Research Article **CODEN: AJPAD7** **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

DIRECT CHIRAL SEPARATION AND QUANTITATIVE DETERMINATION OF S-AMLODIPINE BY RP-HPLC-PDA METHOD

R. Sathiyasundar*[1], K. Selvakumar[1], K. Valliappan[1], R. Suresh[1], P. Venkatesan[1], M. Swamivelmanickam[1]

[1*]Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar, Tamil Nadu, India-608002.

ABSTRACT

The simple, sensitive, rapid, direct chiral method for the quantitative determination of chiral purity in S-Amlodipine by HPLC-PDA. The stationary phase was Lux-2 chiral column and mobile phase was Acetonitrile: Triethylamine: Acetic acid (100: 0.1: 0.2 % v/v/v) and measurement was carried out in PDA at a flow rate of 1.0 ml min^{-1}. This method was validated as per the ICH guideline and the method was accurate, precise, specify, linear and sensitive. In enantiomeric separation of the chromatographic run time were less than 7.0 min and R- form eluted at 5.87 and S- form eluted at 6.79 min respectively. The calibration plots were linear over the concentration ranges 1.0-5.0 µg ml^{-1} for both S and R-Amlodipine. The proposed method was successfully applied to separate (S)-enantiomers from (±)-Amlodipine and was proven to be reproducible and accurate for the quantitative estimation of (S)-enantiomers in bulk drugs and pharmaceutical formulation. The proposed method can be applied to identify and quantitative estimation chiral purity of both recemic amlodipine and S-amlodipine.

KEY WORDS

(±)-Amlodipine, S- amlodipine, HPLC-PDA and Direct chiral separation.

Author for Correspondence:

Sathiyasundar R,
Department of Pharmacy,
FEAT, Annamalai University,
Annamalai Nagar,
Tamil Nadu, India-608002.

Email: sundaranalysis@gmail.com

INTRODUCTION

Chiral separation in this field has grown itself into a specialized discipline concerned with the three dimensional aspect of drug action and disposition. This approach essentially values each version of the chiral twins as separate chemical species[1,2]. Racemic drugs are not drug combinations in the accepted sense of two or more co-formulated therapeutic agents, but combinations of isomeric substances whose pharmacological activity may well reside predominantly in one specific enantiomeric form[3]. The majority of these are recemic mixtures of synthetic chiral drugs; mixtures of diastereoisomers

(compounds with multichiral centers) are used less frequently. The use of these can be thought of as polypharmacy, with the proportions of the various optical forms presently being dictated by chemical rather than pharmacological or therapeutic criteria[4,5]. Amlodipine (AML) is chemically known as a 3-ethyl 5-methyl-2-[(2-(aminoethoxy methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6methyl-3, 5-pyridinedicarboxylate (Figure No.1), It is mostly used for the treatment of antihypertensive in calcium channel blocker, especially dihydropyridine type[6]. Amlodipine is therapeutically used as racemic mixture; however, more recently S-amlodipine is available in market because S-form is more potent vasodilatation effect then recemic mixture[7]. From, the literature search there are many methods have been cited for the enantio separation of amlodipine in formulation[8], Pharmacokinetic behavior of *R*- and *S*-amlodipine in single enantiomer administration of plasma samples[9-12] and metabolites by HPLC method. There are other methods also reported such as Liquid chromatography–tandem mass spectrometry in chiral study of amlodipine biotransformation in rat hepatocytes[13, 14] and CE[15,16], with Hydroxypropyl-*b*-Cyclodextrin as chiral selector, for separation of the enantiomers of amlodipine in the serum of hypertensionpatients[17] and pharmacokinetic studies by HPTLC[18]. In perversely reported methods are having a few drawbacks and they and all excessive run time as well as utilized complicated procedures i.e. nonpolar solvents and buffer preparations considered. There is need to develop a method to overcome all the above issues.

The main objective of the research was carried out easiest way to separate and quantitative determination of chiral purity in S-amlodipine and (±)-amlodipine by RP-LC-PDA. The optimized method was validated as per the ICH Q2 (R1) guidelines.

