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Short communication

A novel reverse phase stability indicating RP-UPLC method for the quantitative determination of fifteen related substances in Ranolazine drug substance and drug product

Vakamulla Malati [a,b,*], Anumala Raghupati Reddy [a], K. Mukkanti [b], M.V. Suryanarayana [1,c]

[a] Analytical Research and Process Development, Integrated Product Development Operations, Dr. Reddy's Laboratories Ltd, Bachupalli, Qutubullapur, Ranga Reddy District 500 072, Andhra Pradesh, India
[b] Centre for chemical science and technology, Institute of science and technology, J.N.T. University, Kukatpally, Hyderabad-500072, Andhra Pradesh, India
[c] Matrix Laboratories Limited, Plot No.34A, Anrich Industrial Estate, Bollaram, Jinnaram (Mandal), Medak Dist-502 325, Andhra Pradesh, India

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ABSTRACT

A gradient reverse-phase ultra performance liquid chromatographic (RP-UPLC) method was developed for the quantitative determination of Ranolazine and potential process-related impurities (starting materials, positional isomers, degradants and byproducts) at the level of 0.1 μg mL^{-1} to 0.3 μg mL^{-1}. Fifteen potential impurities were identified in the crude samples during the process development. Tentative structures for all the impurities were assigned based on *m/z* values from LC-MS/MS analysis. This method can be used for the quality control of both drug substance and drug product. All these impurities were separated with a gradient UPLC method by using a polar embedded Waters Acquity BEH RP18 100 mm × 2.1 mm,1.7 μm column, monobasic sodium buffer, a basic organic modifier and acetonitrile in the mobile phase. Further, this method is also capable of separating a major oxidative degradant Di-N-oxide. Impurities having electron donating groups(+I effect) on the phenyl ring increased the retention by improved π–π interactions. The drug was subjected to the International Conference on Harmonization (ICH)-prescribed hydrolytic, oxidative, photolytic and thermal stress conditions. The performance of the method was validated according to the present ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.

1. Introduction

Ranolazine is a racemic mixture and chemically described as 1-piperazineacetamide, *N*-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, (±). It has an empirical formula of $C_{24}H_{33}N_3O_4$, a molecular weight of 427.54 g $mole^{-1}$. The U.S. Food and Drug Administration (FDA) has approved a new first line indication for Ranolazine for the treatment of chronic angina. In addition, the new labeling also provides information about reduced arrhythmias including ventricular arrhythmias, new onset atrial fibrillation and potentially dangerous slow heartbeat known as bradycardia in patients with coronary artery disease. Further, it states that Ranexa (Trade name of Ranolazine drug product) reduces hemoglobin A1c (HbA1c) in patients with diabetes. Ranolazine is believed to have its effects via altering the trans-cellular sodium current by altering the intracellular sodium level. Ranolazine effects the sodium-dependent calcium channels during myocardial ischemia [1]. Thus, Ranolazine indirectly prevents the calcium overload that causes cardiac ischemia.

Although Ranolazine is a well-known drug manufactured commonly as a bulk drug, few references were found in literature regarding separation and detection of its related isomers and impurities. Ranolazine has three regio isomers. To the best of our knowledge, no method available in the literature can separate all the impurities presented in this work. Many analytical methods have been reported in the literature for the determination of assay [2–6], for enantiomeric separation [7], four impurities were found to be reported for Ranolazine [8] and a highly sensitive liquid chromatographic-tandem mass spectrometric method (LC-MS-MS) to quantitate Ranolazine and its metabolites in human plasma [9–11]. Compared the effects of Ranolazine with a new fatty acid oxidation inhibitor, CVT-4325 [(*R*)-1-(2-methyl-benzo[*d*]thiazol-5-yloxy)-3-(4-((5-(4-(trifluoromethyl)phenyl)-1,

* Corresponding author at: Dr. Reddy's Laboratories Ltd, Analytical Research and Process Development, Integrated Product Development Operations, Bachupalli, Qutubullapur, Ranga Reddy District 500 072, Andhra Pradesh, India.
Tel.: +91 9866598276.
E-mail addresses: malativ@drreddys.com, malu_1108@yahoo.co.in (V. Malati).
[1] Tel.: +91-40-30492004(D). 08458-279301/02.

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2,4-oxadiazol-3-yl)methyl)-piperazin-1-yl)propan-2-ol], on carbohydrate and fatty acid oxidation [12]. Effects of Ranolazine With Atenolol, Amlodipine, or Diltiazem on Exercise Tolerance and Angina Frequency in Patients With Severe Chronic Angina were described [13]. Determined the efficacy and safety of Ranolazine during long-term treatment of patients with non-ST-elevation ACS [14]. As per stringent regulatory requirements, the impurity profile study has to be carried out for any active pharmaceutical ingredient (API) and for pharmaceutical dosage forms. During the method development of novel process for Ranolazine 15 process related impurities namely Impurity-1 to Impurity-15 were identified in crude samples, whose area percentages were ranging from 0.05 to 2.5% (Refer to Fig. 2(B)). Initially the structures for all the unknown impurities have been tentatively assigned based on mass numbers obtained by liquid chromatography-mass spectrometry, further confirmed by spiking analysis with synthesized and characterized impurities.

There is no stability-indicating UPLC method reported in the literature that can conduct an accurate and quantifiable analysis of Ranolazine, related impurities and its regio isomers. It is, therefore, necessary to develop a new stability-indicating method for the determination and quantitative estimation of Ranolazine and its related impurities. It is also required to identify and characterize any impurity which is more than 0.05% and is essential to control the same in API and drug product as per ICH [15].

Hence, a reproducible stability-indicating RP UPLC method was developed for the quantitative determination of Ranolazine and its impurities, namely Impurity-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, -13, -14 and -15 (Fig. 1).This method was successfully validated according to the ICH guidelines (Validation of Analytical Procedures: Test and Methodology Q2) [15–18].

2. Experimental

2.1. Chemicals and reagents

Samples of Ranolazine, raw materials and impurities were supplied by Process Research Department of Integrated product development operations of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade acetonitrile, Sodium dihydrogen phosphate monohydrate were purchased from Merck, Darmstadt, Germany. Triethylamine was purchased from JT.bakers, Mallinckrodt inc, Phillipsburg, NJ, USA. High pure water was prepared by using Millipore Milli Q plus purification system. Ranexa (Ranolazine) is available as a film-coated, non-scored, extended-release tablet for oral administration. Commercially available Ranexa tablets were purchased.

