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SHORT COMMUNICATION

Novel Spectrofluorimetric Method for the Determination of Perindopril Erbumine Based on Charge Transfer Reaction with 7-Hydroxycoumarin

Hanan Fael[1] · **Amir Al-Haj Sakur**[1]

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Abstract A novel, simple, selective and sensitive spectrofluorimetric method was developed and validated for the determination of perindopril erbumine using 7-hydroxycoumarin. Perindopril erbumine was found to react with 7-hydroxycoumarin in acetonitrile resulting in a new fluorescent product with about 58 nm blue shifted emission. The fluorescence of the complex was measured at 440 nm after excitation at 350 nm in acetonitrile. Under the optimum conditions, the fluorescence intensity was linear over a concentration range of 2.0–16.0 μg/mL (R^2=1) with a detection limit of 0.054 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 proposed and reference method revealed no significant differences in the performance of the two methods regarding the accuracy and precision respectively. The method was shown to be highly specific in the presence of indapamide, a diuretic that is commonly combined with perindopril erbumine. A proposal for the reaction pathway with 7-hydroxycoumarin was postulated.

Keywords Perindopril erbumine · 7-hydroxycoumarin · Charge transfer reaction · Spectrofluorimetry

Introduction

Perindopril erbumine (PDE) 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-1H-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].

Not only are chromatographic methods considered to have disadvantages such as the expensive instrumentation, but also the spectrophotometric methods that lack sensitivity in spite of being simple and economic. In contrast, spectrofluorimetric methods has several advantages, such as sensitivity, simplicity and selectivity. Therefore, it is still significant to make an effort for developing a new simple and sensitive spectrofluorimetric method for the determination of such an important drug, perindopril erbumine.

Derivatives of coumarin, especially 7-amino-4-methylcoumarin, are widely used reagents for labeling protein and nucleic acid conjugates [17]. In addition, 3-carboxy-7-hydroxycoumarin has been used for the determination of

✉ Hanan Fael
hananfael@hotmail.com

[1] Department of Analytical Chemistry, Faculty of Pharmacy, University of Aleppo, Aleppo, Syrian Arab Republic

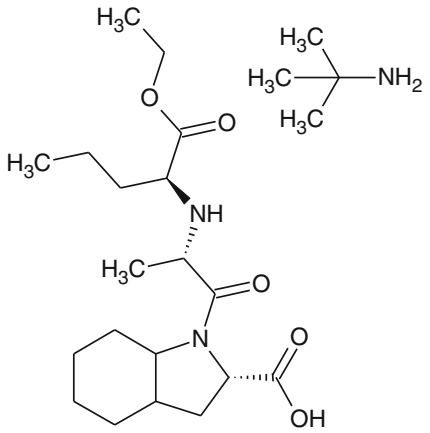


Fig. 1 Perindopril Erbumine structure

amphetamines [18]. To the best of our knowledge, 7-hydroxycoumarin has not been used before for the determination of drugs. In the present work, a novel spectrofluorimetric method has been developed for the determination of PDE using 7-hydroxycoumarin as a 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 350 nm and 440 nm, respectively. 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 0.05 mg/mL was prepared in acetonitrile. 7-Hydroxycoumarin was purchased from Merck, Germany. Solution of 7-Hydroxycoumarin was prepared in acetonitrile at 0.70 mg/mL. PDE and 7-hydroxycoumarin standard solutions were stable at lab temperature. All reagents and solvents were of analytical 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 0.5 mL of 7-hydroxycoumarin solution. Volumes were made up to mark with acetonitrile and mixed before the fluorescence intensity was measured at 440 nm after excitation at 350 nm against reagent blank that had been prepared similarly.

