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Green Chemistry Spectrofluorimetric Assessment of Folic Acid in Pharmaceutical Formulations Using Acriflavine as an Efficient Fluorescent Probe

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Abstract

A sensitive, simple, inexpensive, rapid, and eco-friendly spectrofluorimetric method was created to assess the folic acid (FA) concentration in tablets using acriflavine (ACF) as an eco-friendly photoprobe. Based on its ability to quench the ACF fluorescence intensity in water at pH 8.0 and $\lambda_{ex} = 460$nm. FA concentration was measured by quenching the fluorescence intensity of the ACF at λ_{em} =508 nm within the linear range of 3.5×10^{-6}–30.0×10^{-6} mol L^{-1} with a correlation coefficient $r^2 = 0.9991$. The limit of quantification (LOQ) and the limit of detection (LOD) are 1.159×10^{-6} mol·L^{-1} and 0.383×10^{-6} mol·L^{-1}, respectively. This method was easy to use and accurately and effectively evaluated FA in pharmaceutical tablet samples. No influence was observed from the excipients usually contained in pharmaceutical formulations. The method was verified as valid according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. In addition, its greenness has been estimated using environmental assessment tools.

Keywords

Acriflavine, folic acid, fluorescent assay, greenness evaluation, eco-friendly

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Introduction

Folic acid (FA) or folate (Figure 1a) is a vital nutritional agent for humans and other mammals, playing a role in several events that contribute to DNA synthesis.[1] It is a water-soluble vitamin that cannot be generated or stored in the human body, resulting in its continuous loss, which suggests problems with FA deficiency. It is classified as an anti-anemia and growth agent that can stimulate the production of RBCs. In addition, FA supplementation during pregnancy can lower the risk of newborn congenital heart defects and prevent neural tube defects.[2,3] FA deficiency is linked to several neurological issues, such as depression, stroke, and Alzheimer's disease. Additional symptoms include poor growth, heart palpitations, fatigue, swollen tongue, and hearing loss.[3,4] The European Union has authorized a daily intake of 400 µg for adults. FA can be obtained from medications and dietary supplementation to reduce this risk. Different methods are used for assessing FA, such as electrochemical detection,[5] capillary zone electrophoresis,[6] chemiluminescence,[7] and various liquid chromatographic techniques, e.g., liquid chromatography–mass spectrometry (LC-MS),[8,9] high-performance liquid chromatography (HPLC),[10,11] ultra-HPLC,[12] and fluorescence detection.[13,14]

Most of these methods have some disadvantages. The spectrophotometric methods have a narrow range of determination, extraction, or heating; the reaction takes a long time, and the colored product formed is unstable.[15] Chromatographic methods are expensive and require intricate purification processes and extraction.[16] Also, the electroanalytical technique is poorly sensitive.[8]

Recently, fluorescent sensors have been in greater demand due to their high sensitivity, selectivity, speed, real-time processing capabilities, and ability to identify targets visually. The accessibility of spectrofluorimetric techniques may benefit quality control evaluations and laboratories that lack access to costly or complex methods. Moreover, with no multiple

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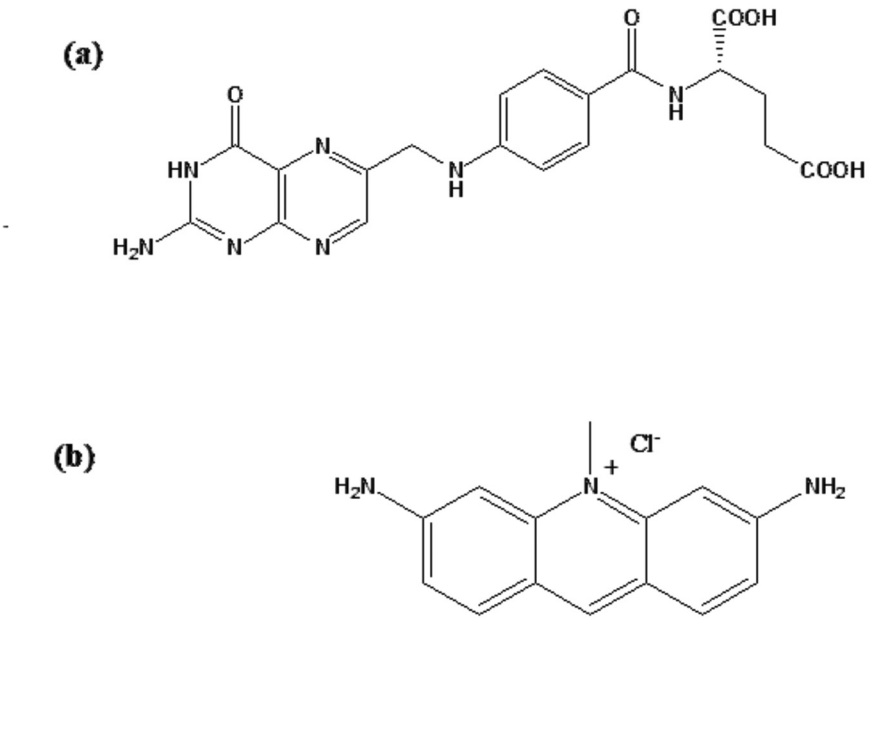


Figure 1. (a) Folic acid structure. (b) Acriflavine structure.

extraction or preprocessing processes, the method's simplicity enhances its reproducibility. The suggested method presented additional benefits, including operational simplicity, cost efficiency, and safety. Spectrofluorometric investigations for pharmaceuticals may serve as a substitute for other complex, time-intensive, costly, and laborious analytical methods.