2.2. Instrumentation

The system used for method development, forced degradation and validation study was Waters Acquity UPLC system equipped with 2996 photodiode array detector. The out put signal was monitored and processed using Empower software (Waters) on Pentium computer (Digital Equipment Co).

2.3. Chromatographic conditions

The stationary phase used was Acquity UPLC BEH RP-18 100 mm, 2.1 mm, and 1.7 μm particles. The mobile phase-A contains acetonitrile-Sodium dihydrogen phosphate (pH 7.3; 0.01 M)-Triethylamine (10:90:0.1, v/v/v), and mobile phase-B contains acetonitrile-mobile phase-A (55:45, v/v). The flow rate was 0.3 mL min^{-1}. The LC gradient program (Time (in min)/%mobile phase-B) was set as 0.01/17, 1.5/17, 3.5/45, 5.5/60, 8/65, 12/70, 13/95, 15/95, 15.5/17 and 18/17. The column temperature was maintained at 27 °C and the absorption was monitored at a wavelength of 223 nm. The injection volume was 1.0 μL, mobile phase-B is used as diluent.

2.4. Preparation of standard solutions

Working solutions containing 3000 μg mL^{-1} and 300 μg mL^{-1} of Ranolazine and its impurities were prepared for the determination of related substances and for the assay determination, respectively. Mixed and individual stock solutions (300 μg mL^{-1}) of the impurities (denoted as Impurity-1 to Impurity-15) were prepared in the mixture of acetonitrile and water, which are in the ratio of 7:3(diluent). Ranexa(500 mg) tablets powder solution equivalent to 3000 μg mL^{-1} of the drug was prepared by dissolving in the diluent and was filtered through a 0.22 μm nylon membrane filter. The resulting solution was analyzed by UPLC for the estimation of related substances.

2.5. LC-MS conditions

The LC-ESI/MS studies were carried out on AB-MDS SCIEX (Applied bio-systems, USA) 4000Q TRAP quadrupole mass spectrometer. The source voltage was kept at 5000 V and capillary temperature at 400 °C. Nitrogen was used as both sheath and auxiliary gas. Mass range was kept at m/z 89–1000 in the positive ionization mode. The instrument was operated in enhanced product ion mode in positive polarity mode with the following settings: collision energy of 40 V, collision energy spread 10 V and declustering potential 10 V. Nitrogen was used as curtain gas at a pressure of 12 psi, and as collision associated dissociation (CAD) gas. Zero air was used as nebulizer gas and heater gas at pressure of 50 psi. The LC part consisted of Waters Acquity UPLC system with quaternary gradient pump with a degasser and an auto sampler. Acquity UPLC BEH RP-18 100 mm, 2.1 mm, and 1.7 μm particles was used for chromatographic separation. The mobile phase consisting of a mixture of ammonium Acetate (Mobile phase-A, pH 7.3; 0.01 M) and mobile phase-B contains acetonitrile: water (8:2, v/v) was used. The flow rate was maintained at 0.3 mL min^{-1} and the injection volume is 3.0 μL. The gradient program as follows: (time in min./%mobile phase -B 0.01/17, 1.5/17, 3.5/45, 5.5/60, 8/65, 12/70, 13/95, 15/95, 15.5/17 and 18/17.

3. Results and discussions:

3.1. Synthetic process and identification of impurities

Method development is started by using crude samples at various stages of synthetic process. A brief synthetic scheme was detailed in Fig. 2(A), where starting material 2-methoxyphenol reacted with epichlorohydrin (Williamson ether synthesis) to form aryloxy epoxide intermediate (Compound-1). This compound-1, upon reaction with piperazine, aryloxy piperazine intermediate (Compound-2) was obtained. Finally, condensation of 2-chloro-N-(2, 6-dimethylphenyl) acetamide (Compound-3) with aryloxy piperazine intermediate in presence of base and solvent afforded Ranolazine dihydrochloride.

As a common practice, efforts should be made to identify and characterize all unknown impurities in the drug substance due to the ever increasing demand from regulatory agencies to manufacture high purity drug substances. Impurity profiling of drugs is the most important issue in modern pharmaceutical analysis [19–24] for developing process technology to manufacture high purity drug substance. Hence, the method should be capable of separating all the impurities is worth emphasizing.

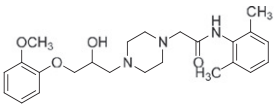
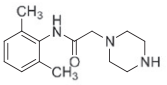
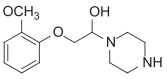
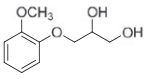
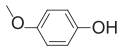
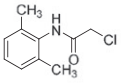
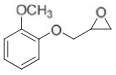
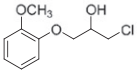
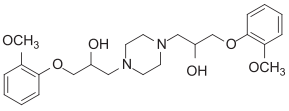
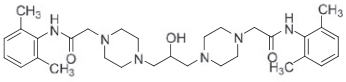
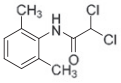
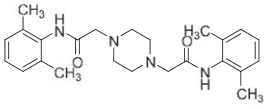
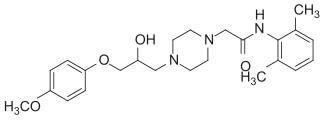
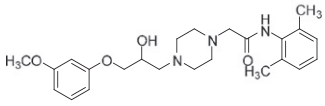
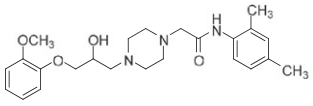
Fig.	Name	Structure	Chemical name
(A)	Ranolazine		(±)-*N*- (2,6-dimethylphenyl) -4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]-1-piperazineacetamide
(B)	Impurity-1		1-[(2,6-Dimethylphenyl)-aminocarbonylmethyl] piperazine
(C)	Impurity-2		1-[3-(2-methoxyphenoxy)-2-hydroxypropyl]-piperazine
(D)	Impurity-3		3-(2-Methoxyphenoxy)propane-1, 2-diol
(E)	Impurity-4		4-Methoxy phenol
(F)	Impurity-5		2-Methoxy phenol
(G)	Impurity-6		[(2,6-Dimethylphenyl)-aminocarbonylmethyl] chloride
(H)	Impurity-7		1-(2-Methoxyphenoxy)-2, 3-epoxy propane
(I)	Impurity-8		1-Chloro-3-(2-methoxyphenoxy) propane-2-ol
(J)	Impurity-9		1,3-Bis-{4-[(2,6-dimethylphenyl)-aminocarbonylmethyl] piperazin-1-yl}-propan-2-ol
(K)	Impurity-10		1-{4-[2-Hydroxy-3-(2-methoxyphenoxy) -propyl]-piperazin-1-yl}-3-(2-methoxyphenoxy)-propan-2-ol
(L)	Impurity-11		2,2-Dichloro-*N*-(2,6-dimethylphenyl) acetamide
(M)	Impurity-12		1,4-Bis-[(2,6-dimethylphenyl)-aminocarbonylmethyl] piperazine
(N)	Impurity-13		(±)-*N*- (2, 6-dimethylphenyl)-4-[2-hydroxy-3-(4-methoxyphenoxy) propyl]-1-piperazineacetamide
(O)	Impurity-14		(±)-N- (2, 6-dimethylphenyl)-4-[2-hydroxy-3-(3-methoxyphenoxy) propyl]-1-piperazineacetamide.
(P)	Impurity-15		(±)-N- (2, 4-dimethylphenyl)-4-[2-hydroxy-3-(3-methoxyphenoxy) propyl]-1-piperazineacetamide

