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Development of a chemometric method in urine sample for newly designed hallucinating psychoactive substances: 5-MeO-MiPT

Halüsinasyon yapan yeni tasarım psikoaktif maddelerden biri olan 5-MeO-MiPTin idrarda tayinine yönelik kemometrik yöntem geliştirilmesi

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ABSTRACT

Aim: In this study, a method was developed for analysis and chemometric optimization for 5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT).

Materials and Methods: Our study was carried out in Ege University Institute on Drug Abuse, Toxicology and Pharmaceutical Science, Addiction Toxicology Laboratory.

Analysis and optimization of the effects of hydrolysis and solid phase extraction processes during the analysis of 5-MeO-MiPT by Gas Chromatography-Mass Spectrometry (GC-MS). For chemometric screenings design, Plackett-Burman was used. The most effective three factors were determined, and a central composite design was applied, and results were evaluated with surface response methodology. The method was validated for selectivity, linearity, the limit of detection, the limit of quantitation, accuracy, intra-day and inter-day repeatability, stability and carry over.

Results: In the chemometric method, the most effective parameters were found sample volume, hydrolysis temperature, and elution volume for 5-MeO-MiPT in urine analysis. Optimum values for these parameters were calculated by surface response methodology and the results were determined 1ml urine volume, 30°C hydrolysis temperature, 3,5 ml elution volume, respectively. The optimized method was validated for selectivity, linearity (25-500 ng/mL), limit of detection (5 ng/mL), limit of quantitation (18 ng/mL), accuracy (72-101%), intra-day and inter-day precisions were measured, respectively (4,43% RSD),(4,27% CV), stability and carry over parameters.

Conclusion: With a chemometric approach, quick, practical and accurate method for the detection of 5-MeO-MiPT has been developed with GC-MS. Working of 5-MeO-MiPT without derivatization in GC-MS analysis has shortened the pre-preparation time and is a pioneer for other analogs. It provides an effective method in the analysis of substances such as synthetic analogues from tryptamines which are added every day, with the use of such classical equipment and new methods.

Keywords: 5-MeO-MiPT, forensic toxicology; Gas Chromatography-Mass Spectrometry, solid-phase extraction, chemometry.

ÖZ

Amaç: *Bu çalışmada yeni psikoaktif maddelerden biri olan 5-metoksi-N-metil-N-izopropiltriptamin (5-MeO-MiPT) için analiz ve kemometrik optimizasyon için bir yöntem geliştirmeyi amaçladık.*

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Gereç ve Yöntem: *Çalışmamız Ege Üniversitesi Madde Bağımlılığı, Toksikoloji ve İlaç Bilimleri Enstitüsü Bağımlılık Toksikolojisi Laboratuvarında gerçekleşmiştir. 5-MeO-MiPT'nin Gaz Kromatografi-Kütle Spektrometrisi (GC-MS) ile analizi sırasında hidroliz ve katı faz ekstraksiyon işlemlerinin etkileri incelendi ve optimizasyonu yapıldı. Kemometrik tarama tasarımı için Plackett-Burman kullanıldı. En etkili üç faktör belirlenerek merkezi bir kompozit tasarım uygulandı ve sonuçlar yüzey tepki metodolojisi ile değerlendirildi. Yöntem, seçicilik, doğrusallık, tespit limiti, miktar tayini limiti, doğruluk, gün içi ve günler-arası tekrarlanabilirlik, stabilite ve taşıma arasında valide edildi.*

Bulgular: *Kemometrik yöntemde, idrar analizinde 5-MeO-MiPT için en etkili parametreler numune hacmi, hidroliz sıcaklığı ve elüsyon hacmi bulundu. Bu parametreler için optimum değerler yüzey tepki metodolojisi ile hesaplandı ve sonuçlar sırasıyla 1 ml idrar hacmi, 30°C hidroliz sıcaklığı, 3,5 ml elüsyon hacmi olarak belirlendi. Optimize edilmiş yöntem seçicilik, doğrusallık (25-500ng/mL), belirtme alt limiti (5,63 ng/mL), tayin alt limiti 18,75 ng/mL), doğruluk (%43,01-%101,47), gün içi tekrarlanabilirlik (100 ng/ml derişimde 3 paralel, %RSD 4,43),günler-arası tekrarlanabilirlik (100 ng/ml derişimde 5 farklı gün 3 tekrarda, %CV 4,27), seyreltme tamlığı, taşınma etkisi ve kararlılık parametreleri incelenmiştir.*

