



The direct immersion solid phase microextraction coupled with the LC–MS method for ex vivo determination of selected date-rape drugs in the human blood samples

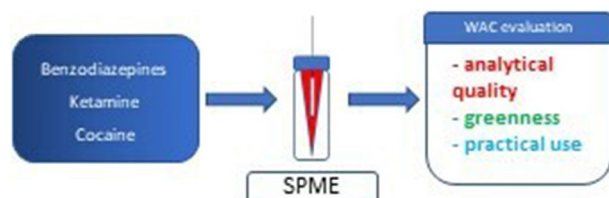
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Abstract

Nowadays, solid phase microextraction is developing rapidly. The use of this extraction technique allowed for the reduction of toxic solvents usage, easy automatization, and integration with other techniques. In this study, the use of DI-SPME/LC–MS to determine selected date-rape drugs (benzodiazepines, ketamine, and cocaine) is presented. The determined values of validation parameters: limits of detection ($LOD = 0.6–4.9 \text{ ng cm}^{-3}$), and quantification ($LOQ = 25 \text{ or } 50 \text{ ng cm}^{-3}$), intra-day and inter-day precision ($CV = 0.87–10.7\%$ and $4.96–16.1\%$, respectively), recovery ($RE = 94.6–106.7\%$) and matrix effect ($81.7–116.5\%$) indicated that the tested method could be used to determine the concentration of date-rape drugs in blood samples. The evaluation of the method according to the principles of White Analytical Chemistry showed that the DI-SPME/LC–MS method was characterized by satisfactory analytical quality, greenness, and economical use. The use of this method met the requirements of Green Chemistry. The significant advantages of this method were the quick analytical procedure, partial automation of the extraction stages, high sensitivity, lower sample, and reagent consumption.

Graphical abstract



Keywords Date-rape drugs · Extraction · Green chemistry · High pressure liquid chromatography · Solid phase microextraction

Introduction

Drug-Facilitated Sexual Assault is defined as an offense in which a victim is subjected to a sexual attack, while at the same time being under the influence of alcohol and/or drugs, therefore being unable to resist or consent [1]. In addition to substances such as ethanol, γ -hydroxybutyric

acid, and ketamine, drugs from the benzodiazepine group are often used. Flunitrazepam is one of the most common substances occurring in rape pills. Other drugs often used by the offender of drug-facilitated sexual assault are, among others, diazepam, temazepam, nitrazepam [2]. Benzodiazepines exhibit anxiolytic, sedative, and amnesic effects, which can lead to the unconsciousness of the rape victim. Due to the short half lives of these drugs, the possibility of rapid detection and quantification in biological samples such as blood and urine is an important matter [3]. The properties of selected date-rape drugs investigated in this work are

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presented in Table 1. The chemical structures of selected benzodiazepines and ketamine are presented in Fig. 1.

