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Stability Indicating RP-HPLC Method for Simultaneous Assay of Bisoprolol and Hydrochlorothiazide in Combined Tablet Dosage Form

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Abstract : A simple, sensitive, precise and accurate stability-indicating HPLC with photodiode array detection method has been developed and validated for simultaneous determination of bisoprolol and hydrochlorothiazide in its bulk and combined tablet dosage form. Chromatographic separation was achieved on an YMC Pack Pro C18 column (250 mm × 4.6 mm; 5 µm particle size, maintained at a temperature of 30 °C) by a mobile phase consisted of 0.1% orthophosphoric acid and acetonitrile (55:45, *v/v*) with a flow rate of 1.0 ml/min. The detection wavelength was set at 259 nm. Bisoprolol and hydrochlorothiazide was subjected to different forced degradation conditions. In all the conditions, the degradation products were well resolved from the pure drugs with different retention time values. The method was linear (R^2 = 0.9999) at a concentration range of 40-120 µg/ml (bisoprolol) and 50-150 µg/ml (hydrochlorothiazide). The limit of quantitation was 0.398 and 0.385 µg/ml for bisoprolol and hydrochlorothiazide, respectively. The precision of the method was satisfactory; the relative standard deviations did not exceed 1%. The accuracy of the method was proved; the mean recovery of both drugs was in the range of 99.67% to 100.28%. The proposed HPLC method would have a significant value when applied in quality control laboratories for the simultaneous assay of bisoprolol and hydrochlorothiazide.
Key words: antihypertensive, Bisoprolol, hydrochlorothiazide, stability-indicating, chromatographic analysis.

Introduction:

Bisoprolol[1-4], chemically known as 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy] propan-2-ol, belongs to the group of beta blockers (class of drugs used primarily in cardiovascular diseases). Bisoprolol selectively blocks cardiac β1-adrenergic receptors with very less action against β2-adrenergic receptors. The two substituents present in the para position of the benzene ring might be the reason for its β1-adrenergic receptor selectivity (Figure 1). Bisoprolol is used in the management of myocardial infarction, congestive heart failure, angina pectoris and mild to moderate hypertension.

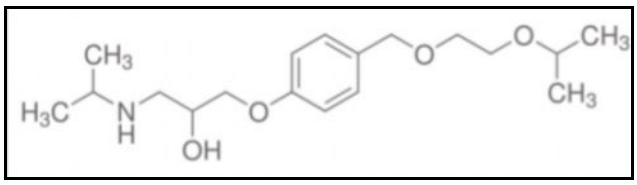

Figure 1: Structure of bisoprolol.

Hydrochlorothiazide[5-8] belongs to a class of drugs called as thiazide diuretics. Thiazide diuretics reduce the electrolytes reabsorption from the renal tubules and helps in the prevention of fluid retention in the body. Chemically, hydrochlorothiazide is designated as 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide (Figure 2). Hydrochlorothiazide is frequently used in the treatment of congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, hypertension, hypoparathyroidism, osteoporosis and the prevention of kidney stones.

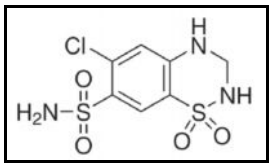

Figure 2: Structure of hydrochlorothiazide.

The low-dose combination of bisoprolol and hydrochlorothiazide is used to treat hypertension and helps in the prevention of strokes, heart attacks and kidney problems[9-10]. This combination is used together when blood pressure is not controlled by one mediation. Together use of these two drugs can also reduce the amount of each drug intake and thus lessening the chances of side effects. Due to the above said advantages, the dosage forms containing bisoprolol and hydrochlorothiazide are became popular medication in cardiovascular therapy.

Several methods have been reported for the simultaneous determination of bisoprolol and hydrochlorothiazide in bulk, tablet dosage forms and biological samples. They include HPTLC [11,12], differential pulse & square wave voltammetry[13], UV spectrophotometry[13-16], RP-UPLC[17], stability indicating UPLC[18] and LC-MS/MS[19,20]. Though the reported methods are sensitive, they are not simple for routine analysis and require expensive or sophisticated instruments. The reported UV spectrophotometry methods are simple but lacks selectively.

