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Investigating the Luminescent Characteristics of Formoterol for Innovative Spectrofluorimetric Determination in Pharmaceutical Formulation: Theoretical Calculations and Greenness Evaluation

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ABSTRACT

This study investigates the fluorescence characteristics of formoterol, emphasizing the influence of various experimental conditions on its emission intensity. The fluorescence intensity of FMT was monitored at 342 nm after being excited at 223 nm. Comprehensive optimization of parameters, including pH, buffer solutions, and diluting solvents, revealed that neutral to basic environments and distilled water maximized the fluorescence intensity. The fluorescence behavior of FMT with pH changes was supported using density functional theory (DFT) calculations. Additionally, the change of fluorescence intensity with pH was utilized to calculate the pKa value of FMT. The method was validated per ICH guidelines, demonstrating high linearity, sensitivity, precision, and accuracy. The fluorescence-concentration plot was rectilinear from 50 to 1000 ng mL^{-1}, with high linearity ($r^2 = 0.9997$). The limits of detection and quantitation were 11.5 and 34.9 ng mL^{-1}, respectively. The selectivity of the method was revealed from the absence of any significant interfering effect from the presence of different pharmaceutical excipients. Applications of the developed method in analyzing two commercially available pharmaceutical formulations showed high recovery percentages (99.18 ± 1.72 and 100.00 ± 0. 89). A comparative analysis with existing methodologies revealed superior sensitivity and cost-effectiveness, whereas the greenness evaluation confirmed its environmental friendliness.

1 | Introduction

Formoterol (FMT, Figure 1) is a highly potent and selective long-acting beta2-adrenoceptor agonist used as a bronchodilator in patients with reversible obstructive airway disease with fast onset of action [1]. For treating nocturnal and exercise-induced asthma, FMT seems to have higher efficiency than short-acting beta2 agonists owing to its longer duration of action [1]. FMT is known chemically as ((RR)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxypheny)-1-methylethyl] amino]ethyl] phenyl]formamide). FMT stimulates the enzyme adenyl cyclase responsible for catalyzing the transformation of adenosine triphosphate (ATP) to cyclic-3′,5′adenosine monophosphate (cyclic AMP). Increased cyclic AMP causes relaxation of the smooth

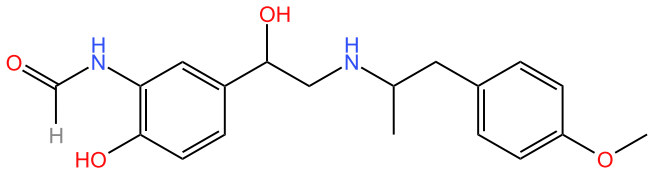

FIGURE 1 | The chemical structure of FMT.

muscles of the bronchi and inhibits the release of mediators of acute hypersensitivity from the cells, especially mast cells.

The FDA approved FMT for the treatment of asthma on July 22, 2006, and it is indicated to maintain long-term management of bronchoconstriction associated with COPD (including emphysema and chronic bronchitis) in the United States [2, 3]. FMT is a safe and effective monotherapy for EIB and an add-on controller therapy for moderate to severe persistent asthma [4].

Several techniques are available for the analysis of FMT, including spectrophotometry [5], spectrofluorimetry [6], capillary electrophoresis [7–9], HPLC [10–12], LC-MS-MS [13], GC-MS [14], and HPTLC [15–17]. However, reports on analytical techniques for determining FMT in biological fluids are limited [12]. Most of these methods are based on HPLC technology, which offered high accuracy and reproducibility, but requires costly equipment and is time consuming due to extensive sample clean-up and analysis. Additionally, HPLC consumes vast amounts of organic solvents of high purity, which increase both analytical costs and environmental risks. LC-MS-MS [13] seems to have high sensitivity but is very expensive and requires skilled personnel. GC-MS [14] has excellent sensitivity but is suitable only for volatile compounds. Although HPTLC can address some of the HPLC's limitation and could provide multiple samples to be analyzed in one batch, it suffers from low sensitivity.

