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CONTENTS

Determination of Racecadotril by HPLC in Capsules

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Prabu, *et al.*: HPLC Determination of Racecadotril

A simple, precise and rapid RP-HPLC method was developed for the determination of racecadotril in a pharmaceutical formulation using gemfibrozil as internal standard. Ratio of the peak area of analyte to internal standard was used for quantification. The chromatographic separation was carried out by using a Reverse Phase C18 column (BDS-Hypersil). The mobile phase consisting of a mixture of 20 mM phosphate buffer (pH 3.5) and acetonitrile in the ratio of (40:60) with detection at 230 nm at a flow rate of 1 ml/min was employed. The method was statistically validated for linearity, accuracy and precision. The elution time was 6.9 min for racecadotril and 9.8 min for gemfibrozil. The simplicity and accuracy of the proposed method ensures its use in routine quality control analysis of pharmaceutical formulations.

Key words: Racecadotril, RP-HPLC estimation

Racecadotril (RAC)[1], is chemically known as [2-{2(acetylsulfanylmethyl)-3-phenyl-propanoyl} amino acetic acid benzyl ester], which is a prodrug of the enkephalinase inhibitor thiorphan (fig. 1). It gets rapidly converted into thiorphan which interacts specifically with the active site of enkephalinase. The drug is used for the treatment of acute diarrhea of bacterial and viral aetiology[2,3]. Since this drug is being marketed in domestic and international market, there is a need to develop a simple assay procedure for the determination of this drug, particularly in its pharmaceutical formulations for quality control purpose. The availability of an HPLC method with high sensitivity and selectivity would be very useful.

RAC (assigned purity 99.8%) was procured as a gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad, India, and gemfibrozil (GEM) was a gift sample from Sun Pharmaceutical Industry Ltd., Vadodara, India. HPLC grade acetonitrile and water procured from Ranbaxy Fine Chemicals Limited, SAS Nagar, India and Qualigens Chemicals, India, respectively were used in this study. Potassium dihydrogen phosphate was obtained from S. D. Fine Chemicals, Mumbai. Commercially available RAC capsules claimed to contain 100 mg of the drug were procured from the local pharmacy (Zedott, Torrent Pharmaceuticals, Ahmedabad, India).

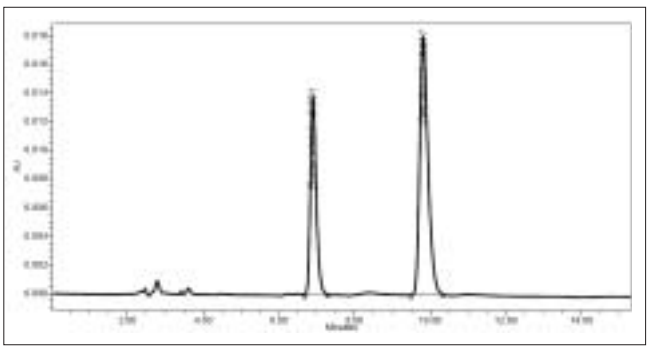

Fig. 1. Typical HPLC chromatogram of racecadotril

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Quantitative HPLC was performed on an isocratic high pressure liquid chromatograph (Shimadzu HPLC Class 10A Series) with two LC-10AT pumps at a fixed wavelength guided by a programmable UV/Vis detector (SPD-10A) which was connected to a BDS-Hypersil RP-C18 column (particle size 5 µ). The HPLC system was equipped with the software Class LC-10AT series version 5.03 (Shimadzu) and a pH meter (Systronics, Mumbai). The contents of the mobile phase were 20 mM phosphate buffer (pH 3.5) and acetonitrile in the ratio of 40:60. They were filtered before use through a 0.45 µm membrane filter, degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs through the column at a flow rate of 1 ml/min, yielding a column back pressure of 140–160 kg/cm^2. The run time was set at 15 min and the column temperature was maintained at 30±2^0. The volume of the injection loop was 20 µl. Prior to injection of the drug solutions, the column was equilibrated for at least 45 min with the mobile phase flowing through the systems. The eluent was monitored at 230 nm and the data acquired was stored and analyzed.

A stock solution of the drug and internal standard was prepared by dissolving 50 mg of RAC and GEM in two separate 50 ml volumetric flask containing acetonitrile, sonicated for about 10 min and then made up to the volume. Aliquots of these stock solutions were suitably diluted with mobile phase to get the working standard solution of drug in the concentration range of 5–15 µg/ml, along with a fixed concentration (10 µg/ml) of GEM as internal standard. Daily working standard solutions of RAC and GEM were prepared by suitable dilution of the stock solution with the mobile phase. Each of this drug solution (20 µl) was injected in triplicate into the column and the peak ratio was recorded.

