www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 1090-1093, Oct-Dec 2009

Development and validation of a rapid RP-HPLC method for the determination of Racecadotril in formulation

Anton Smith .A*, Madhusudhana Reddy Induri, Varaprasad.K[1] and Manavalan .R

Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamilnadu, India
[1]Department of Quality Control, Granules India Limited, Gagillapur, Hyderabad, India

Corresponding author: auantonsmith@yahoo.co.in

ABSTRACT: A simple rapid specific precise and accurate reverse phase high performance liquid chromatographic method was developed for the determination of Racecadotril (RACE) in sachet dosage forms using atorvastatin as internal standard. Ratio of the peak area of analyte to internal standard was used to calculate for quantification. A phenomenex-Luna RP-18, 5mm column having 250x4.6 mm i.d. in isocratic mode, with mobile phase containing acetonitrile: 0.05M phosphate buffer (potassium dihydrogen orthophosphate): triethylemine (80:19.95:0.05) adjusted to pH 3.95 using orthophosphoric acid. The flow rate was 1.0ml/min and effluents were monitored at 231nm. The retention times of atorastatin calcium and racecaclotril were 3.453 min and 4.210 min respectively. Linearity was observed over concentration range of 10-80 µg/ml. The recovery of racecadotril was found to be in the range of 99.6-100.5%. The proposed method was validated successfully and applied to the estimation of a racecadotril in sachet dosage forms.

Keywords: RP-HPLC, Validation, Racecadotril.

1. INTRODUCTION

Racecadotril (RACE) is chemically known as 2(acetyl sulfanyl methyl)-3- phenyl–propanoyl)amino acid benzyl ester, which is a prodrug of the enkephalinase inhibitor thiorphan. It gets rapidly converted into thiorphan which interacts with the active site of enkephalinase. The drug is used for the treatment of acute symptomatic diarrhea [1].

A literature survey regarding quantitative analysis of RACE revealed that attempts were made to develop analytical methods for RACE using spectrophotometric [2, 3], HPLC [3 - 6], LC-MS [7], NMR [8] methods had been reported for its estimation. Present study involves development and validation of RP-HPLC method for the estimation of RACE in sachet dosage forms.

2. MATERIALS AND METHODS

A HPLC instrument (Shimadzu HPLC class VP series) with LC-10 AT vp pump, variable wave length programmable UV/VIS detector SPD-10A VP, phenomenex-Luna RP-18, 5 µm column having 250x4.6 mm i.d. was used. A rheodyne injector with a 20µl loop was used for the injection of sample. RACE (assigned purity 99.8%) and Atorvastatin (ATOR) were procured as a gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad, India and Biocon India Ltd., Bangalore, India respectively.

The sachet formulation containing racecadotril 10mg (Brand Name REDOTIL, manufactured by Dr.Reddy's laboratories Ltd., India and ENUFF, manufactured by Hetero drug Ltd., India) were procured from the local market. HPLC grade acetonitrile (Merck, India) and HPLC grade water (milli-Q-water system) were used in this investigation. Orthophosphoric acid, phosphate buffer, and triethlyamine were of analytical reagent grade obtained from S.D. fine chemicals.

2.1. Preparation of Mobile Phase

The 0.05m phosphate buffer was prepared by dissolving 3.4gms of potassium dihydrogen ortho phosphate in 1000ml volumetric flask and dissolved in distilled water. Then made up the volume with distilled water and sonicated the resulting solution for 10min. The pH of the resulting solution was adjusted to 3.95 by using orthophosoric acid. HPLC experiments were carried out using binary pump. In one solvent reservoir acetonictrile and in another phosphate buffer and triethyl amine mixture (80:19.95:0.05; v/v/v mobile phase) were taken. They were filtered through a 0.45µm membrane filter before use.

2.2. Preparation of stock solutions

A stock solution of the drug and internal standard was prepared by dissolving 50mg of RACE and ATOR in two separate 50 ml volumetric flask containing acetonitrile, sonicated for about 10 min and then made upto the volume with acetonitrile. Aliquots of these stock solutions were suitably diluted with mobile phase to get the working standard solution of drug in the concentration range of 10-80µg/ml, a long with a fixed concentration (20 µg/ml) of ATOR as internal standard.

2.3. Chromatographic conditions

A reverse phase C_{18} column equilibrated with mobile phase adjusted to pH 3.95 was used. The mobile phase was degassed with a helium reservoir through the column at a flow rate of 1ml/min, yielding a column back pressure of 140-160 kg/cm^2. The run time was set as 6 min and the column temperature was maintained at 30 ± 2°C. Prior to the injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The effluents were monitored at 231nm and the date required was stored and analyzed.