Determination of PDE/7-Hydroxycoumarin Stoichiometric Relationship

The composition ratio of the complex product was determined using job's continuous variation method and molar ratio

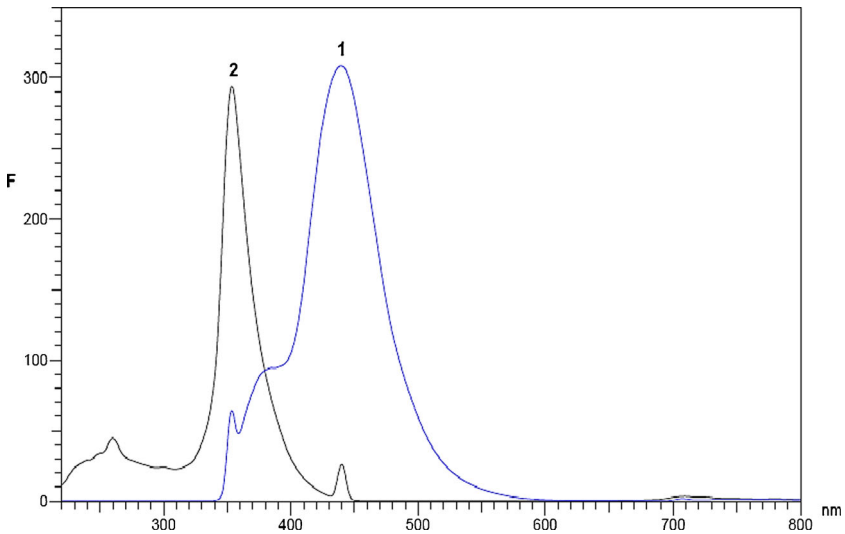


Fig. 2 Emission (1) and excitation (2) spectra of PDE/7-hydroxycoumarin complex in acetonitrile

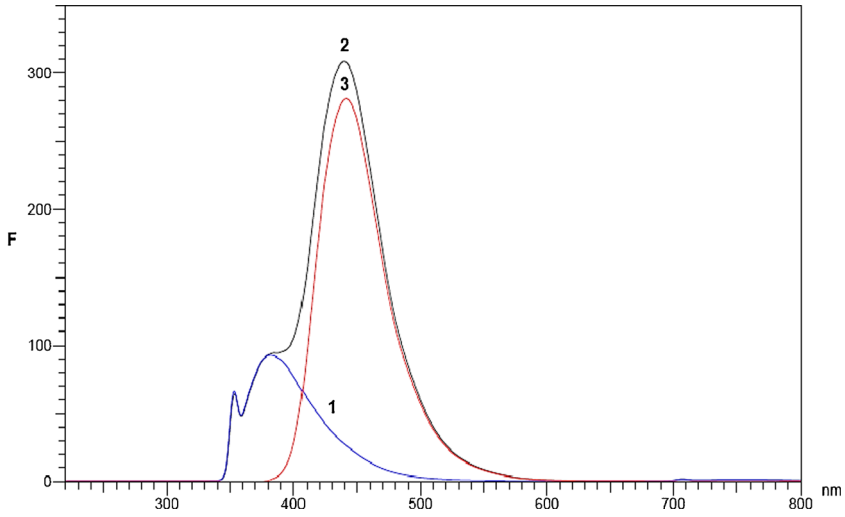


Fig. 3 Emission spectra of: (1) 7-hydroxycoumarin blank solution in acetonitrile, (2) PDE/7-hydroxycoumarin complex, and (3) PDE/7-hydroxycoumarin complex after blank subtraction

method. In job's method, equimolar solutions (5.0×10^{-4} M) of perindopril erbumine and 7-hydroxycoumarin were mixed in which the total moles of reactants were kept at 5.0×10^{-7} mole and steps were completed as described under the general procedure. A plot of fluorescence intensities against the mole fraction of reagent was then constructed.

On the other hand, the molar ratio method was carried out, where increasing volumes of 7-hydroxycoumarin were added to a fixed volume of drug solution. The obtained fluorescence intensities were then plotted against reagent molar ratio.

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 acetonitrile. The content of the flask was sonicated for 20 min then diluted to volume with acetonitrile. 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.