Sonuç: *Kemometrik bir yaklaşımla, GC-MS ile 5-MeO-MiPT tespiti için hızlı, pratik ve doğru bir yöntem geliştirildi. 5-MeO-MiPT'nin GC-MS analizinde türevlendirilmeden çalışması, ön hazırlık süresini kısaltmış ve diğer analoglar için öncü olmuştur. Her gün eklenen triptaminlerin sentetik analogları gibi maddelerin analizinde bu tür klasik cihazlar/sistemler ve yeni yöntemler ile etkin bir yöntem sağlar.*

Anahtar Sözcükler: *5-MeO-MiPT, adli toksikoloji; Gaz Kromatografisi-Kütle Spektrometrisi, katı faz ekstraksiyonu, kemometri.*

INTRODUCTION

New psychoactive substances (NPS) involves synthetic chemicals, also have natural origin including plant or fungal source. These substances are used as recreational drugs by engendering a psychoactive response, and not classified as illegal substances (1). According to the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory report in February 2019, 119 countries and territories were monitored 900 different NPS (2). These substances belong to different chemical classes such as synthetic cannabinoids, cathinone derivatives, piperazines, tryptamines and phenethylamines (3).

Recently, interest in synthetic tryptamines has gained popularity due to its hallucinogenic properties (3). Tryptamines such as N,N-dimethyltryptamine (DMT), 5-methoxy-N,N dimethyltryptamine (5-MeO-DMT), and 4-phorphoryloxy-N,N-dimethyltryptamine (psilocybin) are naturally occurring substances with a classic psychedelic profile (4). The first synthetic analogues of tryptamines were sold on the drug market in the late 1990s and have more popular on the internet. Research on synthetic tryptamines has been described in the book “TIHKAL” that published by Shulgin (5). 5-MeO-MiPT, chemically associated with naturally occurring tryptamine 5-MeO-DMT, is a hallucinogenic and psychedelic drug (Figure-1). It is also an analogue of the synthetic tryptamine 5-methoxy-N, N-diisopropyltryptamine (5-MeO-DiPT) which known as “foxy” or “foxy methoxy”. Therefore, 5-MeO-MiPT is often also referred to as “moxy” (6).

International concerns about tryptamines have been growing in recent years; because of the large number of reports that have been described as new tryptamines intoxication and deaths (7). The lack of literature on the toxicological properties of the new tryptamine hallucinogens hinders the assessment of the actual potential hazards of these substances to public health and safety. According to the first information about NPS-related substance diversity, a member of tryptamines, 5-MeO-MiPT, had the second-highest amount confiscated after 5-F-ADB, a synthetic cannabinoid in Türkiye (8).

Precise and accurate analysis of these NPS in biological samples is a very important issue for the mentioned reasons. The analytical methods can generate masses of datasets even for a single sample. For a large number of samples, the amount of output data will increase tremendously and can become very time consuming for the expert. In addition, manual examination may provide false-positive results. Therefore, advanced chemometric methods are used to analyse large and complex data sets.

Moreover, chemometric methods provide accurate and meaningful results in a short time (9). The optimization of analytical procedures has been carried out by using different multivariate statistic techniques. Among the most used techniques in optimization is response surface methodology (RSM). The RSM includes mathematical and statistical strategies to investigate the experimental space of process variables, empirical modelling techniques used to determine the relationship between the response of the system and the independent variables acting on it (10).

The aim of this study was to develop an analytical method suitable for routine analysis in clinical and forensic toxicology for the detection of 5-MeO-MiPT in urine samples using Gas Chromatography Mass Spectrometry (GC-MS). And we aimed to chemometrically optimized and fully validated of this method.

