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RP-HPLC Method for the Estimation of Racecadotril in Bulk and in Tablets

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A rapid and precise RP-HPLC method has been developed for the estimation of racecadotril in pure form as well as in pharmaceutical dosage forms. The quantification was carried out using an ODS column (250 × 4.6 mm ID), 5 µ particle size with a mobile phase comprising of water and acetonitrile in the ratio 30 : 70 (v/v) in isocratic mode at a flow rate of 1.0 mL/min. The eluent was monitored at 211 nm. The retention time was 5.85 min. Aceclofenac was used as an internal standard. The method was duly validated by evaluation of the required parameters. The calibration curve was linear in the concentration range of 0.5-100 µg/mL and the percentage recovery ranged from 99.8-100.6.

Key Words: RP-HPLC, Racecadotril.

INTRODUCTION

Racecadotril[1-4] is N-[2-(acetylthio)methyl]-1-oxo-3-phenylpropyl]-glycine phenylmethylester. It is lipophlic prodrug of the enkephalinase inhibitor thiorphan. Thiorphan, by inhibiting the membrane bound enkephalinase enzymes increases the availability of endogenous opioids (enkephalins). These enkephalins activate delta receptors in the gastrointestinal tract. This in turn leads to a reduction in c-AMP mucosal levels, resulting in a reduction in the secretion of water and electrolytes into the intestinal lumen which resulted an antidiarrhoel activity. Literature survey reveals that few HPLC[5] methods were reported for the estimation of racecadotril. The proposed method was simple, rapid, sensitive, highly accurate and precise for the estimation of racecadotril in bulk as well as in tablet dosage forms.

EXPERIMENTAL

A Shimadzu LC-10AT high pressure liquid chromatographic instrument provided with an ODS reversed phase column (250 × 4.6 mm ID), 25 mL Hamilton syringe and supported by a Windows software was employed in the study.

Racecadotril received as a gift sample from Dr. Reddy's laboratories, India. HPLC grade acetonitrile (E. Merck India) and milli-Q water were used for preparing the mobile phase.

Chromatographic conditions: The mobile phase used was water and acetonitrile in the ratio 30 : 70 (v/v). Water and acetonitrile were filtered through 0.45 µ membrane filter and sonicated before use. The mobile phase was pumped from solvent reservoir in the ratio of 30 : 70 to the column at a flow rate of 1 mL/min. The run time was set at 10 min. The column was maintained at 30°C and the volume of each injection was 20 µL. Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The detector sensitivity was set at 0.0001 A.U.F.S and eluent monitored at 211 nm.

About 100 mg of pure sample of racecadotril was weighed accurately and transferred to a 100 mL volumetric flask and dissolved in 70 mL of acetonitrile. The solution was sonicated for 20 min and then the volume made up with a further quantity of acetonitrile to get 1 mg/mL solution. Subsequent dilutions of this solution ranging from 0.5-100 µg/mL were made in 10 mL volumetric flasks. The solutions prepared as above were filtered through 0.45 µ membrane filter and then 20 µL of filtrate was injected each time in to the column at a flow rate of 1 mL/min. Each concentration was injected six times into the column and corresponding chromatograms were obtained. Detection of the drug was performed at 211 nm. From the chromatograms, the retention time and mean peak area ratio were recorded for all the concentrations. A calibration curve of peak area ratios versus the respective concentration was plotted. From this, the regression of drug concentration over the peak area ratio was computed using least squares method of analysis. This regression equation was used to estimate the amount of racecadotril in pharmaceutical formulations.

Estimation of racecadotril in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate racecadotril in tablet dosage forms. For this, twenty tablets were weighed and powdered. Accurately weighed portion of the tablet powder equivalent to 100 mg was taken in 100 mL volumetric flask and 50 mL acetonitrile was added, shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug. The mixture was then thoroughly mixed and made up to the mark with acetonitrile and filtered through a 0.45 µ membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. The contents of the flask were made up to the volume with the acetonitrile and mixed well. Each of these solutions was then injected into the column. All the determinations were conducted three times from the peak area ratios. The drug content in the tablets was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The run time of the chromatographic method was set at 10 min and racecadotril was appeared on chromatogram at 5.85 min. This indicates that the present HPLC method is rapid, which in turn shows that the method consumes less volume of HPLC solvents. When the same drug solution was injected 6 times, the retention time of the drug was found to be the same. The retention time for the internal standard (aceclofenac) was observed to be 1.80 min.

The peak area ratios from such different concentrations set up as above were calculated and are shown in Table-1. A good linear relationship was observed between the concentration of racecadotril and the respective peak area ratio. The regression curve was constructed by least squares method and its mathematical expression was Y = 0.8094x - 8.19 (where Y is the peak area ratio and x is the concentration of racecadotril). This regression equation was used to estimate the amount of racecadotril in tablet dosage forms.

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Concentration (µg/mL)	Peak area ratios	Concentration (µg/mL)	Peak area ratios
0.5	0.40	20	16.39
1	0.80	40	32.03
2	1.61	60	49.19
5	3.89	80	64.19
10	7.99	100	80.54

Regression equation from 0.5-100(µg/mL): Y = 0.8094X-8.19 (r = 0.999)

To ensure reliability and accuracy of the method, a known quantity of drug was mixed with preanalyzed sample. Recovery studies were carried out by the proposed method. About 100.1% of racecadotril could be recovered from the preanalyzed samples indicating high accuracy of the proposed HPLC method (Table-2).

TABLE-2
RESULTS OF RECOVERY STUDY

Amount of racecadotril	Recovery from drug solution		Recovery from formulation	
	Mean amount	Mean (%) recovery	Mean amount	Mean (%) recovery
10	9.99	99.90	9.98	99.8
30	29.95	99.83	29.97	99.9

The HPLC method, developed in the present study, has also been used to quantify racecadotril in tablet dosage forms. Racecadotril tablets (containing 100 mg) were quantified using by the proposed method. No

interfering peaks were found in the chromatogram indicating that the tablet excepients did not interfere with the estimation of drug by proposed HPLC method (Table-3). The tablets were found to contain 99.15-100.65 % of the drug. A known amount of drug solution was added to the sample of tablet dosage form and subjected to estimation of drug by proposed method. There was a high recovery of racecadotril (99.8-100.6 %) indicating that the proposed procedure for determination of racecadotril in tablet dosage form is highly accurate.

TABLE-3
ASSAY OF RACECADOTRIL IN TABLET DOSAGE FORMS

Labeled amount (mg)	Mean ± S.D amount (mg) recovered (n = 3)	Mean ± S.D % of recovery (n = 3)
100	99.85 ± 0.21	99.9 ± 0.75
100	99.97 ± 0.17	99.9 ± 0.61

Hence, the proposed HPLC method is simple, precise, accurate and rapid for the determination of racecadotril in dosage forms. It can be easily and conveniently adopted for routine quality control analysis.

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