RP-HPLC method offers several advantages, including minimal sample manipulation before chromatography, rapid analysis and simultaneous analysis of multicomponent mixtures with good specificity, precision & accuracy. There are few reports on the simultaneous determination of bisoprolol and hydrochlorothiazide in tablet dosage forms using RP-HPLC. Patel et al.[21] have applied the isocratic HPLC method for the separation and quantification of bisoprolol and hydrochlorothiazide in tablet dosage form. A lichrospher 100 C-18 analytical column (20 cm × 4.6 mm, 5 µm) with mobile phase containing water, acetonitrile and tetrahydrofuran in the ratio of 80:20:5 *v/v/v* were used. The flow rate was 1 ml/min and effluent was monitored at 225 nm. HPLC analysis of bisoprolol and hydrochlorothiazide in their dosage forms using RP Zorbax Eclipse XDB-C18 analytical column (150 × 4.6 mm, id, 5 µm particle size) with acetonitrile–15 mM phosphate (25:75, *v/v*) as mobile phase at a 1.0 ml/min flow rate has been studied by Bozal et al.[13]. A stability-indicating RP-HPLC method for the determination of bisoprolol and hydrochlorothiazide in its pharmaceutical dosage forms was presented by Joshi et al.[22]. They used Inertsil ODS 3V (25 cm × 4.6 mm) 5 µm column and 0.1 M potassium dihydrogen phosphate buffer: acetonitrile (70:30, *v/v*) as mobile phase with UV detection at 228 nm.

The reported HPLC methods suffer from one or more of the disadvantages such as preparation of buffer, use of triple solvent system, less sensitive, lack of precision and accuracy. The methods of Patel et al.[21] and Bozal et al.[13] are not stability indicating. Though the method of Joshi et al.[22] is stability indicating, peak purity details were not reported. The objective of this work, therefore, was to develop a simple, cost effective, sensitive, precise and accurate stability-indicating RP-HPLC method with photodiode array detector for

quantitative determination of bisoprolol and hydrochlorothiazide simultaneously in bulk and in tablet dosage forms. The developed method was validated in accordance with ICH guidelines[23].

Experimental:

Instrumentation:

- Waters HPLC system, consisted of a binary HPLC pump model 2695, PDA detector model 2998, vacuum degasser and Waters Empower2 software
- YMC Pack pro C18 (250 mm x 4.6 mm, 5 µ particle size) analytical column

Standard, chemicals and reagents:

Bisoprolol and hydrochlorothiazide standards were provided by Lara Drugs Private Limited (Telangana, India). Orthophosphoric acid (Sd Fine Chemicals Ltd., Mumbai , India) and acetonitrile (Merck Pvt Ltd., Mumbai, India) of HPLC grade were used during the present study. Purified water was obtained from a Milli-Q system. Qualiz-5 tablets, labeled to contain bisoprolol-5 mg and hydrochlorothiazide-6.25 mg (Medreich Saimirra Ltd, India), were purchased from the pharmacy market.

Chromatographic conditions:

Separation of bisoprolol and hydrochlorothiazide was achieved on YMC Pack pro C18 (250 mm x 4.6 mm, 5 µ particle size) analytical column as the stationary phase using mobile phase consisted of 0.1% orthophosphoric acid and acetonitrile in the ratio of 55:45 *v/v*. The mobile phase was filtered by passing through a membrane filter prior to use. Isocratic elution was achieved at a flow rate of 1.0 ml/min with a column temperature of 30°C. The injection volume was 10 µl. The chromatograms were recorded at 259 nm using photodiode array detector.

Standard solutions:

The mobile phase was used as the diluent. An accurately weighed amount of about 20 mg of bisoprolol and 25 mg of hydrochlorothiazide were put into a 50 ml volumetric flask, 15 ml of mobile phase was added and the mixture was sonicated to dissolve it. The resulting mixture was made up to volume with the same solvent. Working standard solution was prepared in mobile phase by diluting 5 ml of the above stock solution to 25 ml in a 25 ml volumetric flask to get bisoprolol and hydrochlorothiazide with final concentration of 80 µg/ml and 100 µg/ml, respectively.

General assay procedure:

The working standard solutions were prepared by dilution of the stock solution with diluent to reach the concentration range 40-120 µg/ml and 50-150 µg/ml for bisoprolol and hydrochlorothiazide, respectively. Triplicate injections were made for each concentration. The chromatograms were recorded under the earlier described chromatographic conditions. The peak areas were plotted against the corresponding concentrations to construct the calibration curve.

