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RAPID COMMUNICATION

A Rapid Stability-Indicating RP-HPLC Method for the Determination of Betaxolol Hydrochloride in Pharmaceutical Tablets

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Abstract: A stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for the determination of betaxolol hydrochloride, a drug used in the treatment of hypertension and glaucoma. The desired chromatographic separation was achieved on a Nucleosil C18, 4 µm (150 × 4.6 mm) column, using isocratic elution at a 220 nm detector wavelength. The optimized mobile phase consisted of a 0.02 M potassium dihydrogen phosphate: methanol (40:60, v/v, pH 3.0 adjusted with *o*-phosphoric acid) as solvent. The flow rate was 1.6 mL/min and the retention time of betaxolol hydrochloride was 1.72 min. The linearity for betaxolol hydrochloride was in the range of 25 to 200 µg/mL. Recovery for betaxolol hydrochloride was calculated as 100.01%–101.35%. The stability-indicating capability was established by forced degradation experiments and the separation of unknown degradation products. The developed RP-HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines. This validated method was applied for the estimation of betaxolol hydrochloride in commercially available tablets.

Keywords: betaxolol hydrochloride, method validation, forced degradation, tablet drug product, chromatography

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Introduction

Betaxolol hydrochloride is a cardioselective beta-adrenergic receptor blocking agent used in the treatment of hypertension and glaucoma.[1–4] Betaxolol hydrochloride was approved by the US Food and Drug Administration (FDA) for the treatment of hypertension as 10 mg and 20 mg tablets for oral administration. The initial dose of betaxolol hydrochloride in hypertension is ordinarily 10 mg once daily, either alone or added to diuretic therapy. If the desired response is not achieved, the dose can be doubled after 7 to 14 days.[1,2] Few methods have been reported for the quantitative determination of betaxolol hydrochloride in tablet formulations by high performance liquid chromatography (HPLC) and spectrophotometric procedures.[5–7] These reported methods were not validated as per ICH guidance for specificity and forced degradation studies. Therefore, they are not suitable for the quantification of the betaxolol hydrochloride drug products. The proposed assay is able to separate betaxolol hydrochloride from tablet ingredients and from unknown degradation products within 10 minutes. This assay was validated according to the International Conference on Harmonization (ICH) guidelines.[8–12] The chemical structure and UV spectrum of betaxolol hydrochloride are presented in Figure 1.

Materials and Methods

Materials and reagents

Analytical pure betaxolol hydrochloride was procured from Inresa Pharma (Bartenheim, France). HPLC grade methanol was obtained from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate (Sigma-Aldrich, Saint-Louis, USA) and orthophosphoric acid (VWR International, Leuven, Belgium) were analytical grade and used for the preparation of the mobile phase. Kerlone® 20 mg tablets (containing 20 mg betaxolol hydrochloride per tablet) were purchased from Sanofi-Aventis (Paris, France).

Chromatographic conditions

Chromatographic separations were achieved by using an Ultimate 3000 binary analytical LC system (ThermoScientific, Salt Lake City, UT, USA) comprising a binary pump, thermostated autosampler, thermostated column compartment, photodiode array detector, and Chromeleon 6.8 chromatography data system. The autosampler rack temperature was set to 4 °C and the column compartment temperature to 20 °C; 20 µL of sample was injected into the HPLC system. Separations were performed on the reversed-phase column (250 × 4.6 mm inside diameter) packed with 5 µm Nucleosil C18. The isocratic mobile phase consisted of 40% 0.02 M potassium phosphate buffer and 60% methanol, and adjusted to pH 3 with orthophosphoric acid. The mobile phase was delivered at a flow rate of 1.6 mL/minute (min). Eluate was monitored at 220 nm.

Stock solution preparation

Stock solutions were prepared by accurately weighing 200 mg of betaxolol hydrochloride and transferring to 200 mL volumetric flasks containing 10 mL of deionized water. The flasks were sonicated for 10 min to dissolve the solids. Volumes were made up to the mark with deionized water, which gave 1 mg/mL. Aliquots from stock solutions were appropriately

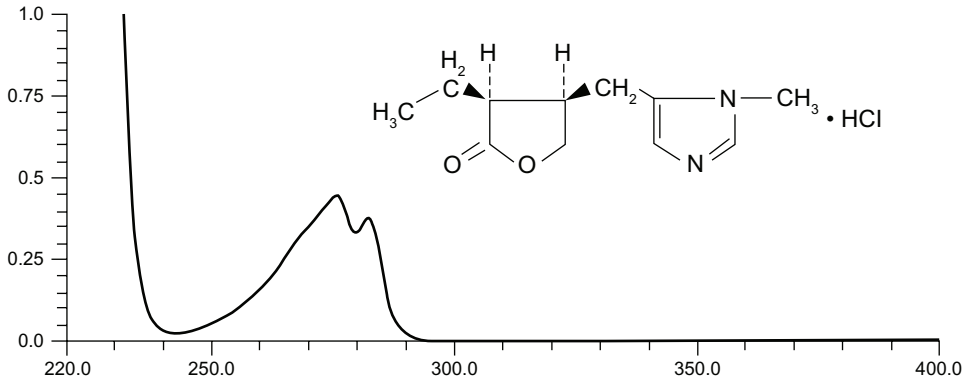

Figure 1. Chemical structure and UV spectrum of betaxolol hydrochloride ((±)-2-propanol, l-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-[(l-methylethyl) amino]-, hydrochloride, (±)).

