WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 13, 1412-1419. Research Article ISSN 2277–7105

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF REBAMIPIDE IN BULK AND TABLET DOSAGE FORM

Bindhyashree K. M.*, Naveen Kumar G. S. and Sowmya H. G.

Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Bharathinagara, K M Doddi, Maddur Taluk, Mandya District, Karnataka, India-571422.

Article Received on
19 May 2024,

Revised on 09 June 2024,
Accepted on 30 June 2024

DOI: 10.20959/wjpr202413-33093

***Corresponding Author**
Bindhyashree K. M.
Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Bharathinagara, K M Doddi, Maddur Taluk, Mandya District, Karnataka, India-571422.

ABSTRACT

Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the estimation of Rebamipide in bulk and pharmaceutical dosage form. The drug shows maximum absorption (λ_{max}) at 235nm in 0.1N NaOH solution and Area under Curve [AUC] in absorption spectra were measured between the wavelength range 230 to 240nm which obeys Beer's law in the concentration range of 1-6µg/ml. The linearity study carried and regression coefficient was found to be 0.9992 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 98.5-100.2. The LOD and LOQ were found to be 0.06 and 0.18µg/ml. The % relative standard deviation were found less than 2. According to ICH guidelines the method has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for reliable quantification of Rebamipide in bulk form and pharmaceutical dosage form.

KEYWORDS: Rebamipide, Area under curve spectroscopy, validation, pharmaceutical formulations.

INTRODUCTION

Rebamipide is used for the treatment of stomach ulcers as an anti-ulcer drug. It is known that the anti-ulcer activity of Rebamipide is due to its enhancement of the protective gastric

mucosal lining by increasing the concentration of prostaglandin E2 in the gastric mucosa and reducing free radical production. Both of which lead to improved gastric ulcer healing. Rebamipide as a mucosal protective drug, can not only increase the production of endogenous prostaglandins but also has the cytoprotective antiulcer effects.[1] Rebamipide is also used as an ophthalmic solution to treat patients suffering with dry eyes.[2] It is classified as a class IV drug according to the biopharmaceutical classification systems (BCS).[3]

Chemically Rebamipide is 2- [(4- chlorobenzoyl) amino]-3-(2-oxo-1H-quinolin-4-yl) propanoic acid]. Rebamipide is a white powder available in amorphous solid form and it is soluble in organic solvents such as Dimethyl sulfoxide, NAOH, slightly soluble in water. Its molecular formula is $C_{19}H_{15}N_2O_4$ and its molecular weight is 370.786g/mol and having melting point in the range of 288 - 290˚C

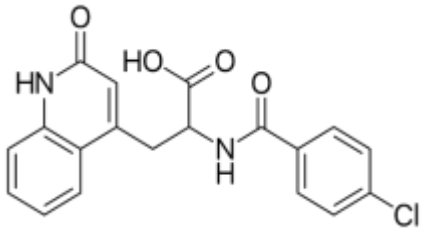

Fig.1: Chemical structure of Rebamipide.

Literature survey revealed that there were few analytical methods have been reported for the determination of rebamipide in pure drug and pharmaceutical dosage forms by using UV[4-6], HPLC[7-10], and HPTLC[11-12] so far. The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Area under curve UV Spectrophotometric method for estimation of Rebamipide in bulk and tablet dosage form.

MATERIALS AND METHODS

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals: Rebamipide pure drug was obtained as a gift sample from Medreich Pharmaceuticals, Bengaluru and its pharmaceutical dosage Rebamipide 20 tablets (Rebagen-100) labelled claim 100mg from local pharmacy manufactured by Macleods pharmaceuticals Ltd.

Solvent: 0.1N NaOH is used as a solvent.

Selection of analytical wavelength: Appropriate dilutions of Rebamipide were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. Area under Curve [AUC] in absorption spectra were measured between the wavelength range of 230 to 240nm as the wavelength for detection (Fig-2).

Preparation of standard stock solution: 100mg of Rebamipide was weighed accurately and transferred into 100ml volumetric flask and diluted in 0.1N NaOH up to mark. From this, the solution was further diluted into 100µg/ml and pipetted 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6ml into 10ml individual volumetric flask and diluted in 0.1N NaOH up to mark, this gives 1, 2, 3, 4, 5, and 6µg/ml concentration.

Preparation of sample solution: 20 tablets of Rebamipide marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Rebamipide was transferred into a 100ml of volumetric flask then it was diluted with 0.1N NaOH and made up to the mark.

