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Stability Indicating Method Development and Validation of Ranolazine Extended Release Tablets

Evin Aycicek DURAK[1], İbrahim KURTGÖZ[2], Burcu MESUT[1], Erdal CEVHER[1], Yıldız ÖZSOY[1*]

1 Istanbul University, Faculty of Pharmacy, Pharmaceutical Technology Dept., Beyazıt, Istanbul, Turkey
2 Biopharma Pharmaceutical, Istanbul, Turkey

ABSTRACT

The objective of this study was to develop and validate the stability-indicating method for newly developed Extended-Release tablet formulation of Ranolazine. First, new Ranolazine tablet formulation was developed. These tablets were analyzed by using a High-Performance Liquid Chromatography system with a UV detector at 220 nm wavelength and by using C8-3 column (150 mm x 4.6 mm i.d; 5 µm particle size). The injection volume of the system was 10 µl. The validation parameters; Selectivity, linearity, accuracy, robustness, precision and limit of quantification and detection parameters were proved good results. A highly sensitive and simple HPLC-UV analytical method of the Ranolazine tablet formulation was developed in accordance with ICH Guideline Q2 and Q3.

Keywords: Ranolazine, HPLC, forced degradation, stability indicating method, validation

INTRODUCTION

Ranolazine, (RNZ, N-(2,6-dimethylphenyl)-2-{4-[2-hydroxy-3-(2-methoxyphenoxy) propyl] piperazine-1-yl}acethamide) is a piperazine derivative drug substance, which has anti-ischemic and antianginal effects[1-3]. The chemical structure of RNZ[4] is given in Figure 1. RNZ is a stereo isomer molecule. The pharmacological activity of enantiomers shows statistically insignificant differences[5, 6].

*Corresponding author:
Yıldız Özsoy
Istanbul University, Faculty of Pharmacy, Pharmaceutical Technology Dept., Beyazıt. yozsoy@istanbul.edu.tr
ORCIDs:
Evin Ayçiçek Durak: 0000-0003-0724-6698
İbrahim Kurtgöz: 0000-0002-0836-8622
Burcu Mesut: 0000-0003-2838-1688
Erdal Cevher: 0000-0002-0486-2252
Yıldız Özsoy: 0000-0002-9110-3704
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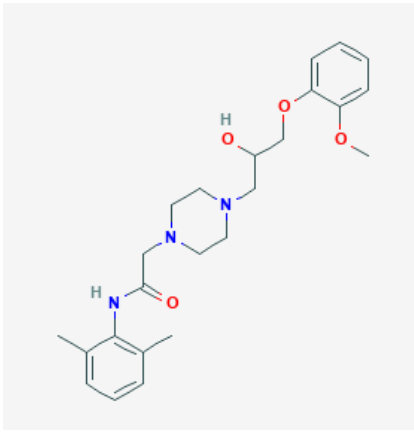

Figure 1. The chemical structure of RNZ

In the past decades, RNZ is the only anti arrhythmic medication developed and marketed for the treatment of chronic angina[7]. RNZ inhibits selectively fast and late sodium current. RNZ is a well-tolerated drug without showing any bradycardia and/or hypotension effect in contrast to β-blockers, Ca^{++} blockers, and nitrates[8, 9]. Although there is no convincing proof, RNZ has been shown to improve glycometabolic homeostasis in rats by enhancing insulin function [10, 11].

Many patients sustain persistent angina, despite the using combined drug therapies[12]. In some cases, the use of additional antianginal agents with a novel mechanism of action, such as RNZ, is unavoidable. RNZ extended release (ER) tablets are developed and marketed due to the short half-life of RNZ immediate release tablets. Because of the increasing consumption of RNZ, separate chromatographic methods for its analysis were reported in the literature. There is no official monograph for RNZ in the pharmacopoeias, in this context developing and validating stability indicating chromatographic method could be useful.