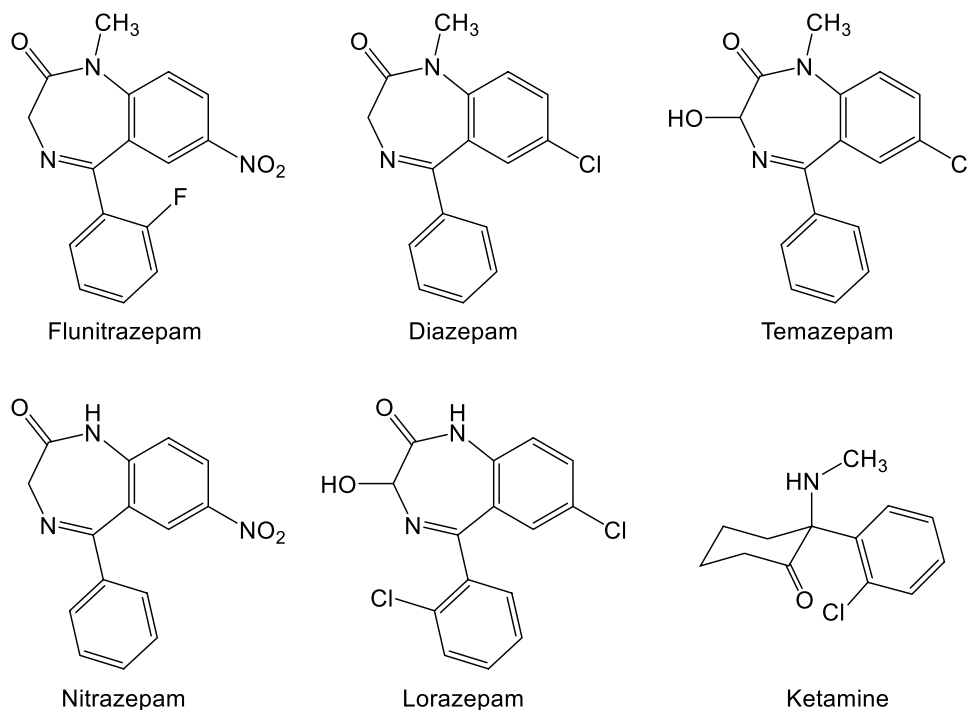
Recently, analysis of date-rape drugs in biological samples, due to their low concentrations in these samples, are focused on using chromatography methods coupled with mass spectrometer [4, 5]. Most of the research presents the possibility of using different extraction and samples preparation and extraction processes, which enable fast purification of biological samples from the matrix substances, increase the extraction efficiency, and make the analytical process faster and less energy consuming. For example, dispersive liquid–liquid microextraction (DLLME) was used for the rapid determination among others benzodiazepines and cocaine in blood samples [6, 7]. The use of DLLME extraction allowed for the miniaturization of liquid–liquid

extraction and reduced the use of extraction agents. Moreover, it enables effective sample purification [6]. Microextraction by packed sorbent (MEPS) used by Vejar-Vivar et al. [8] allowed for the automatization and shortening of the analytical procedure. The use of MEPS extraction enabled reduced solvents and sample consumption. The seven benzodiazepines were determined to follow this procedure in post-mortem blood samples given the good results in the future use of this procedure in a routine forensic toxicological analysis. The solid phase extraction (SPE) procedure was developed to determine ketamine and its metabolites in human blood samples. The SPE extraction combined with ultrafiltration led to an increase the extraction efficiency enabling the determination of targeted analytes at very low concentrations even at a few ng cm^{-3} in blood samples [9].

Table 1 Properties of tested analytes and selected internal standards (IS) with values of monitored ions

Analyte/IS	Abb	IS	$\text{p}K_a$	$\text{Log } P$	Monitored ion (± 0.0050)	Retention time/min
Ketamine	KET	FLU- d_3	7.5	3.1	238.0993	1.65 ± 0.03
Flunitrazepam	FLU	FLU- d_3	1.8	2.1	314.0935	6.65 ± 0.01
Diazepam	DIA	DIA- d_5	3.3	2.8	285.0789	6.91 ± 0.01
Temazepam	TEM	DIA- d_5	1.6	2.2	301.0738	6.70 ± 0.04
Lorazepam	LOR	DIA- d_5	1.3	2.4	321.0192	6.08 ± 0.01
Nitrazepam	NIT	NIT- d_5	3.2	2.3	282.0873	5.83 ± 0.01
Cocaine	COC	FLU- d_3	8.7	2.3	304.1543	3.04 ± 0.04
Flunitrazepam- d_3 (IS)	FLU- d_3	n/a	n/a	n/a	317.1123	6.64 ± 0.02
Diazepam- d_5 (IS)	DIA- d_5	n/a	n/a	n/a	290.1103	6.84 ± 0.01
Nitrazepam- d_5 (IS)	NIT- d_5	n/a	n/a	n/a	287.1187	5.78 ± 0.03

