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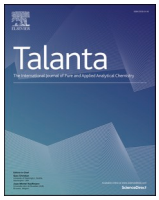

Deep eutectic solvents with low viscosity for automation of liquid-phase microextraction based on lab-in-syringe system: Separation of Sudan dyes

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ABSTRACT

In the work limitations of deep eutectic solvents in the flow-based analysis are discussed. Deep eutectic solvents based on terpenes and fatty acids with low viscosity were studied as extraction solvents for liquid-liquid microextraction into a lab-in-syringe system for the first time. As a result an automated deep eutectic solvent-based microextraction approach was proposed. The procedure involved aspiration of deep eutectic solvent (based on terpene and fatty acid) and aqueous sample solution followed by phases mixing by a magnetic stirrer inside a syringe of flow system. After phase separation the extract phase was transferred from the syringe into a vial followed by analysis by a high-performance liquid chromatography with diode-array detection. The determination of Sudan I, Sudan II and Sudan III in chili-based sauces was considered as an analytical task. The mass-transfer intensification performed by the magnetic stirring inside the syringe allowed to perform fast (2 min) and efficient (extraction recoveries 87–95%) extraction. The limits of detection, calculated from a blank test based on 3σ, were from 0.003 to 0.005 mg kg^{-1}, RSD was <9%. The microextraction procedure did not involve the use of hazardous organic solvents, only 100 μL of natural deep eutectic solvent was required for dyes preconcentration.

1. Introduction

Typically, in the food analysis hazardous, volatile, and flammable organic solvents are used for sample dissolution, analytes separation/preconcentration, and derivatization. In fact, the mass analysis of a large number of samples can lead to the accumulation of a large amount of hazardous waste. To reduce the environmental impact of analytical procedure, its automation and miniaturization and the use of environmentally friendly solvents and reagents are of interest.

Flow-based methods have been recognized as an efficient tool for automatization and miniaturization of analytical procedures. One of the efficient and versatile flow-based approaches is a lab-in-syringe method [1]. This approach has been applied to automate various liquid-phase microextraction procedures, such as dispersive [2], single drop [3], and homogeneous microextraction [4], as well as sorption using various sorbents [5]. The lab-in-syringe method assumes performance of analytical procedures inside a syringe pump of flow system. The phases mixing by the magnetic stirrer inside the syringe pump is typically used in the lab-in-syringe method. Another advantage of this method is the possibility of its combination with various instrumental methods such as chromatographic [6] and spectral [7] methods.

Among environmentally friendly solvents, deep eutectic solvents (DESs) have grown increasingly popular. DESs are mixtures of two or more precursors capable of forming viscous solvents when mixed in certain molar ratios due to interaction through the formation of hydrogen bonds [8]. These solvents have found wide application for solving various problems of analytical chemistry [9], including food analysis [10,11]. Despite the extremely high application of DESs in chemical analysis, only a few works have been devoted to the automation of sample pretreatment based on flow systems with the use of DESs [12,13]. The main reason that limits the use of DESs in flow-based analysis is their high viscosity. The viscosity of hydrophobic DESs based on quaternary ammonium salts (tetrabutylammonium chloride or tetraoctylammonium bromide) and long chain fatty acids is higher than 500 mPa s at 25 °C [14]. Obviously, such high viscosity practically excludes the possibility of aspiration of DES in a flow system without preliminary dilution of the extraction solvent. Another hydrophobic DESs based on natural terpenes and fatty acids have less viscosity at ambient condition (less than 30 mPa s at 25 °C [15]) in comparison with DESs based on quaternary ammonium salts. Nevertheless, flow-based microextraction with the use of DESs based on natural terpenes has not been implemented.

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Currently, only one work illustrates the possibility of using deep eutectic solutions based on terpenes and acids for single-drop automated analysis [16]. In this procedure, a drop of extractant is used for fluoroquinolones separations. However, from the point of view of drop microextraction, the high viscosity of the extractants is a positive feature, since it allows to create a stable droplet. However, the liquid-liquid microextraction variant remains simpler in terms of automation, and when using this approach, it remains important to use low viscosity extractants.

