

Selective Spectrofluorimetric Method for the Determination of Perindopril Erbumine in Bulk and Tablets through Derivatization with *O*-Phthalaldehyde in Presence of 3-Mercaptopropionic Acid

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ABSTRACT: A new, selective and sensitive spectrofluorimetric method was developed and validated for the determination of perindopril erbumine via derivatization with *o*-phthalaldehyde in presence of 3-mercaptopropionic acid in alkaline medium. The resulted derivative has a strong blue fluorescence that was measured at 452 nm after excitation at 340 nm in aqueous solution. The reaction conditions were carefully studied and optimized. Under the optimum conditions, the fluorescence intensity was linear over a concentration range of 1.2 – 8.4 $\mu\text{g/mL}$ ($R^2 = 0.9997$) with a detection limit of 0.097 $\mu\text{g/mL}$. 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 difference in the performance of the two methods regarding the accuracy and precision respectively. A proposal for the reaction pathway with *o*-phthalaldehyde was postulated.

Keywords: Perindopril erbumine, *O*-phthalaldehyde, Derivatization, Spectrofluorimetry.

1. 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 (2*S*,3*aS*,7*aS*)-1-[(2*S*)-2-[[[(1*S*)-1-(ethoxycarbonyl) butyl] amino] propanoyl]octahydro-1*H*-indole-2-carboxylate. Its molecular formula is $\text{C}_{19}\text{H}_{32}\text{N}_2\text{O}_5\text{C}_4\text{H}_{11}\text{N}$ (Fig. 1).

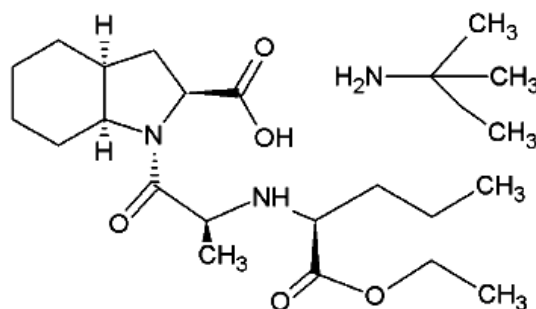


Fig.1 Perindopril Erbumine structure.

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 [BNF, 2014].

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.

Only few analytical methods has been reported for the determination of PDE in its bulk, dosage forms and human plasma, such as high performance liquid chromatography (Raju and Rao [2011]; Zaazaa et al [2013]; Riyaz et al [2012]; Simončič et al [2008]; Chaudhary et al [2010]; Jogia et al [2010]; Joseph et al [2011]; Prajapati et al [2011]), HPLC-MS (Jiana 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 [2011]; Sharma [2011]). However, the chromatographic methods were found to have certain disadvantages, such as the expensive instrumentation and high analysis cost. Spectrophotometric methods, on the other hand, are not such sensitive methods in spite of being simple and economic as technique. Therefore, it is still significant to develop a new simple and sensitive spectrofluorimetric method for the determination of such an important drug, perindopril erbumine.

O-Phthalaldehyde (OPA), in combination with a thiol compound, is known to react with primary amines (Garcia et al [1989]). The reaction of OPA with amino acids in presence of 2-mercaptoethanol was first reported by Roth in 1971 (Dorresteijn et al [1996]). It has been used for the determination of many amino acids (Dorresteijn et al [1996]) as well as cholesterol (Rudel and Morris [1973]). Several pharmaceutical compounds have been also determined through this approach (Vermeij and Edelbroek [2004]; El-Enany et al [2010]; Michail et al [2011]; Ramadan [2014]).

No attempts have been made for the fluorimetric determination of PDE. This paper describes, for the first time, the derivatization of PDE with *o*-phthalaldehyde in presence of 3-mercaptopropionic acid. 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.

2. EXPERIMENTAL

2.1. 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 340 nm and 452 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 μ m, 100 mm \times 4.6 mm).

2.2. Reagents and solutions

Perindopril erbumine (ROLABO outsourcing, S.L. Spain) standard solution of 0.1 mg/mL was prepared in deionized (DI) water. This solution was freshly prepared at time of study. *o*-phthalaldehyde (OPA) and 3-Mercaptopropionic acid (3MPA) were purchased from Sigma Aldrich, Germany. Solutions of OPA and 3MPA were prepared in methanol at 0.02 M and 0.1 M, respectively. These solutions were stable at refrigerator temperature. The OPA and 3MPA derivatization solution was prepared freshly by mixing suitable volumes of OPA and 3MPA methanolic solutions and diluting it with water after adding 2 mL of bicarbonate buffer solution (pH 10.5) to get a final concentration of 1.5×10^{-3} M and 3.0×10^{-3} M of OPA and 3MPA, respectively. The derivatization solution is not stable and should be used within few hours. Bicarbonate buffer (0.1M) solution was prepared in DI water and adjusted to pH 10.5 with 1 M sodium hydroxide. Bicarbonate buffer solution was kept in refrigerator and used within about 5 days. All reagents and solvents were of analytical grade.

