

RESEARCH ARTICLE

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Selective spectrofluorimetric method for the determination of perindopril erbumine in bulk and tablets through derivatization with dansyl chloride

Amir Alhaj Sakur¹, Tamim Chalati² and Hanan Fael^{1*}

Abstract

Background: Perindopril erbumine is an antihypertensive, which belongs to the category of angiotensin-converting enzyme inhibitors (ACE inhibitors) that inhibit the conversion of angiotensin I to angiotensin II.

Methods: A new, selective, and sensitive spectrofluorimetric method was developed for the determination of perindopril erbumine based on the reaction with dansyl chloride in alkaline medium to give a highly fluorescent derivative which was measured at 496 nm after excitation at 340 nm in dichloromethane. The reaction conditions were studied and optimized.

Results: Under the optimum conditions, the fluorescence intensity was linear over a concentration range of 1.0 to 21.0 µg/mL ($R^2 = 0.9997$) with a detection limit of 0.242 µg/mL. In order to validate the method, the results were compared with those obtained by a high performance liquid chromatography method.

Conclusions: The proposed method was successfully applied to the analysis of perindopril erbumine in pure form and tablets with good precision and accuracy as revealed by *t*- and *F* tests. The mechanism of the reaction has also been discussed.

Keywords: Perindopril erbumine; Dansyl chloride; Derivatization; Spectrofluorimetry

Background

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, Figure 1. Its empirical formula is $C_{19}H_{32}N_2O_5C_4H_{11}N$.

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

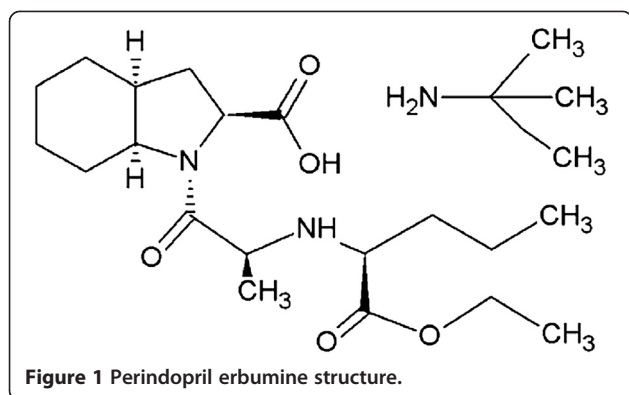
the inhibition of circulating and tissue ACE activity thereby reducing angiotensin II formation and decreasing vasoconstriction. Perindopril erbumine is also indicated for patients with congestive heart failure [British National Formulary BNF 2014].

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 (Raju and Rao 2011; Zaaza et al. 2013; Riyaz et al. 2012; Chaudhary et al. 2010; Jogia et al. 2010; Joseph et al. 2011; Prajapati et al. 2011a), HPLC-MS (Jaina et al. 2006 and Nirogi et al. 2006), high performance thin layer chromatography (Dewani et al. 2011), and spectrophotometry (Neelam et al. 2012; Rahman et al. 2012; Prajapati et al. 2011b). However, the chromatographic methods were found to have certain drawbacks, such as the expensive instrumentation and high analysis cost. Spectrophotometric methods, on the other

* Correspondence: hananfael@hotmail.com

¹Department of Analytical Chemistry, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria

Full list of author information is available at the end of the article



hand, are not such sensitive methods in spite of being simple and economic technique. Therefore, it is still significant to develop a new, simple and sensitive method for the determination of perindopril erbumine.

There seems to be no reports on determination of such important drug, perindopril erbumine, using spectrofluorimetry, which shows several advantages such as high sensitivity, low detection limit, ease of use, and less time consumption comparing with other analytical methods.

Dansyl chloride (DNS) is known to react with primary and secondary amines, phenolic and alcoholic hydroxyl groups, and carboxylic acid groups (Bartzatt 2003). DNS has been used as a fluorogenic reagent for the determination of many pharmaceutical compounds (Aydoğmuş et al. 2012; Abd El Ghaffar et al. 2011; Abdel Fattah et al. 2010; Ulu 2011; Karasakal and Ulu 2013).

This paper describes, for the first time, the derivatization of PDE with dansyl chloride. 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.