In this research an automated hydrophobic deep eutectic solvent-based microextraction approach for food analysis is proposed. DESs based on terpenes (thymol and menthol) and fatty acids (from hexanoic acid to decanoic acid) were studied for liquid-liquid microextraction into a lab-in-syringe system for the first time. The automated microextraction of Sudan I, Sudan II and Sudan III from chili-based sauces and their determination by a high-performance liquid chromatography with diode-array detection (HPLC-DAD) was chosen as analytical task. In recent years, the determination of Sudan dyes in food has become an important task of food quality control. Even though Sudan dyes provide genotoxic carcinogenic effect on the humans, they are still utilized illegally in some daily foodstuffs because of its colorfastness and low cost [17]. It is not surprising that DESs have found application in the separation of Sudan dyes from foods (Table 1). Meanwhile, no automated deep eutectic solvent-based microextraction procedures have been proposed for the Sudan dyes determination due to their high viscosity. DESs based on terpenes and fatty acids with low viscosity have not been studied for Sudan dyes microextraction.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared in ultra-pure water (resistivity 18MΩcm) obtained using a Milli-Q system (Millipore, USA). Hexanoic, heptanoic, octanoic, nonanoic, decanoic acids, methanol, acetonitrile, menthol, thymol, Sudan I, Sudan II, Sudan III, sodium chloride were purchased from Sigma Aldrich, Germany. A stock solution of Sudan dyes (100.0 mg L^{-1}) was prepared by dissolving the dyes in methanol. Working solutions of dyes were prepared by dilution of the stock solution with ultra-pure water.

2.2. DESs preparation

Menthol or thymol were used as hydrogen bond acceptors. Hexanoic, heptanoic, octanoic, nonanoic and decanoic acids acted as hydrogen bond donors. To prepare DESs, the hydrogen bond acceptor and hydrogen bond donor were mixed in molar ratio of 1:2 in glass vessels and were heated and stirred on a magnetic stirrer for 30 min until homogeneous liquids were formed. The prepared eutectic solvents were stored in a closed flask in a refrigerator at 6 °C for at least 3 months. During this time, these solvents were stable. This was confirmed by the absence of extraneous peaks in the chromatographic analysis of blank eutectic solvents.

2.3. Apparatus and flow manifold

The flow manifold (Fig. 1) consists of an eight-way selective valve (Sciware Systems SL, Palma De Mallorca, Spain), a syringe pump (Sciware Systems SL, Palma De Mallorca, Spain) (flow rate from 0.1 to 15 mL min^{-1}) with a 5 mL glass syringe, equipped with a magnetic stirrer (Sciware Systems SL, Palma De Mallorca, Spain) and a miniature magnetic stirrer (length 5 mm, diameter 2 mm) placed inside the syringe. The device of the magnetic stirrer is described in detail in Ref. [18]. PTFE tubes with an inner diameter of 0.8 mm were used for the entire collector.

Chromatographic measurements were performed using a LC-20

Table 1
Analytical procedures for the determination of Sudan dyes in food samples using deep eutectic solvents.

Sample	Analytes	Sample preparation	DES composition (molar ratio) and volume, μL	Sample amount, g	Sample preparation time, min	Microextraction automatization	LODs, mg kg^{-1}	Linear range, mg kg^{-1}	Extraction recovery, %	RSD, %	Ref
Chili, paprika, cumin and sumac spices	Sudan I, Sudan II, Sudan III, Sudan IV	Ultrasound-assisted solid-liquid microextraction	Coumarin/thymol (1:1), 200	0.1	10	No	0.25–0.35	2.5–100	85–99	4	24
Chili oil, chili sauce, duck yolk	Sudan I	Vortex-assisted liquid-liquid microextraction	Choline chloride/sesamol (1:3), 800	1	1	No	0.02	0.2–200	93–118	4.5	25
Tomato chili sauce	Sudan I, Sudan II, Sudan IV, Sudan orange G, Sudan red G	Vortex-assisted liquid-liquid microextraction	Brij-35/hexafluoroisopropanol (1:5), 100	0.2	5	No	0.0045–0.0118	0.04–2	61–74	8	26
Duck blood and chili spices	Sudan I	Vortex-assisted liquid-liquid microextraction	Methyl trioctylammonium chloride/oleic acid (1:2), 150	5	3	No	0.0003	1–500	83–114	5.4	27
Chili sauce, chili spice, ketchup	Sudan red G, Sudan III, Sudan IV	Vortex-assisted liquid-liquid microextraction	Benzyltriethylammonium bromide/eugenol (1:2), 75	0.5	10	No	0.0005–0.001	0.002–1	89–119	6.8	28
Chili sauce	Sudan I, Sudan II, Sudan III,	Liquid-liquid microextraction	Menthol/hexanoic acid (1:2), 100	1	7	Yes	0.003–0.005	0.02–50	87–95	9	This work

