The Analysis of Common Drugs of Abuse in an Oral Fluid Matrix using A New Q TRAP® Platform

QTRAP® 4500 LC/MS/MS System

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Abstract

The multiplexing analysis of several compound classes, such as common prescription drugs along with drugs of abuse is very important. The measurement of these drugs has generally been performed using urine as the sample matrix. However, many research laboratories are finding the need to analyze these compounds in oral fluid matrices. This type of testing requires monitoring compounds using a simple sample preparation protocol allowing for the analysis to be performed in a timely fashion, while at the same time providing excellent data quality where the accuracy and precision are uncompromised. In the present study a fast, robust, and reliable method was developed for the detection of 15 compounds in an oral fluid matrix. The LC-MS/MS methods were performed using Multiple Reaction Monitoring (MRM) along with fast positive negative switching detecting all compounds with Limits of Quantitation (LOQ) in-line with the current recommended cutoffs. Throughput was also considered by using the MPX™-2 high throughput HPLC system where an increase in sample throughput of 142% was observed. In addition to the above the quantitative positive negative switching capabilities were assessed and it was found to work excellent on the QTRAP® 4500 system.

Introduction

Much interest has surrounded the detection of several pain medications in oral fluid matrices. What makes oral fluid an attractive matrix is the fact that it involves a non-invasive collection. The collection of such a matrix typically involves a collection swab that retains the oral fluid matrix. Once collection has been completed, the drugs are then removed from the swab using an extraction and preservation buffer.

Using LC-MS/MS, we have developed a simple dilute and shoot method to simultaneously quantify these compounds in an oral fluid matrix which is suitable for high volume analysis. The compounds analyzed in this method consisted of 9 drugs including: Morphine, 6-MAM, Amphetamine, Benzoylecgonine, Codeine, MDA, MDMA, Methamphetamine and PCP.

Figure 1. AB SCIEX QTRAP® 4500 LC/MS/MS System

In addition to the above compounds monitored in positive mode a separate quantitative method was created using positive/negative switching which incorporated the following barbituates that were monitored in negative mode: Amobarbital, Butalbital, Butabarbital, Phenobarbital, Pentabarbital, Secobarbital.

Materials and Methods

Calibrator Preparation:
An oral fluid matrix was spiked with the above drugs at various levels. The calibration curve extended above and below the cutoff and confirmation levels.

Sample Preparation:

The collection workflow was simulated by diluting the oral fluid matrix with an extraction buffer at the appropriate dilution factor. The samples were further diluted in an equal volume of a methanol:water diluent containing 0.1% formic acid.

HPLC Conditions:

Liquid chromatography was performed using the MPX™-2 high throughput multiplexed HPLC system to achieve maximum throughput. Utilization of the MPX™-2 system afforded analysis times of less than 5 minutes per sample. The column employed was a Phenomenex Kinetex C18 2.6u 50 x 3.0mm one where 10mM Ammonium Formate in H2O was used in mobile phase A and 0.1% formic acid in methanol in mobile phase B. The injection volume was 10µL. A gradient was applied during chromatographic run and the total run time (not multiplexed) per stream was 6 minutes. The acquisition window was set to 3.5 minutes per stream providing an increase in sample throughput of 142% during multiplexed analysis.

MS/MS Conditions:

All samples were then analyzed using a QTRAP® 4500 instrument operating in electrospray ionization mode and utilizing MRM acquisition. In addition to the above a quantitative positive negative switching method was found to also be viable.

Results

Figure 2 shows the separation of all 15 drug analytes using the positive and negative MRM switching method. In Figure 5 quantitative results are shown for Amphetamine for positive mode only. Figure 6, on the other hand illustrates that the quantitative capabilities are not compromised when using the fast positive negative switching capabilities of the QTRAP® 4500 allowing the monitoring of both positively and negatively charged compounds in the same experiment.

Figure 2. Chromatogram of all 15 Analytes using the positive and negative MRM switching method.