Although spectrophotometry is a simple technique, it has low sensitivity and selectivity compared to advanced techniques, making it rarely used for analyzing biological samples. Despite its high separation efficiency, capillary electrophoresis is complex and may require expensive instrumentation.

On the other hand, spectrofluorimetric methods are characterized by their intrinsic ease, low-cost, convenience, ease of sample preparation, and speed. They are considered effective tools for trace-level detection in plasma, urine, and complex matrices. Therefore, spectrofluorimetry plays a very important role in pharmaceutical, clinical, and environmental analysis. However, only one method for determining FMT through fluorescence detection was published [6].

Accordingly, the purpose in this work is to develop a spectrofluorimetric technique that retains these advantages, while enhancing selectivity and environmental friendliness. Thus, developing a new, accurate and highly selective fluorimetric approach for determining FMT in dosage forms is inescapably of prime interest to this work. In addition, the technique can be used with extreme simplicity, and the developed methodology is green, which will be proved with different analytical green tools.

2 | Experimental

2.1 | Instrumentation

A Perkin Elemer LS 45 fluorescent spectrometer was used. This spectrometer has fixed slits for reliable daily use. Unlike conventional light sources, durable and long-life light source, the enhanced xenon lamp reduces photobleaching of samples.

2.2 | Chemicals and Materials

All chemicals and reagents used were of laboratory analytical grades. FMT was a gift from Novartis Pharmaceutical Company (Cairo, Egypt). Formohale 12-μg capsules for inhalation are a product of Chemipharm Pharmaceutical Industries (Cairo, Egypt). Foradil 12-μg capsules for inhalation are a product of Novartis Pharmaceutical Company (Cairo, Egypt). Both products were purchased from the local market in Egypt. Each contains 12 μg of FMT per capsule. Acetonitrile, methanol, and ethanol were acquired from Merk (Darmstadt, Germany). Acetone, cetyltrimethylammonium ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), sodium hydroxide, and sulfuric acid were supplied from El Nasr Pharmaceutical and Chemical Co. (Cairo, Egypt).

2.3 | Standard Solutions

For preparing stock solution, an exactly 10 mg of pure powder of FMT was transferred into a 10-mL calibrated flask and then dissolved in a small volume of methanol. The volume was completed with the same solvent to yield 1.0 mg mL^{-1} FMT standard solution.

A working solution that contains 100 μg mL^{-1} of FMT was obtained by diluting the stock solution with methanol. Later, several standard FMT solutions were prepared through serial dilution using the same solvent. The solutions were stable for a period of 2 weeks when stored in the refrigerator and away from light.

2.4 | General Assay Procedure

Aliquots of standard FMT solutions in 50–1000 ng mL^{-1} concentrations were pipetted into 10-mL volumetric flasks. Each flask was then filled to capacity with distilled water and well shaken. The intensity of fluorescence of the obtained solutions was monitored at 342 nm (e.g., at 223 nm). A blank was performed using the above procedure without using FMT solution.

2.5 | Procedure for Analyzing Commercial Tablets

The content of 20 Formohale 12-μg or Foradile 12-μg capsules was accurately weighed and then subjected to extraction with 70 mL of methanol in 100-mL calibrated flask by the help of sonication for 30 min. The flask was filled to 100 mL with methanol, and the mixture was filtered. The first part of the filtrate was discarded. An aliquot of the obtained filtrate was diluted

with methanol to get solution that had concentration within the linear range. The final solution was assayed according to the general assay procedure in five replicates. The nominal content of the tablet was calculated using the corresponding regression equation.