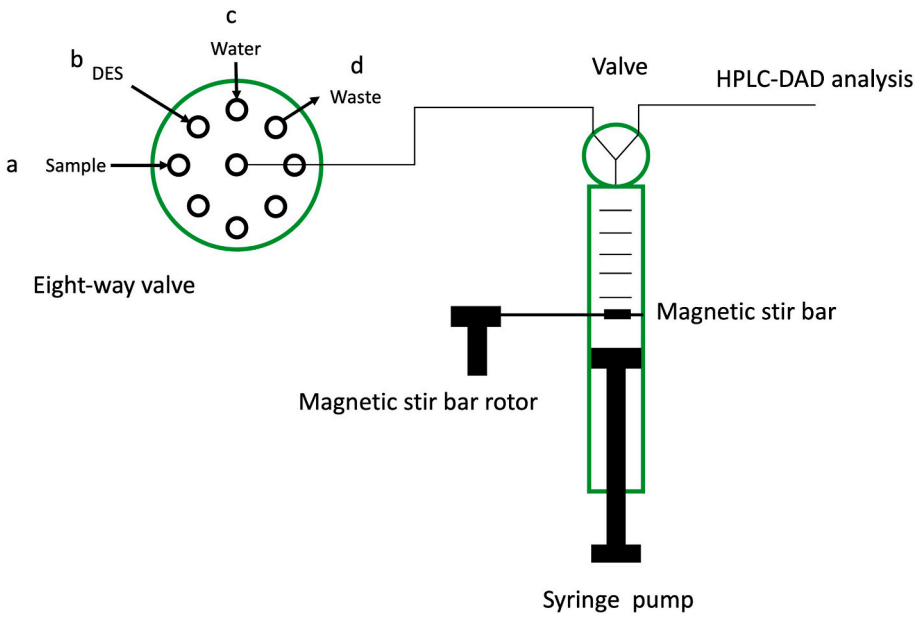

Fig. 1. The lab-in-syringe manifold for automated hydrophobic deep eutectic solvent-based microextraction.

Prominence HPLC-DAD system (Shimadzu, Kyoto, Japan). Density and viscosity were measured by a SVM 1001 kinematic viscometer (Anton Paar, Vienna, Austria).

2.4. Samples and sample preparation

Three chili-based sauces were purchased from a supermarket (St. Petersburg, Russia) and stored in their original packaging in a refrigerator at + 5 ^{0}C.

To prepare spiked sauce sample, 0.10 mL of working solution of dyes was added to 9.90 g of the sauce sample and mixed by a glass rod. The concentration of dyes in spiked samples was determined by reference procedure [19].

As the real food samples contain particulate matter that can block the tubes of the flow system, it is necessary to prepare the sample before beginning the automated analysis. For sample preparation before microextraction, 1.00 ± 0.01 g of the real or spiked sauce sample was mixed with 4.0 mL of sodium chloride solution (10 g L^{-1}) in 5 mL plastic vials and centrifuged for 1 min at 600×*g* to precipitate solid particles. The obtained sample solution (4 mL) was withdrawn and used for microextraction.

2.5. Automated microextraction procedure

According to the proposed procedure (Figs. 1), 100 μL of DES (menthol and hexanoic acid) was aspirated into the syringe pump through the valve channel (a) at speed value of 1 mL min^{-1}. After that, 4.0 mL of the sample solution (b) was aspirated into the syringe pump through the valve at speed value of 5 mL min^{-1}. Then, portion of ultra-pure water (50 μL, c) was aspirated to deliver a sample phase into the syringe pump. The mixture was stirred inside the syringe pump with the magnetic stirrer for 2 min. After the mixing was stopped for 3 min, the upper extract phase (50 μL) was transferred into a vial for the HPLC-DAD analysis and remaining mixture was transferred to the waste (d). Finally, ultra-pure water (4.0 mL) was aspirated into the syringe pump to wash the flow system and then transferred to the waste (d). The washing procedure was repeated three times. This prevented the sample carry-over effect when analyzing a series of samples, so that the signal from the previous sample did not overlap with subsequent samples.