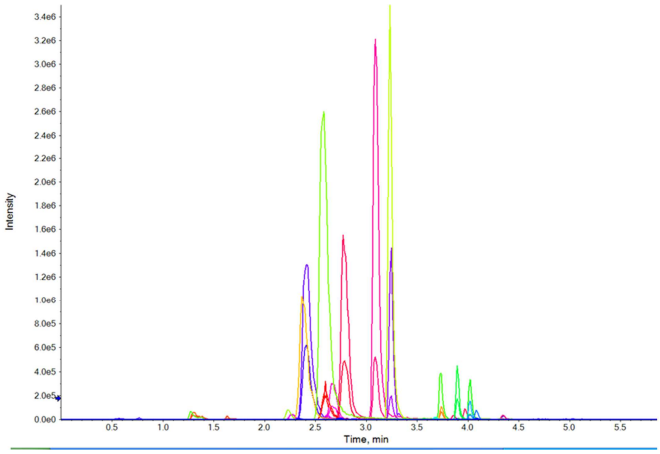

Figure 3. The fast Positive Negative switching capabilities of the AB SCIEX QTRAP® 4500™ LC/MS/MS were explored in MRM mode only are displayed.

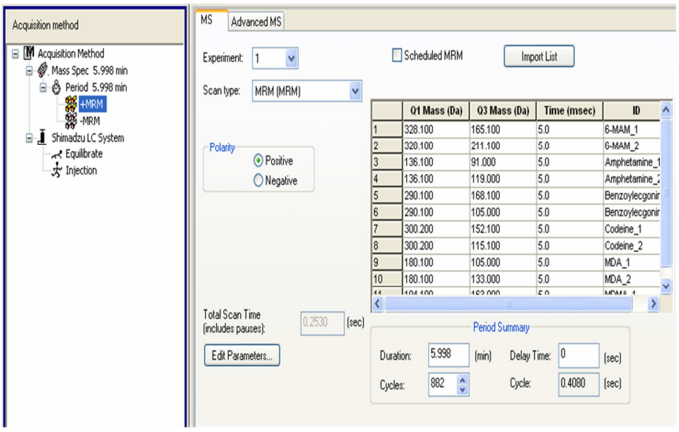

Figure 4. Positive mode only MRM method.

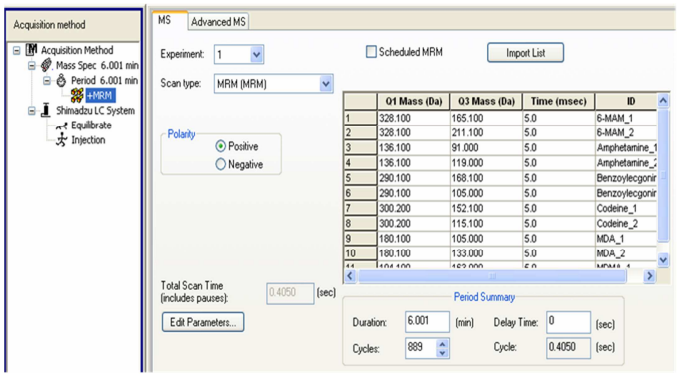

Figure 5. Quantitative example of Amphetamine in an oral fluid matrice while using MRM and in positive mode only.

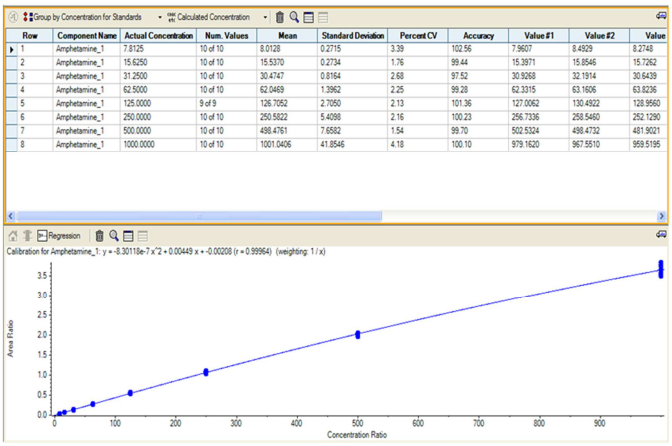

Figure 6. Quantitative example of Amphetamine in an oral fluid matrix while switching between positive and negative MRM mode.

