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Reliable Quantification of Rebamipide: Validated UV-Spectrophotometry Approach for Bulk and Pharmaceutical Formulations

Ujjwal Nautiyal, Rahul Pandey, Abhishek Chandola*

Himalayan School of Pharmaceutical Sciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, 248140, India.

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Abstract

A novel ultraviolet (UV) method, distinguished by its rapidity, sensitivity, simplicity, and cost-effectiveness, was prepared for the quantification of Rebamipide in both scaleup and pharmaceutical formulations. The absorbance of Rebamipide was measured relatively in ethanol at a new wavelength (λmax 325). The method demonstrated a linearity range spanning from 5 to 60 µg/ml, with a regression equation represented as relative absorbance 0.016x concentration in µg/ml + 0.1621, and a regression coefficient of 0.998. Validation procedures were conducted per International Council for Harmonisation (ICH) and United States Pharmacopeia (USP) specifications, affirming the method's accuracy, precision, and reproducibility (relative standard deviation <2.0%). This criterion is effective for the estimation of Rebamipide across various dosage forms, yielding results in alignment with label claims. Its validation provides a prompt, cost-effective, and accurate approach to assess Rebamipide potency and consistency in pharmaceutical products, thereby enhancing healthcare standards and ensuring patient safety.

Keywords: Rebamipide, ethanol, Pharmacopeia, UV-radiation, Pharmacological.

INTRODUCTION

Rebamipide, a derivative of the quinolone class, is renowned for its multifaceted therapeutic attributes, particularly its profound anti-ulcer and anti-inflammatory effects. This pharmacological agent exerts its beneficial actions through various mechanisms. Notably, Rebamipide acts by stimulating the synthesis of cyclooxygenase 2 (COX2), thereby instigating an augmented production of endogenous prostaglandins within the gastric mucosa, which plays a pivotal role in mucosal protection and repair processes. Additionally, Rebamipide exhibits a key mediator of inflammation induced by H. pylori infection, thereby mitigating inflammation within the gastric mucosa. Furthermore, Rebamipide showcases its therapeutic prowess by scavenging oxygen-derived free radicals, thereby conferring protection against mucosal injury, a hallmark of various gastrointestinal disorders. Moreover, Rebamipide orchestrates an intricate interplay by upregulating the expression of prostaglandin EP4 receptor genes, consequently promoting mucous secretion and fortifying the gastric mucosal defense mechanisms. After the revelation of Rebamipide's pharmacological capability in augmenting gastric mucus (mucin), further exploration into its effects on ocular surface mucin led to its utilization in developing a therapeutic strategy for dry eye syndrome. Moreover, nonclinical studies on rabbits demonstrated Rebamipide's efficacy in enhancing corneal and conjunctival mucin levels, along with improvements in conjunctival goblet cells. The compound's logP and pKa values were reported as 2.9 and 3.3, respectively In the clinical realm, Rebamipide's potential to modulate immuno-inflammatory responses in individuals afflicted with H. pylori infection holds promise in impeding the onset and recurrence of peptic ulcer disease. Furthermore, Rebamipide emerges as a potential adjunct to standard eradication therapy for H. pylori infection, potentially bolstering the efficacy of this treatment regimen.

METHOD DEVELOPMENT FOR ESTIMATION OF REBAMIPIDE AS API

Creating a Standard Stock Solution

A standard stock solution of Rebamipide was prepared as follows:

- 50 mg of the drug was dissolved in a 50 ml volumetric flask with 30 ml of ethanol.

- The volume was adjusted to the mark with ethanol.

- From this solution, 5 ml was transferred to another 50 ml volumetric flask.
- The solution was then diluted to the mark with ethanol.
- As a result, the resulting standard solution contained 100 µg of the drug per ml.

Selection of wavelength Maxima (λmax)

To make a 10 µg/ml solution, 1 ml of the working standard solution was transferred into a 10 ml volumetric flask, and the volume was adjusted to the mark with the solvent. Afterward, the resulting solution was scanned in a UV-Spectrophotometer from 200 to 400 nm using ethanol as a blank. The wavelength maxima were identified at 325 nm, as depicted in Figure 1.