Fig. 1. Chemical structures of Ranolazine and its associated impurities.

3.2. Optimization of chromatographic conditions

UPLC is a new category of separation science which built upon well-established principles of liquid chromatography, using sub-2 μm porous particles. These particles operate at elevated mobile phase linear velocities to produce rapid separation with increased sensitivity and increased resolution [25–29]. Drug impurity profiling, i.e., identification, structure elucidation

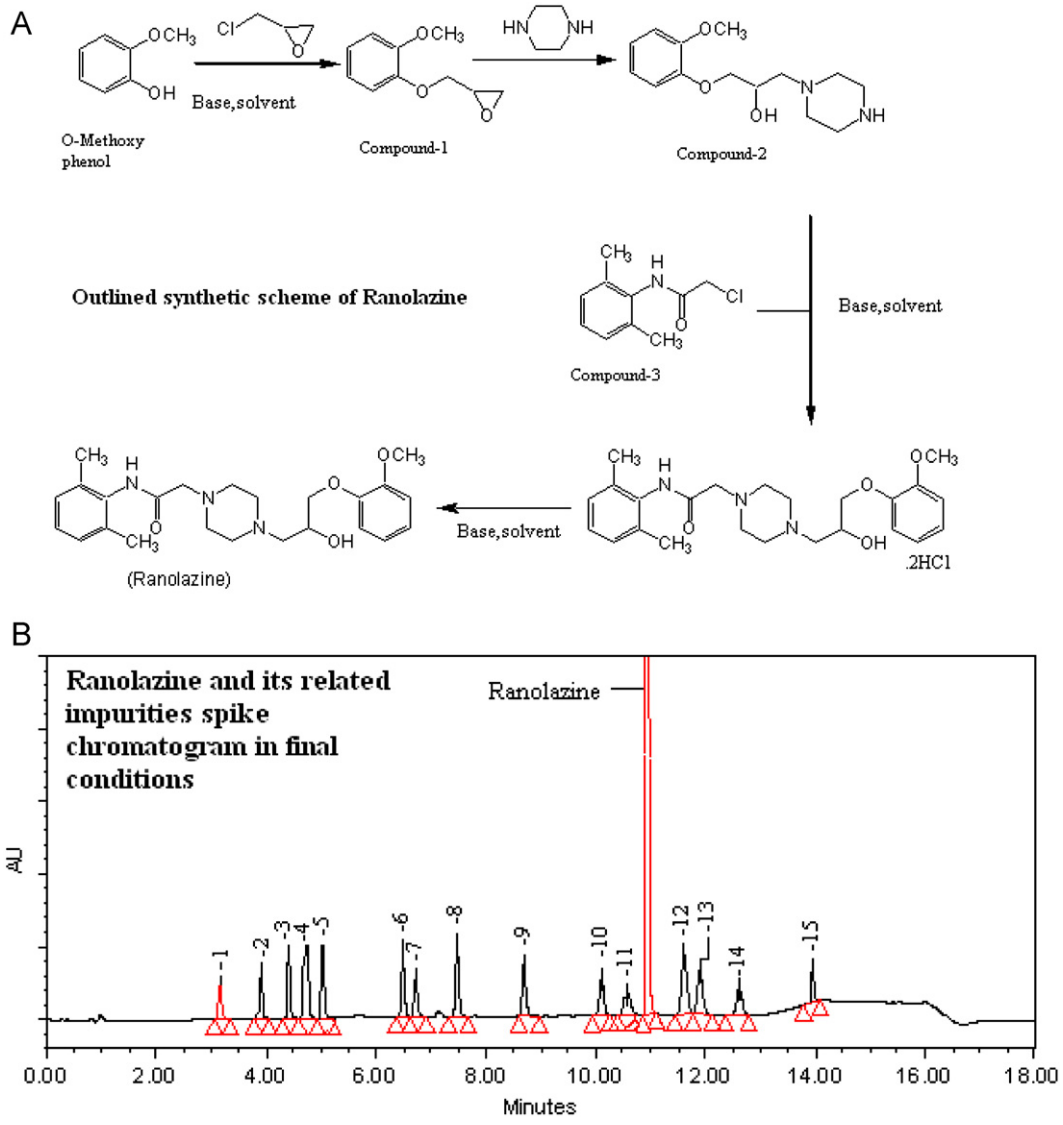


Fig. 2. (A) Schematic presentation of Ranolazine synthesis; (B) blend chromatogram of Ranolazine and its related impurities in final chromatographic conditions (pH 7.3).

Table 1
System suitability parameters.

