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Green Spectrofluorimetric Technique for the Determination of Vildagliptin Through Quenching Lanthanide Luminescence in Pure, Pharmaceutical Dosage Form and Spiked Plasma

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ABSTRACT

Objectives: The objective of the proposed method is to develop a validated sensitive, selective, rapid, and low-cost spectrofluorimetric technique for the estimation of Vildagliptin depending on its quenching effect on the fluorescence intensity of Terbium (Tb^{3+}). **Methods:** The proposed method involves quantitative fluorescence quenching of terbium measured at 477 nm after excitation at 239 nm. Various experimental conditions were investigated as pH, time of reaction, order of mixing of reagents, concentrations of Tb^{3+} and buffer. **Results:** Under optimum conditions, good linearity was obtained within the range 20.0-3000.0 ng/mL with correlation coefficients of 0.9997. LOD and LOQ were 1.8 ng/mL and 19.2 ng/mL, respectively. **Conclusion:** The proposed method accomplished greener parameters; accordingly, it is a green choice for the determination of Vildagliptin in bulk, pharmaceutical dosage form, and spiked plasma.

Keywords: *Spectroflorimetry; Terbium; Vildagliptin*

INTRODUCTION

Vildagliptin (VIL) is known as (2S)-1-[2-[(3-hydroxy-1 adamantlyl) amino] acetyl] pyrrolidine-2-carbonitrile, **Figure 1**. It is used in the treatment of type II diabetes mellitus as it has a selective effect on inhibition of dipeptidyl peptidase-IV. It could be taken alone or with other antidiabetic medications[1]. Various methods have been published for the estimation of VIL using UV spectrophotometric [2,3], spectrofluorimetric [4,5], liquid chromatographic methods[6–11] and voltammetric method[12].

Lanthanide ions (terbium) form stable chelates with organic ligands. Luminescence of lanthanide chelate was identified by large stock shift, narrow emission bands, and long fluorescence lifetimes. Therefore, they improve fluorometric detection of certain organic compounds[13] as fluoroquinolones[14],

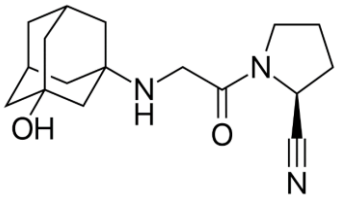


Figure 1. Structure formula of vildagliptin.

fexofenadine[15], and DNA[16]. Different organic compounds can quench the background luminescence of lanthanide ions especially when they are in the form of chloride salts, because the probability of collisions leading to energy transfer is larger for the chloride salts[17]. The proposed method provides a new simple method for quantitative spectrofluorimetric measurement of VIL through quenching the fluorescence of Terbium-Tris complex.

Recently analytical techniques have directed toward substituting existing techniques with greener ones [18]. Green techniques are usually rapid and less consuming in solvents and reagents; accordingly, researchers have established spectroscopic techniques especially fluorometric ones depending on their greenness and high selectivity of the methods[19].

Many greenness assessment tools have been developed. Recently the analytical eco-scale was used to calculate a greenness score for each method by subtracting penalty points for each factor from total of 100 points. A total eco-scale score represents > 75 (=excellent greenness), > 50 (=acceptable greenness), and < 50 (=inadequate greenness) [20]. Another method, the green analytical procedure index (GAPI) was used to assess the greenness of the technique by giving an accurate picture about the effect of each step of the proposed technique [21], and a new assessment called the 'green certificate' classifies the methods by colors and letters from A (the greenest) to G [22].

VIL has no intrinsic fluorescence. Thus, the proposed method depends on developing a valid, green, sensitive and selective spectrofluorimetric technique for the estimation of the drug in bulk powder, pharmaceutical dosage form, and spiked plasma.

MATERIAL AND METHODS

Reference samples

Vildagliptin (VIL) pure samples, kindly supplied by (Mash premiere, Co, Egypt) with % purity 99.6%.

