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

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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 has been developed.

Goal
To evaluate the performance of an automated online sample preparation method for an LC/MS 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.

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 MS2 and MS3 spectra. MS3 spectra bring an additional level of specificity, although in most cases, the analytes can be easily identified by using only the MS2 spectra. However, some analytes may have the same molecular weight, very similar MS2 spectra, and a very close retention time. For these reasons, MS2 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 MS2 spectra (Figure 1). Therefore, by running only MS2 experiments, it is impossible to properly differentiate the two analytes. When MS3 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

<table>
<thead>
<tr>
<th></th>
<th>O-Desmethylvenlafaxine</th>
<th>Tramadol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor mass</td>
<td>264.3</td>
<td>264.3</td>
</tr>
<tr>
<td>MS2 Fragment</td>
<td>246.3</td>
<td>246.3</td>
</tr>
<tr>
<td>Retention Time</td>
<td>10.6 min</td>
<td>10.3 min</td>
</tr>
</tbody>
</table>
**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. MS3 spectra acquisition brings an additional level of specificity for forensic toxicology laboratories.

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