The aim of developing RNZ ER tablets is to reduce the severity and frequency of angina pectoris symptoms and improving the life standard of patient[13]. Common granulation techniques such as wet and dry granulation were used to prepare RNZ ER tablets, indicated in the literature. The effect of different pH-dependent[14-16] and/or pH-independent polymers on the release of RNZ ER tablets were observed[17-19] in these studies.

The objective of this study was developing and validating stability indicating method for prepared ER tablet formulation of RNZ. The developed method was validated RNZ and its impurities. The proposed chromatographic method was applied to the assay of commercial Ranexa ER tablet and prepared RNZ ER tablet.

METHODOLOGY

Chemical, reagents and materials

Ranolazine, Ranolazine Working Standard (WS), Zen-I Impurity WS, Zen-II Impurity WS, and Zen-III Impurity WS were purchased from Aarti Healthcare (Mumbai-India). Acetonitrile, methanol, hydrochloric acid, perchloric acid, phosphoric acid, ammonium dihydrogen phosphate, sodium hydroxide, ethanol, and triethylamine were purchased from J.T.Baker (AP-USA). Fumaric acid was a kind gift from Merck Millipore (Darmstadt-Germany). Povidon (Kollidon® K30) was purchased from BASF (Florham Park-USA). Xanthan gum was purchased from CP-Kelco (USA). Hypromellose (Benecel™ K15M) was a gift from Ashland (Covington-USA). Magnesium stearate was purchased from Faci (Carasco GE Italy).

Preparation of RNZ ER tablets

RNZ 500 mg ER tablets were prepared by wet granulation method. The high shear mixer was used for granulation. The granulation process designed in two stages. At the first stage, fumaric acid and Ranolazin were granulated with granulation solvent consist of purified water and PVP. The wet mass sieved and dried in the oven. Afterward, dried bulk was sieved and this premix blended with hypromellose and xanthan gum. The second granulation was carried out with ethanol. The wet mass sieved and dried in the oven. The dried mass eluted and mixed with magnesium stearate to obtain the final blend. The unit formula and the function of the raw materials of RNZ ER tablets are shown at the Table 1. The final blend was compressed by using rotary tablet press machine (Manesty XSpress).

Table 1. The unit formula and the function of the raw materials of RNZ ER tablet

Raw materials	Function	% w/w
Ranolazine	Active ingredient	63,69
Fumaric acid	Diluent	25,48
Povidon	Binder	2,42
Hypromellose	Controlled release agent	2,04
Xanthan gum	Controlled release agent	5,10
Magnesium stearate	Lubricant	1,27
Purified water*	Granulation solvent	-
Ethanol*	Granulation solvent	-

*purified water and ethanol were used as granulation solvent and do not exist in the finished product.

Instrumentation and analytical conditions

Analysis method of RNZ and its impurities were chromatographic. A Waters *High Performance Liquid Chromatography* (HPLC) system (New Castle-USA) with UV detector was used. The method validation was made in accordance with ICH Guideline Q2 and Q3[20, 21].

Chromatographic condition for quantization of RNZ

Inertsil C8-3 column (150 mm x 4.6 mm i.d; 5 µm particle size) from GL Sciences (Japan), was performed for chromatographic separation of RNZ. The temperature of column was set and maintained at 30°C. The UV detection was fixed to 220 nm wavelength and the injection volume was 10 µl. The mobile phase was consisting of acetonitrile (A) and buffer solution (B) (30:70). Buffer solution was prepared with triethylamine: purified water; 4.2:1000 (v/v) and the pH of solution was adjusted to 3.00 ± 0.05 with phosphoric acid. The flow rate was set to 1.0 ml/min. The injection time was 9 minutes.

