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SHORT COMMUNICATION

# Novel Spectrofluorimetric Method for the Determination of Perindopril Erbumine Based on Fluorescence Quenching of Rhodamine B

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**Abstract** A novel, simple and specific spectrofluorimetric method was developed and validated for the determination of perindopril erbumine (PDE). The method is based on the fluorescence quenching of Rhodamine B upon adding perindopril erbumine. The quenched fluorescence was monitored at 578 nm after excitation at 500 nm. The optimization of the reaction conditions such as the solvent, reagent concentration, and reaction time were investigated. Under the optimum conditions, the fluorescence quenching was linear over a concentration range of 1.0–6.0 μg/mL. The proposed method was fully validated and successfully applied to the analysis of perindopril erbumine in pure form and tablets. Statistical comparison of the results obtained by the developed and reference methods revealed no significant differences between the methods compared in terms of accuracy and precision. The method was shown to be highly specific in the presence of indapamide, a diuretic that is commonly combined with perindopril erbumine. The mechanism of rhodamine B quenching was also discussed.

**Keywords** Perindopril erbumine · Rhodamine B · Fluorescence quenching · Charge transfer reaction · Spectrofluorimetry

## Introduction

Perindopril erbumine is the tert-butylamine salt of perindopril, which is the ethyl ester prodrug of the angiotensin converting enzyme (ACE) inhibitor, perindoprilat. Perindopril erbumine is chemically described as 2-Methylpropan-2-amine (2S,3aS, 7aS)-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl) butyl] amino] propanoyl]octahydro-1 H-indole-2-carboxylate. Its molecular formula is $C_{19}H_{32}N_2O_5C_4H_{11}N$ (Fig. 1).

Perindopril erbumine belongs to the category of Angiotensin converting enzyme inhibitors (ACE inhibitors) that inhibit the conversion of angiotensin I to angiotensin II. Perindopril erbumine is indicated for the treatment of hypertension, this effect appears to result primarily from the inhibition of circulating and tissue ACE activity thereby reducing angiotensin II formation, and decreasing vasoconstriction. Perindopril erbumine is also indicated for patients with congestive heart failure [1].

Up till now no official monograph has been reported for the determination of PDE in pharmaceuticals Therefore, it is very important to develop simple and suitable analytical method for the determination of PDE in bulk and moreover in formulations.

Literature reported only few analytical methods for the determination of PDE in its bulk, dosage forms and human plasma, such as high performance liquid chromatography [2–9], HPLC-MS [10, 11], high performance thin layer chromatography [12], and spectrophotometry [13–16].

However, the reported methods were found to have certain drawbacks. The chromatographic methods require expensive equipment and are complicated in operation. On the other hand, spectrophotometric methods are not such sensitive. In contrast, spectrofluorimetric methods have several advantages, such as sensitivity, simplicity and selectivity. Only one spectrofluoremetric method for the determination of PDE in tablets was reported in our previous work [17]. Therefore, it is still significant to make an effort for developing new, simple and sensitive spectrofluorimetric methods for the determination of such an important drug, perindopril erbumine.

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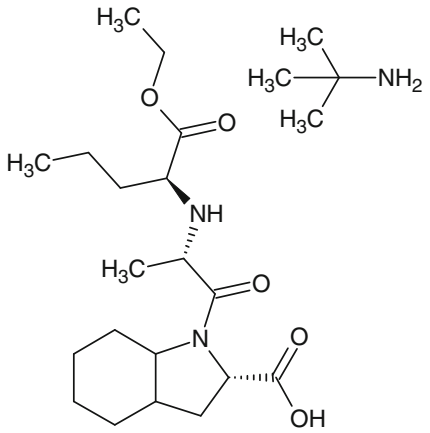


**Fig. 1** Perindopril Erbumine structure

Rhodamine B is a fluorescent dye and it was used as a spectrophotometric reagent in the determination of free fatty acids [18] and metal ions such as gallium, mercury, thallium, palladium, and uranium [19–23].

