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Research Article

Development and validation of a GC-MS method for determination of amphetamine-type stimulants and ketamine in human hair

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ABSTRACT

A solid-phase extraction (SPE) followed by gas chromatography-mass spectrometry (GC-MS) method was developed and validated for determination of seven amphetamine-type stimulants (ATSs) including amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylendioxyethylamphetamine (MDEA), para-methoxyamphetamine (PMA), 4-bromo-2,5-dimethoxyamphetamine (DOB), and ketamine (KET), norketamine (NKT) in human hair. Ten milligrams of human hair was washed by water and methanol followed by soaking in a solution of 1% hydrochloric acid in methanol for 18 hours. The analytes from the methanolic extract were isolated by a SPE procedure before being derivatized using heptafluorobutyric anhydride (HFBA) at 80°C for 40 minutes. The selected ion monitoring (SIM) method was used for the quantification of the derivatized compounds. The linear range was from 0.5-40 ng/mg for all of the analytes with the coefficient of determination (R^2)>0.9971. The intra-day and inter-day accuracies were in the range of 93.63-112.40% and 94.70-110.20%, respectively. The intra-day and inter-day precision (RSD%) were in the range of 8.72 and 9.73, respectively. Limit of detection (LOD) and limit of quantitation (LOQ) for each analyte were less than 0.10 and 0.32 ng/mg, respectively. The recoveries were in the range of 75.18-89.30%. The GC-MS was used for the hair analysis on 51 subjects suspected to be ATSs and ketamine user. The average content of all the analytes was in the range of 1.14-12.70 ng/mg. On the basis of these results, the method was proved to be effective for identification and quantitation of above mentioned nine narcotics.

Keywords:
Amphetamine-type stimulants, Ketamine, GC-MS, Hair

1. INTRODUCTION

The amphetamine-type stimulants (ATSs) such as amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylendioxyethylamphetamine (MDEA), para-methoxyamphetamine (PMA), 4-bromo-2,5-dimethoxyamphetamine (DOB), and ketamine (KET), norketamine (NKT) are medicines that stimulate the central nervous system causing in hallucination[1,2]. These narcotics were handled in accordance with the regulation on the list of narcotic substances and precursors in Vietnam.

In recent years, ecstasy-type (MDMA) and methamphetamine (MA) have been widely used as a recreational drug among the youth[3]. The abuse of MA and MDMA has significantly increased since 1995s and becomes a serious matter in the society. Teenagers are the main ATSs users and their age was more and more declined[4]. Since 2000s KET has been abused and used more and more together with ATSs[5].

Drug testing provides objective evidence on the illegal use of drug by proper detection of the presence of drug and its metabolites in the body. Thus, it is necessary that a method for simultaneous, rapid, and reliable

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analysis at trace level of drug and its metabolites should be developed for the prevention of drug abuse and illicit trafficking and estimation in forensic medicine in Vietnam.

The suitable specimens for estimation of drug use are usually biological such as blood, urine, saliva, sweat or hair, nail. Usually for drug testing, urine and blood specimens are commonly used due to their relevance to absorption and metabolism of drugs in the body. However, in recent years, the analysis of hair for determination of drug has been significantly interested due to its advances against the routine urine or blood testing such as its easy and non-invasive collection[6]. The incorporation of drug into hair is carried out by a number of metabolism at different time in developing cycle of hair including passive diffusion from blood-stream into growing hair cell, from sweat, sebaceous gland, secretory gland and external environment[7-8].

The drug excreted into hair has moved along hair shaft at the rate of 2.8-3.2 mm/week, according to hair growth, without diffusion[9]. ATSs can be accumulated in hair for 3-5 months[10]. There have been many reports on analysis of ATSs, ketamine and opiate in hair[11-12]. These studies were applied LC-MS/MS[11], CE with UV detector[13], GC-MS[14-16]. However, most reports described only the analysis of one or some of the above-mentioned drug. Thus, the aim of this study is to develop a sensitive GC-MS method for simultaneous determination of narcotics which are commonly abused in Vietnam including 7 ATSs and ketamine together with its metabolite of norketamine. The proposed method is applicable for analysis of ATSs and ketamine in hair specimen of drug abuse subjects in Vietnam.

2. MATERIALS AND METHODS

2.1. Chemicals, reagents and test Specimens

AM, MA, PMA, MDA, MDMA, MDEA, DOB, KET, and NKT reference standards and MA-d5 and KET-d4 internal standards were purchased from Cerilliant (Round Rock, USA) at the solution of 1.0 mg/mL in methanol. Figure 1 presents the chemical structure of the studied analytes.

LC-grade methanol and ethyl acetate, analytical-grade dipotassium hydrogen phosphate, potassium dihydrogen phosphate, and ammonia were provided from Merck (Damstadt, Germany). Heptafluorobutyric anhydride (HFBA) was of analytical grade and purchased from Sigma-Aldrich (Missouri, USA).

