

Derivative Spectrophotometric Methods for the Analysis and Stability Studies of Ranolazine in Bulk and Dosage Forms

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ABSTRACT

The present study was aimed to develop simple and accurate derivative spectrophotometric methods (¹D and ²D) for the assay and stability studies of Ranolazine (RAN) in bulk and dosage forms. The methods were based on differentiating the original UV spectrum of RAN solution in methanol to generate the first and second derivative spectra which were measured at 278nm and 283 nm, respectively. The developed methods were then validated as per ICH guidelines and applied for the stability studies. Beer's law was found to be valid over the concentration range 100-600 µg/ml with correlation coefficient (r) not less than 0.999. The obtained limit of detection (LOD) and limit of quantification (LOQ) reflected the sensitivity of the methods (24.0 µg/ml and 73.0 µg/ml, 17.8 µg/ml and 53.6 µg/ml, for ¹D and ²D, respectively). Good results were also obtained from the assay of RAN tablets (98.6 ± 2.3%, n=3). Studying the stability behavior of RAN using the developed methods reflects its instability under alkaline conditions following the first-order kinetics. The statistical validation at a 95% confidence level proves the sensitivity, precision, accuracy, and stability-indicating properties of the developed methods.

Keywords: Ranolazine, Spectrophotometry, ICH standards, Stability, Kinetics.

INTRODUCTION

The need to develop a stability-indicating method using stress degradation has been recommended by International Conference of Harmonization¹. In practice, the effects of pH and temperature changes on drug stability are often used in such studies. The results of such studies are of great importance in the estimation of drug shelf life and the effect of degradation products on decreasing efficacy and causing toxicity. They may also serve as guides for better drug design, drug formulation and drug analysis.

The stability of a pharmaceutical product is defined as the capability of the product to retain its efficacy, properties and characteristics throughout its shelf life². One of the most important types of stability is chemical stability which includes hydrolysis. Amides are generally more stable to hydrolysis than esters. In general, the rate of hydroxyl ion-catalyzed reaction of amides is greater than the rate of proton-catalyzed hydrolysis³.

Ranolazine (RAN, Figure 1) is an amide containing compound, piperazine derivative with anti-anginal and potential antineoplastic activities. The IUPAC name is N-(2,6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]piperazin-1-yl] acetamide. It has the molecular formula (C₂₄H₃₃N₃O₄)⁴. Although its relationship to angina symptoms is uncertain, RAN can inhibit the inactivating component of the sodium current (I (Na)) at therapeutic levels. It also inhibits the rapid inward rectifying current (I (Kr)), thereby prolonging the ventricular action potential⁵.

Although literature revealed different methods (chromatographic and spectroscopic methods) for the assay of RAN⁶⁻²², neither official pharmacopeias' methods nor stability studies utilizing spectrophotometric methods are available. Although the UV spectrophotometric methods might lack selectivity, the present method was found to be superior on the reported methods in terms of simplicity, cost-effectiveness and stability indication. Therefore, the aim of the present work was to develop simple and accurate derivative spectrophotometric methods for the analysis and stability studies of RAN in bulk and dosage forms.

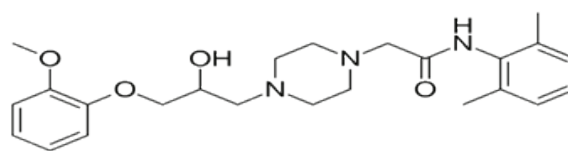


Figure 1. Chemical structure of Ranolazine

MATERIALS and METHODS

Apparatus

UV spectrophotometric studies were carried out on Shimadzu UV- 1800EN240V, double beam, (Kyoto, Japan). The operating conditions were as follows:

- Wavelength range: 240-400 nm.
- Scan speed: Medium, 0.2 nm/s.

Sensitive balance: Kern ALS 120-4, Germany

Reference standard and sample

RAN reference standard was purchased from Lab CO in UAE (98%). RAN tablets: Ranexa 375 mg prolonged release tabs were purchased from UAE.

Chemicals

Methanol Lobalchemie, India. Sodium hydroxide: BDH, Poole, England. Hydrochloric acid: BDH, Poole, England

Preparation of stock solutions

Standard stock solution

Standard stock solution was prepared by dissolving 0.1 g of RAN standard in 100 ml methanol (Solution A; 1000µg/ml).