Methods

Chemicals and materials

All reagents and solvents were of analytical grade.

Perindopril erbumine (ROLABO outsourcing S.L., Spain) stock standard solution of 1.0 mg/mL was prepared in deionized (DI) water. This solution was freshly prepared at time of study. A series of working standard solutions were prepared by diluting aliquots of stock standard solution with DI water. Dansyl chloride (5-dimethylaminonaphthalene-1-sulphonyl chloride) was purchased from Merck, Germany. Solution of DNS was freshly prepared at 1.0 mg/mL in acetonitrile. Bicarbonate buffer (0.1 M) solution was prepared in DI water and adjusted to pH 9.5 with 0.1 M sodium hydroxide. Bicarbonate buffer solution was kept in refrigerator and used within about 5 days.

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

Instrumentation

Fluorescence spectra and measurements were obtained using fluorescence spectrophotometer F-2700 (Hitachi, Japan) equipped with xenon lamp. Excitation and emission wavelengths were set at 340 and 496 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 (HPLC) 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 × 4.6 mm).

Derivatization procedure

A 100 µL of PDE working standard solutions equivalent to a final concentration of 1.0 to 21.0 µg/mL was transferred into a series of 2-mL micro tubes that contain 100 µL of pH 9.5 bicarbonate buffer solution. A 300 µL of dansyl chloride solution was then added, and solution was mixed vigorously; then, tubes were kept in dry block heater at 40°C for 30 min. The tubes were then cooled, and the dansyl derivative was extracted three times with 1.5 mL of dichloromethane by a vortex mean. The organic layer was separated after centrifuging at 5,000 rpm for 1 min to ensure separation of organic-aqueous layers. The combined dichloromethane extracts were adjusted to 5 mL with the same solvent. The fluorescence intensity of the resulting solution was measured at 496 nm after excitation at 340 nm against reagent blank that had been treated similarly.

Determination of stoichiometric relationship

The composition ratio of the derivative product was determined using Job's continuous variation method and molar ratio method. In Job's method, equimolar solutions (2.26×10^{-3} M) of perindopril erbumine and dansyl chloride were mixed in which the total moles of reactants were kept at 4.53×10^{-7} moles. The volume of reaction phase was kept constant at 300 µL; then, steps were completed as described under the derivatization 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. Increasing volumes of dansyl chloride 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 8 mg of PDE was transferred into 25-mL volumetric flask and dissolved in 20 mL of bicarbonate buffer. The content of the flask was sonicated for 20 min then diluted to volume with bicarbonate buffer. Portion of this solution was centrifuged at 5,000 rpm for 10 min. Suitable aliquot of the supernatant was then transferred into micro tubes that contain 100 μ L of DI water. A 300 μ L of dansyl chloride solution was then added, and procedure was continued as mentioned above.

Results and discussion

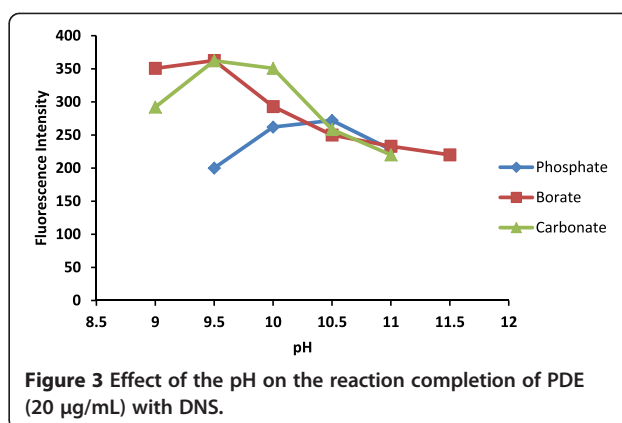
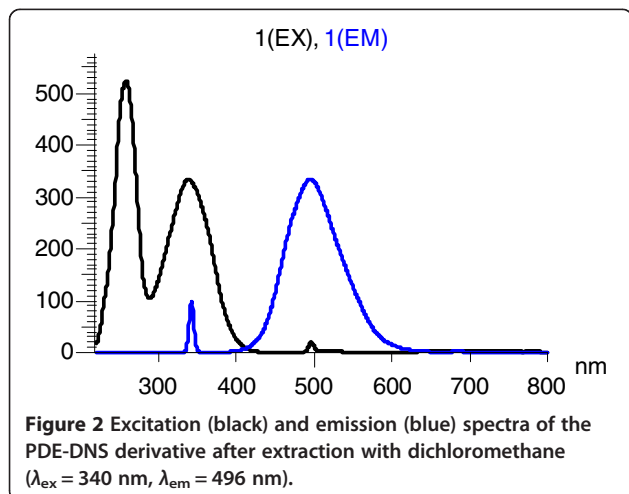
Fluorescence spectra