SPE AccuBond II Evidex (200 mg, 3 mL) and Bond elut empty SPE cartridge 3 mL were obtained from Agilent Technologies (Santa Clara, California, USA).

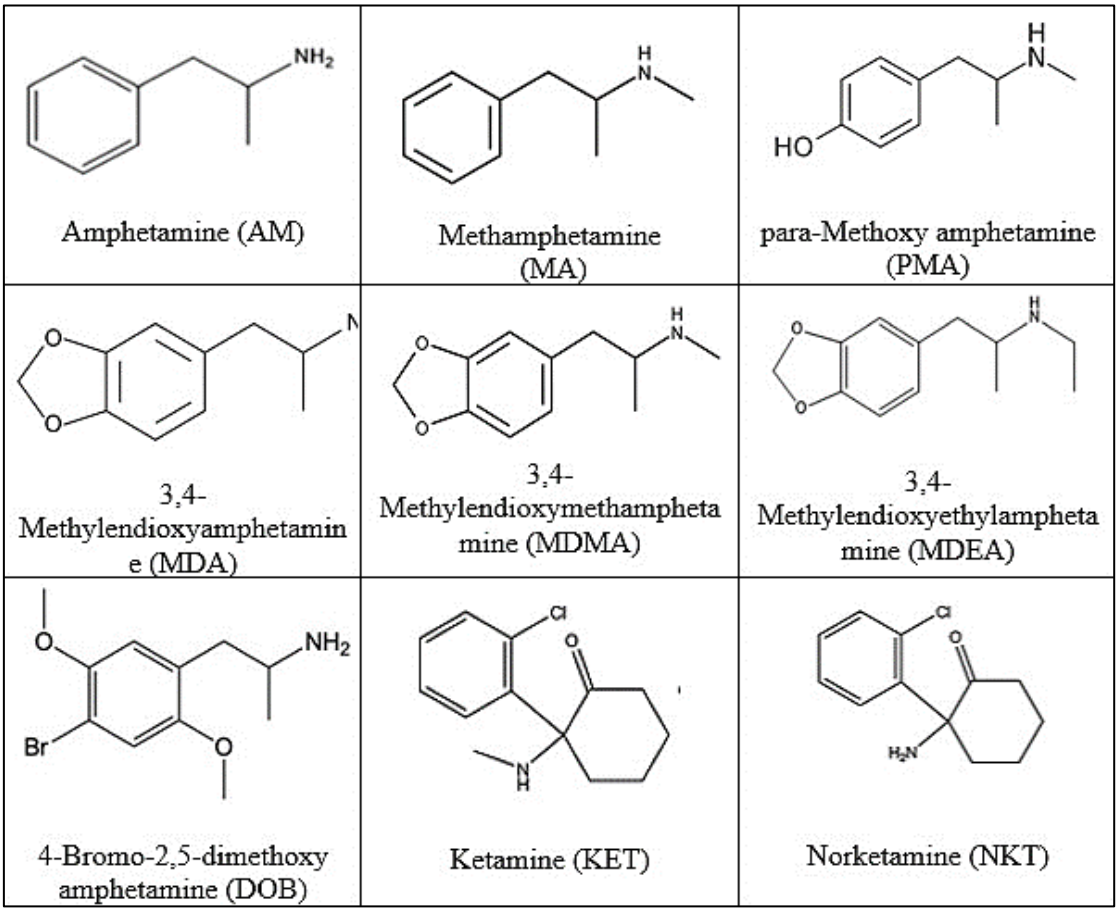

Figure 1. Chemical structure of the studied compounds.

Human hair specimens which are free from ATS and ketamine obtained from the staffs of Forensic Chemistry Department, National Institute of Forensic Medicine (Hanoi, Vietnam) were used for method development and validation.

Fifty one human hair specimens obtained from ATMs or ketamine users who are inpatients in Hanoi Psychiatric hospital were utilized for analysis of the relevant compounds.

2.2. Preparation of standard solution

The standard solutions of AM, MA PMA, MDA, MDMA, MDEA, DOB, KET, and NKT at the concentration of 1.0 mg/mL in methanol were diluted to obtain working standard solutions of 0.1 µg/mL, 1.0 µg/mL and 10.0 µg/mL in methanol. They were stored at 0°C and protected from light.