S.No	Compound	RT (in min)[a]	RRT[b]($n=6$)[a]	USP resolution [c]($n=6$)[a]	USP tailing factor($n=6$)[a]
1.0	Impurity-1	2.58	0.25	–	0.95
2.0	Impurity-2	3.42	0.33	6.05	1.18
3.0	Impurity-3	3.93	0.38	5.75	0.99
4.0	Impurity-4	4.05	0.39	2.70	1.12
5.0	Impurity-5	4.38	0.43	3.56	1.09
6.0	Impurity-6	5.76	0.56	14.75	1.07
7.0	Impurity-7	5.99	0.58	2.35	1.07
8.0	Impurity-8	6.68	0.65	6.94	1.06
9.0	Impurity-9	8.06	0.79	13.04	1.06
10.0	Impurity-10	9.14	0.89	8.24	1.03
11.0	Impurity-11	9.92	0.97	5.57	1.07
12.0	Ranolzine	10.25	1.00	3.17	1.15
13.0	Impurity-12	10.86	1.06	3.64	0.99
14.0	Impurity-13	11.09	1.08	1.34	1.20
15.0	Impurity-14	11.78	1.15	4.13	1.04
16.0	Impurity-15	13.48	1.32	11.89	0.90

RNB, Ranolazine.

[a] Mean ± SD ($n=6$).
[b] Relative retention times (RRT) were calculated against the retention time (RT) of Ranolzine.
[c] Resolutions were calculated between two adjacent components.

and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical formulations is one of the most important fields of activities in modern pharmaceutical analysis [30]. The main goal of this chromatographic method is to get the separation of Ranolazine from all the process related impurities, isomers and degradants. At the beginning of method development, it is generally important to know various physicochemical parameters like pKa, log *P*, solubility, absorptivity and wavelength maximum of the drug. The knowledge of pKa is important as most of the pH-related changes in retention occur at pH values within ± 1.5 units of the pKa value.

The ionization value(pKa) also helps in selecting the pH of the buffer to be used in the mobile phase.

The knowledge of log *P* for the drug and the identified degradation products provides good insight into the separation behavior likely to be obtained on a particular stationary phase. The effect of pH on selectivity was also studied and it was found

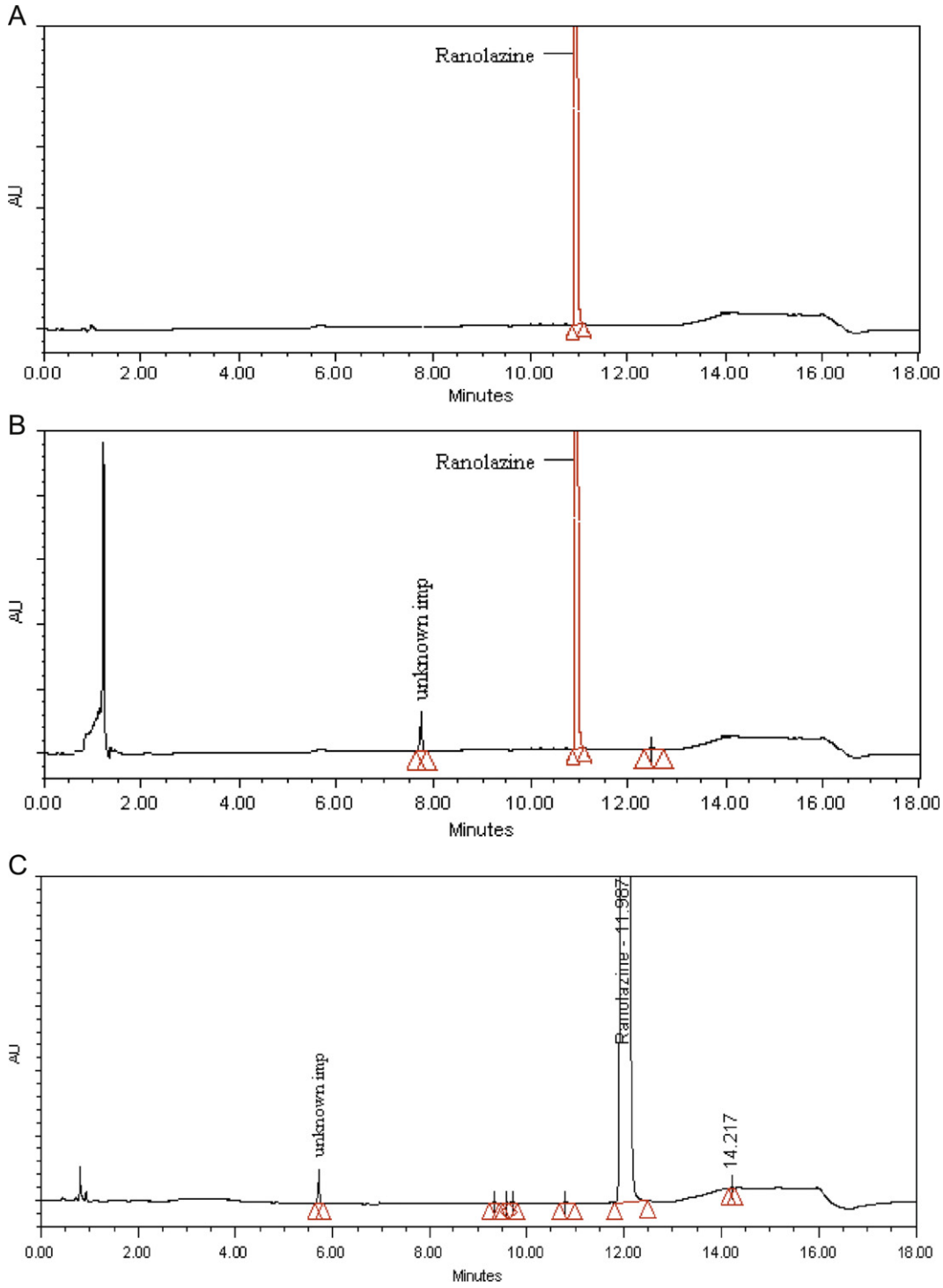


Fig. 3. (A) Ranolazine standard chromatogram; (B) acid hydrolysis of Ranolazine; (C) base hydrolysis of Ranolazine; (D) oxidation chromatogram of Ranolazine; (E) water hydrolysis of Ranolazine; (F) ranexa(Tablet) chromatogram; (G) mass spectrum of N-Oxide impurity of Ranolazine; (H) mass spectrum and fragmentation pattern of Di–N–Oxide impurity of Ranolazine, +Q1 Mass spectrum of Di–N–Oxide of Ranolazine, MS2 spectrum of Di–N–Oxide of Ranolazine, fragmentation of Ranolazine, Di–N–Oxide. (I) Mass spectrum and fragmentation pattern of Ranolazine, +Q1 Mass spectrum of Ranolazine, MS2 spectrum of Ranolazine fragmentation pattern of Ranolazine.

