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New green DI-SPME/CE-MS method for quantitative analysis of selected illicit drugs in human whole blood

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ABSTRACT

The purpose of the research was to develop a new method that would allow the determination of selected drugs (mephedrone, MDMA, and cocaine and its metabolites: benzoylecgonine, norcocaine, and cocaethylene) in whole blood, while meeting not only the criteria of greenness, but also balancing analytical and economic aspects. For this purpose, solid phase microextraction was paired with capillary electrophoresis hyphenated to a mass spectrometer. The method was validated. Parameters such as LOD (1.2–7.2 ng/ml), LOQ (3.7–24.0 ng/ml), intra- (2.24–10.72%) and inter-day (3.97–19.01%) precision, bias (RE=0.1–14.6%), recovery (91.7–105.4%) and matrix effect (-1.8–5.4%, except benzoylecgonine) were determined. In addition to analytical parameters, the greenness of the method and its practicality and cost-effectiveness were also evaluated. The White Analytical Chemistry method was used for this purpose. A high score of 90.6/100.0 was obtained, indicating that the method balances these three aspects very well. On this basis, it was concluded that the developed DI-SPME/CE-MS method could be a useful tool for toxicological analyses.

1. Introduction

Psychoactive substances have been known to mankind since antiquity, originally used for religious and ritual purposes; today, they are used for recreational purposes. From year to year, there is a significant increase in the popularity of addictive substances, a large part of which are stimulants such as cocaine, mephedrone, and ecstasy. The first post-pandemic report, World Drug Report 2022 compiled by the United Nations Office on Drugs and Crime (UNODC), reports that in 2020, 1 in 18 people aged 15 to 64 (estimated 5.6% of the total population) used illicit drugs over the past year. It is alarming that cocaine production in 2020 reached a historic record level (including an increase of 11% compared to 2019) of 1982 tons (at 100% purity). It is estimated that 21.5 million people used cocaine in 2020, which is 0.4% of the world population, and early estimates suggest that this number will increase in the upcoming years. According to the report, ecstasy, also known as MDMA, is a drug that has lost the most popularity as a result of the pandemic [1]. However, the ease of restrictions on movement, travel, and socialisation resulted in a renewed increase in the popularity of this substance [2].

More and more dangerous illicit drugs are the so-called new psychoactive substances (NPS), amongst them synthetic cathinones, often included in the group of so-called "legal highs". The most commonly used synthetic cathinone is mephedrone [3]. 2020 was a breakthrough year for the number of reported seizures of synthetic cathinones: up to 3.3 tons were recorded and in 2019 it was only 0.75 tons. Large seizures of synthetic cathinones continued to be reported in 2021 and 2022 [2].

Due to these disturbing statistics, it is very important to develop new analytical methods for the determination of psychoactive substances in biological samples. The preparation of biological material for toxicological analysis is a key stage in the entire analytical process. Research shows that more than 80% of the analysis time is spent collecting and preparing the sample, which is why this stage has the greatest impact on the success of the analysis. Therefore, the selection of an appropriate technique is particularly important and is dictated by many factors such as precision, efficiency, repeatability, selectivity, costs, access to equipment, methodology and analysis time [4,5].

Until recently, the stage that consumed the largest amounts of solvents during the entire analysis was sample preparation. When in 1991 Paul Anastas introduced the concept of green chemistry, techniques in which the use of organic solvents was limited or eliminated began to gain more popularity [6]. One of the most popular green sample preparation techniques is solid phase micro-extraction (SPME) [4].

SPME is a simple, fast, and efficient sample preparation technique for analysis using small solvent volumes. It was developed and introduced into analytical practice in 1990 by Pawliszyn and Arthur as an effective alternative to routine liquid-liquid extraction (LLE) and ultrasound-assisted extraction (UAE) [7]. The greatest advantages of SPME, apart

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from reducing the use of solvents, are simplicity and speed of implementation and the possibility of automating the entire process. This technique allows trace analysis to be performed on very complex matrices due to its high sensitivity. This technique makes it possible to collect samples in situ and in vivo; as a result, analyses can be carried out on a living organism and the analysis time can be reduced because of the combination of sample collection and preparation stage. Thanks to various extraction methods (direct immersion SPME and headspace SPME), this technique can be applied to liquid, solid, and gaseous samples. An additional advantage of SPME is the possibility of combining it with various instrumental methods such as gas chromatography (GC), liquid chromatography (LC), or capillary electrophoresis (CE) [4,8]. This technique has been widely used in environmental [9], toxicological [10], biological [11] and pharmaceutical [12] analyses, that is, where the matrix composition is very complex [4]. The available literature shows that LC and GC are used most frequently for the analysis of psychoactive substances using the SPME technique. However, few applications of SPME/CE have been found in the literature, for example, for the analysis of ephedrine and pseudoephedrine [13]. This method has been successfully applied to the analysis of residues of oxytetracycline in milk [14], pesticides in food [15], and proteomic analysis [16].

