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Short communication

A validated chiral liquid chromatographic method for the enantiomeric separation of Rivastigmine hydrogen tartarate, a cholinesterase inhibitor

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Abstract

A new and accurate chiral liquid chromatographic method was developed for the enantiomeric resolution of Rivastigmine hydrogen tartarate, (−)*S*-*N*-ethyl-3-[(1-dimethyl-amino)ethyl]-*N*-methylphenyl-carbamate hydrogen tartarate, a cholinesterase inhibitor in bulk drugs. The enantiomers of Rivastigmine hydrogen tartarate were baseline resolved on a Chiralcel OD-H (250 mm × 4.6 mm, 5 μm) column using a mobile phase system containing hexane: isopropanol: trifluoroacetic acid (80:20:0.2, v/v/v). The resolution between the enantiomers was not less than four and interestingly distomer was eluted prior to eutomer in the developed method. The presence of trifluoroacetic acid in the mobile phase has played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. The developed method was extensively validated and proved to be robust. The limit of detection and limit of quantification of (*R*)-enantiomer were found to be 500 and 1500 ng/ml, respectively for 10 μl injection volume. The percentage recovery of (*R*)-enantiomer was ranged from 95.2 to 104.3 in bulk drug samples of Rivastigmine hydrogen tartarate. Rivastigmine hydrogen tartarate sample solution and mobile phase were found to be stable for at least 48 h. The proposed method was found to be suitable and accurate for the quantitative determination of (*R*)-enantiomer in bulk drugs. Chiralcel OJ-H column can also be used as an alternative for the above purpose.

Keywords: Rivastigmine hydrogen tartarate; Alzheimer's disease; Cholinesterase inhibitor; Chiralcel OD-H; Chiral HPLC; Validation; Solution and mobile phase stability

1. Introduction

Dementia is a progressive brain dysfunction, which results in a restriction of daily activities and in most cases leads in the long term to the need for care. Alzheimer's disease (AD) is the most frequent type of dementia in old age [1]. People with AD suffer mainly from impaired memory and orientation, limitations of concentration, planning and judgement, personality changes and later also perceptual, speech and walking disorders. Neuropathologically, AD is characterized by the presence of neurofibrillary tangles and senile plaques, impaired synaptic function and cell loss [2]. Alzheimer's disease is recognized as being one of the most important challenges facing medicine in the 21st century due to aging population and high cost of managing the disease.

Rivastigmine hydrogen tartarate (Exelon) is chemically (−)*S*-*N*-ethyl-3-[(1-dimethyl-amino)ethyl]-*N*-methylphenyl-carbamate hydrogen tartarate, a carbamate inhibitor of acetylcholinesterase is used for the treatment of mild to moderate Alzheimer's disease in adults[3]. Rivastigmine hydrogen tartarate is synthesized as a single enantiomer because (*S*)-enantiomer is pharmacologically more potent than (*R*)-enantiomer.

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Few HPLC methods were reported in the literature for the quantitative determination of Rivastigmine and its major metabolite with atmospheric pressure chemical ionization tandem mass spectroscopy, simultaneous determination of Rivastigmine and its major metabolite NAP 226-90 with electrospray ionization mass spectrometry and for the determination of the dissociation constants of basic acetyl cholinesterase inhibitors [4–6].

A cyclodextrin-modified capillary zone electrophoresis method was reported in the literature for the enantiomeric analysis of Rivastigmine in pharmaceuticals [7].

In the literature, there is no reference for the enantiomeric separation of Rivastigmine hydrogen tartarate in bulk drugs using high performance liquid chromatography.

Enantiomers of racemic drugs often differ in pharmacokinetic behaviour and/or pharmacological action [8].

In recent years, research has been intensified to understand the aspects of the molecular mechanism for stereoselective biological activities of the chiral molecules. The development of analytical methods for the quantitative analysis of chiral materials and for the assessment of enantiomeric purity is extremely challenging due to the fact that enantiomers possess virtually identical properties [9]. Recently, much work has been reported describing the use of chiral stationary phases, in conjunction with HPLC, as a way to separate and thereby individually quantitate the enantiomers of an enantiomeric pair [10–12].

This report describes a chiral LC method for the enantiomeric separation of Rivastigmine hydrogen tartarate using a cellulose-based chiral stationary phase, Chiralcel OD-H. The developed HPLC method was validated for optical purity assessment (determination of (*R*)-enantiomer in Rivastigmine hydrogen tartarate).

