

## A VALIDATED CHIRAL LC METHOD FOR THE ENANTIOMERIC SEPARATION OF ETHYL-METHYL-CARBAMIC ACID-3-[1-HYDROXY-ETHYL]-PHENYL ESTER

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### ABSTRACT

A rapid isocratic HPLC method has been developed for the separation of Ethyl-methyl-carbamic acid-3-[1S-hydroxy-ethyl]-phenyl ester (enzymatically synthesized key intermediate for Rivastigmine tartarate) from its R-Enantiomer. Good resolution viz.  $R_s > 2.5$  between R-and S-forms was achieved with Chiralpak AD-H (250x4.6 mm) column using n-Hexane, Ethanol and Trifluoro acetic acid in the ratio of 95:05:0.1 % v/v/v as mobile phase at 30°C temperature. Flow rate was kept as 1.0 mL/min and elution was monitored at 215 nm. The method was validated as per International Conference on Harmonization (ICH) guidelines in terms of limit of detection (LOD), limit of quantitation (LOQ), linearity, precision, accuracy, specificity.

**Keywords:** Chiral HPLC, validation, enantiomers, ethyl-methyl-carbamic acid -3-[1-hydroxy-ethyl]-phenyl ester.

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### INTRODUCTION

Chirality is a key factor in the efficacy of many drug products and agrochemicals, and thus the production of single enantiomers of chiral intermediates has become increasingly important in the pharmaceutical industry<sup>1</sup>. Most of the pharmaceutical industries are now concentrating towards the study of the therapeutic effects of enantiomers of the existing drug molecules to have a detailed impurity profile. The determination of amounts of different enantiomeric forms in pharmaceuticals is essential in this connection and high performance liquid chromatographic method (HPLC) is generally opted for this purpose.

The developments of analytical methods for the quantitative analysis of chiral compounds are extremely challenging due to the fact that enantiomers possess virtually identical properties<sup>2</sup>. Although many analytical techniques such as capillary electrophoresis (CE), Super fluid liquid chromatography (SFC) can be employed to attain this, the most widely used is liquid chromatography (LC) employing a chiral stationary phase (CSP)<sup>3-5</sup>.

Rivastigmine, (S)-N-Ethyl-N-methyl-3-[1-dimethylamino]ethyl]-phenyl carbamate, marketed in the form of its tartarate salt is a reversible cholinesterase inhibitor, prescribed for the treatment of mild to moderate Alzheimers disease.

Rivastigmine and its pharmaceutical activity is first disclosed in US 4,948,807, wherein racemic Rivastigmine is prepared by reacting  $\alpha$ -m-hydroxy phenylethyldimethylamine with carbamoyl chloride in the presence of NaH.

US 5,602,176 claims hydrogen tartarate salt of Rivastigmine and process for the preparation of Rivastigmine comprises reacting m-hydroxyphenylethyldimethyl amine with N-Ethylmethylcarbamoyl chloride in the presence of NaH to obtain Rivastigmine. It also describes resolution of Rivastigmine using di-O, O'-p-toluoyl tartaric acid.

We have developed a Chiral HPLC method for the separation of R-and S-forms of ethyl-methyl-carbamic acid 3-[1-hydroxy-ethyl]-phenyl ester (which is a key intermediate for Rivastigmine) to know the enantioselectivity of enzyme, purity of our desired product.

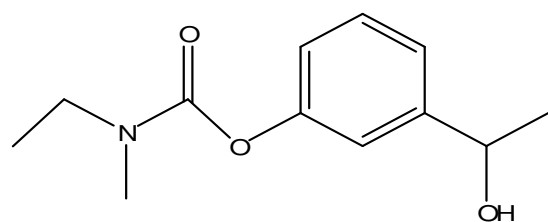
## EXPERIMENTAL

### Chemicals

HPLC grade n-Hexane and Ethanol were procured from Merck, Mumbai, India. Trifluoro acetic acid was purchased from Sigma-Aldrich, Bangalore, India. Racemic compound, S- and R-forms of ethyl-methyl-carbamic acid-3-[1-hydroxy-ethyl]-phenyl ester were prepared at Research and Development laboratory of Matrix laboratories Ltd, Hyderabad, India.

