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RESEARCH ARTICLE

Simultaneous Equation and Area Under the Curve Spectrophotometric Methods for Estimation of Ranolazine Hydrochloride Presence of its Base-induced Degradation Product: A Comparative Study

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ABSTRACT

Two simple spectrophotometric methods were developed and validated for the determination of ranolazine hydrochloride in the presence of its base-induced degradation product, namely simultaneous equation method using two wavelengths of 272 and 249 nm method (A) and area under the curve method using two wavelength ranges of 267–277 nm and 244–254 nm method (B). The accuracy, precision, and linearity ranges of the planned methods were firm. The methods were validated and the specificity was assessed by analyzing synthetic mixtures containing the drug and it's degradant. The two methods were useful for the determination of the cited drug in its pharmaceutical preparation and the obtained results were statistically compared with those of a reported method. The comparison shows that there is no important difference between the proposed methods and the reported method about both accuracy and precision.

Keywords: Base degradation, ranolazine hydrochloride, spectrophotometric methods

INTRODUCTION

Ranolazine hydrochloride (RS)–N-(2,6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy)-propyl]piperazin-1-yl] acetamide [Figure 1] is an antianginal class. Ranolazine HCl is available as tablet dosage form 1 to 2. Ranolazine is not official in pharmacopoeia. A few methods in literature were reported for the determination of ranolazine HCl by ultraviolet (UV)-visible spectroscopy, high-performance liquid chromatography (HPLC), and high-performance thin-layer chromatography method.[1-3] Although these techniques are sufficiently sensitive, they use expensive instrument and time consuming. The present UV method is a simple method and does not include complicated solvent system development as required for liquid chromatography.[4,5] Therefore, this study aimed to develop and validate simple, rapid, accurate and specific, fast, low cost, and selective methods for routine quality control analysis of pharmaceutical product containing ranolazine HCl. UV spectrophotometry is an easy to use and robust method for the quantification of drugs in formulation when there is no interference from excipients.[6]

Experimental

Instruments

SHIMADZU UV-1800 PC dual-beam UV-visible spectrophotometer was used.

Software

UV-Probe personal spectroscopy software version 2.1 (SHIMADZU) was used.

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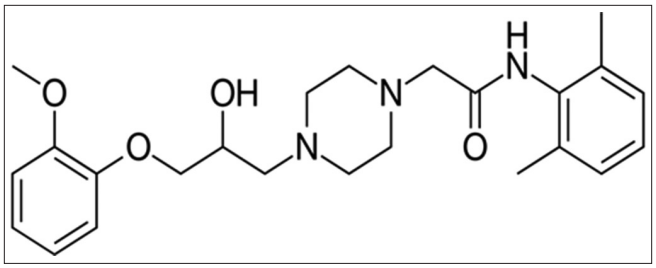

Figure 1: Chemical structure of ranolazine hydrochloride

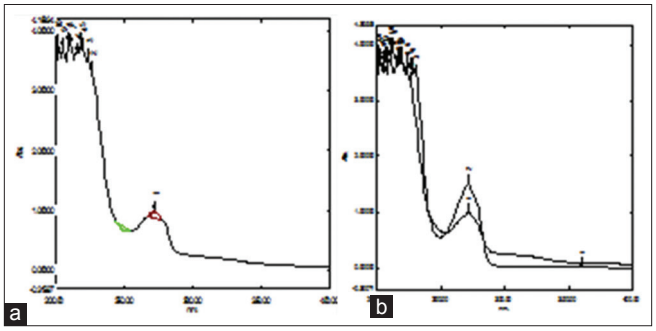

Figure 2: (a) Zero-order spectra of drug and drug degradant (20 µg/ml) (b) Zero-order absorption spectrum of ranolazine HCl (20 µg/ml) showing areas under the curve over the ranges of 244–254 and 267–277 nm