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

2.3. Derivatization procedure

Increasing volumes of PDE working standard solution were transferred into series of 5 mL volumetric flasks that contain 0.5 mL of bicarbonate buffer pH 10.5 and 0.5 mL of OPA/3MPA derivatization solution. Solutions were mixed gently and allowed to stand at room temperature for 15 minutes. Volumes were made up to mark with DI water and mixed before the fluorescence intensity was measured at 452 nm after excitation at 340 nm against reagent blank that had been treated similarly.

2.4. Determination of OPA/3MPA stoichiometric relationship

The composition ratio of the derivatization reagent was determined using molar ratio method, in which fixed volumes of PDE and OPA solutions correspond to 4.5×10^{-8} and 5×10^{-7} moles of PDE and OPA,

respectively, were added. Increasing volumes of 3MPA solution were then added to each solution and procedure was then completed as described above. The obtained fluorescence intensities were then plotted against 3MPA molar ratio.

2.5. Procedure for pharmaceutical samples

Ten individual tablets were weighed and pulverized carefully. An accurately weighed amount of the powder equivalent to 8mg of PDE was transferred into 50 mL volumetric flask and dissolved in 25 mL of bicarbonate buffer. The content of the flask was sonicated for 20 min then diluted to volume with DI water. A portion of this solution was centrifuged at 5000 rpm for 10 minutes. Suitable aliquot of the supernatant was then transferred into 5 mL volumetric flask and procedure was continued as described under the derivatization procedure.

3. Results and Discussion

3.1. Fluorescence spectra

Perindopril erbumine contains primary amino group and can therefore reacts with OPA in presence of 3MPA in alkaline medium to give a strongly fluorescent isoindole derivative. OPA is nonfluorescent itself, and when present in excess does not break down or react to form fluorescent by-products, as shown clearly in figure 2. Under the described experimental conditions, the shiny blue fluorescence was observed at 452 nm after excitation at 340 nm (Fig. 3).

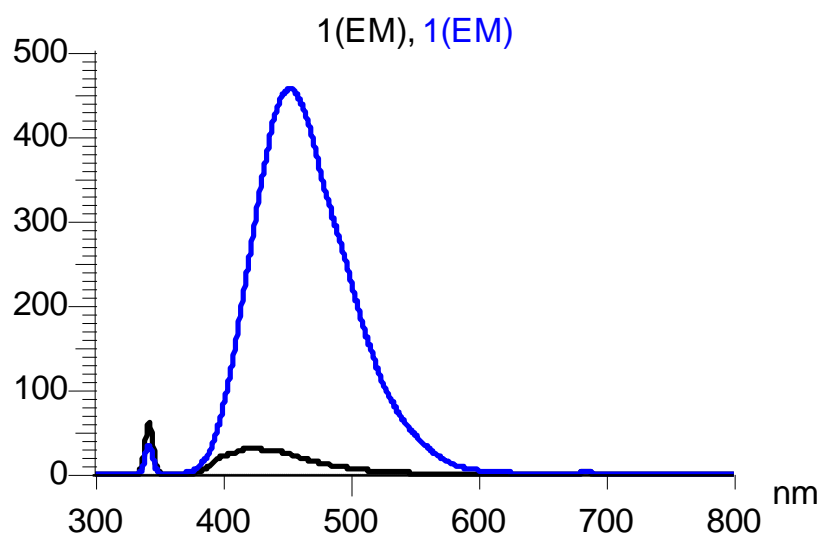


Fig.2 Emission spectra of PDE/OPA derivative (blue) and blank (black) after excitation at $\lambda_{ex}=340$ nm.

