RESEARCH ARTICLE

Development and Validation of simple UV Spectrophotometric Method for the Determination of Racecodotril both in Bulk and Marketed Dosage Formulations

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ABSTRACT

Racecodotril (RCT), (N-[2-[(acetylthio) methyl]-1- oxo- 3- phenyl propyl] -glycine phenylmethyl ester) is an enkephalinase inhibitor. A rapid, specific, and economic UV spectrophotometric two methods have been developed using a solvent composed of methanol to determine the RCT content in bulk and pharmaceutical dosage formulations. Method A is the absorbance maxima method, in which λ_{max} is 231 nm, linearity was observed in the concentration range 10 to 100 µg/mL for all the two methods, and exhibited good correlation coefficient for method A ($r^2 = 0.9991$) and excellent mean recovery (98.22–102.77%). Method B is the area under curve (AUC), in which λ_{max} 226 to 236 nm was selected for estimation of RCT and exhibited a good correlation coefficient for method B ($r^2 = 0.9953$). The method was validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. The obtained results reveal that the method can be employed for the routine analysis of RCT in bulks, as well as, in the commercial formulations.

Keywords: Area under curve, Enkephalinase inhibitor, Racecodotril (RCT), UV-spectrophotometry.

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INTRODUCTION

The RCT, also known as acetorphan, is an antidiarrheal drug that acts as a peripherally acting enkephalinase inhibitor. The other opioid medications used to treat diarrhea, which reduces intestinal motility, RCT has an antisecretory effect and it reduces the secretion of water and electrolytes into the intestine.[1] Chemically, RCT, (N-[2-[(acetylthio) methyl]-1- oxo- 3- phenyl propyl] -glycine phenylmethyl ester) is an enkephalinase inhibitor. Enkephalins are peptides produced by the body that act on opioid receptors with a preference for the δ subtype.[2] Activation of δ receptors inhibits the enzyme adenylyl cyclase, increasing intracellular levels of the messenger molecule cAMP.[3] The active metabolite of RCT, thiorphan, inhibits enkephalinase enzymes in the intestinal epithelium with an IC_{50} of 6.1 nm, protecting enkephalins from being broken down by these enzymes.[4] Racecadotril itself is much less potent at 4,500 nm. These reduce diarrhea-related hypersecretion in the small intestine without influencing basal secretion. Racecadotril also has no influence on the time substances, bacteria, or virus particles stay in the intestine.[5] The drug is commercially available as a capsule for oral administration. In the present work, an attempt has been made to develop three rapid, precise, and accurate spectrophotometric methods for the estimation of RCT in bulk form. Method A is the absorbance maxima method, in which λ_{max} is 231 nm for RCT. Method B is the area under the curve, in which wavelength range 226 to 236 nm was selected for estimation of RCT. The results of the analysis have been validated statistically, which confirm the accuracy and reproducibility of the methods. All the methods were found to be simple, precise, and accurate and can be employed for routine quality control analysis of RCT in bulk, as well as, in its solid dosage form. In this study, efforts were made to develop a simple, easy, and economic UV spectrophotometric method using solvent composed of methanol for the determination of RCT in the raw materials, as well as, in the marketed dosage formulations. The developed method was optimized and validated as per the guidelines of the International Conference on Harmonization (ICH),[6] and demonstrated excellent specificity, linearity, precision, and accuracy for RCT.

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MATERIALS AND METHODS

Materials

UV-visible double beam spectrophotometer, JASCO (Model V-530), with a spectral bandwidth of 1.5 nm, wavelength accuracy of ± 0.3 nm, and a pair of 10 mm matched quartz cells was used. The samples were weighed on an electronic analytical balance (Contech Model CB-50). The commercially available capsule, Redotil (Label claim: RCT, I.P.-100 mg) was procured from the local market.

Selection of Common Solvent

After assessing the solubility of the drug in different solvents, methanol was used as a common solvent for developing spectral characteristics.

Preparation of Standard Stock Solution

The 100 mg of the pure drug was accurately weighed and dissolved in 75 mL methanol and the volume was made up to 100 mL with methanol to give a standard stock solution of 1,000 µg/mL. Aliquots of standard stock solution were pipette out and suitable dilutions were made with methanol to get standard solutions of concentration: 10, 20, 30, and up to 100 µg/mL. Working standard solutions of 10 µg/mL were scanned in the entire UV range of 400 to 200 nm to obtain the absorbance spectra and overlain spectra (Figure 1).

