Original Article

Stress degradation studies of Telmisartan and Metoprolol extended release tablets by a validated stability indicating reverse phase-high performance liquid chromatography method

Abstract

Background and Aim: A sensitive reverse phase high-performance liquid chromatographic method has been developed for the simultaneous determination of Telimisartan and Metoprolol in tablet dosage form. **Materials and Method:** The chromatographic separation was achieved on Inertsil ODS 3V, 150 x 4.6 mm, 5µ analytical column. Mobile phase consisting of mobile phase A- 0.05M sodium dihydrogen phosphate buffer pH 3.0 and mobile phase B–Acetonitrile, with gradient program time in min /Mobile phase B% 0/22, 4/45, 6/45,18/22, 20/22. Detector was set at 222nm. **Results and Conclusion:** The described method shows excellent linearity over a range of 80–2 µg mL^{-1} for Telmisartan and 100-4 µg mL^{-1} for Metoprolol. The correlation coefficient for Telmisartan is 0.9998 and Metoprolol is 0.9999. The proposed method was found to be suitable for determination of Telmisartan and Metoprolol in tablet dosage form. Forced degradation of the drug product was conducted in accordance with the ICH guideline. Acidic, basic, hydrolytic, oxidative, thermal and photolytic degradation was used to assess the stability indicating power of the method. The drug product was found to be stable in acid, oxidation, thermal and photolytic stress condition and found degradation in base hydrolysis stress condition.

Key words:
Forced degradation, Metoprolol, RP-high performance liquid chromatography, Telmisartan

Introduction

Metoprolol succinate chemically is 1-(isopropyl amino)-3-[4-(2-methoxyethyl) pehnoxy] propan-2-ol[1] [Figure 1] is a selective β_1 receptor blocker used in the treatment of several diseases of the cardiovascular system, especially hypertension. Telmisartan chemically is 2-(4-{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl) benzoic acid [Figure 1].[2] It is angiotensin II receptor antagonist, effective in the treatment of hypertension. It is also effective when used alone or in combination with other drugs for the treatment of high blood pressure. A fixed dose combination of 40 mg Telmisartan and 50 mg Metoprolol ER is available commercially as tablets (Telmaxx, Glenmark Pharmaceuticals), are widely used for the treatment of cardiovascular disease. Extended release formula of Metoprolol is designed such that it will delay the release of the Metoprolol in the body over a period of time. A drug of this formula may have effects that last until the next dosage is taken. The two main aspects of drug products that play an important role in shelf life determinations are assay of active drug and degradants generated during the stability study. The literature survey reveals that several high performance liquid chromatography (HPLC)/ultraviolet (UV) methods were reported for the determination

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of Telmisartan individually[3-5] or in combination with Hydrochlorothiazide.[6-9] There are few HPLC methods are available for the simultaneous determination Telmisartan in combination with other drug substances,[10,11] also liquid chromatography-mass spectrometry (LC/MS) methods for the determination of Telmisartan or in combination with other drugs substances in human plasma.[12-14] Some HPLC/UV methods are available for the determination of Metoprolol as individual or in combination with other drug substances.[15-19] There are two UV/HPLC methods available for the determination of Telmisartan and Metoprolol in tablet dosage form,[20,21] but both these methods are not stability indicating also forced degradation study is not conducted. The base degradation of drug product may seriously affect the quality of products, and is usually associated with a reduction of the pharmacological activity and/or the occurrence of side effects. The stress conditions are useful for establishing degradation pathways, developing and validating suitable procedures. Hence present study attempt was made to develop a rapid, economical, precise, accurate and stability indicating method for the simultaneous determination of Metoprolol and Telmisaratan in tablet dosage form in the presence of their degradants. The chemical structures of Telmisartan and Metoprolol is shown in Figure 1.

Materials and Methods

Instrumentation

The chromatographic system used was a Dionex-3000 series comprised of degasser, quaternary pump, auto injector, column compartment, photodiode array detector and the system was controlled through Thermo Scientific, Dionex Chromoleon Software

Materials

Telmisartan and Metoprolol succinate working standards were obtained as gift sample from Sir Sayyed College Roshan Gate, Aurangabad, M.S., India. Sodium dihydrogen phosphate (AR grade), orthophosphoric acid (AR grade), methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from Merck Fine Chemicals (Mumbai, India). The 0.45-um nylon filter was obtained from Advanced Micro Devices Pvt. Ltd. The combination product of Telmisartan and Metoprolol ER tablet (Brand name, Telmaxx, Glenmark pharmaceuticals Ltd. Telmisartan 40 mg and Metoprolol 50 mg) Branded Tablets were purchased from the Indian market. Double distilled water was used throughout the experiment. Other chemicals used were of analytical or LC grade.

