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Application of Iron Oxide Magnetic Nanoparticles Modified by Superhydrophobic Cellulose as a Sorbent for Solid-Phase Extraction of Fat-Soluble Vitamins

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ABSTRACT

Superhydrophobic cellulose-coated magnetic nanoparticles (SCCMNPs) were synthesized and applied as an advanced solid-phase extraction (SPE) sorbent for the simultaneous extraction of fat-soluble vitamins (FSVs) (A, E and D_3). These modified nanoparticles (NPs) improve extraction efficiency and reduce process time through magnetic separation. The cellulose coating, further modified with stearoyl chloride, enhances sorbent stability and specificity, providing strong hydrophobic interactions while preventing NP aggregation. Extracted vitamins were quantified by high-performance liquid chromatography. Optimization of coating materials revealed that 1.0000 g cellulose and 1.00 mL stearoyl chloride per 1.0000 g magnetic NPs (MNPs) were optimal. The NPs were characterized using Fourier-transform infrared spectroscopy, alternating gradient force magnetometry, dynamic light scattering and zeta potential measurements. Optimized extraction conditions included 50.0 mg sorbent, 10.00 mL sample solution (pH = 5.0), 250 µL tetrahydrofuran as the desorption solvent and 1.5-min sorption/desorption times. Calibration curves exhibited excellent linearity ($R^2 \geq 0.999$), with a dynamic linear range of 2.4×10^1–1.0×10^3 µg/L, limits of detection ≤8.6 µg/L, repeatability (%relative standard deviation [RSD] ≤ 4.6) and accuracy (recovery ≥79.60%). This is the first report demonstrating the application of stearoyl-modified cellulose in SPE, enabling rapid, solvent-efficient and highly selective vitamin extraction. The method provides a sustainable, cost-effective and high-performance alternative to conventional SPE, achieving efficient recovery with minimal sorbent amount, attributed to its optimized hydrophobicity from the affordable cellulose support. The application of this method in extracting FSVs from pharmaceutical formulations highlights these NPs as a promising next-generation SPE sorbent, offering an efficient, selective and environmentally benign solution.

1 | Introduction

Vitamins encompass a diverse array of structurally distinct, complex organic compounds that are indispensable for normal metabolism. These substances function as biological catalysts in photochemical processes within living cells. Primarily acting as constituents of enzyme systems, vitamins play a pivotal role in metabolism and enhance immunity against various diseases [1]. On the basis of solubility, vitamins are categorized into two groups: water-soluble vitamins and fat-soluble vitamins (FSVs). The latter includes four main groups—vitamins A, D, E and K. Although small amounts of FSVs are crucial for maintaining normal metabolic functions and overall health, excessive intake can lead to toxicity. Therefore, monitoring

FSV levels in fortified foods and pharmaceuticals is critical to ensure [2].

High-performance liquid chromatography (HPLC), coupled with various detection techniques, is one of the most widely used analytical methods for quantifying vitamins [3–5]. Among these, reverse-phase HPLC with a UV detector is particularly common [2, 6–10]. The detection and quantification of vitamins in medicinal and natural samples are challenging because of their low concentration and their fatty characteristics. Accurate measurement of FSVs necessitates their separation from complex sample matrices and, when required, their pre-concentration. Moreover, FSVs are sensitive to extreme pH, oxygen, light and heat, making an extraction step essential during sample preparation to ensure reliable results [2, 11].

Various methods have been developed to extract FSVs from different matrices such as saponification followed by solvent extraction [12, 13], liquid–liquid extraction (LLE) [14, 15], supercritical fluid extraction (SFE) [16], liquid-phase microextraction [17] and solid-phase extraction (SPE) [18–22]. Among the mentioned methods, SPE remains a widely used sample preparation technique. Compared to conventional solvent extraction methods, SPE offers significant advantages such as reduced equipment complexity, higher pre-concentration efficiency and improved recovery rates. However, traditional SPE techniques face inherent limitations, including high back pressure in packed cartridges, slow throughput and inefficiencies in separation during dispersive modes reliant on centrifugation or filtration. Furthermore, sorbent materials, such as unmodified nanoparticles (NPs), often lack selectivity in complex matrices, leading to potential interferences. Addressing these challenges is essential to meet analytical requirements effectively.

Various solid compounds have been used as adsorbents in the SPE method such as polar adsorbents, non-polar polymer resins, ion exchange adsorbents, activated carbon, molecular sieve and NPs [23–25]. NPs are promising sorbents for SPE due to their high surface-to-volume ratio, which enhances extraction capacity. Additionally, surface modifications can improve their efficiency in selective analyte extraction. However, NPs present limitations, such as high back pressure in packed cartridges and inefficiencies in the separation of the solid phase when using dispersive modes [23, 26].

Magnetic nanoparticles (MNPs) are a key innovation in addressing these issues. These particles are superparamagnetic, meaning they are strongly attracted to a magnetic field but lose all residual magnetism when the field is removed. This property allows for efficient separation without the complications of residual magnetism, such as aggregation or clogging during subsequent handling steps [25, 27]. However, the use of MNPs may still introduce other potential side effects that must be managed. For example, the tendency of pure inorganic NPs (such as Fe_3O_4 and Fe_2O_3) to aggregate in solution can alter their magnetic properties, reduce their effectiveness and complicate their recovery. In addition, these nanometre-sized metal oxides, in their unmodified state, lack target selectivity, meaning they cannot specifically interact with the desired analyte. Instead, they may bind indiscriminately to various substances, leading to non-specific adsorption and poor extraction efficiency. For example, in samples with complicated matrices—such as biological fluids, food or pharmaceutical formulations—these NPs are unable to effectively differentiate between the target analyte and interfering substances. To address these limitations, a suitable coating is essential to enhance their selectivity and stability. Various compounds, including silica, carbon, polymers and cellulose, have been employed for coating and surface modification of MNPs to tailor their interactions with specific targets [20, 28].

Cellulose, the most abundant natural polysaccharide, is a promising biopolymer for NP coating, creating a suitable sorbent for extraction of different species. Its surface chemistry involves a large number of hydroxyl (OH) groups, which are easily hydrogen bonded with water and enable water molecules to spread on the surface. Its surface chemistry and structure can easily change and shift from hydrophilic to hydrophobic/superhydrophobic by acylation of cellulose hydroxyl groups with long-chain alkyl hydrocarbons [29, 30]. Stearoyl chloride is one of the long-chain fatty acid acyl halides which can be replaced with the hydroxyl groups and modifies the surface of cellulose to enhance its hydrophobicity [29, 31, 32].

