Application Note: 467

Screening Drugs and Toxic Compounds with LC-MS/MS: An Alternative to LC-UV for Research Toxicology Labs

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Key Words

- ToxSpec Analyzer
- ToxID software
- LXQ Linear Ion Trap
- Accela UHPLC System

Introduction

Screening for drugs of abuse and other toxic compounds in biological samples has quickly become a routine assay conducted in many research toxicology laboratories. The main challenge is to get rapid and accurate results amidst the generally large number of potential analytes to be identified within complex biological matrices. One of the techniques widely used in this area is high pressure liquid chromatography (HPLC) combined with photo diode array detection (DAD) or ultra-violet (UV) detection. The most popular LC-UV platform has been the Bio-Rad® REMEDi™ HS drug profiling system. When this platform was recently discontinued, a significant technological gap became apparent. Now this gap is rapidly being filled by newer, more effective high pressure liquid chromatography - mass spectrometry (HPLC-MS) technologies.

Here we present the workflow and results obtained by using the Thermo Scientific ToxSpec Analyzer, a new UHPLC-MS system based on ultra-high pressure liquid chromatography and linear ion trap mass spectrometry technology.

Goal

Evaluate an LC-MS/MS method for screening and semi-quantitation of drugs and toxic compounds in serum and urine matrices to determine if this approach can provide an alternative to REMEDi technology for research toxicology.

Experimental

The ToxSpec™ Analyzer combines hardware, software, and screening methodologies designed to significantly simplify and improve the screening assay workflow. LC-MS² data is acquired by using a pre-configured instrument method, and the data is automatically processed, post-acquisition, by Thermo Scientific ToxID automated drug screening software.

The LC-MS screening was performed on Thermo Scientific instrumentation including an LXQ™ linear ion trap mass spectrometer coupled to an Accela™ UHPLC system using a polarity-switching and scan-dependent MS/MS experiment (Figure 1). The MS² spectra generated were processed through ToxID™ software. Using a novel screening algorithm, the software program identifies target analytes through a MS² library search against a large spectral library of known analytes as well as expected retention times. Semi-quantitative data results can also be generated concurrently from the MS² spectral intensity ratios between the target analyte and the corresponding internal standard.

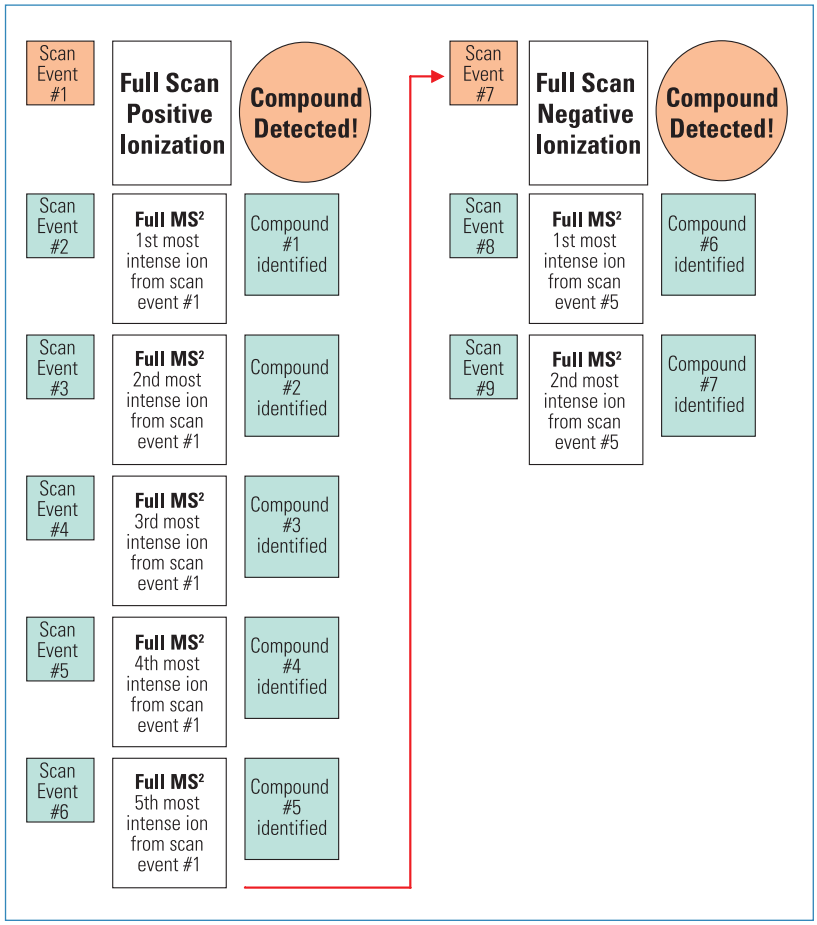

Figure 1: Polarity-switching and scan-dependent MS/MS experiment

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The ToxSpec Analyzer includes a diverse and easily-expandable MS/MS library of 300 compounds that it screens using a single pre-configured method. In our laboratory, we have expanded the library by more than 50 entries to date.