Sample stock solution

Ten tablets of Ranexa (RAN 375 mg) were accurately weighed and grinded. An equivalent amount containing 0.1g RAN was weighed and dissolved in 20 ml methanol and transferred into 100 ml volumetric flask. The volume was completed to mark with methanol. The solution was sonicated for 10 min and filtered (Solution B; 1000µg/ml).

Method validation

Linearity

Serial dilutions were made from solution A by transferring accurately measured volumes (1-6 ml) into a set of 10 ml volumetric flasks. The volumes were then completed to mark with the solvent (methanol), the ¹D and ²D order derivative spectra were recorded over the range 240-400 nm. The procedure was repeated three times. The mean absorbance values were plotted against concentration to construct the calibration curve.

Limits of detection and quantification were determined from the calibration curve using adopted formulae²³:

$$\text{LOD} = 3.3 \text{ SB/Slope}$$

$$\text{LOQ} = 10\text{SB/Slope}$$

Where SB is the standard deviation calculated from the regression analysis data.

Content uniformity

Three mL of solution B was transferred into 10mL volumetric flask. The volume was then completed to mark with the solvent (methanol), and the absorbance values were recorded over the range 240-400 nm. The procedure was repeated three times. The content uniformity of the tablet solutions was then evaluated by the direct comparison of sample/standard absorbance values.

Precision

Serial dilutions from solution A were done as under linearity to obtain concentrations of 100µg/ml, 200µg/ml and 300µg/ml. These solutions were scanned using the derivative modes (¹D and ²D) three times within the same day (inter-day) and at three consecutive days (intra-day). The results obtained were used to evaluate the precision of the developed method in terms of relative standard deviation values (RSD %).

Recovery percentage

Recovery studies were carried out by spiking sample of known concentration with standard solution. The recovery percentage was then determined using the following equation²⁴:

$$\% \text{ recovery} = (C_s - C_u)/C_A \times 100$$

Where:

C_s = concentration of spiked samples; C_u = concentration of unspiked samples; C_A = concentration of analyte added to the test sample

Stability studies

Effect of alkali and acid on the stability of RAN solution

Aliquots of solution A (2 mL) were transferred to four stoppered glass tubes then 1 ml of 0.05 M NaOH was added to each tube. The volumes were then completed to mark with methanol. The first and second derivative spectra of the first tube were recorded at zero time. The other three tubes were heated in a boiling water bath at 15 minutes heating time interval. The solutions were then cooled and the effect of the alkali on the degradation of RAN was monitored using the developed methods.

The same procedure was repeated adding 1mL of 1M HCl instead of NaOH to study the effect of acid on RAN stability.

Light effect

RAN standard methanolic solution (40µg/ml) was prepared and placed in transparent glass tubes. The solutions were then subjected to direct sunlight during mid-day time for 6 hours. The photo-degradation was then monitored using the developed methods.

RESULTS and DISCUSSION

Discovery of new drugs, their synthesis and manufacturing in suitable pharmaceutical forms, is a highly expensive process. Consequently, the development of accurate and precise methods is important for their analysis to ensure their quality.

For any drug stability studies, a validated stability-indicating method that can distinguish the active ingredient from its degradation products is the first and major requirement by the International Conference on Harmonization (ICH) ¹. Direct spectrophotometric methods (zero-order) are widely used in pharmaceutical analysis although they lack selectivity and stability-indicating properties. Prior to the develop-

ment of derivative spectroscopy, chromatography was the most frequent used method for stability studies. Chromatographic techniques have been advanced by introducing various types of columns and detectors which improve the selectivity and efficiency of the procedure and its application in structural elucidation. However, these techniques are still expensive, time-consuming and require sophisticated skills which may not be available in each analytical laboratory especially in the developing countries.

The development of derivative spectroscopy provided simple and accurate tool for the analysis and stability studies of different pharmaceutical dosage forms²⁵⁻²⁸.

Derivative spectrophotometry is based on the mathematical differentiation of spectral curve into a derivative (first- or higher derivatives). This technique usually improves resolution bands, eliminates the influence of background or matrix and provides more defined fingerprints than traditional ordinary or direct absorbance spectra, since it enhances the detectability of minor spectral features²⁹.

