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Article

Simultaneous Quantification of Cocaine, Amphetamines, Opiates and Cannabinoids in Vitreous Humor

Mariana Dadalto Peres[1], Fabrício Souza Pelição[1,2], Bruno Caleffi[3] and Bruno Spinosa De Martinis[3]*

[1]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14050-140, Brazil, [2]Departamento Médico Legal de Vitória, Serviço de Laboratório Médico Legal, Vitória, ES 29045-402, Brazil and [3]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14050-140, Brazil

*Author to whom correspondence should be addressed. Email: martinis@usp.br

A GC–MS method for simultaneous analysis of cocaine (COC), amphetamines (AMPs), opiates, cannabinoids and their metabolites in vitreous humor (VH) was developed and fully validated. VH samples were extracted using solid phase extraction and injected into the GC–MS, using a selected ion monitoring mode. Linearity ranged from 10 to 1000 ng/mL; the exception was anhydroecgonine methyl ester (AEME), for which linearity ranged from 10 to 750 ng/mL. Inter-assay imprecision lay from 1.2 to 10.0%, intra-assay imprecision was <10.4% for all the analytes and accuracy ranged from 95.6 to 104.0%. An limit of quantitation for all drugs was 10 ng/mL and recoveries ranged from 70.4 to 100.1% for basic and neutral compounds; the acid compounds had poor recovery—<40%. The validated method was applied to 10 VH samples taken from individuals whose blood had screened positive for drugs of abuse. All the individuals screened positive for COC in the blood (seven samples) also had positive results in VH; COC concentration ranged from 30.81 to 283.97 ng/mL (mean 186.98 ng/mL) and benzoylecgonine concentration ranged from 11.47 to 460.98 ng/mL (mean 133.91 ng/mL). It was also noticed that, in five cases, cocaethylene was detected. AEME was also quantified in one case. The use of AMP detected by blood analysis was confirmed in the VH of one individual (24.31 ng/mL). However, samples taken from three individuals whose blood tested positive for carboxy-tetrahydrocannabinol presented negative results. The results demonstrated that VH is a suitable alternative biological sample to determine COC, AMPs, opiates and their metabolites.

Introduction

Toxicological analyses are essential in forensic medicine: they clarify the causes of death and help establish whether they are related to overdoses, addictions, accidents or injuries.

Vitreous humor (VH) is the aqueous gel located in the posterior cavity of the eye between the lens and retina (1). VH is a clean fluid that contains less protein than urine, which can be readily collected by direct suction during the autopsy. This fluid consists of a sterile saline solution with low concentration of proteins; therefore, it is a relatively stable environment for drugs and their metabolites during the postmortem interval (2). Given its characteristics, VH is an useful alternative postmortem matrix that can be used to detect drugs and alcohol, particularly during investigations of death, where postmortem blood is not available or is limited in terms of quality or quantity (e.g. after hemorrhagic shock, burns, embalming or decomposition processes) (3).

The analysis of electrolytes, glucose and nitrogen compounds in VH is well established, and VH is routinely employed to test ethanol. More recently, it has been proposed as a matrix to detect drugs of abuse. A wide variety of substances, including opiates, can be determined in this biological fluid. Indeed, VH can be a useful sample, and in some cases, it is the best or the only available sample for forensic toxicological analysis.

Drugs and their metabolites enter the VH by passive diffusion from the blood across the blood-vitreous barrier, which is made up of ciliary epithelium, retinal pigment epithelium and blood vessels of the retina. Analyte concentrations in the VH are often similar to their concentrations in circulating blood (4). Nevertheless, highly protein-bound drugs, such as 9-tetrahydrocannabinol (THC), achieve lower VH concentrations, because only the free fraction can cross the blood-vitreous barrier (5).

The lower protein content, easy accessibility, low contamination and high stability of VH are the main advantages of this matrix over other biological samples (6). VH is located in an isolated compartment, and the osseous structure around the eyeball protects VH from putrefaction (7). Therefore, this matrix can provide useful information in those cases, where other biological samples have suffered extensive chemical changes during the postmortem interval (8). However, the downside of VH is related with its small volume: ~2 mL of VH can be collected from each eye, which can limit the number of assays. Moreover, the database for interpretation of the analytical results is limited (3).

