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Stability-Indicating RP-UPLC Method Development and Validation for the Process Related Impurities of Nebivolol and Structural Characterization of Its Forced Degradation Products by LC-MS/MS

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MK and PD designed the study. Authors PK, PA and BK managed the analyses of the study. Author SK helped in literature searches and synthetic preparation of impurities. Authors PK and SS prepared the first draft. Authors MK and PD made the final corrections in the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present study reports the development and validation of a stability-indicating reverse phase ultra performance liquid chromatographic method for the determination of nebivolol (NEB) in the presence of its process related impurities in bulk drugs and also demonstrates the structural characterization of forced degradation products (DP) of nebivolol by liquid chromatography-tandem

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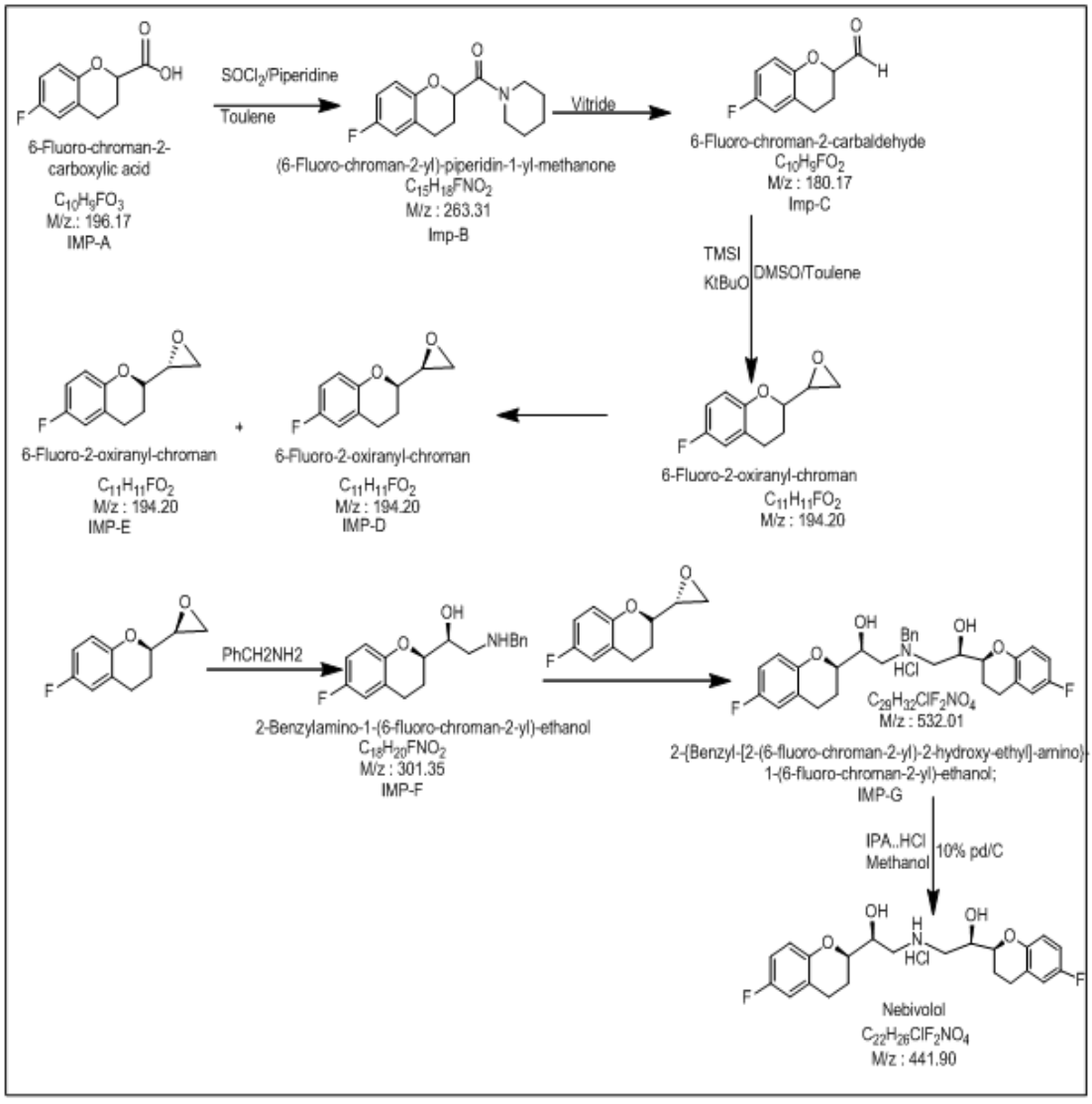
mass spectrometry (LC-MS/MS). Forced decomposition study of nebivolol was carried out and studied under acidic, basic, aqueous, oxidative, photolytic and thermal conditions according to the ICH guidelines. Nebivolol was found to be stable under photolytic, thermal and aqueous conditions applied in our study. However, nebivolol degradation was observed under basic and acidic conditions. Successful chromatographic separation of the drug and its process related impurities and degradation products were achieved on a waters acquity BEHC18 stationary phase (50 × 2.1 mm, 1.7µm) with a gradient elution of 10 mM ammonium acetate and acetonitrile at a flow rate of 0.3 mL min^{-1}. Detection of analytes was carried out using a PDA detector with its wavelength set at 222 nm. The developed UPLC method was validated for all validation parameters according to the ICH guidelines. The degradation products were characterized by LC-MS/MS study and the most possible degradation and fragmentation pathways were proposed.

