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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PERINDOPRIL ERBUMINE AND AMLODIPINE BESYLATE IN TABLET DOSAGE FORM

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ABSTRACT

An isocratic separation was carried out using X-Terra (4.6 ×150mm, 5µm particle size) column and Methanol: Acetonitrile: Water (50:35:15%v/v) as mobile phase with quantification carried out at a wavelength of 215nm. The retention time of the Perindopril erbumine, Amlodipine besylate was 3.234, 1.694 minutes, respectively with theoretical plate count and asymmetry as per the ICH limits. The % assay of Perindopril erbumine and Amlodipine besylate were 99.5% and 98.99%.The flow rate was found to be 1ml/min. The linear regression analysis data for the calibration plots showed a good linear relationship for Perindopril erbumine and Amlodipine besylate over a concentration range of 5-25µg/ml and 6.25-31.25 with correlation co-efficient of 0.999 for Perindopril erbumine and 0.999 for Amlodipine besylate. The limits of detection and quantitation were found to be 1.08, 0.80 and 3.27, 2.45 µg/ml respectively.

KEYWORDS: Perindopril erbumine, Amlodipine besylate, PDA Detector; RP-HPLC; Tablet Dosage forms.

INTRODUCTION

Amlodipine Besylate[1] is chemically (2-[(2-Aminoethoxy) methyl]-4-(2–chlorophenyl)-1, 4–dihydro–6–methyl–3, 5–pyridinedicarboxylic acid 3–ethyl 5–methyl ester.)(fig.1) belongs to the class of Calcium channel blocker, used as anti-anginal. Molecular Formula – $C_{26}H_{31}ClN_2O_8S$,Molecular Weight – 567.1, Solubility - Slightly soluble in water and in isopropyl alcohol, sparingly soluble in dehydrated alcohol, freely soluble in methanol.

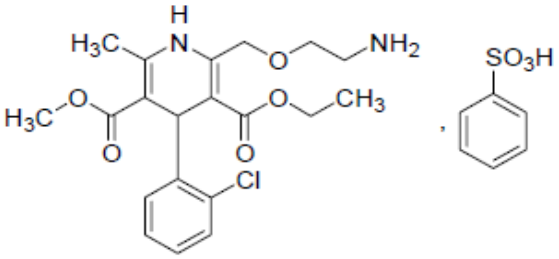

Fig 1 Chemical Structure of Amlodipine Besylate

Perindropil erbumine

Perindopril erbumine Tablets contain the tert-butylamine salt of perindopril, the ethyl ester of a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor. Perindopril erbumine is chemically described as 2-Methyl Propane-2-amine (2S, 3As, 7As)-1-[(2S)-2-2[[(1S)-1-(ethoxycarbonyl) butyl] amine] propanoyl] octahydro-1H-indol-2-carboxylate**.**Its molecular formula is ($C_{23}H_{43}N_3O_{5)}$. Its structural formula is.

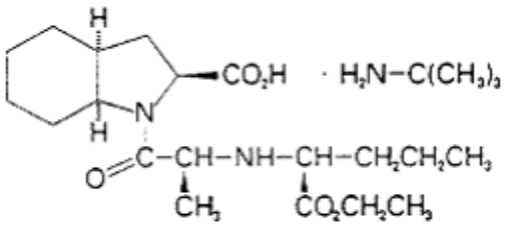

Fig.2: Chemical Structure of Perindropil erbumine

Perindopril erbumine is a white, crystalline powder with a molecular weight of 368.47 (free acid) or 441.61 (salt form). It is freely soluble in water (60% w/w), alcohol and chloroform. Perindopril is the free acid form of perindopril erbumine, is a pro-drug and metabolized in vivo by hydrolysis of the ester group to form perindoprilat, the biologically active metabolite. From the literature survey it was found that many methods are available for determination of Perindopril erbumine and Amlodipine besylate individually and few methods in combination with other drugs. In the proposed study an attempt will be made to develop a HPLC method for simultaneous estimation of Perindopril erbumine and Amlodipine besylate in pharmaceutical formulation (tablet). Pharmaceutical grade of Amlodipine Besylate, and Perindropil erbumine were kindly supplied as gift samples by Sura Labs pvt Ltd ,Dislhuknagar Hyderabada, certified to contain > 99% (w/w) on dried basis. Commercially available *Coversyl-AM* Serdia Pharmaceuticals Ltd,Mumbai, India) tablets claimed to contain 5 mg Amlodipine Besylate and 4 mg Perindropil erbumine have been utilized in the

present work. All chemicals and reagents used were of HPLC grade and were purchased from Agenta Chemicals, Hyderabad, India.