2.3. Preparation of sample

About 10 mg of hair was weighed accurately into each Empty SPE cartridge. The hair was washed orderly with distilled water for three times, each time using 5 mL then with methanol for another three times, each time using 5 mL. The hair was cut to obtain section of about 1 mm, add 100 µL of 1.0 µg/mL internal standard mixture then add 2 mL of a solution of 1% hydrochloric acid in methanol (v/v) and incubate for about 18 hours at room temperature. The supernatant was transferred into another tube and dried using nitrogen stream[17]. The residue was dissolved in 3 mL of 0.1 M phosphate buffer solution pH 6 and purified by SPE. The cartridge was activated by methanol, following by 0.1 M phosphate buffer solution pH 6, 3 mL each at the speed of 2 mL/min. The sample was loaded onto the column at the speed of 2 mL/min. The sample was washed with 10 mL of distilled water, following by 5 mL of 0.1 N hydrochloric acid solution at the speed of 5 mL/min. The sample was continued to be washed by 5 mL of methanol at the speed of 2 mL/min. The cartridge was exhausted for 1 minute. The sample was eluted by a mixture of ethyl acetate, methanol, and ammonia (80:18:2, v/v/v) at the speed of 2 mL/min. The eluate was transferred into a glass tube with cap, added with 50 µL of hydrochloric acid solution in methanol (1:99, v/v) and dried to residue using nitrogen stream[18]. The analytes were derivatized by adding of 100 µL of ethyl acetate and 100 µL of HFBA, following by incubation at 80°C for 40 minutes. The reaction mixture was evaporated to dryness under a stream of nitrogen and reconstituted with 100 µL of ethylacetate. An aliquot (1 µL) of the sample solution was injected into the GC-MS instrument.

2.4. GC-MS conditions

GC-MS was performed with a Agilent Technologies 7890B gas chromatography system coupled with a MS 5977A mass spectrometer, the mass spectrometer was operated at 70 eV in the electron impact (EI) mode. Compound was separated on a 30 m×0.25 mm i.d. capillary column coated with 0.25 µm film of 5% phenyl methylpolysiloxane (HP-5MS). Helium was used as carrier gas of 1.0 mL/min flow rate. The analysis was carried out in the splitless mode. The initial column oven temperature was at 80°C, held for 3 minutes, then programmed at 40°C/min to 200°C and held for 1 minute, finally programmed at 10°C/min to 290°C and held for 10 minutes. The ionization source temperature was 230°C. The Aux-temperature was 280°C. The ionization energy was 70 eV. The analytes were determined by injection of standard solutions and internal standard solutions. The data output was achieved using scanning (SCAN) mode for identification and selected ion monitoring (SIM) mode for quantification using characteristic ions.

2.5. Method validation

The method validation was referred to the guidance of United Nations Office on Drugs and Crime; Guidelines for Testing Drugs under International Control in Hair, Sweat and Oral Fluid.

The calibration curves were built on the basis of matrix to avoid matrix effect. The calibration curve of each analyte was built in the range of 0.5-40.0 ng/mg. Six calibration points were built by adding standard mixture into 10 mg of blank hair at the concentrations of 0.5, 1.0, 5.0, 10.0, 20.0, and 40.0 ng/mg, respectively. The concentration of internal standard was 100.0 ng/mg. Plot the peak response ratios of standard to internal standard versus concentration of analytes in standard solutions in matrix, and obtain a regression line for calibration.

Accuracy and precision of the method were evaluated by analysis of hair samples added standards at the concentration of 1.0, 10.0, and 40.0 ng/mg hair. Estimation of accuracy and intra-day precision by performing five repeated analysis for each concentration. Estimation of accuracy and inter-day precision by performing three repeated analysis for each concentration for five consecutive days. The concentration of analytes in the specimens was determined by using calibration curve. Accuracy is the ratio of concentration determined from the calibration curve to theoretical concentration. Precision is evaluated on the basis of relative standard deviation (RSD) of the analysis outcomes.

Limits of detection (LOD) and limits of quantitation (LOQ) were calculated on the basis of signal to noise ratio (S/N). Analysis of standard adding samples at low concentration provided that signal of the analyte emerged. S/N was calculated by the software of the GC-MS instrument. A signal-to-noise ratio between 3 is generally considered acceptable for estimating the LOD whereas for LOQ, a typical signal-to-noise ratio is 10:1.

Analysis recovery was estimated by comparison of peak response of analytes and one of standards without extraction, and preparation with the same amount. The concentration of 1.0, 10.0, and 40.0 ng/mg were investigated. Each sample was performed for five times.

3. RESULTS

3.1. Selection of derivative agents

Heptafluorobutyric anhydride (HFBA), pentafluoropropionic anhydride (PFPA), and trifluoroacetic anhydride (TFAA) were used as derivative agents for the analytes at the appropriate temperatures. The main characteristic of the derivatizing process is to form a new compound which is larger in molecular weight with various polarity and volatility. This can improve the peak shape and tailing factor. Depending on chemical structure of each analyte, after ionization of the derivatized analytes, different molecular ions were obtained. Thus, some ions were used to confirm the analyte and internal standard. The characteristic ions of each analyte were used as quantified ion and identified ion.