Assay of tablet dosage form:

For the determination of bisoprolol and hydrochlorothiazide in combined tablet dosage forms, 20 Qualiz-5 tablets were weighed and finely powdered. A suitable portion of powder equivalent to 20 mg of bisoprolol and 25 mg of hydrochlorothiazide was accurately weighed and transferred to a 50 ml volumetric flask. The flask was made up to volume with diluent and sonicated for 15 min. The solution was passed through a 0.45 µm membrane filter and diluted appropriately with diluent to reach a final concentration of 80 µg/ml (bisoprolol) and 100 µg/ml (hydrochlorothiazide). The sample solution was treated as described under the general assay procedure. Recovered concentrations of bisoprolol and hydrochlorothiazide were calculated from the corresponding calibration graphs.

Results and Discussion:

Optimization of chromatographic conditions:

The most important phase in the stability indicating RP-HPLC method development is the achievement of adequate resolution of bisoprolol, hydrochlorothiazide and their stress degradants with acceptable peak symmetry in a reasonable analysis time. To accomplish this, several experiments were carried out so as to optimize the stationary phase and mobile phase. For the stationary phase, two analytical columns ACE C8 (150 x 4.6mm, 5 µm particle size) and YMC Pack pro C18 (250 × 4.6 mm; 5 µm particle size) were tested. Successful resolution and acceptable peak symmetry of the drugs was attained by using the YMC Pack pro C18 (250 x 4.6mm, 5 µm particle size) analytical column. Hence it was used in the present study.

Several mobile phase combinations such as 0.1 M NaH_2PO_4 with acetonitrile; 0.1 M ammonium acetate with acetonitrile and; 0.1% orthophosphoric acid with acetonitrile were tested using various proportions and various flow rates. The best resolution of the two drugs within acceptable analysis time was obtained through an isocratic elution using a mobile phase consisting of 0.1% orthophosporic acid and acetonitrile in the ratio 55:45 (*v/v*) at a flow rate of 1 ml/min. Quantification was done using photodiode array detection based on peak area measurement. Bisoprolol and hydrochlorothiazide exhibited considerable absorption at 259 nm. Hence the wavelength 259 nm was selected for quantification of the selected drugs.

The above described chromatographic conditions showed symmetric peaks and sufficient resolution between bisoprolol and hydrochlorothiazide. Figure 3 shows a typical chromatogram for the separation of two drugs. Bisoprolol and hydrochlorothiazide eluted at retention times 3.688 min and 5.824 min, respectively.

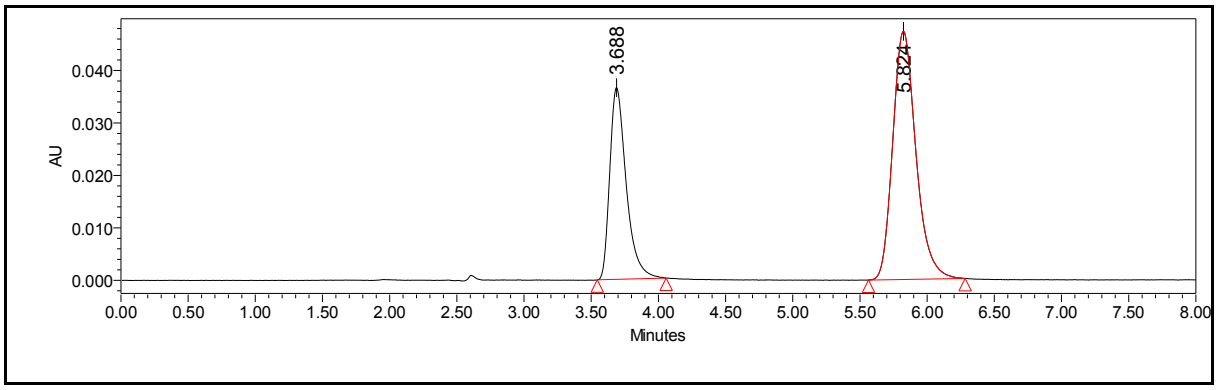

Figure 3: Typical chromatogram of Bisoprolol (tR – 3.688) and Hydrochlorothiazide (tR – 5.824).