Preparation of standard solutions

A solution containing 40 µg/mL of Telmisartan and 50 µg/mL of Metoprolol was prepared in diluent.

Preparation of sample solution

Tablet powder equivalent to 50 mg of Metoprolol and 40 mg of Telmisartan was transferred into a 100 mL volumetric flask, to which 70 mL of methanol was added and sonicated for about 20 min to dissolve and the volume was made up to 100 mL with methanol. Centrifuged 10 mL of solution and further diluted 5 mL to 50 mL with diluent. A typical HPLC chromatogram of the sample solution is shown in Figure 2.

Chromatographic conditions

A Dionex-3000 HPLC comprising online degasser, quaternary pump, auto injector, column compartment and photodiode-array detector was used and controlled through Chromeleon Software. Mobile phase A-0.05 M sodium dihydrogen phosphate buffer pH 3.0 and Mobile phase B-acetonitrile (min: Mobile phase B 0/22, 4/45, 6/45, 18/22, 20/22). At a flow rate of 1.0 mL/min. The column compartment temperature was set at 40°C and the UV spectrum of all components exhibits a relative absorption maximum at 222 nm. Diluent 0.05 M Sodium dihydrogen phosphate buffer pH 3.0 and Acetonitrile in the ratio of 75:25 v/v. Typical HPLC chromatograms are extracted at this wavelength and system suitability results are listed in Table 1.

Method validation

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ),

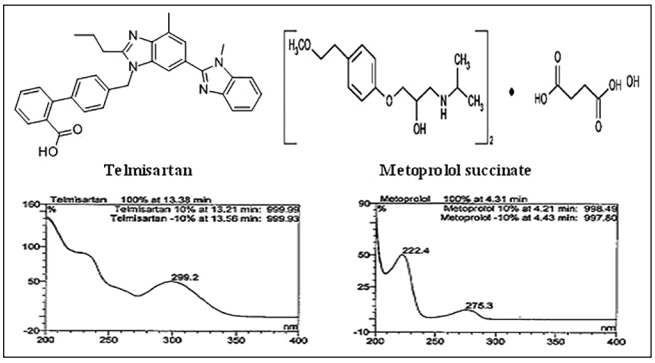

Figure 1: Chemical structure and spectrum of telmisartan and metoprolol succinate

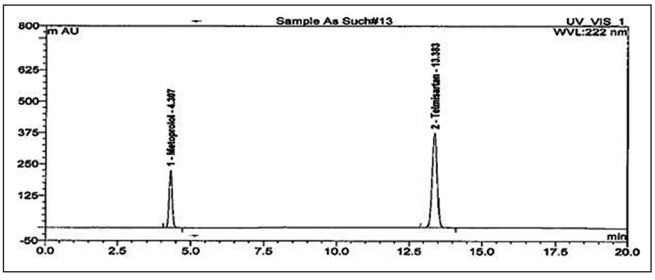

Figure 2: A typical high performance liquid chromatography chromatogram of unstressed sample solution

robustness and system suitability, in accordance with ICH guidelines Q2 (R1).[22]

Specificity

The specificity of the method was checked by injecting solution containing excipient without drug substances, and no chromatographic interference was observed from excipients at the retention time of the analyte peaks. Peak purity was verified by confirming homogeneous spectral data for Metoprolol and Telmisartan. The specificity of the method was also checked by performing the stressed study and no interference from degradation products at the retention time of Metoprolol and Telmisartan was found.

Forced degradation study of drug product

Forced degradation studies was performed to demonstrate the selectivity and stability indicating capability of the proposed method. The powdered samples of tablets was exposed to acidic, alkaline and oxidizing degradation conditions. The stress conditions engaged for degradation studies as per ICH recommendation[23] summary of forced degradation results is captured in Table 2. The stress degradation study has been perform on the drug product as follows.

