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SIMULTANEOUS EQUATION METHOD AND ABSORPTION CORRECTION METHOD FOR THE ESTIMATION OF PERINDOPRIL ERBUMINE AND AMLODIPINE BESYLATE IN BULK AND IN COMBINED TABLET DOSAGE FORM USING UV SPECTOPHOTOMETRY

S. K. Masthannamma*, I. S. Tejaswini, P. Saidulu and G. Rambabu

Department of Pharmaceutical Analysis, University College of Pharmaceutical Science, Acharya Nagarjuna University, Nagarjunanagar, Guntur - 522510 Andhra Pradesh, India

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Correspondence to Author:
S. K. Masthanamma

Assistant Professor,
Department of Pharmaceutical Analysis, University College of pharmaceutical science, Acharya Nagarjuna University, Nagarjunanagar, Guntur–522510 Andhra Pradesh India

E-mail: masthanamma.sk@gmail.com

ABSTRACT: In the present work a simple, accurate and precise method has been developed and validated for the simultaneous estimation of Perindopril Erbumine (PDE), Amlodipine Besylate (AMD)and in their combined dosage form by UV Spectrophotometric Methods. The Method A employs estimation of drugs by simultaneous equation method (SEM) using 298.3 nm and 231.9nm i.e. λmax values of PDE and AMD respectively. Method B employs the estimation of drugs by Absorption Correction method (ACM) at 250.4nm for PDE where absorbance of AMD is zero. Absorbance correction method was based on the property of additivity of absorbances. At 250nm, Perindopril erbumine showed some absorbance while Amlodipine besylate showed zero absorbance. The method involved measurement of absorbance at two wavelengths 250 and 231 nm. For estimation of AMD, corrected absorbance was calculated at 231 nm due to the interference of PDE at this wavelength. Calibration curves were linear with correlation coefficient between 0.999 over the concentration range of 10-150µg/mL and 0.5-10 µg/mL for PDE and AMD respectively. The percent recoveries of the drugs were found nearly 100 % representing the accuracy of the both methods. Validation of the proposed methods was carried out for its accuracy, precision, specificity, linearity and limit of detection according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of perindopril and amlodipine in combined dosage form.

INTRODUCTION: Perindopril Erbumine is chemically 2- Methyl Propane-2-amine (2S, 3As, 7As)-1-[(2S)-2- 2[[(1S)-1-(ethoxycarbonyl) butyl] amine] propanoyl] octahydro-1H-indol-2-carboxylate, with molecular formula- $C_{19}H_{32}N_2O_5.C_4H_{11}N$ and 441.60 mg molecular weight. It is freely soluble in water and sparingly soluble in methaline chloride. Perindopril Erbumine is Angiotensin Converting Enzyme Inhibitor. It is used for the treatment of hypertension. It may be used alone or in combination with other antihypertensive.

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Amlodipine besylate [8] is 3-ethyl 5-methyl 4RS-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5- dicarboxylate benzene sulphonate. Its molecular weight is 567.1 mg. It is slightly soluble in water and proponol, sparingly soluble in ethanol and freely soluble in methanol. It is a calcium channel blocker, used as an anti-hypertensive and in the treatment of angina; it lowers the blood pressure, relaxes heart muscles and dilates the heart blood vessels to prevent spasm.

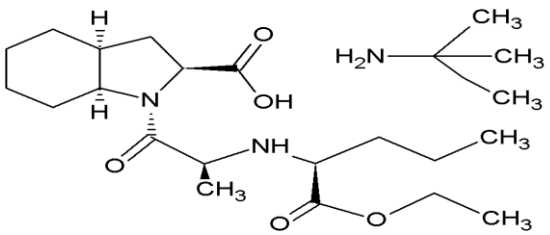

FIG.1: STRUCTURE OF PERINDOPRIL ERBUMINE

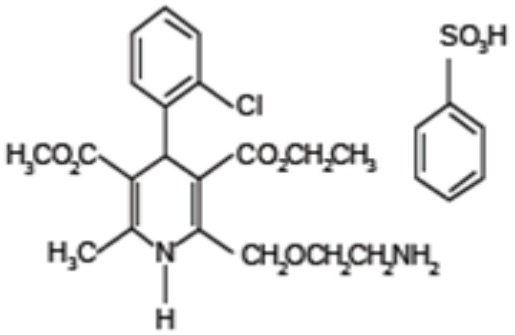

FIG.2 STRUCTURE OF AMLODIPINE BESYLATE

Objective of study:

Literature survey revealed that Methods for the determination of Amlodipine Besylate include (HPLC) and for the determinations of Perindopril erbumine[5] include HPLC, simultaneous spectrophotometric determination and visible spectrophotometric method. The analytical methods lack stability indicating nature in case of Amlodipine besylate and Perindopril erbumine combination using water as solvent. In the present investigation, an attempt was made to develop a simple, rapid, precise and accurate simultaneous equation method and absorption correction method for simultaneous estimation of PDE and AMD using water as solvent by UV Spectrophotometry. The major advantage of the proposed method is economic. This proposed method can be successfully employed for quality control during manufacture and for assessment of the stability of both drugs in bulk samples and their combined tablet dosage forms.

MATERIALS AND METHODS:

Drug substance: Perindopril erbumine and Amlodipine Besylate (working standard 99.10 and 99.70) were obtained as gift sample from Rainbow pharma training lab, Hyderabad, India. Pharmaceutical tablet formulation of COVERSYL-AM4/5 was purchased from local pharmacy. Distilled water was used as a solvent for the entire study.

Instrumentation:

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by shimadzu UV-probe software ,version 2.21 was used for spectral measurements with 10mm matched quartz cells . shimadzu balance (BL-220H) was used for weighing.

Experimental condition:

According to the solubility characteristics, the common solvent for both the drugs was found to be Distilled water. Hence the stock solution was prepared in Distilled water and further dilutions were made up with Distilled water.

Preparation of standard stock solution:

10 mg of PDE, 10 mg of AMD were accurately weighed and transferred in to 10 mL volumetric flasks separately. Dissolved in distilled water and made up to the volume to 10mL with the same. These solutions were observed to contain 1000 µg/mL, for both the drugs. From this stock solution further dilutions are made with distilled water to produce 10, 30, 60, 90, 120µg/mL and 0.5, 2.5, 5, 7.5, 10µg/mL of PDE and AMD respectively.

Determination of λmax:

From the stock solutions, a working standard was prepared. The absorption spectrum for PDE was recorded using the concentration of 10µg/mL and it was found to show absorption maxima at 298.3nm. For AMD, the absorption spectrum was recorded using 10µg/mL solution and the maximum absorption was found to be 231.95nm. The Calibration curves were prepared for PDE and AMD in the concentration range of 10-150 µg/mL and 0.5-10µg/mL at selected wavelengths by diluting aliquot portions of stock solution of each drug. The Calibration plots PDE and AMD are shown in **Fig.3** and **Fig.4** respectively.