System suitability test:

The suitability of the developed method was verified by repeated injections (n=5) of working standard solution (80 µg/ml bisoprolol and 100 µg/ml hydrochlorothiazide). The system suitability parmameters such as USP plate count, USP tailing factor, USP resolution and repeatability of the retention time and peak areas were determined. The results and the acceptable limits are shown in Table 1.

Table 1: System suitability.

Parameters	Bisoprolol	Hydrochlorothiazide	Recommended limits
Retention time	3.687	5.823	-
Peak area	310679 (%RSD – 0.7)	566284 (%RSD – 0.6)	RSD ≤1
USP resolution	-	7.90	> 1.5
USP plate count	4572	4572	> 2000
USP tailing factor	1.46	1.23	≤ 2

Linearity and concentration ranges:

The linearity of the proposed HPLC method was evaluated by analyzing a series of different concentrations (n=5) for each of the two drugs. The linear regression equations were generated by least square treatment of the calibration data. Under the optimized chromatographic conditions, the measured peak areas of ambroxol and hydrochlorothiazide at 259 nm were found to be proportional to their concentrations. Table 2 presents the linear regression equations, concentration ranges, regression coefficients, intercept and slope. Regression analysis shows good linearity as indicated from the correlation coefficient values (>0.9990). The calibration curves are given in Figure 4.

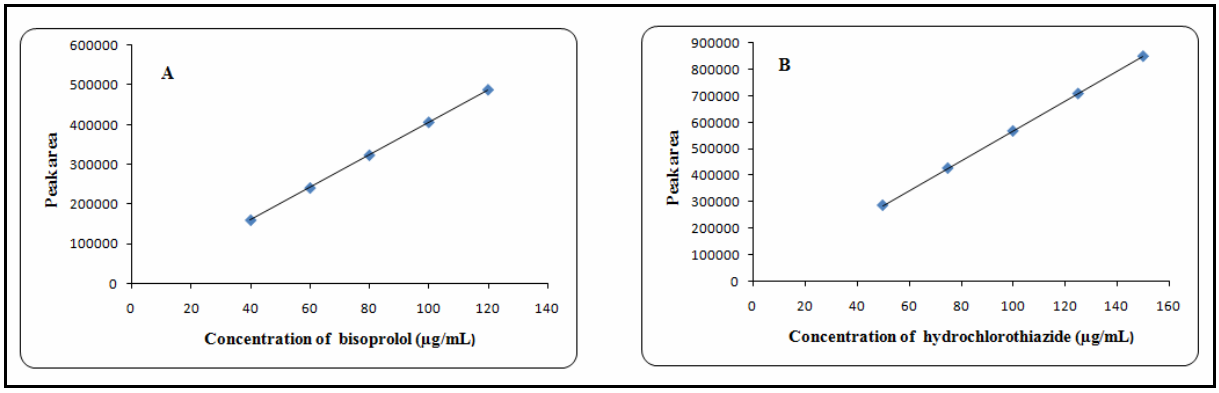

Figure 4: Calibration curve of [A] Bisoprolol [B] Hydrochlorothiazide.

Table 2: Linearity, regression and sensitivity parameters and for the determination of bisoprolol and hydrochlorothiazide.

Parameter	Bisoprolol	Hydrochlorothiazide
Linearity range (µg/ml)	40-120	50-150
Regression equation ($y^a = m\ x^b + c$)	y = 4080.x - 3060	y = 5623.x + 4902.
Slope (m)	4080	5623
Intercept (c)	-3060	4902
Regression coefficient (R^2)	0.9999	0.9999
LOD (µg/ml)	0.398	0.385
LOQ (µg/ml)	1.327	1.283

a – peak area of the drug: b - concentration of drug in µg/ml

Sensitivity:

Sensitivity of the method was assessed by determining limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were calculated as signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ values for bisoprolol and hydrochlorothiazide were calculated and are presented in Table 1.

Precision:

The precision for the proposed method was studied at a concentration of 80 µg/ml bisoprolol and 100 µg/ml hydrochlorothiazide using six replicate determinations. The percentage relative standard deviation (RSD %) did not exceed 1.0% proving the high repeatability of the developed method (Table 3).

Table 3: Precision of the method.