3 | Results and Discussion

The chemical structure of FMT has two phenyl rings, which have extensively conjugated with planner structure; thus, it should be very fluorescent. The first phenyl ring is attached to amide and hydroxyl groups, whereas the second one is attached to methoxy group. Both rings are attached to each other by an aliphatic bridge. Experimental findings show that the fluorescence is highly dependent on pH change, and the maximum values were observed in the alkaline solution. This indicates that the fluorescence of FMT is mainly attributed to the first phenyl ring, which is attached with a strong electron donating group, hydroxyl. Under basic conditions, the hydroxyl group attached to the first phenyl ring can be deprotonated, leading to the formation of a phenoxide ion. This ion can enhance the overall electron density in the system, thereby stabilizing the excited state and hence increasing fluorescence. In addition, deprotonation of the hydroxyl can also lead to an improvement in a more planar conformation, enhancing π–π stacking and raising the likelihood of fluorescence.

The aliphatic bridge possesses a secondary amino group and hydroxyl group between the phenyl ring and the secondary amine group. Theoretically, the presence of the amino group near the phenyl ring is expected to suppress the fluorescence of FMT in alkaline solution via photo-induced electron transfer (PET) phenomenon from the phenyl rings to the secondary amine. In acidic conditions, the secondary amine can get protonated, something that would reduce its ability to undergo PET leading to fluorescence enhancement. However, practical results indicated that fluorescence remains low in acidic media, and there is no fluorescence quenching at alkaline pH. This suggests that PET does not play a significant role in the fluorescence behavior. The presence of aliphatic hydroxyl group in between the amino group and the phenyl ring could efficiently hinder PET process. The hydroxyl group could also stabilize the ground or excited state of the molecule through hydrogen bonding or other interaction and thus definitely inhibits electron transfer.

The fluorescence intensity of FMT was monitored at 342 nm after being excited at 223-nm neutral or alkaline aqueous media (Figure 2). The stock shift here is 119 nm, which is high value. This high stock shift is advantageous, as it minimizes the interference that may be encounter from self-absorption or from the light source of excitation.

3.1 | Optimizing Experimental Variables

The effect of the various experimental variables that may influence the intensity of fluorescence of FMT has been studied to get the optimal values that could produce the highest response.

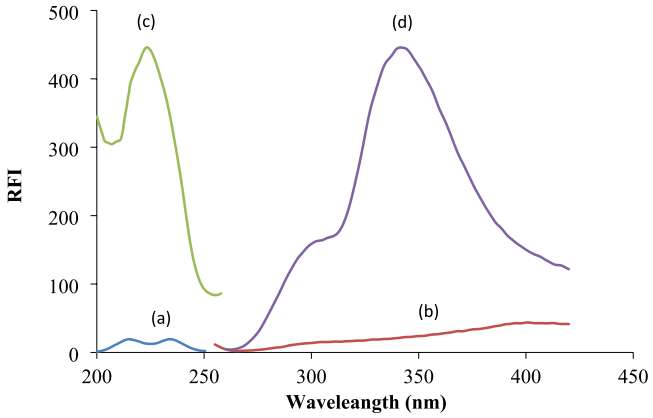

FIGURE 2 | Emission (d) and excitation (c) spectra of 500 ng mL^{-1} FMT and the excitation (a) and emission (b) spectra of blank in water.

3.1.1 | Effect of Buffers and pH Modifier

The intrinsic fluorescence of FMT was explored in a wide range of pH (2.0–12.0) using Teorell buffer solutions as shown in Figure 5. Fluorescence was very poor in the acidic media until pH 6.0. On further increasing the pH of the medium, the fluorescence was increased progressively. The intensity of fluorescence generally starts to increase at pH 6.0 and achieve its maximum value approximately at pH 10.0. The drug's fluorescence intensity at pH 10.0 is fivefold that at pH 4.0. The emission wavelength was not shifted significantly upon alteration of the pH. At low pH, the phenolic hydroxyl group (–OH) is protonated, and this can reduce the fluorescence intensity due to the fact that protonation stabilizes the ground state and can result in nonradiative decay. Protonation declines at higher pH, and the phenolic group becomes deprotonated. This kind of deprotonation can lead to the more fluorescent phenoxide ion to be exist. At alkaline pH 10.0 and higher, the intensity of fluorescence levels off, because maximum fluorescence is reached.

