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Solidified floating organic drop microextraction procedure based on deep eutectic solvent for the determination of melatonin in pharmaceuticals and dietary supplements

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ABSTRACT

A miniaturized clean-up and preconcentration procedure involving deep eutectic solvent-based solidified floating organic drop microextraction was developed for the determination of melatonin in pharmaceuticals and dietary supplements by high-performance liquid chromatography with UV detection. Melatonin is widely used for the treatment of a large spectrum of diseases, and many studies have focused on its efficacy in reducing COVID-19 severity. For the first time, various hydrophobic deep eutectic solvents based on menthol, medium-chain fatty acids, and long-chain alcohols were studied for the microextraction of melatonin. Among the studied solvents, the deep eutectic solvent based on menthol and heptanoic acid provided the highest extraction recovery (90 %). In the developed procedure, a flat magnetic stirrer bar was covered by a microliter amount of the deep eutectic solvent and the sample solution was added under magnetic stirring. In this case, the deep eutectic solvent phase was easily dispersed into the aqueous phase without the use of any organic disperser solvents, resulting in fast analyte extraction (1 min). In the absence of stirring, the aggregation of extract as a floating drop on the surface of the aqueous phase was observed immediately. The low melting/freezing point and low density of the extraction solvent compared with water allowed one to quickly and easily retrieve a low volume of extract (25 μL) in a microextraction procedure by solidification. Validation of the procedure showed that limits of detection and quantification, calculated from the blank tests based on 3σ and 10 σ, were 0.003 mg g^{-1} and 0.01 mg g^{-1}, respectively.

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is an indolamine hormone derived from tryptophan and secreted by the pineal gland of vertebrates. Melatonin is considered a kind of regulator of circadian rhythms that affect most living organisms' physiology, behavior, and metabolism, synchronizing the internal hormonal background and the daily cycle [1]. In the last decade, melatonin has been used for the treatment of the large spectrum of diseases, mainly in sleep disturbances and tumors [2]. Moreover, many studies focused on its efficiency in reducing COVID-19 severity [3]. Currently, many countries produce pharmaceuticals and dietary supplements containing melatonin [4]. Since melatonin is highly bioactive it is necessary to provide quality control of melatonin-containing products.

For the determination of the melatonin in pharmaceutical samples chromatographic [5,6], capillary electrophoresis [7], spectrofluorimetric [8], and electrochemical [9] methods have been utilized (Table 1). Typically, the sample preparation procedures include a dissolution of solid-phase samples in relatively high volume (10–50 mL) of organic solvents such as ethyl acetate, methanol, and ethanol to reduce and eliminate the interferences originally present in the samples. Such procedures are in contradiction to the principles of sustainable "green" practices in modern pharmaceutical laboratories. To provide fast and green quality control of pharmaceuticals and dietary supplements and ensure the safety of manufactured medicine products the development of miniaturized analytical procedures is required.

One of the trends in modern pharmaceutical analysis is the introduction of green solvents to the analytical procedures as alternative

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Table 1
Analytical procedures reported for melatonin determination in pharmaceutical samples.

Detection method	Sample preparation	Solvent type	Sample preparation time, min	Solvent volume, mL	LOD, μg L^{-1}	Reference
GC–MS	Dissolution, solvent evaporation for preconcentration	Ethyl acetate	25	10	0.02	[5]
Spectro- fluorimetry	Dissolution under sonication	Ethanol:water (1:1)	10	50	10	[8]
Voltammetry	Dissolution	Ethanol	–	–	200	[9]
Capillary electrophoresis	Dissolution	- Phosphate buffer solution	10	25	300	[7]
HPLC-UV	Dissolution	Methanol	–	10	3220	[6]
HPLC-UV	Dissolution under sonication, DES-based SFODME	DES based on menthol and heptanoic acid	25	0.025	3 (0.003 mg g^{-1})	This work

HPLC-UV – high performance liquid chromatography with ultraviolet detection; GC–MS – gas chromatography with mass spectrometry detection; DES – deep eutectic solvent; SFODME – solidified floating organic drop microextraction.

solvents to hazardous one [10]. Among other solvents, deep eutectic solvents (DESs) have been assigned to new generation green solvents used for clean-up and preconcentration [11,12]. Typically, DESs are viscous liquids formed by mixing two or three components that are associated within hydrogen bond interactions using a hydrogen bond donor and a hydrogen bond acceptor to obtain an eutectic mixture [13–16]. Indeed, by changing the nature and ratio of their components, DESs can be adapted for extraction of wide a range of analytes. From this point of view, DESs can be considered as «designed» extraction solvents. Usually, components used for the synthesis of DESs are inexpensive, non-toxic, and often biodegradable [17–19]. Additionally, DESs have beneficial characteristics, such as high thermal stability, negligible vapor pressure, and non-flammability [20,21]. Despite the relatively high viscosity of DESs fast mass-transfer can be obtained by intensive phase mixing, ultrasonication, and in the presence of disperser solvents and emulsifier agents [22–25]. DESs have been utilized for effective separation of target substances from liquid- and solid-phase pharmaceutical samples [26].