MATERIALS and METHODS

Chemicals and Standards

5-MeO-MiPT (1.0 mg/mL) reference standard was purchased from CHIRON Chemical (Trondheim, Norway). Methanol, potassium hydroxide, acetone were purchased from Sigma Aldrich (Missouri, USA). Ethyl acetate, sodium monophosphate were purchased from Merck (Darmstadt, Germany). Urea, sodium chloride, potassium diphosphate, creatinine, N,O-bis(trimethylsilyl)trifluroacetamide (with 1% TMCS) were purchased from Sigma Aldrich (Missouri, USA).

Sample Preparation

Synthetic urine was prepared by adding 0.33 M urea, 0.007 M creatinine, 0.016 M potassium diphosphate, 0.004 M sodium monophosphate, and 0.12 M sodium chloride in ultrapure water (11). Briefly, urine samples with 5-MeO-MiPT standard addition (500 ng/mL) were hydrolysed with of 1 M potassium hydroxide solution. Samples were vortexed and incubated. 1 mL hydrolysed synthetic urine was extracted by using Oasis HLB solid-phase extraction cartridges (Waters Cooperation, USA).

Gas Chromatography-Mass Spectrometry Conditions

GC–MS analysis was performed using a Thermo Finnigan TRACE ISQ equipped with a HP- 5MS capillary column (0.25 mm × 0.25 mm × 30 m). The oven temperature was held at initial temperature of 50°C for 1.5 min and ramp to 300°C at a rate of 30°C/min. The temperature of the ion source, interface and injection port was set at 200°C. Helium was used as the carrier gas at a flow rate 1.0 mL/ min. 1 µL of the samples was injected in the splitless mode. The mass spectrometer was operated with a mass scan range of mass to charge ratio (m/z) 44, 86 (quantification), 160.

Chemometric Optimization with Using Plackett–Burman and Central Composite Design (CCD)

The Plackett–Burman design was used to study the effects of seven independent factors sample volume X1, hydrolysis solution volume X2, hydrolysis temperature X3, hydrolysis time X4, conditioning volume X5, washing volume X6 and elution volume X7. The experimental minimum and maximum levels of these variables are shown in (Table-1). Regression analysis was performed according to the field values obtained at the end of the experiments performed according to Table-2 and the most effective three factors were determined after these screening experiments.

Table-1. Screening analysis factors, minimum and maximum levels

	Factors	Units	Min (-1)	Max (+1)
X1	Sample Volume	mL	1	3
X2	Hydrolysis Solution Volume	mL	1	3
X3	Hydrolysis Temperature	°C	20	80
X4	Hydrolysis Time	min	5	60
X5	Conditioning Volume	mL	1	3
X6	Washing Volume	mL	1	3
X7	Elution Volume	mL	1	3

Table-2. Pattern of plackett–burman design.

Number of experiments	Sample volume	Hydrolysis Solution Volume	Hydrolysis Temperature	Hydrolysis Time	Conditioning Volume	Washing Volume	Elution Volume
1	-	-	-	-	-	-	-
2	+	-	-	+	-	+	+
3	+	+	-	-	+	-	+
4	+	+	+	-	-	+	-
5	-	+	+	+	-	-	+
6	+	-	+	+	+	-	-
7	-	+	-	+	+	+	-
8	-	-	+	-	+	+	+

After determining the three most effective factors, a central composite design was applicate. Accordingly, α value was found as $\alpha \rightarrow \sqrt{3} = 1.682$. Values of - α, -1, 0, +1, + α were determined for 3 factors. A quadratic polynomial model (Equation 1) was made to obtain the predicted response from dependent variables in determining the amount of 5-MeO-MiPT in urine samples.

$$y = b_0 + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i=1}^{4} b_i x_i + \sum_{ij=1(i \neq j)}^{6} b_{ij} x_i x_j$$

(Equation 1)

