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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ANALYSIS OF RACECADOTRIL IN PURE AND FORMULATIONS

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ABSTRACT
A rapid, simple and validated reversed-phase high-performance liquid chromatographic method has been developed for analysis of Racecadotril in Tablet dosage form. Racecadotril was separated on a Phenomenex C_{18} column (250 mm length, 4.6 mm internal diameter and particle size 5 µm) with a 60:40 (v/v) mixture of Acetonitrile and Phosphate buffer as mobile phase at a flow rate of 1.0 mL min^{-1}. The effluent was monitored by UV detection at 228 nm. Calibration plots were linear in the range of 10 to 50 µg mL^{-1} and the LOD and LOQ were 0.635 and 1.94 µg mL^{-1}, respectively. The high recovery and low relative standard deviation values confirm the suitability of the method for routine quality control determination of Racecadotril in tablets.

KEYWORDS: Racecadotril, RP-HPLC, Tablet analysis, Validation.

INTRODUCTION
Racecadotril[1] N-[(R,S)-3-acetylmercapto-2-benzyl]-glycine[2], benzyl ester, (Figure-1) is an oral enkephalinase inhibitor for use in the treatment of acute diarrhea. In peripheral tissue membranes Racecadotril is converted is converted into thiorphan, which is the specific inhibitor of enkephalinase, and therefore prolongs the antisecretory effect of the endogenous enkephalins. Enkephalin concentration is increased, as a result of this, leading to activation of opiod receptors and a decrease in Ca,P level. This in turn results in reduced secretion of water and electrolytes into the lumen.[3-6]

Racecadotril (RAC) is not official in any pharmacopoeia. There are some UV[7], HPLC [8-10], NMR[11] and LC-MS[12] methods reported in the literature for the estimation of Racecadotril in bulk drug and pharmaceutical dosage forms. Hence the present research work was aimed to develop and validate[13-14] the simple, specific and sensitive RP-HPLC method for the determination of Racecadotril in pure and its pharmaceutical formulation.

MATERIALS AND METHODS
Potassium dihydrogen ortho phosphate (AR grade, Merck)
Triethyl amine (HPLC grade, Merck)
Ortho phosphoric acid (AR grade, SD fine)
Acetonitrile (HPLC grade, Merck)
Water (HPLC grade, Merck)

Chromatographic System and Conditions
Analysis was performed with a Shimadzu chromatograph comprising an LC-10 AT VP solvent-delivery module, a SPD-10A UV–visible detector, 10 µL sample loop. Racecadotril was chromatographed on a 250 mm × 4.6 mm i.d., 5 µm particle, Phenomenex C18 analytical column under reversed-phase partition conditions. The mobile phase was a 60: 40 (v/v) mixture of Acetonitrile and Phosphate buffer. The flow

rate was 1.0 mL min^{-1} and the analyte was monitored at 228 nm. Before analysis the mobile phase was degassed by use of a sonicator and filtered through a 0.45 μm injection filter. The column was equilibrated before each injection.

Calibration

Calibration plots were constructed by analysis of appropriate working solutions (Concentrations of 10, 20, 30, 40 and 50 μg mL^{-1}) of Racecadotril in the mobile phase and plotting concentration against peak-area response for each injection, Unknown samples were quantified by reference to these calibration plots.

Sample Preparation

Twenty tablets were accurately weighed. An amount of powder equivalent to 50 mg of Racecadotril was accurately weighed and transferred to a 50 mL volumetric flask. Mobile phase (25 mL) was added and the mixture was sonicated for 10 min for complete extraction of the drug and the solution was diluted to volume with mobile phase. The solution was filtered through a 0.45 μm membrane filter. This solution was suitably diluted and injected for HPLC analysis.

RESULTS AND DISCUSSION

Method development and optimization

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), additive strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized, so there was no interference with the Racecadotril peak from solvent or excipients peaks. Other criteria, for example the time required for analysis, assay sensitivity, solvent noise and use of the same solvent system for extraction of the drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase the column was equilibrated by passage of at least twenty column volumes of the new mobile phase. To investigate the appropriate wavelength for determination of Racecadotril, UV–visible spectra in the range 200–400 nm were acquired from a solution of the drug in the mobile phase (Systronics model 2201 spectrophotometer). From the UV spectra obtained the wavelength selected for monitoring the drug was 228 nm. Solutions of the drug in the mobile phase were injected directly for HPLC analysis and the responses (peak area) were recorded at 228 nm. It was observed there was no interference from the mobile phase or baseline disturbance at 228 nm. Therefore, it was, concluded that 228 nm was the most appropriate wavelength for analysis of the substance with suitable sensitivity.

