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A validated chiral HPLC method for the enantiomeric separation of ramipril

G.Ramulu[1,2,*], K.Rama Seshaiah[1], Y.Ravindra Kumar[1], K.Vyas[1], K.Mukkanti[2], M.V.Suryanarayana[1]

[1]Dr. Reddy's Laboratories Ltd. Active pharmaceutical ingredients, IPDO, Bachupally, Hyderabad-500072, A.P., (INDIA)

[2]Department of chemisty, J.N.T.University, Kukatpally, Hyderabad-500072, A.P., (INDIA)

E-mail : ramulug@drreddys.com; gsramu77@yahoo.com

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ABSTRACT

An isocratic chiral HPLC method was developed for the separation of Ramipril enantiomers. The mobile phase consists of n-hexane and 2-Propanol in the ratio of 900: 100(v/v) with 0.2 ml volumes of Tri fluoro acetic acid and 0.1 ml volume of Di ethyl amine. This method is capable of detecting the Ramipril RRRRR-isomer up to 0.3µg/ml. The method was validated interms of Precision, Linearity, Accuracy, and Limit of detection and Limit of Quantification.

KEYWORDS

Pharmaceutical analysis;
Ramipril RRRRR- isomer.

INTRODUCTION

The determination of the stereo isomeric composition of pharmaceuticals is rapidly becoming one of the key issues in the development of new drugs. Among the methods currently used to achieve chiral separation of racemic mixtures, high resolution liquid chromatography systems based on the chiral stationary phases(CSPs), (direct methods) are more rapid and suitable for the resolution of racemic mixtures of pharmacologically active chemical entities[1-3].

Ramipril is a non sulfhydryl angiotensin converting enzyme inhibitor. Ramipril is converted to ramiprilat by hepatic cleavage of the ester group. Its chemical name is (2S,3As,6As)-1[(S)-N-[(S)-1-carboxy-3-phenylpropyl]alanyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid,1-ethyl ester, brand name Altace. Altace is indicated for the treatment of hypertension. It may be used alone or in combination with thiazide diuretics.

Altace is indicated in patients 55 years or older at high risk of developing a major cardiovascular event because of a history of coronary artery diseases, stroke, peripheral vascular disease or diabetes that is accompanied by at least one another cardiovascular risk factor (hypertension, elevated total cholesterol levels, low HDL levels, cigarette smoking), to reduce the risk of myocardial infarction, stroke or death from cardiovascular causes. Altace can be used in addition to other needed treatment such as antihypertensive. Antiplatelet).

The quantitative enantiomeric purity of Ramipril is very important issue for quantitative estimation of RRRRR-isomer. As per our knowledge till date there was no reported validated method in the literature for the chiral separation of Ramipril.

EXPERIMENTAL

Instrumentation

A Waters model alliance 2690-separation module equipped with an auto sampler and waters 996 photo diode array UV detector was used for analysis. The data was recorded using waters millennium software.

Chemicals

Ramipril and RRRRR-isomer (> 99.0%) were supplied from Dr.Reddy's laboratories limited (Hyderabad, India). HPLC grade n-Hexane, 2-Propanol, Diethyl amine and Trifluoro acetic acid were supplied from Merck, Germany. All the chemicals and solvents used were of analytical grade. This study also involves two proprietary Dr. Reddy's Laboratories Pharmaceutical Research & Development compounds.

Preparation of solutions

The stock solutions of Ramipril and RRRRR-isomer were prepared by dissolving 20mg each of the compounds in mobile phase.

Operating conditions

The HPLC separation was conducted on chiral cell OJ-H column with a dimension of 250m x 4.6mm. The mobile phase consists of n-hexane and 2-Propanol in the ratio of 900: 100(v/v) with 0.2 ml volumes of Tri fluoro acetic acid and 0.1 ml volume of Di ethyl amine at a constant flow of 0.5ml/min with column oven temperature 50°C. The elution was monitored at 220nm. The samples were injected with 20 µl.

RESULTS AND DISCUSSION

Method development and optimization

The main challenge was to achieve the desired detection and quantitation limit using the most commonly available instrument, i.e. a HPLC system.

The preliminary trials carried out in reverse phase chiral columns were not fruitful in the separation of these isomers. The separation was not achived presence of diethyl amine and trifluoro acetic acid alone with the combination of n-Hexane and 2-Propanol. However, the resolution for the both isomers was achieved only with the presence of these two modifiers only. Moreover the quantity of trifluoro acitic acid is found to be very crucial in the separation of these isomers.

Different experiments have been conducted by varying the quantity of trifluoro acetic acid and found the interesting observations. If the quantity of acid increased from 0.2ml for 1000ml mobile phase to 0.3, 0.4, 0.5, 0.6ml the retention times increases drastically and peak shapes are broad. On other hand if we decrease acid to 0.1ml the elution time decreases and also resolution is too low.