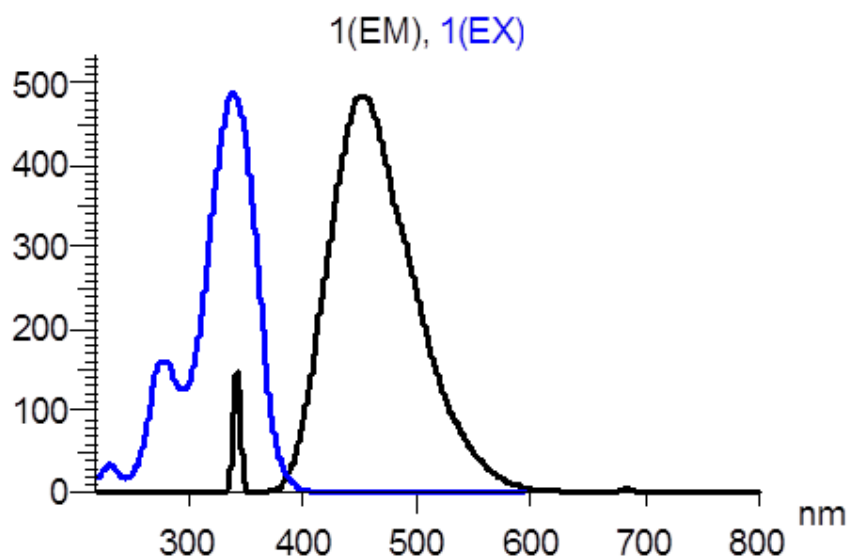
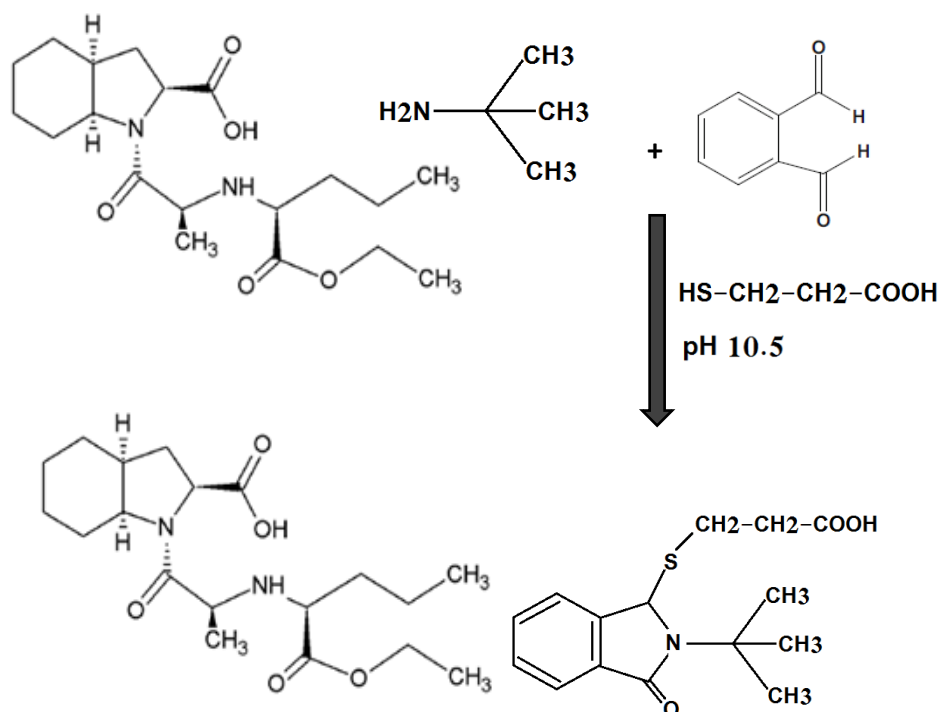


Fig. 3 Excitation (blue) and emission (black) spectra of the PDE/OPA derivative ($\lambda_{\text{ex}}=340\text{ nm}$, $\lambda_{\text{em}}=452\text{ nm}$).

The fluorescent isoindole product was rapidly formed at room temperature, but subsequently degraded to non-fluorescent derivatives. A schematic explanation of the reaction pathway is given in Scheme 1. Fluorescence quantum yield and stability of derivative are dependent to the particular amine, excess of OPA, pH of alkaline medium, and mostly on the nature of the nucleophile (thiol) used in the cyclization reaction. However, 3MPA seems to be the best thiol compound used along with OPA in derivatization reaction, since it produces the most stable amino acid derivatives with fluorescent yields comparable to other thiols. Thus, 3MPA was chosen for this study.



Scheme 1 Proposed reaction pathway between PDE and OPA in presence of 3MPA.

3.2. Optimization of reaction conditions

3.2.1. Effect of pH

To optimize the reaction the pH effect of three different buffers was studied. Bicarbonate, phosphate, and borate buffers (0.1M) were used each at its working range. Buffers with amino groups such as Tris and Triethylamine were excluded, since they react with OPA. The reaction was carried out at room temperature for 10 min. The highest fluorescence intensity was obtained using bicarbonate buffer at pH 10.5 (Fig. 4). A study of needed buffer volume showed that 0.5 mL of bicarbonate buffer was adequate.

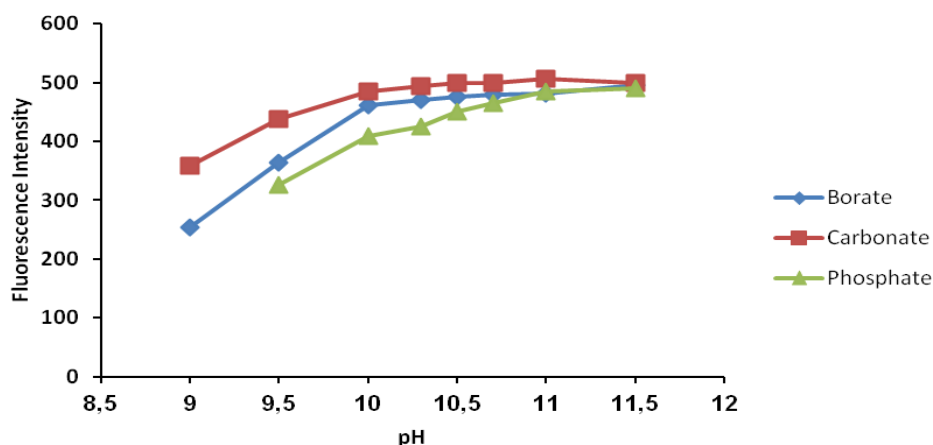


Fig. 4 Effect of the pH on the reaction completion of PDE (10 µg/mL) with OPA/3MPA.

The effect of buffer concentration (0.1-0.5 M) was also investigated and the reaction was monitored within the time to find out whether buffer strength plays any role in the reaction rate and fluorescence stability of isoindole product. As shown in figure 5, a molar concentration of 0.1 was sufficient to get a maximum and steady fluorescence.