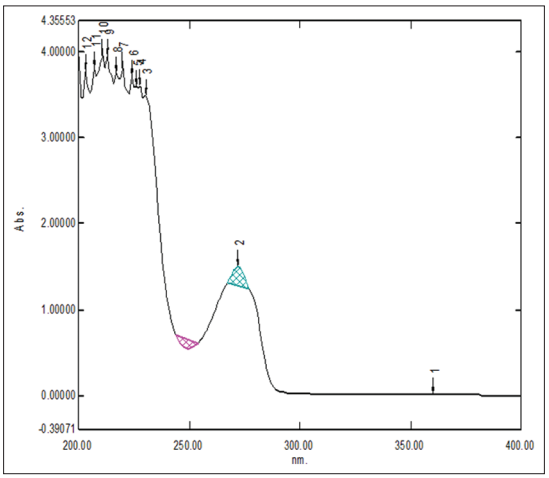

Figure 3: Zero-order absorption spectrum of ranolazine HCl degradant (20 µg/ml) showing areas under the curve over the range of 244–254 and 267–277 nm

Chemicals and reagents

All chemicals and reagents used during the work were of analytical grade and the water used during the procedure was freshly double distilled.

Ranolazine HCl powder was kindly supplied by Glenmark Pharmaceuticals Ltd., Mumbai.

Pharmaceutical preparation: Ranolaz® 500 mg tablet (Batch no. 25317213) manufactured by Torrent Pharmaceuticals Company. It is labeled to contain 500 mg of ranolazine HCl per capsule and purchased from local Pharmacy. Sodium hydroxide and methanol were used as analytical grade.

Standard solutions

Standard solution of ranolazine HCl

A standard solution of ranolazine (100 µg/ml) was prepared by dissolving 10 mg of ranolazine HCl in 50 ml of methanol and volume was made up to 100 ml with methanol.

Standard solution of degradation product

About 10 ml of 100 µg/ml of stock solution was taken in round bottom flask and was refluxed with 10 ml 1 N NaOH for 2 h. From the refluxed solution, 2 ml quantity is transferred to 10 ml volumetric flask and volume was made with water to get a concentration equals to 20 mg/ml of degraded ranolazine HCl.

Procedure

Linearity and construction of calibration curves

From the standard solution (100 µg/ml), the concentrations of 5–30 µg/ml for ranolazine HCl were prepared in distilled water. The absorbance of the solutions was recorded using UV-visible spectrophotometer using water as a blank.

Simultaneous equation method

The absorbances of component were recorded at 249 nm and 272 nm and the absorptivity values were calculated. The absorbance and absorptivity values were used for calculating the concentration of ranolazine HCl using the following equations:

$Cx = (A_2\, a_{y1} - A_1\, a_{y2})/a_{x2}\, a_{y1} - a_{x1}\, a_{y2}$

$Cy = (A_1\, a_{x2} - A_2\, a_{x1})/a_{x2}\, a_{y1} - a_{x1}\, a_{y2}$

where,

Cx and Cy = Concentrations of x and y.

a_{x1} and a_{x2}= Absorptivities of x at λ1 (lmax of x) and λ2 (lmax of y).

a_{y1} and a_{y2}= Absorptivities of y at λ1 (lmax of x) and λ2 (lmax of y).

A_1 and A_2= The absorbance of the diluted samples at λ1 and λ2.

Area under the curve method

The absorption spectra (from 200 to 400 nm) of the solutions were recorded using water as blank. The area under the curve values for each component were recorded over the wavelength ranges of 270–280 nm and 240–250 nm and the calibration graphs were constructed. The area absorptivity values were calculated at each wavelength range for the two components and then the concentration of ranolazine was calculated from the equations:

$A_1 = a_{x1} C(x) + a_{y1} C(y)$ (λ1-λ2) nm

$A_2 = a_{x2} C(x) + a_{y2} C(y)$ (λ3-λ4) nm

$C(x) = [A_2 \times a_{X2} - A_1 \times a_{y2}]/[a_{X2} \times a_{Y1} - a_{X1} \times a_{Y2}]$

$C(y) = A_2 - a_{X2} \times C(x)/a_{Y2}$

where,

a_{x1} and a_{x2} are absorptivities of x at (λ1-λ2) and (λ3-λ4), respectively.

a_{Y1} and a_{Y2} are absorptivities of y at (λ1-λ2) and (λ3-λ4), respectively.