2.6. HPLC-DAD procedure

Chromatographic separation was performed using a C18 column (150 × 4 mm, 5 μm particles size; Phenomenex, USA) operated at 50 °C. A mobile phase consisted of acetonitrile, methanol, and water at a ratio of 63:32:5 was pumped at a flow rate of 0.65 mL min^{-1} in isocratic mode. Twenty μL of extract was introduced into the HPLC-DAD system. The detection wavelength was 490 nm for all Sudan dyes.

2.7. Reference procedure

The reference procedure [19] was used to confirm the accuracy of the obtained results. In this case, the dyes were diluted with acetonitrile. First, 1.00 ± 0.01 g of the real or spiked sauce sample was taken into a 10 mL vial, and 5.0 mL of acetonitrile was added. The resulting mixture was stirred on a shaker for 1 h, and the mixture was centrifuged at 600×*g* for 10 min. Then, 200 μL of the upper phase was collected and analyzed by HPLC-DAD.

3. Results and discussion

3.1. Physical properties investigation

For automation of DES-based microextraction with the use of the lab-in-syringe system, extraction solvent should have low viscosity and density less than water to be separated at the top into the syringe pump. Therefore, such key-parameters as the kinematic viscosity and density were determined for synthesized DESs based on terpenes (thymol and menthol) and fatty acids (from hexanoic acid to decanoic acid). As can be seen from the data obtained (Table 2), the density values of DESs were comparable (0.892–0.941 g cm^{-3} at 25 °C) and were less than density of water (0.997 g cm^{-3} at 25 °C). In this case after phase separation all DESs phase was located on the top of the extraction system.

The type of fatty acid had a significant effect on DES viscosity. In all the cases, with the increase of chain length of fatty acid the viscosity of DES was increased (Table 2). DESs based on decanoic acid had higher viscosity (almost twice high) than DESs based on hexanoic acid. To establish the behavior of the solvents in the flow system, portion of DES (500 μL) and ultra-pure water (4.0 mL) were aspirated into the syringe pump (Fig. 1) through the valve at speed value of 1 mL min^{-1} and mixed for 5 min by the magnetic stirrer. It was established that despite the

Table 2
Physical properties of DESs based on terpenes and fatty acids at 25 °C.

Hydrogen bond acceptor	Hydrogen bond donor	Density, g cm^{-3}	Kinematic viscosity, mPa·s
Menthol	Hexanoic acid	0.911	12.3
	Heptanoic acid	0.906	14.8
	Octanoic acid	0.901	16.6
	Nonanoic acid	0.895	18.3
	Decanoic acid	0.892	20.12
Thymol	Hexanoic acid	0.941	5.13
	Heptanoic acid	0.937	5.8
	Octanoic acid	0.935	7.12
	Nonanoic acid	0.934	9.6
	Decanoic acid	0.932	12.22

difference in the viscosity of DESs, the syringe pump provided aspiration of all studied DESs, and phase separation was observed without centrifugation in all cases.

3.2. Microextraction optimization

3.2.1. Effect of DES composition

The type of terpene had significant effects on DES viscosity and on Sudan dyes extraction. DESs based on menthol were more viscous in comparison with DESs based on thymol (Table 2). To study the effect of nature of terpene on the Sudan dyes, extraction DESs based on menthol or thymol and hexanoic acid with low viscosity were chosen. For extraction, 500 μL of DES and 4.0 mL of working analytes solution (1.0 mg L^{-1}) were mixed in the syringe pump by the magnetic stirrer for 10 min. In accordance with the results obtained (Fig. 2 A), the maximum extraction efficiency was achieved for DES based on menthol. Despite the higher viscosity of DES based on menthol (12.2 mPa s at 25 °C) in comparison with DES based on thymol (5.1 mPa s at 25 °C), this solvent provided higher affinity for analyte extraction. Despite the fact that thymol is an aromatic compound, unlike menthol, this does not contribute to a better extraction of the studied dyes into eutectic solutions based on thymol. Most likely, this is due to the greater hydrophobicity of eutectic solvents based on menthol, since it is less polar than thymol. The hydrophobic interaction between menthol and the aromatic dye system may be the driving force of the extraction. In addition, the menthol-based DES provided faster phase separation into the syringe pump. Thus, menthol was chosen as the hydrogen bond acceptor for further research.

