Simultaneous Estimation of Perindropil and Amlodipine with possible degradants in fixed dose pharmaceutical formulation by UV Spectroscopic method

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***Abstract*: A novel, simple, precise and reproducible UV stability indicating spectroscopic method has been developed and validated according to ICH Q2R1 guidelines for the simultaneous estimation of Perindropil and Amlodipine used as cardiovascular drugs in fixed dose pharmaceutical formulations. Pure drug samples were dissolved in Methanol and found to have absorbance maxima at 209 nm and 229 nm for Perindropil and Amlodipine respectively. Beer's law was obeyed over concentration ranges of 8-40 µg/ml for Perindropil and 10-50 µg/ml for Amlodipine in this method.**
Simultaneous calibration of both drugs in Methanol shows that λmax of one drug does not interfere on the λmax of other drug. The results of analysis have been validated statistically as per ICH guidelines and recovery studies confirmed the accuracy of the proposed methods. The method showed good reproducibility and recovery with % RSD <2. Stability indicating studies were done by subjecting to acid and alkali hydrolysis, oxidation, thermolysis and photolysis. Hence, this proposed method was found to be rapid, specific, precise, and accurate and can be successfully applied for the routine analysis of Perindropil and Amlodipine in fixed dose pharmaceutical formulations.

***Keywords*: UV Spectroscopic method, Validation, Beer's law, Perindropil, Amlodipine.**

INTRODUCTION:

Perindopril and Amlodipine drugs are used alone or in combination with other antihypertensive agents in the treatment of hypertension. They help in lowering of the blood pressure, relaxing heart muscles and dilating the heart blood vessels thus preventing the spasm. Perindopril is chemically (2*S*, 3*aS*, 7*aS*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxopentan-2-yl] amino] propanoyl]-2, 3, 3*a*, 4, 5, 6, 7, 7*a*-octahydroindole-2-carboxylic acid belonging to the angiotensin converting enzyme (ACE) inhibitor class. Amlodipine is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate belonging to the class of calcium antagonists.

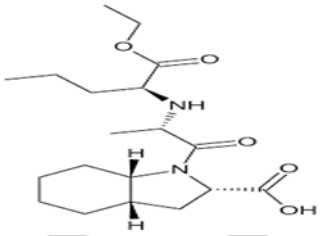

Figure 1: Perindopril

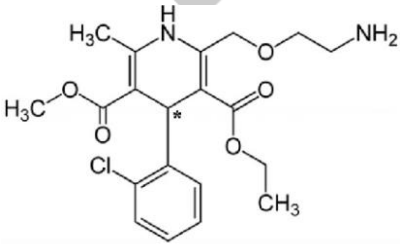

Figure 2: Amlodipine

The present work was an attempt to develop a simple, sensitive and less expensive method for the estimation of Perindopril and Amlodipine and validate it as per ICH guideline along with the stability indicating studies.

MATERIALS AND METHODS

Instrumentation: UV/VIS spectrophotometer- LABINDIA UV 3000$^+$ with 1cm U.V matched quartz cells was used. An electrical balance (Shimadzu ELB 300) for weighing and an ultrasonicator bath (PCI analytics Pvt. Ltd) was used for sonicating the drug samples.

Materials and Methods: Perindopril and Amlodipine pure drugs were a gift from PharmaTrain Labs, Hyderabad. Methanol used was of analytical grade purchased from. COVERSYL AM tablets were procured from the local commercial sources.
All the reagents and chemicals used were of analytical reagent grade. Double-distilled water was used to prepare the required solutions.

Preparation of the Perindopril and Amlodipine Standard and Sample Solutions:

Preparation of standard solution: 20 mg of Perindopril and 25 mg of Amlodipine working standards were accurately weighed and transferred into a 25 ml clean dry volumetric flask and small amount of diluent was added and sonicated to dissolve it completely. Finally the volume was made up to the mark with the same solvent to give the Stock solution of 800 µg/ml and 1000 µg/ml concentration of Perindopril and Amlodipine respectively. Further 0.3 ml of the above stock solution was pipette into a 10 ml volumetric flask and diluted up to the mark with diluent to give a concentration of 24 ppm of Perindopril and 30 ppm of Amlodipine respectively.

Preparation of sample Solution: 20 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 20 mg of Perindopril and 25 mg of Amlodipine sample (763 mg of tablet powder) of active ingredient present in 20 tablets was transferred into a 25 ml clean dry volumetric flask, small amount of diluent was added and sonicated for about 30 minutes by shaking at intervals of five minutes each to dissolve it completely and was diluted up to the mark with diluent to give a concentration of 800 µg/ml of Perindopril and 1000 µg/ml and Amlodipine stock solutions respectively. Further, dilutions were made to give the concentration of 24 ppm of Perindopril and 30 ppm of Amlodipine respectively.