Acriflavine (ACF) is a naturally occurring acridine fluorescence dye with therapeutic application (Figure 1b). Chemically, it consists of a combination of 3,6-diamino-10-methylacridinium chloride (trypaflavin) and 3,6-diaminoacridine (proflavine) and has a history of clinical application. ACF is an FDA-approved medication used topically or systemically as an antibacterial,[17] antimalarial,[18] antiviral,[19] antifungal,[20] antimicrobial,[21] and anti-cancer agent.[22,23] It could be used as a diagnostic tool due to its pharmacological and spectroscopic properties. It was a fluorescent and photometric probe to identify the nucleic acid structure and create a DNA sensor.[24] It was also used as a biosensor for detecting Sudan I–IV azo dyes and staphylococcal enterotoxin B (SEB).[25,26] Furthermore, it is efficiently applied to quantify certain drugs based on their quenching effect on ACF. This quenching effect was attributed to the formation of new fluorescent ion-associated complexes composed of ACF and each of the studied drugs, including tartrazine, ascorbic acid, cephalosporins, ketoprofen, ibuprofen, olsalazine, sulfasalazine, acemetacin, aceclofenac, and furosemide.[27–32] As a result, the concentration might be easily determined by calculating fluorescence intensity differences. Meanwhile, FA contains carboxylic groups that can react with ACF at an appropriate pH. So, the current spectrofluorimetric methodology depends on quenching the native fluorescence intensity of ACF, which leads to the development of easy and rapid methods for determining FA. Enhancing the environmental sustainability of any analytical technique is difficult due to the complexity of the methods or the extensive use of organic solvents. Nonetheless, our approach would be suitable for evaluating environmental sustainability due to its eco-friendly nature. The method was valid following ICH guidelines. In addition, the suitability and evaluation of the current approach for environmental sustainability were done using different tools, including the green analytical procedure index, analytical eco scale, analytical greenness metric approach, and analytical greenness prep metric approach.

As far as we know, the study is the first work that uses acriflavine as a sensitive, environmentally friendly fluorescent probe to determine FA. Compared to other reported methods, the suggested spectrofluorimetric method offers several clear advantages. The method demonstrates excellent accuracy, high reproducibility, sensitivity, a low detection limit, a wide linear dynamic range, cost-effectiveness, and time efficiency. It works at room and physiological temperatures, which is suitable for biological samples. In addition, a simple

sample preparation process lets you analyze pharmaceutical tablets directly without having to extract or derivatize them first. These advantages not only make the analytical process easier but also make the method more useful for routine quality control and pharmaceutical analysis.

Experimental

Materials and Methods

The absorption spectra were obtained using an ultraviolet–visible (UV–Vis) spectrophotometer (Thermo Scientific Evolution 300) running in double-beam mode. The recorded emission spectra were obtained using a fluorescence spectrometer (Meslo-PN 222-263000) Thermo Scientific Lumina) operating within the wavelength of 190–900 nm and 2.5 nm spectral bandwidth utilizing a 1 cm quartz cell. The device is supplied with a xenon flash lamp as a light source and is linked to a Dell computer equipped with Luminous software. The value of pH was recorded using a pH-JAN-WAY 3330 calibrated pH meter.

Pure ACF and FA were obtained from Sigma Aldrich in a pure form. Commercial pharmaceutical tablets (folic acid batch no. 7724) containing 500 µg of FA tablet produced by Mepaco-Medifood Co., Egypt, were purchased from the local market. All solvents, including absolute ethyl alcohol, acetonitrile, dimethyl sulfoxide, and dimethylformamide, were bought from Sigma Aldrich. Britton– Robinson buffer components (sodium hydroxide, acetic acid, hydrochloric acid, phosphoric acid, and boric acid) were obtained from El-Nasr Chemicals Co.

Preparation of Solutions

A stock solution of ACF of 1.0×10^{-2} mol L^{-1} was produced by dissolving 0.026 g of ACF in a small quantity of distilled water in a 10 mL measuring flask. The solution was then diluted to the final concentration using distilled water. More diluted solutions were prepared using the same solvent. Adjustment of pH was achieved by using 1.0 mL of Britton–Robinson buffer solution with a pH range of 2.0 to 12.0, which was created by mixing suitable volumes of (1.0 M phosphoric acid, 1.0 M acetic acid, and 1.0 M boric acid) and using 0.1 M of HCl and NaOH to get the right pH.

