ABSTRACT
A simple, rapid, accurate, precise and sensitive absorbance ratio spectrophotometric method was developed for the simultaneous estimation of Nebivolol HCl (NEB-H) and Hydrochlorothiazide (HCTZ) in bulk as well as in the pharmaceutical formulation. Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the \( \lambda \)-max of one of the two components. Nebivolol HCl and Hydrochlorothiazide have shown an isoabsorptive point at 287 nm in methanol. The second wavelength used is 271 nm, which is \( \lambda \)-max of Hydrochlorothiazide in methanol. Calibration curves were linear in range of 10-80 \( \mu \)g/mL \((r^2=0.999)\) and 2-16 \( \mu \)g/mL \((r^2=0.999)\) for Nebivolol HCl and Hydrochlorothiazide respectively. The method was validated statistically.

Keywords: Hydrochlorothiazide, Nebivolol Hcl, Absorbance ratio method.

INTRODUCTION
Chemically Nebivolol is \( \alpha, \alpha' \)-[iminobis (methylene)] bis [6-flouro-3,4,-dihydro-2H-1-benzopyran-2- methanol hydrochloride with competitive & selective B1-receptor antagonist\(^1\). Chemically Hydrochlorothiazide is 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide with diuretic activity\(^1\). Both of the drugs are used for the treatment of hypertension. A literature survey regarding quantitative analysis of these drugs revealed that attempts were made to develop analytical methods for NEB-H using RP-HPLC\(^2\)-\(^4\), HPTLC\(^5\) and Spectrophotometry\(^6\)-\(^7\). As far as HCTZ is concerned, some reports are available for its estimation in dosage form using spectrophotometry\(^8\)-\(^15\), RP-HPLC\(^10\)-\(^13\),\(^16\)-\(^34\), HPTLC\(^15\)-\(^35\)-\(^38\). This paper describes spectrophotometric method for the determination of NEB-H and HCTZ in mixtures without prior separation. Also the proposed method is shown to be useful in determination of both drugs in combined tablet formulation.

MATERIALS & METHODS
MATERIALS
Spectrophotometric analysis was carried out on a SHIMADZU 2400 series double beam spectrophotometer with a fixed slit width (1nm) attached to the computer with UV-Probe, version 2.21 software for obtaining the spectra. Pure drug samples of Nebivolol Hcl and Hydrochlorothiazide were kindly gifted by Torrent Pharmaceutical Ltd., Ahmedabad-Mehsana Highway, Gujarat-382721, Unichem Laboratories Ltd., Raigad. and Micro Laboratories Ltd., Bangalore. The gift samples were used as standard without further purification. Methanol (AR Grade, CDH® (P) LTD., Sarigam, Gujarat) was used as solvent in this work. The commercial pharmaceutical preparations were procured from the local market.

METHODS
Preparation of Standard Solutions
A 10 mg of standard NEB-H and HCTZ were weighed and transferred to 100 mL separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 \( \mu \)g/mL each of NEB-H and HCTZ.

METHODOLOGY
Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the \( \lambda \)-max
of one of the two components. From the overlay spectra of two drugs, it is evident that NEB-H and HCTZ show an isoabsorptive point at 287 nm. The second wavelength used was 271 nm, which is the λ-max of HCTZ. Working standard solutions having concentration range 10-80 μg/ml for NEB-H and 2-16 μg/ml for HCTZ were prepared in methanol and the absorbance at 271 nm and 287 nm were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

\[ CX = [(QM - QY) / (QX - QY)] \times A1/ax1 \] .... (1)

\[ CY = [(QM - QX) / (QY - QX)] \times A2/ay1 \] ... (2)

Where, A1 and A2 are absorbance of mixture at 271 nm and 287 nm; ax1 and ay1 are absorptivities of HCTZ and NEB-H at 271 nm; ax2 and ay2 are absorptivities of HCTZ and NEB-H respectively at 287 nm; QM = A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1.

**Analysis of Tablet Sample**

A total of 20 tablets were accurately weighed and powdered. Tablet powder equivalent 12.5 mg of HCTZ and 5 mg NEB-H was taken for the analysis and dissolved in 100 mL methanol and were further diluted with methanol to get the solution containing 12.5 μg/mL of HCTZ and 5 μg/mL of NEB-H. The absorbance of solution was measured at 271 nm and 287 nm. Absorbances at two wavelengths were substituted in absorbance ratio equation to calculate the amount of drugs present in tablet.

**Method Validation**

The method was validated for linear range, accuracy, precision, Limit of detection (LOD) and Limit of quantification (LOQ).

(a) **Linearity and range**

solutions of different concentrations were prepared and checked for the linearity.

(b) **Precision**

Precision of the method was measured in terms of the interday and intraday precision. The experiment was repeated for three times in a day using three different concentrations (3×3) for intraday and three different concentrations on three consecutive days for interday precision.

