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Evaluation of the on–off fluorescence method for facile measurement of vilazodone in pharmaceutical dosage form; Application to content uniformity testing and greenness evaluation

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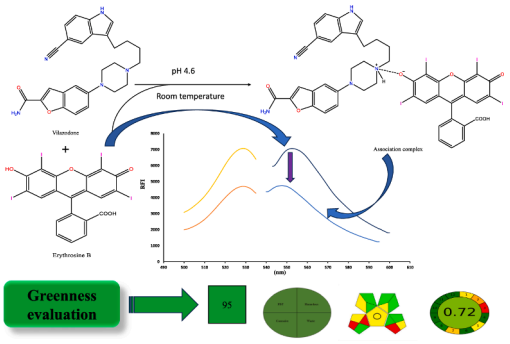
HIGHLIGHTS

- Herrin, the first method for determination of Vilazodone was reported based on quenching mechanism.
- The procedure does not include prolonged stages for sample preparation, extraction or chemical derivatization.
- The suggested method has been effectively applied to confirm the content uniformity of medication tablets.
- Furthermore, the greenness of the procedure was evaluated with metric tools.

GRAPHICAL ABSTRACT



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ABSTRACT

Vilazodone is a recently approved antidepressant medicine used for treating major depressive disorder. A simple, extremely sensitive, accurate and green spectrofluorimetric method was constructed for its determination through formation of ion-pair complex with erythrosine B. The formation of ion-pair complex lowers the dye's native fluorescence emission measured at 552 nm (λ ex = 530 nm). In terms of analysis, the system's parameters for producing the vilazodone-erythrosine B complex have been optimized. The reaction was carried out in Teorell-Stenhagen buffering solution (pH 4.6). The fluorescence emission intensity of the dye decreased linearly in the range of 20 – 600 ng mL^{-1} and the correlation coefficient was 0.9999. The quantitation and detection limit values were 18.5 and 6.1 ng mL^{-1}, respectively. The proposed strategy has been validated according to the ICH criteria. The proposed technique was thoroughly employed for evaluating vilazodone in raw material and pharmaceutical tablet dosage form. Furthermore, it was also successfully used for content uniformity testing. Lastly, using four advanced tools namely the Eco-Scale, the National Environmental Method Index (NEMI), the Green Analytical Procedure Index (GAPI), and the Analytical Greenness metric approach (AGREE), the greenness of the established technique was evaluated.

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1. Introduction

Vilazodone HCl (VZN, Fig. 1) is known chemically as 5-(4-[4-(5-cyano-1H-indol-3-yl)butyl] piperazin-1-yl) benzofuran-2-carboxamide hydrochloride [1]. It represents the inaugural member of a novel category of antidepressants that merge the potency of the selective inhibitors for serotonin reuptake (SSRI) with partial agonist activity at the 5-HT_{1A} receptor. It affects several subtypes of 5-HT (serotonin) receptors [2]. Vilazodone Hydrochloride readily dissolves in methanol and has a pKa of 7.1 [3]. The Food and Drug Administration (FDA) approved it on January 21, 2011, as a therapeutic agent for major depressive disorder (MDD) in adult. Vilazodone functions in the same way as the drugs which selectively inhibit the reuptake of serotonin (SSRIs) by blocking the transporter of serotonin, desensitizing serotonin receptors, and finally increasing serotonergic neurotransmission [4]. Vilazodone's absolute bioavailability in fed circumstances was reported to be 72 %. Additionally, it was shown that eating boosts the drug's bioavailability, hence taking it with food would be beneficial [5]. Vilazodone has a very large volume of distribution and has a high protein binding percentage (96–99 %). It is heavily metabolized in the liver, and only 1 % to 2 % of the administered dose is retrieved in unaltered form in the feces and urine [6]. Vilazodone is strongly metabolized by CYP450 3A4 in the liver. When using strong 3A4 inhibitors concurrently, the dosage of Vilazodone should be decreased. Vilazodone's parent medication is mostly responsible for its therapeutic effects, and no active metabolites are known to exist whatsoever [7]. A review of the literature reveals that VZN has been determined in pharmaceutical and biological matrices via few analytical techniques which involve spectrophotometry [2,3,8,9], spectrofluorimetry [10,11], HPTLC [9,12], HPLC [1,10,13,14], LC/MS [4], and UPLC [5,6] methods. It is essential to point out that HPLC techniques demand the use of plenty of highly purified organic solvents, laborious sample preparation procedures, sophisticated apparatus and expensive detectors. Furthermore, they are considered tedious and highly time-consuming techniques. In addition, the poor sensitivity of spectrophotometric methods was a significant disadvantage. In contrast, spectrofluorimetric procedures utilize a simple instrument, require facile sample preparation, and are highly sensitive as well as highly selective. However, the published spectrofluorimetric methods for VZN analysis had low sensitivity. Therefore, the work should be conducted to design a spectrofluorimetric method that is direct, rapid, simple, accurate and sensitive for evaluating the antidepressant medication, vilazodone. The spectrofluorimetric determination can achieve these objectives by reacting the drug under study with self-fluorescent dyes in a mildly acidic aqueous solution.

Donor-acceptor reaction including charge transfer and ion pair complex formation is an effective approach for the analysis of pharmaceuticals [15 16 17]. Erythrosine B (Fig. 1) is an ion pairing reagent and a food colorant. It is the disodium salt of 2,4,5,7-tetraiodofluorescein, that can also be employed as a fluorogenic reagent [18]. Thanks to its basic moiety (amino group), VZN could react with erythrosine B to form an ion pair complex. The amino group is protonated in an acid media resulting in a cationic molecule that is appropriate for interacting with the negatively charged dye component [19]. The pH of the reaction media was adjusted using Teorell-Stenhagen buffer to enable the complex development. Because the generated complex can quench the inherent fluorescence of erythrosine B, an appropriate and sensitive spectrofluorimetric method can be designed depending on this character. The formed ion pair was easily dissolved in water, allowing direct measurements without the need of extraction with potentially hazardous organic solvents. The suggested procedure offered a very simple and extremely precise way to quantify the drug in both pure and pharmaceutical forms. An inexpensive reagent, an easy-to-use, inexpensive instrument that is commonly available in most quality assurance units are additional advantages of the suggested procedure. Analyzing pharmaceutical formulations using this strategy yielded satisfactory results. The method's analytical performance, including linearity, precision, accuracy, and limits of detection and quantitation, will be thoroughly evaluated and verified in accordance with the regulatory guidelines.