Recently, the interest in alternative samples, especially the VH, to determine drugs has increased. Some reports on the analyses of drugs of abuse in this matrix exist in the literature—cocaine (COC) is the most studied drug (9–14), followed by opiates (7, 9, 11, 14–17). The interest in opiates stems from the fact that 6-acetylmorphine is stable in VH and can confirm heroin abuse. Other drugs that were studied in VH include cannabinoids (18), phencyclidine (18) and benzodiazepines (19).

This study aimed to optimize and validate a GC–MS method that can be applied to the analysis of COC, amphetamines (AMPs), opiates and cannabinoids in VH. To the best of our knowledge, no comprehensive method has been fully validated for the simultaneous analysis of all these compounds in VH.

Methods

Reagents and solutions

The illicit drugs and metabolites studied were the following: AMP; methamphetamine (MAMP); 3,4-methylenedioxyamphetamine (MDA); *N*-methyl-3,4-methylenedioxymethamphetamine (MDMA); *N*-ethyl-3,4-methylenedioxyethylamphetamine (MDEA); cocaine (COC); cocaethylene (COE); benzoylecgonine (BE); AEME; morphine (MOR); codeine (COD); dihydrocodeine

(DIHYD); 6-acetylmorphine (6-AM); methadone (MET); THC; 11-nor-9-carboxy-9-tetrahydrocannabinol (THCCOOH), AMP-d_{11}, MAMP-d_{14}, MDMA-d_5, COC-d_3, BE-d_3, MOR-d_3 and THCCOOH-d_3. All these drugs were purchased from Cerilliant (Round Rock, TX, USA) as solutions in acetonitrile or methanol at a concentration of 1 mg/mL or 100 μg/mL. The silylating *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) reagent was acquired from Sigma-Aldrich (Milwaukee, WI, USA).

Methanol, acetonitrile, acetone, chloroform and dichloromethane were obtained from JT Baker (USA); isopropanol and ammonium hydroxide were provided by Mallinkrodt (USA) and ethyl acetate was supplied from Sigma-Aldrich (USA). All the solvents and reagents were reagent grade or higher. Sodium bicarbonate and anhydrous sodium carbonate were purchased from Synth (Diadema, Brazil).

Working standard solutions (1.0 and 10.0 μg/mL) of each compound were prepared by appropriate dilution with methanol or acetonitrile. Internal standards were prepared in methanol, to give a working standard solution of 10.0 μg/mL. All these solutions were stored at −20°C, protected from light.

Vitreous humor samples

All the samples were obtained by direct puncturing of the eyes; they were collected in gray-top tubes containing 2% of sodium fluoride and frozen at −20°C until analysis. Because VH consists of 95–99% water and it was difficult to obtain enough drug-free VH samples, deionized water was used as drug-free VH during the validation of the analytical methods. Matrix substitution during the validation process did not give rise to any matrix effects for the employed analytical method. Quality control (QC) was added in water and VH had comparable results. QC spiked in water and quantified in VH calibration curve varied <15% and also VH QC quantified in water calibration curve. The case samples were obtained from Departamento Médico Legal de Vitória, Brazil.

This study was approved by the Research Ethics Committee of Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto—University of São Paulo.

Extraction and derivatization procedure

An solid phase extraction (SPE) procedure was developed to isolate target compounds from VH and to concentrate them. Prior to extraction, the VH samples were centrifuged for 5 min at 1048 × *g*. One milliliter of carbonate buffer (pH 9.0) and 400 ng/mL of deuterated internal standards were added to 1 mL of the sample supernatant. The drugs were separated from VH using Trace B® 335 cartrigdes (SPEware, CA, USA), which had previously been conditioned with 1 mL of ethyl acetate followed by 1 mL of methanol and 1 mL of deionized water. The sample was passed through the cartridge with an SPE positive pressure manifold (Cerex® 48™, SPEware). Then, the cartridge was washed with 1 mL of 0.01 M acetic acid and dried for 5 min in the maximum flow. The analytes were eluted in two separate fractions. The first fraction was eluted with 2 mL of acetone/chloroform solution (1 : 1, v/v). After the elution of acidic compounds, the column was rinsed with 1 mL of methanol and the second fraction was eluted with 2 mL of a freshly prepared dichloromethane/isopropanol/ammonium hydroxide solution (78 : 20 : 2, v/v/v). The first fraction was evaporated to dryness at 35°C, under a nitrogen stream; the residue was mixed with the second fraction and submitted to evaporation again. The extract was reconstituted with 40 μL of acetonitrile and derivatized in a closed vial equipped with a 0.20-mL insert, by adding 40 μL of MSTFA and heating the mixture at 90°C for 40 min.