Keywords: Nebivolol; UPLC; process related impurities; stability indicating; validation.

1. INTRODUCTION

Nebivolol (NEB) [α, α'-(iminodimethylene) bis (6-fluoro-2-chromanmethanol)] is a new third-generation β-adrenergic blocker with unique pharmacologic properties compared with other agents in its class. NEB is a vasodilating β-blocker, which can be distinguished from other β-blockers by its haemodynamic profile [1]. It combines β-adrenergic blocking activity with a vasodilating effect mediated by the endothelial L-arginine NO pathway [2-3]. Decreased bioavailability of NO has been demonstrated in essential hypertension and other conditions associated with increased cardiovascular risk, such as diabetes and hypercholesterolemia. NEB therefore, has considerable potential in cardiovascular therapy [4,5]. In addition to its antihypertensive effects, NEB improves arterial compliance [6] and left ventricular (LV) function in patients with heart failure [7]. Treatment with NEB preserves LV function and can reduce LV mass in hypertensive patients with LV hypertrophy [8]. These properties of NEB have considerable therapeutic potential especially in patients with hypertension with coexisting coronary artery disease, a common cause of impaired cardiac function [9]. Endothelial dysfunction occurs early in various forms of CVD and arterial endothelial damage may contribute to the pathogenesis of atherosclerosis in hypertension [10]. A direct action of nebivolol on the vascular endothelium, together with its effectiveness as a β-blocker, may therefore be beneficial in patients with endothelial dysfunction associated with hypertension, diabetes mellitus and hypercholesterolemia. Thus treatment with NEB may slow, or even prevent some of the vascular complications of hypertension. RP-HPLC and HPTLC methods for the estimation of nebivolol hydrochloride in tablet dosage form, simultaneous determination of nebivolol hydrochloride and hydrochlorothiazide in tablets by first-order derivative spectrophotometry and liquid chromatography validation of HPTLC method, with densitometric detection, for quantative analysis of nebivolol hydrochloride in tablet formulations have been discussed [11,12,13]. Liquid chromatographic method development and validation for assay and dissolution of nebivolol hydrochloride in tablet dosage form was reported [14]. Stereo selective analysis of nebivolol isomers in human plasma by HPLC-MS and its application in pharmacokinetics, LC-PDA ultraviolet method for quantification of nebivolol in rat plasma and its application to pharmacokinetic studies, Simultaneous determination of fixed dose combination of nebivolol and valsartan in human plasma by liquid chromatographic-tandem mass spectrometry and its application to pharmacokinetic study have been studied [15,16,17,18]. RP-HPLC determination of nebivolol hydrochloride and its related substances, liquid chromatographic impurity profiling of nebivolol hydrochloride from bulk drug were reported [19-21]. Development and validation of a stability-indicating reverse phase ultra performance liquid chromatographic method for the estimation of nebivolol impurities in active pharmaceutical ingredients and pharmaceutical formulation was reported [22]. A selective and sensitive GC-MS analysis of process related genotoxic impurities of NEB was discussed [23]. Even though the above published methods deals with the method development of related substances of nebivolol hydrochloride the proposed methods were for HPLC, and one UPLC method was proposed but the considered impurities taken for the study was completely different and the characterization of degradation products is a major drawback. In the reported UPLC method nebivolol formulation sample was subjected to the various stress conditions and

significant degradation was observed under peroxide stress condition. However, the above mentioned prior art methods did not disclose the characterization of stress degradation products (DPs) by mass spectrometry. Thus, there is a great scope for development of analytical methods to monitor the levels of all possible impurities in the bulk drugs of NEB during process development and characterization of DPs to ensure its safety in formulations. In the present study we have disclosed a mass compatible stability indicating UPLC method for the determination of nebivolol and its seven process related impurities. Nebivolol was subjected to different stress conditions, as described by the International Council for Harmonization (ICH) guidelines [24,25]. DPs formed under acidic and basic stress conditions were characterized by LC-ESI-MS/MS and probable chemical structures were proposed. The RSs (related substances) were thoroughly investigated using LC–ESI-MS and UPLC–MS/MS techniques and were further confirmed by synthesized reference substances. The information collected from the study plays an important role in providing a detailed chemistry and manufacturing controls package for nebivolol. The synthetic scheme for the preparation of nebivolol was represented in Scheme 1 [26].



Scheme 1. Synthetic route for preparation of Nebivolol

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Analytical-grade reagents were used throughout the method unless stated otherwise. LC-grade acetonitrile and methanol were procured from Merck, Mumbai, India. High purity water was obtained from Milli-Q water purification system, Millipore Pvt. Ltd., Bangalore, India. Analytical-grade HCl and NaOH were procured from SD Fine Chemicals Pvt. Ltd, Mumbai, India. H_2O_2 (27% w/w) was purchased from Acros Organics, India. LR grade H_2SO_4, Ethyl acetate and methanol were procured from RANKEM India Pvt. Limited, Mumbai, India. Reference standard of nebivolol and seven impurities namely Imp-A, Imp-B, Imp-C, Imp- D, Imp-E, Imp-F and Imp-G (Fig. 1) were synthesized and characterized in M/s Green Evolution Laboratories Private Limited, Hyderabad, India.

