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Development of a Magnetic Solid-Phase Extraction Sorbent for Enhanced UV-Vis Spectrophotometric Detection of Riboflavin in Vitamin Tablets

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ABSTRACT

A UV-Vis spectrophotometric method was developed for the determination of riboflavin (Vitamin B2), employing magnetic solid-phase extraction (MSPE) for preconcentration. A novel magnetic material (Fe_3O_4@TEOS-Captopril) was synthesized for this purpose and characterized using Fourier-Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). All parameters affecting the MSPE efficiency, such as sample pH, contact time, and elution conditions, were optimized. The determination of the preconcentrated riboflavin was carried out using a UV-Vis spectrophotometer at three characteristic wavelengths: 268, 370, and 446 nm. The absorbance at 446 nm consistently provided the highest signal intensity and was therefore selected for all quantitative analyses to maximize sensitivity. Under the optimized conditions at this wavelength, the method exhibited a linear range of 125.00–2500.00 ng mL^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 37.88 ng mL^{-1} and 113.64 ng mL^{-1}, respectively. The method's accuracy and robustness against matrix effects were evaluated through recovery studies via the standard addition method on commercial pharmaceutical tablet samples, yielding satisfactory results between 93.7% and 100.4%. The overall procedure was evaluated using the Blue Applicability Grade Index (BAGI), achieving a score of 65. This score reflects a favorable balance, confirming the method's robustness against matrix effects and its relatively green profile, facilitated by the magnetic solid-phase extraction which reduces solvent consumption.

Keywords: Analytical method development, Magnetic solid phase extraction, Pharmaceutical analysis, Riboflavin, UV-VIS

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Introduction

Flavins, whose name originates from the Latin term "flavus" meaning "yellow," are a group of organic compounds built upon a pteridine cyclic base. Their fundamental framework is the tricyclic heterocycle known as isoalloxazine Riboflavin ($C_{17}H_{20}N_4O_6$), also identified as 7,8-dimethyl-10-(1'-D-ribityl)isoalloxazine, is present in three primary states: the free form, and its two vital cofactor derivatives, flavin mononucleotide (FMN, or riboflavin-5'-phosphate) and flavin adenine dinucleotide (FAD)[1]. This molecule is a biochemically significant flavin and serves as the exclusive precursor in the human body for the synthesis of the coenzymes FMN and FAD[2]. Consequently, it is an essential vitamin for metabolic reactions and cellular energy generation. As a vitamin with limited water solubility that cannot be produced internally, it must be consistently obtained from the diet. A partly water-soluble vitamin, it cannot be synthesized endogenously by the human body and must be regularly supplied from exogenous sources. It plays a critical role in metabolic processes by being converted to FMN and FAD in biological systems[3]. However, since riboflavin's storage capacity in living organisms is limited, adequate dietary intake is crucial to prevent clinical symptoms associated with deficiency. Riboflavin deficiency can lead to various metabolic disorders in the body[4]. This can lead to health problems such as growth retardation and skin and mucosal lesions in children. Furthermore, riboflavin deficiency can predispose cancer and other diseases. On the other hand, excessive riboflavin intake can lead to mild oxidative damage in exposed tissues. This can lead to DNA degradation and, due to riboflavin's photosensitivity, increase the formation of lipid peroxides. In this context, assessing riboflavin levels in the body can contribute to clinical diagnostic processes[5]. In food quality monitoring, a decrease in riboflavin content is considered an indicator of certain food spoilage due to riboflavin's sensitivity to light. For example, analysis of riboflavin content can help determine the quality of milk processing and storage.

In the pharmaceutical sector, investigations into the pharmacological properties of riboflavin have become increasingly comprehensive in recent years. Riboflavin shows promise as a therapeutic agent in photodynamic therapy for targeting tumor tissues, managing cardiovascular and cerebrovascular disorders, and enhancing bacterial resistance[6]. Consequently, establishing precise methods for quantifying riboflavin is critical for quality control in pharmaceutical products, advancing pharmacological studies, and monitoring its levels in various samples [7]. Typically, riboflavin status in the body is evaluated through the erythrocyte glutathione reductase activity coefficient (EGRAC), determined by