Thus, our aim in the present work was to develop accurate derivative spectrophotometric methods for the analysis of RAN which will be also applied for its stability studies under stress conditions according to the ICH guidelines. The developed first and second derivative modes (¹D and ²D) were successfully applied for the stability studies and quantification of the degradation products. However, the ²D resulted in better resolution between the drug and its degradation products peaks at longer and shorter wavelengths than the parent drug, which can also be applied as an identification tool. This is also come in favor to the reported chromatographic methods by Gade et al., 2011; Khedkar et al., 2015 in terms of simplicity, accuracy and time saving^{9, 10}.

The zero-order derivative spectrum of RAN shows absorption maximum at 273 nm (Figure 2). Since derivatization of normal spectrum gives rise to reduction of band width, sharper peaks were achieved on the first and second order derivative spectra of RAN showing an absorption maximum at 278nm and 283 nm (Figures 3 and 4).

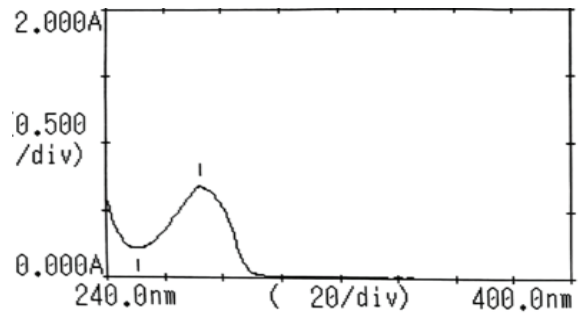


Figure 2. UV spectrum of RAN solution (100µg/ml; 273 nm)

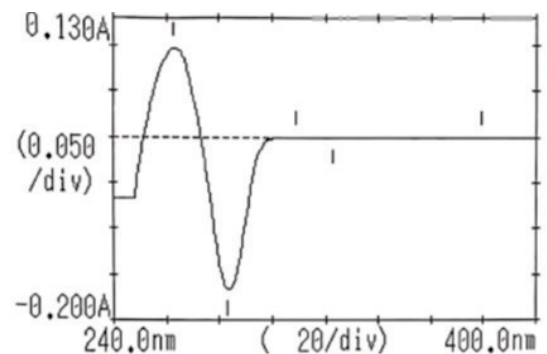


Figure 3. First derivative spectrum of RAN solution (400µg/ml; 283 nm)

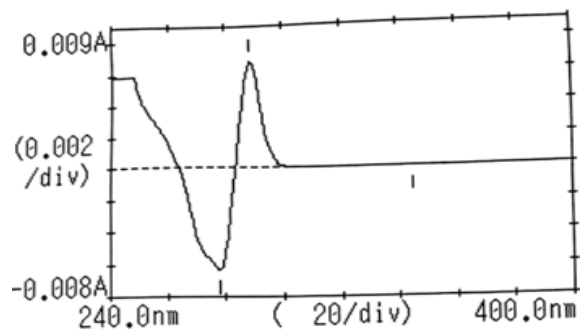


Figure 4. Second derivative spectrum of RAN solution (100 µg/ml; 278 nm)

Linearity

The constructed calibration curves for the ¹D and ²D modes was found to obey Beer's law in a concentration range 100- 600 µg/ml with a good correlation coefficient (not less than 0.999). The regression analysis data was calculated at 95% confidence level for n-2 degrees of freedom using the following formula²³:

$$y = (b \pm ts_b) x + (a \pm ts_a)$$

Results obtained for linearity data of the developed method was summarized in table 1, which reflected the sensitivity and consistency of the constructed curve.

Assay and Validation

The developed methods were applied for the drug uniformity testing in RAN tablets. Good assay results were obtained using ¹D and ²D modes (101.4 ± 1.34%; 98.6 ± 2.3%, n=3, respectively). The validity of the methods was assessed by statistical evaluation of the results obtained using the following formula³⁰:

$$t = \frac{\bar{X} - \mu}{\sqrt{N}} / S$$

where \bar{X} = content % mean at 95% confidence level (99.5%), μ = true mean N = number of measurements, S = standard deviation.