The obtained results after derivatization showed that most of the peaks of interests had high enough signal for qualitative and quantitative analysis. However, the peak intensity of KET derivatives with PFPA and TFAA was not satisfactory enough. HFBA was the best derivative agent, allowing to achieve a high sensitivity analysis.

The selection of reference ion was performed after scanning all analytes to obtain mass spectrum. The selected ions were the ones with high mass and intensity. The target ions were the ones for identification with stable and high intensity. The retention time of derivatized analytes and the target ions for qualitative and quantitative analysis were presented in Table 1. Figure 2 is the GC-MS (SIM mode) chromatograms and quantitative ions of analytes. The different analytes were to form different characteristic ions. The retention time of the analytes fall within the range of 6.4-10.4 minutes. This range was suitable for analysis of the analytes as well as simultaneous determination of ATSs and ketamine in a single chromatographic run.

3.2. Optimization of derivatization process

The optimal conditions were determined by evaluating the ramping rate and start temperature as well as a reasonable run time. The derivatization temperature at 60°C, 70°C, 80°C, 90°C, and 100°C as well as the derivatization time 20, 30, 40, and 50 minutes, were investigated. At the temperature of 60°C and 70°C, the derivatization of all the analytes were not complete. At the temperature of 90°C and 100°C, the chromatograms were not good with noise in the range of retention time for KET and DOB. At the temperature of 80°C, the peak responses were highest, the peak shapes were symmetrical, and the chromatograms were less noise. Data in Figure 3 indicated that at the time of 20 and 30 minutes, the derivatization of all the analytes were not complete, and the peak responses were highest at the time of 40 and 50 minutes. As the results, the optimal time for derivatization was 40 minutes and the best derivatization temperature was 80°C.

3.3. Method validation

The method was validated comprising the following parameters: specificity, linearity range, LOD, LOQ, recovery, accuracy and precision.

Comparison of the SIM chromatograms of test solution, blank solution, and hair specimen sample solutions spiked standards and internal standards showed that the retention time of ATSs, KET, and NKT in the chromatograms of spiked samples were coincident. In the chromatogram of blank solution, no emerged peaks with the retention time that interfere with those of ATSs,

Table 1. Retention time and characteristic ions of analytes and internal standards.

Analyte derivatized with HFBA	Retention time (min)	Molecular ion (m/z)	Quantitative ion (m/z)	Qualitative ion (m/z)	
AM-HFB	6.42	331	240	118	91
MA-HFB	6.90	345	254	118	91
PMA-HFB	7.46	316	148	168	240
MDA-HFB	8.01	375	135	162	77
MDMA-HFB	8.74	389	254	162	210
MDEA-HFB	8.99	403	268	176	135
DOB-HFB	9.95	470	231	258	260
KET-HFB	10.37	434	370	362	236
NKT-HFB	9.21	420	384	340	314
MA-d5-HFB	6.88	350	258	213	-
KET-d4-HFB	10.36	438	374	366	-