comparing FAD-stimulated enzyme activity to the unstimulated baseline. Nevertheless, an elevated EGRAC value does not always directly correspond to the severity of riboflavin deficiency, and the technique depends on the use of fresh erythrocytes and requires consistent standardization across laboratories[2,8]. Fluorometric measurement of 24-hour urinary excretion is another important method for routinely assessing riboflavin levels in the body. In healthy adult individuals, total urinary excretion of riboflavin above 120 mg per day, but below 40 mg, indicates riboflavin deficiency. While this method is not as accurate as EGRAC in reflecting long-term riboflavin status, it has a broader range of applications[9]. From a broader perspective, the Recommended Daily Allowances (RDA) for riboflavin vary among populations, and the riboflavin and matrix content in various samples vary[8,10] As a result, riboflavin analysis varies in biological samples (blood, serum, urine, etc.), food samples, and pharmaceutical samples, depending on the density of the matrix. For example, complex samples with extremely low riboflavin levels, such as blood, are suitable for analysis by mass spectrometry (MS)[11,12]. In food samples, the quantitative analysis of -- riboflavin is effectively performed by High-Performance Liquid Chromatography coupled with a Diode Array Detector (HPLC-DAD), often following a cloud point extraction procedure[13]. Fluorescence and electrochemical detection/sensor methods are preferred for rapid, space-saving, and cost-effective quantification[5,14]. Immunoassay methods are used to increase the specificity of riboflavin analysis[15]. Furthermore, commercial kits based on microbiological methods are available for riboflavin measurement, which require less technical and equipment space. In recent years, an increasing number of articles have been published on the important role of riboflavin in food, pharmaceutical, and biological fields, and further research has been conducted on various new riboflavin determination methods[16]. Riboflavin is an inherently unstable organic compound, exhibiting significant susceptibility to light, elevated temperatures, and alkaline conditions, all of which can cause its degradation and a consequent reduction in biological activity[17]. This inherent instability underscores the need for highly sensitive and robust analytical techniques to precisely determine riboflavin levels across different sample types[18]. In response to these challenges, a variety of analytical approaches have been established. These can be generally classified into several groups, including spectrophotometric, chromatographic, fluorometric, electrochemical, and microbiological methods[14,19–22]. The underlying principle of these techniques often involves exploiting specific characteristics of riboflavin, such as its natural fluorescence or electrochemical redox behavior, to enable its identification and accurate measurement[23,24].

The primary objective of this study was to develop an innovative analytical method for the analysis of riboflavin - that offers high sensitivity, selectivity, and reproducibility[25]. Aiming to overcome some limitations of existing methods (e.g., complex sample preparation, high costs, or low sensitivity), a new approach for the quantitative analysis of riboflavin is proposed[26]. Solid-phase extraction (SPE) is a well-established pretreatment technique for the purification and preconcentration of analytes from complex sample matrices[27,28]. Building upon this foundation, magnetic solid-phase extraction (MSPE) has emerged as a significant advancement in analytical methodology[29]. MSPE utilizes magnetic adsorbents to selectively separate and preconcentrate target analytes, offering advantages such as simpler and faster operation by eliminating the need for centrifugation or filtration steps [30,31] .The method to be developed in this work will be supported by the synthesis of a novel magnetic material, previously unreported for the analysis of riboflavin. Captopril was selected as the functional ligand due to its ability to form hydrogen bonds and electrostatic interactions with the riboflavin molecule, potentially through its carboxylic acid and thiol groups, thereby enhancing the selectivity and adsorption capacity of the sorbent. This innovative adsorbent is anticipated to significantly enhance the analytical sensitivity of the extraction process. This technique is based on the use of magnetic adsorbent materials, enabling rapid and efficient separation by applying a magnetic field. MSPE is widely preferred for the isolation and pre-analytical enrichment of analytes present at low concentrations, particularly in complex sample matrices. This method has become an important research area in recent years due to its faster, more environmentally friendly, and higher efficiency compared to traditional solid-phase extraction techniques.

Experimental

Instrumentation

Absorbance measurements were performed on a UV-Visible spectrophotometer (Shimadzu, model 1800, Japan) using a 1 cm quartz cell, with the instrument operating at a wavelength accuracy of ±0.2 nm and a fixed bandwidth of 2 nm across the 190–1100 nm range. Solution pH was determined using a glass-electrode pH meter (Hanna Instruments, HI 2211 model, Germany).

The functional groups of the synthesized material were identified by FTIR spectroscopy (Bruker, Vertex 70 model, Germany) with KBr pellets, scanning the wavenumber range from 400 to 4000 cm^{-1}. The crystallographic properties of the synthesized material were characterized using X-ray diffraction (XRD, Rigaku MiniFlex 600 model, Japan). Furthermore, the surface morphology was examined with a field-emission scanning electron microscope (model: Tescan Mira3 Xmu, Feg-Sem; manufacturer location: Brno, Czechia).

Reagents

All experimental procedures utilized ultrapure Milli-Q water with a resistivity of 18.2 MΩ·cm, supplied by Millipore Corporation, Turkey. A 500 µg mL^{-1} of Riboflavin solution (Sigma, St. Loius, MO, USA) was prepared by

dissolving in 0.5% ammonium hydroxide solution. Britton-Robinson (BR) buffer solution was used to work at desired pH values. The chemical reagents employed for the synthesis of the magnetic nanoparticles, namely ferric chloride ($FeCl_3$), ferrous chloride ($FeCl_2$), tetraethyl orthosilicate (TEOS), and captopril, were procured from Sigma-Aldrich (St. Louis, USA) and used without further purification.