METHOD AND VALIDATION

The method was validated according to ICH guidelines.[13-15]

RESULTS AND DISCUSSION

METHOD: AREA UNDER CURVE SPECTROSCOPY

Linearity: The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 1-6µg/ml and Area under Curve [AUC] in absorption spectra were measured between the wavelength of 230 to 240nm as absorbance values are shown in table-1 (Fig-3). The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in (Fig-4). Statistical parameter like slope, intercept, regression equation, correlation coefficient and Sandell's sensitivity were determined. (table-2).

Precision: The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision was determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. (table-3).

Accuracy: The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 50%, 100% and 150%. In which the formulation concentration kept constant and varied pure drug concentration. (table-4).

Ruggedness: The ruggedness is defined as the reproducibility of results when the method is performed under the variation in conditions. This includes different analyst, laboratories, instruments, temperature etc. Ruggedness was determined between different analyst, the value of %RSD was found to be less than 2.(table-5)

LOD and LOQ: The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated using formula.

$$LOD = 3.3(SD)/S \text{ and } LOQ = 10(SD)/S$$

LOD and LOQ value of Rebamipide were found be 0.06 and 0.18µg/ml.

Table 1: Results of calibration curve at 230-240nm by Area under curve method.

SL NO	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	1	0.134±0.0020
3	2	0.263±0.0018
4	3	0.403±0.0016
5	4	0.530±0.0018
6	5	0.645±0.0075
7	6	0.802±0.0023

**Average of six determinations.*

Table 2: Regression parameter for Rebamipide at 230-240nm by Area under curve method.

Regression parameter	Results
Range(µg/ml)	1-6
λ_{max}(nm)	230-240
Regression Equation	Y= 0.132X+0.0008
Slope(b)	0.132
Intercept(a)	0.0008
Correlation coefficient(r^2)	0.9992
Sandell's equation	0.00744

Limit of detection(µg/ml)	0.06
Limit of quantitation(µg/ml)	0.18

Table 3: Determination of precision results for Rebamipide at 230-240nm by Area under curve method.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
1	0.134±0.0020	1.40	0.132±0.0013	0.98
2	0.263±0.0018	0.68	0.261±0.0012	0.45
3	0.403±0.0016	0.39	0.403±0.0018	0.44
4	0.530±0.0018	0.33	0.530±0.0016	0.30
5	0.645±0.0075	1.16	0.643±0.0025	0.38
6	0.802±0.0023	0.28	0.804±0.0019	0.23

**Average of six determinations, **percentage relative standard deviation.*

Table 4: Determination of Accuracy results for Rebamipide at 230-240nm by Area under curve method.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
50	3	1.5	4.42	100.2	0.17
100	3	3	5.86	98.5	0.45
150	3	4.5	7.39	100.1	0.08

***Average of six determinations, **percentage relative standard deviation.*

Table 5: Determination of Ruggedness results for Rebamipide at 230-240nm by Area under curve method.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.403	0.403
±Standard deviation*	0.0016	0.0018
%RSD	0.397	0.446