GC–MS analysis

Analytes were determined by GC–MS on an Agilent 7890A gas chromatograph equipped with an Agilent 7693 automated liquid sampler, interfaced with an Agilent 5975C mass-selective detector (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation was achieved using a HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and helium at a constant flow rate of 1.0 mL/min, as carrier gas, which yielded a linear velocity of 39 cm/s. Injections (1 μL) were made in the splitless mode at 280°C. The initial column temperature of 90°C was held for 2 min, ramped to 220°C at 10°C/min and increased to a final temperature of 290°C at 20°C/min that was held for 4 min (run time = 22.50 min).

The MS was operated in an electron impact (EI) mode. The temperature of the MS interface, source and quadrupole were 280, 230 and 150°C, respectively. The selected ion monitoring (SIM) mode was used to monitor the target analytes listed in Table I; the dwell time was 30 m. Three ions for each analyte and two ions for internal standards were acquired.

Data analysis

Analytes were identified by comparing the retention time (±2%) and relative abundance of qualifier ions with the corresponding average values (±20%) of calibrators assayed in the same run. Quantification was based on ratios of the target ion peak areas of the native analyte to the corresponding deuterated internal standard. Calibration with internal standardization was performed using linear regression curve fits with $1/x$ weighting factor for all calibrators. Six multianalyte working calibration

Table I
List of analytes, internal standards, target and qualifier ions as well as retention time

Compound	Target ion (*m*/*z*)	Qualifier ions (*m*/*z*)	Retention time (min)
Amphetamine-d_{11}	120	98	7.495
Amphetamine	116	91, 192	7.593
Metamphetamine-d_{14}	137	98	8.552
Metamphetamine	130	91, 206	8.669
AEME	152	166, 181	8.951
MDA	116	91, 236	11.780
MDMA-d_5	135	255	12.637
MDMA	130	100, 250	12.685
MDEA	144	135, 264	13.713
Cocaine-d_3	185	85	17.306
Cocaine	182	82, 303	17.316
Methadone	296	85, 281	17.418
Cocaethylene	196	82, 318	17.647
Benzoylecgonine-d_3	243	85	17.696
Benzoylecgonine	240	82, 361	17.708
THC	386	315, 371	18.085
Dihydrocodeine	373	236, 282	18.318
Codeine	371	178, 196	18.669
Morphine-d_3	239	432	18.917
Morphine	236	196, 429	18.922
6-AM	399	287, 340	19.282
THCCOOH-d_3	476	374	20.110
THCCOOH	473	371, 488	20.130

standards at 10, 50, 100, 400, 750 and 1000 ng/mL were assayed with each batch. The calculated concentration of each calibrator was compared with the concentration of the target. Calibrator concentrations had to be ±10%, relative to their respective target value.

Experimental design for the optimization of the derivatization procedure

The optimization study was conducted using a response surface methodology. This methodology allowed us to simultaneously optimize the levels of the variables and to observe the interactive effects among them (20). Silylation conditions (volume of MSTFA, temperature and time) were simultaneously evaluated using a Box-Behnken response surface design (Design Expert 8.7.0.1 Trial—Statease, MN, USA). Derivatization assays were carried out with aliquots from a pooled SPE extract corresponding to spiked aliquot VH samples. The Box-Behnken design permitted the optimization of three factors with only 15 experiments. All the variables were tested at three different levels: 20, 30 and 40 μL of MSTFA (final volume was always made to 80 μL); 60, 75 and 90°C; and 20, 40 and 60 min. The experiments were conducted in a random order, to provide an accurate estimation of the experimental error. Finally, the optimum conditions were selected using a desirability function.

