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SOLID-PHASE EXTRACTION OF FOLIC ACID FROM PHARMACEUTICAL FORMULATIONS USING MODIFIED MAGNETIC IRON OXIDE NANOPARTICLES

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ABSTRACT. A new analytical approach was suggested for the extraction and determination of folic acid in pharmaceutical samples using solid-phase microextraction. Magnetic iron oxide nanoparticles modified with sodium dodecylbenzene sulfonate (Fe_3O_4@SDBS) were used for the adsorption of folic acid (FA) from an aqueous solution and determined spectrophotometrically at λ_{max} of 365 nm. The chemical and physical conditions that may affect the efficiency of extraction were studied and optimized, such as the pH of the solution, surfactant and adsorbent amount, extraction time, and desorption factors. A good linearity range of 0.2-6 µg/mL with correlation coefficient higher than 0.999 and limit of detection of 0.08 µg/mL were obtained, in addition to high extraction efficiency of 98% and enrichment factor 15. The method exhibited good accuracy and precision with recoveries ranged 98-102% and intraday precisions of best than 3.5% at all concentrations. The method was successfully applied for the determination of folic acid in pharmaceutical samples within a limited separation time.

KEY WORDS: Folic acid, MNPs, Extraction, SDBS, Spectrophotometry, Solid phase extraction

INTRODUCTION

Folic acid (FA) (N(4-((2-amino-l,4-di-hydro-4-oxo-6-pteridinylmethyl)amino)-benzoyl)-L-glutamic acid) [1], which naturally occurs in cereals, is necessary for humans. It plays an important role in the development of red blood cells, fetal tissue and brain development, the production of RNA and DNA, and the growth of an infant [2]. A literature survey shows that there are several methods available for the assay of folic acid in different matrices, including spectrophotometry [3-5], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [6], ultrahigh performance liquid chromatography [7], voltammetry [8], potentiometry [9], and spectrofluorometry [10]. In addition, different extraction methods of FA from different samples were reported, such as dispersive liquid-liquid microextraction [11], dispersive solid-phase extraction [12], and solid-phase extraction (SPE) [13]. Due to its simplicity and ability to achieve higher extraction efficiency, the MNPS-SPE technique using magnetic nanoparticles (MNPs) proved to be a favorable separation method [14]. Compared to other adsorbents, magnetic nanoparticles provide many advantages, such as large surface area, small size, and stability [15]. The modification processes of nanoparticles' surface using polymer coating, surfactant adsorption, and silanation are commonly used to improve the selectivity and removal efficiency of these particles [16-18]. It is still important to extract trace amounts of pharmaceutical compounds using cheap and green methods engaged with simple techniques such as spectrophotometry. In this study MNPs coated with SDBS were prepared and used for enrichment and quantification of folic acid from aqueous solutions of pharmaceutical forms. Adsorption and desorption of FA were determined spectrophotometrically at 365 nm.

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EXPERIMENTAL

Chemicals and materials

All the chemicals used were of analytical grade, and double-distilled water was used to prepare the solutions for this analysis. $FeCl_3.6H_2O$ (96%), $FeCl_2.2H_2O$ (99.9%) and SDBS were supplied from Merck (Darmstadt, Germany). Sodium hydroxide (NaOH, 99.9%) and hydrochloric acid (HCl, 36% m/m), were supplied from BDH (England). FA (99.0%) was kindly donated by Samara Company for drugs industries (SDI/Iraq). A stock standard solution of FA (100 µg/mL) was prepared by dissolving the required amount of the pure FA in 10 mL of 0.1 M NaOH and completed the volume in 100 mL volumetric flask with double-distilled water. The solution of 1 M of sodium hydroxide was prepared by dissolving a suitable amount of the base in double-distilled water. Also, 1 M of hydrochloric acid was prepared by diluted required volume of concentrated HCl (36% w/w) in 100 mL double-distilled water and standardized. The solution of SDBS (0.2% w/v) was prepared by dissolving 0.5 g of surfactant in 250 mL of double-distilled water.

Pharmaceutical samples preparation

The solutions of two types of pharmaceutical tablets of folic acid (folic acid, 5 mg tablet, Actavis-Indonesia; folic acid, 5 mg tablet, SDI-Iraq) were prepared by taken thirty tablets of each application and weighed then powdered. An amount of the powder (equivalent to 25 mg of standard FA) was weighing and dissolved in 10 mL of 0.1 M NaOH. The contents then transferred to a 100 mL volumetric flask, completed with double-distilled water, shaken, and filtered.

