Research Article

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DEVELOPMENT AND VALIDATION OF ION PAIR-LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF INDAPAMIDE AND AMLODIPINE BESYLATE IN BULK AND MULTICOMPONENT FORMULATION

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Received on: 18/06/12 Revised on: 06/07/12 Accepted on: 19/09/12

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DOI: 10.7897/2277-4343.03531

Published by Moksha Publishing House. Website www.mokshaph.com

ABSTRACT

The present paper report the simple, rapid, accurate and precise ion pair high performance liquid chromatographic method for the simultaneous estimation of Indapamide and Amlodipine Besylate in bulk and multicomponant formulation.

The reverse-phase liquid chromatographic analysis has been performed on a Hypersil C_{18} column (250 mm × 4.6mm i.d., 5 µm particle size) column with mobile phase Methanol: Acetonitrile: 0.1 M Heptane sulphonic acid sodium salt (1.5 ml triethylamine was added and pH adjusted to 3.5 with Ortho phosphoric acid) in the ratio 20:40:40 v/v/v and column temperature at 30°C. The flow rate of the mobile phase was adjusted to 1.0 ml/min and the injection volume was 10 µl. Detection was performed at 237 nm. The retention time for Indapamide and Amlodipine besylate were 4.13 min and 7.64 min respectively. The method was validated and shown to be linear for indapamide and Amlodipine Besylate in 25-150 µg/ml (r^2=0.999) and 100-600 µg/ml (r^2=0.999) respectively. The relative standard deviation of Indapamide and Amlodipine besylate for intra-day was 0.2930 and 0.1218 respectively, inter-day was found to be, 0.5905 and 0.2507 respectively.

KEYWORDS: Indapamide, Amlodipine Besylate, heptanes sulphonic acid sodium salt, RP-HPLC, ion pair liquid chromatography.

INTRODUCTION

Indapamide is chemically 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-sulfamoyl-benzamide (structure **a**). Indapamide is diuretics, belongs to the class of Benzothiadiazines and also used as Antihypertensive Agents[1,2].

Amlodipine Besylate is chemically (RS)-3-ethyl 5-methyl 2- [(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate (Structure b). It is dihydropyridine type calcium channel blocker and widely used as Antihypertensive Agents[1,3].

Upon detailed literature survey it was found that, individually and in combination these drugs have been analyzed by many methods such as spectrophotometric[5,6], HPLC[7-9], HPTLC[10] for indapamide and spectrophotometric[11,12] HPLC[13-15], HPTLC[16], Chiral chromatography[17], LC-MS [18,19] for Amlodipine.

Indapamide API is official in the British Pharmacopoeia[1] and U.S. Pharmacopeia[2], Amlodipine Besylate is also is official in the British Pharmacopoeia[1] and Indian Pharmacopoeia[3]. The combination of Indapamide and Amlodipine Besylate is not included in any pharmacopoeias. Thus, objective of present work describes the simple, accurate, precise, sensitive HPLC method for the determination of Indapamide and Amlodipine Besylate in combination. The method was validated as per the ICH guidelines.

MATERIALS AND METHODS

Chemical and reagents

Acetonitrile (HPLC grade), methanol (HPLC grade), triethylamine (HPLC grade), ortho-phosphoric acid (AR grade), Heptanes Sulphonic acid sodium salt (HPLC grade), water (HPLC grade) were procured from Rankem chemicals, Mumbai, India. Standard drug sample of Indapamide and Amlodipine besylate were procured from Macleoids Pvt. Ltd. Mumbai. Tablets are available in the ratio of 3:10 containing Indapamide S.R. (1.5 mg) and Amlodipine Besylate (5 mg) from Natrilam of Serdia Pharmaceuticals Pvt. Ltd., Mumbai.

Chromatographic condition

Analysis was performed on chromatographic system consisted of Perkin Elimer, Series-200 pump (Quaternary system). Separation was carried out with Hypersil ODS C_{18}, (250x 4.6 mm, 5 µ particle size) column at 30^0 C temperature with an isocratic mobile phase constituting Methanol: Acetonitrile: 0.1 M Heptane Sulphonic Acid sodium salt (1.5 ml triethylamine was added and pH adjusted to 3.5 with o-phosphoric acid (20:40:40 v/v/v).

