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A Ferrofluidic Hydrophobic Deep Eutectic Solvent for the Extraction of Doxycycline from Urine, Blood Plasma and Milk Samples Prior to its Determination by High-Performance Liquid Chromatography-Ultraviolet

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Abstract

The ferrofluid phase was prepared according to mixing magnetic nanoparticle and the hydrophobic deep eutectic solvent as a green microextraction solvent. This new composite was applied for vortex-assisted dispersive liquid–liquid microextraction (VA-HDES-ferrofluid-DLLME) of doxycycline (DOC) residual extraction and determined through high-performance liquid chromatography-ultraviolet (HPLC-UV). The characterization of magnetic nanoparticle was investigated by XRD, TEM and FESEM. The dependency of DOC microextraction to main variables and their interaction and find optimum points were undertaken using response surface methodology with either central composite design (CCD). Thus, the optimum pH, ionic strength, ferrofluid volume and vortex time for DOC extraction are determined to be 3.0, 4%w/v, 150 μ L and 7 min, respectively. According to this condition, linear response is found to be greater than 10–400 ng mL⁻¹, with a correlation coefficient of 0.983. The detection and quantification limits are 3.6 and 8.5 ng mL⁻¹, while the repeatability and reproducibility as precision criteria (RSD%) are 3.74% and 4.15%, respectively. The DOC recoveries in all of the urine, blood plasma and milk samples are between 86.70 and 97.48%, with RSD% lower than 5.72%.

Keywords

Doxycycline, Ferrofluid, High-performance liquid chromatography (HPLC), Hydrophobic deep eutectic solvent, Vortex-assisted dispersive liquid–liquid microextraction

1. Introduction

Doxycycline (DOC) belongs to the tetracycline antibiotic family and has extensive application in human life [1]. DOC is classified as a member of tetracycline, containing β -diketonate structure. It has comprehensive range of antibacterial roles and is recommended for the action of particular contagions because of its impartially dependable absorption and extended half-life—and accordingly, its need for fewer administrations. It is often used to treat pelvic inflammatory disease, chlamydia, syphilis and sinusitis [2]. The monitoring of DOC in biological samples is meaningful owing to high levels of DOC, which leads to bacterial resistance and serious health problems including yellowing of teeth, allergic reactions, damage to the liver and gastrointestinal upset [3,4].

Measurement of DOC in biological samples usually includes extraction, clean-up technique and instrumental analysis [5]. However, the use of advanced devices for the analysis of DOC would allow urgent highly effective sample preparation requirements to design and develop an approach to quantify this compound in complex samples with content lower than their LOD. The most typical protocol for the DOC separation and/or pre-concentration from biological samples contains dispersive solid-phase microextraction (DSPME), dispersive liquid–liquid microextraction (DLLME) [6], liquid–liquid extraction (LLE), solid-phase extraction (SPE), matrix solid-phase dispersion (MSPD) or QuEChERS (which is quick, easy, cheap, effective, rugged and safe) [7].

The improvement of a novel microextraction based sample preparation would provide a rapid, simple and effective strategy, as it uses the smallest volume of organic solvents and sorbent [8]. DLLME is an ecofriendly microextraction procedure that permits effective, rapid and simple analysis without the requirement of special instruments [9,10].

The extracting solvent can be separated and decanted simply from the solid phase via jointing and combination magnetic nanomaterial. The ferrofluid based on the mixture of magnetic nanomaterial with a carrier agent helped to overcome some disadvantages in recovering extractor [11]. Ferrofluid application, as an extractor liquid, is associated with resolving or reducing special equipment, refrigeration and centrifugation. Ferrofluids

are composed of stable colloidal magnetic nanomaterial suspended in a carrier solvent. The magnetic nanomaterial in carrier solvent helps with the accumulation of species because of its dipolar attractive interactions, Van der Waals forces, magnetostatic interactions and more [12,13]. Such innovation in lower scale extractions leads to more rapid phase disengagement rate, with respect to the customary process [14,15].