As the calculated t-value for ¹D and ²D (2.095 and 1.596, respectively) is less than the tabulated one (12.706), this reflects there is no significant difference between the developed method and the true mean value.

Precision and accuracy

The precision results showed acceptable RSD% values ranging between 0.00- 2.3% for inter-day and intra-day precisions, respectively. These low values (less than 2%) reflected the precision of the developed methods.

The accuracy of the developed method and freedom of interference by tablet excipients were confirmed by the good results obtained from recovery testing (96.5 ± 3.05%, n=3) (Table 2)

Table 1. Regression analysis data of the developed method (n=3)

Parameter	¹ D	² D
λ_{max}	283nm	278nm
Concentration range	100-600 µg/ml	100-600 µg/ml
Slope ± tsb*	1.0×10 ⁻⁴ ± 2.24× 10 ⁻⁵	5.6 ×10 ⁻⁵ ± 1.13× 10 ⁻⁵
Intercept ± tsa**	4.0×10 ⁻³ ± 1.8× 10 ⁻²	- 5.3 ×10 ⁻⁴ ± 8.2×10 ⁻³
LOD	24.0	17.8 µg/ml
LOQ	73.0	53.6 µg/ml
R	0.999	0.999

*Standard error of slope at 95% confidence level for (n-2) degrees of freedom, **Standard error of intercept at 95% confidence level for (n-2) degrees of freedom

Table 2. Results of the studied validation parameters (¹D, ²D)

Parameter	Experiment 1	Experiment 2	Experiment 3	Results
Inter-day precision (100-300µg/ml)	0.005-0.018	0.005-0.018	0.005-0.018	RSD%= 0
Intra-day precision (100-300µg/ml)	0.005-0.018	0.005-0.018	0.005-0.017	RSD%= 2.3
Recovery % (300µg/ml)	94.7	94.7	100.0	Mean ±SD 96.50 ± 3.05%
Content uniformity%	100.0	102.5	101.3	Mean ±SD 101.40 ± 1.34

Stability studies

Effect of alkali on the stability of RAN solution

Different NaOH concentrations with different heating time intervals were studied. 0.05M NaOH with 15 minutes heating time interval was found appropriate and selected among the other concentrations for the further studies as it gave the best correlation coefficient.

The ¹D and ²D spectra of RAN solution treated with 0.05M NaOH showed subsequent decrease in its peak and concentration (Figures 5 and 6). It was unveiled from the ¹D and ²D spectra that the parent peaks; at 283nm and 278nm, respectively; are decreased in concentration and changed in shape (²D) with the appearance of degradation products at longer and shorter wavelengths (220nm, 300nm and 311nm). The log % remained drug (Table 3) was obtained as follows:

Plotting the log % values vs time revealed that the degradation rate follows first order kinetic (Figure 7). The degradation rate was found to be [OH⁻] dependent, suggesting that the OH⁻ is playing the role of nucleophile (intermolecular hydrolysis) to give the proposed degradation process shown in Scheme 1.

Testing the effect of hydrochloric acid revealed no change in RAN spectra and concentration even at high temperatures. However, further studies are recommended to obtain the pH profile of RAN in addition to the effect of different temperatures on its stability.

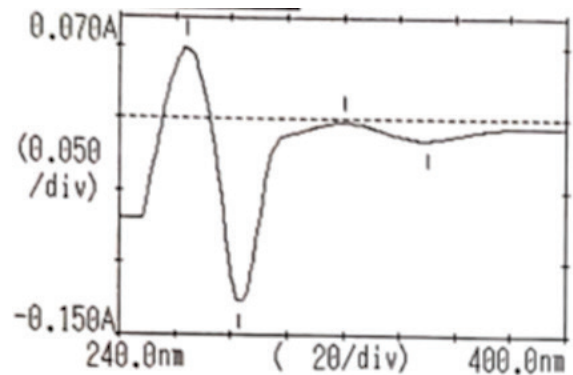


Figure 5. First derivative spectrum for alkali-treated RAN (30 minutes heating, parent drug at 283nm, degradation product at 311nm)

