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RESEARCH ARTICLE

Absorption Correction Method for Estimation of Nebivolol and Hydrochlorothiazide in Combined Tablet Dosage Form

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ABSTRACT
A new, simple, accurate and sensitive UV-spectrophotometric absorption correction method has been developed for simultaneous determination of Nebivolol and Hydrochlorothiazide in combined pharmaceutical dosage form. The method is based upon determination of Nebivolol at 281 nm and Hydrochlorothiazide at 316.5 nm, in aqueous methanol (20 % v/v). . Nebivolol hydrochloride and Hydrochlorothiazide at their respective λ_{max} 281.0 nm and 316.5 nm shows linearity in the concentration range of 5-35 µg/ml and 10-70 µg/ml respectively. The method was validated statistically.

KEY WORDS Nebivolol, Hydrochlorothiazide, absorption correction method, standard addition

INTRODUCTION:
Hydrochlorothiazide (HCTZ), 6-chloro-3,4-dihydro-7-sulfamoyl-2*H*-1,2,4-benzothia- diazine-1,1-dioxide, is a thiazide diuretic. It increases sodium and chloride excretion in distilled convoluted tubule.[1]
Nebivolol (NBV),α,α'-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2*H*-1-benzpyran-2-methanol], is used for treatment of hypertension through vascular endothelial nitric oxide releasing capabilities and β_1-antagonist action.[2, 3, 4]
Literature survey reveals many analytical methods, including spectroscopic and chromatographic methods, for the quantitative determination of HCTZ alone or in combination with other antihypertensive drugs.[5, 6] While NBV have been reported to be estimated using spectroflurometric and HPLC.[7,8] However no method is reported for simultaneous estimation of these two drugs in tablet. Hence the present work was attempted to develop accurate simple and sensitive method for simultaneous estimation of NBV and HCTZ. For this purpose marketed tablets Nebi-H and Nebistar-H containing 5 mg of NBV and 12.5 mg of HCTZ was used. As the absorbance of NBV was very low in the concentration available tablet, standard addition was used by adding 20 mg of NBV.

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MATERIAL AND METHOD:
A UV/Visible double beam spectrophotometer, model SHIMADZU UV-1700 with 1cm UV matched quartz cells was used. One Pan Balance (K-14 super By K Roy) was used for experimental purpose. Methanol used was of analytical grade. M/s Hetero Drugs Limited and M/s Golden Cross, Daman provided the samples of NBV and HCTZ respectively.

Hydrochlorothiazide was standardized by official method reported in Indian Pharmacopoeia and the purity of the sample was found to be 99.75%. The purity of NBV was 99.70%.

Accurately weighed quantities (100 mg) of NBV and HCTZ were dissolved separately in 20 ml methanol and volumes were made up to 100 ml with water (1000 µg/ml). These solutions were used as working standards. Aliquot portions of stock solutions of NBV and HCTZ were diluted appropriately with aqueous methanol (20% v/v) to obtain concentration 20 µg/ml of NBV and 10 µg/ml of HCTZ. The working standard solutions were scanned from 200 to 400 nm to select the wavelengths for estimation. From the overlain spectrum shown in Fig.1, the wavelength selected for estimation of HCTZ was 316.5 nm, where NBV has no absorbance and for NBV it was 281.0 nm, where absorbance of HCTZ is corrected. Different binary mixture solutions of NBV and HCTZ were then run in entire range from 200 to 400 nm. The drug obey Beer's law in the concentration range of 0 to 35 µg/ml. Quantitative estimation of these drugs was carried out by using following formulae's.

$Cy = A_{316.5\ nm} / A\ (1\%, 1cm)_{316.5\ nm\ of\ HCTZ.}$ (1)

$Cx = CAx_{281.0\ nm} / A\ (1\%, 1cm)_{281.0\ nm\ of\ NBV}$ (2)

$CAx_{281.0\ nm} = A_{281.0\ nm} - Ay_{281.0\ nm}$ (3)

$Ay_{281.0\ nm} = Cy \times A\ (1\%, 1cm)_{281.0\ nm\ of\ HCTZ}$ (4)

Where, Cx and Cy are concentration (g /100ml) of NBV and HCTZ, respectively, $A_{316.5nm}$ and $A_{281.0\ nm}$ are absorbance of mixture at 316.5 nm and 281.0 nm, respectively, $CAx_{281.0\ nm}$ is corrected absorbance of NBV and $Ay_{316.5\ nm}$ absorbance of HCTZ at 281.0 nm.