Synthesis of Magnetic Solid Phase Material

Magnetite (Fe_3O_4) nanoparticles were synthesized via the chemical co-precipitation technique, a well-established and commonly employed procedure. The synthesis procedure was initiated by preparing a homogeneous precursor solution. This was achieved by dissolving iron(II) chloride ($FeCl_2$) and iron(III) chloride ($FeCl_3$) in a 2:1 molar ratio into 50 mL of a 0.01 M hydrochloric acid (HCl) medium. The resulting mixture was then agitated at 600 rpm for 30 minutes with simultaneous heating to 65 °C. Once this temperature was stabilized, 100 mL of a 50% ethanol solution was added to the system. Under continuous stirring, the reaction temperature was further elevated to 85 °C. To initiate the precipitation process, 20 mL of 9 M ammonium hydroxide (NH_4OH) was added dropwise over a 10-minute period while maintaining a nitrogen environment. The resulting black Fe_3O_4 nanoparticles were magnetically isolated, purified via three washing cycles with 50% ethanol, and subsequently oven-dried at 60 °C for 4 hours.

In the following stage, 2 grams of the synthesized Fe_3O_4 nanoparticles were uniformly dispersed in 50 mL of a 50% ethanol solution. The mixture was maintained under stirring at 600 rpm and a temperature of 80 °C. Then, 3 mL of 8 M ammonia was introduced into the suspension. After a 30-minute reaction period, 2 mL of tetraethyl orthosilicate (TEOS) was added to the system. In parallel, a separate solution was prepared by dissolving 2 mL of Captopril in 2 mL of ethanol, which was thoroughly mixed under stirring. The combined reaction mixture was then agitated continuously for 6 hours. The final Captopril-functionalized magnetic particles were magnetically separated, washed five times with 50% ethanol, and dried in an oven at 45 °C.

Magnetic Solid Phase Extraction

The magnetic solid-phase extraction procedure (MSPE) was carried out using 40 mg of the synthesized magnetic adsorbent. The material was first conditioned by washing twice with 2 mL of ultrapure water. Then, 1 mL of Britton–Robinson buffer (0.01 M, pH 3) was supplied. To this mixture, 200 µL of a solution (50 mg L^{-1}) was added, and the total volume was adjusted to 10 mL using ultrapure water. The suspension was subjected to adsorption under continuous shaking at 80 rpm for 20 minutes on an orbital shaker. Following this, the magnetic sorbent was isolated from the solution by applying an external neodymium magnet. The aqueous phase was discarded, and desorption was performed by adding 1000 µL of methanol and vortexing for 30 seconds. The resulting methanolic extract was analyzed by UV-Vis spectroscopy using a micro-volume cuvette with methanol as the blank. After each extraction cycle, the magnetic particles were regenerated by washing twice with 1 mL of methanol and dried for subsequent reuse.

A calibration curve for riboflavin was established prior to MSPE, as depicted in Figure 1. The curve correlates the concentration of riboflavin with its absorbance at three characteristic wavelengths (A268 nm, A370 nm, and A446 nm), providing the quantitative basis for its determination

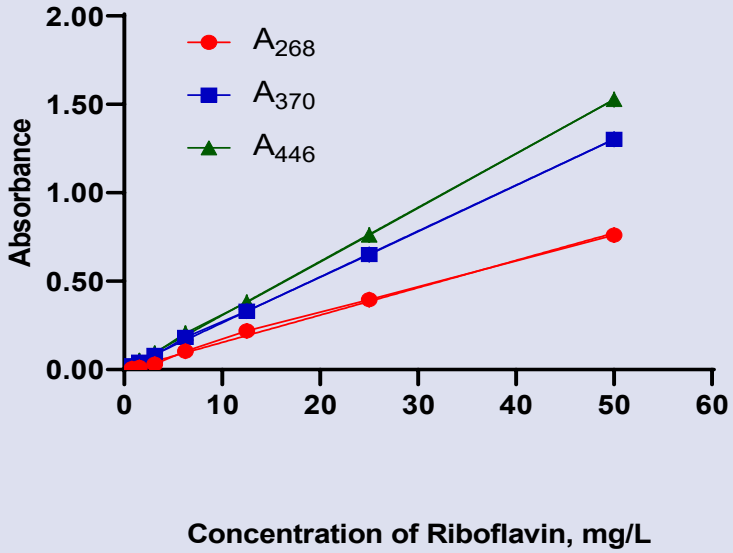

Figure 1. Calibration Curve for riboflavin solution before MSPE

Results and Discussion

Structural and Morphological Characterization of the Synthesized Magnetic Adsorbent

The successful formation of the Fe_3O_4@TEOS-Captopril adsorbent was verified using a combination of analytical techniques, including Fourier-Transform Infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD).