Fig. 1 Chemical structures of selected benzodiazepines and ketamine



Another interesting approach in the procedure of sample preparation seems to be the “QuEChERS” protocol. Anzilotti et al. [10] used this approach to determine a wide range of drugs of abuse, including benzodiazepines. The “QuEChERS” protocol was used for cheap clean-up of blood samples before UHPLC-MS/MS analysis enabled a reduction of the amounts of used solvents and made the sample preparation step faster. The procedures presented in this paragraph are very effective from the views of analytical parameters, economical use, and eco-friendly aspects, although the challenge is still the reduction of the sample volume used for analysis, which is another particularly important aspect in forensic toxicological analyzes. The standard volume of the blood sample necessary for the analysis is from 200 cm³ [8, 9] to even 1000 cm³ [10].

Extraction procedures with solid phases have developed rapidly. These techniques allow easy isolation of analytes, purification, and the concentration of biological samples. The extraction process is performed by the use of the solid phase material to which the analytes can bound. The procedure includes several steps such as conditioning and activation of solid material, after which the sample is loaded onto the sorbent. The washing step allows the removal of potential contaminants. Subsequently, the bound analytes can be evaluated and analyzed [11].

Solid Phase Microextraction (SPME) is currently the fastest developed technique using solid sorbent as the extraction phase. The SPME allows for a quick, sensitive, and economical approach to sample preparation, and it can be easily automated and integrated into other analytical techniques. The most common SPME configuration uses solid phase materials that are applied directly onto a thin fiber made of quartz or steel. The SPME fiber can be placed above a sample (Head Space SPME, HS-SPME) or inserted directly into a tested material (Direct Immersion SPME, DI-SPME) [12]. Additionally, the DI-SPME mode allows use for in vivo applications [13].

In the last 30 years, SPME extraction has been successfully applied, among others, to solvent trace analysis [14], determination of various groups of drugs [15, 16], cannabinoids [17], chemical warfare agents [18], environmental and food analysis [19, 20].

In this work, the application of DI-SPME followed by ultra-high performance liquid chromatography with a mass spectrometer is used in the case of determination of selected date-rape drugs (ketamine, benzodiazepines, cocaine) is presented. The basic validation parameters were determined and the DI-SPME/LC–MS method was fully evaluated according to the White Analytical Chemistry (WAC) principles proposed by Nowak et al. [21].

Results and discussion

Validation process

The validation process was carried out according to the methodology presented by the European Medicines Agency [22] and the Scientific Working Group for Forensic Toxicology [23], which contain general recommendations for the validation of bioanalytical methods. The following validation parameters were determined: linearity, limits of detection (LOD) and quantification (LOQ), precision (CV), recovery (RE), and matrix effect (ME). The validation parameters were determined by analyzing spiked human blood free of the tested analytes.

The linearity of the method was determined in the working range of 25–300 ng cm⁻³. The calibration curves were plotted based on the signals for samples at the 7 concentration levels: 25, 50, 100, 150, 200, 250, and 300 ng cm⁻³ of each analyte. The signal (S) was calculated as a peak area ratio of the analyte and internal standard. The parameters of the calibration curves with values of R-squared (R^2) are presented in Table 2. In the investigated working range, the R^2 values were above 0.98, only for cocaine that had a lower coefficient of about 0.96. The high values of the R^2 coefficients showed a strong linear dependence of the recorded signal on the concentration of the tested analytes.

The detection limits were estimated based on the standard deviation (SD) for three samples containing analyte at a concentration of 25 ng cm⁻³ and the slope of the calibration curve. The LOD was calculated according to the Eq. (1). The LOQ value was considered the lowest concentration of the tested linearity range for which an acceptable inter- and

Table 2 Parameters of calibration curves and values of LOD and LOQ determined for tested analytes (KET – ketamine; FLU – flunitrazepam; DIA – diazepam; TEM – temazepam; LOR – lorazepam; NIT – nitrazepam; COC – cocaine)

