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RESEARCH ARTICLE

Development and validation of HPLC method for the determination of S (-) Amlodipine Besylate and its related substance in tablet formulation by using chiral separation.

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ABSTRACT

A simple, specific and sensitive high-performance liquid chromatographic (HPLC) method for determination of S (-) Amlodipine Besylate (SAB) and its related substance i.e. R (+) Amlodipine Besylate (RAB) in tablet formulation was developed and validated. The isomer separation was carried out using simple isocratic reversed-phase HPLC method using a chiral column. Isocratic elution at a flow rate of 1.0 mL/min was employed on chiral column Ultron-ES-OVM, (250 x 4.6 mm, 5 µm). The mobile phase consisted of (Buffer: ACN) (78:22, v/v) in which buffer is mixture of 0.01M disodium hydrogen phosphate dihydrate and 0.01 M potassium dihydrogen phosphate. The UV detection wavelength was 237 nm and 20 µL of sample was injected. Method was validated with respect to specificity, linearity, precision accuracy and robustness. The method was found to be linear for the concentration range of 0.04 – 0.06 mg/mL of SAB with correlation coefficient r = 0.9998. The proposed method was applied successfully for quantification of SAB and RAB in tablet formulation. The method was found to be simple, accurate, sensitive and reproducible. It can be successfully utilized for routine analysis in quality control laboratory.

KEYWORDS: S (-) Amlodipine Besylate, R (-) Amlodipine Besylate, dihydropyridine, HPLC, validation.

1. INTRODUCTION

Amlodipine is a calcium channel antagonist belonging to the dihydropyridine class. It is chemically 3-ethyl 5-methyl-2-[(2-aminoethoxymethyl]-4-(2-chlorophenyl)- l,4-dihydro-6-methy 1-3,5-pyridinedicarboxylate.[1] Amlodipine is widely used for the treatment of hypertension as well as stable and variant angina and known to be life saving drug.[2]

Amlodipine is therapeutically used as racemic mixture similar to the other calcium channel blocking agents of dihydropyridine type.[3] The S (-) isomer of amlodipine is found to possess greater pharmacological effects than R (+) amlodipine. S (-) amlodipine is 1000 times more potent than the R (+) isomer in binding to the dihydropyridine receptor. In humans, the dominant effects of amlodipine are consequent to vasodilation. S (-) amlodipine lowers peripheral vascular resistance without causing a reflex tachycardia. It is effective as a once daily dosage in the control of hypertension.[4-6] The efficacy and tolerability of 2.5 mg of SAB is almost similar with that of 5 mg of amlodipine besylate in the treatment of mild to moderate hypertension. Pharmacokinetic behaviour of RAB and SAB after single enantiomer administration is comparable to that of each enantiomer after administration of the racemate, no racemization occurs in vivo in human plasma after single enantiomer administration.[1,7,8]

Due to the therapeutic importance of amlodipine, several analytical methods have been reported for its quantitative determination both in pure form as well as in pharmaceutical formulations. Different analytical methods reported for the determination of amlodipine are liquid chromatography [9-14], reversed phase high performance liquid chromatography[15-18], gas chromatography[19,20], high performance thin layer chromatography[21–23], liquid chromatography with tandem mass spectrometry[24] and fluorimetry[25].

In this research work, the objective is to develop and validate HPLC method for determination of SAB and its

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related substance i.e. RAB in tablet formulation by using chiral column separation technique.

MATERIALS AND METHODS

MATERIALS:

S (-) Amlodipine Besylate (SAB) and R (+) Amlodipine Besylate (RAB) working standards were purchased from Glochem industries limited (Hyderabad, AP, India). Acetonitrile HPLC grade, Methanol HPLC grade, Potassium dihydrogen orthophosphate AR and disodium hydrogen orthophosphate dihydrate AR were purchased from Merck limited (Mumbai, India). High purity deionised water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

METHODS:

Instrumentation:

HPLC system (Waters 717 plus auto sampler HPLC system USA.) consisting of pump 515, Injector and 2487 dual wavelength absorbance UV detector were employed for analysis. Chromatographic data was acquired using Empower software.