A better presentation of the microextraction process is achieved by using a mathematical method to obtain a more refined knowledge of the process and supply more exact outcomes [16]. Accordingly, the correlation among different factors and response were revealed and modeled, which consequently optimal conditions is suggested and predicted [17].

The aim of present research is the synthesis of the ferrofluid based on magnetic nanoparticles and hydrophobic deep eutectic solvent, along with its subsequent usage for extraction of DOC based on VA-HDES-ferrofluid-DLLME method. The individual and interactive effect of factors on the microextraction of DOC—including ferrofluid volume, pH, vortex time and ionic strength (NaCl concentration (%w/v))—is examined via CCD. Subsequently, DOC quantification is completed through combination of HPLC-UV with preliminary VA-HDES-ferrofluid-DLLME in biological samples.

2. Experimental

2.1. Reagents and instruments

All reagents—such as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ($\geq 99\%$), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ($\geq 99.0\%$), NH_4OH (30%–33% NH_3 in H_2O), acetone ($\geq 99.9\%$), methanol ($\geq 99.9\%$), acetonitrile (99.8%) octanoic acid ($\geq 98\%$), doxycycline (DOC; $\geq 98\%$), DL-menthol (99%), oleic acid ($\geq 99\%$), NaOH ($\geq 98\%$) and HCl (37%)—were purchased from Merck, Darmstadt, Germany. Ultrapure water was prepared from a MilliQ gradient water purification system. The pH values of all solutions were controlled with a pH/Ion meter (model-686, Metrohm, Switzerland, Swiss). The morphology and average size of the Fe_3O_4 nanoparticles were examined by field emission scanning electron microscopy (FE-SEM: ZEISS, Sigma VP Germany) and transmission electron microscope (TEM, Zeiss model EM 10C, Germany). X-ray diffraction (XRD) pattern was achieved with an automated Philips X'Pert X-ray diffractometer (Philips, Netherland). A vortex agitation system (Retsch, Germany) was utilized to quicken the extraction. An ultrasonic bath (Tecno-GAZ SPA Ultra Sonic System, Italy) at ambient temperature, frequency and power of 40 kHz and 130 W, respectively, was used to disperse the Fe_3O_4 nanoparticles in deep eutectic solvent [18], [19], [20].

HPLC analysis was obtained by an Agilent 1100 liquid chromatography (Wilmington, DE, USA) that contained a micro vacuum degasser (model G1379A), quaternary pump (model G1311A), multiple wavelength detector (model G13658), sample injection valve with a 20 μL sample loop and an Agilent C18 column (4.6 mm i.d. 250 mm, 5 μm). Acetonitrile (25%) and water (75% v/v) adjusted to pH 2.5 with orthophosphoric acid were used as the mobile phase in this analysis. All the measurement operations were carried out at ambient temperature, followed by ultraviolet detector wavelength was operated at 245 nm for the analyte (DOC). STATISTICA software version 10.0 (Stat Soft Inc., Tulsa USA) was utilized for the design of experiments, model fitting and DOC extraction efficiency significance.

2.2. Synthesis of Iron Oxide (Fe_3O_4) nanoparticles coated with oleic acid

An aqueous solution (150 mL distilled water) of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (1.1 g) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.4 g)—in the Fe (III): Fe(II) molar ratio 2:1—was kept at 60°C for 15 min under vigorous stirring. Next, ammonium hydroxide solution (20 mL NH_4OH (25%)), under the previously mentioned conditions, was added until the pH reached ~ 11 , which lead to formation of a black suspension that was subsequently stirred at 50°C for 2 h with N_2 gas. Fe_3O_4 nanoparticles were collected by an external magnet, washed with deionized water several times and dried overnight in a vacuum oven [21]. Then, 0.5 g of Fe_3O_4 nanoparticles were mixed thoroughly with 10% v/v oleic

acid under vigorous stirring at 150 rpm for 2 h [22]. Finally, the suspended particles were washed five times with 10 mL of an acetone–methanol mixture (1:1 v/v) to remove the excess oleic acid. Last, the prepared suspension ($\text{Fe}_3\text{O}_4\text{-OA}$) was separated through magnetic decantation, followed by drying under a vacuum for 24 h [23].