MATERIALS AND METHOD

Apparatus

In this study was performed with a Shimadzu (Japan) chromatograph equipped with an LC-10 AD and LC-10 AD vp solvent-delivery module, an SPD-10A PDA detector, and a Rheodyne model 7125 injector valve fitted with a 20µL sample loop. The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1.11SP1) installed on it. The mobile phase was degassed using Branson sonicator (Branson Ultrasonics Corporation, USA). Absorbance spectra were recorded using an UV-Visible spectrophotometer (Model UV-1601PC, Japan) employing quartz cell of 1.00cm of path length. The chromatographic analyses were done on a cellulose chiral column Lux C-2 (250mm × 4.6mm I.D and 5-µm particle size).

Materials and Reagents

Working standards of amlodipine and S-amlodipine were gifts from Dr.Reddys laboratory Ltd., Hyderabad, Telungana, India. The marketed formulation was purchased from whole saler(Asomex and Amlogard). Acetonitrile (MeCN) of HPLC grade and Triethylamine (TEA), Acetic-acid and other reagents of analytical-reagent grade were from SD Fine Chemicals (Mumbai, India). HPLC-grade water was prepared by use of a Milli-Q Academic water purifier.

Software

The statistical based calculations (ANOVA) were made with the help of Graph padPrism (Trial version 7.0), the chemical structure was drawn in Chem. draw (Trail version 7.0) and other calculations were utilized with help of Microsoft Excel.

Standard Solutions

Standard stock solutions of (±)-amlodipine and S-amlodipine (1mg/ml) were prepared in mobile phase. Working standard solutions were freshly obtained by diluting the standard stock solutions with mobile phase during the analysis time. Calibration curves were plotted in the concentration was established in the range of 1.0-5.0 µg/mL for both R-amlodipine and S-amlodipine respectively.

Sample preparation

Twenty tablets were weighed and mixed thoroughly, an amount of capsule powdered equivalent to 40 mg of recemic amlodipine were accurately weighed and transferred in a 100 ml Volumetric flask added 60 ml of mobile phase. The mixture was subjected to

sonication for 10 min then complete extraction of drugs and the solution was made up to the mark with mobile phase to obtain a concentration of 10 µg/mL. The solution was centrifuged at 2500 rpm for 10 min; the clear supernatant was collected and filtered through a 0.45µm membrane filter (pall tech, India) and 20µl of this solution was injected for HPLC analysis.

RESULTS AND DISCUSSION

Method development

In preliminary study, there are various stationary and mobile phase were tried in different chromatographic conditions like., Organic phase modifier, composition of organic phase, flow rate of mobile phase and type of stationary phase. Initially to tried with different chiral columns amylose and cellulose such as Amylose-2 (Amylose tris (5-chloro-2-methylphenylcarbamate), Lux-2 (Cellulose tris (3-chloro-4-methylphenylcarbamate) and Lux-4(Cellulose tris (4-chloro-3-methylphenylcarbamate) among these Lux-2 is suitable for in this separation because valuable resolution and capacity factor and reasonable retention time.

In initial study were to conduct with HPLC water and other polar organic solvents such as acetonitrile, methanol, ethanol, tetrahydrofuran and isopropanol. Then different ratio of mobile phase were tried with different mobile phase additives (Try ethylamine, Diethylamine, formic acid and acetic acid) it does not influence the enantiomeric separation. Then further study was done with 95 % methanol: 5.0 % acetonitrile with additive (0.1-0.4% TEA and 0.05-0.3% FA) its influence the enantiomeric separation and the peak broadening, less resolution, excessive peak tailing were observed and instead of acetonitrile to be tried with ethanol and isopropanol it does not alter the separation. Then further study was conducted with 100 % methanol in different ratio of additives to get better separation and less chromatographic factors. Then the above composition of mobile phase were tried with acetonitrile instead of methanol, it does not modify the separation, then altered mobile phase additives (instead of formic acid to added acetic acid) to observed well chromatographic separation. Then finally tried with 100 % acetonitrile in 0.1 % TEA and 0.2 % acetic acid as a mobile phase, flow rate at a 1.0 ml/min and ambient room temperature was fixed. The above-mentioned chromatographic condition was its gives high resolution, capacity factor and reasonable run time was less than 8.0 min (Figure No.2).