Chromatographic system and conditions

The HPLC system waters 2695 consisted of Quaternary pump. The Analytical column and Isocratic elution with X-Terra (4.6 ×150mm, 5µm particle size) column and Methanol: Acetonitrile: Water (50:35:15%v/v) as mobile phase with quantification carried out at a wavelength of 215 nm. Before analysis the mobile phase was filtered through a (0.45 µm) membrane and degassed by ultrasonification. And injection volume was 10µl. All the experiments were performed at ambient temperature. Pharmaceutical grade of Amlodipine Besylate and Perindropil erbumine were kindly supplied as gift samples by Sura Labs Pvt. Ltd. Hyderabad, certified to contain > 99% (w/w) on dried basis. Commercially tablets claimed to contain 5 mg Amlodipine Besylate and 4 mg Perindropil erbumine have been utilized in the present work. All chemicals and reagents used were of HPLC grade and were purchased from Agenta Chemicals, India.

Standard solutions and calibration graphs for chromatographic measurement.

Stock standard solutions were prepared by dissolving separately 10 mg of Perindropil erbumine and Amlodipine Besylate in 10 ml mobile phase (1000 µg/ml). The standard calibration solutions were prepared by appropriate dilution of the stock solution with Methanol: Acetonitrile: Water (50:35:15%v/v)) to reach a concentration range of 5-25µg/ml and 6.25-31.25 µg/ml. For Amlodipine Besylate, Perindropil erbumine. 10 injections were made for each concentration and chromatographed under the optimized conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

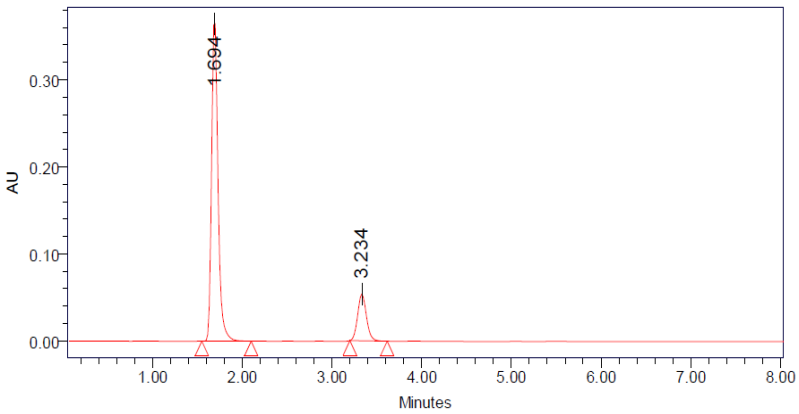

Fig 3: Typical Chromatogram for Amlodipine besylate and Perindropil erbumine

Preparation of Sample Solution

Take average weight of two Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Perindopril erbumine, Amlodipine besylate sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Procedure

Further pipette 0.186 ml of Perindopril erbumine, Amlodipine besylate from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

A 10 µl of volume of sample solution was injected into HPLC, six times. The peak areas for the drugs were measured at 215 nm and amounts of Perindropil erbumine and Amlodipine besylate were determined using the related linear regression equations.

Method validation

The developed method was validated according to the ICH guidelines. The system suitability was evaluated by six replicate analyses of Amlodipine besylate and Perindropil erbumine mixture at a concentration of 50 µg/ml Perindropil erbumine and 25 µg/ml Amlodipine besylate. The acceptance criteria were % R.S.D. of peak areas and retention times less than 2%, Theoretical plate numbers (N) at least 2500 for each peak and tailing factors (T) less than 1% for Perindropil erbumine and Amlodipine besylate. Standard calibration curves were prepared in the mobile phase with six concentrations ranging from 5-25µg/ml and 6.25-31.25 µg/ml for Perindropil erbumine And Amlodipine besylate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. To study the reliability and suitability of the developed method, recovery experiments were carried out at three levels 50, 100 and 150%. Known concentrations of commercial tablets were spiked with known amounts of Perindropil erbumine and Amlodipine besylate. At each level of the amount six determinations were performed and the results obtained were compared with expected results. Recovery for pharmaceutical formulations should be within the range 100±5%. The percent R.S.D. of individual measurements was also determined. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) for 2 consecutive days. Three different concentrations of Perindropil erbumine and Amlodipine besylate were analyzed in six independent series in the same day (intra-day precision) and 3 consecutive days (inter-day

precision).The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of percent RSD.