However, mentioned DES-based sample preparation procedures have several drawbacks. One problem is associated with the incompatibility of hydrophobic DESs with chromatographic separation in water-polar organic solvent mixtures and MS-detection. Usually, DES-based extracts are diluted by polar solvent before chromatographic analysis or back extraction of target substances in an aqueous phase is required to introduce into a MS-detector [27]. Another problem is associated with the relatively high viscosity and low density of DESs. Such features of the solvents make the microliter amount of extract collection difficult and unreproducible. To exclude this drawback a solidified floating organic drop microextraction (SFODME) has been proposed [28]. This approach assumes an application of a small volume of an extraction solvent with melting point near room temperature. Application of polar, water-miscible disperser solvents in SFODME promotes the formation of a large surface area between aqueous and DES phases, resulting in the formation of a cloudy solution and fast mass-transfer. The drop is floated on the surface of an aqueous solution, solidified after extraction and manually picked up for subsequent analysis [29]. DES-based SFODME procedures have been found in application for the separation of various analytes from water [30], foods [31], urine and plasma samples [28,32]. To the best of our knowledge, DES-based SFODME has not yet been used for clean-up and preconcentration in pharmaceutical analysis. Moreover, the extraction of melatonin in DESs has not yet been presented in the literature.

The purpose of the work was to develop a new approach for miniaturized sample pretreatment in pharmaceutical analysis that assumes fast separation of the target analyte from sample matrix into low volume of DES without any emulsifier agents and disperser solvents. In the present research, a miniaturized clean-up and preconcentration procedure assumed DES-based SFODME was developed for the determination of melatonin in pharmaceuticals and dietary supplements by the high-performance liquid chromatography with UV detection (HPLC-UV). For separation of melatonin from aqueous samples hydrophobic DESs based on menthol, medium chain fatty acids, and long-chain alcohols were studied for the first time.

2. Experimental

2.1. Reagents and solutions

All chemicals and reagents were of analytical grade. Ultra-pure water from Millipore Milli-Q RG system (Millipore Corporation, Bedford, USA) was used throughout the work. Medium chain fatty acids (hexanoic acid, heptanoic acid, and octanoic acid), long-chain alcohols (1-hexanol, 1-heptanol, 1-octanol), phosphoric acid, menthol and melatonin were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). Acetonitrile was purchased from J.T. Baker Chemical Company (Phillipsburg, New Jersey, USA). A stock solution of melatonin (100.0 mg L^{-1}) was prepared by dissolving the reagent in 0.1 % phosphoric acid solution and it was stored in a refrigerator (5 °C) and used within 2 weeks. Working analyte solutions were prepared daily by appropriate dilutions of the stock solution with 0.1 % phosphoric acid solution.

The DESs were prepared according to [33] by mixing long-chain alcohol (1-hexanol, 1-heptanol, 1-octanol) or medium chain fatty acid (hexanoic acid, heptanoic acid, and octanoic acid) with menthol in 3:1, 2:1, 1:1, 1:2, 1:3 M ratios under heating and stirring at 50 °C until clear liquids were formed. After cooling, the DESs were stored at room temperature and used for the DES-based SFODME procedure.

2.2. Instrumentation

A Shimadzu LC-20 Prominence liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) with UV detection equipped with a Luna C18 (2) column (250 mm × 4.6 mm, 5 μm; Phenomenex, USA) was used for the melatonin determination.

A Fourier transform-infrared IR Prestige-21 spectrometer (Shimadzu, Japan) was used for the characterization of DES.

A laboratory IKA RH digital stirrer (IKA, Germany) was used for the DES-based SFODME.

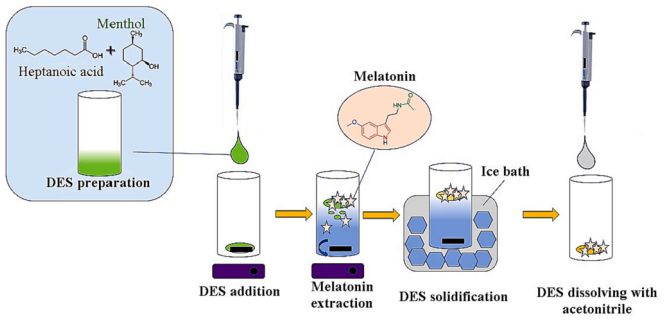

Fig. 1. A deep eutectic solvent-based solidified floating organic drop microextraction procedure for melatonin separation.