Method Validation

Selectivity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, intra-day and inter-day repeatability, stability and carry over parameters were used to validate the method. Selectivity was determined blank sample and all analytes searched for different samples were added to investigate whether they formed interference to distinguish the sought analytes from the sample containing other analytes. Linearity was determined by least-squares regression equations. Acceptable linearity was achieved when the coefficient of determination was at least 0.99 and the calibrators were quantified with precision and accuracy within ±20%. Sensitivity was evaluated by determining the LOD and LOQ of the assay. The LOD was defined as the lowest concentration for which the signal-to-noise ratio for all ions was at least 3 and it was evaluated in three replicates. Chromatography exhibited acceptable peak shape, qualifier ion ratios (within ±20% of the average ion ratios of all calibrators) and retention time (±2% of target). LOQ was defined as the lowest concentration that met LOD criteria and a signal-to-noise ratio of at least 10. The recovery range of 80-120% was within the acceptable range in biological samples. The accuracy of the study was determined with the recovery and relative standard deviation (RSD). The RSD for each concentration should not exceed 20%. Intra-day precision was performed at medium concentration (100 ng/mL) on three repetitions on the same day and expressed as mean coefficient of variation (CV %). Also inter-day precision was measured with medium concentration determined (100 ng/mL) on three repetitions on the 5 different days and expressed as mean (CV%). The stability of analytes can be affected by many variables, including storage conditions and method. Sample in a vial was kept under GC auto analyser at room temperature after 2, 4, 12, 24, 48 hours. Blank matrix samples were analysed immediately after a high concentration sample (1000 ng/ml) to evaluate carryover.

RESULTS

Chemometric Optimization Results

As a result of the Plackett–Burman screening design (Table-2), how much each factor affected the chromatographic area of 5-MeO-MiPT was calculated by regression analysis. Table-3 shows the regression coefficients of these seven factors.

Table-3. Regression Coefficients Obtained According to Plackett-Burman Screening Design.

Factors		Units	Regression Coefficients
X1	Sample Volume	mL	5537.969,875
X2	Hydrolysis Solution Volume	mL	1495.931,875
X3	Hydrolysis Temperature	°C	-1947.485,125
X4	Hydrolysis Time	min	-1223.340,875
X5	Conditioning Volume	mL	967.424,625
X6	Washing Volume	mL	-808.422,125
X7	Elution Volume	mL	3456.129,125

Table-4. Central composite design pattern for three variables at five levels.

Number of experiments	Sample volume	Hydrolysis Temperature	Elution Volume
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1,682	0	0
10	1,682	0	0
11	0	-1,682	0
12	0	1,682	0
13	0	0	-1,682
14	0	0	1,682
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table-5.ANOVA results for resolution obtained by CCD.

Factors	Regression coefficient	T-Values	P-Values
X1	274009	0,60	0,561
X2	-610564	-1,34	0,210
X3	-79408	-0,17	0,865
X1* X1	-324095	-0,73	0,481
X2* X2	772179	1,74	0,112
X3* X3	-107871	-0,24	0,813
X1* X2	588626	0,99	0,346
X1* X3	-526786	-0,89	0,397
X2* X3	-572019	-0,96	0,359

Table-6. Analytical recovery values for optimized method for 5-MeO-MiPT

	25 ng/mL		500 ng/mL	
	Found Value (ng/ml)	% Recovery	Found Value (ng/ml)	% Recovery
1. Parallel Extraction	18,24	72,98	487,08	97,42
2. Parallel Extraction	20,28	81,11	507,36	101,47
3. Parallel Extraction	18,06	72,23	486,35	97,27
Mean Value	12,71	75,44	493,59	98,72
SD	1,23	4,93	11,93	2,39
% RSD	6,53	6,53	2,42	2,42

Table-7.Intra-day and Inter-day repeatability values.

Values found for 100 mg/mL standard	1	2	3	Mean	SD	CV %
1st day	113,83	105,51	105,54	108,29	4,79	4,43
2nd day	115,69	102,74	114,6	111,01	7,18	6,47
3rd day	101,36	99,10	98,31	99,59	1,58	1,59
4th day	97,97	106,80	110,82	105,20	6,57	6,25
5th day	108,65	114,96	99,5	107,70	7,77	7,22

As a result of the screening experiments, it was found that the sample volume, hydrolysis temperature and elution volume parameters were the most effective factors. 5-level, 3-variable and a total of 20 experiments (in Table-4) were employed for CCD. Influences of significant variables and the model efficiency were checked by analysis of variance (ANOVA) analysis and p values (Table-5). Also, the estimated quadratic polynomial model showing the relationship between the obtained resolutions and the investigated variables was found as in Eq. (2):

$$y = 1571894 + 274009x_1 - 610564x_2 - 79408x_3 - 324095x_1^2 + 772179x_2^2 - 107871x_3^2 + 588626x_1x_2 - 526786x_1x_3 - 572019x_2x_3$$