Chromatographic condition for quantization of impurities

Waters XTerra RP18 column (250 mm x 4.6 mm i.d; 5 µm particle size) from Waters (New Castle-USA), was performed for chromatographic separation of Zen-I (2-((2-methoxyphenoxy) methyl) oxirane), Zen-II (2-chloro-N-(2,6-dimethylphenyl) acetamide), and Zen-III, RNZ impurities. The UV detection was fixed to 210 nm wavelength and the injection volume was 20 µl. The mobile phase X was consisting of ammonium dihydrogen phosphate solution (C) and methanol (D) (80:20). The mobile phase Y was consisting of ammonium dihydrogen phosphate solution (C) and methanol (D) (20:80). Ammonium dihydrogen phosphate solution was prepared with ammonium dihydrogen phosphate: purified water; 5.75:1000 (w/v) and filtered (0.45 µm PET filter). The injection time was 40 minutes. The following gradient, at a fixed flow rate of 1.0 ml/min was used: from 0 to 5 min the composition was X: Y, 90:10. From 5 to 15 min the composition was X: Y, 80:20. From 15 to 27 min the composition was X: Y, 40:60. From 27 to 32 min the composition was X: Y, 20:80. From 32 to 40 min the composition was X: Y, 90:10.

Preparation of standard and sample solutions

Preparation of standard and sample solutions for validation of RNZ

Preparation of standard solution: 50 mg RNZ WS was accurately weighed to a 20 ml volumetric flask and then 10 ml 0.1 N HCl solution was added to the flask, mixed 1 min by vortex and then kept in an ultrasonic bath during 15 min to obtain a clear solution. The volumetric flask was complemented to designated

volume with 0.1 N HCl solution. 5.0 ml of this solution precisely was transferred to a 50 ml volumetric flask and complemented to designated volume with the mobile phase. The obtained solution was filtered 0.45 µm PET filter and transferred to HPLC vials (the concentration of solution 0.25 mg/ml).

Preparation of sample solution: 20 RNZ ER tablets were weighed and pulverized by grinding with the help of mortar and pestle. The obtained powder, equivalent to 250 mg RNZ was accurately weighed and transferred to a 100 ml volumetric flask. The volumetric flask was complemented to designated volume with the mobile phase. The obtained solution was filtered 0.45 µm PET filter and transferred to HPLC vials (the concentration of solution 0.25 mg/ml).

Preparation of RNZ standard stock solution: 125 mg RNZ WS was accurately weighed to a 50 ml volumetric flask and, then 25 ml 0.1 N HCl solution was added to the flask, mixed 1 min by vortex and then kept in an ultrasonic bath during 15 min to obtain a clear solution. The volumetric flask was complemented to designated volume with 0.1 N HCl solution (the concentration of solution 2.5 mg/ml).

Preparation of standard and sample solution for validation of impurities

Preparation of RNZ standard solution: 20 mg RNZ WS was accurately weighed to a 50 ml volumetric flask and then 30 ml mobile phase X solution was added to the flask, mixed 1 min by vortex. 5.0 ml of this solution precisely was transferred to a 50 ml volumetric flask and complemented to designated volume with mobile phase X. 5.0 ml of this solution precisely was transferred to a 50 ml volumetric flask and complemented to designated volume with the mobile phase X. The obtained solution was filtered 0.45 µm PET filter and transferred to HPLC vials (the concentration of solution 0.002 mg/ml).

*Preparation of placebo solution:*124 mg RNZ ER tablet placebo was weighed to a 100 ml volumetric flask and, then 70 ml mobile phase X solution was added to the flask, and kept in an ultrasonic bath during 10 min. The volumetric flask was complemented to designated volume with the mobile phase X solution and kept in an ultrasonic bath during 10 min. The obtained solution was filtered 0.45 µm PET filter and transferred to HPLC vials.

Preparation of sample solution: The pulverized RNZ ER tablets, equivalent to 200 mg RNZ was accurately weighed and transferred to a 100 ml volumetric flask and, then 70 ml mobile phase X solution was added to the flask and kept in an ultrasonic bath during 10 min. The volumetric flask was complemented to designated volume with mobile phase X solution and kept in an ultrasonic bath

for 10 min. The obtained solution was filtered 0.45 μm PET filter and transferred to HPLC vials (the concentration of solution 2.0 mg/ml).

