

# ToxSpec Analyzer: A Complete Toxicology Screening Procedure for Drugs and Toxic Compounds in Urine and Plasma Using LC/MS

<sup>1</sup>Taha Rezai, <sup>1</sup>Marta Kozak, <sup>1</sup>Kate Torchilin, <sup>2</sup>Bénédicte Duret

<sup>1</sup>Thermo Fisher Scientific, San Jose, CA, USA; <sup>2</sup>Thermo Fisher Scientific, Les Ulis, France.

## Overview

**Purpose:** To develop a complete general unknown screening (GUS) procedure to support the analytical needs of clinical and toxicology laboratories

**Methods:** Liquid chromatography with electrospray ionization was used for the screening of human urine samples prepared by solid-phase extraction (SPE) against a 300 compound library of MS2 spectra

**Results:** The ion trap LC-MS along with ToxID software and a versatile SPE procedure, allows for the identification of ~300 drugs, toxic compounds and metabolites in urine

## Introduction

The GUS procedure is commonly employed by clinical toxicology laboratories to analyze for drugs of abuse and other compounds in urine and other matrices. Lately, LC-MS has been considered for this application because of this technology's sensitivity, specificity, and ability to detect a broad range of compounds. However, a generic method that supports all of the compounds of interest in a clinical toxicology laboratory, remains to be developed. In this work, we present a generic method, including sample preparation and LC/MS methodology, which supports the analysis of a wide range of compounds. The MS2 spectra, as well as parent masses, the most intense daughter ion and retention times are all used to identify the compounds present in the sample, and the positive hits are automatically reported via ToxID software. In addition, a 13 min LC method has been developed as an alternative to longer LC methods for laboratories that require higher throughput and faster sample turnaround. The GUS application allows additional compounds to be easily added to the screen.

## Methods

**Sample Preparation:** For SPE, 200 mg mixed mode C18 solid phase extraction cartridges (Thermo Fisher Scientific, Hypersep™ Verify™-CX) were utilized. 1 mL of urine was then spiked with 10, 100 and 1000 ng of analytes of interest, as well as 100 ng of three deuterated standards. Basic, acidic and neutral fractions were then collected, combined, evaporated to dryness, reconstituted in 100 µL and injected onto the LCMS.

**LC Conditions:** 13 minute and 30 minute LC methods were developed. Gradients from 5%-95% B were employed, with flow rates of 200 µL/minute.

Solvent A: Water, 10 mM Ammonium Formate w/0.1% Formic Acid  
Solvent B: Acetonitrile, 0.1% Formic Acid

Columns:  
13 min. method: 5 x 2.1 mm, 5 µm, Hypersil™ PFP C18  
30 min. method: 150 x 2.1 mm, 5 µm, Hypersil PFP C18

**MS Conditions:** The SPE prepped samples were analyzed on a Thermo Scientific LXQ™ ion trap with a ESI source, in a scan dependent polarity switching method. Spray Voltage: 5kV, Capillary Temperature: 275 °C