System suitability parameters

Table 1Observation of System Suitability Parameters

S. NO	Parameter	Amlodipine besylate	Perindopril erbumine
1.	Retention Time (min)	1.694	3.234
2.	Theoretical Plates	6993	5735
3.	Tailing factor	1.23	1.12
4.	Area	1429524	300414
5.	Resolution	10.69	

The system suitability parameters were found to be within the specified limits for the proposed method.

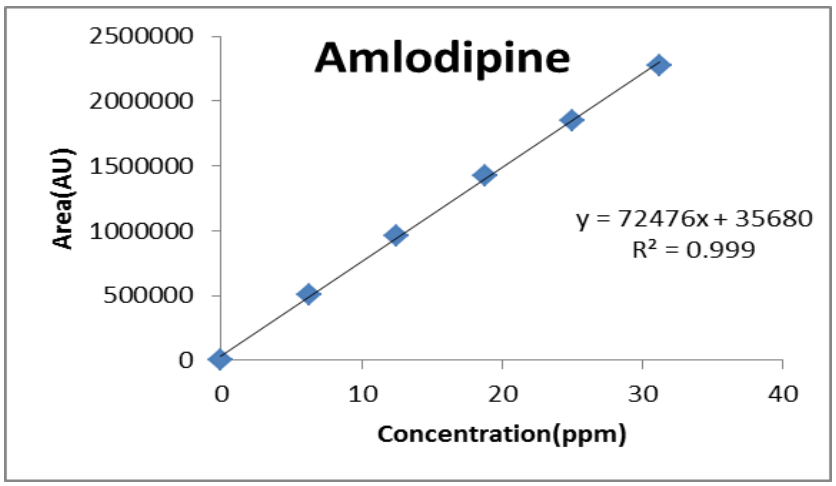

Fig 4: Calibration curve for Amlodipine besylate

Table 2: Linearity Observation of Amlodipine besylate

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1.	I	6.25	504954
2.	II	12.5	958753
3.	III	18.75	1426583
4.	IV	25	1845498
5.	V	31.25	2272948
Correlation coefficient			0.999

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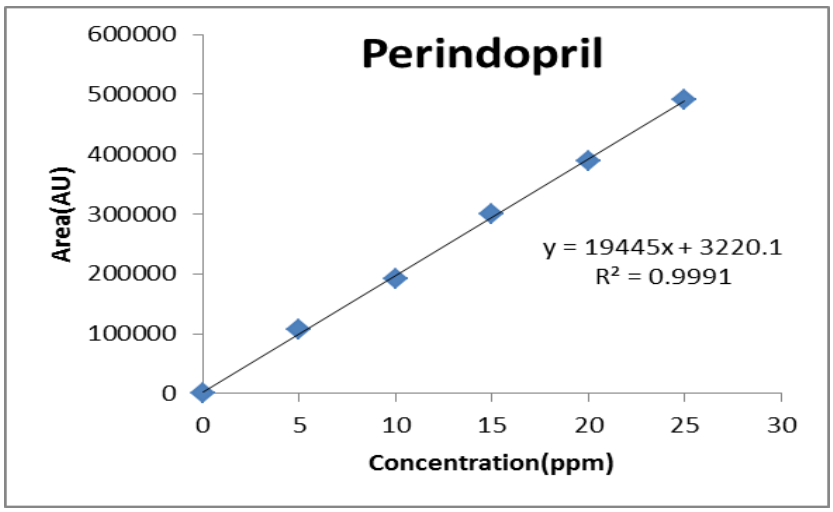

Fig 5: Calibration curve for Perindopril erbumine

Table 4: Linearity Observation of Perindopril erbumine

S.No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	5	107359
2	II	10	191497
3	III	15	300389
4	IV	20	388105
5	V	25	490352
Correlation coefficient			0.999

The linearity range was found to be 50-150 µg/ml for both Amlodipine besylate and Perindopril erbumine. Calibration curve was plotted and correlated Co-efficient for both the drugs found to be 0.999.