Method validation

The method was validated by determining sensitivity, selectivity, specificity, linearity, extraction efficiency, precision (intra-assay and inter-assay), accuracy and stability. To obtain the validation data, QC samples were prepared by spiking deionized water (as drug-free VH sample), which resulted in the following concentrations of the analytes: 15, 400 and 750 ng/mL (QC1, QC2 and QC3).

Limit of detection and limit of quantitation

A series of decreasing concentrations of drug-fortified VH were analyzed. The limit of detection (LOD) was defined as the lowest concentration giving an S/N ratio of at least 3. The limit of quantitation (LOQ) was defined as the lowest concentration giving an S/N ratio of at least 10; acceptable precision (RSD 20%) and accuracy (±20%). LOD and LOQ should still satisfy the predetermined acceptance criteria of qualification (retention time within 2% compared with standards analyzed in the same batch and qualifier ion ratios within ±20% of average calibrator ion ratios) and also furnish an acceptable chromatographic peak shape. A standard sample with the LOQ was included in the calibration curve.

Selectivity/specificity

Matrix effects and method specificity were evaluated by assaying 10 different drug-free VH samples and 23 potential interfering drug compounds. To assess potential interferences, drug-free VH samples were spiked individually, so that they contained 1000 ng/mL of acetylsalicylic acid, pseudoephedrine, acetaminophen, tramadol, fluoxetine, clomipramine, nicotine, caffeine, diazepam, clonazepam, alprazolam, lorazepam, cotinine, ephedrine, norfluoxetine, paroxetine, iron sulfate, dipyrone, phenylephrine, metoclopramide, cannabidiol, lidocaine and sertraline. The concentrations of peaks with retention times equal to those of the components in the method were compared with the LOQ. False−positive results below the LOQ were rejected as interfering peaks.

Linearity

The linearity study was performed by analyzing drug-free samples spiked with all the analytes, to obtain the concentrations of 10, 15, 30, 60, 100, 250, 500, 750 and 1000 ng/mL. The deuterated internal standards were added at a concentration of 400 ng/mL. Calibration curves were obtained by plotting the peak area ratios (analyte/internal standard) against the concentrations, and the linearity, which was expressed as the correlation coefficient (R^2), was determined.

Extraction efficiency

Extraction efficiency was assessed by adding analyte control solution to the blank matrix at low, medium and high control concentrations (15, 400 and 750 ng/mL, respectively) before SPE and a second set after extraction, but prior to the evaporation step. Internal standards were added to both sets prior to SPE. Samples were derivatized and analyzed. The relative extraction efficiency was calculated by comparing the mean analyte peak area of each compound in the first set with the appropriate mean analyte peak area in the second set ($n = 7$).

Precision and accuracy

The precision and accuracy assay were performed by analyzing replicate spiked samples at the target analyte concentrations of 15, 400 and 750 ng/mL. Inter-assay precision was determined on three different days ($n_{total} = 15$). Intra-assay precision was calculated from 10 replicate determinations per concentration in one assay batch. Data were evaluated by examining the variance in each group (intra-assay and inter-assay), which was established by the relative standard deviation (RSD%). Accuracy was determined by comparing measured concentrations with target values over 15 runs and expressed as percent of the target concentration.

Stability

The stability assay was assessed using drug-free VH samples in triplicate fortified with the analytes of interest at 50 ng/mL. The storage conditions studied were: room temperature for 24 h; three freeze−thaw cycles; −20°C for 7 and 30 days. The concentrations obtained after each assayed condition were compared with those from freshly prepared samples.

Autosampler stability was assessed by re-injecting QC specimens after 48 and 72 h and by comparing the calculated concentrations to values obtained against the original calibration curve.

Carryover

To evaluate the carryover, three blanks were analyzed after the highest concentration in the calibration curve.

Case study

To prove the applicability of this method, 10 VH samples coming from forensic samples that had tested positive for COC, AMPs, opiates and/or THC were used.

