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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF BENFOTIAMINE IN BULK AND DOSAGE FORM

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ABSTRACT

A new simple, precise, sensitive and validated RP-HPLC method was developed for the estimation of Benfotiamine in bulk and pharmaceutical dosage form. The chromatographic conditions used for the separation was Phenomenex Luna C18 (4.6x250mm,5µ) and mobile phase comprised of acetonitrile : methanol : water: 0.1% OPA (40:20:35:5 v/v). The flow rate was 1.0 ml/min with detection at 249 nm. The retention time was found to be 3.84 min. The linearity was found to be in the range of 5-35 µg/ml for benfotiamine with correlation coefficient of 0.9999. The proposed method is accurate with 99.278% - 100.791 % recovery and precise (%RSD of repeatability, intra-day and inter-day variations were 0.53, 0.45-0.67, 0.58-0.79).The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.1448 and 0.4388 µg/ml respectively. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from tablet excipients were found.

Key words: Benfotiamine, RP-HPLC, Validation,Acetonitrile, Methanol.

INTRODUCTION

Benfotiamine (*S*-benzoylthiamine O-monophosphate) is a synthetic S-acyl derivative of thiamine (vitamin B1) belonging to the family of compounds known as allithiamines. It is a lipid-soluble form of the Vitamin B-1[(1)].. It may ease pain from neuropathy, retinopathy ,nephropathy. By blocking AGEs (advanced glycation end products), it prevent some complications due to diabetes, such as blood vessel damage and atherosclerosis [(2)].

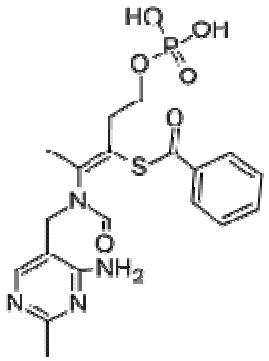


Fig. 1: Structure of benfotiamine

Literature survey reveals that few HPLC [4,5] methods have been reported for the estimation of benfotiamine individually and combination with other drugs. Till now, there is no simple RP-Hplc method has been reported using simple solvents. The main objective of the present study is to develop simple, sensitive, accurate and precise RP-HPLC method for estimation of benfotiamine in bulk and tablet dosage forms. The validation has been carried out as per ICH guidelines.

MATERIALS & METHODS

Apparatus

The chromatography was performed on a Waters HPLC instrument equipped with UV detector and Peak LC 7000 software, Phenomenex Luna C18 (4.6 x 250 mm, 5 µ particle size) was used as stationary phase. Shimadzu Ax200 analytical balance and Sonicator Pci Ultrasonic 3.5L100H were used in the study.

Reagents and materials

The reference sample of benfotiamine was supplied by Gulf exports, Mumbai. The formulation was procured from the local market. HPLC grade methanol, acetonitrile, OPA were purchased from Merck specialities private ltd, Mumbai .

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, acetonitrile: methanol : water : 0.1% OPA [40 :20: 35: 5 % v/v] was chosen with detection wavelength 249 nm, since it gave sharp peak with good symmetry within limits.

Chromatographic conditions

The optimized parameters which were used as a final method for the estimation of benfotiamine represented in the Table 1.

Preparation of standard stock solution

An accurately weighed quantity of benfotiamine (5 mg) was transferred to a 50 ml volumetric flask, dissolved and diluted to the mark with mobile phase to obtain standard stock solution of 100µg/ml.

Preparation of calibration curve

Aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5ml standard stock solution (100 µg/ml) was transferred to 10 ml of volumetric flasks and made up to the mark with mobile phase to get concentration of 5,10,15,20,25,30,35µg/ml. An aliquot (20 µl) of each solution was injected under the operating chromatographic conditions and responses were recorded. Calibration curve was constructed by plotting the peak areas versus the concentration and the regression equation was calculated.

The standard solution was prepared by transferring 1.5 ml of 100µg/ml to the 10 ml of volumetric flask and made up to the mark with mobile phase to get 15µg/ml.

Sample preparation

Tablet powder equivalent to 15mg of benfotiamine is weighed and transferred to 100ml standard flask. Small amount of mobile phase is added to dissolve and then the volume is made up to the mark. Then it is filtered with 0.45 micron filter and sonicated. From this stock solution 1ml is transferred to the 10 ml volumetric flask and volume is made up to mark to prepare 15µg/ml concentration. Prepared sample solution was analysed. (Table 7)

Method validation

The optimized Chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical methods (ICH, 2005) [5].

System suitability test

20 µL of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table 2.