2.3. Preparation of hydrophobic deep eutectic solvent

The hydrophobic deep eutectic solvent was prepared by mixing octanoic acid and DL-menthol with 1:1 molar ratio in a jacketed glass vessel, using a magnetic stirrer at 95 °C–100 °C with a speed of 350 rpm until a homogeneous liquid developed and no solid is observed. This liquid was then cooled down slowly until it reached room temperature.

2.4. Preparation of ferrofluid

50 mg of $\text{Fe}_3\text{O}_4\text{-NP}$ coated with oleic acid, as a magnetic nanomaterial, was diffused in 1 mL of DES. The mixture was then sonicated for about 20 min, leading to formation of stable ferrofluid. This was subsequently applied in the next stage for the VA-HDES-ferrofluid-DLLME procedure.

2.5. Vortex-assisted dispersive liquid–liquid microextraction procedure

A 10 mL of DOC solution (150 ng mL^{-1}), with a pH 3.0 and in the presence of 4 w/v% of NaCl was added to a 15 mL centrifuge tube. Next, 150 μL ferrofluid was added, then the mixture was vigorously shaken by a vortex agitator for 7 min. Afterward, a strong magnet was placed near the centrifuge tube in order to attract and isolate the extraction solvents. Finally, the sample solution was discarded through decanting. After removal of the magnet and introduction of 50 μL methanol (as the precipitation reagent) into the vial, exposure to the magnet at the bottom of the centrifuge tube leads to phase separation. After setting magnetic nanoparticles, the extraction phase was collected into an Eppendorf tube and, consequently, 20 μL was injected into HPLC for quantification and separation of DOC.

2.6. Experimental design

The STATISTICA, along with CCD, provides appropriate analysis of variance when given numerical estimation of main and interaction of variables—including pH (1.5–7.5), ferrofluid volume (50–250 μL), ionic strength (NaCl concentration (1–5%w/v)) and vortex time (1–7 min)—based on conduction of 30 experiments (Table 1). Subsequently, 3D dimensional and predicted versus actual value plots demonstrate usefulness and tendencies of contribution of variables and their interaction on responses.

Table 1. Design matrix for the central composite designs.

Factors		Levels			
		$-\alpha$	Low (-1)	Central (0)	High (+1)
A: pH		1.5	3.0	4.5	6.0
B: NaCl concentration (%w/v)		1	2	3	4
C: Ferrofluid volume (μL)		50	100	150	200
D: Vortex time (min)		1.0	2.5	4.0	5.5
Run	A	B	C	D	ER (%)
1	4.50	3.00	150.00	4.00	96.454
2	3.00	2.00	100.00	5.50	67.985
3	6.00	2.00	200.00	5.50	71.661
4	7.50	3.00	150.00	4.00	76.183
5	6.00	4.00	100.00	2.50	78.669
6	6.00	2.00	200.00	2.50	70.967
7	6.00	2.00	100.00	5.50	67.019
8	4.50	3.00	150.00	4.00	96.703

9	4.50	3.00	150.00	7.00	96.518
10	4.50	3.00	150.00	4.00	97.381
11	4.50	1.00	150.00	4.00	54.402
12	6.00	4.00	200.00	5.50	80.769
13	4.50	3.00	150.00	4.00	95.172
14	3.00	4.00	200.00	2.50	84.371
15	4.50	3.00	50.00	4.00	70.896
16	4.50	3.00	150.00	4.00	95.502
17	1.50	3.00	150.00	4.00	83.591
18	4.50	5.00	150.00	1.00	67.469
19	4.50	3.00	150.00	4.00	98.316
20	4.50	3.00	150.00	4.00	97.545
21	3.00	2.00	100.00	2.50	68.183
22	6.00	2.00	100.00	2.50	71.438
23	3.00	2.00	200.00	2.50	72.957
24	6.00	4.00	200.00	2.50	75.245
25	3.00	2.00	200.00	5.50	79.793
26	6.00	4.00	100.00	5.50	83.285
27	4.50	3.00	250.00	4.00	75.285
28	3.00	4.00	100.00	5.50	93.622
29	3.00	4.00	100.00	2.50	87.073
30	3.00	4.00	200.00	5.50	91.211