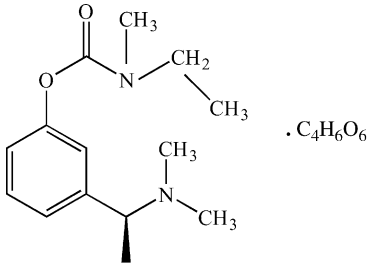
2. Experimental

2.1. Chemicals

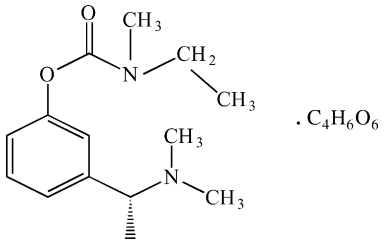
Rivastigmine hydrogen tartarate and (*R*)-enantiomer were kindly supplied by Process Research Department of Dr. Reddy's Laboratories Limited, Hyderabad, India, and the chemical structures were given in Fig. 1. HPLC grade hexane and isopropanol were purchased from Merck, Germany. Laboratory reagent grade trifluoroacetic acid was purchased from Merck, Germany.

2.2. Equipment

A Waters Alliance HPLC system equipped with 2695 separation module with inbuilt auto injector, 270852 thermostatic compartment, and 996 photo diode array detector was utilized for method development and validation. Millennium 32 chromatography manager software (Waters) was used for data acquisition and system suitability calculations.



Rivastigmine hydrogen tartarate



(R)-enantiomer

Fig. 1. Chemical structure of Rivastigmine hydrogen tartarate and (*R*)-enantiomer.

2.3. Sample preparation

Stock solutions of (*R*)-enantiomer (150 μg/ml) and Rivastigmine hydrogen tartarate (5 mg/ml) were prepared by dissolving the appropriate amount of the substances in ethanol. The analyte concentration of Rivastigmine hydrogen tartarate was fixed as 1.0 mg/ml. Working solutions of Rivastigmine hydrogen tartarate and (*R*)-enantiomer were prepared in mobile phase.

2.4. Chromatographic conditions

The chromatographic conditions were optimized using a cellulose-based chiral stationary phase Chiralcel OD-H (250 mm × 4.6 mm, 5 μm, Daicel make), which was safeguarded with a 1 cm long guard column. The mobile phase was hexane:isopropanol:trifluoroacetic acid (80:20:0.2, v/v/v). The flow rate was set at 1.0 ml/min. The column was maintained at 25 °C, and the detection was carried out at a wavelength of 210 nm. The injection volume was 10 μl. Protein-based chiral stationary phase Chiral AGP (ChromTech make), cellulose-based chiral stationary phase Chiralcel OJ-H (Diacel make), amylose-based chiral stationary phase Chiralpak AD-H (Diacel make) and pirkle-based chiral stationary phase Whelk-O-1 (Merck make) were also employed during method development.

2.5. Validation of the method

2.5.1. Method reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between-day precision) of retention times and peak areas for each enantiomer.

In order to determine the repeatability of the method, replicate injections ($n = 6$) of a 1.0 mg/ml solution containing Rivastigmine hydrogen tartarate spiked with (*R*)-enantiomer (0.3%) was carried out. The intermediate precision was also evaluated over 3 days by performing six successive injections each day.

2.5.2. Limit of detection and limit of quantification of (R)-enantiomer

The limit of detection, defined as lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal to noise ratio [13]. The limit of quantitation, defined as lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated as ten times the signal to noise ratio [13]. The limit of detection (LOD) and limit of quantification (LOQ) were achieved by injecting a series of dilute solutions of (*R*)-enantiomer.

The precision of the developed chiral method for (*R*)-enantiomer at limit of quantification was checked by analyzing six test solutions of (*R*)-enantiomer prepared at LOQ level and calculating the percentage relative standard deviation of area.

2.5.3. Linearity of (R)-enantiomer

Detector response linearity was assessed by preparing six calibration sample solutions of (*R*)-enantiomer covering from 1500 (LOQ) to 9000 ng/ml (1500, 3000, 4500, 6000, 7500 and 9000 ng/ml), prepared in mobile phase from (*R*)-enantiomer stock solution.

Regression curve was obtained by plotting peak area versus concentration, using the least squares method. Linearity was checked for 3 consecutive days in the same concentration range from the same stock solution. The percentage relative standard deviation of the slope and *Y*-intercept of the calibration curve was calculated.

