Application Note: 409

A Complete General Unknown Screening Workflow for Analysis of Drugs and Toxic Compounds in Urine Using LC-MS

Taha Rezai[1], Marta Kozak[1], Kate Torchlin[2]
[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Franklin, MA

Key Words

- Surveyor™ HPLC System
- Hypersil GOLD™ Column
- LCQ Fleet™
- LXQ™
- General Unknown Screening
- Toxicology

Introduction

Clinical and forensic laboratories commonly use automated immunoassays, gas chromatography-mass spectrometry (GC-MS) and high pressure liquid chromatography-diode array detector (HPLC-DAD) techniques to perform general unknown screening (GUS) analysis. None of these techniques sufficiently identify all the drugs and toxic compounds that are potentially present in a sample. Complimentary implementation of liquid chromatography-mass spectrometry (LC-MS) for GUS provides specific and sensitive analysis of drugs and toxic substances. The benefits of the LC-MS GUS methodology include a simple sample preparation procedure, ease of adding new compounds to the screening method and fewer limitations based on compound volatility and thermal stability. In addition, a customized software program (ToxID™ 1.0, Thermo Scientific) is able to automatically generate both short and long reports, avoiding the need for manual analysis of each sample chromatogram. This application note describes the use of an ion trap mass spectrometer (LXQ and LCQ Fleet) equipped with an ESI source, Surveyor LC system and CTC autosampler for identification of unknown compounds in human urine.

Goal

To develop a complete LC-MS based general unknown screening procedure which includes sample preparation, use of either 13 or 30 minute LC methods and automated report generation.

Experimental Conditions

An MS/MS spectral library of over 300 drugs and toxic compounds was created. Sample preparation of spiked human urine was carried out using a solid-phase extraction (SPE) cartridge for basic, neutral and acidic compounds.

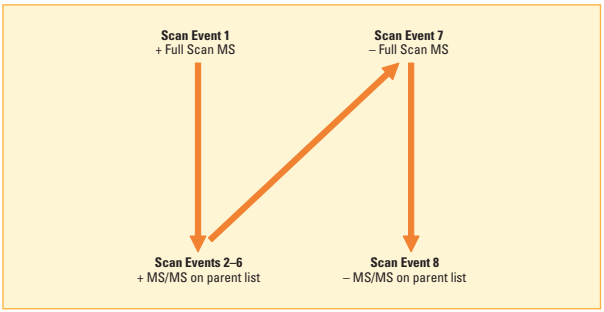

Figure 1: MS Scan Events

The method includes the option of using either 13 or 30 minute HPLC run times. Samples were analyzed using electrospray ionization (ESI) on an ion trap mass spectrometer in polarity switching scan dependent MS/MS experiments (see Figure 1), with retention time windows specified for each listed parent mass. Figure 2 shows the overall application workflow.

Sample Preparation

0.1 mL of an internal standard solution at 100 µg/mL was added to 1 mL of urine, and the resulting mix was extracted with an SPE (Hypersep Verify-CX 200 mg mixed mode cartridges, Thermo Fisher Scientific) procedure prior to injection onto LC-MS. Details of the SPE procedure are described here.

- Add 2 mL of 0.1 M phosphate buffered urine at pH 6 and 100 µL of internal standard spiking solution (1 µg/mL of each Internal Standard in 50% acetonitrile) to 1 mL of urine. Vortex.
- Condition SPE column with 2 mL methanol and then 2 mL phosphate buffer at pH 6
- Pour sample into SPE column
- Wash with 1 mL deionized water, 0.5 mL 0.01M acetic acid, dry 4 min, 50 µL methanol, dry 1 min
- Elute acidic and neutral fractions with 1.5 mL acetone/chloroform 50/50 (v/v), and 1.5 mL acetone/dichloromethane 50/50 (v/v)
- Elute basic fraction with 1.5 mL ethyl acetate/ammonium hydroxide = 98/2 (v/v), 1.5 mL dichloromethane/isopropanol/ammonium hydroxide = 78/20/2 (v/v/v)
- Evaporate to dryness
- Reconstitute in 100 uL deionized water/acetonitrile 1/1 (v/v) and 0.1% formic acid

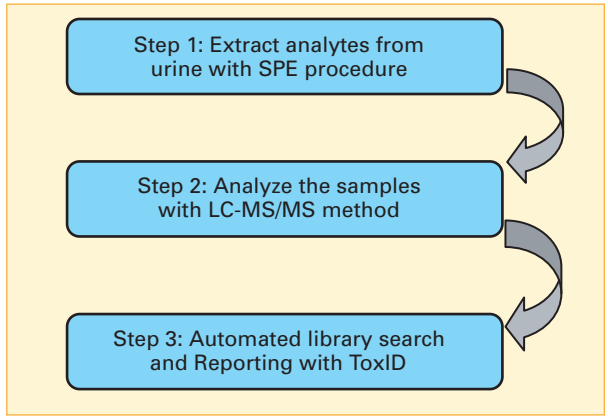

Figure 2: Overall Application Workflow

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Chromatography

HPLC experiments were performed using a Surveyor MS Pump Plus. Two LC methods, 13 and 30 minutes, were developed. The 13 min method utilized a Thermo Scientific Hypersil GOLD PFP™ (perfluorophenyl) 50×2.1 mm, 5 µm column, while the 30 min method used a longer 150×2.1 mm, 5 µm Hypersil GOLD PFP column. Mobile phases for both methods were water (A) at 0.1% formic acid and 10 mM ammonium formate as the aqueous phase and acetonitrile (B) at 0.1% formic acid as the organic phase. Flow rates for both methods were 200 uL/min and the injection volume of SPE prepared urine was 10 uL for all experiments. See Tables 1 and 2 for details of the 13 and 30 minute LC methods.

