ISSN : 0974-7419 Volume 10 Issue 10

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(10) 2011 [695-698]

A validated chiral LC method for enantiomeric separation of intermediate of lopinavir by using cellulose based chiral stationary phase

P.Surya Prakash Rao[1,2]*, A.Nageswari[2], V.Ranga Reddy[1], Mukkanti[2]

[1]Integrated Product Development, Dr. Reddy's Laboratories Limited, Bachupally, Qutubullapur, Rangareddy District - 500 072, Andhra Pradesh, (INDIA)

[2]Department of Chemistry, Institute of Science and Technology, J. N. T. University, Kukatpally, Hyderabad - 500 072, Andhra Pradesh, (INDIA)

E-mail: suryaprp@drreddys.com; surya2880@rediffmail.com

Received: 16th June, 2011 ; Accepted: 16th July, 2011

ABSTRACT

A rapid isocratic chiral LC method has been developed for the separation of 2R-(1-Tetrahydro pyrimid-2-onyl)-3-methyl butanoic acid (R-THPA) from 2S-(1-Tetrahydro pyrimid-2-onyl)-3-methyl butanoic acid. (S- THPA). Good resolution with $R_S > 3$ was obtained using cellulose based chiral stationary phase, chiralcel OD-H column (250 x 4.6 mm, 5µm particle size) and n-hexane, ethanol and trifluoroacetic acid (900:100:2, v/v) as the mobile phase at ambient temperature. Flow rate was kept at 1.2 mL min^{-1} and elution was monitored by UV detection at 210 nm. This method allowed for the detection and quantification of R-THPA of levels at 0.5 and 1.5µg mL^{-1} respectively. The method was validated following ICH guidelines.

KEYWORDS

Liquid chromatography;
Enantiomer;
Method validation;
Chiral stationary phase;
Resolution.

INTRODUCTION

2R-(1-Tetrahydro pyrimid-2-onyl)-3-methyl butanoic acid. (S- THPA) (Figure 1) is a intermediate for Lopinavir (Figure 2). Lopinavir is antiretroviral drugs acting as inhibitors of human immunodeficiency virus (HIV) protease, a class of drugs that has markedly improved morbidity and mortality of HIV infected patients[1]. Within the highly active antiretroviral therapy (HAART) they are used in a fixed combination (33 mg ritonavir + 133 mg lopinavir) called Kaletra® with ritonavir added to boost lopinavir by inhibition of cytochrome P450 (CYP) CYP3A isozymes and active transport by P-glycoprotein[2,3]. Even though HAART has markedly improved the clinical outcome of HIV-infected patients, virological treatment failure often occurs already within the first year of therapy[4,5].

Separation of enantiomers has become very important in analytical chemistry, particularly in the pharmaceutical and biological fields, reflecting the fact that different enantioselective properties can impact on pharmacokinetics and result in a variety of different pharmacological or toxicological effects[6-9]. As a consequence, regulatory authorities insist on stringent investigations when evaluating the safety and the effectiveness of chiral drugs. This places a significant importance on the application of enantiomeric separations to all stages of drug development and the commercialization process. The development of new methods for efficient chiral separations are mainly based on high performance

Full Paper

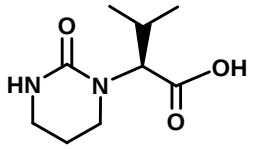

Figure 1 : S-THPA: 2S-(1-Tetrahydro pyrimid-2-onyl)-3-methyl butanoic acid

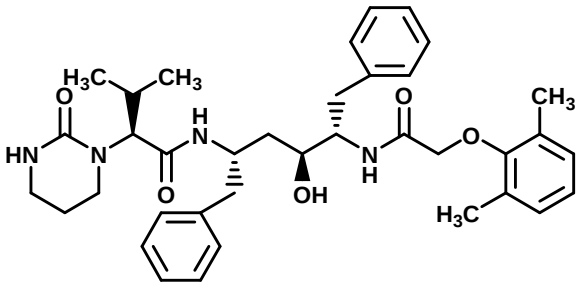

Figure 2 : Lopinavir: (2S, 3S, 5S)-2-(2, 6-Dimethyl phenoxy acetyl)amino-3-hydroxy-5-[2S-(1-tetrahydro-pyrimid-2-onyl)-3-methyl butanoyl] amino-1, 6-diphenylhexane

liquid chromatography (LC), capillary electrophoresis (CE) or gas chromatography (GC). A large number of commonly employed LC methods are based on the use of chiral stationary phases (CSPs) .

