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Analysis of Cathinones in Plasma Using LC-MS/MS

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Introduction

Cathinones better known as bath salts have been listed as illicit drugs since 2011 and are the structural analog of Drug Enforcement Agency (DEA) Schedule I and II substances. They have been banned in numerous countries. Newly synthesized cathinones, which mimic the effects of illegal drugs of abuse and bypass the provisions of drug regulations, are still available. These products have caught concern among law enforcement agencies. Acute toxicity and numerous fatalities have been linked with the abuse of designer cathinones. Despite the increased availability of designer drugs, few studies have focused on the analytical extraction techniques and the matrix effect for their detection and quantification in biological samples. Accurately quantifying the concentration of cathinones in biological samples is important for the regulation of cathinones.
LC-MS/MS is commonly used for detecting cathinones. However, Concheiro *et al*. (2013) showed the matrix suppression was observed in urine sample of cathinones by LC-MS/MS analysis. Endogenous interferences in urine have suppressed cathinone, mephedrone, and 3,4-methylenedioxypyrovalerone (MDPV) detections by LC-MS/MS up to 27%, 11%, and 9%, respectively. This study was aimed to elucidate the plasma matrix effect on the quantification of these three cathinones by LC-MS/MS. We developed LC-MS/MS methods that utilize standard addition without internal standard. A mixture of cathinone and mephedrone in plasma was analyzed.

Method

A UHPLC system with Phenomenex Kinetex™ C18, 2.1x100 mm, 1.7 um column at 30°C with a isocratic of eluent A water/0.05% formic acid in 5 mM ammonium formate buffer and eluent B methanol/0.05 formic acid was used at a flow rate of 500 uL/min. The injection volume was set to 10 uL, and the auto-sampler temperature was set to 15°C.

UHPLC Conditions

Column	: Phenomenex Kinetex™ C18, 2.1x100 mm, 1.7 um
Column heater	: 30 °C
Mobile phase gradient	: Isocratic
M.P.A	: water/0.05% formic acid in 5 mM ammonium formate buffer
M.P.B	: methanol/0.05 formic acid
Needle wash	: Methanol
Flow rate	: 0.5 mL/min.
Auto-sampler	: 15°C, inject 10 µL

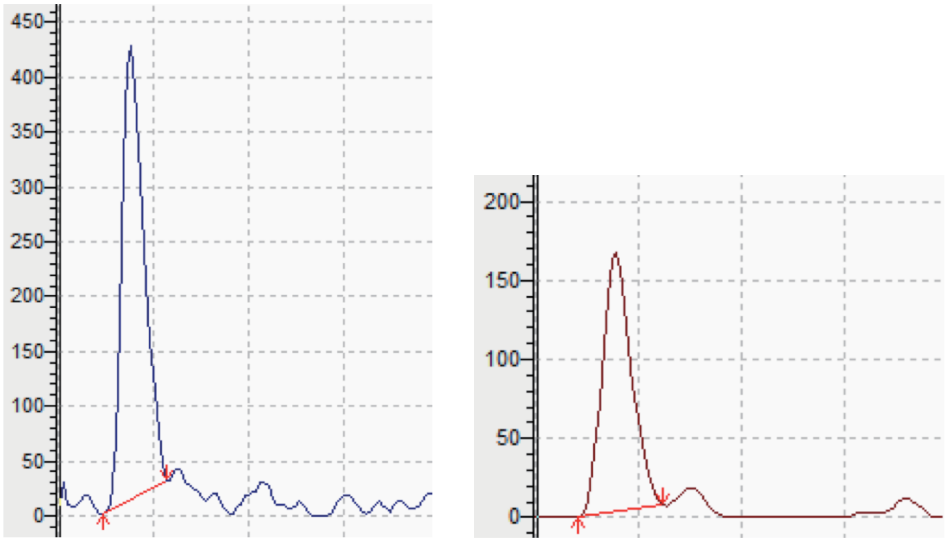
LC-MS/MS Method

Mass spectrometer	: Shimadzu LCMS-8030 Triple Quad Instrument
Interface	: DUIS
Nebulizing gas flow	: 1.5 L/min
DL temperature	: 250 °C
Heat block temperature	: 400 °C
Drying gas flow	: 15 L/min
Mass transitions	: Cathinone: 150.2>132.1 (CE -15); 150.2>117.1 (CE -22)
	Mephedrone: 178.2>159.8 (CE -14); 178.2>145.1 (CE -20)

Sample Preparation

Cathinone and mephedrone standards: 10, 20, 30, 40, 50, 60, 70, 80 pg/uL in sheep plasma; diluted with methanol (1:1) then centrifuged with 14,000 rpm for 10 min.

