# 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<sub>4</sub>/ 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 300 drugs were detected at LODs ranging from 5-100 ng/mL in whole

#### 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<sub>4</sub> 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 with 0.1% ammonia in water (A) and methanol (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. Divert valve is set to waste from 0 to 1 minutes, to mass spectrometer from 1 minute 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 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

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

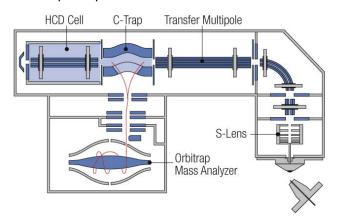


FIGURE 3. Scan Parameters for Exactive Plus Mass Spectrometer

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 positive and negati			

FIGURE 4. Source Parameters for APCI Probe

Value

320

60

350

	Parameter					
	Sheath Gas					
	Aux gas					
	Sweep gas					
	Discharge current					
	Capillary temp					
)	S-Lens RF Level					
	Vaporizer Temp					

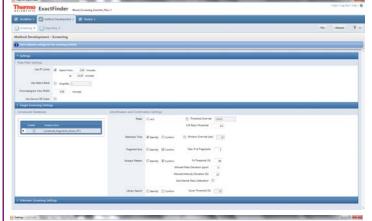
To determine LOD for each compound, aliquots of whole blood were spiked with sets of compound at 5, 10, 20, 50, and 100 ng/mL. Matrix effects were determined by processing a sample spiked in water in place of blood.

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 300 different compounds were tested in this study: 265 were detected at 5 ng/mL, 43 at 10 ng/mL, 15 at 20 ng/mL, 19 at 50 ng/mL, and 3 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

FIGURE 5. ExactFinder processing method and database.