Bisoprolol		Hydrochlorothiazide	
Peak area	**%RSD**	**Peak area**	**%RSD**
321746	0.38	562632	0.30
324580		563474	
323927		562524	
321861		560001	
322363		560180	
324045		564139	

Accuracy:

The accuracy of the proposed method was established by means of the standard addition technique, by adding a known amount of standard drug at three different levels (50%, 100% and 150%) to the preanalyzed sample. Accuracy was expressed as percentage recovery in Table 4. The accuracy of the developed method for the bisoprolol and hydrochlorothiazide ranged from 99.67% to 100.28% indicating acceptable accuracy.

Table 4: Accuracy of the method.

Spiked level	Concentration of drug (µg/ml)		% Recovery	% Mean
	added	found		
Bisoprolol				
50%	40	39.82	99.55	99.81
	40	39.85	99.62	
	40	40.10	100.25	
100%	80	79.90	99.87	100.09
	80	80.23	100.29	
	80	80.08	100.10	
150%	120	119.57	99.64	99.67
	120	119.84	99.87	
	120	119.41	99.51	
Hydrochlorothiazide				
50%	50	50.19	100.38	100.28
	50	50.03	100.06	
	50	50.20	100.40	
100%	100	100.27	100.27	99.98
	100	99.65	99.65	
	100	100.01	100.01	
150%	150	150.08	100.05	100.11
	150	150.40	100.27	
	150	150.00	100.00	

Robustness:

The robustness of the developed method was checked by studying the effect of deliberate changes in the flow rate of mobile phase (± 0.1 ml/min) and column temperature (± 2°C) on the chromatographic system suitability parameters. According to the results shown in Table 5, these small and deliberate variations did not have any significant effect on the measured system suitability parameters.

Table 5: Robustness of the method.

Parameter	Investigated value	Area	USP Plate Count	USP Tailing	USP resolution
Bisoprolol					
Temperature (^{o}C)	30 - 2	442787	4449	1.46	-
	30 + 2	246131	3595	1.40	-
Flow rate (ml/min)	1.0 - 0.1	445358	4444	1.44	-
	1.0 + 0.1	245941	3504	1.41	-
Hydrochlorothiazide					
Temperature (^{o}C)	30 – 2	737271	5464	1.25	7.83
	30 + 2	400712	4134	1.20	7.00
Flow rate (ml/min)	1.0 - 0.1	731360	5474	1.21	7.82
	1.0 + 0.1	394819	4076	1.21	6.96

Specificity (Forced degradation studies):

The forced degradation study was conducted to make sure that the proposed method was able to separate bisoprolol and hydrochlorothiazide from the possible degradants generated during the acid, base, oxidative, sunlight and thermal degradation. The degradation study was carried out using the tablet powder containing bisoprolol and hydrochlorothiazide at a concentration of 80 µg/ml and 100 µg/ml, respectively. Acidic degradation was performed by sonication of sample with 10 ml of 0.1N HCl for 30 minutes. Alkaline degradation was performed by sonication of sample with 10 ml of 0.1N NaOH for 30 minutes. The acid and alkali degraded samples are neutralized with 0.1 N NaOH and 0.1 N HCl, respectively. Oxidative degradation was performed by sonication of sample with 10 ml of 30% hydrogen peroxide for 30 minutes. Thermal degradation was performed by heating the sample at 105 ^{o}C for 30 minutes in oven. The sample was exposed to sunlight for 24 hrs for photolytic degradation. All the forced degraded samples were injecting into the HPLC system. The chromatograms are shown in Figure 5.

Under all degradation conditions, a small percentage of degradation was observed (Table 6). The analysis of the chromatograms of the degraded samples and determination of the peak purity angle values demonstrated that the bisoprolol peak and hydrochlorothiazide peak was pure in all situations. The results of forced degradation studies allowed to conclude that the degradants produced as a result of forced degradation did not interfere with the detection of bisoprolol and hydrochlorothiazide, and the proposed method can hence be regarded as stability-indicating.

Table 6: Forced degradation studies.