Perindopril erbumine contains two amino groups and can therefore react with dansyl chloride in alkaline medium to give a strongly fluorescent product. On contrast, a reagent blank gave a negligible fluorescence signal at the chosen excitation and emission wavelengths. Under the described experimental conditions, the excitation spectra was obtained showing two maximum excitation wavelength at 260 and 340 nm, respectively. The excitation wavelength of 340 was employed, and emission spectra were obtained showing emission wavelength maxima at 496 nm (Figure 2).

Optimization of reaction conditions

Effect of pH

Effect of pH on the derivatization reaction was investigated using three different buffers in the alkaline region. Bicarbonate, phosphate, and borate buffers were studied. Buffers with amino groups such as tris and triethylamine were excluded, since they react with DNS reagent. The reaction was carried out initially at lab temperature for 30 min. The highest fluorescence intensity was obtained using pH 9.5 bicarbonate and borate buffer (Figure 3).



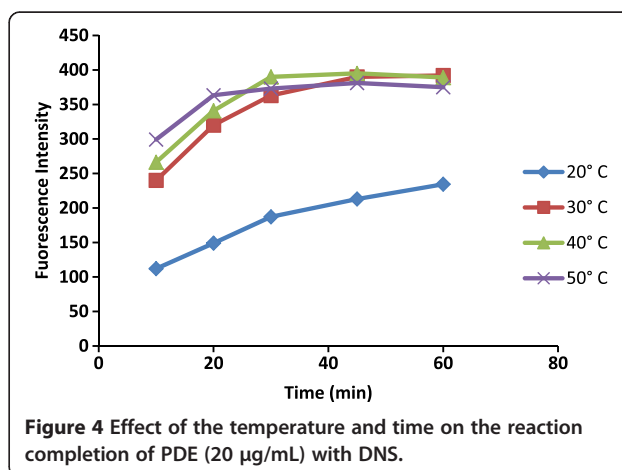
Following that, in the study of temperature effect, bicarbonate and borate buffer were tested to find out which buffer is more appropriate.

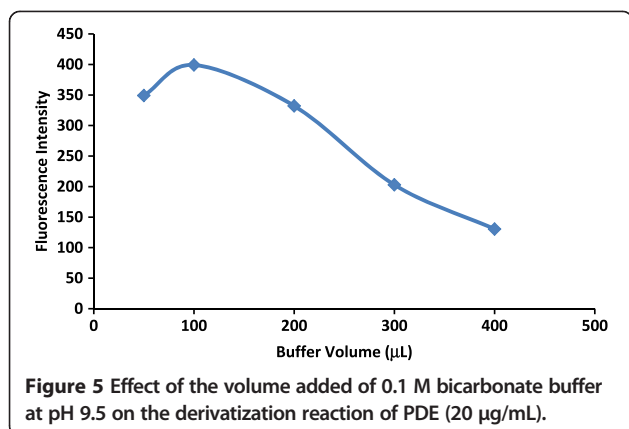
Effect of time and temperature

In this study, the reaction between PDE and dansyl chloride was performed using pH 9.5 bicarbonate buffer at different temperatures (20°C, 30°C, 40°C, and 50°C) for various time intervals (10, 20, 30, 45, and 60 min). As it is seen in Figure 4, the derivatization reaction was found to be completed after 30 min at 40°C. Subsequently, borate buffer was tested at 40°C for 30 min and bicarbonate buffer was preferred over it.