CE is one of the fastest growing analytical techniques. It was introduced to laboratory practice at the turn of the 1980s and 1990s, and since then, because of its numerous advantages and wide applications, it has gained the interest of forensics experts. The high analytical potential of CE makes this technique one of the greatest competitors for high performance liquid chromatography (HPLC), which is commonly used in toxicological studies [17]. The main advantage of capillary electrophoresis is the low consumption of organic reagents and solvents. Due to this advantage, this technique perfectly meets the assumptions of green analytical chemistry [18]. Furthermore, capillary electrophoresis is characterised by a relatively low cost of exploitation (although the cost of the CE system, especially in combination with MS, can be quite high) due to the fact that the capillaries used in CE systems are relatively durable and useful for a long time, and separation is carried out without the use of a pump, at low pressure, usually at room temperature [19]. Due to the many advantages and benefits of combining capillary electrophoresis techniques with a sensitive mass detector, the CE-MS technique has found application in environmental analyses [20], clinical trials [19], pharmacy [21], proteomics [22], and also in toxicological studies [23].

The research carried out aimed to develop the DI-SPME/CE-MS method for the detection and quantification of cocaine and its metabolites: benzoylecgonine, norcocaine and cocaethylene, MDMA and mephedrone in human whole blood. The developed DI-SPME/CE-MS method was validated in order to determine the scope of its usefulness and limitations for the analysis of biological samples. In addition, the developed DI-SPME/CE-MS method was evaluated using an innovative concept for a comprehensive assessment of the analytical method, white analytical chemistry (WAC), and was compared with the SPME/LC-MS method. To the best of our knowledge, this is the first study in which the DI-SPME/CE-MS technique has been used to analyse psychoactive substances and selected metabolites in human blood.

2. Materials and methods

2.1. Chemicals and materials

During the study, standard solutions of 1 mg/ml were used: cocaine (COC), norcocaine (NCOC) and cocaethylene (COCE) in acetonitrile and benzoylecgonine (BE), MDMA, and mephedrone (MEF) in methanol. In this study, internal standards of 1 mg/ml were also used: cocaine-d3 (KOK-D3) in acetonitrile and MDMA-D3 in methanol. All of the standards mentioned above were purchased from Lipomed AG (Arlesheim, Switzerland).

High purity organic solvents such as acetonitrile, propan-2-ol, methanol (Honeywell - Riedel-de Haën, Morristown, USA) were used during the study. Formic acid (Supelco - Merck; Darmstadt, Germany), acetic acid (Sigma-Aldrich, USA), 50% sodium hydroxide solution (Sigma-Aldrich, USA), ammonia (Merck KGaA, Darmstadt, Germany), 10 M ammonium formate (Sigma-Aldrich, USA) and ultrapure water generated by the Mili-Q Plus water purification system (Merck-Milipore, Bedford, USA) were also used.

C18 SPME-LC silica fibres with a coating thickness of 45 μm and a fibre coverage length of 1.3 cm (Supelco - Merck; Darmstadt, Germany), 1.5 ml HPLC vials (VWR, USA); 200 μl vial inserts (VWR, USA), Kimtech dustless wipes (Kimberly-Clark Corporation; Irving, TX, USA) were used during extraction. During the study 1.5 ml electrophoresis vials (VWR, USA), PCR tubes (Axygen, Corning, USA) and 1.5 ml Eppendorf tubes (Eppendorf AG, Germany) were also used.

2.2. Apparatus and conditions

Measurements were performed using a PA 800 capillary electrophoresis system (Beckman Coulter, USA), hyphenated to a MicrOTOF II mass spectrometer with time-of-flight analyser (Bruker, Germany), with electrospray ionisation (ESI). The programme used to control the capillary electrophoresis apparatus was Karat32 (Beckman Coulter, USA). The CE instrument was coupled with MS using an external detector adaptor cartridge for the CE capillary (Beckman Coulter, Brea, USA) and a CE ESI-MS Sprayer Kit (Agilent Technologies, Santa Clara, CA, USA). The separation process was carried out for 10 min using a voltage of +30 kV in a silica capillary (75 µm i.d., 100 cm length, Beckman Coulter). The temperature was set at 25 °C (capillary) and 15 °C (sample storage). Hydrodynamic injection of the samples was used: 10,34 kPa for 10 s. The sheath fluid (a mixture of isopropanol/H2O, 1:1, v/v with 0.2% HCOOH) was delivered to the ESI source (flow 180 µl/h) using a syringe pump (Holliston, USA). MS detection was performed in positive-ion mode. Spectra in the range of 100–1450 m/z were acquired. The following operating conditions of the ESI source were used: nebuliser pressure: 0.4 bar; capillary voltage: 4500 V; flow rate and temperature of drying gas: 4 l/min, 180 °C. According to the procedure described by Bruker, the calibration was performed before the series of measurements and each time after the measurement. For this purpose, a calibrant solution was injected, that is, 10 mM sodium formate in a mixture of water and isopropanol (1:1, v/v), at a rate of 0.18 ml/h. Data collected were processed using Compass DataAnalysis 3.2 software (Bruker).