Type of degradation	Peak area	% Assay	% Degradation	Purity Angle	Purity Threshold
Bisoprolol					
Acid	309046	96.45	3.55	0.121	0.880
Base	308692	96.34	3.66	0.266	0.760
Oxidative	309721	96.66	3.34	1.588	2.106
Heat	309151	96.49	3.51	0.398	0.926
Sunlight	307402	95.94	4.06	0.346	0.687
Hydrochlorothiazide					
Acid	533313	95.10	4.9	0.121	0.880
Base	531126	94.71	5.29	0.100	0.958
Oxidative	539958	96.29	3.71	0.300	0.597
Heat	535062	95.41	4.59	0.281	0.691
Sunlight	534381	95.29	4.71	0.117	0.577

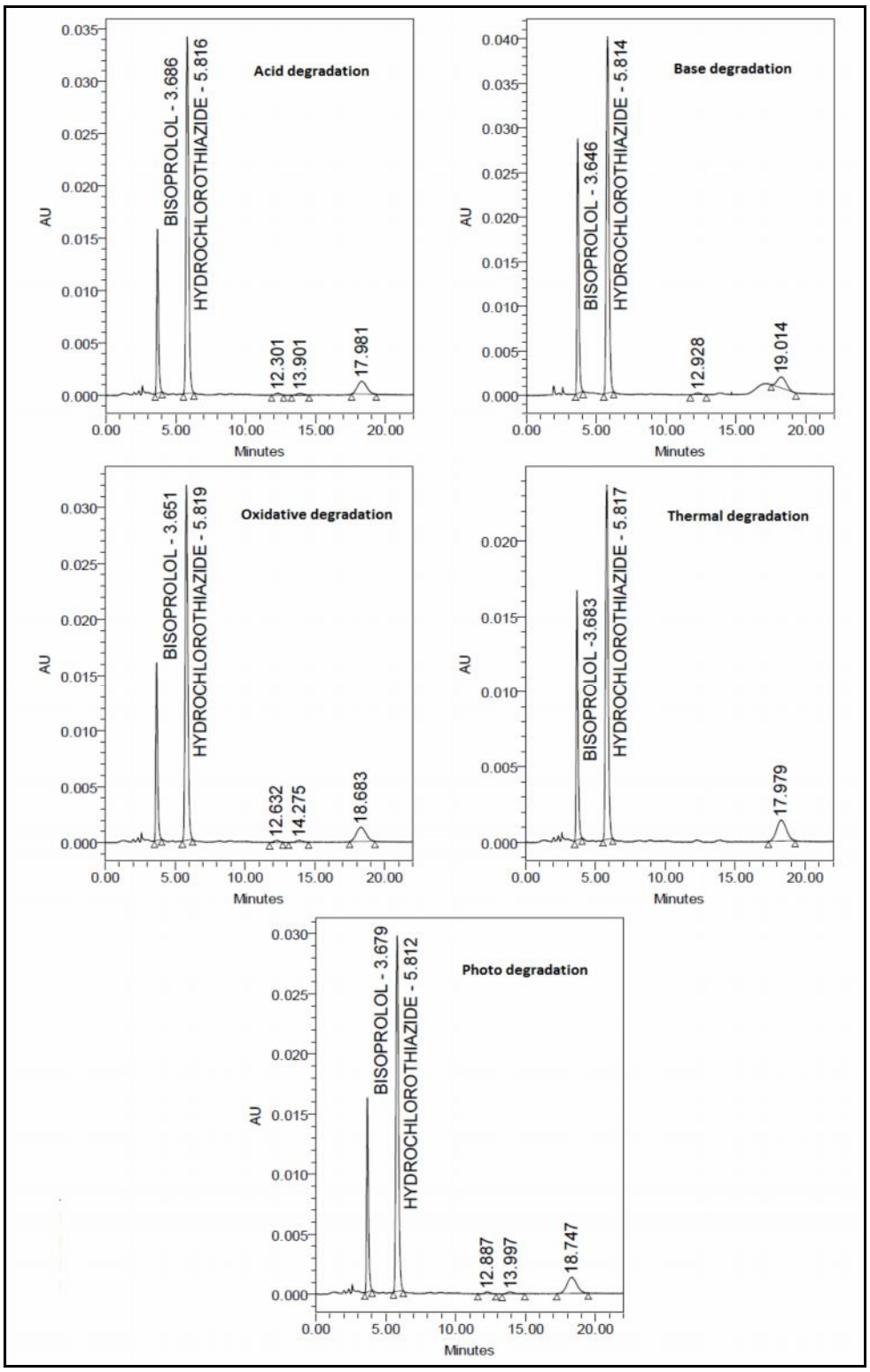

Figure 5: Chromatograms of forced degradation studies.

