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Development and validation of stability indicating RP-HPLC method for Simultaneous determination of Hydrochlorothiazide and Nebivolol Hydrochloride in pharmaceutical dosage form

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ABSTRACT

A stability-indicating RP-HPLC method has been established for determination of Hydrochlorothiazide (HCTZ) and Nebivolol hydrochloride (NBL) in combine dosage form under different stress conditions. The drug substances were subjected to stress by hydrolysis (0.1N HCl and 0.5NaOH), photochemical, and UV degradation (254 nm). Both the drugs were degraded under selected experimental conditions. Successful separation of the drugs from the degradation products was achieved on a Hypersil BDS C18, 150x4.6, 5µ column with 50:50 (v/v) Mixed buffer and Methanol in the ratio 50: 50. 2.6 gms of potassium hydrogen phosphate and 0.6gms of dipotasssium hydrogen phosphate were dissolved in 1000 ml distilled water, pH was adjusted 5.5±0.1 with dilute orthophosphoric acid as mobile phase. The method was linear over the concentration range of 6.250-38.750 µg mL^{-1} ($r^2 > 0.991$), with limits of detection and quantitation (LOD and LOQ) of 0.3061 and 0.9276 µg mL^{-1}, respectively, for HCTZ and 2.500-15.000 µg mL^{-1} ($r^2 > 0.991$) with LOD and LOQ of 0.489 and 1.4815 µg mL^{-1}, respectively, for NBL. The method was validated for specificity, selectivity, accuracy, and precision. This method was reproducible and selective. As the developed method could effectively separate the drugs from their degradation products, it can be used as stability-indicating.

Keywords: Hydrochlorothiazide (HCTZ), Nebivolol hydrochloride (NBL), Stability Indicating Assay Method and RP-HPLC.

INTRODUCTION

Hydrochlorothiazide is chemically (6-Chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2, 4-benzothiadiazin 1, 1- dioxide [1, 2]. It has molecular formula of $C_7H_8ClN_3O_4S_2$ and molecular weight is 297.739g/mol. Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. Normally, the sodium-chloride symporter transports sodium and chloride from the lumen into

the epithelial cell lining the distal convoluted tubule. The energy for this is provided by a sodium gradient established by sodium-potassium ATP ases on the basolateral membrane. Once sodium has entered the cell, it is transported out into the basolateralinterstitium via the sodium-potassium ATPase, causing an increase in the osmolarity of the interstitium, thereby establishing an osmotic gradient for water reabsorption. By blocking the sodium-chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout the nephron.

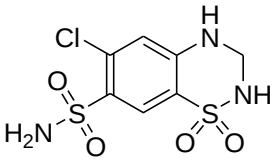


Figure 1: Chemical structure of Hydrochlorothiazide

Nebivolol Hydrochloride is chemically α, α'-(iminodimethylene) bis [6-fluoro-2-chromanmethanol] hydrochloride [3]. It has a molecular formula of $C_{22}H_{25}F_2NO_4$.HCL and molecular weight of 441.9 g/mol. Nebivolol hydrochloride is a selective β 1- adrenoceptor receptor antagonist. Activation of β 1-receptors by epinephrine increases the heart rate and the blood pressure, and the heart consumes more oxygen. Nebivolol blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure. In addition, beta blockers prevent the release of renin, which is a hormone produced by the kidneys which leads to constriction of blood vessels. At high enough concentrations, this drug may also bind beta 2- receptors.

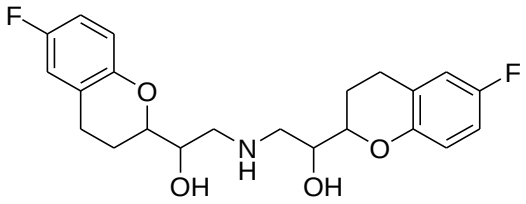


Figure 2: Chemical structure of Nebivolol Hydrochloride

HCTZ in combination with NBL potentiates the antihypertensive activity showing synergestic effect in reducing systolic and diastolic blood pressure. In addition to excess reduction in blood pressure the combination of HCTZ and NBL is safe, well tolerated with lower incidence of adverse effects and a neutral impact on lipid and glucose metabolism. Literature survey reveals that few methods have been reported for the determination of HCTZ or NBL individually in biological fluids or in combination with other drugs in pharmaceutical dosage forms [4-19]. Literature survey also reveals a few analytical methods like spectrophotometric methods[20-23] and HPTLC methods[24-25] in alone or in combination in pharmaceutical dosage forms but no simple stability indicating RP-HPLC method for the simultaneous estimation of Hydrochlorothiazide and Nebivolol Hydrochloride in pharmaceutical dosage forms have been reported so far. The present manuscript describes a sensitive, simple, precise and accurate isocratic stability indicating RP-HPLC method for simultaneous estimation of HCTZ and NBL in combined dosage form with subsequent validation as per ICH guidelines [26-29].