Market samples

Icandra® tablet (Mash premiere, Egypt) 50 mg of VIL per tablet (batch no. M1133320) was purchased from the local market.

Reagents

All solvents used were of HPLC grade and chemicals were of analytical reagent grade. Terbium (III) Chloride hexahydrate 99.9 ٪(Aldrich, Germany) 1×10^{-4} M methanolic solution of terbium was prepared. Tris (hydroxymethyl) aminomethane buffer (Tris) (El-Nasr pharmaceutical company, Cairo, Egypt) 0.1 M methanolic solution of tris buffer was prepared at pH 7 with 1 N HCl. Methanol, acetonitrile, and isopropanol (Fisher chemical, United States), Fresh frozen human plasma samples were obtained from VACSIRA (Cairo, Egypt) and were kept frozen until use after gentle thawing in room temperature.

Standard Solutions

Stock standard solution of 1 mg/mL VIL was prepared by dissolving 10 mg of VIL in methanol in 10 mL volumetric flask.

Working standard solution of 40 µg/mL VIL was prepared from the stock solution. The standard solutions were kept in a refrigerator at approximately 4˚C and remained stable for at least 1 month.

Procedures

Construction of a calibration curve of VIL

1.0 mL of terbium chloride solution (1×10^{-4} M) was transferred into a series of 10.0 mL volumetric flasks containing 1.0 mL of Tris buffer (0.1 M) at pH 7.0 ± 0.2. Different amount of the working standard solution of the drug from 0.2.0-30.0 µg were added, left for 15 min then the volume was completed with methanol. The fluorescence intensity was measured at λ477 nm after excitation at λ 239 nm. The calibration curve was obtained over concentration range 20-3000 ng/mL by plotting concentrations of VIL against the fluorescence quenching (F°/F). Stern-Volmer regression equation was obtained and the concentration of VIL was calculated.

Application to pharmaceutical preparation

10 tablets of Icandra® 50 mg were ground and an accurate weight equivalent to 50 mg was added to 50 mL volumetric flask containing 30 mL of methanol, sonicated for 20 min, then the volume was completed with methanol, filtered using 0.45 µm filter and proceeded as under construction of calibration curve.

Application to spiked plasma

A 0.5 mL aliquot of human plasma was transferred into a series of 5 mL centrifuge tubes and spiked with different amounts of VIL to give a final concentration range of 0.2-10.0 µg/mL, then 4.0 mL acetonitrile were added, vortexed for 30 s, then centrifuged at 6000 rpm for 6 min and the supernatant was separated carefully. 1 mL of the supernatant was taken separately from each centrifugation tube and was treated as under construction of calibration curve.

Apparatus

All the fluorescence spectra were recorded using a JASCO FP-6200 Spectrofluorometer, equipped with 150 W Xenon lamp, grating excitation and emission monochromators, and a recorder. Slit widths for both monochromators were set at 10 nm. A 1.0 cm quartz cell was used. Spectra were evaluated using Spectra Manager FP-6200 Control Driver software, Version 1.54.03, JASCO Corporation.

RESULTS AND DISCUSSION

Fluorescence spectral characteristics

Terbium chloride (Tb^{3+}) does not show characteristic fluorescence while by adding Tris buffer intense fluorescence was observed. When excited at λ 239 nm, two emissions bands at λ 477 and λ 545 nm were obtained as shown in **Figure 2.** By adding VIL to Tb^{3+}/Tris complex, a decrease in the fluorescence intensity was observed based on the quenching effect of the studied drug (**Figure 3)**, the quenching effect was proportional to the concentration of VIL. Quantitative determinations were made by excitation at λ 239 nm and measuring the fluorescence at λ 477 nm. The emission peak at λ 477 nm was selected for the fluorescence quenching measurements as it has higher fluorescence intensity than the emission peak at λ 545 nm leading to greater sensitivity while measuring the concentration of the studied drug, so our study will be at λex/λem = 239/477 nm.