Based on the observed experimental data, the pH should be faintly alkaline or alkaline region. Hence, in order to simplify this approach, in the current study, fluorescence was investigated in distilled water in the absence of either buffer solutions or alkalis.

The experimental data for the effect of pH on the fluorescence intensity were supported by theoretical calculation using density functional theory (DFT). Gaussian 09W program was utilized for fully optimizing FMT and its deprotonated form using B3LYP/3-21G method. HOMO, LUMO, and their energy gap have also been calculated. The energy diagram for FMT and its deprotonated form is presented in Figure 3. The HOMO, LUMO, and gap energies were −4.60, −8.32, and 3.72 eV for FMT and −4.61, −7.85, and 3.24 eV for the deprotonated FMT. It is clear that the energy gap in the case of the deprotonated form is decreased compared to FMT itself.

3.1.2 | Effect of Organized Medium

A widely known approach to improve the fluorescence characteristic of fluorophore is the addition of different type of

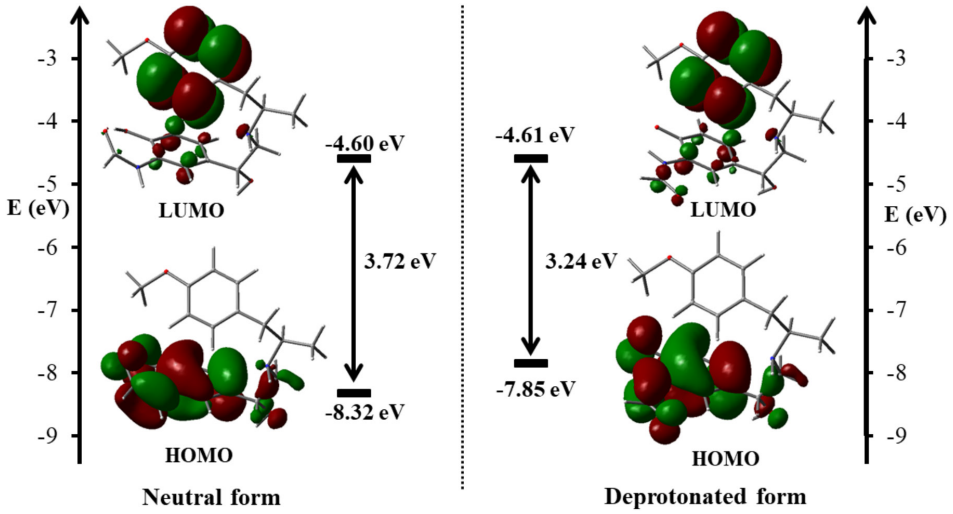

FIGURE 3 | Energy level diagram of the HOMO and LUMO for FMT and its deprotonated forms.

surfactants. Therefore, some organized media were used in the present work to increase the fluorescence of FM. The examined surfactants were SDS (1.0% w/v) as anionic type, CTAB (1.0 w/v) as cationic type, and tween 80 as nonionic type. Unfortunately, none of these reagents increased the fluorescence activity of FMT. The fluorescence intensity in case of CTAB (RFI = 409) was slightly lower than that without surfactant, whereas the value was reduced with SDS (RFI—223). However, tween 80 had a drastic effect on the fluorescence intensity (RFI = 23) of the drug. Hence, none of these reagents were used in this study.

3.1.3 | Effect of Diluting Solvents

The drug solution was diluted with various solvents to determine the most appropriate one. The solvents used were acetone, acetonitrile, ethanol, methanol, and water. The most suitable solvent was found to be distilled water because it gave the highest fluorescence intensity and was therefore employed in the general procedure. Water was also chosen as diluent in this study, as it is highly beneficial due to its low cost, eco-friendly, and easy to handle. Its efficiency as a solvent is accounted for by its ability to accept proton from the solute, which facilitates the formation of hydrogen bonds. The α scale of the solvent, which is the hydrogen-bond acceptor basicity, may be utilized to illustrate the tendency of the solvent to accept a proton from the solute in the solute-solvent hydrogen bonds. A correlation between the fluorescence intensity in the different solvents and their respective α scales gave a straight line (Figure 4) described by the equation: $RFI = 313.85\alpha + 44.338$, $r^2 = 0.8458$. A satisfactory relationship between the fluorescence and α scales was revealed from the high correlation coefficient (r^2).