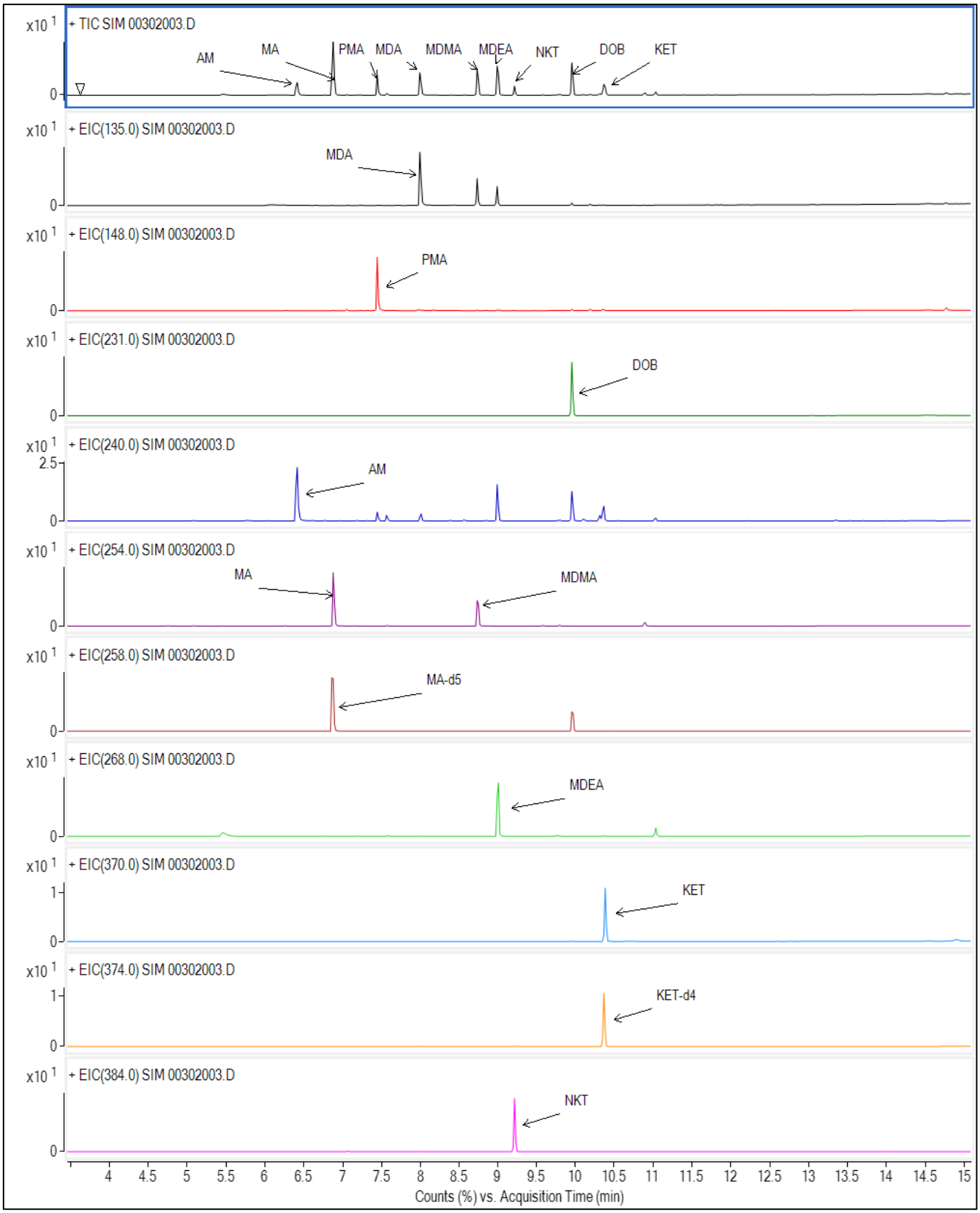

Figure 2. GC-MS (SIM mode) chromatograms and quantitative ions of analytes.

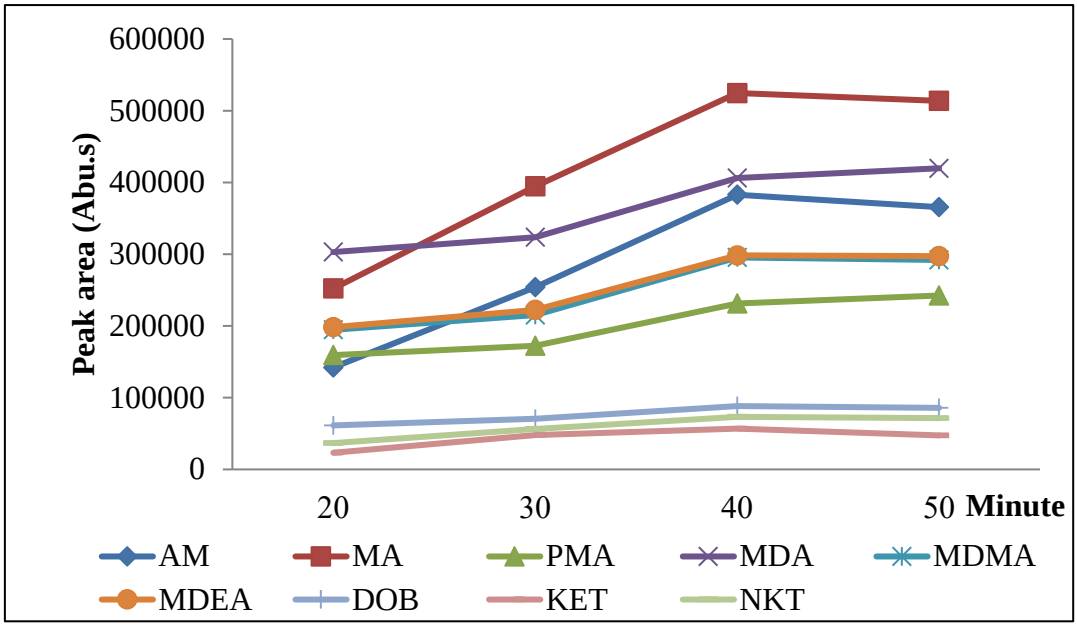

Figure 3. Comparison of time for derivatization of analytes with HFBA.

Table 2. Linearity, limit of detection (LOD), and limit of quantitation (LOQ).

