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# UV and RP-HPLC Estimation of Racecadotril

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Quantitative method for estimating racecadotril, which is a potent enkephalinase inhibitor, in raw material and from dosage form (capsules), using UV spectroscopy and RP-HPLC, were developed. In the UV method, using ethanol, the $\lambda_{max}$ was 231 nm and linearity range, limit of detection, limit of quantification, molar extinction coefficient were calculated. In RP-HPLC method, mobilephase used was methanol, acetonitrile, water. Relevant parameters were determined and these methods were used to assay the drug present in raw material and in the dosage form. The results were subjected to statistical analysis and the methods were validated.

**Key Words: Estimation, RP-HPLC, Ultraviolet, Racecadotril.**

## INTRODUCTION

Racecadotril is chemically N-2[{(acetyl thio)-methyl}-1-oxo-3-phenyl propyl] glycine phenyl methyl ether[1]. It is a potent enkephalinase inhibitor and has selective anti-secretary activity[2]. It is available as capsules and also as pellets (for paediatric use) for treating diarrhoea. A HPLC method is reported for estimating impurities present in racecadotril raw material only[3]. There is no reported method for quantitative estimation of racecadotril. This study reports a simple, reproducible, fast, accurate, sensitive UV spectroscopic method and RP-HPLC method for quantitative estimation of racecadotril in raw material and in oral solid dosage form.

## EXPERIMENTAL

Racecadotril was a gift sample from Dr. Reddy's Labs, Hyderabad. Tablets of racecadotril were purchased from the local market. Ethanol, acetonitile, methanol (all HPLC grade) were purchased from E-Merck (India) Ltd., Mumbai. Instruments used were Shimadzu UV-Visible spectrophotometer model 1601 and high performance liquid chromatograph (HPLC), Shimadzu pumps Lc-10 ATVP equipped with Hamilton 100 (Naduz Schweiz). Rheodyne valve injector with 20 μL fixed loop, UV-Vis detector SPD-10ATVP (Shimadzu).

## UV method

**Determination of $\lambda_{max}$:** 100 mg of racecadotril RS was accurately weighed and dissolved in 100 mL of ethanol to obtain concentration of 1 mg/mL. From this stock solution, suitable dilution was made to obtain a concentration of 50 mcg/mL. This was subjected to UV scanning from 200-400 nm for determining the $\lambda_{max}$.

**Beer's law range determination:** From the stock solution, aliquots were pipetted and suitable dilutions were made to obtain concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80 mcg/mL using ethanol. Absorbances of these solutions were measured at 231 nm which was the detected $\lambda_{max}$. This was used to determine the linearity range. Equation for straight line : $y = 0.00464 + 0.01311x$ was obtained by performing linear regression analysis (LRA) of the data.

Molar extinction coefficient, limit of detection and limit of quantification were calculated[4]. Results reproduced in Table-1.

TABLE-1
PARAMETERS FOR UV METHOD FOR ESTIMATING RACECADOTRIL

| Parameters | Result |
|---|---|
| Linear dynamic range LDR (µg/mL) | 5-80 |
| Slope (b) | 0.013177 |
| Intercept (m) | 0.003973 |
| Correlation coefficient (r) | 0.9993 |
| Molar extinction coefficient (L $mol^{-1}$ $cm^{-1}$) | 5116.31 |
| Limit of detection LOD (µg/mL) | 0.49 |
| Limit of quantification LOQ (µg/mL) | 1.48 |

**Assay:** For the assay, solution of racecadotril in ethanol with concentration of 50 mcg/mL was used as standard. For the sample, content of 20 capsules were accurately weighed and powdered. The powder equivalent to 50 mg of racecadotril was accurately weighed and transferred to a 50 mL volumetric flask. About 25 mL of ethanol was added and sonicated for 15 min. Then the volume was made up to 50 mL with ethanol and 6 such samples were prepared. The absorbance of each solution was measured at 231 nm and the amount of racecadotril present in the dosage form was calculated by using LRA equation. Results are presented in Table-2. To ensure the accuracy of the method, recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples and the whole contents were reanalyzed by the proposed method. Results reproduced in Table-3.