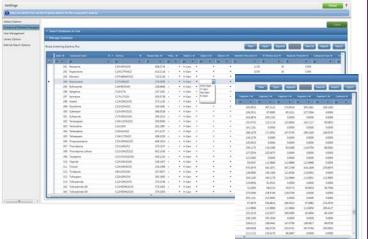


FIGURE 6. Chromatograms reconstructed with a 5 ppm mass window at 5 ng/mL

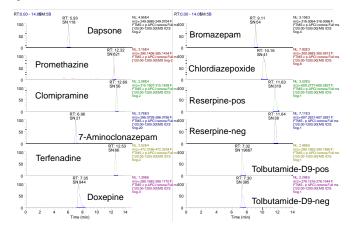


FIGURE 7. LODs for around 350 compounds

Compound	LOD	Compound	LOD	Compound	LOD	Compound	LOD
1-(3-Chlorophenyl)piperazine	5	Des(2-hydroxyethyl)opipramol	5	Loratadine	5	Pentazocine	5
10-hydroxycarbazepine	10	Desalkylflurazepam	5	Lorazepam	5	Perimetazine	5
i-(p-Methylphenyl)-5-phenylhydantoin	20	Desipramine Desmethylcitalogram	5	Lormetazepam	5	Phenacetin	5
6-Acetylcodeine	10		5	Loxapine LSD	5	Phenazone	5
6-Monoacetylmorphine 7-(2,3-dihydroxypropyl)Theophylline	5	Desmethylcitalopram  Desmethylclozapine	50	Maprotiline	5	Phencyclidine Phenylbutazone	10
7-(2,3-dinydroxypropyi) i neopnylline 7-Aminoclonazepam	5	Desmethyldoxepin	5	Maraviroc	5	Pholodine	10
7-Aminoflunitrazepam	5	Desmethylmirtazapine	10	MBDB	10	Pimozide	5
9-Hydroxyrisperidone	5	Desmethylnefopam	5	MDEA	10	Pindolol	5
Acepromazine	5	Desmethyltramadol	10	MDMA	10	Pipamperone	5
Aceprometazine	5	Dexamethasone	5	Mebutamate	20	Pipotiazine	5
Alfentanyl	5	Dextromethorphan	5	Medazepam	5	Posaconazole	5
Alfuzosine	5	Dextropropoxyphene	10	Medifoxamine	5	Pramipexole	5
Alimemazine	5	Dextrorphan	5	Mefloquine	5	Prazepam	5
Alprazolam	5	Diazepam	5	Melatonin	5	Prednisone	5
Amidopyrine	5	Dibenzepine	5	Mepivacaine	5	Primaquine	10
Amiodarone	10	Diltiazem	5	Meprobamate	20	Primidone	10
Amisulpride	5	Dimetotiazine	5	Mescaline	50	Prochlorperazine	5
Amitriptyline	5	Diphenhydramine	5	Mesoridazine	5	Procyclidine	5
Amlodipine	5	Dipyridamole	50	Metenolone	20	Proguanil	5
Amoxapine	5	Disopyramide	10	Methadone	5	Promazine	10
Amprenavir	5	Domperidone	5	Methamphetamine	10	Promethazine	5
Anhydroecgonine methyl ester	10	Dosulepin	5	Methandienone	50	Prometryn	5
Antipyrine	5	Doxepine	10	Methaqualone	5	Propericiazine	5
Apocodeine	5	Droperidol	20	Methotrimeprazine	5	Propizepine	5
Aprindine	5	Dropopizine	5	Methoxsalen	5	Propranolol	5
Asternizole	5	Duloxetine	10	Methoxyamphetamine	50	Protriptyline	5
Atazanavir	5	Efavirenz	5	Methoxymethamphetamine	5	Pyrilamine	5
Atenolol	5	EMDP	5	Methylene Blue	100	Quinidine	5
Atropine	5	Ephedrine	50	Methylphenidate	10	Quinine	5
Benperidol	10	Escitalopram	5	Metobromuron	5	Quinupramine	5
Benzoylecgonine	5	Esmolol	5	Metoclopramide	5	Ranitidine	5
Bepridil	5	Estazolam	5	Metronidazole	5	Reserpine	5
Betaxolol	5	Ethyl Loflazepate	5	Mexiletine	10	Risperidone	5
Bezafibrate	20	Ethylamphetamine	5	Mianserine	5	Ritonavir	10
Biperiden	5	Etifoxine	5	Midazolam	5	Ropivacaine	5
Bisoprolol	5	Etodolac	10	Milnacipram	5	Rufinamide	5
Bromazepam	5	Felbamate	5	Molsidomine	5	Secobarbital	50
Brompheniramine	5	Fenfluramine	5	Morphine	10	Selegiline	50
Bufotenine	50	Fenofibrate	5	Mycophenolic acid	5	Sertraline	5
Bupropion	5	Finasteride	5	Nadolol	10	Sotalol	5
Buspirone	10	Flecainide	5	Naltrexone	5	Strychnine	5
Butorphanol	5	Fluconazole	10	N-Demethyl-trimipramine	5	Sufentanil	5
