Screening Workflows for Anticholinergic Drugs on the QTRAP® 5500 LC/MS/MS System

Quantitative/Qualitative Workflows and Positive/Negative Switching

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Introduction

A combination of comprehensive drug screening, semi-quantitation and confirmation is essential in the anti-doping industry. An ideal screening method should cover a large number of drugs, yield semi-quantitative results, and provide spectral information for confirmation– all in a single analysis.

To fulfill all of the above requirements, several LC/MS/MS methods were developed using a hybrid quadrupole linear ion trap: the AB SCIEX QTRAP® 5500 system. The fast scanning speed and the short pause/settling time (50 msec) for polarity switching on this system are perfectly suited to the development of methods for simultaneous acquisition of quantitative and qualitative information.

A targeted drug screening method was developed, employing a highly sensitive and selective Multiple Reaction Monitoring (MRM) survey scan, followed by Information Dependent Acquisition (IDA) of Enhanced Product Ion (EPI) scans in the linear ion trap. A complementary non-targeted drug screening method was also developed, employing an Enhanced MS (EMS) survey scan, followed by IDA-triggered EPI scans. For both the targeted and non-targeted methods, MS/MS library searching was used for confirmation of detected compounds.

In addition, a purely quantitative method was developed for the simultaneous analysis of both positively and negatively ionized compounds in a single injection. The quantitation integrity of this method was assessed and compared to 'single-polarity' methods, and the robustness of this method was evaluated based on the observed precision, linearity and accuracy.

Method

The LC/MS/MS system consisted of a Shimadzu Prominence HPLC system interfaced to an AB SCIEX QTRAP® 5500 System.

Urine samples were diluted 1:3 with methanol, and centrifuged. 10µL of supernatant was injected directly onto the analytical column (SB-C18, 4.6 x 50 mm, 1.8 µM).

All three methods employed reverse phase chromatography with the following conditions:

- Mobile phase A: Water with 0.01% formic acid
- Mobile phase B: Methanol with 0.01% formic acid
- Flow rate of 0.7 mL/min
- Column temperature of 60°C
- Non-linear gradient ramp from 5% to 80% B in 8 minutes, followed by a hold at 80% B for 3 minutes, followed by a re-equilibration at 5% B
- Total run time: 14 minutes

MRM-EPI method (MRM-Enhanced Product Ion scan): The method consisted of an MRM survey scan with 98 MRM transitions, each with a 3-millisecond dwell time, followed by three EPI scans to provide MS/MS spectra for library searching. This method was used for simultaneous quantitative and qualitative analysis.

EMS-EPI method (Enhanced Mass Spectrum-Enhanced Product Ion scan): The method was created using an inclusion list containing all potential analytes, with their corresponding masses and retention times (RT). This method has the added benefit of being able to identify and confirm the presence of unknown compounds.

Positive/Negative Switching method: A total of 150 positive and negative MRM transitions were monitored in the same period. The dwell time for each transition was 3 milliseconds, with a 50-millisecond pause/settling time for polarity switching.

Results

A group of 20 anticholinergic and other drugs in human urine matrix was used for the evaluation of a combined quantitative and qualitative analysis workflow. Both targeted (MRM-EPI) and non-targeted (EMS-EPI) drug screening methods were evaluated. In each case all 20 analytes were detected, and dependent EPI spectra were acquired and submitted for library

searching against a comprehensive MS/MS spectral library for confirmation of compound IDs.

The MS/MS library was built by augmenting an existing drug screening library containing more than 1400 compounds. New entries were added to the library using data that was acquired by performing MRM-EPI experiments on neat standards.

Upon analysis of the urine samples, library search results for the acquired EPI spectra were automatically generated by the software (Figure 1). The search results yielded Purity scores ranging from 85-100% for the 20 analytes detected (Tables 1 and 2). The ability to confirm compound IDs using MS/MS library searching ensured that no false positives were reported.

Table 1. MS/MS Library Search Results for Compounds Detected in Positive ESI Mode

RT (min)	Compound Name	Purity (%)
2.06	Acetaminophen	94.9
2.50	Procaine HCl	100
2.52	Pseudoephedrine HCl	100
3.08	Atropine Sulfate	98.8
3.99	Clidinium Bromide	98.6
4.18	Butacaine Sulfate	97
4.25	Tetracaine HCl	93.5
4.34	Prednisolone	84.8
4.42	Cortisone	86.6
5.50	Dibucaine HCl	98.5
5.75	Benazepril HCl	98.3
5.79	Reserpine	92.9
7.30	Indomethacin	95.8
7.78	Meclofenamic Acid	97.9
7.89	Tolfenamic Acid	94.2

Table 2. MS/MS Library Search Results for Compounds Detected in Negative ESI Mode

RT (min)	Compound Name	Purity (%)
4.14	Trichlomethiazide	98.6
5.00	Salicyclic Acid	92.5
6.04	Furosemide	91.8
6.76	Sildenafil Citrate	100
8.34	Carprofen	96.3

A positive/negative polarity switching method, containing a total of 150 MRM transitions (Figure 2), was used to perform quantitation of 20 analytes in a urine sample. The robustness of the instrument and the method is demonstrated in Figures 3 and 4, in which the chromatographic peak areas for two of the target compounds, acetaminophen (positive ESI mode) and carprofen (negative ESI mode), are plotted for 40 consecutive injections.

