RESEARCH ARTICLE

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Development and Validation of A Rapid and Sensitive HPLC Method for Estimation of Racecadotril

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Abstracts: A simple, specific, sensitive and rapid reverse phase high performance liquid chromatographic (HPLC) method for the determination of racecadotril (RCD) was developed and validated. Sample preparation involved simple dissolution, followed by dilution with mobile phase to eliminate any chromatographic solvent effects. RCD was quantitated on a C18 column (4.6 mm i.d. × 300 mm length), using a mobile phase composed of acetonitrile: water (65:35 %v/v) which was delivered at a flow rate of 1.0 mL/min. The method was proven to be linear over a concentration range of 5 to 100 µg/mL with a mean correlation coefficient of 0.9991. The intra-day and inter-day precision (coefficient of variation) were in the range of 0.53% to 0.92% and 0.95% to 1.76%, respectively. The intra-day accuracy (relative error) were in the range of 2.53% to 4.47% and the inter-day accuracy were in the range of 3.11% to 4.92%. The limit of detection (LOD) and the limit of quantification (LOQ) of the developed method were determined to be 0.5 µg/mL and 5 µg/mL, respectively. The developed method was established as a rapid analytical tool as it required short retention time, high precision, sensitivity and small volumes of sample for analysis.

INTRODUCTION

Racecadotril (RCD) is Benzyl 2- ({2- [(acetylsulfanyl) methyl] -3- phenyl propanoyl} amino) acetate (Figure 1), is a synthetic antisecretory agents, utilized for the treatment of diarrhea. RCD is a poorly water-soluble drug (BCS Class II) which displays a dissolution rate-limited absorption pattern in humans and animals. Hence, it can be used as a model drug to assess the influence of various physicochemical, physiological, and dosage form factors on the dissolution and subsequently bioavailability of hydrophobic drugs. [1-6]

A variety of methods have been developed for determination of RCD individually or with combination with some other pharmaceutical agents. These includes Ultraviolet (UV)-visible spectrophotometry, [7-8] Nuclear Magnetic Resonance (NMR) and Mass spectroscopy, [9] Liquid chromatography / tandem mass spectrometry method, [10] thin layer chromatography (TLC) and derivative spectroscopy [11] were reported. The objective of the present study is to develop a simple, precise, accurate and economic analytical method with a better detection range for the estimation of RCD in pharmaceutical formulations. Among them liquid chromatographic–tandem mass spectrometric method can provide excellent sensitivity, it is not available for some laboratories because of its special requirements and financial reasons. As described earlier all the reported methods for RCD have various limitations, including time-consuming sample clean-up, laborious extraction steps and long chromatographic elution time. As far as our knowledge is concerned there is no simple HPLC method reported till date for the estimation of RCD. Thus the present study was undertaken to develop and validate a simple, sensitive, accurate, precise and reproducible analytical HPLC method for estimation of RCD.

MATERIALS AND METHODS

Chemicals and Reagents

RCD was procured form Ogene Systems (I) Pvt Ltd, Hyderabad, India. Acetonitrile (HPLC grade), methanol (HPLC grade) and potassium dihydrogen phosphate were purchased from Merck Chemicals, Mumbai, India. Double deionized water was utilized for entire study.

Instrumentation

A High Performance Liquid Chromatography (HPLC) with work station of Shimadzu LC SOLUTION was employed for present investigation. The system consisted Shimadzu UFLC as solvent delivery system, Shimadzu 7D Rheodyne Injector as loop injector and UV detector as a source of detection.

Chromatographic Conditions

A combination of acetonitrile and water (65:35 %v/v) was selected as mobile phase. Samples were separated using Phenomenex Luna® C18 column with a pore size 100 Å, length 300 mm and internal diameter (i.d.) 4.6 mm. The mobile phase was injected to the system using binary pumping mode at a flow rate of 1 mL/min. For all samples, injection volume and run time were fixed as 20 µL and 10 min, respectively.

Preparation of Mobile Phase

For preparing a mobile phase, HPLC grade acetonitrile were filtered through a 0.2 µm membrane filter and subjected to degassing in an ultrasonic bath (Frontline FS-4, Mumbai, India) for a period of 15 min.