The novelty of the current work comes from the fact that it is the first spectrofluorimetric approach for assay of VZN exploiting the quenching effect of the drug on the native fluorescence of a dye. The innovation also involves the use of simple extraction process, since it utilizes a

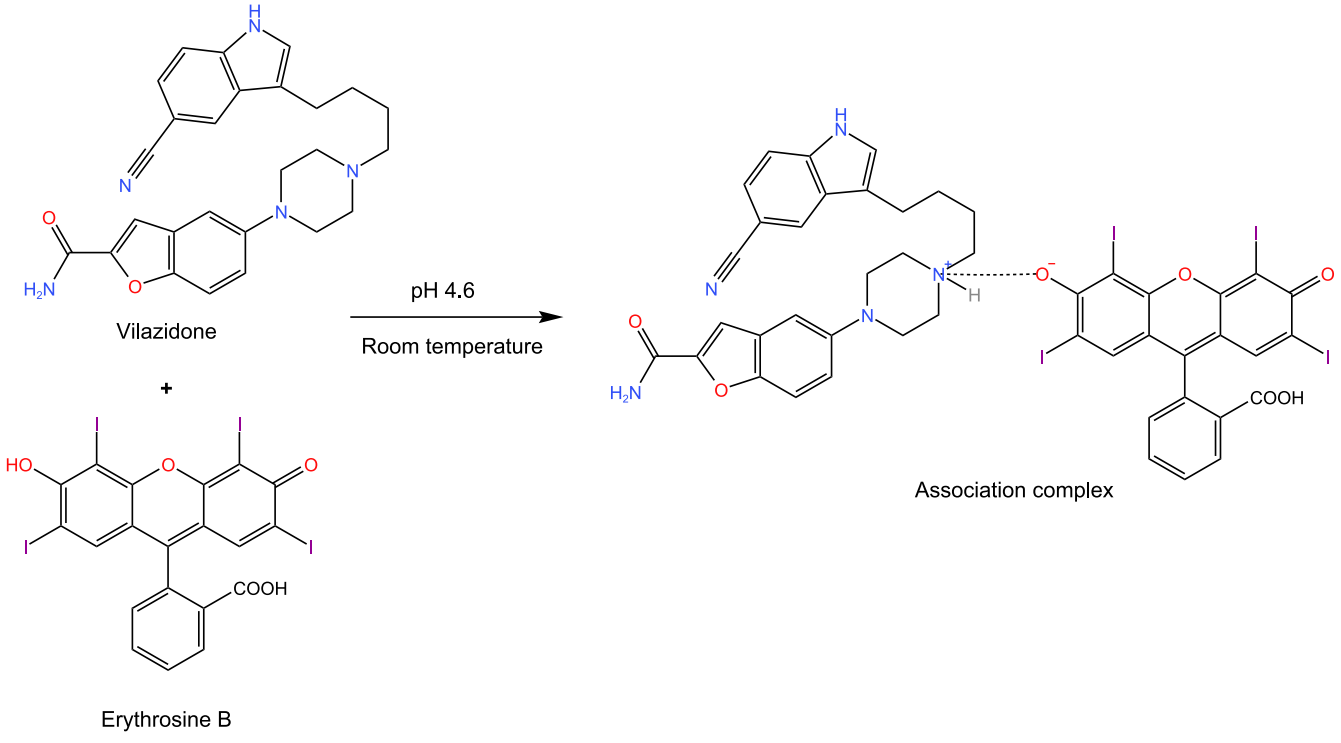

Fig. 1. Chemical structure of VZN, erythrosine B and their association complex.

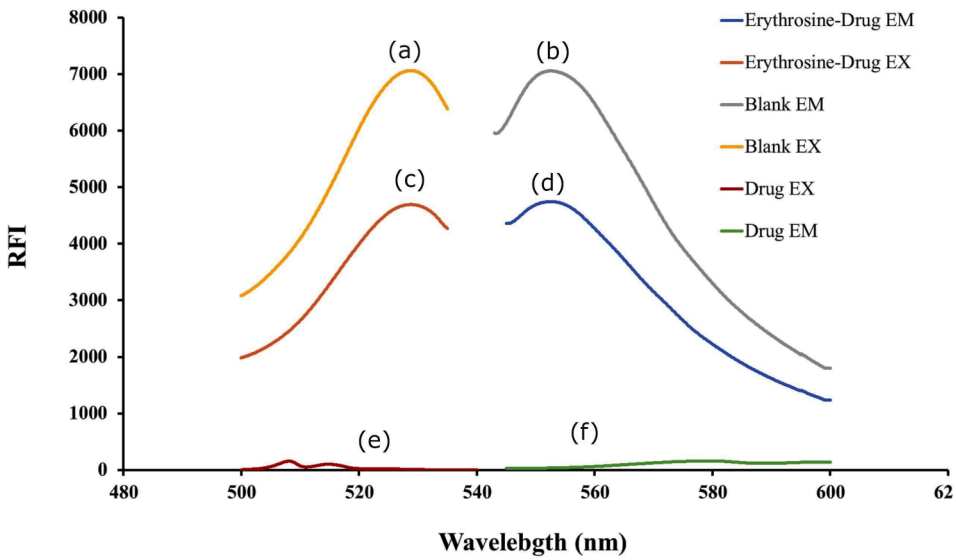

Fig. 2. Excitation (a) and emission (b) spectra of erythrosine B ($5.675\ 10^{-5}$ M) and the excitation (c) and emission (d) spectra of its binary complex with VZN (400 ng mL^{-1}) while (e) is the excitation and (f) is emission spectra of VZN (400 ng mL^{-1}).

single, straightforward procedure for sample preparation rather than using a number of complicated steps. The suggested method has been effectively applied to verify the content uniformity of medication tablet formulations because of its simple manipulation process. Moreover, the process is highly sustainable and in line with the principles of green chemistry because the reagent utilized is an ingredient in foods and water serves as the diluting solvent for the reaction. Four contemporary metrics were used to assess the proposed strategy in order to emphasize the produced approach's sustainable characteristics and greenness.