The fast scanning speed and short pause/settling time for polarity switching resulted in a short total cycle time of 1.0 second for the positive/negative switching method. The short cycle time enabled accurate quantitation by ensuring the acquisition of sufficient data points across chromatographic peaks, even for peaks narrower than 10 seconds. For all analytes, the %CVs obtained for 40 consecutive injections ranged from 2-11% (Table 3). In addition, the semi-quantitative three-point calibration curves demonstrated excellent linearity, with R-values ranging from 0.9960-1.000 for all analytes.

Figure 1. Library Search for Atropine Sulfate in Urine Sample

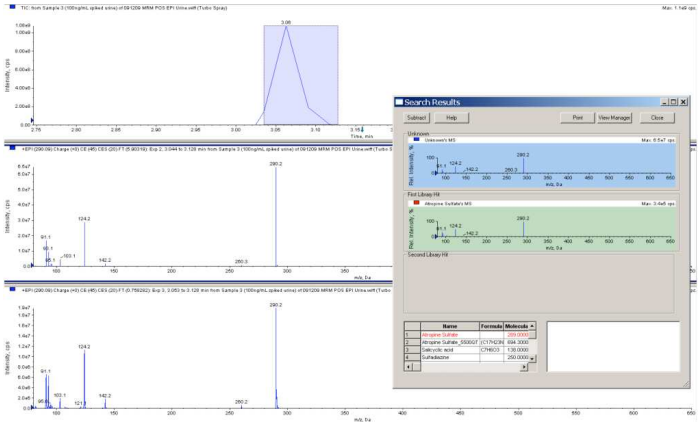


Figure 2. A Positive-Negative Polarity Switching Screening Method for 150 Drugs

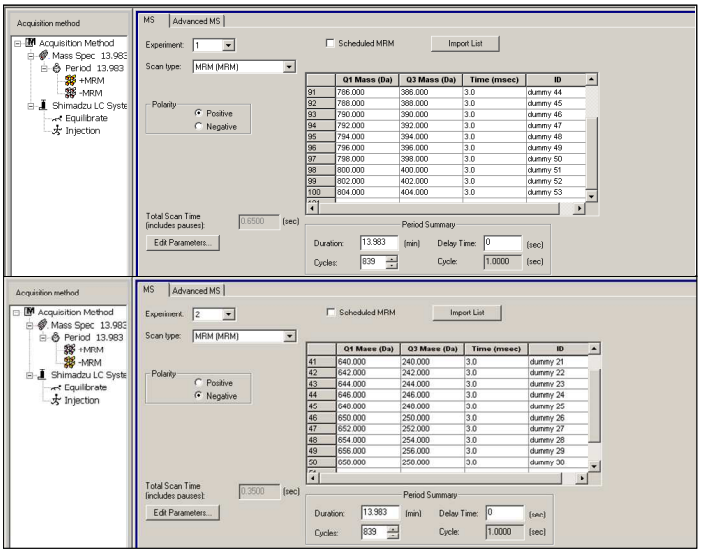


Table 3. %CVs for 40 Injections of Urine Matrix Spiked with 20 Drugs at 25 ng/mL

Compound Name	CV (%)
Acetaminophen 1	4.0
Acetaminophen 2	6.2
Procaine HCl 1	6.6
Procaine HCl 2	7.3
Pseudoephedrine HCl 1	5.1
Pseudoephedrine HCl 2	3.4
Atropine Sulfate 1	4.1
Atropine Sulfate 2	3.0
Clidinium Bromide 1	2.2
Clidinium Bromide 2	3.1
Butacaine Sulfate 1	7.9
Butacaine Sulfate 2	8.9
Tetracaine HCl 1	7.5
Tetracaine HCl 2	6.1
Prednisolone 1	4.6
Prednisolone 2	5.6
Cortisone 1	4.0
Cortisone 2	3.5
Dibucaine HCl 1	8.1
Dibucaine HCl 2	7.9
Benazepril HCl 1	5.3
Benazepril HCl 2	6.6
Reserpine 1	10.3
Reserpine 2	11.2
Indomethacin 1	4.7
Indomethacin 2	5.6
Meclofenamic Acid 1	5.4
Meclofenamic Acid 2	5.6
Tolfenamic Acid 1	4.1
Tolfenamic Acid 2	3.6
Carprofen 1	3.4
Carprofen 2	2.3
Furosemide 1	4.4
Furosemide 2	3.5
Salicyclic acid 1	4.2
Salicyclic acid 2	1.7
Sildenafil citrate 1	5.0
Sildenafil citrate 2	2.7
Trichlomethiazide 1	7.1
Trichlomethiazide 2	6.7

