Application Note: 524

A Fully Automated LC-MS Screening System using Automated Online Sample Preparation for Forensic Toxicology

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Key Words

- Transcend TLX-1 system
- TurboFlow Technology
- LXQ Linear Ion Trap
- Illicit Drugs
- Forensic Toxicology

Introduction

Liquid chromatography-mass spectrometry (LC-MS) is a powerful tool widely used for forensic targeted drug screening. However, the quality of the results is highly affected by the sample preparation. Offline solid phase extraction (SPE) and liquid-liquid extraction (LLE) are widely used, but these methods are often time-consuming and costly. To provide a fast and sensitive approach, an automated online sample preparation method using Thermo Scientific Transcend TLX-1 system powered by TurboFlow™ technology for the forensic toxicological screening of more than 400 acidic, neutral, and basic drugs in urine with LC/MS^n has been developed.

Goal

To evaluate the performance of an automated online sample preparation method for an LC/MS^n screening approach.

Experimental

Sample preparation was performed by an online sample extraction method utilizing Thermo Scientific TurboFlow technology. Two TurboFlow columns (Cyclone, C18XL) were connected in series and used for sample extraction. Urine samples were run both natively and after enzymatic hydrolysis. The eluent was then transferred to the LC column (Thermo Scientific Betasil Phenyl-Hexyl, 100 x 3 mm, 3 µm) for separation.

A 30-minute gradient from 1% to 98% organic was employed for separation of the analyte with flow rates of 300 µL/min. All samples were then analyzed on a Thermo Scientific LXQ linear ion trap mass spectrometer with the atmospheric pressure chemical ionization (APCI) source. A data-dependent polarity switching method was used for data acquisition. MS^2 and MS^3 spectra were acquired. Since polarity switching was used, a single injection of a sample containing unknown compounds was sufficient to detect both substances ionizing in negative and positive mode. The data was automatically processed, post-acquisition, by Thermo Scientific ToxID automated screening software.

Results and Discussion

The method using online extraction has been fully validated. A minor matrix effect (suppression < 5%) was observed for over 98% of the compounds. A recovery of more than 90% was seen in 90% of the substances. The limit of identification (LOI) was below 10 ng/mL for 60% of the substances and 90% could be identified at a concentration of 100 ng/mL. The 400-compound library contains both MS^2 and MS^3 spectra. MS^3 spectra bring an additional level of specificity, although in most cases, the analytes can be easily identified by using only the MS^2 spectra. However, some analytes may have the same molecular weight, very similar MS^2 spectra, and a very close retention time. For these reasons, MS^3 data have to be used for the identification. One example is the isobaric compounds O-desmethylvenlafaxine and tramadol. The two analytes have the same molecular weight, very close retention times (see details in Table 1), and the same MS^2 spectra (Figure 1). Therefore, by running only MS^2 experiments, it is impossible to properly differentiate the two analytes. When MS^3 spectra are recorded, tramadol does not fragment ions while O-desmethylvenlafaxine gives a specific spectrum (Figure 1). Therefore, the analytes can be properly identified. Total run time of the analysis is 30 minutes. An example of a chromatogram obtained from a sample is presented in Figure 2.

Table 1. Tramadol and O-desmethylvenlafaxine information

	O-Desmethylvenlafaxine	Tramadol
Precursor mass	264.3	264.3
MS^2 Fragment	246.3	246.3
Retention Time	10.6 min	10.3 min

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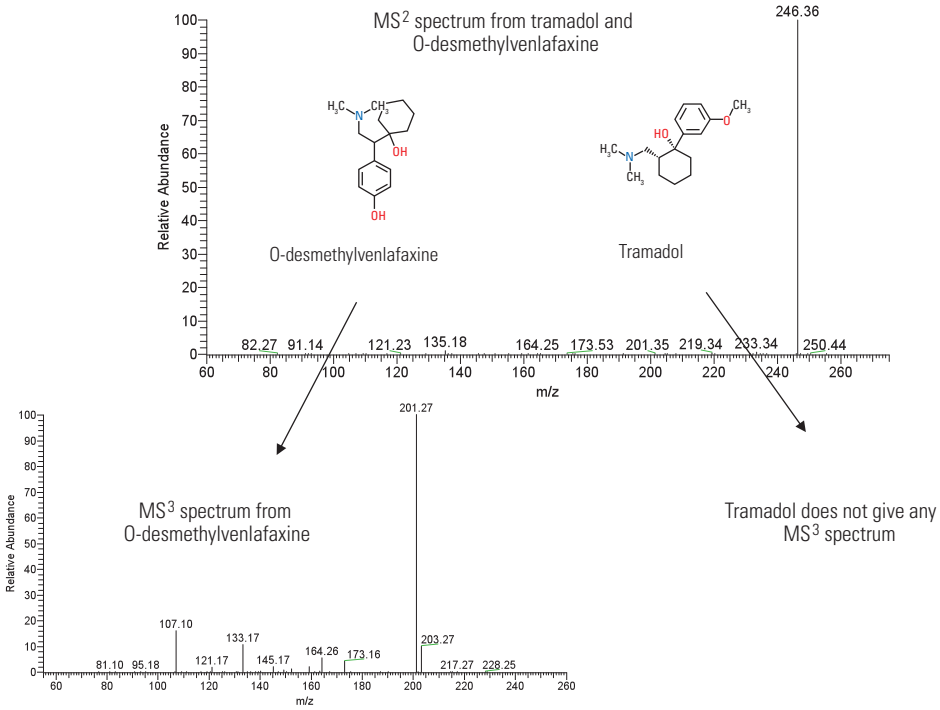

Figure 1. Fragmentation of tramadol and O-desmethylvenlafaxine in MS^2 and MS^3

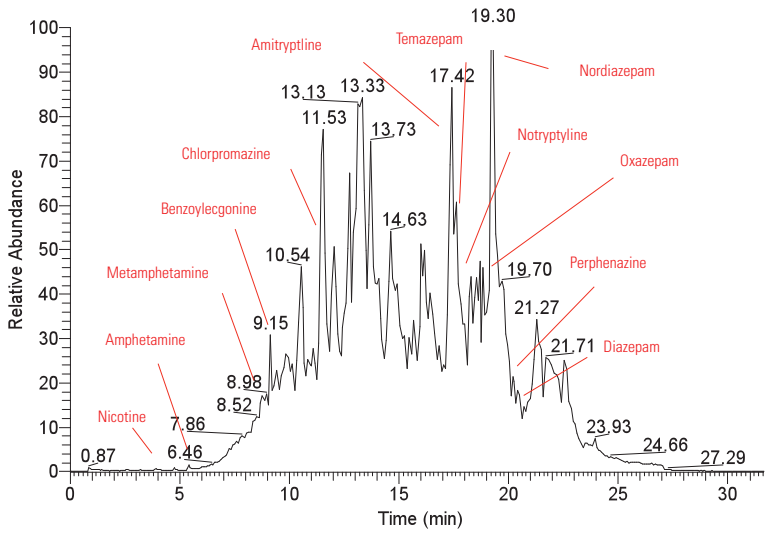

Figure 2. Full scan MS chromatogram of a sample containing 12 different analytes

Conclusion

The automated online TurboFlow method with the LXQ™ linear ion trap mass spectrometer allows a fast and specific approach for the identification of a broad range of compounds in positive and negative mode in a single run. The sample preparation time is 15 minutes with this method as compared to 2 hours with an offline approach. The LOIs are below 100 ng/mL for more than 90% of the analytes. MS^3 spectra acquisition brings an additional level of specificity for forensic toxicology laboratories.

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