Parameter	Analyte						
	KET	FLU	DIA	TEM	LOR	NIT	COC
Slope/S ng cm ⁻³	0.0033	0.0015	0.0083	0.0038	0.0043	0.0127	0.0051
Intercept/S	0.0168	-0.0130	-0.0120	-0.0571	-0.1614	0.0933	0.1655
R^2	0.9949	0.9856	0.9933	0.9893	0.9813	0.9927	0.9687
LOD/ng cm ⁻³	4.9	1.4	3.8	2.9	2.0	0.6	3.5
LOQ/ng cm ⁻³	50.0	25.0	25.0	25.0	25.0	25.0	25.0

intra-day precision values were obtained. The values of LOD and LOQ are summarized in Table 2.

$$LOD = 3.3 \times \frac{SD}{slope} \quad (1)$$

The developed procedure allowed the detection of the analyzed drugs at the level of a few ng cm^{-3} in blood samples. The obtained values of LOD and LOQ for each analyte were satisfactory.

The precision and recovery of the method were determined for three concentration levels: 50 ng cm^{-3} (low concentration), 150 ng cm^{-3} (medium concentration), and 300 ng cm^{-3} (high concentration). The values of the coefficients of variation (CV%) for intra-day precision were determined for the analyses of the three samples at each tested level. The analysis of each sample was repeated three times. The inter-day precision was evaluated based on the repeating of the analysis of three samples on three consecutive days. Moreover, the inter-day precision was evaluated for LOQ level of each analyte. The recovery of the method was calculated for the same concentration levels as for precision. The analysis of four samples for each level was performed and the results of the analysis were compared with the obtained calibration curve and expected concentration to evaluate RE values of the method. The precision and recovery values are summarized in Table 3. The intra-day and inter-day precision of the method for tested concentration levels were in the range of 0.87–10.7% and 4.96–16.1%,

respectively. According to the adopted criteria [23], the CV value should not exceed 15%, which was achieved. Only in the case of inter-day precision for the determination of ketamine at the concentration at 50 ng cm^{-3} , the value exceeds 15% (CV = 16.1%). The criteria allow for the precision of determinations be higher than 15% (up to 20%) for the lowest point of calibration range, therefore in case of ketamine, concentration at 50 ng cm^{-3} was recognized as LOQ. The precision for LOQs for other analytes were lower than 20%. Therefore, it could be concluded that the precision values obtained for all drugs tested were satisfactory. The matrix effect was investigated at 3 concentration levels based on the analysis of spiked blood samples collected from 6 different sources. The values of ME% were in the range of 81.7–116.5%, which were satisfactory. The DI-SPME/LC-MS method could be considered as precise and accurate.

White analytical chemistry assessment

The DI-SPME/LC-MS used in the case of the determination of selected date-rape drugs in blood samples was assessed on the basis of the 12 WAC principles [21]. The WAC approach covers the issues of Green Chemistry (GC) and also takes into account aspects of the quality of the analytical method, practical and economical use. The method presented in this publication was compared with another methods determined for the quantitative analysis of date-rape drugs. The selected methods for WAC principles assessment were based on different sample preparation procedures: the dried blood spot

Table 3 Validation parameters of the DI-SPME/LC-MS method

Parameter	Analyte						
	KET	FLU	DIA	TEM	LOR	NIT	COC
Precision, CV/%:							
Intra-day at LOQ level ¹	4.13	8.35	8.96	14.4	9.29	10.6	8.36
Intra-day (n = 9)							
50 ng cm^{-3}	4.13	5.36	3.94	7.83	7.74	10.7	8.13
150 ng cm^{-3}	4.80	1.92	3.77	1.18	4.17	0.87	4.85
300 ng cm^{-3}	7.65	3.52	2.75	4.12	2.87	3.17	5.37
Inter-day (n = 27)							
50 ng cm^{-3}	16.1	12.7	11.4	9.03	10.7	13.1	10.3
150 ng cm^{-3}	4.96	5.34	8.23	11.4	12.9	13.7	9.32
300 ng cm^{-3}	12.1	8.49	5.97	7.84	10.7	7.13	7.64
Recovery, RE/% (n = 12):							
50 ng cm^{-3}	98.2	105.6	101.9	97.3	94.6	100.0	98.0
150 ng cm^{-3}	98.6	102.1	99.0	106.7	103.1	99.9	104.9
300 ng cm^{-3}	98.5	102.1	98.9	101.2	100.5	102.9	102.5
Matrix effect, ME/% (n = 6)							
50 ng cm^{-3}	81.7	95.7	83.9	90.6	116.5	104.2	99.1
150 ng cm^{-3}	81.8	114.4	88.4	112.9	103.7	110.7	98.1
300 ng cm^{-3}	106.7	102.5	105.7	88.1	107.2	107.5	97.9

