ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Amlodipine and Indapamide in Bulk and Tablet Dosage Form

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Received:
16th Aug 2012
Received in revised form:
9th Sept 2012
Accepted:
12th Sept 2012
Available online:
10th Oct 2012

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

A simple, rapid and precise RP-HPLC method is developed and validated for the simultaneous estimation of amlodipine besylate and indapamide in both bulk drug and combined pharmaceutical-dosage form. The method is based on Ultra Performance Liquid Chromatography (UFLC) on a reversed-phase column, Enable, ODS (C18), 250 mm × 4.6 mm & 5 µm, using a mobile phase of mixed phosphate buffer (pH 4.0) : Acetonitrile (40:60 v/v). The optimum chromatographic conditions to obtain the resolution was flow rate 1.0 mL/min, column temperature at 22˚C and detector wavelength at 247 nm. Both the drugs were well resolved on the stationary phase and the retention times was around 3.5 minute for amlodipine besylate and 4.8 minute for indapamide. The method validation data establish precision, linearity, specificity, limit of detection, limit of quantification and robustness parameters. The linear calibration range was found to be 1 to 10 µg/mL for amlodipine besylate and 0.3 to 3 µg/mL for indapamide. The proposed method can be successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug in combination without any interference by the excipients.

Keywords: Amlodipine Besylate, Indapamide, RP-HPLC, Method development, validation.

1. INTRODUCTION

The present study was aimed to develop simple and precise analytical method for simultaneous estimation of Amlodipine Besylate (AMD) and Indapamide (IND). Amlodipine besylate [1] is chemically 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, is a calcium channel blocking agent. It inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes. The decrease in extracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload. Amlodipine occupies the plasma membrane dihydropyridine receptor and causes competitive blockade of the voltage- operated slow calcium channel.

Indapamide[2] is chemically 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoyl-benzamide. it is an oral antihypertensive agent. The mechanism whereby indapamide exerts its antihypertensive action has not been completely elucidated; both vascular and renal actions have been implicated.

The literature survey reveals that, there have been several publications describing analytical methods for the determination of AMD[3-8] and IND[9-17] individually or with other drugs as combination.

Several methods have been studied for simultaneous determination of Amlodipine and Indapamide, but there is no report on method for combination. So the aim of the present research is to develop and validate simple, fast, accurate and specific reversed phase high performance liquid chromatographic method for

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simultaneous determination of related substances of Amlodipine and Indapamide in bulk drugs and tablet formulation.

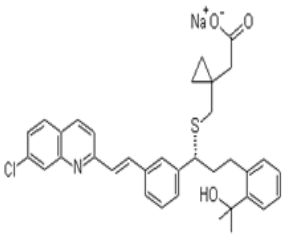

Figure1: Amlodipine Besylate

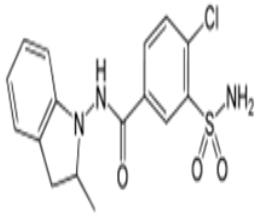

Figure2: Indapamide

Materials and Methods:

2.1. Instrumentation

HPLC system LC SHIMADZU UFLC-2000 ProminanceLC-20AD Binary Gradient System, Shimadzu Corporation, Japan. The column compartment having temperature control and Photodiode Array (PDA) Detector was employed throughout the analysis. Chromatographic data was acquired using LC Solution software. Ultraviolet (UV-1800; 240V) from Shimadzu Corporation, Japan. The Analytical Balance used for weighing, Model-AUX220; Shimadzu Corporation, Japan. The pH meter and Sonicator and Filtration also used for the filtrating the solutions.

2.2. Materials

Pure sample of Amlodipine besylate was obtained as gift sample from Glochem industries limited, Hyderabad and Indapamide was obtained as gift sample from Supra chemicals, Mumbai. Acetonitrile and methanol were of HPLC grade and were purchased SD fine- chem Limited, Mumbai, india.

Potassium dihydrogen ortho phosphate, Disodium hydrogen phosphate, and glacial acetic acid were of analytical-reagent grade and purchased from Thermo fisher Scientific india pvt.Ltd, Mumbai, india. Water was deionised and millipore water. Natrilam tablets were purchased from market. Each tablet was labeled to contain 5 mg amlodipine and 1.5 mg indapamide.

2.3. Methods

2.3.1. Chromatographic Conditions

The method is based on Ultra Performance Liquid Chromatography (UFLC) on a reversed-phase column, enable, ODS (C18), 250 mm × 4.6 mm & 5 µm, using a mobile phase solution of phosphate buffer pH 4.0, mixed (the pH was adjusted to 4.0 ± 0.05 with glacial acetic acid), and acetonitrile (40:60 v/v). The chromatographic conditions are- flow rate of 1.0 ml/min, column temperature at 22˚C and detector wavelength of 247 nm.