A1 and A2 are AUC of mixed standard at (λ1-λ2) and (λ3-λ4), respectively.

C(x) and C(y) are the concentration of x and y, respectively.

Application to laboratory prepared mixtures

The aliquots of ranolazine HCl and its degradant were transferred from their working solutions into a series of 10 ml volumetric flasks to prepare mixtures containing different ratios. The volumes were completed with the methanol. The spectra of the prepared solutions were scanned for 200–400 nm. The concentrations of ranolazine HCl were calculated as described under linearity for each proposed method.

Application to pharmaceutical preparation

A content of 10 tablet of Ranzol 500 tablets was mixed well and accurately weighed; then, an accurate portion equivalent to 10 mg was extracted by shaking with 50 ml of water for 15 min, then filtered into 100 ml volumetric flask and the volume was adjusted with the same solvent. Repeat the general procedure described under linearity using aliquots covering the working concentration range. Determine the drug concentrations from the equations described under linearity.

RESULTS AND DISCUSSION

The spectrophotometric methods have the advantages of being the mainly simple, fast, and applicable in all laboratories, as most of the active compounds show absorbance in the UV region. The compounds susceptible to degradation may present with its degraded form. They may exhibit strongly overlapping spectra that interfere the determination of the main compound. The zero-order spectra of ranolazine HCl and its degradant show severe overlapping. Hence, the cited methods were used to overcome this interference from the degradation product.

Simultaneous equation method

The absorbance of ranolazine HCl and its degradant was recorded at two wavelengths of 272 nm and 249 nm for intact ranolazine HCl and its degradant simultaneously. Absorption value at these two wavelengths (λ1, λ2) of ranolazine HCl and its degradant in the concentration ranges of 5–30 mg/mL for both was calculated. The absorptivity of ranolazine HCl and its degradant was calculated at each wavelength. The concentrations of ranolazine HCl can be obtained by applying Cramer's rule and matrices in following Equations. Concentrations of ranolazine HCl in the presence of its degradant were calculated according to the following equations:

A_1 = 0.13916 Ranolazine + 0.72793R degradate at 272 nm λ1

A_2 = 0.05670 Ranolazine + 0.90905R degradate at 249 nm λ2

Where ranolazine HCl and degradant are the concentrations of ranolazine HCl and its degradant in 20 mg/mL, respectively, 0.13916 and 0.13916 are the absorptivity values of ranolazine HCl at λ1 and λ2, respectively, 0.72793 and 0.90905 are absorptivity values of degradant at λ1 and λ2, respectively. A1 and A2 are the absorption values of sample solutions at the wavelengths (λ1, λ2), respectively. Under the described experimental conditions, the calibration graphs for the method were constructed by plotting the absorbance at the two selected wavelengths

Table 1: Linearity studies and regression equations of the proposed methods

Parameters	Simultaneous Equation Method Values		Area Under Curve Method Values	
Wavelength (nm)	249 nm	272 nm	244–254	267–277
Linearity range (μg/ml)	5–30	5–30	5–30	5–30
Slope	0.0276	0.0546	0.1458	0.2468
Intercept	0.0064	0.0089	0.0060	0.0058
Regression coefficient (r^2)	0.9982	0.9976	0.9989	0.9998
LOD (μg/ml)	0.246	0.358	0.452	0.267
LOQ (μg/ml)	0.9256	0.9742	1.4682	0.8294
Accuracy (mean±%RSD)[a]	98.80±0.564		98.60±0.628	
Precision				
Repeatability (%RSD)[b]	0.652		0.942	
Intermediate precision (RSD)[c]	1.246		1.542	

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation, In simultaneous equation method: working at 2 wavelengths 249 and 272 nm, In AUC method: working at 2 wavelengths ranges (244-254) and (267-277) nm

Table 2: Determination of ranolazine HCl in laboratory prepared mixture with its acid degradation by the proposed methods