Optimization of the experimental conditions

Effect of concentration of Tris buffer

The effect of the concentration of Tris buffer was studied and as shown in **Figure 4**, the quenching in fluorescence intensity reached a maximum at concentration 0.01M Tris buffer. Thus, 1 ml of 0.1 M Tris-HCl in 10 ml volumetric flask was the optimum buffer volume for analysis.

Effect of concentration of terbium

The effect of the concentration of Tb^{3+} was studied and as shown in **Figure 5**, the quenching in fluorescence intensity reached a maximum at concentration of 0.1×10^{-4} M Tb^{3+}. Thus, 1 mL of 1×10^{-4} M Tb^{+3} in 10 mL volumetric flask was selected for further analysis.

Effect of pH

A series of 10 mL volumetric flask containing Tb^{+3}/ Tris complex and 800ng/mL of VIL at different pH values with their corresponding blank solutions were prepared and measured at λex/λem = 239/477 nm. As shown in **Figure 6,** the quenching in fluorescence intensity of the system reached a maximum when pH was 7.0. At higher pH (pH >7.0) the intensity decreased due to the precipitation of terbium hydroxide. Thus, pH 7 was selected for the following measurements.

Effect of reaction time

The time of the reaction was studied at room temperature, and it was found that the complex was formed after 10 min and remained stable up to 50 min. In this method, 15 min was set as the standard time for the reaction.

Effect of different solvent

Different solvents were investigated as acetonitrile, water, isopropanol, and methanol, high fluorescence intensity of Tb^{3+}/Tris complex was only observed when methanol was used as the diluting solvent.

Effect of order of addition of reagents

Series of solution with the same concentrations of reagent but differ in the order of mixing and their corresponding blank solution was prepared and measured at λex/λem = 239/477. The results showed no significant difference can impact fluorescence quenching (F°/F).

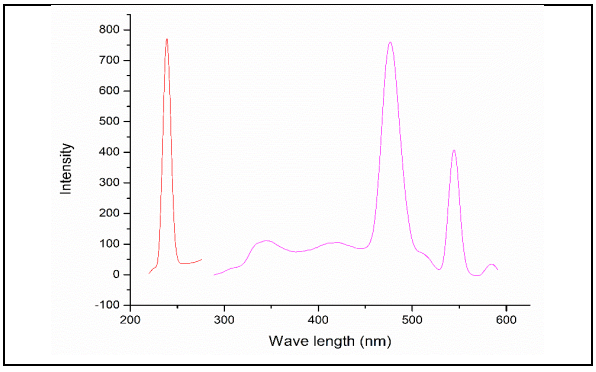


Figure 2. The excitation and emission spectra of Tb^{3+}/Tris complex in methanol.

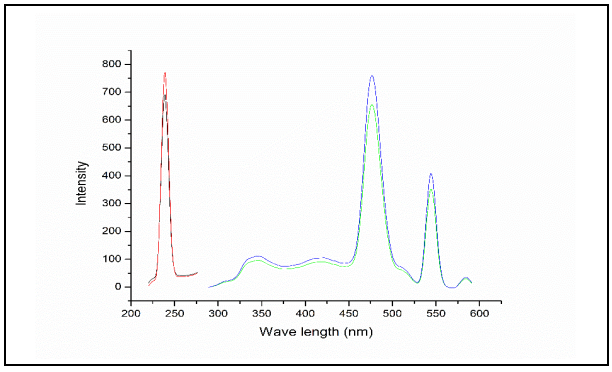


Figure 3. The excitation and emission spectra of: Tb^{3+}/Tris complex in methanol (a,b),and Tb^{3+}/Tris complex in methanol with 800 ng/mL of VIL (c,d) at λ_{ex} = 239 nm and λem = 477 nm.