¹LOQ = 50 ng cm^{-3} for ketamine; LOQ = 25 ng cm^{-3} for other analytes

method coupled with microwave-assisted extraction (DBS/MAE/LC–MS method) [24]; the “QuEChERS” dispersive SPE procedure (QuEChERS/UPLC-MS/MS method) [10]; dispersive liquid–liquid microextraction (DLLME/LC–MS/MS method) [6]; microextraction by packed sorbent (MEPS/ESI-QqTOF-MS method) [8]; solid phase extraction (SPE/microLC–ESI–sMRM) [9].

The final result of the WAC assessment is presented in Table 4. A calculation sheet with the evaluation of methods according to the WAC methodology is included as supplementary information (Supplementary file1). The details of the evaluation of all WAC principles and the procedure for justifying them were as follows. R1: Scope of application—all methods had a similar scope of application. However, some evaluated procedures enable the determination of more substances and these methods scored higher. The SPE procedure received less points due to the fact that it was developed only for ketamine and its metabolites determination. R2: LOD and LOQ – this principle was evaluated mainly on the LOQ values due to the fact that these values were confirmed experimentally. All procedures received similar amounts of points, the DBS/MAE procedure scored the lowest, because the obtained LOQ values were significantly higher than for other procedures. R3: Precision and R4: Accuracy—was evaluated on the basis of the fact if these values are acceptable for bioanalysis. All methods met the satisfactory criteria, therefore received 100 points, except the QuEChERS procedure for which the precision for selected benzodiazepines was not satisfactory (procedure received 90 points). G1: Toxicity of reagents—the methods were ranked based on using toxic or non-toxic reagents and organic or water

solutions. G2: Amount of reagents and waste—different numbers of points for compared methods were awarded on the basis of the reagent consumption that was used during the sample preparation step. G3: Consumption of energy and other media—evaluation of these principles required knowledge of the specifications of the used apparatus. Due to the lack of data, the impact of this issue was not assessed. G4: Direct impacts—the evaluated methods were considered as safe for personnel, all methods were awarded 100 points (the methods do not pose any additional hazards). B1: Cost efficiency—evaluated based on the estimated total cost of the analysis. The cost of analysis was estimated for all methods based on the used reagents and materials for sample preparation. The scores were influenced by whether specific reagents were used and whether the used materials could be reused. B2: Time efficiency—the compared methods were ranked based on the estimated time of perform sample preparation procedure. B3: Requirements—the volume of the used sample was mainly influenced by requirements assessment. The sample consumption for the DBS method was 4 times smaller than for the SPME method. Therefore, sample volume in this method was still lower than in DLLME or QuEChERS procedures. B4: Operational simplicity—the assessed procedures were not fully miniaturized or automated. However, the potential of these aspects was estimated and evaluated.