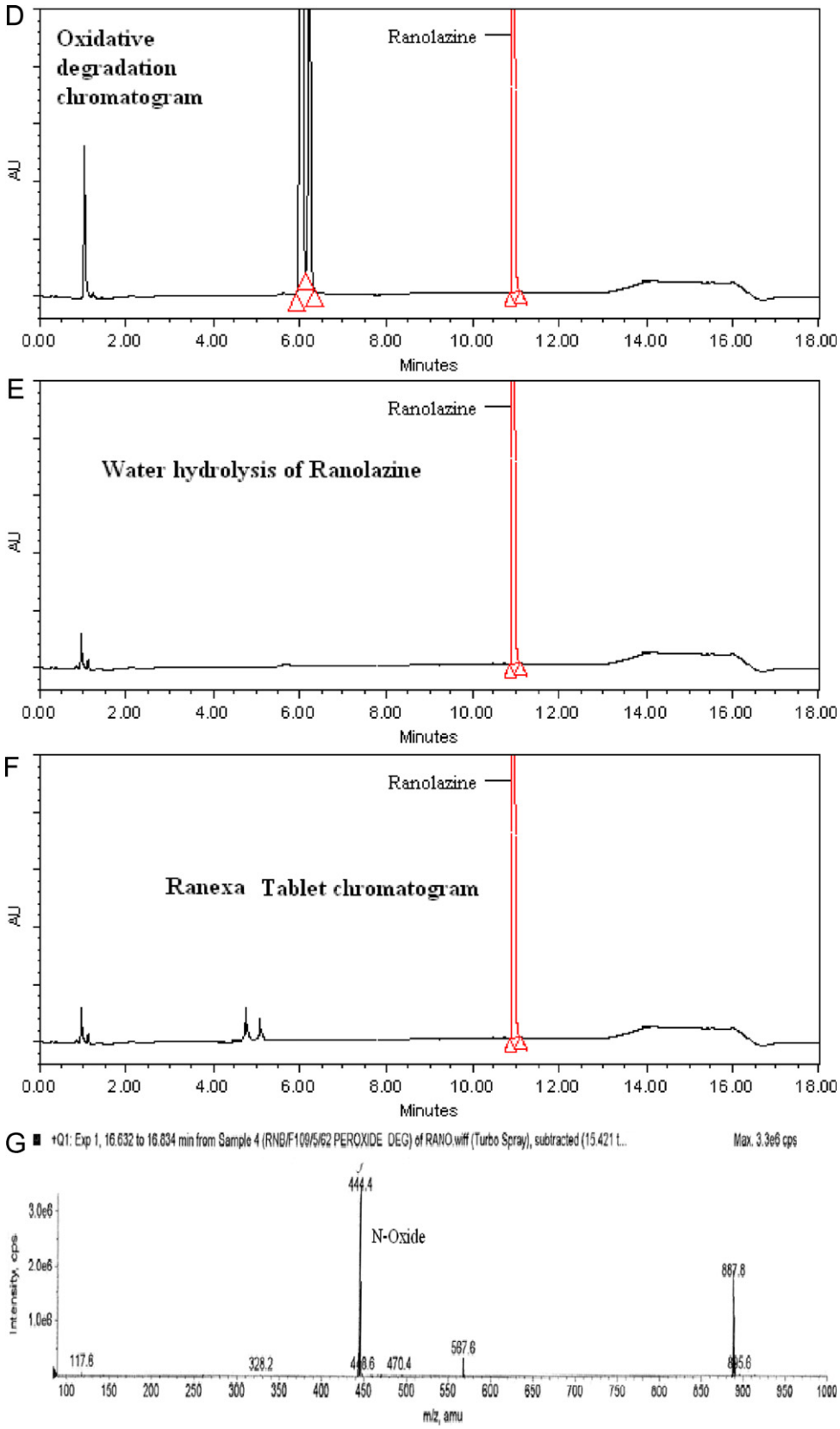


Fig. 3. (*continued*)

that pH of 7.3 shown good results over other pH's (Fig. 2(B)) At pH 2.3, impurity-11,-13 and -15,-8 were co eluted. At pH 5.0, impurity-6 and impurity-8 were co eluted. The resolution between some impurities at pH 2.3 and pH 5.0 are not appropriate (Fig. 2(C) and (D)). pH was found to have statistically significant effect on *N*(theoretical plates) and *T*(Tailing factor) as it was observed. The affinity of all impurities towards different commercially available column were also evaluated to get the