2.2 Preparation of Standard and Sample Solutions

Stock solutions of NEB were prepared by dissolving in minimum amount of methanol and made up to the volume with diluent. The specification concentrations of NEB are 200 µg. $mL^{-}1$ and 100 µg mL^{-1} for related substances and assay methods, respectively. Working solutions of NEB at 200 µg mL^{-1} spiked with and 100 µg mL^{-1} (unspiked) were prepared from the above stock solutions for the related substances and assay determinations, respectively. The spiked solutions were prepared by taking all impurities in the mobile phase from stock solutions for the evaluation of the LOD, LOQ and linearity in accordance with ICH guidelines. Nebivolol sample solution from stock was spiked with 0.8µg mL^{-1} of all impurities to establish accuracy, precision and robustness. The quantities of impurities were calculated from their peak areas.

The stock solutions of NEB and impurities were adequately mixed and diluted to study accuracy, precision, linearity, robustness, limits of detection and limits of quantification.

2.3 Instrumentation

The analyses were performed on a Quattro Premier XE Mass Spectrometer with an ACQUITY UPLC, which was equipped with an auto injector, PDA detector and a binary pump (Waters Corporation, Milford, MA-01757, and USA). The analytes were separated on a Waters Acquity BEH C18 stationary phase (50 ×2.1 mm, 1.7 µm). The output signal was monitored and processed using the Empower-2 software (Waters). The acquisition and Processing of the mass data was controlled by the mass lynx software. A splitter was placed before the ESI/APCI analysis was performed on a Waters Acquity UPLC system equipped with an auto injector, PDA detector and a binary pump (Waters Corporation, Milford, MA-01757, USA). For optimization of chromatographic conditions, the effects of various method parameters such as mobile phase, column, flow rate and solvent ratio were studied and the chromatographic parameters such as asymmetric factor, resolution and column efficiency were calculated. Mobile phase was a gradient elution of 10 mM Ammonium acetate acid and Acetonitrile. Source to limit the entry of the chromatographic eluent to 40%. The typical operating source conditions for the MS scans in both the positive and negative modes of the ESI/APCI were optimized to the following: the fragment voltage was 30 V; the capillary voltage was 3 kV; the source temperature was 150°C, the gas flow was 1800 L h^{-1}.

2.4 Chromatographic Conditions

The analytes were separated on a Waters Acquity BEH C8 stationary phase (50 ×2.1 mm, 1.7 µm). The mobile phase was prepared by mixing 10 mM Ammonium acetate in water and Acetonitrile filtered through a 0.22 µm PTFE filter and degassed by using an ultra-sonicator for 15 min prior to use.The sample injection volume taken was 0.3 µl. The system was equilibrated for 30 min and analysis was carried out under gradient conditions using a flow rate of 0.3 mL min^{-1} at a temperature of 35°C. Chromatograms were recorded at 222 nm. Same chromatographic conditions were used for LC-MS/MS studies for the identification and characterization of DPs.

2.5 Analytical Method Validation

The validation of the UPLC method was carried out for the determination of related substances (i.e., Imp-A to Imp-G) and assay of NEB as per ICH guidelines to demonstrate that the method is appropriate for its intended use.

2.5.1 System suitability

The system suitability tests were conducted throughout the validation studies by injecting 200 µg. ml^{-1} of NEB solution containing 0.8 µg. ml^{-1}. (0.4% of each impurity) of all related substances

and 100 µg. ml^{-1} of NEB solution for related substance and assay methods, respectively.

2.5.2 Precision

The system precisions were checked by analyzing six replicates of standard solutions for both assay (NEB 100 µg.ml^{-1}) and related substances (NEB 200 µg. ml^{-1} spiked with 0.4% of each impurity) methods individually. The method precisions for assay and related substances were evaluated by injecting six individual test preparations of NEB (100 µg. ml^{-1}), and NEB (200 µg. ml^{-1}) spiked with 0.4% of each impurity, respectively.

The intermediate precision were evaluated with same concentration solutions used for methods precision prepared separately on a different day by different analysts using different instruments located within the same laboratory. Precision at LOQ levels was also determined by injecting six individual preparations of mixtures of all impurities spiked into NEB (200 µg. ml^{-1}) at their LOQ level. The % RSD (relative standard deviation) values of the areas of each impurity and NEB were calculated for precision studies.

2.5.3 Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ values for NEB and related substances (Imp-A to Imp-G) were determined at signal-to-noise ratios of 3: 1 and 10: 1, respectively, by injecting a series of dilute solutions with known concentrations.

2.5.4 Accuracy

Accuracy of the related substance method was evaluated by spiking known amounts of the impurities into the test sample, analyzing the same and calculating the percent recovered. For related substances, the recovery studies were performed in triplicate at three concentration levels (50, 100 and 150%) to specification level (0.4%) of impurities with respect to NEB drug substance concentration 200 µg ml^{-1}. The accuracy of the NEB assay was evaluated in triplicate at the three concentration levels 50, 100 and 150% (i.e., 50, 100 and 150 µg. ml^{-1}) to NEB concentration 100 µg ml^{-1} and the recovery was calculated for each concentration.