To study the effect of pH, the standard solution was analyzed by using two different buffer solutions of varying pH (phosphate buffer of pH 3.0, 3.2, 3.5, 4.0, 6.0 and 7.0 and acetate buffer pH 5.0) with acetonitrile in the ratio of (40:60) as the mobile phase, at a flow rate of 1 ml/min. A BDS-Hypersil C_{18} column was used as the stationary phase. The retention time of RAC did not change with increase in pH of the buffer solution in mobile phase but changes were observed in retention time of GEM (decrease in retention time). This may be due to unionized state of racecadotril at every pH.

Further, twenty capsule contents were weighed to obtain the average capsule content weight and the contents were mixed. A sample of the mixed capsule content, claimed to contain 50 mg of the active ingredient RAC was taken in a 50 ml clean volumetric flask. The contents were dissolved in acetonitrile and the final volume was made up. This mixture was shaken well and was then filtered through a 0.45 µm membrane filter. An appropriate dilution was made with mobile phase to get a concentration of 10 µg/ml RAC along with a fixed concentration (10 µg/ml) of GEM as internal standard. The system precision was evaluated by performing six consecutive injections of RAC and GEM standard solution. Method precision was evaluated by six repeated assays of the same lot of commercial formulation.

Recovery studies were done at three different levels. The pre-analyzed samples were spiked with 80%, 100% and 120% of the standard racecadotril and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate.

Drug–capsule excipient interactions were performed by the following method. Physical mixtures of the drug with the excipients (1:1) were transferred into a 50 ml volumetric flask and dissolved in acetonitrile (HPLC grade) and sonicated for 10 min. Then finally volume was made up to 50 ml with acetonitrile. An appropriate dilution was made with mobile phase to get a concentration of 10 µg/ml, and it was analyzed by the proposed method.

Stability studies were performed using standard solutions of RAC and GEM by keeping the solutions at room temperature for 24 h. The solutions were analyzed at intervals of 0, 6, 12, 18 and 24 h. All chromatograms run at the above mentioned period were free from additional peaks and did not show

TABLE 1: INTER AND INTRA-DAY PRECISION STUDIES

Observed peak area ratio of RAC					
Concentration		Intraday		Interday	
of RAC µg/ml		Mean	% RSD	Mean	% RSD
10	Day I	0.629	0.469	0.624	0.503
		0.630	0.843		
		0.628	0.587		
	Day II	0.629	0.16	0.617	0.258
		0.630	0.40		
		0.630	0.32		
	Day III	0.628	0.40	0.620	0.752
		0.631	0.60		
		0.627	0.28		

TABLE 2: ASSAY AND RECOVERY OF RACECADOTRIL

Labeled amount (mg/capsule)	Observed amount (mg/capsule)	% Purity	Amount of drug added (µg/ml) to pre analyzed capsule formulation	% Recovery
100	99.45±0.19	99.45±0.32	8	98.38
	99.24±0.20	99.24±0.45	10	98.20
	100.28±0.24	100.28±0.74	12	99.50

any change in peak area ratio which indicates that the solutions were stable up to 24 h at room temperature.

The main objective of this investigation was to develop a simple, precise and rapid RP-HPLC method for the analysis of RAC in its capsule dosage form, using the most commonly employed RP C-18 column with UV detection. Retention times (t_r) of RAC and internal standard GEM were found to be 6.9 and 9.8 min, respectively. A typical HPLC chromatogram for analysis of RAC and internal standard GEM is shown in fig.1. A system suitability test was performed to check various parameters such as number of theoretical plates, peak tailing, capacity factor and resolution. Theoretical plates were found to be 11458.7 and 10582.6 per meter for RAC and GEM respectively. Peak tailing was found to be 1.05 and 1.31 for RAC and GEM, respectively.

Capacity factor was found to be 2.04 and 2.98 for RAC and GEM, respectively and the resolution was found to be 7.98. The calibration curve of RAC was constructed by plotting the peak area ratio of the RAC and GEM against the concentration. It was found to be linear with a correlation coefficient of 0.999, the representative linear regression equation being Y=0.0633X – 0.0045. This method was also validated for its intraday and inter-day precision at a concentration of 10 µg/ml racecadotril and gemfibrozil as internal standard, which was expressed as relative standard deviation based on the peak ratio for six injections. The intra day precision was found to be between 0.469% and 0.843% and inter-day precision was found to be between 0.258% and 0.752% (Table 1). Stability studies indicated that the prepared solutions were stable up to 24 h.

The present study was used to quantify RAC in capsule form. RAC capsules (100 mg) were analyzed and the results given in Table 2. The average drug content was found to be 99.66% of the labeled amount. Drug excipients interaction studies were carried out with the common capsule excipients. No interfering peaks were observed in the chromatogram, which indicates that the capsule excipients did not interfere with the estimation of the drug in the proposed HPLC method. Recovery studies were performed by adding known amount of drug solutions to the pre-analyzed sample solutions and the recovery ranged from 98.20 % to 99.52% (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) was found to be 50 ng/ml and 100 ng/ml respectively indicating that the proposed procedure is highly accurate. The results of the study showed that the present method is simple, rapid, precise and accurate.

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