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 (Tb³⁺). 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 Tb³⁺ 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: Spectrofluorimetry; Terbium; Vildagliptin

INTRODUCTION

Vildagliptin (VIL) is known as (2S)-1-[2-[(3-hydroxy-1 adamantyl) 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. Various methods have been published for the estimation of VIL using UV spectrophotometric, spectrofluorimetric, liquid chromatographic, and voltammetric methods. 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 as fluoroquinolones.
fexofenadine\textsuperscript{15}, and DNA\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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)\textsuperscript{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\textsuperscript{21}, and a new assessment called the ‘green certificate’ classifies the methods by colors and letters from A (the greenest) to G\textsuperscript{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\textsuperscript{®} 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\textsuperscript{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\textsuperscript{-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 λ 477 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\textsuperscript{®} 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 0.2.0-10.0 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.

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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 \(\lambda = 239\) nm, two emissions bands at \(\lambda = 477\) and \(\lambda = 545\) 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 \(\lambda = 239\) nm and measuring the fluorescence at \(\lambda = 477\) nm. The emission peak at \(\lambda = 477\) nm was selected for the fluorescence quenching measurements as it has higher fluorescence intensity than the emission peak at \(\lambda = 545\) nm leading to greater sensitivity while measuring the concentration of the studied drug, so our study will be at \(\lambda_{ex}/\lambda_{em} = 239/477\) 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 0.01 M 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 \(1 \times 10^{-4}\) M Tb\(^{3+}\). Thus, 1 mL of \(1 \times 10^{-4}\) 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 800 ng/mL of VIL at different pH values with their corresponding blank solutions were prepared and measured at \(\lambda_{ex}/\lambda_{em} = 239/477\) 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 acetonitride, 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 \(\lambda_{ex}/\lambda_{em} = 239/477\). 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 \(\lambda_{ex} = 239\) nm and \(\lambda_{em} = 477\) nm.

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Method validation

Linearity

Under the above condition, the calibration curve was constructed by plotting $F^0/F$ 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. The results were shown in Table 1. $LOQ = 10\sigma / S$, $LOD = 3.3\sigma / S$, Where $\sigma$ = 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-CI | 0.03–0.37 μg mL⁻¹ | 0.003 μg mL⁻¹ | 4          |
| Spectrophotometric NQS NBD-CI | 4.5–35 μg mL⁻¹ | 1.84 μg mL⁻¹ | 4          |
| 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 precision | Inter-day precision |
|------------|---------------------|---------------------|
| Conc (ng/mL) | 100 | 500 | 1000 | 100 | 500 | 1000 |
| Recovery % | 99.7 | 100.6 | 100.6 | 99.8 | 100.2 | 100.5 |
| Mean | 100.2 | 99.4 | 100.4 | 98.7 | 99.8 | 100.6 |
| ±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.

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Figure 7. Emission spectra of Tb\textsuperscript{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\textsuperscript{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\textsuperscript{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.

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Table 5. Quantitative determination of VIL in drug product

| Parameters | Proposed method | Reported method |
|------------|-----------------|-----------------|
| % 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) | K_{SV} |
|-----------|-----------------|------------------------|----------------------------|--------|
| 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    |

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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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