Effect of buffer volume and concentration

Under the above described experimental conditions, the volume required of 0.1 M bicarbonate buffer (pH 9.5) was tested. As shown in Figure 5, a volume of 100 μ L has given a maximum fluorescence intensity; thus, it was chosen to proceed the reaction. In addition, the buffer concentration was investigated at three different molarities (0.05, 0.1, and 0.2 M). It was found that 0.1 M has given the best results; thus, it was used for the next experiments.





Effect of dansyl chloride volume

The influence of the volume of dansyl chloride solution was examined by addition of different volumes of 0.1% w/v reagent in the range of 50 to 500 µL (Figure 6). A maximum and steady fluorescence intensity was obtained when more than 200 µL of dansyl chloride solution was utilized. Thus, a fixed volume of 300 µL was used in the optimal procedure

Effect of extraction solvent

The aqueous reaction medium contains, in addition to the derivatization product, a highly fluorescent secondary product: dansyl hydroxide (Bartzatt 2003). In order to avoid the interference of this compound, an extraction step has been performed, so that the polar dansyl hydroxide remained in the aqueous phase and derivatization product moved to the immiscible organic solvent. For this purpose, different solvents including dichloromethane, chloroform, diethyl ether, and ethyl acetate were tested.

Ethyl acetate was rejected, since the blank value was very high when it was employed as an extractant. However, the highest fluorescence was obtained upon using dichloromethane (Table 1). The emission and excitation

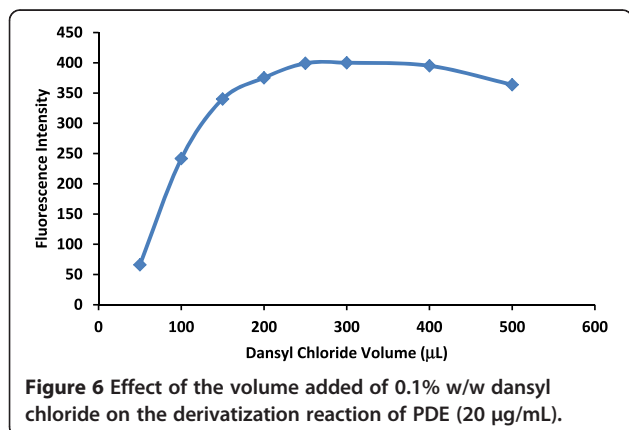


Table 1 The maximum excitation and emission wavelengths of the PDE-DNS derivatization product and its fluorescence intensities in different organic extraction solvents

Extraction solvent	$\lambda_{ex}/\lambda_{em}$ (nm)	Fluorescence intensity ^a	
		Sample	Blank
Dichloromethane	340/496	540.3	3.6
Chloroform	337/494	432.7	3.5
Diethyl ether	330/489	391.5	3.0
Ethyl acetate	323/459	876.9	636.3

^aPDE concentration = 20 µg/mL.

spectra of derivatization product of perindopril erbumine in dichloromethane are shown in Figure 2.

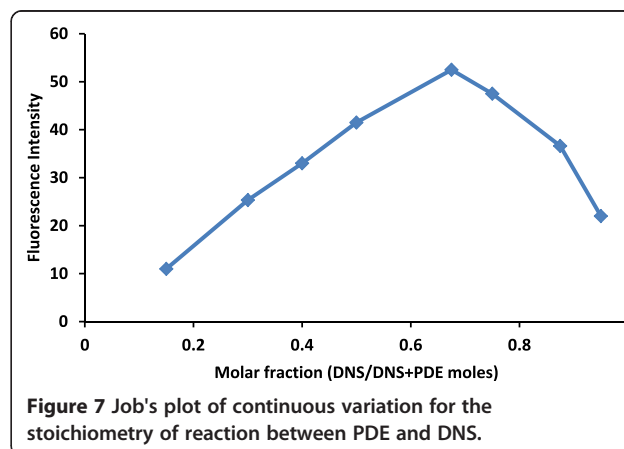
Stoichiometric relationship of the reaction

Under the described conditions, the stoichiometry of the reaction between the drug and DNS was studied by Job's method of continuous variation and molar ratio method. As shown in Figures 7 and 8, the stoichiometry of the reaction was found to be 2:1 ratio (DNS/drug), confirming that one molecule of PDE reacts with two molecules of DNS. Perindopril erbumine contains a secondary amine in the perindopril moiety and a primary aliphatic amine in the erbumine molecule. In alkaline medium, these amine groups become more basic, and thus the electron pairs on the nitrogen atoms are free and could be involved easily in nucleophilic reactions. The reaction may be illustrated by the attack of these nucleophilic groups into the sulfonyl chloride group of DNS. A schematic proposal of the reaction pathway is given in Scheme 1.