Time (min)	%A	%B
0	95	5
0.5	95	5
5.5	5	95
8.5	5	95
8.6	95	5
13	95	5

Table 1: 13 minute LC method

Time (min)	%A	%B
0	95	5
5	55	45
18	30	70
20	5	95
25	5	95
25.1	95	5
30	95	5

Table 2: 30 minute LC method

MS Conditions

Instrument: LXQ or LCQ Fleet ion trap mass spectrometer
Ionization: ESI, Ion Max™ source
Capillary temperature: 275 °C
Spray voltage: 5.0 kV
Sheath gas: 30
Aux gas: 8
Data acquisition mode: Polarity switching scan dependent experiment
Microscans: 1
Wideband: Activated
Step Collision Energy™: 35% ± 10%

Results

Table 3 (pages 2 through 6) lists the concentrations at which each analyte in the general unknown screen is present. The validated concentration levels were at 10, 100 and 1000 ng/mL (clinically relevant values). The presence of an analyte at 10, 100 or 1000 ng/mL implies that the limit of detection (LOD) is likely below that value. Of the 275 compounds analyzed 70% were present at 10 ng/mL, 20% at 100 ng/mL, 8% at 1000 ng/mL and 2% were present at a concentration above 1000 ng/mL.

	LXQ – 30 min method Concentration Tested (ng/mL)			LXQ – 13 min method Concentration Tested (ng/mL)		
Compound	**10**	**100**	**1000**	**10**	**100**	**1000**
11-Hydroxy-delta-9-THC	N	P	P	N	N	>1000
11-nor-9-carboxy-Delta-9-THC	N	P	P	N	N	P
2-Bromo-Alpha-Ergocryptine	P	P	P	P	P	P
2-Hydroxyethylflunitrazepam	N	P	P	N	P	P
3-Hydroxystanozolol	N	N	P	N	N	>1000
4-Hydroxynordiazepam	N	P	P	N	P	P
6-Acetylcodeine	P	P	P	P	P	P
6-Acetylmorphine (6-MAM)	P	P	P	P	P	P
7-Amino-Clonazepam	P	P	P	P	P	P
7-Amino-Flunitrozepam	P	P	P	P	P	P
Acebutolol	P	P	P	P	P	P
a-Hydroxy-Alprazolam	P	P	P	P	P	P
a-Hydroxy-Triazolam	P	P	P	P	P	P
Albuterol	P	P	P	P	P	P
alpha-Hydroxymidazolam	P	P	P	N	P	P
Alprazolam	P	P	P	P	P	P
Alprenolol	P	P	P	P	P	P
Aminorex	N	P	P	N	P	P
Amiodarone	P	P	P	P	P	P
Amitriptyline	P	P	P	P	P	P
Amlodipine	N	P	P	N	N	P
Amobarbital	P	P	P	P	P	P
Amoxapine	P	P	P	P	P	P
Amphetamine	P	P	P	P	P	P
Anhydroecgonine MethylEster	N	P	P	N	P	P
Antipyrine	N	N	P	N	N	>1000
Apomorphine	N	N	P	N	N	>1000
Astemizole	N	N	>1000	N	P	P
Atenolol	P	P	P	P	P	P
Atropine	N	P	P	N	P	P
BDB	N	P	P	N	P	P
Benzocaine	N	P	P	N	N	P
Benzoylecgonine	P	P	P	N	P	P
Betaxolol	P	P	P	P	P	P
Bisacodyl	P	P	P	P	P	P

