DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE RATIO METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN MARKETED FORMULATIONS BY DIFFERENCE SPECTROPHOTOMETRY

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ABSTRACT

A simple Absorbance ratio method was developed and validated for the simultaneous estimation of telmisartan (TELM) and hydrochlorothiazide (HCTZ) in fixed dose combination tablets by difference spectrophotometry technique in the UV region. The absorbance ratio method uses the ratio of absorbance at two selected wavelengths; one is the isoabsorptive point of two components and the other is the λ-max of one of the two components. The difference in spectral characteristics for both the drugs was observed in 0.01N acetic acid and 0.01N NaOH using methanol as solvent. The difference absorbance was measured at 240nm and 308nm for HCTZ and TELM respectively. The method was validated according to ICH guidelines.

Linearity range was observed over the concentration range of 5-25µg/ml for hydrochlorothiazide and 10-50 µg/ml for telmisartan. The accuracy and precision of the method were determined and validated statistically. The method showed good repeatability with a %RSD less than 2. The limit of detection and limit of quantization was found to be 0.9µg/ml and 2.7µg/ml for HCTZ and 2.3µg/ml and 7.2µg/ml for TELM at 240nm and 308nm respectively. The validated method was successfully applied for the analysis of hydrochlorothiazide and telmisartan in fixed dose combination tablets.

KEYWORDS: UV region, Hydrochlorothiazide, Telmisartan, Absorbance ratio method, repeatability, ICH guidelines.
INTRODUCTION

Hypertension is a very common disorder, is an important risk factor for the future development of cardiovascular diseases. Antihypertensive drugs comprise several classes of compounds with the therapeutic intention of preventing, controlling, or treating hypertension. The classes of antihypertensive drugs differ both structurally and functionally.

Telmisartan (TELM), is an angiotensin receptor antagonist (ARB) used in the treatment of hypertension. Telmisartan is 4'-(1, 4'-dimethyl-2'-propyl [2, 6'-bi-1H benzimidazol]-1’-yl) methyl]-[1, 1’-biphenyl]-2-carboxylic acid as antihypertensive agent. TELM blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Generally, angiotensin receptor blockers (ARBs) such as telmisartan bind to the angiotensin type 1 (AT1) receptors with high affinity, causing the action of angiotensin on the vascular muscle, leading to a reduction in arterial blood pressure.

Hydrochlorothiazide (HCTZ) belongs to thiazide class of diuretics. Chemically it is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide1, 1 dioxide. Hydrochlorothiazide acts on the distal convoluted tubules and inhibits the sodium chloride co-transport system and reduces blood volume and vascular resistance.\(^\text{[1,2]}\)

![Chemical structure of Telmisartan and Hydrochlorothiazide](image)

**Fig. 1:** Chemical structure of (a) Telmisartan (b) Hydrochlorothiazide.
UV spectrophotometric methods are commonly used methods for quantitative analysis of drug substances and their formulations as they are simple, easy to develop, and give accurate and precise results. From the literature review, it was reported that there are few UV, HPLC, and HPTLC methods for simultaneous estimation of telmisartan and hydrochlorothiazide in bulk and tablet dosage forms but many methods were reported for the individual drugs.\textsuperscript{[3-25]}

Besides conventional methods, we can also develop derivative and difference spectroscopic methods for simultaneous estimation of two drugs in fixed dose combination formulations based on their absorption characteristics. No difference spectrophotometric methods were using the Q-absorbance ratio method reported so far. In this article, the development of a new Q-absorbance ratio method by difference spectrophotometry for the simultaneous estimation of telmisartan and hydrochlorothiazide in fixed dose combination is presented.

**MATERIALS AND METHODS**

All of the materials and reagents used in this experiment were of analytical grade quality. Hetero Labs, Hyderabad provided the gift samples of telmisartan and hydrochlorothiazide for carrying out this research work. Three brands of formulations (Telmikind-H, Telmiwock-H, and Venpress-H) were obtained from a local pharmacy store.