Chromatography

Symmetrical peaks were obtained for Racecadotril. Typical chromatograms obtained from a blank and from a solution of the drug are illustrated in **Figure-3(a & b).** The retention time of Milnacipran was 6.48 min and the overall chromatographic run time was 10.0 min.

Method Validation

Linearity

The linearity of the method was tested using the calibration solutions described above. Plot of concentrations against responses were linear in the range of 10-50 μg mL^{-1} **(Figure-2).** The mean regression equation was $y = 23.84x + 4.85$. The correlation coefficient was 0.9998. The system suitability parameters are given in **Table-1**

Limits of detection and quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal-to-noise ratio (SNR) is 3:1. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy. It is usually regarded as the amount for which the SNR is 10:1. Two types of solution, blank solution and solutions containing known, progressively decreasing concentrations of the analyte were prepared and analyzed. LOD and LOQ were 0.635 and 1.94 μg mL^{-1}, respectively.

Accuracy

Recovery studies were performed in triplicate by adding a known amount of standard solution of pure drug to a preanalyzed sample solution (10 μg mL^{-1}) at three different levels (10, 20 and 30 μg mL^{-1} of standard solution). The results obtained **(Table-2)** indicate that recovery were excellent, not less than 98%, not more than 102% and that relative standard deviations also less than 2%.

Precision

Intra-day precision was calculated from results obtained from six-fold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on three consecutive days. The results obtained are listed in **Table-3**.

Specificity

The specificity of the method was tested by chromatographing a mixture of commonly used tablet excipients, for example starch, lactose and magnesium stearate (blank placebo) and comparing the chromatogram with that obtained from a mixture of drug and the same Additives (placebo). The chromatograms obtained showed separation of the analyte from the excipients was complete, i.e. there was no interference from the excipients under the chromatographic conditions used for the analysis.

Application of the Method to the Formulations

The method was used for determination of Racecadotril in a tablet formulation. The results obtained **(Table-4)** showed the amount found was that expected and RSD (%) values were low, which confirms the method is suitable for routine analysis of the compound in pharmaceutical preparations. A typical chromatogram obtained from analysis of a tablet formulation is shown in **Figure-4.**

CONCLUSION

This RP-HPLC method for analysis of Racecadotril in formulations is very simple, sensitive, and accurate. The run time is 10.0 min only; so many samples can also be processed and analyzed in a short period of time. The procedure described is suitable for the routine estimation of Racecadotril in pharmaceutical formulations.

REFERENCES

1. Goodman LS and Gilman AG. The Pharmacological Basis of Therapeutics, 9th Edn. By Hardman, J.G., Limbard, L.E., Editors in chief, McGraw – Hill, 1996.
2. Matheoson AJ, Noble S. Drugs 2000; 59(4): 829-835
3. Scand PD. J Gastroenterol 2002; 37(6):656-661.
4. Vetal JM, Barard H, Fretault N, Lecomte JN. Aliment pharmacol ther 1999; 13(6):21-26.
5. Lecomte JM. Int J Antimicrob Agents 2000;14(1):81-87.
6. Alam NH, Ashraf H, Khan WA, Karim MN, Fuchs GJ. Gut 2003; 52(10):1419-1423.
7. CH Narasimha Raju BH, G Devala Rao and Ramanjaneyulu Sikharam. Biosciences, Biotechnology Research Asia 2008; 5(2):747-752.
8. JVN Seshagiri Rao, P Bhanu Prakash, M Murali Krishna and P Ravi Kumar. Asion J chemistry 2007; 19(4): 2623-2626.
9. P Srinivasa Rao and M Nappinnai. Asian J of Chemistry 2007; 19(5):3697-3702.
10. T Srinivasa Rao and M Nappinnai. Asian J of Chemistry 2007; 19(5): 3697-3702.
11. Reddy K, Babu J, Sudhakar P, Sharma M, Reddy G, Vyas K. Structural studies of racecadotril and its process impurities by NMR and mass spectroscopy pharmazie 2006;61(12):994-8.
12. Xu Y, Huang J, Liu F, Gao S, Guo Q. J Chromatogr *B* Analyt Technol Biomed Life Sci. 2007;852(1-2):101-7.
13. USP United States pharmacopoeia, 31st edition NF 26. United States Pharmacopeia Convention, Asian edition, Rockville 2008; pp. 683-687.
14. ICH, Q2R1 Validation of Analytical Procedures: Text and Methodology; International Conference on Harmonization, Geneva; 1996.