Based on the results of the evaluation of 12 WAC principles, the analytical quality (red) of assessed methods was found to be similar. There are differences between the methods in the case of LOD and LOQ. The higher LOD and LOQ values were obtained for DBS/MAE procedure. The

Table 4 The evaluation of the selected methods by the WAC approach

WAC principle	Extraction procedure					
	DI-SPME	DBS/MAE	QuEChERS	DLLME	MEPS	SPE
R1: Scope of application	90.0	100.0	100.0	100.0	90.0	60.0
R2: LOD and LOQ	90.0	80.0	100.0	100.0	90.0	100.0
R3: Precision	100.0	100.0	90.0	100.0	100.0	100.0
R4: Accuracy	100.0	100.0	100.0	100.0	100.0	100.0
Red/%	95.0	95.0	97.5	100.0	95.0	90.0
G1: Toxicity of reagents	100.0	70.0	70.0	100.0	80.0	90.0
G2: Amount of reagents and waste	100.0	75.0	75.0	90.0	90.0	60.0
G3: Consumption of energy and other media	100.0	100.0	100.0	100.0	100.0	100.0
G4: Direct impacts	100.0	100.0	100.0	100.0	100.0	100.0
Green/%	100.0	86.3	86.3	97.5	92.5	87.5
B1: Cost efficiency	85.0	85.0	90.0	100.0	85.0	80.0
B2: Time efficiency	90.0	90.0	95.0	100.0	80.0	85.0
B3: Requirements	90.0	100.0	70.0	80.0	90.0	90.0
B4: Operational simplicity	90.0	70.0	65.0	73.0	100.0	80.0
Blue/%	88.8	86.3	80.0	88.3	88.8	83.8
Whiteness/%	94.6	89.2	87.9	95.3	92.1	87.1

best greenness (green) of the method was achieved for the DI-SPME/LC–MS method. This is due to the use of less toxic and more aqueous reagents. However, DLLME procedure received also very high rate which was caused by reduction of using organic solvents. The economic issues (blue) of evaluated methods seem to be very similar. The consumption of sample (B3) to perform the analysis had the highest impact on the assessment of economic issues. The selected methods required wide range of sample volume—from 50 mm³ (DBS/MAE procedure) to 1000 mm³ (QuEChERS procedure).

Individual comparison of each principal could indicate when the application of which analytical method would be more appropriate for the purpose. The performed WAC assessment enables to choose the best method based, for example, on the sample volume or the expected concentration of the analyte. The evaluation could be also helpful in finding the advantages and disadvantages of the developed method in relation to other methods routinely used in the same laboratory or published in the literature. In the case of performed evaluation, it can be underlined that presented DI-SPME/LC–MS compared to other methods used fewer reagents and enable reduction of organic and toxic solvents (method can be assumed as green and eco-friendly). However, the results indicate that the analytical parameters of the method could be improved in future research.

Conclusion

The tested DI-SPME/LC–MS method could be successfully used in the quantitative analysis of selected substances from the date-rape drug group in blood samples. The estimated validation parameters indicated that it was possible to detect and determine the tested analytes even at concentrations of few ng cm⁻³ in the sample. The precision of the determinations was satisfactory at each tested concentration level. The results of the validation process indicate that this method was precise and accurate for the tested drugs.

Evaluation of the method using the WAC approach was used to determine the advantages and disadvantages of the DI-SPME/LC–MS method in relation to the other sample preparation procedure, which were used for the determination of the same analytes as in the presented study. In general result, the SPME extraction seemed to be more suitable for the implementation of Green Chemistry assumptions. The use of SPME extraction allowed to reduce the usage of toxic reagents. The tested method obtained satisfactory results for aspects related to analytical quality, greenness, and economical use. The DI-SPME/LC–MS method can be successfully used for forensic toxicology analysis. The possibility of ex vivo analysis enables the performance of the

SPME extraction procedure for blood sample taken from the rape victim in the crime scene investigation.

Experimental

Chemicals and Materials

The following reagents and standard substances were used. Hypergrade reagents for LC–MS: acetonitrile and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade formic acid was provided by Merck (Darmstadt, Germany). Ultrapure water (< 18.2 MΩ cm⁻¹, TOC < 5 ppb) was produced using the Mili-Q Plus system from Millipore (Bedford, MA, USA). Standard substances (1 mg cm⁻³ in methanol) of ketamine, flunitrazepam, diazepam, nitrazepam, lorazepam, temazepam, and cocaine, as well as their selected deuterated forms, which were used as internal standards: flunitrazepam-*d*₃, diazepam-*d*₅, and nitrazepam-*d*₅ were purchased from Lipomed AG (Arlesheim, Switzerland).