Figure 2 illustrates the FTIR spectrum of the developed magnetic adsorbent. FTIR spectrum of pure Fe_3O_4 nanoparticles (black curve) exhibits a strong and characteristic absorption band around 580 cm^{-1}, which corresponds to the stretching vibration of the Fe–O bond in the spinel structure of magnetite. This confirms the successful formation of Fe_3O_4. In the spectrum of Fe_3O_4@TEOS–Captopril (red curve), the Fe–O vibration band is still visible but slightly shifted and reduced in intensity, indicating that the Fe_3O_4 core remains intact after surface modification. In addition, several new absorption bands appear in the modified sample. The broad peak observed around 3400 cm^{-1} can be attributed to the O–H stretching vibration of surface hydroxyl groups and adsorbed water molecules. The bands near 1080 cm^{-1} and 800 cm^{-1} are characteristic of Si–O–Si and Si–O–Fe stretching vibrations, confirming the successful coating of Fe_3O_4 with a silica layer derived from TEOS. Furthermore, weak absorption bands around 1650–1700 cm^{-1} and 2550–2600 cm^{-1} correspond to the C=O and S–H stretching vibrations, respectively, originating from the captopril molecules attached to the silica surface. These new functional groups provide clear evidence that captopril has been successfully immobilized onto the Fe_3O_4@TEOS surface through chemical bonding or hydrogen interactions. Overall, the FTIR spectra confirm the successful stepwise modification of Fe_3O_4 nanoparticles — first with a silica coating and then with captopril functionalization — while maintaining the structural integrity of the Fe_3O_4 core.

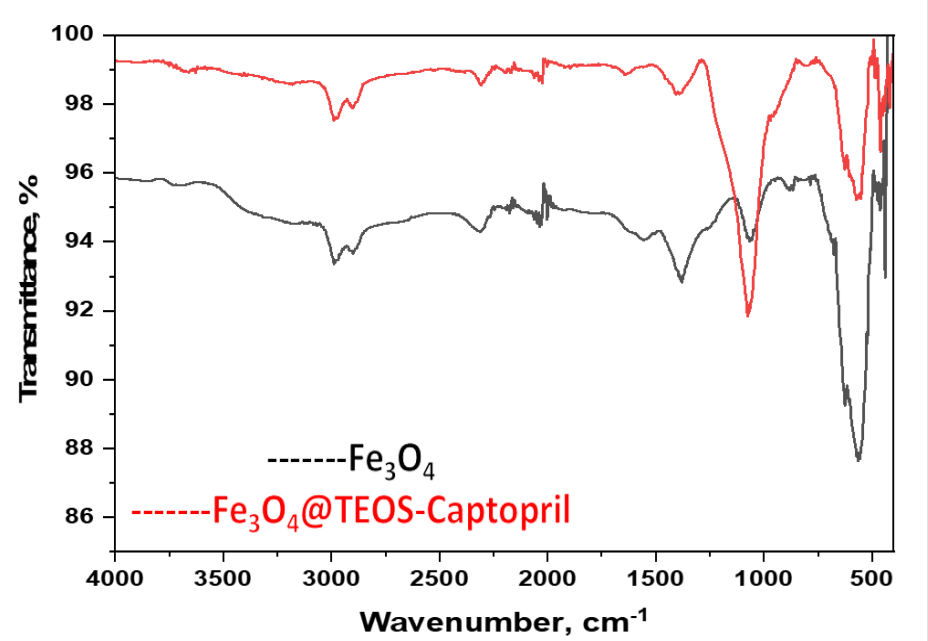

Figure 2. FTIR spectrum of the synthesized Fe_3O_4@TEOS-Captopril material

The surface morphological properties of developed magnetic sorbent were shown in Figure 3 by means of SEM images. Figure 3A shows the morphology of the as-synthesized Fe_3O_4 nanoparticles. The particles appear to be nearly spherical and densely packed, exhibiting a relatively uniform size distribution. The surface texture is rough, and the nanoparticles tend to aggregate, which is typical due to the strong magnetic dipole–dipole interactions among Fe_3O_4 particles. The overall morphology indicates successful formation of nanosized magnetite particles. Figure 3B presents the SEM image of Fe_3O_4@TEOS–Captopril, obtained after surface modification with tetraethyl orthosilicate (TEOS) and functionalization with captopril. Compared with the bare Fe_3O_4 nanoparticles, the modified sample exhibits a noticeable change in morphology. The surface becomes smoother and less compact, with some larger aggregates visible. This morphological change suggests the successful coating of Fe_3O_4 with a silica layer derived from TEOS, followed by captopril immobilization. The apparent increase in particle size and the more defined outer layer is consistent with the formation of a core–shell structure, confirming that the functionalization process was effective.