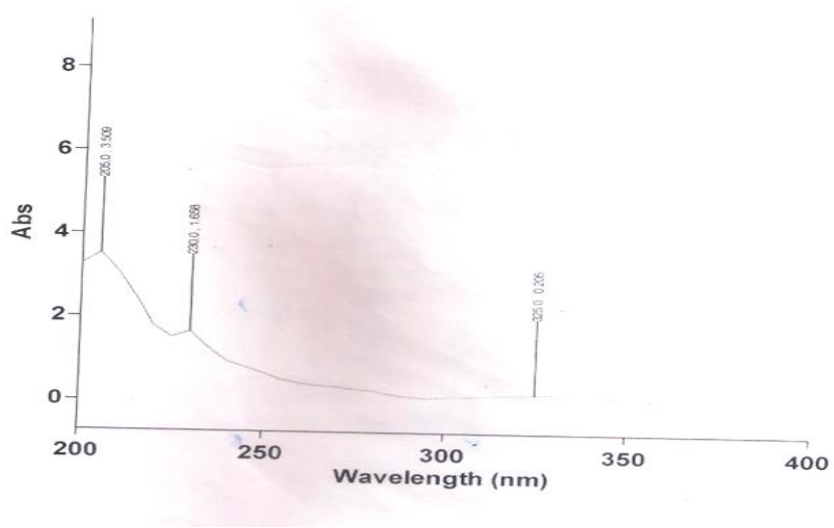

Figure 1. Wavelength maxima of Rebamipiode in ethanol

Preparation of Calibration curve

To construct a comprehensive calibration curve, measured quantities of 0.5, 1, 2, 3, 4, 5, and 6 milliliters of the working standard solution were meticulously dispensed into seven distinct 10 milliliter volumetric flasks. Subsequently, each individual flask was meticulously supplemented with ethanol until reaching the calibrated mark, thus yielding concentrations of 5, 10, 20, 30, 40, 50, and 60 micrograms per milliliter, separately. Following this precise preparation, the absorbance of each resultant solution was meticulously assessed at a

wavelength of 325 nanometers (nm), with ethanol serving as the reference blank solution. Subsequently, a graphical representation was meticulously charted, correlating the meticulously measured concentrations to their respective absorbance values. It is noteworthy to highlight that throughout the meticulous investigation spanning the range of 5 - 60 µg/ml, the drug's response demonstrated remarkable linearity. The culmination of this meticulous experimentation yielded a meticulous calibration equation, represented as y = 0.016x + 0.1621, showcasing an exemplary correlation coefficient of 0.998, meticulously detailed in Table 1.

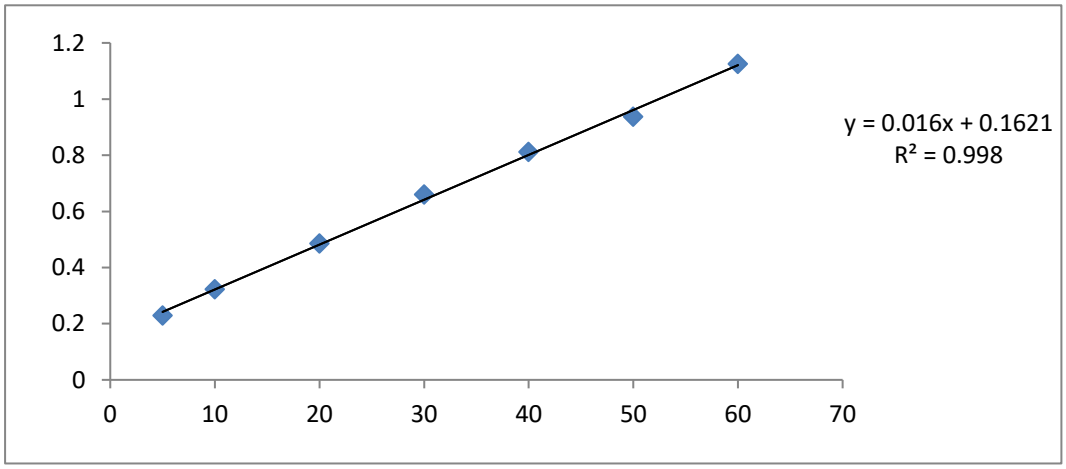

Figure 2. Calibration curve of Rebamipide in ethanol

Table 1. Concentration and absorptivity measurement

S.No.	Concentration (µg/ml)	Absorbance at 325nm	$E^{1\%}_{1\ CM}$	Absorptivity	Molar Absorptivity
1	5	0.2290	458	45.8	16981.9988
2	10	0.3229	322.9	32.29	11972.6799
3	20	0.4851	242.55	24.255	8986.1379
4	30	0.6603	220.1	22.01	8160.999
5	40	0.8117	202.925	20.2925	7524.174905
6	50	0.9364	187.28	18.728	6944.0802
7	60	1.1248	187.46666	18.746	6950.7543

		Mean	260.1745	26.01745	29947820.001

Repeatability

To create a series of solutions at concentrations of 10, 20, and 30 µg/ml, volumes of 1, 2, and 3 ml from the standard solution were accurately pipetted into nine individual 10 ml volumetric flasks. Each flask was then filled to the mark with ethanol. After the precise preparation, the absorbance of each solution was measured at 325 nm, using ethanol as the reference blank. The collected data was diligently compiled and presented in Table 2.