Selection of analytical wavelength: Solubility and stability of Perindopril and Amlodipine was checked in different analytical solvents and the drugs gave good spectral characters with methanol. Standard and sample solutions were kept in the UV system and were scanned in the wavelength range of 200-400 nm. A good spectrum of λmax at 209 nm and 229 nm was observed which was taken as the λmax for further studies and the % Assay was calculated.

Method I (Simultaneous equation method):

Two wavelengths selected for the method are 209 nm and 229 nm that are absorption maximas of Perindropil and Amlodipine respectively in methanol. The stock solutions of both the drugs were further diluted separately to get a series of standard solutions of 8-40 µg/ml for Perindropil and 10-50 µg/ml for Amlodipine concentrations. The absorbance of these dilutions were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

$A_1 = a_{X1}bc_x + a_{Y1}bc_Y$ ……1

$A_2 = a_{X2}bc_x + a_{Y2}bc_Y$ …….. 2

Where, A1 and A2 are absorbances of mixture at 209 nm and 229 nm respectively, a_{x1} and a_{x2} are absorptivities of Perindropil at 209 nm and 229 nm respectively and a_{y1} and a_{y2} are absorptivities of Amlodipine at 209 nm and 229 nm respectively. C_x and C_y are concentrations of Perindropil and Amlodipine respectively.

Method II (Absorbance ratio or Q-analysis method):

From the overlain spectrum of Perindropil and Amlodipine, two wavelengths were selected one at 207 nm which is the isoabsorptive point for both the drugs and the other at 229 nm which is λ_{max} of Amlodipine. The absorbances of the sample solutions prepared in a similar manner as in the previous method were measured and the absorptivity values for both drugs at the selected wavelengths were also calculated. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula**,**

For AB,

$C_X == (Q_M - Q_Y)/(Q_X - Q_Y) * A_1/a_{X1}$ ……3

For PE,

$C_Y = (Q_X - Q_Y)/(Q_Y - Q_X) * A_1/a_{Y1}$……4

$$Q_m = \frac{\text{Absorbance of sample at 207 nm}}{\text{Absorbance of sample at 229 nm}}$$

$$Q_x = \frac{\text{Absorptivity of Nebivolol at 207 nm}}{\text{Absorptivity of Nebivolol at 229 nm}}$$

$$Q_y = \frac{\text{Absorptivity of Valsartan at 207 nm}}{\text{Absorptivity of Valsartan at 229 nm}}$$

A = Absorbance of sample at isoabsorptive point, a_1 and a_2 = Absorptivities of Perindropil and Amlodipine respectively at isoabsorptive point.

Validation of the proposed method:

The UV spectrometric method was validated with respect to specificity, linearity, precision, accuracy, limit of detection (LoD), limit of quantitation (LoQ), and robustness following the ICH guidelines.

Specificity: Specificity was evaluated by analyzing solutions of standard, sample, blank and placebo of Perindropil and Amlodipine. The system response was examined for the presence of any interferences or overlaps.

Linearity: The linearity of the analytical procedure is its ability to obtain the best results which is directly proportional to the concentration of analyte in the sample. To determine linearity range for the drugs, a series of working standard solutions were prepared from the respective stock solutions of drugs. A volume of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml of stock solutions of Perindropil and Amlodipine was pipetted into 10 ml volumetric flask and volume was made up with methanol to obtain solutions in concentration range of 8-40 µg/ml for Perindropil and 10-50 µg/ ml for Amlodipine. Absorbances of the solutions were recorded at predetermined wavelengths and calibration curves of absorbance vs. concentration were plotted. The linear regression equations and correlation coefficients (r^2) were determined.

Accuracy: The accuracy was evaluated analysing of the in house mixture of the excipients with known amounts of the drug having 50, 100, and 150% of the nominal analytical concentration, recovery studies were carried out by adding a known amount of drug to the pre analysed tablet powder and percentage recoveries were calculated.

The results of recovery studies were found to be satisfactory and were presented in table no

Precision: The absorbance of known concentration solutions of Perindropil and Amlodipine were measured on different days. Intraday precision was performed for three times on same day and interday precision was performed three times on different days. The %RSD of the sample solution was calculated and were presented in table no

LoD and LoQ: The LoD and LoQ were calculated based on the standard deviation of the response (y-intercepts of regression lines) and the slope using three independent analytical curves. LoD and LoQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Robustness: Robustness was performed by making deliberate minor changes in the experimental conditions such as use of different UV spectrophotometer; alteration in the diluent composition and percentage assay values were determined.