Method validation

The method was validated as per the ICH guideline for specificity, recovery, precision, linearity and the method validation to conform the characteristics of the method to fulfill the requirements of the application. The specificity of the method was clarified to compared with the API, formulated samples and blank placebo; there is no interference between the samples and placebo. The linearity of the calibration curve was made from 1.0 - 5.0 µg/ml of R and S-AML for each concentration levels of the analyte were triplicate and the regression coefficient values more than 0.999, which showed reproducibility (Figure No.3). The LOD and LOQ were estimated at 0.59 ng/ml, 1.63 ng/ml and 0.29 ng/ml, 0.85 ng/ml for R-AML and S- AML was founded respectively. The ruggedness of the method to be tested in varies conditions, such as different days, laboratories, analysts, instruments, lot of reagents, samples and the test results were to be obtained within limit. The robustness of analytical method were present for the assay estimated them it gives the variation less than 1.0 % RSD. The solution stability of the final concentration sample was tested in both bench top and freeze thaw conditions at 24 hours with 4-hour interval and its gives acceptable limit. The detailed validation parameter and system suitability were presented on Table No.1.

Table No.1: Validation parameters and system suitability of R-AML and S-AML

S.No	Parameters	R-AML	S-AML
1	Linearity range (µg/ml)	1.0 – 5.0 µg/ml	1.0 – 5.0 µg/ml
2	Slope	1.079x	1.080x
3	Intercept	0.050	0.059
4	Correlation coefficient R^2	0.998	0.999
5	Accuracy (n=3) 80 % w/w 100 % w/w 120 % w/w (mean % recovery, % CV) (n=9)	 99.62 99.23 99.54 99.46, 0.37	 99.87 99.75 99.89 99.83, 0.29
6	Precision (% CV) (n=6) Intra-day precision (n=6) 0.5 2.5 5.0 Inter-day precision (n=6) 0.5 2.5 5.0	 1.25 0.98 1.52 0.39 0.79 0.88	 0.98 1.32 1.22 0.76 0.58 0.86
7	LOD	1.69 ng/ml	1.57 ng/mL
8	LOQ	5.61 ng/ml	4.66 ng/mL
9	Theoretical plate (USP)	2567	2984
10	Retention time	5.87 min	6.79 min
11	Tailing factor	0.62	0.58

