IJPSR (2021), Volume 12, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 December 2020; received in revised form, 26 March 2021; accepted, 01 October 2021; published 01 November 2021

DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR THE ANALYSIS AND STABILITY STUDIES OF RANOLAZINE IN BULK AND DOSAGE FORMS

A. A. Noon Kamil [1, 2], W. Shaza Shantier [* 2] and A. Elrasheed Gadkariem [2]

Department of Pharmacy, Fatima College of Health Sciences [1], United Arab Emirates.
Department of Pharmaceutical Chemistry [2], Faculty of Pharmacy, University of Khartoum, Sudan.

Keywords:

Ranolazine, Derivative Spectrophotometry, Validation, Stability, Kinetics

Correspondence to Author:
Mr. Shaza Shantier

Associate professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Sudan.

E-mail: sshantier@yahoo.com

ABSTRACT: Objectives: The present study aimed to develop simple and accurate first and second-order derivative spectrophotometric methods (1D and 2D) for the assay and stability studies of Ranolazine (RAN) in bulk and dosage forms. **Methods:** The original UV spectrum of RAN solution in methanol was differentiated instrumentally to generate the first and second derivative spectra which were measured at 278 nm and 283 nm, respectively. The developed methods were then validated as per ICH guidelines and applied for the stability studies. **Results:** Beer's law was found to be valid over the concentration range 100-600µg/ml with a correlation coefficient (r) not less than 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 24µg/ ml and 73 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. **Conclusion:** The statistical validation at a 95% confidence level proves the developed methods' sensitivity, precision, accuracy and stability-indicating properties.

INTRODUCTION: The need to develop a stability-indicating method using stress degradation has been recommended by the International Conference of Harmonization [1]. In practice, such studies often use the effects of pH and temperature changes on drug stability. 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.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(11).5827-32

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(11).5827-32

They may also serve as guides for better drug design, drug formulation, and drug analysis. The stability of as pharmaceutical product is defined as the capability of the product to retain its efficacy, properties and characteristics throughout its shelf life [2]. 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 hydrolysis [3]. Ranolazine (RAN, **Fig. 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 ($C_{24}H_{33}N_3O_4$) [4]. Although its relationship to angina symptoms is uncertain, RAN can inhibit the sodium current (I (Na) inactivating component at therapeutic levels. It also inhibits the rapid inward rectifying current (I (Kr)), thereby prolonging the ventricular action potential [5].

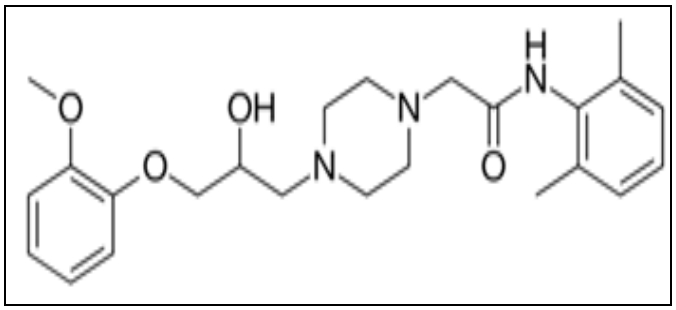


FIG. 1: CHEMICAL STRUCTURE OF RANOLAZINE

Although literature revealed different methods (chromatographic and spectroscopic methods) for the assay of RAN [6, 15], neither official pharmacopeias' methods nor stability studies using spectrophotometric methods are available. Therefore, the present work aimed to develop simple and accurate derivative spectrophotometric methods for the analysis and stability studies of RAN in bulk and dosage forms.

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; 1000 µg/ml).

Sample Stock Solution: Ten tablets of Ranexa (RAN 375 mg) were accurately weighed and grinded. An equivalent amount containing 0.1g RAN 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; 1000 µg/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), the1D and 2D order derivative spectra were recorded over the 240-400 nm range. 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 formulae 16:

$$LOD = 3.3\ SB / Slope,\ LOQ = 10\ SB/Slope$$

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

Content Uniformity: The procedure under linearity was repeated using solutions B instead of solution A. 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 to obtain concentrations of 100 µg/ml, 200 µg/ml, and 300 µg/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 result obtained were used to evaluate the precision of the developed method in terms of relative standard deviation values (RSD %).

