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STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF RACECADOTRIL IN SOLID DOSAGE FORM

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ABSTRACT

A simple, rapid and accurate RP-HPLC method was developed for the determination of racecadotril in solid pharmaceutical dosage form. The method showed a linear response for concentrations in the range 10 - 200 □g/ml using methanol: water (80:20 v/v) as the mobile phase with detection at 231 nm and a flow rate of 1.2 ml/min. Solution concentrations were measured at a weight basis to avoid the use of an internal standard. The method was statistically validated for accuracy, precision, linearity, range, ruggedness, robustness, forced degradation, solution stability, and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed; the % RSD from recovery studies was found to be 0.53. Due to the simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.

Key words: *Racecadotril; HPLC; Degradation studies.*

INTRODUCTION

Racecadotril, (2-[2-(acetyl sulfanyl methyl)-3-phenyl-propanoyl] aminoacetic acid benzoyl ester)[1], is a lipophilic diesterified prodrug of the enkephalinase inhibitor, thiorphan, which interacts specifically with the active site of enkephalinase and is used in the treatment of acute diarrhoea of bacterial and viral aetiology[2,3]. So far, no assay procedure has been reported for the determination of this drug from pharmaceutical formulations. However, analytical methods for determination of the metabolite of racecadotril in human plasma by LC/MS[4], structural studies of racecadotril and its process impurities by NMR and MS[5] have been reported. The objective of the present study is to develop an HPLC method and validate it for the rapid and accurate determination of racecadotril in solid dosage form.

EXPERIMENTAL

Instrumentation

Quantitative HPLC analysis was performed using a high performance liquid chromatograph with reciprocating dual pistons in two LC-10AT VP pumps from Shimadzu Corp., Japan (Shimadzu Class LC-10A VP), with a programmable fixed wavelength UV/Vis detector (SPD-10A VP), Phenomenex RP-C18 (250 mm X 4.6 mm i.d., particle size 5□) was the column used for the analysis. The HPLC system was equipped with Class VP software, Version 5.03 (Shimadzu).

Reagents

Racecadotril was received as a gift sample form Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC grade methanol (Ranbaxy Fine Chemicals, SAS Nagar, India) and water (Qualigens Chemicals, India) were used in the study. Commercially available racecadotril capsules, claimed to contain 100mg of the drug were procured from the local market.

HPLC Conditions

The experiment was performed using a 250x 4.6 mm 5 □m particle size Phenomenex C18 stainless steel column with methanol: water (80:20 v/v) as the mobile phase which was filtered through a nylon membrane (pore size 0.22 □m) and degassed before use. The analysis was performed at room temperature, with a flow rate of 1.2 ml/min, and the run time was 10 min. The injection volumes for samples and standards were 20 □l. Prior to injection of the drug solutions, the column was equilibrated for at least 45 min with the mobile phase flowing through the system. The eluents were monitored at 231 nm and the data was acquired, stored and analyzed using Class VP software, Version 5.03 (Shimadzu).

Recommended procedure

A standard stock solution of racecadotril was prepared by dissolving 100 mg of racecadotril in a 100 ml volumetric flask containing methanol (HPLC grade), sonicated for about 10 min and then made up to volume with methanol. Seven sets of the drug solution in the concentration range of 10 – 200 □g/ml were prepared and suitably diluted using the mobile phase. Each of these drug solutions (20 □l) was injected in triplicate into the column and the peak area and retention time

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was recorded. The working standard solutions of racecadotril were prepared daily by suitable from the stock solution with the mobile phase.

Procedure for pharmaceutical formulation

Twenty capsules, each containing 100mg, were weighed and the average weight was calculated. A quantity of powder equivalent to 100 mg of racecadotril was weighed accurately, transferred to a 100ml volumetric flask and a small quantity of methanol was added to dissolve the contents of the flask. The final volume was made up with further addition of methanol, thereby giving a stock solution of 1000 □g/ml concentration. The stock solution was filtered using Whatmann filter paper No.1. An aliquot of the filtrate was then suitably diluted to obtain concentrations in the desired linearity range. 20 □l each of these solutions in triplicate were injected into the column and the chromatograms were recorded.