Acid hydrolysis

Tablet powder equivalent to 50 mg of Metoprolol and 40 mg of Telmisartan was transferred into a 100 mL volumetric flask, to which 70 mL of methanol was added and sonicated for about 20 min to dissolve, further added 5 mL of 2 M HCl and solution was heated at 60°C for 1 h, cool the solution to room temperature and neutralized with 5 mL of 2 M NaOH and the volume was made up to 100 mL with methanol. Centrifuged 10 mL of solution and further diluted 5 mL to 50 mL with diluents and mix.

Table 1: System suitability studies

Parameter	Telmisartan	Metoprolol
Theoretical plates	28328	7039
Resolution	15.53	–
Asymmetry factor	1.0	1.1
%RSD (peak area)	0.10	0.10

RSD: Relative standard deviation

Base hydrolysis

Tablet powder equivalent to 50 mg of Metoprolol and 40 mg of Telmisartan was transferred into a 100 mL volumetric flask, to which 70 mL of methanol was added and sonicated for about 20 min to dissolve, further added 5 mL of 2 M NaOH and solution was heated at 60°C for 1 h, cool the solution to room temperature and neutralized with 5 mL of 2 M HCl and the volume was made up to 100 mL with methanol. Centrifuged 10 mL of solution and further diluted 5 mL to 50 mL with diluents and mix.

Peroxide degradation

Tablet powder equivalent to 50 mg of Metoprolol and 40 mg of Telmisartan was transferred into a 100 mL volumetric flask, to which 70 mL of methanol was added and sonicated for about 20 min to dissolve, further added 5 mL of 10% H_2O_2 and solution was kept on bench top for 1 h, and the volume was made up to 100 mL with methanol. Centrifuged 10 mL of solution and further diluted 5 mL to 50 mL with diluents and mix.

Thermal degradation

Tablet powder was exposed to thermal degradation at 60°C for 24 h. Further weigh transferred tablet powder equivalent to 50 mg of Metoprolol and 40 mg of Telmisartan to 100 mL volumetric flask, to which 70 mL of methanol was added and sonicated for about 20 min to dissolve and the volume was made up to 100 mL with methanol. Centrifuged 10 mL of solution and further diluted 5 mL to 50 mL with diluents and mix.

Photolytic degradation

Drug product exposed UV and visible light so has to complete 1.2 million lux hours and 200 Wh/m^2 and measured with the help of Lux meter and UV meter respectively. Tablet powder was exposed to thermal degradation at 60°C for 24 h. Further weigh transferred tablet powder equivalent to 50 mg of Metoprolol and 40 mg of Telmisartan to 100 mL volumetric flask, to which 70 mL of methanol was added and sonicated for about 20 min to dissolve and the volume was made up to 100 mL with methanol. Centrifuged 10 mL of solution and further diluted 5 mL to 50 mL with diluents and mix.

Table 2: Forced degradation study

Stress condition/media/duration	Telmisartan assay (%)	Telmisartan degradation (%)	Metoprolol assay (%)	Metoprolol degradation (%)
Unstressed sample	99.8	–	99.5	–
Acidic/2 N HCl/60°C/60 min	99.3	0.5	98.7	0.8
Alkaline/2N NaOH/60°C/60 min	97.3	2.5	91.3	8.2
Oxidative/10% H_2O_2/ambient/60 min	98.3	1.5	97.4	2.1
Heat/60°C/24 h	99.0	0.8	99.2	0.3
Humidity/40°C, 75% RH for 7 days	99.6	0.2	98.8	0.7
Photostability/1.2 million lux hours and 200 watt h/m^2	99.5	0.3	99.0	0.5

The purity of Metoprolol and Telmisartan both peaks estimated using the peak purity match factor, which can be calculated by Chromeleon® Chromatography Data System (CDS) software. All stress study results are summarized [Table 2].

Linearity

Linearity of the method was tested from 10% to 200% of the targeted level of the assay concentration (Temisartan 40 ug/mL, Metoprolol 50 ug/mL) for both analytes. Mixed standard solutions contaning 4-80 ug/mL of Telmisartan and 5-100 ug/mL of Metoprolol. Linearity solutions were injected. The calibration graphs were obtained by plotting peak area ratio against the concentration of the drugs. The equations of the calibration curves for Telmisartan and Metoprolol obtained was y = 11306x – 2629 and y = 11773x – 805.4 respectively. In the simultaneous determination, the calibration graphs were found to be linear in the aforementioned concentrations with correlation for Telmisartan is 0.9998 and Metoprolol is 0.9999. Linearity plot is as shown in Figure 3.