EXPERIMENTAL

Materials and Methods Chemicals and Reagents

Dipotasssium hydrogen phosphate and potassium hydrogen phosphate and orthophosphoric acid were bought from SR Scientifics - Tirupati, India. Acetonitrile (HPLC grade) purchased from SR Scientifics - Tirupati, India. Bio Leo Labs pLtd. Hyderabad, Telangana, India was kind enough and supplied the reference standards of HCTZ and NBL for this research work. All the chemicals used throughout the research work were of analytical grade. Commercial tablets of HCTZ (25 mg) and NBL (10 mg) were purchased from local market manufactured by Bio Leo Labs pLtd. Hyderabad, India

Instrumentation

Waters HPLC 2 2695 series consisting pump, Auto sampler, UV-Vis detector, Thermostat column compartment connected with Waters(alliance) Empower software. In addition, an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40) were used in this present study.

Chromatographic conditions

The chromatographic separation was performed on Hypersil BDS C18, 150x4.6, 5µ. The column temperature was kept at 30ºC. Separations were performed in isocratic mode using a mobile phase consisting of mixed buffer and methanol in the ratio of 50: 50. (P^H was adjusted to 5.5 ±0.1 by using Ortho-phosphoric acid) with a flow rate of 1.0 ml/minute. The detection wavelength was set at 254 nm.

ANALYTICAL METHODOLOGY

Preparation of Reagents and Standards

Mobile phase

Precisely weighed and dissolved 2.6gms of potassium hydrogen phosphate and 0.6gms of dipotasssium hydrogen phosphate in 1000 ml distilled water, adjusted ph 5.5±0.1 with dilute orthophosphoric acid solution. The above prepared buffer and methanol were mixed in the proportion of 50:50 v/v. The mobile phase was then duly filtered through 0.45 µm nylon membrane vacuum filtration and duly degassed by sonication.

Preparation of Hydrochlorothiazide and Nebivolol hydrochloride Stock and standard solutions

25mg of Hydrochlorothiazide and 10 mg of Nebivolol hydrochloride were weighed accurately and transferred in to 100 ml volumetric flasks separately. 30 ml of diluents were added and sonicated to dissolve the compounds. These were made up to marks with diluent which yields concentrations of Hydrochlorothiazide and Nebivolol hydrochloride 250µgml^{-1} & 100µgml^{-1} respectively (stock solution A). 10 ml of above solutions were pipetted out into 100ml volumetric flasks and volumes were made up to mark with diluent which gave concentrations of Hydrochlorothiazide and Nebivolol hydrochloride 25µgml^{-1} & 10µgml^{-1} respectively (stock solution B). The standard solutions ranging from 2.5-15 mL were transferred into a series of 100 ml volumetric flasks to provide a final concentration range of Hydrochlorothiazide 6.25-38.75 µg/ml and Nebivolol 2.5-15 µg/ml and the contents of each flask was made up to the mark with diluent.

Preparation of Formulation Test Solutions

Twenty tablets containing Hydrochlorothiazide and twenty tablets containing Nebivolol were weighed and finely powered. An accurately weighed portion of the powder equivalent to 25 mg of Hydrochlorothiazide and 10 mg of Nebivolol was transferred into 100 ml volumetric flask. 10 ml of diluent was added and shaken for 20 minutes by manually and further sonicated for 10 minutes. This was diluted up to the mark with diluent. This solution was centrifuged at 8000 rpm for 10 minutes. The solution was filtered through 0.45 µm Nylon membrane filter paper. The supernatant solution was decanted into another test tube which yields concentrations of Hydrochlorothiazide and Nebivolol hydrochloride 250µgml^{-1} & 100µgml^{-1} respectively.10 ml of supernatant solution was transferred into another 100 ml volumetric flask and made up to the mark with diluent which gave concentrations of Hydrochlorothiazide and Nebivolol hydrochloride 25µgml^{-1} & 10µgml^{-1} respectively . 2.5-15mL of solutions were transferred into 100ml volumetric flasks separately and made up to the mark with diluent. Hydrochlorothiazide concentration range was 6.25 – 38.75 µg/mL and Nebivolol concentration range was 2.5-15µg/mL. 20µL of blank solution, placebo solution, three times of standard solutions

were injected, disregarding peaks due to blank and placebo.

Assay procedure

The column was equilibrated for at least 30 minutes with mobile phase flowing through the system with a flow rate of 1.0 mL/min. Detector was set at a wavelength of 254 nm. Twelve sets of the drug solutions were prepared in diluent containing Hydrochlorothiazide and Nebivolol at a concentration range of 6.25 – 38.75 µg/mL and 2.5-15 µg/mL. Then 20 µl of each standard and sample solution were injected for Six times separately. The retention time for Hydrochlorothiazide and Nebivolol were found to be 2.625 and 6.060 min (Fig-3). The peak areas of the drug concentrations were calculated.

System suitability solution

Hydrochlorothiazide and Nebivolol standard working solution was used as system suitability solution.