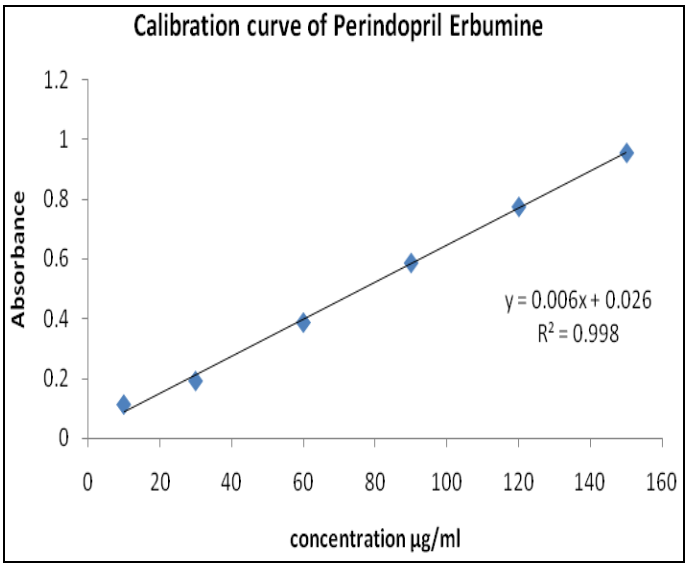

FIG.3.CALIBRATION PLOT OF PDE

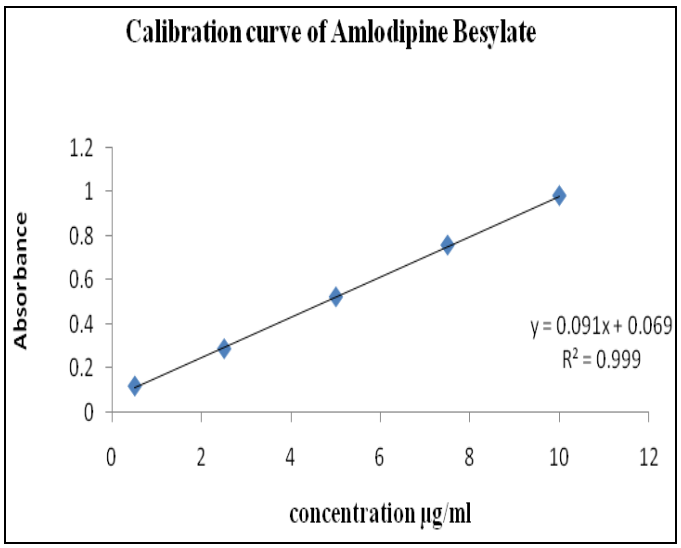

FIG.4: CALIBRATION PLOT OF AMD

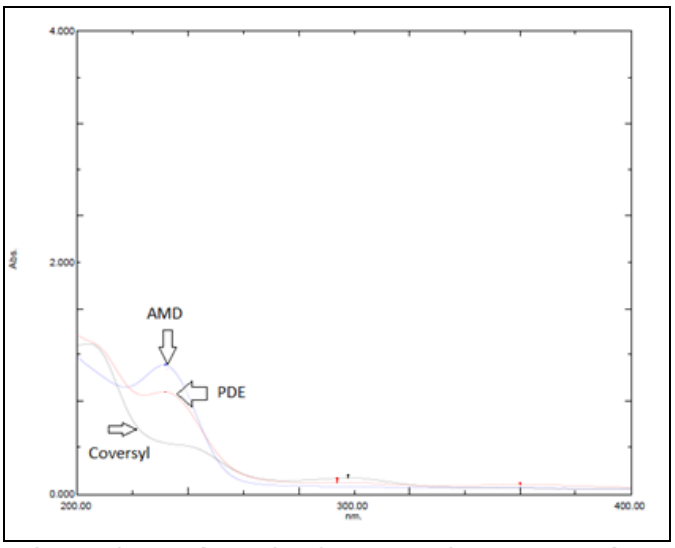

FIG.5: OVERLAY SPECTRUM OF PDE, AMD, FORMULATION (COVERSYL)

Method A: Simultaneous Equation Method (SEM) [1]:

In quantitative estimation of two components by Simultaneous Equation method two wavelengths i.e.298nm of PDE and 231nm of AMD were selected as their respective λmax from the overlain spectrum (**Fig.5**) at which both drugs have absorbance. A set of two simultaneous equations were formed using absorptivity coefficients at selected wavelengths. The concentrations of two drugs in the mixture were calculated using the following two simultaneous equations. Statistical parameters like the slope, intercept coefficient correlation, standard deviation and relative standard deviation were calculated (**Table 1**).

a) For estimation of PDE:

$$C_x = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

b) For estimation of AMD:

$$C_y = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

A1 and A2 are absorbances of diluted mixture at 298.3 and 231.95 nm respectively. Cx and Cy concentrations of PDE and AMD respectively (g/100 mL). ax1, ax2, ay1, ay2 are absorptivities of PDE and AMD at 298.3 and 231.95nm respectively.