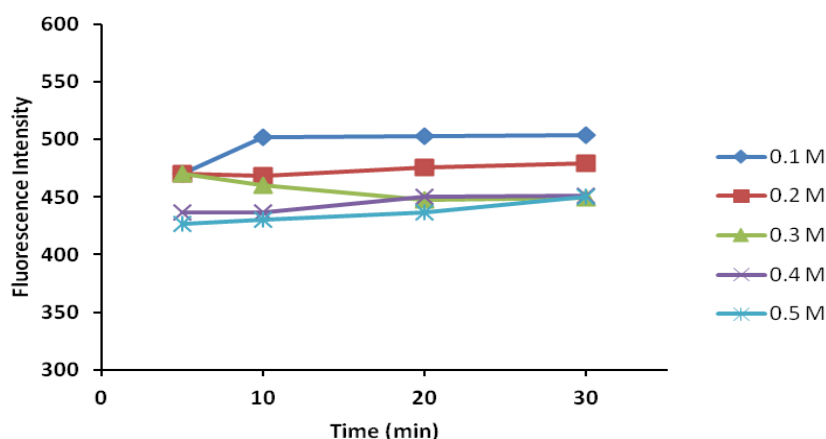


Fig. 5 Effect of the bicarbonate buffer strength on the reaction of PDE (10 µg/mL) with OPA/3MPA over time.

3.2.2. Effect of time and temperature

In this study, the reaction between PDE and OPA/3MPA was performed using pH 10.5 bicarbonate buffer at different temperatures (20° C, 25° C, 30° C, 40° C, 50° C and 60° C) at seven time intervals (5, 10, 15, 20, 30, 45, and 60 minutes). A steady and maximum fluorescence was noticed at room temperature (25 ± 2°C)

within 10 - 30 min, thus 15 min was an adequate reaction time. A slight increase in the fluorescence was observed when the reaction was left up to one hour. High temperature up to 60 °C has affected the reaction negatively, which can be due to the instability of the isoindole compound (Fig. 6).

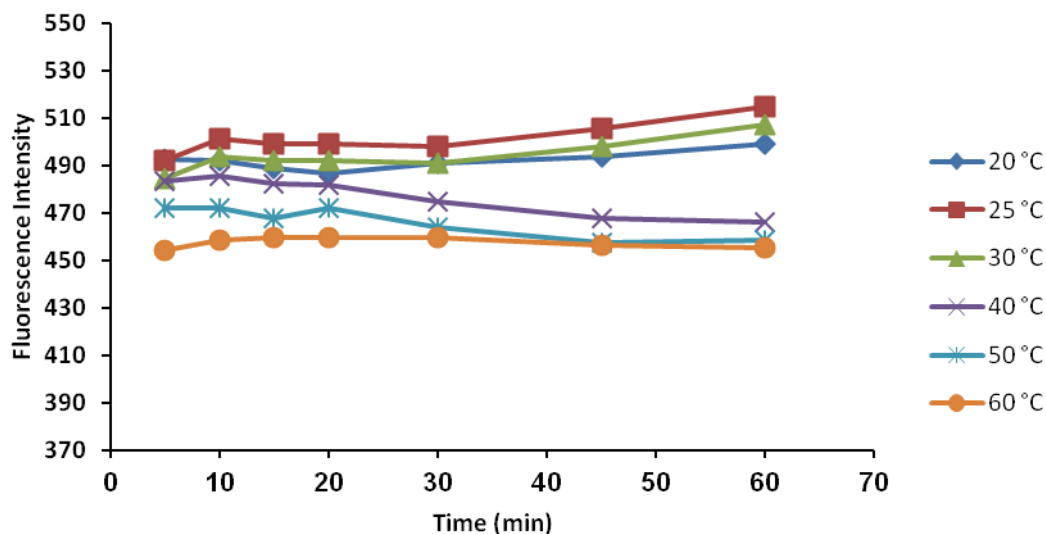


Fig. 6 Effect of the temperature and time on the reaction completion of PDE (10 µg/mL) with OPA/3MPA.

3.2.3. Effect of OPA reagent volume

The influence of the volume of reagent solution was examined by addition of increasing volumes of OPA/3MPA (1.5×10^{-3} M and 3.0×10^{-3} M, OPA/3MPA) derivatization solution. Volumes were ranged from 0.1 – 0.7 mL. High fluorescence intensities were attained when more than 0.3 mL of the reagent solution was added. Thus a fixed volume of 0.5 mL was used in the optimal procedure. (Fig. 7).