The nature of fatty acid can also affect the Sudan dyes extraction. Thus, DESs based on menthol and fatty acids from hexanoic acid to decanoic acid were investigated for the Sudan dyes extraction. For this purpose, 400 μL of DES and 4.0 mL of analytes solution (1.0 mg L^{-1}) were mixed in the syringe pump. The phases were stirred for 10 min with the magnetic stirrer, and after phase separation (5 min), the extracted phase was analyzed by HPLC-DAD.

The results showed that with an increase in hydrocarbon chain of fatty acid the extraction recoveries were decreased (Fig. 2 B). The most efficient extraction was achieved using DES based on menthol and hexanoic acid (extraction recoveries 87–95%). The low viscosity of the solvent provided higher diffusion coefficients and more effective mass-transfer in comparison with viscous solvents. DES based on menthol and decanoic acid had maximum viscosity (20.1 mPa s at 25 °C, Table 2) and provided minimum extraction recoveries for all analytes (42–71%). Thus, DES based on menthol and hexanoic acid was chosen as the most effective extraction solvent.

3.2.2. Effect of DES volume

The volume of extraction solvent can affect the analytes preconcentration. To achieve maximum preconcentration, the effect of the DES volume on preconcentration at a fixed sample solution volume was studied. The capacity of the syringe pump was 5.0 mL. The sample solution volume was fixed at 4.0 mL (1.0 mg L^{-1}). The volume of the DES varied from 100 to 400 μL. Stirring was carried out for 10 min, and after phase separation, the upper phase was transferred to HPLC-DAD analysis. It was found that with an increase in the volume of the DES, there is no significant change in the extraction recoveries (ESM Fig. 1). However, increase of the volume of the DES leads to a dilution effect and a decrease in enrichment factor values. Maximum enrichment factors (35–38) were obtained for 100 μL of the DES phase. Smaller volumes of the DES did not provide reproducible aspiration of the extraction solvent in the flow system.

3.2.3. Effect of extraction and phase separation time

Since the DES is viscous and mass-transfer can have slow kinetics, it was necessary to study the effect of the phases mixing time and phases separation time in the syringe pump. For this 100 μL of the DES and 4.0 mL of the working analytes solution (1.0 mg L^{-1}) were mixed in the syringe pump with a magnetic stirrer from 1 to 7 min. It was found that 2 min was sufficient to completely extract the dyes into the DES phase (Fig. 2C). In all cases 3 min was required to achieve the separation of phases.

3.3. Evaluation of the matrix effect

Chili-based sauces have complex matrices (containing water, sugar, chili pepper, proteins, fructose, starch, garlic, citric acid, onion, acetic acid, acetic acid, lactic acid, sodium acetate), and it was necessary to evaluate a possible matrix effect. For this purpose, all chili-based sauces samples were spiked at 0.02 mg kg^{-1} of dyes. To reduce viscosity and precipitate solid particles (garlic and onion) of chili-based sauce 1.00 ± 0.01 g of the spiked sauce sample was mixed with 4.0 mL of diluent (ultra-pure water) and centrifuged for 1 min at 600×*g*. The obtained sample solution was withdrawn and used for microextraction.

The response for extracts obtained for standard solution (0.005 mg L^{-1}) and spiked sample solution (0.02 mg kg^{-1}) has been received and the matrix effect was evaluated according to the formula [20]:

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

It was found that the matrix effect was significant, and it ranged from – 65.9 to – 52.6% (Table 3). To reduce matrix effect electrolyte addition was studied. In the presence of the electrolyte (sodium chloride) more effective precipitation of solid particles was observed and the obtained aqueous phase was more transparent due to the proteins precipitation. The sodium chloride concentration in diluent varied from 1 to 20 g L^{-1}. It can be seen from the data obtained (Table 3) that the salt content of 10 g of L^{-1} in diluent provided significant reduction in the matrix effect from −1.1 to −3.6%.

3.4. Procedure validation

For analytical procedure validation, linear ranges, determination coefficients, limits of detection (LODs), limits of quantification (LOQs), precision, extraction efficiency and accuracy were determined (Table 4).

To construct calibration curves, spiked chili-based sauce samples were used. The linearity of the calibration curve ranged from 0.01 to 50 mg kg^{-1} for Sudan I and II and from 0.02 to 50 mg kg^{-1} for Sudan III. The determination coefficients were from 0.995 to 0.997.

The LODs and LOQs calculated from a blank test based on 3σ and 10σ, were from 0.003 to 0.005 mg kg^{-1} and from 0.01 to 0.02 mg kg^{-1}. The developed procedure allows to determine the concentration of the Sudan dyes in foods at levels less than the Maximum Residue Level (0.5 mg kg^{-1} [21]).