To the best of our knowledge, rhodamine B has not been used before for the determination of drugs. In the present work, novel spectrofluorimetric method has been developed for the determination of PDE using rhodamine B as fluorescence probe. The proposed method is sensitive, accurate, simple and selective. It was applied for the determination of PDE in bulk and as well as in pharmaceutical preparations.

## Experimental

### Apparatus

Fluorescence spectra and measurements were obtained using fluorescence spectrophotometer F-2700 (Hitachi, Japan) equipped with xenon lamp. Excitation and emission wavelengths were set at 500 nm and 578 nm. The slit widths for excitation and emission monochromators were fixed at 5 nm. All measurements were performed in 1 cm quartz cell at room temperature.

Chromatographic analysis was performed on (Agilent 1200 series, Agilent Technologies, Germany) apparatus equipped with UV detector, autosampler, and column oven. Chromatographic separation was achieved on C18 column (5 μm, 100 mm × 4.6 mm).

### Reagents and Solutions

Perindopril erbumine (ROLABO outsourcing, S.L. Spain) standard solution of 0.25 mg/mL was prepared in acetone. Rhodamine B was purchased from Riedel-de Haen AG, Hannover, Germany. Solution of rhodamine B was prepared at 0.462 mg/mL in acetonitrile. PDE, and rhodamine B standard solutions were stable at lab temperature. All reagents and solvents were of extra pure grade.

Perindopril erbumine tablets, Revosyl® (Ibn Al-Hayhtam Pharma. Industries Co. Syria), containing 4 mg and 8 mg, were purchased from local medical stores.

### General Procedure

Increasing volumes of PDE working standard solution were transferred into series of 5 mL volumetric flasks that contain 0.65 μL of rhodamine B solution. Volumes were made up to mark with acetone and mixed before the fluorescence intensity was measured at 578 nm after excitation at 500 nm. The fluorescence intensity for complex product (F) and reagent blank ($F_0$) was measured and the quenched fluorescence intensity was calculated $\Delta F = F_0 - F$.

### Procedure for Pharmaceutical Samples

Ten individual tablets were weighed and pulverized carefully. An accurately weighed amount of the powder equivalent to 4 mg of PDE was transferred into 25 mL volumetric flask and dissolved in 20 mL of acetone. The content of the flask was sonicated for 20 min then diluted to volume with acetone. A portion of this solution was centrifuged at 5000 rpm for 10 min. Suitable aliquot of the supernatant was then transferred into 5 mL volumetric flask and procedure was continued as described under the general procedure.

## Results and Discussion

### Fluorescence Spectra

Rhodamine B has native fluorescence in acetone, whereas PDE does not have any fluorescence. It was observed that addition of drug to rhodamine B solution causes a decrease in the fluorescence intensity of rhodamine B with an insignificant shift (2 nm) from 580 nm to 578 nm (Fig. 2). This

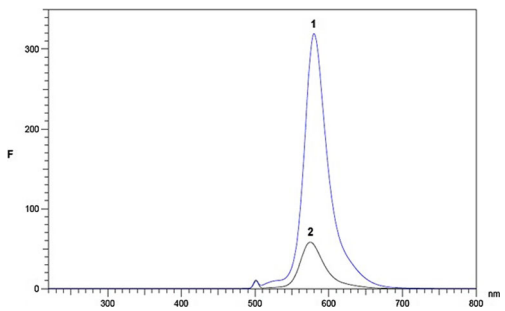


**Fig. 2** Emission spectra of rhodamine B 6 μg/mL in acetone: without PDE (1) and with 5 μg/mL of PDE (2), after excitation at 500 nm

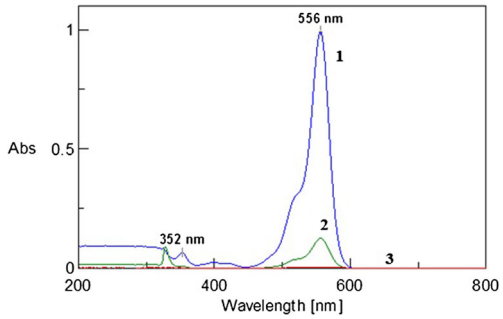


**Fig. 3** Absorption spectra of rhodamine B 6 μg/mL in acetone: without PDE (1), with 5 μg/mL of PDE (2), and PDE 5 μg/mL (3)

fluorescence quenching of rhodamine B by the drug was found to be directly proportional to drug concentration in a certain range.