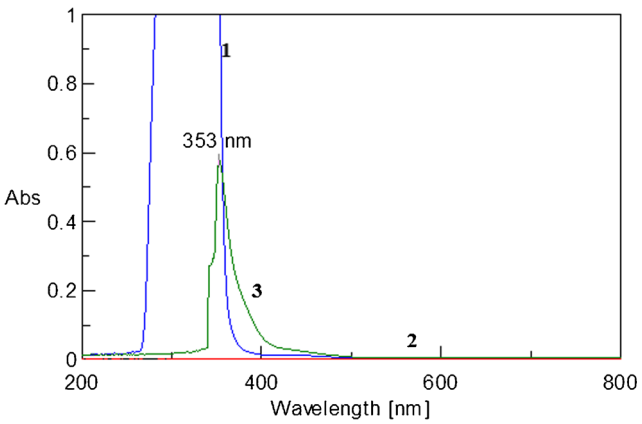


Fig. 4 Absorbance spectra of: (1) 7-hydroxycoumarin blank solution in acetonitrile, (2) PDE solution in acetonitrile, and (3) PDE/7-hydroxycoumarin complex

Results and Discussion

Fluorescence Spectra

The complex formed between PDE and 7-hydroxycoumarin was found to give a strong emission peak at 440 nm after excitation at 350 nm. This excitation wavelength was specific for the complex emission rather than for 7-hydroxycoumarin reagent blank (Fig. 2).

Although, 7-Hydroxycoumarin solution in acetonitrile exhibits a fluorescence peak at 382 nm after excitation at 350 nm, the complex formed between PDE and 7-hydroxycoumarin was found to fluoresce at 440 nm after excitation at 350 nm. This significant blue shifting (about 58 nm) has resulted from the formation of charge transfer complex and has allowed the measurement of PDE with only a small contribution of the reagent that was added in excess in the medium as shown in Fig. 3.

Table 1 The maximum emission wavelengths and fluorescence intensities of the PDE-7hydroxycoumarin complex in different organic solvents

Extraction solvent	$\lambda_{ex}/\lambda_{em}$ (nm)	Fluorescence intensity*
Dichloromethane	350/431	126
Acetone	350/419	52
Acetonitrile	350/440	340

* PDE concentration=20 μg/mL

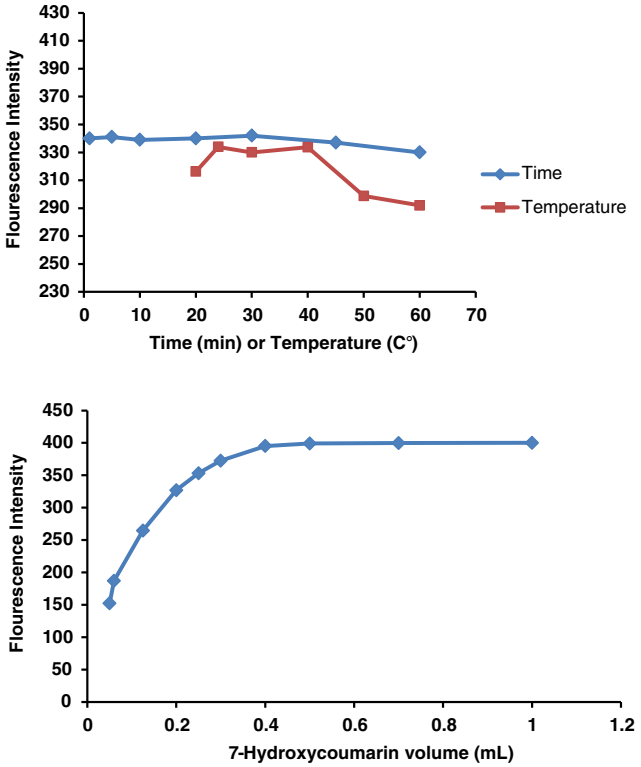


Fig. 5 **a**. Effect of the time and temperature on the reaction completion of PDE (20 μg/mL) with 7-hydroxycoumarin in acetonitrile **b**. Effect of the volume added of 7-hydroxycoumarin (0.7 mg/mL) on the complex formation with PDE (20 μg/mL)

Absorption Spectra

The complex formed between PDE and 7-hydroxycoumarin has a very light yellow color and exhibits a new absorption bands around 350 nm and 415 nm. In contrast, the blank solution of 7-hydroxycoumarin in acetonitrile is transparent and does not show any absorbance at the visible region, and absorbs maximally at 332 nm. Whereas, PDE solution does not absorb at any of these wavelengths (Fig. 4). The rise of new absorption bands confirms the formation of charge transfer complex between PDE and 7-hydroxycoumarin.