(c) **Accuracy**

Accuracy of the method was determined by performing recovery study from previously analyzed tablet powder by standard addition method at three different levels.

(d) **LOD and LOQ**

LOD and LOQ were found out by the following equations.

LOD = 3.3 Sa / b and LOQ = 10 Sa / b, where Sa = standard deviation of the intercept and b = slope of calibration curve.

**RESULTS AND DISCUSSION**

Methanol was a solvent of choice owing to high solubility of NEB-H and HCTZ and also there was no shift in the absorbance maxima of NEB-H and HCTZ in methanol. The overlain UV absorption spectrum of HCTZ and NEB-H showing isoabsorptive point (287 nm) in methanol is shown in [Figure 1]. The validation parameters were studied at all the wavelengths for the proposed method. The calibration curves for NEX– H and HCTZ at both the selected wavelengths were obtained by plotting the absorbance Vs the concentration. NEB–H and HCTZ were found to be linear in the range of 10-18 μg/mL and 2-16 μg/mL with \( r^2 = 0.999 \) and 0.999 at both the wavelengths respectively [Figure 2,3,4,5]. The regression analysis of the calibration curves is shown in [table 1]. The recoveries of NEB – H and HCTZ were found to be 100.67 - 102.25 and 98.67 -101.87 respectively, which were satisfactory [Table 2]. The validation parameters are summarized in [Table 1]. The proposed spectroscopic method was applied to combined dosage form (Tablet). The results obtained for NEB-H and HCTZ were comparable with the corresponding label claim percentage [Table 4].

**ACKNOWLEDGEMENTS**

The authors are grateful to Torrent Pharmaceuticals Ltd., Unichem Laboratories Ltd. and Micro Laboratories Ltd. for providing gift samples of reference drugs.

**CONCLUSION**

The proposed Absorbance spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of NEB-H and HCTZ in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of both drugs hence the method was also economic for estimation of NEB-H and HCTZ from synthetic mixture and may be successfully applied in laboratories for the determination of NEB-H and HCTZ in combined dosage form. High recoveries show that the method is free from the interference from excipients used in the commercial pharmaceutical preparations.
Table 1: Regression analysis and summary of validation parameters for HCTZ and NEB-H

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HCTZ</th>
<th>NEB-H</th>
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<tbody>
<tr>
<td>Wavelength</td>
<td>271</td>
<td>287</td>
</tr>
<tr>
<td>Linear range (μg/ml) 2-16</td>
<td>2-16</td>
<td>2-16</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Slope</td>
<td>0.065</td>
<td>0.0095</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0021</td>
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</tr>
<tr>
<td>Intraday Precision</td>
<td>0.782</td>
<td>0.888</td>
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<tr>
<td>Interday Precision</td>
<td>1.526</td>
<td>1.255</td>
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<tr>
<td>LOD</td>
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<td>0.650</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.432</td>
<td>1.969</td>
</tr>
<tr>
<td>% Recovery</td>
<td>98.67-</td>
<td>100.67-</td>
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<tr>
<td></td>
<td>101.87</td>
<td>102.25</td>
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</table>

Table 2: Result of recovery study

<table>
<thead>
<tr>
<th>Sample Concentration (μg/mL)</th>
<th>Amt. of Drug Added (μg/mL)</th>
<th>Amt. recovered (μg/mL)</th>
<th>% Recovery*</th>
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<tbody>
<tr>
<td>HCTZ</td>
<td>NEB-H</td>
<td>HCTZ</td>
<td>NEB-H</td>
</tr>
<tr>
<td>12.5</td>
<td>5.0</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>12.5</td>
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<td>6.00</td>
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* Mean of three determinations

Table 3: Assay result

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Label claim (mg)</th>
<th>% Assay* ± SD</th>
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<tr>
<td></td>
<td>HCTZ</td>
<td>NEB-H</td>
</tr>
<tr>
<td>Brand A</td>
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<td>5.00</td>
</tr>
</tbody>
</table>

* Average of three determinations

Fig. 1: Overlaid spectra of HCTZ (10 μg/mL) and NEB-H (10 μg/mL)
Fig. 2: Calibration Curve of HCTZ at 271.0 nm

Fig. 3: Calibration Curve of HCTZ at 287.0 nm

Fig. 4: Calibration Curve of NEB-H at 271.0 nm
Calibration curve of NEB at 287.0 nm

\[ y = 0.0095x - 0.0051 \]

\[ R^2 = 0.9995 \]

Fig. 5 Calibration Curve of NEB-H at 287.0 nm

REFERENCES


36. Shah NJ, Suhagia BN, Shah RR and Patel NM. Development and validation of a HPTLC method for the simultaneous