Procedure

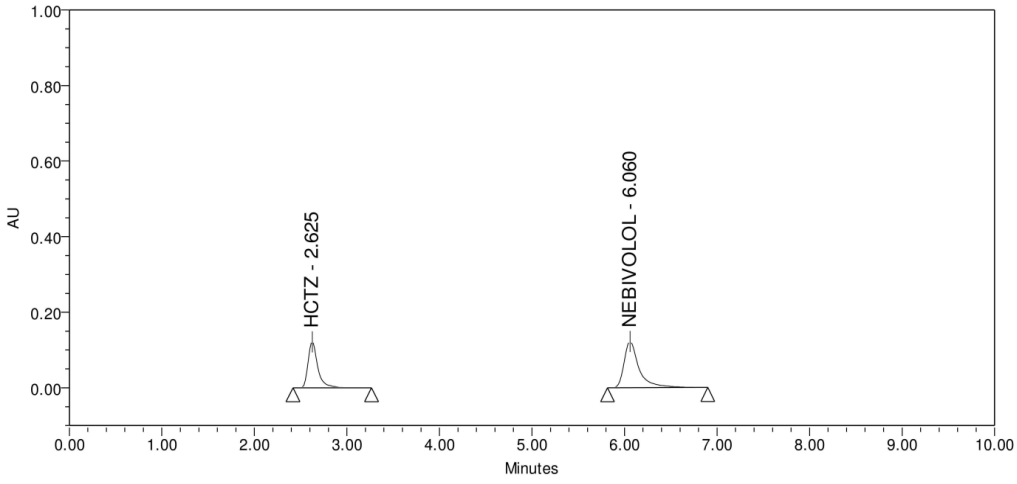
Equal volumes of blank were injected and twelve replicate injections of system suitability solutions in to column (Hydrochlorothiazide and Nebivolol standard working solution). The chromatograms were recorded. Disregarded any peaks due to blank in the test solution. % RSD of twelve replicate injections of system was calculated (Hydrochlorothiazide and Nebivolol standard working solution). Tailing factor and theoretical plates of the peak in the chromatogram obtained with 12th injection of system suitability solution (Hydrochlorothiazide and Nebivolol standard working solution) were checked.

System suitability requirements from SST solution:

a) Tailing factor : NMT 2.0
b) Theoretical Plates : NLT 2000
c) Resolution : NLT 2.0

Linearity and Construction of Calibration Curve

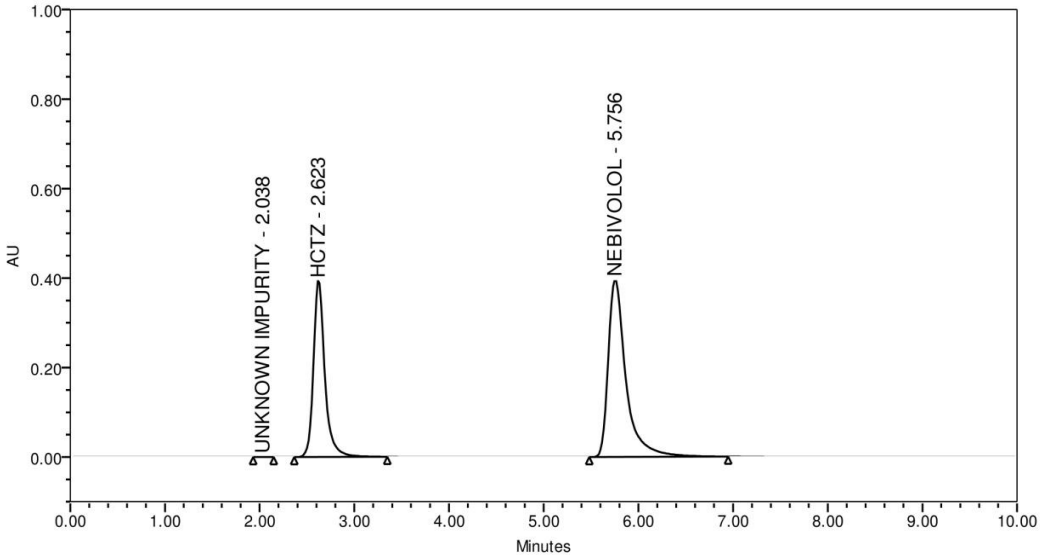
Linearity of the peak area response was determined by taking measurements at twelve concentrations of working standard of Hydrochlorothiazide and Nebivolol solutions in the range of 6.25-38.75 µg/ml and 2.5-15 µg/ml. 20µL quantity of the solution was injected each time in to the column. The drug elutes were monitored at 254 nm at a column temperature of 30ºC and the corresponding chromatograms were recorded. The Linearity of the calibration curve was plotted between the mean peak areas versus respective Concentration in figs-8 &9.



