Cheng et al. Journal of Analytical Science and Technology (2017) 8:19
DOI 10.1186/s40543-017-0128-7

Journal of Analytical Science and Technology

SHORT REPORT Open Access

Detection of cathinone and mephedrone in plasma by LC-MS/MS using standard addition quantification technique

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Abstract

Background: Cathinones better known as bath salts have been listed as illicit drugs since 2011. Few studies have focused on the analytical extraction techniques and the matrix effect affecting their detection and quantification in biological samples. Matrix suppression of the signal of cathinones has been previously observed in urine sample by LC-MS/MS. This study is aimed to use the standard addition method to overcome the plasma matrix effect on the quantification of cathinone and mephedrone by LC-MS/MS.

Findings: The results showed the matrix effect for cathinone at lowest tested concentration (10 ng/ml) was significantly reduced from 210.9 to 133.7%, but not for mephedrone (from 196.8 to 191.9%) by using standard addition method. At the higher tested concentrations of samples, the matrix effects were significantly reduced for both cathinone and mephedrone by using standard addition technique.

Conclusions: Standard addition quantitative technique can serve as an alternative quantitative method for LC-MS/MS when suitable internal standards are not available.

Keywords: Cathinone, Mephedrone, LC-MS/MS, Plasma, Standard addition

Introduction

Cathinones are also known as bath salts and have been listed as illicit drugs since 2011. The Administrator of the Drug Enforcement Administration (DEA) issued the order to temporarily schedule three synthetic cathinones as schedule I substances under the Controlled Substances Act (CSA) (Drug Enforcement Administrations 2011; 2014). Internationally, cathinones have been banned in numerous other countries. Designer cathinones, which are newly synthesized derivatives of existing cathinones to mimic the effects of existing illegal drugs and at the same time bypass the existing controlled drug regulations, are still available (Kelly 2011). These products have caught the attention of law enforcement due to incidents of acute toxicity and numerous fatalities that have been linked with the use of these designer cathinones.

Accurately, quantifying the concentration of cathinones in biological samples is important for the regulation of cathinones. A problem for law enforcement and clinical laboratories occurs due to the difficulties of validation and maintenance of multiple analytical methods used in the accurate and precise detection of these compounds. Despite the increased availability of designer drugs, few studies have focused on the analytical extraction techniques and the matrix effects affecting their detection and quantification in biological samples.

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. By IUPAC definition, matrix effects are “the combined effect of all components of the sample other than the analyte on the measurement of the quantity” (Guilbault and Hjelm 1989). Many studies have been conducted to

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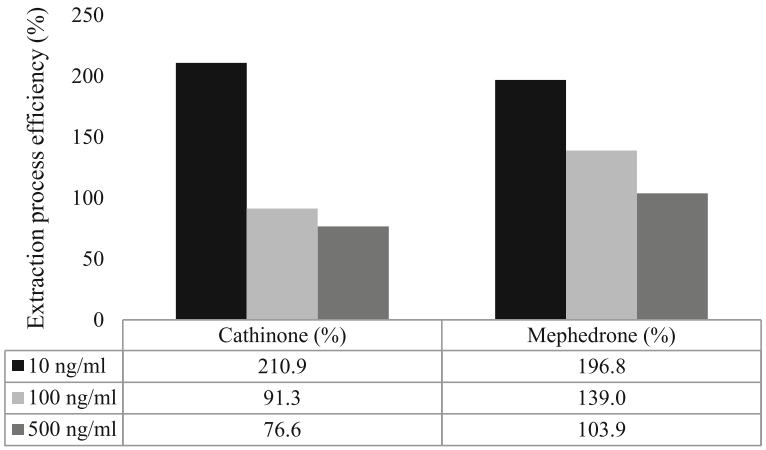

	Cathinone (%)	Mephedrone (%)
10 ng/ml	210.9	196.8
100 ng/ml	91.3	139.0
500 ng/ml	76.6	103.9

Fig. 1 Extraction process efficiency of cathinone and mephedrone by solid phase extraction using Strata X Drug B cartridge and Shimadzu LC-MS/MS 8030