2.4. Construction of calibration curve

Calibration curve was prepared by taking appropriate aliquots of RACE and ATOR stock solutions were taken in different 10ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration of 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml of RACE and 20 µg/ml of ATOR respectively. The solutions were injected using a 20µl fixed loop system and chromatograms were recorded. The calibration curve was constructed by plotting average response factor versus concentration and regression equation was computed for RACE.

2.5. Determination of RACE in sachet dosage forms

A total powder from 20 sachets was accurately weighed and an amount equivalent to 100 mg was taken and dissolved in 70 ml of acetonitrile and sonicated for five minutes. The mixture was shaking well for 2 minutes and transferred to a 100ml volumetric flask through what Mann No. 40 Filter paper. The residue was washed thrice with 10ml acetonitrile l and the combined filtrate was made up to the mark with acetonitrile. The sample solution thus prepared was diluted with acetonitrile to get the solutions containing different concentrations of *RACE*. Appropriate volume of the aliquots was transferred to a 50ml volumetric flask and the volume was made up to the mark with mobile phase to obtained 20 µg/ml of RACE. The solution was sonicated for 10min. The solution was injected at above chromatographic conditions and peak areas were recorded. The method was validated for accuracy, precision, LOD, LOQ and robustness.

2.6. Accuracy

The accuracy of the method was determined by calculating recoveries of RACE by method of standard additions. Known amount of RACE (8, 10, 12 µg/ml) and ATOR (20 µg/ml) was added to a pre-quantified sample solution and the amount of RACE was estimated by measuring the response factor and by fitting these values to the straight line equation of calibration curve.

2.7. Precision

The intraday and inter day precision study of RACE was carried out by estimating the corresponding responses three times on the same day and on three different days for five different concentrations of RACE (10, 20, 40, 60, 80 µg/ml) and the results are reported in terms of relative standard deviation (RSD, Table-2).

2.8. Detection limit and quantification limit

A calibration curve was prepared using concentrations in the range of 10-80 µg/ml for RACE. The standard deviation of y-intercepts of regression lines were determined and kept in following equation for the determination of detection limit and quantification limit.

Detection limit = $\frac{3.3\sigma}{s}$; quantification limit = 10σ/s;

where σ is the standard deviation of y-intercepts of regression lines and S is the slope of calibration curve.

2.9. Robustness

Robustness of the method was studied changing the composition of organic phase by ±5% flow rate by ± 0.2 ml and the pH by ± 0.2.

3. RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both RACE and ATOR. The mobile phase acetonitrile: 0.05M phosphate buffer: triethylamine (80:19.91:0.05) adjust to pH 3.95 using ortho phosphoric acid was found to be satisfactory and gave two symmetric and well-result peaks for ATOR and RACE. The resolution between ATOR and RACE was found to be 2.861, which indicates good separation of both the compounds.

The retention time for ATOR and RACE were 3.453 mm and 4.210 mm responsively (Figure-1) overlain UV spectra of RACE showed that the drug absorbs appreciably at 231nm hence, 231nm was sleeted as the detection wavelength in liquid-chromatography (Figure-2). The calibration curve for RACE was obtained by plotting the Response factor versus the concentration of RACE over the range of 10-80 µg/ml , and it was found to be linear with r =0.9996. The data of the regression analysis of the calibration curve are shown in (Table-1). The validation parameters were summarized in (Table-2).

The recovery of RACE was found to be in the range of 99-6 to100.5 %. The system suitability test parameters were shown in (Table-3). The results for RACE was comparable with the corresponding labeled amounts (Table-4)

4. CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of RACE using simple mobile phase with low buffer concentration. This method gives good resolution between internal standard (ATOR) and RACE with a short analysis time (26min). This method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of RACE in commercial dosage forms.

Table 1: Regression analysis of the Calibration curve for the proposed method

Parameters	RACE
Linearity range (µg/ml)	10-80
Slope	0.0338
Correlation coefficient	0.9996

Table 2: Summary of validation parameters

Parameters	RACE
Detection limit (mg/ml)	0.0328
Quantization limit (mg/ml)	0.0995
Accuracy (%)	99.6-100.5
Precision (R.S.D. %)	
Intraday	0.12-1.81
Intraday	0.08-0.18

Table 3: System Suitability test parameters for RACE

Parameters	RACE
Retention time (min)	4.210
Resolution	2.861
Theoretical plates	5523
Tailing factor	1.18

Table 4: Assay Results

Formulation	Ladled amount (mg)	Amount Mean ± SD	abstained	% purity
A	10	999±1.0264		99.98
B	10	9.94±0.9251		99.45

* average of three reading. Mean ± SA standard deviation of three determinations. Sachet A was REDOTIL, Manufactured by Dr. Reddy's laboratories Ltd., India, and Sachet B was ENUFF, Manufactured by Heterodrugs Ltd., India, both containing Racecadotril 10ng.

