SIMULTANEOUS DETERMINATION OF CARVEDILOL AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM BY FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRY

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ABSTRACT

Objective: To develop an accurate, precise and linear UV spectrophotometry method for simultaneous determination of Carvedilol and Hydrochlorothiazide in Co-Dilatrol® tablet and validate as per ICH guidelines.

Methods: Derivative spectrophotometric methods: The amplitudes in simultaneous determination of the first order derivative of the resultant spectra at 301 nm and 278 nm were selected to find out Carvedilol and Hydrochlorothiazide respectively in its tablet dosage form by using methanol as a solvent.

Results: The linearity was found to be 5-25 μg/ml for Carvedilol and Hydrochlorothiazide. The mean % recoveries were found to be 101.13% and 99.02% for simultaneous determination of first order derivatives method of Carvedilol and Hydrochlorothiazide. For Incraday precision, Inter day precision % RSD was found to be 0.0082, 0.6304 and 0.0096, 0.6354 for Carvedilol and 0.0085, 0.6257 and 0.0083, 0.6398 for Hydrochlorothiazide respectively. Limit of Detection and Quantitation was found to be 0.7852μg/ml and 2.2539μg/ml for Carvedilol and 0.7859μg/ml and 2.3571μg/ml for Hydrochlorothiazide. Assay results of market formulation were found to be 100.89% for simultaneous determination of the first order derivatives method of Carvedilol and Hydrochlorothiazide. The proposed method has been validated as per ICH guidelines and successfully applied to the simultaneous determination of the first order derivatives method of Carvedilol and Hydrochlorothiazide in its tablet dosage form.

Conclusion: A simple, accurate, precise, linear and rapid UV spectrophotometry method was developed for simultaneous determination of Carvedilol and Hydrochlorothiazide in Co-Dilatrol® tablet and validated as per ICH guidelines. Hence it can be used for the routine analysis of Carvedilol and Hydrochlorothiazide in tablets in various pharmaceutical industries.

Keywords: Carvedilol, Hydrochlorothiazide, UV visible spectrophotometry, Method Validation, First order derivative method.

INTRODUCTION

Carvedilol is a combined alpha-and nonselective beta-blocker. Carvedilol chemically, 2-Propanol, 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxy phenoxy) ethyl amino]-2-propanol. It is a non-selective beta blocker indicated in the treatment of mild to moderate congestive heart failure [CHF]. [1, 2] It blocks beta-1 and beta-2 adrenergic receptors as well as the alpha-1 adrenergic receptors. Carvedilol is official drug in British Pharmacopeia. It has been prescribed as an antihypertensive agent and an angina agent. It is first beta blocker labeled in United States especially for the treatment of heart failure of ischemic or cardio myopathic origin with significant antioxidant activity. Relative to other beta blocker, carvedilol (CAR) has minimal inverse agonist indicating a reduced negative chronotropic and inotropic effect, which decrease its potential to worsen symptoms of heart failure. At high dosage, it exerts calcium channel blocking activity. The benefits of using CAR in patient with CHF in both single center and multi center trial have been reported in the literature. It prevents vitamin E, glutathione and SH protein depletion induced by oxidation stress, the main defense mechanism against tissue injury caused by free radical. Literature survey revealed the estimation methods of Carvedilol or with other drugs by UV spectrophotometry [7-9] HPLC [10, 11] calorimetric method, flow injection analysis and HPTLC.

Hydrochlorothiazide chemically known as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzoathiadizine-7-sulphonamide-1,1-dioxide is a moderately potent thiazide diuretic [9, 10]. It exerts its effect by reducing the reabsorption of electrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions, and consequently of water. Hydrochlorothiazide is used in the treatment of hypertension either alone or with other antihypertensives [6]. Literature survey revealed the estimation methods of Hydrochlorothiazide or with other drugs by UV spectrophotometry [7-9] HPLC [10, 11] calorimetric method, flow injection analysis and HPTLC.

Application of derivative technique of spectrophotometry offers a powerful tool for quantitative analysis of multi-component mixtures. When derivatives, the maxima and minima of the original function take zero values, and the inflections are converted into maxima or minima, respectively. The derivative curves are more structured than the original spectra, thus enabling very tiny differences between the original spectra to be identified. Derivative spectrophotometry provides selectivity and offers a solution in resolving the overlapping spectra in multi-component analysis without previous chemical separation.

In the last decades, this technique has rapidly gained an application in the field of pharmaceutical analysis to overcome the problem of interference, due to substances other than analytes, commonly present in pharmaceutical formulations or for the combination of two or more drug substances.

