RP-HPLC Method Development and Validation for the Simultaneous Estimation of Imidapril HCl and Amlodipine Besylate in Bulk and Tablet

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Summary: A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Imidapril HCl and Amlodipine Besylate in combination. The separation was carried out using a mobile phase consisting of 0.01M potassium dihydorgen phosphate buffer (adjusted with 0.1% phosphoric acid to pH 3.0), acetonitrile and methanol in the ratio of 50: 35: 15. The column used was Lichrospher C18, 5μm, 25 cm × 4.6 mm maintained at 40°C with flow rate of 1 ml / min using PDA detection at 210 and 237 nm for Imidapril HCl and Amlodipine Besylate respectively. The described method was linear over a concentration range of 10-100 μg/ml and 14-140 μg/ml for the assay of Imidapril HCl and Amlodipine Besylate respectively. The retention times of Imidapril HCl and Amlodipine Besylate were found to be 4.3 and 6.7 min respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and limit of quantification (LOQ) for Imidapril HCl and Amlodipine Besylate were found to be 0.13 and 0.28 μg/ml and 0.24 and 1.47 μg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Amlodipine Besylate and Imidapril HCl bulk drug and in its pharmaceutical dosage form.

Keywords: HPLC [Hitachi PDA], Amlodipine Besylate, Imidapril HCl.

Introduction

Imidapril HCl (IH) is chemically described as (-)-(4S)-3-[2(S)-[N-[1(S)-(Ethoxycarbonyl)-3-phenyl-propyl] amino] propionyl]-1-methyl-2-oxoimidazolidine-4-carboxylic acid hydrochloride. Its empirical formula is $C_{20}H_{27}N_3O_6HCl$.
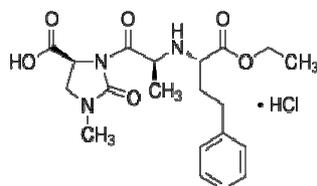
Amlodipine Besylate (AB) is chemically described as 3-Ethyl-5-methyl (±)-2-[(2-amino-ethoxy) methyl]-4-(o-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylate, monobenzenesulphonate. Its empirical formula is $C_{20}H_{25}ClN_2O_5C_6H_6O_3S$.
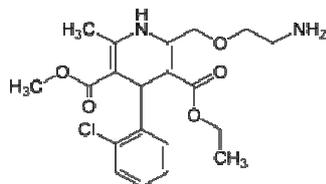
Several methods are cited in literature for determination of IH and AB individually by UV-Vis spectrophotometry [1-4] HPLC [5], GC [6] and HPLC-ESI-MS-MS [7] for individual drug but for combination not only a single method is reported. Hence the objective of work is to develop a simple, accurate, and precise analytical method for this combination in commercial dosage form like tablet. This paper describes validated RP-HPLC method for simultaneous estimation of AB and IH in combination using a mobile phase consisting of 0.01M potassium dihydorgen phosphate buffer (adjusted with 0.1%phosphoric acid to a pH of 3.0), acetonitrile and methanol in the ratio of 50: 35: 15. The column used was Lichrospher C18, 5μ, 25 cm × 4.6 mm maintained at 40°C with flow rate of 1 ml / min using PDA detection at 237 and 210 nm for AB and IH respectively.

Results and Discussion

An attempt was made to develop RP-HPLC method for simultaneous estimation of IH and AB from combined dosage form utilizing C18 column and 0.01M potassium dihydrogen phosphate buffer pH 3, acetonitrile and methanol as mobile phase. Detection of eluent was carried out using PDA detector at 210 and 237 nm for IH and AB respectively. The run time per sample is just 10 min. The excipients in the formulation did not interfere in the accurate estimation of Imidapril HCl and Amlodipine Besylate. The method is validated as per ICH guidelines with respect to linearity, range, accuracy, specificity and precision and results are shown in [Table–1].

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Table-1: Validation Results.

Validation Parameters	IH	AB
Linearity range (μg / ml)	10-100	14-140
r^2	0.99997	0.99997
LOD (μg /ml)	0.13	0.28
LOQ (μg /ml)	0.24	1.47
System Repeatability* (% RSD)	0.762	0.740
Method Repeatability* (% RSD)	1.043	1.135
Accuracy*	100.91± 0.784	99.08 ± 0.541
Specificity	No interference of mobile phase and placebo with AB and IH	
Peak purity index	1.000000	1.000000
Resolution factor (Rs)	-	8.18053
No. of theoretical plates (N)	5552	5843
Capacity factor (K')	-	1.50864
Tailing factor (Asymmetry)	1.11038	1.32063

Each value is a mean of six observations.

Experimental

Materials and Methods

Standard bulk drug sample IH and AB were provided by Mitsubishi Tanabe Seiyaku Co. Ltd. Japan and Dr. Reddy's Laboratories, India. Tablets of combined dosage form were received from product development department of Highnoon Laboratories Limited. All other reagents used were of HPLC grade. HPLC (Hitachi PDA) method was developed using Lichrospher column C18, 5μ, 25 cm × 4.6 mm maintained at 40°C. Mobile phase selected for this method contained 0.01M potassium dihydorgen phosphate buffer of pH 3.0 (adjusted with 0.1% orthophosphoric acid that was filtered through 0.45-micron membrane filter), acetonitrile and methanol in the ratio of 50: 35: 15. Flow rate employed was 1 ml/min. Detection of eluent was carried out at 210 and 237 nm using PDA detector for IH and AB respectively. Standard stock solution of pure drugs was made in mobile phase containing 50 μg /ml of IH and 70 μg /ml of AB and filtered through a 0.45μ membrane filter. Each solution was injected and a chromatogram was recorded. Mean retention times for IH and AB were found to be 4.3 and 6.7 min respectively.