Results and discussion

GC–MS analysis

All analytes were successfully extracted and separated using this method. All the ion peaks were Gaussian-shaped and demonstrated baseline resolution. Figure 1A–C show a drug-free VH

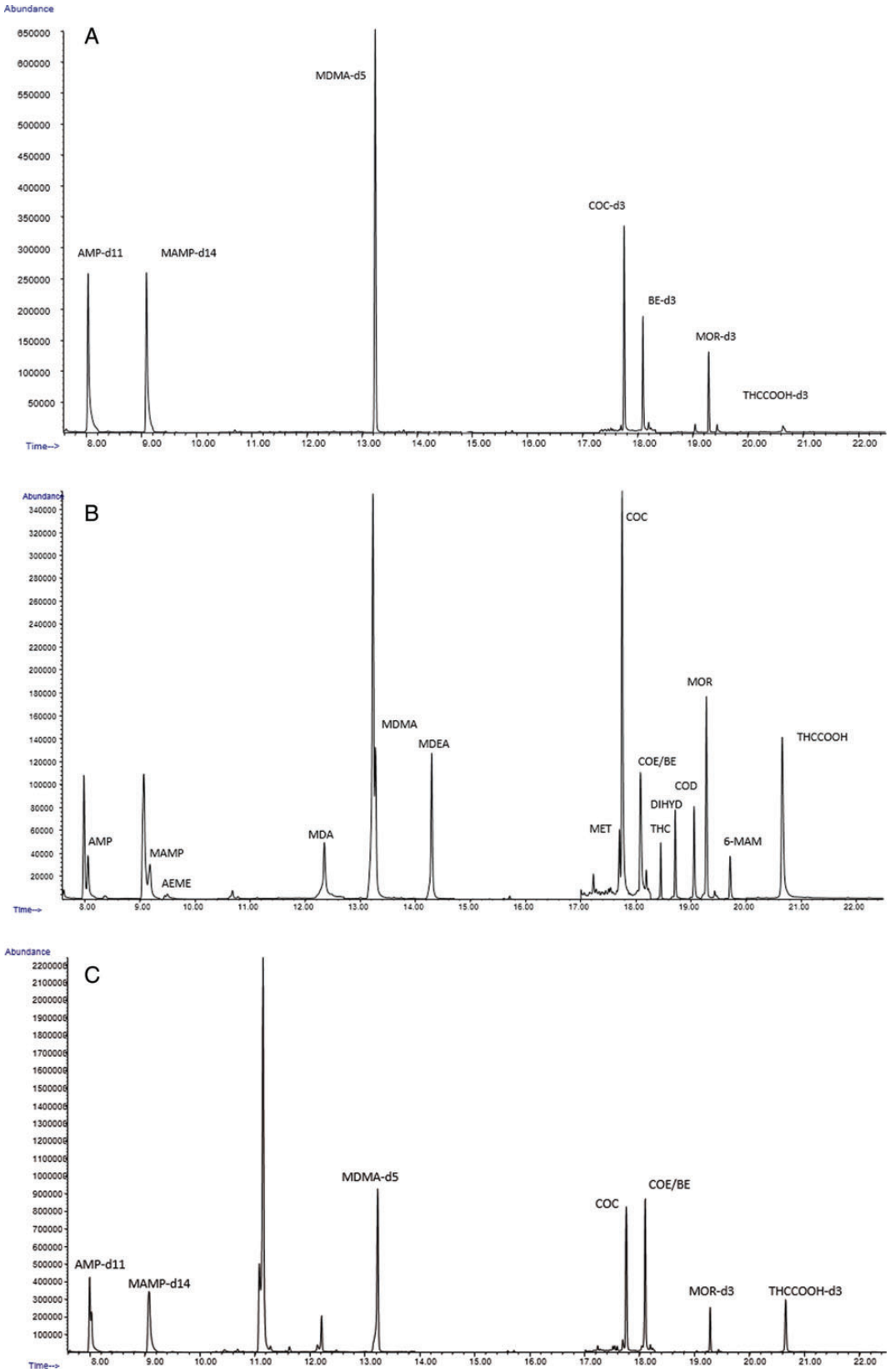

Figure 1. Chromatogram obtained at a drug-free VH sample (A), spiked with all the analytes under investigation at 100 ng/mL (B) and the chromatogram obtained in case 7 (C).