Validation of the proposed method

Linearity

Under the optimum experimental conditions, standard calibration curve was constructed at eight concentration levels ($n = 5$). The correlation coefficient was 0.9997, indicating good linearity over the concentration range of 1.0 to 21.0 µg/mL. The intercept, slope, limit of



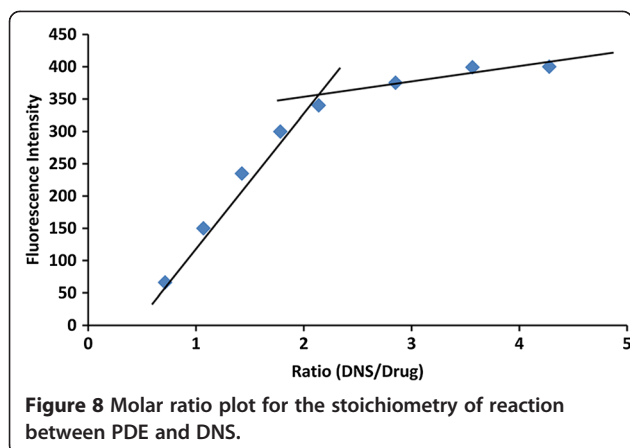


Table 2 Statistics and analytical parameters of PDE determination

Parameter	Result
$\lambda_{ex}/\lambda_{em}$ (nm)	340/496
Linear range ($\mu\text{g/mL}$)	1.0 to 21.0
Slope	27.164
Standard deviation in the slope	0.178
Intercept	4.5958
Standard deviation in the intercept	1.992
Correlation coefficient	0.9997
Limit of detection ($\mu\text{g/mL}$)	0.242
Limit of quantification ($\mu\text{g/mL}$)	0.733

detection (LOD), and limit of quantitation (LOQ) are summarized in Table 2. LOD and LOQ values were calculated according to ICH Q2B using the following equations:

$$\text{LOQ} = 10 \sigma / S$$

$$\text{LOD} = 3.3 \sigma / S$$

where σ is the standard deviation of intercept of regression line and S is the slope of the calibration curve (Table 2).

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 (PVP k30), talc, and magnesium stearate. None of the studied excipients has given any fluorescent product. So, the proposed method is suitable for analysis of perindopril erbumine in its dosage forms and application in quality control laboratories.

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 4.92% for the smallest concentration, indicating good precision (Table 3).

