

Online Sample Extraction Technique for LC/MS Forensic Toxicology Screening

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Overview

Purpose: Evaluation and comparison of three sample preparation methods implemented in forensic screening application on ion trap mass spectrometer

Methods: Three sample preparation methods, Thermo Scientific TurboFlow method, solid phase extraction (SPE) and liquid liquid extraction (LLE), were evaluated to find the most robust, easy to use and efficient method to acquire quality MS/MS data with an ion trap mass spectrometer.

Results: The online TurboFlow™ method with the Thermo Scientific LXQ ion trap mass spectrometer allows for the identification of about 300 compounds. Compared to traditional sample preparation methods (SPE, LLE), the online TurboFlow method provides competent extraction performance.

Introduction

LC/MS is a powerful tool for forensic drug screening. New, sensitive MS/MS systems enable the detection of drugs at low levels. However, the quality of LC/MS data collected in screening applications is largely affected by sample preparation methods. Offline SPE and LLE are the most used sample preparation methods. Online sample preparation provides an automatic platform for forensic drug screening, which is convenient and labor-saving. TurboFlow technology has a reputation in sample preparation for online extraction with a variety of biological matrices; for example, plasma, blood, urine, etc. In this contribution, three different urine sample preparation methods implemented in forensic toxicology screening (TurboFlow technology, SPE, LLE) were evaluated on a set of 300 basic, neutral, and acidic compounds using ES/LC/MS on an ion trap mass spectrometer.

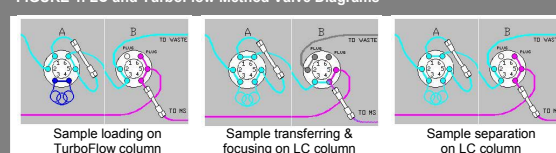
Table 1. Columns and Mobile Phases

TurboFlow Columns	0.5x50mm
LC Column	Thermo Scientific Hypersil GOLD PFP, 3.0x100mm, 3µm
Loading Pump	Quaternary Pump
Solvent A	Water:ACN (1:1) + 10mM Ammonium Formate + 0.1% FA
Solvent B	Water + 1% FA
Solvent C	ACN:IPA:Acetone 7:2:1
Solvent D	Water
Eluting Pump	Binary Pump
Solvent A	Water + 10 mM Ammonium Formate + 0.1% FA
Solvent B	ACN + 0.1% Formic Acid

Table 2. LC and TurboFlow Method Gradients

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Te	Loop	Flow	Grad	%A	%B
1	00:00	60	2.00	Step	-	-	-	100.0	=====	out	0.60	Step	99.0	1.0
2	01:00	120	0.10	Step	-	-	-	100.0	=====	in	0.60	Step	99.0	1.0
3	03:00	180	0.50	Step	-	100.0	-	-	=====	in	0.60	Ramp	65.0	35.0
4	06:00	60	0.50	Step	100.0	-	-	-	=====	in	0.60	Ramp	44.0	56.0
5	07:00	60	0.50	Step	-	-	-	100.0	=====	in	0.60	Ramp	25.0	75.0
6	08:00	30	2.50	Step	100.0	-	-	-	=====	in	0.60	Step	5.0	95.0
7	08:30	30	2.50	Step	95.0	5.0	-	-	=====	in	0.60	Step	99.0	1.0
8	09:00	180	2.00	Step	-	-	-	100.0	=====	out	0.60	Step	99.0	1.0

FIGURE 1. LC and TurboFlow Method Valve Diagrams

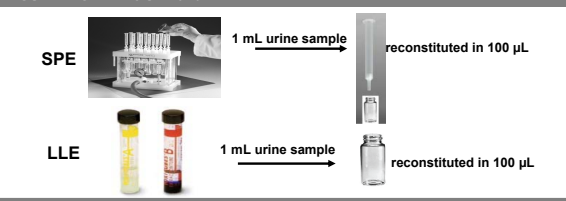


Methods and Conditions

Online sample extraction with a TurboFlow method: An online sample extraction method utilizing TurboFlow technology was developed. With this method, the online sample extraction and LC separation were achieved within 12 minutes. Tables 1 and 2 list detailed LC and TurboFlow method conditions. Figure 1 shows the appropriate valve diagrams.