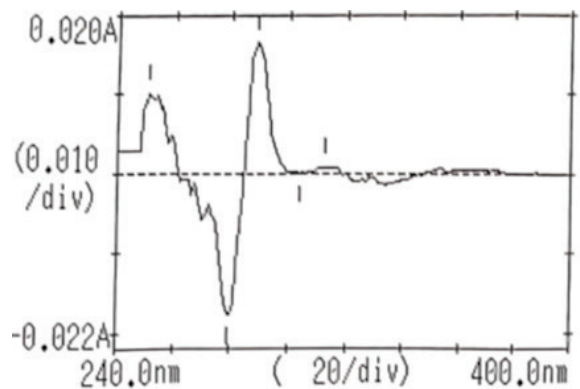


Figure 6. Second derivative spectrum for alkali-treated RAN (30 minutes heating, parent drug at 278nm, degradation products at 220nm and 311nm)

Table 3. Effect of 0.05M NaOH on the stability of RAN

Time (min.)	Parent value	% Remained	Log % drug remained
0	0.012	100.0	2
15	0.008	70.8	1.85
30	0.006	52.5	1.72
45	0.004	35.5	1.55
60	0.003	24.0	1.38

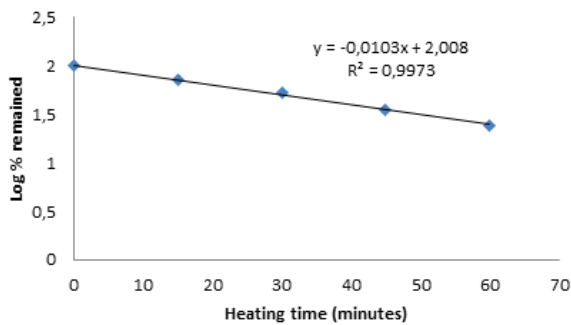
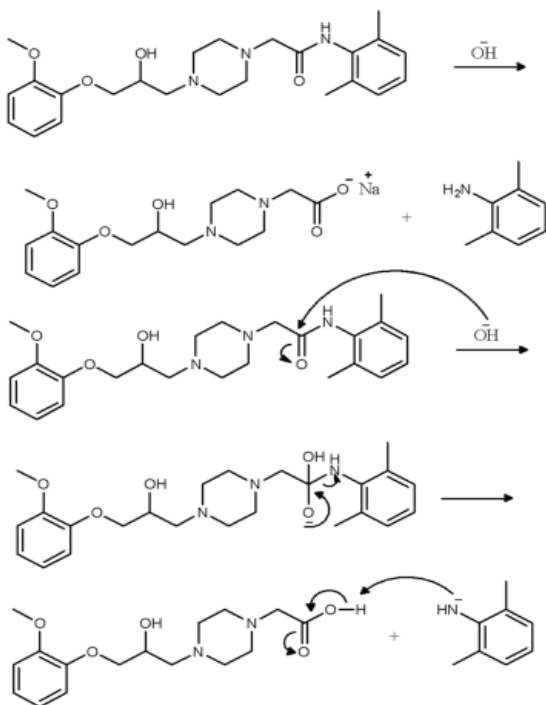


Figure 7. Effect of 0.05M NaOH on RAN degradation at 100 °C



Scheme 1: Proposed pathway of RAN alkaline hydrolysis

Light effect

RAN solution remained stable upon exposure to sunlight as there was no reduction of drug absorbance or change in the derivative spectrum. This reflected the stability of RAN solution under these conditions.

Conclusion

The developed methods proved to be simple, rapid, accurate and precise for the determination of RAN in bulk and dosage forms. In addition, the procedure of

the developed methods does not require neither extraction step nor chemicals and thus can be used for routine quality control analysis of the drug in pharmaceutical industry. The stability indicating properties of the developed methods were successfully proven. Results showed that the drug is unstable under alkaline and heating conditions as it undergoes degradation following the first order kinetics and it was found to be stable in outdoor conditions.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest

DATA AVAILABILITY

All the data supporting the findings of the present work are available with the article text.

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The authors received no financial support and all the research was self-funded.

AUTHORS CONTRIBUTION

N.K. conducted the practical work and contributed in the writing the first draft; S.S. wrote the original draft and approved the final draft version; E.G supervised and revised the final draft.

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