2.3. Samples and sample preparation

Pharmaceuticals and dietary supplements containing melatonin were purchased in a local pharmacy (St. Petersburg, Russia). Before analysis, each table of the samples was weighted and homogenized in a mortar to a powder state. After, total amount of the powdered table was dissolved in 200 mL of phosphoric acid solution (0.1 %) under sonication for 25 min at 30 °C. Then, 10 mL of the sample solution was filtered through a hydrophilic filter (0.45 μm).

2.4. Microextraction procedure

In a 10 mL vial, the flat magnetic stirrer bar was placed and 25 μL of DES (heptanoic acid-menthol, molar ratio 3:1) was spread on the magnetic stirrer bar (Fig. 1). The magnetic stirrer was switched on and 2 mL of the sample solution was inserted into the vial. Immediately the DES droplets were dispersed into the sample solution and the extraction of the analyte for 1 min was performed with stirring rate of 150 rpm. Then, the magnetic stirrer was switched off and the aggregation of DES as the floating drop on the surface of sample solution was observed immediately. The vial was transferred to an ice bath for 5 min to provide the solidification of the extract drop. After that, the aqueous phase was withdrawn. The DES phase contained menthol that has limited solubility in the used mobile phase. To increase the solubility of menthol in the mobile phase the dilution of extract in the acetonitrile (100 μL) was required. The resulting solution was directly injected into the HPLC-UV system.

2.5. HPLC-UV procedure

The chromatographic system was operated at 40 °C. The injection volume was 20 μL. The gradient elution program was carried out as follows: mobile phase of 0.1 % phosphoric acid (A) and acetonitrile (B) at a flow-rate of 1.0 mL min^{-1}. The concentration of solvent B was 30 % to 7 min, increased linearly to 90 % from 7 to 8 min and kept constant to 11 min, then decreased to 30 % to 20 min and kept constant to 25 min. The analytical wavelength was 278 nm.