ILLUSTRATION

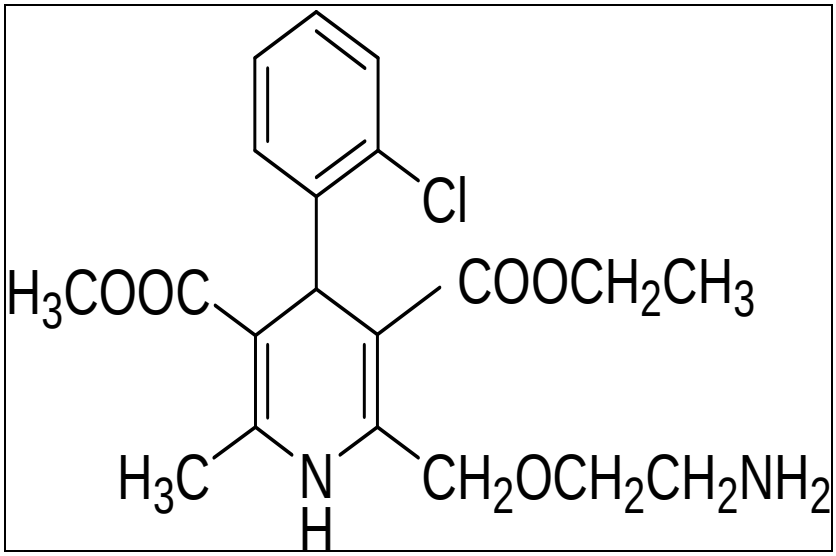

Figure No.1: Chemical Structure of (±)- Amlodipine

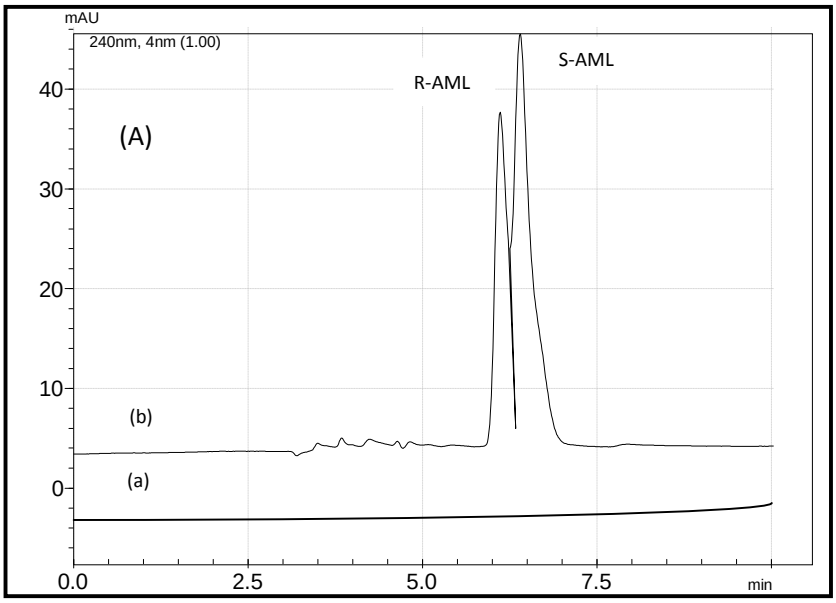

Figure No.2: Representative Chromatogram of (A)(±)Amlodipine (a) placebo peak (b) Marketed formulation of recemic amlodipine

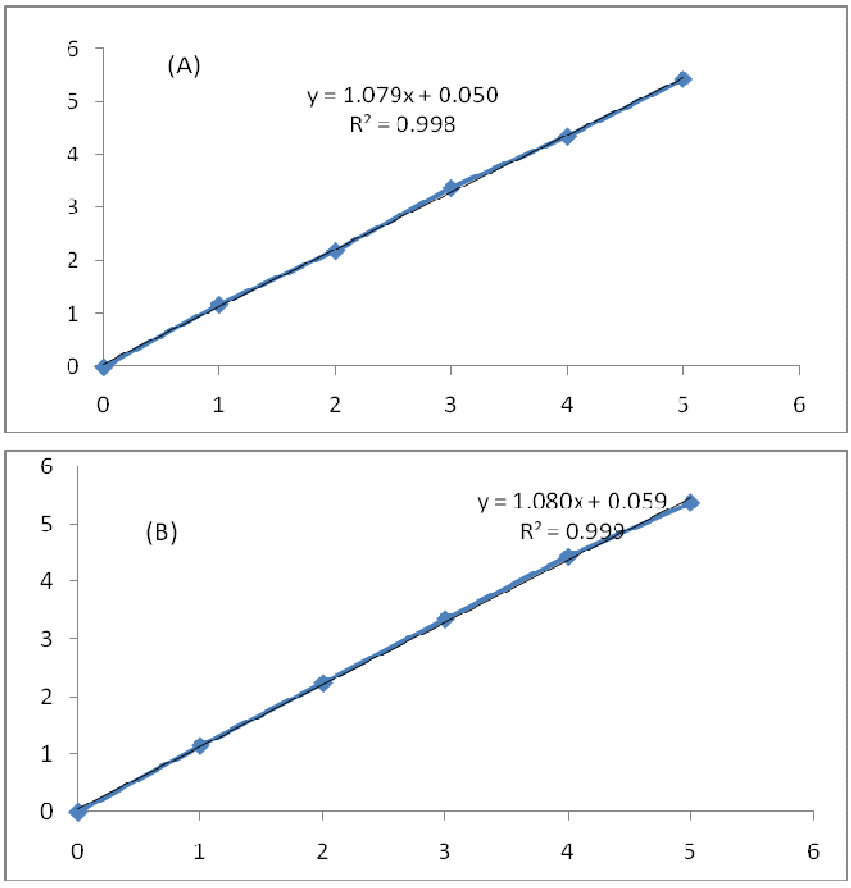

Figure No.3: Linearity curve for (A) R-amlodipine and (B) S-amlodipine peak area vs drug concentration

CONCLUSION

The above-proposed RP-HPLC-PDA method has been developed and validated, it was simple, rapid and it can be able to detect the chiral purities in both (±) - amlodipine and S-amlodipine. The method is economically because polar organic mobile phase used and the total run time was less than 7.0 min. The method has been exhibited a good accurate, precise, linear, rugged and robust. This method applied for the routine quality control analysis of the Active Pharmaceutical Ingredient and tablet dosage forms.