Cafeine	5	Flunarizine	10	N-Desmethyl-clomipramine	5	Sultopride	5
Cannabidiol	50	Flunitrazepam	5	N-Desmethyl-clobazam	5	Temazepam	5
Carbamazepine	5	Flunixin	20	N-Desmethyl-trimipramine	5	Terbinafine	5
Carbamazepine epoxyde	5	Fluoxetine	5	N-Desmethyl-venlafaxine	5	Terfenadine	5
Carbutamide	5	Fluoxymesterone	5	Nebivolol	20	Tetrazepam	5
Carpipramine	5	Flupentixol	5	Nefazodone	5	THCA	10
Celiprolol	5	Fluphenazine	5	Nelfinavir	10	Thioproperazine	5
Cetirizine	5	Flurazepam	5	Nelfinavir/M8	5	Thioridazine	5
Chlordiazepoxide	5	Fluvoxamine	5	Nevirapine	5	Thioridazine sulfone	5
Chlormezanone	20	Galantamine	5	Nialamide	50	Tianeptine	50
Chloroquine	5	Glafenine	5	Niaprazine	5	Tiapride	5
Chlorpheniramine	5	Glibornuride	5	Nicardipine	5	Timolol	5
Chlorproethazine	5	Gliclazide	100	Nicotine	5	Tinidazole	5
Chlorpromazine	5	Glipizide	10	Niflumic Acid	20	Tofisopam	5
Chlorpropamide	5	Haloperidol	5	Nitrazepam	5	Tolbutamide	5
Chlorprothixene	5	Hydrocodone	5	Nitrendipine	5	Tramadol	10
Cilazapril	50	Hydromorphone	10	Nomifensine	5	Trazodone	5
Cinchonine	5	Hydroquinine	5	Norcocaine	10	Triazolam	5
Cinnarizine	5	Hydroxychloroquine	5	Norcodeine	5	Trifluoperazine	5
Cisapride	5	Hydroxyltraconazole	5	Nordazepam	5	Trifluperidal	5
Clenbuterol	10	Hydroxyzine	5	Norethandrolone	50	Triflupromazine	5
Clobazam	5	Ibogaine	5	Norfluoxetine	10	Trihexyphenidyl	5
Clofibrate	5	Imatinib	5	Norfluvoxamine	5	Trimeprazine	5
Clomethiazole	5	Imipramine	5	Norketamine	10	Trimetazidine	5
Clomipramine	5	Indalpine	5	Nor-LSD	5	Trimethoprim	5
Clonazepam	5	Indinavir	5	Normaprotiline	5	Trimipramine	5
Clonidine	5	Indometacin	50	Normeperidine	50	Tropatepine	5
Clotiazepam	5	Iproniazid	20	Normorphine	5	Urapidil	5
Clozapine	5	Isocarboxazide	5	Noroxycodone	5	Valpromide	50
Clozapine N-oxide	5	Itraconazole	5	Northiaden	5	Venlafaxine	5
Cocaethylene	10	Ketamine	5	Nortriptyline	5	Verapamil	5
Cocaine	20	Ketoconazole	10	Norverapamil	5	Viloxazine	10
Codeine	5	Ketoprofen	50	Olanzapine	5	Vincristine	5
Colchicine	5	Ketotifen	5	Opipramol	5	Voriconazole	5
Cotinine	5	Lacidipine	100	Orphenadrine	5	Yohimbine	5
Coumatetralyl	50	Lacosamide	5	Oxatomide	5	Zimelidine	5
Cyamemazine	5	Lamotrigine	5	Oxazepam	5	Ziprasidone	20
Cyclobenzaprine	5	LAMPA	5	Oxcarbazepine	10	Zolpidem	5
Cyclophosphamide	5	Levomepromazine	5	Oxprenolol	5	Zonisamide	10
Cyproheptadine	5	Levorphanol	5	Oxycodone	5	Zopiclone	5
Dapsone	5	Lidocaine	5	Oxymorphone	20	Zuclopenthixol	5
Darunavir	5	Linezolid	5	Papaverine	5		
	5	Lopinavir	5	Paroxetine	5		
Deacetyl Diltiazem							

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 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.

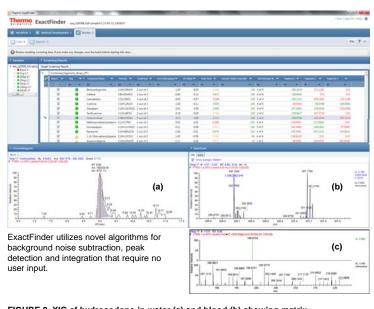
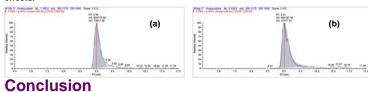


FIGURE 9. XIC of hydrocodone in water (a) and blood (b) showing matrix effects.



- We have developed a screening method for over 300 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

## **Acknowledgements**

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