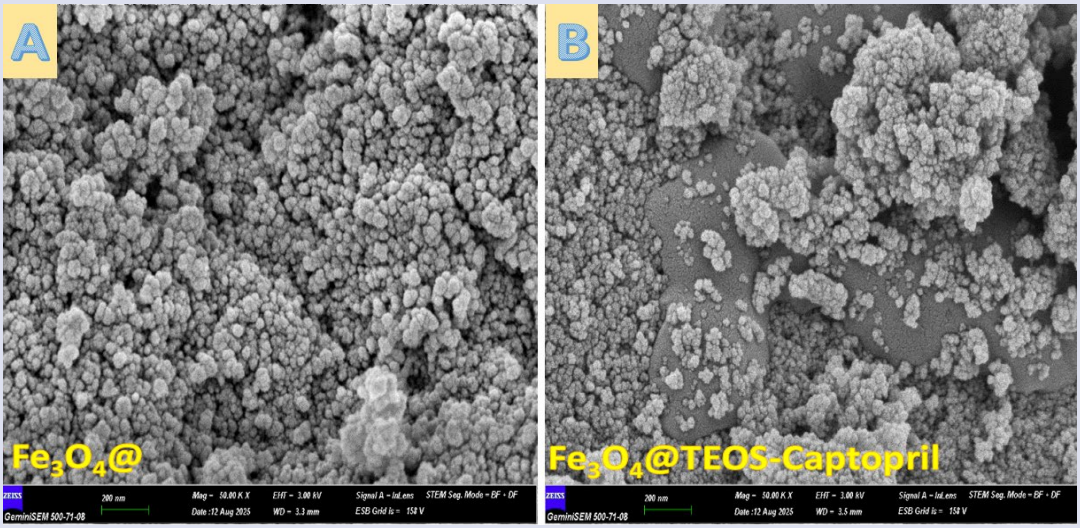

Figure 3. SEM image of the synthesized Fe_3O_4@TEOS-Captopril material

Another characterization data was provided by XRD analysis as can be seen in Figure 4. The XRD pattern of the pure Fe_3O_4 nanoparticles (black curve) exhibits distinct diffraction peaks at approximately 2θ = 30.2°, 35.5°, 43.1°, 53.4°, 57.0°, and 62.6°, which correspond to the (220), (311), (400), (422), (511), and (440) crystal planes, respectively. These reflections are characteristic of the spinel cubic structure of magnetite (Fe_3O_4), and their presence confirms the successful formation of crystalline magnetite according to the JCPDS card no. 19-0629. The sharp and intense peaks indicate high crystallinity of the synthesized Fe_3O_4 nanoparticles.

For the Fe_3O_4@TEOS–Captopril composite (red curve), the main diffraction peaks of Fe_3O_4 remain visible, indicating that the core magnetite structure is preserved after surface modification. However, a slight decrease in peak intensity and broadening of reflections are observed. This reduction in crystallinity can be attributed to the coating of Fe_3O_4 nanoparticles with an amorphous silica (SiO_2) layer derived from TEOS and the subsequent immobilization of captopril molecules. The presence of a weak broad hump around 2θ ≈ 20–25° further supports the existence of amorphous silica on the particle surface. Overall, the XRD results confirm that the Fe_3O_4 core retains its spinel structure after modification, while the formation of an amorphous outer layer demonstrates the successful coating and functionalization process

Evaluation of the pH Effect on the MSPE Procedure

Considering the effect of the pH value of the medium on the interactions between riboflavin - and the adsorbent (Fe3O4@TEOS-Captopril) and the presence of other solvents, it is of great importance that this optimization process is carried out meticulously. It is critical that pH optimization be performed with high precision and accuracy, as it directly affects the enrichment factor. Therefore, a pH scan was performed with BR buffer, ranging from 2 to 11 to comprehensively investigate the adsorption behavior across a wide pH range, especially to confirm the significant drop in efficiency under alkaline conditions. As illustrated in Figure 5, the maximum absorbance signal for riboflavin was achieved at pH 3, indicating optimal adsorption efficiency at this pH. Consequently, a pH 3 buffer was selected for all subsequent experiments. As a result of these measurements, the maximum retention between - riboflavin and the Fe_3O_4@TEOS-Captopril adsorbent was achieved at pH 3. After selecting the pH, studies were conducted in the range of 0-2 mL using a pH 3 buffer to investigate the pH volume. it was observed that the ideal conditions were achieved at a volume of 1 mL.