A stock solution of 0.1 mol L^{-1} of pure FA was formed in a 10 mL measuring flask by dissolving 0.44 g of FA in a tiny quantity of 0.1 M NaOH solution with stirring, then diluting the mixture using distilled water. The practical solutions were achieved by suitable dilution with distilled water to generate more dilutions ($1.0 \times 10^{-2} - 1.0 \times 10^{-7}$ mol L^{-1}) from the standard stock solution. Storage temperatures for stocks and working solutions range from 2 to 8 °C when not being used.

Calibration Curve

As previously stated, various standard solutions of FA in water were prepared; 1.0 mL of 1.0×10^{-5} mol L^{-1} ACF fluorescence sensor solution with pH 8.0, using 1.0 mL of Britton–Robbins buffer solution, was mixed with each standard FA solution in the spectrofluorimetric device cell. The spectra of fluorescence were measured at room temperature at $\lambda_{ex}/\lambda_{em}$ of 460/508 nm. The calibration curve was created by plotting the resulting values from the division of the fluorescence intensity of ACF alone (F_o) by the fluorescence intensity of ACF in the presence of known concentrations of FA (F) on the y-axis and plotting the concentration of FA on the x-axis in mol L^{-1}.

Determination of FA in Tablets

Twenty tablets of folic acid 500 µg from Mepaco-Medifood Co. were weighed and ground into a tiny homogeneous powder. A weighed amount containing 500 µg of FA was dissolved in 4 mL of 0.1 M sodium hydroxide (NaOH). Then, the solution was filtered using 12 mm filter paper and washed with water. The filtrate was diluted in a separate measuring flask to 10 mL with water. More diluted solutions were created by diluting a fraction of the filtrate with distilled water to get the desired concentration within the working range. In the spectrofluorometer cell, a filtrate volume was individually added to the 1.0 mL of the ACF sensor. Then, fluorescence spectra were obtained at $\lambda_{ex}/\lambda_{em} = 460/508$ nm after the solutions were mixed and scanned. Various concentrations from the matching calibration plot were used to determine the drug concentration.

Results and Discussion

Fluorescence Sensor Spectra

Generally, quenching describes the non-radiative transfer of energy from one excited molecule to another. The spectrofluorimetric approach has been used as a foundation for drug determination. The molecular composition of FA shows that it contains carboxylic groups (Figure 1a). This study aimed to use ACF dye to determine FA in a sensitive and selective method by forming an ion association complex. ACF demonstrates a natural fluorescence at 508 nm in excitation at 460 nm, and its interaction with FA reduces this fluorescence. Different parameters affecting the reaction between ACF and FA were examined to achieve optimal conditions. Figure 2a illustrates the excitation and emission spectra of ACF. Figure 2b illustrates the absorption spectrum of FA in Curve 1. Curve 2 shows the absorption spectrum of ACF, which displays significant peaks at 262 nm and 450 nm because of the $\pi \rightarrow \pi^*$ transition. Curves 3–6 demonstrate the ACF absorption spectra at varying concentrations of FA. Figure 3 displays the emission spectra of the ACF at different FA concentrations with a dynamic range of $0–30 \times 10^{-6}$ mol L^{-1}. The intensity of the unique peak at $\lambda_{em} = 508$ nm was quenched after various amounts of FA were added to ACF in water. This was because energy was transferred from the fluorescence sensor to FA.

