Application Note: 517

Screening for Drugs and Toxic Compounds: Comparison between LC-MS/MS, HPLC-DAD, and Immunoassay

C. Gechtman[1], A. Masarin[1], S. Scurati[2]; [1]A.O. Ospedale Niguarda Cà Granda, Milano, Italy; [2]Thermo Fisher Scientific, Milano, Italy

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Key Words

- ToxID software
- LXQ Linear Ion Trap
- Drugs of Abuse
- Toxicology Screening

Introduction

Screening of biological samples for drugs of abuse and other toxic compounds is a critical feature of forensic toxicology laboratories. The main challenge is to provide rapid and accurate results despite the large number of target molecules and the complexity of biological matrices. The classical approach is based on immunoassay or high pressure liquid chromatography-diode-array detection (HPLC-DAD). However, the advent of newer and more effective liquid chromatography-tandem mass spectrometry (LC-MS/MS) technologies can lead to a significant improvement in non-targeted screening.

Goal

Evaluate the Thermo Scientific ToxSpec Analyzer, LC-MS solution for forensic toxicology screening, for non-targeted screening of several compounds in human urine. LC-MS technology is used to increase the confidence of identification and to simplify the workflow in a forensic toxicology laboratory when compared with the classical screening approaches.

Experimental

Sample Preparation

Urine was stored at -20 °C for the analysis. After thawing, the sample was diluted 1:10 with water. For the analysis, 20 µL of diluted urine were directly injected.

Chromatography and Mass Spectrometry

The ToxSpec™ Analyzer was used for the analysis. Briefly, for the LC separation a Thermo Scientific Hypersil GOLD PFP analytical column (50 x 2.1, 5 µm) was used, with mobile phase A (10 mM ammonium formate in 0.1% formic acid) and B (ACN containing 0.1% formic acid). The gradient was from 95% A to 95% B in about 5 minutes with a flow rate of 200 µL/min. For the MS analysis, a Thermo Scientific LXQ linear ion trap mass spectrometer equipped with an electrospray ionization (ESI) source utilizing polarity switching was employed. A data dependent scan collected MS/MS spectra of all the compounds eluted. Data generated were processed with Thermo Scientific ToxID automated screening software. ToxID™ software identifies compounds on the basis of retention time, precursor ion, and MS/MS spectrum. Samples screened by LC-MS/MS were previously analyzed also with immunoassay or HPLC-DAD, allowing a comparison between methods.

Results and Discussion

The ToxSpec Analyzer is able to process a sample in about 15 minutes, which allows the performance of routine screening analysis. Data obtained are highly specific and reliable because the identification of compounds is based on three peculiar characteristics of the molecules: retention time, precursor ion, and MS/MS spectrum. Figure 1 shows a report generated by ToxID software after the analysis of a urine sample that tested positive for LSD.

The comparison of results obtained by analyzing the same urine samples with different screening approaches has given interesting results (see Table 1). The ToxSpec Analyzer confirmed, for the most part (Urine 1-4), the results obtained with HPLC-DAD or an immunoassay, but also identified additional compounds, such as metabolites or other minor components that were not recognized with other screening approaches.

Surprisingly, in Urine 5, the results are clearly not in agreement. Particularly, the immunoassay identified amphetamines, while the ToxSpec Analyzer method identified ranitidine and metoclopramide, two therapeutics drugs often used in combination. To better understand the difference between the techniques, we compared the MS/MS spectra of the molecules detected in Urine 5 with those present in the library.

Table 1. Comparison of results obtained analyzing the same urine samples using different screening techniques.

Sample	HPLC-DAD or Immunoassay	ToxSpec Analyzer	Results Comparison
Urine 1	Cocaine	Cocaine, Benzoylecgonine, Cocaethylene, Nicotine	√
Urine 2	Ketamine	Ketamine, Norketamine	√
Urine 3	Quetiapine	Lidocaine, Quetiapine	√
Urine 4	LSD	OH-LSD	√
Urine 5	Amphetamines	Ranitidine, Metoclopramide	X

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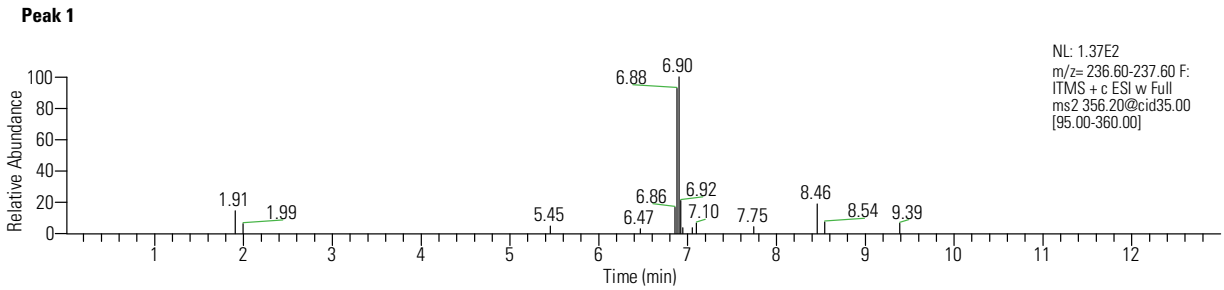