3. Result and discussion

3.1. Characterization

The Fe₃O₄ nanoparticle investigation with FE-SEM (Fig. 1a) reveals particles of similar size and spherical shape, while TEM evaluation (Fig. 1b) reveals the unified size of many nanoparticles within the size range of 10–30 nm. The XRD analysis of Fe₃O₄ is composed of peaks related to (Fig. 1c) intensities of its pure form without impurity. This material (Fig. 1c) has eight X-ray diffraction peaks appearing at $2\theta = 30.0^\circ$, 35.6° , 37.0° , 43.1° , 47.0° , 53.4° , 57.3° and 62.8° (JCPDS NO. 88–0866), which are assigned to the (2 2 0), (3 1 1), (2 2 2), (4 0 0), (3 3 1), (4 2 2), (5 1 1) and (4 4 0) crystal planes, respectively.

Fig. 1. (a) SEM image, (b) TEM image and (c) XRD pattern of the Fe₃O₄ nanoparticle.

3.2. Experimental design and data analysis

The CCD, as best optimization tools, was applied to investigate the correlation among variables, including ferrofluid volume, pH, vortex time, ionic strength (NaCl concentration (%w/v)) and DOC extraction (response). Based on selected levels, according to Table 1, the recommended experimental factors were undertaken, and their consequent response is represented in Table 2. The interaction and main effects at 95% confidence level were estimated using ANOVA according to judgment based on F-test and their respective *p*-value. The obtained construct between the ER% and independent factors (Eq. (1)) based on numerical value are mentioned as follows. The positive sign indicates synergistic effects, while the negative sign shows the antagonistic effects [24].

(1)

$$\begin{aligned}
 \text{ER}(\%) = & -121.52345 + 22.43710A + 59.62849B + 0.85833C \\
 & + 2.30473D - 1.26983AB - 0.011032AC \\
 & - 0.37811AD - 0.039757BC + 0.83145BD \\
 & + 0.011122CD - 1.90597A^2 - 7.46748B^2 \\
 & - 2.39502E - 003C^2 - 0.45438D^2
 \end{aligned}$$

Table 2. Analysis of variance (ANOVA) for determination of DOC.

Factor	Sums of squares	Df*	Mean squares	F-value	P-value
Model	4220.86	14	301.49	126.68	< 0.0001
A	154.83	1	154.83	65.05	< 0.0001
B	800.43	1	800.43	336.32	< 0.0001
C	14.23	1	14.23	5.98	0.0273
D	54.99	1	54.99	23.10	0.0002
AB	58.05	1	58.05	24.39	0.0002
AC	10.95	1	10.95	4.60	0.0487
AD	11.58	1	11.58	4.87	0.0434
BC	63.23	1	63.23	26.57	0.0001
BD	32.81	1	32.81	13.78	0.0021
CD	11.13	1	11.13	4.68	0.0471
A ²	508.60	1	508.60	213.70	< 0.0001
B ²	1005.36	1	1005.36	422.42	< 0.0001
C ²	991.48	1	991.48	416.59	< 0.0001
D ²	18.84	1	18.84	7.92	0.0131
Residual	35.70	15	2.38		
Lack of Fit	28.08	9	3.12	2.46	0.1429
Pure Error	7.62	6	1.27		
Cor Total	4256.56	29			
Model Summary Statistics					
Std. Dev.		1.54	R-Squared		0.9916
Mean		81.52	Adj-R-Squared		0.9838
C.V.%		1.89	Pred-R-Squared		0.9565
PRESS		185.32	Adeq-Precision		39.247

*Degrees of freedom.