Sample preparation

The extraction procedure was performed by using liquid/liquid extraction (LLE) with Toxi-Tube A® (Varian, les Ulis, France). Details of the procedure are described below.

- Vortex the Toxi-Tube A for 10 seconds.
- Add 1 mL of serum or urine into the Toxi-Tube A.
- Add 200 µL of a solution of internal standard [haloperidol-d4, chlorpromazine-d3, and prazepam-d5 at the following concentrations: 100 ng/mL, 1 µg/mL and 100 ng/mL, respectively, in 70/30 of A/B (A: water containing 10 mM ammonium acetate and 0.1% formic acid; B: acetonitrile containing 0.1% formic acid)].
- Add 5 mL of water.
- Vortex for 10 seconds.
- Mix for 5 minutes.
- Centrifuge for 5 minutes at 2700 rpm.
- Transfer the upper layer to a tube and evaporate to dryness at 40 °C.
- Reconstitute the sample in 200 µL of 70/30 of A/B.

HPLC Conditions

Chromatographic analyses were performed using the Thermo Scientific Accela UHPLC system. The chromatographic conditions were as follows:

Column:	Thermo Scientific Hypersil GOLD PFP 5 µm, 150 x 2.1 mm		
Flow rate:	0.2 mL/min		
Mobile phase:	A: water containing 10 mM ammonium acetate and 0.1% formic acid; B: acetonitrile containing 0.1% formic acid		
Injection volume:	10 µL		
Gradient:	T (min)	A (%)	B (%)
	0.0	95	5
	5.0	55	45
	18.0	30	70
	20.0	5	95
	27.0	5	95
	27.1	95	5
	32.0	95	5

Mass Spectrometry Conditions

MS analysis was carried out on a our LXQ linear ion trap mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

Ion polarity:	Polarity-switching scan-dependent experiment
Spray voltage:	5000 V
Sheath gas (N_2) pressure:	30 (arbitrary units)
Auxiliary gas (N_2) pressure:	8 (arbitrary units)
Capillary temperature:	275 °C
Microscan:	1
Wideband Activation™:	Activated
Stepped normalized collision energy:	35% ± 10%

Results and Discussion

More than 150 real laboratory samples (serum and urine) have been analyzed. Table 1 reports some of the data obtained from both the REMEDi HS LC/UV system and the ToxSpec Analyzer UHPLC/MS system. Among the 12 samples reported here, 22 compounds have been identified using both the REMEDi HS and the ToxSpec Analyzer. Notably however, the ToxSpec Analyzer system identified 24 additional compounds that were not detected with the REMEDi HS due in most cases to a lack of sensitivity, specificity, and coelution capability.

The ToxSpec Analyzer also provided a better response for some classes of compounds, like benzodiazepines. With the REMEDi HS system, the retention time for this class of compounds was close to the dead volume of the column. For that reason, the signals that interfered with matrix components were rather difficult to identify. It was also observed that haloperidol (sample #5) and paroxetine (sample #10) gave a much better signal with the ToxSpec Analyzer.

Sample #	Compounds identified using ToxSpec Analyzer	Compounds identified using REMEDi HS
1	Acetaminophen	**Not detected**
	Nortriptyline	**Not detected**
	Amitriptyline	Amitriptyline
	Oxazepam	**Not detected**
2	Nordiazepam	Nordiazepam
	Alprazolam	**Not detected**
	Cyamemazine	Cyamemazine
3	Acetaminophen	**Not detected**
	Nordiazepam	Nordiazepam
	Venlafaxine	Venlafaxine
	Oxazepam	Oxazepam
	Alprazolam	**Not detected**
4	Nordiazepam	**Not detected**
	Diazepam	Diazepam
	Oxazepam	**Not detected**
	Temazepam	**Not detected**
	Levomepromazine	Levomepromazine
	Zopiclone	Zopiclone
5	Oxazepam	**Not detected**
	Clomipramine	Clomipramine
	Quinidine	Quinine
	Haloperidol	**Not detected**
	Clonazepam	**Not detected**
6	Acetaminophen	**Not detected**
	Bisoprolol	Bisoprolol
7	Venlafaxine	Venlafaxine
	Risperidone	**Not detected**
8	Quinine	Quinine
	Hydromorphone	Hydromorphone
	Morphine	Morphine
9	Lidocaine	**Not detected**
	Nortriptyline	**Not detected**
	Mirtazapine	**Not detected**
	Amitriptyline	Amitriptyline
	Cyamemazine	Cyamemazine
	Levomepromazine	Levomepromazine
	Zopiclone	**Not detected**
10	Bromazepam	Bromazepam
	Paroxetine	**Not detected**
11	Sertraline	**Not detected**
	Hydrocortisone	**Not detected**
12	Acetaminophen	**Not detected**
	Alprazolam	Alprazolam
	Prednisolone	**Not detected**
	Hydroxyzine	Hydroxyzine
	Fexofenadine	**Not detected**
TOTAL	46 Molecules	22 molecules

