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Research Article

A VALIDATED STABILITY INDICATING REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD OF RANOLAZINE DIHYDROCHLORIDE AND CHARACTERIZATION OF ITS DEGRADATION PRODUCTS

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ABSTRACT

The present paper deals with the development of stability indicating reversed phase high-performance liquid chromatographic (RP-HPLC) method for ranolazine dihydrochloride, an antianginal drug in presence of its degradation products formed during forced decomposition studies. Forced degradation studies were performed on the bulk drug by using acid (0.1 N hydrochloric acid), base (0.1 N sodium hydroxide), water (neutral hydrolysis), 3% v/v hydrogen peroxide (oxidation), dry heat (60 ^{o}C) and UV light (254 nm). Degradation was observed for ranolazine dihydrochloride in all cases excepting wet heat and UV light exposure. The formed degradation products were found to be o-Methoxyphenol (degradation product-1) and 2,6-Dimethylaniline (degradation product-2). Successful separation of the drug from the degradation products formed under different stress conditions was achieved on a Novapak C18 column (150 mm ×3.9 mm, 4 µm particle size) using methanol- acetonitrile-phosphate buffer (pH 3.6; 6.3 mM) (4:3:3, v/v/v) as the mobile phase at a flow rate of 1ml/min. The detection wavelength was 220 nm. The developed method was completely validated and proved to be robust. As the method could effectively separate the drug from its degradation products, it can be employed for analysis of the samples of stability study.

Keywords: Ranolazine dihydrochloride, Stability indicating, Degradation, Validation, RP-HPLC.

INTRODUCTION

Ranolazine dihydrochloride [N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl-1-piperazineacetamide dihydrochloride], is a new antianginal agent approved for the treatment of chronic stable angina pectoris[1]. Ranolazine dihydrochloride has antianginal and anti-ischemic effects that does not depend on reduction in heart rate or blood pressure[2]. Ranolazine dihydrochloride is available as bulk material and it is marketed as tablet. The stability assessment of any promising drug candidate plays a vital role in its preformulation study. Many environmental conditions such as heat, light, moisture as well as the inherent chemical susceptibility of a substance to hydrolysis or oxidation can play an important role in pharmaceutical stability. So, this study also helps to define storage and handling conditions. The exposition of the drug substance to extreme external conditions helps to reveal and identify the likely degradation products which will open a new scope of research on toxicity study. The findings of toxicity study will help in scrupulous determination of expiry, adverse effects etc.

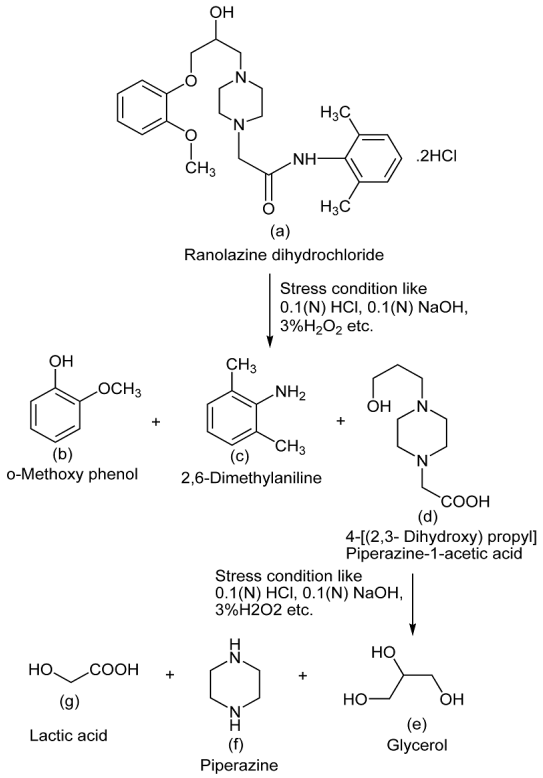

Fig. 1: Degradation pathway of Ranolazine dihydrochloride

Ranolazine dihydrochloride has not appeared in any pharmacopoeia yet. Few spectrophotometric[3], HPLC[4-6], LC-MS[7-11] and LC-MS-MS[12-14] methods were reported in literature for determination of Ranolazine dihydrochloride. However, most of these reported methods were related to the quantitative assay of ranolazine dihydrochloride in human or dog plasma. To our current knowledge, no article related to the stability indicating high performance liquid chromatographic (HPLC) determination of ranolazine dihydrochloride and characterization of its degradation products has been reported yet as revealed by literature survey. Therefore the aim of this study was to develop and validate stability-indicating reversed phase high performance liquid chromatography (RP-HPLC) method for determination of ranolazine dihydrochloride in presence of its degradation products formed under different stress conditions.

