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Research Article

Simultaneous Quantitative Determination of Synthetic Cathinone Enantiomers in Urine by GC-NCI-MS/MS using Menthylchloroformate as Chiral Derivatization Reagent

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ABSTRACT

Development and validation of a new analytical method for enantioseparation and quantitation of synthetic cathinones is reported using a chiral derivatization agent (CDA) and GC-MS triple quadrupole mass spectrometry with negative chemical ionization (NCI) mode. Indirect separation of ten synthetic cathinone compounds has been achieved using a commercially available optically pure CDA called (1*R*)-(-)-menthylchloroformate (MCF). MCF was capable of converting synthetic cathinone enantiomers into diastereoisomers, which were later separated with good resolution on an ultra-inert 60 m achiral stationary phase column. An internal standard (IS), 3-methylmethcathinone (3-MMC) was used for quantitation of synthetic cathinone diastereoisomers. Method validation in terms of calibration curve linearity, sensitivity in terms of limits of detections (LODs), limits of quantitation (LOQs), inter-day and intra-day reproducibility, and recoveries has been obtained for the ten synthetic cathinone compounds that were analyzed simultaneously as a mixture after being spiked in urine. It was found that LOD's and LOQ's for the ten synthetic cathinones mixture in urine were in the ranges of 0.004-3.678 ppm and 0.012-11.14 ppm respectively. The results of four synthetic cathinones (Buphedrone, 3-MethylBP,3,4-DMEC, and Butylone) derivatized with MCF were compared with the same compounds derivatized with N-trifluoroacetyl-L-prolyl chloride (L-TPC). It was found that the later was more sensitive in terms of LOD and LOQ than the former method.

Keywords: Synthetic cathinones; (1*R*)-(-)-menthylchloroformate; Derivatization; Quantitation, Urine.

INTRODUCTION

Cathinone is a psychoactive phenylalkylamines class of alkaloids found in khat [1-5], has an amphetamine-like structure and stimulant effects. Amphetamine is a well-known classical drug of abuse worldwide. Therefore, cathinone is considered to be a natural beta-keto amphetamine analogue found in the leaves of khat plant [1,6].

Traditionally, the main psychoactive component of khat plant, cathinone is extracted during the chewing process of the fresh green leaves of khat. On the other hand, a new generation of modified cathinones have been synthesized and abused in different regions around the world known as New Designer Drugs (NDD) or Novel Psychoactive Drugs (NPD) [7-10]. This new strategy taken by drug dealers to sell their products as "novel psychoactive substances," drugs which contain at least one chemical substance that has similar biological effects as of the illegal natural cathinone allowing them to offer legal alternatives to the controlled substance. Those modified or synthesized cathinones are considered as "legal highs' and usually labeled as "not for human consumption" under the name of "bath salts" and other commercial names to circumvent drug abuse legislation and to become widely spread. Synthetic cathinones or bath salts as known in the United States are mostly sold over the internet and in head shops as white powder, crystalline mixture or tablets. These compounds are usually snorted, smoked, injected intravenously or taken orally [11]. Mephedrone (4-methylmethcathinone) is the first synthesized cathinone, became one of the most popular abused drugs in Europe since 2007 [12]. This cathinone derivative has produced many serious intoxications and some deaths in different countries. Due to the high concerns about the abuse of the cathinone-related derivatives and other novel psychoactive substances in Europe, the UK government and European Monitoring Center for Drugs and Drug Addiction (EMCDDA) banned all cathinone derivatives in April 2010 [13].

Like amphetamine, cocaine and other stimulation-induced drugs, synthetic cathinones stimulate the central nervous system (CNS) by increasing synaptic concentration of serotonin, dopamine and norepinephrine. This increase of different neurotransmitters

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in CNS results in desired effects such as: increase in energy, sociability, and sexual desire. On the other hand, undesired effects such as insomnia, muscle twitching, increase heart rate, confusion, dizziness, prolonged panic attack and suicidal thoughts have been reported by users [14].

In recent years, synthetic cathinones have spread across many regions around the world as an important class of the new designer drugs (NDD). These proliferations of synthetic cathinones have been associated with multiple criminal acts, intoxications and fatalities. From a forensic standpoint, this proliferation makes scientists in an ongoing challenge to develop new analytical methods for synthetic cathinones detection to keep up with the high pace of new NDD production trends.