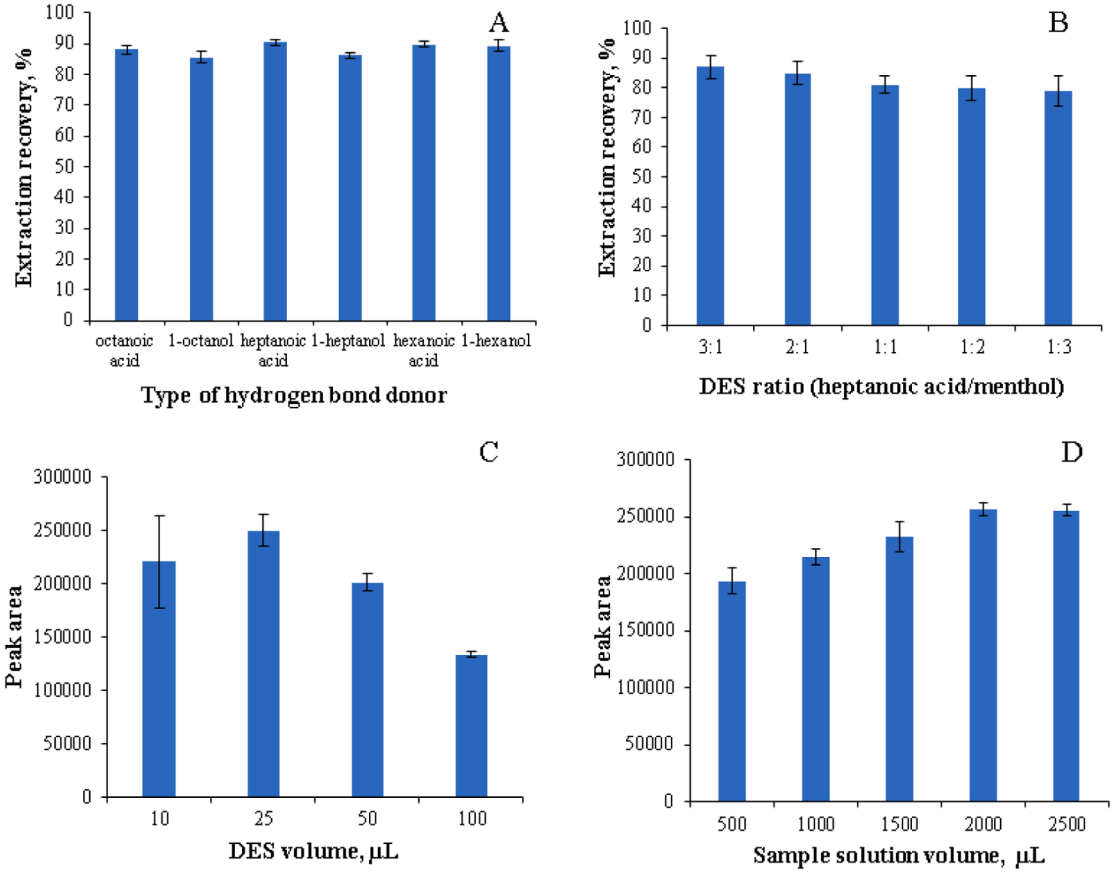

Fig. 2. Optimization of microextraction (Cmelatonin = 10 mg L^{-1}, n = 3): (A) Effect of hydrogen bond donor type (Vsample = 1000 μL, VDES = 100 μL, hydrogen bond donor/menthol molar ratio of 2:1); (B) Effect of DES components ratio (Vsample = 1000 μL, VDES = 100 μL); (C) Effect of DES volume (Vsample = 1000 μL, heptanoic acid/menthol molar ratio of 3:1); (D) Effect of sample solution volume (VDES = 25 μL, heptanoic acid/menthol molar ratio of 3:1).

3. Results and discussion

3.1. Preliminary studies

In this research hydrophobic DESs based on menthol, medium chain fatty acids, and long-chain alcohols were studied for the extraction of melatonin. Natural monoterpenoid (menthol) was chosen as the hydrogen bond acceptor for DES-based SFODME since it has low melting point (39–40 °C [34]). Hexanoic, heptanoic, and octanoic acids, as well as 1-hexanol, 1-heptanol, and 1-octanol were chosen as the hydrogen bond donors for hydrophobic DESs synthesis because such hydrogen bond donors provide formation of liquid at room temperature DESs [19]. In the preliminary studies, all DESs were prepared at the hydrogen bond donor/menthol molar ratio of 2:1.

The nature of DES precursor can affect the extraction efficiency. To study the effect of DES composition 1000 μL of working melatonin solution (10 mg L^{-1}) was mixed with 100 μL of DES. Extraction mixture was stirred for 10 min, centrifugated and upper organic phase was withdrawn for analysis. It was found that melatonin can be extracted from all studied DESs. Extraction recovery values were in the range of 85 to 90 % (Fig. 2 A). Melatonin is a relatively polar molecule (log Kow = 1.18) and its mass-transfer into the most hydrophobic DESs based on octanoic acid (log Kow = 3.05 [35]) and 1-octanol (log Kow = 3.00 [35]) was less effective. More polar precursors such as hexanoic acid (log Kow = 1.88 [35]) and heptanoic acid (log Kow = 2.41 [35]) allowed to slightly increase in the extraction efficiency. At the same time, heptanoic acid is less soluble in water than hexanoic acid, which ensured the higher stability of DES in the aqueous phase. The stability of DES is especially important for SFODME assumed the use of the microliter amounts of extractants. Thus, for further experiments DES based on menthol and heptanoic acid was chosen due to its stability in the aqueous phase and excellent extraction properties.

To confirm the DES formation, the FT-IR spectra of pure menthol, heptanoic acid, and DES were obtained. The O—H and C—O vibrations of pure menthol are positioned at 3243 cm^{-1} and 1044 cm^{-1}, respectively. In the FT-IR spectrum of pure heptanoic acid, absorptions belonging to O—H (3044 cm^{-1}) and C═O (1705 cm^{-1}) were observed. The characteristic peaks of both heptanoic acid and menthol are presented in the DES spectrum. In this spectrum, the O—H vibration of pure heptanoic acid and menthol was not observed. This may be due to the transfer of electrons from the oxygen atom to the hydrogen bond leading to a decrease in the force constant. Thus, this proves the existence of a hydrogen bond between heptanoic acid and menthol when the DES is formed.

3.2. The microextraction procedure optimization

3.2.1. Effect of DES components ratio

On the one hand, molar ratio of heptanoic acid and menthol in DES phase effected on DES hydrophobicity and its affinity to melatonin. On the other hand, menthol as a component with melting point near room temperature effected on solidification temperature of DES phase.

Initially, the effect of molar ratio of heptanoic acid and menthol in DES phase on the extraction recovery was investigated. For this, the molar ratio of heptanoic acid and menthol was varied from 1:3 to 3:1 at constant volumes of working melatonin solution (10 mg L^{-1}, 1000 μL) and DES (100 μL). The highest extraction recovery values were observed for DESs with the heptanoic acid and menthol molar ratios of 2:1 and 3:1 (Fig. 2 B). For further experiments DES based on menthol and hexanoic acid at molar ratios of 3:1 was chosen due to less contain of menthol and its less effect on chromatographic separation.

The solidification of obtained extracts at the molar ratio of 3:1 (heptanoic acid menthol) was studied. It was found that the DES solidification was observed in a conventional ice bath for 5 min.

