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# Reverse Phase High Performance Liquid Chromatographic Method for the Analysis of Racecadotril in Pharmaceutical Dosage Forms

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A rapid and sensitive reverse phase HPLC method is applied for the qualitative and quantitative assay of racecadotril in pharmaceutical dosage forms. Racecadotril was chromatographed on a reverse phase $C_{18}$ column with a mobile phase consisting of acetonitrile:methanol:water in the ratio of 50:40:10 (v/v/v). The mobile phase was pumped at a flow rate of 1 mL/min. Sildenafil was used as an internal standard and the eluents were monitored at 217 nm. The retention time of the drug was 3.168 min. With this method, excellent linearity was observed in the concentration range of 10-70 µg/mL. LOD (lower limit of detection) and LOQ (lower limit of quantification) were found to be 0.05 and 0.14 ppm, respectively. The recovery of analytes after extraction from formulations using the described method was 99.65 ± 0.77 %. The method was found to be applicable for analysis of drug in tablets. The results of the analysis were validated statistically.

**Key Words: Racecadotril, Reverse phase HPLC, Tablets.**

## INTRODUCTION

Chemical name of racecadotril[1] is N-[2-[(acetylthio)methyl]-1-oxo-3-phenylpropyl]-glycine phenylmethyl ester. Racecadotril is an oral enkephalinase inhibitor used in the treatment of acute diarrhoea[2]. It prevents the degradation of endogenous opioids (enkephalins), thereby reducing hypersecretion of water and electrolytes into the intestinal lumen[3]. A few methods of analysis of racecadotril have been reported using different techniques such as high performance liquid chromatography (HPLC)[4], high performance thin layer chromatography[5] and spectrophotometry[6]. Most of these methods are considered tedious. The HPLC methods using the most commonly available columns and detector like UV are preferred. The present

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study describes the determination of racecadotril in pharmaceutical dosage forms by using RP-$C_{18}$ column with UV detectors. Owing to the widespread use of HPLC in routine analysis, it is important that validated HPLC methods are to be developed for estimating racecadotril. The aim of this study is to develop a simple, precise, rapid and accurate reverse phase HPLC method for the estimation of racecadotril in different pharmaceutical dosage forms.

## EXPERIMENTAL

The pure racecadotril used for the development of analytical method, was gifted by Dr. Reddy's Lab. Ltd, Hyderabad. Acetonitrile, methanol and water were of HPLC grade (Merck). All other reagents were of AR grade. An isocratic HPLC (Waters India, USA) with a single Waters 510 Pump, Waters 486 tunable absorbance detector and RP-$C_{18}$ column (Bondapak $C_{18}$, 250 × 4.6 mm, packed with 5 µm particle size) was used. The HPLC system was equipped with Millennium32 software.

**Chromatographic conditions:** The mobile phase consists of acetonitrile: methanol:water in the ratio of 50:40:10 v/v. The mobile phase was filtered before use through a 0.45 µm membrane filter and degassed for 15 min. The components of the mobile phase were pumped from the solvent reservoir to the column at a flow rate of 1 mL/min that produced column back pressure 140-150 kg/cm$^2$. Ambient column temperature was maintained. The eluents were monitored at 217 nm.

**Drug and internal standard solution:** A pure sample of racecadotril procured from Dr. Reddy's Laboratories Ltd, Hyderabad was used as reference standard in the study. About 50 mg of racecadotril was weighed accurately and transferred into a 50 mL volumetric flask and dissolved in 25 mL of the mobile phase. Then the volume was made up with a further quantity of the mobile phase to get 1 mg/mL solution. Following this, the solution was sonicated for 15 min to ensure complete solubility of the drug. Subsequent dilutions of this solution ranging from 10 to 70 µg/mL were made in 10 mL volumetric flasks after addition of 0.5 mL sildenafil solution (50 µg/mL) as an internal standard to each dilution. 20 µL of the solution was injected each time into the stream of mobile system at a flow rate of 1 mL/min. Each of the dilutions was injected 6 times into the column and the corresponding chromatograms were obtained. From these chromatograms, the area under the peaks of the drug and the internal standard were noted. Using these values, the mean ratio of peak area of the drug to that of the internal standard for each dilution was calculated. The regression of the drug concentration over these ratios was computed. This regression equation was used to estimate the amount of racecadotril in the pharmaceutical dosage forms.

The solutions containing 10, 30 and 70 μg/mL of racecadotril were subjected to the proposed HPLC analysis to check the intra-day and inter-day variation of the method. The recovery studies were carried out by adding known amounts of racecadotril to the preanalyzed samples and then analyzing them by the proposed HPLC method.