The acceptability of a model is based on ANOVA (Table 2), and the respective results imply the model *F*-value was 126.68. The corresponding *p*-value was < 0.0001, and both of these values confirm its suitability for well representation of data. The model was evaluated by its coefficient of determination (*R*²) (0.9916); *R*² was adjusted and predicted with value of 0.9838 and 0.9565, respectively. The value of *R*² indicates that 99.16% of the response change is attributed to parameters effect. Fig. 2a shows acceptable and good agreement among experimental recoveries. The significant variables investigation by Pareto plat (Fig. 2b) represents and emphasizes the significant parameters exceeding the vertical line (*P* = 0.05) [25]. The low value of coefficient of variance (CV) (1.89%) shows the precision of the proposed model and confirms its high reliability and applicability for fitting experimental results. Adequate precision measured the signal to noise ratio and ratio (39.247), which confirms adequate signal.

Fig. 2. (a) Correlation of experimental and predicted values, (b) Pareto chart for determination of DOC.

3.3. 3D-dimensional plots

Fig. 3 indicates the effects of all parameters on the extraction percentage, and as expected, DOC mass transfer and its respective mechanism strongly depend on solution pH. The solution pH control at suitable pH suppresses the ionization of acidic or basic DOC species. Meanwhile, pK_a of DOC ($pK_a = 3.09$) at high pH causes higher ionization, and basic compounds interfere with DOC extraction by the ferrofluid phase, which encourages us to investigate pH contribution in the range of 1.5–7.5. The optimal extraction efficiency was achieved at pH 3.0, and it was demonstrated that increasing or decreasing the pH decreases the extraction percentage.

Fig. 3. Three-dimensional plot for determination of DOC.

Vortex time is an effective factor on extraction, as raising its respective time from 1 to 7 min leads to higher DOC extraction due to more intense binding interfaces of the ferrofluid phase and aqueous phase, which consequently improves and facilitate the migration and penetration of DOC molecules. The DOC extraction efficiency is improved at higher ferrofluid volume (Fig. 3a) up to 150 μL , and higher volume leads to sharp decrease in extraction percentage, which is attributed to the DOC dilution effect. Moreover, the ionic strength affects the DOC solubility in the aqueous phase, and accordingly, NaCl content contribution on the DOC extraction in the range of 1–5 w/v% examination (Fig. 3b) indicates that its raising from 1 to 4 w/v% leads increase of the extraction efficiency. Further increase (above 4% w/v) causes a rise in solution viscosity, making mass transfer difficult and resulting in lower extraction efficiency.

3.4. Optimization

Fig. 4 shows the suggested optimum value of each factor on DOC extraction by CCD, which accordingly is set as pH of 3, 150 μL of ferrofluid, 4%w/v NaCl and vortex time 7 min, resulting in 99.36% extraction efficiency.

Fig. 4. Optimization plot for the determination of DOC.

3.5. Method validation

Analytical performance of the present ferrofluid liquid-phase microextraction method was investigated under optimum conditions—pH of 3, 150 μL of ferrofluid volume, ionic strength (NaCl concentration (%w/v)) of 4%w/v and 7 min vortex time (Table 3). The calibration graph was achieved after pre-concentration of standard DOC solutions between 10 and 400 ng mL^{-1} with R^2 of 0.983. The enrichment factor, based on the calibration slope ratio after to before pre-concentration [26], is 83.61. The suitable closeness of pre-concentration factor (66.67) to enrichment factor confirms DOC quantitative extraction and recovery. The detection and quantification limits based on $3S_b$ and $10S_b$ division to calibration curve slope, while S_b represents the blank standard deviation ($n = 10$) [27,28] were 3.6 ng mL^{-1} and 8.5 ng mL^{-1} , respectively. The repeatability and reproducibility of the proposed method were verified by five replicates at 150 ng mL^{-1} DOC at same day and two consecutive days [9], and were $97.52 \pm 3.74\%$ for repeatability (intra-day) and $95.60 \pm 4.15\%$ reproducibility (inter-day). These results show high accuracy and reliability of the suggested method.

Table 3. Figures of merit for determination of DOC.