Selectivity:

The chromatograms of mobile phase blank, placebo blank, tablet sample and standard sample were compared to establish the selectivity of method. Placebo blank solution was prepared in the same way of the tablet sample solution by common excipients of the tablet dosage form but without bisoprolol and hydrochlorothiazide. The chromatograms are presented in Figure 6. The method was selective for the

simultaneous assay of bisoprolol and hydrochlorothiazide, since common excipients of the tablet dosage form and components of the mobile phase did not interfere with the peaks of bisoprolol and hydrochlorothiazide.

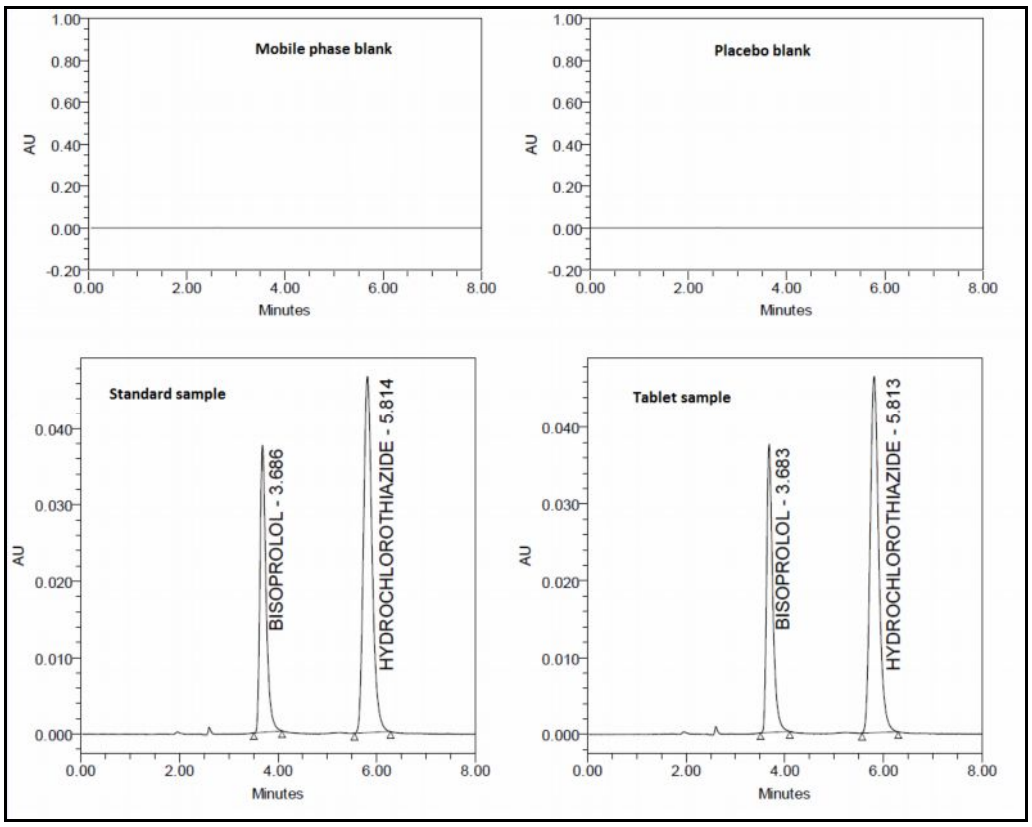

Figure 6: Chromatograms of method selectivity.

Conclusion:

A simple, rapid, accurate, precise and sensitive HPLC method with photodiode array detection was developed for the simultaneous determination of bisoprolol and hydrochlorothiazide in bulk and in combined tablets. The method was validated for linearity, limit of detection, limit of quantitation, precision, accuracy, robustness, specificity and selectivity as indicated by the ICH guidelines. The retention time of less than 6 minutes for both the drugs enables rapid determination of drugs. The proposed method is adequate to separate the peaks of bisoprolol and hydrochlorothiazide from the degradants produced during forced degradation studies. Hence, it can be recommended for use in quality control laboratories for the simultaneous assay of bisoprolol and hydrochlorothiazide in the presence of its degradants.

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