Preparation of 0.1 M Heptane Sulphonic acid sodium salt

About 2.0225 g of heptane sulphonic acid sodium salt were accurately weighed and transferred to 100 ml volumetric flask and total volume was made up to 100 ml with HPLC grade water to prepare 0.1 M heptane sulphonic acid sodium salt solution.

Preparation of Standard solution

10 mg of Indapamide and 50 mg of Amlodipine besylate were weighed accurately and transferred to 50 ml

volumetric flask and dissolved in 25 ml of mobile phase and then volume was made up to the mark with mobile phase to get 200 µg/ml and 1000 µg/ml of stock solution of Indapamide and Amlodipine besylate respectively. A figure 2 and 3 represents the typical chromatogram of standard Indapamide and Amlodipine besylate respectively.

Preparation of sample solution

Twenty tablets, labeled as containing 1.5 mg of Indapamide, and 5 mg of Amlodipine besylate together with excipients, was accurately weighed, and finely powdered. A weight of powder equivalent to 1.5 mg of Indapamide and 5 mg of Amlodipine besylate was weighed accurately and transferred into 25 ml volumetric flask and volume was made up to 25 ml with Mobile phase. The contents were sonicated for 10 minutes and filtered through 0.45 µm membrane filter paper. The final solution was prepared by appropriate dilution of this solution to get the concentration 30 µg and 100 µg of Indapamide and Amlodipine besylate respectively. A typical chromatogram of Indapamide and Amlodipine besylate are shown in figure 4.

Table 1: Validation parameter for Indapamide and Amlodipine besylate

Validation Parameter	Indapamide	Amlodipine besylate
Linearity (conc.)	25-150 µg/ml	100-600 µg/ml
Accuracy (mean, n=3)		
80%	99.99	100.04
100%	99.63	99.83
120%	100.02	100.19
Mean	99.90	100.05
RSD	0.277	0.232
Precision		
Repeatability (n=6), % RSD	0.2805	0.2743
Intraday	0.2930	0.1218
Interday	0.5905	0.2507
LOD	0.0000319	0.000087
LOQ	0.0000967	0.00026
System Suitability Parameter		
Retention time (min)	4.13	7.64
Resolution	11	
Asymmetry Factor	1.442	1.579
Theoretical plate	10497.49	7522.68
Peak area	4409096	5300362
Analysis of marketed Formulation		
% Mean	99.90	100.05
RSD	0.1832	0.1233

Table 2: Robustness data for Indapamide and Amlodipine besylate

Robust Condition		**Retention time (RT)**		**Tailing factor**		**% assay**	
flow rate							
ml/min		Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate
0.8	-0.2	5.11	7.99	1.50	1.60	101.54	101.73
1	0	4.01	6.80	1.44	1.552	99.99	100.06
1.2	0.2	3.66	5.30	1.32	1.421	99.99	99.86
		RT		**Tailing factor**		**% assay**	
temp		Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate
28	-2	4.11	6.52	1.57	1.68	100.67	101.15
30	0	4.01	6.80	1.44	1.55	99.99	100.04
32	+2	4.03	6.28	1.54	1.52	100.10	99.42
		RT		**Tailing factor**		**% assay**	
mobile phase ratio		Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate
18:38:44	-	5.04	2.44	1.54	1.53	99.50	99.74
20:40:40	0	4.01	6.72	1.44	1.55	100.01	100.05
22:36:42	-	3.77	6.80	1.47	1.57	100.38	101.58
		RT		**Tailing factor**		**% assay**	
pH		Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate	Indapamide	Amlodipine besylate
3.3	-0.2	5.2	11.68	1.52	1.587	99.39	99.52
3.5	0	4.01	6.80	1.44	1.552	99.99	100.03
3.7	+0.2	4.22	7.13	1.49	1.604	101.85	101.98