### Absorption Spectra

Rhodamine B solution in acetone has a maximum absorption wavelength at 556 nm. Once a charge transfer complex forms between rhodamine B and PDE, the absorbance intensity at 556 nm drops significantly as shown from Fig. 3.

### Effect of Solvent Nature

Different solvents with varied polarities and dielectric constants were examined (methanol, ethanol, isopropanol, acetonitrile, acetone, dimethylsulfoxide, dichloromethane, chloroform and n-hexan). Interestingly, the complex with rhodamine B was formed in acetone, acetonitrile, DCM and chloroform. Whereas, the complex was not formed in the protic solvents, and the rhodamine B was found to be non-fluorescent in DMSO and n-hexan. The quenched fluorescence intensity percent was found to be increased when changing from non-polar solvents: dichloromethane and chloroform (about 50 %) to the polar aprotic solvents: acetone and acetonitrile (about 80 %). Thus, acetone, a cheaper solvent than acetonitrile, was chosen for continuing the study (Fig. 4).

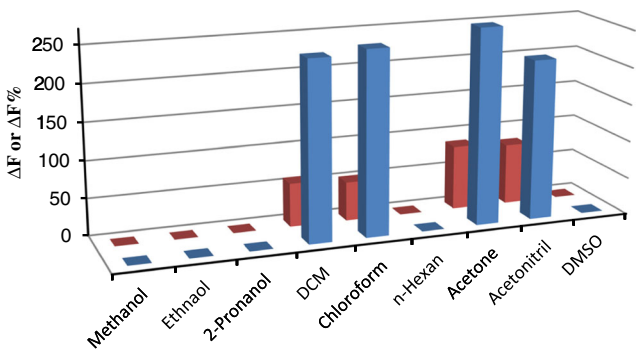


**Fig. 4** Solvent effect on fluorescence quenching of rhodamine B (6 μg/mL) in presence of PDE (5 μg/mL)

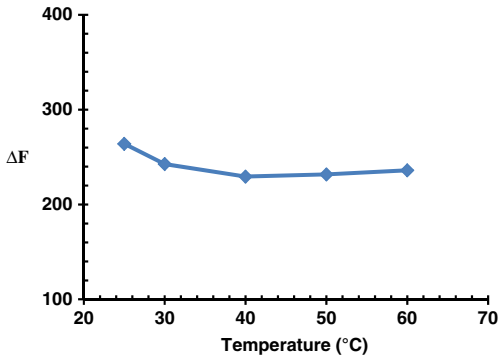


**Fig. 5** Effect of temperature on the reaction completion of PDE (5 μg/mL) with rhodamine B in acetone

### Effect of Time

The optimum reaction time was studied by following the fluorescence intensity at 578 nm. It was observed that the complex formed and got stabilized immediately after mixing and fluorescence intensity remained stable for at least 24 h.

### Effect of Temperature

The effect of temperature on complex formation with rhodamine B and ΔF values was also investigated. For that, reaction was left for 10 min at room temperature (about 25 °C), in addition to four different temperatures of 30 °C, 40 °C, 50 °C and 60 °C. Solutions were cooled and balanced with lab temperature before measuring the fluorescence intensity. It was observed that ΔF was slightly decreased at high temperatures (Fig. 5) which may due to a dissociation of complex formed between reagents and drug. Therefore further experiments were carried out at lab temperature (25 ± 2 °C.).