Preparation of Zen-I, Zen-II, and Zen-III standard stock solution: 7.5 mg Zen-I WS was accurately weighed to a 25 ml volumetric flask and then 10 ml mobile phase X solution was added to the flask, and kept in an ultrasonic bath during 15 min. The volumetric flask was complemented to designated volume with mobile phase X solution and mixed by a vortex (the concentration of solution 0.3 mg/ml). The same procedure was applied to obtain Zen-II and Zen-III standard stock solutions.

Preparation of 100 % preparative sample solution: The pulverized RNZ ER tablets, equivalent to 200 mg RNZ was accurately weighed and transferred to a 100 ml volumetric flask and then 70 ml mobile phase X solution was added to the flask and kept in an ultrasonic bath during 10 min. From each standard stock solutions respectively Zen-I, Zen-II, and Zen-III, 1.0 ml solution were added to the flask and complemented to designated volume with the mobile phase X solution and kept in an ultrasonic bath during 10 min. The obtained solution was filtered 0.45 μm PET filter, and transferred to HPLC vials (C_{RNZ}:2.0 mg/ml, C_{ZEN-I}:0.003 mg/ml, C_{ZEN-II}: 0.003 mg/ml, $C_{ZEN-III}$:0.003 mg/ml).

Preparation of 200 % preparative sample solution: The pulverized RNZ ER tablets, equivalent to 200 mg RNZ was accurately weighed and transferred to a 100 ml volumetric flask and then 70 ml mobile phase X solution was added to the flask and kept in an ultrasonic bath during 10 min. From each standard stock solution respectively Zen-I, Zen-II, and Zen-III, 2.0 ml solution were added to the flask and complemented to designated volume with mobile phase X solution and kept in a ultrasonic bath during 10 min. The obtained solution was filtered 0.45 μm PET filter, and transferred to HPLC vials (C_{RNZ}:2.0 mg/ml, C_{ZEN-I}:0.006 mg/ml, C_{ZEN-II}: 0.006 mg/ml, $C_{ZEN-III}$:0.006 mg/ml).

Method validation protocol

Analytical method validation is done to demonstrate that the obtained results are precise and valid and to show the applicability of the method. The stability-indicating method was validated according to ICH Guidelines Q2 and Q3. In this context linearity, selectivity, accuracy, robustness, precision, the limit of quantification and detection were evaluated.

Forced degradation studies

Forced degradation studies were performed to prove the stability indicating capability of the method. Prepared samples and placebos were exposed to:

Acidic condition (1N HCl / room temperature for 24 hours)

Basic condition (1 N NaOH / room temperature for 24 hours)

Oxidative condition (H_2O_2 30% / room temperature for 24 hours)

Thermal condition (60 ºC ± 2 ºC / for 1 week)

Light (1.2 million lux hours)

Acidic condition

The pulverized RNZ ER tablets, equivalent to 200 mg RNZ was weighed to a 100 ml volumetric flask and then 20 ml ammonium dihydrogen phosphate solution was added to the flask and kept in an ultrasonic bath during 15 min. Then 10 ml methanol was added to the flask and kept in ultrasonic bath for 1 min. Afterward 15.0 ml 1 N HCl was added. The prepared solution-maintained room temperature for 24 hours. At the end of time the solution was neutralized with 15.0 ml 1 N NaOH and the volumetric flask was complemented with ammonium dihydrogen phosphate solution to designated volume. The flask was kept in an ultrasonic bath for 15 min. The obtained solution was filtered 0.45 µm PET filter and transferred to HPLC vials (the concentration of solution 2.0 mg/ml). The same procedure was applied to the placebo.