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 5 mg NBV and 12.5 mg HCTZ was accurately weighed and as per standard addition method,[9, 10] 20 mg of pure NBV was added to achieve 2:1 ratio of NBV and HCTZ. The powder was transferred to 100 ml volumetric flask and shaken vigorously with methanol for 15 min and filtered. Necessary dilutions are made with aqueous methanol to give final concentration 20µg/ml and 10 µg/ml of NBV and HCTZrespectively. The absorbance's values were read at 281 and 316.5 nm and concentration was obtained by solving the absorption correction equations. Results of analysis of tablet formulation are shown in Table No. 1.

The proposed method was validated on the basis of parameters namely accuracy, precision, specificity, ruggedness and linearity and range. The accuracy of the proposed method was ascertained by carrying out recovery studies using standard addition method. The recovery study was performed to determine if there was any positive or negative interference from excipients present in the formulation. Precision of an analytical method is expressed as SD or RSD of a series of measurements. It was ascertained by replicate estimation of drug by the proposed method.

The specificity is the ability to assess unequivocally the analyte for the presence of interesting components that may be expected to be present, such as impurities, degradation product and matrix components. During the specificity study, accurately weighed quantities of tablet powder equivalent to 12.5 mg of HCTZ were taken in different 100 ml volumetric flasks. To them 20 mg of pure drug Nebivolol was added and stored for 24 hours under different conditions, like room temperature (normal), at 50º after addition of 1.0 ml of 0.1 N of hydrochloric acid , at 50º after the addition of 1.0 ml of 0.1 N of sodium hydroxide, at 50º after the addition of 3 % of hydrogen peroxide and at 60º . After 24 hours, the contents of the flask were subjected to same procedures as previously described. Test for ruggedness was carried out by repeating the procedure under different conditions, i.e., on different days, at different time and by different analysts.

Linearity and range study was done by preparing concentration in the range of 80 -120 % of test concentration and absorbance values were recorded at 281.0 nm and at 316.5 nm. The plot of linearity and range is shown in Fig. 2.

RESULTS AND DISCUSSION:

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of NBV and HCTZ in their combined dosage form. In this method drugs obey Beer's law in the concentration range of 0 to 35 µg/ml. The absorption additivity study was carried out to see whether the drugs in mixture showed additivity or not. It was observed that both the drugs showed additivity of absorbance at selected wavelengths indicating that both the drugs do not interact with each other in the solvent system used. A (1%, 1cm) values were also calculated for both the drugs. For NBV, A (1%, 1cm) was found to be 130.4 ±0.8944 at wavelength 281 nm and for HCTZ, it was 119 ±0.7071, 354.4 ±0.8944 at wavelength 316.5 nm and 281 nm respectively. The result of percentage estimation of drug is shown in Table No.1. The method was validated as per the ICH and USP guidelines. The results of recovery study were found to be within the prescribed limit of 98 - 102 %, proving the accuracy and showing that the method is free from interference from excipients. The results are shown in Table No. 2. For precision, replicate estimation of both NBV and HCTZ in the same batch of tablets were done by proposed method, which yielded quite concurrent results, indicating reliability of the method. The values of SD or RSD are within the prescribed limit of 2 %, showing high precision of the method, as shown in Table No. 1. In the specificity study, the sample was exposed to different stress conditions like acid, alkali, Peroxide and heat. The study showed degradation of NBV under peroxide, but the exact nature of degradation of drug could not be found out. For ruggedness the proposed method was repeated under different conditions like different time, on different day and by different analyst. The results shown in Table No. 4, prove that the method is reproducible. During the linearity study it was observed that absorbance values of NBV and HCTZ in the marketed formulation were linear in the range of 80 % to 120 % of the test concentration with R^2 close to one for this method of analysis.

From the study of validation parameters namely accuracy, precision (SD and RSD), ruggedness (interday, intraday and different analyst), specificity, linearity and range, it was observed that the method is specific, accurate, precise, reproducible and rugged. Hence both the methods can be employed for routine analysis of tablet dosage form.

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