As per the requirement of various regulatory authorities, chiral method development for this vital intermediate, THPA is very essential. To the best of our knowledge a chiral LC method has not yet been reported any literature for the separation of THPA enantiomers. In this study, a simple and efficient chiral LC method was developed and validated as per the ICH guidelines[10,11].

EXPERIMENTAL

Chemicals and reagents

HPLC grade n-hexane and ethanol were purchased from Merck (India) and trifluoroacetic acid (TFA), was procured from Fluka (India). THPA enantiomers were obtained from the process development laboratory of Dr.Reddy's Laboratories, IPDO, Hyderabad, India.

Instrumentation and chromatographic conditions

The resolution of the enantiomers was performed on Waters HPLC system, Alliance 2690 separation module consisting quaternary HPLC pump, equipped with an auto sampler and 2487 Dual channel absorbance detector (Waters Corporation, Milford, USA). The column used for the analytical separation was the cellulose tris (3,5-dimethylphenylcarbamate) coated on 5µm silica-gel particles known as Chiralcel OD-H (250 x 4.6 mm, 5 µm) procured from Diacel Chemical Industries (Japan).

The mobile phase consisted of n-hexane, ethanol and Trifluoroaceticacid (900:100:2, v/v). The flow rate was 1.2 mL min^{-1}, the sample injection volume was 15 µL.

The appropriate wavelength for the detection of enantiomers was determined by wavelength scanning over the range of 200-400nm using Waters 2996 photodiode array detector and the chromatograms were monitored by UV detection at a wavelength of 210nm.

Preparation of solutions

A stock solutions of both the enantiomers of THPA were prepared at 2000 µg mL^{-1} by dissolving appropriate amount in the mobile phase as diluent.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

In order to get optimum resolution and selectivity for the two enantiomers of THPA various experiments were conducted by using various CSPs containing cellulose and amylase derivatives. Enantiomeric separation is known to be achieved by the formation transient diastereomeric complexes, mostly based on hydrogen bonding, dipole-dipole and π-π interactions[12].

Various proportions of n-hexane / 2-propanol and n-hexane / ethanol were used as organic modifiers in our initial efforts to achieve normal-phase separation. But, it was found that addition of the Trifluoroacetic acid led to the gaussian peak. Attempts to separate enantiomers on an amylase carbamate derivatized CSP column (Chiralpak AD-H) and cellulose ester derivatized columns (Chiralcel OJ and Chiralcel OB) were not successful. Further trials were made on Cellulose carbamate derivatized column (Chiralcel OD-H) using n- hexane, ethanol, and trifluoroacetic acid in the ratio of 900:100:2 (v/v) as mobile phase with flow rate of 1.2 mL min-1. Hence, the chiral separation was optimized using isocratic conditions as these offers more rapid analysis attributable to the presence of column re-equilibration steps. The typical retention times of R-THPA and S-THPA were about 6.2 and 9.7 min (Figure 3), respectively.

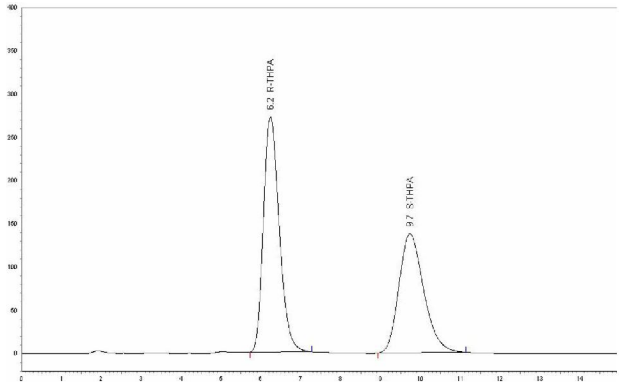

Figure 3 : Typical LC chromatogram of system suitability solution

Limit of detection and quantification

The lowest LOD and the LOQ were determined based on signal-to-noise ratios using analytical responses of 3 and 10 times the background noise, respectively[13]. The LOD and the LOQ for R-THPA were calculated to be 0.5 and 1.5 μg mL^{-1}, respectively.

Linearity

Under the optimized working conditions, plotted standard calibration curve for R-THPA was linear over the concentration range of 1.5 μg mL^{-1} (LOQ) to 9.0 μg mL^{-1}. The results of the statistical analysis of the experimental data, such as slope, the intercept and the correlation efficient obtained by the least squares treatment of the results were 5100135, 322 and 0.9991, respectively. The standard calibration curve was linear with the linear regression equation

y = 322 + 5100135x

Precision

The repeatability (intra-day) and the intermediate precision (inter-day) of the method was evaluated by the determination of peak area percentage RSD of R-THPA for six replicate injections of spiked sample at the levels of 1.5 μg mL^{-1} (LOQ) and 6 μg mL^{-1}. The intermediate precision (Ruggedness) of the method was evaluated by different analyst using different column and different instrument in the same laboratory. The results were summarized in the TABLE 1.