Specificity

Specificity of the HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities, degradants or

excipients. A volume of 20µl of working placebo sample solution was injected and the chromatogram was recorded. No peaks were found at retention time of 3.84 min. Hence, the proposed method was specific for benfotiamine.

Linearity

The linearity of calibration curve in pure solution, over the concentration range of 5-35 µg/ml through proposed HPLC method. The data was represented in Table 3.

Precision

The precision of the method was determined by repeatability and intermediate precision (intra-day and inter-day).

Repeatability

The repeatability of the proposed method was ascertained by injecting six replicates of fixed concentration within the Beer's range and finding out the peak area by the proposed method. From this peak area %RSD was calculated. (Table 4)

Intra-day precision

Intra–day precision was determined by injecting three different concentrations (90 %, 100% and 110%) for three times in the same day. Peak area was measured and %RSD was calculated. (Table 4)

Inter-day precision

Inter-day precision was determined by injecting three different concentrations (90 %, 100% and 110%) for three days in a week. Peak area was measured and %RSD was calculated. (Table 4)

Accuracy

For the accuracy of proposed method, recovery studies were performed by standard addition method at three different levels (50%, 100% and 150% of final concentration).A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory and reported in Table 5.

Robustness

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the pH of the mobile phase (± 0.2), organic solvent content (± 5%),wave length(±5nm) None of these alterations caused a significant change in peak area RSD, tailing factor and theoretical plates. Although the changes in the retention time were significant, yet quantitation was possible. The results were represented in Table 6.

Limit of detection and Limit of quantification

Limit of detection (LOD) and Limit of Quantification (LOQ) were determined by using the formula based on the standard deviation of the response and the slope.LOD and LOQ were calculated by using equations, LOD=3.3 xσ/s and LOQ=10 x σ/s, where σ = standard deviation, S= slope of the calibration curve.(Table 4)

Results and discussions

To develop simple and economical RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with Phenomenex Luna C18 (4.6 x 250 mm, 5µ) column and mobile phase comprising of acetonitrile : methanol : water : 0.1% OPA (40:20:35:5 v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 249 nm based on peak area. The retention time was found to be 3.84 min. The optimised method was validated as per ICH guidelines. The system suitability parameters observed by using this optimised conditions were reported in Table2. A linearity range of 5-35 µg/ml with correlation coefficient 0.9999 was established. The result of recovery study by standard addition method ranging from 99.278 % to 100.791 % suggested the good accuracy. The precision of the proposed method was carried in terms of the repeatability, inter-day and intra-day time periods. The low % RSD values of repeatability (0.53%), inter-day (0.58% - 0.79%) and intra-day (0.45 % – 0.67 %) variations reveal that the proposed method is precise. The LOD, LOQ values were found

to be 0.1448µg/ml and 0.4388µg/ml respectively. The absence of interference peak, indicates that method can be used for routine analysis of benfotiamine in pharmaceutical dosage form.

CONCLUSION

A specific, precise, accurate, rapid and reliable RP-HPLC method has been developed and validated. It has short runtime 10 min and retention time 3.84 allows analysis of large number of samples in a short period of time. In this method, there was no interference from tablet excipients. So this RP-HPLC method can be used in the quality control department.

Table 1: Optimised chromatographic conditions

Mobile phase	Acetonitrile: methanol: water : 0.1% OPA[40 :20: 35: 5 % v/v]
Stationary phase	Phenomenex Luna C18 (4.6 x 250 mm ,5 µ particle size)
Wavelength	249nm
Run time	10 min
pH of the mobile phase	4.6
Flow rate	1 ml/min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Table 2: System Suitability Test Parameters

System suitability parameters	Results
Retention time	3.84
Area	178135.1
Theoretical plate number	30976
Tailing factor	1.92

Table 3: Linearity data for benfotiamine

Concentration (µg/ml)	Peak area
5	55915.8
10	118024.5
15	178292.4
20	242973.3
25	307853.6
30	368942.2
35	434392.6

Table 4: Validation parameters of the proposed method

Parameter	Results
Linearity (µg/ml)	5-35
Slope(b)	12620.19
Intercept(a)	-8633.22
Correlation co efficient (r)	0.9999
Regression equation(y=mx+c)	Y=12620.19x-8633.22
Precision	
Repeatability(%RSD, n=6)	0.53
Intraday precision(%RSD n=6)	0.45 -0.67
Interday precision(%RSD ,n=6)	0.58-0.79
% Recovery	99.278 – 100.791
Robustness	Robustted
LOD(µg/ml)	0.1448
LOQ(µg/ml)	0.4388