Accuracy as recovery studies

The accuracy of the assay was evaluated for 3 amounts (80, 100, 120 □g); at each level of the amount, six determinations were performed by spiking the preanalyzed sample with 80-120% of the concentration present, by addition of standard.

Precision

Intra-day and inter-day accuracy and precision of the assay samples containing racecadotril (40, 80 and 120 □g/ml) were analyzed six times in the same day (intraday), and for three consecutive days by different analysts. Precision was calculated as inter and intra-day coefficient of variation [% C.V. = S.D/mean) X 100] (Table 1).

Table 1. *Intra and inter day precision of HPLC method*[a]

Actual conc. (µg/ml)	Intra-day precision			Inter-day precision		
	Observed conc. (µg/ml)	S.D.	% R.S.D.	Observed conc. (µg/ml)	S.D.	% R.S.D.
80	79.73	0.29	0.73	79.79	0.35	0.87
100	99.82	0.34	0.42	99.67	0.42	0.52
120	119.68	0.75	0.63	119.84	0.87	0.73

[a] *n= 6*

Robustness

By introducing small but deliberate changes in the mobile phase pH (□ 0.1), mobile phase composition (□ 2.0%), detection wavelength (□ 5.0 nm), flow rate (□ 10.0 % of absolute value) robustness of the described method was studied. The robustness of the method was assessed for 3 different amounts of the calibration plot (80, 100, 120 □g).

Sensitivity

The sensitivity of the method was determined with respect to LOD, LOQ. Both LOD and LOQ were calculated with amounts in the lowest part of the linear range of the calibration plot; LOD was calculated as (3.3 x S.D)/b and LOQ was calculated as (10 x S.D)/b, where b is the slope obtained in the linearity study and S.D is the blank response.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was attempted using acidic, basic and hydrogen peroxide induced oxidation. Degradation samples were prepared by taking 100 mg of racecadotril in a 100 ml volumetric flask followed by addition of 50 ml of HPLC grade methanol, racecadotril was dissolved properly by shaking and sonicating the mixture and the final volume was adjusted to 100 ml with methanol. 10 ml of the above solution was taken in 3 different 50 ml round bottomed flasks, and to one of the flasks 10 ml of 0.01N HCl was added for acidic degradation, to the second flask 10 ml of 3 % H_2O_2 was added for oxidative degradation, and to the third flask 10 ml of 0.01 N NaOH was added for basic degradation. All the flasks were kept in the dark to exclude the possible degradative effect of light. Acidic and oxidative degradation were carried out by refluxing for 3 hours, while basic degradation was carried out by refluxing for 1 hour. The pH's of the degraded samples was adjusted to 7.0 with dilute HCl and dilute NaOH. The volumes in all the three flasks was adjusted to 50 ml with methanol, the samples were injected and analyzed against control samples (lacking of degradation treatment).

RESULTS AND DISCUSSION

The goal of this study was to develop a stability indicating HPLC method for the analysis of racecadotril in its pharmaceutical dosage form. A mobile phase composed of methanol: water (80:20 v/v) gave a well resolved, sharp peak for racecadotril with a retention time of 5.10 minutes (Fig. 1). A system and method suitability test was applied to the representative chromatograms, to check various parameters such as column efficiency, peak tailing and capacity factor, these were found to be 7680, 1.39 and 3.32 respectively. A set of seven solutions of racecadotril at concentrations ranging from 10 to 200 µg/ml were analyzed in triplicate; calibration curve was constructed by plotting the peak area against concentration using linear regression analysis. The correlation coefficient was found to be 0.9999, indicating an excellent linearity.