Each time before the series of measurements, the capillary was conditioned with 0.1 M NaOH (5 min), water (5 min), MeOH (5 min) and water (5 min) at a pressure of 137.9 kPa. After the separation process of each sample, the capillary was rinsed with water (2 min; 172.37 kPa), 1 M NaOH (0.5 min; 10.34 kPa), water (2 min; 172.37 kPa), MeOH (2.5 min; 206.84 kPa) and water (5 min; 137.90 kPa). After a series of measurements, the capillary was subjected to a cleaning procedure with methanol (5 min, 137.90 kPa), water (5 min, 137.90 kPa), and air (2 min, 137.90 kPa).

Thermal Shake Touch from VWR (Randor, PA, USA), Concentrator plus from Eppendorf AG (Hamburg, Germany), SevenExcellence pH metre (Mettler-Toledo, Switzerland), Allegra X-30R centrifuge (Beckman Coulter, USA) and Microfuge 16 Centrifuge (Beckman Coulter, USA) were used during extraction and preparation of samples and reagents.

2.3. Blood sample collection

The biological sample used to develop the method was blood free from the tested analytes, obtained from the Centre for Blood Donation and Hemotherapy in Kraków. The samples used in the matrix effect study were provided by volunteers.

Table 1
List of analytes.

Analyte	Abbreviation	Formula	*m/z*
Cocaine	COC	$C_{17}H_{21}NO_4$	304.1543 ± 0.0050
Benzoylecgonine	BE	$C_{16}H_{19}NO_4$	290.1387 ± 0.0050
Norcocaine	NCOC	$C_{16}H_{19}NO_4$	290.1387 ± 0.0050
Cocaethylene	COCE	$C_{18}H_{23}NO_4$	318.1700 ± 0.0050
Mephedrone	MEPH	$C_{11}H_{15}NO$	194.1158 ± 0.0050
MDMA	MDMA	$C_{11}H_{15}NO_2$	194.1176 ± 0.0050
Cocaine-d3	COC-d3	$C_{17}H_{18}D_3NO_4$	307.1362 ± 0.0050
MDMA-d3	MDMA-d3	$C_{11}H_{12}D_3NO_2$	197.1119 ± 0.0050

2.4. Sample preparation

When optimising the composition of the separation buffer, a solution containing all analytes and internal standards was used at a concentration of 500 ng/ml in the buffer.

The 500 ng/ml intermediate drug solution needed during validation was prepared in methanol and stored in the freezer at −20 ˚C. The list of analytes, the abbreviations of their names used in the publication, the formulas of the analytes, and the ion masses characteristic of them are summarised in Table 1.

To obtain solutions with concentrations of 25, 50, 100, 150, 200 and 250 ng/ml used during the validation of the method, appropriate amounts of the intermediate solution containing the analytes at a concentration of 500 ng/ml and an appropriate amount of the solution of the internal standards used (so that their concentration in the sample is 125 ng/ml) were pipetted into the inserts. The solvents were then evaporated using a concentrator and 200 μl of blood free of the tested compounds was added to each insert. The sample prepared this way were mixed for 10 min using a thermoshaker (2200 rpm, room temperature). Samples were prepared the day before analysis and stored in the freezer at −20 ˚C.

The extraction procedure used in the study presented here was developed by Majda et al. for the extraction of selected antidepressants from blood [10]. It was also successfully used by Świądro-Piętoń et al. to extract selected drugs from the same biological matrix [24]. The procedure consists of three steps. The first is conditioning the sorbent by immersing it for 45 min in a 1.5 ml methanol:water solution (*v:v,* 1:1) and shaking it at room temperature at 2200 rpm. The fibres are then transferred to blood samples, for which the preparation procedure was described above. The vials are shaken at room temperature for 60 min at 2200 rpm. After the sorption step, it is necessary to manually clean the fibres of blood residues. For this purpose, the fibre is wiped with dust-free tissue, then dipped in a vial of ultrapure water and stirred for 10 s in Vortex. The final step involves placing the fibre in an insert vial containing 200 ul of acetonitrile: methanol: 0.1% formic acid solution (*v:v:v;* 2:2:1) and shaking for 30 min at room temperature at 2200 rpm. The solutions obtained are then evaporated for 45 min at 45 ˚Celsius, after which the residue is reconstituted by adding 50 μl of separation buffer. The sample prepared this way is stirred (20 s) with Vortex and transferred to a PCR-type vial, centrifuged and analysed with a CE-TOF-MS system. Meanwhile, the fibers are subjected to a cleaning procedure in a methanol:water:isopropanol solution (*v:v:v*; 2:2:1; room temperature, 60 min, 2200 rpm) to allow reuse. The described procedure is shown graphically in Fig. 1.

2.5. Validation study

During the validation of the method, the guidelines formulated by the Scientific Working Group for Forensic Toxicology (SWGTOX) for the validation of the methods developed for forensic toxicology [25] were followed. Whole blood without the analytes under study was used throughout the method validation process. It was spiked with appropriate amounts of the aforementioned compounds.