TABLE1: SIMULTANEOUS EQUATION VALUES

Drug	Absorbance maxima(λ_1)	Absorbance maxima(λ_2)
Perindopril Erbumine	0.541(ax_1)	0.229(ax_2)
Amlodipine Besylate	0.041(ay_1)	0.512(ay_2)
Formulation(Coversyl-AM)	0.442(A_1)	0.492(A_2)

Method B: Application of Absorption Correction Method:

From the overlain spectrum of PDE and AMD in water (**Fig.6**), it was observed that AMD has zero absorbance at 250nm, where as PDE has substantial absorbance. Therefore, PDE was estimated at 250nm with no interference from AMD. To estimate AMD, absorbance of PDE was measured at 231nm using standard solution of PDE (10µg/mL). The contribution of PDE was deducted from the total absorbance of sample mixture at 231nm.The calculated absorbance for AMD was called as 'Corrected Absorbance' for AMD. The concentration of AMD was determined from calibration curve at 231nm using corrected absorbance.

Corrected Absorbance =

Total Absorbance -Interfering Absorbance

Analysis of Tablet formulations:

Ten tablets were accurately weighed and average weight was calculated. The tablets were triturated to produce a fine powder. An accurately weighed quantity of powder equivalent to 4.0 mg of PDE was transferred to 10mL volumetric flask and dissolved in water by shaking and volume was

made up to 10mL with water as solvent. The solution was filtered through Whatman filter paper No. 41 and aliquot portion of filtrate was diluted to obtain a solution of 10µg/mL of PDE and 10.24µg/mL of AMD respectively. The absorbance of sample solution was measured at selected wavelengths.

The content of PDE and AMD in sample solution of tablet was calculated by using SEM method (equations 1 and 2, **Table 1**) and absorption correction method. The analysis procedure was repeated six times and the result of analysis are shown in **Table 2**. curve (n = 6) and s is slope of regression equation.

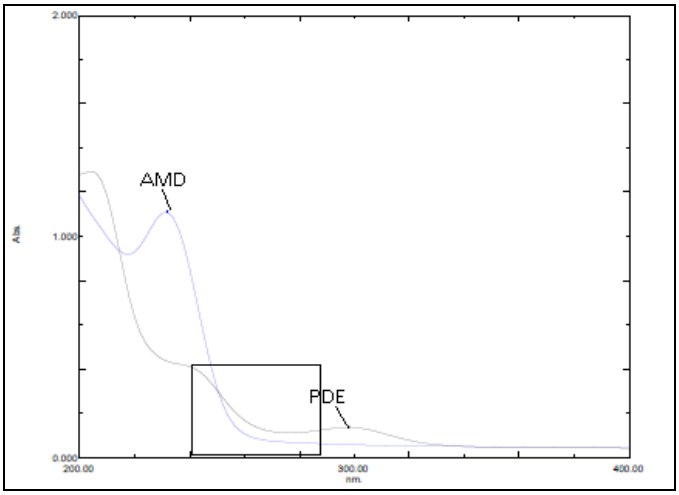

FIG.6: OVERLAY FOR ABSORPTION EQUATION METHOD

TABLE 2: ESTIMATION OF MARKETED FORMULATION

Drug name	Labelled claim (mg)	Test conc. (µg/mL)	Simultaneos equation method	Absorption correction method
			% ± SD (n=6)	% ± SD (n=6)
PDE	4	10	99.85 ± 0.455	101.43 ± 0.76
AMD	5	10.12	99.81 ± 0.233	100.05 ± 0.344

Validation of methods:

The methods were validated with respects to linearity, accuracy, precision, LOD (Limit of detection), LOQ (Limit of quantitation).