To assess the precision of the developed procedure, the intra-day and inter-day repeatability studies were carried out. The experiments were repeated 5 times using spiked chili-based sauce samples with analytes

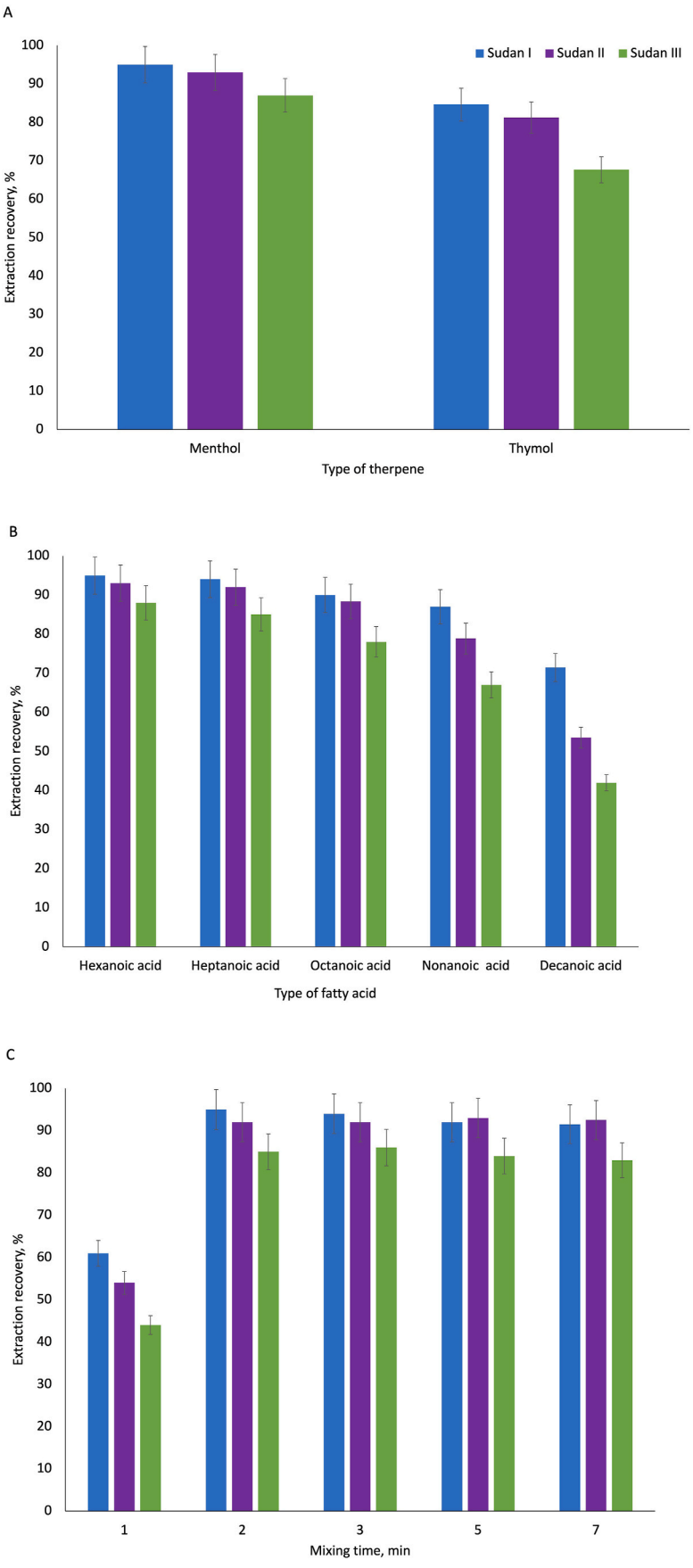

Fig. 2. Investigation of appropriate experimental conditions for Sudan dyes separation (Cdyes 1.0 mg L^{-1}, sample volume 4.0 mL): (A) Effect of hydrogen bond acceptor type on extraction recovery (hydrogen bond donor – hexanoic acid, DES volume 500 μL, mixing time 10 min); (B) Effect of hydrogen bond donor type on extraction recovery (hydrogen bond acceptor – menthol, DES volume 500 μL, mixing time 10 min); (C) Effect of mixing time on extraction recovery (DES based on menthol and hexanoic acid, DES volume 100 μL).