Basic condition

The pulverized RNZ ER tablets, equivalent to 200 mg RNZ was weighed to a 100 ml volumetric flask and, then 20 ml ammonium dihydrogen phosphate solution was added to the flask and kept in an ultrasonic bath for 15 min. Then 10 ml methanol was added to flask and kept in ultrasonic bath for 1 min. Afterward 15.0 ml 1 N NaOH was added. The prepared solution-maintained room temperature for 24 hours. At the end of time the solution was neutralized with 15.0 ml 1 N HCl and the volumetric flask was complemented with ammonium dihydrogen phosphate solution to designated volume. The flask was kept in an ultrasonic bath for 15 min. The obtained solution was filtered through 0.45 µm PET filter and transferred to HPLC vials (the concentration of solution 2.0 mg/ml). The same procedure was applied to the placebo.

Oxidative condition

The pulverized RNZ ER tablets, equivalent to 200 mg RNZ was weighed to a 100 ml volumetric flask and then 20 ml ammonium dihydrogen phosphate solution was added to the flask, and kept in an ultrasonic bath for 15 min. Then 10 ml methanol was added to the flask and kept in ultrasonic bath for 1 min. Afterward 15.0 ml H_2O_2 30% was added. The prepared solution-maintained room

temperature for 24 hours. At the end of time the volumetric flask was complemented with ammonium dihydrogen phosphate solution to designated volume. The flask was kept in an ultrasonic bath for 15 min. The obtained solution was filtered 0.45 μm PET filter and transferred to HPLC vials (the concentration of solution 2.0 mg/ml). The same procedure was applied to the placebo.

Thermal condition

RNZ ER tablets and placebo were kept at 60 °C ± 2 °C in the oven for a week. At the end of the time the pulverized RNZ ER tablets, equivalent to 200 mg RNZ was weighed to a 100 ml volumetric flask, and then 70 ml ammonium dihydrogen phosphate solution was added to the flask and kept in an ultrasonic bath for 10 min. The solution was kept at room temperature for 5 min and complemented with ammonium dihydrogen phosphate solution to designated volume. The flask was kept in an ultrasonic bath for 10 min. The obtained solution was filtered 0.45 μm PET filter and transferred to HPLC vials (the concentration of solution 2.0 mg/ml). The same procedure was applied to the placebo.

Light (1.2 million lux hours)

RNZ ER tablets and placebo were exposed to light for 24 hours (1.2 million lux hours). The procedure applied according to ICH guideline Q1B. At the end of the time the pulverized RNZ ER tablets, equivalent to 200 mg RNZ was weighed to a 100 ml volumetric flask and then 70 ml ammonium dihydrogen phosphate solution was added to the flask and kept in an ultrasonic bath for 10 min. The solution was kept at room temperature for 5 min and complemented with ammonium dihydrogen phosphate solution to designated volume. The flask was kept in an ultrasonic bath for 10 min. The obtained solution was filtered 0.45 μm PET filter and transferred to HPLC vials (the concentration of solution 2.0 mg/ml). The same procedure was applied to the placebo.

RESULTS and DISCUSSION

Method optimization

In order to optimize the assay method, several mobile phase solvents were tested. In this context 0.1 N HCl, pH 6.8 phosphate buffer, acetonitrile: buffer (ammonium dihydrogen phosphate) (80:20), methanol: water (80:20), acetonitrile: water (80:20), acetonitrile: pH 6.8 phosphate buffer (80:20), acetonitrile: 0.1 N HCl (80:20) were tried and the condition that enabled a suitable resolution and shape of peaks was acetonitrile: buffer (80:20). Also different sample and standard preparation solvents: acetonitrile + 0.1 N HCl (1:10); water + 0.1 N HCl (1:5); mobile phase and different sample preparation processes (mixing with vor-

tex, keeping in an ultrasonic bath) were tried. The results of the analysis of these changes showed that for sample preparation RNZ ER tablet using mobile phase and for standard preparation using 0.1 N HCl as described at *2.4. Preparation of standard and sample solutions* were the best way of solving RNZ.