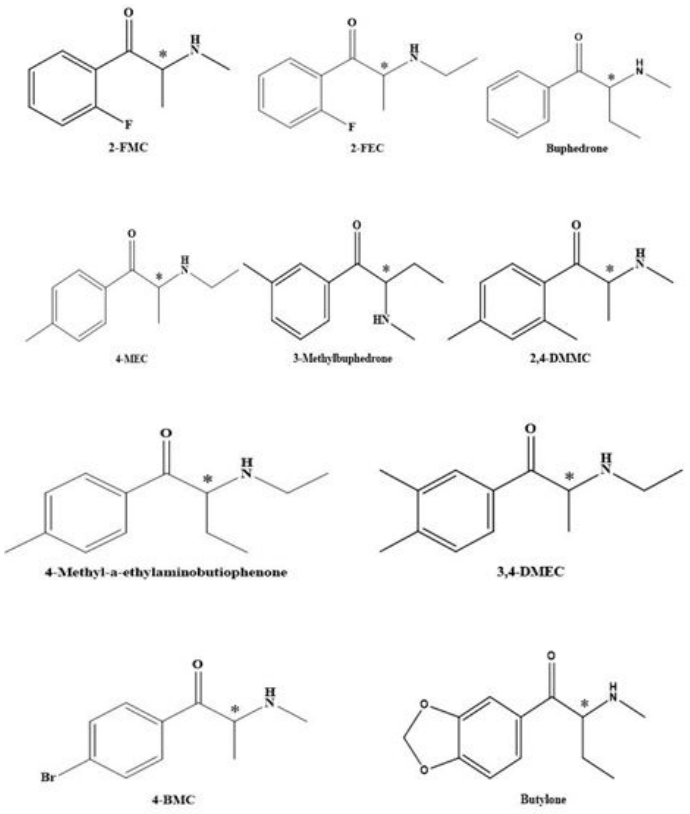

Figure 1: Chemical Structures of the Analyzed Synthetic Cathinones.

All synthetic cathinone derivatives consist of a chiral center and usually sold out as a racemic mixture. Usually, one of the two enantiomers will have stronger stimulatory activity on the central nervous system (CNS) and the other one may have no significant CNS activity or addictive properties [15]. Therefore, it is important to develop an analytical method capable of separating and detecting the synthetic cathinone enantiomers to provide information regarding the sources of these drugs and the raw materials used to create them to help law enforcement agencies with the drug tracking.

In general, there are two main approaches used within the pharmaceutical studies to separate chiral compounds: A direct method, where the enantiomers are separated inside a column with a chiral stationary phase (CSP) or the enantiomers are separated using a chiral selector in a mobile phase with an achiral stationary phase. The other approach is the indirect method which is based on the formation of diastereomers by reacting the chiral compounds with derivatizing agent (CDA) and separating them on an achiral stationary phase [16]. The low cost, commercial availability of CDA and easier method development make the indirect approach an efficient technique for the enantioseparation of chiral compounds.

There are no reports in literature that discuss the use of indirect chiral separation of synthetic cathinones using (1R)-(-)-menthylchloroformate (MCF) as chiral derivatization agent (CDA). In fact, MCF was used for indirect chiral separation of amphetamine derivatives and it was found to be less satisfying in terms of resolution compared to other CDA such as (R)-(+)-α-methoxy-α-trifluoro methyl phenyl acetic acid (MTPA) [17].

On the other hand, only one literature is found that discusses the use of GC-MS in negative chemical ionization mode for chiral separation and quantitative determination of synthetic cathinones spiked in urine and plasma after being derivatized using (S)-(-)-N-(Trifluoroacetyl)pyrrolidine-2-carbonyl chloride (L-TPC) as CDA. It was reported that GC-MS with negative chemical ionization mass spectrometry increased the sensitivity of detection and resolution compared to previously reported methods that use electron impact (EI) and positive chemical ionization (PCI) mass spectrometry [13].

In this work, a sensitive and selective analytical method has been developed to analyze ten synthetic cathinones using gas chromatography mass spectrometry (GC-MS) with negative chemical ionization (NCI) and multiple reaction monitoring (MRM) mode. The studied synthetic cathinones were converted into their diastereoisomers after a reaction with MCF chiral derivatizing agent. A racemic mixture of the ten synthetic cathinones was qualitatively and quantitatively analyzed in spiked urine samples as well as method validation. Figure 1 shows the structures of the ten studied synthetic cathinones in this study.