MATERIALS AND METHODS

Apparatus

Experiments were performed using a Waters (India) 510 HPLC system with Waters 486 tunable absorbance detector. The samples were injected manually using a 20 µl sample loop. The Millennium[32] software was used for quantification and data processing.

Chemicals and reagents

Pure ranolazine dihydrochloride (Fig.1a) was provided by Sun Pharmaceutical Pvt. Ltd. Gujarat, India and its degradation products namely o-methoxyphenol (degradation product -1, Fig.1b) and 2, 6-dimethylaniline (degradation product -2, Fig.1c) were procured from Hi Media Laboratories Pvt. Ltd. Mumbai, India. All chemicals and reagents used were of HPLC grade and were purchased from Merck (India) Ltd., Mumbai.Two commercial formulations of ranolazine dihydrochloride [RANOLAZ (Torrent India Limited) and RANOZEX (Sun Pharmaceutical Pvt. Limited)] were used in this study.

Chromatographic conditions

The experiment was performed on a Novapak C18 (150 ×3.9 mm, 4µm) column using methanol-acetonitrile-phosphate buffer (pH 3.6; 6.3 mM) (4:3:3, v/v/v) as mobile phase. The mobile phase was filtered through a nylon membrane filter paper (pore size 0.45 µm) and degassed with a sonicator for 10 min. The chromatography was performed at room temperature at a flow rate of 1 ml/min. The column temperature was maintained at 25 °C and eluents were monitored at a wavelength of 220 nm.The volume of each injection was 20 µl.

Sample preparation

The standard and sample stock solutions of ranolazine dihydrochloride were prepared separately in a solvent mixture of methanol- acetonitrile- phosphate buffer (pH 3.6; 6.3 mM) (4:3:3, v/v/v) at a concentration of 1000 µg/ml.Working standard solutions were prepared by diluting the standard stock solution with above solvent to get solutions of concentration in the range of 5-200 µg/ml. A stock solution of degradation product-1 and 2 (500 µg/ml) were also prepared separately in mobile phase.

Degradation studies

All the degradation studies were carried out with drug solution of 100 µg/ml concentration. For acidic and alkaline hydrolysis studies, the drug solution was mixed with 0.1(N) HCl and 0.1(N) NaOH separately. These mixtures were refluxed on a water bath for 4 h at 60°C. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light. The resulting solution was neutralized by base and acid, respectively to avoid any interference of acid or base in the following steps. 20 µl of resulting solution were injected into HPLC and the chromatograms were recorded. About 4.24% and 9.75% degradation of drug were observed for acid and basic media, respectively. In acid and basic hydrolysis similar type of degradation was observed. In both the cases degradation product-2 was (about 1.52% and 3.82% respectively) observed at RT 1.564 min. When ranolazine dihydrochloride was subjected to oxidative degradation by treating with 3% hydrogen peroxide solution for 24 h at ambient temperature, the extent of degradation was about 19.02 %, out of which 2.3% corresponded to degradation product-1 (RT 1.341) and 16.72 % was degradation product-2 (RT 1.564). The drug was stored in incubator at 60 °C for 72h for dry heat degradation study, the extent of degradation was about 12.5%, out of which 4.12% corresponded to degradation product-1. The aqueous solution of ranolazine dihydrochloride refluxed for 6 h on a water bath set at 60 °C for wet heat degradation study, no degradation was observed. Photo chemical stability of the drug was also studied by exposing the drug to direct UV light (254 nm) for 24 h and no degradation was observed. In all cases, degradation product-1 and 2 were first assumed from degradation pathway (Fig. 1) and then confirmed by co-injection.