The proposed method utilizing superhydrophobic cellulose-coated MNPs (SCCMNPs) addresses these limitations effectively. Magnetic separation not only simplifies the extraction workflow by eliminating the need for centrifugation but also ensures a rapid and clean separation process. The innovative surface modification with cellulose and stearoyl chloride enhances both selectivity and stability, providing strong hydrophobic interactions with FSVs while preventing NP aggregation. These advancements result in reduced sorbent quantities, shorter extraction times and higher recoveries, underscoring the efficiency of SCCMNPs as a next-generation sorbent for SPE. Finally, the FSVs content in pharmaceutical samples was determined using proposed methods, and results showed good agreement with those reported by manufacturers.

2 | Experimental Procedures

2.1 | Reagents and Standard Solutions

Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), microcrystalline cellulose, boric acid, phosphoric acid (85%), ethanol, dichloromethane, tetrahydrofuran (THF), pyridine, butylated hydroxytoluene (BHT) and HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). 2-Propanol, ammonia, hydrochloric acid (37%) and acetic acid were purchased from AppliChem GmbH (Darmstadt, Germany). Stearoyl chloride was acquired from Fluka (Buchs, Switzerland). Vitamin A (retinol palmitate), vitamin D_3 (cholecalciferol) and vitamin E (α-tocopherol acetate) were generously donated by Amin Pharmaceutical Co. (Isfahan, Iran). All reagents were analytical grade and used as supplied. Ultrapure water was employed in all experiments.

Standard stock solutions of each vitamin were prepared at 10.0 mg/mL: Vitamins E and D_3 were dissolved in methanol, whereas vitamin A was dissolved in 2-propanol containing 0.5%

BHT. These stock solutions were stored under dark and cold conditions to ensure stability. Working standard solutions containing the three FSVs were prepared by diluting appropriate volumes of the stock solutions with a universal buffer solution at pH = 5. The universal buffer consists of a mixture of 0.04 M·H_3BO_3, 0.04 M·H_3PO_4 and 0.04 M·CH_3COOH that has been titrated to the desired pH with 0.2 M NaOH.

2.2 | Apparatus

The HPLC measurements were performed using a reciprocating HPLC pump (Smartline model 1050, Knauer, Germany), a Rheodyne Model 7225 manual injector (Cotati, CA) with a 20 µL loop, an inline degasser (Knauer, model 5050, Germany) and a diode array detector (Smartline model 2600, Knauer, Germany). A reverse-phase H5MOS Hypersil dimethyl(octyl)silane (C_8) column (5 µm, 250 × 4.6 mm^2) supplied by Unicam (UK) was used. The mobile phase, consisting of methanol and water (95:5), was delivered under isocratic elution at a flow rate of 1 mL/min. Detection wavelengths were set at 285 nm for vitamins E and D_3 and 330 nm for vitamin A. Data acquisition and analysis were conducted using EZChrom Elite software (Knauer, Germany). The Fourier-transform infrared (FT-IR) spectra were recorded using the JASCO 6300 spectrometer (Japan). Particle size and dynamic light scattering (DLS) analyses were performed with the HORIBA SZ-100 particle size analyser (Japan). The magnetic properties of the sorbent were characterized using an alternating gradient force magnetometer (AGFM) manufactured by Kavir Kashan Company (Iran).

2.3 | Synthesis of Iron Oxide NPs

According to Zhang et al. [33], 3.5000 g of $FeCl_2{\cdot}4H_2O$ and 8.5000 g of $FeCl_3{\cdot}6H_2O$, were dissolved in 38.00 mL of 0.4 M HCl (degassed beforehand). Then added quickly to 375.00 mL of 0.8 M NH_3 under $Ar_{(g)}$ protection with vigorous stirring (non-magnetic) at room temperature. After 30 min, the black precipitate of Fe_3O_4·MNPs was isolated from the reaction medium by magnetic force. The magnet used in this work is called a 4-tesla neodymium magnet with dimensions of 50 × 40 × 20 mm^3. The precipitates were washed three times with ultrapure water to remove impurities and then re-suspended in 150.00 mL ultrapure water.

2.4 | Synthesis of SCCMNPs

To synthesize the cellulose-coated MNPs (CCMNPs), 0.5000 g cellulose was gradually added to 50.00 mL 10% NaOH solution, until complete saturation. Next, 0.5000 g of synthesized iron oxide NPs were collected from the suspension using an external magnet. Then, they were added to the cellulose alkaline solution and stirred in an ultrasonic bath for half an hour in order to complete the coating process. After that, the synthesized CCMNPs were collected using an external magnet and washed multiple times with ultrapure water until neutral pH was achieved. Finally, the NPs were washed with ethanol and dried under vacuum conditions.

To modify the CCMNPs, 25.00 mL of pyridine and 1.000 g of CCMNPs were mixed in l00 mL round-bottom flask and heated under an argon atmosphere to 100°C under reflux conditions. After stabilization of the system, 1.00 mL of stearoyl chloride was added dropwise to the flask content. The mixture was stirred under the argon atmosphere and refluxed at 100°C–110°C for 1 h. Finally, the stearoyl-modified CCMNPs were collected from solution using a magnet, washed three times with 20.00 mL methanol and dried in a vacuum oven. This process resulted in the successful synthesis of SCCMNPs.

2.5 | SCCMNPs MNPs-SPE Procedure

A total of 50.0 mg SCCMNPs and 10.00 mL of test solution (or diluted multivitamin formulation) adjusted to pH = 5 were transferred to a 15 mL screw-capped tube. The mixture was shaken on a vortex mixer for 1.5 min to facilitate adsorption. Then, the SCCMNPs were collected at the bottom of the tube using magnetic force, and the supernatant was carefully decanted. The isolated SCCMNPs were dried in a vacuum oven, and then, 250.0 µL of THF was added to the dry SCCMNPs sorbent. To hasten the desorption, the mixture was vortexed for 1.5 min, followed by separation of the SCCMNPs from desorbing solvent using an external magnetic force. Finally, the supernatant was collected and injected into the HPLC system. Figure 1 provides a schematic representation of the SPE procedure using SCCMNPs as the sorbent.

2.6 | Sample Preparation

A multivitamin syrup produced by Alhavi Pharmaceutical Company (Tehran, Iran) and a vitamin D_3 capsule manufactured by Dana Pharmaceutical Company (Tabriz, Iran) were analysed to determine their FSVs content and evaluate the accuracy of the proposed method. The multivitamin syrup was diluted 10,000 times with a pH = 5 universal buffer, and 10.00 mL of resulting solution was subjected to extraction under optimal conditions. The contents of a vitamin D capsule were emptied into a 20.00 mL volumetric flask and made up to volume with 2-propanol. After that, the resulting solution was further diluted 200 times with the pH = 5 universal buffer, and 10.00 mL of this resulting solution was extracted under optimal conditions.