Analyte	Linearity range (ng/mg)	Regression equation	Coefficient of determination (R^2)	LOD (ng/mg)	LOQ (ng/mg)
AM	0.5 - 40.0	y = 0.0058x - 0.0011	0.9990	0.07	0.23
MA	0.5 - 40.0	y = 0.0114x - 0.0350	0.9981	0.04	0.12
PMA	0.5 - 40.0	y = 0.0044x - 0.0189	0.9991	0.05	0.17
MDA	0.5 - 40.0	y= 0.0080x - 0.0227	0.9993	0.10	0.32
MDMA	0.5 - 40.0	y = 0.0045x - 0.0036	0.9971	0.05	0.18
MDEA	0.5 - 40.0	y = 0.0048x + 0.0057	0.9987	0.02	0.07
DOB	0.5 - 40.0	y = 0.0015x - 0.0007	0.9985	0.06	0.21
KET	0.5 - 40.0	y = 0.0105x - 0.0240	0.9982	0.03	0.09
NKT	0.5 - 40.0	y = 0.0183x + 0.0169	0.9990	0.05	0.18

KET and NKT. Thus, the method was proved to be specific for ATSs and KET.

Table 2 presents the linearity range, data of LOD and LOQ. The calibration curve was built with 6 points for 9 compounds. Each concentration was injected in triplicate. The coefficient of determination (R^2) was more than 0.9971 for all the analytes indicated a good linearity. The sensitivity of the method was evaluated by determination of LOD and LOQ of each analyte. LOD and LOQ fall within the range of 0.02-0.10 ng/mg and 0.07-0.32 ng/mg, respectively.

Table 3 presents the recovery of the analyte in hair. The recovery of ATSs and KET at low, medium, and high concentration levels were within the range of 75.18-89.30% with RSD below 15%.

Table 4 presents the validation data of accuracy and precision. The intra- and inter-day accuracy were within the range of 93.63% to 112.4%, and 94.70% to 110.20%, respectively. The intra- and inter-day precision (RSD%) were 8.72 and 9.73%, respectively.

The validation results proved that the GC-MS method can be applied for analysis of the analytes in real hair specimen samples.

3.4. Application of the validated method for analysis of hair specimen of suspected drug users

In order to prove the applicability of the method for analysis of real specimen, this method was used to analyze hair specimens collected from suspected drug users. Total of 51 hair specimens which had been collected for 3 months, were analyzed and quantified. Figure 4 is the representative chromatogram of hair specimen collected from one MA user. Table 5 presents the positive results with ATSs and ketamine. MA was detected in 49 hair specimens. There were 34 positive results for MDMA whereas there were only 12 positive results for KET. There were only one positive result for MDEA which occupies the lowest positive rate among the analytes in hair. The application of the proposed method allows identification and simultaneous quantitation of ATSs and ketamine in hair specimen. The success on method development for determination of trace level of ATSs and ketamine in hair is a great contribution in the struggle for prevention of drug use in Vietnam.

Table 3. Recoveries of analytes in hair.

Analyte	Concentration level (ng/mg)	Recovery (%)	RSD (%)
AM	1.0	77.98	8.05
	10.0	78.57	4.84
	40.0	80.09	7.84
MA	1.0	87.15	3.73
	10.0	84.39	5.48
	40.0	85.74	4.07
PMA	1.0	85.20	4.31
	10.0	85.04	4.84
	40.0	82.70	6.42
MDA	1.0	87.51	8.75
	10.0	84.99	5.32
	40.0	85.99	9.61
MDMA	1.0	87.36	5.66
	10.0	88.23	3.24
	40.0	83.57	4.26
MDEA	1.0	78.16	7.77
	10.0	80.50	4.00
	40.0	83.33	7.81
DOB	1.0	85.64	4.37
	10.0	82.58	7.16
	40.0	83.49	3.32
KET	1.0	75.18	8.16
	10.0	77.75	7.63
	40.0	79.24	7.02
NKT	1.0	87.24	4.59
	10.0	89.30	4.25
	40.0	85.07	4.58

Table 4. Validation data of accuracy and precision.