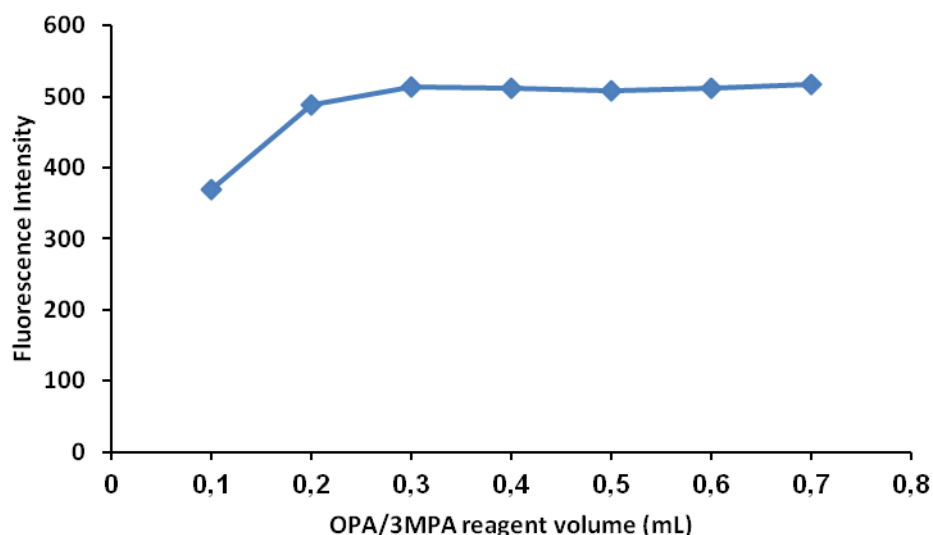


Fig. 7 Effect of the volume added of OPA/3MPA solution on the derivatization reaction of PDE (10 µg/mL).

3.2.4. Effect of solvent

Effect of diluting solvent was also investigated. Six different solvents (methanol, ethanol, isopropanol, acetone, acetonitrile, and dimethylsulfoxide) have been tested and compared to water as diluting solvent. Higher blank value in addition to lower and less stable fluorescence intensity was observed in methanol and acetonitrile in comparison with water, with an emission peak shifted to 440 nm (Figure 8). However, cloudy solutions were generated with other solvents. Thus, water was chosen as a perfect solvent to perform this reaction.

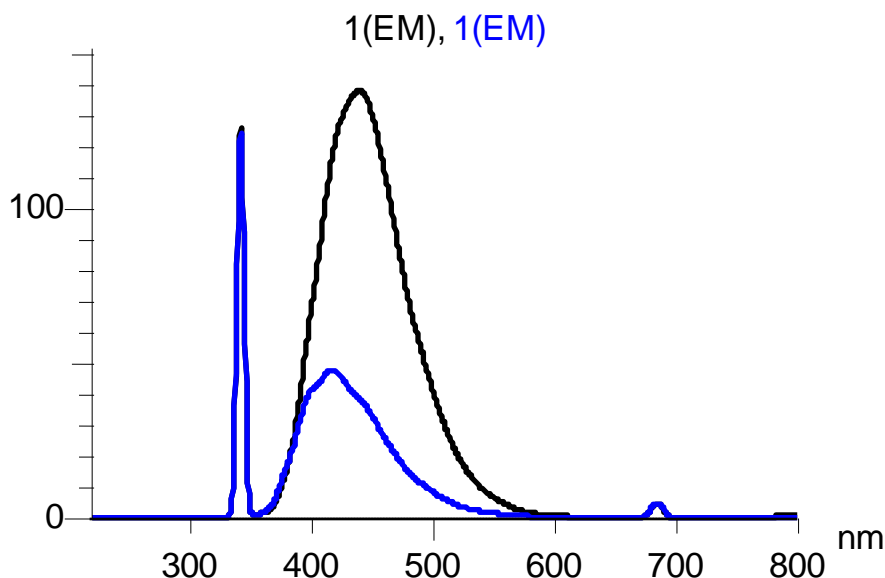


Fig. 8 Emission spectra of PDE/OPA derivative (black) and blank (blue) in acetonitrile after excitation at $\lambda_{ex}=340$ nm.

3.2.5. Effect of adding β -Cyclodextrin

Cyclodextrins play an important role in fluorescent systems. It can enhance the fluorescence intensity of some fluorescent moieties (Lakowicz [2006]). Effect of β -cyclodextrin on the fluorescence intensity of the formed isoindole product was investigated using fixed concentration of PDE with varied moles of β -cyclodextrin which covered molar ratios of cyclodextrin:drug correspond to 1:2, 1:1, 2:1 and 3:1. It was found that β -cyclodextrin has not significantly affected the fluorescence intensity of the derivatization product.

3.2.6. Stoichiometric relationship of the derivatization reagent

The stoichiometric relationship between OPA and 3MPA during the derivatization reaction with PDE was determined by molar ratio method. In this method PDE and OPA moles were kept at a fixed concentration so that the OPA moles were 10 times greater than PDE moles to insure a complete reaction of drug with reagent at any OPA/3MPA ratio.

As seen from Figure 9, OPA/3MPA ratio was found to be 1:1, confirming that one molecule of OPA reacts with one molecule of 3MPA during the derivatization reaction with PDE. Thus a ratio of 1:2 OPA/3MPA has been reserved during our study to ensure maximum derivatization process.