2.3.2. Selection of Wavelength

The wavelength of maximum absorption for Amlodipine besylate was 360 nm and Indapamide was at 242 nm. A single wavelength has selected for estimation of Amlodipine besylate and Indapamide as 247 nm as both the peaks have the significant response. Overlaid spectrum of Amlodipine Besylate and Indapamide is given in **Figure 3**.

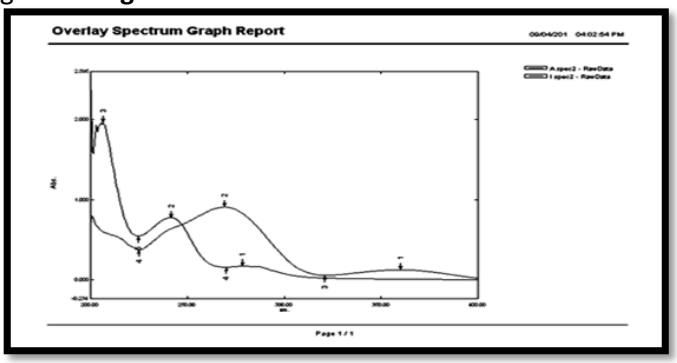

Figure 3. Overlay spectra of Amlodipine besylate and Indapamide.

2.3.3. Mobile Phase

For preparation of the mobile phase, 5.04 gm of Disodium hydrogen phosphate and 3.01 gm of potassium dihydrogen ortho phosphate were dissolved in 900 ml of millipore water. The buffer solution was shaked manually to dissolve and finally make the volume up to 1000 ml with the solution. The pH of phosphate buffer 4.0, mixed was adjusted to 4.0 ± 0.05 with glacial acetic acid. A mixture of phosphate buffer and acetonitrile in the ratio of 40:60 was prepared. Finally the mobile phase was filtered through a 0.45 µm membrane filter and degassed for 15 minutes.

2.3.4 Preparation of Standard solutions:

Standard stock solutions were prepared by dissolving 10 mg each of Amlodipine Besylate and

Indapamide in 10 ml of mobile phase and then 0.1 ml of the above solution was further diluted to

10 ml with the same mobile phase, to get a solution of concentration 10 µg/ml each of Amlodipine besylate and Indapamide then from above solutions 5 µg/ml of Amlodipine Besylate and 3 µg/ml of Indapamide were prepared. A typical chromatogram is given in **Figure 4**.

2.3.6. Estimation of Amlodipine besylate and Indapamide from Tablet Formulation

Randomly picked twenty tablets were weighed accurately and powered. Tablet powder equivalent to 5 mg of Amlodipine Besylate and 1.5 mg of Indapamide were weighed into clean and dry 10 mL volumetric flask. The powder was first dissolved in sufficient volume of mobile phase by sonication and the volume was made to10 mL with mobile phase. Further, filtered through whatman 0.45µ filter paper and 1ml of resultant solution was diluted to 100ml with mobile phase. Then

1mL of above solution was diluted to 100mL with mobile phase. The sample solution (20µl) was injected and the chromatogram was recorded. The peak area of the drugs was calculated and the amount of each drug present per tablet was estimated from the respective regression equation and the is presented in Table 1.

2.3.7. System Suitability Tests

System suitability was verified by injecting standard solution. The percent RSD was verified from the replicate injections of standard solution. Various parameters such as tailing factor and resolution between the peaks of Amlodipine besylate and Indapamide were obtained.

2.4 Method Validation [18-20]: The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1).

2.4.1 Linearity and Range: Linearity was determined by plotting the standard curve in the concentration range of 1-10µg/mL for AMD and 0.3-3µg/mL for IND (Fig. 5**)**. The linearity of the methods was evaluated by linear regression analysis, using least square method Table-1.

2.4.2. Accuracy

This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (50%, 100% and 150%) and the % recovery of AMD and IND was calculated and data presented in **Table-2**.

2.4.3. Precision

2.4.3.1 Injection repeatability (System precision):

The injection repeatability was established by six replicate injections of the standard solution containing both the analytes of interest.

2.4.3.2 Sample repeatability (Method precision):

The sample repeatability was established by carrying out the analysis of the analytes six times.