Linearity:

Linearity was checked by diluting standard stock solution at six different concentrations. PDE was linear with the concentration range of 10–150 µg/mL at 298nm (method A), 250nm (method B) shown in fig.7 and AMD showed the linearity in the range of 0.5 –10µg/mL at 231nm for absorption correction method[1] and simultaneous equation method. The results are tabulated in **Table 3.**

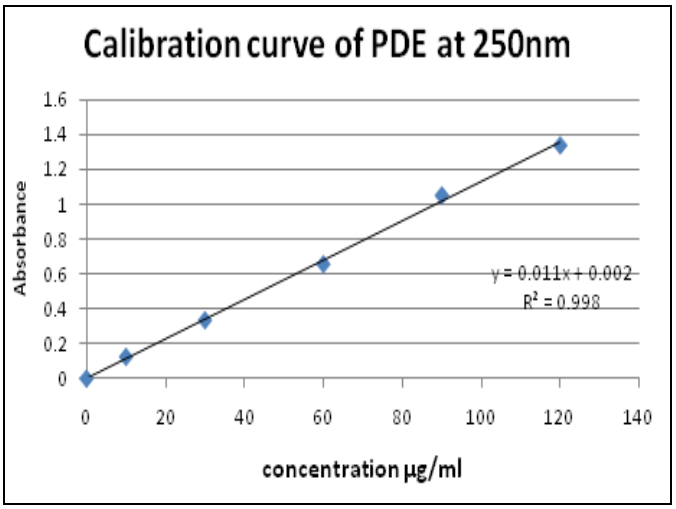

FIG.7: LINEARITY OF PDE AT 250nm (METHOD B)

TABLE 3: LINEARITY VALUES FOR METHOD A &B

Parameter	Simultaneous equation method		Absorption correction method	
	PDE	AMD	PDE	AMD
λmax(nm)	298	231	250	231
Linearity Range(µg/mL)	10-150	0.5-10	10-150	0.5-10
Regression Equation(y=mx+c)	y=0.006x+0.026	y=0.0911x+0.0692	y=0.0113x+0.0029	y=0.0911x+0.0692
Slope (m)	0.006	0.0911	0.0113	0.0911
Intercept (c)	0.026	0.0692	0.0029	0.0692
Correlation Coefficient (r2)	0.998	0.999	0.998	0.999

Accuracy: To check the accuracy of the developed methods and to study the interference of excipients in formulation, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was

calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated and the results are shown in **Table 4** for both the methods respectively.

TABLE 4: %RECOVERY STUDIES FOR PDE AND AMD IN BOTH METHODS

Concentration taken (µg/mL)	Spiked level(%)	Amount added (mg)	Amt found (µg) n=6		%Recovery	
			A	B	A	B
Perindopril Erbumine						
10	50	5	14.83	15.48	98.8	101.4
10	100	10	19.83	20.07	99.16	101.6
10	150	15	24.66	24.69	98.6	101.3
Amlodipine Besylate						
2.5	50	1.25	3.74	3.76	99.7	100.2
2.5	100	2.5	5.058	4.98	101.6	99.6
2.5	150	5.75	5.71	5.73	99.3	99.65

Precision:

The precision of the methods was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation was repeated for six times .The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The Intermediate precision of the methods was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated. The results are shown in **Table 5** for both the methods respectively.

TABLE 5: PRECISON FOR BOTH METHODS

Day of Analysis	%Recovery±SD;(n=3)			%RSD
Intraday Precision				
PDE (µg/mL)	10	30	60	
Day0	99.01±0.171	99.33±0.76	98.01±0.03	
Day1	98.13±0.08	99.56±1.46	98.13±0.08	0.23
Day2	99±0.22	98.2±0.91	99±0.04	
AMD(µg/mL)	2.5	5	7.5	
Day0	99.33±0.72	98.33±0.76	97.33±0.28	
Day1	100.56±1.52	98.56±1.53	98.56±1.54	0.12
Day2	99.3±0.83	99.2±0.85	99.2±0.83	
Interday Precision				
PDE (µg/mL)	10	30	60	
Day0,1,2	99.13±0.09	98.2±0.91	97.13±0.94	0.25
AMD (µg/mL)	2.5	5	7.5	
Day0,1,2	98.57±1.52	99.54±1.55	99.4±0.85	0.12

Sensitivity:

The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations;

$$\mathbf{LOD=3.3\sigma/\ s}$$

$$\mathbf{LOQ = 10\sigma/\ s}$$

where σ is standard deviation of y intercept of calibration times with the same concentration.