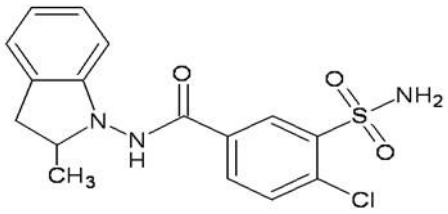

Figure 1(a): Indapamide

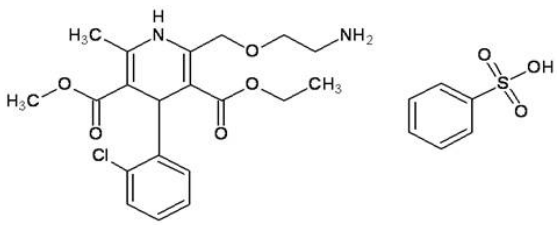

Figure 1(b): Amlodipine Besylate

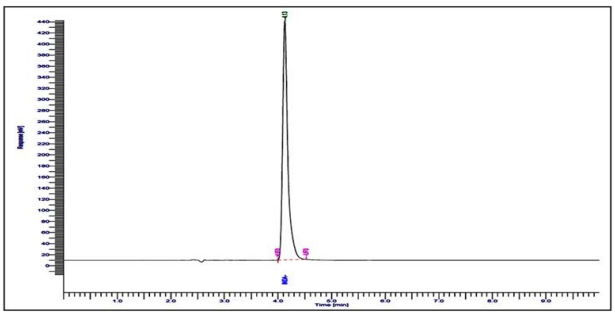

Figure 2: HPLC chromatogram of standard Indapamide

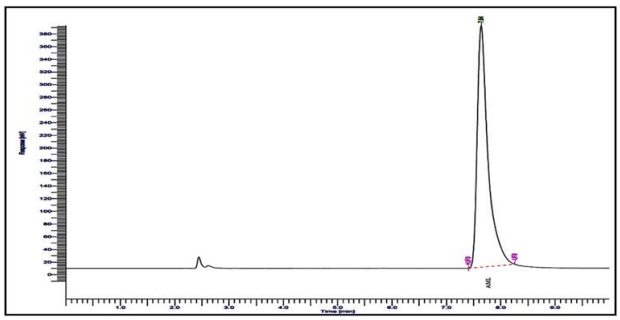

Figure 3: HPLC chromatogram of standard Amlodipine Besylate

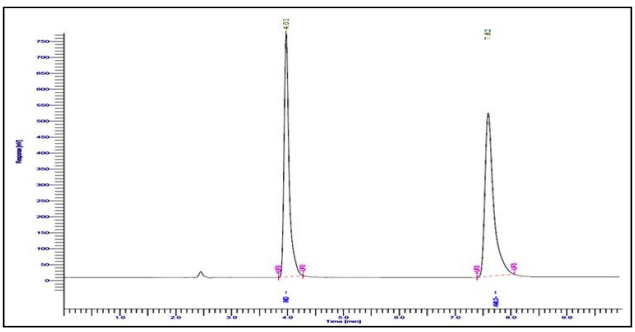

Figure 4: Typical Chromatogram of Indapamide and Amlodipine besylate mixture

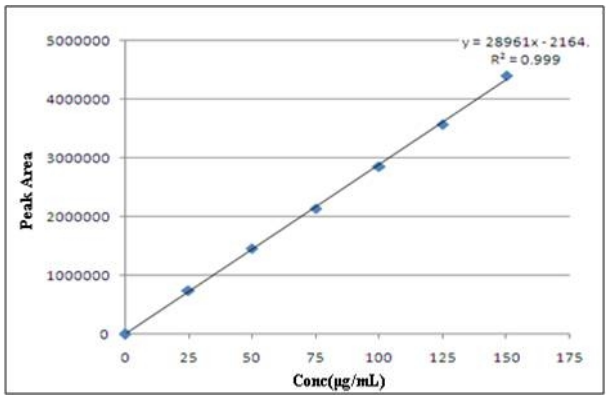

Figure 5: Calibration curve for standard Indapamide

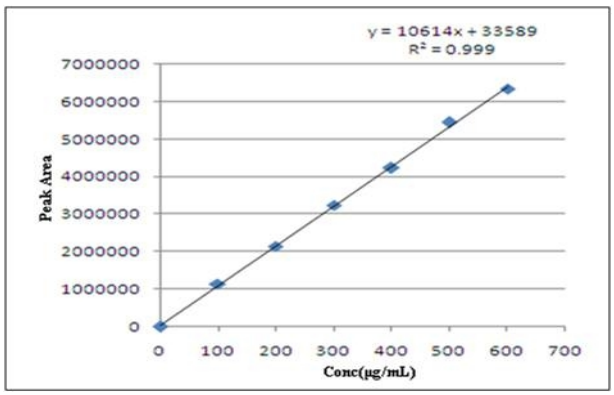

Figure 6: Calibration curve for standard Amlodipine besylate

RESULTS

Calibration and Linearity

Linearity was determined by calculating the regression line using a mathematical treatment of the results (response) versus analyte concentration. Solutions were injected in triplicate. It was determined for Indapamide in the range of 25-150 µg/ml (Figure 5) and 100-600 µg/ml for Amlodipine besylate (Figure 6). The linear regression data for both the analyte concentration was within range i.e.≤ 1.

