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Simultaneous quantification of nebivolol hydrochloride and hydrochlorothiazide by first derivative UV- Spectroscopy

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ABSTRACT

A new sensitive, simple, rapid, accurate and precise method for simultaneous quantification of nebivolol HCl (NEB) and hydrochlorothiazide (HCT) in combined tablet dosage form has been developed. The method is based on the derivative spectrophotometric method at zero-crossing wavelengths. Two wavelengths 280nm (zero crossing point for NEB) and 272.2nm (zero crossing point for HCT) were selected for the quantification of hydrochlorothiazide and nebivolol HCl respectively, using phosphate buffer pH 3.6 as solvent. The first derivative amplitude-concentration plots were rectilinear over the range of 4-24 µg mL^{-1} and 2-32 µg mL^{-1} with detection limits of 0.127 and 0.230 µg mL^{-1} and quantification limits of 0.425 and 0.769 µg $mL^{-1}$for hydrochlorothiazide and nebivolol HCl respectively. The proposed method was statistically validated as per ICH guidelines. The percentage recovery was within the range between 97-101 and % relative standard deviation for precision and accuracy of the method was found to be less than 2%. The proposed method was effectively applied to routine quality control analysis of studied drugs in their tablet formulations.

Key Words: Hydrochlorothiazide, nebivolol HCl, derivative spectrophotometry.

INTRODUCTION

Nebivolol HCl is chemically known as α,α′-[Iminobis(methylene)]bis[6–fluoro–3,4–dihydro–2H-1–benzopyran–2–methanol] (figure 1). It is white powder, soluble in methanol and practically insoluble in water. It is a selective beta-1 antagonist, used in the management of hypertension [1]. Hydrochlorothiazide is chemically 6-Chloro–3,4–dihydro–2*H*-1,2,4 benzothiadiazine–7–sulfonamide1,1–dioxide, white crystalline powder and practically insoluble in water (figure 2). It is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia, acts as diuretic [2-4]. Nebivolol HCl and hydrochlorothiazide have been formulated in a fixed dose tablet dosage form and used in the treatment of hypertension.

A detailed literature review indicate that few analytical methods include UV-spectrophotometry, high performance liquid chromatography (HPLC) and HPTLC methods available for simultaneous quantification of nebivolol HCl and hydrochlorothiazide [5-11]. To the best of our knowledge, only one method reported on the use of derivative spectrophotometry for the simultaneous quantification of nebivolol HCl and hydrochlorothiazide in methanol as solvent, but methanol is environmental toxic and expensive than aqueous buffer. Moreover there was no simple, eco-friendly and economical method available for estimation of nebivolol HCl and hydrochlorothiazide either in bulk drug or in formulation by first derivative spectroscopy using aqueous buffer as solvent.

Derivative spectroscopy provides a greater selectivity and spectral discrimination than common spectroscopy. It is the dominant approach for resolution of one analyte whose peak is hidden by a large overlapping peak of another

analyte in multi component analysis. The first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ_{max} of the absorption band [12, 13].

This investigation describes a simple, eco-friendly and economical first –derivative spectrophotometric method for simultaneous quantification of nebivolol HCl and hydrochlorothiazide either in bulk drug or in tablet dosage form using phosphate buffer, pH 3.6 as solvent and method was stastically validated as per ICH guidelines.