Results



Representative chromatograms of cathinone and mephedrone at 2 pg/uL

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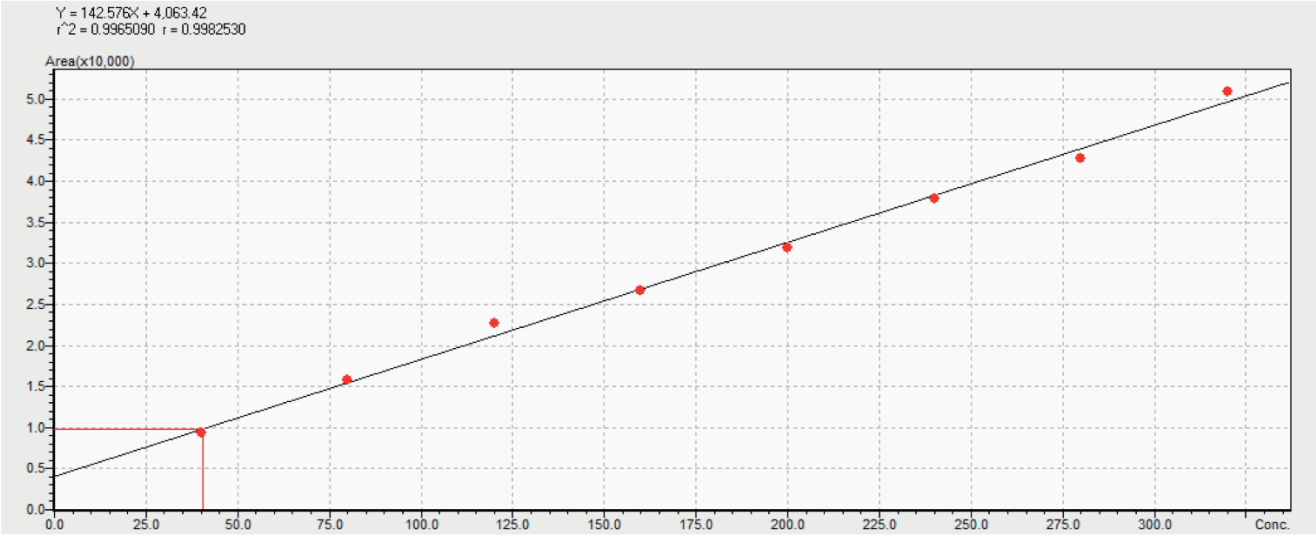
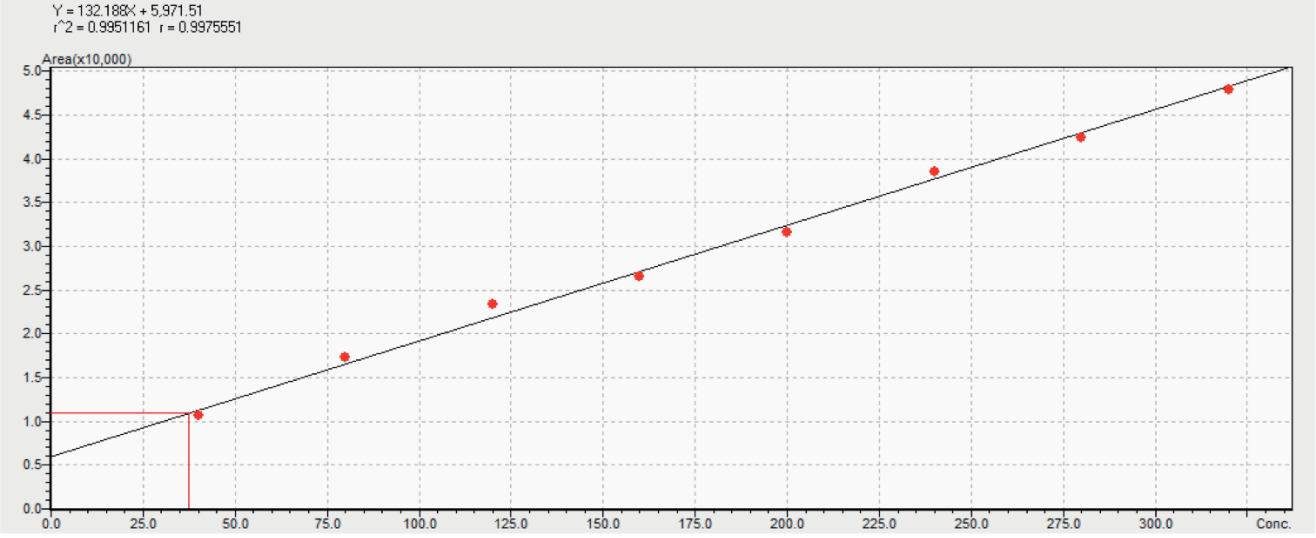