	LXQ – 30 min method Concentration Tested (ng/mL)			LXQ – 13 min method Concentration Tested (ng/mL)		
Compound	**10**	**100**	**1000**	**10**	**100**	**1000**
Bisoprolol	P	P	P	P	P	P
Bromazepam	P	P	P	P	P	P
Brompheniramine	P	P	P	P	P	P
Bupivocaine	P	P	P	P	P	P
Buprenorphine	P	P	P	P	P	P
Bupropion	P	P	P	P	P	P
Buspirone	P	P	P	P	P	P
Butalbital	N	P	P	N	P	P
Butorphanol	P	P	P	P	P	P
Cannabidiol	N	N	P	N	N	>1000
Cannabinol	N	N	P	N	N	>1000
Captopril	N	N	P	N	N	P
Carbamazepine	P	P	P	P	P	P
Carbinoxamine	N	N	P	N	P	P
Carisoprodol	N	N	P	N	N	P
Cathinone	N	N	P	N	N	P
Chlordiazepoxide	P	P	P	P	P	P
Chlorothiazide	N	P	P	N	P	P
Chlorpheniramine	P	P	P	P	P	P
Chlorpromazine	P	P	P	P	P	P
Chlorpromazine-D3	N	P	P	N	P	P
Chlorprothixene	N	P	P	N	N	>1000
Cinnarizine	P	P	P	P	P	P
cis-4-Methylaminorex	N	P	P	N	P	P
Cisapride	P	P	P	N	P	P
Citalopram	P	P	P	P	P	P
Clenbuterol	P	P	P	P	P	P
Clenbuterol	N	P	P	N	P	P
Clobazam	P	P	P	N	P	P
Clomipramine	P	P	P	P	P	P
Clonazepam	P	P	P	P	P	P
Clonidine	P	P	P	P	P	P
Clopidogrel	P	P	P	P	P	P
Clozapine	P	P	P	P	P	P
Cocaethylene	P	P	P	P	P	P
Cocaine	P	P	P	P	P	P
Codeine	P	P	P	P	P	P
Cyclobenzaprine	P	P	P	P	P	P
Delta9-THC	N	P	P	N	P	P
Desalkylflurazepam	P	P	P	N	P	P
Desipramine	P	P	P	N	P	P
Desmethyldoxepin	P	P	P	P	P	P
Dextromethorphan	P	P	P	P	P	P
Diazepam	P	P	P	P	P	P
Diflunisal	N	P	P	P	P	P
Digoxin	N	N	P	N	N	P
Dihydrocodeine	P	P	P	P	P	P
Dihydroergotamine	P	P	P	P	P	P
Diltiazem	P	P	P	P	P	P
Diphenhydramine	P	P	P	P	P	P
Dipyridamole	P	P	P	N	N	P
Disopyramide	P	P	P	P	P	P
Dothiepin	N	N	P	N	P	P
Doxepin	P	P	P	P	P	P
Doxylamine	P	P	P	P	P	P
Ecgonine-Methyl-Ester	N	N	P	N	N	P
EDDP	P	P	P	P	P	P
EMDP	P	P	P	P	P	P
Enalapril	N	P	P	P	P	P
Ephedrine	N	P	P	N	P	P
Ergotamine	P	P	P	P	P	P
Estazolam	P	P	P	N	P	P

	LXQ – 30 min method Concentration Tested (ng/mL)			LXQ – 13 min method Concentration Tested (ng/mL)		
Compound	**10**	**100**	**1000**	**10**	**100**	**1000**
Felcainide	P	P	P	P	P	P
Fendiline	N	P	P	P	P	P
Fenfluramine	P	P	P	P	P	P
Fentanyl	P	P	P	P	P	P
Fexofenadine	P	P	P	P	P	P
Flumethasone	N	P	P	N	N	P
Flunitrazepam	P	P	P	P	P	P
Flunixin	P	P	P	N	P	P
Fluoxetine	P	P	P	P	P	P
Fluoxymesterone	P	P	P	N	P	P
Fluphenazine	N	P	P	P	P	P
Flurazepam	P	P	P	P	P	P
Fluvoxamine	P	P	P	P	P	P
Furosemide	P	P	P	N	P	P
Gabapentin	N	N	P	N	N	P
Gliclazide	P	P	P	N	N	P
Glimepiride	P	P	P	N	P	P
Glipizide	P	P	P	P	P	P
Glyburide	P	P	P	P	P	P
Haloperidol	P	P	P	P	P	P
Haloperidol-D4	N	P	P	N	P	P
Heroin	P	P	P	P	P	P
HMMA	N	N	P	N	N	>1000
Hydrochlorothiazide	N	P	P	N	N	P
Hydrocodone	P	P	P	P	P	P
Hydromorphone	N	P	P	P	P	P
Hydroxyzine	P	P	P	N	P	P
Imipramine	P	P	P	P	P	P
Indomethacin	P	P	P	N	N	>1000
Isradipine	P	P	P	P	P	P
Ketamine	P	P	P	P	P	P
Ketoconazole	P	P	P	P	P	P
Ketoprofen	N	P	P	N	N	>1000
Ketorolac	N	P	P	N	N	>1000
Labetolol	P	P	P	N	P	P
Lamotrigine	P	P	P	P	P	P
LAMPA	P	P	P	P	P	P
Lidocaine	P	P	P	P	P	P
Lometazepam	P	P	P	N	P	P
Loratadine	P	P	P	P	P	P
Lorazepam	P	P	P	P	P	P
LSD	P	P	P	P	P	P
Maprotiline	P	P	P	P	P	P
MBDB	P	P	P	N	P	P
MDA	P	P	P	P	P	P
MDEA	N	N	P	N	P	P
MDMA	P	P	P	P	P	P
Melatonin	N	N	P	N	N	>1000
Meperidine	P	P	P	P	P	P
Mepivocaine	N	P	P	N	P	P
Meprobamate	N	P	P	N	P	P
Mescaline	N	P	P	P	P	P
Mesoridazine	P	P	P	P	P	P
Metaprolol	P	P	P	P	P	P
Methadienone	P	P	P	P	P	P
Methadone	P	P	P	P	P	P
Methamphetamine	P	P	P	P	P	P
Methaqualone	N	N	P	N	N	>1000
Methcathinone	N	P	P	N	N	P
Methenolone	P	P	P	P	P	P
Methohexital	P	P	P	P	P	P
Methoxyverapmil	P	P	P	P	P	P