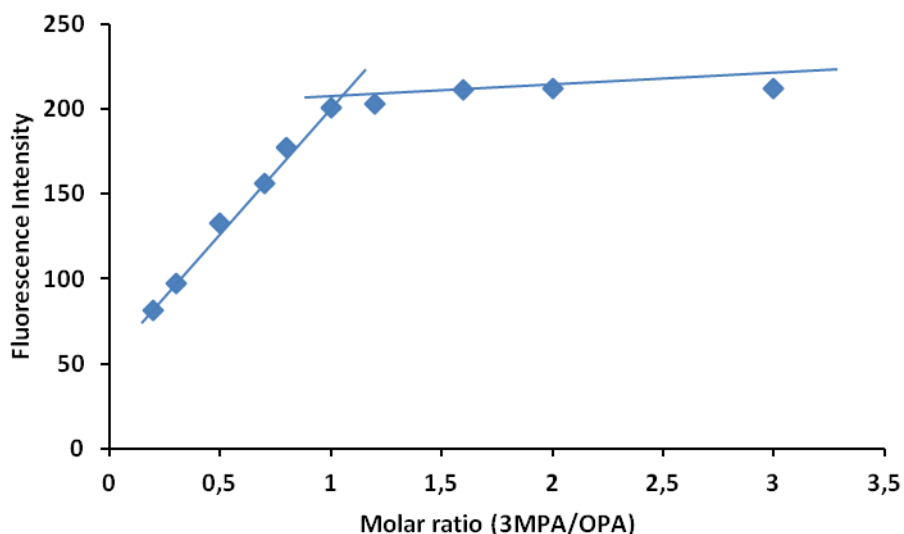


Fig. 9 Molar ratio plot for the stoichiometric relationship between OPA and 3MPA.

3.2.7. Mechanism of the reaction with *o*-phthalaldehyde

The stoichiometry of the reaction between PDE and *o*-phthalaldehyde was studied adopting the limiting logarithmic method. The fluorescence of the reaction product was measured in presence of excess of the drug and the reagent (Harvey [2009]). Plots of log fluorescence versus log [OPA] and log [PDE] gave two straight lines, the values of their slopes were 1.0624/0.9894 for OPA and PDE, respectively (Fig.10). Therefore, it is concluded that, the reaction proceeds in the ratio of 1:1, confirming that one molecule of the drug condenses with one molecule of *o*-phthalaldehyde, as illustrated before in Scheme 1.

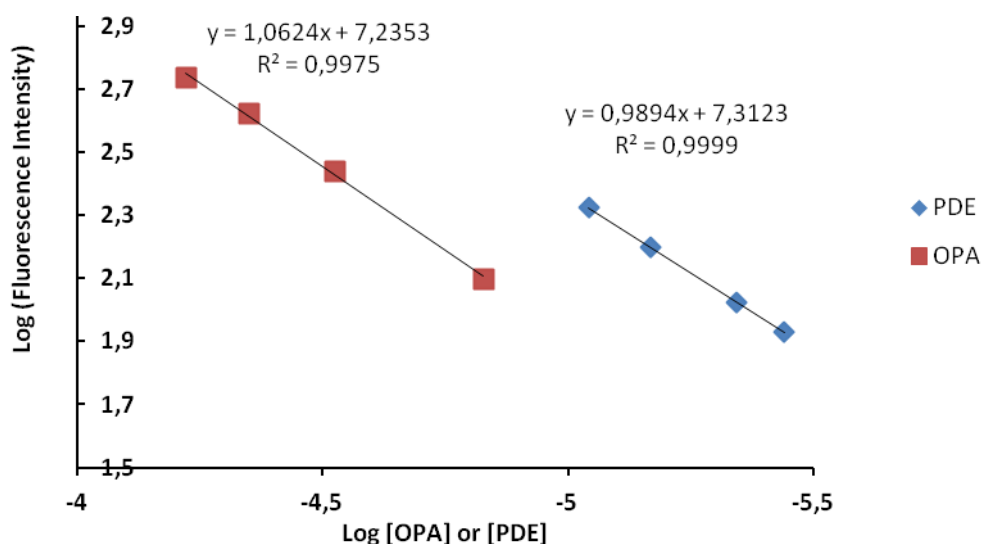


Fig. 10 Limiting logarithmic plots for the molar ratio. a Log fluorescence intensity vs. Log [PDE] or Log [OPA]

3.3. Validation of the Proposed Method

3.3.1. Linearity

Under the optimum experimental conditions, standard calibration curve was constructed at nine concentration levels (n=5). The correlation coefficient was 0.9997 indicating very good linearity, over the concentration range of 0.8 – 8.4 µg/mL (Fig. 10). The intercept, slope, limit of detection (LOD), and limit of quantitation (LOQ) are summarized in Table 1. 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 [Harvey [2009]; ICH [2005]] (Table 1).

Table 1. Statistics and analytical parameters of PDE determination.

Parameter	Result
$\lambda_{ex}/\lambda_{em}$ (nm)	340/452
Linear range (µg/mL)	1.2 – 8.4
Slope	51.638
Standard deviation in the slope	0.310
Intercept	2.5508
Standard deviation in the intercept	1.511
Correlation coefficient	0.9997
Limit of detection (µg/mL)	0.097
Limit of quantification (µg/mL)	0.293

3.3.2. 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.

3.3.3. 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 0.47% to 1.86% for the smallest determined concentration, indicating good precision (Table 2).

Table 2. Precision and accuracy for determination of PDE in pure form using proposed method.