2.5.5 Linearity

Linearity of the related substance method was established by analyzing series of dilute solutions at six different concentration levels ranging from 25 to 150% to the specification level of impurities (A-G) spiked into NEB drug substance (200 µg. ml^{-1}). The calibration curves were drawn by plotting the peak areas of impurities against their corresponding concentrations. Similarly, assay method linearity was established by injecting NEB at six different concentration levels ranging from 25 to 150% (25, 50, 75, 100, 125, and 150%) to NEB concentration 100 µg ml^{-1}. The correlation coefficients (r^2), slopes and Y-intercepts of impurities and NEB were determined from their respective calibration plots.

2.5.6 Robustness

The robustness study was carried out to evaluate the influence of small variations in the optimized chromatographic conditions. The factors chosen for this study were temperature of the column (± 2°C), mobile phase pH (± 0.2) and flow rate (± 0.1 ml min^{-1}). System suitability parameters and changes in assay of NEB and recoveries of impurities were checked. In all the above deliberately altered experimental conditions, the components of the mobile phases were held constant.

2.5.7 Degradation studies

Specificity is the ability of the UPLC method to measure the analyte NEB response unequivocally in the presence of its potential process related impurities and degradation products. Forced degradation studies of NEB drug substance can help to identify the possible DPs (degradation products), which in turn can help to establish the degradation pathways and the intrinsic stability of the molecule and to validate the stability indicating nature of the developed analytical method. The specificity of the developed LC method for NEB was carried out in the presence of its DPs. About 10 mg of NEB (1.0 mg ml^{-1}) solution was subjected to stress degradation such as acid (1 N HCl, 1 h), alkaline (1N NaOH, 1 h), neutral hydrolysis (60°C, 8 h) and oxidation (10% H_2O_2, 24 h) conditions. NEB drug substance was also exposed to heat (60°C, 7 days) and UV light (254 nm, 7 days) degradation in solid state. Different stress conditions were carried out to achieve significant degradation. The collected degradation samples were neutralized (for acidic and basic hydrolyzed) and diluted five times prior to analysis. Peak purity tests were carried out to check purity and homogeneity of NEB peak in stressed samples by using PDA detector. Assay

of NEB in stressed samples was performed by comparison with qualified reference standard and the mass balance (% assay + % impurities + % degradation products) was calculated. The specificity of the method was also checked by comparing the assays of NEB in both spiked (with 0.4% impurities), and unspiked samples.

3. RESULTS AND DISCUSSION

3.1 Development and Optimization of the UPLC Method

The structure of NEB contains polar amine and hydroxyl functional groups and, on the basis of this, UPLC development trials commenced with a mobile phase containing acidic pH buffer to retain the analyte in its unionized form. Acetonitrile was used as a high polarity solvent with a low UV cut-off. Detection at a low UV wavelength makes the stability-indicating method more sensitive. A number of development trials were performed to optimize the separation by varying the factors such as flow (from 0.1to 0.5 mL min^{-1}) and various ratios of acetonitrile to 0.05 M potassium phosphate buffer (between pH 2.5 and 4.5). To obtain a rapid method, short length UPLC columns (C-18 or C-8) were exercised.

Desired separation was achieved on the sample solution spiked with all impurities on a 50-mm C18 Alltima column at a flow rate of 0.3 mL min^{-1} with a mobile phase of pH 3.5 buffer: acetonitrile, 30:70 v/v. For method development, C18 and C8 stationary phases were tested for separating the analytes applying several combinations of the proposed mobile phase and using acetonitrile or methanol as a diluent. The results were compared and good separation was achieved with Waters acquity BEH C18 stationary phase (50 × 2.1 mm, 1.7µm) with a mobile phase consisted of a mixture of 10 mM ammonium acetate and acetonitrile in a gradient mode. The PDA detector was set at 222 nm. Injection volume was 0.3 µL and data acquisition time was 10 min. Column oven temperature was maintained at 35°C. Gradient elution program was as follows: time (min)/: B (v/v); 0.01/25, 3.0/80, 7/80, 8.5/25, 10/25. Peak separation was satisfied and resolution values (R) were greater than 2 for all peaks, Fig. 1.

3.2 Method Validation

System suitability data indicating that the system was suitable for use as the tailing factor for all the analytes was less than 1.6 and the resolution between any of the two adjacently eluting analytes was greater than 2. It also confirms the good selectivity of the method.

3.2.1 Specificity

Specificity was established by injecting a mixture of nebivolol (200 µg. ml^{-1}) with impurities 0.8 µg.ml^{-1} (0.4% of each impurity). No interference occurred between Nebivolol and its impurities, which proved that the method is sufficiently specific, Table 2.

3.2.2 Precision

The % RSD values of peak areas obtained were below 0.3-2.2 and 0.3-0.5 for related substances and NEB, respectively, indicating good precision of the method.