Instrumentation

Single-beam UV–Vis spectrophotometer (Shimadzu-1260, Japan) was used for the absorbance and spectra measurements supplied by quartz cell of 1 cm path length. Metrohm (914 pH, Switzerland) pH/mV meter was used for pH measurement. A magnetic stirrer (IKA, USA) and a strong magnet (1.4 T magnetic fields and 1×5×4 cm) were used for the preparation and active separation of MNPs. The FTIR spectra were recorded by Perkin-Elmer RX-I spectrophotometer (France). The morphology of the magnetic nanoparticles before and after coated was recorded by a scanning electron microscope (SEM) (Philips XL30, Netherlands).

Synthesis of Fe_3O_4 magnetic nanoparticles

Coprecipitation is common method used for the synthesis of iron oxide nanoparticles [19]. It involves the preparation of 50 mL of an aqueous solution results from mixing and dissolved 10.4 g of $FeCl_3 \cdot 6H_2O$, 3.27 g of $FeCl_2 \cdot 2H_2O$, and 1.7 mL of 12 M HCl. Formation MNPs was accomplished by adding the previous solution slowly using a dropping funnel to the alkaline solution (500 mL of 1.5 M of NaOH) heated and keeping the temperature to 80 ◦C with stirring for 30 min. The formed magnetic nanoparticles were rapidly collected from the reaction medium by an external magnet field, washed three times, and resuspended with double-distilled water. Suspension solution of a concentration of 10 mg/mL (pH = 11) remained stable for at least one month.

Extraction FA using Fe_3O_4 @SDBS

Solid-phase microextraction and back-extraction of folic acid using synthesized Fe_3O_4 @SDBS were performed by the following procedure. A 50 mL portion of the sample containing 0.2–6

µg/mL FA was transferred into a 75 mL beaker, and adjusted the pH by adding 1.0 mL of 1 M HCl. Then 2.0 mL of Fe_3O_4 MNPs and 2.0 mL of SDBS (0.2% w/v) were directly added to the beaker and put on a stirrer for 10 min. The magnetic particles of adsorbents were rapidly collected from the aqueous medium using an external magnet and the percentage of removal was estimated by measuring the supernatant's absorption. The supernatant was removed and the sorbed FA was back-extracted from the MNPs with 2.0 mL of 0.1 M NaOH under stirring for 2.0 min. The magnetic sorbents were collected again by a strong external magnet and the eluent absorbance was measured at 365 nm. The removal efficiency (ER%) was estimated using the following equation:

$$ER\% = (C_o - C_r)/C_o \times 100 \quad (1)$$

where: C_o and C_r are the initial and remaining amount of the FA in the solution (µg/mL), respectively. All the optimization experiments were performed using 6 µg/mL of FA solution at room temperature

RESULTS AND DISCUSSION

Adsorbent characterization

Sorbent characterization and morphology with and without surfactant coating were explored by Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM) respectively. Two characteristic bands at 576 and 582 cm^{-1} associated with the metal-oxygen vibrational bands (Fe-O) were observed in both spectra (with and without coated SDBS) [20]. Also, the peaks that appeared at 3429 cm^{-1} and 1618 cm^{-1} in the spectrum of uncoated Fe_3O_4, are attributed to the O–H stretching and H–O–H bending modes of vibration, respectively of adsorbent water molecules [21]. In comparison with the spectrum of pure MNPs, the spectrum of coated particles with SDBS showed characteristic bands. The band at 1631 cm^{-1} is due to the presence of the aromatic group. The bands at 2925 and 2856 cm^{-1} could be attributed to the stretching vibration of C–H, while the bands 1542, 1514, and 1400 cm^{-1} are attributed to carbon-carbon stretches in the aromatic ring of the SDBS molecule. According to this information, it was hypothesized that SDBS was related to physical adsorption to the magnetite particles. The adsorption bands at 1340 and 1180 cm^{-1} could be attributed to strong asymmetric and symmetric stretching vibration of the S=O band, which indicated the coated of the surface of Fe_3O_4 MNPs with SDBS. The description of surface morphology for nanoparticles of iron oxide is shown in Figure 1. For the accumulation of numerous nanoparticles with a mean diameter of 40 nm, an SEM image of the magnetic nanoparticles with and without SDBS-coating was produced.