(Equation 2)

From the Pareto chart was given in Figure-2, the effects of the coefficients in the obtained equation, standardized according to the central composite design. From the obtained regression model, the dual effects of sample volume, hydrolysis temperature and elution volume factors in central composite design, surface areas and contour graphs are shown in Figure-3 and Figure-5.

As a result of these chemometric studies, the developed analytical method for 5-MeO-MiPT was as follows: 1 mL of urine sample was hydrolysed with 1 mL of 1 M potassium hydroxide for 30 minutes at 30°C.. However, in the elution step, it was eluted with 3,5 mL acetone: methanol (70:30 v/v) mixture. The extracts were evaporated to dryness under nitrogen, dissolved in 100 µL ethyl acetate, transferred into GC-MS vial and injected into the device.

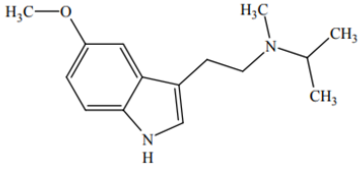

Figure-1. 5-MeO-MIPT molecular structure.

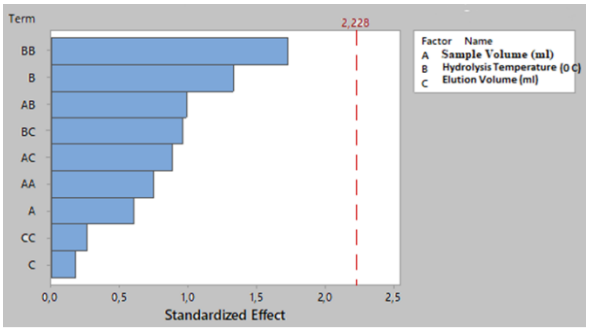

Figure-2. Pareto chart of standardized effects.

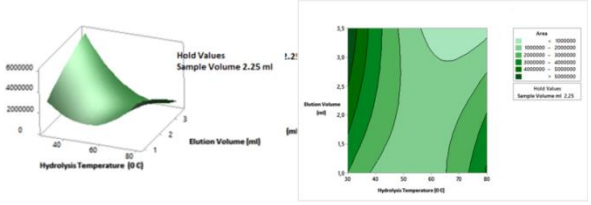

Figure-3.Dual Effect of Elution Volume and Hydrolysis Temperature (Left-Surface Plot, Right-Contour Plot).

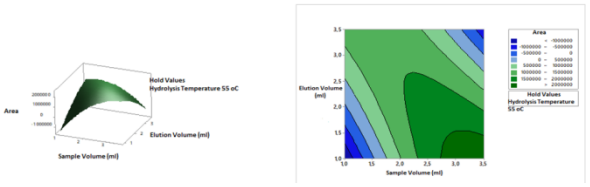

Figure-4. Dual Effect of Elution Volume and Sample Volume (Left-Surface Plot, Right- Contour Plot).

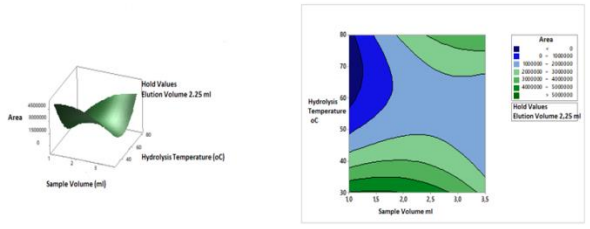

Figure-5. Dual Effect of Sample Volume and Hydrolysis Temperature (Left-Surface Plot, Right- Contour Plot).