**Average of six determinations, **percentage relative standard deviation.*

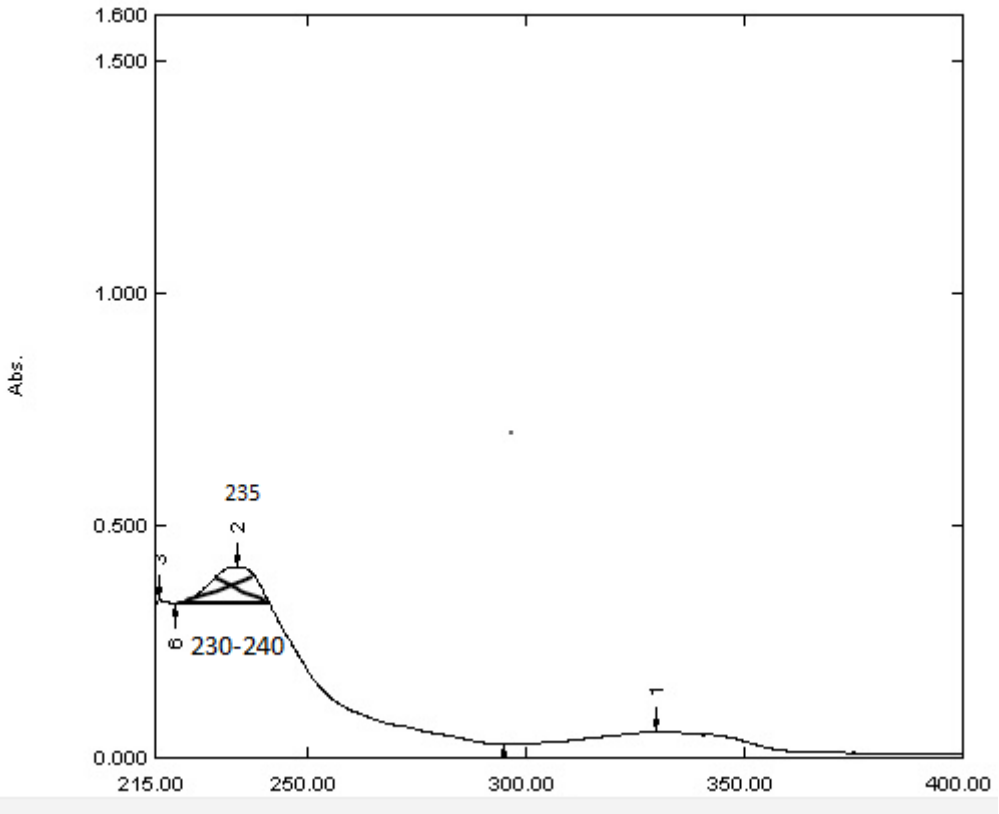

Fig.2: Area under curve spectrum of Rebamipide at 230-240nm.

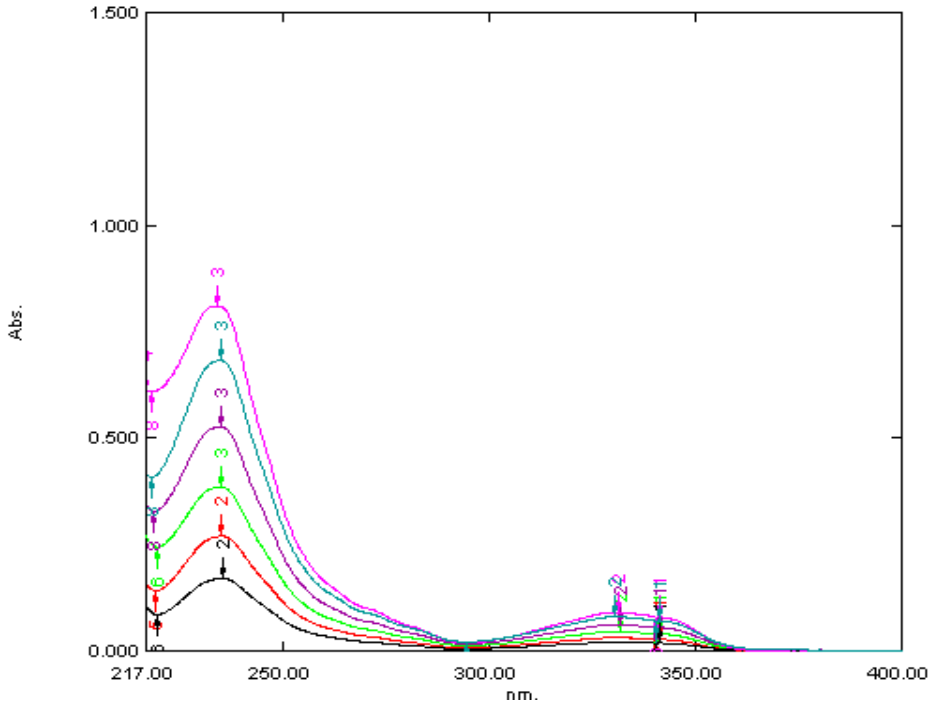

Fig.3: Area under curve overlain spectra of Rebamipide showing absorbance at 230-240nm.