Optimizations MNPS-SPE variables

Optimization of all conditions that can affect the separation process has been developed to achieve successful extraction and maximum recovery percent. Figure 2 illustrates the spectrum of FA determined before and after the extraction process. Maximum absorbance of FA was recorded at a wavelength of 365 nm against the reagent blank (Figure 2) and was used in all experiments. There were two main steps in the present extraction process: adsorption and desorption (back-extraction) of the vitamin. A folic acid molecule containing an aromatic amino group in addition to other types of amino groups. These groups are easily converted to ammonium group that carries a positive charge in acidic medium, on the other hand and in the same medium the surface of MNPs is carrying a positive charge. To obtain a successful extraction and increase the affinity of FA to MNPs surface, SDBS (anionic surfactant carries a negative charge) was used for coating magnetic particles and facilitate the adsorption process.

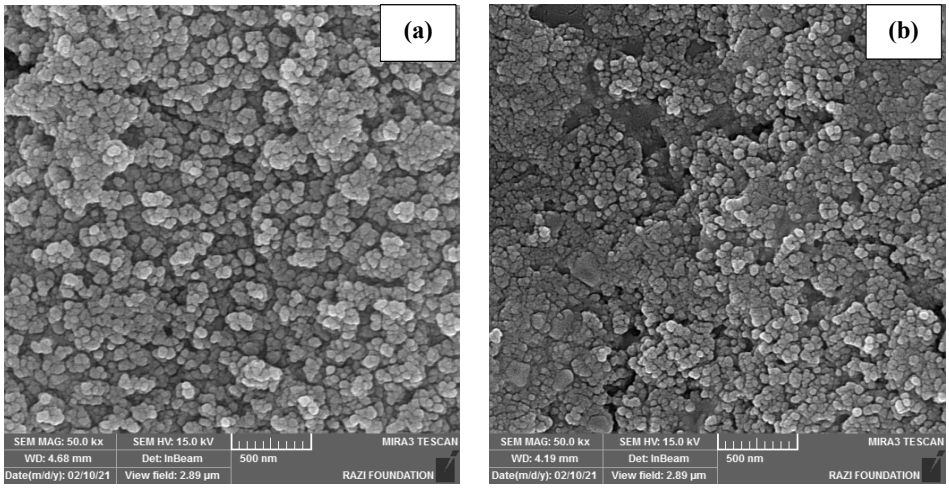


Figure 1. SEM image of (a) Fe_3O_4 nanoparticles and (b) SDBS-coated Fe_3O_4 nanoparticles.

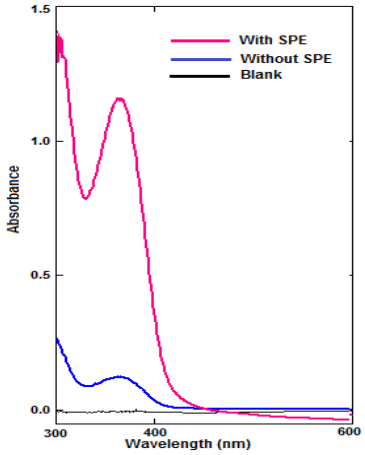


Figure 2. The spectra of FA measured against blank before and after SPE.

Effect of SDBS concentration

The adsorption process on the adsorbent surface is based on both hydrophobic interactions and electrostatic attraction in the mixed-hemimicelles phase. Under acidic conditions, both the FA (containing the aromatic amine group) and the surface of the MNPs bear a positive charge. The repulsion force between two positive charges of FA and the surface of MNPs, making it impossible to extract the analyte without altering the surface of MNPs. The anionic surfactant SDBS, with a negative sulfonate group, increases the FA adsorption and attraction on the surfaces of positively charged MNPs in an acidic medium. The effect of SDBS (0.2% w/v) concentration required for modification of the surface of MNPs was considered using different volumes (1-4

mL) of surfactant (Figure 3 a). Figure 4a showed that with the increase of the amount of SDBS, the extraction efficiency of the drug has been changed slightly, because of the formation of mixed hemimicelles on the MNPs' surface. The absorbance was progressively reduced after 2 mL because the surfactant molecules form micelles molecules in an aqueous solution allowing reorganization of the extracted drug molecules [19]. So, volume 2 mL of SDBS was selected as the optimum volume.