Effect of Solvent Nature

Different solvents with varied polarities and dielectric constants were examined (water, methanol, ethanol, isopropanol, acetonitrile, acetone, dimethylsulfoxide, dichloromethane, chloroform and n-hexan). Interestingly, the complex was formed only in three media: acetonitrile, acetone and dichloromethane. As shown from Table 1, the greatest fluorescence shifting and the highest intensity was observed in acetonitrile. Thus acetonitrile was chosen for continuing the study (Table 1).

Effect of Time and Temperature

The optimum reaction time was studied by following the fluorescence intensity at 440 nm for 1 h. It was observed that the complex got stabilized immediately after mixing and fluorescence intensity remained stable for at least 45 min (Figs. 5a, b).

In addition, the effect of temperature was also investigated. In this study, the reaction between PDE and 7-hydroxycoumarin was monitored at different temperatures (20 ° C, 24 ° C, 30 ° C, 40 ° C, 50 ° C and 60 ° C) for 10 min. The reaction was then balanced to lab temperature and fluorescence signal was obtained. A steady and maximum fluorescence was noticed at room temperature (24±2 °C) with a decrease in the fluorescence at temperatures higher than 40 ° C. Thus all measurements were carried out after mixing the reagent at laboratory ambient temperature (Fig. 5a, b).

Effect of 7-Hydroxycoumarin Reagent Volume

The influence of the volume of reagent solution was examined by adding an increasing volumes ranged from 0.1 to 1.0 mL of 0.7 mg/mL 7-hydroxycoumarin solution to a fixed amount of

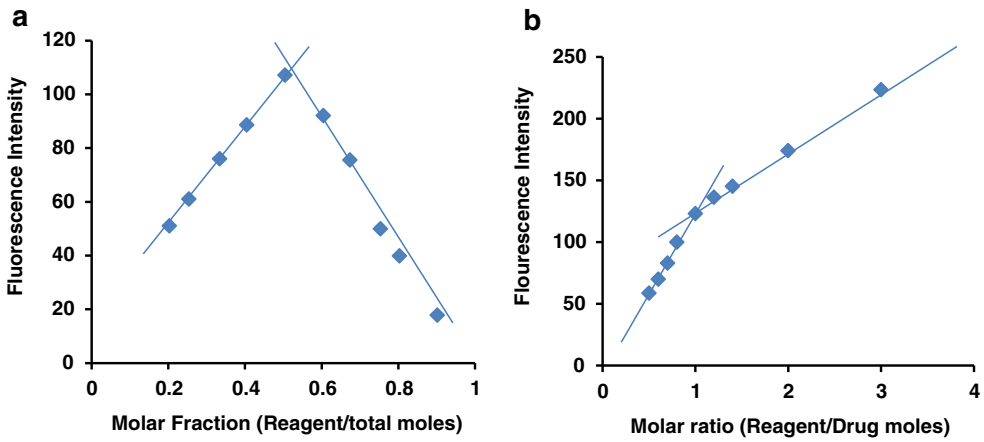
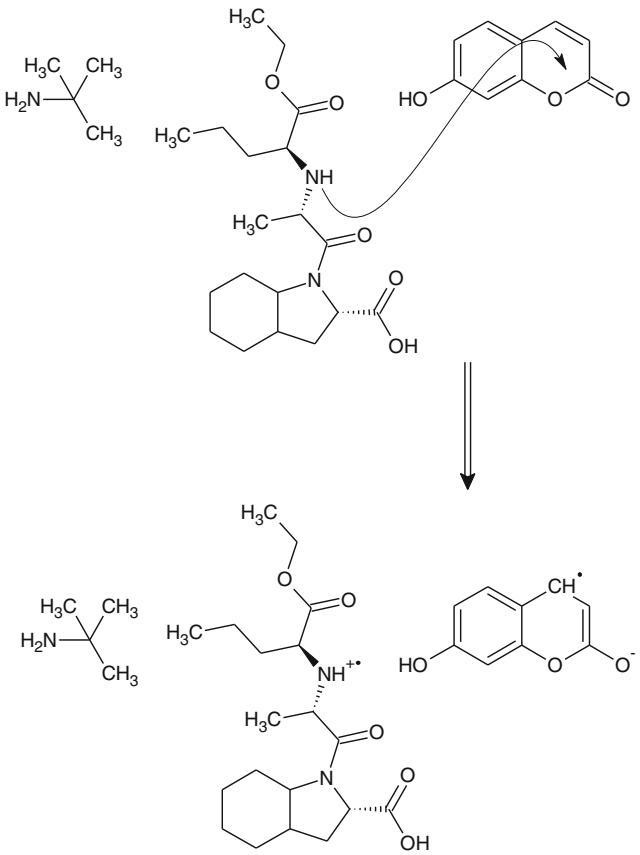


