Simultaneous detection and quantification of 15 drugs of abuse in whole blood by online solid phase extraction and LC-MS/MS

**3200 QTRAP® LC/MS/MS system**

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**Introduction**

A new method for quantification of drugs of abuse in whole blood was developed with a simple sample pre-treatment, online solid-phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Fifteen drugs of abuse and metabolites were measured in a single chromatographic run. These included amphetamine, metamphetamine, MDMA, MDA, MDEA, MBDB, mephedrone, 6-MAM, morphine, codeine, dihydrocodeine, ethylmorphine, cocaine, BEG and cocaethylene.

**Materials and Methods**

**Sample Preparation**

For quantitative determination, 14 deuterated analogues were used as internal standards. After protein precipitation of 250 µL whole blood with ZnSO₄-methanol spiked with a mixture of internal standards deuterated, samples were mixed and centrifuged; the supernatant was evaporated to dryness, and reconstituted with mobile phase, before injection in the chromatographic system as described below.

- Added 250 µL whole blood to a microcentrifuge tube
- Added 500 µL ZnSO₄·7H₂O 0.2 M - MeOH (1:4) + 14 internal standards
- Vortexed followed by centrifugation
- Removed the supernatant and placed in HPLC vial and dried under nitrogen
- Added 100 µL of mobile phase A

**HPLC Conditions**

Analytes were firstly loaded on the extraction column (Strata-X, Phenomenex) with a 2 mM ammonium formate buffer, diverting salts and unwanted components to waste. A switching valve was then triggered to back-flush with mobile phase and elute analytes from the extraction column and diverting the flow onto the analytical column (Kinetex PFP, Phenomenex) kept at 60°C. The compounds were well separated in a total run time of 15 minutes. A gradient was performed with the aqueous phase A (2 mM ammonium formate and 0.2% formic acid), and the organic phase B (2 mM ammonium formate with methanol-acetonitrile (70-30) and 0.2% formic acid) (figure 1).

**MS/MS Conditions**

The mass spectrometer used was an AB SCIEX 3200 QTRAP® LC/MS/MS System. The method was developed using the scheduled MRM™ algorithm, with two transitions per compound. All analytes were detected in positive ionization mode (figure 2).
Analyte Internal Standard | Q1 (m/z) | Q3 (m/z) | DP (V) | CE (V)  
--- | --- | --- | --- | ---  
Amphetamine | 136.1 | 91 | 24 | 25  
D5-Amphetamine | 141 | 93.1 | 40 | 20  
Metamphetamine | 150.1 | 91 | 40 | 27  
D5-Metamphetamine | 155 | 92.1 | 40 | 20  
MDMA | 194.1 | 163.1 | 31 | 34  
D5-MDMA | 199.1 | 165.1 | 31 | 17  
MDA | 180.1 | 105.1 | 21 | 23  
D5-MDA | 185.1 | 110.1 | 88 | 30  
MDEA | 208.1 | 105.1 | 26 | 17  
D5-MDEA | 213.1 | 163.2 | 76 | 17  
MBDB | 208 | 77 | 26 | 57  
D5-MBDB | 213.1 | 136.1 | 40 | 20  
Mephedrone | 178.1 | 160.2 | 26 | 15  
D3-Mephedrone | 181.1 | 148.1 | 26 | 23  

Analyte Internal Standard | Q1 (m/z) | Q3 (m/z) | DP (V) | CE (V)  
--- | --- | --- | --- | ---  
6-MAM | 328.2 | 165.1 | 56 | 45  
D3-6-MAM | 331.1 | 165.1 | 56 | 53  
Morphine | 286.1 | 152.2 | 56 | 73  
D3-Morphine | 289 | 165.2 | 40 | 50  
Codeine | 300.2 | 165.2 | 56 | 61  
D3-Codeine | 303 | 215.2 | 40 | 35  
Dihydrocodeine | 302.2 | 199.2 | 43 | 46  
D6-Dihydrocodeine | 308.1 | 202.2 | 61 | 43  
Ethylmorphine | 314.1 | 152.2 | 66 | 89  
Cocaïne | 304.2 | 182.2 | 66 | 89  
D3-Cocaïne | 307 | 185.2 | 40 | 101  
Benzoylcegonine | 290.1 | 105.1 | 25 | 39  
D3-Benzoylecgonine | 293 | 171.2 | 40 | 20  
Cocaethylene | 318 | 196.2 | 41 | 25  
D3-Cocaethylene | 321.1 | 199.3 | 36 | 25  

Figure 2. MRM transitions and compound- dependent parameters

Results

The dynamic range of the assay for 6-MAM and Cocaine was demonstrated to be from 0 to 50 ng/mL with linearity up to 250 ng/mL. All other compounds showed a dynamic range of 0-200 ng/mL, linear up to 1000 ng/mL. To assess precision and repeatability, 5 replicate injections of 3 QC levels were performed. For reproducibility, the extraction was performed on 5 separate days. For all 3 QC levels the coefficient of variations (CVs) were less than 15% and accuracies of between 85 and 115% for all experiments. Limit of quantitation for 6-MAM and Cocaine were both 1.25 ng/mL and all other compounds was 5 ng/mL.

Recoveries measured with addition before and after precipitation were all greater than 75%. Compounds after precipitation were shown to be stable for 72 hours at 10 °C and cross contamination was shown to be less than 0.6%. Matrix effect was evaluated using six different whole bloods at 2 concentration levels with a result of coefficient of variation of less than 15%. A good correlation was demonstrated when the results of the developed LC/MS/MS were compared to the results of a GC/MS method that consisted of a liquid/liquid extraction followed by derivitization.
Conclusion

An LC/MS/MS method has been developed that enables the simultaneous analysis of amphetamines, cocaine, opiates and mephedrone from whole blood. The method has a simple and fast sample preparation with a short chromatographic separation. It provides an alternative approach of the lengthy liquid/liquid extraction and derivitization procedure required for GC/MS analysis, using low volume of sample and with no matrix effects.