During the experiments, the following laboratory equipment devices were used. The HPLC vials (1.5 cm³) and inserts (200 mm³) were purchased from VWR (Radnor, PA, USA). The SPME fibers with C18-SiO₂ (*d* = 45 μm) coating (Supelco) were provided by Merck (Darmstadt, Germany).

Apparatus and conditions

The following devices were utilized during the experiments. The Digital Vortex Mixer and Thermal Shake Toucher were purchased from VWR (Radnor, PA, USA). The vacuum concentrator, Concentrator Plus, was provided by Eppendorf AG (Hamburg, Germany). The UHPLC process was performed using the Ultimate 3000 RS system (Dionex, Sunnyvale, CA, USA) with Hypersil Gold Phenyl column (50 × 2.1 mm, 1.9 μm) provided by Thermo Scientific (Bremen, Germany). The UHPLC system was coupled to a mass spectrometer (MS) with electrospray ionization (ESI) and time-of-flight analyzer (TOF) purchased from Bruker (Bremen, Germany). Collection and data processing was performed using Chromeleon 6.8 (Dionex), HyStar 3.2, MicrTOFcontrol, and Compass Data Analysis (Bruker). The masses of extracted ions of analytes and internal standards were calculated using IsotopePattern software (Bruker).

The settings of the mass detector, the gradient program, and the composition of the mobile phase were chosen based on previous research on psychoactive substances [25]. The mobile phase consisted of 0.1% formic acid in water (phase A) and acetonitrile (phase B). Separation was carried out in a gradient program. The mobile phase flow rate was 0.3 cm³ min⁻¹ and the column temperature was set to 35 °C during the entire measurement. Eluents A and B were

mixed during the analysis according to the following gradient. First, the content of eluent B increased from 15 to 40% (0.0–4.0 min). For the next 3 min, the content of eluent B remained constant at 40% (4.0–7.0 min) and then increased to 70% in 3 min (7.0–10.0 min). Then, the content of eluent B was decreased to 15% in 2.5 min (10.0–12.5 min) and held for 4.5 min (12.5–17.0 min) to stabilize the column before the next injection. The injection volume was 5 mm³.

Analysis was carried out with the use of a mass spectrometer. The ionization process was carried out using an ESI system with positive ionization mode, a voltage of 4.5 kV, a nebulizer pressure of 2.5 bar, a gas flow rate of 5.5 dm³ min⁻¹, and a gas temperature of 200 °C. The ions were monitored in the range of $m/z = 50$ –800.

Blood sample collection

Human blood, without tested analytes, was purchased from a local blood bank (Cracow, Poland). Blood was stored in the freezer at –20 °C.

Spiked blood sample preparation

Blood samples with analytes were prepared one day before analysis. The appropriate volumes of mixes of analytes and internal standards in methanol were pipetted into 1.5 cm³ Eppendorf vials. The contents of the vials were then evaporated with the use of a vacuum concentrator at a temperature of 45 °C. Then, the unspiked blood was added to the vials. The contents of the vials were then mixed with the use of a vortex for 7 min at a speed of around 2100 RPM. Finally, the spiked blood samples were stored in a freezer until analysis.