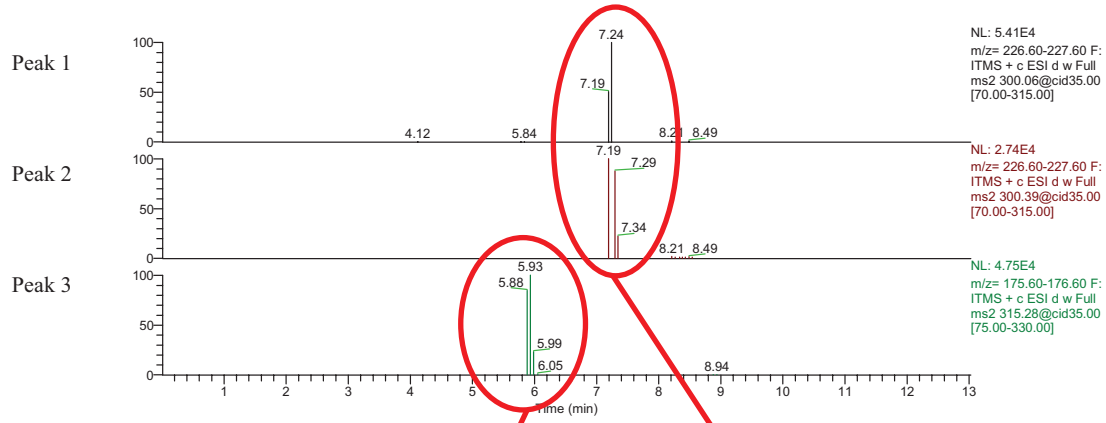
Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Actual RT	Intensity	Library Name
1	OH-LSD	m	756	922	356.20	6.20	6.90	15461	Tox_Library_LXQ

Figure 1. Example ToxID software short report showing ion chromatogram and a compound detected in urine positive for LSD.

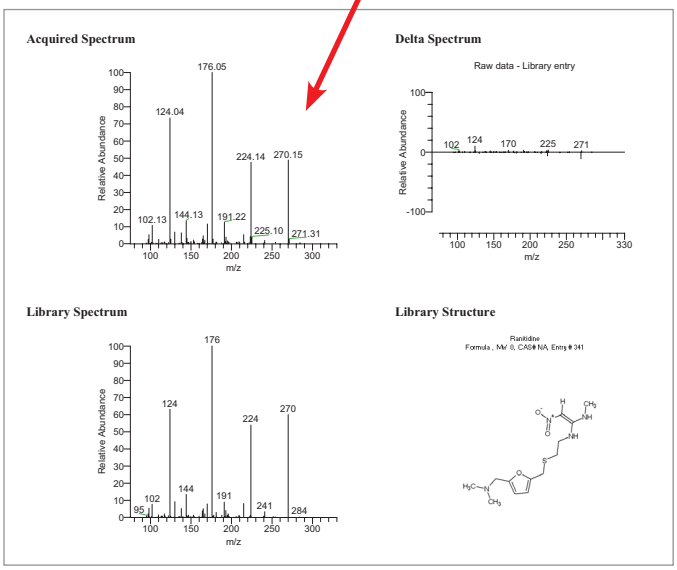
We confirmed the presence of ranitidine and metoclopramide through the mass spectra, which are very similar to that present in the library and the measured retention times are very similar to the expected (Figure 2). Moreover, some immunoassays are known to give cross-reactivity between ranitidine and amphetamines. As a consequence, we established that a false positive was found by the immunoassay and the ToxSpec Analyzer that identified the cross-reacting molecule as ranitidine.

Conclusion

The ToxSpec Analyzer was used to screen toxic compounds and their metabolites in urine based on LC-MS/MS. This method has been compared with other classical screening techniques such as HPLC-DAD and an immunoassay. LC-MS/MS demonstrated more reliable results than other techniques. In conclusion, the LC-MS/MS method provides rapid sample preparation, ease-of-use, sensitivity, specificity and a low cost per sample analysis, making the ToxSpec Analyzer an appropriate tool for non-targeted screening in a forensic toxicology laboratory.



Peak Number	Compound Name	Code	SI	RSI	*m/z*	Expected RT	Real RT	Intensity	Library Name
1	Metoclopramide	p	958	958	300.20	7.20	7.24	54117	Tox_Library_LXQ
2	Metoclopramide	p	984	984	300.20	7.20	7.19	27361	Tox_Library_LXQ
3	Ranitidine	p	792	802	315.20	5.45	5.93	47525	Tox_Library_LXQ



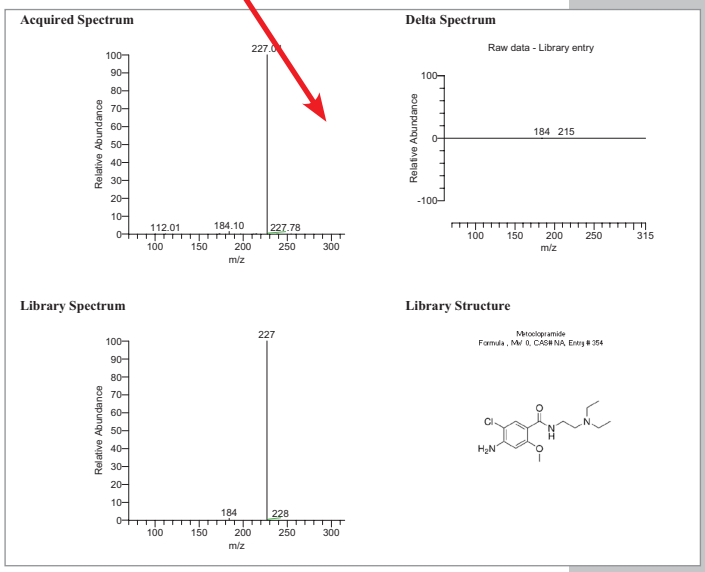


Figure 2. ToxID software long report showing ion chromatograms and MS/MS spectra of compounds detected in Urine 5. Mass spectra recorded for ranitidine and metoclopramide show a perfect match when compared with spectra from the database.

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