Forced degradation studies: To assess the stability of Perindropil and Amlodipine, forced degradation was performed under acidic, basic, oxidative, thermal and photolytic conditions; absorbance values obtained were compared with those of freshly prepared solutions.

Results and Discussion

Method development and optimization:

The choice of the diluent was based upon the solubility of the drugs. Both the drugs were completely soluble in methanol. Moreover, during the development phase, use of methanol as the diluent resulted in preferable outcome in UV analysis. Hence methanol was used as diluent to get optimized method. The analytical wavelengths were selected by scanning the standard solutions of Perindropil and Amlodipine (80 µg/ml & 100 µg/ml each) in the range of 200-400 nm against blank. From the overlain spectra the wavelengths chosen for analysis of Perindropil and Amlodipine were 209 nm and 229 nm.

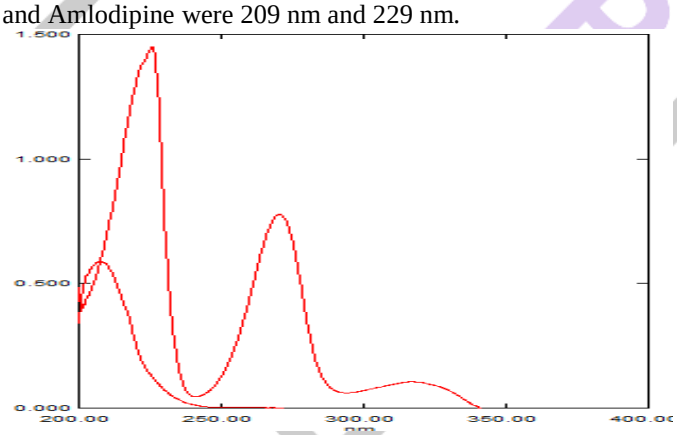

Fig. 3: Overlain UV spectra of Perindropil and Amlodipine for optimized method

Method validation

Linearity: Linearity range for each drug was obtained by plotting calibration curves at predetermined wavelengths. The calibration plots for the drugs were displayed. Good correlation was observed between the absorbance and concentration of the drugs for the concentration range 8-40 µg/ml for Perindropil, 10-50 µg/ml for Amlodipine. The results of linearity for Perindropil and Amlodipine were given in Table no. 1 & 2 respectively.

