Application Note: 449

A Complete Toxicology Screening Procedure for Drugs and Toxic Compounds in Urine and Plasma Using LC-MS/MS

Marta Kozak, Taha Rezai, Thermo Fisher Scientific, San Jose, CA

Key Words

- ToxSpec Analyzer
- ToxID Software
- LXQ Linear Ion Trap
- Clinical Toxicology
- General Unknown Screening

Introduction

Toxicology laboratories commonly use automated immunoassays, gas chromatography-mass spectrometry (GC-MS) and high pressure liquid chromatography-diode array detector (HPLC-DAD) techniques to perform toxicology screening analyses. None of these techniques are able to identify all the drugs and toxic compounds that are potentially present in a sample. Implementation of liquid chromatography-mass spectrometry (LC-MS) for toxicology screening provides specific and sensitive analysis of drugs and toxic substances. The benefits of the LC-MS/MS screening 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, Thermo Scientific ToxID automated toxicology screening software is able to automatically generate both Summary and Long Reports, avoiding the need for manual analysis of each sample chromatogram. This application note describes the use of the Thermo Scientific LXQ ion trap mass spectrometer equipped with an ESI source and HPLC for identification of unknown compounds in human urine and human plasma.

Goal

To develop a complete LC-MS/MS screening methodology which includes a sample preparation method, LC-MS method, spectra library, and data processing and reporting software.

Experimental Conditions

An MS/MS spectral library of 275 drugs and toxic compounds was created. Sample preparation of spiked human urine or human plasma was carried out using a solid-phase extraction (SPE) cartridge for basic, neutral and acidic compounds. A 13-minute LC method implementing a Perfluorophenyl (PFP) column was developed. 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. The method allows acquisition of MS^2 spectra for co-eluting compounds and analysis of positively and negatively ionized compounds with a single run. Figure 2 shows the overall application workflow.

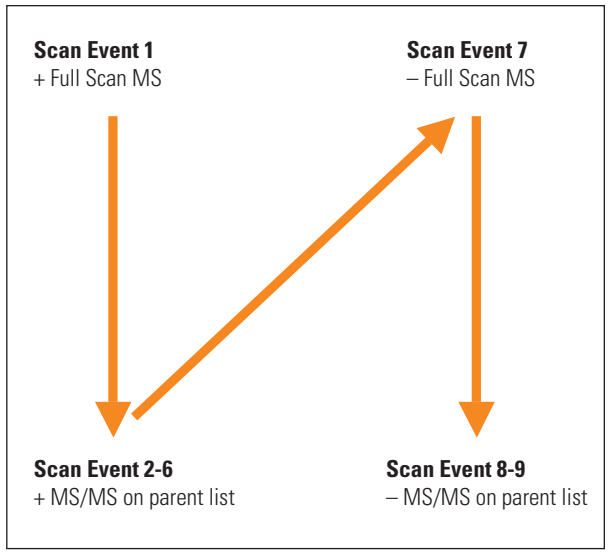

Figure 1: MS scan events

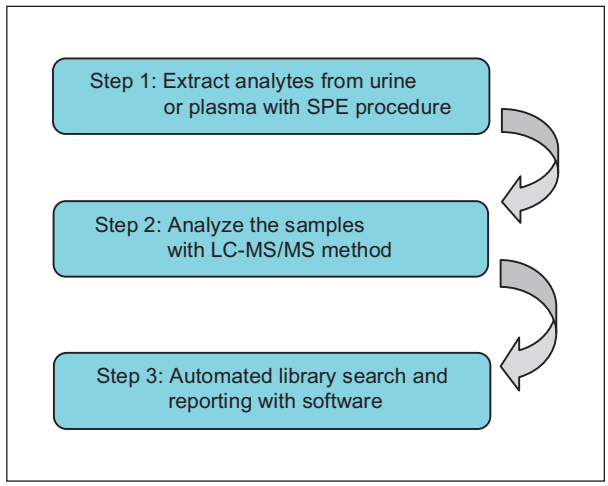

Figure 2: Step-by-step application workflow

Sample Preparation

Samples (1 mL of urine or 0.5 mL of plasma) were spiked with 0.1 mL of an internal standard solution at a concentration of 1 µg/mL (Chlorpromazine-D3, Haloperidol-D4 and Prazepam-D5) and diluted with 2 mL of 0.1 M phosphate buffer pH 6.0. The resulting mix was extracted with an SPE (Thermo Scientific Hypersep Verify-CX 200 mg mixed mode cartridges) procedure prior to injection onto LC-MS.