Peak Results

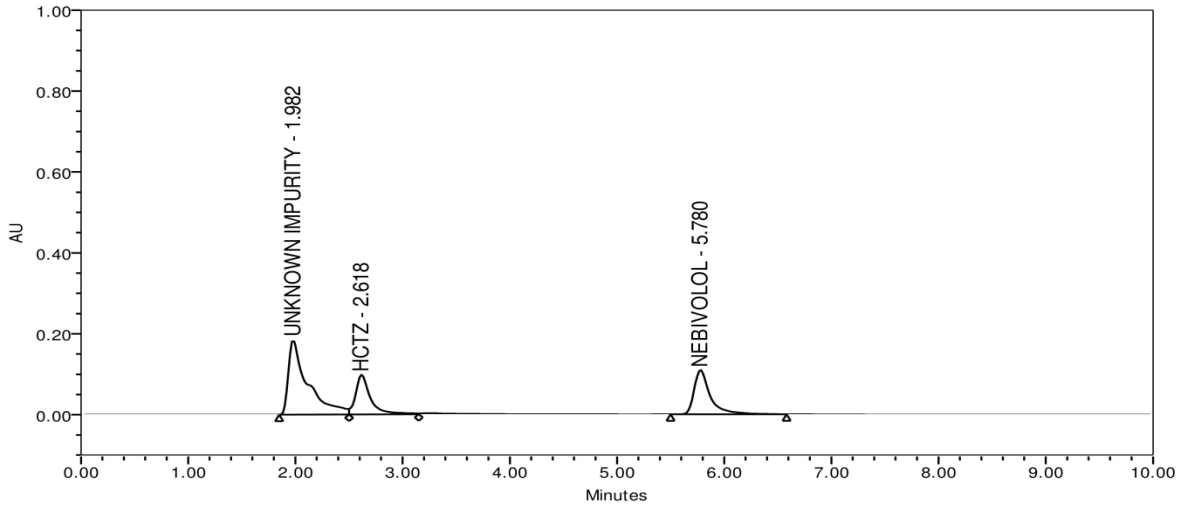
	Name	RT	Area	Height	USP Tailing	USP Plate Count	% Area	USP Resolution
1	HCTZ	2.625	889695	122982	1.5	3423.5	39.06	
2	NEBIVOLOL	6.060	1388360	123300	1.7	8429.0	60.94	15.075750

Fig.3: Chromatograms of Hydrochlorothiazide and Nebivolol



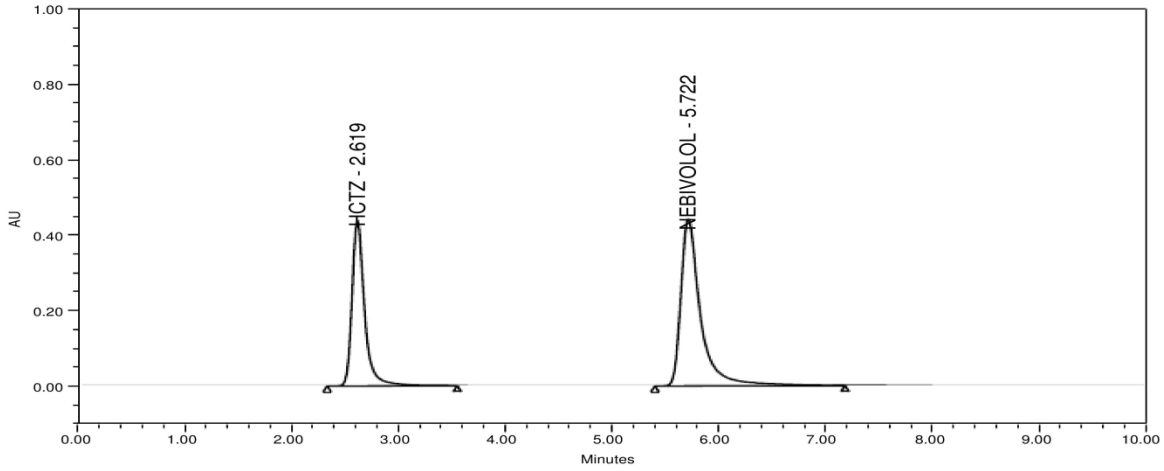
	Peak Name	RT	Area	% Area	Resolution	USP Plate Count	USP Tailing
1	UNKNOWN IMPURITY	2.038	850	0.01		1603.97	1.01
2	HCTZ	2.623	3289052	38.91	2.69	2655.62	1.28
3	NEBIVOLOL	5.756	5163225	61.08	12.40	5439.59	1.70