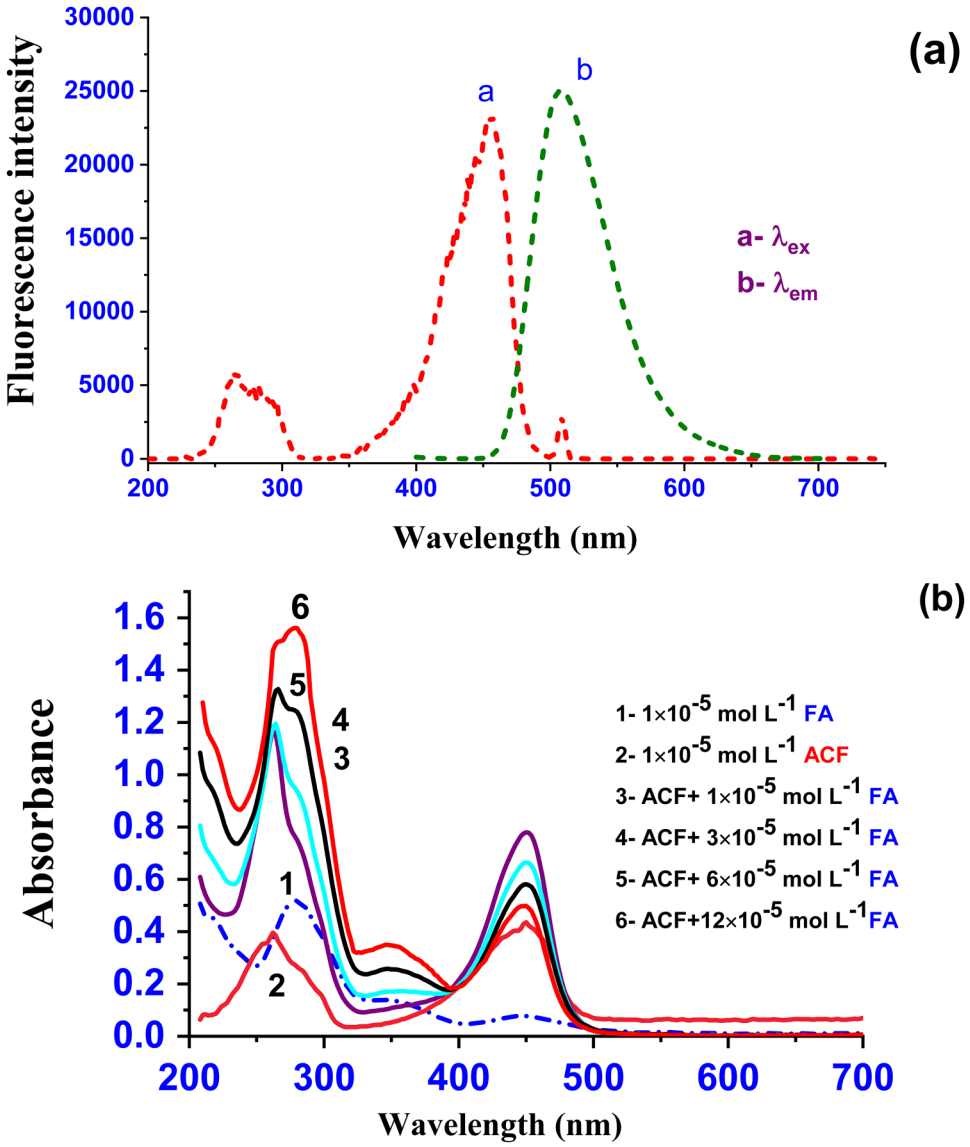


Figure 2. (a) For excitation (red line) and emission (green line) spectra of ACF, and (b) For absorption spectra of 1.0×10^{-5} mol L^{-1} FA, 1.0×10^{-5} mol L^{-1} of ACF sensor, and the sensor with FA.

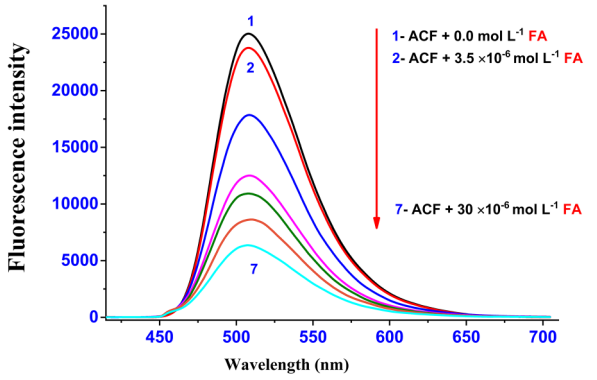


Figure 3. ACF sensor fluorescence emission spectra at $\lambda_{ex} = 460$ nm with various concentrations of FA in water and pH 8.0.

Impact of Varying Experimental Variables: Influence of the Solvent

Different standard solutions of 1.0×10^{-5} mol L^{-1} of ACF in numerous solvent solutions were prepared in multiple solvent solutions, including water, EtOH, acetonitrile, DMF, and DMSO. Then, the fluorescence intensity was evaluated under the previous conditions to study how different solvents affected the sensor intensity. Figure 4a indicates that the emission of ACF is amplified in water, an eco-friendly solvent.

Impact of pH and Volume of Buffer

The impact of the pH of the Britton–Robinson buffer solution on the quenching of the fluorescence intensity of ACF

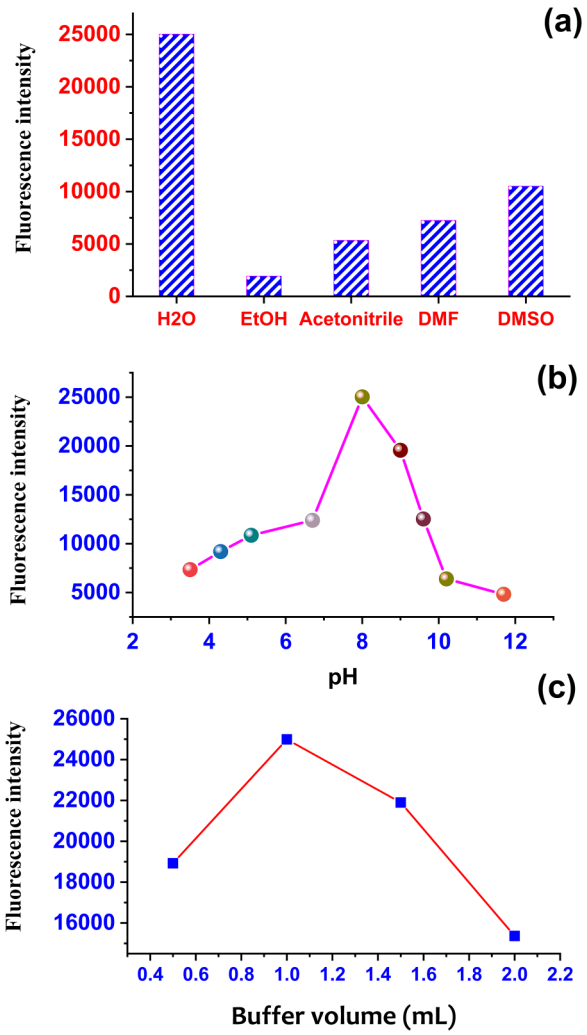


Figure 4. (a) For the impact on the fluorescence emission spectra of the ACF of solvents at pH = 8.0 and λ_{ex} =460 nm. (b) For the impact of pH in water at λ_{ex} =460 nm, and (c) The effect of buffer volume in water at λ_{ex} =460 nm.