Lack of any published method for simultaneous spectrophotometric determination of Carvedilol and Hydrochlorothiazide, therefore, provoked us to investigate the application of derivative spectrophotometry for simultaneous determination of these compounds in pharmaceutical dosage forms using zero-crossing method[12, 13].

MATERIALS AND METHODS

Apparatus and instrumentation
A Shimadzu 1800 U/VIS double beam spectrophotometer with 1 cm matched quartz cells were used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an
Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

Materials
Reference standard of Carvedilol and Hydrochlorothiazide API was supplied as gift sample by Lupin Laboratory park Aurangabad, Maharashtra, India. The commercial formulation Co-Dilatrol® as purchased from the local market Solapur, Maharashtra, India.

Method development
Preparation of standard stock solution
Stock solution was prepared by diluting 10 mg of each drug in sufficient quantity of methanol in separate volumetric flask and volume was made up to 100 ml to get the concentrations of 100 μg/ml for each drug. Dilutions from stock solution were prepared in the range of 5 - 25 μg/ml for Carvedilol and 5 - 25 μg/ml for Hydrochlorothiazide. Methanol was used as a blank solution.

Spectrophotometric measurements
Zero-order spectra of standard solutions of Carvedilol (20 μg/ml) and Hydrochlorothiazide (20 μg/ml) versus their solvent blank were recorded in the range of 200-400 nm (fig. 3). The first order derivative spectra of these solutions were obtained in the same range of wavelength against their blanks (fig. 4).

The values of first order derivative amplitudes for Carvedilol in the presence of Hydrochlorothiazide and vice versa were measured at 301 nm (zero-crossing of Carvedilol) and 278 nm (zero-crossing of Hydrochlorothiazide), respectively. The calibration curves for derivative spectrophotometry were constructed by plotting the drug concentration versus the absorbance values of the first order derivative spectrum, at 301 nm for Carvedilol and at 278 nm for Hydrochlorothiazide [14, 15].

Analysis of commercial tablet formulation
Contents of 20 tablets were weighed and their average weight was determined and powdered. Accurately weighed powder equivalent to fill weight of one tablet was transferred to 100 ml calibrated flask containing 50 ml of methanol and sonicated for 30 minutes. The volume was then made up to the mark with methanol. The resulting solution was then filtered through whatmann filter paper (#41). From this solution, 1 ml was transferred to another 10 ml calibrated flask and diluted up to 10 ml which gives 200 μg/ml concentration of the solution. Then 1 ml of this solution was further diluted to 10 ml to get approximate concentration 20 μg/ml of Carvedilol and 20 μg/ml of Hydrochlorothiazide.

Table 1: Assay of tablet dosage form

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample solution concentration (μg/ml)</th>
<th>Amount found (%)</th>
<th>Mean % found</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>99.47</td>
<td>100.89</td>
<td>0.5421</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>102.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>100.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *n=3, % RSD = % Relative standard deviation.

RESULTS AND DISCUSSION
The proposed simultaneous determination method provides simple, specific, precise, accurate and reproducible quantitative analysis for determination of Carvedilol and Hydrochlorothiazide in Co-Dilatrol® tablets. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of Carvedilol and Hydrochlorothiazide in bulk and tablet formulations.

Linearity and range

Linearity
Calibration curves were constructed using six replicates of Carvedilol solutions between 5-25 μg/ml in the presence of 5-25 μg/ml of Hydrochlorothiazide. The same procedure was used for solutions containing Hydrochlorothiazide 5-25 μg/ml in the presence of 5-25 μg/ml of Carvedilol. The calibration curves were constructed (fig. 5 and fig. 6) and statistical analysis were
performed. The regression equations of calibration curves were 
y=0.017x+0.001 \((r^2=0.9997)\) at 301 nm for Carvedilol and 
y=0.016x+0.005 \((r^2=0.9994)\) at 278 nm for Hydrochlorothiazide for 
first order derivative spectrophotometry methods. The range was 
found to be 5-25 \(\mu g/ml\) for both drugs for second order 
spectrophotometry methods.