Table 1: System suitability parameters

Parameter	Results
Retention Time (Rt) (Min)	6.48
Theoretical Plates (n)	13777
Peak asymmetry	1.182
Linearity range (µg mL^{-1})	10-50
Limit of Detection (µg mL^{-1})	0.635
Limit of Quantification (µg mL^{-1})	1.94

Table 2: Accuracy of the method

Sample solution	Amount of standard drug added µg mL^{-1}	% Recovery ± SD (*n* = 3)	% RSD
Racecadotril 10 µg mL^{-1}	10	98.94 ± 0.36	0.28
	20	100.08 ± 0.53	0.57
	30	99.81 ± 0.61	0.46

Table 3: Intra-day and inter-day precision of the method.

Concentration Added, µg mL^{-1}	Intra-day precision		Inter-day precision	
	Mean amount found, µg mL^{-1} (n = 6)	% RSD (n = 6)	Mean amount found, µg mL^{-1} (n = 3)	% RSD (n = 3)
20	19.06 ± 0.64	0.81	19.26 ± 0.27	0.76
30	28.98 ± 0.80	0.75	29.73 ± 0.45	0.59
40	39.84 ± 0.36	0.72	39.62 ± 0.59	0.84

Table 4: Results from analysis of Racecadotril in tablets

Label claim. mg per tablet	30
Average Amount found, (mg per tablet) (n = 6)	29.47 ± 0.51
% RSD (n = 6)	0.73

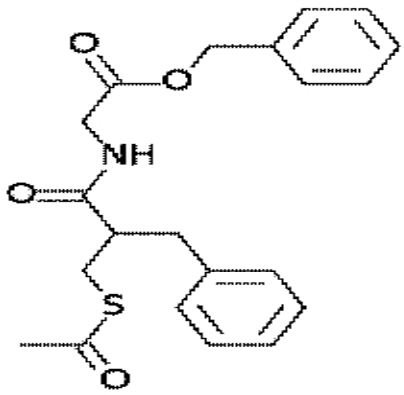

Figure 1: Structure of Racecadotril

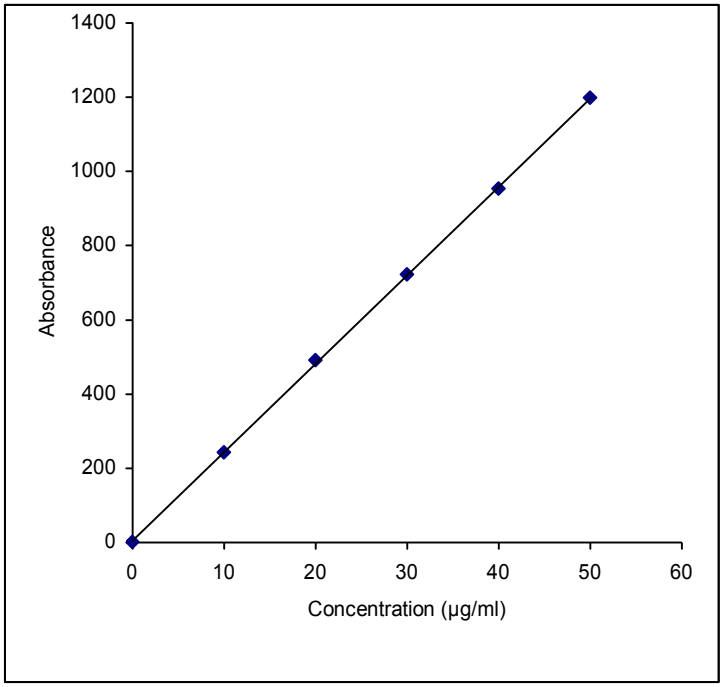

Figure 2: Calibration plot for Racecadotril

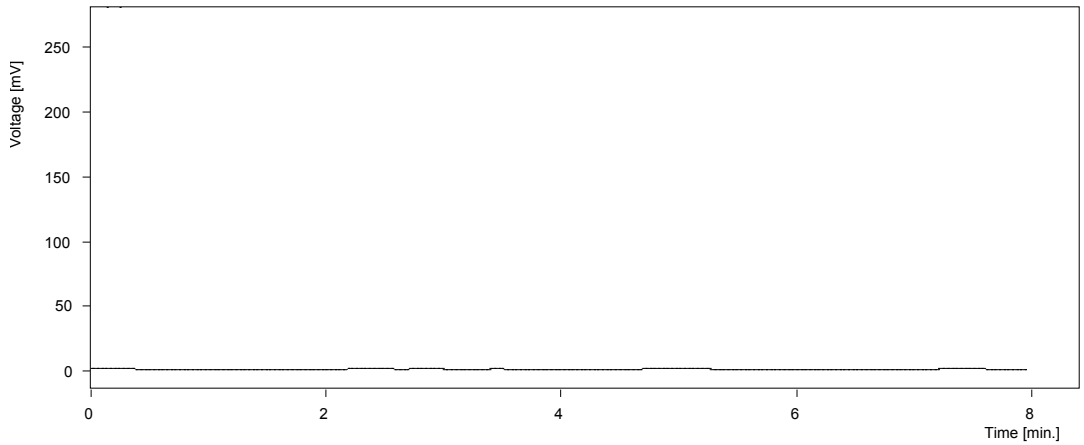

Figure 3a

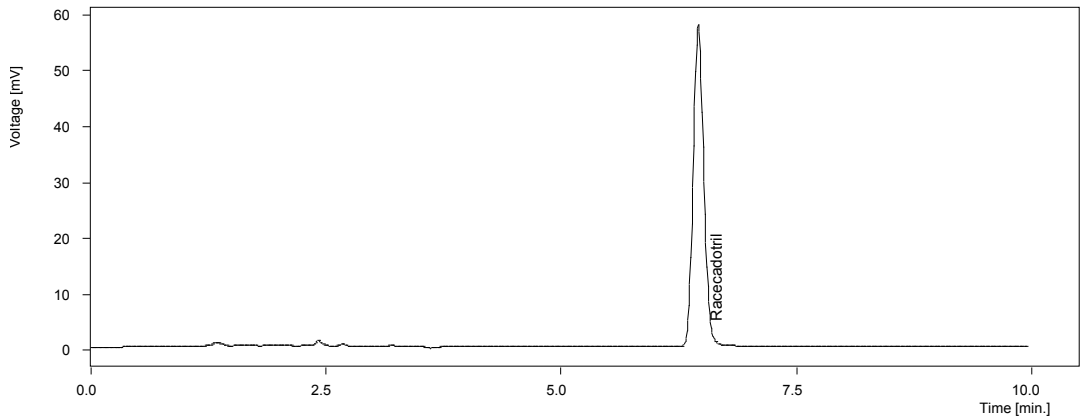

Figure 3b

Figure 3(a): Typical chromatograms obtained from blank and (b): Racecadotril solution

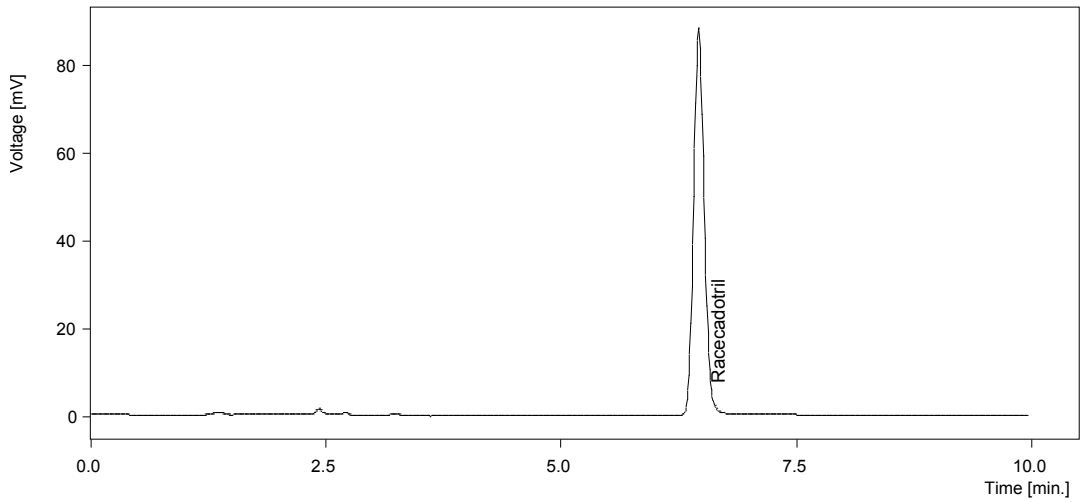

Figure 4: Typical chromatogram obtained from Racecadotril sample solution (tablets)

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