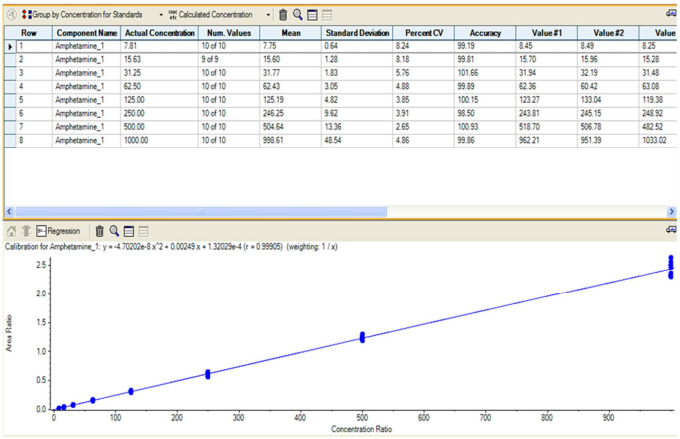

Figure 7. Quantitative example of Amobarbital (negative mode) in an oral fluid matrix while switching between positive and negative MRM mode.

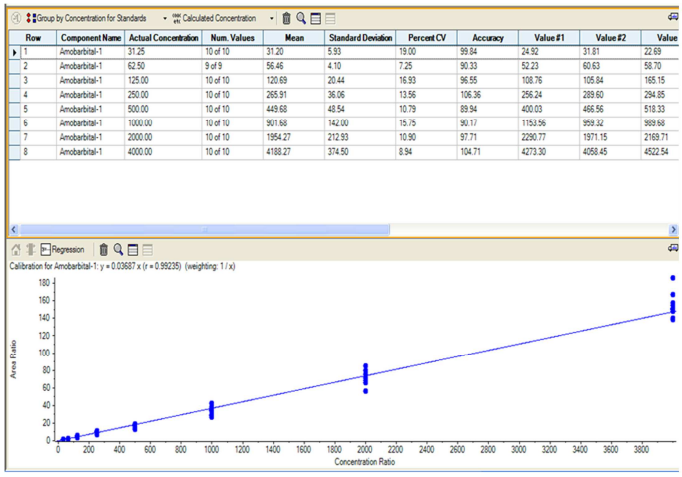

Table 1 Limits of quantitation attained in this effort.

Compounds monitored in positive mode.

Compounds monitored in negative mode.

Compound Name	LOQ (ng/mL)	ULOQ (ng/mL)
6-MAM	0.16	20
Amphetamine	7.8125	1000
Benzoylecgonine	2.3438	300
Codeine	1.5625	200
MDA	3.9062	500
MDEA	3.9062	250
MDMA	3.9062	500
Methamphetamine	7.8125	1000
Morphine	1.5625	200
PCP	0.3906	50
Amobarbital	31.3	4000
Butalbital	31.3	4000
Butabarbital	31.3	4000
Phenobarbital	31.3	4000
Pentabarbital	31.3	4000
Secobarbital	31.3	4000

***Note that the lower sensitivity level for the barbituates was not assessed below 31.3 ng/mL**

Conclusions

A simple and robust method, for the detection of 16 drugs in an oral fluid matrix was developed on the AB SCIEX QTRAP® 4500 LC/MS/MS system. This method utilized a simple protein precipitation procedure followed by a dilution step to analyze all of the analytes. When performing quantitation from the positive and negative switching mode, the linearity and precision observed were excellent in comparison to performing positive mode only.

This contribution solidifies the quantitative capabilities of the AB SCIEX QTRAP® 4500 LC/MS/MS system where the fast electronics allow for quantitation in both positive and negative mode without compromising data quality.

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