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Chromatography

HPLC separation was performed with a Thermo Scientific Accela pump using a Thermo Scientific Hypersil GOLD PFP column (50 x 2.1 mm; 5 µm particles). Flow rate was set to 200 µL/min. The gradient is summarized in Table 1 (solvent A = water/0.1% formic acid/10 mM ammonium formate, solvent B = acteonitrile/0.1% formic acid). Injection volume was 10 µL.

Table 1. Thirteen-minute LC method

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

MS Conditions

Instrument:	LXQ ion trap mass spectrometer
Ionization:	ESI, Thermo Scientific 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 Activation™:	On
Stepped Normalized Collision Energy:	35% ± 10%

Method Validation and Results:

The method was prequalified by processing and analyzing urine samples spiked with 10 randomly selected compounds in concentrations of 10 ng/mL, 100 ng/mL and 1000 ng/mL. Table 2 lists the concentration at which each analyte in the toxicology screen for urine samples is identified. The presence of an analyte at 10, 100 or 1000 ng/mL implies that the limit of detection is likely below that value. Of the 275 compounds analyzed, 70% were detected at 10 ng/mL, 20% at 100 ng/mL, 8% at 1000 ng/mL and 2% were detected at a concentration above 1000 ng/mL.

Table 2. Results for spiked urine samples in toxicology screen by LC-MS/MS

LXQ – 13 min method Compound	**Concentration Tested (ng/mL)** 10	100	1000
All barbiturates require an APCI source for detection.	*P=Drug present.*	*N=Drug not present.*	
11-Hydroxy-delta-9-THC	N	N	>1000
11-nor-9-carboxy-Delta-9-THC	N	N	P
2-Bromo-Alpha-Ergocryptine	P	P	P
2-Hydroxyethylflunitrazepam	N	P	P
3-Hydroxystanozolol	N	N	>1000
4-Hydroxynordiazepam	N	P	P
6-Acetylcodeine	P	P	P
6-Acetylmorphine (6-MAM)	P	P	P
7-Amino-Clonazepam	P	P	P
7-Amino-Flunitrozepam	P	P	P
Acebutolol	P	P	P
a-Hydroxy-Alprazolam	P	P	P
a-Hydroxy-Triazolam	P	P	P
Albuterol	P	P	P
alpha-Hydroxymidazolam	N	P	P
Alprazolam	P	P	P
Alprenolol	P	P	P
Aminorex	N	P	P
Amiodarone	P	P	P
Amitriptyline	P	P	P
Amlodipine	N	N	P
Amobarbital	P	P	P
Amoxapine	P	P	P
Amphetamine	P	P	P
Anhydroecgonine MethylEster	N	P	P
Antipyrine	N	N	>1000
Apomorphine	N	N	>1000

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

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

LXQ – 13 min method Compound
Mianserin
Miconazole
Midazolam
Mirtazapine
Molsidomine
Morphine
Morphine-3-b-glucuronide
Nalbuphine
Nalorphine
Naloxone
Naltrexone
NAPA
N-DemethylTrimipramine
N-Desmethyl-cis-tramadol
N-Desmethylflunitrazepam
N-Desmethylselegiline
N-DesmthylClomipramine
N-Ethylamphetamine
Nicardipine
Nicotine
Nitrazepam
Nitrendipine
Nizatidine
Norbenzoylecgonine
Norbuprenorphine
Norclomipramine
Norcocaethylene
Norcocaine
Norcodeine
Nordiazepam
Nordoxepin
Norethandrolone
Norfentanyl
Norfluoxetine
Norketamine
NOR-LSD
Normeperidine
Normorphine
Noroxycodone
Noroxymorphone
Norproproxyphene
Nortriptyline
Noscapine
OH-LSD
Ondansetron
Opipramol
Oxazepam
Oxcarbazepine
Oxycodone
Oxymorphone
Papaverine
Paraxanthine
Paroxetine
PCP
Pentazocine
Pentobarbital
Perphenazine
Pheniramine
Phenobarbital
Phenolphthalein
Phentermine
Phenylbutazone
Phenyltoloxamine
Physostigmine
Pindolol
Piroxicam
PMA
PMMA

Concentration Tested (ng/mL)		
10	100	1000
P	P	P
P	P	P
P	P	P
P	P	P
N	N	>1000
N	P	P
N	N	>1000
P	P	P
P	P	P
P	P	P
P	P	P
P	P	P
P	P	P
N	N	P
N	P	P
N	P	P
N	P	P
N	P	P
P	P	P
P	P	P
N	N	>1000
P	P	P
N	N	P
N	N	>1000
N	N	>1000
P	P	P
P	P	P
P	P	P
N	P	P
P	P	P
P	P	P
N	P	P
N	P	P
P	P	P
N	P	P
P	P	P
P	P	P
N	N	P
N	P	P
N	N	>1000
P	P	P
P	P	P
P	P	P
N	P	P
P	P	P
P	P	P
P	P	P
N	N	P
P	P	P
N	P	P
P	P	P
N	N	>1000
N	P	P
P	P	P
P	P	P
P	P	P
P	P	P
N	P	P
P	P	P
P	P	P
N	N	P
N	N	P
N	N	P
P	P	P
N	N	P
P	P	P
N	N	P
N	P	P