Chromatographic conditions:

Ultron ES-OVM, (250mm x 4.6mm, 5 µm) chiral column was used as a stationary phase. The isocratic mobile phase consists of mixture of buffer and acetonitrile in the ratio of 78:22 (v/v). The buffer is mixture of 0.01M disodium hydrogen phosphate dihydrate and 0.01M potassium dihydrogen phosphate. The flow rate of the mobile phase was 1.0 mL/min and the injection volume was 20 µL. Detector signal was monitored at a wavelength of 237 nm.

Solution preparation:

Standard solution:

Accurately weighed quantity of powder (37.02 mg of Amlodipine Besylate) equivalent to 25 mg of S (-) Amlodipine was taken in 50ml volumetric flask. Thirty millilitres of mobile phase was added into the flask followed by sonication of 5 minute in order to obtain clear solution. Volume was made up to 50 ml with mobile phase. The solution was filtered by 0.45 µm membrane filter.

Sample solution:

Tablets (n=20) were finely grinded in a porcelain mortar. The average mass of the 20 tablets taken was determined. In a 50 ml volumetric flask, accurately weighed quantity of powder equivalent to about 25 mg of S (-) amlodipine was taken. Thirty millilitres of mobile phase was added into the flask and it was sonicated to obtain clear solution. Volume was made up to 50 ml with mobile phase. The solution was filtered by 0.45 µm membrane filter.

Validation procedure:

The specificity of the method was determined by injecting the sample solution containing excipients with and without drugs having concentration same as that of the standard. Five standard solutions were prepared for the linearity test of SAB in the range of 0.04-0.06 mg/ml and for the linearity test of RAB in the range of 0.03-0.07 mg/ml in mobile phase. Each solution was injected in three replicates and linear regression analysis for SAB and RAB was performed.

The limit of detection (LOD) was determined from the regression data of calibration curve by using following formula (Equation 1)

$$LOD = 3.3 \times \frac{\sigma}{S} \quad (1)$$

Where, σ is the average residual standard deviation and S is slope of the calibration line.

Limit of quantification (LOQ) was calculated using following formula (Equation 2).

$$LOQ = 10 \times \frac{\sigma}{S} \quad (2)$$

The accuracy of the method was carried out by adding known amount of each drug corresponding to three concentration levels 50%, 100% and 150% of the label claim along with the excipients in triplicate. The samples were given the same treatment as mentioned for the sample solution preparation. Recovery of SAB was determined in presence of excipients.

Precision of the method was checked by carrying out six independent assays of test samples against standard. Intermediate precision was performed by analyzing the samples by different analyst on different day.

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.8 mL/min and 1.2 mL/min. The organic strength was varied by ±2 % units. Standard solution was injected six times in replicate for each change.

Respective peak areas, dilution factors, sample and standard weights were taken into account to quantitate the amounts of SAB in milligram per tablet.

Market sample evaluation:

Three commercial lots of the S-Numlo 2.5 mg Emcure Pharma limited (Pune, Maharashtra, India) tablets were analyzed for content of RAB.

RESULT AND DISCUSSION

Selection of column and sample preparation:

Preliminary experiments have been performed based on various HPLC methods available in the literature for the analysis of amlodipine racemate. But the separation of isomers on normal silica gel analytical column is very difficult, so we have selected chiral analytical column, Ultron-ES-OVM, (250 x 4.6 mm, 5 µm) in order to get good resolution between SAB and RAB. The sample preparation and concentration selection for analysis was very critical. It was found that at higher concentration both isomers were found to be merged into single peak during the analysis.

Therefore, we have taken 0.05 mg/ml as a standard concentration for analysis.

Optimization of the chromatographic conditions:

The effect of composition of the mobile phase on the retention time of SAB and RAB was thoroughly investigated. Mobile phase composition of buffer: Acetonitrile (78:22 v/v) was found to be optimum to separate the SAB and its related compound peaks i.e. RAB peak. The optimum pH of mobile phase is 6.98 to 7.02, which was adjusted by any one the salt used in buffer. Mobile phase flow rate of 1 mL/min was found to be optimum for separation of SAB and RAB with satisfactory resolution. The chromatogram was recorded at 237 nm. The elution order was found to be for related substance i.e. RAB (RT = 6.12 min), SAB (RT= 6.93 min, resolution = 1.43) at optimized chromatographic condition as shown in Figure 1.