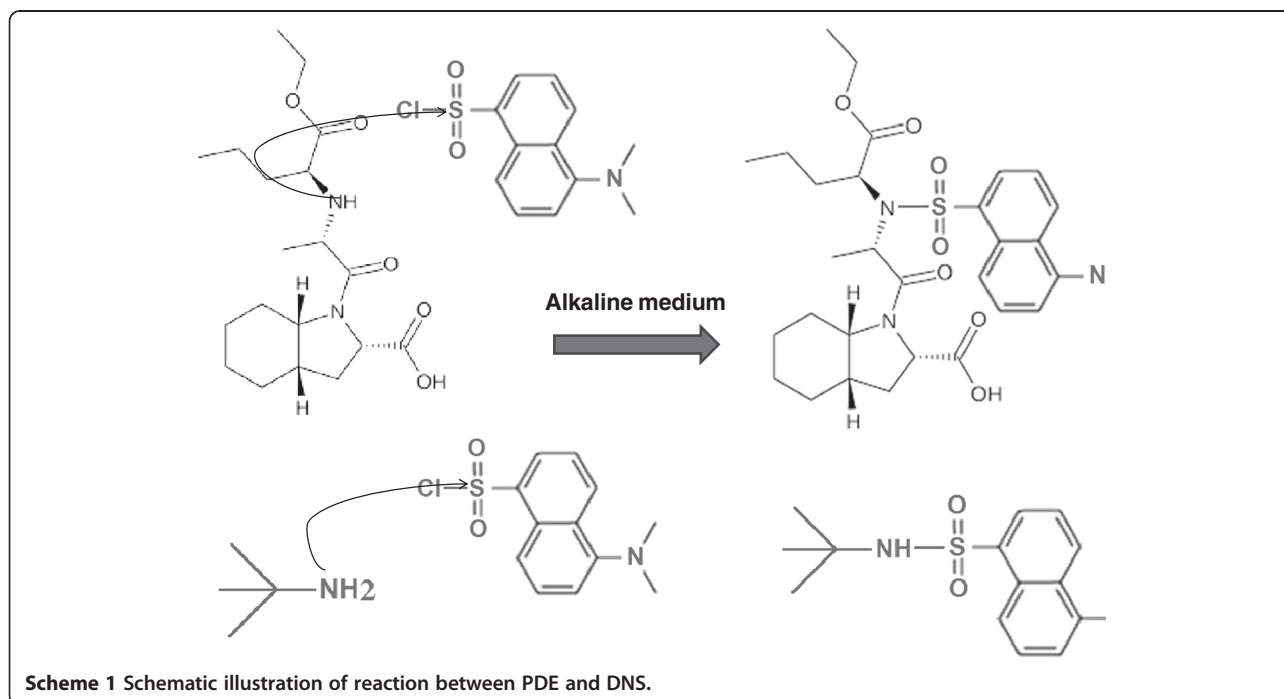


Table 3 Precision and accuracy for determination of PDE in pure form

Perindopril erbumine ($\mu\text{g/mL}$)		SD	RSD%	Recovery %	t -test ^b
Taken	Found ^a				
1.00	0.976 \pm 0.059	0.048	4.92	97.60	1.118
2.20	2.195 \pm 0.074	0.060	2.73	99.77	0.186
4.50	4.590 \pm 0.104	0.084	1.83	102.00	2.395
6.70	6.733 \pm 0.118	0.095	1.41	100.49	0.776
8.70	8.770 \pm 0.156	0.126	1.44	100.80	1.242
11.20	11.169 \pm 0.125	0.101	0.90	99.72	0.686
16.80	16.940 \pm 0.182	0.147	0.86	100.83	2.129
21.00	20.930 \pm 0.160	0.129	0.61	99.66	1.213

^aAverage of five determination \pm confidence limit.

^bThe tabulated t -value at 95% confidence limit for 4 degrees of freedom ($n = 5$) is 2.78.

Accuracy

The proposed method was applied on the available commercial tablets that contain PDE, 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 (Harvey 2009):

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

As shown in Table 3, the calculated t -value is less than tabulated t (0.05, 4) value (2.78), and thus there are 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 97.60% to 102.00%. (2) The method's accuracy is also judged by comparing the results obtained from the presently proposed method with those obtained from a reference method such as high performance liquid chromatography (HPLC) (Raju and Rao 2011). The obtained results were statistically compared with each other (Table 4) using

t - and F -tests. t_{exp} was calculated using the following equation (Harvey 2009):

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

Where \bar{X}_A and \bar{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

Robustness was investigated by evaluating the influence of minute variations in the experimental conditions such as volume of reagent ($\pm 10 \mu\text{L}$), volume of bicarbonate solution ($\pm 5 \mu\text{L}$), and reaction time ($\pm 5 \text{ min}$). These minor changes that may happen during the analysis did not have any significant effect on fluorescence intensity of the reaction product.

Application to tablets

The proposed method was successfully applied to analysis of two different commercial tablets (Revosyl[®] and Neomeril[®] tablets) were labeled to contain 4 and 8 mg of PDE. The mean recovery values were ranged from 96.50 to 104.25, which were identical to the recoveries recorded by the reference method (HPLC) as revealed by t - and F test (Table 4).