The linearity of the method was checked using standard blood solutions with the following drug concentrations: 25, 50, 100, 150, 200 and 250 ng/ml. During the study, the internal standard interpolation method was used. The curves were created by fitting a linear regression model taking into account the ratios of the analyte areas divided by the migration time to the areas of the corresponding internal standards divided by their migration time. Deuterated standards of cocaine (KOK-d3) and MDMA (MDMA-d3) were used at a concentration of 125 ng/ml.

The limit of detection is defined as the lowest concentration of an analyte in a sample that can be reliably distinguished from a blank matrix and identified by an analytical method. The limit of quantification is the lowest concentration of an analyte that can be determinable by a given analytical method with acceptable accuracy and precision. In the study described here, these parameters were determined from the signal-to-noise ratio obtained for a standard sample with the lowest drug concentration. The limit of detection was defined as the concentration for which this ratio was 3, while for the limit of quantification, it was 10.

Another parameter that was determined as part of the method validation was precision. This parameter was calculated for each of the compounds tested at three concentration levels: low (50 ng/ml), medium (150 ng/ml) and high (250 ng/ml). The intra-day precision of the method for each concentration was calculated based on measurements for three samples prepared separately on the same day ($n = 3$). The precision between days was determined by measurements taken over five days ($n = 15$). In both cases, this parameter was expressed as the coefficient of variation (CV,%). Using the same data on which the precision between days was calculated ($n = 15$), the relative error (RE,%) of the method was also calculated.

Recovery of the method was determined for each analyte based on three samples at each of three concentration levels (50, 150, 250 ng/ml). This parameter was calculated as the ratio of the amount of analyte detected in blood samples spiked before the extraction procedure to the amount determined in blood samples spiked after the extraction step.

The matrix effect was determined using ten blood samples provided by ten volunteers. The matrix effect was calculated by comparing the response of the analytes in the solution containing the matrix, subsidised with the analytes after extraction (A), with the response of the analytes in pure separation buffer (B). ME was calculated by the following formula: ME=(A-B)*100%/B [26]. All samples contained analytes at a concentration of 250 ng/ml.

The study also included a check for a carryover effect. For this purpose, a blank sample was measured three consecutive days immediately after measurement for the sample with the highest concentration amongst the standard curve (standard 250 ng/ml).

3. Results and discussion

3.1. Selection of the background electrolyte solution

The first step of the study was the selection of a separation buffer. amongst the analytes (with theoretical *m/z* values for $[M + H]^+$) were cocaine (*m/z* = 304.1543), cocaethylene (*m/z* = 318.1700), norcocaine and benzoylecgonine (which are isomers, *m/z* = 290.1387), as well as MDMA (194.1176) and mephedrone (whose monitored ion was not the pseudomolecular ion $[M + H]^+$, but the experimentally determined ion with *m/z* = 194.1158). Two internal standards were also used in the study: MDMA-d3 (*m/z* = 197.1119) and COC-d3 (*m/z* = 307.1362). Therefore, the priority was to find a buffer that would allow the separation of peaks from NCOC and BE, as well as MDMA and MEPH (for which the resolution of the mass spectrometer was not sufficient). Because of the specificity of the MS detector, which allows for independent detection of analytes with different masses, separation of the rest of the analytes was less important.

The first type of buffer tested was formic acid-based solutions at two different concentrations (100 mM and 200 mM) with the addition of