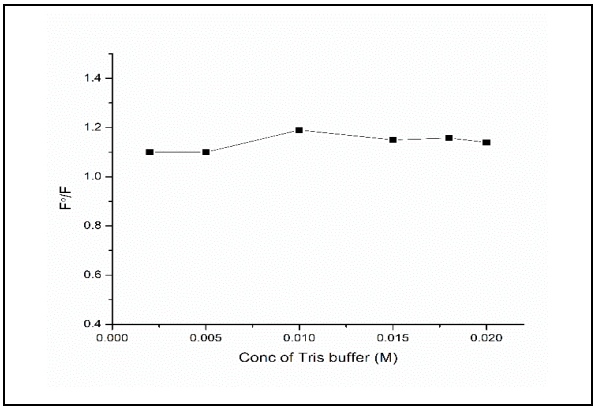


Figure 4. Effect of Tris buffer concentration with 0.1×10^{-4} M terbium and 800ng/mL of VIL at λex/λem = 239/477 nm.

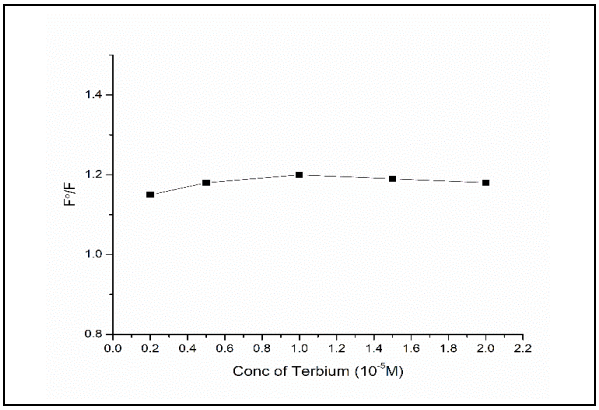


Figure 5. Effect of terbium concentration with 0.01 M Tris and 800ng/mL of VIL at λex/λem = 239/477 nm.

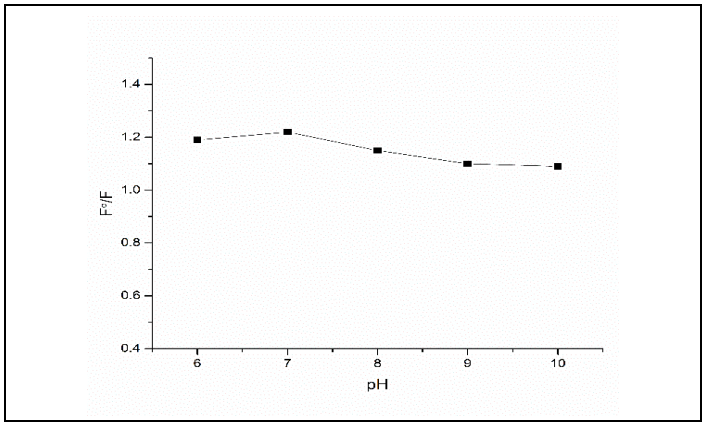


Figure 6. Effect of pH on complex formation between Tb^{3+}/Tris complex and 800ng/mL of VIL at λex/λem = 239/477 nm.

Method validation

Linearity

Under the above condition, the calibration curve was constructed by plotting F°/F against VIL concentration (**Figure 7)**. A linear relationship was obtained over concentration range 20-3000 ng/mL. The limit of quantitation (LOQ) and the limit of detection (LOD) were calculated according to ICH guidelines[23]. The results were shown in **Table 1**. $LOQ = 10\sigma/S$, LOD $= 3.3\sigma/S$, Where σ = the standard deviation of response and S = slope of the calibration curve.

A comparison of the analytical performances of the previously reported methods and the proposed method for the determination of VIL is summarized in **Table 2**. Compared with the previous spectroflourimetric methods, the proposed method was relatively simple with higher sensitivity and lower detection limit that can detect minor amount of VIL in spiked plasma.