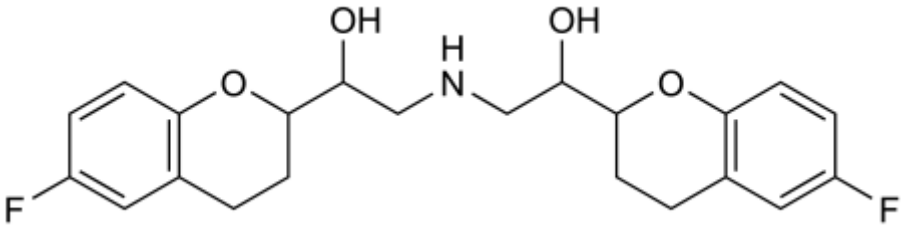

Figure 1: Chemical structure of nebivolol HCl

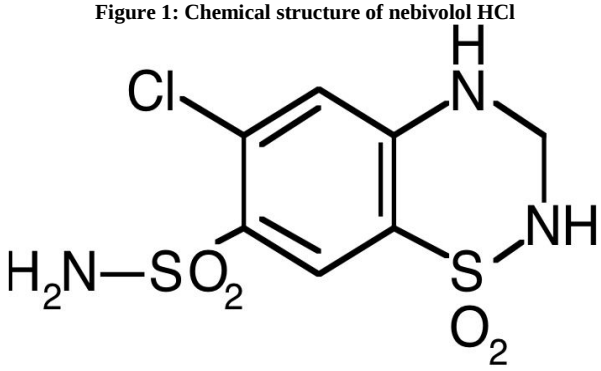

Figure 2: Chemical structure of hydrochlorothiazide

MATERIALS AND METHODS

Instrumentation:
Shimadzu (Japan) UV-Visible spectrophotometer (UV-1800) with 1cm matched quartz cells was used for spectrophotometric analysis, a calibrated electronic single pan balance (Shimadzu ,Aux-220), ultra sonic cleaner (SONICA, Italy) and pH meter (Elico) were used during the analysis.

Reagents and chemicals:
Nebivolol HCl and hydrochlorothiazide bulk drugs were obtained as gift samples from Dr.Reddy's laboratories Ltd, Hyderabad, India. Tablets (Nebicard-H and Nebistar-H) were procured from local pharmacies. Di-sodium hydrogen ortho phosphate, anhydrous citric acid and methanol AR grade were purchased from Hi-media, Mumbai.

Preparation of standard stock solutions
Each of standard nebivolol HCl (10mg) and hydrochlorothiazide (10mg) were weighed and transferred into two separate 10 ml volumetric flasks and dissolved in 5ml methanol. The flasks were shaken and volume was made up to the mark with phosphate buffer, pH 3.6. From this 1 ml solution was diluted to 10 ml with phosphate buffer, pH 3.6 to obtain a standard solution of nebivolol HCl and hydrochlorothiazide having final concentration of 100 µg mL^{-1} of each.

Selection of wavelengths:
Standard solution of nebivolol HCl (NEB) and hydrochlorothiazide (HCT) were diluted appropriately with pH 3.6 phosphate buffer to obtain solution containing nebivolol HCl (8 µg mL^{-1}) and hydrochlorothiazide (8 µg mL^{-1}). Spectra of these diluted solutions were scanned in the spectrum mode between 200 nm to 400 nm using phosphate buffer, pH 3.6 as a blank. The zero-order spectra of nebivolol hydrochloride and hydrochlorothiazide were transformed to corresponding first-derivative spectra in the range of 200 - 400 nm. The overlaid spectra (zero and first order) of nebivolol HCl (NEB) and hydrochlorothiazide (HCT) are shown in figure 3 and 4.

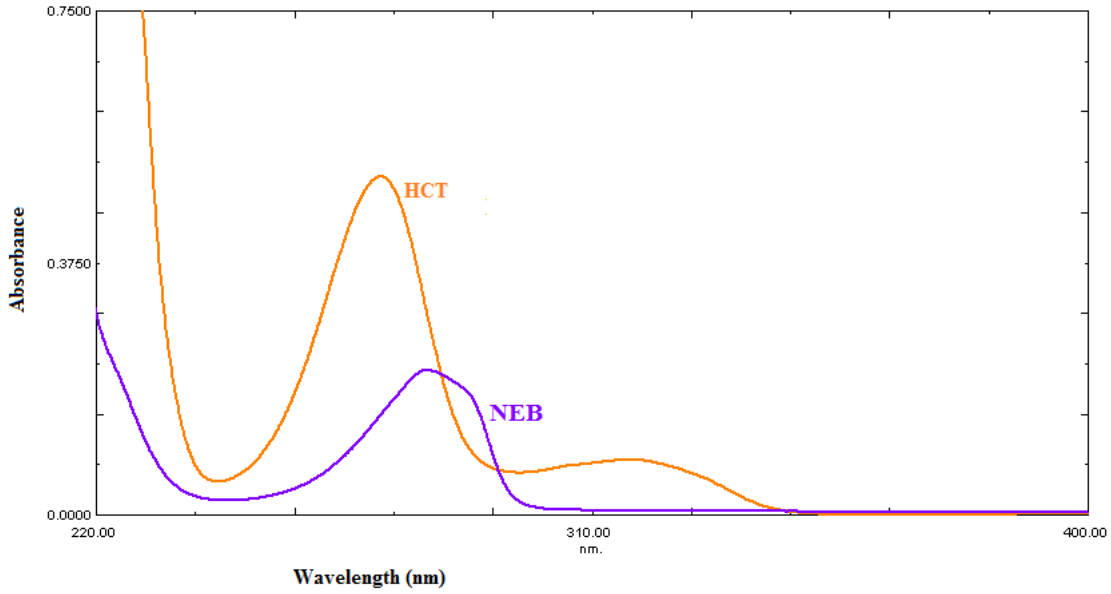

Figure 3: Zero-order UV overlaid spectrum of nebivolol HCl (NEB) (8 µg mL^{-1}) and hydrochlorothiazide (HCT) (8 µg mL^{-1})