### Chromatographic conditions

Waters make HPLC (Alliance 2695 Model, Waters Corporation, Milford, USA) equipped with an auto sampler and 2996-photodiode array detector was used for the analysis. The data was recorded using Waters Empower software. The separation and quantification were performed on Chiralpak AD-H column (250x4.6 mm, Make: Diacel Chemical Industries Ltd. Japan) with the mobile phase containing n-Hexane, Ethanol and Trifluoro acetic acid in the ratio of 95:05:0.1 (%v/v/v) at 30°C temperature. Flow rate was kept at 1.0 mL/min and the elution was monitored at 215nm.



3-(1-hydroxyethyl)phenyl ethylmethylcarbamate

Fig.-1: Structure of the desired molecule

### Sample preparation

5.0 mg of chemically synthesized racemic compound of ethyl-methyl-carbamic acid-3-[1-hydroxy-ethyl]-phenyl ester was dissolved in 10 mL of ethanol to get concentration of 0.5 mg/mL solution. This solution was used for enantiomeric separation. Ethanol as blank was injected.

## RESULTS AND DISCUSSION

### Method Development

Initial experiments were carried out using Chiralpak OD-H column as n-Hexane: Isopropyl alcohol (70:30 %v/v), and n-Hexane: Ethanol (90:10 %v/v) at 1.0 mL/min flow. But in both the experiments no resolution between the isomers was observed. Literature survey reveals that the critical chiral separations were achieved by using amylose tris (3, 5-dimethyl phenyl carbamate) or cellulose tris (3,5-dimethylphenylcarbamate) packing materials. Hence experiments were carried out with Chiralpak AD-H column containing amylose tris (3, 5-dimethyl phenyl carbamate).

When Chiralpak AD-H column was used as mobile phase n-Hexane: Isopropyl alcohol in the ratio of 70:30 %v/v with 1.0 mL/min flow rate, the isomers were separated, but only resolution 1.07 is obtained. In order to get better resolution mobile phase was changed to n-Hexane: Ethanol in the ratio of 90:10 %v/v with same column was used. Resolution was increased to 1.43. When 0.1% Trifluoro acetic acid was added to mobile phase containing n-Hexane: Ethanol in the ratio of 90:10 %v/v resolution was increased to 1.59. Finally good resolution of >2.5 was obtained with Chiralpak AD-H column, as n-Hexane, Ethanol and Trifluoro acetic acid in the ratio of 95:05:0.1 %v/v/v with flow rate 1.0ml/min at 30°C temperature. The elution was monitored at 215 nm. However, dead volumes were not considered as the retention times are 13.8 and 15.5 for R-and S-forms of ethyl-methyl-carbamic acid-3-[1-hydroxy-ethyl]-phenyl ester respectively with resolution more than 2.5.

### Method Validation

The method was validated in accordance with ICH guidelines<sup>6-7</sup>.

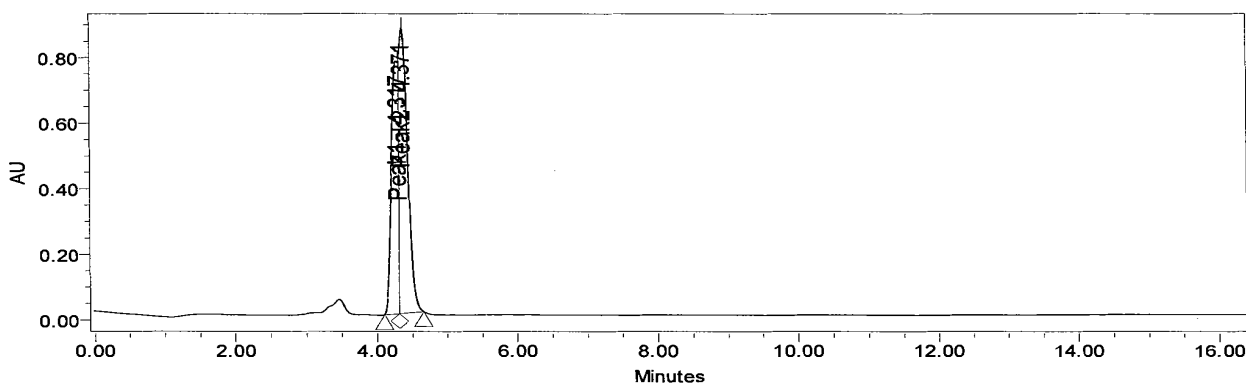


Fig.-2: n-Hexane: Ethanol (90:10), Chiralcel OD-H (250x4.6mm;5.0um) column, no resolution of Isomers.