Effect of extraction medium

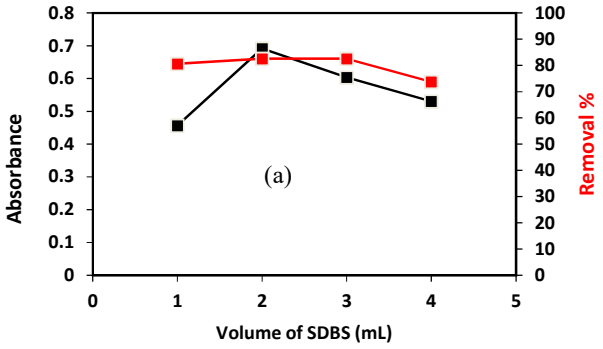
The pH of the extraction medium is mainly affected the extraction efficiency by controlling the charges of the analyte and MNPs' surface. Preliminary experiments of the extraction process of FA indicated the necessity to be conducted in an acidic medium. In an acidic medium (pH of the sample is lower than the pH zero point of charge (6.5)) both the surfaces of the MNPs and FA bear a positive charge. The positively charged surface of MNPs in the solution provided favorable conditions for increased anionic surfactant adsorption, resulting in increased cationic FA removal. The effect of various volumes of HCl (1 M) on FA extraction efficiency was investigated in the range of 0.5–3 mL (Figure 3b). Using 1mL of HCl solution, maximum absorbance value of desorbed FA with removal percentage (82%) was achieved and therefore was chosen for further work.

Influence of amount of nanosorbent

The optimum dose of MNPs causing efficient extraction of analyte was investigated by adding various volumes ranging from 0.5–4.0 mL of the MNPs suspension (10 mg/mL) to the sample (Figure 3c). Due to the increase of the available sites for extraction with increasing the amount of MNPs, the removal percentage was increased from 74.9 to 86.4% with the increasing amount of sorbent up to 2 mL. After 2 mL the extraction efficiency significantly not changed, while the absorbance of desorbed FA was extremely decreased. As a result, 2 mL of the magnetic sorbent was chosen for the next experiments.

Influence of contact time

To investigate the effect of contact time on the extraction efficiency of FA onto Fe_3O_4@SDBS, experiments were conducted at various stirring times. The effect of extraction time was investigated in the range of 4–15 min, and the findings showed enhanced extraction efficiency with time and maximum extraction efficiency of 96.1% was obtained with 10 min and then decreased gradually. This depression may be linked to nanoparticles' shorter diffusion path; therefore, a contact time of 10 min was chosen for the extraction process (Figure 3d).