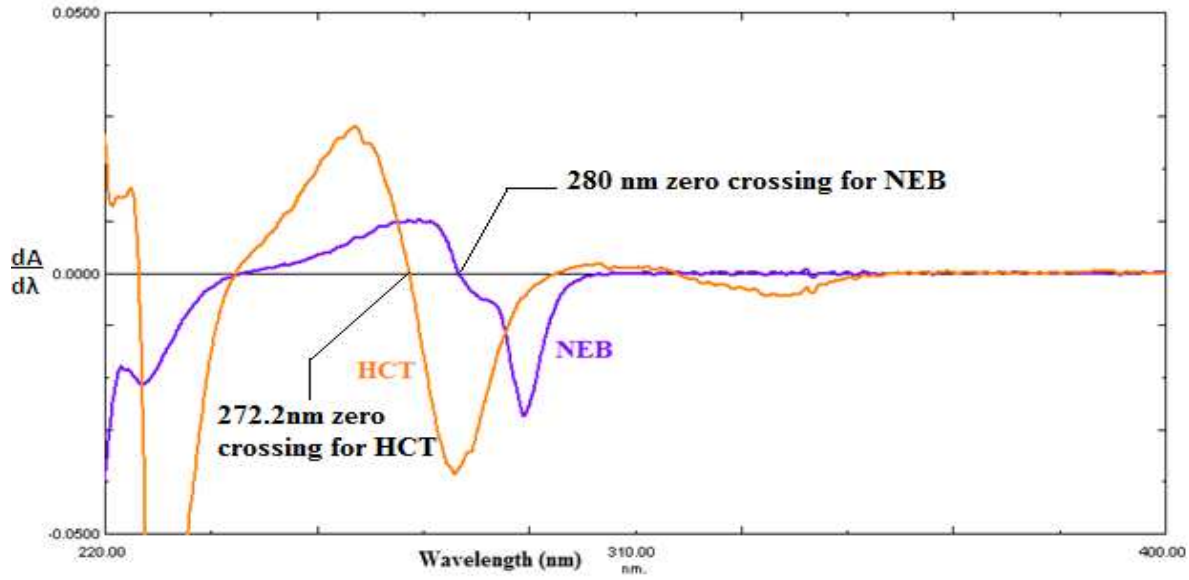

Figure 4: First-order UV overlaid spectrum of nebivolol HCl (NEB) (8 µg mL^{-1}) and hydrochlorothiazide (HCT) (8 µg mL^{-1})

Derivative conditions:
First-order derivative spectra of nebivolol HCl and hydrochlorothiazide were overlapped. The wavelength 272.2 nm was selected for the quantification of nebivolol HCl (where the derivative response for hydrochlorothiazide was zero). Similarly, 280 nm was selected for the quantification of hydrochlorothiazide (where the derivative response for nebivolol HCl was zero). Characteristic wavelengths (zero-crossing points) for nebivolol HCl and hydrochlorothiazide were confirmed by varying the concentrations of both drugs.

Calibration curves for nebivolol HCl and hydrochlorothiazide:
The standard solution of nebivolol hydrochloride (100 µg mL^{-1}) and hydrochlorothiazide (100 µg mL^{-1}) were used to prepare two different sets of working standard solutions of nebivolol HCl (2-32 µg mL^{-1}) and hydrochlorothiazide (2-24 µg mL^{-1}).

The first-derivative spectra were recorded using the prepared solutions against pH 3.6 phosphate buffer as blank. The values of first-derivative absorbance were plotted against corresponding concentrations to construct the calibration curves. First derivative spectra of working standard dilutions and calibration curves are shown in figure 5, 6 and 7.