2.5.4. Quantification of (R)-enantiomer in bulk sample

The Rivastigmine hydrogen tartarate bulk sample, provided by Process Research Department of Dr. Reddy's Laboratories, showed the presence of 0.06% of (*R*)-enantiomer. Standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of (*R*)-enantiomer in bulk drug samples.

The study was carried out in triplicate at 0.3, 0.6 and 0.9% of the Rivastigmine hydrogen tartarate target analyte concentration. The recovery of (*R*)-enantiomer was calculated from the slope and *Y*-intercept of the calibration curve obtained in section 2.5.3.

2.5.5. Robustness

The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate, mobile phase composition and column temperature. To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between Rivastigmine and (*R*)-enantiomer was evaluated.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution of enantiomers, it was changed by 0.2 units from 0.8 to 1.2 ml/min. The effect of change in percent isopropanol on resolution was studied by varying from −1 to +1%, while the other mobile phase components were held constant as stated in section 2.4. The effect of column temperature on resolution was studied at 20 and 30 °C instead of 25 °C, while the other mobile phase components were held constant as stated in section 2.4.

2.5.6. Solution stability and mobile phase stability

Stability of Rivastigmine hydrogen tartarate in solution at analyte concentration was studied by keeping the solution in tightly capped volumetric flask at room temperature on a laboratory bench for 2 days. Content of (*R*)-enantiomer was checked for 6 h interval upto the study period.

Mobile phase stability was carried out by evaluating the content of (*R*)-enantiomer in Rivastigmine hydrogen tartarate sample solutions prepared freshly at 6 h interval for 2 days. Same mobile phase was used during the study period.

3. Results and discussion

3.1. Method development

The aim of this work is to separate the enantiomers of Rivastigmine hydrogen tartarate and accurate quantification of (*R*)-enantiomer. A 0.5 mg/ml solution of racemic mixture prepared in mobile phase was used in the method development. To develop a rugged and suitable LC method for the separation of Rivastigmine enantiomers, different mobile phases and stationary phases were employed. In an attempt to separate the enantiomers of Rivastigmine hydrogen tartarate, various chiral columns namely Chiralcel OD-H, Chiralcel OJ-H, Chiralpak AD-H of Diacel, Chiral AGP of ChromTech and Whelk-O-1 of Merck were employed. Various experiments were conducted to select the best stationary and mobile phases that would give optimum resolution and selectivity for the two enantiomers. No separation was found on Chiralpak AD-H, Chiral AGP and Whelk-O-1 columns using different possible mobile phases. There is an indication of separation on Chiralcel OJ-H and Chiralcel OD-H columns using a mobile phase consisting of hexane: isopropanol (80:20,v/v) and the peak shapes were very broad. Introduction of trifluoroacetic acid in the mobile phase enhanced the chromatographic efficiency and resolution between the enantiomers. Very good separation was achieved on Chiralcel OD-H (Resolution greater than 4) and Chiralcel OJ-H (Resolution greater than 2.5) columns using the mobile phase system hexane: isopropanol: trifluoroacetic acid (80:20:0.2, v/v/v). Interestingly distomer was eluted prior to eutomer in Chiralcel OD-H column where as elution order of enantiomers was reversed on Chiralcel OJ-H column.