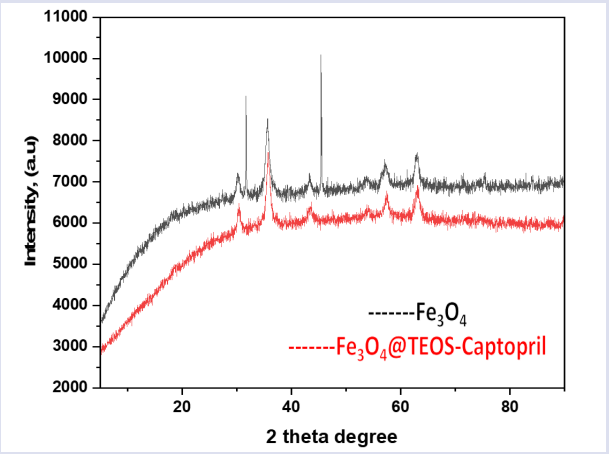

Figure 4. XRD analysis of the synthesized Fe_3O_4@TEOS-Captopril material

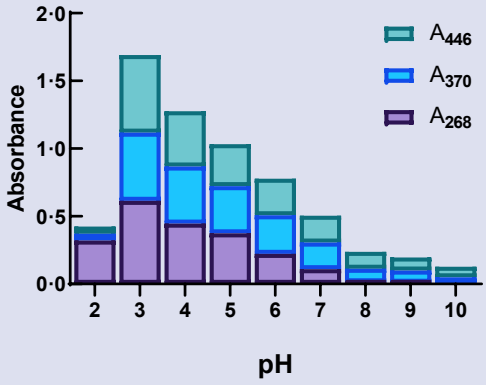

Figure 5. Effect of sample pH on the adsorption efficiency of riboflavin.

Optimization of Desorption Conditions: Solvent Selection and Volume

The selection of an appropriate stripping solvent is critical to ensure the efficient release of the target analyte from the adsorbent and to maximize the pre-concentration factor. Various solvents, including methanol (MeOH), ethanol (EtOH), isopropanol, acetonitrile (ACN), acetone, water, and n-hexane, were evaluated. As shown in Figure 6, methanol provided the highest recovery of riboflavin from the Fe_3O_4@TEOS-Captopril adsorbent and was therefore chosen as the eluent in all subsequent experiments.

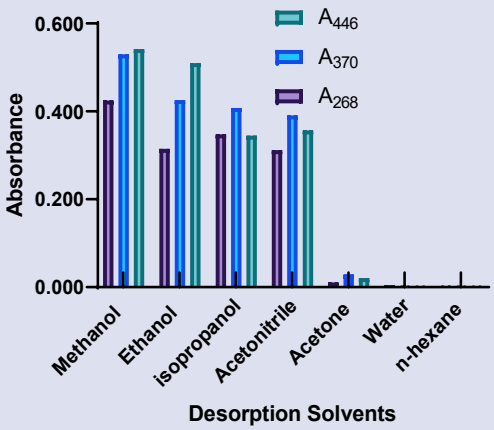

Figure 6. Effect of different desorption solvents on the recovery of riboflavin.

Following the selection of methanol as the optimal eluent, its volume was optimized to maximize the pre-concentration factor (PF). The PF is critically dependent on eluent volume, as dilution increases with larger volumes. As shown in Figure 7, the elution efficiency was evaluated across a range of 400–2000 µL by monitoring the absorbance of riboflavin at its characteristic wavelengths (268, 370, and 446 nm). A volume of 600 µL of methanol was found to provide complete elution while minimizing dilution, yielding the maximum analytical signal. This optimized volume was employed in all subsequent experiments.

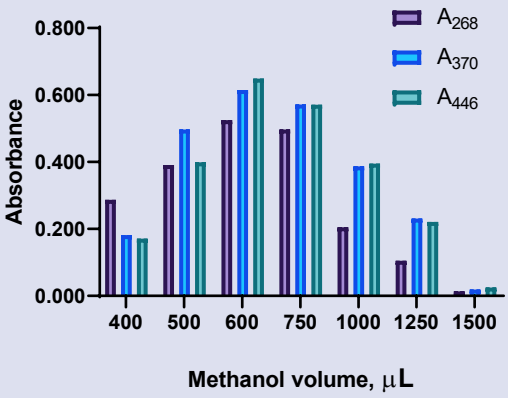

Figure 7. Optimization of desorption solvent volume for riboflavin

Effect of Contact Time on the MSPE Performance

This study highlights the important role of adsorption time in the MSPE and reveals the critical importance of this time in optimizing the interaction of the analyte with the adsorbent material [32] . Adsorption directly affects the interaction between the analyte and the magnetic material, as insufficient time may lead to incomplete adsorption, resulting in lower sensitivity and accuracy of the enrichment factor. Conversely, overly prolonged retention can introduce complications such as matrix effects or the potential for analyte degradation. The adsorption time optimization step of MSPE was carried out by using orbital shaker at room temperature. In this study, the influence of adsorption time was evaluated over a 5 to 60-minute interval using model solutions containing 200 ng mL^{-1} of riboflavin. The experiments were conducted on an orbital shaker operating at 100 rpm under ambient temperature. As illustrated in Figure 8, a contact time of 30 minutes was determined to be optimal, ensuring quantitative adsorption of riboflavin onto the magnetic sorbent.