	LXQ – 30 min method Concentration Tested (ng/mL)			LXQ – 13 min method Concentration Tested (ng/mL)		
Compound	**10**	**100**	**1000**	**10**	**100**	**1000**
Methylphenidate	P	P	P	P	P	P
Metoclopramide	P	P	P	P	P	P
Metronidazole	N	P	P	N	P	P
Mexiletine	N	P	P	N	N	>1000
Mianserin	P	P	P	P	P	P
Miconazole	P	P	P	P	P	P
Midazolam	P	P	P	P	P	P
Mirtazapine	P	P	P	P	P	P
Molsidomine	N	N	P	N	N	>1000
Morphine	N	P	P	N	P	P
Morphine-3-b-glucuronide	N	N	>1000	N	N	>1000
Nalbuphine	P	P	P	P	P	P
Nalorphine	P	P	P	P	P	P
Naloxone	P	P	P	P	P	P
Naltrexone	P	P	P	P	P	P
NAPA	P	P	P	P	P	P
N-DemethylTrimipramine	N	N	P	P	P	P
N-Desmethyl-cis-tramadol	N	N	P	N	N	P
N-Desmethylflunitrazepam	P	P	P	N	P	P
N-Desmethylselegiline	N	P	P	N	P	P
N-DesmthylClomipramine	N	N	P	N	P	P
N-Ethylamphetamine	N	P	P	N	P	P
Nicardipine	P	P	P	P	P	P
Nicotine	P	P	P	P	P	P
Nitrazepam	N	P	P	N	N	>1000
Nitrendipine	P	P	P	P	P	P
Nizatidine	N	P	P	N	N	P
Norbenzoylecgonine	N	N	P	N	N	>1000
Norbuprenorphine	N	N	P	N	N	>1000
Norclomipramine	P	P	P	P	P	P
Norcocaethylene	P	P	P	P	P	P
Norcocaine	P	P	P	P	P	P
Norcodeine	P	P	P	N	P	P
Nordiazepam	P	P	P	P	P	P
Nordoxepin	P	P	P	P	P	P
Norethandrolone	P	P	P	N	P	P
Norfentanyl	N	P	P	N	P	P
Norfluoxetine	N	P	P	P	P	P
Norketamine	N	P	P	N	P	P
NOR-LSD	P	P	P	P	P	P
Normeperidine	P	P	P	P	P	P
Normorphine	N	P	P	N	N	P
Noroxycodone	N	P	P	N	P	P
Noroxymorphone	N	N	P	N	N	>1000
Norproproxyphene	P	P	P	P	P	P
Nortriptyline	P	P	P	P	P	P
Noscapine	P	P	P	P	P	P
OH-LSD	N	P	P	N	P	P
Ondansetron	P	P	P	P	P	P
Opipramol	P	P	P	P	P	P
Oxazepam	P	P	P	P	P	P
Oxcarbazepine	P	P	P	N	N	P
Oxycodone	P	P	P	P	P	P
Oxymorphone	N	P	P	N	P	P
Papaverine	P	P	P	P	P	P
Paraxanthine	P	P	P	N	N	>1000
Paroxetine	P	P	P	N	P	P
PCP	P	P	P	P	P	P
Pentazocine	P	P	P	P	P	P
Pentobarbital	P	P	P	P	P	P
Perphenazine	P	P	P	P	P	P
Pheniramine	N	N	P	N	P	P