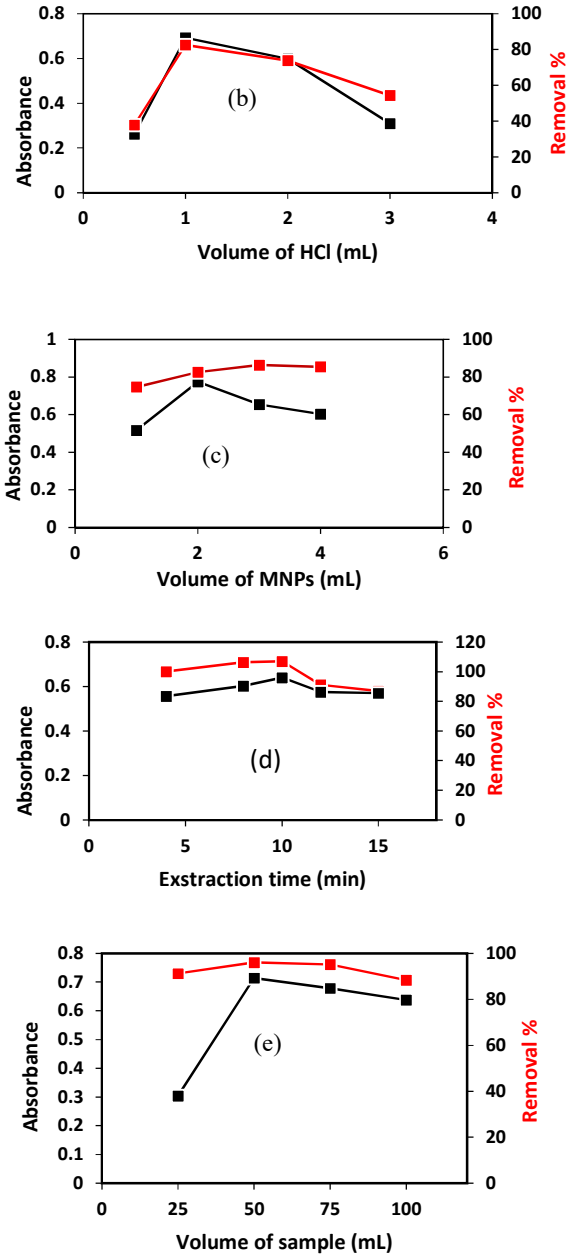


Figure 3. Effect of (a) sodium dodecylbenzene sulfonate (0.2%w/v), (b) hydrochloric acid (1 M), (c) volume of MNPs, (d) extraction time, and (e) volume of sample on adsorption and desorption the FA.

Study of desorption conditions

Type and volume of desorption solvent

Back-extraction of FA from the surface of sorbent can be established by disturbing the hemimicelles' structure with an organic solvent. To achieve high recovery efficiency, a variety of solvents including methanol, ethanol, acetone, and sodium hydroxide (0.1 M) were studied. The results showed that sodium hydroxide had the maximum response (Figure 4a), so it was selected as the best solvent. Different volumes of sodium hydroxide (0.1 M) were used for selected the best volume of eluent needed for effective FA extraction, and the results showed that 2 mL volume yielding the highest absorbance (Figure 4b). Based on this result the preconcentration factor for this procedure was determined to be 25.

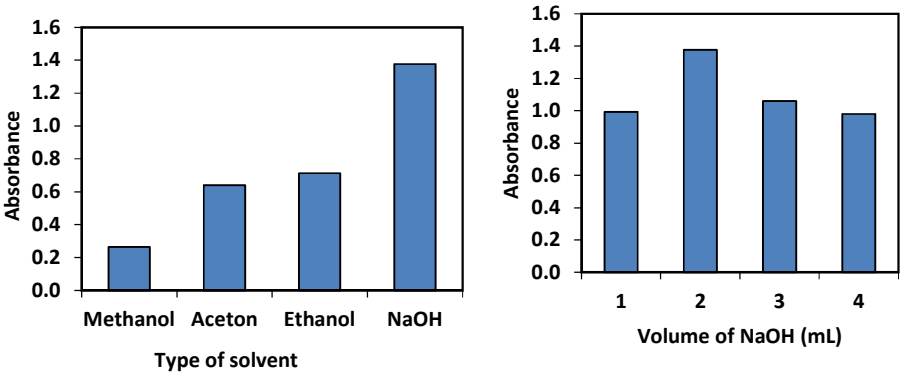


Figure 4. Effect of (a) type of eluent, (b) volume of eluent on desorption. Conditions: 50 mL of 6 µg/mL FA; 1 mL of 1 M HCl; 2 mL of MNPs; 2 mL of 0.2% (w/v) SDBS; extraction time is 10 min.

Influence of elution time and adsorbent reusability

To obtain effective analyte back-extraction from sorbent, the time required for elution FA was investigated in the range of 1-4 min. As shown in Figure 5, the absorbance value of the desorbing FA was increased with the elution time up to 2 min, and then decreased slightly. Thus, 2 min was selected as optimum desorption time. Also, the ability of adsorbent (Fe_2O_3@SDBS) of adsorbing 6 µg/mL FA during six consecutive extraction cycles has been studied. The reusability of MNPs was investigated by re-used the same nanoparticles for several consecutive extractions. The extraction efficiency slightly decreased from 96.1 to 90.3 % after repeating the process four times and reduced to 80 % by the fifth cycle. This means the possibility of used synthesized NPs 4 times with acceptable recovery and negligible decrease in removal efficiency.