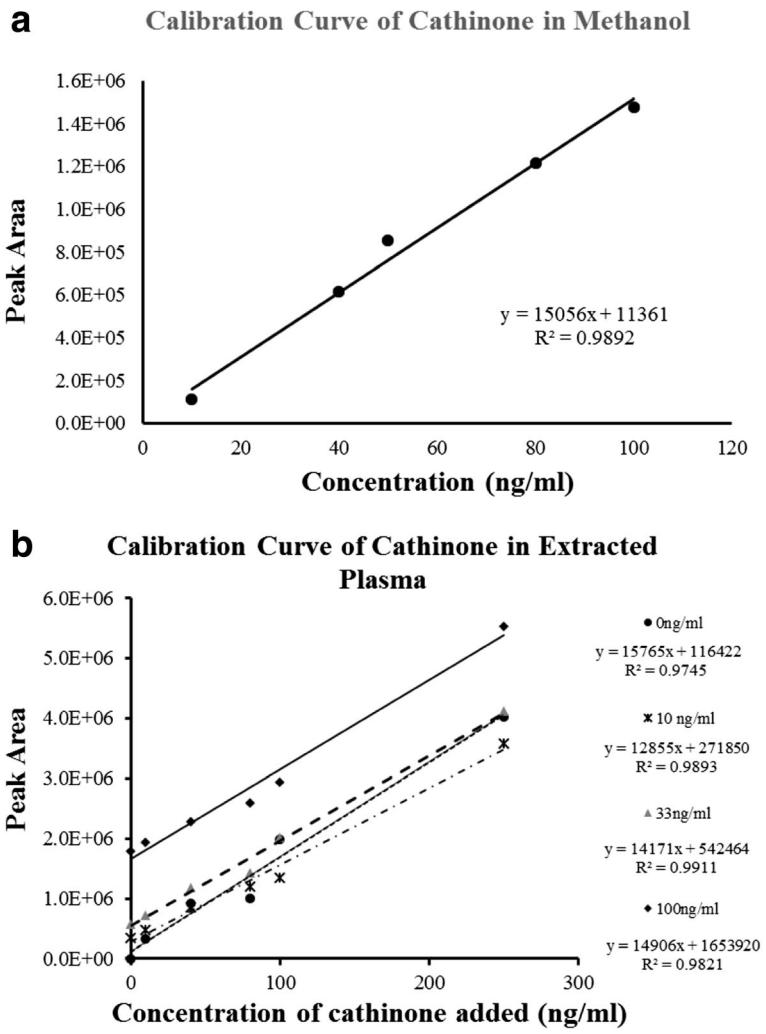

Fig. 2 Calibration curves of cathinone in methanol (**a**) and in extracted plasma (**b**)

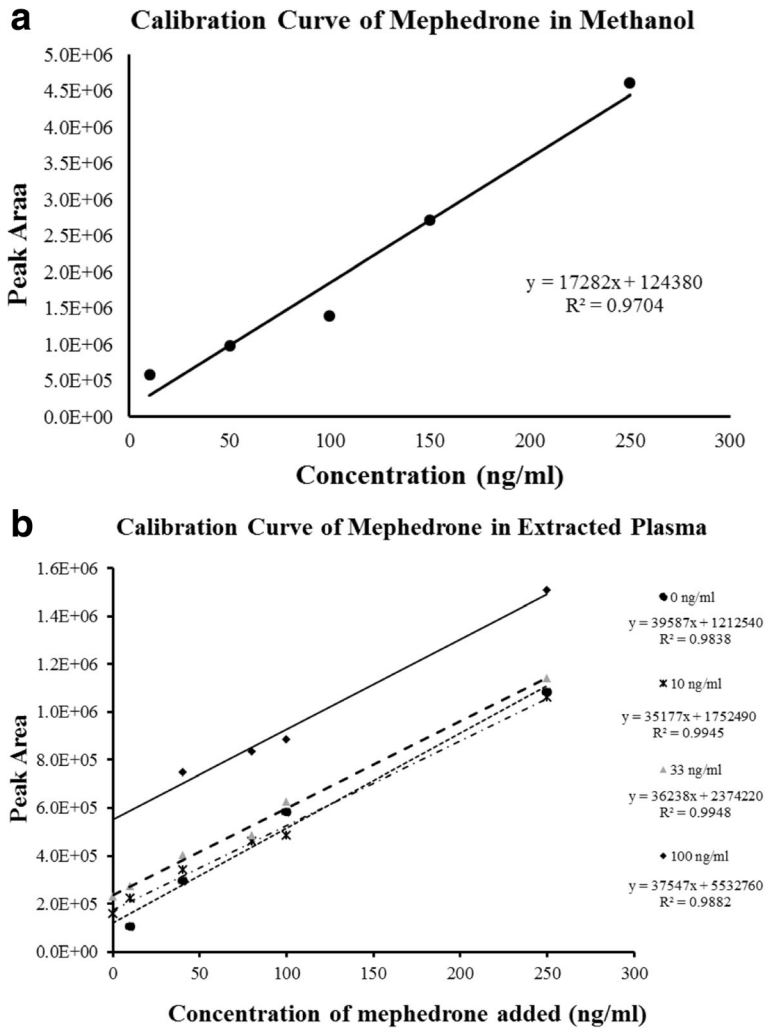

Fig. 3 Calibration curves of mephedrone in methanol (**a**) and in extracted plasma (**b**)

provide the solution to overcome the matrix effects (Lehotay et al. 2010; Stahnke et al. 2012). This study was aimed to explore the standard addition method as a means to overcome the plasma matrix effects on the quantification of cathinone and mephedrone by LC-MS/MS. We developed LC-MS/MS methods that utilized standard addition without internal standard (IS).