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × S.D / Slope

Table 5: LOD Results of the method

Drug	Amount(µg/ml)
Amlodipine besylate	0.80
Perindopril erbumine	1.08

From the above, the LOD values of Amlodipine besylate and Perindopril erbumine were found to be 0.80 and 1.081 µg/ml respectively.

LIMIT OF QUANTITATION (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10× S.D / Slope

Table 6: LOQ Results of the Method

Drug	Amount(µg/ml)
Amlodipine besylate	2.45
Perindopril erbumine	3.27

From the above, the LOQ values of Amlodipine besylate and Perindopril erbumine were found to be 2.45 and 3.27 µg/ml respectively.

Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.2 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Perindopril erbumine, Amlodipine besylate. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Perindopril erbumine, Amlodipine besylate were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

All chromatgrams were examined to determine if compounds of interest co-eluted with each other or with any additional excipients peaks. Marketed formulations were analyzed to determine the specificity of the optimized method in the presence of common tablet excipients. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ were calculated using 3.3σ/s and 10σ/s formulae, respectively, where, σ is the standard deviation of the peak areas and *s* is the slope of the

corresponding calibration curve. To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate.

RESULTS AND DISCUSSION

During the optimization of HPLC method, columns (X-Bridge (4.6 ×150mm, 5µm particle size), Symmetry (4.6 ×150mm, 5µm particle size), X-Terra (4.6 ×150mm, 5µm particle size), two organic solvents (acetonitrile,methanol and water Initially methanol:water, acetonitrile:water, The Amlodipine besylate eluted with these Different ratios of various mobile phases were tried but Perindropil erbumine was retained. Then, with Methanol :Acetonitrile: Water all the two drugs eluted. The mobile phase conditions were optimized so the peak from the first-eluting compound did not interfere with those from the solvent, excipients. Other criteria, *viz*. time required for analysis, appropriate k range (1<k<10) for eluted peaks, assay sensitivity, solvent noise were also considered. Finally a mobile phase consisting of a mixture of Methanol: Acetonitrile: Water (50:35:15%v/v) was selected as mobile phase to achieve maximum separation and sensitivity. Flow rates between 0.8 to 1.3 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed phase C18 (X-Terra (4.6 ×150mm, 5µm particle size) column, the retention times for Amlodipine besylate and Perindropil erbumine were observed to be 1.694 and 3.234 min. respectively. Total time of analysis was less than 10 min. The chromatogram at 215 nm showed a complete resolution of all peaks.

Representative chromatograms of standard solutions (a) standard solution of Perindropil erbumine (50 µg/ml); (b) standard solution of Amlodipine besylate (25 µg/ml) and (c) a standard solution containing 50 µg/ml Perindropil erbumine, 25 µg/ml Amlodipine besylate.

Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability test. All critical parameters tested met the acceptance criteria on all days. As shown in the chromatogram, all three analytes are eluted by forming symmetrical single peaks well separated from the solvent front.

Excellent linearity was obtained for all the two drugs in the range of 5-25µg/ml and 6.25-31.25 for Perindropil erbumine and Amlodiine besylate. The correlation coefficients (r^2) were found to be greater than 0.999 (n=6) in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for Perindropil erbumine and Amlodipine besylate tablet. Results obtained from recovery studies presented

in Table 2, indicate that this assay procedure can be used for routine quality control analysis of this ternary mixture in tablet. Precision of the analytical method was found to be reliable based on % RSD (< 2%) corresponding to the peak areas and retention times. The % RSD values were less than 2, for intra-day and inter-day precision. Hence, the method was found to be precise for all the two drugs.

The chromatograms were checked for the appearance of any extra peaks. It was observed that single peak for Amlodipine besylate 1.694 and Perindropil erbumine , 3.234 were obtained under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with the standard and % purity calculated was found to be within the limits. These results demonstrate the specificity of the method

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