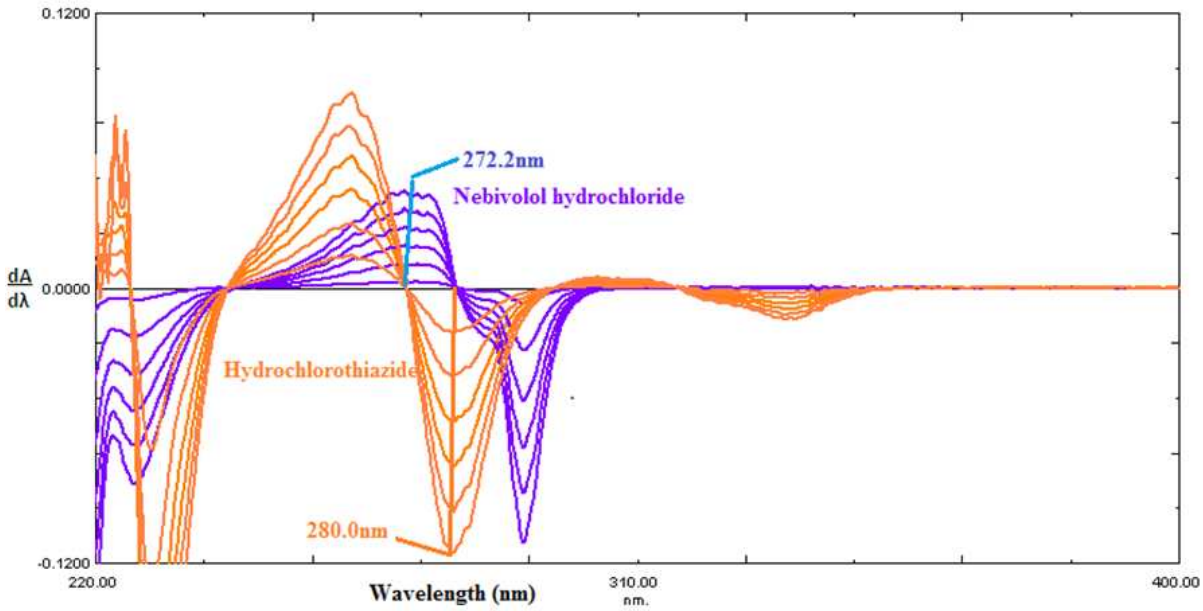

Figure 5: UV first-derivative linearity range of nebivolol HCl (2-32 µg mL^{-1}) and hydrochlorothiazide (4-24 µg mL^{-1})