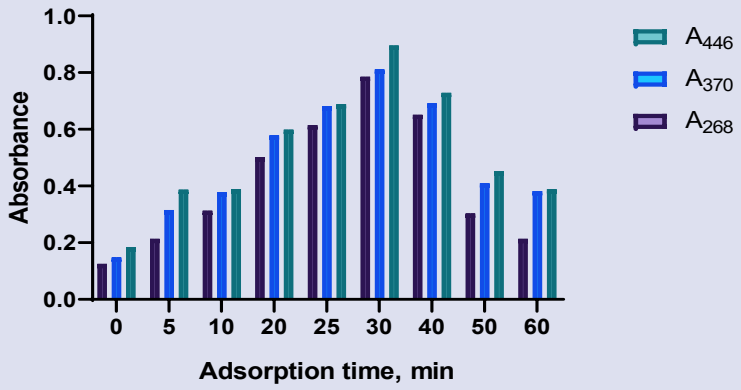

Figure 8. Effect of adsorption time on the extraction efficiency of riboflavin.

Adsorbent Reusability and Regeneration

One of the critical attributes of the newly developed Fe_3O_4@TEOS-Captopril adsorbent is its reusability, which significantly impacts the overall analysis cost. In MSPE methods, the ability to reuse the sorbent phase contributes to a reduction in analytical expenses. Furthermore, the reusability of the material reflects the stability and robustness of the synthesized adsorbent.

In this study, the Fe_3O_4@TEOS-Captopril adsorbent was weighed and subjected to optimized extraction conditions for riboflavin. After each extraction cycle, the quantity of adsorbed riboflavin molecules was compared to the previous measurements. The experiments were designed to ensure that the change in the measured amount of riboflavin did not exceed 5.0%. It was observed that there was no significant change in the absorbance signal up to 10 uses of the adsorbent. After each use, the magnetic material was thoroughly washed with 2.0 mL of MeOH to maintain its extraction efficiency.

Blue Applicability Grade Index (BAGI) for Riboflavin Analysis

The practical aspects of the proposed MSPE method for riboflavin were evaluated using the Blue Applicability Grade Index (BAGI). This tool quantifies methodological practicality on a scale from 25 to 100, facilitating the identification of a procedure's strong points and potential drawbacks for routine application. As shown in Figure 9 below the attained score of 65 for this method indicates a favorable level of practicality and positions it as a competitive and user-friendly alternative for the analysis of riboflavin.

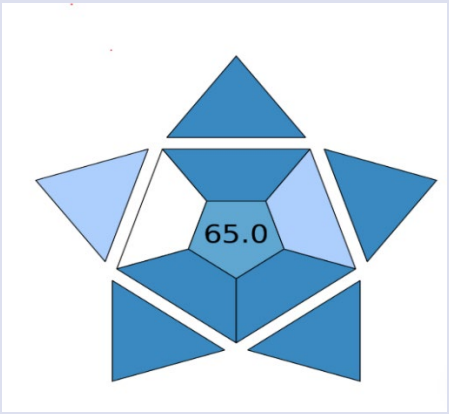

Figure 9. The practical applicability score of the developed MSPE method, as determined by the Blue Applicability Grade Index (BAGI) for riboflavin.

Analytical Characteristics

An analytical evaluation of the proposed MSPE methodology was conducted to assess its performance for riboflavin quantification under the established optimal conditions. The absorption characteristics of the extracted analyte were examined across different concentration levels. As shown in Figure 10, the absorption spectra of riboflavin after MSPE exhibits three characteristic maxima at 268, 370, and 446 nm, with absorbance intensities increasing progressively with concentration, confirming the effective extraction and pre-concentration of the target molecule.

The analytical merits of the proposed method were systematically evaluated, focusing on aspects such as the regression equation, linear range, limits of detection (LOD) and quantification (LOQ), repeatability, and pre-concentration and enrichment factor.

The developed method demonstrated a linear response across the concentration range of 125 to 2500 ng mL^{-1}. The LOD and LOQ were determined to be 37.88 ng mL^{-1} and 113.64 ng mL^{-1}, respectively, based on measurements at 446 nm. A preconcentration factor of 16.7 was achieved, calculated as the ratio of the initial sample volume (10 mL) to the final eluent volume (0.6 mL) following the MSPE procedure. Additionally, the enrichment factor, derived from the ratio of the calibration graph slopes after and before MSPE, was found to be 65.3. A summary of these analytical characteristics is provided in Table 1.