Quantitative analysis	Values
Sample volume (mL)	10
Extraction solvent (μL)	150
Linear range (ng mL^{-1})	10.0–400.0
Coefficient of determination (R^2)	0.983
Limit of detections (LOD) (ng mL^{-1})	3.6
limit of quantification (LOQ) (ng mL^{-1})	8.5
Enrichment factors (EF)	83.61
Pre-concentration factor (PF)	66.67
Repeatability (ER $\pm$ RSD,%)	$97.52 \pm 3.74\%$

Quantitative analysis	Values
Reproducibility (ER±RSD,%)	95.60 ± 4.15%

3.6. Analysis of real sample

The applicability of the proposed method for the recovery of DOC in the biological sample spiked with known amount of DOC was examined. The accumulated urine samples were centrifuged at 4000 rpm for 30 min, filtered through 0.45 µm membrane filters and stored at 4°C until subsequent application. Also, 5 mL of the blood sample was poured into centrifuge tubes containing sodium citrate (4% w/v), then the mixture was placed into a centrifuge for 10 min at 4000 rpm. Finally, the supernatant transparent solution or plasma blood was separated, diluted to 10 mL and used for the next analyses.

An ultrasonication-assisted digestion method was used to treat bovine milk samples. First, 100 µL of hydrogen peroxide was added to 30 mL of milk sample, then sonicated for 15 min. Then, 5 mL of hydrochloric acid and 5 mL of acetic acid was added into the above solution and sonicated for 10 min. After that, the final mixture was centrifuged at 5000 rpm for 15 min, and finally, the supernatant was collected and filtered using a cellulose membrane with pore size of 0.22 µm and diluted to 50 mL ultra-pure water. Next, 10 mL of this diluted solution was utilized for the extraction process [29], and this entire process was replicated 5 times.

The accuracy of the method was verified by spiking known content of analyte and calculating their relative recovery (RR%), which is listed in Table 4. As seen, DOC is not detected in all biological samples. Regarding the known content addition, the DOC is quantified with recoveries in the interval of 86.7%–97.5%, which confirms the successful application of the present approach for real sample and analysis with RSD < 5.7%.

Table 4. Spiked recoveries (%) of urine, blood plasma and milk samples at optimum conditions.

Samples	DOC Added (ng mL ⁻¹)	DOC Found (ng mL ⁻¹)	RR% ± RSD (%)
Urine 1	0.0	N.D*	—
	10	9.441	94.41 ± 2.80
	100	97.48	97.48 ± 3.17
	250	240.63	96.25 ± 3.57
Urine 2	0.0	N.D*	—
	10	9.28	92.80 ± 4.31
	100	95.51	95.51 ± 3.85
	250	236.4	94.56 ± 3.44
Urine 3	0.0	N.D*	—
	10	9.501	95.01 ± 1.63
	100	96.22	96.22 ± 2.26
	250	235.73	94.29 ± 2.41
Urine 4	0.0	N.D*	—
	10	8.816	88.16 ± 5.28
	100	91.45	91.45 ± 4.69
	250	234.88	93.95 ± 5.72
Urine 5	0.0	N.D*	—
	10	9.463	94.63 ± 2.94
	100	95.71	95.71 ± 3.75
	250	236.33	94.53 ± 3.35
Blood Plasma 1	0.0	N.D*	—
	10	9.376	93.76 ± 1.89

	100	92.2	92.20 ± 4.55
	250	239.91	95.96 ± 4.30
Blood Plasma 2	0.0	N.D*	—
	10	8.85	88.51 ± 3.50
	100	90.62	90.62 ± 3.08
	250	224.86	89.94 ± 2.96
Blood Plasma 3	0.0	9.59	—
	10	18.52	89.30 ± 3.85
	100	101.09	91.50 ± 4.91
	250	235.71	90.45 ± 3.55
Blood Plasma 4	0.0	8.85	—
	10	17.94	90.90 ± 2.13
	100	102.57	93.72 ± 2.49
	250	244.6	94.30 ± 3.27
Blood Plasma 5	0.0	N.D*	—
	10	9.19	91.90 ± 3.16
	100	93.05	93.05 ± 4.81
	250	224.9	89.96 ± 3.64
Milk 1	0	20.53	—
	10	29.5	89.70 ± 4.77
	100	110.82	90.29 ± 5.39
	250	254.39	93.54 ± 4.50
Milk 2	0	27.7	—
	10	36.51	88.10 ± 4.63
	100	119.28	91.58 ± 5.42
	250	249.78	88.83 ± 5.11
Milk 3	0	14.2	—
	10	22.95	87.50 ± 3.19
	100	104.33	90.13 ± 3.80
	250	237.31	89.24 ± 3.65
Milk 4	0	18.13	—
	10	27.44	93.10 ± 4.05
	100	112.62	94.49 ± 4.30
	250	247.37	91.70 ± 4.74
Milk 5	0	12.95	—
	10	22.16	92.10 ± 4.27
	100	102.09	89.14 ± 3.91
	250	229.71	86.70 ± 3.52