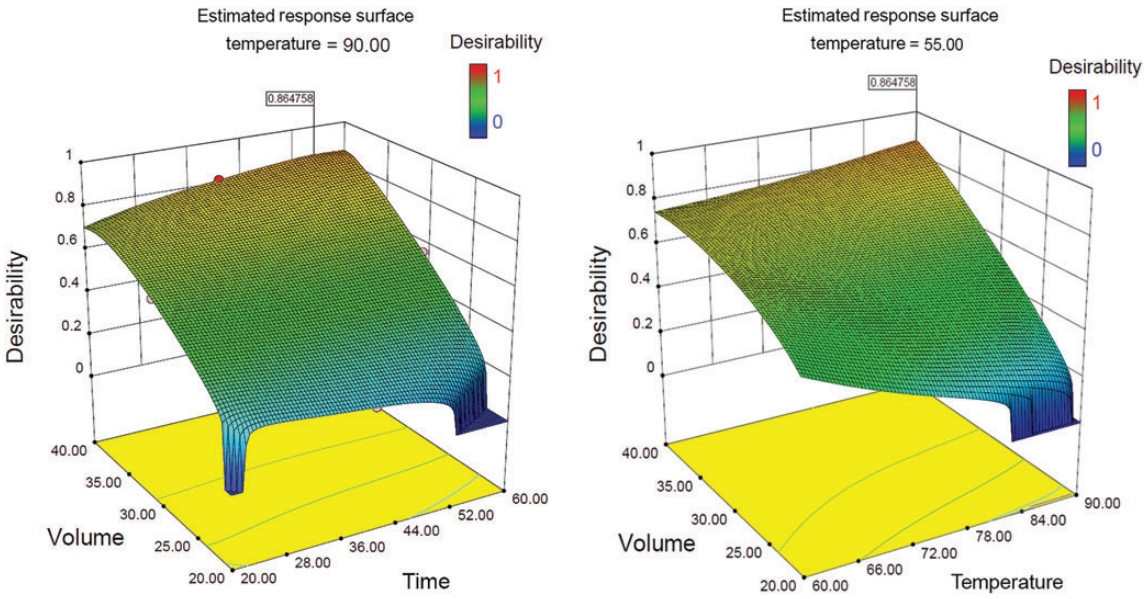

Figure 2. Desirability function plots.

sample, a VH sample spiked with all the analytes and a real sample, respectively.

Optimization of the extraction procedure

To obtain good extraction recovery for all the analytes with different physicochemical properties, the SPE procedure was optimized. The Trace-B column consists of a hydrophilic polymer with a cation exchanger, which allows the extraction of compounds of different polarities.

Initially, the effect of sample pH (5, 6, 7 and 9) on the retention of the analytes was investigated. Aliquots of deionized water were spiked with 50 ng/mL of AMP, MAMP, COC, BE, MET, COD, THC and THCCOOH; the compounds were extracted as described previously. Lower pH reduced the recoveries of opiates (represented by COD and MET) and AMPs (AMP and MAMP)—these compounds have high pKa (8.2–9.9) and protonation makes them too polar to retain on the sorbent. pH 9 gives the best overall recovery (data not showed), which agrees with the results of Gonzalez-Marino *et al.* (21).

Finally, different volumes were considered for the elution step, bearing in mind that the column pore size is 200 Å and small volumes could be used for extraction. Elution volumes of 2, 3 and 4 mL were tested; there was not a statistically significant difference [two-way analysis of variance (ANOVA), Bonferroni post hoc test] between these volumes, and then, the small volume (2 mL) was chosen.

Optimization of the derivatization procedure

The optimum conditions were selected using a desirability function, calculated by considering the derivatization reactive compounds only (Figure 2). The optimum values predicted by this function were 40 µL of MSTFA, 90°C and 55 min. However, to reduce time consumed in the process, statistical analysis (two-way ANOVA, Bonferroni post hoc test) using 30, 35, 40, 45 and 55 min was conducted. Because the derivatization times 40 and 55 min did not differ significantly and both resulted in complete derivatization, the shortest period (40 min) was selected.

Method validation

The drug-free VH samples did not present any interfering peaks. None of the 23 potential exogenous interferences fortified at 1000 ng/mL produced a transition ratio or failure of quantification criteria.