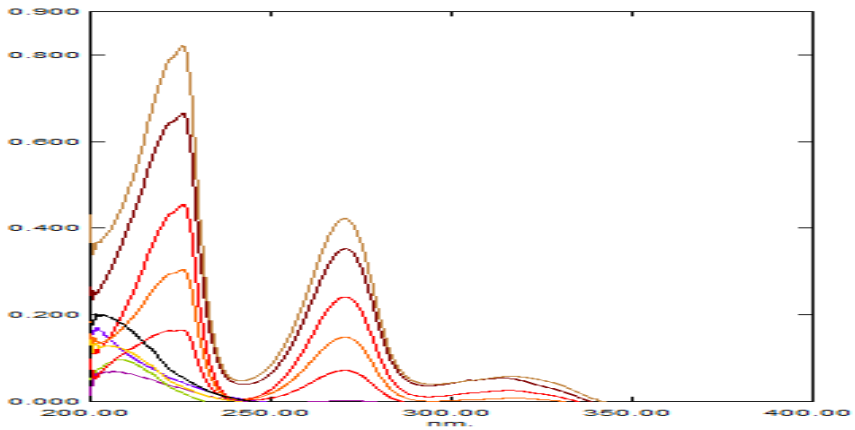

Fig no.4: Overlain UV spectra of Perindropil and Amlodipine for all Linearity

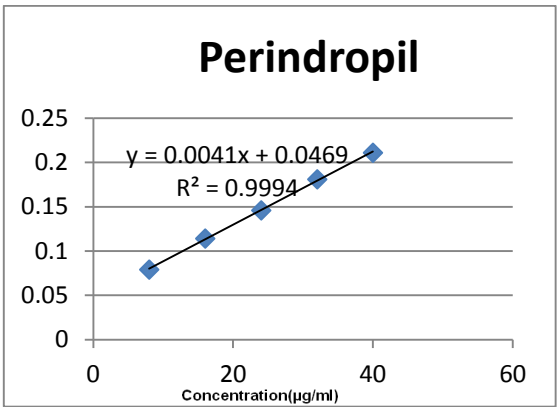

Fig no.5: Calibration curve of Perindropil

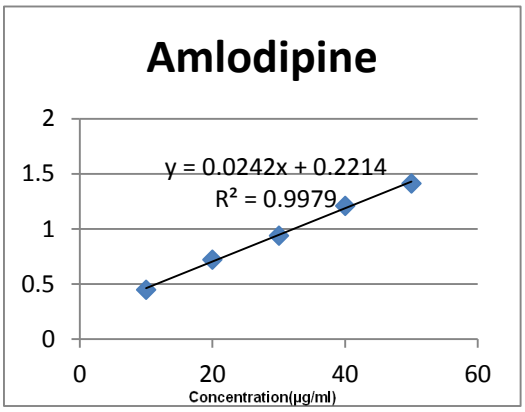

Fig no.6: Calibration curve of Amlodipine

S. No	Linearity Level	Concentration(µg/ml)	Absorbance at 209 nm
1	I	8	0.079
2	II	16	0.114
3	III	24	0.146
4	IV	32	0.181
5	V	40	0.211
Correlation Coefficient			0.9994

Table no.1: Linearity results for Perindropil

S. No	Linearity Level	Concentration(µg/ml)	Absorbance at 229 nm
1	I	10	0.449
2	II	20	0.721
3	III	30	0.939
4	IV	40	1.209
5	V	50	1.413
Correlation Coefficient			0.9979

Table no.2: Linearity results for Amlodipine

Accuracy: The accuracy of the method was determined by performing percentage recovery at three levels: 50 %, 100 % and 150 %. As seen in **Table 3 and 4** the mean percentage recoveries for Perindropil and Amlodipine were found to be within the acceptance limit of 98-102 %. Hence the developed method was found to be accurate.

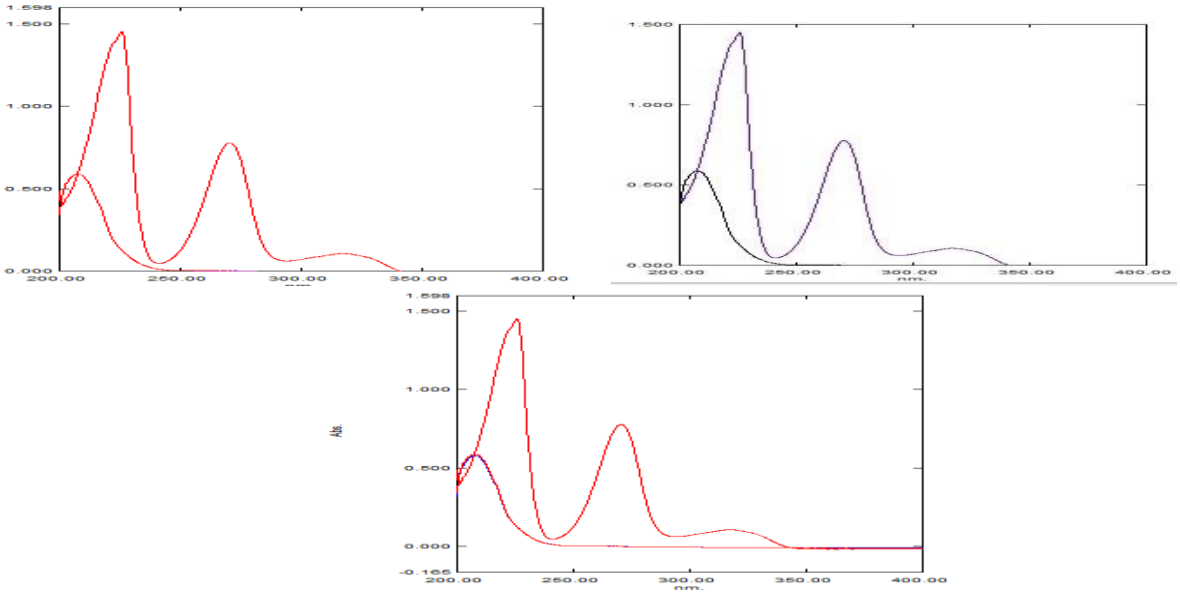

Fig no.5: Overlaid UV spectra of Perindropil and Amlodipine of Accuracy

%Concentration (at specification Level)	Area	Amount taken (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	0.069	10	9.98	99.82	100.19
100%	0.149	20	20.02	100.41	
150%	0.238	40	40.01	100.34	

Table no.3: Accuracy results for Perindropil

%Concentration (at specification Level)	Area	Amount taken (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	0.471	12.5	12.51	100.14	100.3
100%	0.941	25	25.01	100.21	
150%	1.391	50	50.20	100.57	

Table no.4: Accuracy results for Amlodipine

Precision: Precision was carried out by analysing the mixed standard solution of drugs in triplicate against the blank thrice a day for intraday studies and on three consecutive days in triplicate for interday precision analysis. The results as depicted in **Table 5 and 6** gave percentage relative standard deviation (RSD) values of less than 2 %.