MATERIALS AND METHOD

Chromatographic Conditions

Chromatographic separation was performed on an Agilent 7890A GC equipped with a 7693B autosampler and 7000 Series triple quadrupole (QQQ) mass spectrometer detector, MSD system (Agilent, USA). An Agilent Ultra Inert 60 m capillary column consisting of (5%-Phenyl)-methylpolysiloxane, with 0.25 mm inner diameter and a 0.25 µm film thickness was used as stationary phase. Chemical ionization (CI) with methane gas (40%, 2.0 mL/min) was employed in the negative ion mode at a voltage of 110 eV. Helium was used as carrier gas at a constant flow rate of 1.0 mL/min. Injection volume of 3 µL of sample solution was performed automatically in splitless mode. The injector and GC-MS interface temperature were set at 250 and 280°C, respectively. Data collection was performed in Multiple Reaction Monitoring (MRM) mode starting at 42 min after injection (i.e., filament delay). Table 1 summarizes the MRM conditions. The column temperature program was as follows: starting at 160°C and hold for 5 min, followed by heating to 270°C with a heating rate of 2°C/min. The final temperature was held at 270°C for 5 minutes. The obtained data was analyzed using the Agilent Mass Hunter Workstation software B.06.00.

No.	Compound	Precursor Ion (m/z)	Product Ion (m/z)	Collision energy (v)	Time (min)
1	2-FMC	363.5	212, 155	15	44.00-45.55
2	2-FEC	377.5	226, 155	15	45.55-47.20
3	Buphedrone	359.8	344, 212, 155, 145	20	47.20-49.00
IS	3-MMC	359.8	202, 188	15	
4	4-MEC	373.8	344, 188, 155	15	49.00-51.65
5	3-MethylBP	373.8	212, 174	20	
6	2,4-DMMC	373.5	217, 173	15	51.65-53.70
7	4- Methyl-α- ethylaminobutiophenone	387.8	226, 155	15	
8	3,4-DMEC	387.5	231, 188	20	
9	4-BMC	424.7	211, 81	15	55.10-58.00
10	Butylone	403.5	212, 190, 175, 155	15	58.00-65.00

Table 1: MRM acquisition parameters for the analysis of cathinones mixture in urine.

Chemicals and Reagents

All chemicals were of analytical grade. Triethylamine, acetonitrile, methanol, 2-propanol, hexane, dichloromethane, ammonium hydroxide, (1R)-(-)-Menthylchloroformate (99% purity), and sodium phosphate were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Doubly deionized water was obtained from ultra-pure Millipore system (MS, USA). All synthetic cathinones standards shown in Table 1 were supplied as racemic mixtures of R and S enantiomers and were purchased from Cayman chemicals (Michigan, USA).

Sample Preparation

Samples: This work conforms to the UAE community guidelines for the use of humans in experiments. The Human Ethics committee at the Dubai police approved this study. Urine samples were collected with the consent of the subject.

Urine Spiking and Solid Phase Extraction (SPE): Solid phase extraction (SPE) was carried manually and offline. The SPE column was 200 MG clean screen CSDAU203 from FluoroChem, (Hadfield, UK). Urine sample were diluted with doubly deionized water in 1:1 ratio. 2 mL of diluted urine was spiked with 1 mL of 50 ppm synthetic cathinones mixture and internal standard (IS) 3-Methylmethcathinone (3-MMC) in addition to 1 mL of 0.1 M phosphate buffer (pH 6.0). The mixture was mixed and vortexed for 1 minute. For SPE cartridge conditioning, 1 mL of methanol and 1 mL of doubly deionized water were used. 2 mL of spiked urine sample was loaded to the cartridge followed by sequential washing using 1 mL 0.1 M acetic acid and 1 mL methanol. The cartridge was left for drying under soft air stream for 1 minute. Finally, 3 mL of the eluate mixture, which is made of dichloromethane, hexane, isopropanol, and ammonium hydroxide with a relative ratio of (39:39:20:2) was used to collect the analyte. The collected sample was evaporated to dryness under soft air stream.

Derivatization: Derivatization reaction was done for the spiked urine samples after the evaporation step. The obtained dried and water free sample was dissolved in 50 µL of Triethylamine and 850 µL Acetonitrile to obtain final concentration of 50 ppm. 500 µL of the 50-ppm sample was reacted with 35 µL of menthylchloroformate CDA and vortexed for 10 minutes at room temperature. The derivatized sample was stored in the freezer for 24 hours prior to injection in GC-MS instrument. Derivatization of Buphedrone, 3-MethylBP, 3,4-DMEC, Butylone with L-TPC was performed using the same procedure reported before [13,18].