was examined within the pH range 2–12. Figure 4b shows that rising pH values led to a subsequent increase in ΔF at pH 8.0. After this, a reduction in ΔF was achieved. Hence, the Britton–Robinson buffer solution with pH 8.0 was the optimal pH for the duration of the study (Figure 4b). The volume of the buffer solution was also investigated, and findings show that enhancing the fluorescence intensity of the ACF at a volume of 1.0 mL (Figure 4c).

Influence of ACF Concentration

The effect of different concentrations of ACF solution was examined in the range 1.0×10^{-2}–1.0×10^{-7} mol L^{-1}. Figure 5a shows that 1.0×10^{-5} mol L^{-1} is the optimum concentration that exhibits the optimal fluorescence intensity.

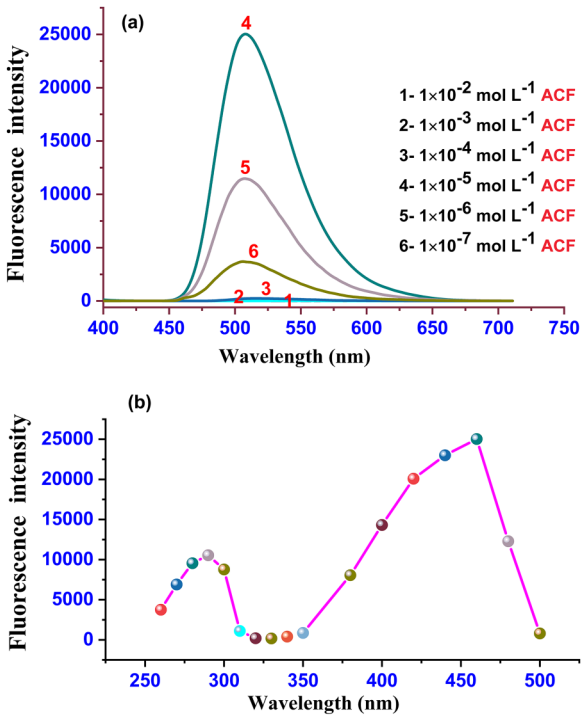


Figure 5. (a) Different concentrations of the ACF sensor affect its fluorescence emission spectra in water and pH 8.0 at λ_{ex} =460 nm, (b) Different wavelengths affect the fluorescence intensity of the ACF.

Impact of Wavelength

The influence of change wavelengths on the sensor intensity was studied by synthesizing 1.0×10^{-5} mol L^{-1} of ACF in water and pH 8.0. Then, fluorescence intensity was measured under the previous conditions. Figure 5b indicates that the emission of ACF was enhanced at λ_{ex} =460 nm.

Influence of ACF Temperature

The temperature impact was examined under the optimal conditions at a range of 0–100 °C, and the optimum temperature is 20–25 °C, in which the ACF sensor has a high intensity (Figure 6a).

Reaction Time

A standard solution of FA (1.0×10^{-5} mol L^{-1}) was mixed with ACF, and the fluorescence intensity was recorded at 1–6 min under the optimum conditions. It was observed that the maximum quenching effect of FA was obtained immediately after mixing with the sensor, after which ΔF was not affected (Figure 6b). Overall, the optimum conditions for the spectrofluorimetric determination of FA using ACF as a fluorescence sensor are shown (Table S1, Supplemental Material).

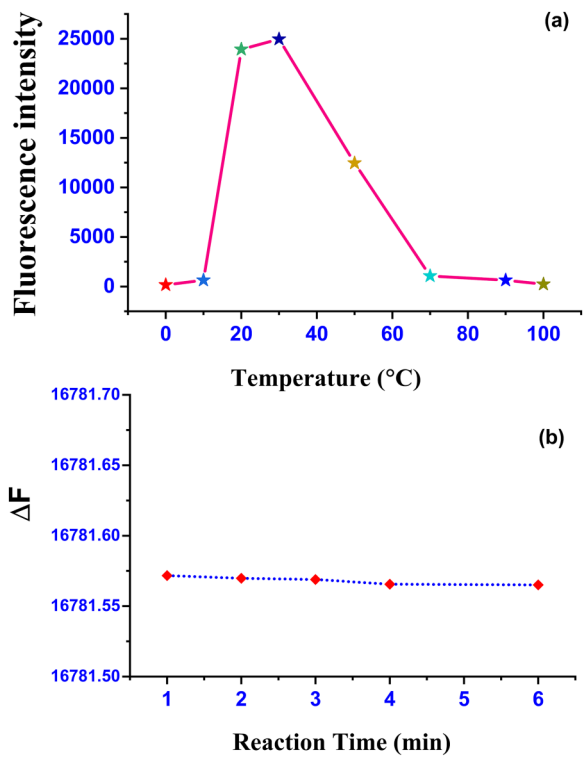


Figure 6. (a) Temperature impact on the ACF sensor fluorescence intensity, (b) Effect of time of reaction between ACF sensor and FA.