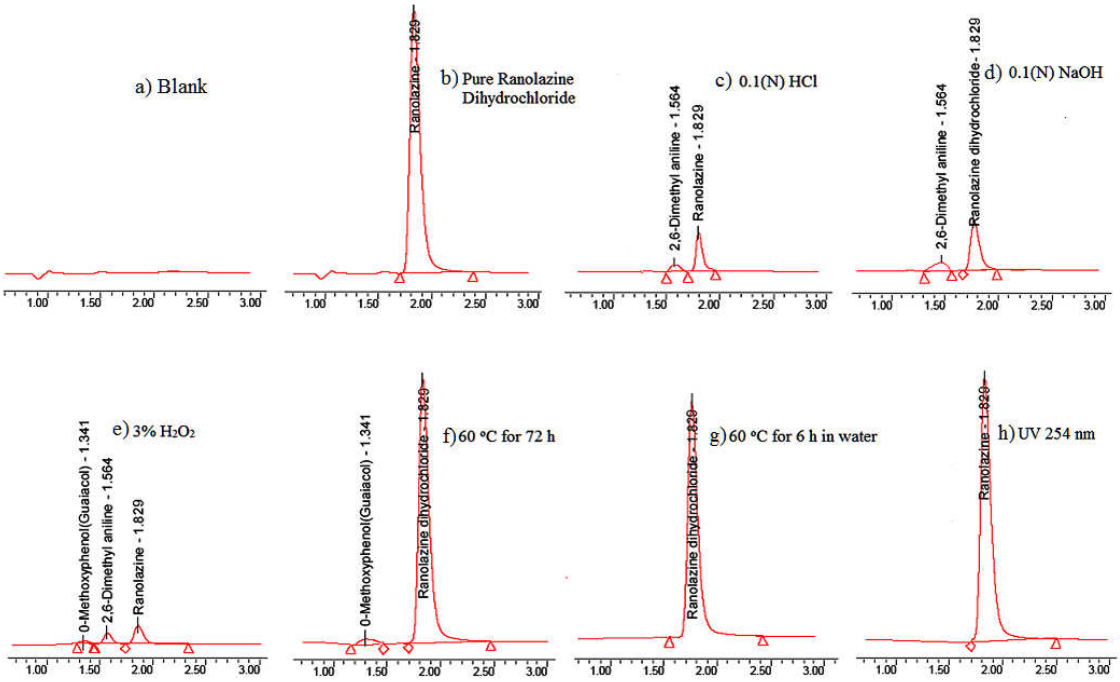

Fig. 4: Representative HPLC chromatograms of ranolazine dihydrochloride pure and stressed samples

Analysis of marketed formulation

To determine the content of ranolazine dihydrochloride in conventional tablets (label claim 500 mg/tablet), the tablets were powdered and the powder equivalent to 100 mg of ranolazine dihydrochloride was taken in a 100 ml volumetric flask and approximately 80 ml of suitable diluent [methanol- acetonitrile-phosphate buffer (pH 3.6; 6.3 mM) (4:3:3, v/v/v)] was added to it. The content of the flask was then allowed to stand for 15min with intermittent sonication to ensure that the drug was completely dissolved and then filtered using 0.45 µm nylon membrane filter (Whatman). Final volume was made up to 100 ml with the above diluent to prepare a stock solution of 1000 µg/ml. 1.0 ml of the resulting solution was transferred into a 10 ml volumetric flask and the volume was made up to the mark with diluent. A sample of 20 µl of the resulting solution was directly injected. The average drug content of the formulations was determined from the corresponding regression equation.

Validation of the analytical method

The developed chromatographic method was validated by evaluating the following analytical parameters: specificity, Precision, Limit of detection (LOD), Limit of quantification (LOQ), Accuracy, Linearity and Robustness.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential degradation products. Testing of the drug substance under stress conditions can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule.