To optimize and validate the analytical method of RNZ impurities for RNZ ER tablets, data obtained from *AARTI Healthcare Related Substances Analytical Method* was modified and used.

The obtained features provided the chromatogram reported in Figure.2. a good peak shape and peak resolution were obtained within acceptable analysis time (20 min).

Method validation

Selectivity

To assess the selectivity of the method, placebo solution, mobile phases, sample solution of RNZ ER tablet, RNZ WS, Zen-I, Zen-II, and Zen-III impurity solutions were injected in duplicate and ability of chromatographic separation of each sample was evaluated. The chromatogram shown Figure. 2 was obtained. Five peaks were obtained from the chromatogram. Between the peaks of RNZ, solvent, mobile phases, placebo and impurities did not observe any interference. All peaks completely separated from another one.

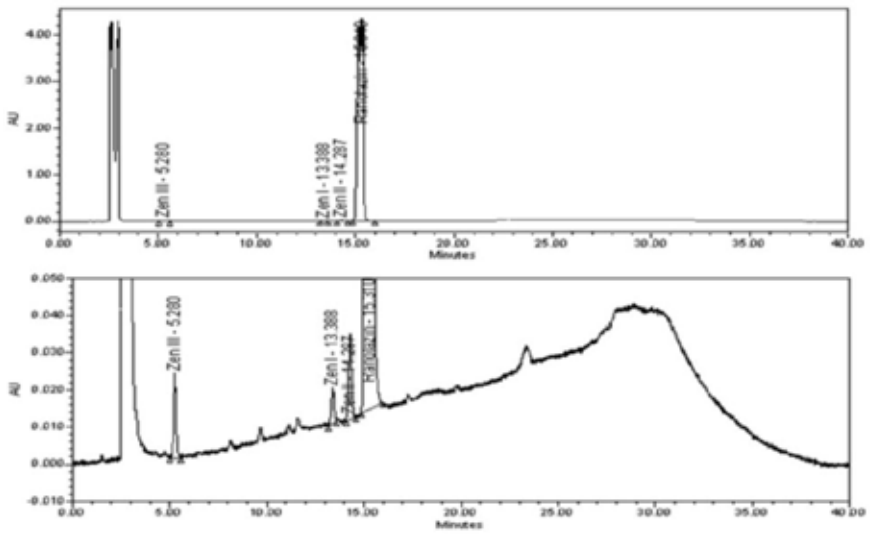

Figure 2. HPLC chromatogram (λ=220 nm) of a solution containing RNZ ER tablet, impurities Zen-I, Zen-II and Zen-III.

Linearity

The linearity of the method was evaluated for five concentration levels by diluting the standard stock solution corresponding to 20, 50, 80, 100, and 120 %. The prepared solutions were filtered and injected in triplicate. For impurities, response and correction factor were calculated using the formula shown below:

$$RRF = \frac{m_i}{m_s} \qquad CF = \frac{1}{RRF}$$

RRF: Response factor

CF: Correction factor

m_i: the slope value obtained from the calibration curve of the impurity standard solutions

m_s: the slope value obtained from the calibration curve of the active substance standard solutions

The obtained data showed that in the expected concentration RNZ and its impurities are linear. The summary of the results is reported in Table 2. The determination coefficient of all compounds was found greater than 0,9999, these results show the precision of the proposed methods[22].

Table 2. The summary of five levels calibration graphs for RNZ, Zen-I, Zen-II and Zen-III (y=ax+b), three replicates for each level (n=15).