Method Validation

The proposed spectrofluorimetric method was evaluated for validity depending on the ICH Q2 (R1) guideline for assessing linearity, accuracy, selectivity, robustness, repeatability, and intermediate precision.[33]

Linearity

A linear correlation was generated using the Stern–Völmer Eq. 1 by plotting the (F_0/F) values at $\lambda_{em} = 508$ nm and the concentration of FA. It was found to be linear in the range given in 3.5×10^{-6}–30.0×10^{-6} mol L^{-1} and a correlation coefficient (r^2) of 0.9991 (Figure 7a, Table I).

$$F_o/F = 1 + K_{sv}[Q] \quad (1)$$

The limit of detection (LOD) and quantitation (LOQ) were calculated using equations LOD = 3.3 S/b and LOQ = 10 S/b mol L^{-1}, where S is the standard deviation of the intercept, and b is the slope of the calibration plot (Table I). The low LOD value suggests that the suggested method exhibits more sensitivity than previous methods. Table S2 (Supplemental Material) [7,9,12,13,34–36]compares this spectrofluorimetric method with various reported techniques for determining FA. In addition, the most recent analytical methods that employed acriflavine as a fluorescent probe are displayed (Table S3). Although Table S3 [25,27–32,37–39]shows the suitability and versatility of acriflavine as a fluorescent probe, which has been used for other pharmaceutical compounds, its application for FA determination has not been previously reported, which exhibits the novelty of our work.

Accuracy and Precision

The study involved using three distinct concentrations of FA. The accuracy and precision were assessed by replicating the investigations described in the general procedures three times a day to evaluate intra-day precision and on different days for inter-day precision determination. The results are presented in relative standard deviation (RSD). The method exhibits good precision, as demonstrated by the low RSD values of intra- and inter-day readings (Table II). The accuracy reflects the computed value's proximity to the sample's actual value. Different drug concentrations were prepared from the stock solution and then analyzed to assess the accuracy. Additionally, the accuracy of the proposed method was verified by utilizing an additional standard method. It included mixing different amounts of the pure FA with a previously examined dilution. After that, the suggested method was used to measure the whole concentration. The accuracy was evaluated by computing the percentage relative error (%RE) between the measured mean concentrations and the known values of FA. The bias percentage for each concentration was calculated by applying the equation: bias% = [(observed concentration − known concentration) × 100 / known concentration]. The low %RE indicates the great accuracy of the suggested method (Table II).

Selectivity

The method's selectivity was evaluated through an examination of the spectrum of commonly used excipients in tablets. The impact of various common foreign substances present in standard pharmaceutical products, including talc, starch, lactose, potassium chloride, calcium carbonate, sodium alginate, citric acid, and magnesium stearate, was evaluated under optimal conditions and utilizing the prescribed procedure for the spectrofluorimetric method. Different amounts of interfering substances were added separately to a standard sample solution of pure FA. The tolerance limit was identified as the concentration that resulted in an error of not more than 5% in the determination of the FA. No interferences were detected from the presence of the foreign species in the ratios usually used in pharmaceutical formulations (Figure 7b).

Robustness

The method's robustness was assessed by introducing gradual and slight changes to the ACF concentrations, time of

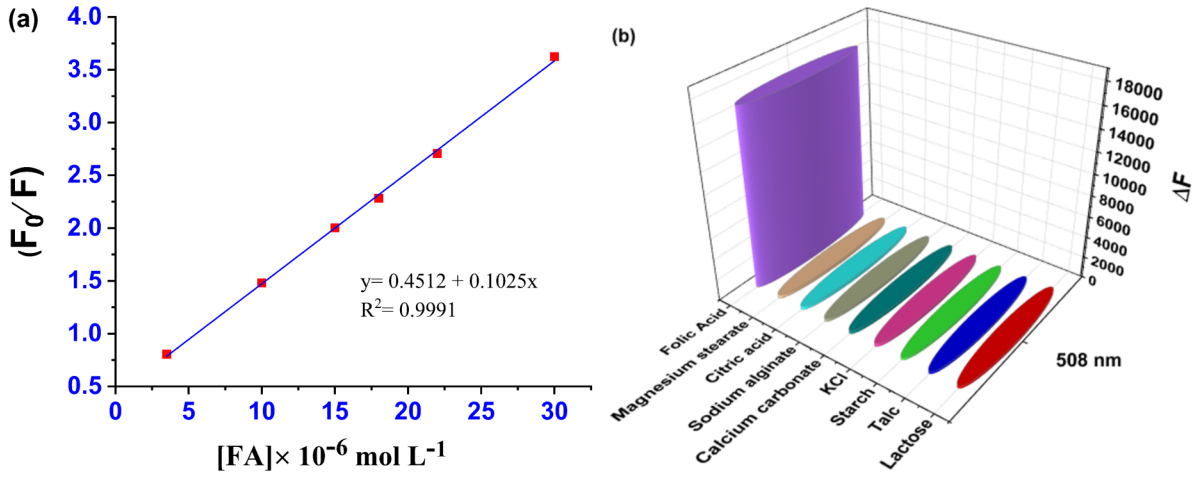


Figure 7. (a) For the calibration plot of the proposed method, and (b) for the selectivity study of the suggested method.