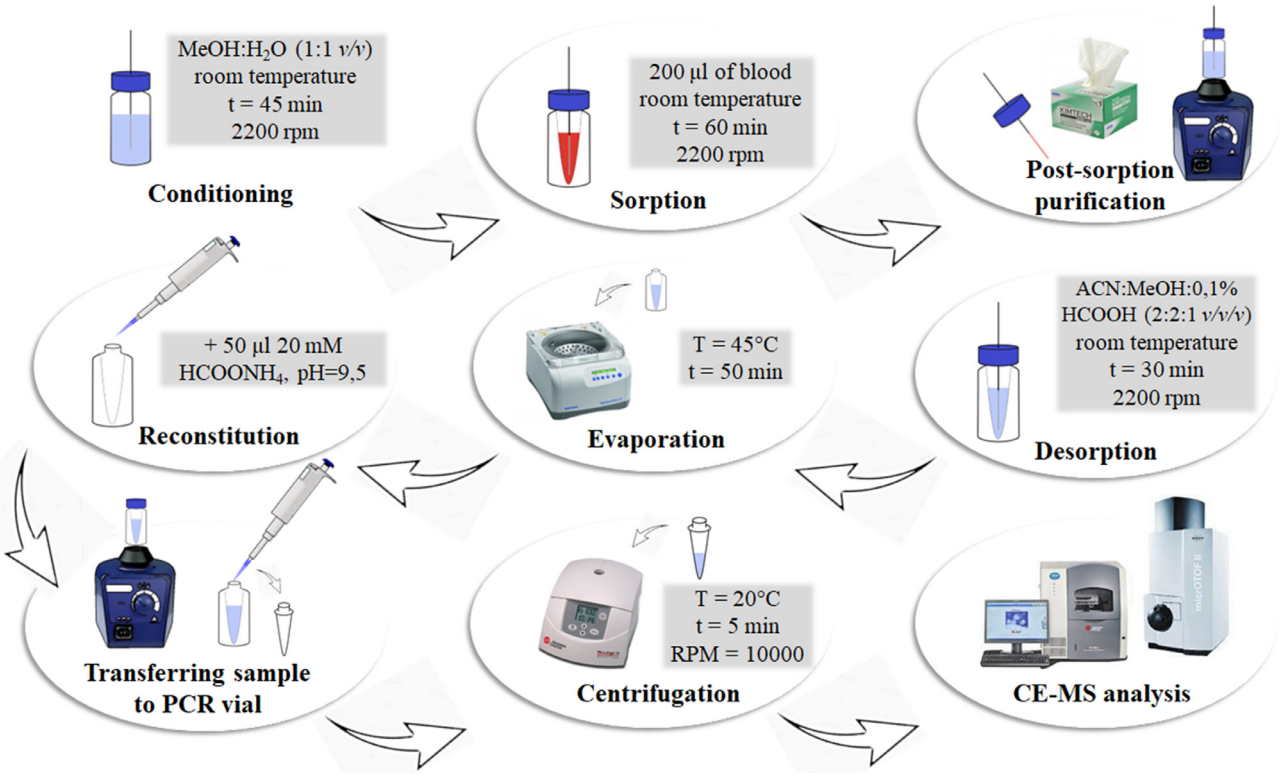

Fig. 1. Sample preparation procedure

Table 2
Results of the examination of different BGEs based on ammonium formate with regard to functions F_1 and F_2 (X - criterion not considered due to rejection of buffer in the first stage).

No.	Ammonium formate [mmol/l]	pH	F_1	D_{194}	D_{290}	F_2
1	10	7.5	5	0	1.6	X
2	10	8.5	6	0.3	0.7	1
3	10	9.5	5	0.6	0	X
4	20	7.5	5	0	1.4	X
5	20	8.5	5	0	0.9	X
6	20	9.5	6	0.8	0.3	1.1

acetonitrile or methanol in different ratios (60:40 and 80:20). The possibility of using analogous buffers based on acetic acid was also tested. The third type of buffer used in the test was ammonium formate solutions at two different concentrations (10 mM and 20 mM) at three pH levels (7.5; 8.5; 9.5) whose application to a similar group of analytes has already been described by Gottardo et al. [27].

The first and most important criterion used for the selection of the separation buffer was the number of peaks (n) visible on the electropherograms (F_1=*n*). At this stage, all eight formic acid-based buffers and eight acetic acid-based buffers that yielded seven of the eight peaks were rejected. Of the ammonium formate solutions, four of the six solutions tested were rejected for the same reason. In the next step, the difference between the migration times of BE and NCOC (D_{290}) and the difference between the migration times of MEPH and MDMA (D_{194}) were taken into account. To choose the better of the two buffers, another response function was created: $F_2 = D_{194}+D_{290}$. The buffer for which the sum of differences in migration times was greater was selected. A list of the ammonium formate-based buffers tested, along with the response values for each buffer and the F_1,F_2-function values, is shown in Table 2.

For buffers number 2 (10 mM; pH=8.5) and 6 (20 mM; pH=9.5), all eight peaks were successfully obtained. Due to the higher value of the F_2 function, buffer 6 was finally selected for further study. Electropherograms obtained with the selected buffer for the sample acquired after the extraction of analytes from blood are shown in Fig. 2.

3.2. Validation of the DI-SPME/CE-MS method

The validation of the developed DI-SPME/CE-MS method included determination of the following parameters: linearity range, calibration model, limit of detection (LOD), limit of quantification (LOQ), intra-day and inter-day precision (CV), bias (RE), recovery (REC) and matrix effect (ME). It was also investigated whether there is a carryover effect. The manner in which the aforementioned parameters were determined is described in Section 2.5, and the results are shown in Table 3.

Identification of the particular analyte was performed by comparing the accurately measured ion mass with the calculated mass, and (especially for BE/NCOC and MDMA/MEPH) on the basis of observed migration times.

The linearity of the method was checked in the same concentration range (25 to 250 ng/ml) for all analytes. The relatively low LOD and LOQ values of 1.2 to 7.2 ng/ml and 3.7 to 24.0 ng/ml, respectively, suggest the applicability of the method in clinical cases or during screening studies when blood analyte concentrations are relatively low. For example, in their publication, Cosby et al. reported that the average blood mephedrone concentration amongst the 32 driver cases they analysed was 210 ng/ml [28]. Garcia et al. found that the "recreational" doses of MDMA generate blood concentrations in the range of 100–250 ng/ml [29], and in Clarke's Analysis of Drugs and Poison one can find information that therapeutic concentrations of cocaine start at 50 ng/ml [30].

