracaine	5
Carbutamide	5	Glafenine	5	Niflumic Acid	5	Tetrazepam	5
Carpipramine	5	Glibenclamide	5	Nimodipine	5	Theophylline	10
Carvedilol	5	Glibornuride	5	Nitrazepam	5	Theophylline-7-acetic acid	5
Celiprolol	5	Gliclazide	5	Nitrendipine	5	Thiopental	5
Cetirizine	5	Glipizide	5	Nizatidine	5	Thioproperazine	5
Chloramphenicol	10	Glyburide	50	Nomifensine	5	Thioridazine	20
Chlordiazepoxide	5	Haloperidol	5	Norbenzoylecgonine	5	Thioridazine sulfone	5
Chlormezanone	20	Haloperidol-d4	5	Norcocaine	10	Tianeptine	5
Chloroquine	10	Heptabarbital	5	Norcodeine	5	Tiapride	5
Chlorpheniramine	10	Heroin	10	Nordazepam	5	Ticlopidine	5
Chlorproethazine	5	Hexobarbital	5	Norpropoxyphene	50	Timolol	5
Chlorpromazine	10	Hydrochlorothiazide	5	Norethandrolone	50	Tinidazole	5
Chlorpropamide	5	Hydrocodone	5	Norfloxacin	5	Tofisopam	5
Chlorprothixene	5	Hydromorphone	5	Norfluoxetine	5	Tolbutamide	5
Chlortalidone	5	Hydroquinine	5	Norfluvoxamine	5	Tolmetin	5
Cilazapril	5	Hydroxychloroquine	5	Norketamine	5	Tramadol	5
Cimetidine	5	HydroxyItraconazole	5	Nor-LSD	5	Trans-2-Phenylcyclopropylamine	10
Cinchonine	5	Hydroxyzine	5	Normaprotiline	5	Trazodone	5
Cinnarizine	5	Ibogaine	5	Normeperidine	20	Triamterene	5
Ciprofloxacin	5	Imatinib	5	Normesuximide	5	Triazolam	5
Cisapride	5	Imipramine	5	Normorphine	10	Trifluoperazine	50
Clemastine	5	Indalpine	5	Noroxycodone	5	Trifluperidol	5
Clenbuterol	5	Indinavir	5	Northiaden	5	Triflupromazine	5
Clindamycin	50	Indometacin	5	Nortriptyline	5	Trihexyphenidyl	5
Clobazam	5	Irebsartan	5	Norverapamil	5	Trimeprazine	5
Clomethiazole	5	Isocarboxazide	5	Noscapine	5	Trimetazidine	5
Clomipramine	5	Itraconazole	5	Ofloxacin	5	Trimethoprim	5
Clonidine	5	Ketamine	5	Olanzapine	5	Trimipramine	5
Clopidogrel	5	Ketoconazole	10	Ondansetron	5	Tropatepine	5
Clotiazepam	5	Ketoprofen	5	Opipramol	5	Urapidil	5
Cloxacillin	100	Ketorolac	5	Orphenadrine	5	Valsartan	50
Clozapine	5	Ketotifen	5	Oxatomide	5	Vecuronium	100
Clozapine N-oxide	5	Lacidipine	50	Oxazepam	5	Venlafaxine	5
Cocaethylene	5	Lacosamide	5	Oxcarbazepine	5	Verapamil	5
Cocaine	10	Lamotrigine	5	Oxprenolol	5	Viloxazine	5
Codeine	5	LAMPA	5	Oxycodone	5	Vinbarbital	10
Colchicine	5	Levofloxacin	5	Oxymorphone	10	Vincristine	5
Cotinine	5	Levomepromazine	10	Pancuronium	50	Vinylbital	5
Cyamemazine	5	Levorphanol	10	Papaverine	5	Voriconazole	5
Cyclobenzaprine	5	Lidocaine	5	Paroxetine	5	Yohimbine	5
Cyclophosphamide	5	Linezolid	5	Penbutolol	5	Zimelidine	5
Cyproheptadine	5	Lisinopril	100	Penfluridol	5	Ziprasidone	5
Danazol	5	Lopinavir	5	Pentamidine	5	Zolpidem	5
Dantrolene	100	Loprazolam	5	Pentazocine	5	Zonisamide	10
Dapsone	5	Loratadine	5	Pentobarbital	5	Zopiclone	5
Darunavir	5	Lorazepam	5	Perazine	5	Zuclopenthixol	5

For targeted screening, ExactFinder uses parameters set in processing method to identify and confirm the presence of compound based on database values. Figure 8 shows data review results for one donor sample. In this method, compounds were identified by accurate mass within 5 ppm and retention time. Identity was further confirmed by isotopic pattern and presence of known fragments.

Matrix effects were observed to be compound dependent and were generally within ±50%. Figure 9 shows peaks for hydrocodone in water and blood to compare effect of matrix on signal intensity.

FIGURE 8. ExactFinder results page showing XIC chromatogram reconstructed wit h 5 ppm mass window (a), isotopic pattern (b) and fragment ion confirmation (c). Fragment mass deviation is 1.5 ppm.



ExactFinder utilizes novel algorithms for background noise subtraction, peak detection and integration that require no user input.

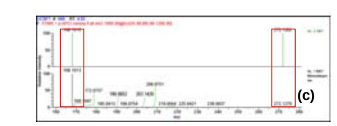
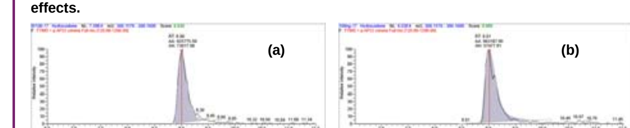


FIGURE 9. XIC of hydrocodone in water (a) and blood (b) showing matrix effects.



Conclusion

- We have developed a screening method for over 400 compounds in whole blood with LODs of 5-100 ng/mL.
- Matrix effects are compound dependent and ±50%.
- Combination of high-resolution, accurate mass and fast polarity switching allows for rapid and confident identification in whole blood matrix for forensic toxicology purposes.

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