Fig. 6 Job's plot (**a**) and molar ratio plot (**b**) for the stoichiometric relation between PDE and 7-hydroxycoumarin

PDE. High fluorescence intensities were attained when more than 0.4 mL of the reagent solution was added (Fig. 6). Thus a fixed volume of 0.5 mL was used in the optimal procedure, which corresponds to a final reagent concentration of about 70 μg/mL (corresponds to a minimum molar ratio of 1:7 Drug/Reagent at the highest drug molar concentration of the linearity).

Stoichiometric Relationship of PDE/7-Hydroxycoumarin Complex

The stoichiometrirc relationship between PDE and 7-hydroxycoumarin was determined by Job's method of continuous variation and molar ratio method. As shown in Fig. 6, the stoichiometry of the reaction was found to be 1:1 ratio (7-hydroxycoumarin /PDE), confirming that one molecule of PDE reacts with one molecule of 7-hydroxycoumarin. It is more likely to form the complex though a charge transfer reaction between the drug acting as n-donor and 7-hydroxycoumarin as π-acceptor. The secondary amino group in the drug molecule has free electron pair and it is most probably involved in the complex formation. However, the carbonyl group at the heterocyclic section acts as a withdrawing group,



Scheme 1 Schematic illustration of reaction between PDE and 7-hydroxycoumarin in acetonitrile

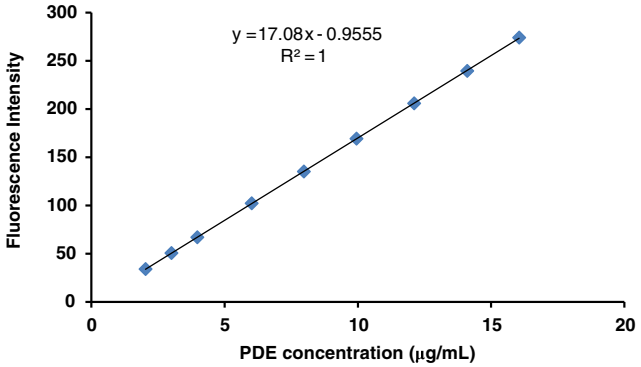


Fig. 7 Plot of fluorescence intensity versus PDE concentration

based on that, the reaction mechanism for PDE/7-hydroxycoumarin complex is proposed and given in Scheme 1.

Validation of the Proposed Method

Linearity

Under the optimum experimental conditions, standard calibration curve was constructed at nine concentration levels (n=5). The correlation coefficient was 1 indicating very good linearity over the concentration range of 2.0–16.0 μg/mL (Fig. 7). The intercept, slope, limit of detection (LOD), and limit of quantitation (LOQ) are summarized in Table 2. LOD and LOQ 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 [19, 20].