The evaluation of intra-day and inter-day precision and standard error was followed by the guidelines described by the Scientific Working Group for Forensic Toxicology [25]. For both CV and RE, the SWGTOX report states that the values must not exceed 20%. The best precision within a day was found for cocaine at the highest concentration level. The worst intra-day precision was obtained for mephedrone (10.72 ng/ml) at a concentration level of 50 ng/ml. For precision be-

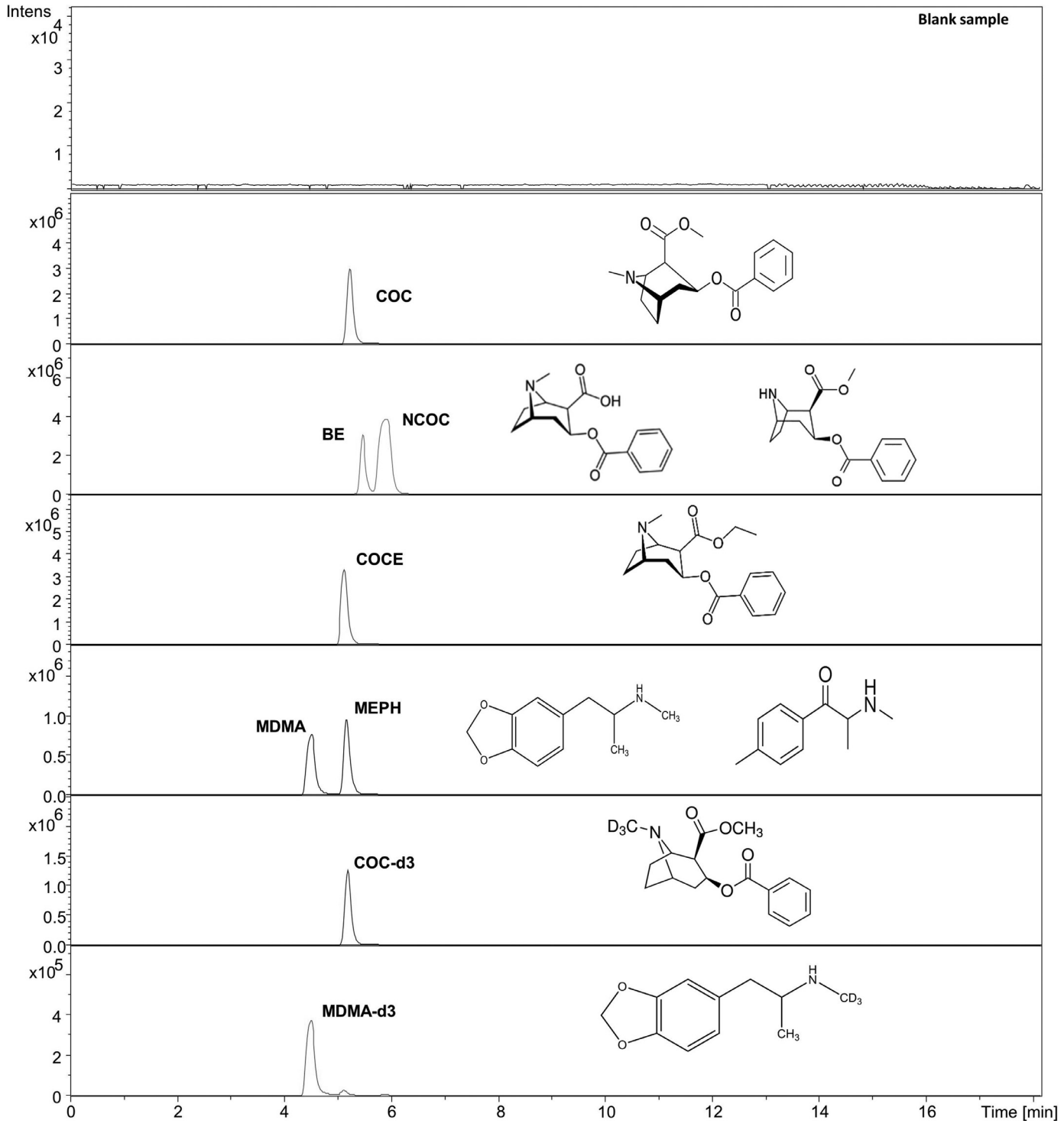

Fig. 2. Electropherograms obtained by applying the DI-SPME/CE-MS method to a blank blood sample and a blood sample containing analysed narcotic drugs and their metabolites at a concentration of 250 ng/mL. Peak assignment: MDMA (t_m = 4.52 min); MDMA-d3 (t_m = 4.54 min); cocaethylene COCE (t_m = 5.19 min); mephedrone MEPH (t_m = 5.26 min); cocaine COC (t_m = 5.29 min); cocaine-d3 COC-d3 (t_m = 5.31 min); benzoylecgonine BE (t_m = 5.51 min); norcocaine NCOC (t_m = 5,85 min).

tween days, the best results were also observed for cocaine at concentrations of 250 ng/ml, while the worst precision was achieved for mephedrone at 250 ng/ml (19.01 ng/ml). At the same time, it should be noted that all results were within the established acceptance criteria and were considered satisfactory. In case of bias, the highest RE value was obtained for mephedrone at the highest concentration level (more than 14%), while the rest of the results were within the range of up to +/- 5%. Thus, the acceptance criteria mentioned above were also met for this parameter.

