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 $ZnSO_4$-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 $ZnSO_4$,$7H_2O$ 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).

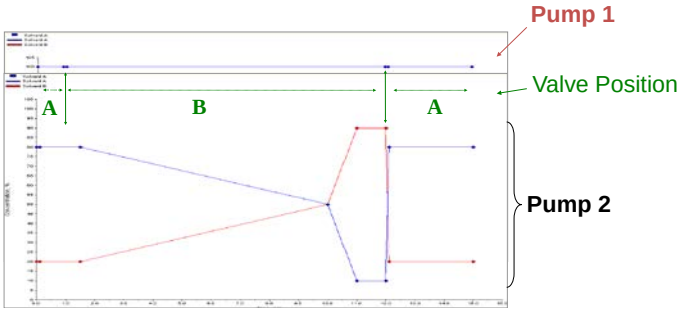

Figure 1. Chromatographic gradient profile

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
		65		50
D5-Amphetamine	141	93.1	40	20
Metamphetamine	150.1	91	40	27
		65		50
D5-Metamphetamine	155	92.1	40	20
MDMA	194.1	163.1	31	17
		105.1		34
D5-MDMA	199.1	165.1	31	17
MDA	180.1	105.1	21	27
		135		23
D5-MDA	185.1	110.1	88	30
MDEA	208.1	105.1	26	33
		163.2		17
D5-MDEA	213.1	163.2	76	17
MBDB	208	77	26	57
		51		79
D5-MBDB	213.1	136.1	40	20
Mephedrone	178.1	160.2	26	15
		144.2		35
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
		211.2		31
D3-6-MAM	331.1	165.1	56	53
Morphine	286.1	152.2	56	73
		165.2		47
D3-Morphine	289	165.2	40	50
Codeine	300.2	165.2	56	61
		215.2		34
D3-Codeine	303	215.2	40	35
Dihydrocodeine	302.2	199.2	43	46
		171.2		57
D6-Dihydrocodeine	308.1	202.2	61	43
Ethylmorphine	314.1	152.2	66	89
		115.1		101
Cocaïne	304.2	182.2	66	89
		105.1		101
D3-Cocaïne	307	185.2	40	20
Benzoylecgonine	290.1	168.2	40	25
		105.1		39
D3-Benzoylecgonine	293	171.2	40	20
Cocaethylene	318	196.2	41	25
		82.1		43
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.

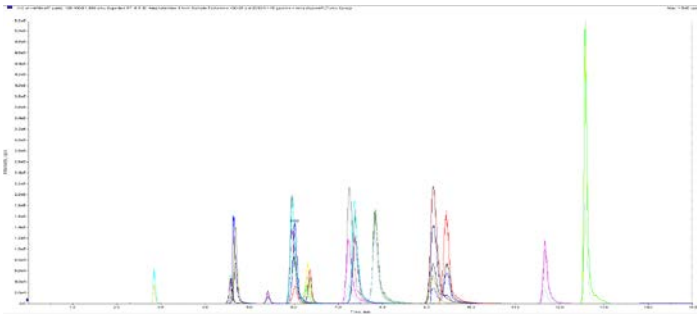

Figure 3. Example Calibrator Chromatogram

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.

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