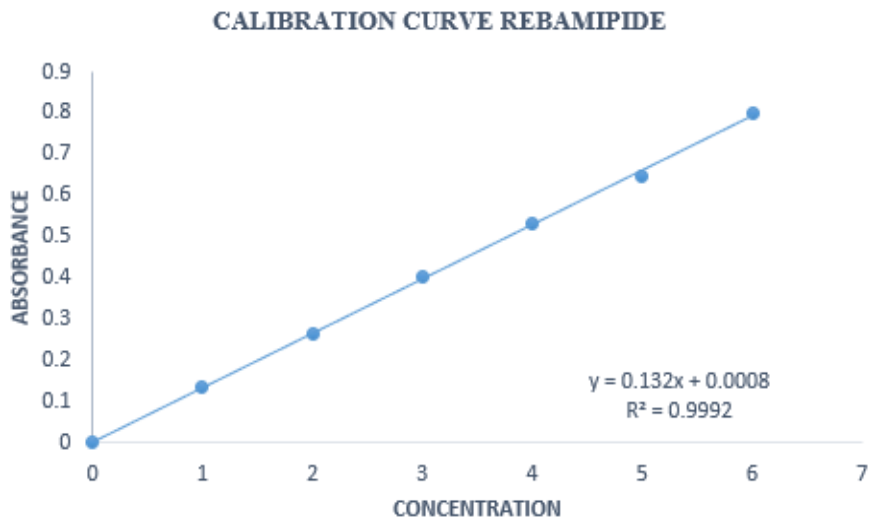

Fig.4: Calibration curve of Rebamipide at 230-240nm by Area under curve method.

CONCLUSION

As per ICH guidelines, the present analytical was carried and met the acceptance criteria. It was concluded that the developed analytical method was simple, specific, accurate, economical and sensitive and can be used for routine analysis of Rebamipide in bulk drug and in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

We authors wish to gratitude to our management, principal of Bharathi college of pharmacy for providing all facilities and also, we extend our thanks to all.

REFERENCES

1. Liu J, Xiong Z, Geng X, Cui M, Adinortey MB. Rebamipide with proton pump inhibitors (PPIs) versus PPIs alone for the treatment of endoscopic submucosal dissection-induced ulcers: a meta-analysis. BioMed Research International, Sep. 28, 2020; 2020: 1-0.
2. Shrivastava, S., Patkar, P., Ramakrishnan, R., Kanhere, M., & Riaz, Z. Efficacy of rebamipide 2% opthtalmic solution in the treatment of eyes. Oman J Ophthalmol, Sep-Dec., 2018; 11(3): 207-212.
3. Nagai N, Ishii M, Seiriki R, Ogata F, Otake H, Nakazawa Y, Okamoto N, Kanai K, Kawasaki N. Novel sustained-release drug delivery system for dry eye therapy by rebamipide nanoparticles. Pharmaceutics, Feb. 14, 2020; 12(2): 155.
4. Srivastava PK, Roy A. Development and validation of a new UV method for the analysis of rebamipide. Int J Pharmtech Res., 2011; 3(3): 1270-4.

5. Khaggeswar B, Reddy R, Bharadwaj R. New validation, UV Spectrophotometric method for the estimation of rebamipide and tramadol in bulk and dosage form. Der Pharmacia Letter., 2011; 3(1): 298-306.
6. Alqarni MA, Moatamed RS, Naguib IA, El Ghobashy MR, Farid NF. Development and validation of four spectrophotometric methods for assay of rebamipide and its impurity: application to tablet dosage form. J of AOAC Int., Jan.1, 2022; 105(1): 299-308.
7. Manglani UR, Khan IJ, Soni K, Loya P, Saraf MN. Development and validation of HPLC-UV method for the estimation of rebamipide in human plasma. Ind J of pharm sci., Jul. 1, 2006; 68(4): 475-478.
8. Jeoung MK, Kim CS, Kim NH, Hong JT, Chung YB, Park Y, Kim KS, Moon DC. Determination of rebamipide in human plasma by HPLC. J of Liq Chromatogr and Rel Tech., Jan. 1, 2004; 27(12): 1925-35.
9. Son DC, Thuong N, Park ES, Chi SC. High performance liquid chromatographic analysis of rebamipide in human plasma. Anal Lett., Apr.1, 2005; 38(6): 997-1005.
10. Sonawane S, Gide P. Optimization of forced degradation using experimental design and development of a stability-indicating liquid chromatographic assay method for rebamipide in bulk and tablet dosage form. Sci Pharm., Mar. 2011; 79(1): 85-96.
11. Patel p, poshiya p, chauhan g. Development and validation of high-performance thin layer chromatographic method for determination of rebamipide from its tablet dosage form. Int J Pharm Pharm Sci., Jan. 2014; 6(5): 606-610.
12. Shirkhedkar AA, Dhumal DM, Surana SJ. A sensitive and specific high-performance thin- layer chromatography—densitometry method for determination of rebamipide in the bulk material and in pharmaceutical formulation. J Planar Chromat Modern TLC., Aug. 2012; 25: 368-73.
13. ICH, Q2A text on validation of analytical procedures; 1994. https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf
14. ICH, Q2B validation of analytical methodology, 1996.
15. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q2b-validation-analytical- procedures-methodology
16. ICH, Q2 (R1) validation of analytical procedures: text and methodology, 2005. https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf