

DEVELOPMENT AND VALIDATION OF NEW UV SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF BENFOTIAMINE IN BULK AND SOLID DOSAGE FORM

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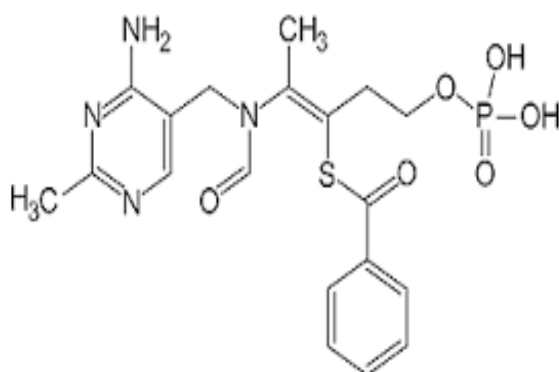
ABSTRACT

Benfotiamine is the precursor of thiamine which is a water soluble vitamin. Benfotiamine is fat soluble, a characteristic that allows it to enter cells more readily than thiamine and thus helps to prevent diabetes-related dysfunction within the cells. This enhanced bioavailability makes benfotiamine particularly effective in treating hyperglycemia related damage to tissues and organs. Two simple, precise, accurate UVspectrophotometric methods namely dual wavelength spectrophotometric method and difference UV spectrophotometric method have been carried out using 0.1 N HCl as solvent. The drug obeyed Beer's law in the range between 4-20µg/mL. The overall 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.

KEYWORDS: Benfotiamine, precursor, UV spectroscopy method, Beer's law, recovery.**INTRODUCTION**

Benfotiamine (synonym S-benzoylthiamine-O-monophosphate) is a so called "allithiamine", a member of the class of lipophilic thiamine derivatives first identified in heated garlic in 1950 (Fujiwara *et al.*, 1954). It was later confirmed that similar compounds could be formed using other allium vegetables from compounds similar to allicin, and a study in rabbits appeared to show that allithiamines are formed *in situ* in the intestine in the presence of garlic and thiamine (Fujiwara, 1976). Reaction with allicin and other sulphur compounds in allium vegetables open thiamine's thiazole

ring, leading to a lipophilic molecule which readily diffuses across cell membranes.^[1,2] It contains an open thiazole ring that raises thiamine levels in blood and tissues to a much higher degree than the water soluble salts. It inhibits three major biochemical pathways implicated in diabetes and it has been shown to have beneficial effect in end stage renal disease and alcoholic neuropathy.^[3] It significantly decreases pro-inflammatory mediators.^[4,5] Difference spectrophotometry and dual wavelength spectrophotometric methods have been proposed which is not reported till date



CAS Number 22457-89-2

Empirical formula C₁₉H₂₃N₄O₆PS

Molecular weight 466.45

Figure 1: Structure of benfotiamine

MATERIALS AND METHODS

All the standard and sample solutions were freshly prepared with 0.1M HCl and 0.1M NaOH. Bulk material was obtained as a gift sample from Franco Indian Pharmaceuticals, Mumbai and benfotiamine tablets were purchased from the local market. All spectral measurements were made on Shimadzu UV-Visible spectrophotometer-model 1800 with 1cm matched quartz cells.

Difference spectrophotometry

The criteria for applying difference spectrophotometry to the assay of the substance in the presence of other absorbing substances are that reproducible changes may be introduced in the spectrum of the analyte by the addition of one or more reagents and the absorbance of the interfering substances is not altered by that reagent.

The simplest and the most commonly employed techniques for altering the spectral properties of the analyte is the adjustment of the pH by means of aqueous solutions of acids, alkalies or buffers. The difference absorption spectrum is a plot of difference between the solution at alkaline pH and acidic pH against wavelength. The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferents may be markedly improved by the technique of difference spectrophotometry.^[6-9]

Dual wavelength spectrophotometry

Dual wavelength method “also known as two wavelengths method” facilitates analyzing a component in the presence of an interfering component by measuring the absorbance difference A between two points in the mixture spectrum. The basis for such method is the selection of two wavelengths where the

interfering component of interest shows significant difference in absorbance with concentration between two points is directly proportional to the concentration of the component of interest independent of interfering component. In dual wavelength spectrophotometry the absorbance is measured at two wavelengths at which the analyte must absorb strongly at one of these wavelengths than the other. The differential absorbance is then directly proportional to the analyte concentration.^[6-9]

Preparation of standard stock solution

Aliquot quantity of standard benfotiamine was accurately weighed and transferred into 100ml standard flask. Sufficient quantity of 0.1M HCl was added to dissolve the drug and the volume was made up with 0.1M HCl. From the above standard stock solution different concentrations in the range of 4-20 µg/mL were prepared using 0.1M HCl.

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.

Recovery studies

The recovery studies^[10] were carried out on spiked samples by adding predetermined amount of amount of standard drugs to respective sample. About 50% and 100% of standard drug were added to the sample and the absorbance was measured.

RESULTS AND DISCUSSION

Table I: Recovery studies of Benfotiamine.

S.no	Method	Label claim	Amount of drug added(%)	Amount of drug recovered(%)	% Recovery
1.	Difference spectrophotometry method	100mg	50	50.68	101.36
			100	100.70	100.70
2.	Dual wavelength spectrophotometry method	100mg	50	49.92	99.84
			100	100.10	100.10

Table II: Optical parameters proposed for the method.