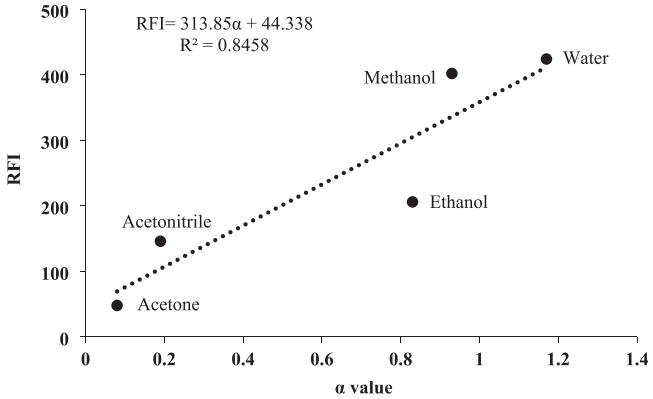

FIGURE 4 | Relationship between the value of α factor of the diluting solvent and the measured fluorescence intensity of FMT (500 ng mL^{-1}).

3.2 | Method Validation

The validation of the analytical method was done according to the guiding principles provided by the International Council for Harmonization, ICH [18]. The parameters involved in the validation include linearity, range, accuracy, precision, robustness, selectivity, limit of detection, and limit of quantitation.

3.2.1 | Linearity and Range

Linearity of the technique was evaluated by analyzing several standard solutions with different concentrations of FMT using the recommended analytical procedure. The observed intensity of fluorescence was correlated with the FMT concentration to construct the calibration curve. It was found that the linearity range of the presented fluorimetric approach is 50–1000 ng mL^{-1} with a high correlation coefficient (r) that indicated that the current method is highly linear. The data were also analyzed using linear regression, and the computed statistical and validation parameters for determining FMT are presented in Table 1.

3.2.2 | Quantitation (LOQ) and Detection (LOD) Limit

In the calculation of LOD and LOQ values, recommendations given by ICH were used, which are based on slope of calibration plot, s, and the intercept's standard deviation, σ. The limits have been calculated in order to constitute a criterion of the

TABLE 1 | Regression equation and validation parameters for the proposed spectrofluorimetric method.

Parameter	Value
Leaner range (ng mL^{-1})	50–1000
Slope (b)	0.956
Standard deviation of slope (s_b)	0.007
Intercept (a)	1.6756
Standard deviation of intercept (s_a)	3.33
Correlation coefficient (r)	0.9997
Determination coefficient (r^2)	0.9996
Number of determinations	5
Limit of detection (LOD, ng mL^{-1})	11.5
Limit of quantitation (LOQ, ng mL^{-1})	34.9

TABLE 3 | Effect of different pharmaceutical excipients on the determination of FMT using the proposed spectrofluorimetric method.

Substance added	Amount of excipient added (mg mL^{-1})	Drug taken μg mL^{-1}	% recovery ± SD[a]
Lactose monohydrate	1.0	0.5	100.22 ± 0.48
Magnesium stearate	1.0	0.5	99.31 ± 0.70
Gelatin	1.0	0.5	100.74 ± 0.88
Titanium dioxide	**1.0**	0.5	99.83 ± 0.54

Abbreviation: SD, standard deviation.
[a]Mean of five measurements.

TABLE 2 | Evaluation of accuracy and intraday and interday precisions for the FMT determination with the proposed method.