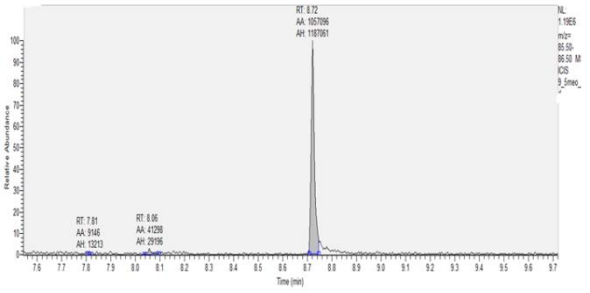

Figure-6. Chromatograms of 5-MeO-MiPT in urine.

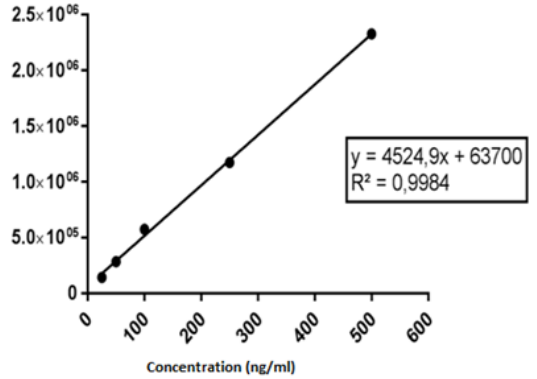

Figure-7. 5-point calibration curve, equation, and correlation coefficient of 5-MeO-MiPT.

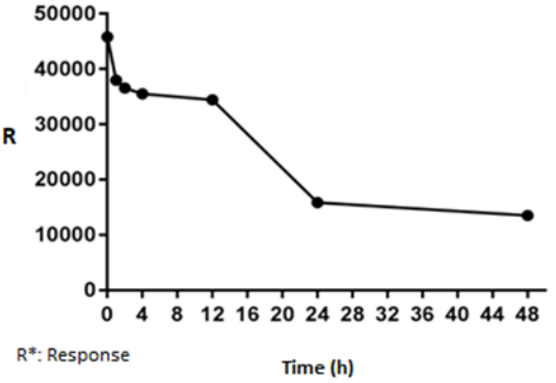

Figure-8. Stability results

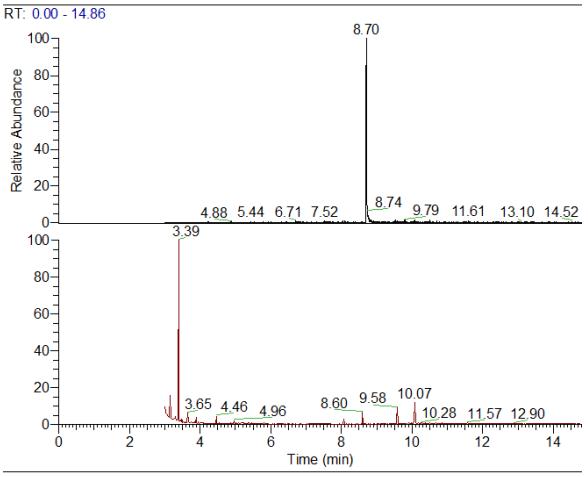

Figure-9. Chromatogram of high concentration sample followed by blank sample for carry-over effect

Validation Results

The developed method was validated for all analytes at different concentrations (linear between 25-500 ng/mL) in triplicate under the selective GC-MS conditions (Figure-6-9). The calibration curve was linear with a determination coefficient, R^2 = 0.9984 based on the measurement of the analyte peak areas obtained by GC-MS (Figure-7). The LOD and LOQ calculated 5 ng/mL and 18 ng/mL for the compound, respectively.

Accuracy was expressed in % recovery through standard addition method at two different levels as given in (Table-6). Responses found for method in a short time interval, on the three different day (100 ng/mL) and with the same instrument were within the desired ranges to assure analytical reliability. Inter-day repeatability was measured with medium concentration

determined (100 ng/mL) on three repetitions on the 5 different days and expressed as mean coefficient of variation (CV%4.27) values given in (Table-7).

For dilution integrity, sample of 1000 ng/mL was diluted 1:10, a result of 94 ng/ml was obtained. The result report can be given by multiplying this value by 10. Samples prepared as a result of the extraction process (250 ng/mL, n = 3) were injected after 0, 2, 4, 12, 24, 48 hours and a stability study were carried out. Stability results are shown in (Figure-8).