The specificity of the developed HPLC method for ranolazine dihydrochloride was determined in the presence of its degradation products, namely degradation product-1 and 2. Degradation was attempted under stress conditions like acid (0.1N HCl at 60°C for 4 h), base (0.1N NaOH at 60 °C for 4 h), oxidation (3% H_2O_2 at ambient temperature for 24 h), dry heat (60 °C for 72 h), wet heat (60 °C for 6 h) and UV light (254 nm for 24 h) to evaluate the ability of the proposed method to separate ranolazine dihydrochloride from its degradation products. Peak purity test was performed for ranolazine dihydrochloride peak by using tunable absorbance UV Visible detector in samples exposed to stress conditions. Assay studies were performed for stress samples against qualified ranolazine dihydrochloride reference standard. Assay was also made for ranolazine dihydrochloride bulk sample by spiking both the degradation products (1 and 2) at the specification level (i.e., 0.12 %).

Precision

a. Intra Assay Precision

Intra assay precision was evaluated by performing six independent assays of the ranolazine dihydrochloride at three concentration levels 80, 100, and 120% of assay analyte concentration(100 µg/ml) i.e., 80,100 and 120 µg /ml on the same day against qualified reference standard and calculating the % CV of assay.

The intra assay precision of the analytical method of related substances was checked by injecting six individual preparations of stress ranolazine dihydrochloride (100 µg/ml) spiked with 0.06 %, 0.12 % and 0.18 % each of degradation product-1 and 2 with respect to stress ranolazine dihydrochloride analyte concentration on the same day and calculating the % CV of degradation product-1 and 2.

b. Inter Assay Precision

Inter assay precision was evaluated by performing six independent assay of the ranolazine dihydrochloride at three concentration levels 80, 100 and 120% of assay analyte concentration (100 µg/ml) i.e., 80, 100 and 120 µg/ml for 6 days against qualified reference standard and calculating the % CV of assay.

The inter assay precision of the analytical method of related substances was checked by injecting six individual preparations of stress ranolazine dihydrochloride (100 µg/ml) spiked with 0.06%, 0.12 % and 0.18 % each of degradation product-1 and 2 with respect to stress ranolazine dihydrochloride analyte concentration for 6 days and calculating the % CV of degradation product-1 and 2.

Limit of detection (LOD) and Limit of quantification (LOQ)[15]

The LOD and LOQ for ranolazine dihydrochloride, degradation product-1 and 2 were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of dilute solutions with known concentration (ICH 1995). Precision study was also performed at the LOQ level by injecting six individual preparations of ranolazine dihydrochloride, degradation product-1 and 2 by calculating the % CV of the area.

Accuracy

The accuracy of the assay method was evaluated at three concentration levels i.e. 40 µg/ml, 50 µg/ml and 60 µg/ml in bulk drug sample. The percentage of recoveries was calculated from the slope and Y-intercept of the calibration curve obtained during linearity study of assay method. Accuracy/recovery experiment was repeated six times.

Standard addition and recovery experiments were conducted to determine accuracy of the related substance method for the quantification of degradation product-1 and 2 in ranolazine dihydrochloride stress samples.

The study was conducted six times at 0.06%, 0.12 % and 0.18 % of stress analyte concentration (100 µg/ml). The percentage of recoveries for degradation product-1 and 2 were calculated from slope and Y- intercept of the calibration curve obtained.

Linearity

Linearity test solutions for assay method were prepared from stock solution at eleven concentration levels starting from 5 to 200 µg/ml.The peak area versus concentration data was performed by least-squares linear regression analysis. Linearity test solutions for related substance method were prepared by diluting stock solution to the required concentrations. The solutions were prepared at eleven concentration levels from LOQ to 200 % of the specification level.

Linearity test was performed for three consecutive days in same concentration range for both assay and related substance method. The % RSD, value of the slope and Y-intercept of the calibration curve were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution between ranolazine dihydrochloride and degradation products was evaluated.

The flow rate of the mobile was 1 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 ml/min. The effect of the column temperature on resolution was studied at 20 °C and 30 °C instead of 25 °C. The effect of the present organic strength on resolution was studied by varying acetonitrile by -3 to +3 % while other mobile phase components were held constant.

Solution stability and mobile phase stability

The solution stability of ranolazine dihydrochloride in the assay method was performed by leaving both the test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were analyzed for 6 h interval up to the study period. Furthermore, mobile phase stability was also performed by analyzing the sample solutions against freshly prepared reference standard solution for 6 h interval up to 48 h. Mobile phase prepared was kept constant during the study period. The % RSD for the assay of ranolazine dihydrochloride was calculated during mobile phase and solution stability experiments.