A Double beam UV-Visible spectrophotometer (SHIMADZU, Japan) 1800 series was used. Data collection and integration were accomplished using UV Probe, 2.43 version software. Sonicator (PCI Analytics, 6.5lit 200H) and electronic balance (Shimadzu, BL220H) were also used in the method development and validation work.

**Preparation of Standard and Sample solutions**

Standard stock solutions of 1000µg/ml of TELM and HTZ were prepared in methanol.

**Preparation of sample solution**

Twenty tablets (Telmakind-H, containing 12.5mg hydrochlorothiazide 40mg telmisartan) were weighed; average weight was determined and finely powdered. An accurately weighed quantity of powder equivalent to 12.5mg hydrochlorothiazide and 40mg telmisartan was transferred in a 10ml volumetric flask and dissolved in methanol and sonicated for 10min. After sonication, the volume was made up to the mark with methanol. The solution was then filtered through Whatman filter paper. The sample stock solution was further diluted to get the concentration of 12.5µg/ml HCTZ and 40µg/ml TELM.
Development and optimization of Q-absorbance ratio method\textsuperscript{26-30}

The difference absorbance (Δa) or amplitude between an adjacent maximum or minimum between two equimolar solutions of the analyte in different chemical forms exhibiting different spectral characteristics is the basic element of a difference spectrophotometric assay. pH adjustment is employed in this technique development since it is the simplest way to change the spectral characteristics of the analyte. It is accomplished by utilizing acetic acid and sodium hydroxide for the acidic and alkaline pH ranges, respectively.

Selection of Solvent and Strength of Acid and Alkali

Trails were performed with different strengths of acids and bases for selecting a suitable strength and type of acid and alkali desired for bridging differences in spectral characteristics of both drugs using methanol as solvent. Hydrochloric acid, sulphuric acid, acetic acid, and nitric acid were used to maintain acidic pH and Sodium hydroxide for alkaline pH. The difference absorption spectra of telmisartan and hydrochlorothiazide of optimized trial were shown in Fig.2

Determination of absorption maximum

50µg/ml standard solutions of telmisartan and hydrochlorothiazide were taken from the respective standard stock solutions and the differential absorbance of solutions were scanned in the range 200-400nm by taking the drug in 0.01N NaOH in the sample compartment and drug in 0.01N acetic acid as a reference in UV region. The difference absorption spectra and the overlay spectrum of both the drugs are shown in Fig.2

Selection of analytical concentration ranges

From the standard stock solution of hydrochlorothiazide appropriate volumes, 0.05, 0.1, 0.15, 0.2, and 0.25 were transferred from the stock solution to obtain concentrations of 5, 10, 15, 20, and 25µg/ml into two sets of 10ml volumetric flasks separately and the volume was made up to the mark with 0.01N acetic acid in one set and 0.01N NaOH in another set. The difference absorbance of all standard solutions was measured at 240 nm.

For telmisartan, appropriate dilutions were made by transferring 0.1, 0.2, 0.3, 0.4, and 0.5 from the stock solution to obtain the concentrations of 10, 20, 30, 40, and 50µg/ml into two sets of 10ml volumetric flasks separately and the volume was made up to the mark with 0.01N acetic acid in one set and 0.01N NaOH in another set. The difference absorbance of the prepared series of standard solutions was measured at 308 nm.
Q- Absorbance ratio method