2.4.4. Ruggedness (Intermediate Precision)

The ruggedness of the method was demonstrated by analysis of the sample by using variations like different days, different analysts and different equipment. For proposed method intermediate precision different days like interday (in between the days) and intraday (with-in the day) was determined. The percent relative standard deviation (% RSD) of two sets of data indicates the ruggedness of the method and data presented in Table-3.

2.4.5. Specificity/Selectivity

The placebo sample solution was prepared with commonly used excipient for tablet formulation like Lactose, Magnesium carbonate, CMC, Mannitol and starch were selected for specificity study. Accurately weighed 85 mg lactose, 3 mg carbonate, 2 mg CMC, 5 mg Mannitol and 5 mg starch were suspended with mobile phase in 100 ml volumetric flask. The resulting solution was filtered through whatman filter paper. Working placebo sample solution was prepared by diluting 0.5 ml of filtrate up to 10 mL with mobile phase and chromatogram presented in Fig.No.4.

2.4.6. Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) for AMD and IND were determined from standard deviation of the response and the slope.

LOD= σ/S X 3.3

LOQ= σ/S X 10

2.4.7. Robustness

The robustness of the method was determined as a measure of the analytical method capability to be unaffected by small variations in method parameters. The different variations such as variation in flow rate by ± 0.1 ml/minute, variation in composition of mobile phase by ± 10 v/v. At these changed conditions, the standard solutions were injected. The amounts of AMD and IND were calculated (% assay) in each varied condition and data ptesented in Table-4.

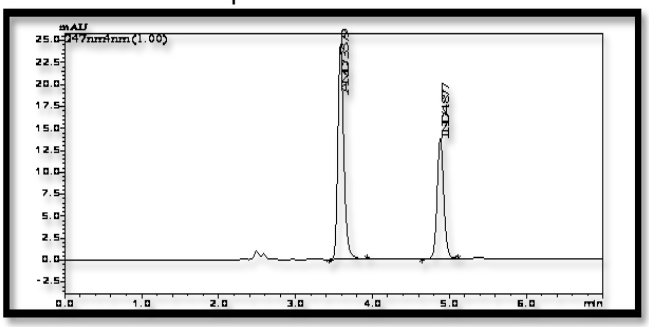

Figure 4.A typical chromatogram of standard solutions of AMD and IND

Results

3.1 Method development:

A variety of mobile phases were investigated in the development of LC method for the analysis of AMD and IND in tablet dosage form. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay.

The maximum absorption wavelength of the reference drug solution was found to be 247 nm. This was observed from the UV absorption spectra (Fig.No-3) and was selected as detection wavelength for LC analysis.

During the optimization of the method, different columns (Inertsil C8, 250mm×4.6mm, 5µm; Zorbax C18 250mm×4.6mm, 5µm; Symmetry C18 250mm×4.6mm, 5µm; Enable C18 G 250mm x 4.6mm, 5µm) and two organic solvents (acetonitrile and methanol) were tested. The chromatographic conditions were also optimized by using different buffers like phosphate, acetate and citrate for mobile phase preparation.

After a series of screening experiments, it was concluded that phosphate buffers gave better peak shapes than their acetate and citrate counter parts. With methanol as solvent both the peaks shows less theoretical plates and more retention time compared to acetonitrile.

While assessing the effect of pH on the retention time of analytes, the peak of AMD with phosphate buffer (pH 2.5) and acetonitrile in the ratio of 40:60 (% v/v), revealed from the PDA analysis, was not pure which suggested co-elution of some impurity peak(s). Further on increase of pH of mobile phase to pH 4.0 MIxed phosphate buffer helped to sharpen the AMD peak, probably due to increase in hydrophobic interactions between stationary phase and less unionized analyte. After several trials, using Enable C18 G (250mm x 4.6mm, 5µm) analytical column and the mobile phase consisting Mixed phosphate buffer (pH 4.0) and ACN (40:60% v/v), and the flow rate of 1.0ml/min was considered optimum to achieve adequate retention time and sharp peaks of both the drugs and their impurities. System suitability parameters (Tailing factor, HETP, Resolution, Theoretical Plates, Asymmetry) for analyte peaks were evaluated and presented in the table-5.