### Effect of Reagent Volume

The influence of the volume of reagent solution was examined by adding increasing volumes ranged from 25 to 100 μL of 0.462 mg/mL rhodamine B solution. A volume of 65 μL of rhodamine B solution was found to be sufficient for producing

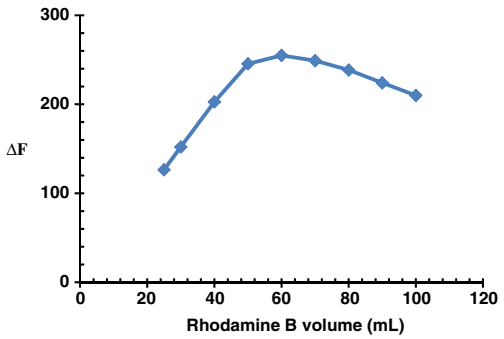


**Fig. 6** Effect of the volume added of rhodamine B (0.462 mg/mL) on the quenched fluorescence intensity at a fixed concentration of PDE (6 μg/mL)

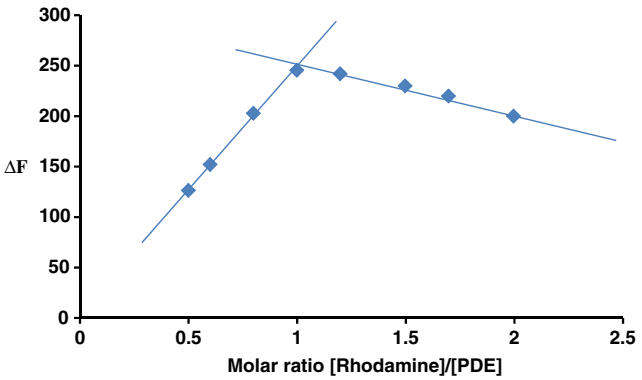


**Fig. 7** Molar ratio plot for the stoichiometry of reaction between rhodamine B and PDE

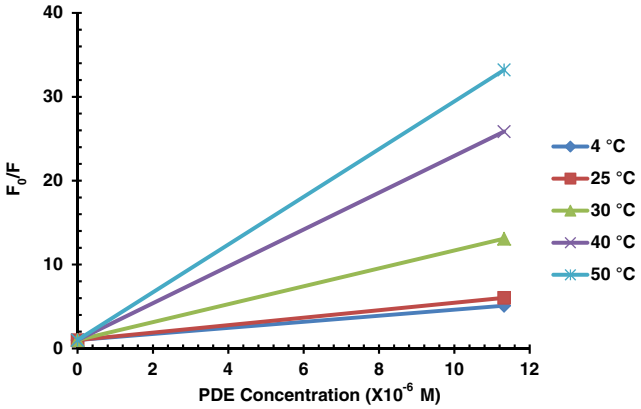


**Fig. 8** Effect of temperature as an indicator of quenching mechanism (Rhodamine B : 6 μg/mL with PDE: 5 μg/mL)

maximum, steady and reproducible quenched fluorescence intensity (ΔF) with low inner filter effect that usually observed at higher reagent concentration (Fig. 6). Thus a fixed volume of 65 μL of rhodamine B solution was used in the optimal procedure, which corresponds to a minimum molar ratio of 1:1 Drug/Rhodamine B at the highest drug molar concentration of the linearity.

## Stoichiometric Relationship of PDE/Rhodamine B Complex

The composition ratio of the complex was determined using molar ratio method (Fig. 7). From the obtained plot, it was concluded that rhodamine:perindopril ratio is 1:1. This confirms that only one site of interaction was involved in the formation of the complex. The quenching effect is most probably due to the formation of non-fluorescent charge transfer complex between the drug acting as n-donor (D) and rhodamine B, as π-acceptor (A):

$$D^{\cdot\cdot} + A \leftrightarrow [D^{\cdot\cdot} \rightarrow A] \quad \leftrightarrow \quad D^{\cdot +} + A^{\cdot -} \text{DA complexradical anion}$$

The dissociation of DA complex is promoted by the high dielectric constant of the solvent, acetone, (20.7). This radical anion absorbs light and immediately returns