Recovery Percentage: The freedom of interference by the tablets' excipients was confirmed by results obtained for recovery testing of the added amount of authentic RAN to the sample solution in the ratio of 1:1. Two ml of each solution A and B were transferred to separate stoppered glass tubes. Another 2 ml of solution B

was mixed with 2 ml of solution A in a third tube. The above solutions were scanned in the two modes. The recovery percentage was determined using the following equation 17:

$$\text{Percent recovery} = A_{mix} - A_{sam} / A_{std} \times 100$$

Where Amixis the absorbance of mixture, Asam is the absorbance of sample, Astdis the absorbance of the standard.

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 min heating time intervals. 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 1 mL of 1MHCl instead of NaOH to study the effect of acid on RAN stability.

Light Effect: RAN standard methanolic solution (40 µg/ml) was prepared and placed in transparent glass tubes. The solutions were then subjected to direct sunlight during mid-day time for 6 h. The photodegradation 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. However, they lack selectivity and did not prove in most cases to be a useful tool in the stability-indicating procedure. Prior to the development of derivative spectroscopy, chromatography was the most frequently 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 developing countries. The development of derivative spectroscopy presented the analyst with a tool suited for the analysis of different pharmaceutical dosage forms as it has proved to be an accurate stability-indicating method and applied for the analysis of various drugs [18, 22].

Derivative spectrophotometry is an analytical technique, which differentiates the normal spectrum by mathematical transformation 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 features [23].

Thus, our aim in the present work was to develop accurate derivative spectrophotometric methods for the analysis of RAN, which will also be 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 also comes in favor of 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 an absorption maximum at 273 nm **Fig. 2.** Since derivatization of the normal spectrum gives rise to a reduction of bandwidth, sharper peaks were achieved on the first and second-order derivative spectra of RAN, showing an absorption maximum at 278 nm and283 nm **Fig. 3** and **4.**

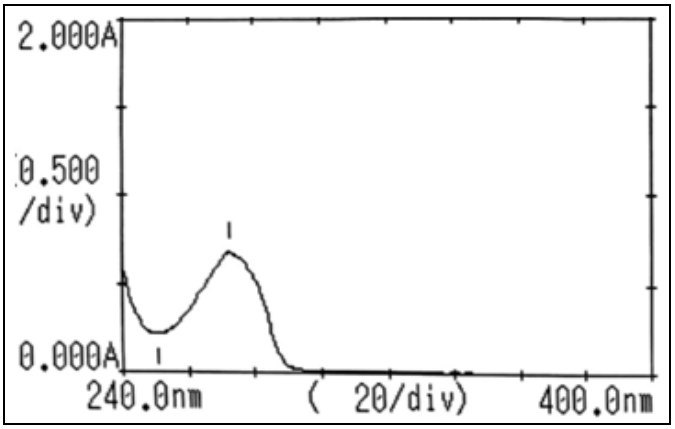


FIG. 2: UV SPECTRUM OF RAN SOLUTION (100 µG/ML; 273 NM)

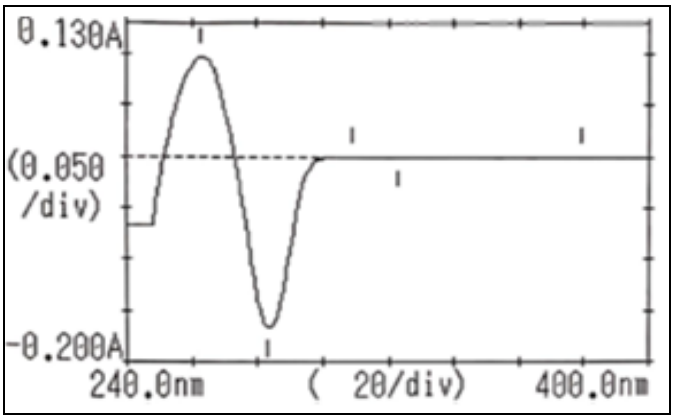


FIG. 3: FIRST DERIVATIVE SPECTRUM OF RAN SOLUTION (400 µG/ML; 283 NM)