Specificity and Selectivity

The specificity of the HPLC method was determined by complete separation of Indapamide and Amlodipine Besylate by peak purity with the PDA detector. The results of the peak purity analysis show that the peaks of the analytes were pure and also the formation of excipients were not interfering with the analyte peaks.

The selectivity of an analytical method was measured accurately by the analyte of interest in the presence of components that may be expected to be present in the sample matrix. The absence of interfering endogenous components at the retention times of the both the drug was demonstrated by the chromatograph. The average retention time ± standard deviation (S.D) for Indapamide and Amlodipine Besylate were found to be 4.09 ± 0.046 and 7.64±0.076 respectively.

Accuracy

Accuracy was performed by recovery studies. The recovery studies were carried out at three concentration level 80%, 100%, 120% by standard addition method. The percentage recovery and standard deviation were calculated and presented in Table 1.

Precision

The precision studies were carried out as repeatability, intra-day precision and inter-day precision. The studies were performed by six replicate of the same solution using standard drug. The intra-day studies were carried at different time within same day while the inter-day precision was carried at the same time on different day. The intra- and inter-day variation (S.D. and relative standard deviation RSD) method throughout the linear range of concentrations is shown in Table 1.

Robustness

The robustness study was carried out by variable experimental condition as change pH, flow rate, organic solvent ratio, and temperature. The result of robustness studies are shown in Table 2.

LOD and LOQ

The LOD and LOQ were separately determined which is based on calibration curve. The residual standard deviation of regression line or the standard deviation of y-intercept of regression line may be used as standard deviation. The lower limit of Indapamide and Amlodipine besylate were found to be 0.0000319 and 0.000087 µg/mL respectively. Limit of detection were found to be 0.0000967 and 0.00026 µg/mL for Indapamide and Amlodipine besylate respectively.

System suitability

The system suitability was checked using parameters such as tailing factor, resolution, theoretical plate number, peak area, retention time. These parameters were found to be within limit. The results of system suitability are given in Table 1.

DISCUSSION

The objective of the work was to develop the simple, accurate, precise and sensitive HPLC method for the estimation of Indapamide and Amlodipine besylate in bulk and multicomponent formulation. Both the drugs are strong basic in nature thus the efforts were made to separate these component with shorter retention time, asymmetry factor and better resolution. To achieve this goal an ion pairing agent heptane sulphonic acid sodium salt was used in mixture of mobile phase. Ion pairing agent is bind to the solute molecules by ionic interaction to increase the hydrophobicity of the solute molecule and change selectivity. Better resolution has been achieved by keeping the column temperature at 30^0C and flow rate at 1.0 ml/min. The developed method is applicable for analysis of the drug combination (Indapamide and Amlodipine besylate) in pharmaceutical formulation as this combination is not included in pharmacopoeias.

CONCLUSION

From the results obtained by all validation parameters, it is concluded that developed isocratic ion pairing RP-HPLC method is suitable for the simultaneous estimation of Indapamide and Amlodipine besylate in bulk and multicomponent formulation. Therefore, the method is found to be suitable for routine analysis of marketed tablet formulations in quality control laboratories.

ACKNOWLEDGEMENT

Authors are thankful to Director of school of pharmacy, S. R. T. M., University, Nanded for their support and for providing necessary facilities to carry out this work.

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Cite this article as:

Kalyankar TM, Khadkutkar PK, Kakde RB. Development and validation of Ion Pair-Liquid Chromatographic method for the simultaneous estimation of Indapamide and Amlodipine besylate in bulk and multicomponent formulation. Int. J. Res. Ayur. Pharm. 2012; 3(5):729-732

Source of support: Nil, Conflict of interest: None Declared