Selectivity

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

Table 2 Statistics and analytical parameters of PDE determination

Parameter	Result
$\lambda_{ex}/\lambda_{em}$ (nm)	350/440
Linear range (μg/mL)	2.0–16.0
Slope	17.08
Standard deviation in the slope	0.029
Intercept	–0.9555
Standard deviation in the intercept	0.2797
Correlation coefficient	1
Limit of detection (μg/mL)	0.054
Limit of quantification (μg/mL)	0.164

Table 3 Precision and accuracy for determination of PDE in pure form using proposed method

Perindopril erbumine (μg/mL)		SD (μg/mL)	RSD%	Recovery %	*t*-test[b]
Taken	Found[a]				
2.000	2.020±0.072	0.058	2.87	101.00	0.77
3.000	3.019±0.060	0.049	1.62	100.63	0.87
4.000	3.999±0.083	0.067	1.67	99.97	0.03
6.000	5.974±0.073	0.059	0.99	99.56	0.98
8.000	7.989±0.115	0.093	1.16	99.86	0.26
10.000	9.933±0.094	0.076	0.76	99.33	1.97
12.000	12.012±0.112	0.090	0.75	100.10	0.30
14.000	13.965±0.115	0.093	0.66	99.75	0.84
16.000	15.965±0.121	0.098	0.61	99.78	0.80

[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

(PVP k30), talc, and magnesium stearate. None of the studied excipients has given a significant fluorescence intensity and the maximum interference did not exceed 1.08 %. Unfortunately, coloring agents has been found to interfere as they reduce the fluorescence intensity of the complex. As a result, the proposed method is suitable for perindopril erbumine determination in its dosage forms that do not contain colors. Possible effect of indapamide, a diuretic which is commonly combined with perindopril erbumine in tablets, was thoroughly studied without interference at the emission wavelength of the complex. Thus, the method can be considered selective, and can be applied for the determination of perindopril erbumine in presence of indapamide.

Precision

The repeatability of 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%) ranged from 0.61 to 2.87 % for the smallest determined concentration, indicating good precision (Table 3).

Accuracy

The proposed method was applied on the available commercial tablets at three different concentration levels, 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 μ [19]:

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

Table 4 Application of the proposed method 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	6.40	6.45	100.83
		8.00	8.15	101.87
		9.60	9.71	101.17
Mean found%				**101.29**
RSD%				**0.52**
Revosyl	8 mg	6.40	6.70	104.70
		8.00	8.41	105.16
		9.60	10.14	105.64
Mean found%				**105.17**
RSD%				**0.45**

[a] Average of three determinations

Table 5 Application of the proposed method to a synthetic mixture spiked with PDE standard solution

Synthetic mixture	Amount spiked (μg/mL)	Amount found[a] (μg/mL)	Recovery %
Excipient solution[b]	6.40	6.49	101.43
	8.00	8.13	101.57
	9.60	9.75	101.60
			101.53
			0.08

[a] Average of three determinations

[b] prepared as mentioned under procedure for pharmaceutical sample

Where t is an absolute value. As shown in Table 2, 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 2) which varied from 99.33 to 101.00 %. (2) preparing a synthetic mixture of common excipients and spike it with known quantities of PDE then calculating recoveries percent that did not exceed 101.60 % (Table 5). (3) 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 6) using t- and F-tests. t_{exp} was calculated using the following equation [19]:

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

Where $\overline{X}_A$ and $\overline{X}_B$ 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 proposed method involves adding an excess amount of 7-hydroxycoumarin, even though only a small contribution comes from reagent molecules at the selected emission wavelength. Similarly, other experimental conditions such as time and temperature have no significant effect on fluorescence intensity of the reaction product. Thus, 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 method was 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 ranged from 102.60 to 106.45, which were similar to the recoveries recorded by the reference method (HPLC) as revealed by t- and F-test (Table 6).

Table 6 Precision and accuracy for determination of PDE in tablets

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	4.104±0.023 (102.60)	4.060±0.024 (101.50)	2.38, 0.94
	8 mg	8.516±0.048 (106.45)	8.440±0.040 (105.50)	2.39, 1.48

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

Conclusion

Novel, simple, selective and extremely rapid spectrofluorimetric method for the determination of PDE has been successfully developed and validated. The method involved the formation of a fluorescent charge transfer complex between PDE and 7-hydroxycoumarin. The proposed method was specific, precise and accurate with a comparable low detection limit value of 0.054 μg/mL. The method was effectively applied for determining PDE in pure form and in tablets without interference with the excipients. Therefore, the developed method can be suitable for routine analysis of PDE in quality control laboratories.

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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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