diluted with mobile phase to obtain working standards of 100 µg/mL of drug.

Calibration curves for betaxolol

Betaxolol hydrochloride calibration solutions were prepared by diluting the stock solutions to different concentrations. Betaxolol hydrochloride working standard solutions were taken in different 20 mL volumetric flasks. The volume was made up to the mark with mobile phase to obtain final concentrations of 25, 50, 75, 100, and 200 µg/mL of betaxolol hydrochloride, respectively. Calibration curves were constructed by plotting peak area versus concentrations of the drug. Regression analyses were computed for betaxolol hydrochloride.

Analysis of labeled tablets

Five 20 mg betaxolol hydrochloride tablets were finely powdered. Tablet powder equivalent to 100 mg betaxolol hydrochloride was transferred to a 100 mL volumetric flask. A few mL of deionized water were added to the flask and it was sonicated for 10 min. The appropriate volume of the aliquot was transferred to a 100 mL volumetric flask and the remainder was filled with the mobile phase to obtain a solution containing 100 µg/mL of betaxolol hydrochloride. One mL of this solution was added to polypropylene tubes. The tubes were then centrifuged for 10 min at 15,000 rpm. The supernatant was injected as per above chromatographic conditions.

Accuracy

The known amount of betaxolol hydrochloride (0, 25, 100, 150 µg/mL) was added to sample solutions. The amount of betaxolol hydrochloride was estimated by measuring the peak area and fitting these values to the linear equation of the calibration curve.

Precision

The instrument precision was evaluated by injecting the 100 µg/mL betaxolol hydrochloride solution, three times repeatedly. The intra-day and inter-day study of betaxolol hydrochloride was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations of betaxolol hydrochloride (30, 90, 180 µg/mL).

Specificity

The specificity was estimated by spiking excipients such as lactose, sodium starch glycolate, microcrystalline cellulose, colloidal silica, and magnesium stearate into a pre-weighed quantity of drug.

Limit of detection (LOD) and quantification (LOQ)

The LOD and LOQ were calculated using the ICH guidelines equation as LOD = 3.3 xσ/S and LOQ = 10 xσ/S, where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness

The optimum HPLC conditions set for this method have been slightly modified for samples of betaxolol hydrochloride as a mean to evaluate the method robustness. The small changes made include the flow rate, the detection wavelength, the column temperature and the organic content in the mobile phase.

Forced degradation study

Control samples were used in each condition. A minimum 10% degradation of the initial concentration remaining was considered to indicate a significant loss in terms of stability.

Alkali hydrolysis

To the 20 mL volumetric flask, 20 mg of betaxolol hydrochloride was added and 4 mL of 5N NaOH was added to perform base hydrolysis. The flask was stored at room temperature for 24 hours (h). The solution was neutralized with 5N HCl and the remainder of the flask was filled with deionized water.

Acid hydrolysis

To the 20 mL volumetric flask, 20 mg of betaxolol hydrochloride was added and 4 mL of 5M H2SO4 was added to perform acid hydrolysis. The flask was stored at room temperature for 24 h. Solution was neutralized with 5N NaOH and the remainder of the flask was filled with deionized water.

Oxidative stress degradation

To the 20 mL volumetric flask, 20 mg of betaxolol hydrochloride was added and 4 mL of 3% hydrogen peroxide was added. The flask was stored at room

temperature for 24 h. The remainder of the flask was filled with deionized water.

Sunlight degradation

The drug was exposed to sunlight for 7 days. The powder was transferred to 10 mL volumetric flask and dissolve in deionized water. The remainder of the flask was filled with deionized water.

Results and Discussion

Validation of the method

Linearity

The linearity was determined by preparing standard solutions at five different concentrations levels ranging from 25 to 200 µg/mL. The regression equation of calibration curves was obtained as $y = 0.4434x + 0.0767$ with a correlation coefficient of 0.9999.