Offline SPE and LLE method: Mixed mode solid phase extraction cartridges (Thermo Scientific HyperSep Verify-CX, 200 mg) were utilized for offline SPE. ToxiTube A & B (Varian) were utilized for offline LLE. A 13-minute LC method was developed for SPE and LLE samples. Gradient from 5% to 95% B was employed, with flow rates of 200 µL/minute.

FIGURE 2. Off-Line SPE and LLE



Mass Spectrometer (MS) Conditions: All samples were analyzed on a LXQ™ ion trap mass spectrometer with the electrospray ionization (ESI) source and a scan-dependent polarity switching method. There are 300 compounds on the parent mass list in the acquisition method (Table 3). The parent mass list allows the instrument to only search for masses on the list within retention time windows specific to each compound. This greatly conserves instrument resources, by preventing the search for all masses throughout the entire method.

Table 3. Parent Mass List in MS Method

The overall workflow for forensic toxicology screening is shown in Figure 3. After sample preparation with TurboFlow technology, SPE, or LLE, samples were analyzed with LC-MS/MS. Reports were produced with Thermo Scientific ToxiD automated screening software – including lists of identified compounds and their MS/MS spectrum.

FIGURE 3. Overall Application Workflow

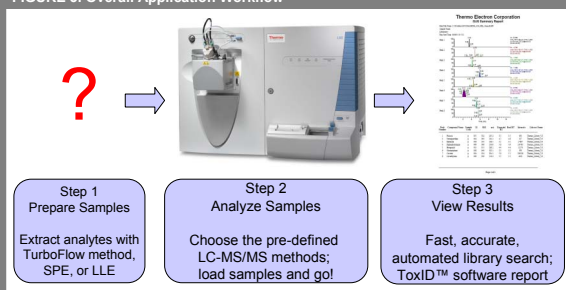


FIGURE 4. Scan-dependent Method Flow Chart

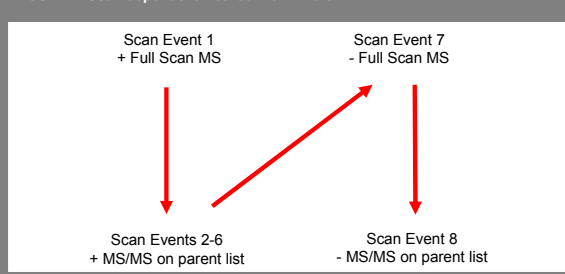


Figure 4 shows a flow chart of the MS method used for forensic toxicology screening. A full scan of the sample is initially used to find compounds on the parent mass list (300 compounds). MS/MS data-dependent experiments, which are triggered by the presence of the masses from the parent list, are executed for compound confirmations based on their MS/MS spectrum. In the positive mode, one full scan and five data-dependent experiments are conducted. In the negative mode, one full scan and one data-dependent MS/MS experiment are conducted.

The ToxiD software executes the library search based on the MS/MS spectrum collected and reports a list of compounds identified in the sample based on the user defined search index value, m/z range, retention time window and parent/product ion intensity threshold. The report also includes LC chromatograms and MS/MS spectra.

Results and Discussion

Figure 5 shows the typical LC-MS/MS chromatograms collected with the TurboFlow method. With a polarity switch in the MS method, both positive charged and negative charged compounds (Piroxicam and Nimodipine) are identified in a single run.

FIGURE 5. LC-MS/MS Chromatograms for Urine-Spiked Samples at 100 ng/mL with TurboFlow sample extraction method