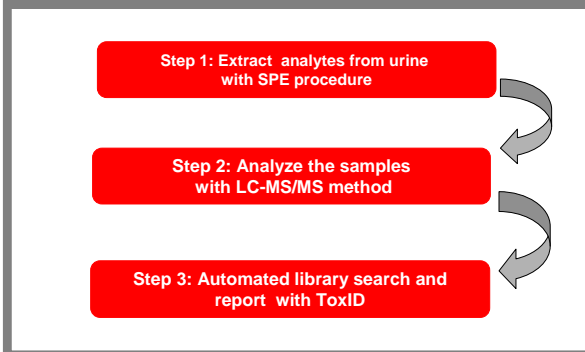
## Results

Table 1 shows a simple workflow for adding new analytes to the GUS method

TABLE 1. Simple Workflow for Adding New Analytes

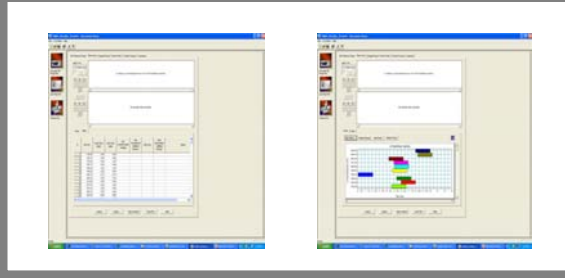
Step	Description	Time
STEP 1:	Directly infuse analyte to obtain MS2 spectra, then add spectra to the library	10 Minutes
STEP 2:	Run analyte on column to obtain retention times	13 or 30 min – depending on LC method
STEP 3:	Update Parent Mass Table in instrument method with parent masses and retention times	2 Minute
STEP 4:	Update ToxID with name, parent masses, the most intense daughter ion and retention times	2 Minutes

FIGURE 1. Overall Application Workflow



The overall workflow for GUS is shown in Figure 1. After sample preparation with SPE, samples are analyzed with LC-MS/MS, and reports are produced with ToxID.

FIGURE 2. Parent Mass Table/Chart



The Parent Mass Table allows the instrument to only search for masses within retention time windows specific to each compound. This greatly conserves instrument resources, by preventing the search for all masses throughout the entire method.

FIGURE 3. Scan Dependent Method Flow Chart

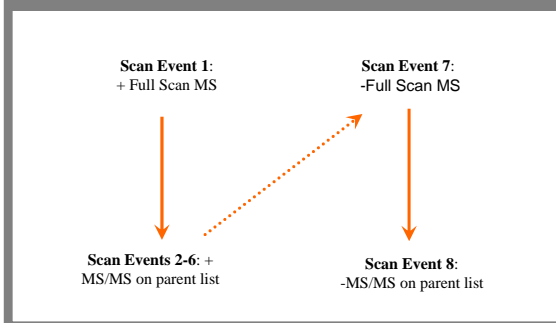
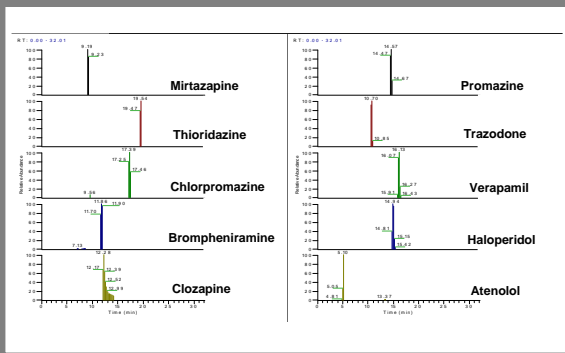


Figure 3 shows a flow chart for the GUS analysis instrument method. In the positive mode, one full scan is followed by 5 scan dependent MS/MS experiments, which are triggered by the presence of masses from the parent list. In the negative mode, one full scan is followed by one scan dependent MS/MS experiment.

FIGURE 4. Example of TIC Chromatograms for SPE-Prepped Urine-Spiked Samples at 10 ng/mL



The ToxID short report shown in Figure 5, includes LC chromatograms and TIC data, as well as a list of analytes that were found and identified in the sample. With ToxID search index cutoffs, m/z ranges, retention time ranges and parent/product ion intensity thresholds are defined by the user.

FIGURE 5. ToxID Short Report

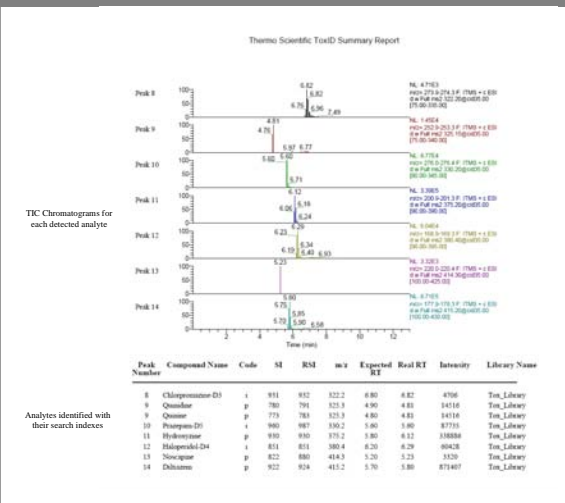
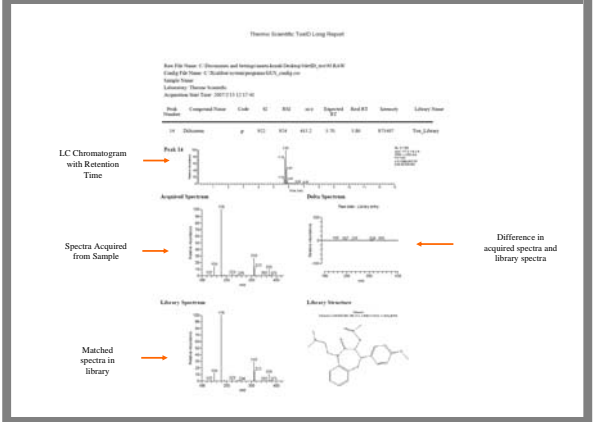