3 | Results and Discussion

3.1 | Characterization of MNPs

FT-IR spectra of Fe_3O_4 MNPs, cellulose, CCMNPs and SCCMNPs were recorded to confirm the surface modification of Fe_3O_4 MNPs with cellulose and with stearoyl chloride groups (Figure 2). As shown in Figure 2a, an absorption band at 568.838 cm^{-1}, resulted from the Fe–O–Fe vibration of Fe_3O_4. Figure 2b shows the FT-IR spectrum of pure cellulose. A wide peak at 3349.75 cm^{-1} is related to the stretching vibrations of the hydroxyl (–OH) groups; an absorption band observed at 2898.49 cm^{-1} corresponds to the stretching vibrations of C–H bonds, and absorption bands observed in the range of 1000–1300 cm^{-1} are associated with the stretching vibrations of the C–O bonds.

Figure 2c shows the FT-IR spectrum of the Fe_3O_4 NPs after the cellulose coating process. As it is obvious, all the correspondence

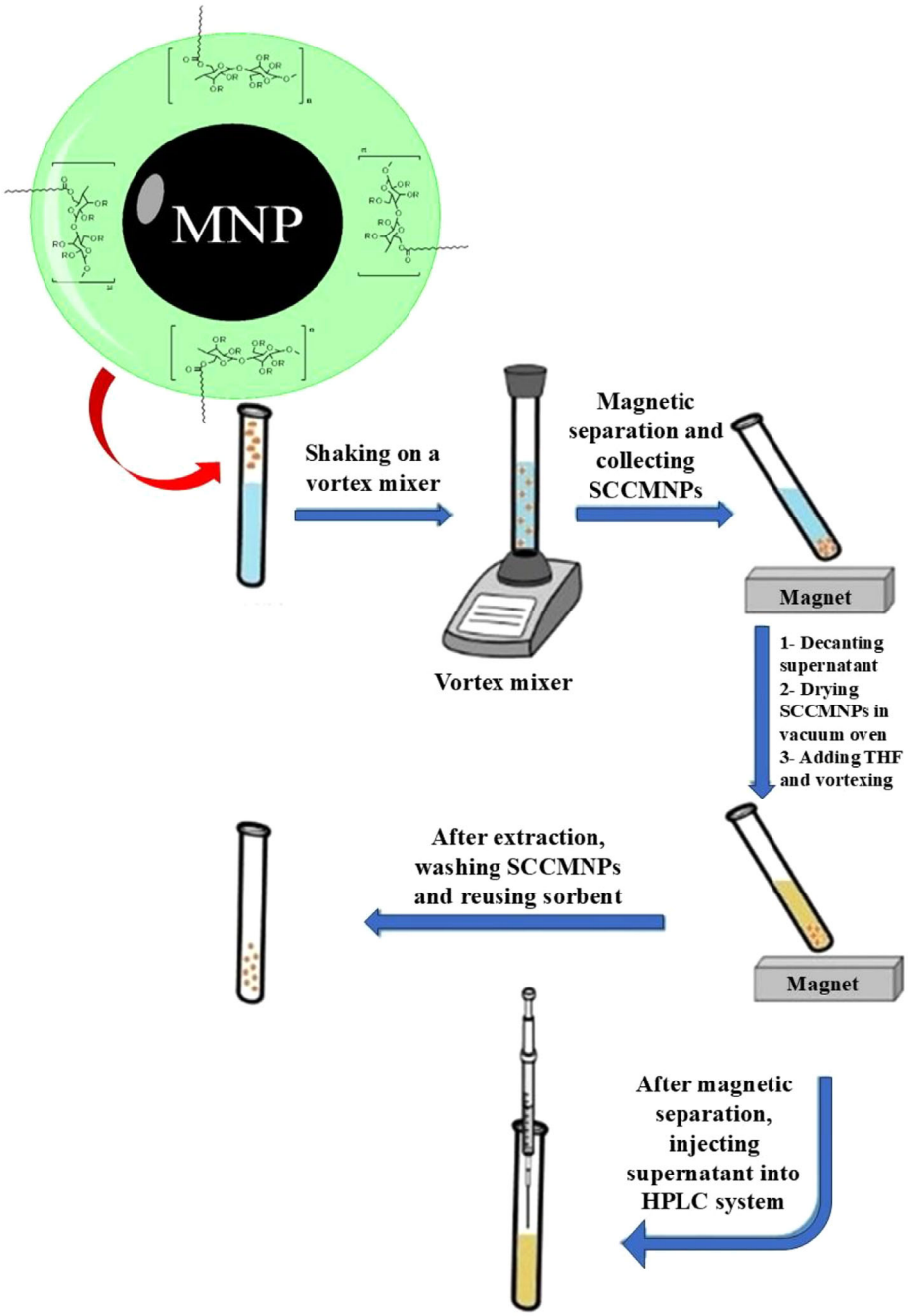


FIGURE 1 | Schematic representation of the solid-phase extraction (SPE) procedure using SCCMNP sorbent. The process includes (1) dispersing superhydrophobic cellulose-coated magnetic nanoparticles (SCCMNPs) in the sample solution and shaking on a vortex mixer, (2) magnetic separation to collect SCCMNPs, (3) decanting the supernatant, drying the sorbent in a vacuum oven and re-dispersing in THF with vortex mixing and (4) magnetic separation of the extract, followed by (5) injecting the supernatant into the HPLC system for analysis. The SCCMNPs are washed and reused after extraction.

characteristic absorption peaks of cellulose are observed in CCMNPs (a wide peak at 3369.03 cm^{-1}, the absorption band at 2898.49 cm^{-1} and bonds observed in the range of 1000–1300 cm^{-1}). Figure 2d shows the FT-IR spectrum of SCCMNPs. The absorption bond seen at 1738.51 cm^{-1} is related to the stretching vibration of the C═O bonds in the ester groups. Due to the addition of the sp^3 carbon chain, which belongs to the ester functional group of structure framework, the intensified absorption bonds appeared at 2851.17 and 2933.59 cm^{-1}, attributed to the stretching vibrations of the C–H bonds.

By comparing these spectra, the successful completion of both the cellulose coating and the esterification steps is clearly confirmed.