Precision

The repeatability of the analytical method was evaluated by assaying six samples solutions of telmisratn 40 ug/mL and Metoprolol 50 ug/mL, during the same day, under the same experimental conditions. Intermediate precision was evaluated by assaying solutions on different days. Peak areas were determined and compared. Precision was expressed as percentage relative %RSD <2. From the data obtained, the developed RP-HPLC method was found to be precise [Table 3].

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments was performed by spiking solution of known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (40 mg of Ttelimisartan and 50 mg of Metoprolol). Placebo equivalent to one tablet was transferred into a 200 mL volumetric flask and the amounts of Telmisartan and Metoprolol at 50, 100 and 150% of the label claim of the tablet was added. Three samples were prepared for each recovery level. The solutions was then analyzed, and the percentage recoveries was calculated. The recovery values for Telmisartan and Metoprolol are as shown in Table 3.

LOD and LOQ

The LOD and LOQ for Telmisartan and Metoprolol was determined at a signal to-noise ratio of 3:1 and 10:1, respectively by injecting a series of dilute solutions with known concentrations. The LOD and LOQ are as shown in Table 3.

Robustness

The robustness of a method is the ability to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered and tailing factor, % relative standard deviation (%RSD) of five replicate injection of Telmisartan and Metoprolol standard solution were evaluated. The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on the tailing factor and %RSD of five replicate injection of Telmisartan and Metoprolol standard solution, it was changed to 0.2 units from 1.0 to 1.2 mL/min and 0.8 mL/min. The effect of column temperature on the tailing factor, %RSD of five replicate injection of Telmisartan and Metoprolol standard solution was studied at 35 and 45°C instead of 40°C. The effect of pH variation of buffer on the tailing factor and %RSD of five replicate injection of Telmisartan and Metoprolol standard solution was studied at pH 2.8 and pH 3.2 instead of pH 3.0.

Result and Discussion

Method development and optimization

The main criteria for development of a successful HPLC method for determination of Telmisartan and Metoprolol in tablet dosage form was the method should be able to determine

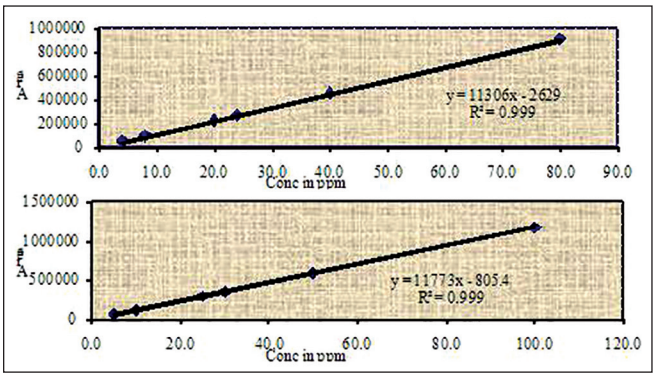

Figure 3: Linearity graph of Telmisartan and Metoprolol

Table 3: Results from validation studies

Parameter	Telmisartan	Metoprolol
Linearity range (µg/mL)	4-80	5-100
Correlation coefficient	0.9998	0.9999
LOD (ug/mL)	0.0033	0.007
LOQ (ug/mL)	0.01	0.021
Accuracy (%) (n=6)		
50	99.5	100.2
100	99.8	100.5
150	100.3	100.0
Precision RSD (%) (n=6) (RSD% < 2)		
Repeatability day 1	0.26	0.52
Intermediate precision	0.35	0.64

LOD – Limit of detection; LOQ – Limit of quantification; RSD – Relative standard deviation

assay of both drugs in single run. Developed method should be accurate, reproducible, robust, stability indicating, free of interference from degradation products, and straight forward enough for routine use in the quality control laboratory.

Our objective of the chromatographic method development was to achieve a peak tailing factor <2, Run time up to 20 min, along with a resolution between Telmisartan and Metoprolol >5. In order to optimize the LC separation of Telmisartan and Metoprolol initially the retention behavior of both the components was studied in the pH range of 2.5-6.8, using mobile phases of buffer (pH 2.5-6.8) and acetonitrile, methanol as organic modifier.