The Q-absorbance ratio method uses the ratio of absorbance at two selected wavelengths; one is an isoabsorptive point and the other is the $\lambda_{max}$ of one of the two drugs. From the overlay spectrum of the two drugs, it is evident that HCTZ and TELM show an iso-absorptive point at 258 nm. The second wavelength used is 240 nm, which is the $\lambda_{max}$ of HCTZ. Standard solutions of TELM having concentration 10, 20, 30, 40, and 50µg/ml and 5, 10, 15, 20, and 25µg/ml of HCTZ were prepared in 0.01 N Acetic acid and 0.01N sodium hydroxide by appropriate dilutions from their respective standard stock solutions and their corresponding difference absorbance values were measured at 258 nm and 240 nm and absorptivity coefficients were calculated using Beer-Lambert law. The concentration of TELM and HCTZ were calculated by using the following equations:

$CX = \frac{[QYM - QY]}{[QXM - QY]} \times A1/ax1 \ldots$ Equation (3)
$CY = \frac{[QYM - QX]}{[QYM - QX]} \times A1/ay1 \ldots$ Equation (4)

Where,
- $\Delta A1$ and $\Delta A2$ are difference absorbance values of the mixture at 240 nm and 258 nm;
- $ax1$ and $ay1$ are absorptivities of X and Y at 240 nm;
- $ax2$ and $ay2$ are absorptivities of X and Y respectively at 258 nm;
- $QM = \Delta A2 / \Delta A1$, $QX = ax2 / ax1$ and $QY = ay2 / ay1$.

Method validation

The developed method was validated according to the ICH guidelines Q2 (R1): validation of analytical procedures: Text and methodology for the following validation parameters for the following parameters linearity, LOD, and LOQ, precision and accuracy for drug substance and drug product.\[30\]

Linearity and Range

For constructing the calibration curve, working standard solutions were prepared in the concentration range of 5-25µg/ml for hydrochlorothiazide and 10-50µg/ml for telmisartan and the difference absorbance values were measured at 240nm and 308nm for both the drugs, HCTZ and TELM. The calibration curves and data of both drugs are shown in Table 1 and Fig. 3-6
Limit of Detection and Limit of quantification
The limit of detection and limit of quantitation values were calculated and reported using standard deviation method using the following equations, LOD = 3.3σ / S and LOQ = 10σ / S, Where σ = the standard deviation of the y-intercept of the calibration curve and S = the slope of the calibration curve.

Precision
The precision of the methods was reported in terms of repeatability and intermediate precision.

Repeatability was performed by analyzing the six replicates of a single concentration of 10µg/ml of HCTZ and 20µg/ml of TELM. Difference absorbance of samples was measured at 240nm and 308nm and the % Relative Standard Deviation (RSD) was calculated. The intra-day and inter-day precision of the analytical method was determined for both the drugs at different concentration levels (10µg/ml, 15µg/ml, 20µg/ml for HCTZ and 20µg/ml, 30µg/ml, 40µg/ml for TELM) by analyzing the three replicates of each on the same day at different time intervals and different days at 240nm and 308nm. The %RSD was calculated and the results are shown in Table 1.

Accuracy
The accuracy of the method was determined for both the drug substance and the drug product. For drug substance was determined on samples of drug solutions at varying concentration levels in the range of 80-120% by analyzing the three replicates of each sample as a batch in a single assay. Recovery studies were carried out for reporting the accuracy of the drug product. It was determined on samples at different concentration levels in the range of 80-120% by adding a known amount of TELM (30µg/ml) and HCTZ (15µg/ml) standard drugs. The % recovery was calculated and reported in Table 1.

Analysis of fixed dose combination tablets
Three brands of tablets were analyzed using the developed Q-Absorbance method. (Telmakind – H manufactured by mankind pharma ltd, Telmiwock-H by Wockhardt. ltd.and Venpress-H by Leeford. Health care ltd.)

Twenty fixed dose combination tablets containing 12.5 mg of hydrochlorothiazide and 40mg of telmisartan were taken and accurately weighed and triturated to a fine powder. The powder
sample equivalent to 12.5mg of hydrochlorothiazide and 40mg of telmisartan was transferred to a 10ml volumetric flasks containing methanol and sonicated for 10min. Then the solution was filtered through Whatman filter paper and the volume was made up to the mark.