Representative standard addition curves for cathinone and mephedrone

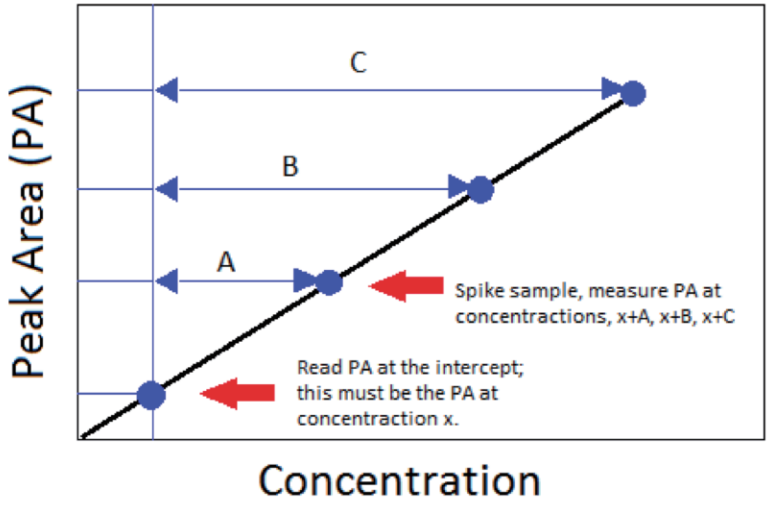
CV% and recovery%

Cathinone (pg/uL)	% CV	% recovery
40	6.1	99.3
80	4.0	101.2
120	3.5	104.0
160	16.9	94.3
200	4.2	92.4
240	6.0	111.6
280	2.1	101.9
320	2.4	95.7
Average	**5.6**	**98.4**

Methedrone (pg/uL)	% CV	% recovery
40	6.1	93.7
80	4.0	103.0
120	3.5	108.5
160	16.9	99.1
200	4.2	97.8
240	6.3	98.7
280	0.7	96.8
320	2.4	102.5
Average	**5.4**	**100.2**

Discussion

SPE is the most commonly used technique for forensic sample preparation. Several types of SPE sorbents have been developed for chemical extraction. On-going researches at John Jay College are using many SPE techniques, such as polymeric sorbents have a broader pH stability range and higher analyte retention for polar compounds than traditional bonded silica sorbents. Chemically modified resins with different functional groups have also been developed to include the ion exchange mechanism. In this study, however, we developed the standard addition method for quantitation without using internal standards. The most attractive aspect of this method is the simplicity of the sample prep; it is a dilute-and-shoot method.
The mechanism for standard addition is illustrated below.



This method has shown that it has a good accuracy and reproducibility for sheep plasma samples containing cathinone and mephedrone.
Research involved quantitation with internal standards using various SPE techniques are in progress for many cathinones, such as cathinone, methcathinone, methedrone, butylone, mephedrone, methylethcathinone, and MDPV. The comparison of those methods will be reported in due course.

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