TABLE-2
RESULTS OF ASSAY PERFORMED BY UV METHOD

| Sample | Assay % w/w* | Standard deviation (SD) | Relative standard deviation (% RSD) | Standard error of mean (SE) |
|---|---|---|---|---|
| Sample-A | 98.62 | 0.0019493 | 0.58449 | 0.0007957 |
| Sample-B | 98.61 | 0.0019289 | 0.58713 | 0.0007953 |

*Each value is a mean of six readings. Label claim 100 mg/capsule

TABLE-3
RESULTS OF PERCENTAGE RECOVERY TEST

| Sample | Concentration of pre analyzed sample (μg/5mL) | Concentration of standard added (μg/5mL) | Amount found theoretically (μg/mL) | Amount found practically (μg/mL) | Percentage recovery (% w/w) |
|---|---|---|---|---|---|
| Sample-A | 248.95 | 100 | 34.89 | 34.75 | 99.62 |
| | | 150 | 39.89 | 39.60 | 99.27 |
| | | 200 | 44.89 | 44.18 | 98.42 |
| Sample-B | 253.35 | 100 | 35.33 | 34.72 | 98.27 |
| | | 150 | 40.33 | 40.01 | 99.20 |
| | | 200 | 45.33 | 44.70 | 98.61 |

Percentage recovery was calculated by using following formula:

$$\% \text{ Recovery} = \frac{\text{Concentration detected practically}}{\text{Concentration calculated theoretically}} \times 100$$

**RP-HPLC method**

**Chromatographic conditions:** The column used was Hypersil ODS $C_{18}$ 250 × 4.6 mm i.d. The mobile phase used was methanol, water and acetonitrile in the ratio of 1:3:1. The mobile phase was filtered through 0.45 μ nylon membrane filter and degassed. The flow rate was 1.2 mL/min. The column was maintained at ambient temperature and the elute was monitored at a wavelength of 231 nm. Volume injected was 20 μL and the mode of operation was isocratic.

**Standard preparation:** About 100 mg of racecadotril RS was weighed accurately and dissolved in 100 mL of the diluent. From this stock solution, dilutions were made to get concentration of 100, 200, 300, 400 and 500 μg/mL. These were used to determine the linearity by applying the above mentioned chromatographic conditions. From the data obtained, correlation coefficient, Y-intercept and slope were calculated to provide mathematical estimates of the degree of linearity. Results shown in Table-4.

TABLE-4
ANALYTICAL PERFORMANCE PARAMETERS OF RACECADOTRIL RS (HPLC)

| Parameters | |
|---|---|
| Linear dynamic range (μg/mL) | 100-500 |
| Correlation coefficient (r) | 0.9997 |
| Slope (b) | 8566.128 |
| Intercept (c) | 23341010 |

TABLE-5
ASSAY OF RACECADOTRIL BY HPLC

| Sample | *Percentage detected (% w/w) | Standard deviation (SD) | Relative standard deviation (% RSD) | Standard error of mean (SE) |
|---|---|---|---|---|
| Sample-A | 100.35 | 0.3015 | 0.30044 | 0.1230 |
| Sample-B | 100.34 | 0.3014 | 0.32272 | 0.1321 |

*Each value is an average of six readings.

**Assay:** The content of twenty capsules were accurately weighed. The powder equivalent to 50 mg was accurately weighed and transferred to a 50 mL volumetric flask. About 30 mL of methanol was added and kept in ultrasonic bath for 15 min. This solution was filtered through a membrane filter and the volume was made up to the mark to get the stock solution. From this suitable dilutions were made to obtain the concentration of 300 μg/mL. 20 μL of the sample solution was injected under the chromatographic conditions described above. Each solution was run 6 times at an interval of 15 min to ensure the complete elution of the previous one. The amount of drug present in capsule formulation was calculated by comparing the peak area ratio from the standard.

$$\text{Amount of drug content present in the sample} = \frac{\text{Sample peak area}}{\text{Standard peak area}} \times \frac{\text{Standard dilution}}{\text{Sample dilutions}} \times \text{A.Wt.}$$

A.Wt. = Average weight of content of one capsule.