**Scheme 1** Schematic illustration of reaction between PDE and rhodamine B in acetone

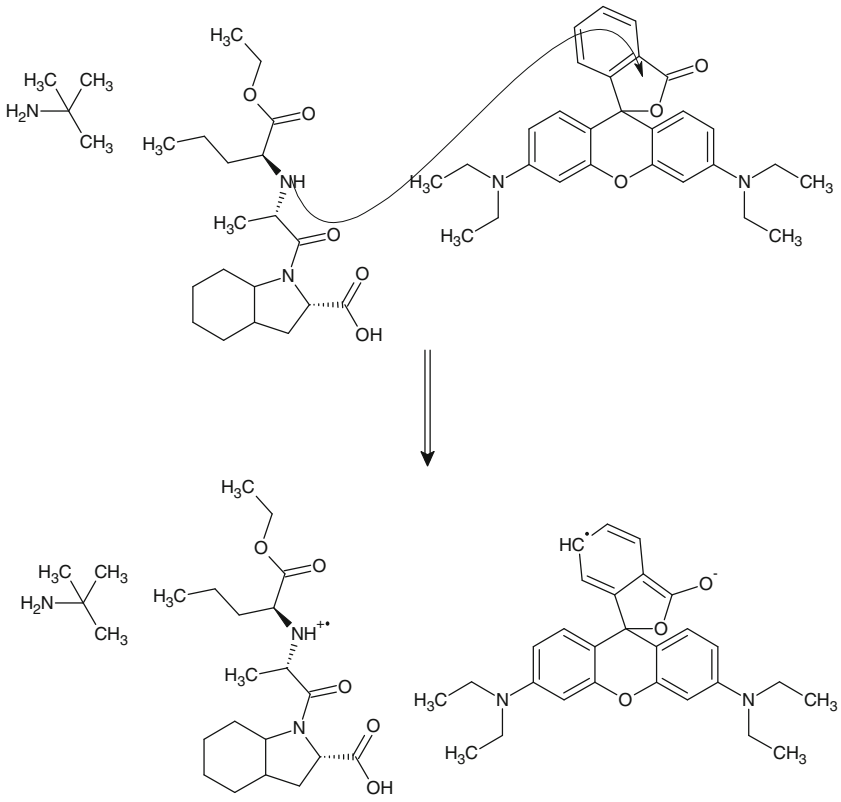

**Table 1** Statistics and analytical parameters of PDE determination using rhodamine B

| Parameter | |
|---|---|
| $\lambda_{ex}/\lambda_{em}$ (nm) | 500/578 |
| Linear range (μg/mL) | 1.0–6.0 |
| Slope | 52.768 |
| Standard deviation in the slope | 0.288 |
| Intercept | - 0.9463 |
| Standard deviation in the intercept | 1.128 |
| Correlation coefficient | 0.9999 |
| Detection limit (μg/mL) | 0.070 |
| Quantitation limit (μg/mL) | 0.214 |

to the ground state without emission of photon, as a result only the un-complexed fluorophore gives the fluorescence emission. A schematic proposal of the reaction pathway is given in Scheme 1.

## Mechanism of Quenching

There are three mechanisms of fluorescence quenching include: static quenching, dynamic quenching, and resonance energy transfer [24, 25]. Resonance energy transfer is definitely not our case, simply because there is no interference between the absorption spectra of PDE and emission spectra of rhodamine B. In dynamic quenching, ΔF value increases with increasing temperature, which is due to enhancement of collisions between molecules at high temperatures. On contrast, increasing temperature decreases ΔF in static quenching due to complex dissociation [25]. Based on this principle, the temperature effect on ΔF was investigated at five different degrees (4, 25, 30, 40, and 50 °C). The fluorescence intensities were carefully measured immediately without losing of heat. As shown in Fig. 8, the value of ΔF rises significantly as temperature increases from 4 to 50 °C and thus, fluorescence quenching is considered to be dynamic. On the other hand, the change in absorption spectra and decrease of rhodamine B absorbance at $\lambda_{max}$ 556 nm is evidence to a static quenching (Fig. 3). Thus, the mechanism of quenching is considered to be a combination of both dynamic and static quenching.