Accuracy

The accuracy of the proposed method was determined by the standard addition method after spiking with three-level concentrations of 50,100,150 ng/mL of VIL solution. The percentage of recoveries based on the average of triplicate determinations are shown in table 3. The results prove the accuracy of the proposed method for the determination of VIL as indicated by high values of recovery percentages.

Precision

The intra-day and inter-day precision were investigated through triplicate analysis of three concentration on the same day and on 3 successive days, respectively. The results are shown in **Table 4.**

Table 1. Performance data of VIL by the proposed fluorescence quenching method

Parameters	The Proposed method
Linearity (ng/mL)	20.0-3000.0
slope	0.21
intercept	1.056
Correlation coefficient (r)	0.9997
LOD (ng/mL)	1.8
LOQ (ng/mL)	19.2

Table 2. Comparison between the proposed method and other reported methods used for determination of VIL

Method	Linearity	Detection limit	references
Spectrofluorimetric NBD-Cl	0.03–0.37 μg mL^{-1}	0.003 μg mL^{-1}	[4]
Spectrophotometric			[4]
NQS	4.5–35 μg mL^{-1}	1.84 μg mL^{-1}	
NBD-Cl	7–45 μg mL^{-1}	1.04 μg mL^{-1}	
Spectrofluorimetric (dansyl chloride)	100-600 μg mL^{-1}	15.05 μg mL^{-1}	[5]
HPLC	5-75 μg mL^{-1}	0.87 μg mL^{-1}	[7]
Gc-MS	3.5–300 ng mL^{-1}	1.5 ng mL^{-1}	[24]
Proposed method	20-3000 ng mL^{-1}	1.9 ng mL^{-1}	

Table 3. Quantitative determination of VIL in a drug product by the proposed method using the standard addition

Parameters	Proposed method			
	Amount taken from sample (ng/mL)	Amount of VIL added (ng/mL)	Amount found (ng/mL)	Recovery percentage %
	100	50	149.9	99.90
		100	198.9	99.45
		150	249.3	99.72
mean				99.7
±SD				0.198
RSD%				0.199

Table 4. Precision data of the proposed method for the determination of VIL in a drug substance

Parameters	Intra-day (Repeatability*)		precision	Inter-day (Intermediate precision**)		precision
Conc (ng/mL)	**100**	**500**	**1000**	**100**	**500**	**1000**
	99.7	100.6	100.6	99.8	100.2	100.5
Recovery %	100.2	99.4	100.4	98.7	99.8	100.6
	100.6	100.5	100.1	99.8	100.9	100.1
Mean	100.2	100.1	100.4	99.4	100.3	100.4
±SD	0.37	0.54	0.2	0.52	0.45	0.21
RSD %	0.37	0.54	0.2	0.52	0.45	0.21

**The intraday (n = 9), an average of three different concentrations repeated three times within day.*
***The interday (n = 9), an average of three different concentrations repeated three times in three successive days.*

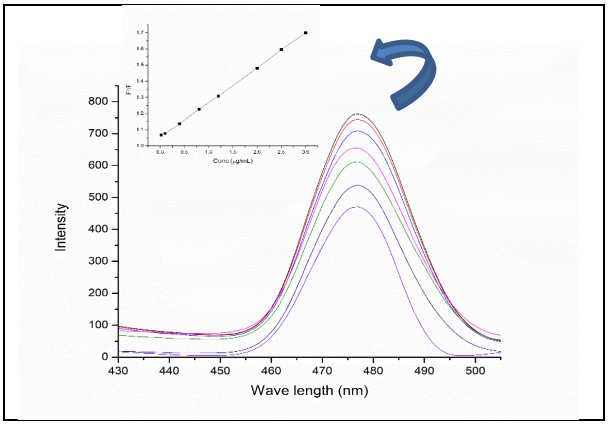


Figure 7. Emission spectra of Tb^{3+}/Tris complex. (a), +VIL 100 ng/mL (b), 400 ng/mL (c), 800 ng/mL (d), 1200 ng/mL (e), 2000 ng/mL, 3000 ng/mL. The inset is the linear relationship between the concentration of VIL and the quenching of the fluorescence of Terbium-Tris complex.

