DEVELOPMENT AND VALIDATION OF AREA UNDER CURVE AND FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF BENFOTIAMINE IN BULK AND SOLID DOSAGE FORM

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ABSTRACT
Simple, sensitive, efficient and economical spectrophotometric methods have been developed for the estimation of benfotiamine in bulk as well as in tablet dosage form. The first order derivative spectrophotometry method and area under curve method obeys Beer’s law concentration in the range of 4-20µg/mL. The overall percentage purity for the sample by both the methods was found to be between 99-100% and the percentage recovery was found to be 99.101% which indicates the accuracy of the proposed method. The methods have been validated as per ICH guidelines and could be applied in future analysis of Benfotiamine formulations. Both the methods showed good reproducibility and recovery. The results of the methods were validated statistically and found to be satisfactory.

KEYWORDS: Simple, economical first order derivative method, area under curve method.

INTRODUCTION
Benfotiamine (S-benzoylthiamine O-monophosphate) is a lipid soluble form of thiamine which alleviates and may even reverse diabetic neuropathy, kidney disease, cardiac impairment, endothelial dysfunction, peripheral vascular disease, and diabetic retinopathy. With its proven ability to confer broad spectrum support for the blood vessels, nerves, kidneys, eyes, and heart, benfotiamine should be considered a first-line defence against the debilitating consequences of diabetes to control blood glucose level. Two simple, precise, accurate UV-spectrophotometric methods namely first order derivative method and area under the curve method have been carried out using 0.1 M HCl as solvent. The drug obeyed Beer’s law in the range between 4-20µg/mL.

First order derivative spectrophotometry
Derivative spectrophotometry involves the transformation of absorption spectra into first, second or higher order derivative spectroscopy. In derivative spectroscopy the first or higher derivative of absorbance or transmittance with respect to wavelength is recorded versus the wavelength.

The first derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength i.e., a plot of the slope of the fundamental spectrum against wavelength or a plot of dA/dλ vs λ. For quantification, peak heights (in mm) are usually measured. The amplitude is the distance from the maximum to the minimum at the λ max (which is the zero crossing in the spectrum) in first order. In this method, use is made of the fact the amplitude of positive peak adjacent to the cross over point is directly proportional to the concentration.

A calibration graph was obtained by plotting concentration versus amplitude. The amplitude obtained was then interpolated on the calibration graph and the concentration of benfotiamine in the sample was then determined.

ADVANTAGES
- In derivative spectrophotometry the ability to detect and to measure minor spectral features is considerably enhanced.
- It can be used in quantitative analysis to measure the concentration of an analyte whose peak is obscured by a large overlapping peak.
- Accurate determination of λ max is possible.
- Increased resolution permits the selective determination of certain absorbing substances.
- Concentration measurement of an analyte in the presence of an interference can be made more easily and accurately.

Area under curve method
The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of area between the two selected wavelengths 229 and...
261 nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. The spectrum obtained from zero order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (4-20 μg/mL) versus area.

The inbuilt software calculates the area bound by the curve and the horizontal axis. In this study area was integrated between wavelength ranges from 229 to 261 nm.

Preparation of standard stock solution
Aliquot quantity of standard benfotiamine was accurately weighed and transferred into 100ml standard flask.

Preparation of sample stock solution
Twenty tablets were weighed and powdered. Aliquot quantity of weighed tablet powder equivalent to 50mg of benfotiamine was accurately weighed and transferred into 100ml standard flask and shaken well with 0.1M HCl to dissolve the active ingredient and made up to the volume. The solution was then filtered, first few ml of filtrate was discarded and the filtrate was used for further dilution matching standard concentration.

RESULTS AND DISCUSSION

a) First order derivative

<table>
<thead>
<tr>
<th>Concentration (mg)</th>
<th>Amplitude (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
</tr>
</tbody>
</table>

b) AUC

<table>
<thead>
<tr>
<th>Concentration (mg)</th>
<th>area</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.668</td>
</tr>
<tr>
<td>8</td>
<td>1.371</td>
</tr>
<tr>
<td>12</td>
<td>2.066</td>
</tr>
<tr>
<td>16</td>
<td>2.672</td>
</tr>
<tr>
<td>20</td>
<td>3.349</td>
</tr>
</tbody>
</table>

UV Spectrum

1) First order derivative method

2) Area under the curve method
Linearity graph

1) First order derivative method
2) Area under the curve method

Table I: Assay and Recovery studies of Benfotiamine

<table>
<thead>
<tr>
<th>S.no</th>
<th>Method</th>
<th>Label claim</th>
<th>Amount of drug in each tablet(mg)</th>
<th>Percentage purity of sample(%)</th>
<th>Amount of drug added(%)</th>
<th>Amount of drug recovered(%)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>First order derivative spectrophotometry method</td>
<td>100mg</td>
<td>99.47</td>
<td>99.47</td>
<td>50</td>
<td>104.42</td>
<td>101.24</td>
</tr>
<tr>
<td>2.</td>
<td>Area under the curve method</td>
<td>100mg</td>
<td>99.85</td>
<td>99.85</td>
<td>50</td>
<td>49.92</td>
<td>100.10</td>
</tr>
</tbody>
</table>

* indicates each value is a mean of three readings.

Table II: Optical parameters proposed for the method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>First order derivative spectrophotometry method</th>
<th>Area under the curve method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength range</td>
<td>200-400nm</td>
<td>200-400nm</td>
</tr>
<tr>
<td>Beer’s law limits</td>
<td>4-20µg</td>
<td>4-20µg</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y=0.571x+0.285</td>
<td>Y=0.167x+0.012</td>
</tr>
<tr>
<td>Slope(m)</td>
<td>0.571</td>
<td>0.1675</td>
</tr>
<tr>
<td>Intercept(c)</td>
<td>0.285</td>
<td>0.0012</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.991</td>
<td>0.9979</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.3779</td>
<td>0.02827</td>
</tr>
</tbody>
</table>

The standard solutions of benfotiamine were scanned separately in the UV range and First-order spectra and spectrum with area under the curve were recorded. The first order derivative absorption at 235 nm (zero cross point) was used. Peak area between 229 and 261 nm was recorded. For area under curve method, Beer’s concentration was found to be between 4.20 µg/ml. The correlation coefficient for both the methods were found to be between 0.991 and 0.995 respectively. Relative standard deviation was found to be less than 2 %, which indicates that proposed method is repeatable. and also reveal that the proposed method is precise. The recovery experiments were performed by the standard addition method. The results of recovery studies indicate that the proposed method is highly accurate. Table II. The validation parameters are summarized in Table III. The proposed validated spectrophotometric methods could be successfully applied to the analysis of solid dosage form.

CONCLUSION
First order UV spectrophotometric derivative technique and area under the curve methods are quite simple, accurate, precise, reproducible and sensitive. Both the method have been developed for quantification of benfotiamine in tablet formulation which is free from interference due to additives and excipients. It can also be used in routine quality control of the raw materials as well as formulations containing benfotiamine.

ACKNOWLEDGEMENT
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