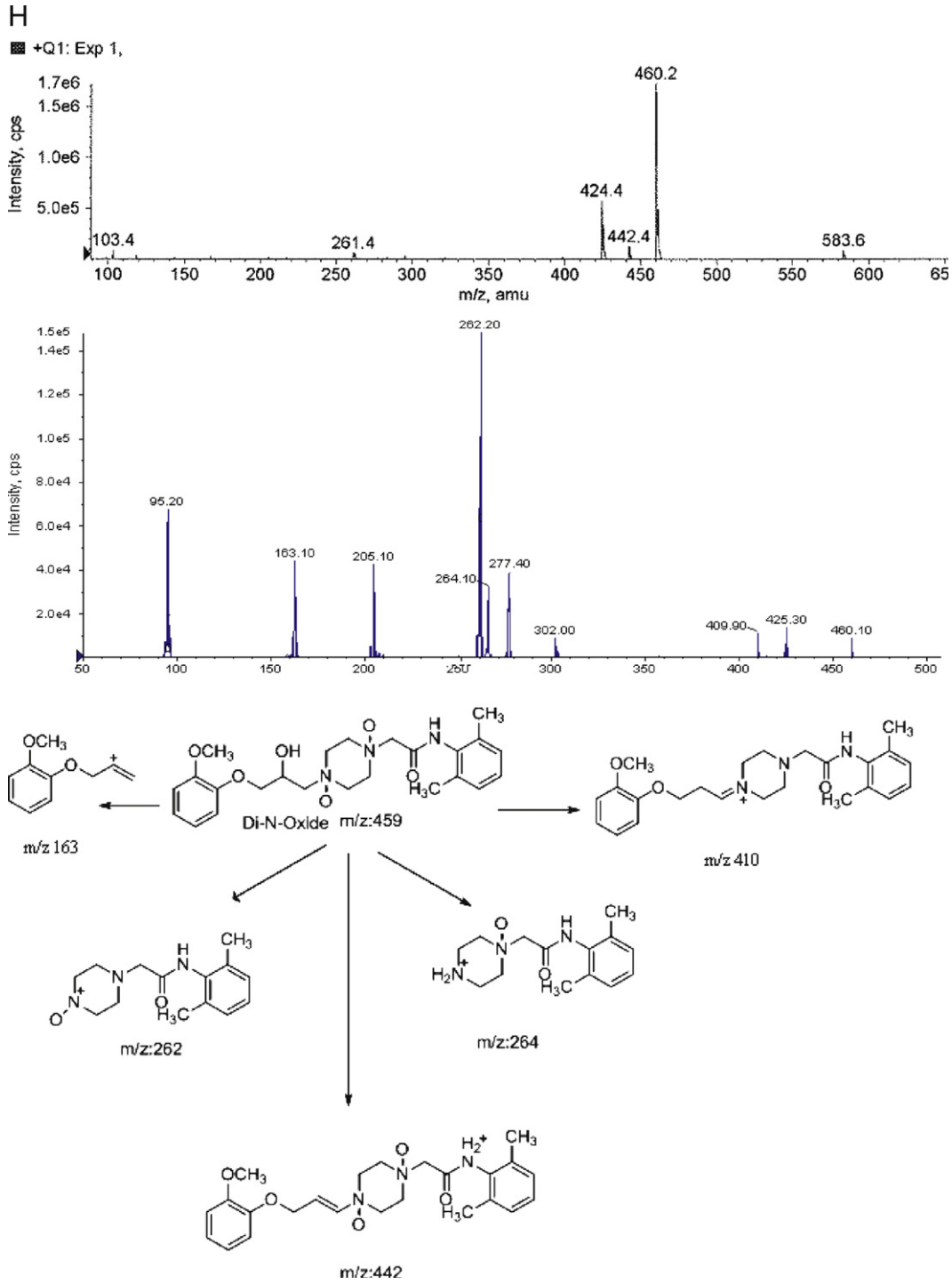


Fig. 3. (*continued*)

best resolution (Fig. 2(E) and (F)). Affect of pH and different stationary phases are graphically represented in Fig. 4(A) and (B). Finally, polar (-CONH-) embedded *C* 18 column at pH 7.3 was selected to get the good resolution between the critical pairs, which is due to its high selectivity, less hydrophobicity, high reproducibility and suitability for widely varying polar compounds compared with other commercially available columns. In this optimized method all the peaks were symmetrical and show the tailing factor –1.0 and the resolution –2.0 from blend chromatogram of system suitability. System suitability parameters were described in Table 1.

3.3. Specificity

Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21CFR section 211 requires the development and validation of stability indicating method. The specificity of the developed UPLC method for Ranolazine was carried out in the presence of its impurities.The current guidance documents do not indicate detailed degradation conditions in stress testing. However, the forced degradation conditions were found to affect the degradation preferably not less than 5% and not complete degradation of drug substance. Intentional degradation was attempted to get