Fig. 5: Calibration curve for carvedilol at 301 nm

![Calibration curve for carvedilol at 301 nm](image)

Fig. 6: Calibration curve for hydrochlorothiazide at 278 nm

![Calibration curve for hydrochlorothiazide at 278 nm](image)

Table 2: Statistical data for the calibration graphs for 
determination of carvedilol and hydrochlorothiazide by 
proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Carvedilol</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range ((\mu g/ml)^*)</td>
<td>5-25</td>
<td>5-25</td>
</tr>
<tr>
<td>(r^2\pm SD^*)</td>
<td>0.9997</td>
<td>0.9995</td>
</tr>
</tbody>
</table>

Note:*n=3, SD: Standard deviation

Table 3: Results of drug content and analytical recovery of carvedilol and hydrochlorothiazide

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Carvedilol</th>
<th>% RSD</th>
<th>Hydrochlorothiazide</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled claim</td>
<td>25 mg</td>
<td>-</td>
<td>12.5 mg</td>
<td>-</td>
</tr>
<tr>
<td>% Drug content(\pm SD)</td>
<td>101.13±0.1302</td>
<td>0.36</td>
<td>99.02±0.1982</td>
<td>0.39</td>
</tr>
<tr>
<td>Analytical recovery at 80 %(\pm SD)</td>
<td>99.58±0.1285</td>
<td>0.14</td>
<td>100.34±0.5966</td>
<td>0.17</td>
</tr>
<tr>
<td>Analytical recovery at 100 %(\pm SD)</td>
<td>100.02±0.1355</td>
<td>0.24</td>
<td>100.01±0.4258</td>
<td>0.29</td>
</tr>
<tr>
<td>Analytical recovery at 120%(\pm SD)</td>
<td>101.11±0.5279</td>
<td>0.19</td>
<td>99.07±0.5811</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 4: Results of intra and inter day precision

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Carvedilol</th>
<th>% RSD</th>
<th>Hydrochlorothiazide</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday precision</td>
<td>SD*</td>
<td>0.0082</td>
<td>0.6304</td>
<td>0.0096</td>
</tr>
<tr>
<td>Inter day precision</td>
<td>% RSD*</td>
<td>0.6257</td>
<td>0.0083</td>
<td>0.6398</td>
</tr>
</tbody>
</table>

Note:*n=3, SD: standard deviation

Sensitivity

The limit of detection \((LOD)\) and limit of quantification \((LOQ)\) were 
calculated by using the equations \(LOD = 3\sigma/S\) and \(LOQ = 10\sigma/S\), 
where \(\sigma\) is the standard deviation of intercept, \(S\) is the slope.

The \(LOD\) and \(LOQ\) were found to be 0.7852\(\mu g/ml\) and 2.3539\(\mu g/ml\) 
respectively of Carvedilol for first order derivative and 0.7859\(\mu g/ml\) 
and 2.3571\(\mu g/ml\) respectively of Hydrochlorothiazide for the first 
order derivative.

Analysis of the marketed formulation

There was no interference from the excipients commonly present in 
the tablets. The drug content was found to be 100.89% first order 
spectrophotometric methods. It may therefore be inferred that 
degradation of Carvedilol and Hydrochlorothiazide had not occurred 
in the marketed formulations that were analysed by this method. 
The low % RSD value indicated the suitability of this method for 
routine analysis of Carvedilol and Hydrochlorothiazide in 
pharmaceutical dosage form.
Table 5: Summary of validation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Carvedilol</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda) range</td>
<td>200–400 nm</td>
<td>200–400 nm</td>
</tr>
<tr>
<td>Regression Equation (y=mx+c)</td>
<td>(y=0.017x+0.01)</td>
<td>(y=0.016x+0.005)</td>
</tr>
<tr>
<td>Measured wavelength</td>
<td>301 nm</td>
<td>278 nm</td>
</tr>
<tr>
<td>Linearity range</td>
<td>5–25 (\mu g/ml)</td>
<td>5–25 (\mu g/ml)</td>
</tr>
<tr>
<td>Slope</td>
<td>0.017</td>
<td>0.016</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Correlation coefficient (R^2)</td>
<td>0.9997</td>
<td>0.9994</td>
</tr>
<tr>
<td>Limit of Detection (LOD) (\mu g/ml)</td>
<td>0.7852</td>
<td>0.7859</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ) (\mu g/ml)</td>
<td>2.2539</td>
<td>2.3571</td>
</tr>
<tr>
<td>Accuracy (Mean % Recovery)</td>
<td>101.13</td>
<td>99.02</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td>0.36</td>
<td>0.39</td>
</tr>
</tbody>
</table>

CONCLUSION

From the results of this study it can be concluded that the proposed first order derivative spectrophotometric method can be used for simultaneous determination of carvedilol and hydrochlorothiazide. This method is simple, rapid, practical, reliable and inexpensive and can be used for routine analysis of simultaneous determination of these compounds without any prior separation in quality control laboratories.

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CONFLICT OF INTERESTS

Declared None

REFERENCES