Selection of Analytical Wavelength

Selected dilutions were scanned and absorbance maxima 210 and 237 nm was selected for analysis of IH and AB respectively. [Fig. 1 and 2]

Calibration Curve for Working Standards

Area of prepared dilutions was reported at 2107 and 237 nm respectively and graph was plotted. The coefficient of correlation (r^2) of 0.99997 for IH and 0.99997 for AB was obtained. [Graph 1 and 2]
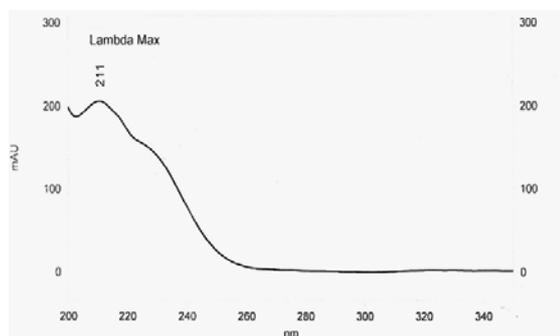

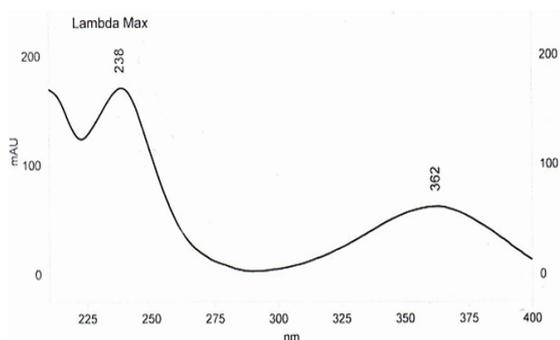Fig. 1:   Spectrum of Imidapril HCI (IH).

Fig. 2:   Spectrum of Amlodipine Besylate (AB).

Analysis of Formulation

Twenty tablets of the formulation were weighed and the average weight per tablet was calculated. Twenty tablets were crushed and ground to a fine powder. Powder equivalent to 25 mg each of IH and AB was weighed and transferred to a 100 ml volumetric flask .The tablet powder was dissolved in the mobile phase and filtered through a membrane filter (0.45μ). The sample solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, 20 μl of the tablet sample solution was injected by a variable injection port and a chromatogram was recorded. The

injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in [Fig. 3]. From the peak areas the amount of each drug present per tablet was estimated from the respective calibration curves. The result of analysis reported in [Table–2].
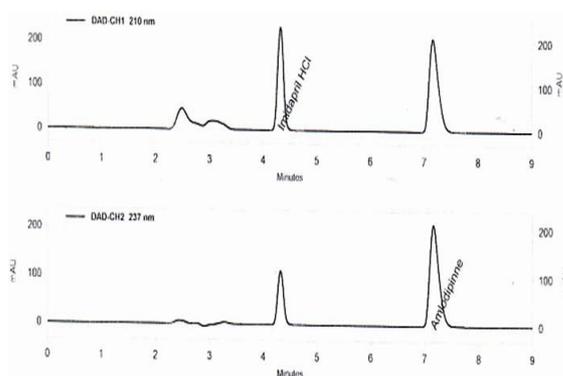

Fig. 3: Chromatogram of sample solution of Imidapril HCI (4.3min) and Amlodipine Besylate (7.1min).

Method Validation

The method was validated as per ICH guidelines [8].

Specificity

The specificity of the method was investigated by observing any interference encountered from the excipient of the tablet. It was shown that these excipients do not interfere with the proposed method.

Precision

The precision was determined at two levels, i.e. system repeatability and method repeatability. System repeatability determined by measurement of six replicates of bulk. Method repeatability determined by measurement of six replicates of sample.

Linearity and Range

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law were found to be 10–100μg/ml for IH (r^2 = 0.99997) and 14-140μg/ml for AB (r^2 = 0.99997).The standard calibration curves are given in [Graph 3 and 4].

Accuracy

Accuracy studies were performed at a level of active ingredient equal to 100% of the established label concentration of the product tested and reported as the difference between the average value found in the analyses and the theoretical value.

Table-2: Results of formulated analysis.

Drugs	Labeled Amount (mg)	Amount taken for assay (µg/ml)	Amount found* (mg)	% label claim	%Recovery*
IH	5	50	50.10 ± 0.095	100.20	100.91 ± 0.784
AB	5	50	49.95 ± 0.242	99.91	99.08 ± 0.541

Each value is a mean of six observations.

Conclusion

The proposed method was validated as per ICH guidelines. The standard deviation and standard error mean calculated for the method are low, indicating high degree of precision of the method. Since none of the methods is reported for simultaneous estimation of IH and AB from combined dosage form, this developed method can be used for routine analysis of two components in formulation.

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