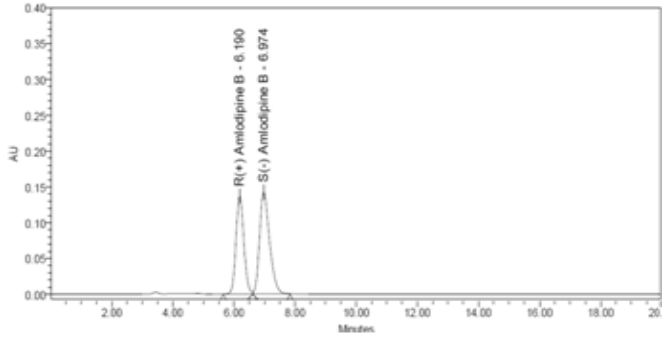


Figure 1: Chromatogram obtained from the analysis of SAB and RAB showing good resolution

METHOD VALIDATION:

Specificity:

The HPLC chromatogram recorded for the mixture of the excipients revealed no peak (Figure 2). The chromatogram recorded for mixture of SAB, RAB and excipients shows distinguishing peaks for the two isomers as shown in Figure 1. None of the excipients interfered with the analyte of interest and hence method was found to be specific.

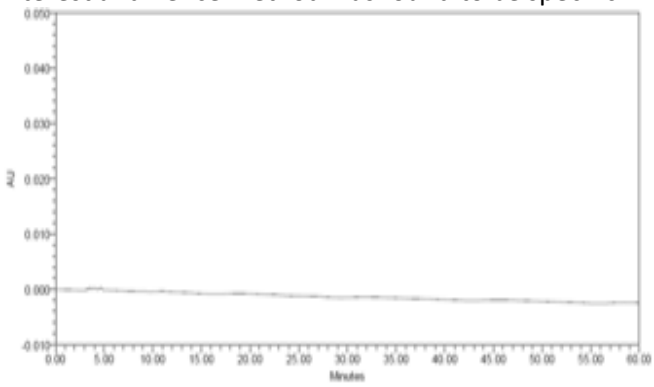
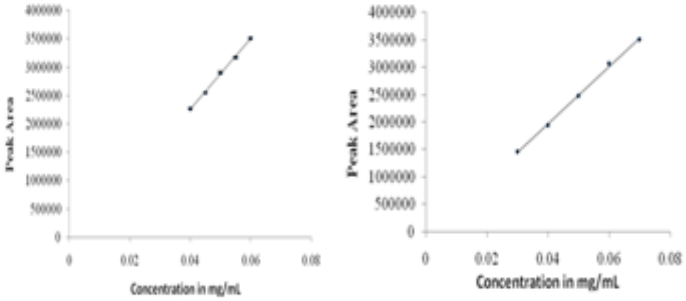


Figure 2: Chromatogram obtained from analysis of placebo tablet formulation

Linearity, Limit of detection (LOD) and Limit of quantitation (LOQ):

Calibration curve with five points were constructed covering a concentration range 0.04-0.06 mg/mL for SAB and 0.03- 0.07mg/mL for RAB (Figure 3a & Figure 3b). Three independent determinations were performed at each concentration. Linearity equation and correlation coefficient were found to be: y = 62271.185x - 232827.161; r=0.9998 for SAB. Similarly linearity equation and correlation coefficient for RAB were y= 50564x-312; r = 0.9999. LOD and LOQ for RAB were found to be 0.5 µg/mL and 1.5 µg/mL respectively.



(a) **(b)**

Figure 3: (a) Calibration graphs of SAB (n=3), showed linear relationship (y = 62271.185x - 232827.161; r=0.9998) and (b) Calibration graphs of RAB (n=3), showed linear relationship (y= 50564x-312; r = 0.9999.)

Accuracy:

The data for accuracy were expressed in terms of percentage recoveries of SAB from tablet formulation. The results are summarized in Table 1. The mean recovery data of SAB in real sample were within the range of 98.34% and 104.60 %. Mean percent relative standard deviation (RSD) was 2.45 %, satisfying the acceptance criteria for the study. Assay results of formulation are shown in Table 2.