Table 3
Effect of the matrix effect on the determination the Sudan dyes (0.02 mg kg^{-1}) in chili-based sauce.

Diluent	Matrix effect, %		
	Sudan I	Sudan II	Sudan III
Ultra-pure water	−52.6	−56.8	−65.9
1 g L^{-1} NaCl	−35.8	−41.1	−62.4
5 g L^{-1} NaCl	−17.4	−25.0	−34.9
10 g L^{-1} NaCl	−2.2	−1.1	−3.6
15 g L^{-1} NaCl	−1.1	−1.1	−2.4
20 g L^{-1} NaCl	−1.1	−1.1	0.6

concentration levels of 0.02 and 50 mg kg^{-1}. The intra-day and inter-day repeatability values (RSDs) ranged from 3 to 5% and from 7 to 9%, respectively.

To evaluate the extraction efficiency the extraction recovery values were established. The extraction recovery values were from 87 to 95%.

To survey the accuracy, the added-found method and the reference procedure [19] were applied. Three chili-based sauces were analyzed: Tabasco, Sriracha and Caucasian sauces. Only in Caucasian sauce Sudan I was found by developed (2.30 ± 0.05 mg kg^{-1}) and reference (2.50 ± 0.04 mg kg^{-}) procedures (Table 5). The concentration of Sudan I in Caucasian sauce was higher than Maximum Residue Level (0.5 mg kg^{-1} [21]. Recovery values were determined at the analytes levels of 5 and 50 mg kg^{-1}. It was shown that recovery values for analytes were in the range of 92–104%. The insignificant bias obtained can be explained by the possible matrix effect. In accordance with [22] acceptable relative recovery values for analyte concentration levels of 1 mg kg^{-1} and higher are in the range from 80 to 110%. Additionally, no interfering peaks were observed during chromatographic analysis of real samples (ESM Fig. 2). Moreover, as can be seen from Table 4, the results of the analysis are in good agreement with the results obtained by the reference procedure [19]. F-values less than 5.05 indicate little difference in accuracy between both methods at a 95% confidence level. *t*-test values less than 2.77 indicate little difference between the results obtained using these procedures (n = 5).

3.5. Green analytical procedure index and comparison with developed procedures

To assess the environmental friendliness of the procedure, the GAPI method [23] was involved. The GAPI method assumes consideration of the environmental impact of sampling and preservation of samples, sample preparation, the reagents and materials used, as well as the influence of the instrumental method. As can be seen from the results obtained (Fig. 3), the developed procedure corresponds to the highest environmental friendliness class in terms of the reagents used, since volatile, combustible and toxic substances are not used at the sample preparation stage. Environmentally friendly DES and an aqueous solution of sodium chloride are used in sample preparation. In fact, only three red zones were obtained on the diagram. The central one corresponds to the fact that this procedure requires sample preparation (extraction). The second red zone is responsible for sample centrifugation. The third is responsible for the consumption of methanol and acetonitrile used for the mobile phase preparation.

The LODs of the developed procedure are comparable with the LODs of the procedures described in literature (Table 1). The sample throughput (8 samples $hour^{-1}$) was comparable with procedures that assumed the extraction of many samples simultaneously with the use of an orbital shaker. Nevertheless, the automated procedure eliminated the centrifugation step for phase separation and thus made a procedure less time and labor consuming. Moreover, in comparison with other procedures the developed procedure is automated and miniaturized and implies low extraction solvent consumption. It is the first automated DES-based microextraction procedure for the Sudan dyes determination [24–28].

Table 4
Analytical characteristics of the developed procedure.

Analyte	Linear range, mg kg^{-1}	r^2	LOD, mg kg^{-1}	LOQ, mg kg^{-1}	intra-day repeatability, RSD, % (0.02/ 50 mg kg^{-1})	inter-day repeatability, RSD, % (0.02/ 50 mg kg^{-1})	Extraction recovery, %
Sudan I	0.01–50	0.996	0.003	0.01	5/3	9/7	95
Sudan II	0.01–50	0.995	0.003	0.01	5/3	9/7	92
Sudan III	0.02–50	0.997	0.005	0.02	5/3	9/7	87

Table 5
Determination of Sudan dyes in souses samples (n = 5, P = 0.95, F = 5.05, t = 2.77).