Analyte	Concentration level (ng/mg)	Intra-day			Inter-day		
		Found concentration (ng/mg)	Accuracy (%)	RSD (%)	Found concentration (ng/mg)	Accuracy (%)	RSD (%)
AM	1.0	1.11	110.80	4.84	1.10	110.20	4.94
	10.0	10.36	103.59	5.00	10.07	100.72	9.73
	40.0	38.84	97.11	4.11	38.73	96.82	4.07
MA	1.0	1.07	107.40	8.29	1.05	104.50	6.47
	10.0	9.87	98.68	7.29	9.96	99.61	7.93
	40.0	39.78	99.45	5.67	39.57	98.94	5.74
PMA	1.0	1.14	113.70	7.05	1.12	112.20	6.86
	10.0	10.67	106.70	4.25	10.27	102.68	3.82
	40.0	40.70	101.75	4.24	39.49	98.72	7.17
MDA	1.0	1.06	105.80	8.16	1.01	101.20	7.22
	10.0	10.18	101.81	4.47	10.08	100.76	3.00
	40.0	40.14	100.36	3.93	39.56	98.90	4.63
MDMA	1.0	1.09	108.70	6.16	1.05	105.00	5.97
	10.0	9.76	97.64	7.09	9.59	95.93	4.32
	40.0	38.97	97.44	4.33	38.30	95.75	3.59
MDEA	1.0	0.97	97.10	4.33	0.98	98.50	5.42
	10.0	9.43	94.33	3.88	9.74	97.38	2.57
	40.0	37.45	93.63	3.05	37.88	94.70	5.68
DOB	1.0	1.01	101.40	1.41	0.95	94.80	8.21
	10.0	10.33	103.29	7.50	10.36	103.63	7.94
	40.0	39.45	98.64	6.19	39.68	99.19	6.50
KET	1.0	1.12	112.40	8.72	1.06	105.70	5.55
	10.0	9.94	99.35	6.09	9.58	95.76	4.95
	40.0	37.78	94.45	3.59	38.00	95.01	4.24
NKT	1.0	1.03	103.10	4.32	1.01	101.10	4.93
	10.0	9.99	99.86	5.18	10.17	101.68	7.28
	40.0	39.60	99.01	6.37	39.63	99.08	6.35

Table 5. Concentration of ATSs and ketamine in positive specimens.

Analyte	Number of positive specimen	Concentration (ng/mg)	Average concentration (ng/mg)
AM	38	0.23 - 16.57	2.60
MA	49	0.53 -108.00	12.70
PMA	9	0.51 - 14.40	2.57
MDA	20	0.33 - 47.93	3.47
MDMA	34	0.19 - 91.26	10.75
MDEA	1	0.29	-
DOB	5	0.61 - 5.68	1.14
KET	12	0.15 - 10.13	2.53
NKT	3	3.94 - 11.73	7.07

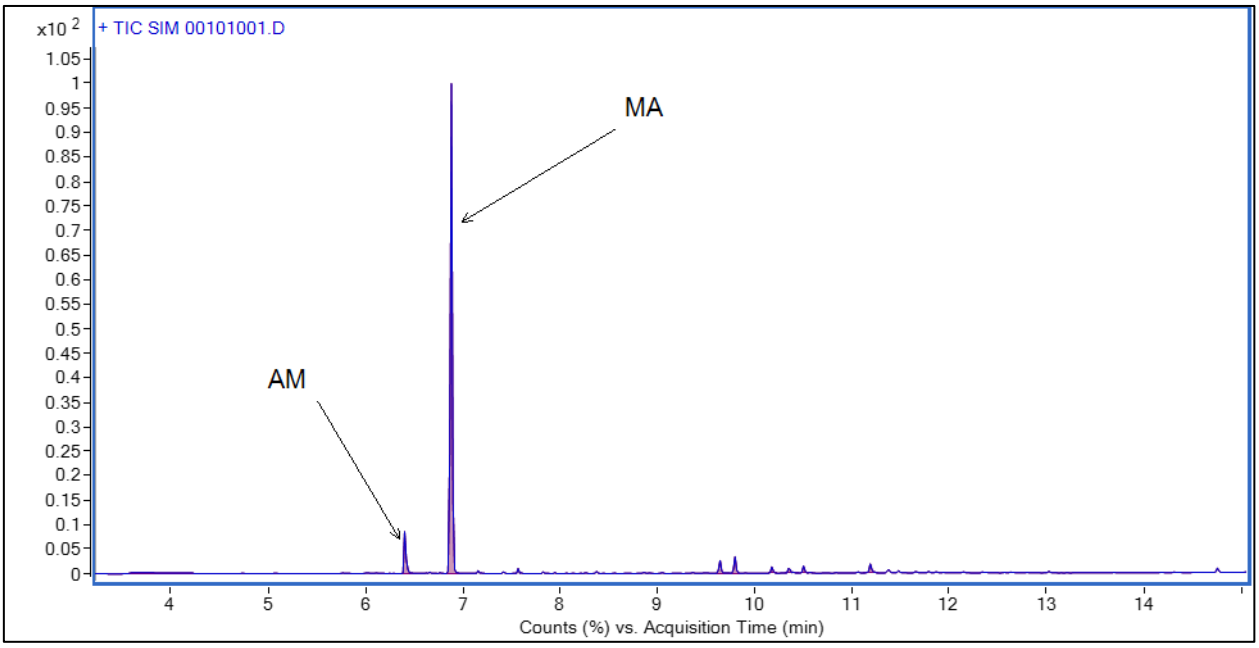

Figure 4. Chromatogram of human hair specimen containing AM and MA.