Perindopril erbumine (µg/mL)		SD (µg/mL)	RSD%	Recovery %	<i>t</i> -test ^b
Taken	Found ^a				
1.200	1.178 ± 0.027	0.022	1.86	98.16	2.23
1.600	1.584 ± 0.023	0.019	1.20	99.00	1.88
2.000	1.992 ± 0.027	0.022	1.10	99.60	0.81
3.000	2.976 ± 0.041	0.033	1.11	99.20	1.63
4.000	4.028 ± 0.046	0.037	0.92	100.70	1.69
5.000	5.006 ± 0.052	0.042	0.83	100.12	0.32
6.000	6.011 ± 0.062	0.050	0.83	100.18	0.49
7.000	6.972 ± 0.065	0.053	0.76	99.60	1.18
8.400	8.360 ± 0.050	0.040	0.47	99.52	2.23

^a Average of five determinations ± Confidence limit.

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

3.3.4. Accuracy

The proposed method was applied on the available commercial tablets at three different concentration levels (80%, 100%, and 120%) and recoveries are mentioned in Table 3. 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 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 98.16 to 100.70%. (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 (Raju and Rao [2011]). The resulted values were statistically compared with each other (Table 4) using t - and F -tests. t_{exp} was calculated using the following equation (Harvey [2009]):

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

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.

Table 3. Application of the proposed method to the determination of PDE in tablets.

Tablets	Labeled amount of PDE	Amount taken ($\mu\text{g/mL}$)	Amount found ^a ($\mu\text{g/mL}$)	Recovery %
Revosyl	4 mg	3.84	3.89	101.30
		4.80	4.88	101.66
		5.76	5.78	100.34
Mean found%				101.11
RSD%				0.69
Revosyl	8 mg	3.84	4.04	105.21
		4.80	5.05	105.21
		5.76	6.05	105.03
Mean found%				105.16
RSD%				0.11
Neomeril	4 mg	3.84	3.73	97.13
		4.80	4.70	97.91
		5.76	5.63	97.74
Mean found%				97.61
RSD%				0.43
Neomeril	8 mg	3.84	3.80	98.96
		4.80	4.75	98.95
		5.76	5.72	99.30
Mean found%				99.04
RSD%				0.23

^a Average of three determinations

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		<i>t</i> - and <i>F</i> - test ^c
		Proposed method	Reference method ^d	
Revosyl	4 mg	4.07 \pm 0.025 (101.68)	4.06 \pm 0.024 (101.50)	0.275, 1.087
	8 mg	8.38 \pm 0.037 (104.78)	8.44 \pm 0.040 (105.50)	2.030, 0.848
Neomeril	4 mg	3.88 \pm 0.021 (96.99)	3.90 \pm 0.026 (97.50)	1.364, 0.600
	8 mg	7.89 \pm 0.051 (98.66)	7.87 \pm 0.042 (98.37)	0.623, 1.492

^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 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.

^d HPLC method (Raju and Rao [2011]).

3.3.5. Robustness

Robustness was examined by evaluating the influence of small variations in the experimental conditions such as volume of reagent (± 0.1 mL), volume of buffer solution (± 0.1 mL) and reaction time (± 5 min). These minor changes that may happen during the analysis did not have any significant effect on fluorescence intensity of the reaction product.

3.4. Application to Tablets

The proposed method was successfully applied to the analysis of two different commercial tablets (Revosyl[®] and Neomeril[®] Tablets) labeled to contain 4 and 8 mg of perindopril erbumine. The mean recovery values were ranged from 96.99 to 104.78, which were identical to the recoveries recorded by the reference method (HPLC) as revealed by *t*- and *F*-test (Table 4).

4. Conclusion

New, simple, and rapid spectrofluorimetric method for the determination of PDE has been successfully developed and validated. The method involved the formation of a fluorescent isoindole derivative resulted from the reaction of PDE with OPA in presence of 3MPA. The proposed method was specific, precise and accurate with a comparable low detection limit value of 0.097 μ g/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.

References

1. Joint Formulary Committee, 2014. British National Formulary. 67th edition. London: British Medical Association and Royal Pharmaceutical Society of Great Britain.
2. Raju, V.B., Rao, A.L., 2011. Development And Validation of New HPLC Method For The Estimation of Perindopril in Tablet Dosage Forms. *Rasayan J.Chem.* 4, 113-116.
3. Zaazaa, H.E., Abbas, S.S., M. Essam, H.A., El Bardicy, M.G., 2013. Validated Chromatographic Methods for Determination of Perindopril and Amlodipine in Pharmaceutical Formulation in the Presence of their Degradation Products. *Science & Mathematics Journal of Chromatographic Science.* 51, 533-543.
4. Riyaz, S.M.D., Vasanth, P.M., Ramesh, M., Ramesh, R., Ramesh, T., 2012. A Sensitive RP-HPLC Method Development and Validation for the Simultaneous Estimation of Perindopril Erbuminee and Amlodipine Besilate in Tablet Dosage Form. *International journal of chemical and life science.* 1, 1033-1038.
5. Simončić, Z., Roškar, R., Gartner, A., Kogej, K., Kmetec, V., 2008. The use of microcalorimetry and HPLC for the determination of degradation kinetics and thermodynamic parameters of Perindopril Erbuminee in aqueous solutions. *International Journal of Pharmaceutics.* 356, 200-205.
6. Chaudhary, A.B., Patel, R.K., Chaudhary, S.A., 2010. Determination of Losartan Potassium and Perindopril Erbumine in Tablet Formulations by Reversed-Phase HPLC. *International Journal of ChemTech Research.* 2, 1141-1146.