Table 1: List of psychoactive molecules identified in real laboratory samples using the ToxSpec Analyzer compared to the REMEDi HS system

Our aim was to quickly and confidently identify toxic compounds in the samples by spectral library searching while performing a semi-quantification calculation for identified compounds. To perform the semi-quantification, a response factor that correlated the intensity of the MS^2 spectra to a concentration was calculated for each molecule present in the library using internal standards. The semi-quantification result was automatically calculated using ToxID software. An example of the automatically-generated report can be seen in Figure 2. The report includes a list of compounds identified in a real laboratory sample and their respective calculated concentrations.

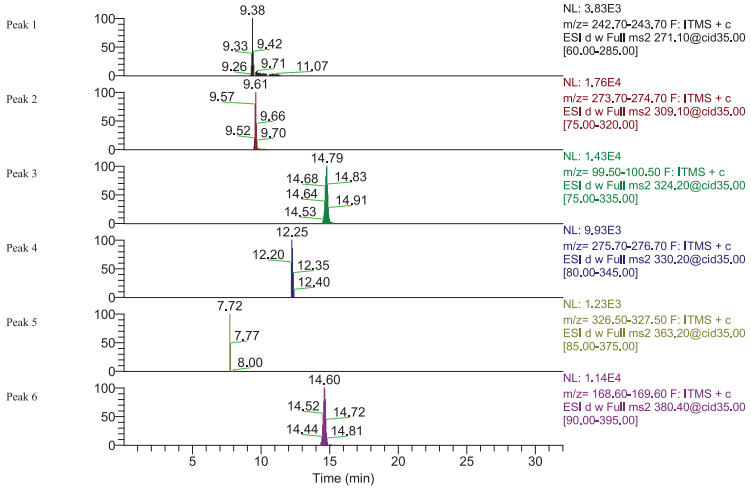
One important aspect of this method is the ability to reprocess data retrospectively from the MS spectra. The ToxID report is based on MS^2 spectra library searching. This means that if the entry corresponding to the compound is not currently available in the library, ToxID will not be able to identify the analyte. However, as data are acquired in MS mode, it is possible to reprocess the MS trace and check that all major ions have been identified by ToxID. If not, it is then possible to re-inject the sample and perform MS^2 acquisition on specific ions.

Conclusion

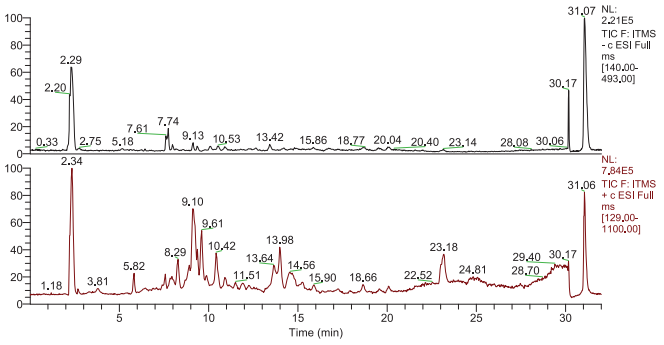
The ToxSpec Analyzer is a good replacement for the REMEDi HS system in research toxicology laboratories because it offers increased sensitivity, greater specificity, and lower cost-per sample analysis.

Centre Hospitalier Lyon Sud
Summary Report

Raw File Name: C:\Xcalibur\Validation REMEDI\Data\Real samples\090107\375489.RAW
Config File Name: C:\Xcalibur\Validation REMEDI\CSV fi...\LXQ_GUS_config_30min_TOXID Semi-quanti.csv
Sample Name: Laboratory: Laboratoire de Toxicologie
Acquistion Start Time: 1/7/2009 1:23:18 PM
Screening Conditions: Based on Full MS2 scans. m/z window (amu): 0.50, RT window (min): 2.00, MS2 Search libraries: Tox_Library_LXQ,Use full MS scan to confirm.



Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Actual RT	Concentration mg/l	Library Name
1	Nordiazepam	p	700	708	271.10	8.60	9.38	1.05	Tox_Library_LXQ
2	Alprazolam	p	811	814	309.10	9.10	9.61	0.84	Tox_Library_LXQ
3	Cyamemazine	p	818	819	324.20	15.00	14.79	0.25	Tox_Library_LXQ
4	Prazepam-D5	i	890	894	330.20	11.70	12.25	0.87	Tox_Library_LXQ
5	Hydrocortisone	p	863	865	363.20	7.50	7.72	0.11	Tox_Library_LXQ
6	Haloperidol-D4	i	845	861	380.40	14.90	14.60	1.00	Tox_Library_LXQ



TIC Full Scans

Figure 2: ToxID report – short summary style

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