The magnetic properties of Fe_3O_4·MNPs, both before and after modification with superhydrophobic cellulose, were evaluated. The AGFM diagrams indicated that all three types of NPs exhibit superparamagnetic behaviour, as no residual magnetism was observed once the external field (H = 0) was removed. A key factor in these properties is the high saturation magnetization, which reflects the maximum magnetic power. According to Figure S1,

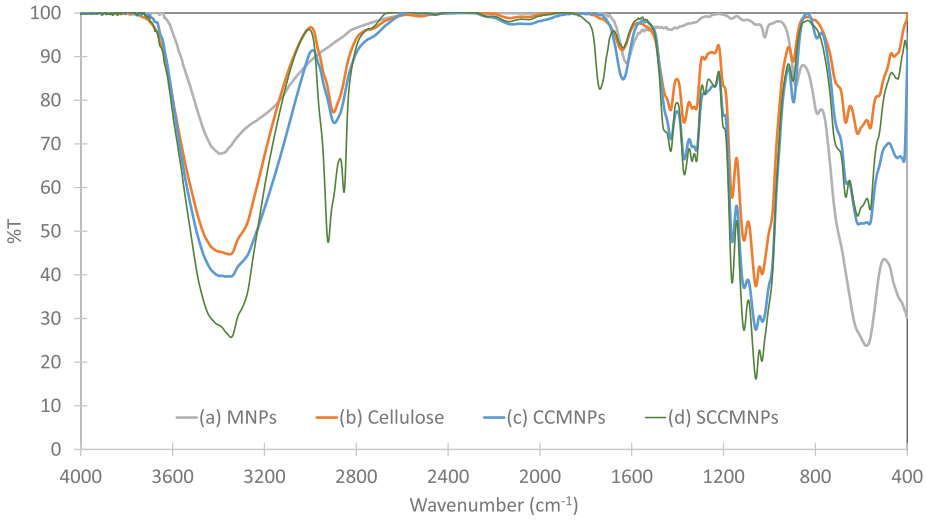


FIGURE 2 | FT-IR spectra of (a) magnetic Fe_3O_4 nanoparticles (MNPs), (b) cellulose, (c) cellulose-coated magnetic nanoparticles (CCMNPs) and (d) superhydrophobic cellulose-coated magnetic nanoparticles (SCCMNPs). FT-IR, Fourier-transform infrared.

the saturation magnetization values for Fe_3O_4·MNPs, CCMNPs and SCCMNPs were 49.60, 8.02 and 6.29 emu/g, respectively. Despite the significant reduction in saturation magnetization following the cellulose coating (a non-magnetic material), the SCCMNPs could still be separated from the solution within minutes using an external magnetic field. The magnetization measurements were conducted using an AGFM instrument at room temperature.

DLS was employed to determine the hydrodynamic particle size and assess the colloidal stability of NPs in the liquid phase. The NPs examined in this project were dispersed in ultrapure water. Generally, NPs have a tendency to aggregate in fluids, forming clusters. The fewer aggregates formed in the system, the more accurate the particle size measurements. In systems where aggregation occurs, the measured particle size will be larger than the actual size of the individual NPs.

As shown in Figure S2, the hydrodynamic size of the synthesized Fe_3O_4 MNPs was 141 nm, whereas the size of CCMNPs was 275 nm. This increase in size is attributed to the presence of multiple hydroxyl groups and hydrogen bonding, which promote aggregation among the CCMNPs. In the final stage, applying a stearoyl coating rendered the particles highly hydrophobic. This hydrophobicity reduces aggregation by promoting separation in water, allowing for the determination of their true size. The hydrodynamic size of the SCCMNPs was measured to be 85 nm (Figure S2), which is significantly smaller than both the uncoated and cellulose-coated NPs. This reduction in size, despite the increased thickness of the cellulose and stearoyl coatings, suggests decreased particle aggregation in the system. This hypothesis is further supported by zeta potential measurements and colloidal stability analyses, which confirm the improved dispersion and reduced tendency for particle agglomeration in the SCCMNPs.

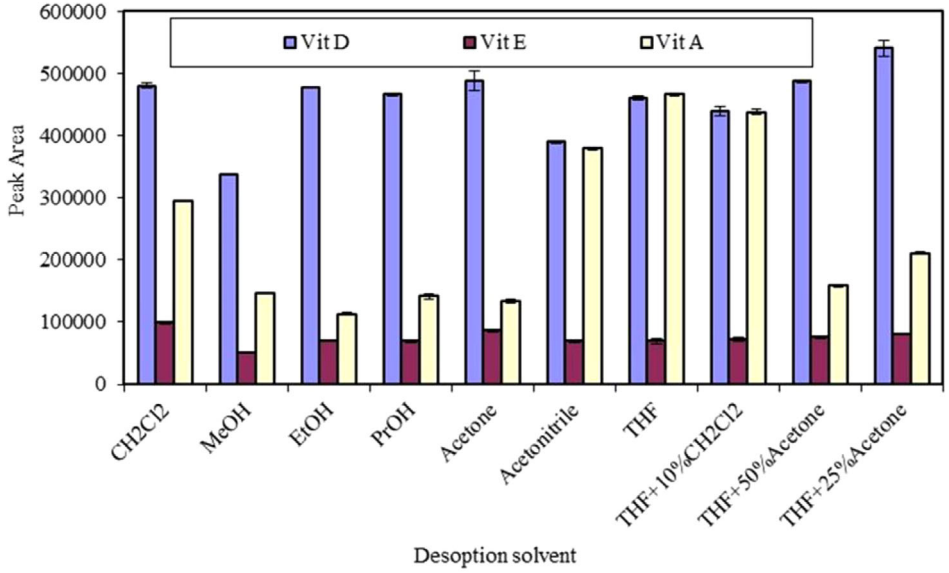
Zeta potential is a measure of the electrostatic potential in a double layer surrounding NPs in solution. It is an essential parameter for evaluating the stability of colloidal systems. A low zeta potential indicates that gravitational forces can overcome electrostatic repulsion, leading to aggregation or clotting. Conversely, NPs with a high zeta potential are electrically stable, resulting in better dispersion and minimal aggregation in the system. When the zeta potential falls within the range of 0 to ±5 mV, rapid aggregation is likely. Stability is significantly improved at zeta potentials of ±30 mV or higher, as increased repulsive forces prevent particle aggregation (Table S1) [34]. The Figure S3 shows the zeta potential distribution of the NPs coated with superhydrophobic cellulose. The observed zeta potential of approximately ±50 mV for these NPs indicates a highly stable colloidal suspension in an aqueous environment. This high zeta potential value supports the stability of the system and corroborates the results of the particle size distribution analysis, further validating the effectiveness of the coating process in minimizing particle aggregation.