2. Experimental

2.1. Apparatus

The spectrofluorimetric measurements were obtained using a JASCO FP-8350 spectrofluorometer. A medium-sensitivity PMT voltage and a 150 W Xe-arc lamp were included in the instrument. The slit width was 5 nm for the emission and excitation monochromators, and a scan rate of 1000 nm min^{-1} was used.

2.2. Chemicals and Reagents

Vilazodone was provided by RAMEDA Co. for Pharmaceutical Industries & Diagnostic Reagents, 6th of October City, Egypt. Vilaphoria® tablets containing 20 mg Vilazodone hydrochloride per tablet, a product of RAMEDA (6th of October City, Egypt), was purchased from local market. Erythrosine B (MP Biomedicals LLC, Illkirch, France) solution with a concentration of $5.675\ 10^{-5}$ M was generated by dissolving 25 mg in 500 ml of double-distilled water. Teorell-Stenhagen buffer solutions have been produced by combining 0.1 M of citric acid, 0.1 M of sodium hydroxide, and 0.1 M of phosphoric acid in various ratios. A standard VZN solution was prepared by placing 10 mg of VZN in a 100 mL volumetric flask, dissolving it in an appropriate amount of methanol, and then completing to the maximum capacity with the same solvent. By mixing a suitable volume of the stock solution with methanol, a working standard solution was produced. To enhance the chemical stability, the solutions were stored in the fridge.

2.3. General assay procedure

Various quantities ranging from 20 to 600 ng/mL from the VZN standard solution were transferred to 10 mL volumetric flask series, followed by adding 0.5 mL of 0.1 M Teorell-Stenhagen buffer (pH = 4.6), and 0.8 mL of erythrosine B solution. Then the volume was finalized with distilled water. After excitation at 530 nm, the produced solution's emission was measured at 552 nm. The differences in relative fluorescence intensity (ΔRFI = Erythrosine B solution − binary mixture of erythrosine B and VZN solution) were calculated and plotted versus VZN concentrations.

2.4. Procedure for VZN assay in dosage form

Ten Vilaphoria 20 mg tablets were carefully grounded in a mortar to very fine powder. A quantity of the tablet powder containing 20 mg of VZN was dissolved in a volumetric flask with 50 mL methanol by sonication for 30 min. Whatman grade No. 1 filter paper was then used to filter the finished solution discarding the first part. An appropriate volume of the filtrate was diluted quantitatively to produce working solution with the suitable concentration. Five estimations of the same VZN concentration were performed using the general procedure. The content of VZN in the tablets was calculated using the relevant linear regression equation.

2.5. Procedure for content uniformity test

The test was conducted in accordance with USP regulations (Chap. 905) to determine the content uniformity (CU) for VZN in tablet formulations [20]. The preceding procedures listed under "Procedure for **VZN** assay in dosage form " were applied to each tablet to determine whether the contents of ten units of Vilaphoria® 20 mg tablets were homogeneous.

3. Results and discussion

3.1. The system spectra

Spectrofluorometric determination of compounds having amino groups is possible through the formation of binary complexes with the acidic dye, erythrosine B. At pH = 4.6, in the presence of Teorell-Stenhagen buffer, electrostatic interaction between the vilazodone protonated amino group and erythrosine B anion resulted in the formation of the complex. The interaction would alter the conjugated system of erythrosine B and distribute its π electron cloud. This would affect the absorption spectra of the dye and quench its fluorescence [19].

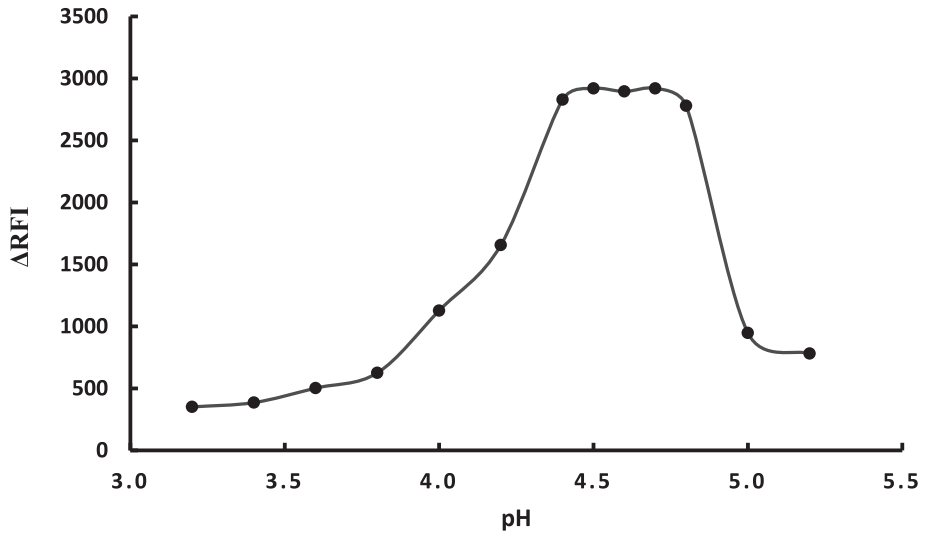

Fig. 3. Effect of pH on the RFI of the association complex formed between VZN (500 ng mL^{-1}) and erythrosine B.