3.2.2. Effect of DES volume

To achieve less extraction solvent consumption its volume was optimized. DES volume was varied from 10 to 100 μL and volume of the working melatonin solution (10 mg L^{-1}, 1000 μL) was constant. At DES volume of 10 μL, low reproducibility of results was observed (RSD 20 %), while further increasing of DES volume resulted in increase of extract phase volume and dilution effect. The minimum RSD (<5%) and the maximum analytical signal were observed for 25 μL of DES (Fig. 2 C).

3.2.3. Effect of sample solution volume

The sample solution volume affects on phase ratio and obtained sensitivity. The volume of the aqueous melatonin solution was varied from 500 to 2500 μL and DES volume (25 μL) was constant. The analytical signal was increased with the increase of sample solution volume. It was established that sample solution volumes of 2000 and 2500 μL provided comparable extraction efficiency (Fig. 2 D). Thus, the volume of the aqueous phase (2000 μL) was chosen as optimal and focused on minimum waste generation.

3.2.4. Effect of extraction time

To obtain maximum extraction recovery an extraction equilibrium should be achieved. Intensive stirring of the extraction system was utilized to increase mass-transfer and obtain the extraction equilibrium. The extraction system stirring was carried out from 1 to 10 min with stirring rate of 150 rpm. The data obtained indicated that the stirring for 1 min was enough to approach equilibrium.

3.3. Validation

For validation, the following parameters were evaluated: linearity and sensitivity, precision, accuracy, and recoveries. The extraction recovery was equal to 90 %.

3.3.1. Linearity and sensitivity

Under the optimized condition of DES-based SFODME procedure, the calibration curve for the melatonin determination was constructed from ten data points using the standard solution of the analyte. The calibration graph was linear over the concentration range of 0.1–150 mg L^{-1} (0.01–15 mg g^{-1}) with a regression coefficient of 0.999. The sensitivity was characterized by the limit of detection (LOD). It was measured by the standard IUPAC method as 3•standard deviation of the blank (3 s). The LOD was 0.003 mg g^{-1}. The limit of quantification was measured as 10•standard deviation of the blank (10 s) and was 0.01 mg g^{-1}.

3.3.2. Precision

The precision of the procedure was evaluated concerning its repeatability and reproducibility.

The repeatability of the developed procedure was determined by analyzing of 5 replicates of solutions with different melatonin concentrations (0.1 mg L^{-1} or 150.0 mg L^{-1}). The RSD values were < 4.0 % and 1.0 % for 0.1 and 150.0 mg L^{-1} of melatonin, respectively.

The reproducibility was assessed by applying the developed procedure with the usage of 2 different HPLC-UV systems at 2 different times. Results obtained with the laboratory-to-laboratory and day-to-day variations were found to be reproducible because the RSD values under conditions were < 7.0 % and 5.0 % for 0.1 and 150.0 mg L^{-1} of melatonin, respectively.

This level of precision is typical for HPLC-UV analysis of pharmaceutical samples.

3.3.3. Accuracy

The available solid dosage form of pharmaceuticals and dietary supplements were analyzed by the proposed procedure and by reference procedure [36]. The obtained results represent no significant differences in suggested and reference procedures (Table 1). Student's *t*-test of statistical hypotheses about the equality of the means was used to test

Table 2
Determination of melatonin in pharmaceuticals and dietary supplements samples (n = 3, Fcr. = 99,01, tcr. = 4.60, P = 0.99).

Sample	Label concentration value, mg g^{-1}	Found concentration, mg g^{-1}		F-test	*t*-test	Relative recovery, %
		Developed procedure	Reference procedure			
Dietary supplement 1	8.3	8.6 ± 0.4	9.1 ± 0.2	2.14	4.37	104 ± 4
Dietary supplement 2	8.3	7.1 ± 1.4	8.4 ± 0.5	4.91	4.37	85 ± 14
Pharmaceutical 1	12.0	11.8 ± 1.8	10.2 ± 0.9	3.80	3.57	98 ± 4
Pharmaceutical 2	0.3	0.32 ± 0.05	0.30 ± 0.02	5.32	1.43	105 ± 16

the equality of the means of the experimental data obtained by developed procedure and by the reference procedure. Hypothesis about the equality of the means of the experimental data obtained by the developed procedure and by the reference procedure was taken as the null hypothesis (H0). Hypothesis about the difference between mentioned means was taken as an alternative hypothesis (H1). The significance was taken equal to the 0.05 level. Based on the obtained results, the observed differences were not contrary to hypothesis H0 and the obtained discrepancies with the 0.05 significance level could be considered insignificant.

3.3.4. Recovery

Relative recovery values were determined by comparison of the received result of melatonin content and those to be claimed and indicated in a pharmaceutical instruction sheet (label concentration value). The investigation was performed for 3 replicates of four samples. It is shown in Table 2 that relative recovery values are between 85 and 105 %.