Fig. 4: Chromatograms of Hydrochlorothiazide and Nebivolol in acid degradation



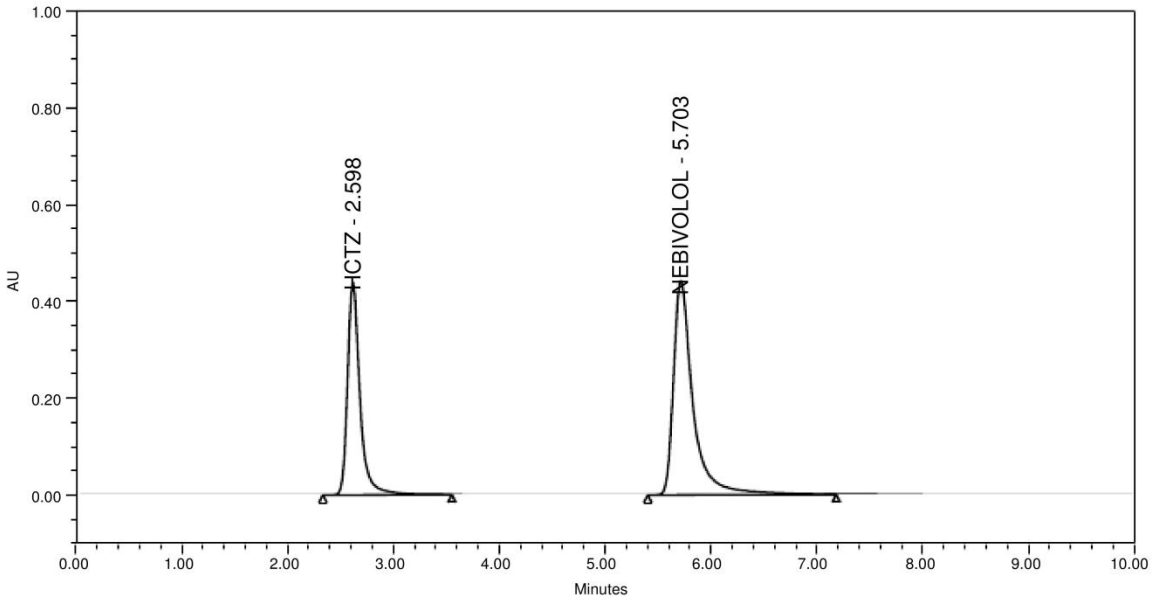
	Peak Name	RT	Area	% Area	Resolution	USP Plate Count	USP Tailing
1	UNKNOWN IMPURITY	1.982	2301244	52.60		907.22	
2	HCTZ	2.618	933279	21.33	2.81	2181.41	
3	NEBIVOLOL	5.780	1140524	26.07	13.91	8962.45	1.71

Fig. 5: Chromatograms of Hydrochlorothiazide and Nebivolol in base degradation



	Peak Name	RT	Area	% Area	Resolution	USP Plate Count	USP Tailing
1	HCTZ	2.619	3375362	38.79		2745.52	1.35
2	NEBIVOLOL	5.722	5365842	61.21	12.76	5822.14	1.70

Fig. 6: Chromatograms of Hydrochlorothiazide and Nebivolol in UVdegradation



	Peak Name	RT	Area	% Area	Resolution	USP Plate Count	USP Tailing
1	HCTZ	2.598	3451326	38.82		2754.20	1.36
2	NEBIVOLOL	5.703	5399840	61.18	12.74	5833.43	1.71