	RNZ	Zen-I	Zen-II	Zen-III
Range (µg/ml)	50 – 300	0.09 – 5.76	0.07 – 5.97	0.07 – 6.01
Slope (a)	20801294.3595	40503993.06	83381036.74	63496097.58
Intercept (b)	5977.0612	2263.45	1162.55	879.75
r^2	0.9998	1.00	1.00	1.00
RRF	-	0.70	1.44	1.09
CF	-	1.43	0.70	0.91
StDev*	229	303	401	179
95% CI for µ*	(114617; 115135)	(111394; 112081)	(247206; 248114)	(190099; 190505)
SE Mean*	132	175	232	103

* 100% concentration data

Accuracy

To assess the accuracy of the method, three parallel samples are prepared by spiking the placebo with known amounts of RNZ WS 80, 100, 120 %. For impurities with the known amounts Zen-I WS, Zen-II WS, and Zen-III WS, LOQ, 100, and 200 % solutions were prepared. Three injections from each sample were run on HPLC. The recovery values were calculated according to the standard solution. The obtained results of accuracy studies were shown in Table 3.

Table 3. The summary of accuracy studies for RNZ and its impurities Zen-I, Zen-II and Zen-III

RNZ Level %	**Concentration (mg/ml)**	**Recovery %**	**Mean %**	**Zen-I Level %**	**Concentration (µg/ml)**	**Recovery %**	**Mean %**
80	0.20	100.5 100.1 100.8	100.5	LOQ	0.096	98.6 98.0 97.1	97.9
100	0.25	99.9 99.9 100.2	100.0	100	3.000	93.3 92.9 90.1	92.1
120	0.30	100.2 99.9 98.8	99.6	200	6.000	84.6 90.3 90.5	88.47
Zen-II Level %	Concentration (µg/ml)	Recovery %	Mean %	**Zen-III** Level %	Concentration (µg/ml)	Recovery %	Mean %
LOQ	0.096	85.6 98.0 84.6	89.4	LOQ	0.096	102.6 103.6 103.2	103.1
100	3.000	100.9 102.2 98.7	100.6	100	3.000	96.4 96.6 95.2	96.1
200	6.000	92.6 99.2 100.0	97.3	200	6.000	91.3 99.1 99.5	96.6

Robustness

To determine the robustness of the method, the effect of different flow rates (0.8 – 1.2 ml/min), column temperature (28 – 32 °C), and filter (Nylon – PET – RC) were evaluated. The consistency between the results without changing the analysis conditions and the results found after the change was evaluated. For the method of RNZ impurities the effect of different wavelength (208 nm-212 nm) and filter (RC – PET) were evaluated. The obtained data of robustness studies summarized in Table 4 and 5.

Table 4. The summary of robustness studies for RNZ

Factor	Flow rate	Column Temperature ºC	Filter	RRT (min)	*Conformity %
Flow Rate	1.0 mg/ml	30	PET	4.204	-
	0.8 mg/ml			5.159	99.0
	1.2 mg/ml			3.464	99.3
Column Temperature	1.0 mg/ml	30	PET	4.204	-
		28		4.164	99.0
		32		4.151	99.3
Filter	1.0 mg/ml	30	PET	4.204	-
			Nylon		100.9
			RC		100.5

* conformity is a description of how the change rate fits with the routine of the study

Table 5. The summary of robustness studies for Zen-I, Zen-II and Zen-III

Factor	Flow rate (mg/ml)	Column Tempera-ture (ºC)	Filter	λ (nm)	Conformity Zen-I %*	Conformity Zen-II %*	Conformity Zen-III %*
Wavelength	1.0	30	PET	210	-	-	-
				208	100.8	98.6	102.0
				212	102.2	99.5	98.0
Filter	1.0	30	PET	210	-	-	-
			Nylon		100.1	101.9	101.8
			RC		100.0	100.0	100.0

* conformity is a description of how the change rate fits with the routine of the study

Precision

The precision of the method was evaluated at three levels: repeatability, intermediate precision and, reproducibility. To assess the reproducibility six separate sample solutions were run on HPLC. Two injections were made for each sample. To assess the repeatability of the method six separate standard solutions were run on HPLC. Field values were evaluated as a result of sequential injections. To evaluate the intermediate precision of the method, the samples identified in the method accuracy were prepared by a different analyst. The samples were injected into a different HPLC system. Quantity values were calculated for each sample. The RSD values obtained from twelve samples were evaluated. The low RSD values of the precision studies indicate that the method is precise[23]. As a System Suitability Parameters, the RSD value of the peak areas

obtained from six measurements of the standard solution should be a maximum of 5.0%. The results are given in the Table 6-8.