contact, pH value, and buffer volume. The impact of these changes on the fluorescence intensity of the ACF was then evaluated. The adjustments did not significantly influence the study findings, as evidenced by the low %RSD values. The findings demonstrate that the within-day RSD values were below 0.84%, indicating that the proposed method shows good robustness and ruggedness.

Application to Formulations

The suggested method was used to estimate the FA in Folic acid MEPACO tablets containing 500 µg of FA produced by MEPACO Egypt Pharmaceutical, bought from the market. (Table III) demonstrates that the method successfully determined FA in the dosage forms without interference. The findings were compared statistically using the method of the official British Pharmacopoeia (BP).[40]

Recovery Evaluation

Recovery studies were performed utilizing the standard addition method to evaluate the accuracy. To conduct the investigation, pure FA was mixed with the pre-analyzed tablet powder at three concentrations (4.0, 5.0, and 8.0) $\times 10^{-5}$ mol L^{-1} of the tablet powder. The total sum was determined following the proposed method. Three replications of the experiment were performed. The approximation of the results to 100% confirms the great accuracy of the procedure for tablet samples (Table III). The average recovery and RSD% were estimated to be 100.33 $\pm$ 0.56%. For comparison, the (BP) method displayed an average recovery of 99.9% and an RSD of 0.055%. These values were compared and are in excellent agreement with the results of the suggested method.

Table I. Regression parameters for photoprobe.

Parameter	Method
λ_{em} nm	508
Linear range, mol L^{-1}	3.5×10^{-6}–30.0×10^{-6}
Slope (b)	0.1025
SD of slope	0.000785
Intercept (a)	0.451253
SD of intercept	0.011884
LOD, mol L^{-1}	0.383×10^{-6}
LOQ, mol L^{-1}	1.159×10^{-6}
Regression coefficient (r^2)	0.9991
Regression equation, Y*	Y = a + Bx

Greenness of the Suggested Method

A principle extensively used in different areas is green analytical chemistry (GAC). Employing the green principle in chemical analysis enhances the environmental and economic features of the analytical approach. This can be done by utilizing chemicals that are safe for the environment and staying away from dangerous materials or methods.[41] Different greenness metrics and software were used to investigate the greenness of the developed probe. The environmentally friendly nature of the suggested analytical methods derives from the utilization of ACF, an eco-sustainable, readily accessible, and inexpensive reagent with medical applications characterized by low toxicity, employed through low-energy, cost-effective spectrofluorimetric methods. In addition to dependence on a mix-and-read analytical method, the exclusion of organic solvents and harsh chemicals supports the method's greenness. The findings indicated the substantial potential of this proposed eco-friendly, safe, cost-effective, and sustainable method for pharmaceutical and biological applications. Four different tools were applied to determine the greenness of the suggested method, including the Green Analytical Procedure Index (GAPI), Analytical Eco-Scale, Analytical Greenness Prep (AGREE Prep) Metric Approach, and Analytical Greenness metric (AGREE).

Table II. Assessment of accuracy and precision for the proposed method.

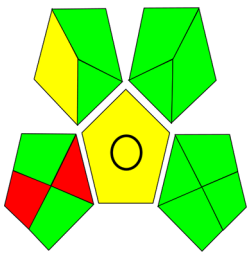
Drug taken $\times 10^{-6}$ mol L^{-1}	Intra-day accuracy and precision ($n = 3$)			Inter-day accuracy and precision ($n = 3$)		
	Average Found	%RE	%RSD	Average found	%RE	%RSD
4.0	4.006 ± 0.063	0.17	0.63	4.013 ± 0.038	0.33	0.38
6.0	6.027 ± 0.062	0.44	0.42	6.012 ± 0.041	0.20	0.227
9.0	9.000 ± 0.038	0.11	0.29	9.020 ± 0.052	0.26	0.231

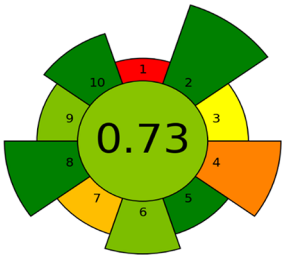
Table III. Standard addition method results of recovery studies.

Sample	Drug taken $\times 10^{-6}$ mol L^{-1}	Found $\times 10^{-6}$ mol L^{-1}	Average	Average recovery ± RSD (%)	BP
Tablet, 500 µg of FA MEPACO	4.0	4.03, 4.02, 4.04	4.03	100.75 ± 0.25	99.9 ± 0.055
	5.0	5.02, 4.97, 5.01	5.00	100.00 ± 0.66	
	8.0	8.03, 8.01, 8.02	8.02	100.25 ± 0.13	

Table IV. Greenness assessment of the suggested method.