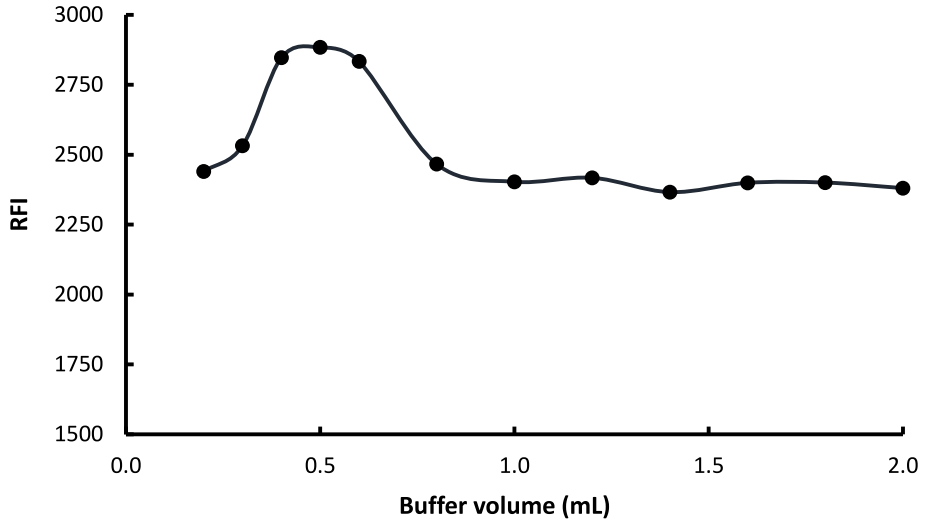

Fig. 4. Effect of buffer volume on the RFI of the association complex formed between VZN (500 ng mL^{-1}) and erythrosine B.

As seen in Fig. 2, there is a reduction in the intensity of erythrosine's native fluorescence measured at 552 nm after excitation at 530 nm. There was a linear correlation between the concentration of drug and the degree to which the relative fluorescence intensity (RFI) of the dye decreased. The procedure developed was the first to yield a highly sensitive and accurate spectrofluorimetric determination of vilazodone.

3.2. Method optimization

To achieve the most favorable outcomes for the suggested spectrofluorimetric approach, the parameters of the reaction that were possibly anticipated to have an effect on the system's reaction (the quenching of the emission intensity of erythrosine B reagent) were investigated and optimized.

3.2.1. Effect of pH

The pH of the medium was studied as a critical variable that must be adequately adjusted to reach an optimal value suitable for ion pair association between VZN and erythrosine B. Teorell-Stenhagen buffer with pH varied from 3.2 to 5.2 was evaluated. The pH 4.6 was the appropriate value since the highest fluorescence quenching values were attained in the pH range of 4.4 – 4.8, as shown in Fig. 3. At pH (4.6), the drug is fully protonated to give its corresponding cation ($HVZN^{+}$). In addition, the dye will be in the monovalent anionic form (HL^{-}). Thus, both the drug cation and the dye anion interact by the action of hydrophobic force and electrostatic attraction forming ion pair association complex.

3.2.2. Effect of the volume of buffer

Teorell-Stenhagen buffer solution (pH 4.6) was used, and the impact of buffer solution volume was examined over a range of 0.2–2.0 mL. The fluorescence quenching values increased regularly with increasing buffer volumes. The optimal quenching effects were noticed when 0.4–0.6 mL of the buffer was utilized. Low readings have been recorded with smaller volumes because the pH of the medium could not be adequately adjusted. Larger buffer volumes also reduced fluorescence quenching because phosphate anion start to compete with erythrosine B for binding with the drug cation. For the current investigation, 0.5 mL of Teorell-Stenhagen buffer solution was recommended as an optimum volume (Fig. 4).

3.2.3. Effect of the erythrosine B volume

To determine the optimal volume of the reagent, several volumes of erythrosine B were tested while the fluorescence quenching was calculated. The produced quenching value was increased linearly as the

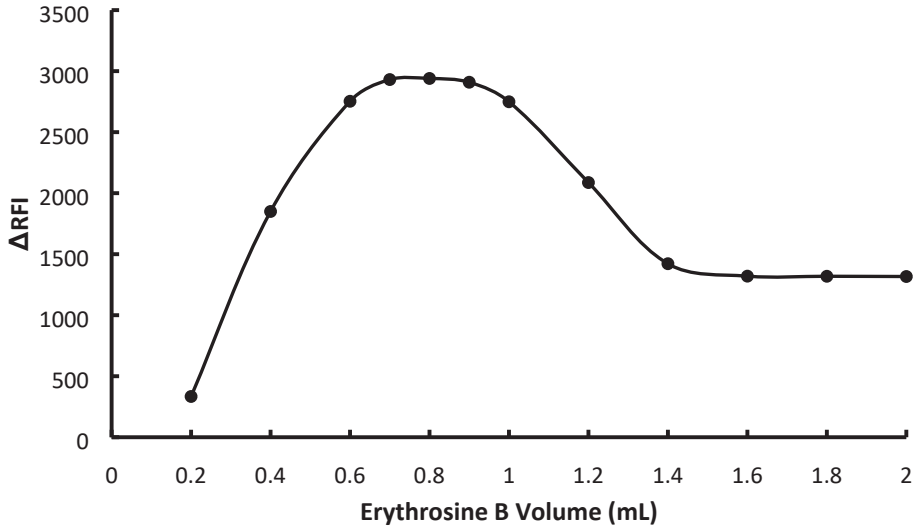

Fig. 5. Effect of erythrosine B volume (5.675 10^{-5} M) on the RFI of the association complex formed with VZN (500 ng mL^{-1}).