Table 6. Results of reproducibility studies for RNZ and its impurities Zen-I, Zen-II and Zen-III

Sample No	RNZ	Zen-I	Zen-II	Zen-III
	Result (%)	Recovery (%)	Recovery (%)	Recovery (%)
1	96.7	96.1	101.4	101.8
2	97.4	96.5	102.7	102.9
3	97.5	94.2	98.6	98.7
4	98.6	94.7	99.5	99.9
5	97.5	100.4	104.6	106.0
6	97.9	99.7	104.3	104.1
Mean	97.6	96.9	101.9	102.2
RSD %	0.7	2.7	2.4	2.6

Table 7. Results of repeatability studies for RNZ and its impurity standard

Sample No	RNZ	RNZ Impurity Standard
	Peak Area	Peak Area
1	5123875	115881
2	5096835	115681
3	5109472	115363
4	5092544	114367
5	5094318	115862
6	5109169	115198

Table 8. Results of intermediate precision studies for RNZ and its impurities Zen-I, Zen-II and Zen-III

Sample No	RNZ Result (%)		Zen-I Recovery (%)		Zen-II Recovery (%)		Zen-III Recovery (%)	
	1st analyst	2nd analyst	1st analyst	2nd analyst	1st analyst	2nd analyst	1st analyst	2nd analyst
	Instrument A	Instrument B	Instrument A	Instrument B	Instrument A	Instrument B	Instrument A	Instrument B
1	96.7	96.8	96.1	94.5	101.4	100.9	101.8	99.4
2	97.4	95.6	96.5	94.8	102.7	103.5	102.9	100.4
3	97.5	96.9	94.2	94.3	98.6	100.3	98.7	99.1
4	98.6	95.8	94.7	94.6	99.5	98.8	99.9	100.9
5	97.5	95.1	100.4	94.4	104.6	99.4	106.0	99.1
6	97.9	96.6	99.7	94.3	104.3	99.6	104.1	101.9
Mean	97.6	96.1	96.9	94.5	101.9	100.4	102.2	100.1
RSD %	0.7	1.0	2.7	0.2	2.4	1.7	2.6	1.1

Limit of quantification and detection

The LOD and LOQ concentrations were obtained from the linearity studies. It is found by calculating the signal to noise ratio (LOD: signal to noise ratio=3; LOQ: signal to noise ratio=10). Data obtained of LOD and LOQ for RNZ and its impurities Zen-I, Zen-II, and Zen-III were showed in Table 9.

Table 9. Results of LOD and LOQ studies for RNZ and its impurities Zen-I, Zen-II and Zen-III

Sample	LOD %	LOQ Mean Peak Area / RSD %	LOQ (mg/mL)	LOD (mg/mL)
RNZ	0.002	2357 / 5.6	0,0000399	0,000012
Zen-I	0.005	3607 / 2.9	0,0000960	0,000029
Zen-II	0.004	4753 / 3.1	0,0000717	0,000022
Zen-III	0.004	4619 / 1.8	0,0000722	0,000022

Forced degradation

RNZ ER tablets were exposed to heat, light, acid, base, and oxidation. Impurity analyses were performed before and after exposure. The analysis results were given in Table 10.

A comprehensive stability study was performed including forced degradation studies. A highly sensitive, simple HPLC-UV method developed and validated for the determination of ranolazine and its impurities. The method validation was performed in accordance with ICH guidelines. The highest degradation occurred in the H_2O_2 environment, indicating that Ranolazine is highly sensitive to the oxygen[24].

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