Method Validation

The adopted analytical approach and performance of the method was validated in terms of linearity, sensitivity [Limit of Detection (LOD), Limits of Quantitation (LOQ)], inter-day and intra-day reproducibility, and recovery for all tested spiked urine samples according to international criteria.

RESULTS

The analytical method that has been developed is based on the conversion of synthetic cathinones to MCF derivatives. The reaction took place between the acid chlorine in the derivatizing agent and the amine group of the targeted compound. The obtained diastereomers of each of the ten synthetic compounds were separated and analyzed on GC-NCI-MS/MS using MRM mode.

The GC-MS/MS database was developed by creating a quantitative analysis method, which consist of the following steps: (i) precursor ions scanning and determination, (ii) creation of acquisition data file for the determined precursor ions, (iii) precursor ions fragmentation at different collision energies, (iv) product ion determination for each compound, (v) determination of the most suitable collision energies, (vi) MRM method creation. Table 1 shows the compound name, the determined precursor ion of each compound, the resulted product ions for each compound, the suitable collision energy and the retention time of each. Figure 2 shows the separation of the (R) and (S) enantiomers of Butylone drug after derivatization with MCF.

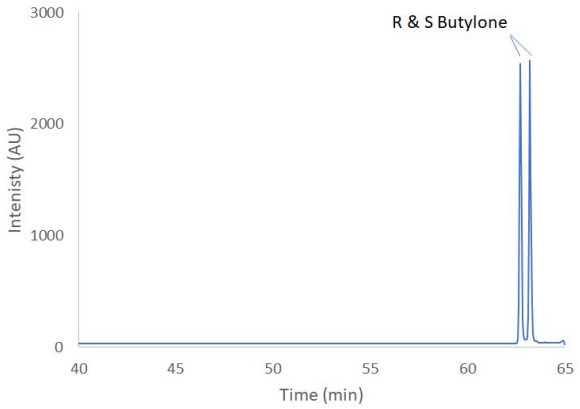

Figure 2: GC Chromatogram for separation of the R and S enantiomers of Butylone drug in acetonitrile after derivatization with MCF.

Table 2 shows the retention time, resolution values and selectivity factor of the separated enantiomers for the ten analyzed synthetic cathinones. All compounds in Table 2 were analyzed individually on GC-NCI-MS using MRM mode, after being derivatized with MCF.

Figure 3 shows the GC total ion chromatogram for the analyzed synthetic cathinones mixture after being spiked in urine followed by SPE and derivatization step using MCF. A good separation can be observed for most of the mixture components. GC chromatograms of compounds with similar retention time such as 4-MEC and 3-MethylBP as shown in Figures 4 and 5 respectively are obtained by extracting the exact chromatogram of each compound. In addition, 2,4-DMMC has one of its enantiomer overlapping with 4-Methyl-α-ethylaminobutiophenone in terms of retention time. 2,4-DMMC is extracted from the TIC as shown in Figure 6. To our knowledge, this is the first example in the literature that shows a simultaneous separation of ten pairs of MCF cathinone derivatives mixture in complex matrix of urine.

#	Compound Name	Abbreviation	Time (min)		Resolution	Selectivity Factor (α)
			tR1	tR2		
1	2-Fluoromethcathinone	2-FMC	44.65	45.40	3.19	1.017
2	2-Fluoroethcathinone	2-FEC	46.10	46.98	6.78	1.019
3	Buphedrone	-	47.40	48.10	11.67	1.015
4	4-Methylethcathinone	4-MEC	50.00	51.45	23.2	1.029
5	3-Methylbuphedrone	3-MethylBP	50.63	51.40	15.4	1.015
6	2,4-Dimethylmethcathinone	2,4-DMMC	51.99	52.81	6.56	1.016
7	4-Methyl-α- ethylaminobutiophenone	-	52.40	52.95	6.11	1.01
8	3,4-Dimethylethcathinone	3,4-DMEC	53.10	53.68	2.42	1.011
9	4-Bromoethcathinone	4-BMC	55.21	55.95	6.43	1.013
10	Butylone	-	62.40	63.65	9.26	1.01

Table 2: List of the analyzed synthetic cathinones and their synonyms, in addition to the retention times of the separated two diastereoisomers for each compound analyzed on GC-MS using MRM mode.

