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Development and Validation of a New Uv Method for the Analysis of Rebamipide

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Abstract: A new, rapid sensitive, simple and cost effective UV method was developed for the estimation of Rebamipide in bulk as well as in pharmaceutical formulations. The relative absorbance of Rebamipide was measured in phosphate buffer (pH 7.4) at new wave length (λ_{max} 227). The linearity range was found to be 2.5-12.5 µg/ml with regression, relative absorbance =0.1061X concentration in µg/ml+0.0009 with regression coefficient 0.9997. The method was tested and validated for various parameters as per ICH and USP specification. The detection and quantification limit was found to be 0.73µg/ml and 2.21 µg/ml respectively. The result demonstrates that the developed procedure is accurate, precise and reproducible (relative standard deviation <2.0%). Proposed method is applicable for the estimation of Rebamipide in different dosage forms and results are in good agreement with label claim.
Key words: Rebamipide, Phosphate buffer (pH 7.4), λ_{max} 227 nm, U V method.

INTRODUCTION:

Rebamipide {2-(4-chlorobenzolylamino)-3-[2(1H)-quinolinon-4-yl] propionic acid} (BCS Class IV drug) is an anti ulcer drug used for the treatment of gastric ulcer. It is also an excellent drug for the treatment of dry eye. Its antiulcer activity has been reported as to increase gastric mucosal blood flow and prostaglandin E2 synthesis and secretion of gastric mucus. Oxygen free radicals can be removed to promote inflammation and peptic ulcer healing improvement.

Rebamipide belongs to BCS class IV drug. Along with it's poor water solubility it also have poor solubility in most of organic solvents, but it have pH dependent solubility. Because of it's poor solubility profile there is no UV method to estimate the Rebamipide in there routine analysis and from their formulation. Available literature states only HPLC method of estimation of Rebamipide at 280nm [1-3]. Though HPLC method is highly sensitive and accurate but it is time consuming (Processing time) and demands lot of expertise with higher cost. Thus there is need to develop simple rapid and cost effective method for routine analysis.

The objective of present study was to develop simple, sensitive, accurate rapid and cost effective method for estimation of Rebamipide. Analytical method was developed in phosphate buffer (pH 7.4) using UV spectrophotometer. The developed method was statistically validated as per ICH and USP specification [4-7].

MATERIALS AND METHODS:

Rebamipide was obtained from Formulation department, Pharmacy group (BITS Pilani). All other chemicals used were of analytical grade and excipients used were of pharmaceutical grade, obtained from central chemical store (BITS Pilani). All spectrometric measurement was done at UV-spectrophotometer (Jasco V-570, Japan), using 10mm quartz cell.

Rebamipide is very poorly soluble drug showing pH dependent solubility. Different solvent systems were used for selection of media. The criteria employed for

media selection was solubility of drug, sensitivity, simplicity, applicability of method and cost

For the preparation of pH 7.4 phosphate buffer solution, 27.22 g of monobasic potassium phosphate was dissolve in distilled water and dilute it up to 1000 ml to get 0.2 M solution (Solution A). 8.0 g of sodium hydroxide pellets were dissolved in distilled water and made up to 1000ml to get 0.2M sodium hydroxide solution (Solution B). Solution A (250 ml) was placed in 1000ml volumetric flask and Solution B (195.5 ml) was added in that. Make up the volume up to 1000 ml to get 7.4 pH Phosphate Buffer (pH 7.4 adjustment was done by using 1.0 M hydrochloric acid solution and/or 10 M sodium hydroxide solution).

For the stock solution 10 mg of Rebamipide was dissolve in pH 7.4 buffer, under sonication for 15 min. to get a clear solution and made up to 100 ml by using pH 7.5 phosphate buffer to get final concentration of 100 µg/ml of Rebamipide as primary stock.

Five working solutions of different concentration (2.5, 5.0, 7.5, 10 and 12.5 µg/ml) were prepared from the above stock solution by serial dilutions. To find the λ_{max} of drug 10µg/ml of working solution was scanned in between 200 nm to 400 nm wavelength, to select the λ_{max} of Rebamipide (**Fig 1).**

A calibration curve was prepared by measuring the absorbance of working solutions at 227nm (obtained from scan) against blank. For establishment of linearity and specificity of proposed method, working solutions (n=6) were prepared and analyzed. Least square regression analysis was carried out to obtain the data.

Specificity and Selectivity of proposed method was assessed by preparing a drug concentration (10 µg/ml) from pure drug stock and analyzed. Solutions of different excipients were prepared with and without drug and analyzed for any interference with spectra of Rebamipide .

Rebamipide solution of 10 µg/ml was prepared in selected media. Both blank (7.4 pH phosphate buffer) and drug solutions were scanned at 200 nm/min in between 200 to 400 nm separately. The effect of solvent on absorbance of drug was checked by overlaying the two scans of drug and solvent.