The separation for these isomers was tried with the combination of triethyl amine and acetic acid with n-hexane and 2-propanol with chiral cell OJ-H column but the separation was found to be very selective with respect to the acid presence.

The separation for these isomers was tried with the combination of n-hexane and 2-Propanol in the ratio of 900: 100(v/v) with 0.1 ml volume of Di ethyl amine and 0.2 ml volumes of Tri fluoro acetic acid also with other chiral stationary phase columns (Chiral cell AD, AD-H, Chiral cell OD and OD-H) but the separation was found to be very selective with respect to the chiral stationary phase also.

The separation was achieved only with 0.2 ml volumes of Tri fluoro acetic acid and 0.1 ml volumes of Di ethyl amine and with the mobile phase consisting of n-hexane and 2-Propanol in the ratio of 900: 100 (v/v). Chiral cell OJ-H column with a dimension of 250m x 4.6mm was used at oven temperature 50°C. Flow rate was kept at 0.5ml/min. The elution was monitored at wave length λ=220nm. The resolution was found to be 3.0 for the two isomers. The structures of Ramipril (SSSSS-isomer) and RRRRR-isomer are displayed in Figure 1 and 2 respectively. The chromatogram of mixture of Ramipril (SSSSS-isomer) and RRRRR isomer are displayed in Figure 3.

As per the ICH guidelines the method was validated interms of following parameters.[4,5]

Structure of ramipril

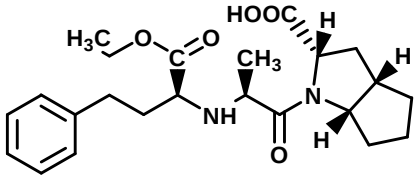

Figure 1 : Ramipril

Structure of ramipril RRRRR isomer

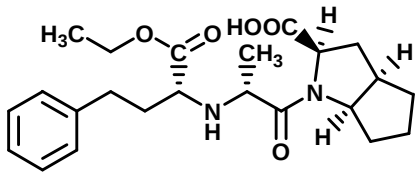

Figure 2 : RRRRR-isomer

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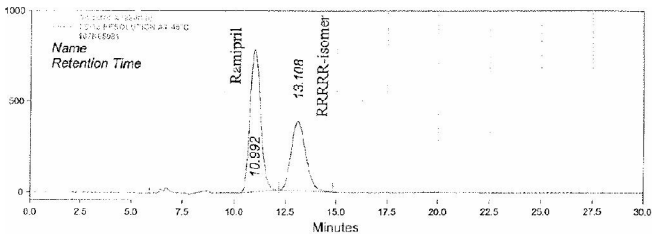

Figure 3 : Chromatogram of mixture of ramipril (SSSSS-isomer) and RRRRR isomer

Method validation

A Critical parameters of validation was done for the developed work. The validated method parameters include establishment of limit of detection, limit of quantification, precession at limit of quantification and accuracy at limit of quantification was done.

The detection limit (LOD) of the method for the RRRRR isomer was estimated from a chromatogram of a solution containing about 0.3µg/ml. From the chromatogram, a signal-to-noise ratio of 3.0 was obtained. In the pharmaceutical industry, the quantitation limit (LOQ) was defined as the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The LOQ was determined to be 0.9µg/ml based on the precision, linearity and accuracy data discussed below.

The experimental results also show that this method has excellent precision without using an internal standard. Multiple injections were made for the RRRRR isomer containing 0.9µg/ml. For six injections of the solution, the R.S.D. of the peak area of RRRRR isomer was 4.3%. The results are listed in TABLE 1.

TABLE 1 : Precision at LOQ level

	RRRRR-isomer area
Sample Prep.1	19982
Sample Prep.2	18904
Sample Prep.3	20494
Sample Prep.4	21057
Sample Prep.5	18978
Sample Prep.6	19845
Mean	19876
% RSD	4.23

The linearity study carried out spiking of RRRRR isomer with Ramipril samples with the concentrations of 0.5µg/ml, 1.0µg/ml, 2.5µg/ml, 5.0µg/ml of target analyte concentrations. The correlation coefficient was observed as 0.999. The results are listed in TABLE 2.

TABLE 2 : Linearity

S.No.	Con. of RRRRR- isomer in µg/ml	RRRRR-isomer area
1.	0.05	17267
2	0.10	38547
3	0.25	93239
4	0.50	175964
	Correlation coefficient	0.999

Accuracy of the method was determined by analyzing drug substance samples spiked with limit of quantification amount of Ramipril RRRRR isomer. The recovery was 99.5% for RRRRR isomer.

CONCLUSION

A simple and sensitive HPLC method has been developed and validated for the trace analysis of RRRRR isomer in pharmaceuticals. The validation was performed. Compared with the previously reported methodologies, this method utilizes a UV detector, which is readily available in most of the quality control testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect 0.3µg/ml of Ramipril RRRRR isomer and quantify 0.9µg/ml of Ramipril RRRRR isomer in pharmaceutical products.

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