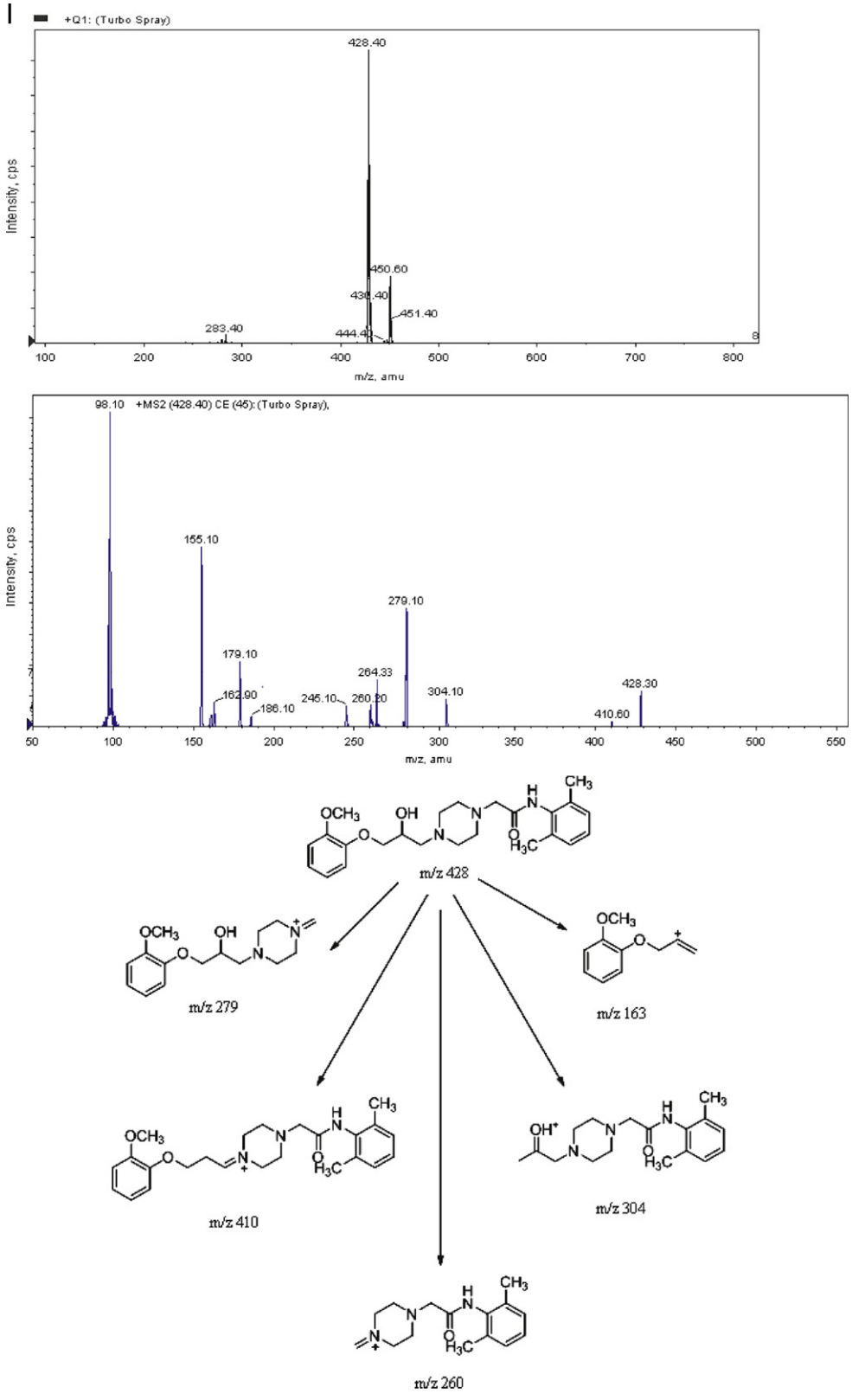


Fig. 3. (*continued*)

degradation not more than 20% (in order to get a mass balance between purity and assay) under stress conditions of UV light (254 nm for 10days), thermal degradation (drug substance exposed to 90 °C for 10days), acid hydrolysis (using 5 N HCl for 48 h at 70 °C), base hydrolysis (using 2.0 N NaOH for 3 h at 70 °C), water hydrolysis (for 48 h at 70 °C) and oxidative degradation (using 0.2% H2O2, immediate). Stress chromatograms are illustrated in Fig. 3(B)–(E). Samples were withdrawn at appropriate time and were analyzed after appropriate dilution to evaluate the ability of the proposed method to separate Ranolazine from all

related impurities and its degradation products. Peak purity test was carried out on the stressed samples of Ranolazine by using PDA detector. Assay studies were carried out for stress samples against qualified reference standard and the mass balance (% assay+% degradation) was calculated (Table 3). Assay was also calculated for bulk sample by spiking all 15 impurities at the specification level (i.e., 0.05%). A major degradation product obtained by oxidation and was confirmed as Di–N–oxide (M+H 460) based on its mass number and fragmentation(Fig. 3(H)). A detailed fragmentation of Ranolazine was shown in Fig. 3(I).

3.4. Method validation

3.4.1. Precision

The repeatability of the method was checked by six fold analysis of 3000 μg mL^{-1} of Ranolazine by spiking with impurities at the level of 0.05%. The %RSD for the area of each peak was calculated. Precision of the method was also evaluated using different analyst and a different instrument in the same laboratory. Fig. 4.

Inter day precision was determined by six-fold analysis of Ranolazine spiked with 1.5 μg mL^{-1} of each of the impurities (Table 2). The same protocol was followed for three different days

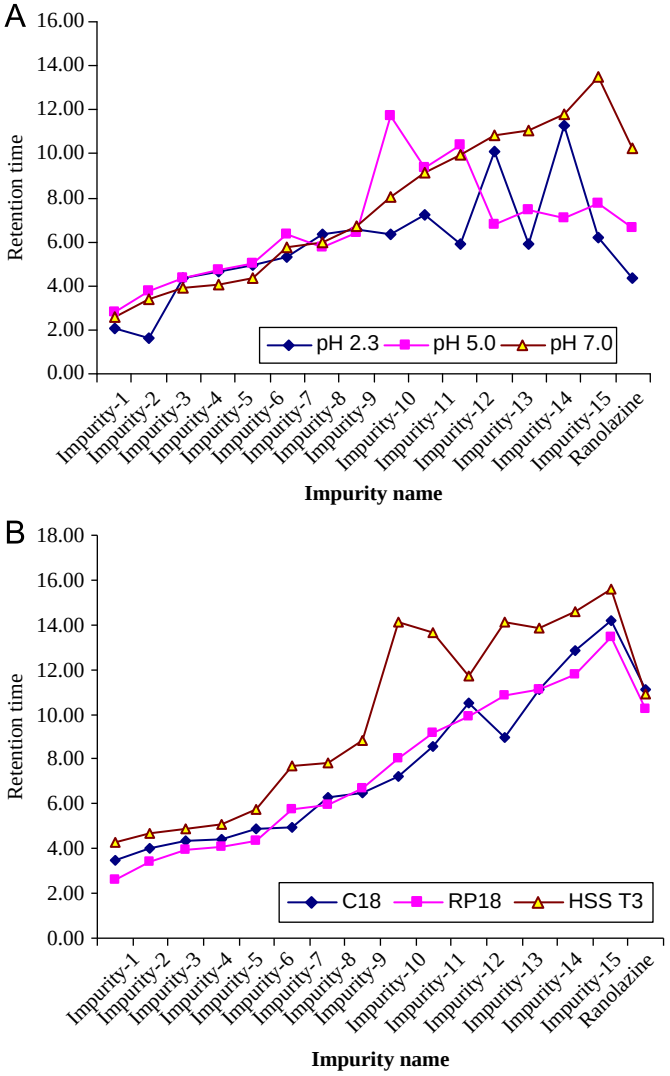


Fig. 4. (A) Effect of pH on selectivity of Ranolzine and its related impurities; (B) selectivity differences of Ranolazine and its associated impurities by using different stationary phases.

Table 2
LOD, LOQ, regression and precision data.