3.2 | Optimization of SCCMNPs-Based SPE Conditions

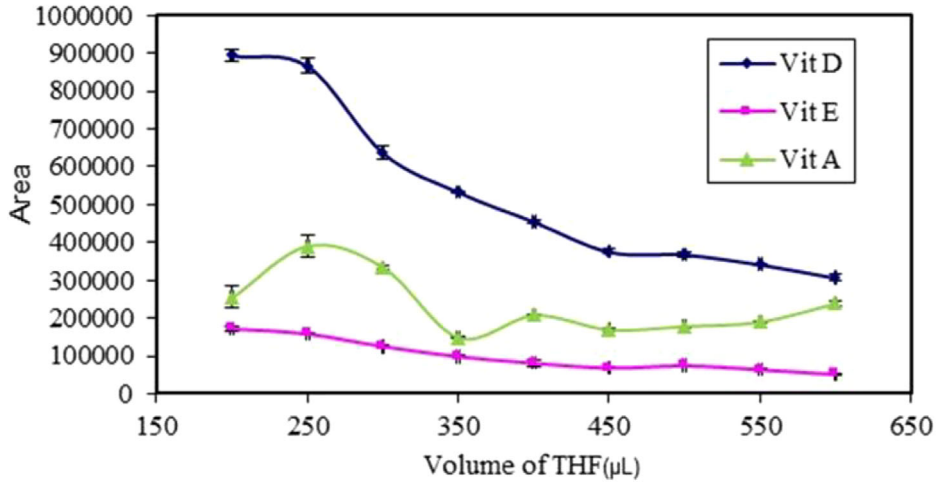
3.2.1 | Effect of Cellulose Shell Thickness and the Amount of Stearoyl Ester Used in the Synthesis of SCCMNPs

MNPs serve various purposes, such as preventing oxidation, enhancing stability, dispersing particles in suspensions and modifying the chemical nature of their surface. As previously discussed, cellulose is a versatile material for coating MNPs. Controlling the thickness of the cellulose shell is crucial, as it significantly impacts the efficiency of SCCMNPs in extracting FSVs. A thicker cellulose shell increases the diameter of MNPs and reduces their magnetic properties, leading to decreased extraction efficiency. Conversely, increasing the surface coverage of stearoyl groups on the cellulose coating enhances extraction efficiency. This improvement is due to the high lipophilicity of FSVs, as more stearoyl groups increase the hydrophobicity of the sorbent, thereby boosting FSV adsorption.

To optimize the cellulose coating thickness, CCMNPs were synthesized with different amounts of cellulose (0.2500–2.0000 g),



(a)



(b)

FIGURE 3 | Effect of (a) type and (b) volume of desorption solvent on extraction efficiencies in 10.00 mL of standard solution with the same concentration 1.0 mg/L of each vitamin, 50.0 mg sorbent, pH = 5.7 (pH of ultrapure water) and 2.5 min sorption and desorption times.

producing five types of SCCMNPs. These sorbents were then evaluated for their FSV extraction efficiency. As shown in Figure S4a, CCMNPs prepared with 1.0000 g of cellulose demonstrated the highest extraction efficiency. Sorbents with less than 1.0000 g of cellulose provided insufficient sites for attaching stearoyl groups during subsequent synthesis steps, resulting in lower extraction capabilities. Conversely, CCMNPs synthesized with more than 1.000 g of cellulose exhibited reduced extraction efficiency due to increased shell thickness, reduced surface area-to-volume ratio and higher polarity.

To evaluate the effect of stearoyl functional groups on extraction performance, four types of SCCMNPs were synthesized using a consistent cellulose shell coating (1.0000 g of cellulose) but different amounts of stearoyl chloride (0.50–2.00 mL). As shown in Figure S4b, SCCMNPs prepared with 1.00 mL of stearoyl chloride achieved the highest extraction efficiency. Insufficient stearoyl chloride resulted in poor FSV extraction, whereas excessive stearoyl chloride increased particle size and sorbent hydrophobicity. This overcoating reduced wettability and ultimately decreased extraction performance.

3.2.2 | Effect of Type and Volume of Desorption Solvent

The desorption solvent should possess several key features to effectively desorb FSVs from SCCMNPs. It must suspend the SCCMNPs, effectively desorb and dissolve FSVs, be compatible with the chromatographic mobile phase and exhibit chromatographic peaks that do not interfere with the analyte peaks. Additionally, the dielectric constant and polarity of the solvent significantly influence desorption efficiency. Given the lipophilic nature of FSVs, non-polar solvents are generally more suitable for their desorption (Table S2). To evaluate desorption efficiency, various solvents were tested, including dichloromethane, methanol, ethanol, isopropanol, acetonitrile, acetone and THF. The results (Figure 3a) indicated that vitamin D_3 exhibited the highest extraction efficiencies with the solvents in the order:

acetone > dichloromethane > methanol > THF. For vitamin E, the order was as follows: dichloromethane > acetone > THF, whereas for vitamin A, it was as follows: THF > acetonitrile > dichloromethane. Despite its high desorption efficiency, dichloromethane—a non-polar solvent—was incompatible with the mobile phase and required drying and re-dissolution in methanol before HPLC injection. Acetone, another highly effective solvent, exhibited UV absorbance that resulted in a prominent solvent peak at the beginning of the chromatogram, overlapping with the vitamin D_3 peak.

To mitigate these challenges while maintaining the advantages of acetone and dichloromethane, solvent mixtures (THF as the base solvent, with small additions of acetone or dichloromethane) were used as the desorption solvent. However, as shown in Figure 3a, the extraction efficiency of vitamin A was significantly lower with the solvent mixtures compared to THF alone. The extraction efficiencies for vitamins E and D_3 with THF were also acceptable and did not show significant reductions compared to dichloromethane and acetone. Therefore, THF was selected as the optimal solvent for desorption due to its balanced performance, compatibility with the chromatographic system and minimal interference with analyte peaks.

To evaluate the effect of desorption solvent volume (THF) on the efficiency of vitamin extraction, varying volumes of THF, ranging from 200.0 to 600.0 μL, were tested for desorbing FSVs from SCCMNPs. As shown in Figure 3b, increasing the volume of THF up to 250.0 μL significantly enhanced extraction efficiency. However, further increases in THF volume beyond 250.0 μL had minimal impact and even led to a slight reduction in the extraction percentage. This decline may be attributed to over-dilution of the analyte, which reduces its effective concentration.

3.2.3 | Effect of Sample Solution pH

The pH of the sample solution significantly influences the forms of analytes and the charge density of the sorbent, thereby playing a critical role in the adsorption process. To evaluate the effect of sample solution pH on extraction efficiencies of FSVs, solutions with various pH values, ranging from 2 to 9, were prepared, and vitamins were extracted by SCCMNPs.