4. DISCUSSION

The analysis of narcotics in biological specimen has a great significance in identification of drug user. Blood and urine are the specimens of priority choice. However, human hair is more and more commonly used for drug testing due to its ease of collection, sampling technique is simple, storage and moving conditions are not special[8]. Hair can be used to elucidate the long intake history of narcotics compared with biological fluid[19].

The content of narcotics in hair is usually very low. Thus, it is necessary to develop an appropriate analytical procedure for analysis at trace level. The SPE-GC-MS procedure was applied for determination of narcotics including AM, MA, MDA, MDMA, MDEA, PMA, DOB, KET, and NKT due to its simplicity, practicability, and sensitivity. However, without derivatization, although KET and NKT were easily detected but ATSs were difficult to be detected. The derivatization converts AM, MA, MDA, MDMA, MDEA, PMA, DOB, KET, and NKT into more volatilized and stabilized compounds. The derivative agents may decrease the chemical polarity by replacing active hydrogen with an alkyl group or acyl of ester, ether, and alkyl amine or acyl halogenid[20]. The appropriate derivative reagents for AM, MA, MDA, MDMA, MDEA, PMA, DOB, KET, and NKT are acylation reagent. For instance TFAA[21], PFPA[22], pentafluorobenzoyl chloride (PFBC)[23], N-methyl-bis(trifluoroacetamide) (MBTFA)[24], HFBA[25], and silylation reagent such as N-methyl-N-t-butyldimethylsilyl trifluoroacetamide (MTBSTFA)[26]. Most of the above derivatizing reagents react well with ATSs, except ketamine. HFBA reacts well with all the analytes with derivatizing time of 40 min at 80°C. Thus. HFBA was used in this study as derivatizing reagent for all the analytes of interest.

The analytical procedure described the soaking of hair in a solution of 1% hydrochloric acid solution in methanol. This process can extract the analytes in a salt form. Thus, it has little effect on the degradation and volatility of the analytes. Solid phase extraction was performed on Evidex cartridge which is a combination of a reversed phase C18 and a cation exchanger. This is suitable for extraction of narcotics in biological fluids. Samples were cleaner after the SPE procedure. There-

fore, effect of impurity and baseline noise can be minimized.

The GC-MS method for analysis of toxics requires a high sensitivity and precision. The results of system suitability parameters such as retention time and peak area with RSD of less than 2.0% indicated the precision of the instrument that can be used for routine analysis. The method shows its high specificity with the good peak shape for all the analytes. Method validation revealed the intra-day and inter-day accuracy of within 93.63%-112.40% and 94.70%-110.20%, respectively. The recovery were within 75.18-89.30%. The LOD and LOQ for each of analytes were less than 0.10 and 0.32 ng/mg, respectively. The method is suitable for analysis of trace amount of AM, MA, MDA, MDMA, MDEA, PMA, DOB, KET, and NKT in hair.

The method was applied for analysis of 51 real specimens. 49 out of 51 specimens were positive with MA within the range of 0.53-108.00 ng/mg whereas 38 out of 51 specimens were positive with AM within the range of 0.23-16.57 ng/mg. 34 out of 51 specimens were positive with MDMA within the range of 0.19-91.26 ng/mg. PMA was detected in 9 specimens whereas DOB was detected in 5 specimens. Only 1 specimen was positive with MDEA implying that this compound keeps the lowest rate on positive case among the investigated compounds. The number of cases using MA and MDMA were the highest with the average MA and MDMA concentration of 12.70 ng/mg and 10.75 ng/mg, respectively. KET was detected in 12 specimens within the range of 0.15-10.13 ng/mg. All the specimens were concomitantly positive with KET and ATSs indicated that the patients had either used both ATSs and KET or mixture drug containing ATSs and KET.

The detection of ATSs and ketamine in human hair suggests that hair may be the optimal specimen for analysis of narcotic in cases where biological fluids such as blood and urine cannot be obtained.

5. CONCLUSION

The proposed gas chromatography-mass spectrometric method for the determination of ATSs and ketamine was validated. The hair specimen was soaked in 1% hydrochloride acid soluiton in methanol, purified by SPE, and derivatized by HFBA for qualitative and quantitative analysis. The method was applied for analysis of ATSs and ketamine containing in hair of 51 Vietnamese people suspected to be synthetic narcotic users. The results showed that the method was fit for studying on determination ATSs and ketamine in human hair.

6. ACKNOWLEDGEMENT

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Conflict of interest
None to declare.

Funding
None to declare.

Ethics approval
None to declare.

Author contribution
TVH developed the idea of the study. TVH, PQC performed the analysis. HMH, PTTH, NDN, NDT check the results. NDT, HMH drafted the manuscript, revised the manuscript. All authors were responsible for the final content.

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