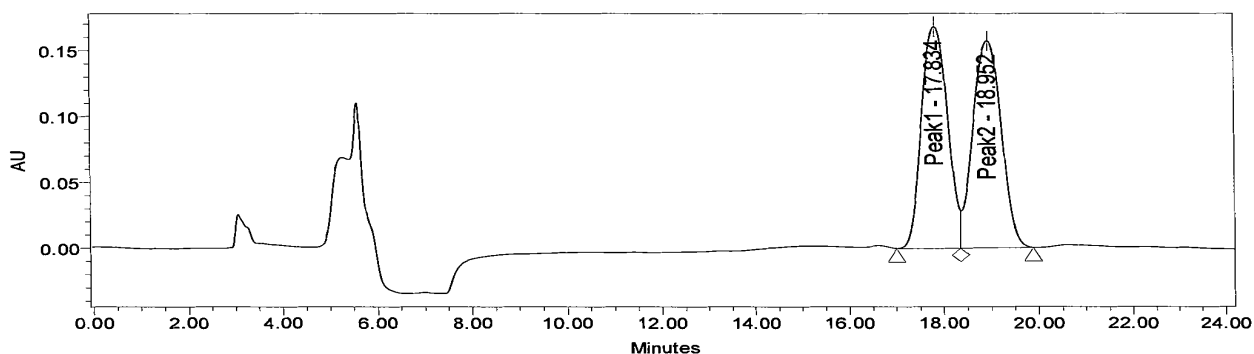


Fig.-3: n-Hexane:Ethanol (70:30), Chiralpak AD-H (250x4.6mm;5.0um) column, resolution of Isomers:1.07.

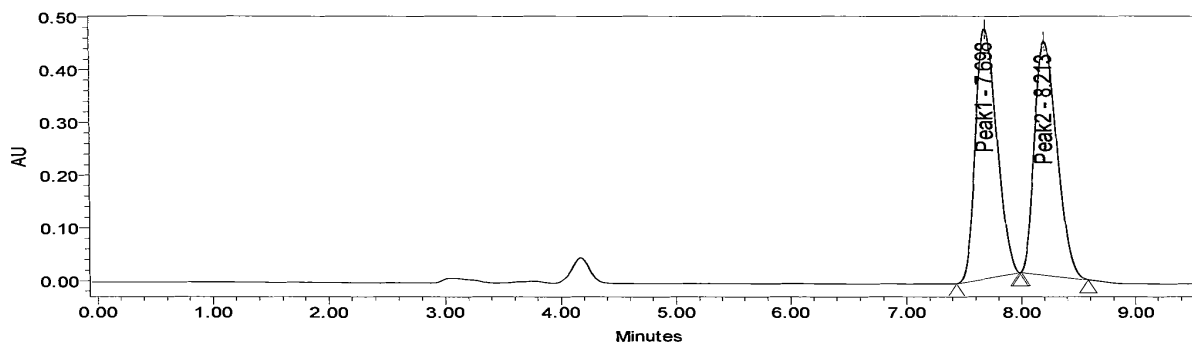


Fig.-4: n-Hexane:Ethanol (90:10), Chiralpak AD-H (250x4.6mm;5.0um) column, resolution of Isomers:1.43.

### Limit of detection and limit of Quantification

The limit of detection (LOD) represents the concentration of analyte that would yield an S/N (signal to noise) ratio of 3. LOD for ethyl-methyl-carbamic acid -3-[1R-hydroxy-ethyl]-phenyl ester was found to be  $0.21 \mu\text{g mL}^{-1}$ . The limit of quantification (LOQ) represents the concentration of analyte that would yield S/N ratio of 10. LOQ for ethyl-methyl-carbamic acid -3-[1R-hydroxy-ethyl]-phenyl ester was found as  $0.84 \mu\text{g mL}^{-1}$ .