Application

Application on analysis of pharmaceutical dosage form

The proposed method was applied successfully for the determination of VIL in bulk and dosage form **Table 5.** The results of our method were compared with those of the reported method. No significant difference was observed between the two methods regarding accuracy as indicated by student's t test and precision as indicated by F test.

Application in spiked plasma

The proposed method was efficiently applied for the determination of VIL in spiked plasma (**Table 6)**. The results prove the efficiency of the proposed method for the determination of VIL in spiked plasma by high values of recovery percentages.

Proposed mechanism of the reaction

The intense lanthanide-sensitized luminescence originates from an intramolecular energy transfer through the excited triplet state of the ligand to the emitting resonance level of the ion followed by radiative emission from the cation. The efficiency of the energy transfer depends on the matching between the triplet level of the organic compound and the resonance level of the ion. The energy of the triplet level should be close to but higher than, that of the resonance level of the ion. In some instances, when the organic compound has a triplet state level below the excited state-level of the lanthanide ion the organic compound can quench the background luminescence of the ion[17]. The proposed mechanism between terbium and VIL is that VIL is coordinated to the metal ion via the amino group and Carbonyl-O groups (**Figure 8)**.

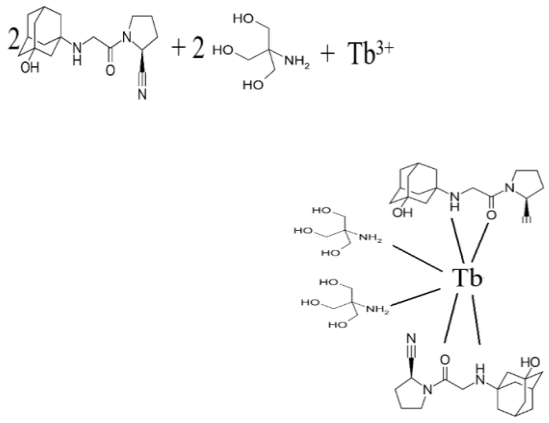


Figure 8. The proposed mechanism of the reaction.

Quenching mechanism

The quenching mechanism was studied using stern Volmer equation [26]

$F_0/F=1+Q\ K_{SV}$

Where F_0/F is the emission intensity in the absence and the presence of the quencher, Q is quencher concentration and K_{SV} is stern volmer constant and calculated From the slope by plotting F_0/F vs concentration of quencher. K_{sv} was found to decrease with increasing the temperature (298,308 K) which is a property for static quenching (**Figure 10, Table 7)**.

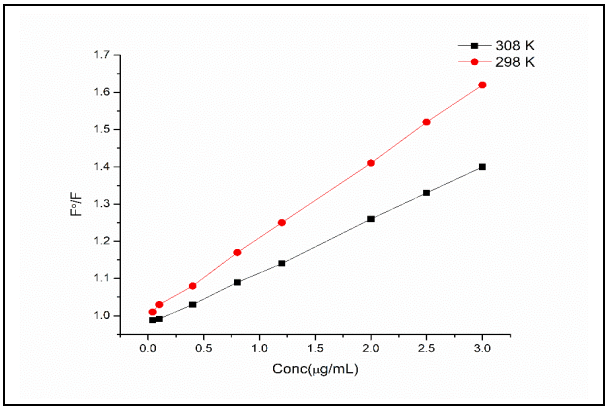


Figure 9. Stern–Volmer plots of the terbium tris buffer with 800ng/mL VIL at different temperatures (298 k, 308 k) at λex/λem = 239/477 nm.

Assessment of method greenness

Assessment of method greenness was determined through the quantitative analytical eco-scale, GAPI and green certificate methods. As shown in **Table 8 and Figure 10**, the proposed method has excellent greenness.