Precision

System precision was determined on six replicate injections of the standard preparation. The percentage relative standard deviation (RSD) of the area counts of six replicate injections was found to be 0.41%. The precision of the assay method was evaluated by carrying out six independent determinations of betaxolol hydrochloride test samples against the qualified working standard. The RSD of repeatability (n = 6) was found to be 0.53%. The reproducibility was checked by analyzing the samples with a different analyst, using a different chromatographic system and column, on a different day. The analysis was conducted in the same manner as the method precision and the % RSD of all six sets of sample preparations was determined. The RSD of reproducibility was found to be 0.31%. The low RSD values indicate that the method is precise.

Accuracy

The accuracy of the method was carried out by adding known amounts of betaxolol hydrochloride corresponding to three concentration levels; 50%, 100%, and 150% of the label claim along with the excipients in triplicate. The percentage recoveries of betaxolol hydrochloride at each level and each replicate were determined. The recoveries found to be 100.01%–101.35% for betaxolol hydrochloride (Table 1). It was confirmed from results that the method is highly accurate.

Limit of detection and limit of quantification

The detection limit and quantification for betaxolol hydrochloride was 1.30 µg/mL and 20 µg/mL, respectively.

Specificity

The specificity study was carried out to check the interference from the excipients used in the tablets by preparing a synthetic mixture containing the drug and excipients. The chromatogram showed peak for betaxolol hydrochloride without any interfering peak.

Robustness

Table 3 shows that the percent recoveries of betaxolol hydrochloride were good under most conditions and did not show a significant change when the critical parameters were modified. Considering

Table 1. Accuracy study for betaxolol hydrochloride proposed LC method.

Amount of sample (µg/mL)	Sets	Amount drug of spiked (µg/mL)	Calculated amount recovered (µg/mL)	% recovery	Mean % recovery	% RSD
100	1	0	101.13	101.13		
	2	0	99.74	99.74	100.48	0.69
	3	0	100.56	100.56		
100	1	25	25.36	101.44		
	2	25	25.45	101.80	101.35	0.49
	3	25	25.20	100.81		
100	1	100	99.91	99.91		
	2	100	100.00	100.00	100.09	0.24
	3	100	100.36	100.6		
100	1	150	149.97	99.98		
	2	150	150.47	100.32	100.01	0.28
	3	150	149.63	99.75		

Table 2. Summary of forced degradation results.

Stress condition	Time (h)	% recovery betaxolol hydrochloride	retention time of degradation product
Acid hydrolysis (5N H2SO4)	24	20.8	0.950, 2.092
Alkaline hydrolysis (5N NaOH)	24	12.5	0.892
Oxidation (3% H2O2)	24	67.7	1.383
Sunlight	24	99.5	–

the modification in the system suitability parameters, we conclude that the method conditions are robust.

Forced degradation study

Forced degradation studies were performed to demonstrate selectivity and stability-indicating capability of the proposed RP-HPLC method (Table 2). A chromatogram of acid hydrolysis performed at room temperature for 24 h showed degradation of betaxolol hydrochloride with degradation product peak at retention time 0.950 min and 2.092 min (Fig. 3). A chromatogram of base hydrolysis performed at room temperature for 24 h showed degradation of betaxolol hydrochloride with a degradation product peak at a retention time of 0.892 min (Fig. 4). The chromatogram of oxidized betaxolol hydrochloride with 3% hydrogen peroxide for 24 h showed degradation of betaxolol hydrochloride with a degradation product peak at a retention time of 1.383 min (Fig. 5). No degradation of betaxolol hydrochloride exposed to sunlight at room temperature for 7 days

Table 3. Robustness.

Parameters	Modification	% recovery
Flow rate (mL/min)	1.4	100.8
	1.5	99.6
	1.7	100.6
Wavelength of detection (nm)	222	99.8
	225	100.4
	230	98.9
Column temperature (°C)	22	100.5
	25	99.4
	30	98.8
Organic content in mobile phase	–2%	100.1
	+2%	98.6

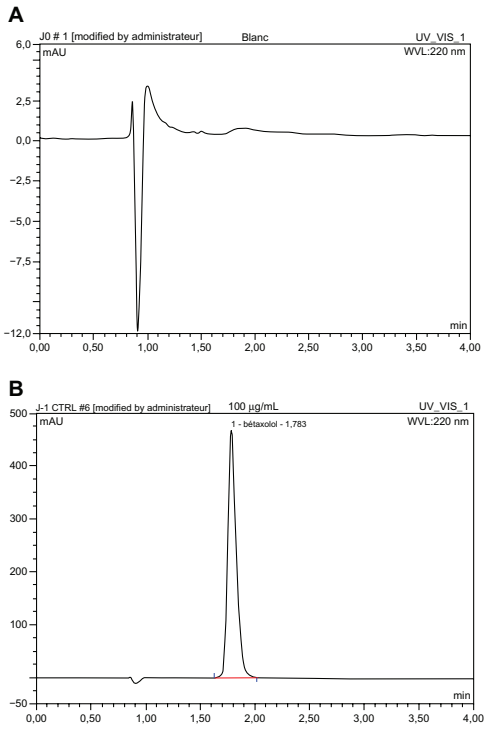

Figure 2. (**A**) Liquid chromatogram of blank on C18 Nucleosil column using 0.01 M potassium dihydrogen phosphate buffer: methanol (pH 3.0 adjusted with orthophosphoric acid) (40:60, v/v) as the mobile phase. (**B**) Liquid chromatogram of betaxolol hydrochloride (retention time = 1.783 min).