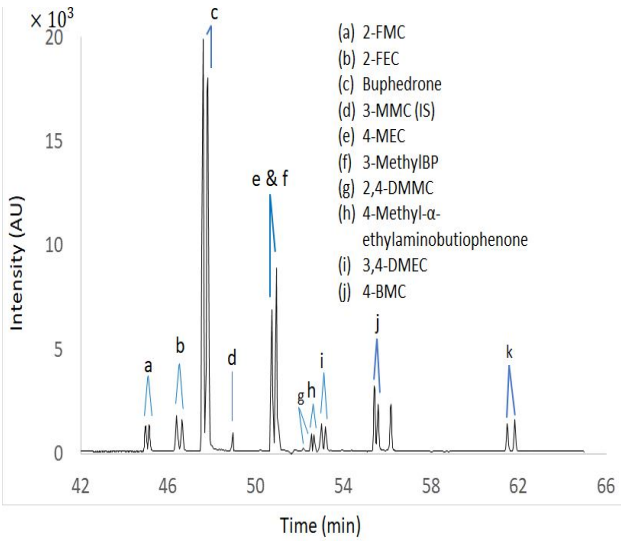

Figure 3: Total Ion Chromatogram (TIC) of the simultaneous chiral separation of ten synthetic cathinone compounds. The mixture was spiked in urine and derivatized using MCF.

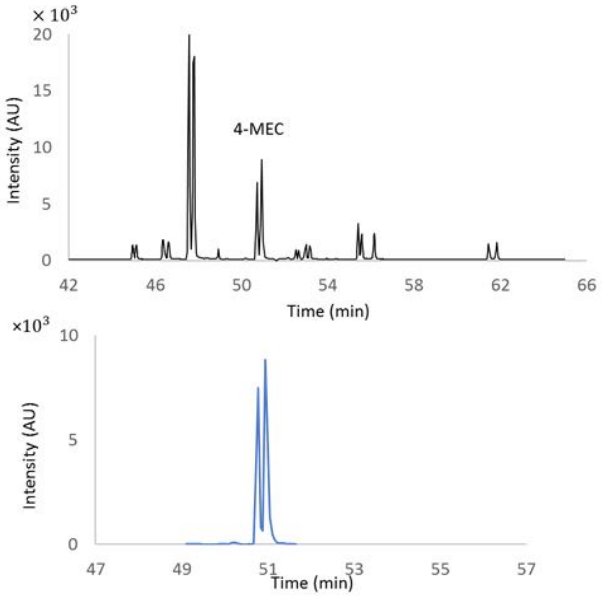

Figure 4: Extracted Ion Chromatogram (EIC) of R and S enantiomers of 4-MEC.

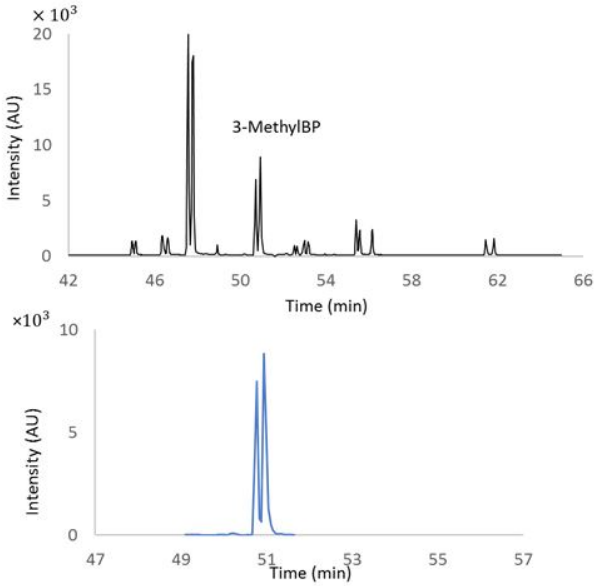

Figure 5: Extracted Ion Chromatogram (EIC) of R and S enantiomers of 3-MethylBP.

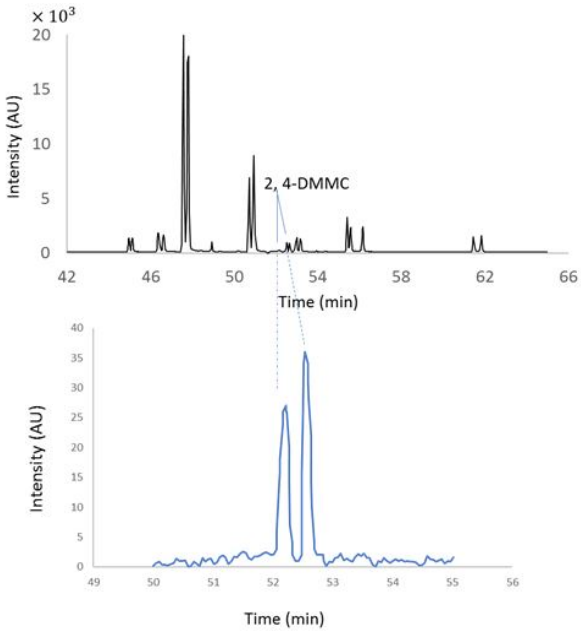

Figure 6: Extracted Ion Chromatogram (EIC) of R and S enantiomers of 2,4-DMMC.