For determination of accuracy of proposed method, within the linearity range three level of drug concentration in triplicate (LQC-3.0 µg/ml, MQC-6.0 µg/ml and HQC-12.0 µg/ml) were prepared from stock solution and analyzed at fixed wavelength 227 nm. Accuracy was assessed as the mean percentage recovery and percentage relative error (Table 2). The accuracy was also checked by standard addition method. For this study, different concentration of pure drug (2, 4, 6 and 9 µg/ml) was added to a known preanalyzed drug solution (3 µg/ml) and total concentration was calculated from calibration curve. From these concentrations, percent recovery of added drug was calculated as,

The percentage recovery = [(Ct-Cp)/Ca]*100;

where Ct is drug concentration after standard addition; Cp is drug concentration in preanalyzed sample and Ca is drug concentration added in preanalyzed drug solution.

Repeatability of proposed method was determined by using different level of drug concentration (as mentioned in accuracy), prepared from independent stock solution and analyzed. Inter-day and intra-day variation and analyst variation were studied to determined intermediate precision of proposed method, different set of drug concentration (2.5 µg/ml to 12.5 µg/ml) were prepared at two different time point in a day for intra-day precision and same protocol was followed at two different days for inter-day precision. The precision was determined in terms of percent relative standard deviation (**Table 3).**

The limit of detection (LOD) and limit of quantification (LOQ) of proposed method were determined by using calibration curve. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively, where S is the slope of the calibration curve and σ is the standard deviation of Y-intercept (ICH guidelines, 1996).

Robustness of proposed method was studied to find out effect of small change in method parameter. Robustness of proposed method was determined by Changing of pH (7.4 ± 0.1) unit and 24 hrs. stability of drug in selected media at room temperature. The three different concentrations (LQC, MQC and HQC) were prepared in different pH of same buffer system and determined the percent recovery and percent RSD.

To check the reproducibility of proposed method Rebamipide was estimated from different formulation such as tablet and suspension. For Tablets: Twenty tablets (in house developed) were weighed and triturated. Amount of powder equivalent to 10 mg Rebamipide was taken and extracted with pH 7.4 phosphate buffer under sonication for 30 min. The solution was made up to 100ml to obtain the concentration of 100µg/ml as primary stock. This solution was filtered with 0.45 µ nylon filter and from the filtrate. 10 µg/ml concentration solutions was prepared (n=6) as a working stock. Samples were analyzed using proposed method.

For suspensions aliquot amount of Rebamipide suspension (in house) equivalent to 10 mg was taken and extracted in selected media (7.4 pH phosphate buffer) under sonication for 30 min. Solution was diluted suitably to prepare 100 µg/ml as a primary stock. Extracted solution was filtered through 0.45 µm nylon filter and from the filtrate 10 µg/ml concentration solutions was prepared (n=6) as a working stock. Samples were analyzed using proposed method.

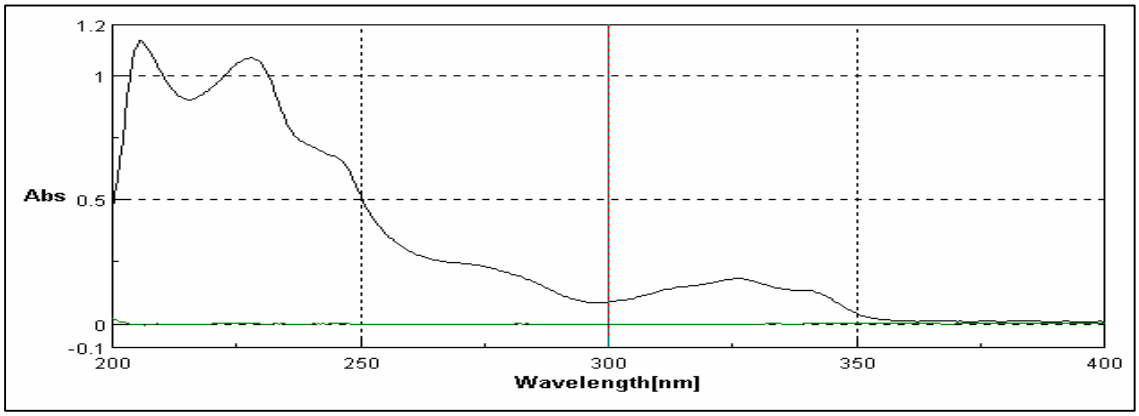

Fig. 1: Absorption spectra of Rebamipide.
Concentration of Rebamipide is 10µg/ml in pH 7.4 phosphate buffer. 227nm was selected as λ max for the estimation of Rebamipide in pH 7.4 phosphate buffer

TABLE 1. CALIBRATION DATA OF REBAMIPIDE

Concentration (µg/ml)	Mean Relative Absorbance (±SD*)	% RSD#	Predicted Concentration (µg/ml)
2.5	0.2718 (±0.003)	0.93	2.55
5.0	0.5301 (±0.005)	0.88	4.99
7.5	0.7878 (±0.007)	0.92	7.42
10.0	1.0618 (±0.011)	1.02	10.00
12.5	1.3325 (±0.019)	1.41	12.55
15.0**	1.5831 (±0.098)	6.18	14.91

(n=6), *Standard deviation, # percent Relative Standard Deviation, **15.0 µg/ml strength excluded from calibration curve because of it's high %Relative Standard Deviation.