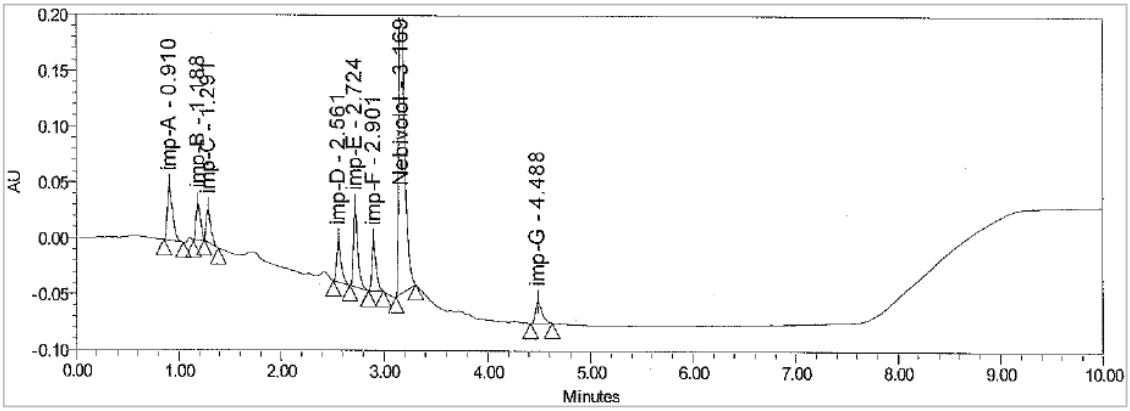


Fig. 1. Chromatogram of Nebivolol spiked with seven process related impurities under optimized chromatographic conditions

3.2.3 Accuracy

The percentage recoveries of related substances ranged from 98.04 to 103.11 and NEB from 98.93 to 100.79 determining the accuracy of the method.

3.2.4 Linearity and range

Linearity results of NEB and related substances showed the existence of an excellent correlation (r^2 > 0.998) between the peak area and the concentrations.

3.2.5 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of NEB and its impurities were established as signal to noise ratio of three times and ten times greater than the background noise, respectively. LOD and LOQ results of NEB and its related substances were obtained in the ranges of 0.01–0.2 µg. ml^{-1} and 0.02–0.61 µg. ml^{-1}, respectively, indicating the higher sensitivity of the method. The results of system suitability, LOD, LOQ, precision, accuracy and linearity are summarized in Table 1.

3.2.6 Robustness

In all deliberately altered chromatographic conditions (flow rate, pH and column temperature), all analytes were adequately resolved and elution order remains unchanged. The resolutions between any two adjacent analytes were obtained greater than 2.0 and tailing factors of all analytes were obtained less than 1.8. The variability in the estimation of NEB assay and related substances was within ± 2 and ± 9%, respectively, indicating the robustness of the method.

Table 1. Validation results

Parameter	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	NEB	Imp-G
RT	0.97	1.26	1.36	2.61	2.73	2.93	3.2	4.51
RRT	0.3	0.39	0.43	0.81	0.86	0.93		1.42
Rs		4	1.5	19.9	2.1	3.7	3.7	12
N	10254	11986	17241	16214	19875	23128	18419	20146
Asymmetry factor	1.2	1.1	1.1	1.2	1.1	1.1	1	1.2
Selectivity	NA	3.2	1.4	13.9	2.1	3.6	3.1	12.9
Linearity/Range	0.2-1.2	0.2-1.2	0.2-1.2	0.2-1.2	0.2-1.2	0.2-1.2	50-300	0.2-1.2
Slope	20859	12735	11797	14373	26819	16061	6068	10882
r^2	0.999	0.999	0.999	0.998	0.999	0.999	0.999	0.999
Intercept	582	-29.46	25.8	-54.46	-31.6	20.6	119.36	98.13
Limit of detection (µg. mL^{-1})	0.01	0.01	0.06	0.05	0.04	0.05	0.2	0.05
Limit of quantification (µg. mL^{-1})	0.02	0.02	0.17	0.15	0.13	0.16	0.61	0.15
RRF	3.44	2.09	1.94	2.36	4.41	2.64		2.78
Precision % RSD (n=6)								
System	1.89	1.71	1.24	1.09	1.58	1.23	0.44	0.39
Method	1.45	1.33	1.47	1.04	1.08	1.3	0.3	2.12
Intermediate	1.08	1.45	2.2	0.477	1.84	1.54	0.53	0.39
Accuracy at 50% level								
Amount added (µg. mL^{-1})	0.4	0.4	0.4	0.4	0.4	0.4	100	0.4
Amount recovered (µg. mL^{-1})	0.396	0.399	0.4	0.396	0.4	0.41	101.78	0.39
% recovery	99.22	99.89	101	99.04	100.19	102.74	101.79	99.71
Accuracy at 100% level								
Amount added (µg. mL^{-1})	0.8	0.8	0.8	0.8	0.8	0.8	200	0.8
Amount recovered (µg. mL^{-1})	0.824	0.784	0.81	0.79	0.8	0.79	200.62	0.8
% recovery	103.11	98.04	101.72	99.65	100.79	98.89	100.31	100.21
Accuracy at 150% level								
Amount added (µg. mL^{-1})	1.2	1.2	1.2	1.2	1.2	1.2	300	1.2
Amount recovered (µg. mL^{-1})	1.21	1.19	1.2	1.21	1.21	1.19	296.8	1.2
% recovery	101.16	99.49	100.1	101.39	101.51	99.72	98.93	100.15

3.2.7 Results of forced degradation studies

Degradation was not observed in NEB samples when subjected to stress conditions like photolytic, thermal and aqueous hydrolysis (Fig. 2). NEB was degraded to Imp-B under base hydrolysis and was degraded to Imp-B and Imp-C under oxidation. Peak purity test results obtained by using a PDA detector confirmed that the nebivolol peak is homogenous and pure in all the analyzed stress samples. The assay of NEB is unaffected in the presence of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G and its degradation products confirm the stability indicating power of the method. The summary of forced degradation studies is given in Table 2.