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

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

Table 3. Results for spiked plasma samples in toxicology screen by LC-MS/MS

LXQ – 13 min method Compound	Concentration Tested (ng/mL) 10	100	1000
BDB	N	P	P
Benzocaine	N	P	P
Benzoylecgonine	P	P	P
Betaxolol	P	P	P
Bisacodyl	P	P	P
Bisoprolol	P	P	P
Bromazepam	N	P	P
Brompheniramine	N	P	P
Bufotenine	N	P	P
Bupivocaine	P	P	P
Buprenorphine	P	P	P
Bupropion	N	P	P
Buspirone	P	P	P
Butorphanol	P	P	P
Cannabidol	N	P	P
Cannabinol	N	P	P
Captopril	N	N	>1000
Estazolam	N	P	P
Carbamazepine	P	P	P
Carbinoxamine	P	P	P
Carisoprodol	N	P	P
Cathinone	N	N	>1000
Chlordiazepoxide	N	P	P
Chloroquine	N	P	P
Chlorpheniramine	P	P	P
Chlorpromazine	N	P	P
Chlorprotixene	P	P	P
Clozapine N-Oxide	N	P	P

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

For selected sets of compounds the method was also prequalified by processing and analyzing spiked plasma samples. Table 3 lists the concentration at which each analyte in the toxicology screen for plasma samples is identified. In general, detection limits for urine and plasma are comparable.

In addition, the assay performance was verified by analyzing patient urine samples obtained from the Johns Hopkins University Hospital Clinical Laboratory and data were compared to the results from established LC-UV and immunoassay analytical techniques. The result is shown in Table 4. The LC-MS/MS method has consistently identified more analytes present in the sample than either LC-UV or immunoassays.

Table 4. Urine sample analyzed with LC-MS/MS, LC-UV and Immunoassay methods

LC-MS	LC-UV	Immunoassay
Nortriptyline	Nortriptyline	Barbiturates
Amitriptyline	Amitriptyline	Benzodiazepines
Benzoylecgonine	Benzoylecgonine	Cocaine
Cocaine	Cocaine	Opiates
Norcocaethylene	Cocaethylene	THC
Norbenzoylecgonine	-	-
Morphine	-	-
Norcocaine	-	-
Quinidine/Quinine	-	-
Hydroxyzine	-	-
Noskapine	-	-
Diltiazem	-	-
Morphine-3-beta-Glucuronide	-	-

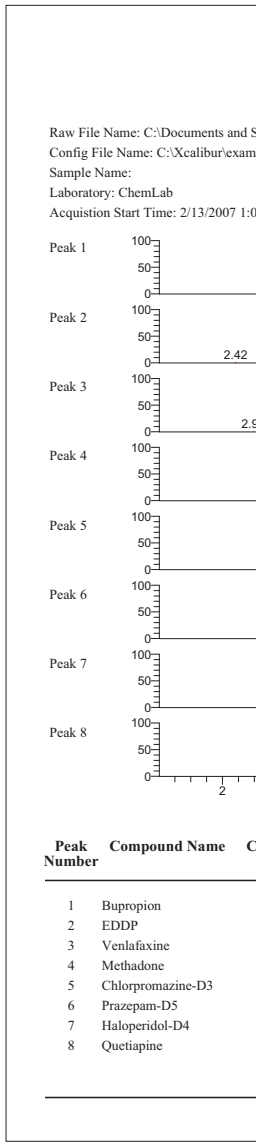
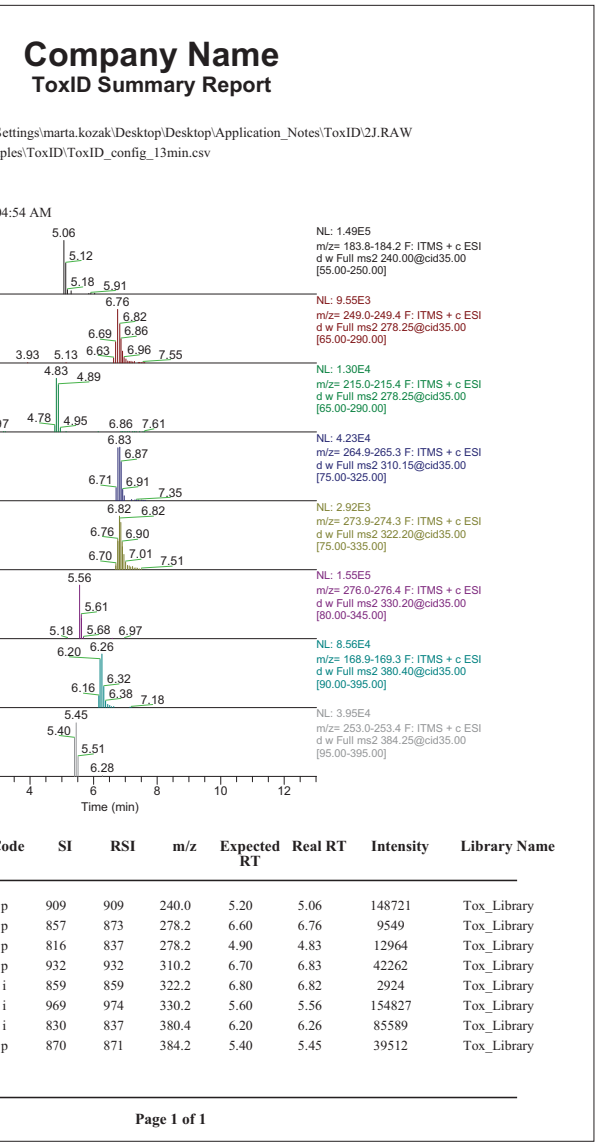