TABLE 2. ACCURACY AND PRECISION OF PROPOSED METHOD

Level	Concentration (µg/ml)	Calculated Concentration (µg/ml)[a]			Mean % recovery (±SD)	Accuracy [b] (%)
		Range	Mean (±SD)	%RSD		
LQC	3	2.97 - 3.1	3.03± 0.1	1.8	101.12±1.8	–0.0112
MQC	6	5.87 - 6.15	6.02± 0.1	2.2	100.42±2.2	–0.0042
HQC	12	11.89 - 12.25	12.00± 0.2	1.3	100.02±1.3	–0.0002

(n=6), [a] concentrations of Rebamipide were calculated by using linear regression equation, [b] accuracy is given in relative percent error [= 100 x (calculated concentration- theoretical concentration)/theoretical concentration].

TABLE 3. RESULT OF INTERMEDIATE PRECISION STUDY

Level	Intra-Day Repeatability %RSD (n=6)			Inter day Repeatability %RSD (n =12)
	Day 1	Day 2	Day 3	
LQC	0.74	1.81	1.24	1.36
MQC	1.01	0.58	0.91	1.66
	1.91	1.03	1.45	
HQC	0.85	1.87	1.48	1.17
	0.79	1.31	0.2	
	0.9	0.75	0.66	

TABLE 4. RESULTS OF ASSAY OF REBAMIPIDE FORMULATION:

Formulations (In-house developed)[a]	Amount Calculated (Mean ±SD)	% Assay (Mean ±SD)
Rebamipide Tablet (400 mg)	200.42 ± 2.23	100.21 ± 1.16
Rebamipide suspension (0.2% w/v = 2 mg/ml)	2.00 ± 0.02	99.98 ± 0.90

(n=6), [a] Formulations were developed in laboratory.

RESULT AND DISCUSSION:

For the media selection various aqueous based media like 0.1M HCl solution, phosphate buffer (pH 5.5-8.0) solution, 0.1M NaOH solution and DMSO with distilled water in different proportion were used for media optimization. Additions of organic solvents like methanol, acetonitrile in different proportion with different media were also carried out but did not improve the sensitivity of method.

Maximum sensitivity was found with phosphate buffer (pH 7.4) media so phosphate buffer (pH 7.4) was selected as media. The drug scan in this media is shown in Fig. 1.

From the drug scan it was found that the maximum drug UV absorbance occurs at 227nm which was used as λ_{max} for the method development.

In method development, different drug concentration and there relative absorbance were shown in Table 1.

All the concentration levels gave acceptable values of the standard deviation and the percentage relative standard deviation (%RSD) was found to be below 2.0 %. The calculated values were in sync with the theoretical concentrations. In the selected media, the linearity range was found to be between 2.5 µg/ml to 12.5 µg/ml. The linear regression analysis slop (± SD) and intercept (± SD) were found to be 0.1061 (± 0.002) and 0.0009 (± 0.008) respectively. These mean values were found to be within the 95% confidence interval limit (confidence interval limit of slope: 0.098 to 0.11; confidence interval of intercept from -0.0071 to 0.0089). The regression coefficient value was found to be (0.9997) which support the best fit of the regression equation. The standard deviation of slope and intercept was very low which indicate the high precession of proposed method.

The relative absorbance of Rebamipide was not affected at λ_{max} in presence of common excipients in selected media. When the absorbance of pure drug sample and sample from different in house formulation were compared by one way ANOVA, low calculated F value (F calculated (2,16) value 0.089 and F critical value 3.69 at P = 0.05 confidence interval), indicate that there is no significant difference between mean relative absorbance. Therefore the proposed method was specific and selective for Rebamipide. All the three concentration level (Table 2) shows accuracy range between -0.0211 to -0.0002%.

The high mean percent recovery and there low standard deviations (SD < 2.5) indicate the accuracy of method. In standard addition method the mean percent recovery (±SD) for 2, 4, 6 and 9 µg/ml concentrations were found 99.91 (±1.46), 99.96 (±1.3), 99.98 (±1.37) and 99.99 (±1.45). These results reveal the repeatability of proposed method. In repeatability study, the %RSD was in the range from 0.18 to 2.2% (Table 2). At all three concentration levels, precision shows satisfactory result. In this study the percent RSD were found not more than 1.91% (Table 3), which indicates the high repeatability and intermediate precision of proposed method.

The LOD and LOQ were found 0.73 µg/ml and 2.21 µg/ml respectively. Variation in pH of selected media by ±0.1 did not show any significant effect on relative

absorbance indicating the high robustness of proposed method.

The proposed method was evaluated by estimation of Rebamipide from different in house formulation (Table 4).

The recovery assay value of Rebamipide ranged from 99.98% to 100.21% with SD value not more than 1.5 which indicates that there is no significant interference of excipient matrix in the estimation of Rebamipide. Results indicate that no organic solvent is required for the extraction of Rebamipide from formulation which reduces the cost of estimation.

Hence the proposed method is simple, sensitive, rapid, accurate, precise and cost effective which can be used for routine analysis of Rebamipide in bulk and different formulations.

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