**Estimation of racecadotril in the tablet dosage form:** Two commercial brands of tablets (Zedott of Torrent and Racy of Sarabhai Piramal) were chosen for testing suitability of the proposed method to estimate racecadotril in tablet formulations. 20 Tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 50 mg of racecadotril was transferred to a 50 mL volumetric flask containing 25 mL of the mobile phase. The contents of the flask were allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug. The mixture was made upto the volume with mobile phase, throughly mixed and then filtered through 0.45 μm membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. These solutions were spiked with suitable volume of the internal standard solution, such that the concentration of each solution was 50 μg/mL. The contents of the flasks was made up to the volume with the mobile phase and mixed well. Each of these solutions (20 μL) was then injected 6 times into the column. The mean peak area ratio of the drug to the internal standard of 6 such determinations was calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

## RESULTS AND DISCUSSION

To achieve sharp peaks with good resolution, under isocratic conditions, mixtures of acetonitrile, methanol and water in different combinations were tested as mobile phase on a $C_{18}$ stationary phase. A ternary mixture of acetonitrile, methanol and water in 50:40:10 (v/v/v) proportions was proved to be the most suitable of all combination, since the chromatographic peaks were better defined and resolved and free from tailing with this system. Though the structure of sildenafil is not similar to racecadotril, it was chosen as an internal standard, because it showed better peak shape and peak location compared to other potential internal standards such as cefdinir and metformin, in this perspective. Under the above mentioned chromatographic conditions, the retention time obtained for racecadotril and the internal standard were 3.168 and 5.867 min, respectively.

Each of the samples was injected 6 times and almost same retention times were observed in all cases. The ratio of peak area of racecadotril to peak area of internal standard for different concentrations set up as above were calculated and the average values for 6 such determinations are shown in Table-1. The peak areas of both the drug and internal standard were

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

| Concentration of racecadotril (μg/mL) | Mean peak area ratio (n = 6) | Coefficient of variance (%) |
|---|---|---|
| 10 | 0.145587 | 0.01 |
| 20 | 0.286334 | 0.18 |
| 30 | 0.430556 | 0.23 |
| 40 | 0.566854 | 0.64 |
| 50 | 0.732317 | 0.27 |
| 60 | 0.873565 | 0.39 |
| 70 | 1.007881 | 0.08 |

Regression equation (from 10 to 70 μg/mL): y = 0.0145x-0.0017, (r = 0.9998).

reproducible as indicated by low coefficient of variation (0.64 %). A good linear relationship (r = 0.9998) was observed between the concentration of racecadotril and the respective ratio of peak areas.

The regression curve was constructed by linear regression, fitting into mathematical expression, y = 0.0145x–0.0017 (where y is ratio of area under the curve of the drug to that of the internal standard and x is the corresponding concentration of racecadotril). When racecadotril solutions containing 10, 30 and 70 μg/mL were analyzed by the proposed method for finding out intra- and inter-day variations, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise. A recovery of 99.78 ± 0.31 % of racecadotril from the preanalyzed samples (Table-3) shows that the present method is highly accurate.

TABLE-2
PRECISION OF THE PROPOSED METHOD

| Concentration of racecadotril (μg/mL) | Observed concentration of racecadotril (μg/mL) | | | |
|---|---|---|---|---|
| | Intra day | | Inter-day | |
| | Mean (n = 6) | Coefficient of variation (%) | Mean (n = 6) | Coefficient of variation (%) |
| 10 | 10.01 | 0.02 | 10.14 | 0.03 |
| 30 | 29.86 | 0.31 | 29.91 | 0.29 |
| 70 | 69.64 | 0.11 | 69.91 | 0.07 |

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of racecadotril in two different brands of tablet dosage form is shown in Table-4. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients

TABLE-3
RECOVERY DATA OF RACECADOTRIL
(ACCORDING TO ICH GUIDELINES)

| Amount of drug added (µg) to solutions of pure drug/tablet formulation | Recovery from drug solution | | Recovery from tablet formulation | |
|---|---|---|---|---|
| | Mean (± SD) Amount (µg) found (n = 6) | Mean (± SD) % Recovery (n = 6) | Mean (± SD) Amount (µg) found (n = 6) | Mean (± SD) % Recovery (n = 6) |
| 16 | 15.97±0.51 | 99.78±0.31 | 15.93±0.74 | 99.65±0.77 |
| 20 | 19.87±0.08 | 99.31±0.13 | 19.88±0.12 | 99.48±0.46 |
| 24 | 23.61±0.62 | 98.45±0.14 | 23.81±0.43 | 99.23±0.15 |

TABLE-4
ASSAY OF RACECADOTRIL DOSAGE FORMS

| Brand name of the tablet | Labeled amount of drug (mg) | Mean (± SD) Amount (mg) Found by the proposed method (n = 6) | % Mean (± SD) Labeled amount (n = 6) |
|---|---|---|---|
| Zedott | 100 | 99.96 ± 0.01 | 99.96 ± 0.01 |
| Racy | 100 | 99.93 ± 0.03 | 99.93 ± 0.03 |

used in the tablets. The tablets were found to contain 99.93 to 99.96% of the labeled amount of the drug. The low coefficient of variation indicates the reproducibility of the assay of racecadotril in tablets. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of racecadotril in pharmaceutical dosage forms within a short analysis time.

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