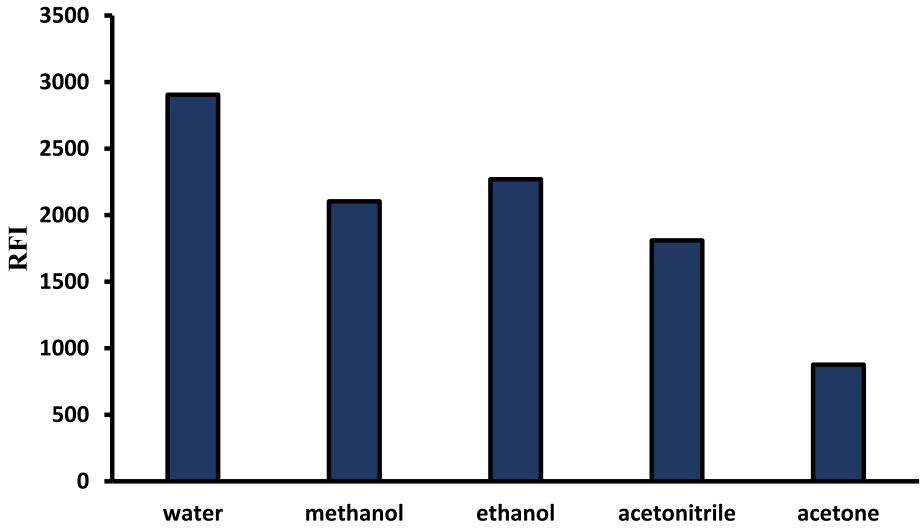

Fig. 6. Effect of diluting solvent on the RFI of the association complex formed between VZN (500 ng mL^{-1}) and erythrosine B.

erythrosine B volume was raised up to 0.6 mL. Then no significant difference was detected until 1.0 mL. After that, a decrease in the quenching was observed with further increase in the dye volume. As indicated in (Fig. 5), a volume of 0.8 mL of erythrosine B was chosen to be the best and was used in the spectrofluorometric approach. The low results upon using volumes of the dye less than 0.6 ml are due to the insufficient dye for the reaction. On the other hand, the use of volume higher than 1.0 mL also reduce the resultowing to self-quenching of between the dye molecules.

3.2.4. *Effect of diluting solvents*

Several solvents were tested as diluting solvent for the reaction product including water, methanol, ethanol, acetonitrile and acetone. It was observed that water was the best diluting solvent. Fig. 6 illustrates that, the remaining solvents yielded less favorable results. Due to its affordability, accessibility, and ecologic benefits, water considered the ideal solvent.

3.3. *Stoichiometry of the reaction*

The ratio between EB and VZN in the formed complex has been estimated by using Job's continuous variation method. A series of solutions with different molar ratios of EB and the drug were prepared and their fluorescence intensities were measured. In all solutions the total moles of both EB and the drug were kept constant. The obtained intensity of fluorescence for each mixture was subtracted from the fluorescence intensity of the solutions that contain a similar quantity of the EB alone under the same conditions. Job's plot was obtained by plotting the obtained difference against the corresponding mole fraction of the drug in the mixtures. The obtained curve showed a maximum at a point, which indicates the molar ratio of EB to drug in the complex. The method revealed a 1:1 ratio for EB**:** VZN complex (Suppl. 1).

3.4. *The suggested reaction mechanism*

Erythrosine B (H_2L) is an acidic dye with two pka values (3.9 and 5.0) [21]. Consequently, at pH 4.6, the most predominant species of erythrosine B was the monovalent anionic form (HL^-). Despite the fact that two groups in erythrosine B could be ionized, the hydroxyl dissociation was more predominant than the carboxyl dissociation. The xanthene nucleus had a powerful electron withdrawing group (iodine atoms) nearby the hydroxyl moiety, leading to reduction of the electron

density of oxygen of the hydroxyl group, making it much simpler to dissociate than the carboxylic group [22,23]. The charge distribution and the geometry for the system was fully optimized by quantum chemistry calculation using the B3LYP functional with 6-31G. The calculation results of AM1 quantum chemistry method show that the enthalpy changes for the ionization of hydroxyl on xanthene group and carboxylic group attached to the phenyl moiety are −285.8 and −165.5 kJ/mol, respectively. Thus, ionization of the hydroxyl could liberate higher quantity of energy (120.3 kJ/mol) than that of the carboxylic group. Thus, higher stability of the system could be achieved upon hydroxyl dissociation [24].

Although VZV contains five nitrogen functionalities, only the two nitrogen atoms in the piperazine ring exhibit basic properties. The electron lone pairs are not available in the remaining nitrogen atoms due to either involvement in sp orbitals (cyano), incorporation into an aromatic ring (indole), or delocalization with oxygen through resonance (amide). The lone pair of the nitrogen atom (N_1) attached to the benzofuran ring is significantly influenced by the aromatic system. Consequently, the other nitrogen atom in the piperazine group (N_2), which is connected to the aliphatic butyl chain, is more basic and is more easily protonated to combine with H^+ giving the quaternary ammonium cationic ($VZNH^+$).

In weakly acidic aqueous solutions (pH 4.6), the hydroxyl group of the EB molecule ionizes, forming a monovalent anion (HL^-). Similarly, under this acidic condition the tertiary amino group of the drug can easily be protonated, giving the drug cation. Consequently, the ionized drug cation ($VZNH^+$) interacts with the dye anion (HL^-) through electrostatic attraction and strong hydrophobic interactions, to give an ion pair associate in the ratio of 1:1, as depicted in Fig. 1.