The solution stability of ranolazine dihydrochloride and its degradation products in the related substance method were performed by leaving spiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Content of degradation products 1 and 2 were determined for every 6 h interval up to the study period. Mobile phase stability was also performed for 48 h by injecting the freshly prepared sample solutions for every 6 h interval.

RESULT AND DISCUSSION

Optimization of chromatographic conditions

The main target of the chromatographic method is to get the separation of critical closely eluting degradation products, namely degradation product-1 and 2. Degradation products were co eluted by using different stationary phases like C18, C8, phenyl and cyano and different mobile phases. The chromatographic separation was achieved on a Novapak C18 (150 mm × 3.9 mm, 4µm) column using methanol-acetonitrile- phosphate buffer (pH 3.6; 6.3 mM) (4:3:3, v/v/v) as a mobile phase. The flow rate of the mobile phase was 1 ml/min, at 25^{o}C column temperature, the peak shape of ranolazine dihydrochloride was found symmetrical. In optimized chromatographic conditions ranolazine dihydrochloride, degradation product-1 and 2 were well separated, typical retention times were shown in Fig. 2e. The developed HPLC method was found to be specific for ranolazine dihydrochloride and its degradation products.

Results of forced degradation studies

Ranolazine dihydrochloride was degraded in all conditions except wet heat and light (Fig. 2). Peak purity test results confirmed that the ranolazine dihydrochloride peak is homogenous and pure in all the analyzed stress samples. The assay of ranolazine dihydrochloride is unaffected in the presence of its degradation products, confirms the stability indicating power of the developed method. The summary of forced degradation studies is given in Table 1.

Precision

The intra and inter assay coefficient of variation for assay of ranolazine dihydrochloride during assay method precision study were within 0.37 and 0.36%, respectively (Table 2).

The intra assay and inter assay coefficient of variation for degradation product-1 and 2 in related substance method precision study were within 0.31 and 0.34%, respectively (Table 3), conforming good precision of the method.

Table 1: Summary report of forced degradation study.

Stress condition	Time (h)	% Assay of active substance	% Assay of degradation products	Mass balance* (%)
Acid Hydrolysis (0.1N HCl)	4	95.95	4.24	100.19
Basic Hydrolysis (0.1N NaOH)	4	90	9.75	99.75
Oxidation (3% H_2O_2)	24	80	19.02	99.02
Dry Heat (60 ^{0}C)	72	86	12.5	98.5
Wet Heat (60 ^{0}C)	6	54.43	45.57	100
UV (254 nm)	24	99.99	-	99.99

*It is the summation of assay of active substance and degradation products

Table 2: Intra and Inter assay precision of ranolazine dihydrochloride.

Conc. of ranolazine dihydrochloride (µg/ml)	Observed conc. of ranolazine dihydrochloride (µg/ml) by the proposed method.			
	Intra-Day		Inter-Day	
	Mean (n=6)	% CV	Mean (n=6)	% CV
80	79.81	0.37	79.69	0.27
100	99.01	0.31	99.30	0.36
120	119.91	0.26	119.75	0.31

n= Number of determinations.

Table 3: Intra and Inter assay precision of degradation products

Sample	Actual concentration (µg/ml)	Intra-Day		Inter-Day	
		Mean(n=6)	%CV	Mean (n=6)	% CV
Degradation product -1	0.06	0.061	0.18	0.062	0.15
	0.12	0.121	0.21	0.122	0.16
	0.18	0.179	0.12	0.181	0.27
Degradation product -2	0.06	0.599	0.19	0.061	0.22
	0.12	0.122	0.31	0.121	0.22
	0.18	0.181	0.23	0.180	0.34

n= Number of determinations.

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of ranolazine dihydrochloride was 0.04 µg/ml and 0.12 µg/ml respectively for 20 µl injection volume. The coefficient of variation of ranolazine dihydrochloride area at LOQ concentration was below 2.9%.