ACKNOWLEDGEMENT

The author is grateful to University Grants Commission (UGC), New Delhi, India, for providing financial assistance through UGC-BSR fellowship and to UGC SAP-DRS Phase II sponsored, Department of Pharmacy, Annamalai University, Tamilnadu, India for providing the facilities to carry this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Gerald Gubit Z, Martin G, Schmid. Chiral Separations Methods and Protocols, *Humana Press Inc., Totowa, NJ*, 1995, 243.
2. Thomas E, Beesley and Raymond P W. Scott, Chiral Chromatography, John Wiley and Sons Ltd, Baffins Lane, Chichester. West Sussex PO19 1UD, *England*, 1998.
3. Andrisano V, Bertucci C, Cavrini V, Recanatini M, Cavalli A, Varoli, Wainer. Stereo selective binding of 2,3-substituted 3-hydroxypropionic acids on an immobilised human serum albumin chiral stationary phase: stereochemical characterisation and quantitative structure-retention relationship study, *J. Chromatogr,A*, 876, 2000, 75-86.
4. Jonathan M, Daniels, Earle R. Nestmann, Alexkerr. Development of stereoisomeric (chiral) drugs: a brief review of scientific and regulatory considerations, *Drug Information Journal*, 31, 1997, 639-646.
5. Michael Lammerhofer. Liquid chromatographic enantiomer separation with special focus on zwitterionic chiral ion-exchangers, *Anal Bioanal Chem.*, 406, 2014, 6095-6103.
6. Mohammadi A, Rezanour N, Ansari M, Ghorbani B F, Hashem M, Walker A. Stability-indicating high performance liquid chromatographic assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets, *J.Chromatogr. B,* 846, 2007, 215-221.
7. Chakravarth B K, Pandya K K, Milan Satia, Gandhi, Modi I R. Detection and determination of total amlodipine by high performance thin-layer chromatography: a useful technique for pharmacokinetic studies, *J. Chromatogr. B,* 667, 1995, 315-320.
8. Luksa J, Josic J, Kremser, Kopitar, Milutinovic. Pharmacokinetic behaviour of R-(+) and S-(-)-amlodipine after single enantiomer administration, *J. Chromatogr. B,* 703, 1997, 185- 193.
9. Elijan A, Qiang, Fang Q, Ronglu, Aiping, Aiguo, Chang. In situ polymerization preparation of chiral molecular imprinting polymers monolithic column for amlodipine and its recognition properties study, *J.Polym Res*, 17, 2010, 401-409.
10. Jacek Bojarski. Stereoselective chromatography of cardiovascular drugs: an update, *J. Biochem, Biophys, Methods*, 54, 2002, 197-220.
11. Josic D J, Luksaa, Podobnika B, Furlana B, Kremser. Semi-preparative chromatographic purification of the enantiomers *S*-(2)-amlodipine and *R*-(1)-amlodipine, *J. Chromatogr. B,* 693, 1997, 367-375.
12. Rong Wang, Zheng-ping, Jun-Jie, Li-Ren, Hua Xie, Jun Ma, Qiang Zhang, Yan Ao, Juan Wang C E. With Hydroxypropyl-*b*-Cyclodextrin as Chiral Selector, for Separation and Determination of the Enantiomers of Amlodipine in the Serum of Hypertension Patients, *Chromatographia*, 65, 2007, 575-579.
13. Bohumila Suchanova, Ludek Sispera, Vladimir Wso I. Liquid chromatography - tandem mass spectrometry in chiral study of amlodipine

biotransformation in rat hepatocytes, *Analytica Chimica Acta*, 573, 2006, 273-283.

14. Ceccato A, Streela B, Laine, Zimmera, Sibenalerb. Enantiomeric determination of amlodipine in human plasma by liquid chromatography coupled to tandem mass spectrometry, *J. Biochem, Biophys, Methods*, 54, 2002, 357-368.
15. Peter Mikus, Katarına, Jozef Marakb, Igor Nemeca, Iva Valaskovaa, Emil Havranek. Direct quantitative determination of amlodipine enantiomers in urine samples for pharmacokinetic study using on-line coupled isotachophoresis-capillary zone electrophoresis separation method with diode array detection, *J. Chromatogr, B*, 875, 2008, 266-272.
16. Hairul Nazirah, Maizatul Akmam, Azalina Mohamed, Muhammad Syarhabil, Akmal Hadi, Zulfakar Mokhtar. Enantio selective Extraction of Racemic Amlodipine Using Tartaric Acid Derivatives and Cyclodextrin Derivatives as Chiral Selectors, *The open conference proceedings journal*, 2, 2011, 17-20.

Please cite this article in press as: R. Sathiyasundar *et al.* Direct chiral separation and quantitative determination of S-Amlodipine by RP-HPLC-PDA method, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 3(3), 2015, 174 - 180.