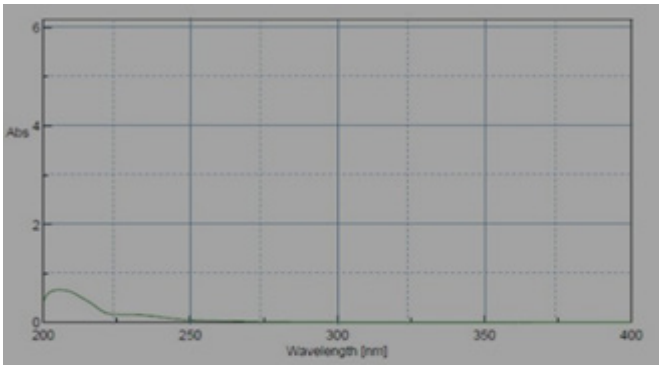

Figure 1: Standard solution of 10 µg/mL

Method A: Absorbance Maxima Method

The selections of analytical wavelength standard solutions of RCT were prepared and a series of dilutions of standard solutions of RCT were prepared by using methanol and were scanned from 400 to 200 nm. The spectra of drug obtained after scanning of the standard solution of RCT, absorbance were measured in the range of 226 to 236 nm was selected for the analysis, the calibration curve was plotted concentration *vs.* absorbance and the regression equation was calculated.

Method B: AUC Method

The selection of analytical wavelength, 10 µg/mL solution of RCT were prepared by appropriate dilution of the standard stock solution and scanned in the spectrum mode from 200 to 400 nm. The AUC method involves the calculation of the integrated value of absorbance with respect to the wavelength between two selected wavelengths 226 to 236 nm. The area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between AUC and concentration. From this, regression equation was calculated (Table 1).

Analysis of Marketed Formulation

Capsules were procured from the local market (Redotil by Reddy's Laboratories Ltd.) and the average weight was

Table 1: Calibration data of RCT (AUC)

Concentration (µg/mL)	*Area under curve (AUC)*	*Concentration (µg/mL)*	*Area under curve (AUC)*
10	1.6007	60	6.9295
20	2.49	70	7.3666
30	3.8466	80	8.5979
40	4.4009	90	9.2827
50	5.7787	100	10.4772

Table 2: Analysis of pure drug

S. No.	*Amount taken (µg/mL)*	*AUC*	*Amount of drug found (µg/mL)*	*% amount found*
1	50	5.7392	49.67	99.34
2	50	5.7582	49.6	99.2
3	50	5.7438	49.53	99.06
4	50	5.7628	49.55	99
5	50	5.7529	49.54	99
6	50	5.7617	49.56	99.12

Table 3: Statistical evaluation of pure drug

*% mean**	*±S.D.**	*% RSD**
99.12	0.05244	0.1057

determined. The powder equivalent to 100 mg of RCT was weighed accurately and dissolved in 100 mL of methanol and filtered using Whatman filter paper. The filtrate was appropriately diluted with methanol to give a standard stock solution of 1,000 µg/mL. Further dilutions were made using methanol to give 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/mL. The area in the range of 226 to 236 nm was measured (Table 2-4).

Validation of Method

The developed method was validated as per ICH guidelines.

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of RCT. Beer's law was obeyed in the concentration range 10 to 100 µg/mL. The correlation coefficient was found to be 0.999 (Table 5).

Precision

100 mg of RCT was weighed accurately and dissolved in 100 mL of methanol to give a concentration of 1,000 µg/mL. From the standard stock solution, an appropriate quantity of solution was taken further dilutions were made with methanol to give 50 µg/mL. AUC was measured in the range of 226 to 236 nm. This procedure was carried out six times (Table 6).

Accuracy

Accuracy studies were carried out by standard addition method. The standard sample of RCT was added at different levels, i.e., 80, 100, and 120% to drug sample present in capsule dosage form (100 mg RCT in each capsule).

Recovery Studies

To capsule stock solution in three different volumetric flasks, aliquots of 8, 10, and 12 mL of the standard stock solution were added, volume was made up to 10 mL with methanol to give a concentration of 18 µg/mL (80%), 20 µg/mL (100%) and 22 µg/mL (120%). AUC was measured in the range of 226 to 236 nm. The procedure was repeated three times for 80, 100, and 120% for recovery (Table 7-9).

