INTRODUCTION

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for simultaneous quantitation of multiple compounds in forensic samples. Multiple Reaction Monitoring (MRM) detection is the gold standard for quantitation purposes because of its speed, specificity, and sensitivity. All these attributes are critical for quantitative analysis of a comprehensive forensic compound panel. However, as the number of analytes in a panel increases and the same total cycle time is maintained, the scanning time of each individual MRM will inevitably decrease, affecting data quality. Therefore, we have employed the Scheduled MRM™ algorithm to intelligently monitor MRMs only during the appropriate retention time windows, thus decreasing the number of concurrent MRMs monitored at any point in time, allowing both the cycle time and dwell time to remain optimal.

In this study we present a rapid, robust and sensitive analysis of a comprehensive forensic panel consisting of 93 compounds in human urine using the QTRAP®/Triple Quad™ 4500 LC-MS/MS system. Owing to the inclusion of several barbiturates in the panel which ionize preferentially in negative mode, a polarity switching method has been implemented. Due to a high number of MRM transitions (212 MRMs in total, including the internal standards) and a short LC runtime (6.5 min), a newly optimized Scheduled MRM™ algorithm is used.

MATERIALS and METHODS

Compound list and spiking solutions:

Table 1 lists all the compounds and internal standards in the panel. The total number of monitored analytes is 93 (regular panel: 72; extended panel: 21 in blue font). Internal standards are shown in grey background.

Sample preparation

Blank human urine was used to prepare calibrators. Urine sample was hydrolyzed at 55°C. After hydrolysis, methanol and water were added to the mixture. The mixture was then centrifuged and the supernatant was transferred to glass vial for LC-MS/MS analysis.

LC-MS/MS

Phenomenex Kinetex Phenyl-hexyl column were used. Mobile phase A (MPA) was ammonium formate in water and mobile phase B (MPB) was formic acid in methanol. The LC flow rate was 1 ml/min and the LC runtime was 6.5 min. Injection volume was 5 μL.

Data acquisition was done with Analyst 1.6.3 using Scheduled MRM™ and polarity switch. Table 2 shows the MRMs in the method (212 in total).

3. We observed strong MRM signal at lowest calibration level, suggesting the possibility of reaching even lower LOQ (Figure 2). Excellent linearity and reproducibility was observed throughout the dynamic range assessed in this effort.

4. It was essential to utilize polarity switching to accommodate more than 200 MRMs within the one short data acquisition method. In the current panel with 93 compounds, the LC runtime is 6.5 minutes (Figure 3). With a smaller panel (e.g. 72 compounds), we can easily reduce the LC runtime to 5.5 minutes.

RESULTS and DISCUSSION

1. We achieved fast separation of various isobaric compounds in the panel despite short LC runtime (Figure 1).

2. We achieved a minimum of 10 data points across the LC peak when the majority of the MRM transitions had over 15 or more data points.

CONCLUSIONS

A rapid and sensitive method for the LC-MS/MS analysis of a 93-compound forensic panel in human urine was developed using the SCIEX ExtronLC™ AC HPLC system and the SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS system. The method takes advantage of the re-optimized Scheduled MRM™ algorithm, and the fast polarity switching capability of the 4500 series, to deliver high throughput and high-quality data. This method utilized a dilute-and-shoot sample preparation procedure. Excellent linearity and precision were observed for all the compounds across the relevant calibration range.

 TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pty. Ltd. or their respective owners. AB SCIEX™ is being used under license.

© 2016 AB SCIEX. RUO-MKT-10-2875-A

For Research Use Only. Not for use in diagnostic procedures.