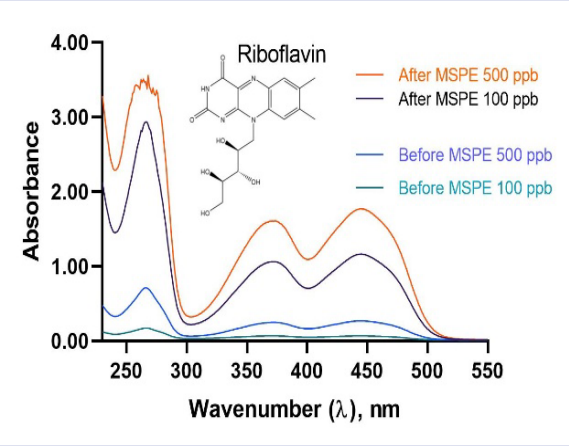

Figure 10. Absorption spectrum of riboflavin solutions in different concentrations before and after MSPE

Table 1. Analytical characteristics of the developed MSPE method for riboflavin determination.

Parameters	The Values	
	Before MSPE	After MSPE
Linear range	0.7–50.0 µg mL^{-1}	125–2500 ng mL^{-1}
Calibration curve equation	y = 0.03048x + 0.002607	y = 1.99171x + 0.021411
Slope(λ_{446})	0.0305	1.9917
Correlation coefficient (r^2)	0.9999	0.9985
RSD (%)	4.2 (at 10.00 µg L^{-1}, n: 3)	5.3 (at 200 ng mL^{-1}, n: 3)
LOD	0.21 µg L^{-1}	37.88 ng mL^{-1}
LOQ	0.63 µg L^{-1}	113.64 ng mL^{-1}
Preconcentration factor[a]	-	16.7
Enrichment factor[b]	-	65.3

Note: All quantitative data reported in this table were obtained from measurements at the optimal wavelength of 446 nm.

[a] Preconcentration factor = $V_{solution}$ / V_{eluent}

[b] Enrichment factor = $Slope_{after\ mspe}$ / $Slope_{before\ mspe}$

Preperation and Determinationof Riboflavinin Vitamin Tablets

The developed MSPE method was applied to determine the riboflavin content in two commercially available multivitamin tablets to evaluate its practical applicability. The sample preparation procedure was as follows: approximately 1.0 g of powdered tablets were accurately weighed and transferred into a 500 mL amber volumetric flask. Then, 30 mL of a 0.5% ammonium hydroxide solution was added, and the volume was completed to the mark with the same solvent. The mixture was sonicated (320 W, 50 Hz) at 25 °C for 25 minutes to ensure complete extraction. The resulting solution was diluted 200-fold, and a 5 mL aliquot of the diluted sample was subjected to the optimized MSPE procedure [33].

The accuracy and precision of the method were assessed through recovery studies by spiking the samples with known concentrations of riboflavin (250 and 500 ng mL^{-1}). Each analysis was performed in triplicate. The results, summarized in Table 2, demonstrate satisfactory recovery rates and repeatability, confirming the method's reliability for determining riboflavin in real pharmaceutical samples.

Table 2. Determination results of riboflavin in commercial multivitamin tablets (n = 3).

Sample	Added (ng mL^{-1})	Founded (ng mL^{-1})	Recovery %	RSD
	-	985.3±54.2	98.5	5.5
Multi Vitamin 1	250.00	1235.4±71.5	98.8	5.8
	500.00	1495.6±99.6	99.7	6.7
	-	975.3±65.2	97.5	6.7
Multi Vitamin 2	250.00	1255.4±89.8	100.4	7.2
	500.00	1405.6±98.5	93.7	7.0

Conclusions

This study presents a novel and environmentally friendly method for determining riboflavin. The approach combines MSPE with UV-Vis spectroscopy, utilizing a newly synthesized Fe_3O_4@TEOS-Captopril sorbent. The specific design of this core-shell material promotes a highly selective interaction with riboflavin, which is crucial for the method's enhanced sensitivity. The practical applicability of the method was successfully demonstrated through the analysis of commercial multivitamin tablets, yielding excellent recovery rates ranging from 93.7% to 100.4% and satisfactory precision (RSD < 7.2%). The procedure is notable for its speed, low cost, and avoidance of organic solvents, aligning with green chemistry principles. It thus presents a viable and practical solution for laboratories that require accurate riboflavin determination but have limited access to advanced chromatographic systems. The method's alignment with Green Analytical Chemistry principles was quantitatively validated by a Blue Applicability Grade Index (BAGI) score of 65, highlighting its good practicality, minimal solvent consumption, and energy efficiency. Consequently, this method stands as a rapid, cost-effective, and sustainable alternative for the accurate determination of riboflavin, proving particularly valuable for laboratories with limited access to sophisticated instrumentation.

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