Parameters	Difference spectrophotometry	Dual wavelength spectrophotometry
Wavelength range	200-400nm	200-400nm
Beer's law limits	4-20µg	4-20µg
Regression equation	$Y=0.009x+0.003$	$Y=0.009x+0.005$
Slope(m)	0.0092	0.0098
Intercept(c)	0.003	0.0050
Correlation coefficient	0.9987	0.9969
Standard error	0.0040266	0.0005989

UV Spectrum of benfotiamine

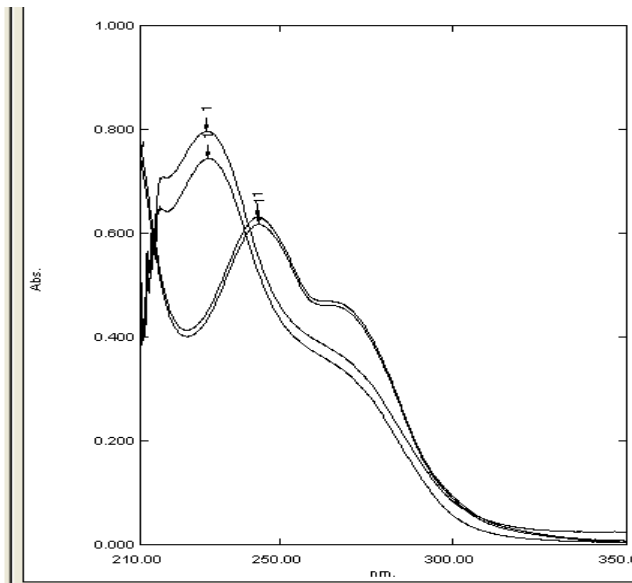


Figure 2: Difference spectrophotometry

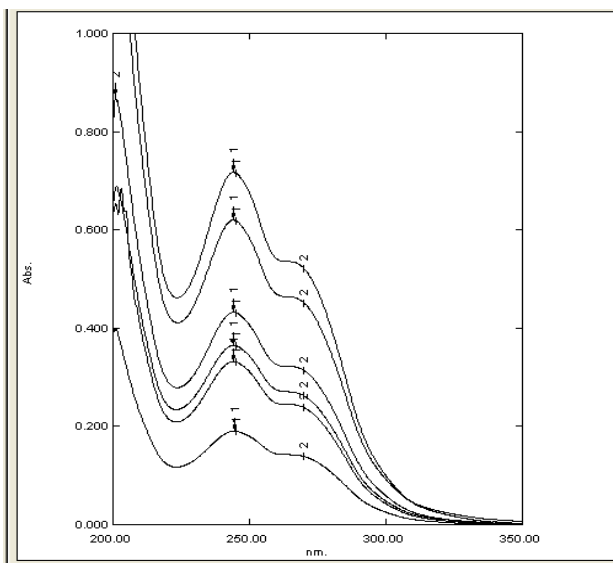


Figure 3: Dual wavelength spectrophotometry
Linearity graph

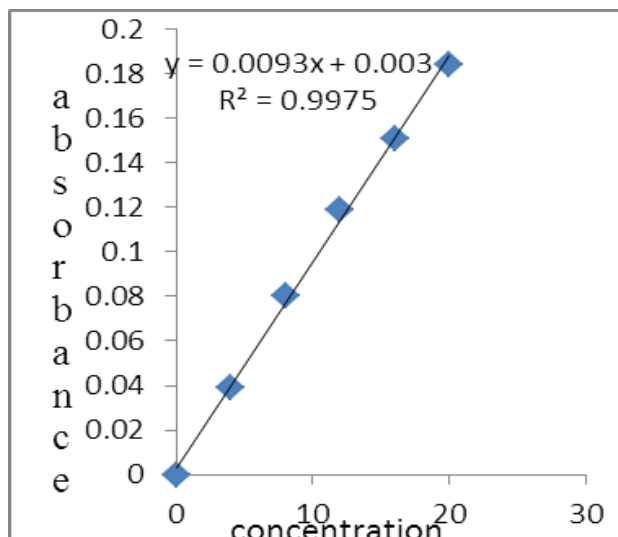


Figure 4: Difference spectrophotometry

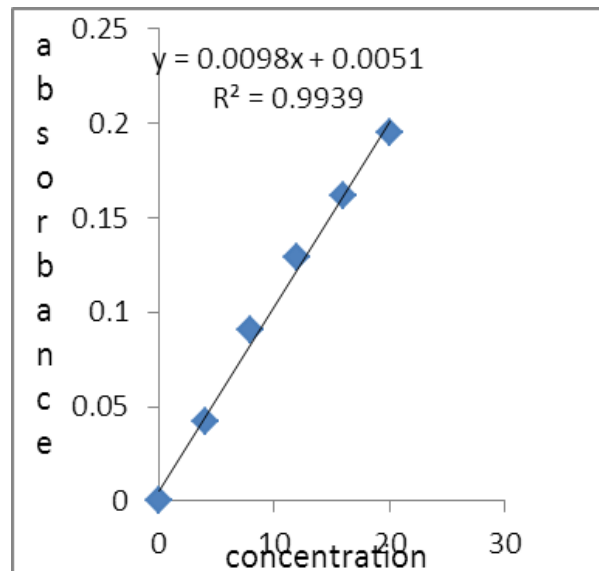


Figure 5: Dual wavelength spectrophotometry

The optical characteristics such as regression equation, correlation coefficient, slope and intercept, recovery studies for the methods were calculated and the results are summarized in Table I & II. Recovery studies revealed that the excipients and additives did not interfere and also reproducibility of the results indicate that this method is valid with percentage purity 99-101%. Linearity of the graph with correlation coefficient 0.99 indicates that the results are accurate.

CONCLUSION

The proposed methods are simple, accurate, precise and selective for the estimation of Benfotiamine in bulk and oral dosage form. Both methods are economical and do not require any sophisticated instruments like HPLC. Hence it can be effectively applied for the routine analysis of Benfotiamine in bulk and pharmaceutical dosage form.

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