Fig. 7: Chromatograms of Hydrochlorothiazide and Nebivolol in light degradation

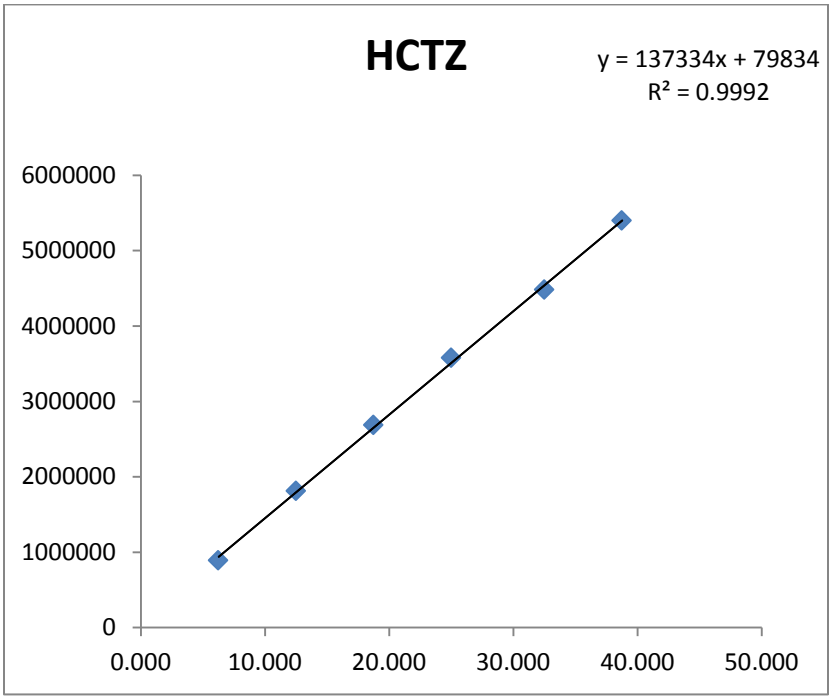


Fig. 8: Linearity Chromatogram of Hydrochlorothiazide

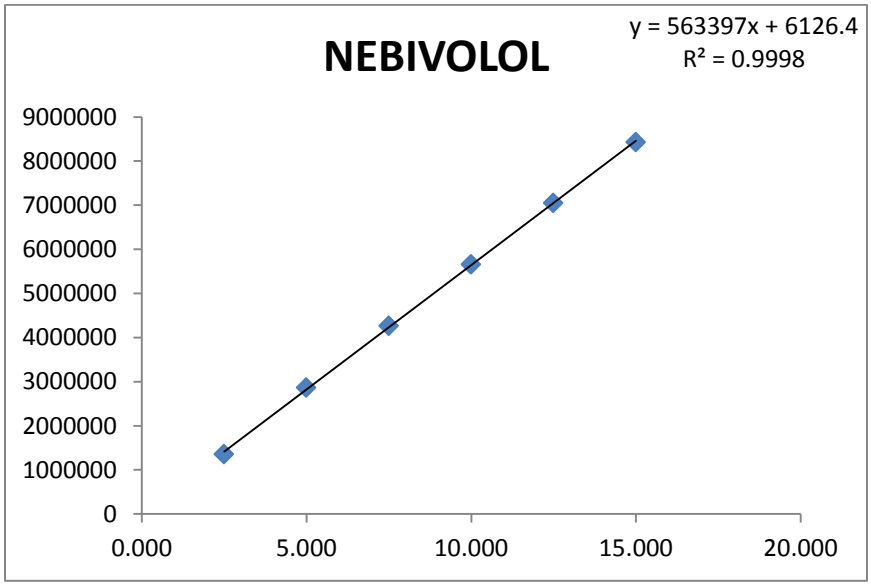


Fig. 9: Linearity Chromatogram of Nebivolol

Table -1: Performance calculations, detection characteristics precision and accuracy of the proposed method for Hydrochlorothiazide and Nebivolol hydrochloride

Parameter	HPLC method for Hydrochlorothiazide	HPLC method for Nebivolol hydrochloride
Wavelength (nm)	254	254
Retention times (t) min	2.625	6.06
Linearity range (µg ml^{-1})	6.25-38.75 µg mL-1	2.5 - 15

LOD (µg ml^{-1})	0.3061	0.489
LOQ (µg ml^{-1})	0.9276	1.4815
Regression equation	(y=bc+a)	(y=bc+a)
Slope (b)	137334	563397
Intercept (a)	79834	6126.4
Correlation coefficient(r^2)	0.9992	0.9998
Relative Standard deviation (%RSD)	0.58	0.4
Intermediate Precision (%RSD)	0.09	0.172

%RSD of five independent determinations

Table-2: Results of linearity

Hydrochlorothiazide		**Nebivolol hydrochloride**	
Conc(µg)	**Area**	**Conc(µg)**	**Area**
6.25	891317	2.5	1357349
12.5	1811058	5	2862859
18.75	2685722	7.5	4264728
25	3579850	10	5655334
32.5	4480216	12.5	7046084
38.75	5399237	15	8428737

Table -3: System precision and system suitability

S No	**Hydrochlorothiazide**		**Nebivolol hydrochloride**	
	RT	Area	RT	Area
1	2.612	3448062	5.689	5301548
2	2.613	3449241	5.691	5303452
3	2.615	3450231	5.694	5289534
4	2.613	3451140	5.696	5284315
5	2.61	3448956	5.693	5289089
6	2.611	3451245	5.695	5284750
Avg	2.612	3449813	5.693	5292115
Std Dev	0.0018	1273.88	0.0026	8347.58
%RSD	0.067	0.037	0.046	0.158

Table -4: Method precision

S No	**HCTZ**		**NBL**	
	RT	**Area**	**RT**	**Area**
1	2.616	3493248	5.682	5277235
2	2.616	3457212	5.682	5299991
3	2.614	3459056	5.681	5292735

4	2.616	3447455	5.682	5292735
5	2.616	3494944	5.686	5332319
6	2.616	3477956	5.686	5324657
Avg	2.6156	3471645	5.683	5303278
Std Dev	0.00082	20006.6	0.00223	21032.3
%RSD	0.03122	0.58	0.03924	0.40

Table-5: Ruggedness of Hydrochlorothiazide Day 1 and Day 2

S No	Name	RT	Area
1	Injection-1	2.612	3581253
2	Injection-2	2.61	3580985
3	Injection-3	2.615	3579862
4	Injection-4	2.616	3576824
5	Injection-5	2.613	3579085
6	Injection-6	2.611	3569870
7	Injection-7	2.617	3576856
8	Injection-8	2.617	3581162
9	Injectoion-9	2.613	3580125
10	Injection-10	2.617	3579850
11	Injection-11	2.614	3576056
12	Injection-12	2.612	3580492
	AVG	2.613917	3578535
	STDEV	0.00246	3267.136
	%RSD	0.09	0.09