	LXQ – 30 min method Concentration Tested (ng/mL)			LXQ – 13 min method Concentration Tested (ng/mL)		
Compound	**10**	**100**	**1000**	**10**	**100**	**1000**
Phenobarbital	P	P	P	P	P	P
Phenolphthalein	P	P	P	P	P	P
Phentermine	N	P	P	N	N	P
Phenylbutazone	N	N	>1000	N	N	P
Phenyltoloxamine	N	N	P	N	N	P
Physostigmine	P	P	P	P	P	P
Pindolol	N	N	>1000	N	N	P
Piroxicam	P	P	P	P	P	P
PMA	N	N	P	N	N	P
PMMA	P	P	P	N	P	P
Prazepam-D5	N	P	P	N	P	P
Prazosin	P	P	P	P	P	P
Prilocaine	P	P	P	N	N	P
Procainamide	P	P	P	N	P	P
Promazine	P	P	P	P	P	P
Promethazine	P	P	P	N	P	P
Prometryn	P	P	P	N	P	P
Propafenone	P	P	P	P	P	P
Propoxyphene	P	P	P	P	P	P
Propranolol	P	P	P	P	P	P
Protriptyline	P	P	P	P	P	P
Psilocin	N	N	500	N	P	P
Pyrilamine	P	P	P	P	P	P
Quetiapine	P	P	P	P	P	P
Quinidine	P	P	P	P	P	P
Quinine	P	P	P	N	P	P
Ranitidine	N	P	P	N	N	P
Risperidone	P	P	P	P	P	P
Scopolamine	P	P	P	P	P	P
Secobarbital	P	P	P	P	P	P
Selegiline	N	P	P	N	P	P
Sertraline	P	P	P	P	P	P
Sotalol	P	P	P	N	P	P
Spironolactone	N	P	P	N	P	P
Stanozolol	N	P	P	N	P	P
Telmisartan	P	P	P	P	P	P
Temazepam	P	P	P	P	P	P
Terfenadine	P	P	P	P	P	P
Tetracine	P	P	P	P	P	P
Thiamylal	N	P	P	N	P	P
Thiopental	P	P	P	P	P	P
Thioridazine	P	P	P	P	P	P
Thiothixene	P	P	P	P	P	P
Timolol	P	P	P	P	P	P
Topiramate	N	P	P	P	P	P
Trazodone	P	P	P	P	P	P
Triazolam	P	P	P	P	P	P
Trimethoprim	P	P	P	P	P	P
Trimipramine	P	P	P	P	P	P
Venlafaxine	P	P	P	P	P	P
Verapamil	P	P	P	P	P	P
Vincristine	P	P	P	P	P	P
Warfarin	P	P	P	P	P	P
Zimelidine	N	P	P	P	P	P
Zolpidem	P	P	P	P	P	P
Zopiclone	N	P	P	N	N	P

All barbiturates require an APCI source for detection. *P=Drug present.* *N=Drug not present.*

Table 3: Results for 13 and 30 minute HPLC methods on the LXQ

Method Validation

The LC-MS method was validated by processing and analyzing urine samples spiked with 10 randomly selected compounds at concentrations of 10 ng/mL, 100 ng/mL and 1000 ng/mL. A mix of three deuterated internal standards (Chlorpromazine-D3, Haloperidol-D4, and Prazepam-D5) at a concentration of 100 ng/mL, were added to each urine sample prior to SPE. In addition, the assay performance was verified by analyzing patient urine samples obtained from the Johns Hopkins University Hospital Clinical Laboratory and data was compared to results from established LC-UV and immunoassay analytical techniques. The comparison of LC-MS, LC-UV and immunoassay analysis is shown in Table 4. Both 13 and 30 minute LC-MS methods have consistently identified more analytes than either LC-UV or immunoassays. The 30 min LC-MS method showed more resolved peaks and detected the presence of lower intensity analytes, resulting in more confirmed target compounds than the 13 min LC-MS method.

LXQ 30 Minute	LXQ 13 Minute	LC-UV	Immunoassay
Nortriptyline	Nortriptyline	Nortriptyline	Barbiturates
Amitriptyline	Amitriptyline	Amitriptyline	Benzodiazepines
Benzoylecgonine	Benzoylecgonine	Benzoylecgonine	Cocaine
Cocaine	Cocaine	Cocaine	Opiates
Cocaethylene	—	Cocaethylene	THC
Cyclobenzaprine	—	—	—
Norbenzoylecgonine	Norbenzoylecgonine	—	—
Morphine	Morphine	—	—
Norcocaine	Norcocaine	—	—
Codeine	—	—	—
Norcocaethylene	Norcocaethylene	—	—
Methadone	—	—	—
Quinidine/Quinine	Quinidine/Quinine	—	—
Hydroxyzine	Hydroxyzine	—	—
Noskapine	Noskapine	—	—
Diltiazem	Diltiazem	—	—
Morphine-3-beta-Glucuronide	Morphine-3-beta-Glucuronide	—	—

Table 4: Real patient samples analyzed with LC-MS, LC-UV and Immunoassay Methods

ToxID Software and Automated Reporting

ToxID software identifies compounds present in the sample based on MS/MS spectra and retention times. Positive hits are automatically reported via ToxID 1.0 software or can be processed individually with a simple user interface (Figure 3). An example of a short summary 1-page report is shown in Figure 4 (page 10). A long report with one page per detected compound is shown in Figure 5 (page 11).