Table 5. Quantitative determination of VIL in drug product

Parameters	Proposed method	Reported method[25 b]
	% Recovery	% Recovery
	100.4	99.8
	99.9	100.1
	100.3	99.5
	100.1	99.89
	99.4	100.06
Mean	100.03	99.82
±SD	0.35	0.21
RSD %	0.35	0.21
t- test	0.49 (2.306)[a]	
F-test	0.36 (6.39)[a]	

[a] *tabulated values of t and F at n=3 and p = 0.05.*
[b] *Reported HPLC/UV method using hypersil ODS C18 column 250 x 4.6mm 5µ, mobile phase 0.1M Potassium hydro phosphate and Acetonitrile at the ratio (60:40%v/v) of pH:7.0, flow rate 1ml/min at 263 nm*

Table 6. Evaluation of accuracy and precision of the proposed method for the determination of VIL in spiked plasma samples.

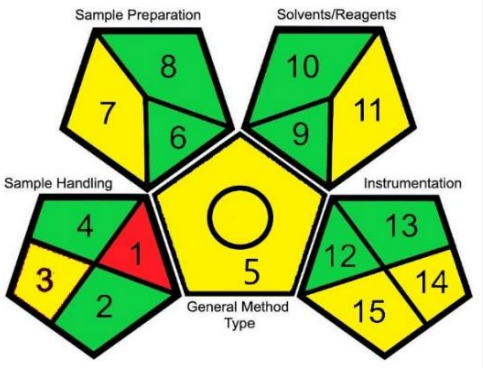
Parameters	Proposed method		
Conc (ng/mL)	Amount of VIL (ng/mL) added	Amount of VIL (ng/mL) found	Recovery percentage %
	100	99.16	99.15
	500	505.52	101.1
	1000	1000.51	100.05
Mean			100.1
±SD			0.79
RSD %			0.79

Table 7. Stern–Volmer and thermodynamic parameters of terbium tris buffer with 800ng/mL VIL

Parameter	Temperature (K)	Stern–Volmer equation	Correlation coefficient (r)	Ksv
Stern–Volmer parameters	298	ΔF= 0.205x + 1.0053	0.9997	0.205
	308	ΔF= 0.1407x + 0.9771	0.9994	0.1407

Table 8. Greenness assessment of the proposed spectrofluorimetric method

	Analytical eco-scale		GAPI
	Eco-scale parameter	Penalty points	
Reagents	Methanol	6	
Instrument	Spectrofluorimeter (≤ 0.1 kWh)	0	
	Occupational hazard	0	
	Waste (< 10 mL, without treatment)	5	
Σ penalty points		11	
Total score		89	



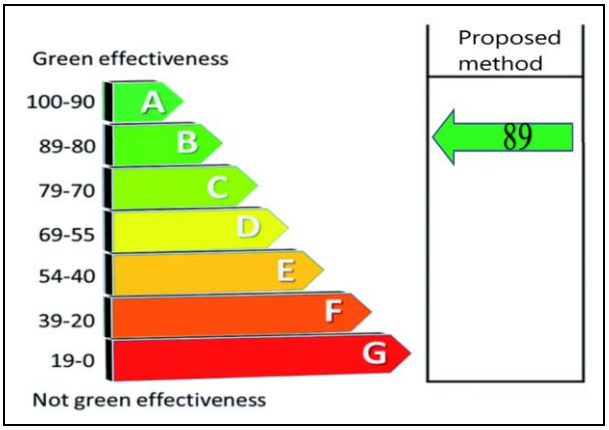


Figure 10. Green effectiveness of the proposed method used for determination of VIL.

CONCLUSION

A validated, rapid, and low-cost spectrofluorimetric method was developed for the quantitative determination of VIL in pure form and pharmaceutical dosage form. The suggested method is economical compared to the reported HPLC method as there is no need to consume a large amount of solvents. The proposed method could be successfully used for routine analysis of the cited drug.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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