Figure 3: The ToxID Summary Re



Company Name
ToxID Summary Report

Settings\marta.kozak\Desktop\Desktop\Application_Notes\ToxID\2J.RAW
ples\ToxID\ToxID_config_13min.csv

04:54 AM

NL: 1.49E5
m/z= 183.8-184.2 F: ITMS + c ESI d w Full ms2 240.00@cid35.00 [55.00-250.00]

NL: 9.55E3
m/z= 249.0-249.4 F: ITMS + c ESI d w Full ms2 278.25@cid35.00 [65.00-290.00]

NL: 1.30E4
m/z= 215.0-215.4 F: ITMS + c ESI d w Full ms2 278.25@cid35.00 [65.00-290.00]

NL: 4.23E4
m/z= 264.9-265.3 F: ITMS + c ESI d w Full ms2 310.15@cid35.00 [75.00-325.00]

NL: 2.92E3
m/z= 273.9-274.3 F: ITMS + c ESI d w Full ms2 322.20@cid35.00 [75.00-335.00]

NL: 1.55E5
m/z= 276.0-276.4 F: ITMS + c ESI d w Full ms2 330.20@cid35.00 [80.00-345.00]

NL: 8.56E4
m/z= 168.9-169.3 F: ITMS + c ESI d w Full ms2 380.40@cid35.00 [90.00-395.00]

NL: 3.95E4
m/z= 253.0-253.4 F: ITMS + c ESI d w Full ms2 384.25@cid35.00 [95.00-395.00]

Time (min)

ode	SI	RSI	m/z	Expected RT	Real RT	Intensity	Library Name
p	909	909	240.0	5.20	5.06	148721	Tox_Library
p	857	873	278.2	6.60	6.76	9549	Tox_Library
p	816	837	278.2	4.90	4.83	12964	Tox_Library
p	932	932	310.2	6.70	6.83	42262	Tox_Library
i	859	859	322.2	6.80	6.82	2924	Tox_Library
i	969	974	330.2	5.60	5.56	154827	Tox_Library
i	830	837	380.4	6.20	6.26	85589	Tox_Library
p	870	871	384.2	5.40	5.45	39512	Tox_Library