### Linearity

The calibration curve was drawn between the peak areas of ethyl-methyl-carbamic acid -3-[1R-hydroxy-ethyl]-phenyl ester versus its concentration in the range of  $0.84$  to  $15 \mu\text{g mL}^{-1}$  of ethyl-methyl-carbamic acid -3-[1R-hydroxy-ethyl]-phenyl ester. The slope, intercept and correlation coefficient derived

from linear leastsquare regression analysis and found to be 161.37, 17.267 and 0.9999 respectively reveals that an excellent correlation existed between the peak area and concentration of the ethyl-methyl-carbamic acid -3-[1R-hydroxy-ethyl]-phenyl ester.

### Precision

The precision was evaluated by calculating the relative standard deviation (RSD%) of six replicate determinations by injecting six freshly prepared solutions containing ethyl-methyl-carbamic acid -3-[1R-hydroxy-ethyl]-phenyl ester at three concentration levels viz. 0.84, 10 and 15  $\mu\text{g mL}^{-1}$  on the same day and RSD% values were found to be 1.57, 2.10, 0.98 respectively. The low RSD% values confirm the good precision of the developed method.

### Accuracy and Specificity

The accuracy of the method was determined by spiking ethyl-methyl-carbamic acid -3-[1R-hydroxy-ethyl]-phenyl ester at 0.84 (LOQ), 10 and 15  $\mu\text{g mL}^{-1}$  levels with three batches of ethyl-methyl-carbamic acid -3-[1S-hydroxy-ethyl]-phenyl ester. Each determination was carried three times. The recovery data (98.4-99.0%) indicates the accuracy of the method.

The specificity defined as the ability of the method to measure the analyte accurately and specifically in the presence of components present in the sample matrix, was determined by analyzing pharmaceutical samples. 0.1mg/ml each of R- and S-forms of ethyl-methyl-carbamic acid -3-[1-hydroxy-ethyl]-phenyl ester and the starting material for the preparation (3-Hydroxy acetophenone, ethyl-methyl-carbamic acid -3-acetyl phenyl ester and ethyl-methyl-carbamic acid -3-[1S-hydroxy-ethyl]-phenyl ester were injected separately and all spiked to a sample of ethyl-methyl-carbamic acid -3-[1S-hydroxy-ethyl]-phenyl ester. In addition, the peak purities of both the forms were studied by using PDA detector and found to be homogeneous with no detectable impurities embedded in them.

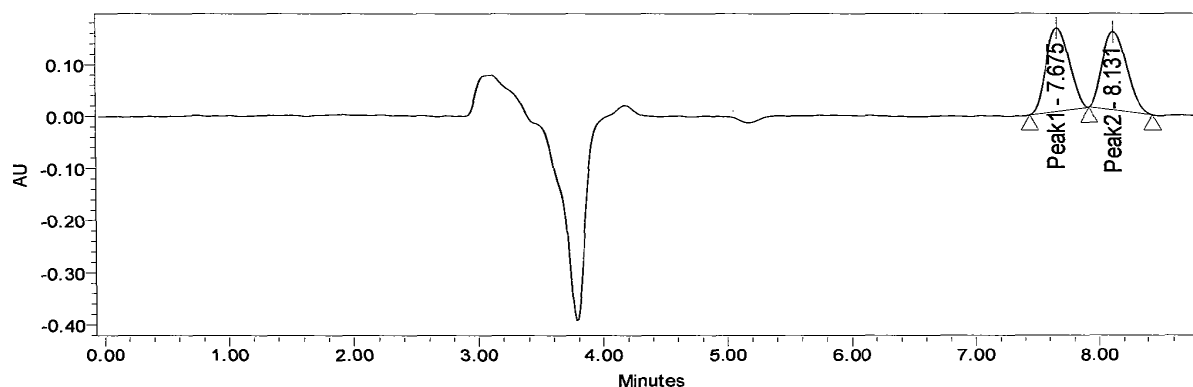


Fig.-5: n-Hexane:Ethanol:DEA (90:10:0.1), Chiralpak AD-H (250x4.6mm;5.0um) column, resolution of Isomers:1.25

### Stability of the sample in the diluent

The stability of the sample in mobile phase was demonstrated by injecting sample solution at different intervals viz. 0, 3, 6, 9, 12, 24, and 48 h of time intervals. Upto 24 hours, no degradants were observed in the chromatogram and the RSD% for recovery of R-form at LOQ level was found to be 0.91 indicating the stability of the sample in the diluent.