As shown in Figure 4, the highest extraction efficiencies were achieved at pH = 5 for vitamins A and E and at pH = 2 for vitamin D_3. Because the analysed FSVs lack ionizable groups in their molecular structure, their primary adsorption mechanism involves hydrophobic interactions with the stearoyl groups on the sorbent, which remain unaffected by pH. The only factor that can be described as the pH effect is less absorption of sample matrix components and non-competition with vitamins at optimal pH.

The data demonstrate that the peak areas for vitamins A and E reach their maximum at pH = 5. In contrast, the peak area for vitamin D_3 at pH = 5 is approximately half of its maximum value, which occurs at pH = 2. However, even at pH 5, the peak area of vitamin D_3 remains nearly twice as high as the peak areas for vitamins A and E, providing sufficient sensitivity for analysis. Consequently, pH = 5 was selected as the optimal pH for the extraction process.

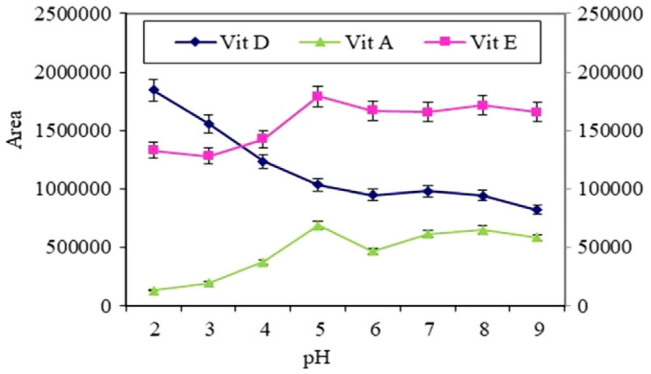


FIGURE 4 | Effect of pH of sample solution on extraction efficiencies in 10.00 mL of standard solution with the same concentration 1.0 mg/L of each vitamin with 250.0 μL THF as desorption solvent, 50.0 mg sorbent and 2.5 min sorption and desorption times.

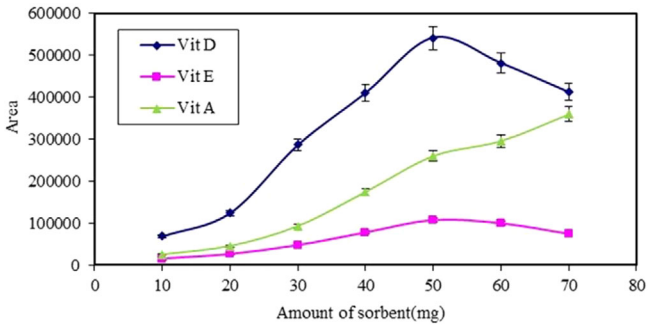


FIGURE 5 | Effect of amount of sorbent on extraction efficiencies in 10.00 mL of standard solution with the same concentration 1.0 mg/L of each vitamin with 250.0 μL desorption solvent, pH = 5 and 2.5 min sorption and desorption times.

3.2.4 | Effect of Sorbent Amount

To ensure quantitative and effective recovery of analytes from the sample matrix, it is essential to use a sufficient amount of sorbent proportional to the mass of analytes. Standard solutions containing 1.0 mg/L of each vitamin were prepared, and 10.00 mL of these solutions were extracted using varying amounts of sorbents ranging from 10.0000 to 70.0 mg (Figure 5).

The results indicate that SCCMNP amounts below 50 mg are insufficient for the effective extraction of FSVs. Increasing the sorbent amount beyond 50 mg does not lead to a significant improvement in extraction efficiency and, in some cases, even results in a decrease. This reduction in extraction efficiency, despite the increased sorbent amount, can be attributed to the potential for irreversible adsorption of analytes onto the sorbent surface.

3.2.5 | Effects of Sorption and Desorption Times

The effect of sorption time on extraction efficiencies was investigated by varying the extraction duration from 0.5 to 3.5 min. As shown in Figure 6a, a sorption time of 1.5 min was found to