Preparation of Standard Solutions

A primary stock solution (1 mg/mL) was prepared by dissolving 10 mg of RCD in 10 mL of HPLC grade methanol. The stock solution was suitably diluted with HPLC grade methanol to obtain working range of standard solutions. The working standard solutions were prepared in duplicates, out of which one set was used to prepare

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Table 1: Chromatographic Conditions for Analytical Method Development

Chromatographic Conditions	
Mobile Phase	Acetonitrile and water (65:35 %v/v)
Column	Phenomenex Luna® C18 Pore size - 100 Å, Length - 300 mm, i.d. - 4.6 mm
λmax	231 nm
Flow Rate	1 mL/min
Injection Volume	20 µL
Run Time	10 min

Table 2: Calibration Data of RCD

Concentration (µg/mL)	Peak Area
05	64054.67 ± 1015.23
10	129689.33 ± 3584.51
20	256992.83 ± 4326.73
30	386895.50 ± 5765.07
40	501184.67 ± 9059.54
50	636482.83 ± 10112.32
60	781154.33 ± 10492.33
70	888712.43 ± 10654.41
80	1035564.17 ± 11686.28
90	1166887.67± 14894.76
100	1256589.83 ± 11984.25

The results are of mean ± SD (n=6)

Table 3: Intra And Inter-Day Accuracy and Precision of Analytical Method

Nominal	Mean ± SD (n=6)		%RE		% RSD	
Concentrations	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
LQC	14.87 ± 1.25	14.74 ± 1.75	4.47	4.92	0.92	1.76
MQC	50.71 ± 0.97	49.23 ± 1.32	3.91	4.04	0.82	1.59
HQC	90.14 ± 0.59	89.33 ± 0.74	2.53	3.11	0.53	0.95

Table 4: Robustness Study of Analytical Method

Flow Rate (mL/min)	0.8	1.0	1.2
Mean	636785.75	636482.83	636305.33
%RSD	1.95	1.53	1.87
Mobile Phase Ratio	60:40	65:35	70:30
Mean	636015.51	636482.83	636925.07
%RSD	1.82	1.03	1.65

calibration curve and the other was used to generate quality control (QC) samples. The calibration curve samples were prepared by diluted working standard solution to obtain final concentrations of 5, 10, 20, 30, 40, 50, 60, 70, 80 90 and 100 µg/mL. The QC samples [15 µg/mL (LQC), 50 µg/mL (MQC) and 90 µg/mL (HQC)] were prepared. All samples were stored at refrigerated cold conditions (2-8°C) and equilibrated to room temperature prior to use. [12]

Construction of Calibration Curves

The values of peak areas were plotted against their respective concentrations in order to construct the calibration curve for RCD. Linear regression analysis was performed for each set of data using Microsoft Excel® version 2010 (Microsoft Corporation, Washington, USA).

Validation Parameters

Linearity and range

The linearity and range of calibration curve was evaluated with eleven calibration standards containing different concentrations of RCD (5, 10, 20, 30, 40, 50, 60, 70, 80 90 and 100 µg/mL). The study was repeated six times to confirm reproducibility of results. The concentrations of the test samples were back-calculated using linear regression analysis.

Specificity

Specificity is the ability of an analytical method to differentiate and quantify analyte in the presence of other components of the sample. The specificity of the method was established through the study of resolution factors of the drug peak from the nearest resolving peak. Specificity was established by the determination of purity of the drug peak using a UV detector.

Accuracy and Precision

Intraday precision and accuracy of developed method was

Table 5. Summary of Validation Parameters of Analytical Method

Parameters	Results
Linearity Range (µg/mL)	05-80
Co-relation Coefficient(R2)	0.9991
Slope	12725
Intercept	2884.7
Limit of Detection (LOD) (µg/mL)	0.5
Limit of Quantification (LOQ) (µg/mL)	5
Retention time (Rt) (min)	7.8