3.5. Investigating the mechanism of quenching

The intrinsic fluorescence of EB may be quenched by a variety of mechanisms as a result of complex formation. These mechanisms include excited state reactions, collisional quenching, and energy transfer. Fluorescence quenching may be caused by the fluorophore's characteristics, conjugation mode, and surrounding environment. As a result, the quenching process was studied using the Stern–Volmer equation [25].

$$F_o/F = 1 + K_{SV}[Q]$$

where [Q] is the quencher (VZN) concentration in moles, K_{SV} is the Stern-Volmer constant, and F and F_o are the relative fluorescence intensities of EB in the presence and absence of the quencher, respectively. The Stern-Volmer plot (Suppl. 2), which is a straight line, is produced when the ratio F_o / F is plotted against the drug concentration (quencher). The graph had a slope of K_{SV} (0.001193) and an intercept of around 1 (1.0161). Multiplication of the natural radiation lifetime (the lifespan in the absence of a quencher), τ0, by the quenching rate constant, kq, gives the Stern-Volmer constant ($K_{SV} = K_q\ \tau_0$). The latter formula allows the rate constant of quenching to be calculated in a simple manner. According to reports, erythrosine B has a fluorescence lifetime of 89 picoseconds [26]. The anticipated quenching rate constant (K_q), based on the previously provided data, is 6.41×10^{15} L mol^{-1} S^{-1}. Note that 2×10^{10} L mol^{-1} S^{-1} is the maximum known value of Kq for collisional quenching [27]. The K_q values obtained is significantly higher than this number. As a result, it that fluorescence quenching of erythrosine B upon paring with VZN is believed to be static quenching process.

3.6. Rate constant and free energy of the reaction

The following equation was used to estimate the number of binding sites (n) for the medication mentioned as well as the reaction's rate constant (*Ka*) [28].

Table 1
Regression equation and validation parameters for the proposed spectrofluorimetric method. LOD is limit of detection and LOQ is limit of quantitation.

Parameters	Vilazodone
Linear range (ng mL^{-1})	20–600
Slope	5.16
Standard deviation of slope (S_b)	0.03
Intercept	306.79
Standard deviation of intercept (S_a)	9.52
Correlation coefficient (r)	0.9999
Determination coefficient (r^2)	0.9998
Number of determinations	5
LOD (ng mL^{-1})	6.1
LOQ (ng mL^{-1})	18.5

$$Log((Fo - F)/F) = \log ka + n\log[D]$$

where [D] represent the total molar concentration of the drug. The estimated number of binding sites (n) was about one (1.24), indicating that the dye and medication had a molar ratio of 1:1. In addition to the Job's approach for continuous variation, this provides further prove of the ratio. Using the values of the slope and the intercept, the rate constants could be driven and found to be 2.18×10^7.

Using the formula, $\Delta G^\circ = -RT \ln K_a$, Gibb's free energy ($\Delta G^\circ$) for the reaction was computed using the previously estimated value of the rate constant. R is the universal gas constant (8.314 J K^{-1} mol^{-1}) and T is the absolute temperature in Kelvin. The found values for ΔG° was –41.9 KJ mol^{-1}. The strong reaction spontaneity between the mentioned medication and the dye at room temperature is indicated by the negatively large values of the Gibbs free energy changes.

3.7. Validation of the developed method

Once every variable was optimized, the developed approach was validated as per the International Council on Harmonization (ICH) requirements [29]. The linearity and range, detection and quantitation limits, precision, accuracy, and robustness are all significant features.

3.7.1. Linearity and range

The suggested approach was used to determine a number of standard drug solutions with varying concentrations under the prescribed experimental conditions. The relative fluorescence quenching values (ΔRFI) have been calculated and correlated with the medication concentrations. A linear relationship was found between (ΔRFI) and the drug's concentration in the range of 20–600 ng mL^{-1}. Table 1 provides a summary of the various analytical parameters. The closeness of the obtained correlation coefficient to the unit (0.9999) confirmed the method's excellent linearity. The linear regression equation was ΔRFI = 5.16C + 306.79, where C is the final drug concentration in ng mL^{-1}.

3.7.2. Limits of quantitation and detection

The sensitivity of the devised approach was assessed by calculation of the quantitation and detection limits. The limits were calculated statistically based on the slope (S) and intercept standard deviation (σ) of the calibration curve. The formula limit = n σ /S was utilized to compute the limit, where n corresponded to 10 for quantitation limits and 3.3 for detection limits. The obtained LOD value for VZN was 6.1 and LOQ was 18.5 ng mL^{-1}. These numbers demonstrate the great degree of sensitivity of the recommended approach.

3.7.3. Accuracy

In order to assess the accuracy of the present approach, three replicates analysis using the standard methodology were used to determine five concentrations of VZN (50, 100, 200, 400 and 600 ng mL^{-1}). Percentages of recovery and standard deviation are displayed for the results

Table 2
Evaluation of accuracy of the analytical procedure for the determination of VZN.

Amount taken (ng mL^{-1})	Amount found (ng mL^{-1})	% Recovery ± SD*
50	50.89	101.78 ± 1.94
100	100.56	100.56 ± 1.35
200	199.22	99.61 ± 0.97
400	401.08	100.27 ± 1.77
600	595.74	99.29 ± 0.68

* Mean of three determinations, SD, standard deviation.

Table 3
Evaluation of intra-day and inter-day precisions of the proposed method for VZN determination.

Conc. (ng mL^{-1})	Intra-day precision % recovery ± SD*	Inter-day precision % recovery ± SD*
50	101.78 ± 1.94	101.53 ± 1.85
200	99.61 ± 0.97	99.29 ± 1.22
600	99.29 ± 0.68	100.38 ± 1.15

* Mean of three determinations, SD, standard deviation.

Table 4
Robustness study of the proposed method for determination of VZN (200 ng mL^{-1}) in pure form.

Parameter		% recovery ± SD*
pH of solution	4.5	100.32 ± 1.14
	4.7	99.64 ± 0.79
Volume of erythrosine B (ml)	0.7	98.38 ± 1.55
	0.9	99.51 ± 1.74
Volume of buffer (mL)	0.4	100.28 ± 0.98
	0.6	99.52 ± 1.32

* Mean of three determinations, SD, standard deviation

in Table 2. The recovery percentage was very close to 100 %, and the standard deviation was low suggesting that the accuracy of the suggested method is reasonable.