Ranolazine concentration (μg/ml)	Degradant concentration (μg/ml)	Degradant %	Simultaneous equation	Area under the curve
30	5	10	98.90	99.89
25	10	20	97.68	97.85
20	15	30	99.50	98.96
15	20	40	96.58	97.56
10	25	50	98.67	98.23
5	30	60	99.68	99.58
Mean			98.52	99.62
SD			0.854	0.026

Table 3: Application of standard addition technique to the analysis of Ranolaz 500 mg tablets by applying the proposed method

Pharmaceuticals taken(μg/ml)	Standard added (μg/ml)	Simultaneous equation	Area under the curve
1 ml	0.5	100.56	100.68
	1	100.23	100.62
	1.5	99.54	99.32
Mean±RSD%		99.56±0.235	100.23±0.145

Table 4: Statistical comparison between the results obtained by applying the proposed and reported method for determination of ranolazine in Ranolaz 500 mg tablet

Parameter	Simultaneous equation	Area under the curve
Mean	99.45	99.56
N*	5	5
SD	0.4556	0.3514
%RSD	0.4256	0.3645
t**	0.896	0.954
F**	1.2354	1.2546

*No of experimental, **The values in the parenthesis are tabulated values of t and F at (p¼0.05).

versus drug concentrations in μg/ml. The yielded statistical results are summarized.

Area under the curve method

The area under the curve for ranolazine HCl and its degradant was recorded over the ranges of 244–254 and 267–277 nm for intact ranolazine and for its degradant in the concentration range of 5–30 mg/mL for both ranolazine and its degradant. The absorptivity "Y" values of ranolazine and its degradant

were calculated at each wavelength range. The concentrations of ranolazine HCl can be obtained by applying Cramer's rule and matrices. Concentrations of ranolazine in the presence of its degradant were calculated according to the following equations:

A1 = 0.55219 Ranolazine + 0.90905R degradate at 244–254 nm (λ1-λ2)

A2 = 1.50606 Ranolazine + 0.72793R degradate at 267–277 nm (λ3-λ4)

Where ranolazine HCl and ranolazine HCl degradant are the concentrations of ranolazine and its degradant in mg/mL, respectively, 0.55219 and 1.50606 are the absorptivity values of ranolazine at (λ1-λ2) and (λ3-λ4), respectively, 0.90905 and 0.72793 are absorptivity values of degradant at (λ1-λ2) and (λ3-λ4), respectively. The proposed area under the curve method was successfully applied for the determination of ranolazine HCl in the presence of its degradant in their laboratory prepared mixtures. Under the described experimental conditions, the calibration graphs for the method were constructed by plotting the area under the curve over the two selected wavelength ranges versus drug concentrations in mg/ml. The yielded statistical results are summarized

Methods validation

The planned methods were validated in compliance with the ICH guidelines. Linearity, range limit of detection, limit of quantification, accuracy, and precision of the proposed methods are shown in Table 1 while Table 2 shows the specificity; recovery of the laboratory prepared mixture of ranolazine with its base degradation product. The validity of the proposed procedures is further assessed by applying the standard addition technique showing no excipients interference.

Application to pharmaceutical preparation

The proposed methods were applied for the determination of ranolazine HCl in Ranolaz® 500 mg tablet. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method. No significant differences were found by applying *t*-test and F-test at 95% confidence level (Tables 3 and 4.), indicating good accuracy and precision of the proposed methods for the analysis of the studied drug in its pharmaceutical dosage form.

CONCLUSION

Unlike the mostly recommended HPLC procedures, the proposed mathematical methods are simple and not expensive. The reagents used in the proposed methods are cheap and readily available. The procedures applied in each method do not involve any critical reactions or tedious sample preparations. The cited methods offer distinct possibility of assaying ranolazine in its pharmaceutical formulation without interference due to the excipients or the degradation products. The suggested methods are found to be simple, accurate, selective, and equally sensitive with no significant difference of the precision compared with the reference method. They could be applied for routine analysis of pure drug or in its pharmaceutical formulation (either alone or in the presence of its degradation product).

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