3.3.5. Matrix effect

Pharmaceuticals and dietary supplements usually contain excipients that can have interfering effects. According to the instruction sheets studied pharmaceuticals contain cellulose microcrystalline, calcium hydrogen phosphate, sodium croscarmellose, silicon dioxide colloidal, magnesium stearate, titanium dioxide, polysorbate 80, polyethylene glycol, talcum. Dietary supplements additionally contain vitamins such as pyridoxine and thiamine. To evaluate the matrix effect, comparison of the analytical signals for extracts obtained for the standard analyte solution and sample solution (with the same analyte concentrations) was performed. To prepare the sample solutions the tablets were dissolved in 50, 100 or 200 mL of 0.1 % phosphoric acid. The matrix effect was calculated according to the formula [37]:

$$Matrix\ effect\ (\%) = \left(\frac{peak\ areas\ of\ the\ \ sample}{peak\ areas\ of\ the\ standart\ solution} - 1\right) \times 100$$

It was found that the sample solutions obtained by the pharmaceuticals/dietary supplements dissolution in 50 and 100 mL of 0.1 % phosphoric acid had a significant matrix effect (from 13 to 23 % for 50 mL and from 9 to 17 % for 100 mL). A minimum matrix effect (3–4 %) was observed when 200 mL of 0.1 % phosphoric acid was used.

4. Conclusion

The present study demonstrates a new miniaturized clean-up and preconcentration procedure for pharmaceutical analysis based on solidified floating organic drop microextraction with the use of deep eutectic solvent. Magnetic stirring of deep eutectic solvent droplets into the aqueous sample phase allows obtaining fast mas-transfer without the use any of disperser solvents and emulsifier agents. For melatonin microextraction it was shown that the phases stirring for 1 min were enough to approach equilibrium with extraction recovery of 90 %. Moreover, the solidified floating organic drop approach allowed one to retrieve low volume of extract (25 μL) reproducibly. In this research hydrophobic deep eutectic solvents based on menthol, medium chain fatty acids and long-chain alcohols were investigated for the extraction of melatonin for the first time. Deep eutectic solvent based on menthol and heptanoic acid was characterized by stability in the aqueous phase and excellent extraction properties for melatonin. The microextraction procedure for the separation of melatonin from the sample matrices was coupled with HPLC-UV method and applied to the determination of the analyte in real pharmaceutical and dietary supplement samples. The proposed procedure is rapid, cheap, and environmentally friendly and assumes low consumption of the extraction solvent.

CRediT authorship contribution statement

Zilya Yakupova: Investigation, Writing – original draft. **Artyom Yakubenko:** Investigation. **Polina Bogdanova:** Investigation. **Pavel Godunov:** Investigation. **Christina Vakh:** Methodology, Writing – original draft. **Sergey Garmonov:** Writing – review & editing. **Andrey Bulatov:** Methodology, Writing – review & editing.

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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References

[1] D. Dawson, N. Encel, Melatonin and sleep in humans, J. Pineal Res. 15 (1993) 1–12, https://doi.org/10.1111/j.1600-079X.1993.tb00503.x.
[2] Z. Xie, F. Chen, W.A. Li, X. Geng, C. Li, X. Meng, Y. Feng, W. Liu, F. Yu, A review of sleep disorders and melatonin, Neurol. Res. 39 (2017) 559–565, https://doi.org/10.1080/01616412.2017.1315864.
[3] S. Lan, H. Lee, C. Chao, S. Chang, L. Lu, C. Lai, Efficacy of melatonin in the treatment of patients with COVID-19: A systematic review and meta-analysis of randomized controlled trials, J. Med. Virol. 94 (2022) 2102–2107, https://doi.org/10.1002/jmv.27595.
[4] T.B. Grivas, O.D. Savvidou, Melatonin the "light of night" in human biology and adolescent idiopathic scoliosis, Scoliosis 2 (2007) 6, https://doi.org/10.1186/1748-7161-2-6.
[5] A. Covaci, C. Doneanu, H.Y. Aboul-Enein, P. Schepens, Determination of melatonin in pharmaceutical formulations and human plasma by gas chromatography-electron impact mass spectrometry, Biomed. Chromatogr. 13 (1999) 431–436, https://doi.org/10.1002/(SICI)1099-0801(199910)13:6<431::AID-BMC906>3.0.CO;2-O.
[6] J. Lin, C. Zhang, Y. Gao, X. Zhao, X. Li, Jian; Zhang, Chunchan; Gao, Yaoxiang; Zhao, Xiaojun; Li, A validated HPLC method for determining melatonin incapsule dosage form, Spat. DD 2 (3) (2012) 147.
[7] G. Chen, X. Ding, Z. Cao, J. Ye, Determination of melatonin and pyridoxine in pharmaceutical preparations for health-caring purposes by capillary electrophoresis with electrochemical detection, Anal. Chim. Acta 408 (2000) 249–256, https://doi.org/10.1016/S0003-2670(99)00809-0.