Precision	Perindropil at 209nm	Amlodipine at 229nm	Perindropil and Amlodipine at 207nm
Precision-1	100.74	100.32	100.45
Precision-2	100.43	100.43	99.03
Precision-3	100.52	99.65	100.53
Precision-4	99.63	99.82	99.56
Precision-5	100.14	100.31	100.16
Precision-6	99.76	100.61	99.96
Average	100.20	100.19	99.94
SD	0.44	0.37	0.57
%RSD	0.44	0.37	0.57

Table no.5: Absorbance values of Perindropil & Amlodipine for Precision –I

Precision	Perindropil at 209nm	Amlodipine at 229nm	Perindropil and Amlodipine at 207nm
Precision-1	0.259	0.638	0.238
Precision-2	0.243	0.678	0.239
Precision-3	0.219	0.657	0.238
Precision-4	0.249	0.649	0.237
Precision-5	0.252	0.651	0.236
Precision-6	0.258	0.632	0.237
Average	0.246	0.650	0.2375
SD	0.014	0.016	0.001
%RSD	0.5	0.2	0.4

Table no.6: Absorbance values of Perindropil and Amlodipine for Precision -II

LoD and LoQ: The Limit of Detection and Limit of Quantification were calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve. LoD was found to be 4.9 for Perindropil and 5.2 for Amlodipine. LoQ was found to be 12.6 Perindropil and 15.7 for Amlodipine.

Robustness: On evaluation of the Robustness results, it can be concluded that the variation in wave length has not affected the method significantly. Hence it indicates that the method is robust even by change in the wave length ±2 nm.
The results of robustness for effect of variation in wavelength were reported in **Table no: 7**. The Spectra were recorded as **Fig. 8**.

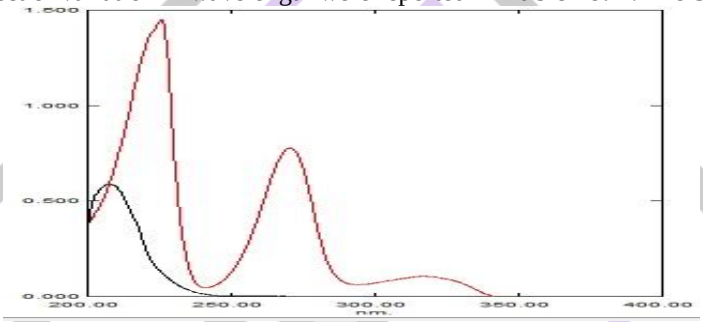

Fig no.6: Overlaid UV spectra for Robustness of Perindropil and Amlodipine.

S. No.	Perindropil		Amlodipine		Perindropil&Amlodipine	
	Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
1	207	0.257	227	0.658	205	0.237
2	209	0.259	229	0.657	207	0.238
3	211	0.261	231	0.659	209	0.239

Table no.7: Robustness results for Perindropil and Amlodipine

Forced Degradation Studies: The results of the stress studies indicated the specificity of the method that has been developed. Forced degradation studies were carried out in order to evaluate the specificity and stability-indicating properties of the method, by exposing samples of the drug test solutions to stress conditions of Acid degradation, Base degradation, Peroxide degradation, Thermal degradation and Photolytic degradation**.** The drug solutions of Perindropil and Amlodipine were relatively stable and there was no evidence of degradation when exposed to various stress conditions. Although the degradation products of the stressed conditions had not been identified, the method had been able to detect the changes due to stress condition.

Conclusion

The developed UV spectroscopic method was novel, simple and cost effective with high reproducibility and precision. The parameters were validated as per ICHQ2R1 guidelines. It can be easily used in routine quality control aspects and for quantitative estimation of Perindropil and Amlodipine from bulk and pharmaceutical dosage formulations.

Conflict of Interests

The work is an extract from PhD Thesis. The authors declare no conflict of interest.

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