Method validation

The analytical parameters of the current method for extracting FA were studied under the optimized conditions outlined in Table 1. The Figures of merit of the MNPS-SPE extraction method such as linear range, limits of detection and quantification, regression equation, correlation coefficient, and preconcentration factor were calculated and documented in Table 2. The linearity of the method was ranged from 0.2–6 µg/mL of FA, with detection and quantitation limits of about 0.08 and 0.26 µg/mL, respectively. Also, the enrichment factor (EF) was estimated

by dividing the slopes of calibration curves with and without the extraction process. An evaluation of the extraction method was developed by extracting different amounts of FA in five replicates. The obtained values of relative standard deviation (RSD 3.5%) and recovery percent (98-102%), exhibited high accuracy and repeatability of the proposed extraction method (Table 3).

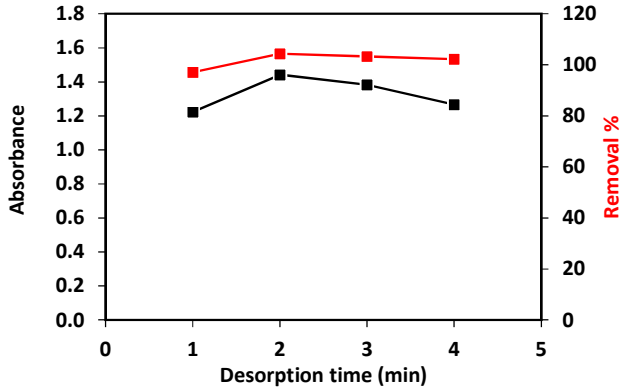


Figure 5. Study of desorption time. Conditions: 50 mL of 6 µg/mL FA; 1 mL of 1 M HCl; 2 mL of MNPs; 2 mL of 0.2% (w/v) SDBS; extraction time is 10 min.

Table 1. Optimum extraction conditions.

Variable	Studied range	Optimum value
Amount of SDBS (0.2% w/v), mL	1-4	2
Volume of HCl (1M), mL	0.5-3	1
Amount of MNPs, mL	1-4	2
Contact time, min	4-15	10
Volume of sample, mL	25-100	50
Type of desorption solvent	Different solvent	NaOH
Volume of NaOH (0.1 M), mL	1-4	2
Desorption time, min	1-4	2

Extraction and estimation of FA in pharmaceutical forms

The extraction of various amounts of FA in pharmaceutical forms was investigated to verify the efficacy of Fe_3O_4 MNPs coated with SDBS sorbent. By spiking two types of FA tablets with varying concentrations of FA at two concentration levels in five replicate and analyzing using the extraction technique, recovery testing validated the applicability of the proposed method. The obtained spiked recoveries values (98.3–104%) and RSDs values (2.3–3.5%) are shown in Table 4, indicating the method's accuracy, independence from the matrix effect, and high reproducibility. Student's t and F tests [22], were used to compare the obtained results to those reported using the official pharmacopeia method [1]. The statistical values indicating a non-significant difference in accuracy between the two methods.

Comparison MNPS-SPE with other literature methods

The proposed approach was compared to several published FA preconcentration-determination approaches [5, 7, 23-26], and Table 5 summarizes the main features for the proposed and the literature methods. According to the comparison in Table 5, it was clear the possibility

replacement of the simple and cheap instrument like spectrophotometer with several expensive techniques such as HPLC, UPLC-IC, and LC-MS/MS with the identical degree of sensitivity. Also, the proposed approach is more accurate (recoveries values) [7, 24-26] and more precise [23, 26] as compared with other literature methods. MNPs can also be easily synthesized using cost-effective materials.

Table 2. Analytical parameters for assay of FA with and without and extraction.

Parameter	Value	
	With extraction	Without extraction
Maximum wavelength, nm	365	365
Calibration equation	y = 0.2712x + 0.0200	y = 0.0181x - 0.0248
Correlation coefficient, r	0.9996	0.9996
Linearity range (µg/mL)	0.2-6	2-60
Limit of detection, LOD (µg/mL)	0.08	0.43
Limit of quantification, LOQ (µg/mL)	0.26	1.43
Average of recovery %	100.3	98.85
RSD (%)	< 3.47	< 2.13
Molar absorptivity (L/mol.cm)	1.19×10^5	7.99×10^3
Sandell's sensitivity, S (µg/cm^2)	3.71×10^{-3}	5.52×10^{-2}
Slope, b (mL/µg)	0.2712	0.0181
Intercept, a	0.0200	-0.0248
$S_{y/x}$	1.94×10^{-2}	1.18×10^{-2}
S_b	3.41×10^{-3}	1.83×10^{-4}
S_a	1.15×10^{-2}	5.35×10^{-3}
Preconcentration factor	15	-
Enrichment factor	25	-