Table II summarizes the LOD results and the linearity experiments. Initial experiments were conducted with nine sets of calibration curves fit via unweighted linear least squares and linear least squares with $1/x$ and $1/x^2$ weighting factors, to identify the most appropriate calibration model. Inspection of residuals indicated that linear regression with $1/x$ weighting factor produced the best fit for the calibration data. The linearity ranged from 10 to 1000 ng/mL for all analytes, except for AEME, for which linearity ranged from 10 to 750 ng/mL. All correlation coefficients exceeded 0.99.

Limits of detection and quantification, intra-assay and inter-assay precision as well as accuracy were adequate for the purposes of the present study. Precision and accuracy were evaluated at three concentrations; Tables III and IV present the corresponding data. Inter-assay precision ranged from 1.1 to 10.0% (RSD) for all the analytes ($n = 15$). Intra-assay precision ($n = 10$) was <10.4 for all the analytes at all QC concentrations (range 0.9–10.3%). Accuracy, calculated as the percentage of target concentration, ranged from 95.6 to 104.0% ($n = 15$).

Extraction efficiency was assessed at three concentrations across the linear dynamic range. The absolute analytical recoveries obtained after SPE extraction at different concentration levels showed that they did not depend on the tested concentration. Extraction efficiency varied for different analytes (Table V): for AMP, MAMP, MDMA, MDEA, BE and MET, the mean extraction efficiency was >90%; for MOR, 6-MAM, COD, DIHYD, COC, COE, AEME and MDA, extraction efficiencies were <90% but >70%.

Table II
LOD, linearity range and coefficient of determination (R^2) for the analyzed drugs and metabolites

Compound	LOD (ng/mL)	Coefficient of determination	Range (ng/mL)
AMP	1.0	0.9985	10–1000
MAMP	1.0	0.9967	10–1000
AEME	1.0	0.9919	10–750
MDA	1.0	0.9990	10–1000
MDMA	2.0	0.9977	10–1000
MDEA	1.0	0.9984	10–1000
MET	1.0	0.9985	10–1000
COC	2.0	0.9965	10–1000
CE	2.0	0.9961	10–1000
BE	2.0	0.9997	10–1000
THC	2.0	0.9974	10–1000
DIHYD	1.0	0.9969	10–1000
COD	2.0	0.9997	10–1000
MOR	2.0	0.9983	10–1000
6-MAM	1.0	0.9980	10–1000
THCCOOH	2.0	0.9956	10–1000

Table III
Precision data for GC–MS quantification of the analytes in VH samples

Analyte	Intra-assay imprecision (%RSD, $n = 10$)			Inter-assay imprecision (%RSD, $n = 15$)		
	15 ng/mL	400 ng/mL	750 ng/mL	15 ng/mL	400 ng/mL	750 ng/mL
AMP	7.3	2.8	5.1	6.4	2.4	2.6
MAMP	3.9	2.5	1.5	5.0	2.6	6.0
AEME	10.3	5.5	5.3	8.7	6.7	6.3
MDA	4.4	4.0	8.7	2.9	9.5	4.6
MDMA	1.4	3.4	2.3	2.4	4.7	4.5
MDEA	2.2	1.7	1.4	4.0	2.2	1.7
MET	5.2	5.9	5.4	3.2	3.1	4.2
COC	1.5	2.8	1.5	2.5	2.1	1.9
CE	2.8	3.7	5.5	5.0	7.0	5.9
BE	1.7	2.0	4.3	5.1	3.0	1.2
THC	4.0	1.4	0.9	4.9	3.5	1.3
DIHYD	2.0	3.2	2.2	4.5	2.9	2.6
COD	7.8	3.4	2.3	7.3	4.9	2.5
MOR	9.2	2.8	1.6	7.6	2.6	2.1
6-MAM	3.3	2.2	3.6	10.0	2.7	2.7
THCCOOH	6.8	4.5	1.2	5.2	1.9	1.1