3.2.8 Degradation

The degraded samples were analysed under optimised chromatographic conditions. The degradative compounds were identified based on the molecular weight and probable chemical structures were proposed. Percentage of degradation was calculated and peak purity test was carried out for the parent drug and degradative compounds and the results are shown in Table 2. Identity confirmation of degradative compounds was confirmed by co-injection of the degradative mixtures and its standard solutions, leading in enhancing the peaks of similar impurities.

To confirm the presence of degradation products MS detectors are helpful, because of its high sensitivity and selectivity. With an ability to separate and identify components with similar structures and close retention times, LC–MS/MS is especially useful in analysing the degradation products. However, LC methods must be able to separate the targeted products. During our study, NEB and its two DPs (degradation products) (DP-1 and DP-2) were well separated by the LC method. The mass spectra showed that NEB, DP-1, DP-2 produced abundant deprotonated molecular ions ($[M+H]^+$) in positive ionization mode. These DPs were characterized using ESI-MS/MS to study their fragmentation patterns. The ESI-MS/MS spectra of NEB and the two DPS are shown in Fig. 3.

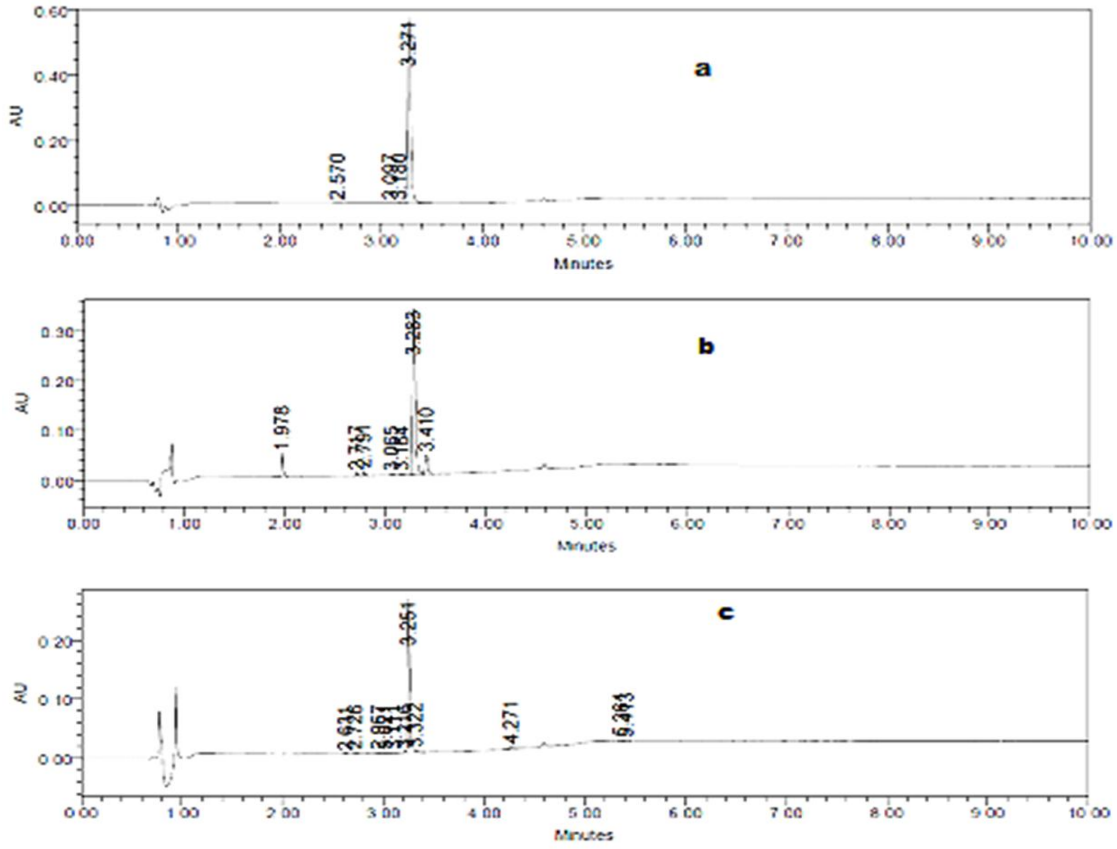


Fig. 2. Chromatograms of NEB: (a) As such (b) Acidic (c) Basic stress samples

Table 2. Summary of forced degradation of Nebivolol

Parameters	RT	% Assay	% Degradation	Mass balance (Assay + IMP)	Purity angle	Purity threshold
As such	3.271	98.2	NA	98.2	0.21	0.31
Acid degradation (5 mL 1N HCl/2h, 60°C)	3.251	80.44	19.49	99.98	0.625	2.695
Base degradation (5 mL 1N NaOH/2h,60°C)	3.251	94.31	4.88	99.19	0.321	0.338
Peroxide degradation (5 mL 1% H2O2/1h, 60°C)	3.275	98.07	1.34	99.41	0.165	0.171
Photolytic degradation (1.2 mn Lux h, 200 W)	3.271	99.8	NA	99.8	0.221	0.312
Thermal degradation (60°C temperature, 3 h)	3.271	99.79	0.03	99.82	0.211	0.315
Humidity degradation (40°C,75% RH, 24 h)	3.272	99.8	NA	99.8	0.224	0.314