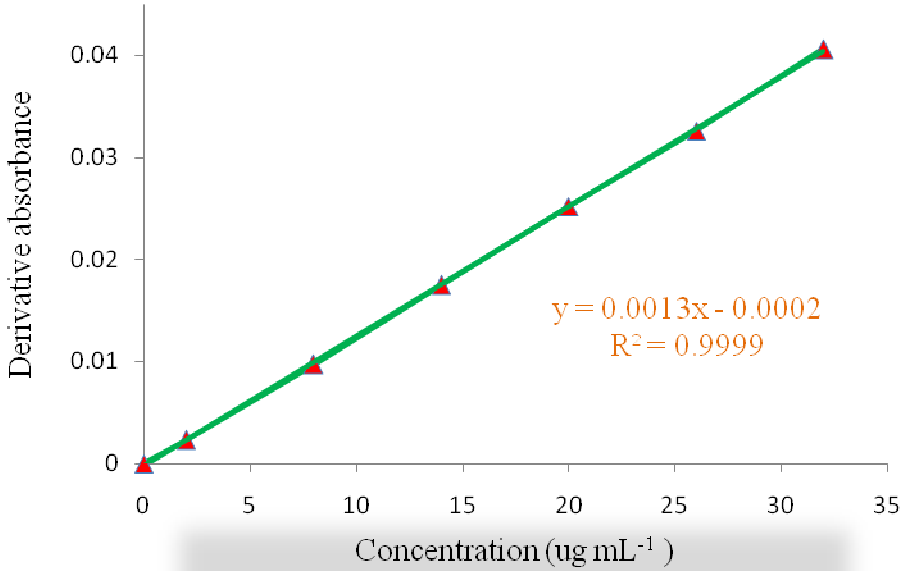

Figure 6: Standard plot of nebivolol HCl in pH 3.6 phosphate buffer at 272.2 nm

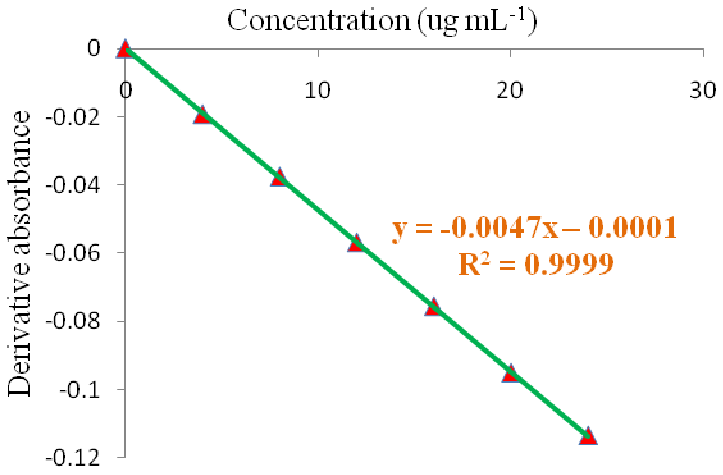

Figure 7: Standard plot of hydrochlorothiazide in pH 3.6 phosphate buffer at 280 nm

Determination of nebivolol hydrochloride and hydrochlorothiazide in their combined dosage form (assay):
Twenty tablets of each marketed formulation (Nebistar-H and Nebicard-H), each containing 5 mg of nebivolol HCl and 12.5 mg of hydrochlorothiazide were taken and accurately weighed. Average weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 5 mg nebivolol hydrochloride and 12.5 mg hydrochlorothiazide was transferred to volumetric flask of 10 ml capacity. Methanol (5 ml) was added to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with pH3.6 phosphate buffer. The above solution was filtered through whatmann filter paper (No.41). From the filtrate 1 ml was transferred into volumetric flask of 10 ml capacity. Volume was made up to the mark with 3.6 phosphate buffer, from this 1.6ml was diluted to 10ml with buffer to give 8 µg mL^{-1} nebivolol HCl and 20 µg mL^{-1} hydrochlorothiazide. These solutions were used for the estimation of nebivolol HCl and hydrochlorothiazide.

The amount of nebivolol HCl and hydrochlorothiazide present in the sample solution were determined by substituting derivative responses into the equation of the straight line representing the calibration curves for nebivolol HCl and hydrochlorothiazide with correction for dilution

METHOD VALIDATION
The method was validated for accuracy, precision, specificity, linearity, LOD and LOQ by the following procedures:

Accuracy:
The accuracy of the method was determined by calculating recoveries of nebivolol .hydrochloride and hydrochlorothiazide by the method of standard additions. Known amounts of nebivolol HCl and hydrochlorothiazide (80%, 100% and 120%) levels were added to a pre quantified sample solutions. The recovery was verified by estimation of drug in triplicate preparations at each specified concentration level and calculated % RSD [14.15].

Precision:
The intra-day and inter-day precision of the proposed first-derivative spectrophotometric simultaneous method was determined by estimating the corresponding response three times on the same day (intra- day) and for three consecutive days (inter-day) for three different concentrations of nebivolol HCl (2, 14 and 32 µg mL^{-1}) and hydrochlorothiazide (4, 16 and 24 µg mL^{-1}). The results are reported in terms of relative standard deviation (% RSD).

Selectivity:
Selectivity is the ability of the method to accurately measure a compound in the presence of other components such as impurities, degradation products and matrix components. The selectivity of the proposed method was evaluated through the analysis of a placebo solution, which was prepared with the common excipients of the pharmaceutical formulation. Thus, the mixture of component inert was prepared in their usual concentration employed in tablets (concentrations were determined based in Handbook of Pharmaceutical Excipients and calculated for medium weight of content) [16]. The developed method was applied in order to check if any component of the formulation could generate a response with emission band similar to the drugs.

Sensitivity:
The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on standard calibration curve.

RESULTS AND DISCUSSION

A simple, eco-friendly and economic first derivative spectrophotometric method was developed for the simultaneous quantification of nebivolol HCl and hydrochlorothiazide and bulk drug and formulations using aqueous buffer, pH 3.6 as solvent and was also validated as per ICH guidelines. The calibration curves shows that, the developed method was linear in the concentration range of 2-32 µg mL^{-1} and 4-24 µg mL^{-1} for nebivolol HCl and hydrochlorothiazide. Limit of detection and limit of quantification values were indicated that the method shows high sensitivity. The optimized conditions for developed method are shown in Table 1. No significant difference between intra-day and inter-day precision, revealed that the method is reproducible (Table 2). The % recovery was within the range between 98-102 (Table 3) and %RSD for commercial formulation was shown less than 2 (Table 3). This indicates that the method is accurate and reliable.