Extraction procedure

The DI-SPME extraction procedure was performed based on previous research by Majda et al. [16]. The procedure was as follows. First, the SPME fiber was conditioned for 45 min in methanol:water (1:1, v/v) solution. Then, 200 mm³ of the spiked blood sample was pipetted into the insert, which was placed into a 1.5 cm³ HPLC vial. After the conditioning step, the SPME fiber was placed directly into the blood sample. The content was vortexed for 60 min at a speed of 2200 RPM. The SPME fiber was then removed and carefully cleaned by being wiped with a dust-free tissue and washed in ultrapure water for 5 s with the vortex at a speed of 2100 RPM. After the cleaning step, the desorption process was performed. The fiber was placed in a desorption solution of acetonitrile:methanol:0.1% formic acid in water (2:2:1, v/v/v) and vortexed for 30 min at a speed of 2200 RPM. Subsequently, the fiber was removed and the solvent was evaporated in a vacuum concentrator for 45 min at a temperature of 45 °C. Finally, 50 mm³ of 0.1% formic acid

(eluent A) was added to the residue in the vial. The resultant solution was then used for LC–MS analysis.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00706-022-02962-4>.

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References

- Grela A, Gautam L, Cole MD (2018) *Forensic Sci Int* 292:50
- Busardò FP, Vari MR, Trana ADI, Malaca S, Carlier J, Luca NMDI (2019) *Eur Rev Med Pharmacol Sci* 23(24):10577
- García MG, Pérez-Cárceles MD, Osuna E, Legaz I (2021) *J Forensic Leg Med* 79:102151
- Sauve EN, Langødegård M, Ekeberg D, Øiestad AML (2012) *J Chromatogr B Anal Technol Biomed Life Sci* 883:177
- Adamowicz P, Kała M (2010) *Forensic Sci Int* 198(1–3):39
- Fisichella M, Odoardi S, Strano-Rossi S (2015) *Microchem J* 123:33
- De Boeck M, Missotten S, Dehaen W, Tytgat J, Cuyppers E (2017) *Forensic Sci Int* 274:44
- Vejar-Vivar C, Bustamante L, Lucena R, Ortega C, Valenzuela M, Mardones C (2021) *Microchem J* 171:106797
- Kurzweil L, Danyeli L, Şen ZD, Fejtova A, Walter M, Gensberger-Reigl S (2020) *J Chromatogr B Anal Technol Biomed Life Sci* 1152:122214
- Anzillotti L, Odoardi S, Strano-Rossi S (2014) *Forensic Sci Int* 243:99
- Andrade-Eiroa A, Canle M, Leroy-Cancellieri V, Cerdà V (2016) *TrAC - Trends Anal Chem* 80:641
- Souza-Silva ÉA, Jiang R, Rodríguez-Lafuente A, Gionfriddo E, Pawliszyn J (2015) *TrAC - Trends Anal Chem* 71:224
- Lord HL, Zhang X, Musteata FM, Vuckovic D, Pawliszyn J (2011) *Nat Protoc* 6.6:896
- Camarasu CC (2000) *J Pharm Biomed Anal* 23(1):197
- Huang W, Shao W, Ji Y, Li H, Chen J, Lin Z (2022) *Talanta* 243:123341
- Majda A, Mrochem K, Wietecha-Postuszny R, Zapotoczny S, Zawadzki M (2020) *Talanta* 209:120533
- Anzillotti L, Castrignanò E, Rossi SS, Chiarotti M (2014) *Sci Justice* 54(6):421
- Nawała J, Czupryński K, Popiel S, Dziedzic D, Bełdowski J (2016) *Anal Chim Acta* 933:103
- Tuzen M, Altunay N, Hazer B, Mogaddam MRA (2022) *Food Chem* 369:130973
- Werner J, Zgoła-Grzeškowiak A, Grzeškowiak T (2022) *J Sep Sci* 45(7):1374

21. Nowak PM, Wietecha-Posłuszny R, Pawliszyn J (2021) *TrAC - Trends Anal Chem* 138:116223
22. Smith G (2012) *Bioanalysis* 4(8):865
23. Scientific working group for forensic toxicology (2013) *J Anal Toxicol* 37(7):452
24. Stelmaszczyk P, Gacek E, Wietecha-Posłuszny R (2021) *Separations* 8(12):249
25. Wietecha-Posłuszny R, Lendor S, Garnysz M, Zawadzki M, Koscielniak P (2017) *J Chromatogr B Anal Technol Biomed Life Sci* 1061:459

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