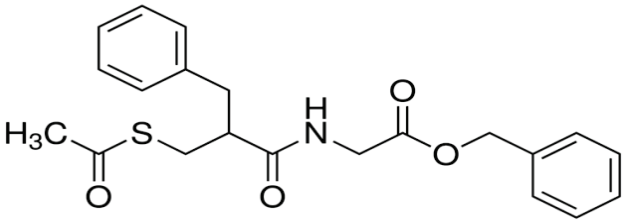

Figure 1: Structure of Racecadotril

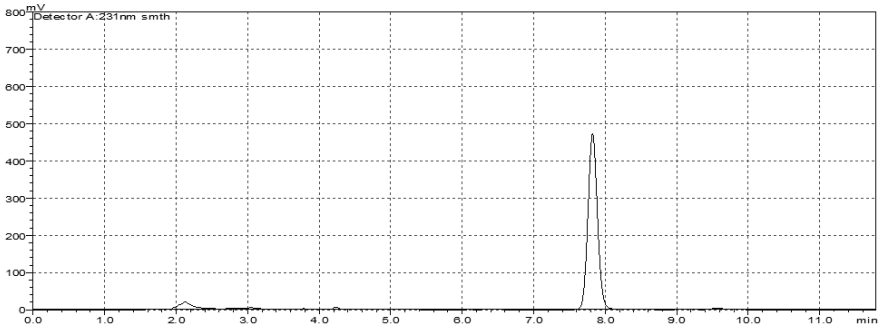

Figure 2: HPLC Chromatogram of RCD

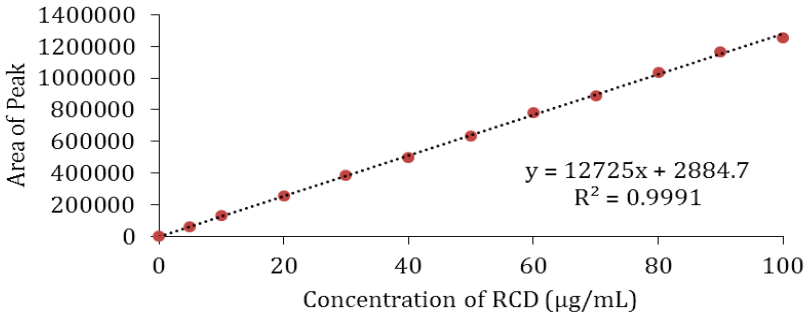

Figure 3: Calibration curve of RCD

determined by analyzing six replicates of QC samples at three concentrations in a single sequence. Similarly, for interday precision and accuracy, six replicates QC samples at three concentrations were analyzed on three consecutive days. The accuracy of method was determined by calculating % relative error (%RE) whereas the precision was determined by calculating % relative standard deviation (%RSD). [12]

Robustness

The robustness of developed method was studied by evaluating the effect of small but deliberate variations in chromatographic conditions. The parameters studied were flow rate and mobile phase composition. [12]

Limits of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ of developed method were estimated on the basis of standard deviation and slope of the calibration curve as 3.3 δ/m and 10 δ/m, respectively. Where δ was the regression standard deviation of intercept and m was the slope of calibration curve. [12]

RESULTS AND DISCUSSIONS

Analysis is an important component in formulation and development of any drug molecule. A suitable and validated method has to be available for the analysis of drug(s) in bulk, drug delivery systems, *in vitro* and *in vivo*. If such suitable method for specific need is not available then it

becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples. The present investigation was aimed to develop and validate analytical HPLC method for estimation of RCD. [12-15]

Selection of Chromatographic Conditions

The chromatographic conditions were selected on the basis of backpressure, peak resolutions, peak shapes and day-to-day reproducibility of the retention time (Table 1). For selecting a mobile phase initial trial was taken using acetonitrile and potassium dihydrogen phosphate buffer (pH 3.5) in various phase ratios but the peaks were not of a good shape. Utilization of water in place of phosphate buffer improved the peak shapes and hence, 65:35 of water: acetonitrile was selected as mobile phase for further trials (Figure 2).

Validation Parameters

Linearity and Range

The mean regression equation of three standard curves for RCD was y = 12725x + 2884.7. Where y presented the peak area of drug and x was the concentration of drug. The precisions of the slope and intercept were less than 2% for RCD which indicated minimum variations. [12] The calibration curve was linear over the studied concentration range (5–100 µg/mL) with a mean correlation coefficient more than 0.9991 (Table 2 and Figure 3).

Specificity

It is the ability of an analytical method to differentiate and quantify analyte in the presence of other components of the samples. Blank sample was evaluated for interference with respective drug. [12] The results revealed that the analyte (RCD) was well separated from co-extracted material under the adopted chromatographic conditions. The retention time (R_t) was 7.8 min (Figure 2).

Accuracy and Precision

The accuracy of an analytical method describes the closeness of test results to the true concentration of analytes whereas the precision is measure of degree of reproducibility of analytical method. [12] The results of intraday and interday precisions are summarized in Table 3 revealed that developed method was accurate and precise for quantification of RCD. Presented results comply with the criterion given by ICH: relative standard deviation not higher than 10% (12).

Robustness

The low values of %RSD for each of drug proposed that during all deliberate variations, assay value of test preparation (MQC) was not affected and it was in accordance with that of actual (Table 4). Hence, developed analytical method was considered to be robust. [12]

Limits of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ were observed as 0.5 µg/mL and 5 µg/mL, respectively. These low values were indicative of high sensitivity of developed method. The relative error was 14.34% being below the one allowed for the LOD (20%). LOQ was determined showing the relative error of 11.19% for RCD. LOD and LOQ were calculated according to the approach based on the standard deviation of the response. [12] Summary of all validation parameters of analytical method developed is given in Table 5.

CONCLUSIONS

A rapid and reliable isocratic HPLC method for determination of RCD has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including accuracy, linearity, and precision. The chromatographic run time of 8 min allows the analysis of a large number of samples in a short period of time. Therefore, the method is also suitable for analysis of sample during routine analysis of formulations as well as raw materials and accelerated stability studies.

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