**Recovery studies:** To ensure the reliability and accuracy of the method[5], recovery studies were carried out by mixing a known quantity of standard drug with the preanalysed sample formulation and the contents were reanalyzed by the proposed method.

**Stability study:** The standard and sample solution was injected after 24 h to study the stability and there was no appreciable change in the peak area of the standard and sample solution. Hence it indicates that both standard and sample is stable for 1 d on benchtop[5].

## RESULTS AND DISCUSSION

The UV-spectrum of racecadotril in ethanol shows absorption maxima at 231 nm. The linearity range of racecadotril was established by plotting various concentrations against absorbances at the $\lambda_{max}$. It was observed that racecadotril in the concentration range of 5-80 µg/mL were found to obey Beer-Lambert's law with the correlation coefficient (r) of 0.9993. The molar extinction coefficient was found to be 5116 which shows that the drug is highly absorbing. The limit of detection and limit of quantification was found to be 0.49 and 1.48 µg/mL. This indicates the sensitivity of the method. Subjecting the assay values to t-test ($p < 0.05$) shows no significant difference between assay value and the label claim. Hence this report relates to precision of adopted method. The accuracy of the proposed method was verified by recovery studies. The mean percentage recovery range was found to be 98.86 ± 1.1 % for both the samples.

TABLE-6
RECOVERY STUDIES FOR RACECADOTRIL (HPLC)

| Sample | Concentration of pre analyzed sample (µg/5mL) | Concentration of standard added (µg/5mL) | Amount calculated theoretically (µg/mL) | Amount detected practically (µg/mL) | Percentage recovery (% w/w) |
|---|---|---|---|---|---|
| Sample-A | 1501.3 | 500 | 200.13 | 198.62 | 99.24 |
| | | 1000 | 300.13 | 296.04 | 98.63 |
| | | 2000 | 400.13 | 395.40 | 98.81 |
| Sample-B | 1531.05 | 500 | 203.105 | 201.56 | 99.24 |
| | | 1000 | 253.105 | 250.55 | 98.99 |
| | | 2000 | 353.105 | 348.94 | 98.82 |

TABLE-7
COMPARISON OF THE TWO METHODS

| Method | Sample | Label claim (mg/cap) | *Amount found (mg/cap) | Percentage recovery (% w/w) | Relative standard deviation |
|---|---|---|---|---|---|
| UV Spectroscopy | Sample - A | 100 | 98.62 | 98.77 | 0.58449 |
| | Sample – B | | 98.61 | 98.69 | 0.58713 |
| RP-HPLC | Sample - A | 100 | 100.35 | 98.89 | 0.30044 |
| | Sample – B | | 100.34 | 99.01 | 0.32272 |

In the HPLC method, the proposed mobile phase comprising of methanol, water and acetonitrile in the ratio of 1:3:1 gave appreciable resolution and sensitivity. The detection was carried out by using UV-Visible detector

at 231 nm. The corresponding peak was identified at the retention time which was around 4.97 min. Linearity range was observed in the concentration range of 100-500 µg/mL with the correlation coefficient of 0.9997. The flow rate of 1.2 mL was the optimum with respect to location and resolution of analytical peaks. The symmetry factor or the tailing factor was found to be 1.07, which indicates symmetrical nature of the peak. The number of theoretical plates was found to be 6793, which indicates efficient performance of the column. The retention time of racecadotril was found to be within the limits of 0-9 min. The limit of detection and limit of quantification was found to be 0.00012 and 0.00036 µg/mL. This indicates the sensitivity of the method. Quantitative estimation of racecadotril in marketed dosage forms by HPLC method was carried out in both samples-A and B, and were found to be 100.32 ± 0.3 % w/w and 100.34 ± 0.3 % w/w, respectively. RSD value obtained below 1 indicates the precision of the method. The accuracy of the proposed method was verified by recovery studies. The percentage recovery range was found to be 98.76 ± 1.2 % for sample-A 98.68 ± 1.3 % for sample-B capsules.

To conclude, both the methods are suitable for evaluating racecadotril in raw material and also from dosage forms.

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