**Table 3** Precision and accuracy for determination of PDE in pure form using method A and B

| Perindopril erbumine (μg/mL) | | SD (μg/mL) | RSD% | Recovery % | *t*-test[b] |
|---|---|---|---|---|---|
| Taken | Found[a] | | | | |
| 1.000 | 1.006 ± 0.029 | 0.024 | 2.38 | 100.60 | 0.55 |
| 2.000 | 1.992 ± 0.043 | 0.035 | 1.75 | 99.60 | 0.51 |
| 3.000 | 3.998 ± 0.057 | 0.046 | 1.53 | 99.93 | 0.09 |
| 4.000 | 4.004 ± 0.059 | 0.048 | 1.19 | 100.10 | 0.18 |
| 5.000 | 5.027 ± 0.092 | 0.074 | 1.47 | 100.54 | 0.81 |
| 6.000 | 5.983 ± 0.031 | 0.025 | 0.41 | 99.72 | 1.52 |

[a] Average of five determinations ± Confidence limit

[b] The tabulated *t*- value at 95 % confidence limit for 4° of freedom ($n = 5$) is 2.78

## Validation of the Proposed Method

### *Linearity*

Under the optimum experimental conditions, standard calibration curve was constructed at several concentration levels ($n = 5$). The correlation coefficient was 0.9999 indicating very good linearity over the concentration range of 1.0–6.0 μg/mL. The intercept, slope, detection limit (DL), and quantitation limit (QL) are summarized in Table 1. DL and QL values were calculated as $3.3S_b$/m and $10S_b$/m, respectively. Where $S_b$ is the standard deviation of intercept of regression line and m is the slope of the calibration curve [26, 27].

### *Specificity*

The effects of some common excipients used in pharmaceutical preparations were studied by analyzing solutions containing suggested amounts of each excipient. Frequently encountered excipients or additives were studied such as lactose, microcrystalline cellulose (Avicel), soluble starch, polyvinylpyrrolidone (PVP k30), talc, and magnesium stearate. None of the studied excipients has given significant fluorescence intensity and the maximum interference did not exceed 0.2 %. Unfortunately, coloring agents has been found to interfere with this method. Thus, the proposed method is suitable for perindopril erbumine determination in dosage forms that do not contain colors.

Potential effect of indapamide, a diuretic which is commonly combined with perindopril erbumine in tablets, was

**Table 2** Selectivity in the determination of PDE using rhodamine B

| Amount taken (μg/mL) | Indapamide added (μg/mL) | Amount found* (μg/mL) | Recovery % | RSD% |
|---|---|---|---|---|
| 4.00 | 1.25 | 3.93 | 98.25 | 0.77 |

* Average of three determinations

**Table 4** Application of the proposed methods to the determination of PDE in tablets

| Tablets | Labeled amount of PDE | Amount taken (μg/mL) | Amount found[a] (μg/mL) | Recovery % |
|---|---|---|---|---|
| Revosyl | 4 mg | 3.00 | 3.08 | 102.66 |
| | | 4.00 | 4.09 | 102.25 |
| | | 5.00 | 5.09 | 101.80 |
| Mean found% | | | | 02.24 |
| RSD% | | | | 0.42 |
| Revosyl | 8 mg | 3.00 | 3.17 | 105.66 |
| | | 4.00 | 4.26 | 106.50 |
| | | 5.00 | 5.30 | 106.00 |
| Mean found% | | | | 106.05 |
| RSD% | | | | 0.40 |

[a] Average of three determinations

thoroughly studied with small interference shown at the emission wavelength of rhodamine B (Table 2). As a result, the method can be considered selective, and can be applied for the determination of perindopril erbumine in presence of indapamide.

*Precision*

The repeatability of the proposed method was estimated by measuring five replicate samples of each concentration of perindopril erbumine prepared in one laboratory on the same day. The precision expressed as the relative standard deviation (RSD%) that did not exceed 2.38 % for the smallest determined concentration which indicating good precision (Tables 3).