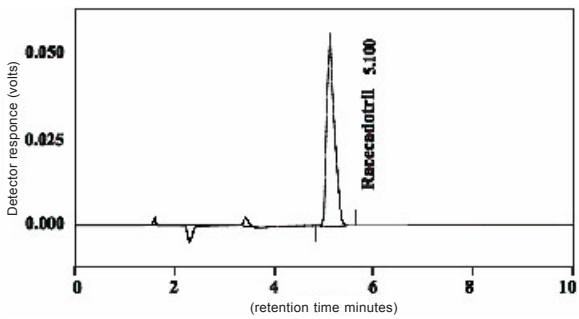

Fig.1. *Typical Chromatogram of standard Racecadotril, RT =5.10*

The percent relative standard deviations of the peak areas of three replicate injections were found to be less than 2.0%. The proposed method was validated for intra and inter-day precision studies in the concentration range of 10 – 200 □g/ml, the RSD of the peak area for 6 injections on the same day (intra day) was found to be 0.73 %, 0.42% and 0.63%. The inter day precision (3 days, n=6) was found to be 0.87%, 0.52 % and 0.73%.

The standard deviation of peak areas was calculated for each parameter such as small changes in the variations of pH of the mobile phase (± 0.1), mobile phase composition (± 2.0%), wavelength of detection (± 5.0 nm), flow rate (± 10.0% of absolute value). The % R.S.D. was found to be less than 2%. The low values of % R.S.D. indicated robustness of the method.

Under the experimental conditions employed, the lowest amount of drug which could be detected was found to be 50 ng/ml and the lowest amount of drug which could be quantified was found to be 100 ng/ml. The developed method was used to quantify racecadotril in capsule. Capsules of 100 mg label claim were analyzed and the average drug content was found to be 99.26% of the labeled amount. No interfering peaks were found in the chromatogram indicating that the excipients in the formulation did not interfere with the estimation of the drug. Recovery studies, at three different concentration levels (80, 100, 120□g), the percentage recovery was found to be 98.83%, 99.24% and 99.68% respectively.

The chromatogram of samples degraded with acid, base and hydrogen peroxide showed some degradation products. The peaks of degraded product were well resolved from the drug peak, the chromatograms are shown in Figure 2, 3 and 4.

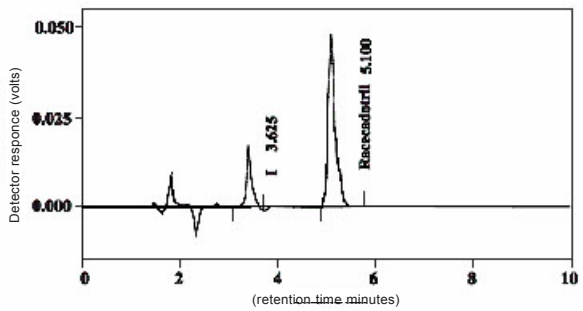

Fig. 2. *Acid degradation of Racecadotril, peak-I. correspond to degradation product*

CONCLUSION

The results of this study show that the proposed RP-HPLC method will be useful for the determination of racecadotril in its pharmaceutical dosage forms as it is simple, rapid, precise, and accurate.

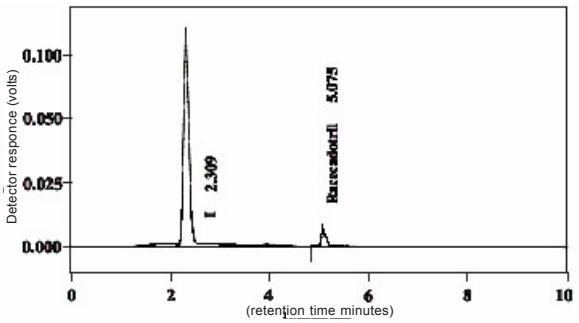

Fig. 3. *Oxidative degradation of Racecadotril, peak-I correspond to degradation product*

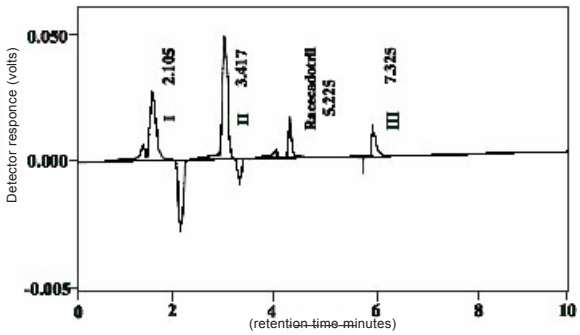

Fig. 4. *Base degradation of Racecadotril, peak I, II, and III are degradation product*

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