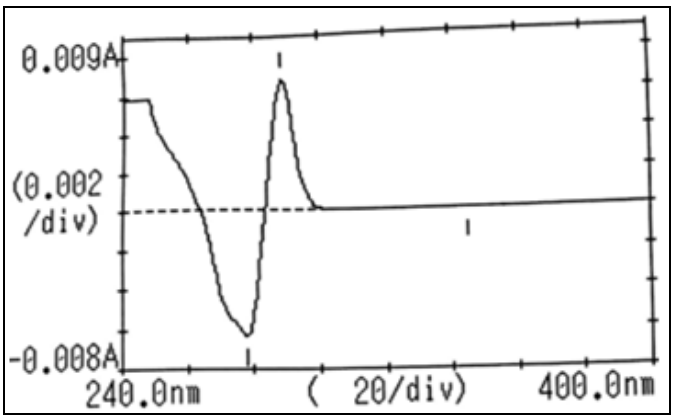


FIG. 4: SECOND DERIVATIVE SPECTRUM OF RAN SOLUTION (100 µG/ML; 278 NM)

Linearity: The calibration curves, relating RAN concentrations in a range 100-600 µg/ml to the mean absorbance values, were constructed for the 1D and 2D modes. Linearity was found to obey Beer's law 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 formula 16:

$$y = (b \pm tsb) \times + (a \pm tsa)$$

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

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*	$1.0\times10^{-}4$ $\pm2.24\times 10^{-5}$	$5.6\times10^{-5} \pm 1.13\times 10^{-5}$
Intercept ± tsa**	$4.0\times10^{-3}\pm 1.8\times 10^{-2}$	$- 5.3\times10^{-4} \pm 8.2\times10^{-3}$
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

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 formula 24:

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

Where 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 the accuracy of the developed methods.

Precision and Accuracy: The precision results showed acceptable RSD% values ranging between 0.00-2.85% for inter-day and intra-day precisions, respectively. The accuracy of the developed method and freedom of interference by tablet excipients was confirmed by the good results obtained from recovery testing (96.5±3.05%, n=3).

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 min heating time interval was found appropriate to give a good correlation coefficient.

The 1D and 2D spectra of RAN solution treated with 1 M NaOH showed a subsequent decrease in its peak and concentration **Fig. 5** and **6.** Plotting the log %remained drug *vs.* time revealed that the degradation rate follows first-order kinetic **Fig. 7.** The degradation rate was found to be [OH-] dependant, suggesting that the OH- is playing the role of nucleophile (intermolecular hydrolysis) to give the proposed degradation process shown in **Scheme 1.**

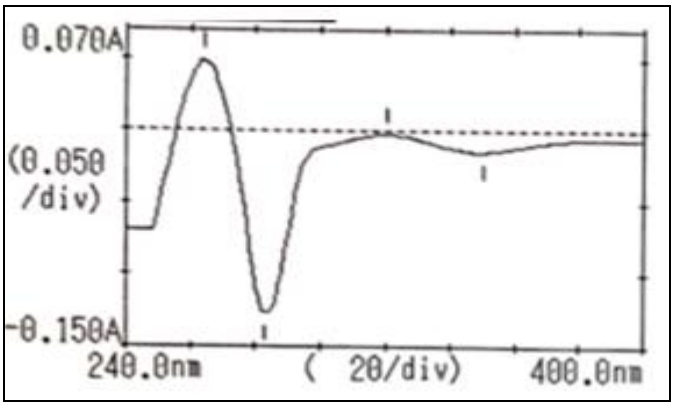


FIG. 5: FIRST DERIVATIVE SPECTRUM FOR ALKALI-TREATED RAN (0.05M NAOH; 30 MIN HEATING)

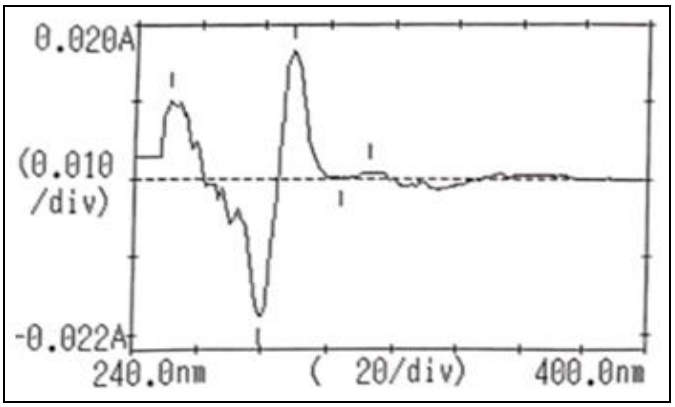


FIG. 6: SECOND DERIVATIVE SPECTRUM FOR ALKALI-TREATED RAN (0.05M NAOH; 30 MIN HEATING)