	Accuracy	Intraday precision	Interday precision
Drug concentration (ng mL^{-1})	% recovery ± SD[a]	% recovery ± RSD[a]	% recovery ± SD[a]
100	98.65 ± 1.20	101.78 ± 0.81	99.69 ± 1.87
400	99.80 ± 0.54	98.39 ± 1.38	99.44 ± 0.99
800	101.20 ± 0.58	101.00 ± 0.88	100.00 ± 1.37
200	98.40 ± 1.50	—	—
600	101.60 ± 0.58	—	—

Abbreviations: RSD, relative standard deviation; SD, standard deviation.
[a]Mean of three determinations.

sensitivity level for the developed approach. The utilized formulae were LOD = 3.3 σ/s and LOQ = 10 σ/s. The obtained LOD and LOQ values were 11.5 and 34.9 ng mL^{-1}, respectively. These values provided an indication of the high sensitivity of the present methodology for FMT assay.

3.2.3 | Accuracy

Accuracy was checked by analyzing various standard FMT solutions containing five different concentrations (100, 200, 400, 600, and 800 ng mL^{-1}). The procedure of the general assay for the indicated approach was carried out in triplicate, and the values of both the standard deviation and percent recoveries were calculated. It could be seen from Table 2 that the found %recovery values were near to 100% with low values of relative standard deviation (RSD %), which point out the enough accuracy of the developed approach.

3.2.4 | Precision

Two degrees of precision were examined: intraday and interday analysis. The general assay procedure was applied to analyze three standard FMT solutions with varying concentration (100, 400, and 800 ng mL^{-1}) at three different times during 1 day to examine intraday level of precision. The three aforementioned standard solutions were also subjected to analysis in three repeated days to assess the interday level. Each experiment was run three times, and the results were expressed as percentage recovery and relative standard deviation (RSD). The obtained values were presented in Table 2, which showed the high repeatability and reproducibility of the proposed method as indicated from low values of RSD.

3.2.5 | Selectivity

To assess the selectivity of the current method, the effects of various tablet additives used in the production of FMT pharmaceutical tablets were examined. The study involved lactose monohydrate, magnesium stearate, gelatin, and titanium dioxide. Each interfering substance was mixed individually with a fixed concentration of FMT. The outcomes of the evaluation were expressed as percentage recoveries and standard deviations. The results are summarized in Table 3, which indicate that these substances did not significantly affect the specificity of the proposed method. This lack of interference could be attributed to the absence of any fluorophoric moiety such phenolic groups in these tablet excipients.

3.2.6 | Robustness

As previously noted in the procedure of the general assay, it was evident that the procedure was very straightforward and that the only experimental variable that could be varied to assess the robustness of the procedure was the measurement wavelength. The percentage recovery obtained did not vary significantly with a slight change of ±2 nm in the excitation or emission wavelengths. Hence, there is the possibility of reaching the conclusion that the current method is extremely resilient.

3.3 | Applications of the Proposed Method

3.3.1 | Analysis of Pharmaceutical Formulations

Two commercially available pharmaceutical products were analyzed using the current spectrofluorimetric technique: Foradil 12-μg capsules for inhalation and Formohale 12-μg capsules for inhalation. The percentages found were 99.18 ± 1.72 and 100 ± 0.89, for the two products, respectively. The high recovery percentages demonstrated that there was no observable effect from the excipients of the tablet. Additionally, previously reported method was used to analyze the same dose formulations [5]. The outcomes of both procedures were statistically compared for precision and accuracy using the *t* and *F* tests. No detectable difference was found in the accuracy and precision between the reported method and the proposed method as the calculated values of the *F* and *t* tests were less than their tabular values at the 95% confidence level (Table 4). Therefore, the proposed method can be applied for the analysis of the drug content in commercial FMT dosage forms.