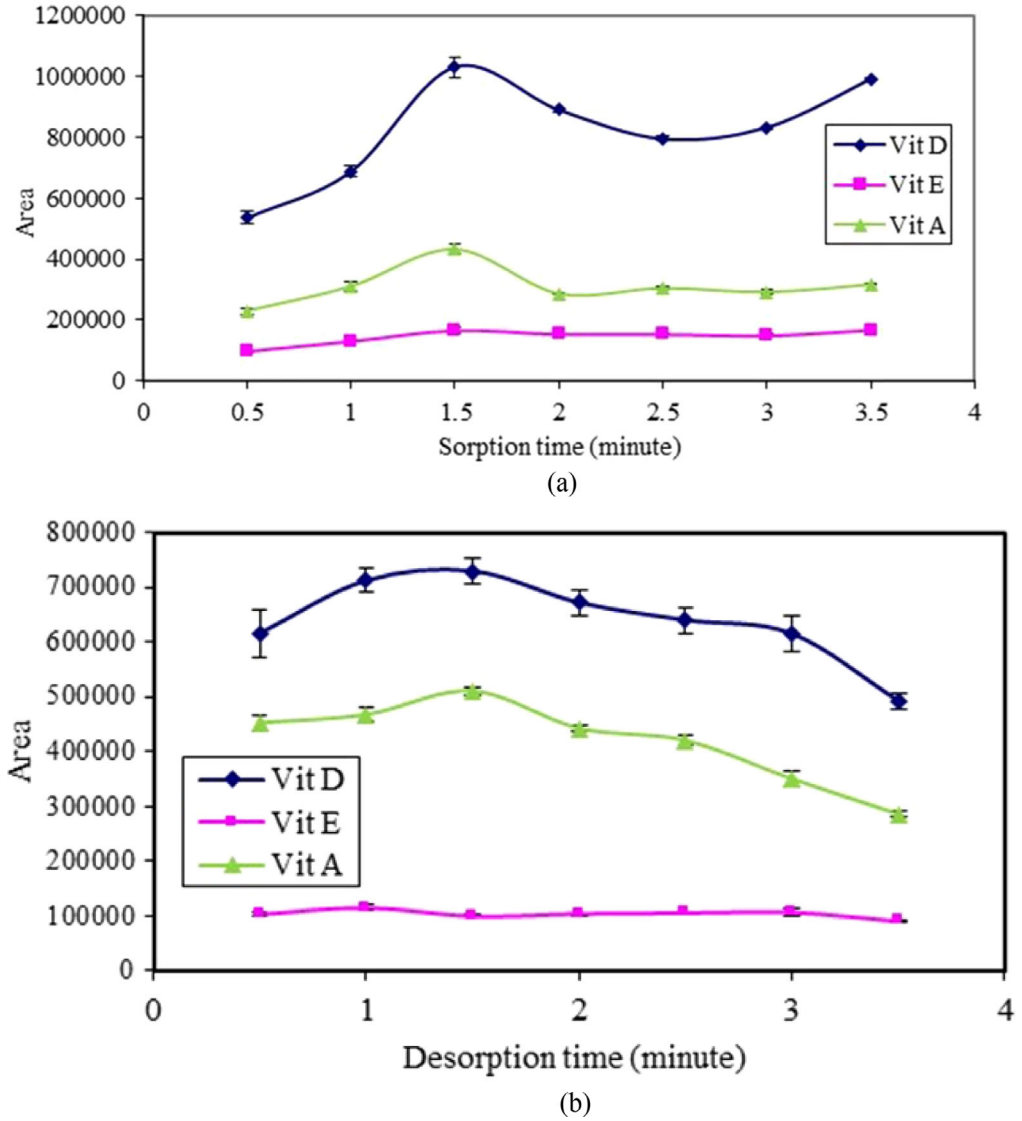


FIGURE 6 | Effect of extraction time: (a) sorption and (b) desorption on extraction efficiencies in 10.00 mL of standard solution with the same concentration 1.0 mg/L of each vitamin with 250.0 μL desorption solvent (THF) and 50.0 mg sorbent and pH = 5.

be optimal. Sorption times shorter than 1.5 min did not allow sufficient time for complete analyte sorption, whereas longer sorption times increased the likelihood of analytes returning to the bulk solution, thereby reducing extraction efficiency.

Similarly, the effect of desorption time was evaluated: Experimental results (Figure 6b) indicated that 1.5 min was sufficient for complete desorption of vitamins from SCCMNPs. Short desorption times resulted in partial elution of analytes, whereas longer times led to back-extraction of analytes into the sorbent, ultimately decreasing extraction efficiency.

3.3 | Performance of the Analytical Procedure

Under the optimized extraction conditions, calibration plots for each vitamin were obtained. The slope, intercept and the linearity range of these plots are presented in Table 1. The calibration plots of the three vitamins showed excellent linearity across working range, with coefficient of determination (R^2) exceeding 0.9989 (Table 1). The method precision was also evaluated, and the results showed repeatability below 4.6%, reflecting the high precision of the proposed method for vitamin determination. The limits of detection (LOD), calculated using the 3σ criterion (with Sy/x substituted for σ and the intercept used instead of the blank signal), were determined to be 5.2, 8.6 and 2.3 mg/mL for vitamins D_3, E and A, respectively. These LOD values were well below the required thresholds for analysing FSVs in pharmaceutical formulations. Extraction efficiencies and enrichment factors were also calculated, with extraction efficiencies of 30.4%, 66.4% and 15.5% and the enrichment factors of 12.2, 19.0 and 6.2 for vitamins D_3, E and A, respectively (Table 1).

To assess method accuracy, recoveries of each vitamin were evaluated by adding certain amounts of a standard solution containing vitamins D_3, E and A to an exact amount of multivitamin syrup (Alhavi Pharmaceutical Company) and extracting under optimal conditions. The average recoveries ranged from 79.6% to 100.8% (Table 2), with relative standard deviations (RSDs) below 5.5%. These results confirm that the proposed method enables the

TABLE 1 | Analytical performance parameter for the three fat-soluble vitamins (D_3, A and E) under optimized extraction conditions, providing a comprehensive comparison of their analytical characteristics.

Parameter	Vitamin		
	A	**E**	**D_3**
Slope	675.27	7.087	758.7
Intercept	16651	18009	−1289.5
Coefficient of determination (R^2)	0.9992	0.9989	0.9994
Linear range (μg/L)	2.4×10^1–8.0×10^2	2.4×10^1–1.0×10^3	2.4×10^1–1.0×10^3
Detection limit (μg/L)	2.3	8.6	5.2
Repeatability (%RSD)	4.2	1.8	4.6
Extraction efficiency	15.5	66.4	30.4
Enrichment factor	6.2	19.0	12.2

TABLE 2 | Recovery study results for vitamins D_3, A and E added to a multivitamin syrup sample: The table presents the added and measured amounts of each vitamin, along with their corresponding recovery percentages and %RSD, assessing the accuracy and precision of the analytical method.

Vitamin	Added amount (mg)	Founded amount (mg)	%Recovery	%RSD
E	2.5	2.5	100.8	0.5
A	0.5	0.4	79.6	1.1
D_3	8.4×10^{-2}	7.1×10^{-2}	84.7	5.5

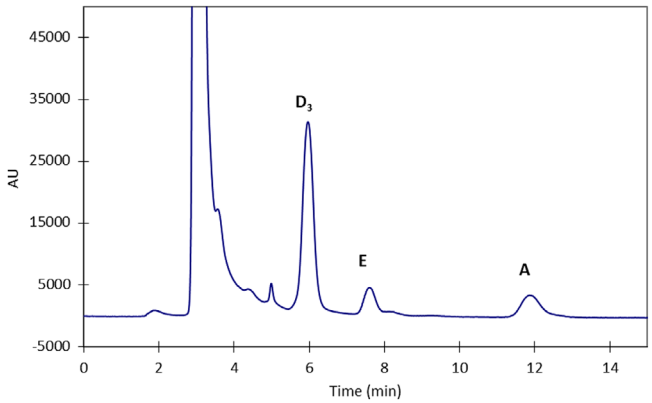


FIGURE 7 | Chromatograms of vitamins E, A and D in multivitamin syrup after extraction with SCCMNPs under optimal conditions (10.00 mL of standard solution with the same concentration 1.0 mg/L of each vitamin with 250.0 μL THF as desorption solvent, 50.0 mg sorbent, pH = 5 and 1.5 min sorption and desorption times). SCCMNPs, superhydrophobic cellulose-coated magnetic nanoparticles.

accurate extraction and quantification of FSVs in pharmaceutical samples.

A chromatogram of a standard solution of FSVs, obtained following extraction using the optimized magnetic SPE (MSPE) method with SCCMNPs, is shown in Figure 7.

3.4 | Real Sample Analysis

To evaluate the applicability of the proposed method for real sample analysis, the FSV content was determined in a multivitamin syrup (Alhavi Pharmaceutical Company) and vitamin D_3 capsule (Dana Pharma Pharmaceutical Company) under the optimum conditions. As shown in Table 3, the extraction was performed with acceptable recovery percentages, and there was good agreement between the vitamin content measured using this method and the values reported by manufacturers.