RESULTS AND DISCUSSION

The methanol was selected as the solvent for RCT because it provides the highest solubility and AUC measurements. Figure 2 shows the absorption spectrum of RCT (10 µg / mL) in methanol for the method. The intra-day and inter-day precision values (% RSD) were calculated (Table 6)

Table 4: Statistical evaluation of marketed formulation

*% mean**	*±S.D.**	*% RSD**
99.72	0.1735	0.3479

Table 5: Parameters from calibration curve

Parameters	*Observations*
Calibration curve	Linear
Expression	Abs = A + B × conc. or y = mx + c
Factor	A = 0.7024
Factor	B = 0.0977
Coefficient (r^2)	0.9953

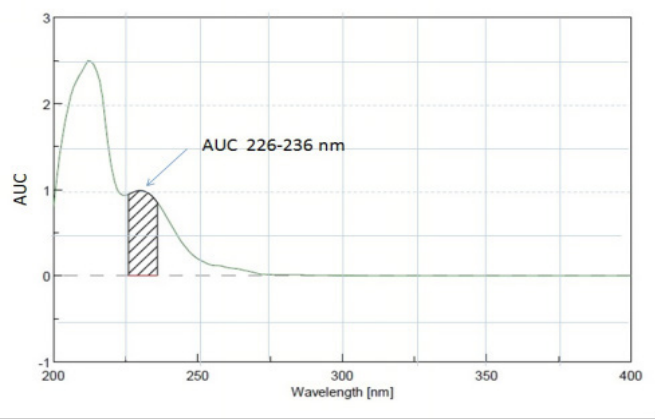

Figure 2: Spectrum of RCT for AUC concentration 10µg/mL

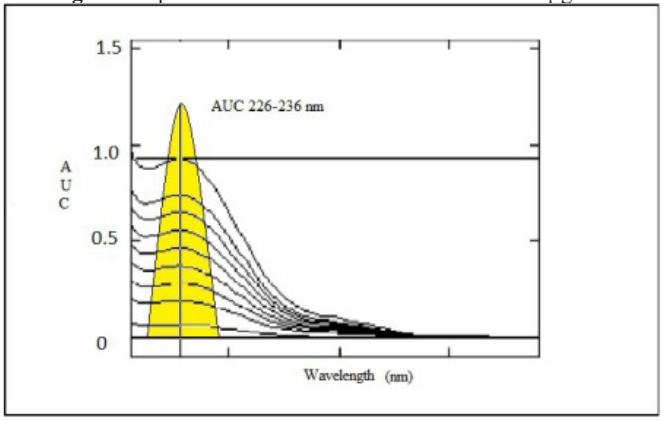

Figure 3: Overlay spectra of RCT for AUC *(*10–100 *µ*g/mL)

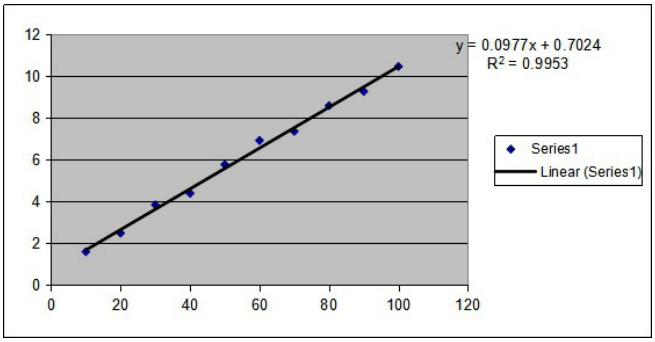

Figure 4: Calibration curve of RCT for AUC

Table 6: Statistical evaluation of inter-day and intra-day precision studies

Parameter	*% mean**	*±S.D.**	*% RSD**
Interday	99.5	0.08571	0.1722
Intraday	99.49	0.08571	0.1722

Table 7: Statistical evaluation of recovery studies

% recovery	*% mean recovery**	*±S.D.*	*% RSD*
80	102.66	0.0251	0.136
100	102.3	0.01	0.0488
120	99.1	0.1677	0.769

Table 8: Results of marketed formulation analysis for RCT

Method	*% mean**	*±S.D.**	*% RSD**
Area under curve	99.72	0.1735	0.3479

Table 9: Results of recovery studies

Method	*% recovery ± S.D.*[*]		
	80%	*100%*	*120%*
Area under curve	102.66 ± 0.02516	102.3 ± 0.01	99.1 ± 0.1677

Table 10: Data of RCT

Parameters	*Method (area under curve)*
λ_{max} (nm)	226–236
Beer's law limit (µg/mL)	10–100
Regression Equation:	
Slope	0.0977
Intercept	0.7024
Regression coefficient (r^2)	0.9953
Precision	
Inter-day	99.5
Intra-day	99.49
Recovery	
80%	102.66%
100%	102.3%
120%	99.1%

for RCT. The accuracy of RCT which was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% was found to be in the acceptable limits ≤ acceptable limits (2%) (Table 9). This indicates that there was no ≤ interference from the excipients present in the dosage form. The results were evaluated by calculating the % RSD value and lying within the range (Figure 3 and 4).

CONCLUSION

The proposed methods were found to be rapid, simple, economical, precise, reproducible, and accurate for the determination of RCT in bulk and solid dosage form. Thus, it can be easily and conveniently adopted for routine quality control analysis.

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