There are three commonly used techniques used to quantify the unknown samples: (1) external calibration with solvent standards, (2) external calibration with matrix matched standards, and (3) standard addition. Internal standard (IS) is important for using the external calibration to determine the chemical concentration. Isotopically labeled IS is the ideal

Table 1 Accuracy of quantifying cathinone in plasma using standard addition method

Matrix	Calibration curve	*X* value when *Y* = 0	Calculated drug concentration (ng/ml)	% Recovery
Solvent	*Y* = 15,056× + 11,361	0.755 ng/ml		
Plasma with 0 ng/ml cathinone	*Y* = 15,765 *X* + 116,422	7.38 ng/ml		
Plasma with 10 ng/ml cathinone	*Y* = 12,855 *X* + 271,850	21.15 ng/ml	21.15–7.38 = 13.77 ng/ml	13.77/10 = 137.7%
Plasma with 33 ng/ml cathinone	*Y* = 14,171 *X* + 542,465	38.28 ng/ml	38.28–7.38 = 30.90 ng/ml	30.90/33 = 93.6%
Plasma with 100 ng/ml cathinone	*Y* = 14,906 *X* + 1,653,920	110.96 ng/ml	110.96–7.38 = 103.57 ng/ml	103.57/100 = 103.6%

Table 2 Accuracy of quantifying mephedrone in plasma using standard addition method

Matrix	Calibration curve	*X* value when *Y* = 0	Calculated drug concentration (ng/ml)	% Recovery
Solvent	$Y = 17{,}282\ X + 124{,}380$	7.20 ng/ml		
Plasma with 0 ng/ml mephedrone	$Y = 39{,}587\ X + 1{,}212{,}540$	30.63 ng/ml		
Plasma with 10 ng/ml mephedrone	$Y = 35{,}177\ X + 1{,}752{,}490$	49.82 ng/ml	49.82–30.63 = 19.19 ng/ml	19.19/10 = 191.9%
Plasma with 33 ng/ml mephedrone	$Y = 36{,}238\ X + 2{,}374{,}220$	65.52 ng/ml	65.52–30.63 = 34.89 ng/ml	34.89/33 = 105.7%
Plasma with 100 ng/ml mephedrone	$Y = 37{,}547\ X + 5{,}532{,}760$	147.36 ng/ml	147.37–30.63 = 116.73 ng/ml	116.73/100 = 116.7%

standard to overcome the matrix effects and accurately quantify the known chemicals in the samples. When isotopically labeled IS is not available, a non-isotopically labeled IS can be used. However, the non-isotopically labeled IS should not be present in any of the tested samples and also should be as similar in physiochemical properties of the analyte. But these ideal internal standards are not always available especially for those new designer drugs. Standard addition (post-extraction spike) has been shown to compensate for the matrix effects contributed by biological matrices (Van Eeckhaut et al. 2009). Standard addition is commonly used for atomic absorption spectroscopy and gas chromatography analyses (Bonilla 1978; Wurita et al. 2014). However, there are few studies using the standard addition method for mass spectroscopy (Hasegawa and Suzuki 2014; Zenkevich and Morozova 2014). This study used the standard addition technique to determine cathinone and mephedrone concentrations in plasma by LC-MS/MS.

Table 3 Matrix effect for cathinone and mephedrone in plasma

Cathinone (pg/μl)	% CV	% Recovery
A		
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
Mephedrone (pg/ul)	% CV	% Recovery
B		
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

Findings

he results showed the matrix effect for cathinone at lowest tested concentration (10 ng/ml) was significantly reduced from 210.9 to 133.7%, but not for mephedrone (from 196.8 to 191.9%) by using standard addition method. At the higher tested concentrations of samples, the matrix effects were significantly reduced for both cathinone and mephedrone by using standard addition technique. Standard addition quantitation technique can be used to overcome the matrix effect when appropriate internal standard is not available.

Materials and methods

Materials

Solvents, cathinone (1 mg/ml in methanol), and mephedrone (1 mg/ml in methanol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sheep plasma was obtained from Carolina (Burlington, NC). Cathinone and mephedrone were diluted either in methanol as "solvent standard" or in plasma with known concentrations (0, 10, 33, and 100 ng/ml) of drugs as "standard addition" to at least five different calibration points (0–250 ng/ml). Solid phase extraction (SPE) was performed using Strat-X™ Drug B cartridges (Phenomenex).