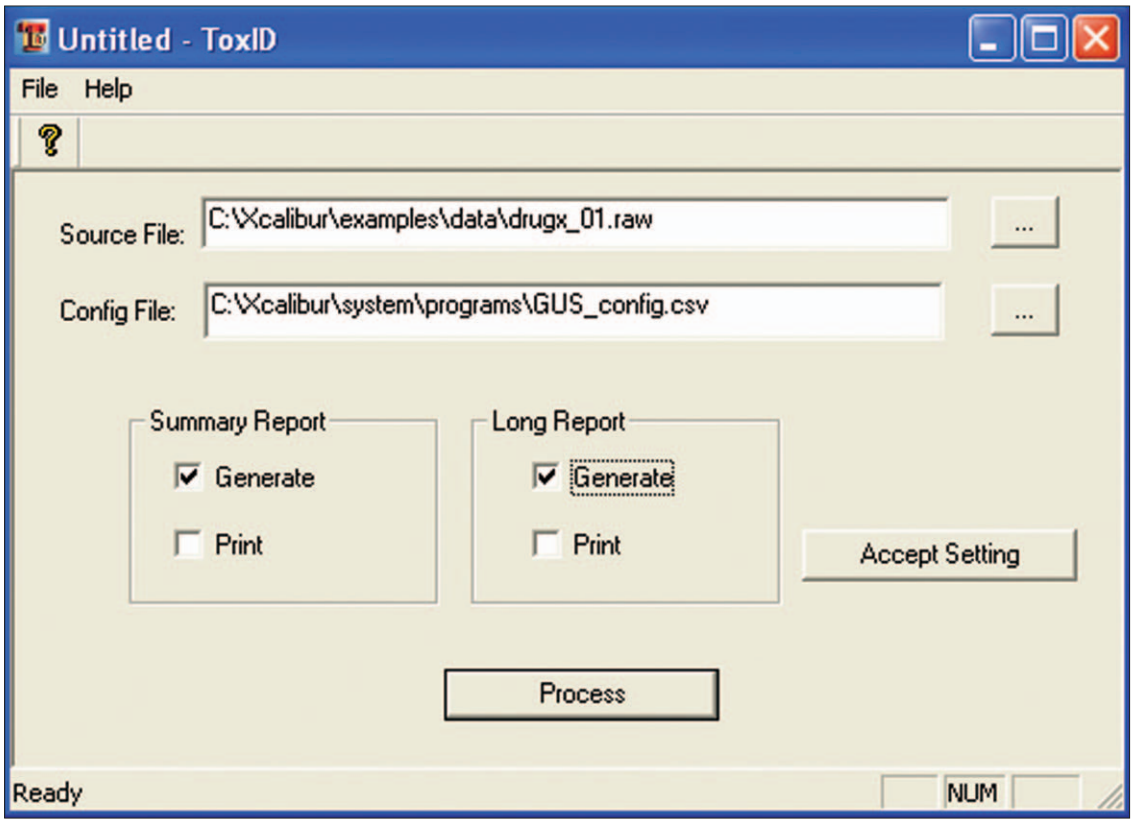


Figure 3: Simple user interface for manual processing

Supplemental

Table 5 is a comparison of a sample set of data acquired on the LCQ Fleet and LXQ. The data for the two instruments show that the analytes are present at similar concentration levels. The LXQ, however, will have better limits of detection.