3.7.4. Precision

Two forms of precision; intra- and inter-day precision, were examined for evaluating the reliability of the suggested strategy. For intra-day precision, three replicates of three standard solutions of the medication at various concentration levels covering the whole linear range of the method (low, medium and high) were analyzed during the same day. The replicate analysis was conducted in three distinct days in sequence to assess the inter-day precision. The relative standard deviation values were remarkably low (less than 2.0 %) suggesting that the method's precision was adequate at both repeatability and reproducibility levels, (Table 3).

3.7.5. Robustness

The impact of slight alteration in the analysis's experimental conditions on the fluorescence quenching was examined in order to assess the robustness of the method. Results were expressed as a percentage of recovery and a standard deviation after changing three independent parameters (Table 4). Solution pH, buffer volume and erythrosine B volume were the factors that were examined. Since the standard deviations were all less than 2 %, it was concluded that none of the parameters under study had a substantial effect on the procedure's analytical result for the established method. This indicates the suggested approach's consistency when used regularly. As a result, the developed spectrofluorimetric method is thought to be reliable and robust.

Table 5
Analysis of VZN in dosage form by reported and proposed method.

Dosage form	Proposed method % recovery ± SD[a]	Reported method % recovery ± SD[a]	*t*-test[b] value	F-test[b] value
Vilaphoria 20 mg	99.46 ± 1.01	99.59 ± 1.22	0.18	1.45

[a] Mean of five measurements.
[b] Tabulated value at 95 % confidence limit, F = 6.388 and t = 2.306.

Table 6
Application of the proposed method for the content uniformity test of Vilaphoria tablets.

Tablet number	% Recovery
1	101.65
2	98.16
3	103.59
4	101.94
5	99.52
6	103.88
7	100.00
8	101.75
9	97.48
10	100.68
Mean % Recovery	100.87
S (standard deviation)	2.12
AV*	5.76
L1*	15

* L1: maximum allowed acceptance value, AV: acceptance value.

3.8. Application

3.8.1. Application to pharmaceutical preparations

The established analytical procedure was utilized for the analysis of a commercially available medication formulation comprising the investigated medicine after it had been properly optimized and validated. Concurrently, the same dosage form was analyzed using another reported method [2]. To assess technique accuracy and precision, the results of both approaches have been compared statistically employing student's t- and variance f- tests (Table 5). The estimated results of both tests were not significantly greater than the tabulated values, indicating that there was no significant difference in accuracy and precision between the two approaches. The proposed approach is sensitive, precise, and accurate. As a result, it might be used in the quality control laboratory to test the aforementioned medication in their pharmaceutical formulations.

3.8.2. Assessing the uniformity of the content

When the drug content in the tablet dosage form as a whole is less than 25 mg or when its proportion to the other constituents of the tablet is less than 25 %, it is advisable to evaluate the drug uniformity within the tablets. To guarantee uniformity in the composition of the tablet in accordance with USP requirements [20]. Ten units of the marketed Vilaphoria® 20 mg tablets formulation were examined individually using the current methodology. Due to its simplicity, the proposed approach could be easily used to examine whether the tablets' content was uniform. In accordance with the guidelines of the US Pharmacopoeia, the acceptability value can be calculated using the formula: $AV=|M-\overline{X}|+KS$, where M is a reference value, K is the acceptability constant (equal to 2.4 in the case of 10 tablets), $\overline{X}$ is the mean of the % recovery of individual contents and S is the standard deviation. The AV should be lower than the maximum allowed AV (L1 = 15). The above equation would be modified according to the value of $\overline{X}$.

Table 7
Evaluation of the greenness of the proposed method using Eco score scale method.

Item	Parameter	Word sign	PPT score
Technique	Fluorimetry		0
Reagent	Erythrosine B	LSH	1
Amount of reagent	< 10 ml		1
Solvent(s)	Water	Green solvent	0
Heating	—		0
Temperature	25 °C		0
Cooling	—		0
pH	4.6		0
Energy (kWh per sample)	< 1.0		0
Waste	1–10 (mL)		3
Occupational hazards	(Analytical process hermitization)		0
Total penalty points			5
analytical eco-scale total score [a]			95

[a] If the score is greater than 75, it represents excellent green analysis. If the score is greater than 50, it represents acceptable green analysis. If the score is less than 50, it represents inadequate green analysis. LSH is an abbreviation for less severe hazard.

$$\text{If } 98.5\% \leqslant \overline{X} \leqslant 101.5\% \text{ , then M } = \text{ , then M} = \overline{X}(\text{AV } = \text{ KS})$$

$$\text{If } \overline{X} < 98.5\% \text{ , then M } = 98.5\%, \text{then M} = 98.5\{\% \text{ (AV } = 98.5 - \overline{X} + \text{KS})$$

$$\text{If } \overline{X} > 101.5\%, \text{ then M} = 101.5\%(\text{AV} = \overline{X} - 101.5 + \text{KS})$$

As shown in Table 6. the estimated AV value using the proposed methodology was less than the maximum permissible AV value (L1 = 15), indicating that the contents of examined Vilaphoria® 20 mg tablets were uniform.

3.9. System greenness evaluation

A green analysis is described as having no or minimal usage of dangerous chemicals, the elimination of waste, and a decrease in energy consumption. To assess the degree of greenness of the proposed spectrofluorometric method, four cutting-edge metrics were used.