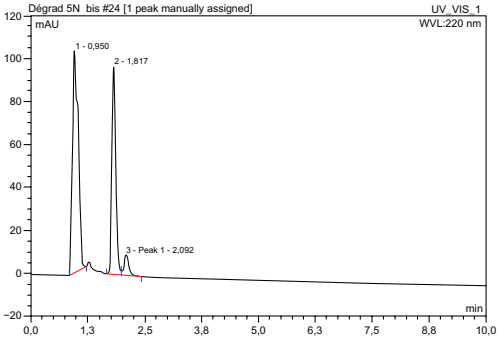

Figure 3. Chromatogram of 5N H2SO4 treated betaxolol hydrochloride at room temperature for 24 h.

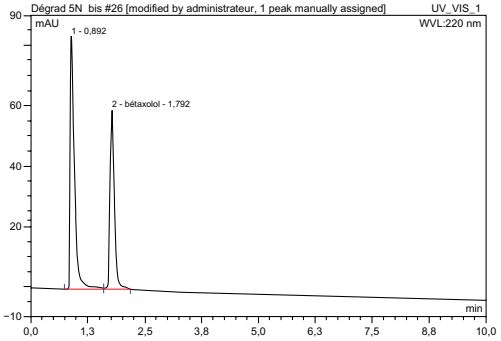

Figure 4. Chromatogram of 5N NaOH treated betaxolol hydrochloride at room temperature for 24 h.

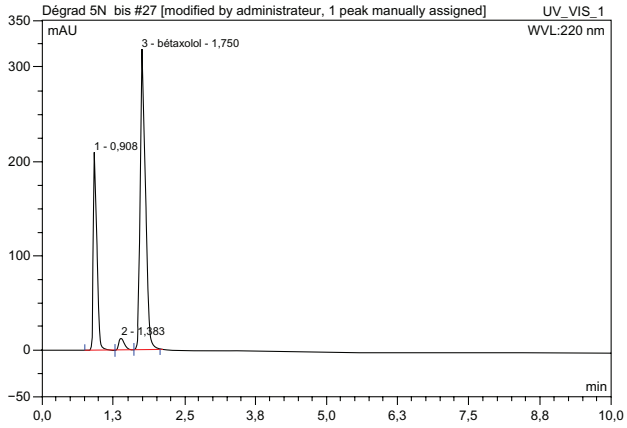

Figure 5. Chromatogram of 3% hydrogen peroxide treated betaxolol hydrochloride at room temperature for 24 h.

was observed. The compound was particularly stable at sunlight condition.

Stability of sample in diluent

Stability of the sample solution was established by storage of the sample solution at room temperature for 24 h. The results from the solution stability experiments confirmed that the sample solution was stable for up to 24 h during assay determination, while the relative standard deviation was found to be below 1.0%.

Application of the method to dosage forms

The present method is applied to the estimation of betaxolol hydrochloride in their commercially available tablets. The % recovery for betaxolol hydrochloride was found to be 99.42 ± 0.67 (mean value ± standard deviation) of three determinations, which was comparable to the corresponding labeled amounts.

Conclusion

A rapid, RP-HPLC method was successfully developed for the determination of betaxolol hydrochloride in the pharmaceutical tablets. The developed method is selective, precise, accurate, and linear. Forced degradation data proved that the method is specific for the analytes and free from the interference of blank and unknown degradation products. The run time (<4 min) enables rapid determination of the tablet dosage form. Also, the results indicate the suitability of the method for acid, base, oxidation, and sunlight degradation studies. The method is suitable for the analysis of stability samples and the routine analysis of betaxolol hydrochloride in tablets.

Author Contributions

Conceived and designed the experiments: SA, FC, SC, J-EF, JS. Analysed the data: SA, FC, SC, J-EF, JS. Wrote the first draft of the manuscript: SA, FC, SC, J-EF, JS. Contributed to the writing of the manuscript: SA, FC, SC, J-EF, JS. Agree with manuscript results and conclusions: SA, FC, SC, J-EF, JS. Jointly developed the structure and arguments for the paper: SA, FC, SC, J-EF, JS. Made critical revisions and approved final version: SA, FC, SC, J-EF, JS. All authors reviewed and approved of the final manuscript.

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Competing Interests

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Disclosures and Ethics

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