*Not detected.

3.7. Comparison with other studies

In comparison with other DOC extraction approaches (Table 5), the suggested method requires a lower volume of hazardous solvent owing to the fact that DES can be produced from non-toxic and ecofriendly reagents. As shown in Table 5, the suggested VA-HDES-ferrofluid-DLLME method supplies good analytical efficiency for DOC extraction with lower LOD, in comparison to other studies. Also, this technique offers highly recommended DOC extraction because of its simple pre-concentration process, application nontoxic DES as an extraction solvent and low cost. The analytical efficiency demonstrates that VA-HDES-ferrofluid-DLLME technique consists of an

effective DOC extraction method in urine, blood plasma and milk samples. The DOC can be measured with the suggested technique at ng mL^{-1} levels in intricate sample matrices.

Table 5. Comparison of different methods for determination of DOC with proposed method.

Extraction Method	Linear Range (ng mL^{-1})	LOD (ng mL^{-1})	RSD (%)	Ref.
Precipitation	100–1000	87	6.65	[30]
SPE	50–500	24.9	6.2	[31]
SPE	5–1000	0.0508	< 11.5	[32]
MSPE-DLLME	10.0–200.0	1.8–2.9	< 8.8%	[33]
MEPS	15–110	0.03–0.21	19%	[34]
MIP-SPE	5.0–500	1.17	< 3.8	[35]
VA-HDES-ferrofluid-DLLME	10–400	3.6	< 5.8	This study

4. Conclusion

A hydrophobic deep eutectic solvent based on ferrofluid was synthesized and applied for VA-HDES-ferrofluid-DLLME and subsequent determination of DOC prior to HPLC-UV. The ferrofluid as green compound was comfortably made from the mixture of magnetic nanoparticles and a hydrophobic deep eutectic solvent. This solvent acted as a carrier liquid to hinder nanoparticles from accumulating by offering shielding layer on all sides of magnetic nanoparticles, consequently enhancing the ferrofluid stability. Unlike most green solvents with hydrophilic features, this solvent not soluble in water. Due to hydrophobic substance of DOC, the hydrophobic interactions between DOC substance and the DES phase in ferrofluid likely act as the main driving force for DOC molecular extraction. Also, vortex-assisted improves the dispersion of the ferrofluid in solution and increases the process of DOC mass transfer to the surface of the ferrofluid, which as a result, propels to higher percentage of DOC extraction in short time.

Meanwhile, several important parameters—such as pH (3), ionic strength (4%w/v of NaCl concentration), 150 μL ferrofluid and 7 min vortex—were optimized using CCD. Under optimum conditions, the limit of detection and quantitative is 3.6 and 8.5 ng mL^{-1} , respectively. The DOC extraction percentage in urine and blood plasma samples are between 86.70% and 97.48%. The enrichment factor and pre-concentration factor of 83.61 and 66.67 are achieved, respectively. The suitable closeness of pre-concentration factor to enrichment factor acknowledges the quantitatively of the suggested extraction technique. The novel VA-HDES-ferrofluid-DLLME method is rapid, simple, eco-friendly and highly efficient for the DOC extraction in real biological samples.

CRedit authorship contribution statement

Ebrahim Alipanahpour Dil: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Mehrorang Ghaedi:** Supervision, Conceptualization, Writing - review & editing. **Arash Asfaram:** Investigation, Formal analysis. **Lobat Tayebi:** Writing - review & editing. **Fatemeh Mehrabi:** Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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