A sample with a high concentration (1000 ng/mL) was then evaluated by injecting a blank sample. It has been observed that there is no carry over effect.

DISCUSSION

NPS are synthetic substances that are rapidly being developed to evade the law, and the change of their structure poses challenges for public health and law enforcement authorities around the world. (12). Changes in the molecular structure of NPS create difficulties about identification of substance for the forensic specialists (13). Thus, time-effective techniques for the detection and control of NPS is necessary for anti-drug effort (14). In this presented study, a validated method was developed after chemometrically optimization for the detection of 5-MeO-MiPT in urine samples using GC-MS.

Regarding tryptamine derivatives, *in vitro* and *in vivo* metabolism studies (1, 15), hallucination and acute effect studies in mice and rats (16, 17), intoxication case reports (18, 19), real case urine, hair and blood sample studies (6, 20–24) are included in the literature. In the metabolism study was about 5-MeO-MiPT in 2017, the obtained blood and urine samples were analysed for in vivo metabolites of 5-MeO-MiPT using liquid chromatography–high resolution tandem mass spectrometry (1). 5-MeO-MiPT was found to be at a concentration of 3380 ng/mL in the urine sample. According to the results of this study, they recommended metabolites 5-hydroxy- N-methyl-N-isopropyltryptamine (5-OH-MiPT), 5-methoxy-N-isopropyltryptamine (5-MeO-NiPT), hydroxyl-5-methoxy-N-methyl-N-isopropyltryptamine (OH-5-MeO-MiPT) and 5-methoxy-N-methyl-N-isopropyltryptamine-N-oxide (5-MeO-MiPT-N-oxide) as biomarkers for the development of new methods for 5-MeO-MiPT consumption (15). In a study conducted by Meyer et al. in 2013, 37 tryptamine derivatives were analysed in full validated blood and urine samples, and a device with a combination of ultra-high performance liquid chromatography (UHPLC) and an ion trap mass spectrometer was used. In our study, we used GC-MS for the detection. Compared to liquid chromatography-mass spectrometry (LC-MS), GC-MS has standard spectral libraries, which makes it easier to characterize compounds by comparing sample spectra with those in standard libraries. Wilson et al. reported a GC-MS method used to quantify 5-MeO-DiPT in the serum and urine of drug abusers and simultaneously identified metabolites in urine (19). Based on the characteristics that GC-MS is easier to characterize and LC-MS has a wider dynamic range, some researchers combined them with qualitative and quantitative research. A screening approach that was used to analyse designer phenethylamines and tryptamines in blood and urine by LC-MS and GC-MS (21).

In contrast to many publications in our study, SPE method was used for this analyte. Much stronger elution was achieved with the acetone-methanol mixture used in the elution. Derivative agents are generally used in illicit substance analysis in GC systems. However, it is not necessary to use derivatives for this substance. Its molecular weight and volatility are suitable for Gas Chromatography. This contributed to the shorter analysis time.

In the chemometric approach, unlike classical optimization techniques, more effective results can be obtained with fewer experiments. This situation provides the chemometry cost and time effective. Thereby, the proposed method can be employed for routine analysis of these compounds in similar formulations. An experimental design such as CCD is a good alternative to study the effect of variables and their interactions on the resolution due to the small number of experiments required. A statistically based CCD was used for the development of a fast, accurate, efficient, precise and robust GC-MS method for the determination of 5-MeO-MiPT. After optimization, the method was validated. The RSD values were <5% which indicate high degree precision of the developed method. It has been determined because of the analysis that the method is selective, linear, sensitive and, but it was determined that the stability of the analyte changed within one hour.

CONCLUSION

Forensic toxicology laboratories are equipped with advanced techniques to effectively analyse substances nowadays. These analytical methods alone are not sufficient when complex results are obtained in many forensic cases. The chemometric methods give better resolution or separation quality of the samples by chromatographic techniques in recent times. With a chemometric approach, a quick, practical and accurate method has been developed with GC-MS. It provides an effective method in the analysis of substances such as synthetic analogues from tryptamines which are added every day, with the use of such classical equipment and current new methods.

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Declaration of Interest Statement

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