The limit of detection of degradation product-1 and 2 were 0.04 µg/ml for 20 µl injection volume. The limit of quantification of degradation product-1 and 2 were 0.12 µg/ml for 20 µl injection volume. The coefficient of variation at LOQ concentration for degradation product-1 and 2 were below 0.5%.

Table 4: Recovery result of ranolazine dihydrochloride

Amount of drug added (µg) to solution of tablet formulation	Recovery from tablet formulation	
	Mean (±SD) amount (µg) found* (n=6)	Mean (±SD)% recovery*(n=6)
40	39.94 (±1.32)	99.88 (±0.21)
50	50.04 (±0.84)	100.05 (±0.12)
60	59.05 (±0.68)	98.47 (±0.06)

*Each data represent the average of 6 reading

Table 5: Recovery data for degradation products

Sample	Amount spiked (mg/ml)	Amount recovered (mg/ml)	Mean recovery* (%) (n=6)	CV (%)
At 40% level				
Degradation product-1	0.06	0.061	101.1	0.27
Degradation product-2	0.06	0.059	99.1	0.43
At 80% level				
Degradation product-1	0.12	0.121	100.3	0.35
Degradation product-2	0.12	0.121	100.1	0.61
At 120% level				
Degradation product-1	0.18	0.18	99.99	0.45
Degradation product-2	0.18	0.182	101.8	0.13

*Each data represent the average of 6 reading

Accuracy

The percentage recovery of ranolazine dihydrochloride in formulation samples was ranged from 98.47 to 100.05 (Table 4).

The percentage recovery of degradation product-1 in stress sample was ranged from 99.99 to 100.3. The percentage recovery of degradation product-2 in stress sample was ranged from 99.1to 101.8 (Table 5).

Linearity

Linearity calibration plot for assay method of ranolazine dihydrochloride was obtained over the calibration ranges tested, i.e., 5-200 µg/ml.The average peak areas were plotted versus the concentrations injected. The equation for the resultant calibration curve was y =17485x + 29629 and correlation coefficient obtained was greater than 0.999. Linearity was checked for assay method over the same concentration range for three consecutive days. The % RSD values of peak areas were within 0.11 and 0.48%. The result shows that an excellent correlation existed between the peak areas and concentrations of analyte.

Linear calibration plot for related substances method was obtained over the calibration ranges tested i.e. LOQ to 200% of the specification level for degradation product-1and 2.The correlation coefficient obtained was greater than 0.998. Linearity was checked for related substance method over the same concentration range for 3 consecutive days. The % RSD values of peak areas were within 0.16 to 0.47%. The result shows an excellent correlation existed between the peak areas and concentration of degradation product-1and 2.

Robustness

In all the deliberately varied chromatographic conditions performed (flow rate, column temperature and organic solvent), the resolution of degradation products in the stress sample mixture and ranolazine dihydrochloride remains unchanged, indicating that the analytical method was appropriately robust.

Solution stability and mobile phase stability

The % RSD of assay of ranolazine dihydrochloride during solution stability experiment was within 1.3%. No significant changes were observed in the content of degradation product-1 and 2 during solution stability and mobile phase stability experiments when performed using related substances method. The solution stability and mobile phase stability experiments data confirm that sample solutions and mobile phase used during assay and related substance determination were stable up to 48hrs.

Assay of ranolazine dihydrochloride marketed formulation

The assay of Ranolaz and Ranozex tablets containing ranolazine dihydrochloride was found to be 99.82% and 99.78% (Table 6).

Table 6: Assay of ranolazine dihydrochloride in dosage form

Brand name of tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method* (n = 6)	% mean (± SD) labeled amount* (n = 6)
Ranolaz	500	499.91 (±0.31)	99.82 (±0.21)
Ranozex	500	499.01 (±0.12)	99.78 (±0.32)

*Each data represent the average of 6 reading.

CONCLUSION

A novel stability indicating method was developed, which separates all degradation products formed under varieties of conditions. The product was found to be stable under wet heat and photolytic conditions. The procedure has been evaluated with regard to linearity, accuracy, precision, specificity and robustness in order to ascertain the suitability of the analytical and stability-indicating method. The method can be conveniently used in quality control laboratories for product release and can also be adopted for analysis of sample stability.

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