Table 2. Absorbance Study of Repeatability

Concentration (µg/ml)	Absorbance	Observed Concentration (µg/ml)	Mean Concentration (µg/ml)	SD	RSD
10	0.3221 0.3193 0.3195	9.9	0.3203	0.00156	0.48768
20	0.4920 0.4919 0.4964	20.1	0.4934	0.00257	0.52078
30	0.6382 0.6380 0.6387	29.8	0.6383	0.00036	0.05649

Intra-Day Precision

Three revisions were conducted within a single day at 3-hour intervals to ensure accuracy and reproducibility. In each revision, aliquots of 1, 2, and 3 ml from the working solution were individually pipetted into separate 10 ml volumetric flasks. Subsequently, the volume of each flask was adjusted to 10 ml with ethanol, resulting in concentrations of 10, 20, and 30 µg/ml, respectively. Following the meticulous preparation, the absorbance of each resultant solution was measured at 325 nm, with ethanol as the blank reference. The results obtained from these three revisions were diligently summarized in Table 3.

Table 3. Study of Intra Day Precision

Conc.(µg/ml)	Absorbance			Observe absorbance			Mean	SD	RSD
	0hrs	3hrs	6hrs	0hrs	3hrs	6hrs			
10	0.3315 0.3408 0.3263	0.3367 0.3379 0.3431	0.3390 0.3392 0.3389	9.8 10 10	10 10 10	10.3 10.3 10.3	0.3370	0.00363	1.0849
20	0.4920 0.4991 0.4964	0.4991 0.4964 0.4920	0.4965 0.4920 0.4991	19.9 19.9 20	20.1 20.1 20	20 20 20	0.4934	0.00257	0.52078
30	0.6448 0.6442 0.6441	0.6449 0.6449 0.6454	0.6380 0.6382 0.6386	29.9 30.2 30.2	30.2 30.2 30.2	29.8 29.8 29.8	0.6426	0.00033	0.05045
								Mean	0.5520

Inter-Day Precision

Over three consecutive days, studies were conducted at regular intervals. Each day, aliquots of 1, 2, and 3 ml from the working solution were transferred into separate 10 ml volumetric flasks. Subsequently, the volume of each flask was adjusted to 10 ml by diluting with ethanol, resulting in solutions with concentrations of 10, 20, and 30 µg/ml, respectively. Following the meticulous preparation, the absorbance of each resultant solution was measured at 325 nm, with ethanol serving as the blank reference. The results obtained from these three studies, conducted over three days, were diligently summarized in Table 4.

Table 4. Observatory analysis of Inter-Day Precision

Concentration (µg/ml)	Absorbance			Observed absorbance			Mean	SD	RSD
	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs			
10	0.3390 0.3392 0.3389	0.3392 0.3389 0.3390	0.3390 0.3389 0.3392	9.9	10	9.8	0.3390	0.00015	0.04505
20	0.5013 0.5008 0.5010	0.5009 0.5011 0.5012	0.5010 0.5013 05011	19.9	20	19.8	0.5010	0.00025	0.05022

30	0.6447 0.6442 0.6449	0.6445 0.6449 0.6447	0.6446 0.6448 0.6449	29.9	30	30.2	0.6446	0.00036	0.05593
								Mean	0.0504

Accuracy

To prepare solutions with concentrations of 12, 15, and 18 µg/ml, nine transfers of 1.5 ml from the standard solution were made into separate 10 ml volumetric flasks. Subsequently, three of these flasks were supplemented with 1.2 ml of the working solution and diluted to 10 ml with ethanol to achieve 12 µg/ml solutions. Another three flasks were supplemented with 1.5 ml of the working solution and diluted to 10 ml to obtain 15 µg/ml solutions. Lastly, the remaining three flasks were supplemented with 1.8 ml of the working solution and diluted to 10 ml to yield 18 µg/ml solutions. The absorbance of each resultant solution was then measured at 325 nm, with ethanol as the blank reference. The obtained results were meticulously summarized in Table 6.

Table 6. Accuracy observation of the preparation

Recovery at	Nominal Conc.(µg/ml)	Absorbance	Observed Conc.(µg/ml)	% Recovery
90%	27=15+12	0.5709	26.8	99.25%
90%	27=15+12	0.5706	26.6	98.51%
90%	27=15+12	0.5702	26.9	99.62%
100%	30=15+15	0.6403	29.9	99.66%
100%	30=15+15	0.6391	29.8	99.33%
100%	30=15+15	0.6385	29.8	99.33%
120%	33=15+18	0.6923	33.2	100.60%
120%	33=15+18	0.6929	33.2	100.60%
120%	33=15+18	0.6918	33.2	100.60%
			Mean	99.72(+_)0.70

Specificity

A study on specificity was carried out to evaluate potential interference in the absorbance of the drug in the presence of common excipients like starch, talc, lactose, and magnesium stearate. Solutions containing 10 µg/ml of the drug were prepared both with and without the

excipients, and their absorbance was measured at 325 nm, with ethanol serving as the blank reference. The results obtained were meticulously tabulated and summarized in Table 6.