Parameter	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Imp-6	Imp-7	Imp-8	Imp-9	Imp-10	Imp-11	Ranolazine	Imp-12	Imp-13	Imp-14	Imp-15
LOD (μg mL^{-1})	0.7	0.09	0.07	0.05	0.05	0.04	0.07	0.04	0.04	0.07	0.07	0.06	0.07	0.07	0.07	0.05
LOQ (μg mL^{-1})	0.25	0.31	0.15	0.13	0.12	0.12	0.22	0.12	0.12	0.23	0.22	0.15	0.20	0.18	0.19	0.15
Precision (% RSD) (at LOQ level)	5.8	6.7	3.2	4.7	6.3	8.1	3.0	5.2	2.7	2.7	6.2	4.1	3.9	2.8	4.0	3.5
Signal/noise (at LOQ)	9.6	9.7	10.2	10.3	9.8	9.6	10.0	10.4	9.9	9.5	10.1	9.7	9.6	10.3	9.9	10.4
Regression equation (y) Slope (b)	11,609,725	13,240,538	21,305,771	25,767,131	26,081,254	23,009,651	17,202,050	17,711,200	18,845,253	12562070	15510644	18169666	16471184	20115066	14787637	16326581
Intercept (a) Correlation Coefficient	567.9	192.5	237.9	1025	−454.9	1096	883	551	397	−1765	−2681	1974	837.3	−797.4	−104	489
R^2 value	0.99998	0.99998	0.99999	0.99996	0.9999	0.99996	0.99997	0.99997	0.99997	0.9998	0.9997	0.9994	0.9998	0.99996	0.9999	0.9999
Precision (% RSD) Intermediate precision (% RSD)	0.99996	0.99997	0.99997	0.99992	0.9999	0.99993	0.99994	0.9999	0.9999	0.9995	0.9994	0.9988	0.9997	0.9997	0.9997	0.9998
	1.2	1.5	0.8	1.2	1.3	1.5	1.7	2.2	0.8	0.9	3.5	1.5	3.2	1.8	2.1	1.8
	1.8	1.2	0.6	0.8	1.0	1.8	1.2	1.8	0.6	0.5	3.1	1.1	3.0	1.2	1.6	1.3

Table 3
Summary of forced degradation studies.

% imp formed																				
Parameter	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Imp-6	Imp-7	Imp-8	Imp-9	Imp-10	Imp-11	Imp-12	Imp-13	Imp-14	Imp-15	%SMI[a]	%TUI[b]	%Degradation	%Assay	[c]Mass balance
Acid	ND	ND	ND	ND	ND	ND	ND	0.03	ND	ND	ND	ND	ND	ND	ND	3.67	4.04	7.74	90.5	98.09
Base	ND	ND	ND	ND	ND	ND	ND	0.024	ND	ND	ND	ND	ND	ND	ND	8.84	8.91	8.91	90.7	98.90
Peroxide	ND	ND	ND	ND	ND	ND	ND	0.015	ND	ND	ND	ND	ND	ND	ND	8.29	8.43	8.43	90.8	99.16
Water	ND	ND	ND	ND	ND	ND	ND	0.03	ND	ND	ND	ND	ND	ND	ND	0.09	0.17	0.17	98.6	98.70

Imp: Impurity; ND, not detected.

[a] SMI: (single maximum unknown impurity).
[b] TI: (total impurities).
[c] Mass balance: (% assay+% sum of all compounds+% sum of all degradants).

to study inter-day variation ($n=18$). Different analysts prepared different solutions on different days. The precision of the assay was evaluated by performing six ($n=6$) independent assays of Ranolazine test sample against the qualified reference standard. The RSD (%) of the peak areas of each impurity and six results of assay was also calculated.

3.4.2. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ values for all impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentration. Precision study was also carried out at LOQ level by injecting six individual preparations of impurities and calculated the %RSD of the each impurity peak area. For LOQ, the signal to noise ratio's for all the impurities were ranging from 9.5 to 10.4 (Table-2). The limit of detection (LOD) and limit of quantification(LOQ) values of Impurity-1 to Impurity-15 were tabulated (Table 2). The % recoveries were calculated for related substances at their LOQ level and those are ranging from 90% to 110%.

3.4.3. Linearity

Linearity test solutions were prepared from impurity stock solution at six different concentration levels ranging from LOQ, 0.9 $\mu g\,mL^{-1}$, 1.5 $\mu g\,mL^{-1}$, 3.0 $\mu g\,mL^{-1}$, 6.0 $\mu g\,mL^{-1}$, 9.0 $\mu g\,mL^{-1}$ and 15 $\mu g\,mL^{-1}$. The calibration curve was drawn by plotting impurity area versus the concentration. The correlation coefficient obtained was greater than 0.999 for all the impurities (Table 2).

3.4.4. Accuracy

Accuracy expresses the closeness of the agreement between the true value and the value obtained.

ccuracy of the method was evaluated at three concentration levels (in triplicate) i.e., 0.75 $\mu g\,mL^{-1}$, 1.50 $\mu g\,mL^{-1}$ and 2.25 $\mu g\,mL^{-1}$ in bulk drug sample and in dosage form. The % recoveries were calculated for related substances and those are ranging from 95% to 108%. The % recovery of Ranolazine in bulk drug sample was ranging from 98.2% to 100.4% w/w in its assay method (Table 3).

3.4.5. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolutions of all the impurities were evaluated. The flow rate of the mobile phase was changed from 0.30 mL min^{-1} to 0.28 mL min^{-1} and 0.32 mL min^{-1}. The affect of pH on resolution of impurities was also studied by varying ± 0.2 pH units (at 7.1 and 7.5 buffer pH instead of 7.3). The affect of column temperature on resolution was studied at 22 °C and 32 °C instead of 27 °C. In all of the above varied conditions, the components of the mobile phase were held constant as per the method. There is no significant change was observed by varying flow rate, pH and column temperature), which confirms the robustness of the method.

3.4.6. Solution stability and mobile phase stability

The solution stability and the mobile phase stability of Ranolazine was tested up to 48 h period, by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions for every 6 h interval up to two days. Mobile phase prepared was kept constant during the study period. The % RSD of assay of Ranolazine was calculated and was less than 1.0. The solution stability and mobile phase stability experiments data confirms that sample solution and mobile phase used is stable up to 48 h.

4. Conclusions

The simple, rugged and robust gradient RP-UPLC method has been developed for separation and quantitative determination of Ranolazine, its regio isomers and its related impurities. The method is precise, accurate and selective. The method was completely validated and showing satisfactory data for all the method validation parameters tested. The developed method is stability-indicating and can be used for assessing the stability of bulk samples, dosage forms of Ranolazine and also for monitoring the synthetic procedures of Ranolazine.

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