Table 3. Accuracy and repeatability for assay of FA using MNPS-SPE method

Concentration of FA, µg/mL		Error %	Rec.%	RSD% (n = 5)
Added	Found			
2	1.96	-2.00	98.00	3.47
3	3.04	1.33	101.3	2.38
4	4.06	1.50	101.5	2.67

Table 4. Estimation of FA in pharmaceutical forms using suggested and official methods.

Dosage form	MNPS-SPE method						Official method					
	Taken conc. (µg/mL)	Spiked conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[a]	Mean (%Rec. ± SD)	RSD (%)[a]	Taken conc. (µg/mL)	Spiked conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[b]	Mean (%Rec. ± SD)	RSD (%)[b]
Actavis® Tablets (5 mg)	1	1	2.07	103.5	102.4±0.9	3.44	10	5	14.71	98.07	98.20±1.13	1.91
		2	3.08	102.7		2.70		10	19.52	97.60		1.59
	2	1	3.04	101.3		2.41	20	10	29.19	97.30		2.07
		2	4.09	102.3		2.84		20	39.93	99.83		1.15
Folic acid® Tablets (5 mg)	1	1	1.99	99.50	99.27±2.04	3.50	10	5	14.92	99.47	99.43±0.77	1.49
		2	2.95	98.33		2.34		10	20.09	100.5		1.01
	2	1	3.06	102.0		2.97	20	10	29.76	99.20		2.09
		2	3.89	97.25		2.34		20	39.44	98.60		0.61
Pure FA					100.3±2.0						98.85±0.97	
t (2.776) [c] F (19.00)[c]	1.832 6.926		$(n_1-1)=2$, $(n_2-1)=2$, $(n_1+n_2-2)=4$									

a, (Average of five determinations); b, (Average of five determinations); c, Theoretical value.

Table 5. Comparison the suggested extraction method with some reported methods.

Analytical technique	Enrichment method	Application	Linear range (µg/mL)	LOD (µg/mL)	Rec. %	RSD%	Ref.
Spectrophotometry	SLE	Salt and solution	1- 25	0.011	99-101.7 98.2-104.1	-	[5]
UHPLC-IC	-	Pharmaceutical samples	0.1- 10	0.0061	92.4- 107.4	0.49- 1.5	[7]
HPLC	SPE	Pharmaceutical samples	0.2- 2	-	97.0	4.9	[23]
HPLC	Enzymatic Extraction	Fortified Rice and Wheat Flour	0.05– 0.8	0.02	90.9 and 80.5%	0.55	[24]
LC-MS/MS	SLE	Wheat Flour	0.001- 0.05	0.06*	85	3	[25]
LC-MS	SPE	Pharmaceutical samples	0.05- 0.6	0.0125	91	8	[26]
Spectrophotometry	SPE	Pharmaceutical samples	0.2- 6	0.08	100.3	3.5	Present work

LC-MS/MS: liquid chromatography with tandem triple quadrupole mass spectrometry, LC-MS: high-performance liquid chromatography–mass spectrometry, SPE: solid-phase extraction, HPLC: high performance liquid chromatography, UHPLC-IC: ultra-high performance liquid chromatography-ionic chromatography. LLE: liquid-liquid extraction, *(µg g^{-1}).

CONCLUSION

Simple MNPS-SPE method was developed for the preconcentration-determination of FA based on used MNPs modified with SDBS as sorbent. The proposed extraction method is rapid, sensitive and cost-effective with high accuracy and precision. Microgram quantities of the vitamin were extracted successfully from pharmaceuticals samples using this procedure. Back-extraction of FA was performed with a small volume of sodium hydroxide as eluent rather than toxic organic solvents. The developed MNPS-SPE method for FA extraction proved to be effective (extraction efficiency 98%) and provided good recoveries (100.28) and precision (3%). According to this research, SDBS coated MNPs have a high analytical capacity for preconcentration-determination of trace quantities of essential species.

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