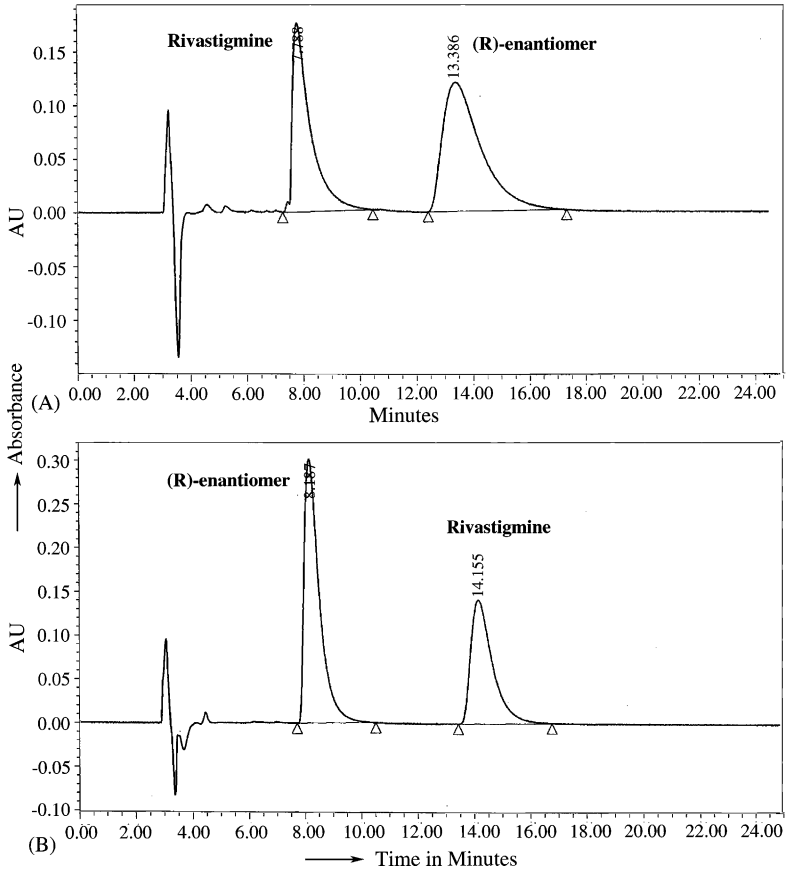


Fig. 2. Enantiomeric resolution of racemic Rivastigmine hydrogen tartarate on (A) Chiralcel OJ-H Column (B) Chiralcel OD-H column. Mobile phase consisted of hexane:isopropanol:trifluoroacetic acid (80:20:0.2, v/v/v); flow rate, 1.0 ml/min; UV, 210 nm; Column temperature, 25 °C.

The reversal of elution order of Rivastigmine hydrogen tartarate enantiomers on Chiralcel OJ-H column could be due to the alteration of steric environment of the chiral cavities. Due to the better chromatographic results obtained on the Chiralcel OD-H column, the method validation was carried out on the same. Chiralcel OJ-H can be used as an alternative column for the quantification of (*R*)-enantiomer.

In the optimized method, the typical retention times of (*R*)-enantiomer and Rivastigmine were about 8.2 and 14.2 min, respectively. The enantiomeic separation of Rivastigmine hydrogen tartarate on Chiralcel OD-H and Chiralcel OJ-H columns was shown in Fig. 2. The system suitability test results of the chiral LC method on Chiralcel OD-H and Chiralcel OJ-H columns were presented in Table 1.

3.2. Validation results of the method

In the repeatability study, the relative standard deviation (R.S.D.) was better than 0.5% for the retention times of both the enantiomers, 1.0% for Rivastigmine peak area and 4.5% for (*R*)-enantiomer peak area (Table 2). In the intermediate precision study, results show that R.S.D. values were in the same order of magnitude than those obtained for repeatability (Table 2).

The limit of detection and limit of quantification concentrations were estimated to be 500 and 1500 ng/ml for (*R*)-enantiomer, when a signal-to-noise ratio of 3 and 10 were used as the criteria. The method precision for (*R*)-enantiomer at limit of quantification was less than 7% R.S.D. (Table 2).

Good linearity was observed for (*R*)-enantiomer over the concentration range of 1500–9000 ng/ml, with the linear re-

Table 1
System-suitability report

Column name	Compound ($n = 3$)	k	R_S	N	T	$\propto$
Chiralcel OD-H	(*R*)-Enantiomer	7.2	5.0	2000	2.0	
	Rivastigmine	13.2		2200	1.8	1.8
Chiralcel OJ-H	Rivastigmine	6.8	3.1	1000	2.6	
	(*R*)-Enantiomer	12.4		650	2.2	1.8

$n = 3$ determinations; k, capacity factor; R_S, USP resolution; N, number of theoretical plates (USP tangent method); T, USP tailing factor, $\propto$, enantioselectivity.

Table 2
Validation results of the developed chiral LC method

Validation parameter	Results
Repeatability ($n = 6$, % R.S.D.)	
Retention time (*R*-enantiomer)	0.3
Retention time (*S*-enantiomer)	0.4
Area (*R*-enantiomer)	4.3
Area (*S*-enantiomer)	0.8
Intermediate precision ($n = 18$, % R.S.D.)	
Retention time (*R*-enantiomer)	0.5
Retention time (*S*-enantiomer)	0.7
Area (*R*-enantiomer)	4.9
Area (*S*-enantiomer)	1.1
LOD-LOQ (*R*-enantiomer)	
Limit of detection (ng/ml)	500
Limit of quantification (ng/ml)	1500
Precision at LOQ (% R.S.D.)	6.7
Linearity (*R*-enantiomer)	
Calibration range (ng/ml)	1500–9000
Calibration points	6
Correlation coefficient	0.998
Slope (% R.S.D.)	2.3
Intercept (% R.S.D.)	13

gression equation $y = 12015X + 256$ (correlation coefficient, $R = 0.998$). Linearity was checked for (*R*)-enantiomer over the same concentration range for 3 consecutive days. The percentage relative standard deviation of the slope and *Y*-intercept of the calibration curve were 2.3 and 13, respectively (Table 2).