From the filtrate of the above sample stock solution, 0.1ml was transferred into two 10ml volumetric flasks separately and the volume was made up to the mark with 0.01N acetic acid in one tube and 0.01N NaOH in the other tube to get the final concentration of 12.5 µg/ml of hydrochlorothiazide and 40 µg/ml of telmisartan respectively. The samples were analyzed in three replicates and difference absorbance values were measured at 240nm and 258nm. The concentration of the drugs in the tablet sample mixture was calculated using the Q-Absorbance ratio method and the amount of drugs present in the formulation was calculated using the following formula.

\[
\text{% Purity} = \frac{\text{Concentration (µg/ml)} \times \text{Dilution factor} \times \text{Average wt of the capsule}}{\text{Weight of sample powder taken (mg)} \times \text{Label claim}} \times 100
\]

RESULTS AND DISCUSSION

Q-Absorbance ratio method was developed and validated for the estimation of hydrochlorothiazide and telmisartan in fixed dose combination tablets by difference spectrophotometry. In conventional UV method for simultaneous estimation of telmisartan and hydrochlorothiazide does not obey the criteria of the simultaneous equation method so a difference spectrophotometric technique was selected for developing a new Q-Absorbance ratio method. The drug with maximum absorbance at its \(\lambda_{\text{max}}\) should have the least absorbance at the \(\lambda_{\text{max}}\) of another drug. In conventional method hydrochlorothiazide has least absorbance for telmisartan at its \(\lambda_{\text{max}}\).

All the standard stock solutions of two drugs were prepared in methanol as solvent. In the trials performed for selecting the strength and type acid and alkali, the difference absorption spectrum of telmisartan and hydrochlorothiazide taken in 0.01 N Acetic acid and 0.01 N Sodium hydroxide was good and met the requirements for multicomponent analysis by Q-absorbance ratio method.
Fig. 2: Difference absorption spectrum of TELM and HCTZ in 0.01N acetic acid and 0.01N sodium hydroxide (methanol as solvent).

The absorption maximum of hydrochlorothiazide and telmisartan were found to be 240nm and 308nm. All the absorbance measurements were carried out at these wavelengths. In the concentration range 5-25µg/ml of hydrochlorothiazide and 10-50µg/ml of telmisartan there was a linear relationship between concentration and difference absorbance values. This range was selected for the construction of the calibration curve at 240nm and 308nm.

The method was validated according to ICH guidelines and all the parameters met acceptance criteria. The linearity of the method was proven statistically by linear regression analysis. A calibration curve was constructed by plotting the difference absorbance vs concentration of HCTZ and TELM and the $r^2$ value was found to be 0.999 for hydrochlorothiazide and 0.998 for telmisartan (Table 2 & Fig 4, 6).

Table 2: Standard calibration curve data of Hydrochlorothiazide and Telmisartan.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Difference absorbance* (ΔA) at 240nm</th>
<th>Difference absorbance* (ΔA) at 308nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.114</td>
<td>0.067</td>
</tr>
<tr>
<td>10</td>
<td>0.236</td>
<td>0.111</td>
</tr>
<tr>
<td>15</td>
<td>0.345</td>
<td>0.165</td>
</tr>
<tr>
<td>20</td>
<td>0.453</td>
<td>0.205</td>
</tr>
<tr>
<td>25</td>
<td>0.566</td>
<td>0.253</td>
</tr>
</tbody>
</table>

* Average of three determinations
Fig. 3: Overlay of the difference absorption spectrum of hydrochlorothiazide (5-25µg/ml).

Fig. 4: Calibration curve of hydrochlorothiazide at 240nm.

Fig. 5: Overlay of the difference absorption spectrum of telmisartan (10-50µg/ml).
The accuracy of the method was determined by calculating % recovery at three different levels (80%, 100%, and 120%) in pre-analyzed samples using the standard addition method. The results of recovery studies are reported in Table 3. The % recovery values for HCTZ and TELM were within 98 - 102% limits, assuring that the developed method can estimate the drugs successfully in presence of excipients.