[8] M.L. Pola, M. Algarra, A. Becerra, M. Hernandez, Cyclodextrin enhanced spectrofluorimetric determination of melatonin in pharmaceuticals and urine, Anal. Lett. 33 (2000) 891–903, https://doi.org/10.1080/00032710008543097.
[9] I.M. Apetrei, C. Apetrei, Voltammetric determination of melatonin using a graphene-based sensor in pharmaceutical products, Int. J. Nanomed. (2016) 1859–1866, https://doi.org/10.2147/IJN.S104941.
[10] G. Jiménez-Skrzypek, C. Ortega-Zamora, J. González-Sálamo, J. Hernández-Borges, Miniaturized green sample preparation approaches for pharmaceutical analysis, J. Pharm. Biomed. Anal. 207 (2022), 114405, https://doi.org/10.1016/j.jpba.2021.114405.
[11] M. Soylak, E. Yilmaz,New Generation Green Solvents for Separation and Preconcentration of Organic and Inorganic Species, Elsevier Inc, 2020. doi: 10.1016/C2018-0-03964-X.
[12] V. Andruch, P. Makoś-Chełstowska, J. Płotka-Wasylka, Remarks on use of the term "deep eutectic solvent" in analytical chemistry, Microchem. J. 179 (2022), 107498, https://doi.org/10.1016/j.microc.2022.107498.
[13] M.R. Afshar Mogaddam, M.A. Farajzadeh, S. Azadmard Damirchi, M. Nemati, Dispersive solid phase extraction combined with solidification of floating organic drop–liquid–liquid microextraction using in situ formation of deep eutectic solvent for extraction of phytosterols from edible oil samples, J. Chromatogr. A 1630 (2020) 461523.
[14] A. Shishov, A. Pochivalov, L. Nugbienyo, V. Andruch, A. Bulatov, Deep eutectic solvents are not only effective extractants, TrAC Trends Anal. Chem. 129 (2020), 115956, https://doi.org/10.1016/j.trac.2020.115956.
[15] A. Huang, W. Deng, X. Li, Q. Zheng, X. Wang, Y. Xiao, Long-chain alkanol–alkyl carboxylic acid-based low-viscosity hydrophobic deep eutectic solvents for one-pot extraction of anthraquinones from Rhei Radix et Rhizoma, J. Pharm. Anal. 12 (2022) 87–95, https://doi.org/10.1016/j.jpha.2021.03.002.
[16] S. Şahin, Tailor-designed deep eutectic liquids as a sustainable extraction media: An alternative to ionic liquids, J. Pharm. Biomed. Anal. 174 (2019) 324–329, https://doi.org/10.1016/j.jpba.2019.05.059.
[17] Y. Cui, C. Li, J. Yin, S. Li, Y. Jia, M. Bao, Design, synthesis and properties of acidic deep eutectic solvents based on choline chloride, J. Mol. Liq. 236 (2017) 338–343, https://doi.org/10.1016/j.molliq.2017.04.052.
[18] B. Kudłak, K. Owczarek, J. Namieśnik, Selected issues related to the toxicity of ionic liquids and deep eutectic solvents—a review, Environ. Sci. Pollut. Res. 22 (2015) 11975–11992, https://doi.org/10.1007/s11356-015-4794-y.
[19] A. Shishov, A. Bulatov, M. Locatelli, S. Carradori, V. Andruch, Application of deep eutectic solvents in analytical chemistry. A review, Microchem. J. 135 (2017) 33–38, https://doi.org/10.1016/j.microc.2017.07.015.
[20] M. Cvjetko Bubalo, S. Vidović, I. Radojčić Redovniković, S. Jokić, Green solvents for green technologies, J. Chem. Technol. Biotechnol. 90 (2015) 1631–1639, https://doi.org/10.1002/jctb.4668.
[21] B. Zhuang, L.-L. Dou, P. Li, E.-H. Liu, Deep eutectic solvents as green media for extraction of flavonoid glycosides and aglycones from Platycladi Cacumen, J. Pharm. Biomed. Anal. 134 (2017) 214–219, https://doi.org/10.1016/j.jpba.2016.11.049.
[22] F. Aydin, E. Yilmaz, M. Soylak, A simple and novel deep eutectic solvent based ultrasound-assisted emulsification liquid phase microextraction method for malachite green in farmed and ornamental aquarium fish water samples, Microchem. J. 132 (2017) 280–285, https://doi.org/10.1016/j.microc.2017.02.014.
[23] M.B. Arain, E. Yilmaz, M. Soylak, Deep eutectic solvent based ultrasonic assisted liquid phase microextraction for the FAAS determination of cobalt, J. Mol. Liq. 224 (2016) 538–543, https://doi.org/10.1016/j.molliq.2016.10.005.