Results are tabulated in **Table 6.**

TABLE 6: LOD AND LOQ

Drug name	Absorbance	LOD	LOQ
PDE	0.0158	0.17(µg/mL)	0.51(µg/mL)
AMD	748669	0.05(µg/mL)	0.165(µg/mL)

RESULTS AND DISCUSSION: The proposed two methods are based on spectrophotometric simultaneous estimation of PDE and AMD in UV region using water as solvent.The absorbance spectral analysis shows the maximum absorbance (λmax) at 298nm for PDE and 231nm for AMD.

Method A is based on simultaneous equation method which involves generation and solving of simultaneous equations using absorptivity coefficient(A_1,A_2) values and absorbance at 298 and 231 nm for estimation of PDE and AMD in standard and sample mixture. Method B is based on absorbance correction method which involves correction of absorbance at 231 nm for estimation of AMD and the estimation of PDE was done at 250 nm directly with no interference of AMD which can be understood from calibration curve plotted for PDE at 250nm. The corrected absorbance of AMD was found to be 0.114 and 0.491µg/mL concentration was obtained using y=mx+c equation, which falls in linearity range of AMD at 231nm.

Beer's law obeyed in the concentration range of 10-150µg/mL and 0.5-10µg/mL for PDE and AMD respectively. The correlation coefficients were found to be in between 0.999 which shows the good linear relationship for both components. The results of optical characteristics such as Beer's law limits, correlation coefficient, slope, intercept and absorptivity coefficient values were summarized in **Table 7** for method A and method B. The tablet assay results obtained by proposed methods were very close to labelled claim and low value of standard deviation, suggesting that the developed methods has high precision.

In order to check the accuracy of the developed methods, known quantities of standard drugs of PDE and AMD in three different levels were added to its pre-analyzed tablet sample and analyzed by the developed methods. The results of recovery studies are shown in **Table 4**. The mean percentage recoveries were found in the range of 99.0- 101.0 and it indicated the non interference of the excipients in the tablet formulation.

TABLE 7: SUMMARY OF VALIDATION RESULTS

Parameter		Method A	Method B		Method A&B
λ_{max}		298 nm	250nm		231nm
Beer's limit (µg/mL)	**PDE**	10-150	10-150	**AMD**	0.5-10
Linearity indicated by correlation coefficient		0.999	0.998		0.999
Precision indicated by % RSD		0.24	0.23		0.12
Accuracy indicated by % recovery		98.85	101.43		99.81

Validation data of the proposed methods:

Linearity*:* Linear correlation was obtained between absorbance vs concentration of PDE and AMD in the range of 10-150 & 0.5-10µg/mL respectively. The linearity of the calibration curves was validated by the high value of correlation coefficients of regression (**Table 3**).

Accuracy:

The recovery studies were carried out using standard addition method. The mean recovery obtained was 99.81 ± 0.45 and 98.85 ± 0.40 % for PDE and AMD, respectively (**Table 4**). The high values indicate that the method is accurate.

Precision: The low RSD values of interday (0.23 % and 0.12 %) and intraday (0.25 % and 0.12%) variations for PDE and AMD respectively reveal that the proposed method is precise (**Table 5**).

LOD and LOQ: LOD for PDE and AMD were found to be 0.17µg/mL and 0.05µg/mL, respectively. LOQ for PDE and AMD were found to be 0.561µg/mL and 0.165µg/mL, respectively (**Table 6**).

Assay of the pharmaceutical formulation:

The proposed validated methods can be successfully employed to determine PDE and AMD in their combined dosage form. The results obtained for PDE and AMD were comparable with the corresponding labelled amounts of marketed formulation. (**Table 2**).

CONCLUSION: The proposed two analytical UV spectrophotometric methods were developed and validated thoroughly for quantitative determination of PDE and AMD in tablets. The developed methods were found to be simple, rapid, accurate, precise and economical. Which can be directly and easily applied to the analysis of PDE and AMD in pharmaceutical tablet formulations.

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