The discrepancies observed between the measured results and the manufacturer-reported values for the multivitamin syrup can be attributed to the addition of excess FSVs beyond the labelled amounts. In the case of vitamin D_3 in multivitamin formulation, the concentration did not fall within the calibration linear range. Further dilution did not improve the results, as the concentration of vitamin D_3 was too low. Additionally, the high concentrations of vitamins A and E in the formulation led to sorbent saturation, limiting extraction performance.

Figure 8 represents the chromatograms of the multivitamin syrup (a) and vitamin D_3 capsule (b) following extraction with SCCMNPs, respectively.

4 | Conclusion

In this study, Fe_3O_4 MNPs were successfully synthesized, coated with cellulose and further modified with stearoyl ester to produce SCCMNPs. The prepared SCCMNPs were used as an innovative and efficient sorbent for the extraction of FSVs (A, D_3 and E) from aqueous solutions via MSPE. Compared to conventional SPE methods, the SCCMNPs offered significant advantages, including reduced sorbent usage and shorter extraction times, attributed to the high surface area of the NPs. Additionally, the use of a magnet for sorbent separation simplified the process, enhancing operational efficiency.

TABLE 3 | Analysis results of the multivitamin syrup and vitamin D_3 capsule with superhydrophobic cellulose-coated magnetic nanoparticles (SCCMNPs): The table compares the manufacturer-reported and experimentally obtained vitamin quantities (IU/mL) and their relative errors (%), evaluating the method's accuracy.

Vitamin	Multivitamin syrup			Vitamin D_3 capsule		
	Quantity reported by manufacturer (IU/mL)	The amount obtained (IU/mL)	Relative error (%)	Quantity reported by manufacturer (IU/mL)	The amount obtained (IU/mL)	Relative error (%)
E	5	5.9	18.4	—	—	—
A	1500	1638.1	9.2	—	—	—
D_3	400	—	—	1000	1045.5	4.5

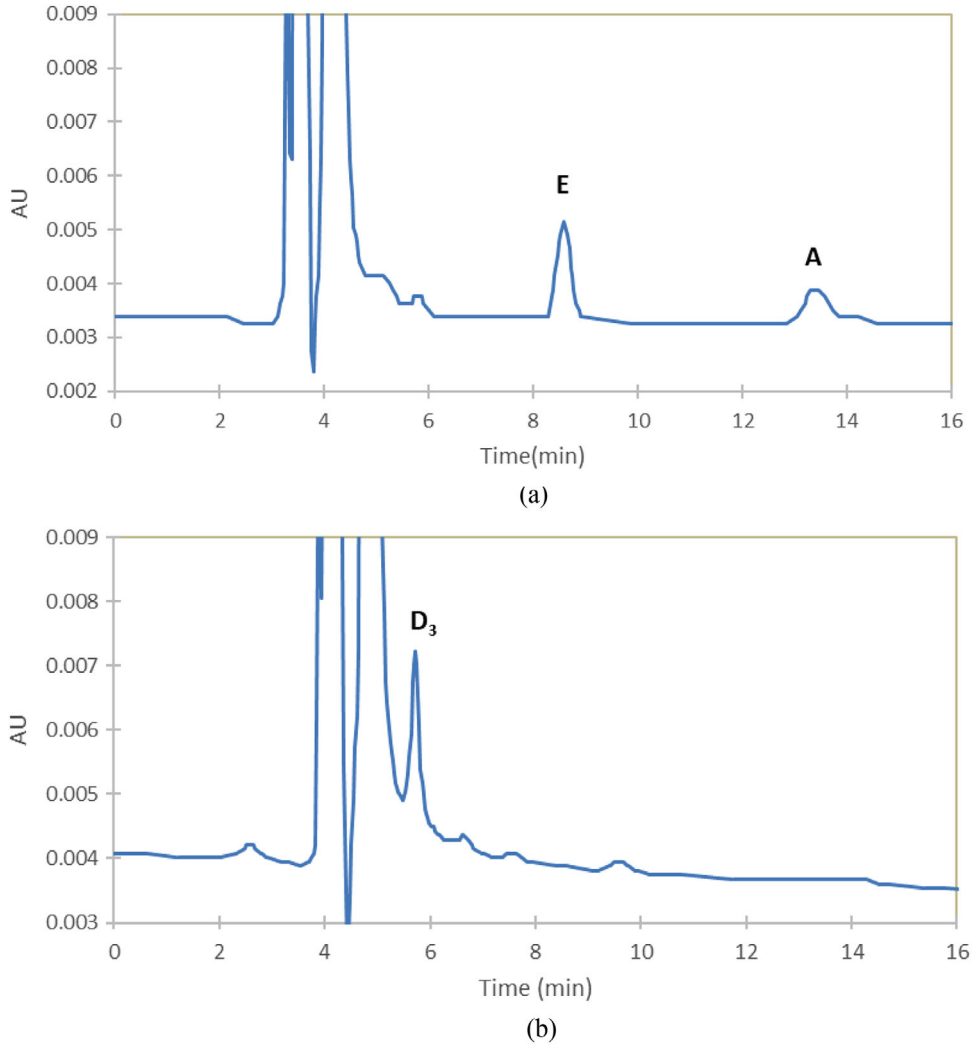


FIGURE 8 | Chromatograms of (a) multivitamin syrup produced by Alhavi Pharmaceutical Company and (b) vitamin D_3 capsule produced by Dana Pharma Pharmaceutical Company after extraction with SCCMNPs under optimal conditions as described in Figure 7. SCCMNPs, superhydrophobic cellulose-coated magnetic nanoparticles.

The results confirmed that the proposed method is environmentally friendly, straightforward and offers desirable analytical performance, with appropriate linear ranges (2.4×10^1–1.0×10^3 μg/L), low LODs (≤8.6 μg/L), acceptable precision—expressed as RSD—of less than 4.6% for all analytes and high accuracy (with recoveries ≥79.60%). The proposed method proved environmentally friendly and straightforward, meeting the demands of modern analytical workflows.

This approach was successfully applied to the determination of the content of FSVs in pharmaceutical formulations, highlighting its potential for routine analysis in quality control laboratories.

However, future studies should explore the use of SCCMNPs in more complex matrices such as biological fluids or fortified food products to assess their robustness and applicability in diverse fields. Additionally, optimizing the scalability of SCCMNP synthesis and their use in automated systems could further enhance their practicality. The integration of this method into multi-analyte platforms for simultaneous detection of vitamins and other nutrients also represents a promising direction for future research.

Author Contributions

Fariborz Momenbeik: writing – review and editing, supervision, conceptualization. **Arezoo Khatam**: data curation, formal analysis, writing – original draft, methodology.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.