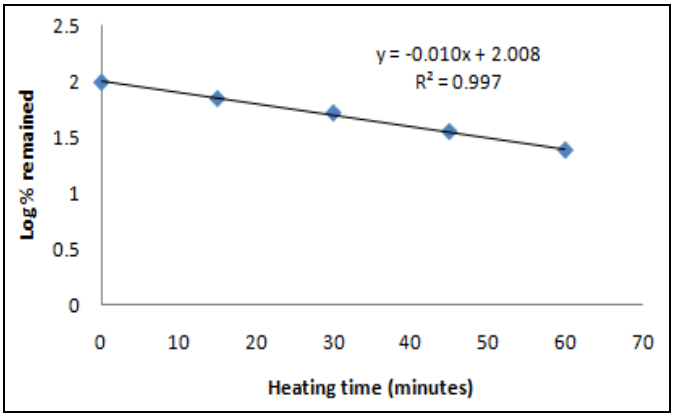
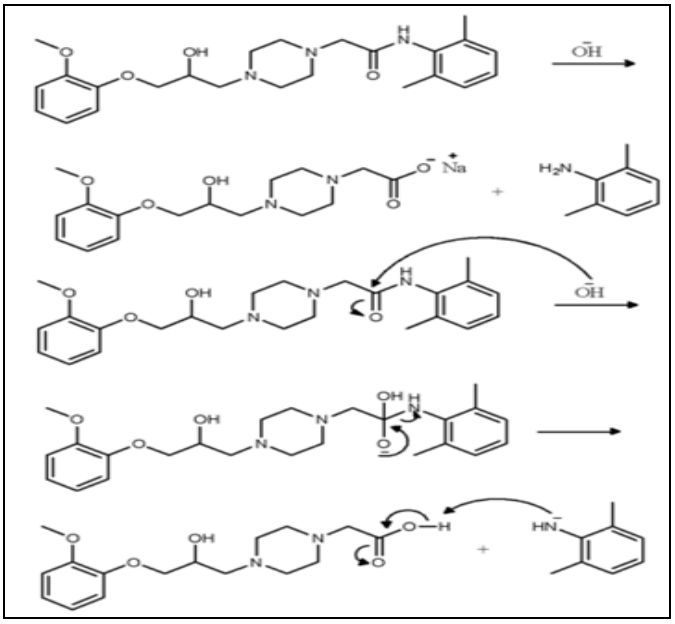


FIG. 7: EFFECT OF 0.05M NaOH ON RAN DEGRADATION AT 100 ºC



SCHEME 1: PROPOSED PATHWAY OF RAN ALKALINE HYDROLYSIS

Using hydrochloric acid, the concentration and derivative spectra of RAN were not affected by the different concentrations of acid, even at high temperatures.

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 the RAN solution under these conditions.

CONCLUSION: The developed methods proved to be simple, rapid, accurate and precise to determine RAN in bulk and dosage forms. In addition, the procedure of the developed methods does require neither extraction step nor chemicals and thus can be used for routine quality control analysis of the drug in the 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. It undergoes degradation following the first-order kinetics and was found to be stable in outdoor conditions.

ACKNOWLEDGMENT: The authors would like to thank the technical staff of the pharmaceutical chemistry lab, the University of Khartoum, for their support.

CONFLICTS 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.

Financial Support: The authors received no financial support, and all the research was self-funded.

REFERENCES:

1. ICH: harmonized tripartite guideline, stability testing of new drug substances and products. 2003; 1-15.
2. Lund W: The pharmaceutical codex. Principals and Practice of Pharmaceutics. 12th Ed London The pharmaceutical Press 1994; 69: 277-33.
3. Genaro AR Remington: The science and practice of Pharmacy, 20th ed. Philadelphia Lippincott Williams and Wilkins 2000; 986.
4. https://pubchem.ncbi.nlm.nih.gov/compound/Ranolazine
5. Product Information: RANEXA(R) extended-release oral tablets, ranolazine extended-release oral tablets. CV Therapeutics Inc Palo Alto CA 2007.
6. Bidada JP, Gonjari ID, Raut CS abd Bhutada CJ: Novel spectrophotometric method for estimation of ranolazine in bulk and pharmaceutical dosage form. Der Pharma Chemica 2011; 3(2): 1-4.
7. Doppa DVS, Konidala SK and Khanabhi S: Development and validation of UV spectroscopic method for the determination of ranolazine in bulk and formulation. Research Jour of Pharm and Tech 2019; 12(10): 5007-10.
8. Ganji R, Ramachandran D, Srinivas G, Gowardhane J, Rao P and Srilakshmi V: Development and validation of stability indicating RP-LC method for estimation of ranolazine in bulk and its pharmaceutical formulations. American Journal of Analytical Chemis 2012; 3: 378-84.
9. Gade BR, Bandhakavi SR, Satyanarayana MV and Peddi P: Development and validation of stability indicating RP-LC method forestimation of ranalozine in bulk and its pharmaceutical formulations. Journal of Chemical and Pharmaceutical Research 2015, 7(8): 454-61.
10. Khedkar AN, Veer SU, Rakh MS and Rao JR: Stability indicating method development and validation of ranolazine hydrochloride in bulk and tablet dosage form by HPTLC. IJPCR 2015; 7(1): 77-83.
11. Sharma A, Prakash D and Singh SK: Development and validation of UV spectrophotometric method for the estimation of ranolazine in bulk drug and pharmaceutical formulation, IJCRGG 2010; 2(4): 1945-48.
12. Sharma T, Si SC and Gowrisankar D: Development and validation of spectrophotometric methods for determination of ranolazine hydrochloride in pharmaceutical dosage forms. Der Pharmacia Lettre 2014; 6(4): 420-32.
13. Suresh CV, Sambasiva R and Vidya KRS SGL: Development of new visible Spectrophotometric methods for the determination of Ranolazine in pharmaceutical dosage forms. International Journal of Pharmacy and Technology 2011; 3(4): 3710-14.
14. Vetrichelvi S, Aruna A, Niraimathi V and Suresh AJ: Spectrophotometric estimation of ranolazine in bulk and tablet formulation. Int J Chem Sci 2009; 7(3): 1889-92.
15. Vetrichelvi S, Aruna A, Niraimathi V and Suresh AJ: Spectrophotometric estimation of ranolazine in tablet dosage form. Asian Journal of Chemistry 2009; 21(9): 7443-45.
16. Miller JC and Miller JN: Statistics and chemometrics for analytical chemistry. 5th ed. London, UK. Pearson Education Limited 2005.
17. Elimam MM, Shantier SW, Gadkariem EA and Mohamed MA: Derivative Spectrophotometric methods for the analysis and stability studies of colistin sulphate. Journal of Chemistry 2015; 6243165.
18. Daabees HG: Selective differential spectrophotometric methods for determination of Niclosamide and Drotaverine hydrochloride. Analytical Letters 2010; 33(4): 639-56
19. Shaza W Shantier, Elrasheed A Gadkriem, Mohamed O Adam and Magdi A Mohamed: Development of stability-indicating methods for cefquinome sulphate. International Journal of Biomedical Sciences 2013; 9(3): 161-67.
20. Shaza Wagiealla Shantier, Elrashied Ali Elobaid and Elrasheed Ahmed Gadkariem: Stability studies on Niclosamide using derivative spectroscopic and chromatigraphic methods. World Journal of Pharmaceutical Research 2015; 4(1): 122-32.
21. Mohammed M Elimam, Shaza W Shantier, Elrasheed A Gadkariem, Magdi A Mohamed and Zuheir Osman: Stability studies on florfenicol using developed derivative spectrophotometric methods. Annales Pharmaceutiques Francaises 2017; 5: 40-44.
22. Ruaa Mohamed Akode, Shaza W Shantier, Magdi A Mohamed and Elrasheed A Gadkariem: Simultaneous determination and stability studies on diaminazinediaceturate and phenazine using developed first derivative spectrophotometry. International Journal of Analytical Chemistry 2017; (2017): 4269587.
23. Sanchez F and Rojas CBO: Recent development in derivative ultra-violet/visible absorption spectrophotometry. Anal Chim Acta 2009; 635: 22-44.
24. Shantier SW, Gadkariem EA and Ibrahim KA: A colorimetric method for the determination of tobramycin. International Journal of Drug Formulation and Research 2011; 2(4): 260-72.

How to cite this article:
Noon AAK, Shaza WS and Elrasheed AG: Derivative spectrophotometric methods for the analysis and stability studies of ranolazine in bulk and dosage forms. Int J Pharm Sci & Res 2021; 12(11): 5827-32. doi: 10.13040/IJPSR.0975-8232.12(11).5827-32.