Table1: Optimum conditions for proposed method

S.No.	Parameter	Nebivolol HCl	Hydrochlorothiazide
1	Absorption maxima (nm)	272.2	280
2	Beer's Law Limit(µg mL^{-1})	2-32	4 – 24
3	slope	0.0013	-0.0008
4	Intercept	-0.0002	-0.0001
5	Correlation coefficient	0.9999	0.9999
6	Regression equation	y = 0.0013x -0.0002	y = -0.0047x -0.0001
7	LOD (µg mL^{-1})	0.230	0.127
8	LOQ(µg mL^{-1})	0.769	0.425

Table 2: Precision of the method

Concentration (mcg/ml)	Intra-day precision		Inter-day precision	
	Concentration estimated (mcg/ml) (AM ± SD) (n=3)	% RSD	Concentration estimated (mcg/ml) (AM ± SD) (n=3)	%RSD
Nebivolol HCl				
2	1.974 ± 0.088	0.04	2.024 ± 0.078	0.038
14	13.709 ± 0.133	0.966	14.001 ± 0.094	0.671
32	31.45 ± 0.333	1.049	31.245 ± 0.245	0.784
Hydrochlorothiazide				
4	4.105 ± 0.056	0.013	4.024 ± 0.024	0.596
16	16.099 ± 0.025	0.155	16.056 ± 0.056	0.348
24	24.120 ± 0.065	0.269	23.985 ± 0.094	0.391

Table 3: Accuracy of the method (Recovery studies)

Formulation	Recovery level (%)	Recovery of analyte	Theoretical content (mg)	Amount found (mg) (AM ± SD) (n=3)	Recovery (%)	% RSD
Nebistar-H	0	NEB	5	4.853± 0.025	97.06	0.515
		HCT	12.5	12.36 ± 0.054	98.88	0.436
	80	NEB	9	9.024 ± 0.124	100.26	1.374
		HCT	22.5	22.02 ± 0.224	97.86	1.017
	100	NEB	10	9.854 ± 0.054	98.54	0.548
		HCT	25	24.94 ± 0.454	99.76	1.828
	120	NEB	11	11.02 ± 0.096	100.12	0.870
		HCT	27.5	27.54 ± 0.122	100.14	0.442
Nebicard-H	0	NEB	5	4.724 ± 0.012	98.48	0.243
		HCT	12.5	12.45 ± 0.101	99.60	0.811
	80	NEB	9	9.245 ± 0.075	102.72	0.811
		HCT	22.5	22.05 ± 0.262	98.00	1.188
	100	NEB	10	10.054 ± 0.16	100.54	1.631
		HCT	25	24.96 ± 0.456	99.84	1.826
	120	NEB	11	10.98 ± 0.102	99.81	0.928
		HCT	27.5	27.57 ± 0.094	100.25	0.340

Table 4: Analysis of commercial tablets (assay)

Formulation	Nebivolol hydrochloride				Hydrochlorothiazide			
	Label claim (mg)	Amount found (mg) (AM ± SD) (n=3)	% Recovery	% RSD	Label claim (mg)	Amount found (mg) (AM ± SD) (n=3)	% Recovery	% RSD
Nebistar-H	5	4.724 ± 0.012	94.48	0.245	12.5	12.45 ±0.104	99.6	0.436
Nebicard-H	5	4.853 ± 0.025	97.06	0.515	12.5	12.36 ±0.054	98.88	0.436

CONCLUSION

Now a day's, usage of organic solvents in development of analytical methods for quantification of drugs might cause environmental toxicity. Hence there is a need of analytical methods in the presence of eco-friendly solvents like water, hydrotropic agents and aqueous buffers. Considering all these criteria's an eco-friendly, simple, sensitive and economic first derivative spectrophotometric method has been proposed for simultaneous quantification nebivolol HCl and hydrochlorothiazide in pure form and in tablet dosage forms by using phosphate buffer, pH 3.6 as solvent. The assay values were in good concurrence with their respective labeled claim, which suggested no interference of formulation excipients in the estimation and obtained results from validation proved the proposed method was scientifically sound. Therefore, the developed method can be readily adopted by pharmaceutical quality control laboratory for routine analysis.

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