To ensure resolution between Telmisartan and Metoprolol not <5, the method was as fast as possible, a gradient run was optimized by using the mobile phase consisted of mobile phase A-0.05M sodium dihydrogen phosphate buffer pH 3.0 and mobile phase B-Acetonitrile (min/Mobile phase B% 0/22, 4/45, 6/45,18/22, 20/22). Gradient elution at flow rate of 1.0 mL/min and column temperature at 40°C. By considering the UV spectrum of both the analytes which gives good response at 222 nm, detector wavelength was kept 222 nm using a photodiode array detector. The analytes of this combination had adequate retentions, peak shape, less tailing, more resolution and the chromatographic analysis time was 20 min.

Stress degradation behavior of drug product

After the finalization of chromatographic condition stability indicating capability of method has been determined by carrying out forced degradation of drug product. The purity of Metoprolol and Telmisartan both peaks estimated using the peak purity match factor, which can be calculated by Chromeleon® CDS software. All the degradation product generated during the forced degradation study are well separated from the principle peaks. Match factor value of principle peaks obtained from all stressed condition is not <990 indicates that there is no any peak interfering or co-eluting with the principle peak, also indicates that both the peaks are pure. Major degradation was observed when drug product was subjected to base hydrolysis stress conditions compare to acid and peroxide stress condition [Figures 4-6]. Less degradation of drug product is observed when exposed to heat, humidity and photolytic stress condition. Metoprolol was sensitive in basic conditions. Metoprolol and Telmisartan peaks obtained from all stressed conditions are pure and unaffected by the presence of the degradation products generated during stress study, confirming the stability-indicating power of the method.

Conclusion

Stress degradation study was considered as tool to check stability indicating capability of developed method and useful to establish the degradation pathways. The developed simple LC method for assay determination of Telmisartan and Metoprolol is linear, precise, accurate and specific. The method was validated to the requirements of ICH and the results were satisfactory. The developed stability-indicating analytical method can be used for the routine analysis of production samples, where sample load is higher and high throughput is essential for faster delivery of results.

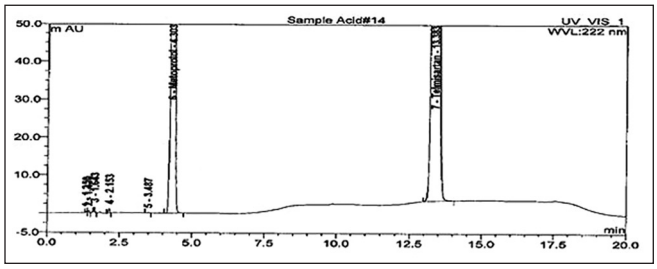

Figure 4: Chromatogram of the acid hydrolysis degradation sample solution

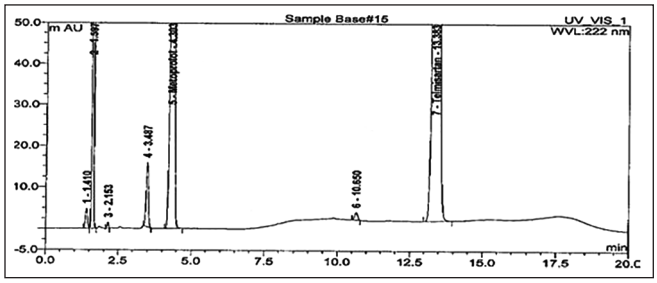

Figure 5: Chromatogram of the base hydrolysis degradation sample solution

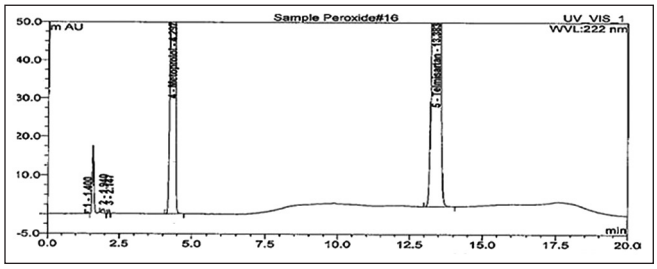

Figure 6: Chromatogram of the peroxide degradation sample solution

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