[24] S.N. Shah, F. Uzcan, M. Soylak, Ultrasound-assisted deep eutectic solvent microextraction procedure for traces Ponceau 4R in water and cosmetic samples, Int. J. Environ. Sci. Technol. 19 (2022) 189–196, https://doi.org/10.1007/s13762-021-03154-z.
[25] A. Shishov, A. Gerasimov, D. Nechaeva, N. Volodina, E. Bessonova, A. Bulatov, An effervescence-assisted dispersive liquid–liquid microextraction based on deep eutectic solvent decomposition: Determination of ketoprofen and diclofenac in liver, Microchem. J. 156 (2020), 104837, https://doi.org/10.1016/j.microc.2020.104837.
[26] A. Dalmaz, S. Sivrikaya Özak, Green deep eutectic solvent assisted liquid–liquid microextraction procedure for Sunset Yellow dye in effervescent vitamin C tablets, J. Iran. Chem. Soc. 19 (6) (2022) 2359–2366.
[27] K.D. Cherkashina, A.S. Pochivalov, F.M. Shakirova, A.Y. Shishov, A.V. Bulatov, Microextraction of Tetracyclines from Milk to Deep Eutectic Solvents for the Subsequent Determination by High-Performance Liquid Chromatography-Tandem Mass Spectrometry, J. Anal. Chem. 77 (2022) 334–341, https://doi.org/10.1134/S1061934822030042.
[28] R.G. Ingle, S. Zeng, H. Jiang, W.-J. Fang, Current development of bioanalytical sample preparation techniques in pharmaceuticals, J. Pharm. Anal. (2022), https://doi.org/10.1016/j.jpha.2022.03.001.
[29] A. Jain, K.K. Verma, Recent advances in applications of single-drop microextraction: A review, Anal. Chim. Acta 706 (2011) 37–65, https://doi.org/10.1016/j.aca.2011.08.022.
[30] M.S. Bidabadi, S. Dadfarnia, A.M.H. Shabani, Solidified floating organic drop microextraction (SFODME) for simultaneous separation/preconcentration and determination of cobalt and nickel by graphite furnace atomic absorption spectrometry (GFAAS), J. Hazard. Mater. 166 (2009) 291–296, https://doi.org/10.1016/j.jhazmat.2008.11.052.
[31] F. de S. Dias, L.B. Peixoto, L.A. Meira, J.A. Barreto, Ultrasound-assisted emulsification of solidified floating organic drop microextracted for pre-concentration of cadmium in food and water samples, Anal. Methods 10 (35) (2018) 4257–4263.
[32] A. Amiri Pebdani, S. Dadfarnia, A.M. Haji Shabani, S. Khodadoust, M. S. Talebianpoor, Modified dispersive liquid-phase microextraction based on sequential injection solidified floating organic drop combined with HPLC for the determination of phenobarbital and phenytoin, J. Sep. Sci. 41 (2018) 509–517, https://doi.org/10.1002/jssc.201701111.
[33] P. Pidenko, C. Vakh, A. Shishov, J. Skibina, N. Burmistrova, A. Bulatov, Microstructured optical fibers sensor modified by deep eutectic solvent: Liquid-phase microextraction and detection in one analytical device, Talanta 232 (2021), 122305, https://doi.org/10.1016/j.talanta.2021.122305.
[34] I. Timofeeva, D. Kanashina, D. Kirsanov, A. Bulatov, A heating-assisted liquid-liquid microextraction approach using menthol: Separation of benzoic acid in juice samples followed by HPLC-UV determination, J. Mol. Liq. 261 (2018) 265–270, https://doi.org/10.1016/j.molliq.2018.04.040.
[35] Y.C. Martin, Exploring QSAR: Hydrophobic, Electronic, and Steric Constants C. Hansch, A. Leo, and D. Hoekman. American Chemical Society, Washington, DC. 1995. Xix + 348 pp. 22 × 28.5 cm. Exploring QSAR: Fundamentals and Applications in Chemistry and Biology . C. Hansc, J. Med. Chem. 39 (1996) 1189–1190. doi: 10.1021/jm950902o.
[36] J. Smajdor, R. Piech, M. Pięk, B. Paczosa-Bator, Carbon black as a glassy carbon electrode modifier for high sensitive melatonin determination, J. Electroanal. Chem. 799 (2017) 278–284, https://doi.org/10.1016/j.jelechem.2017.06.013.
[37] S. Li, S. Feng, A. Van Schepdael, X. Wang, Hollow fiber membrane-protected amino/hydroxyl bifunctional microporous organic network fiber for solid-phase microextraction of bisphenols A, F, S, and triclosan in breast milk and infant formula, Food Chem. 390 (2022), 133217, https://doi.org/10.1016/j.foodchem.2022.133217.