Sample	Analyte	Added, mg kg^{-1}	Found, mg kg^{-1}		Relative recovery, %	t-criterion	F-criterion
			Development procedure	Reference procedure, [19]			
Sriracha	Sudan I	0	<LOD	<LOD	–	–	–
		5.0	4.60 ± 0.15	4.70 ± 0.13	92	1.23	3.54
	Sudan II	0	<LOD	<LOD	–	–	–
		5.0	5.10 ± 0.20	5.00 ± 0.11	102	0.67	4.34
	Sudan III	0	<LOD	<LOD	–	–	–
		5.0	5.20 ± 0.16	5.10 ± 0.13	104	1.27	4.34
Caucasian	Sudan I	0	2.30 ± 0.05	2.50 ± 0.04	–	2.12	3.45
		5.0	7.40 ± 0.13	7.70 ± 0.16	101	2.33	4.48
	Sudan II	0	<LOD	<LOD	–	–	–
		5.0	4.90 ± 0.20	5.00 ± 0.13	98	1.35	4.11
	Sudan III	0	<LOD	<LOD	–	–	–
		5.0	4.80 ± 0.16	5.20 ± 0.19	96	1.16	4.48
Tabasco sauce	Sudan I	0	<LOD	<LOD	–	–	–
		10.0	10.10 ± 0.21	9.70 ± 0.23	101	1.12	4.97
	Sudan II	0	<LOD	<LOD	–	–	–
		10.0	10.3 ± 0.3	10.40 ± 0.25	103	0.74	4.14
	Sudan III	0	<LOD	<LOD	–	–	–
		10.0	9.80 ± 0.21	9.9 ± 0.3	98	0.66	4.43

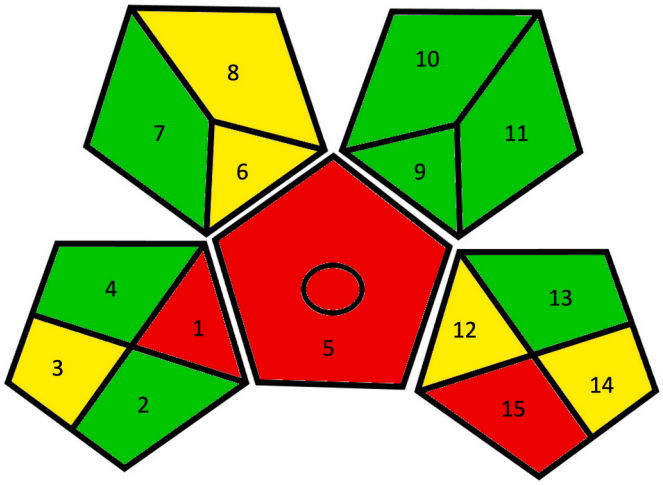

Fig. 3. Green Analytical Procedure Index for procedure.. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Conclusion

In this work, an automated hydrophobic deep eutectic solvent-based microextraction approach was developed and applied to the separation and preconcentration of Sudan I, Sudan II and Sudan III from chili-based sauces. Low viscosity and high hydrophobicity of the deep eutectic solvents based on terpene (thymol and menthol) and fatty acid (from hexanoic acid to decanoic acid) allow to perform automated microextraction of target analytes from aqueous sample phase into the lab-in-syringe system. In this case all studied deep eutectic solvents can be aspirated into the lab-in-syringe system due to their low viscosity, and provide phase separation without centrifugation. The developed microextraction approach can be considered as a versatile approach for preconcentration of various hydrophobic analytes from aqueous samples with neutral and acidic media. In alkaline medium an ionization of fatty acid can be observed resulting in deep eutectic solvent decomposition.

Effect of deep eutectic solvent composition on Sudan dyes preconcentration was studied. It was shown that deep eutectic solvent based on menthol and hexanoic acid provided more effective mass-transfer due to less extraction solvent viscosity and possible hydrophobic interactions between menthol and the aromatic systems of Sudan dyes. The use of extraction solvent based on natural components (menthol and hexanoic acid) allowed the development of an environmentally friendly analytical procedure. The environmentally friendliness of the procedure was confirmed by the GAPI index. The developed procedure allows to determine the Sudan dyes in chili-based sauces at levels less than the Maximum Residue Level.

Credit author statement

Andrey Shishov: Conceptualization. Ivan Dubrovsky: Investigation. Aleksei Pochivalov: Validation. Andrey Bulatov: Writing - Original Draft.

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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2022.124243.

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