The SPME technique is described as a non-exhaustive extraction technique in which extraction is carried out to equilibrium. Thus, it was considered that an important validation parameter in this case would be recovery. The results obtained were within the range of 91.7 to 105.4%. Ideally, the recovery of the method should be 100%, but even

Table 3
Validation results.

		Analytes					
		COC	BE	NCOC	COCE	MEPH	MDMA
Internal standard		COC-d3	COC-d3	COC-d3	COC-d3	MDMA-d3	MDMA-d3
t_m [min]		5.29±0.13	5.51±0.14	5.85±0.17	5.19±0.13	5.26±0.14	4.52±0.11
Linearity range		LOQ-300					
Slope		0.0094	0.0050	0.0071	0.0172	0.0071	0.0068
R^2		0.9947	0.9960	0.9912	0.9919	0.9877	0.9991
LOD [ng/ml]		1.3	1.2	4.5	7.2	2.6	3.7
LOQ [ng/ml]		4.1	3.7	14.2	24.0	8.3	12.1
Precision, CV [%]							
50 ng/ml	Intraday	3.92	3.57	5.62	3.82	10.72	6.29
	Inter-day	5.69	17.27	8.95	5.22	9.18	6.11
150 ng/ml	Intraday	3.69	8.34	5.95	5.10	9.16	6.52
	Inter-day	7.42	14.04	8.97	7.90	11.45	6.28
250 ng/ml	Intraday	2.24	9.13	8.96	2.65	2.40	4.67
	Inter-day	3.97	12.71	9.20	7.51	19.01	7.16
Bias, RE [%]							
50 ng/ml		1.4	−4.1	2.4	4.4	2.7	1.1
150 ng/ml		−1.6	−7.0	0.3	−2.4	3.9	−0.6
250 ng/ml		−0,7	−2.7	0.1	1.1	14.6	0.7
Recovery, REC [%]							
50 ng/ml		103.1	104.7	100.9	102.3	102.8	105.4
150 ng/ml		97.7	91.7	99.1	100.2	103.9	96.7
250 ng/ml		100.8	99.7	104.3	100.2	99.0	101.0
Matrix effect [%]		−1.8 ± 3.6	−31.1 ± 6.3	5.2 ± 8.2	5.4 ± 8.2	3.6 ± 8.8	0.0 ± 5.2

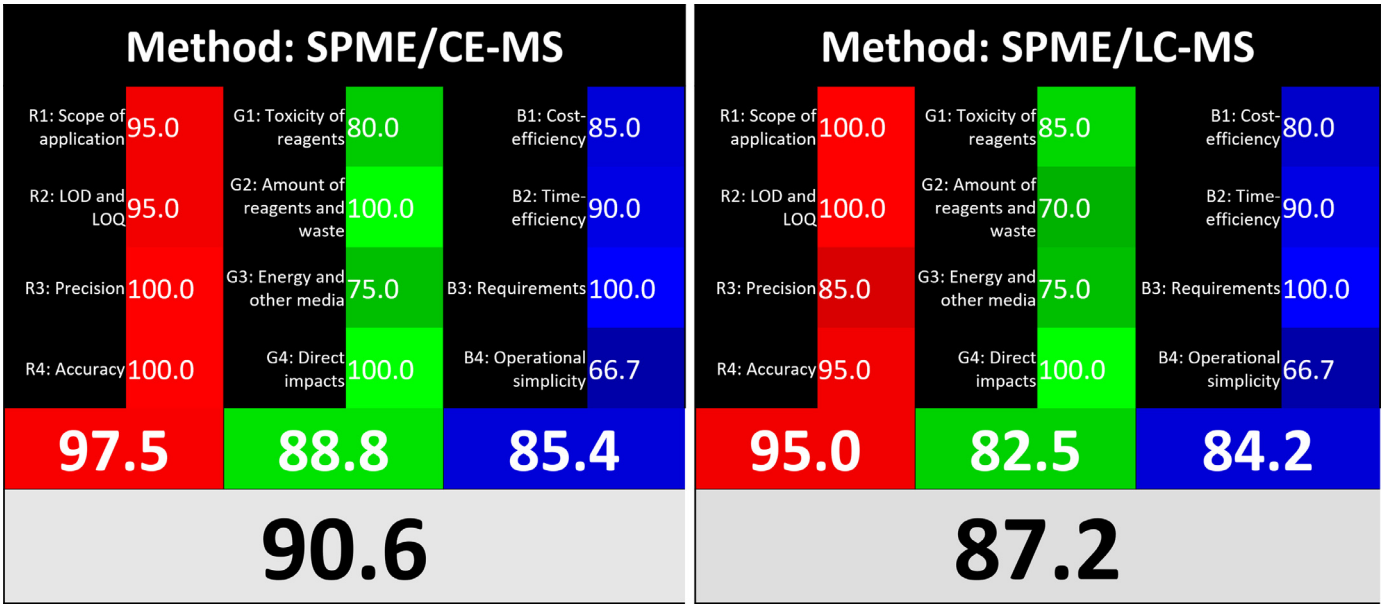

Fig. 3. Evaluation of the SPME/CE-MS and SPME/LC-MS methods with WAC model.

decidedly lower results do not necessarily adversely affect the usefulness of the method, as long as its sensitivity is high enough [31]. However, in the case presented here, recovery of the method was considered sufficient.