Eco-Scale (spectrofluorimetric)			
Parameters			Penalty points
Reagents	ACF	Amount	1
		Hazard	1
Solvents	Water	Amount	0
		Hazard	0
	BRB buffer	Amount	1
		Hazard	0
Instruments	Energy		0
	Occupational hazard		0
	Waste	Amount	3
		Treatments	0
Total penalty points			5
Analytical Eco-Scale (Total score)			100 − 5 = 95
GAPI	AGREE		AGREE prep





Evaluation by Analytical Eco-Scale

This approach assessed solvent usage, energy usage, hazardous circumstances, and processing methods for environmental sustainability. This evaluation depends on determining the penalty points for the suggested spectrofluorimetric method by multiplying the subtotal penalty points for a given volume and hazard and then deducting these points from 100. A method with a value of the Eco-Scale of more than 75 represents an excellent green one. Furthermore, the method is considered acceptable for an Eco-Scale value above 50 and unsustainable if it is below 50.[42–44] For the suggested method, the Eco-Scale value was assessed at 95. The calculated Eco-Scale score indicates that the suggested method is an excellent green approach for the study of the investigated drug (Table IV).

Evaluation by GAPI

The GABI described all stages in the analytical procedure in great detail, involving how the samples were prepared and how they were handled (collecting, preserving, transporting,

and storing), and also the chemicals and the apparatus that were utilized. Five pentagrams were used to determine the environmental hazards of each step of the analytical procedures. The colors green, yellow, and red were employed to signify low, medium, and high harmful environmental effects for each of the analytical processes.[45] The GAPI evaluation of the suggested approach indicated green color in 11 fields, yellow in two, and two red fields, illustrating compliance with the green criteria (Table IV).

Evaluation by AGREE

In this approach, 12 principles are determined on a scale (0–1), and the method is greener if its score is close to 1.[46] A technique is classified as green if its score exceeds 0.6. The performance was illustrated using a red-yellow-green scale for each of the 12 GAC principles. Because of all the analytical processes related to hand sampling, preparing the sample for investigation required considerable time to get the results. Thus, sample collection was regarded as an offline process. Additionally, transportation and storage are necessary, as the pharmaceutical form was manufactured at the manufacturing site and delivered to laboratories. The proposed method uses simple procedures for sample preparation by using a suitable solvent without employing any extraction processes. Furthermore, no additional treatments were applied to the sample. The analytical approach was the spectrofluorimetric method; hence, the instrument's energy usage was regarded as ≤0.1 kWh per sample. Approximately ≤10 ml of waste was generated, and no treatment was conducted. The application of the AGREE approach to the suggested method obtained a score of 0.72 (Table IV).

Evaluation by AGREE Prep

Evaluation of the greenness of sample preparation depends on the 10 principles of GAC. That involves solvent choosing, material use, waste generation, consumption of energy, sample quantity, and sample throughput, and the score varies from 0 to 1.[47] AGREE Prep produces a circular pictogram that graphically represents the procedure's greenness. Different colors indicate different greenness levels (red for low, green for high). Applying the AGREE prep method and its parameters to the proposed method revealed a score of 0.73 (Table IV).

Limitations and Future Perspectives

Although the suggested method demonstrates simplicity, environmental sustainability, and satisfactory analytical performance for the determination of FA in pharmaceutical tablet samples, significant limitations persist. When compared with fluorescence systems that use nanomaterials or surfactants, the method's sensitivity is moderate. Future work may focus on improving the fluorescence signal through micellar or nanoparticle-assisted environments and validating the method across a wider range of biological and environmental samples.

Conclusion

The ACF reagent was employed to establish a green spectrofluorimetric method for determining FA. This complex exhibits a highly sensitive and distinctive peak at 508 nm in the presence of FA. Energy transfer from the ACF sensor causes the fluorescence intensity of this peak to be quenched as the FA concentration increases. The suggested method followed the ICH requirements for validation and was determined to be precise, accurate, specific, and devoid of interferences. The proposed method has a broad linearity (3.5×10^{-6}–30.0×10^{-6}) mol L^{-1} and an excellent correlation coefficient (r^2) of 0.9991. It has a detection limit of 0.383×10^{-6} mol L^{-1} and a quantification limit of 1.159×10^{-6} mol L^{-1}. Due to its cost-effectiveness and applicability, the method is an excellent choice for the routine analysis of FA with high greenness in pharmaceutical preparation.

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CRediT Authorship

Huda M. Younis: Supervision, Project administration, Writing—review and editing.
Mohamed A. Abdel-Lateef: Formal analysis, Writing—review and editing.
Amal A. Mohamed: Project administration, Supervision, Visualization, Methodology, Validation, Formal Analysis, Data Curation, Conceptualization, Software, Writing—original draft, Writing—review and editing.

Data Availability

The data that support the study's findings are included in the main text of the manuscript. Raw data can be obtained from the corresponding authors upon request.

Declaration of Competing Interests

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Supplemental Material

All supplemental material mentioned in the text accompanies this paper online.

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