SAB concentration (mg/ml)	Mean recovery (%)	RSD (%)
12.5	98.35	1.14
25	98.91	1.10
50	104.60	0.90
62.5	100.76	0.60
75	100.25	0.45

Table 1: Accuracy study for SAB (n = 15)

Batch	S (-) Amlodipine label claim (mg)	Assay (mg/tablet)	Assay (%)
01	2.5.00	2.490	99.60
02	2.5.00	2.495	99.80
03	2.5.00	2.498	99.92

Table 2: Assay results of S (-) Amlodipine 2.5mg tablets

Precision:

The repeatability study and intermediate precision was performed (Table 3). The results were well within the acceptance limit (R.S.D. < 2%) indicating a good system and method precision.

Test sample	Day 1	Day 2	Day 3
1	100.67	100.1	98.34
2	100.1	99.63	101.93
3	100.85	100.91	101.92
4	98.7	98.78	99.48
5	100.62	100.41	101.12
6	98.76	99.32	100.07
Average	99.95	99.77	100.47
SD	0.977	0.770	1.430
% RSD	0.978	0.770	1.430
Ovarall % RDS	0.612		

Table 3: Repeatability and intermediate precision for S (-) amlodipine Besylate

Robustness:

In all deliberately varied conditions, the RSD of peak areas of SAB found to be well within the acceptable limit of 2% and the symmetry factor was found to be <1.5.

Market sample evaluation:

Validated method has been applied for determination of related substance i.e. RAB in the three commercial lots of the formulation. The results are presented in Table 4. The results complies the label claim very well reflecting the reproducibility of the proposed method. It was found that all the formulation contains less than 1% of RAB in the formulation.

Batch No.	Mean Area of RAB	Mean Area of SAB	Percentage of RAB
LKBO-7005	29370	27696386	0.1052
ECBO - 7027	29152	27780076	0.105
ECCO - 7015	29187	27797523	0.1051
Mean	29236.33	27757995	0.1051
SDV	117.0741	54063.399	0.0001
%RSD	0.400	0.19476695	0.095147

Table 4: Percentage of R (+) amlodipine besylate impurity in marketed formulation

CONCLUSION

A validated HPLC method has been developed for quantification of SAB and RAB in tablet formulation. The developed method is simple, specific, accurate, precise and robust. The proposed method can be utilized for routine analysis and quality control of SAB and RAB in tablet formulation.

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REFERENCES

1. Luksa J, Josic DJ, Kremser M, Kopitar Z, Milutinovic S. Pharmacokinetic behaviour of R-(+)- and S-(−)-amlodipine after single enantiomer administration. Journal of Chromatography B: Biomedical Sciences and Applications 1997; 703(1–2):185-193.

2. Nafisur R, Manisha S, Md. Nasrul H. Application of oxidants to the spectrophotometric determination of amlodipine besylate in pharmaceutical formulations. IL Farmaco 2004; 59:913-919.

3. Streela B, Laine C, Zimmera C, Sibenalerb R, Ceccato A. Enantiomeric determination of amlodipine in human plasma by liquid chromatography coupled to tandem mass spectrometry. Jounal of Biochemical and Biophysical Methods 2002; 54: 357-368.

4. Zhang XP, Loke KB, Mital S, Chahwala S, Hintze TH. Paradoxical Release of Nitric Oxide by an L-Type Calcium Channel Antagonist, the R+ Enantiomer of Amlodipine. Journal of Cardiovascular Pharmacology 2002; 39(2): 208-214.

5. Goldmann S, Stoltefuss J, Born L. Determination of the absolute configuration of the active amlodipine enantiomer as (-)-S: a correction. Journal of Medicinal Chemistry 1992; 35(18): 3341-3344.

6. Kim SA, Chug N, Lim DS, Yang JY, Oh BH, Tahk SJ, Ahn TH. Efficacy and safety profiles of a new S(—)-amlodipine nicotinate formulation versus racemic amlodipine besylate in adult Korean patients with mild to moderate hypertension: An 8-week, multicenter, randomized, double-blind, double-dummy, parallel-group, phase III, noninferiority clinical trial. Clinical Therapeutics 2008; 30(5): 845-857.

7. Ohmoii M, Arakawa M, Takasaki K, Hifumi S, Miyamori I, Fujimura A. Stereoselective pharmacokinetics of amlodipine in elderly hypertensive patients. American Journal of Therapeutics 2003; 10(1): 29-31.

8. Luksa J, Josic D, Podobnik B, Furlan B, Kremser ML. Semi-preparative chromatographic purification of the

enantiomers S-(−)-amlodipine and R-(+)-amlodipine. Journal of Chromatography B: Biomedical Sciences and Applications 1997; 693(2): 367-375.