Figure 3. Peak Area for 40 Injections of Acetaminophen (Positive ESI) Spiked in Urine at 25 ng/mL

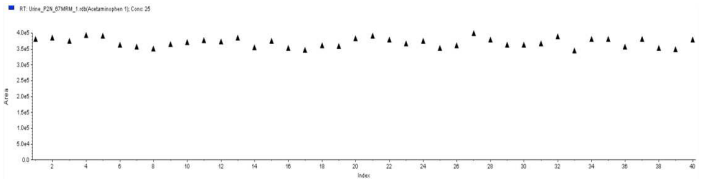


Figure 4. Peak Area for 40 Injections of Carprofen (Negative ESI) Spiked in Urine at 25 ng/mL

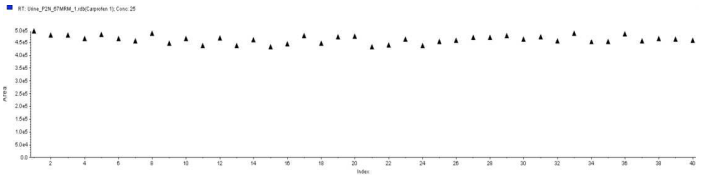


Figure 5. Positive/Negative Method Versus Positive-Only Method for Acetominophen at 10 ng/mL

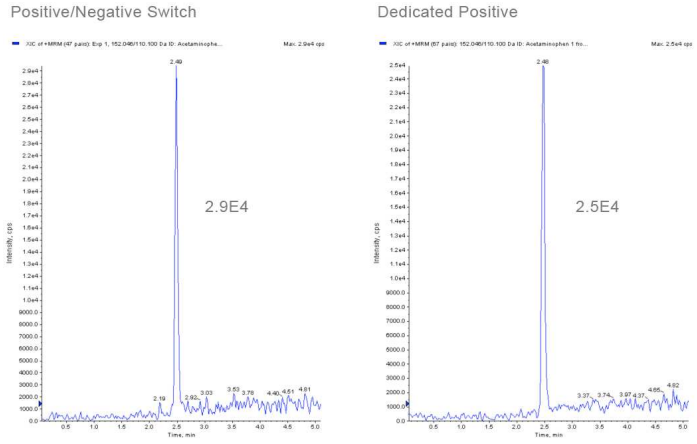
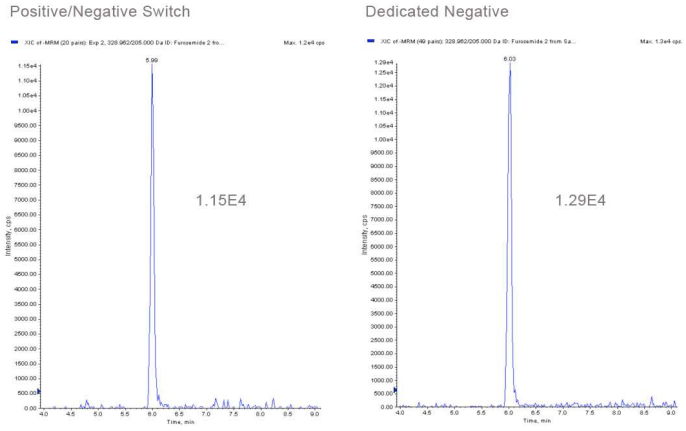


Figure 6. Positive/Negative Method Versus Negative-Only Method for Furosemide at 10 ng/mL



Figures 5 and 6 demonstrate that the sensitivity, quantitative integrity and chromatographic quality were not compromised when a positive/negative switching method was used in place of a dedicated positive-only or negative-only method.

Conclusion

We have developed a HPLC/MS/MS method suitable for drug screening that covers a large number of analytes, providing semi-quantitative results and MS/MS spectral confirmation in a single run. By using an information dependent acquisition method, product ion spectra are automatically acquired for every compound detected, providing the diagnostic information necessary to eliminate false positives.

The fast scanning speed and short polarity-switching time of the QTRAP® 5500 system reduce the overall cycle times, and enable a single-injection workflow for the detection of both positive and negative compounds. When compared to single-polarity screening methods, positive/negative switching methods exhibit no compromise in sensitivity, quantitative integrity and chromatographic quality.

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