Table-6: Ruggedness of Nebivolol hydrochloride Day 1 and Day 2

S No	Name	RT	Area
1	Injection-1	5.713	5661254
2	Injection-2	5.711	5670154
3	Injection-3	5.716	5672103
4	Injection-4	5.718	5670056
5	Injection-5	5.715	5669879
6	Injection-6	5.715	5673542
7	Injection-7	5.716	5644273
8	Injection-8	5.716	5652718
9	Injectoion-9	5.714	5649052
10	Injection-10	5.716	5655334
11	Injection-11	5.713	5660532
12	Injection-12	5.714	5659906
	AVG	5.71475	5661567
	STDEV	0.001865	9751.866
	%RSD	0.03	0.17224

Table-7: Robustness study of Hydrochlorothiazide and Nebivolol hydrochloride

S.No	Peak Name	RT	Area	% Area	USP Plate Count	USP Resolution	USP Tailing
1	HCTZ-256nm	2.61	3452053	39.53	2759.21	---	1.21
	NBL-256nm	5.694	5281201	60.47	5916.54	12.74	1.5
2	HCTZ-252nm	2.613	3449865	39.51	2769.53	---	1.2
	NBL -252nm	5.698	5279980	60.49	5921.43	12.76	1.51
3	HCTZ Tem-25˚C	2.616	3447455	39.38	2774.03	---	1.22
	NBL Tem-25 ˚C	5.682	5307049	60.62	5929.81	12.75	1.54
4	HCTZ Tem-35˚C	2.616	3486573	39.54	2840.16	---	1.24
	NBL Tem-35˚C	5.686	5332319	60.46	5999.03	12.86	1.56

Table-8: Degradation study of Hydrochlorothiazide and Nebivolol hydrochloride

Stress conditions	Time	Area		Assay of active		Deg%		Peak purity	
		HCTZ	NBL	HCTZ	NBL	HCTZ	NBL	HCTZ	NBL
Acid	24 hrs	3289052	5163225	95.4	97.61	4.6	2.39	1	1
Base	24 hrs	933279	1140524	27.06	21.56	72.94	78.44	1	1
UV	7 days	3375362	5365842	100	100	0	0	1	1
Light	10 days	3451326	5399840	97.8	100.1	2.2	0	1	1

Table-9: Assay Results of Hydrochlorothiazide and Nebivolol hydrochloride

Drug	Amount present/tablet(mg)	Amount Found /tablet(mg)	% of Assay
Hydrochlorothiazide	25	25.1233	100.5
Nebivolol hydrochloride	10	10.01	100.09

Table-10: Accuracy data (Triplicate values at 50,100 &150 percent levels) of Hydrochlorothiazide

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
1	50%	1799631	12.5	12.5587	100.47	100	0.436
		1805346	12.5	12.4512	99.61		
		1807415	12.5	12.49	99.92		
2	100%	3576856	25	24.975	99.9	99.723	0.205
		3581162	25	24.9425	99.77		
		3580125	25	24.875	99.5		
3	150%	5384867	38.75	38.808	100.15	100.14	0.065
		5380520	38.75	38.777	100.07		
		5387934	38.75	38.8275	100.2		

Table-11: Accuracy data (Triplicate values at 50,100 &150 percent levels) of Nebivolol hydrochloride

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
1	50%	2860523	5	5.0405	100.81	100.183	0.55
		2864342	5	4.988	99.76		
		2865154	5	4.999	99.98		
2	100%	5644273	10	9.95	99.5	99.33	0.211
		5652718	10	9.941	99.41		
		5649052	10	9.91	99.1		
3	150%	8418130	15	14.823	98.82	98.92	0.089
		8420156	15	14.8425	98.95		
		8423418	15	14.8485	98.99		

METHOD VALIDATION

Specificity study

Mobile phase along with placebo were injected to check the interference at the retention time of HCTZ and NBL in the established chromatographic condition and no interference were observed at the designated Retention Time which was established by peak purity of the chromatogram by UV-Vis detector.

Stress studies

Acid, Alkaline, UV and light degradation studies were conducted and HCTZ and NBL were subjected to this condition. 0.1N HCl, 0.5N NaOH, UV and light (120 lux hours) were used for stress testing studies. The samples are neutralized before injecting into the system for acid and alkaline samples .UV and light samples were injected after proper dilution as such. Placebo and mobile phase were also subjected to same treatment as sample to check for interferences.

Precision

ICH describes precision as closeness of individual measure of analytes when the procedure is applied repeatedly to multiple times interday and intraday precision has been established in the method.

Accuracy

It was evaluated at three levels of 50%, 100% and 150% of test concentration by adding known amount of drug to placebo and extracting the sample. Three sets were prepared and analyzed.

Solution stability

HCTZ and NBL and their formulation stabilities were carried out for a period of 48 hours at auto sampler at 25^0C temperature.

Robustness

Varying conditions of column temperature and wavelength were carried out as per ICH guidelines to estimate the effects on the method.