The analytical performance of the suggested approach was compared with other methods, such as spectrophotometry [5], spectrofluorimetry [6], and chromatography [7–17]. The findings indicate that the present method has a lower detection limit (11.5 ng mL^{-1}), which demonstrates that it is more sensitive than most reported techniques, which have deletion limits around 0.22 μg mL^{-1}. Although a spectrofluorimetric method [6] for FMT determination has been reported, the proposed method offers various advantages. It utilized water as a diluting solvent, which is considered green solvent and more environmentally friendly. In contrast, the previously published one used methanol as diluting solvent and β-cyclodextrin as fluorescence enhancer. Thus, it was more expensive and less environmentally friendly. Therefore, the proposed method is simpler, more cost effective, and greener. Besides, the proposed method is characterized by its speed of analysis, simplicity, and high degree of selectivity. Because no costly reagents or solvents were needed, it is inexpensive and does not require a lengthy sample treatment. Given these advantages, the newly developed method is highly appropriate for routine analysis in quality control units.

3.3.2 | Calculation of *pKa* Value for FMT

As mentioned under the effect of pH, the relative fluorescence intensity (RFI) was affected by the variation of the pH of the medium. The relationship has a sigmoid shape as shown in Figure 5, where the low values appeared in the acidic range and the highest values were observed in the alkaline range. Thus, the value of the *pKa* of the studied drug could be obtained through fitting the data to nonlinear symmetrical sigmoid curve's fitting according to the following equation:

$$RFI = d + \frac{a - d}{1 + \left(\frac{pH}{pk_a}\right)^b}.$$

In the previous equation, *a*, *b*, and *d* in addition to *pKa* are constants. The estimated value of *pKa* is 7.68 with a correlation coefficient (r^2) of 0.9936. The obtained value is in good agreement with the previously reported *pKa* value for FMT, which was 7.4 [19].

3.4 | Greenness Evaluation of the Proposed Technique

It is advisable to assess the available analytical techniques through specific tools to verify their greenness. The analytical

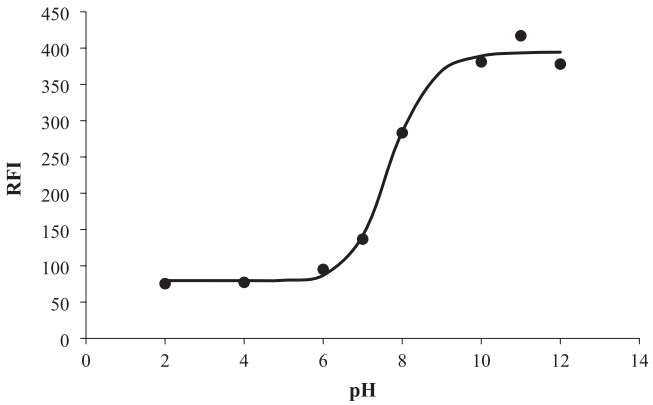

FIGURE 5 | Effect of different pH on the relative fluorescence intensity of 500 ng mL^{-1} FMT.

TABLE 4 | Analysis of FMT in its commercial tablets' dosage forms by proposed spectrofluorimetric and reported methods.

Dosage form	% recovery ± SD[a]		*t* value[b]	*F* value[b]
	Proposed method	Reported method		
Foradil 12 μg	99.18 ± 1.72	99.00 ± 0.75	0.57	1.50
Formohale 12 μg	100.00 ± 0. 89	99.65 ± 0.53	0.077	1.09

Abbreviation: SD, standard deviation.
[a]Mean of five measurements.
[b]Tabulated value at 95% confidence limit, $F = 4.384$ and $t = 2.179$.

TABLE 5 | Evaluation of the greenness of the proposed method using eco-score scale method.

Parameter		Penalty points
Reagents	None	0
Instrument	Spectrofluorometer	0
Solvent	Water	0
Energy consumption	Less than 0.1 kWh per sample	0
Occupational hazard	Analytical process hermitization	0
Waste	10 mL, no treatment	6
Total penalty points		6
Analytical eco-scale total score *a*		94