Solid phase extraction

Plasma samples (1 ml) were mixed with 1 ml of 1 M acetic acid, then centrifuged. After centrifugation, the supernatant was subjected for SPE extraction. SPE cartridges were conditioned with 2 ml of methanol and then 2 ml of water. After loading sample, the SPE cartridges were washed separately with 2 ml of 1 M acetic acid and then 2 ml of methanol. Two milliliters of elution solvent (methylene chloride to isopropanol to ammonium hydroxide = 80:18:2) was passed through the SPE column and collected. After extraction, SPE extracted plasma with cathinone and mephedrone samples were either analyzed directly by LC-MS/MS or first spiked with known concentrations (0–250 ng/ml) of cathinone and mephedrone as

"standard addition" samples. Two hundred (200) microliters of HCl in methanol (2%) was added to samples before drying with a Turbovap N_2 evaporator. After drying, 200 ul of mobile phase A was used to reconstituent samples for LC-MS/MS analysis.

LC-MS/MS analysis

UPLC-MS/MS (Shimadzu LCMS 8030) with a Phenomenex Kinetex™ C18 column (2.1 × 100 mm, 1.7 um) was used to separate compounds under the following condition: gradient mobile phase system (A: 0.1% formic acid in water to methanol and B: 0.1% formic acid in acetonitrile; 0 min 5% B, 3 min 40% B, 3.5 min 90% B, 4 min 5% B); column heater: 30 °C; 10 ul injection volume; flow rate 0.5 ml/min). The Shimadzu LCMS 8030 triple quad instrument was used with the following settings: 1.5 L/min nebulizing gas flow, 250 °C DL temperature; 400 °C heat block temperature; 15 L/min drying gas flow). Mass transitions are as following: cathinone 150.1 > 117.0 (Q1–13, CE – 23, Q3–30); 150.1 > 104.85 (Q1–13, CE – 23, Q3–27) and mephedrone 178.0 > 145.0 (Q1–13, CE – 23, Q3–14); 178.0 > 90.95 (Q1–15, CE – 37, Q3–17).

Results and discussion

Plasma samples containing cathinone and mephedrone (10, 100, and 500 ng/ml) were extracted using Strat-X™ Drug B cartridge and analyzed by LC-MS/MS together with solvent standards. The matrix effects on the extracted samples were significantly observed in low concentration (10 ng/ml) samples (Fig. 1). Since the cutoff concentration for cathinone and mephedrone is 25 ng/ml, the matrix effect will make a possible false positive determination. In order to overcome this matrix effect, extracted plasma samples with cathinone and mephedrone (0, 10, 33, and 100 ng/ml) were spiked with various concentrations of standards (0–250 ng/ml) and analyzed by LC-MS/MS. Calibration curves were established and shown in Figs. 2 and 3 (A solvent standard, B standard addition) for cathinone and mephedrone, respectively.

Concentrations of cathinone and mephedrone were then calculated by subtracting the value of *X*-axis intercept of solvent standard calibration from the value of *X*-axis intercept of standard addition calibration (Tables 1 and 2). The results show the matrix effect for cathinone at 10 ng/ml level was significantly reduced from 210.9 to 137.7%, but not for mephedrone (196.8 vs 191.9%). At higher concentration samples, the matrix effect for both cathinone and mephedrone was not significant compared with 10 ng/ml samples (Table 3).

Conclusions

The study here using standard addition quantitative technique showed a significant reduction of plasma matrix effect on cathinones analysis by LC-MS/MS. However, this standard addition technique for quantifying plasma samples by LC-MS/MS requires sufficient amount of sample to set the standard addition calibration curve and more work (two calibration curves) is needed. Therefore, this technique can serve as an alternative quantitative method to overcome the matrix effect when no appropriate internal standard is available.

Abbreviations

DEA: Drug Enforcement Administration; IS: Internal standard; LC-MS/MS: Liquid chromatography tandem mass spectrometry; SPE: Solid phase extraction

Acknowledgements

We specially thank Argeliz Pomales and Teeshavi Narayne for excellent technical assistance and Scott A. Cassidy and William Orlando from Shimadzu for guidance and support.

Funding

The work is supported by the PRISM fund at CUNY John Jay College. The PRISM is the Program for Research Initiatives for Science Majors at John Jay College and funded by the Title V, HSI-STEM, and MSEIP programs within the US Department of Education; the PAESMEM program through the National Science Foundation; and New York State's Graduate Research and Teaching Initiative.

Availability of data and materials

The data of this project can be shared.

Authors' contributions

TNAQ designed the study and carried out the analysis. BE and JH supervised the experimental work in the study. SYC and JH supervised the analysis of results of the study. SYC contributed to the draft version of the manuscript. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Received: 25 July 2017 Accepted: 15 September 2017
Published online: 23 September 2017

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