*Accuracy*

The proposed method was applied on the available commercial tablets at three different concentration levels (75 %, 100 % and 125 %), and recoveries are mentioned in Table 4. However, the method's accuracy is judged by (1) determining the average amount of PDE in pure form at several levels, and using a significance test to compare it with actual amount $\mu$ [26]:

$$t = \frac{|\overline{X} - \mu|}{SD\sqrt{n}}$$

As shown in Table 3, the calculated *t*-value is less than tabulated $t(0.05,4)$ value (2.78), and thus there is no significant differences between the taken and found concentration at 95 % confidence level. Accuracy was indicated as well by analyzing the recoveries of known different amounts of PDE (Table 3) which varied from 99.60 to 100.60 %. (2) comparing the results obtained from the presently proposed method, that has been applied on commercial tablets, with those obtained from a reference method such as HPLC [2]. The resulted values were statistically compared with each other (Table 5) using *t*- and *F*-tests. $t_{exp.}$ was calculated using the following equation [26]:

$$t_{exp} = \frac{\left|\overline{X}_A - \overline{X}_B\right|}{\sqrt{\left(S_A^2/n_A\right) + \left(S_B^2/n_B\right)}}$$

**Table 5** Precision and accuracy for determination of PDE in tablets using rhodamine B

| Tablets | Labeled amount of PDE | Average PDE found (mg/tablet) ± SD[a] (Recovery%)[b] | | *t*- and *F*- test[c] |
|---|---|---|---|---|
| | | Proposed method | Reference method[d] | |
| Revosyl | 4 mg | 4.088 ± 0.045 (102.20) | 4.060 ± 0.024 (101.50) | 1.04, 3.48 |
| | 8 mg | 8.540 ± 0.081 (106.75) | 8.440 ± 0.040 (105.50) | 2.48, 4.13 |

[a] Average and standard deviation of five determinations for the proposed methods, and three determinations for the reference method

[b] Recoveries were calculated considering the labeled amount reported by the manufacturer

[c] the tabulated *t* value at 95 % confidence limit for 4° of freedom ($n = 5$) is 2.78 and the tabulated *F* value at 95 % confidence limit for (4, 2) degrees of freedom for the proposed and reference methods, respectively, is 19.25

[d] HPLC [2]

Where $X_A$ and $X_B$ are PDE mean values in each pharmaceutical product using the proposed and reference methods, respectively. $S$ and $n$ are the standard deviation and the number of replicate trials conducted on samples, respectively. With respect to $t$- and $F$-tests, no significant differences were found between the calculated values of both the proposed and the reported methods at 95 % confidence level.

*Robustness*

The experimental conditions such as time and temperature have no significant effect on fluorescence intensity of the reaction product. Rhodamine B reagent volume should be added precisely because its signal is engaged in the concentration calculations as we calculate the quenched fluorescence value. However, the proposed method is considered to be robust and can be applied easily in quality control laboratories with no regards to minor changes.

### Application to Tablets

The proposed methods were successfully applied to the analysis of commercial tablets (Revosyl® tablets) labeled to contain 4 and 8 mg of perindopril erbumine. The mean recovery values were similar to the recoveries recorded by the reference method (HPLC) as revealed by $t$- and $F$-test (Table 5).

## Conclusion

Novel, new, simple, and extremely rapid spectrofluorimetric method for the determination of PDE has been successfully developed and validated. The method involved the formation of a quenched charge transfer complex between PDE and rhodamine B. The proposed method was specific, precise and accurate with a comparable low detection limit value of 0.070 μg/mL. The method was effectively applied for determining PDE in pure form and in tablets without interference with the excipients. Furthermore, the method was shown to be highly selective for perindopril erbumine with no interference by indapamide which is commonly combined with perindopril in tablets.

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**Conflict of Interest** The authors declare that they have no conflict of interest.

**Authors' Contributions** Hanan Fael has performed the experimental and analytical work and prepared the draft of the manuscript. The supervision of this work was provided by Prof. Amir Al-Haj Sakur. All authors read and approved the final manuscript.

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