3.2 Method validation:

The calibration plot for the method was linear over the concentration range of 1-10µg/mL for both AMD and 0.3-3 µg/mL for IND. The determination of coefficients (r^2) was 0.999 for both drugs. Values of the method Accuracy was calculated by recovery studies for AMD and IND at three levels and found to be 98.81% to 102.75% and 99.83% and 103.85% respectively. For precision and intermediate precision, % RSD of AMD and IND were within 2.0% thus confirm good precision of the analytical method development. In Specificity there was no any interference at the retention time of AMD and IND in the chromatogram of placebo solution. In peak purity analysis with photo diode detector, purity angle was less than purity threshold for both the analytes. The LOD and LOQ of AMD and IND were found to be 0.2 ng/mL, 0.41 ng/mL and 0.1 ng/mL, 0.21 ng/mL respectively. Robustness of the method was performed by making deliberate changes in flow rate and composition of mobile phase and it was by calculating established % RSD values and was within acceptance criteria range of 2.0%.

Linearity (n=5)	AMD	IND
Range	1-10µg/mL	0.3-3µg/mL
Mean 'r^2' value	0.999	0.999
Regression equation	Y = 13615x + 3602	Y = 26258x + 4360.7

Table 1.Linearity data for AMD and IND

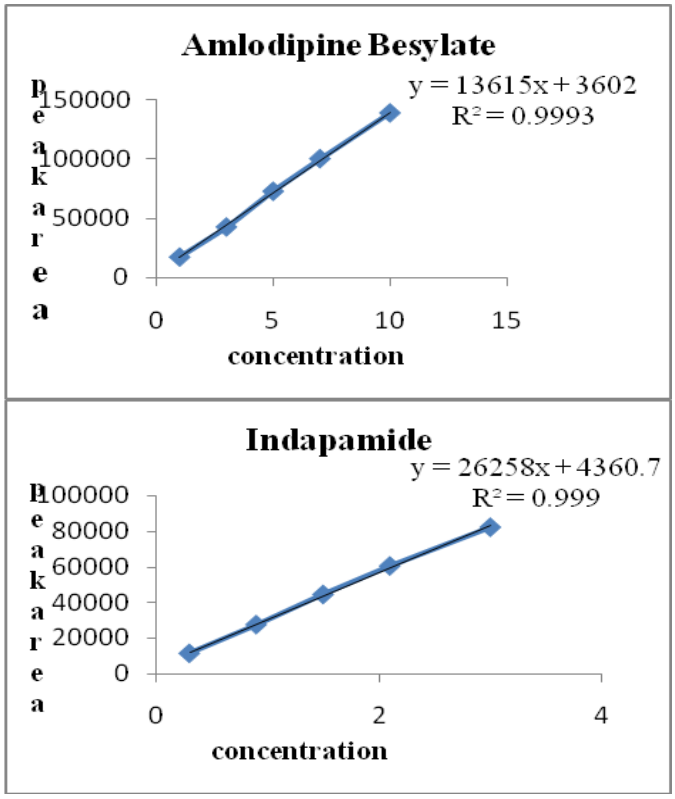

Figure 5.Calibration curve of AMD and IND

Drugs	Levels	Mean recovery	±SD	% RSD
TEL	L_1	102.30	1.37	1.36
	L_2	97.53	0.71	0.73
	L_3	98.17	1.62	1.63
HCTZ	L_1	101.11	1.47	1.45
	L_2	98.51	1.07	1.07
	L_3	104.75	1.17121	1.25

Table 2.Results of recovery analysis of AMD and IND

Compound	Intra-day precision		Inter-day precision	
	% of Label	% RSD	% of Label	% RSD
AMD	99.83	0.16	103.82	0.13
IND	99.66	0.007	100.34	0.06

Table 3. Results of Intermediate precision (intra-day and inter-day) experiment using proposed method

Changing Factor	Level	AMD (n=3) Mean % assay % RSD	IND (n=3) Mean % assay % RSD
Flow rate	0.8 mL	102.13(0.88)	100.60(1.30)
	1.2 mL	101.40(1.59)	99.89 (0.86)
Composition of mobile phase	50:50 (v/v)	98.45(0.83)	98.55 (1.04)
	30:70 (v/v)	100.41 (1.5)	101.50 (0.48)

Table 4. Results of Robustness data for change in flow rate and wavelength

Compound	Retention time (minutes)	Tailing factor	Theoretical plate/meter	Resolution
AMD	3.54	1.14	28,719	1.33
IND	4.87	1.35	52,1225	

Table 5.System suitability data for TEL and HCTZ using proposed method

4. CONCLUSION

The developed HPLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of both AMD and IND in bulk and pharmaceutical formulation without any interference from the excipients. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase, lack of extraction procedures, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of AMD and IND in bulk drugs and in pharmaceutical dosage forms.

Conflict of Interest: None Declared

Acknowledgement:

Authors are thankful to Glochem Industries Limited and Supra Chemicals for providing the gift sample of Amlodipine Besylate and Indapamide.

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