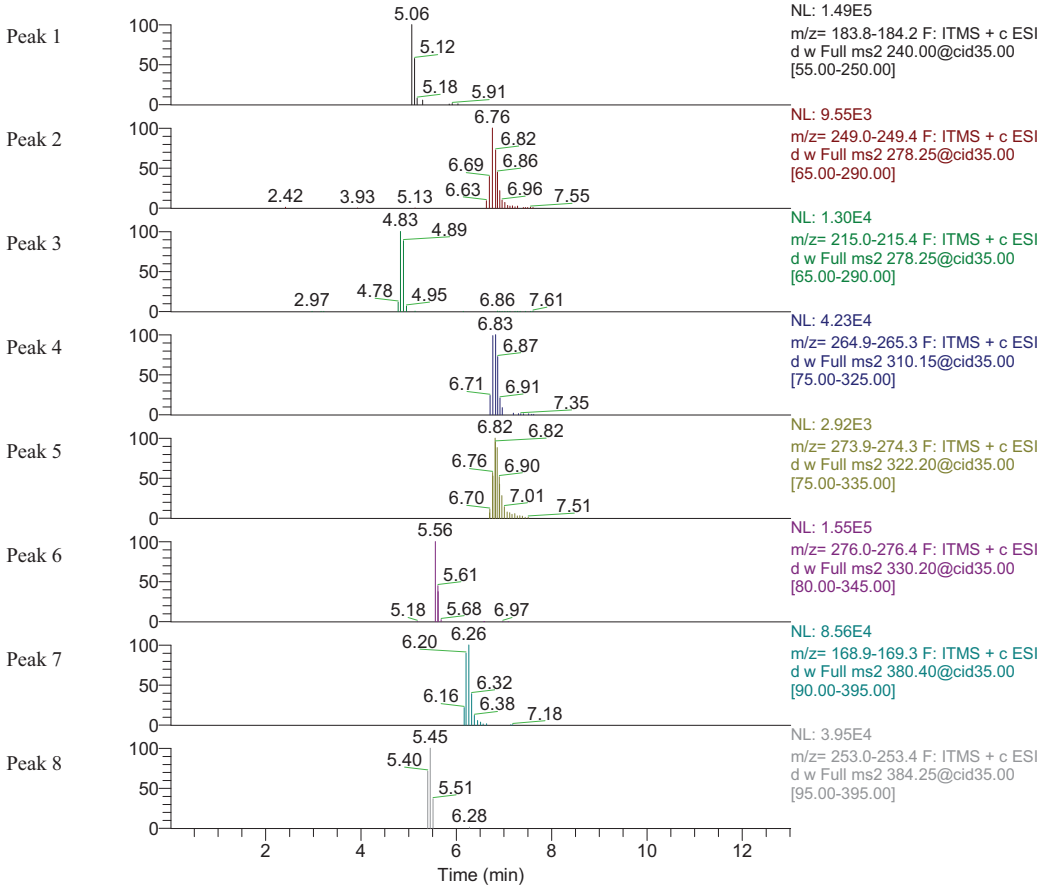
	LXQ – 30 min method			LXQ – 13 min method			LCQ Fleet – 30 min method			LCQ Fleet – 13 min method		
	Concentration Tested (ng/mL)			Concentration Tested (ng/mL)			Concentration Tested (ng/mL)			Concentration Tested (ng/mL)		
Compound	**10**	**100**	**1000**	**10**	**100**	**1000**	**10**	**100**	**1000**	**10**	**100**	**1000**
Amitriptyline	P	P	P	P	P	P	P	P	P	P	P	P
Benzoylecgonine	P	P	P	N	P	P	N	P	P	N	P	P
Bisoprolol	P	P	P	P	P	P	P	P	P	N	P	P
Brompheniramine	P	P	P	P	P	P	P	P	P	P	P	P
Bupivocaine	P	P	P	P	P	P	P	P	P	P	P	P
Buprenorphine	P	P	P	P	P	P	P	P	P	P	P	P
Chlorpheniramine	P	P	P	P	P	P	P	P	P	P	P	P
Citalopram	P	P	P	P	P	P	P	P	P	P	P	P
Clozapine	P	P	P	P	P	P	P	P	P	P	P	P
Cocaethylene	P	P	P	P	P	P	P	P	P	P	P	P
Cocaine	P	P	P	P	P	P	P	P	P	P	P	P
Cyclobenzaprine	P	P	P	P	P	P	P	P	P	P	P	P
Desmethyldoxepin	P	P	P	P	P	P	P	P	P	P	P	P
Dextromethorphan	P	P	P	P	P	P	P	P	P	P	P	P
Diltiazem	P	P	P	P	P	P	P	P	P	P	P	P
Diphenhydramine	P	P	P	P	P	P	P	P	P	P	P	P
Disopyramide	P	P	P	P	P	P	P	P	P	P	P	P
Doxepin	P	P	P	P	P	P	P	P	P	P	P	P
Fenfluramine	P	P	P	P	P	P	P	P	P	P	P	P
Fentanyl	P	P	P	P	P	P	P	P	P	P	P	P
Fexofenadine	P	P	P	P	P	P	P	P	P	P	P	P
Flurazepam	P	P	P	P	P	P	P	P	P	P	P	P
Haloperidol	P	P	P	P	P	P	P	P	P	P	P	P
Imipramine	P	P	P	P	P	P	P	P	P	P	P	P
Ketoconazole	P	P	P	P	P	P	P	P	P	P	P	P
LSD	P	P	P	P	P	P	P	P	P	P	P	P
Meperidine	P	P	P	P	P	P	P	P	P	N	P	P
Methadone	P	P	P	P	P	P	P	P	P	P	P	P
Methamphetamine	P	P	P	P	P	P	P	P	P	N	P	P
NAPA	P	P	P	P	P	P	P	P	P	P	P	P
Nordoxepin	P	P	P	P	P	P	P	P	P	P	P	P
Normeperidine	P	P	P	P	P	P	P	P	P	N	P	P
Nortriptyline	P	P	P	P	P	P	P	P	P	P	P	P
Papaverine	P	P	P	P	P	P	P	P	P	P	P	P
PCP	P	P	P	P	P	P	P	P	P	P	P	P
Pentazocine	P	P	P	P	P	P	P	P	P	P	P	P
Phenolphthalein	P	P	P	P	P	P	P	P	P	P	P	P
Physostigmine	P	P	P	P	P	P	P	P	P	N	P	P
Propranolol	P	P	P	P	P	P	P	P	P	P	P	P
Quetiapine	P	P	P	P	P	P	P	P	P	P	P	P
Quinidine	P	P	P	P	P	P	P	P	P	P	P	P
Risperidone	P	P	P	P	P	P	P	P	P	P	P	P
Sertraline	P	P	P	P	P	P	P	P	P	P	P	P
Temazepam	P	P	P	P	P	P	P	P	P	N	P	P
Trazodone	P	P	P	P	P	P	P	P	P	P	P	P
Triazolam	P	P	P	P	P	P	P	P	P	N	P	P
Trimipramine	P	P	P	P	P	P	P	P	P	P	P	P
Verapamil	P	P	P	P	P	P	P	P	P	P	P	P
Vincristine	P	P	P	P	P	P	N	P	P	P	P	P
Warfarin	P	P	P	P	P	P	P	P	P	P	P	P
Zolpidem	P	P	P	P	P	P	P	P	P	P	P	P

P=Drug present. *N=Drug not present.*

Table 5: A comparison of a sample set of data acquired on the LCQ Fleet and LXQ

Company Name
ToxID Summary Report

Raw File Name: C:\Documents and Settings\marta.kozak\Desktop\Desktop\Application_Notes\ToxID\2J.RAW
Config File Name: C:\Xcalibur\examples\ToxID\ToxID_config_13min.csv
Sample Name:
Laboratory: ChemLab
Acquistion Start Time: 2/13/2007 1:04:54 AM



Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Real RT	Intensity	Library Name
1	Bupropion	p	909	909	240.0	5.20	5.06	148721	Tox_Library
2	EDDP	p	857	873	278.2	6.60	6.76	9549	Tox_Library
3	Venlafaxine	p	816	837	278.2	4.90	4.83	12964	Tox_Library
4	Methadone	p	932	932	310.2	6.70	6.83	42262	Tox_Library
5	Chlorpromazine-D3	i	859	859	322.2	6.80	6.82	2924	Tox_Library
6	Prazepam-D5	i	969	974	330.2	5.60	5.56	154827	Tox_Library
7	Haloperidol-D4	i	830	837	380.4	6.20	6.26	85589	Tox_Library
8	Quetiapine	p	870	871	384.2	5.40	5.45	39512	Tox_Library

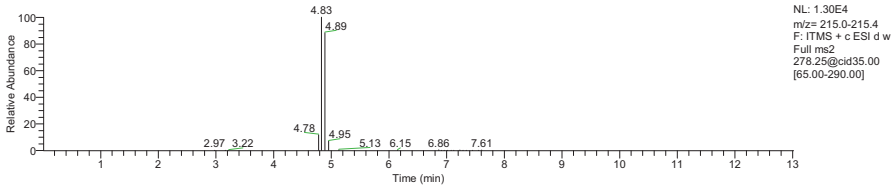
Figure 4: Short Report

Company Name ToxID Long Report

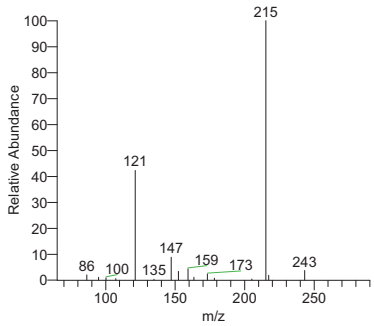
Raw File Name: C:\Documents and Settings\marta.kozak\Desktop\Desktop\Application_Notes\ToxID\2J.RAW
Config File Name: C:\Xcalibur\examples\ToxID\ToxID_config_13min.csv
Sample Name:
Laboratory: ChemLab
Acquistion Start Time: 2/13/2007 1:04:54 AM

Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Real RT	Intensity	Library Name
3	Venlafaxine	p	816	837	278.2	4.90	4.83	12964	Tox_Library

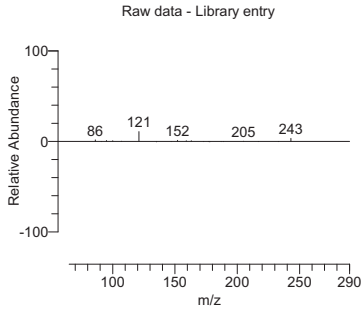
Peak 3



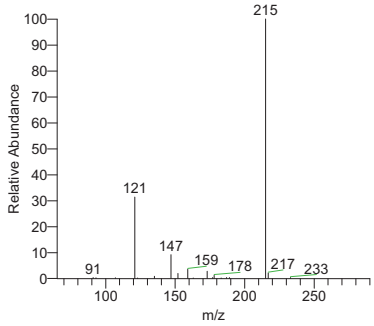
Acquired Spectrum



Delta Spectrum



Library Spectrum



Library Structure

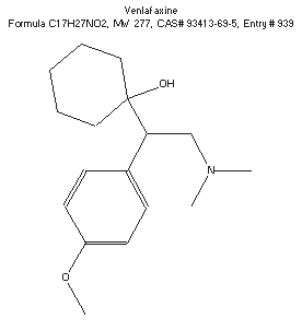


Figure 5: Long Report

Conclusion

The complete GUS method described in this note utilizes either an LXQ or LCQ Fleet ion trap and includes an SPE procedure and two LC methodologies which allow for the identification of over 275 compounds in human urine. The 30 minute LC method is suggested for the most thorough investigation of all analytes present in the sample, while the 13 minute method is recommended for laboratories that operate in environments which require short turn around times. Accompanying ToxID software allows for automatic data analysis and reporting, thereby eliminating the need for manual data interpretation and increasing confidence in compound identification. It is worth noting that when compared to other general unknown screening methods, both the 13 and 30 minute LC-MS based methods identify more analytes.

An important feature of the GUS workflow is the ease of adding new analytes to the screening method, highlighted in Table 6. This aspect of the application is very important for general unknown screening, where new target compounds are continually being added to the target list.

STEP 1: Directly infuse analyte to obtain MS/MS spectra, then add spectra to the library	10 minutes
STEP 2: Run analyte on column to obtain retention times	13 or 30 minutes depending on LC method
STEP 3: Update Parent Mass Table in instrument method with parent masses and retention times	2 minutes
STEP 4: Update ToxID with name, parent masses, the most intense daughter ion and retention times	2 minutes

Table 6: Simple workflow for adding new analytes

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