9. Shimooka K,Sawada Y, Tatematsu H. Analysis of amlodipine by a sensitive high performance liquid chromatography method with amperometric detection. Journal of Pharmaceutical and Biomedical Analysis 1989; 7:1267-1272.

10. Yeung PKF, Mosher SJ, Pollack PT. High performance liquid chromatography assay for amlodipine: chemical stability and pharmacokinetics in rabbits. Journal of Pharmaceutical and Biomedical Analysis 1991; 9: 565-571.

11. Bhushan R, Gupta D, Singh SK. Liquid chromatographic separation and UV determination of certain antihypertensive agents. Biomedical Chromatography 2006; 20(2): 217-224.

12. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Validated HPLC method for determination of amlodipine in human plasma and its application to pharmacokinetic studies. IL Farmaco 2005; 60(9): 789-792.

13. Baranda AB, Jimenez RM, Alonso RM. Simultaneous determination of five 1,4-dihydropyridines in pharmaceutical formulations by high-performance liquid chromatography–amperometric detection. Journal of Chromatography A 2004; 1031(1–2): 275-280.

14. Shimooka K, Sawada Y, Tatematsu H. Analysis of amlodipine in serum by a sensitive high-performance liquid chromatographic method with amperometric detection. Journal of Pharmaceutical and Biomedical Analysis 1989; 7(11): 1267-1272.

15. Patel YP, Patil S, Bhoir IC, Sundaresan M. Isocratic, simultaneous reversed-phase high-performance liquid chromatographic estimation of six drugs for combined hypertension therapy. Journal of Chromatography A 1998; 828(1-2): 283-286.

16. Avadhanulu AB, Srinivas JS, Anjaneyulu Y. Reversed phase HPLC determination of amlodipine in drugs and its pharmaceutical dosage forms. Indian Drugs 1996; 33:36.

17. Sankar SR, Nanjan MJ, Vasudevan M, Shaat N, Suresh B. Simultaneous estimation of atenolol and amlodipine in formulations by reversed phase-HPLC. Indian Journal of Pharmaceutical Sciences 1997; 59:171-173.

18. Dhorda VJ, Shetkar NB. Reversed phase liquid chromatographic determination of ramipril and amlodipine in tablets. Indian Drugs 1999; 36: 638.

19. Bresford AP, Marcrac PV, Stopher DA, Wood BA. Analysis of amlodipine in human plasma by gas chromatography. Journal of Chromatography 1987; 420:178-183.

20. Scharpf F, Riedel KD, Laufen H, Leitold M. Enantioselective gas chromatographic assay with electron-capture detection for amlodipine in biological samples. Journal of Chromatography B: Biomedical Sciences and Applications 1994; 655(2): 225-233.

21. Pandya KK, Satia M, Gandhi TP, Modi IA, Modi RI, Chakarvarthy BK. Detection and determination of total amlodipine by high performance thin layer chromatography: a useful technique for pharmacokinetic studies. Journal of Chromatography B; Biomedical Sciences and Applications 1995; 667(2): 315-320.

22. Ilango K, Kumar PB, Prasad VRV. Simple and rapid high performance thin layer chromatographic determination of amlodipine in pharmaceutical dosage forms. Indian Journal of Pharmaceutical Sciences 1997; 59(6): 336-337.

23. Agrekar AP, Powar SG. Simultaneous determination of atenolol and amlodipine in tablets by high performance thin layer chromatography. Journal of Pharmaceutical and Biomedical Analysis 2000; 21(6):1137-1142.

24. Baranda AB, Mueller CA, Alonso RM, Jimenez RM, Weinmann W. Quantitative determination of the Calcium Channel Antagonists Amlodipine, Lercanidipine, Nitrendipine, Felodipine, and Lacidipine in Human Plasma Using Liquid Chromatography-Tandem Mass Spectrometry. Therapeutic Drug Monitoring 2005; 27(1): 44-52.

25. Mohamed YE, Naglaa MEK, Bahia AM, Nasshwa GM. Fluorimetric determination of amiodrone, amlodipine and propafenone. Bulletin of Faculty of Pharmacy 1998; 36: 1-9.

Conflict of Interest: None Declared