RESULTS AND DISCUSSIONS

Method development and optimization

Actual chromatographic conditions were established after number of preliminary experiments for selecting the proper mobile phase system. Different mobile phase systems were tested, and selection of the proper system depended on its ability to give good separation between the pure drugs and their possible degradation products. Acceptable separation was achieved on Hypersil BDS C18, 150x4.6, 5µ using a mobile phase composed of mixed buffer and Methanol in the ratio 50: 50. (pH was adjusted to 5.5 ±0.1 by using Ortho-phosphoric acid). Mixed buffer and Methanol in ratio of (50:50 v/v %) pumped with a flow rate of 1.0 ml/min the column temperature was kept constant at 30ºC. under these chromatographic conditions, the run time sample was 10 min, and the retention times of HCTZ and NBL 2.625 and 6.060 min. The representative chromatogram is shown in fig-3.

Performance calculations, detection characteristics precision and accuracy of the proposed method for of HCTZ and NBL were reported in the table-1.

System suitability

System suitability parameters like theoretical plates per meter, tailing factor, percentage relative standard deviation of area and retention time of twelve injections were carried out and the values are well within the limits as shown in Table-3.

Linearity and sensitivity

A linear calibration plot of HCTZ and NBL was constructed at nine point concentration levels 6.250-38.750 µg/ml and 2.5-15 µg/ml in duplicate. Average peak area of HCTZ and NBL were plotted against respective concentrations and linear regression analysis was performed. Correlation coefficient was found to be r^2=0.9992 (n=6) and r^2=0.9998 respectively. Limit of detection (LOD) and limit of quantification (LOQ) values for Hydrochlorothiazide were 0.3061 and 0.9276 µg/ml and for Nebivolol were 0.489 and 1.4815 µg/ml respectively.

Precision

The precision of the assay method was evaluated for repeatability and intermediate precision. For intra-day precision and inter-day precision, the percentage relative standard deviation of HCTZ and NBL was found to be 0.58% and 0.40% respectively. These values were well within the acceptable limit of 2%, as per USP. Result is given in table- 4.

Accuracy

Known amount of standard was spiked in 50%, 100%, 150% concentration in triplicate to test solution and recovery of drug was calculated. The accuracy of method was established at three concentration levels at 12.5, 25 and 38.75 µg/ml of HCTZ and 5, 10 and 15µg/ml of NBL standards. The recoveries at three different concentrations were found to be within the range of 95.0 to 105 % as per ICH guidelines. Mean % recovery was 98.92 to 100.183. The results are given in tables- 10 & 11.

Robustness

The robustness of assay method was studied by incorporating small but deliberate changes in the analytical method (variations in column temperature and wave length) and also by observing the stability of the drugs for 24 hours at room temperature in the dilution solvent. In all the varied chromatographic conditions, there was no significant change in chromatographic parameters. Result is given in table- 7.

STRESS STUDIES

Stress testing of the drug substance can help in identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule.

Acid degradation studies

25mg of the HCTZ and 10mg of NBL were weighed accurately and transferred to 100 ml volumetric flask, added 50 ml of freshly prepared 0.1N HCl and kept at room temperature for 24 hours. After keeping the solution for 24 hrs at room temperature, filtered and then neutralized the solution up to the volume with 0.5 N NaOH. Diluted 10 ml of the above solution to 100 ml with diluent. 20µl of the solution was injected into the chromatographic system and the chromatogram was recorded as shown in figure- 4. The results are given in table-8.

Base degradation studies

25mg of the HCTZ and 10mg of NBL were weighed accurately and transferred to 100 ml volumetric flask, added 50 ml of freshly prepared 0.5 NaOH and kept at room temperature for 24 hours. After keeping the solution for 24 hrs at room temperature, filtered and then neutralized the solution up to the volume with 0.1 N HCl. Diluted 10 ml of the above solution to 100 ml with diluent. 20µl of the solution was injected into the chromatographic system and the chromatogram was recorded as shown in figure -5. The results are given in table-8.

UV degradation

Sufficient amount of Hydrochlorothiazide and Nebivolol powder was transferred into petridish spread evenly for NMT 1mm thickness and kept inside hot air oven at room temperature for 7 days. Samples were collected at different time intervals and final dilution were done with the mobile phase and loaded into HPLC system. The representative chromatogram is shown in fig-6. The results are given in table-8

Light degradation

Hydrochlorothiazide and Nebivolol hydrochloride samples were degraded with 120

lux light for 10 days. 10ml of sample was collected on 10th day and injected into the HPLC system. The representative chromatogram is shown in fig-7. The results are given in table-8

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of HCTZ and NBL bulk drugs and pharmaceutical dosage form. The developed method is specific, selective, robust, rugged and precise. This method can be conveniently used for assessing stability assay of selected substances and dissolution of tablets containing HCTZ and NBL in quality control laboratory. The study showed that the drugs are highly degraded in base (72.94% & 78.44%) conditions , moderately degraded in acid (4.6% & 2.39%) conditions, least degraded in the light (2.2% & 0%) conditions and no degradation for the UV conditions .

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