Method validation was performed on the ten analyzed compounds spiked in urine matrix. The method was validated in terms of calibration curve linearity, sensitivity (Limit of Detection (LOD), Limits of Quantitation (LOQ)), inter-day and intraday reproducibility, and spike recoveries as shown in Tables 3-5.

#	Name	R2 ± SD		LOQ ± SD (ppm)		LOD ± SD (ppm)	
		E1	E2	E1	E2	E1	E2
1	2-FMC	0.9350 ± 0.0134	0.9121 ± 0.0134	0.127 ± 0.0014	0.121 ± 0.0009	0.042 ± 0.0004	0.040 ± 0.0003
2	2-FEC	0.9652± 0.0304	0.9117 ± 0.0335	0.056 ± 0.0007	0.056 ± 0.0005	0.018 ± 0.0002	0.018 ± 0.0002
3	Buphedrone	0.9777 ± 0.2181	0.9237 ± 0.176	0.013 ± 0.0004	0.012 ± 0.0005	0.004 ± 0.0001	0.004 ± 0.0002
4	4-MEC	0.9709 ± 0.1083	0.9605 ± 0.1002	0.029 ± 0.0005	0.026 ± 0.0008	0.009 ± 0.0002	0.009 ± 0.0003
5	3-MethylBP	0.9829 ± 0.0078	0.9369 ± 0.007	0.343 ± 0.1992	0.617 ± 0.0546	0.113 ± 0.0657	0.204 ± 0.0180
6	2,4-DMMC	0.9034 ± 0.0011	0.9619 ± 0.002	2.594 ± 0.0007	1.771 ± 0.0005	0.856 ± 0.0002	0.584 ± 0.0002
7	4-Methyl-α-ethylaminobu-tiophenone	0.9545 ± 0.0096	0.9092 ± 0.0188	0.416 ± 0.0101	0.222 ± 0.004	0.137 ± 0.0033	0.073 ± 0.0013
8	3,4-DMEC	0.9592 ± 0.0003	0.9109 ± 0.0008	11.14 ± 0.3337	3.982 ± 0.009	3.678± 0.1101	1.314 ± 0.0030
9	4-BMC	0.9817 ± 0.0893	0.9585 ± 0.0557	0.056 ± 0.0014	0.0879 ± 0.0009	0.0184 ± 0.0005	0.0290± 0.0003
10	Butylone	0.9118 ± 0.0471	0.9263 ± 0.0519	0.096 ± 0.0011	0.091 ± 0.0020	0.032 ± 0.0004	0.029 ± 0.0007

Table 3: Regression coefficient R2 values, Limits of Quantitation (LOQ) and Limits of Detection (LOD) for the ten synthetic cathinone compounds spiked in urine.

#	Name	CV% Interday				CV% Intraday			
		10 ppm		25 ppm		10 ppm		25 ppm	
		E1	E2	E1	E2	E1	E2	E1	E2
1	2-FMC	2.33	3.18	2.56	2.4	1.4	2.4	6.47	2.09
2	2-FEC	3.77	3.22	3.71	2.97	7.57	0.89	1.01	6.14
3	Buphedrone	3.68	2.74	8.51	2.92	3.15	4.77	1.73	1.23
4	4-MEC	10.46	2.61	2.57	7.26	6.71	7.41	1.36	4.56
5	3-MethylBP	4.03	6.62	1.1	2.81	12.24	1.15	2.22	3.7
6	2,4-DMMC	3.63	2.69	9.85	5.93	2.57	2.33	4.82	10.04
7	4-Methyl-α-thylamino-butiophe-none	6.27	4.36	1.21	7.83	9.35	6.21	4.18	7.67
8	3,4-DMEC	3.6	11.87	6.3	7.14	4.57	7.77	7.04	2.13
9	4-BMC	1.71	1.95	1.38	2.4	3.45	3.98	1.64	4.88
10	Butylone	2.85	1.29	2.82	1.19	2.56	1.53	1.45	1.94

Table 4: Interday and intraday reproducibility results for the ten synthetic cathinone compounds spiked in urine at two different concentration levels for the two enantiomers of each compound.