The standard addition and recovery experiments were conducted for (*R*)-enantiomer in bulk samples in triplicate at 0.3, 0.6 and 0.9% of analyte concentration. Recovery was calculated from slope and *Y*-intercept of the calibration curve obtained in linearity study and percentage recovery was ranged from 95.2 to 104.3 (Table 3).

A HPLC chromatogram of spiked (*R*)-enantiomer at 0.6% level in Rivastigmine bulk drug sample was shown in Fig. 3.

The chromatographic resolution of Rivastigmine and (*R*)-enantiomer peaks was used to evaluate the method robustness under modified conditions. The resolution between Rivastigmine and (*R*)-enantiomer was greater than 4.0, under all separation conditions tested (Table 4), demonstrating sufficient robustness.

No significant change in the (*R*)-enantiomer content was observed in Rivastigmine sample during solution stability and mobile phase stability experiments. Hence, Rivastigmine sample solution and mobile phase are stable for at least 48 h.

Table 3
Recovery results of (*R*)-enantiomer in bulk drugs

Added (ng)	Recovered (ng)	% Recovery	% R.S.D.
3002	2858	95.2	6.2
6004	6263	104.3	4.1
9006	9195	102.1	3.3

$n = 3$ determinations.

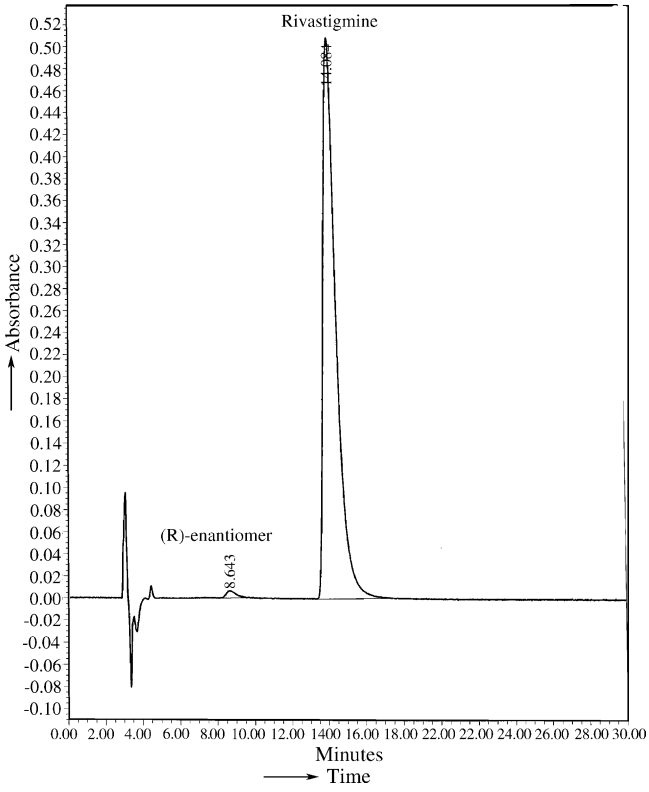


Fig. 3. Typical HPLC chromatogram of Rivastigmine hydrogen tartarate bulk sample (1.0 mg/ml) spiked with (*R*)-enantiomer (0.6%).

Table 4
Robustness of the chiral LC method

Parameter	USP resolution between Rivastigmine and (*R*)-enantiomer
Flow rate (ml/min)	
0.8	5.4
1.0	5.0
1.2	4.4
Column temperature (°C)	
20	5.3
25	5.0
30	4.5
Isopropanol percentage in mobile phase	
19	5.3
20	5.0
21	4.8

4. Conclusion

A new and accurate normal phase chiral LC method was described for the enantiomeric separation of Rivastigmine hydrogen tartarate. Cellulose-based chiral columns Chiralcel OD-H and Chiralcel OJ-H columns were found to be selective for the enantiomers of Rivastigmine hydrogen tartarate. Method validation was carried out using Chiralcel OD-H column due to the better chromatographic results achieved on the column. The method was completely validated showing satisfactory data for all the method validation parameters

tested. The developed method is stability indicating and can be used for the quantitative determination of chiral impurity ((*R*)-enantiomer) in bulk materials.

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