The method was evaluated related to the National Environmental Method Index (NEMI) [30]. It depends on the use of non-permanent, bioaccumulative, and toxic solvents (PBT). Methanol used in the developed spectrofluorometric method is not a PBT solvent. Also, the pH of Teorell-Stenhagen buffer used in the proposed method was 4.6 which is not judged as corrosive. Furthermore, the volume of the waste was not greater than 50 ml. According to these results, the proposed fluorometric method conserved solvents while producing low amounts of waste. As a result of these factors, it achieved each of the four quadrants of the greenness profile and is regarded as an eco-friendly green approach, as shown in (Table 7).

The Eco-scale is a straightforward method that can be used in practice to assess the greenness of analytical methodology. The subsequent equation (analytical Eco-Scale score = 100 − total penalty) is employed to calculate the penalty point value for each of the procedure's defined parameters, such as the quantity of chemicals used, dangers to employees, waste products, and energy consumption [31,32]. If the score is greater than 75, the analytical method is considered green. The Eco-Scale score of the developed fluorometric method was found to be (90) which is regarded as environmentally friendly, as demonstrated in Table 7.

Another trend, called GAPI, can be used to assess how environmentally friendly an analytical process is, from collecting samples to final analysis. The greenness of each stage in the process of analysis is evaluated using a pictogram, which has three color levels: green, yellow and red, in the GAPI tool [33,34]. The suggested technique received 5 yellow, 7 green, and 3 red areas. These regions are related to toxicity and solvent volume used. As a result, the suggested strategy has a minimal environmental impact, (Fig. 7).

The most recent metric is AGREE. The submitted criteria for the AGREE metric are flexible and can be weighted a different way, drawing design ideas from the 12 significance principles. Twelve input variables are all graded from 0 to 1 [35]. The total of each principle's assessment results is the final evaluation result. The outcome is a graph with a clock-like appearance with the overall score and a color representation in the middle. The evaluation can be carried out with software, which also generates a report and an auto-generated graph. The AGREE evaluation reveals that the suggested method depends on numerous variables, including each solvent's number, volume used, quantity of compounds throughout each run, and solvent toxicity to individuals and the surrounding environment. Its score was calculated to be (0.72), as depicted in (Fig. 7).

4. Conclusion

In this study, an easy-to-use, sensitive and green spectrofluorimetric method for the VZN assessment has been presented. Erythrosine B and

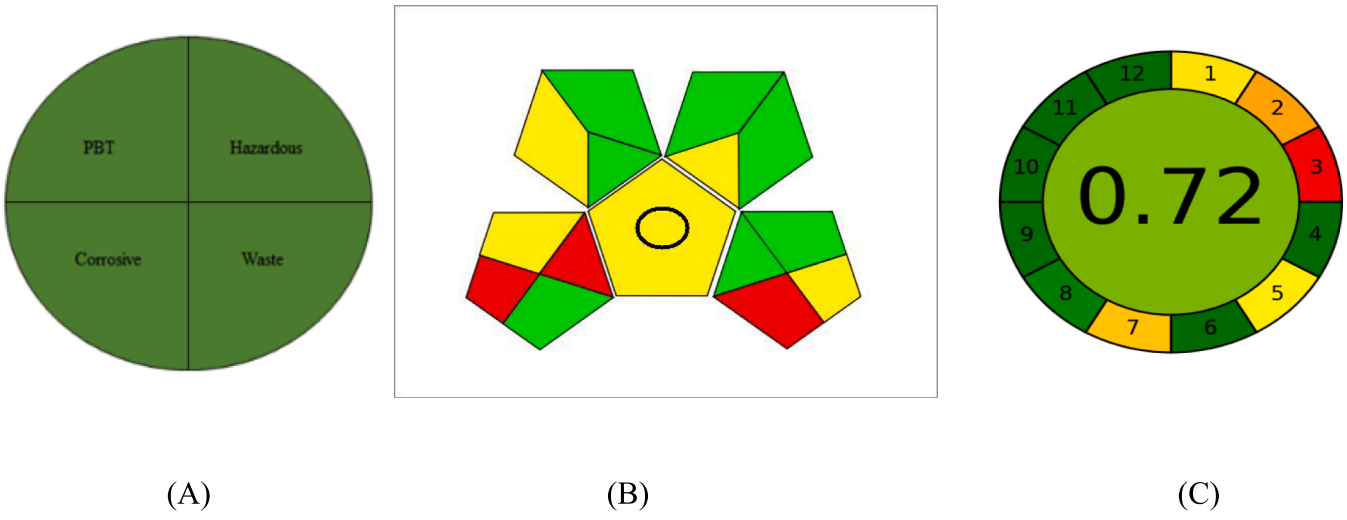

Fig. 7. Evaluation of the greenness of the proposed spectrofluorometric method using NEMI (A) GAPI (B) and AGREE (C) methods.

VZN form an ion pair-related complex between their opposing charges in a moderately acidic solution as part of the current process. The native fluorescence quenching effect of erythrosine B was assessed in the spectrofluorimetric approach as a signal of quantitation. The method could determine accurately VZN in the range of 20–600 ng mL^{-1}. The procedure's main benefit was simplicity because the sample was extracted with water and the generated ion pair could be simply manipulated in the aqueous solution without the use of vaporous solvents, that further contributes to its environmental safety. The use of less dangerous reagent (Erythrosine B), the absence of tedious extraction process, the absence of laborious procedures, the reduction of the analysis time, and the reduced cost are additional advantages of the present approach. Moreover, the proposed method was successfully used for drug analysis in dosage form and to evaluate the content uniformity of tablet formulation. Using various innovative tools, the method's greenness was assessed, and the results revealed a high greenness score. This method can therefore be used to evaluate the quality of this drug in research labs, clinical trials, and the pharmaceutical industry.

CRediT authorship contribution statement

Al Amir S. Zaafan: Writing – original draft, Software, Methodology, Conceptualization. **Sayed M. Derayea:** Writing – review & editing, Supervision, Conceptualization. **Dalia M. Nagy:** Visualization, Data curation. **Mohamed Oraby:** Writing – review & editing, Validation, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.saa.2024.124519.

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