#	Name	Error%			
		10 ppm		25 ppm	
		E1	E2	E1	E2
1	2-FMC	2.36	6.23	8.77	11.57
2	2-FEC	3.07	4.46	5.19	4.12
3	Buphedrone	5.14	6.23	8.77	11.7
4	4-MEC	7.52	3.69	3.64	4.91
5	3-MethylBP	3.34	5.72	4.04	4.88
6	2,4-DMMC	4.98	3.74	7.41	9.09
7	4-Methyl-α- ethylami-nobutiophenone	9.57	2.64	7.92	10.64
8	3,4-DMEC	5.19	6.76	8.46	4.17
9	4-BMC	2.39	3.14	2.29	4.98
10	Butylone	3.95	2.86	3.91	2.98

Table 5: Recovery measurements expressed in percent errors for the ten synthetic cathinone compounds spiked in urine at two different concentration levels for the two enantiomers of each compound.

The calibration graphs for the ten enantiomer pairs of the synthetic cathinones were obtained in a linear form within the tested range of 0.005 to 50 ppm in urine with mean regression coefficient (R2; n=5) of 0.90 or higher. Table 3 summarizes the regression coefficient, LOD and LOQ values of the separated enantiomers of every analyzed compound spiked as a mixture in urine.

For the accuracy and reproducibility validation of this study, inter-day and intra-day reproducibility were obtained by testing two different concentration levels (10 and 25 ppm) for the two separated enantiomers of each of the ten analyzed synthetic cathinone compounds in urine as shown in Table 4. The reproducibility results were obtained in term of coefficient of variance for the ten analyzed synthetic cathinone compounds in urine.

For the recovery study, an evaluation of the percent error has been done for the spiked mixture in urine at two different concentration levels (10 and 25 ppm) as shown in Table 5.

A comparison between the LOD and LOQ results obtained for four synthetic cathinones (Buphedrone, 3-MethylBP, 3,4-DMEC, Butylone) derivatized with MCF and N-trifluoroacetyl-L-prolyl chloride (L-TPC) chiral derivatizing agents is shown in Table 6. It is clear that the results obtained for these drugs with L-TPC were more sensitive.

#	Name	Synthetic cathinones+MCF				Synthetic cathinones+L-TPC (Alremeithi et al., 2018)			
		LOQ (ppm)		LOD (ppm)		LOQ (ppm)		LOD (ppm)	
		E1	E2	E1	E2	E1	E2	E1	E2
1	Buphedrone	0.013	0.012	0.013	0.012	9.6×10^{-4}	9.2×10^{-4}	2.9×10^{-4}	2.8×10^{-4}
2	3-MethylBP	0.347	0.621	0.347	0.621	1.02×10^{-3}	9.9×10^{-4}	3.1×10^{-4}	3.0×10^{-4}
3	3,4-DMEC	9.536	3.429	9.536	3.429	1.12×10^{-3}	1.39×10^{-3}	3.4×10^{-4}	4.2×10^{-4}
4	Butylone	0.094	0.091	0.094	0.091	8.6×10^{-4}	9.8×10^{-4}	2.6×10^{-4}	2.7×10^{-4}

Table 6: Data comparison of LOQs and LODs for four synthetic cathinones with MCF and L-TPC derivatizing agent.