Variations of results within the same day (Intraday) and between days (Interday) were analyzed. The Intraday and Interday precision was determined by analyzing three different concentrations of FEN (3, 6 and 9 µg/mL) and NIC (10, 20, and 30 µg/mL), obtained by dilution from stock solutions three times in a day (Intraday) and for three consecutive days (Interday). The % RSD value of < 2% suggests that the developed methods are precise (Table 3).

Table 3: Summary of validation parameter of Q-absorbance ratio method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hydrochlorothiazide</th>
<th>Telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>5-25 µg/mL</td>
<td>10-50 µg/mL</td>
</tr>
<tr>
<td>Regression equation and Correlation coefficient</td>
<td>y=0.022x+0.006 0.999</td>
<td>y=0.004x+0.020 0.998</td>
</tr>
<tr>
<td>Repeatability (%RSD)</td>
<td>1.87</td>
<td>1.55</td>
</tr>
<tr>
<td>Intra-day Precision (%RSD) n=3</td>
<td>0.6 – 0.9</td>
<td>1.8 – 1.5</td>
</tr>
<tr>
<td>Inter-day Precision (%RSD) n=3</td>
<td>0.6 – 1.0</td>
<td>1.2 – 1.8</td>
</tr>
<tr>
<td>LOD</td>
<td>0.9 µg/mL</td>
<td>2.3 µg/mL</td>
</tr>
<tr>
<td>LOQ</td>
<td>2.79 µg/mL</td>
<td>7.24 µg/mL</td>
</tr>
<tr>
<td>% Recovery (100% level of sample concentration)</td>
<td>99</td>
<td>109</td>
</tr>
</tbody>
</table>

The developed and validated Q-Absorbance ratio method was applied for the analysis of three brands of fixed dose combination tablets. (TELMIKIND-H, TELMIWOCK-H and VENPRESS-H) The QM, QX, and QY values were calculated from the difference absorbance
values of the sample mixture solution at 258 nm and 240 nm. The concentration of HCTZ and TELM were determined using equations (3) and (4) mentioned under methods. The results of the assay of formulations are tabulated and shown in Table 4. The results were found to be within the acceptable limits of 90-110%

Table 4: Assay values of HCTZ and TELM in fixed dose combination tablets.

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Drug</th>
<th></th>
<th></th>
<th>Concentration µg/mL</th>
<th>Calculated amount</th>
<th>Assay %</th>
<th>Concentration µg/mL</th>
<th>Calculated amount</th>
<th>Assay %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TELMIKIND-H</td>
<td>Temisartan</td>
<td>38</td>
<td>38.6mg</td>
<td>102%</td>
<td></td>
<td></td>
<td>11</td>
<td>11mg</td>
<td>98%</td>
</tr>
<tr>
<td>TELMIWOCK-H</td>
<td>Hydrochlorothiazide</td>
<td>37</td>
<td>37.9mg</td>
<td>94.8%</td>
<td></td>
<td></td>
<td>12</td>
<td>11.8mg</td>
<td>98.3%</td>
</tr>
<tr>
<td>VENPRESS-H</td>
<td></td>
<td>36</td>
<td>37.1mg</td>
<td>92.8%</td>
<td></td>
<td></td>
<td>10</td>
<td>10.9mg</td>
<td>90.8%</td>
</tr>
</tbody>
</table>

CONCLUSION
The developed and validated UV difference spectrophotometric method for the simultaneous estimation of telmisartan and hydrochlorothiazide in the tablet dosage form is simple, accurate and precise. The developed method can be employed for routine analysis of telmisartan and hydrochlorothiazide in marketed formulations in quality control assay or QC laboratories. This method compensates the spectral interferences of other absorbing components.

REFERENCES