7. Jogia, H., Khandelwal, U., Gandhi, T., Singh, S., Modi, D., 2010. Development and Validation of a Stability-Indicating Assay Method for Simultaneous Determination of Perindopril and Indapamide in Combined Dosage Form by Reversed-Phase High-Performance Liquid Chromatography. *Journal of AOAC International*. 93, 108-115.
8. Joseph, J., Philip, B., Sundarapandian, M., 2011. Method Development and Validation For Simultaneous Estimation of Perindopril Erbumine and Indapamide By RP-HPLC in Pharmaceutical Dosage Forms. *International Journal of Pharmacy and Pharmaceutical Sciences*. 3, 288-293.
9. Prajapati, J., Patel, A., Patel, M. B., Prajapati, N., Prajapati, R., 2011. Analytical method development and validation of Amlodipine besylate and Perindopril erbumine in combine dosage form by RP-HPLC *International Journal of PharmTech Research*. 3, 801-808.
10. Jaina, D.S., Subbaiah, G., Sanya, M., Pande, U.C., Shrivastav, P., 2006. First LC–MS/MS electrospray ionization validated method for the quantification of perindopril and its metabolite perindoprilat in human plasma and its application to bioequivalence study. *Journal of Chromatography B*. 837, 92-100.
11. Nirogi, R.V.S., Kandikere, V.N., Shukla, M., Mudigonda, K., Maurya, S., Komarneni, P., 2006. High-throughput quantification of perindopril in human plasma by liquid chromatography/tandem mass spectrometry: application to a bioequivalence study. *Rapid Communications in Mass Spectrometry*. 20, 1864–1870.
12. Dewani, M.G., Bothara, K.G., Madgulkar, A.R., Damle, M.C., 2011. Simultaneous Estimation of Perindopril Erbumine And Indapamide in Bulk Drug And Tablet Dosage Form By HPTLC. *International Journal Of Comprehensive Pharmacy*. 2, 1-4.
13. Neelam, S., Gopiseti, J., Chandra, B.S., 2012. Spectrophotometric analysis of Perindopril Erbumine in bulk and tablets using bromophenol blue. *Der Pharmacia Lettre*. 4, 159-169
14. Rahman, N., Rahman, H., Khatoon, A., 2012. Development of Spectrophotometric Method For The Determination of Perindopril Erbumine in Pharmaceutical Formulations Using 2, 4 Dinitrofluorobenzene. *Journal of The Chilean Chemical Society*. 57, 1069-1073.
15. Prajapati, J., Patel, M.B., Prajapati, N., Prajapati, R., 2011. Simultaneous Determination of Perindopril Erbumine And Amlodipine Besylate By Absorption Factor Method. *International Journal of Applied Biology And Pharmaceutical Technology*. 2, 230-233.
16. Sharma, S., Sharma, M.C., 2011. UV-Spectrophotometric Method for the Perindopril Erbumine in Pharmaceutical Formulations Using Indigo Carmine. *American-Eurasian Journal of Scientific Research*. 6, 210-216.
17. Garcia Alvarez-Coque, M.C., Medina Hernandez, M.J., Villanueva Camaiias, R.M., Mongay Fernhdez, C., 1989. Formation and Instability of *o*-Phthalaldehyde Derivatives of Amino Acids. *Analytical Biochemistry*. 178, 1-7.
18. Dorresteyjn, R.C., Berwald, L.G., Zomer, G., de Gooijer, C.D., Wieten, G., Beuvery, E.C., 1996. Determination of amino acids using *o*-phthalaldehyde-2-mercaptoethanol derivatization Effect of reaction conditions. *Journal of chromatography A*. 724, 159-167.
19. Rudel, L.L., Morris, M.D., 1973. Determination of cholesterol using *o*-phthalaldehyde. *J Lipid Res*. 14, 364-366.
20. Vermeij, T.A., Edelbroek, P.M., 2004. Simultaneous high-performance liquid chromatographic analysis of pregabalin, gabapentin and vigabatrin in human serum by precolumn derivatization with *o*-phthalaldehyde and fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci*. 810, 297-303.
21. El-Enany, N.M., El-Sherbiny, D.T., Abdelal, A.A., Belal, F.F., 2010. Validated spectrofluorimetric method for the determination of lamotrigine in tablets and human plasma through derivatization with *o*-phthalaldehyde. *J Fluoresc*. 20, 463–472.
22. Michail, K., Aabees, H., Beltagy, Y., Abdelkhalek, M., Khamis, M., 2011. Spectrophotometric and spectrofluorimetric determination of memantine hydrochloride in bulk and pharmaceutical preparations. *Int J Pharm Pharm Sci*. 3, 180-185.
23. Ramadan, M., 2014. *O*-phthalaldehyde based spectrophotometric method for determination of sitagliptin in tablets. *Int J Pharm Pharm Sci*. 6, 125-129.
24. Lakowicz, J. R., 2006. Principles of fluorescence spectroscopy. 3rd ed. Springer.
25. Harvey, D., 2009. Modern analytical chemistry. Second ed., David Harvey.
26. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1) (2005).