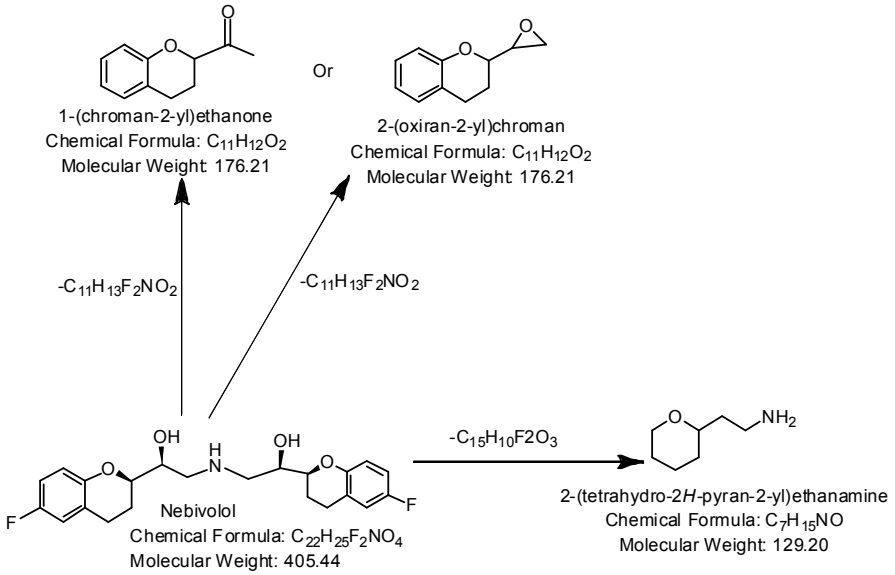


Fig. 3. Proposed fragmentation pathways for Nebivolol

3.2.9 MS/MS of NEB (m/z 406)

To elucidate the degradation patterns of NEB an ESI-MS spectrum of its deprotonated molecular ion ($[M+H]^+$) at m/z 406 was examined. The ESI-MS/MS spectrum of this ($[M+H]^+$) ion showed a high abundance of a product ion at m/z 177; this signal might be attributed to the formation of (2-oxiran-2-yl-chroman) or 1-(chroman-2-yl)ethanone formed due to the (loss of (R)-2-amino-1-((S)-6-(flourochroman-2-yl-)ethanol from Nebivolol. The spectrum also showed two low-abundance peak at m/z 130 may be due to the formation of 2-(tetrahydro-2H-pyran-2-yl) ethanamine.

3.2.10 MS/MS of DP-1 (m/z 161)

The ESI-MS spectrum of its abundant deprotonated molecular ion ($[M+H]^+$) at m/z 161 m/z 161 (1-chroman-2-yl-)ethan-1-ylium) formed in acidic stress conditions was examined to elucidate the degradation behaviour of degradation product 1. The ESI-MS/MS spectrum of ($[M+H]^+$) ion of DP-1 showed low abundant product ion peaks at m/z 115, and m/z 114 which might be due to the formation of 2-ethyltetrahydro-2H-pyran and 1-(tetrahydro-2H-pyran-2-yl)ethan-1-ylium. 6-ethyltetrahydro- 2H-pyran-3-ylium.

3.2.11 MS/MS of Dp-2 (m/z 279.17)

The ion of m/z 279 might have formed due to the loss of m/z 127 from nebivolol under the basic stress conditions. The ESI-MS/MS spectrum of m/z 279 may be due to the formation of 1-((2-(chroman-2-yl)-2-hydroxyethyl)amino)-2-hydroxypentan-3-ylium. The ion at 178 (M+191) might be due to the formation of 2-(chroman-2-yl)- 2-hydroxyethan-1-ylium. In addition, the spectrum showed low-abundance peaks at m/z 177 and m/z 113. The ion at m/z 177 might be due to the formation of 2-isopropylchroman. The ion at m/z 113 might be due to the formation of 1-(tetrahydro-2H-pyran-2-yl)ethan-1-ylium.