Comparison with reported analytical methods

The analytical methods reported in the literature suffered from one or more disadvantages like poor sensitivity, use of expensive chemicals, and/or complicated instruments, as can be seen from Table 5. However, the proposed method was found to be more sensitive than spectrophotometric and chromatographic methods (except for LC-MS);

Table 4 Precision and accuracy for determination of PDE in tablets

Tablets	Labeled amount of PDE	Average PDE found (mg/tablet) \pm SD ^a (recovery %) ^b		t - and F test ^c
		Proposed method	Reference method ^d	
Revosyl	4 mg	4.10 \pm 0.036 (102.50)	4.06 \pm 0.024 (101.50)	1.734, 2.207
	8 mg	8.34 \pm 0.064 (104.25)	8.44 \pm 0.040 (105.50)	2.464, 2.612
Neomeril	4 mg	3.86 \pm 0.035 (96.50)	3.90 \pm 0.026 (97.50)	1.604, 1.830
	8 mg	7.77 \pm 0.075 (97.12)	7.87 \pm 0.042 (98.37)	2.299, 3.196

^aAverage and standard deviation of five determinations for the proposed method and three determinations for the reference method.

^bRecoveries were calculated considering the labeled amount reported by the manufacturer.

^cThe tabulated t -value at 95% confidence limit for 4 degrees 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 6.944.

^dHPLC (Raju and Rao 2011).

Table 5 Performance characteristics of the existing methods used for the determination of PDE

No	Method	Medium	Application	Linear range	Reference
1	HPLC	Phosphate buffer: acetonitrile (65:35)	Tablets	20 to 100 µg/mL	Raju and Rao 2011
2	HPLC	Phosphate buffer: acetonitrile: Tetrahydrofuran (60:40:0.1)	Tablets (combination with amlodipine)	10 to 100 µg/mL	Zaazaa et al. 2013
3	HPLC	Phosphate buffer: acetonitrile (55:45)	Tablets (combination with amlodipine)	16 to 96 µg/mL	Riyaz et al. 2012
4	HPLC	Acetonitrile: acidic water (50:50)	Tablets (combination with losartan potassium)	1 to 30 µg/mL	Chaudhary et al. 2010
5	HPLC	Phosphate buffer: acetonitrile (75:25)	Dosage forms (combination with indapamide)	24 to 56 µg/mL	Jogia et al. 2010
6	HPLC	Phosphate buffer: acetonitrile (60:40)	Dosage forms (combination with indapamide)	8 to 24 µg/mL	Joseph et al. (2011)
7	HPLC	Phosphate buffer: acetonitrile (65:35)	Tablets (combination with amlodipine)	8 to 60 µg/mL	Prajapati et al. 2011a
8	LC-MS/MS	-	Human plasma	0.5 to 350 ng/mL	Jaina et al. 2006
9	LC/MS	-	Human plasma	0.1 to 100 ng/mL	Nirogi et al. [2006]
10	HPTLC	Dichloromethane: methanol: glacial acetic acid (9.5:0.5:0.1)	Dosage forms (combination with indapamide)	1 to 5 µg/band	Dewani et al. 2011
11	Spectrophotometry	Chloroform	Tablets	5 to 125 µg/mL	Neelam et al. 2012
12	Spectrophotometry	Dimethylsulfoxide	Tablets	2.5 to 25 µg/mL	Rahman et al. 2012
13	Spectrophotometry	Methanol	Tablets (combination with amlodipine)	4 to 12 µg/mL	Prajapati et al. 2011b
14	Spectrofluorimetry	Dichloromethane	Tablets	1 to 21 µg/mL	This work

in addition to less time-consuming compared with HPLC methods.

Conclusion

New, simple, and sensitive spectrofluorimetric method for the determination of PDE has been successfully developed and validated. The method involved the formation of a fluorescent derivatization product resulted from the reaction of PDE with DNS in alkaline medium. The proposed method was specific, precise, and accurate with a comparable low detection limit value of 0.242 µg/mL. The method was effectively applied for determining PDE in pure form and in tablets without any interference with the excipients. Therefore, the developed method can be suitable for routine analysis of PDE in quality control laboratories.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HF has performed the experimental and analytical work and prepared the draft of the manuscript. The guidelines and supervision of this work was provided by AAS. AAS and TC read and modified the manuscript. All authors read and approved the final manuscript.

Author details

¹Department of Analytical Chemistry, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria. ²Department of Pharmaceutics, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria.

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