TABLE 1 : Results of intermediate precision

S. No.	Parameter	Variation	%RSD for % Area of R-THPA	%RSD for RT of R-THPA	Resolution Between enantiomers
1	Different	a. Agilent 1100 series PDA	0.4	0.8	3.4
	system	b. Waters 2487 VWD	0.8	0.7	3.5
2	Different	Column-1	0.4	0.8	3.4
	column	Column-2	0.7	0.8	3.3
3	Different	Analyst-1	0.4	0.8	3.4
	analyst	Analyst-2	0.6	0.6	3.5

Accuracy

Accuracy of the method was demonstrated at the four different concentration levels in triplicate. The analysis was carried out at the concentration levels of 1.5 μg mL^{-1} (LOQ), 6μg mL^{-1} and 9 μg mL^{-1}. The percentage recoveries were in between 98 and 104.

Robustness

The Robustness of the method was studied by varying number of method parameter. The experimental conditions were deliberately varied in order to determine the impact on resolution of enanatiomers. The flow rate on resolution was studied by varying the flow rate by ±0.2 mL min^{-1}. The temperature effect was studies by varying ±5°C. In addition, the percentage of ethanol in the mobile phase was varied ±5%. No significant change observed in the resolution of enantiomers, results were shown in the TABLE 2, illustrating the robustness of the developed method.

TABLE 2 : Results of robustness study

Parameter	Variation	USP resolution (Rs)
Flow rate	1.0 mL min^{-1}	3.7
	1.4 mL min^{-1}	3.3
Temperature	22 °C	3.4
	32°C	3.7
Organic ratio	90 mL Ethanol	3.3
	100 mL Ethanol	3.4
	110 mL Ethanol	3.1

CONCLUSION

An isocratic enantioselective HPLC method that enables sensitive determination of R-THPA in S-THPA was developed. The method was found to be simple, sensitive, precise, accurate and robust. Hence, this method can be used in quality control laboratories for the routine analysis.

Full Paper

ACKNOWLEDGEMENTS

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd, for supporting this work. Authors wish to acknowledge the process research group for providing the samples. And the author wishes to gratefully acknowledge helpful suggestions from Mr.K.S.V.Raghavacharyulu and Dr.Y.Ravindra kumar.

REFERENCES

[1] F.J.Palella, K.M.Delaney, A.C.Moorman, M.O.Loveless, J.Fuhrer, G.A.Satten, D.J.Aschman, S.D.Holmberg; N.Engl.J.Med., **338**, 853 **(1998)**.

[2] C.S.Ernest, S.D.Hall, D.R.Jones; J.Pharmacol. Exp.Ther., **312**, 583 **(2005)**.

[3] C.G.Lee, M.M.Gottesman, C.O.Cardarelli, M.Ramachandra, K.T.Jeang, S.V.Ambudkar, I.Pastan, S.Dey; Biochemistry, **37**, 3594 **(1998)**.

[4] R.E.Aarnoutse, J.M.Schapiro, C.A.B.Boucher, Y.A.Hekster, D.M.Burger; Drugs, **63**, 741 **(2003)**.

[5] R.P.G.van Heeswijk; Ther.Drug Monit., **24**, 323 **(2002)**.

[6] FDA Policy Statement for the Development of New Stereoisomeric Drugs, Washington, DC, May **(1992)**.

[7] E.J.Ariens; Eur.J.Clin.Pharmacol., **26**, 663-668 **(1984)**.

[8] E.J.Ariens; Med.Res.Rev., **6**, 451-456 **(1986)**.

[9] E.J.Ariens, E.W.Wuins; Clin.Pharmacol.Ther., **42**, 361-363 **(1987)**.

[10] European Medicines Agency; ICH Topic Q 2 (R1), Note for Guidance on Validation of Analytical Procedures: Text and Methodology, CPMP/ICH/381/95.

[11] The European Agency for the Evaluation of Medicinal Products, ICH Topic Q 2 B, Note for Guidance on Validation of Analytical Procedures: Text and Methodology. CPMP/ICH/281/95.

[12] Y.Okamoto, Y.Kaida; J.Chromatogr.A, **666**, 403-419 **(1994)**.

[13] The United State Pharmacopeia; 24th Edition, United State Pharmacopeial Convention, Rockville, MD, 2150 **(2000)**.