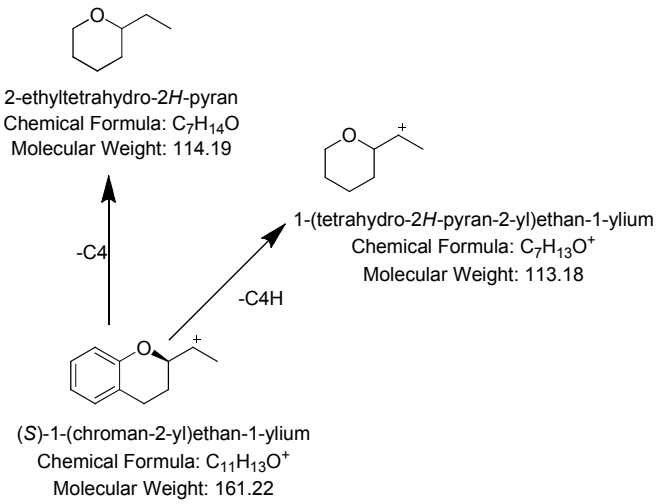


Fig. 4. Proposed fragmentation pathways for Dp-1

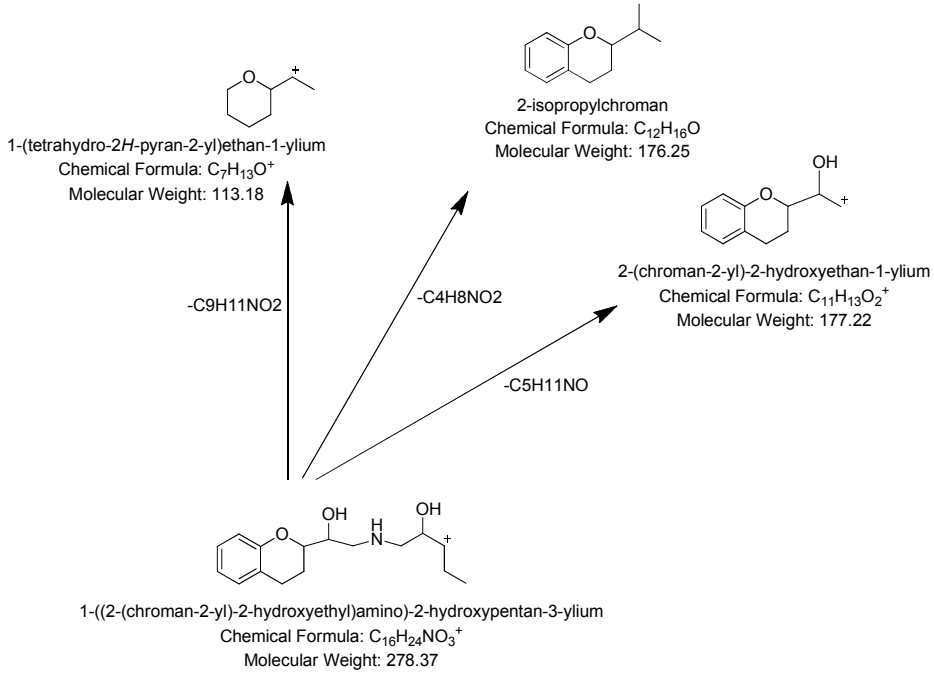


Fig. 5. Proposed fragmentation pathways for Dp-2

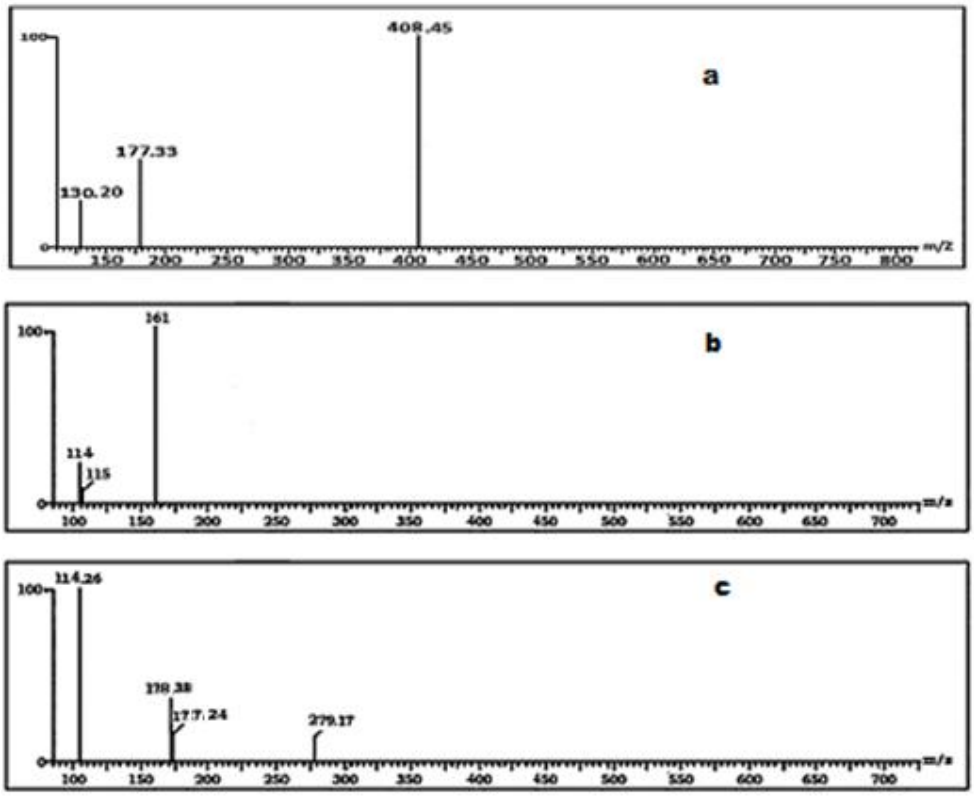


Fig. 6. ESI-MS/MS spectra of molecular ions of (a) NEB (b) DP-1(c) DP-2

4. CONCLUSION

Gradient stability indicating RP-UPLC method was developed for the separation and determination of process related impurities in nebivolol, the degradation behaviour of nebivolol was studied under various stress conditions as per International Conference on Harmonization (ICH) prescribed guidelines. In total, two degradation products were formed and characterized by liquid chromatography and tandem mass spectrometry. The proposed method is simple, selective, precise and sensitive. Therefore, this method can be used for routine testing as well as stability analysis of nebivolol drug and its process-related impurities. All statistical results (mean, %RSD and %recovery) were within the acceptance criteria. Full validation of the method furnished satisfactory results for all the method validation data tested. The method was stability-indicating and can be conveniently used by quality control departments for analysis of related substances, assay of nebivolol samples, and sample stability testing.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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