## CONCLUSIONS

A rapid isocratic chiral HPLC method was developed for the enantiomeric separation of R-isomer from S-form of ethyl-methyl-carbamic acid -3-[1-hydroxy-ethyl]-phenyl ester. The limit of quantification is 0.84  $\mu\text{g mL}^{-1}$ . This is also stability indicating method because S-form is stable in diluent for 24 hours. The method is validated as per ICH guidelines and found to be linear, precise, accurate, specific for the determination of R-form in pure ethyl-methyl-carbamic acid -3-[1-hydroxy-ethyl]-phenyl ester. Hence this method can be used for the routine analysis.

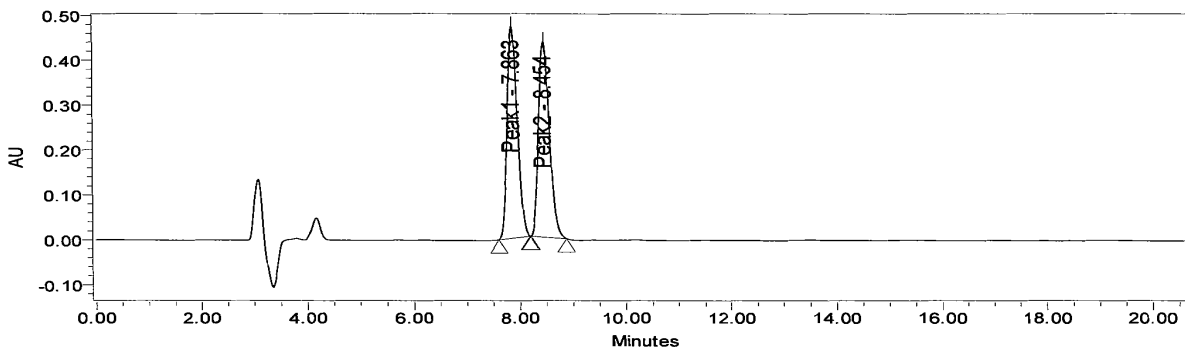


Fig.-6: n-Hexane:Ethanol:TFA (90:10:0.1), Chiralpak AD-H (250x4.6mm;5.0um) column, resolution of Isomers:1.59.

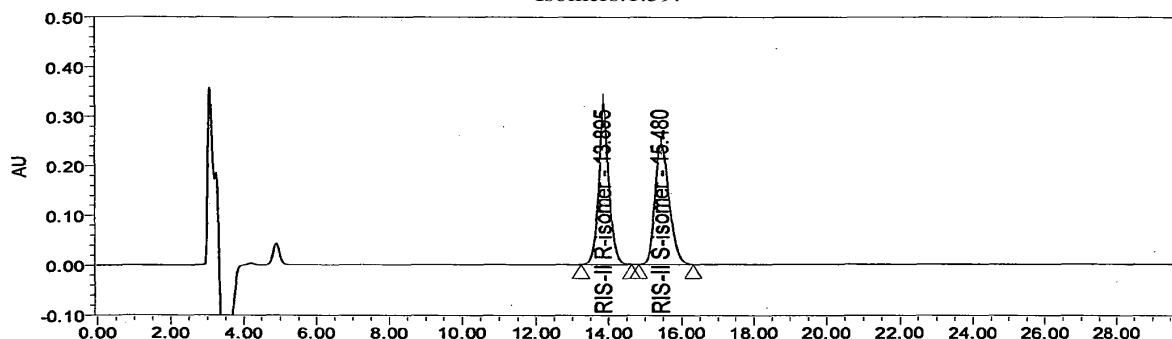


Fig.-7: n-Hexane:Ethanol:TFA (95:05:0.1), Chiralpak AD-H (250x4.6mm;5.0um) column, resolution of Isomers more than 2.5.

### ACKNOWLEDGEMENTS

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