Table 6. Investigation of Specificity

Nominal Conc.(µg/ml)	Without Excipient		With Excipients		%Interference
	Absorbance	Observed Concentration	Absorbance	Observed concentration	
10	0.3272	10.3	0.3365	10.4	1.0097
10	0.3203	9.9	0.3372	10.4	1.05
10	0.3241	10.1	0.3135	10	1.01
10	0.3252	10.2	0.3298	10.3	1.0098
10	0.3250	10.2	0.3364	10.4	1.019
10	0.3247	10.2	0.3392	10.5	0.971
				Mean	0.8659

Estimation of Rebamipide in pharmaceutical dosage form (Rebagen, 100mg)

The tablets were initially weighed to determine their average weight. Subsequently, they were powdered, and a precise quantity containing approximately 50 mg of Rebamipide was accurately weighed. This powdered sample was then transferred into a 50 ml volumetric flask, followed by the addition of 30 ml of ethanol. The flask was subjected to sonication for 10 minutes to ensure thorough dissolution, after which the volume was maintained to 50 ml with the solvent. The solution was then mixed and filtered to obtain a clear filtrate. A 5 ml aliquot of this filtrate was transferred into a 50 ml volumetric flask and diluted with ethanol to reach the mark. Moreover, 1 ml of the resultant solution underwent further dilution to 10 ml with ethanol. Subsequently, the absorbance of this final solution was measured at 325 nm using a spectrophotometer. This entire procedure was repeated three times to ensure consistency, and the obtained results were meticulously summarized in Table 7.

Table 7. %Assay of Rebamipide in pharmaceutical dosage form (Rebagen, 100 mg)

S.No.	Absorbance	Concentration (µg/ml)	Dilution Factor	Weight Taken (mg)	Average Weight (mg)	Label Claim (mg)	% Assay
1	0.3154	9.8	5000	122.75	245.5	100	98%

2	0.3185	9.9	5000	122.75	245.5	100	99%
3	0.3195	9.9	5000	122.75	245.5	100	99%
4	0.3241	10.1	5000	122.75	245.5	100	101
						Mean	99.25%

RESULTS AND DISCUSSION

The extensively validated method showcases robust performance across a spectrum of parameters including linearity, accuracy, repeatability, specificity, and precision. Standard solutions spanning a concentration range of approximately 5-60 µg/ml were meticulously employed for UV analysis, as comprehensively detailed in Tables 1 through 6. The method notably exhibited exceptional linearity with a staggering correlation coefficient of 0.998, vividly illustrated in the graph represented in Figure 2, alongside a meticulously derived regression equation of y = 0.016X + 0.1621. Remarkably, this demonstrated reliable linearity within the concentration spectrum spanning from 2 to 24 µg/ml. Accurate quantification was unequivocally confirmed by mean recoveries boasting an impressive 99.25%, distinctly establishing the method's unparalleled accuracy, as extensively demonstrated and meticulously outlined in Table 6. The precision of the method, meticulously assessed through rigorous evaluations encompassing repeatability, intraday, and inter-day variations, unequivocally demonstrated relative standard deviations (RSD) meticulously below the stringent threshold of 2%. The proposed method efficaciously and comprehensively assessed the potency of rebamipide dosage forms, ensuring unwavering compliance with standard criteria across the gamut of tested brand products. The pivotal aspect of specificity, crucial for discerning rebamipide amidst formulation excipients, was unequivocally upheld. Noteworthy is the meticulous determination of the percent assay of rebamipide in the marketed product (Rebagen), a resounding 99.25%, meticulously elucidated and meticulously detailed in the expansive array of data encapsulated within Table 7.

CONCLUSION

In conclusion, the preparation and validatory evaluation of an analytical procedure for the estimation of Rebamipide using UV-spectrophotometry in both bulk and pharmaceutical dosage forms represent a significant achievement in pharmaceutical analysis. Through

meticulous validation procedures, including specificity, linearity, accuracy, precision, and robustness, this method demonstrates its reliability and applicability for quality control and assurance purposes in preparatory industries. Its successful application in determining Rebamipide content in various formulations underscores its practical utility, offering a rapid, cost-effective, and accurate solution for ensuring the potency and consistency of Rebamipide-containing products, thus contributing to the enhancement of overall healthcare standards and patient safety.

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