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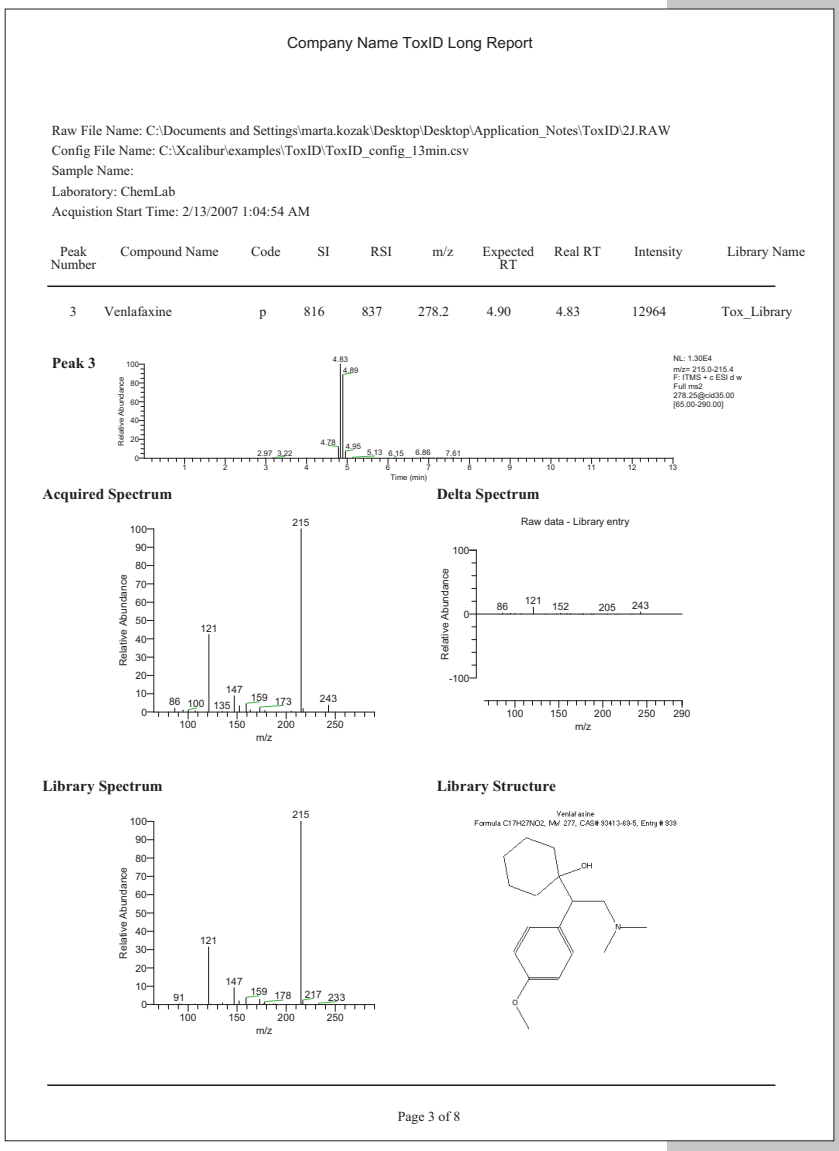
port is designed for a quick synopsis of the data.



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

NL: 1.30E4
m/z= 215.0-215.4 F: ITMS + c ESI d w Full ms2 278.25@cid35.00 [65.00-290.00]

Relative Abundance
Time (min)

Acquired Spectrum

Relative Abundance
m/z

Delta Spectrum

Raw data - Library entry

Relative Abundance
m/z

Library Spectrum

Relative Abundance
m/z

Library Structure

Venlafaxine
Formula C17H27NO2, MW 277, CAS# 93413-69-5, Entry # 939

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Figure 4: The ToxID Long Report is designed for a more thorough examination of the data.

Table 5. Simple workflow for adding new analytes

STEP 1: Directly infuse analyte to obtain MS^2 spectra, then add spectra to the library	10 Minutes
STEP 2: Run analyte on column to obtain retention times	13 Minutes
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 product ion and retention times	2 Minutes

ToxID™ Software Automates Reporting, Reduces Manual Analysis

ToxID software identifies compounds present in the sample based on MS/MS spectra and retention times. Positive hits are automatically reported via ToxID software. Reports are automatically generated, reducing the time necessary for manual analysis of each sample chromatogram. An example of a Summary Report is shown in Figure 3. A Long Report with one page per detected compound is shown in Figure 4.

Adding New Compounds to the Application

This LC-MS/MS workflow allows the user to quickly and easily add new analytes to the screening method. This feature is very important for toxicology screening because new target compounds are continually being added to the target list. As shown in Table 5, new compounds can typically be added in less than 1 hour.

Conclusion

The comprehensive, turn-key toxicology screening methodology described in this application note utilizes an LXQ ion trap, and includes an SPE procedure and LC method that enables the identification of 275 compounds in human urine and human plasma. Accompanying ToxID software performs automatic data analysis and reporting. This eliminates the need for manual data interpretation and increases confidence in compound identification. It is worth noting that when compared to other screening methods, the LC-MS/MS screening methodology identifies more analytes.

Mass Spectrometers are general purpose laboratory instruments. They have not been cleared or approved by the United States Food and Drug Administration, the European IVD Directive or any other agency for diagnostic, clinical or other medical use.

View additional Thermo Scientific LC/MS application notes at: **www.thermo.com/appnotes**

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