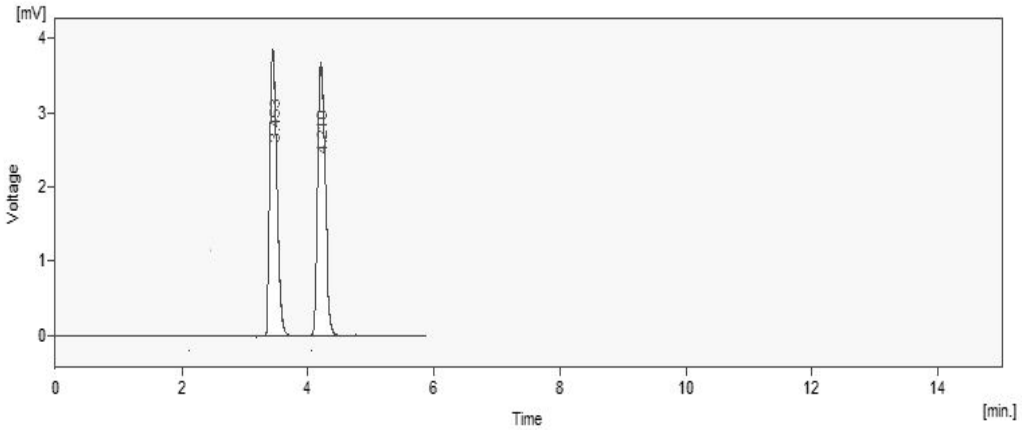

Fig.1 Typical chromatogram of RACE

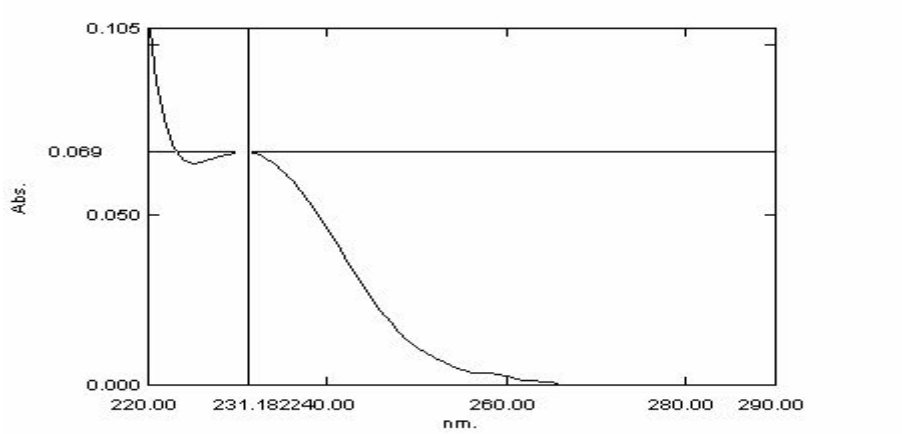

Fig.2 UV overlain spectra of RACE

ACKNOWLEDGEMENTS

The authors are grateful to M/S Dr. Reddy's laboratories Ltd., Hyderabad for providing gift sample of Raeecadotnl and M/S Biocon laboratories Ltd., Bangalore for providing gift sample of aspirin.

REFERENCES

1. Budavari.S, Eds., In; the Merck Index, 13th Edn., Merck research Laboratories, Division of merck and Co., Inc., NJ.,2001, 1450.
2. Ventrichelvan.T, Prabakaran.S, New spectrophotometric methods for the determination of racecadotril in bulk drug and capsular, Indian J.Pharm.Sci., 2007, 69(2), 307-9.
3. Srinivasa Rao.P, Nappinnai.M, UV and RP-HPLC estimation of Racecadotril. , Asian J.chem., 2007, 19(5), 3697-702.
4. Xu.F, Yang.L, Xu.G, A rapid and validated HPLC method to quantify racecadotril metabolite, thiorphan, in human plasma using solid-phase ectraction., J.chromatogr B.Analyt Technol Biomed life Sci., 2007, 861(1), 130-5.
5. Seshagiri Rao.L.N.Jr, Bhanu Prakash.P, Muralikrishna.M, and Ravikumar.P, RP-HPLC method for the estimation of Racecadotril in bulk and in tablets, 2007, 19(4), 2623-26.
6. Prabu.S.L, Singh.T, Joseph.A, Dinesh.C, Determination of Racecadotril by HPLC in capsules, Indian J.pharm sci., 2007, 69(6), 819-821.
7. Xu.Y, Huang.J, Liu.F, Guo.S, Guo.Q, Quantitative analysis of Racecadotril metabolic in human plasma using a liquid chromatography /Tandem mass spectrometry, J.Chromatogr B Analyt Technol Biomed life Sci., 2007, 812(1-2), 101-7.
8. Mallikarjun Reddy.K, Moses Basu., Structural studies of Racecadotril and its process impurities by NMR and mass spectro scopy, Pharmazie, 2006, 61(12), 994-08.

*****

.