FIGURE 6. ToxID Long Report



The ToxID long report (shown above) shows an LC chromatogram (w/ retention times), the MS2 spectra acquired from the sample, the matched spectra in the library, and a delta spectrum. The delta spectrum is the difference between the acquired spectra and the library spectra. In addition, search indexes and structures are also included. Each analyte has one full page dedicated to it in the long report.

TABLE 2. Limits of Detection for 10 Representative Analytes

Analyte	LOD 30 min LC Method* (ng/mL of urine)	LOD 13 min LC Method* (ng/mL of urine)
Heroin	10	10
Codeine	10	10
Cocaine	10	10
Amphetamine	10	10
Benzoylcegonine	10	10
Diazepam	10	10
Fexofenadine	10	10
Furosemide	10	10
Ketamine	10	10
LSD	10	10

\*at least 10 ng/mL, lower detection limits may be possible

Table 2 shows LOD's for a representative set of 10 analytes from both the 30-minute and 13-minute LC method. 10 ng/mL was the lowest concentration validated, lower detection limits may be possible. Of the 300 compounds in the method, 70% of the compounds have LOD's of 10 ng/mL, 20% have LOD's of 100 ng/mL, 8% have LOD's of at least 1000 ng/mL, and 2% have LOD's greater than 1000 ng/mL.

TABLE 3. Patient Sample Analyzed with ToxID, LC-UV and Immunoassay Methods

ToxID 30 Minute	ToxID 13 Minute	LC-UV	Immunoassay
Nortriptyline	Nortriptyline	Nortriptyline	Barbiturates
Amitriptyline	Amitriptyline	Amitriptyline	Benzodiazepines
Benzoylcegonine	Benzoylcegonine	Benzoylcegonine	Cocaine
Cocaine	Cocaine	Cocaine	Opiates
Cocacethylene	-	Cocacethylene	THC
Cyclobenzaprine	-	-	-
Norbenzoylcegonine	Norbenzoylcegonine	-	-
Morphine	Morphine	-	-
Norcocaine	Norcocaine	-	-
Codeine	-	-	-
Norcocacethylene	Norcocacethylene	-	-
Methodone	-	-	-
Quinidine/Quinine	Quinidine/Quinine	-	-
Hydroxyzine	Hydroxyzine	-	-
Noskapine	Noskapine	-	-
Diltiazem	Diltiazem	-	-
Morphine-3-beta-Glucuronide	Morphine-3-beta-Glucuronide	-	-

Table 3 shows the results of the ToxID, LC-UV and Immunoassay screens. The 30-minute ToxID method is able to resolve peaks better and detect the presence of lower intensity analytes, resulting in more hits than in the 13-minute method. LC-UV and Immunoassay results show some overlap with ToxID results. The ToxID method, however, clearly identifies more analytes than either of the other two methods.

## Conclusions

The ToxSpec Analyzer allows for the identification of ~300 compounds, with LOD's ranging from at least 10 to >1000 ng per mL of urine. Further, ToxID software allows for automatic data analysis and report production, thereby eliminating the need for manual spectral examination. In addition, when compared to other general unknown screening methods, ToxID was able to identify more analytes. Also, analytes of interest can be easily added to the screening method.

## Acknowledgements

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