The matrix effect was also checked during the study. For five of the six analytes, values very close to 0% (−1.8–5.4%)%) were obtained. It can be assumed that such fluctuations are of random origin and that the presence of matrix elements in the samples did not affect the analytical signals from these analytes. However, in the case of benzoylecgonine, a significant reduction in the analytical signal was observed in the presence of the matrix. Such a low value of the ME parameter may indicate the presence of ion suppression. A popular way to compensate for the matrix effect is to perform calibrations using a matrix as close to the sample matrix as possible. This method is often used for the analysis of biological samples [32].

In addition, no carryover effect was observed during the study.

Based on the results of the validation process, it was concluded that the developed method is a reliable analytical tool for toxicological analysis.

3.3. Evaluation of the DI-SPME/CE-MS method based on the WAC concept

The developed and validated DI-SPME/CE-MS method was compared with the SPME/LC-MS method for the determination of cocaine, amphetamine, and mephedrone in blood, as described by Świądro et al. [24]. The White analytical chemistry model [33] was used for this purpose. This approach involves a comprehensive evaluation of the analytical method, taking into account not only the commonly considered "greenness" of the method (green), but also its analytical parameters

(red), and practical and economic aspects (blue). The results of this evaluation are shown in Fig. 3.

The details of the evaluation of the various parameters are as follows. For the scope of application (R1), the number of analytes for which the method was developed was taken into account. The evaluation of the analytical parameters was performed using cocaine as an example. Since both methods are characterised by parameters that allow them to be used within the assumed scope, in this case, the method characterised by the better result received 100 points and the other received correspondingly fewer points. For analytical parameters, LOQ (R2), CV-intraday (R3), and RE (R4) were considered. In the case of the SPME/LC-MS method, the authors did not determine this parameter, therefore, scoring was given by estimating it based on the in-laboratory test performed.

For category G1, the toxicity of the reactants was estimated based on the number of pictograms. For the consumption of reagents (G2), the differentiating factor was the volumes of the mobile phase used in the case of LC-MS and the sheath fluid and solutions required for capillary electrophoresis in the case of CE-MS. For energy consumption (G3), both methods were given the same number of points, since the use of mass spectrometry was considered the limiting factor in this case. The same was done for the G4 category (direct impacts – in this case, only user safety was considered, as the other aspects - use of animals and GMOs - were not applicable to the evaluation of these methods), as it was considered that the similarity of the two methods did not allow differentiation of this parameter.

Regarding the assessment of the practicality and cost-effectiveness of the methods, for costs (B1) it was found that the costs of using the apparatus are similar (generated mainly by the energy intensity of MS), so the assessment was influenced by the amounts of reagents consumed. For time consumption (B2), the same number of points was awarded due to the same sample preparation procedure, which is the longest step in this case. There were no differences in the case of parameters in groups B3 (requirements understood as the need for specialised apparatus and/or the need for specialised skills to perform the procedure) and B4 (operation simplicity which includes aspects such as integration, automation, and portability).

Based on the evaluation results shown in Fig. 3, it was concluded that the newly developed SPME/CE-MS method scored higher (90.6 out of 100.0) in the overall evaluation than the SPME/LC-MS method (87.2 out of 100.0). The scores of both methods can be considered very good, but it should be noted that the method using capillary electrophoresis was rated higher both in terms of analytical parameters and considering practical and economic aspects. However, its most significant advantage over the chromatographic method is its higher assessment in terms of greenness, which results from the use of the capillary electrophoresis technique.

4. Conclusions

The study developed a new DI-SPME/CE-MS method for the determination of cocaine and its selected metabolites, mephedrone, and MDMA in whole blood. To our knowledge, this is the first method that combines SPME extraction with CE-MS that can be applied to the toxicological analysis of a complex matrix such as whole blood. When a selective detector, such as a mass spectrometer, was combined with capillary electrophoresis, it was possible to reduce the amount of waste generated during analysis. This allowed the method to be described as green, which was supported by the results of the evaluation performed using the WAC method. The validation parameters determined confirmed the suitability of the method for toxicological analysis, and the low limits of quantification indicated that it can also be used to test samples taken during routine inspections, when expected concentrations are low. On the basis of the validation results and the comprehensive evaluation performed, it was concluded that the described method may be a useful tool to analyse blood samples for the presence of selected illicit drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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