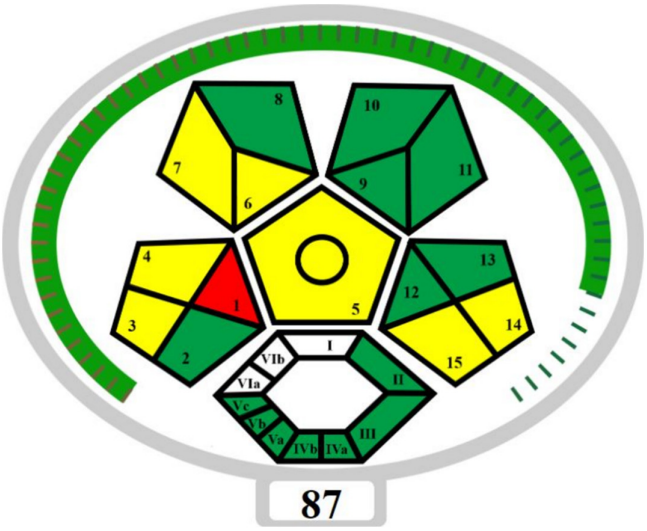

FIGURE 6 | Pictograms for the evaluation of the greenness of the proposed method using complex modified GAPI tools.

methodology was treated as completely green, as no hazardous solvents were used along with no derivatization methods and there was low energy consumption. The method also generates low amount of waste and requires low amounts of nontoxic materials. The modified National Environmental Technique Index (modified NEMI), eco-scale score, and ComplexMoGAPI tools were employed to appraise the environmental profile of the proposed method.

In the modified NEMI metric, five different aspects were evaluated. Health risks, safety hazards, environmental hazards, energy, and waste are the five sections of this qualitative system [20]. Each segment could be either red, yellow, or green, depending on how green it is. Water was used as a solvent in the developed method, and the waste volume did not exceed 50 mg or mL, The generated pictogram according to modified NEMI showed that the proposed method meets all its criteria to be classified as a green method. Because of these aspects, the newly developed spectrofluorimetric method is considered green.

The eco-scale score: This environmental scale score is calculated using the following formula, which adds up the penalty points for each aspect of the examined procedure: generation of waste, energy consumption, reagent consumption, and hazards in the workplace (score = 100 minus the total penalty pints) [21]. An analytical process is deemed to be superior green if its score is above 75. As presented in Table 5, the developed spectrofluorimetric procedures showed an eco-scale score of 94, proving its great environmental friendliness.

GAPI is among the tools that fit the green evaluation trend, using five colored pentagons to analyze the environmental impact of the analysis process at different levels. A presentation of the ComplexGAPI tool was constructed by incorporating additional information about operations that were undertaken before the procedure in question in an effort to advance GAPI. Due to the absence of an overall rating system for individual methods in ComplexGAPI, process comparison is more difficult. This led to the development of the ComplexMoGAPI tool [22], which combines accurate overall ratings with the visual presentation of ComplexGAPI. A supporting program makes usage easy to allow for quicker and easier evaluations which could be freely downloaded online (at bit.ly/ComplexMoGAPI). As can be observed in Figure 6, the tool had three color codes: green, yellow, and red, apart from a numerical overall score. With nine being marked in green, three yellow, and three red, thus, formed pentagrams showed that the current system achieves a brilliant green status, with an overall score of 87.

4 | Conclusion

A spectrofluorimetric method was established for FMT determination. The optimization of experimental conditions has led to enhanced fluorescence intensity, with distilled water identified as the ideal solvent. Validation results confirm the method's high accuracy, precision, and robustness, whereas application to commercial formulations yielded excellent recovery percentages without interference from excipients. Furthermore, the method's eco-friendly profile aligns with contemporary analytical demands, underscoring its potential as a sustainable alternative to traditional techniques. The approach demonstrates remarkable advantages in terms of sensitivity, accuracy, and simplicity, making it suitable for routine quality control in pharmaceutical laboratories. The findings advocate for the broader application of this method in pharmaceutical analysis, contributing to enhanced drug quality assurance.

Author Contributions

Sayed M. Derayea: supervision, conceptualization, writing – review and editing. **Dalia M. Nagy:** data curation, validation. **Sara I Badry:** methodology, investigation, formal analysis, writing – original draft. **Mohamed Oraby:** validation, visualization.

Acknowledgments

The authors have nothing to report.

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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