DISCUSSION

This study adopts the indirect chiral separation method using Menthylchloroformate (MCF) as a chiral derivatizing agent (CDA). MCF reacted with secondary amine enantiomers of the ten analyzed synthetic cathinones (Table 1) resulting in two unique corresponding diastereomers of each of the ten compounds. The resulted diastereoisomers from the reaction of synthetic cathinones with MCF interacted differently with the achiral stationary phase column and where presented in different retention time on the gas chromatography (GC) chromatogram. The total ion chromatogram (TIC) of the synthetic cathinones mixture spiked in urine showed different corresponding pairs of diastereomers with different resolutions and intensity of the products. This difference is suggested to be caused by the difference of physicochemical properties such as stereochemistry and stability of the analyzed compound on the achiral column. Moreover, the structure difference, the distance between the two asymmetric centers in the substrate and CDA, and the conformational rigidity around the chiral center might influenced the difference in resolution and intensity of the resulted corresponding diastereomers [19]. Using the Multiple Reaction Monitoring (MRM) mode for the collection of data, it was possible to separate the ten analyzed synthetic cathinone compounds simultaneously in one chromatogram after spiking the mixture in urine (Figure 3). The obtained TIC had two synthetic cathinone compounds (4-MEC and 3-MethylBP) sharing the similar retention time. By extracting the specific chromatogram of each of the two compounds, it was possible to determine and quantify them separately (Figures 4 and 5). In addition, one compound (2,4-DMMC) had one of its enantiomer overlapping with4-Methyl-α-ethylaminobutiophenone in term of retention time (Figure 6). It was possible to determine and quantify the compound by extracting its exact ion chromatogram (EIC). Calibration curves of the ten analyzed synthetic cathinone compounds in urine were constructed based on the diastereoisomers peak areas of each compound in five different concentrations (0.005, 0.05, 0.1, 1, 5, 10, 20, 25, and 50 ppm). 3-Methylmethcathinone (3-MMC) was added as an internal standard (IS) to the ten analyzed synthetic cathinones mixture. 3-MMC was chosen due to its similar structure to the studied synthetic cathinone compound and its good stability. 3-MMC was previously investigated and found to show a reaction when derivatized with MCF but no enantiomeric separation of the two diastereomers. All constructed calibration graphs showed good linearity and good correlation coefficient (R2) values higher than 0.90 as shown in Table 3. Table 3 also shows the Limits of Quantitation (LOQ) and Limits of Detection (LOD) values for each enantiomer for the ten analyzed synthetic cathinone compounds mixture spiked in urine. For LOQ in urine, the range was 0.012-11.14 ppm and for LOD the range was 0.004-3.678 ppm.

Interday and intraday reproducibility of the ten analyzed synthetic cathinones mixture were evaluated and reported at two different concentration levels (10 and 25 ppm) as shown in table 4. The method reproducibility was evaluated based on the coefficient of variance values and all of the analyzed enantiomers showed values below 12.5% in urine suggesting a good reproducibility of the adopted method.

For the evaluation of the adopted solid phase extraction (SPE), the method recovery has been studied by calculating the percent error for the ten analyzed synthetic cathinones mixture in urine at two different concentrations (10 and 25 ppm). The percent error values were within the acceptable range for most of the enantiomers in urine as shown in Table 5.

We were expecting to obtain better results in terms of resolutions, LOQs, and LODs by using menthylchloroformate (MCF) as chiral derivatizing agent (CDA). However, the comparison of the results of the MCF derivatives with the L-TPC derivatives showed that the L-TPC was better. It is possible that the purity of MCF had affected the overall reaction with the targeted synthetic cathinone compounds. Moreover, the derivatization reaction took place under controlled anhydrous condition due to high reactivity of MCF with water and to maximize the reaction efficiency. Despite the adequate care given to minimize any possible experimental errors, there are still errors such as instrumental setup, samples purity, derivatizing agent reactivity, extraction efficiency and recovery might affect the experimental design. Moreover, there are some reports that have pointed out the lower performance of MCF as a derivatizing agent in terms of low resolving power of the separated two diastereoisomers and poor peak shape [17].

CONCLUSION

A sensitive and selective analytical method for separation and quantitative determination of synthetic cathinones in urine using gas chromatography tandem mass spectrometry (GC-MSMS) was successfully developed. The developed method adopts the indirect chiral separation technique using menthylchloroformate (MCF) as chiral derivatizing agent. A mixture of ten synthetic cathinones was spiked in urine followed by solid phase extraction (SPE) and derivatization using MCF. 3-Methylmethcathinone (3-MMC) was used as an internal standard (IS) in synthetic cathinones quantitation due to its stability and similar structure to the analyzed compounds. The ten derivatized synthetic cathinones mixture in urine were separated as their optical enantiomers successfully using a 60 m achiral stationary phase column in GC. Calibration curves of the ten analyzed synthetic cathinones in urine were constructed based on the diastereoisomers peak areas of each compound in five different concentrations (0.005, 0.05, 0.1, 1, 5, 10, 20, and 50 ppm). The analytical method was validated in terms of calibration curve linearity, LOQ, LOD, inter-day and intraday reproducibility, and spike recovery for all ten synthetic cathinones mixture spiked in urine. It was found that LOQ's and LOD's of the ten synthetic cathinones in urine were in the ranges of 0.012-11.14 ppm and

0.004-3.678 ppm respectively.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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