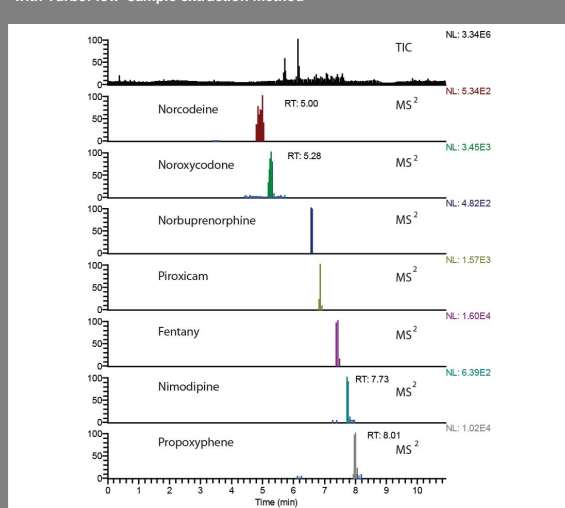


FIGURE 6. Extraction efficiency using a TurboFlow method

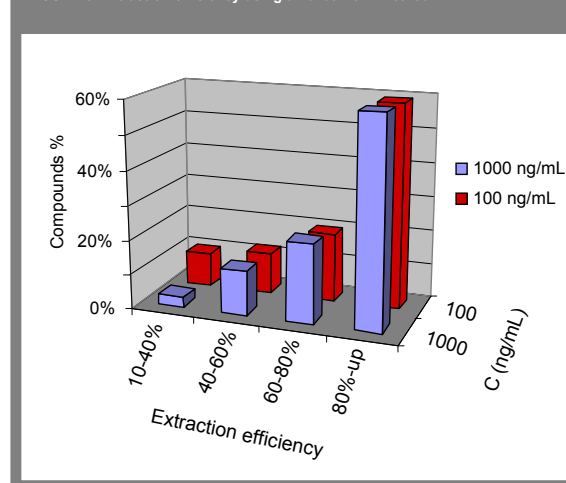


Figure 6 shows the extraction efficiencies of the online TurboFlow method for a random selected group of 50 compounds at two concentration levels. More than 50% of the compounds showed the extraction efficiency of 80% and up at both concentrations. About 20% of them showed the extraction efficiency of 60-80%. Less than 10% fell in the extraction efficiency range of 40-60% and 10-40%.

Table 4 shows limit of identifications for a representative set of 10 compounds from a TurboFlow method, SPE and LLE. Ten ng/mL was the lowest concentration validated. All the three methods showed comparable limit of identifications.

Table 5 lists the sample injection volumes for three sample preparation methods. With an online TurboFlow extraction method, the sample quantity loaded on the column was 1/4 of the SPE method, 1/8 of the LLE method.

TABLE 4. Comparison of Limit of Identifications for 10 representative compounds with three different sample preparation methods

Compounds	TurboFlow Method (ng/mL in urine)	SPE Method (ng/mL in urine)	LLE Method (ng/mL in urine)
Heroin	100	10	10
Delta9-THC	100	100	10
Cocaine	10	10	10
Amphetamine	10	10	1000
Stanozolol	100	100	10
Diazepam	10	10	10
Fexofenadine	10	10	100
Furosemide	100	100	100
Ketamine	10	10	100
LSD	10	10	10

TABLE 5. Comparison of urine sample injection volume of three sample preparation methods

Sample Preparation	TurboFlow Method	SPE Method	LLE Method
Injection volume (µL)	50.0	10.0	20.0
Preconcentrating factor	0.5	10.0	10.0
Equivalent urine sample injection volume (µL)	25.0	100.0	200.0

Figure 7. Limit of identifications of 300 compounds with three sample preparation methods

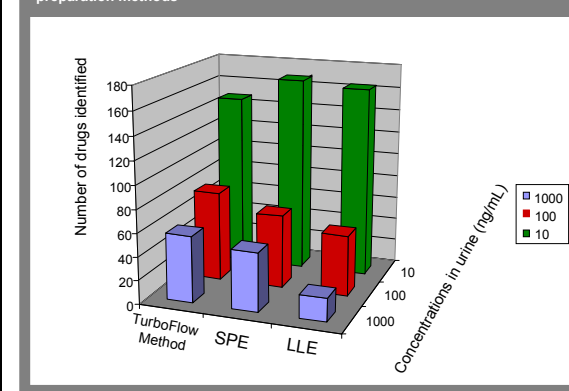


Figure 7 shows the results of the identification limits of 300 compounds with three sample preparation methods: TurboFlow method, SPE and LLE. With the TurboFlow method, 150 compounds have LODs less than 10 ng/mL; 77 compounds have LODs between 10 and 100 ng/mL; 57 compounds have LODs between 100 and 1000 ng/mL; and 20 compounds have LODs greater than 1000 ng/mL. Compared to the traditional sample preparation methods, the online TurboFlow method provides a competent extraction performance with automated sample preparation and less sample consumption.

Conclusions

The online TurboFlow method with the LXQ ion trap mass spectrometer allows for the identification of about 300 compounds, with LODs ranging from less than 10 to greater than 1000 ng per mL of urine. Compared to traditional sample preparation methods (SPE, LLE), the online TurboFlow method provides an automated sample preparation platform for forensic toxicology screening with competent extraction performance and limits of identifications. Implementation of a TurboFlow method allows for significantly lower sample volume consumption in comparison to SPE and LLE methods.

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