Table IV
Accuracy data for GC–MS quantification of the analytes in VH samples

Analyte	Accuracy (% target, $n = 15$)		
	15 ng/mL	400 ng/mL	750 ng/mL
AMP	97.2	99.2	100.1
MAMP	97.3	98.7	101.9
AEME	101.0	101.2	102.7
MDA	99.6	100.5	101.2
MDMA	100.0	95.6	100.7
MDEA	104.6	99.3	101.6
MET	100.5	97.9	98.2
COC	101.9	98.4	101.1
CE	101.7	95.8	98.2
BE	101.9	98.6	100.5
THC	97.1	97.6	99.9
DIHYD	97.6	98.5	99.2
COD	104.0	98.7	99.7
MOR	102.0	97.6	99.5
6-MAM	103.1	98.9	100.2
THCCOOH	100.1	100.1	100.2

Cannabinoids, especially THC, demonstrated poor recovery; nevertheless, these low recoveries were sufficient to quantitatively determine the compounds at the lowest calibration level.

The stability experiments in the VH samples did not demonstrate degradation in the conditions studied.

Table V
Extraction efficiency data for GC–MS quantification of the analytes in VH samples

Analyte	Extraction efficiency (%, $n = 7$)		
	15 ng/mL	400 ng/mL	750 ng/ mL
AMP	85.4	89.4	96.4
MAMP	81.9	90.8	97.9
AEME	83.7	78.0	72.3
MDA	94.2	85.5	89.0
MDMA	94.7	89.4	95.0
MDEA	91.5	88.4	95.7
MET	86.9	91.8	93.3
COC	77.5	83.6	85.1
CE	80.7	86.4	100.1
BE	94.3	100.1	98.5
THC	36.9	24.5	17.8
DIHYD	86.5	81.9	75.8
COD	96.5	82.3	78.6
MOR	76.3	70.4	69.5
6-MAM	74.4	70.9	68.0
THCCOOH	61.0	62.1	59.9

Table VI
Results of postmortem VH sample analyses

Case	Blood screen	Drug	Concentration (ng/mL)
1	Cocaine	Cocaine	267.64
		Benzoylecgonine	142.66
		Cocaethylene	115.32
2	Cocaine	Cocaine	103.03
		Benzoylecgonine	12.99
		Cocaethylene	48.45
3	Cocaine	Cocaine	213.08
		Benzoylecgonine	136.60
		AEME	37.05
4	Cocaine	Cocaine	187.11
		Benzoylecgonine	144.81
		Cocaethylene	33.97
5	Cocaine	Cocaine	30.81
		Benzoylecgonine	11.47
6	Cocaine	Cocaine	283.97
		Benzoylecgonine	27.89
		Cocaethylene	92.65
7	Cocaine	Cocaine	223.21
		Benzoylecgonine	460.98
		Cocaethylene	41.41
8	THC	Negative	–
9	THC	Negative	–
10	Amphetamine	Amphetamine	24.31
	THC	Negative	–

Real-case samples

The validated method was applied to 10 VH samples from individuals who screened positive for drugs of abuse in the blood. Results can be seen in Table VI. In seven cases, COC and their metabolites were detected; COC concentration ranged from 30.81 to 283.97 ng/mL (mean 186.98 ng/mL). BE was found in all cases that screened positive for COC (range 11.47–460.98 ng/mL, mean 133.91 ng/mL). It was also noticed that, in five cases, COE were detected. AEME was also quantified in one case. Case 7 presented the highest BE level (460.98 ng/mL), illustrated in Figure 1C.

Three samples were positive for THC and THCCOOH in blood; however, it was not detected in VH. THC binds strongly to plasma proteins being less extensively transferred into the VH. Other authors had the same result, and Lin and Lin (5) investigated cannabinoid concentration in the VH of victims involved in motor vehicle fatalities—all the samples contained

<10 ng/mL. Jenkins and Oblock (18) analyzed 50 VH specimens from individuals screened positive for cannabinoids in urine or blood. Only one VH sample was positive for THCCOOH at the LOD concentration (2 ng/mL) when analyzed by GC–MS.

Amphetamine usage was also identified in VH. The sensitivity was good enough to ensure reliable determination of all the drugs at concentrations usually found in real samples.

Conclusions

For the first time, a GC–MS method that simultaneously quantified the principal recreational drugs of abuse in VH is described. Our results demonstrate the suitability of VH as an alternative biological sample for the determination of COC, AMPs and opiates and the effective use of this method.

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