Table 5: Recovery data

Drug name	Levels	Amount added(µg/ml)	Amount recovered (µg/ml)	%Recovery	
Benfotiamine	50 %	7.5	7.485	99.8	Mean %Recovery 99.956 SD 0.7685
	100%	15	14.891	99.278	
	150%	22.5	22.677	100.79	

Table 6: Results for robustness test of Benfotiamine

Parameters	Changes	% Recovery
Organic phase variation	60%	99.6
	55%	99.1
pH of the Mobile phase variation	4.8	98.3
	4.4	98.9
Wavelength variation	244	98.2
	254	98.4

Table 7: Analysis of formulation

Brand Name	Drug	Amount labelled (mg)	Amount found (mg)	%recovery	%RSD
Benforce	Benfotiamine	150	149.97	99.97	0.47

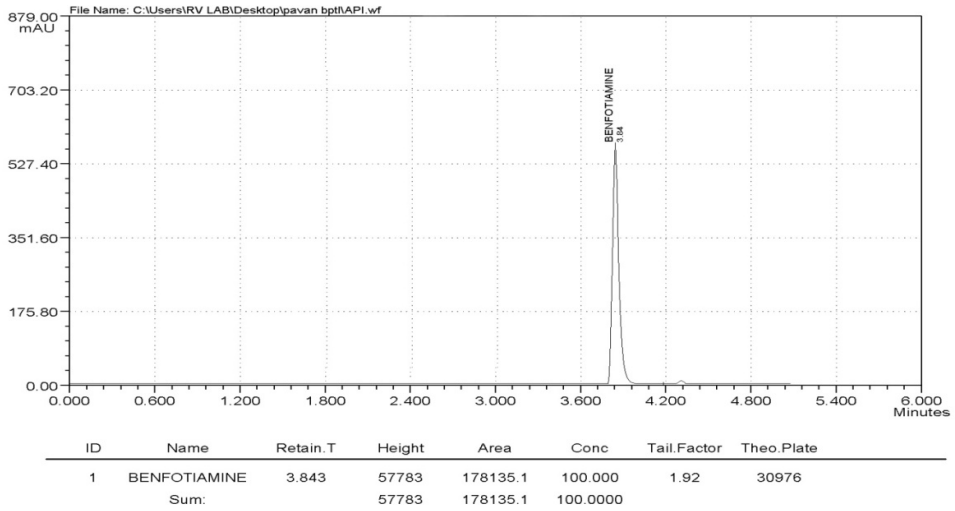
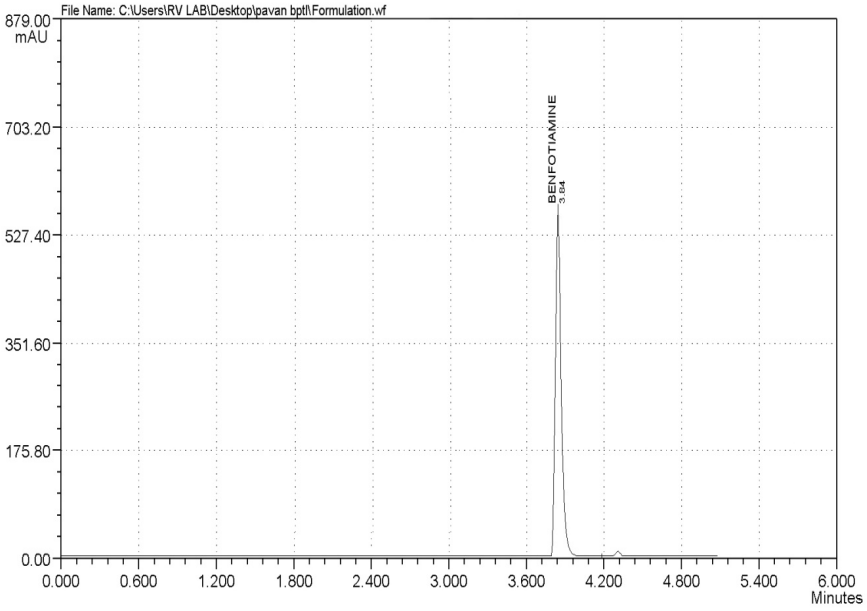


Fig. 2: Benfotiamine API peak



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	BENFOTIAMINE	3.843	57783	178093.2	100.000	1.92	30992
	Sum:		57783	178093.2	100.0000		

Fig. 3: Benfotiamine formulation peak

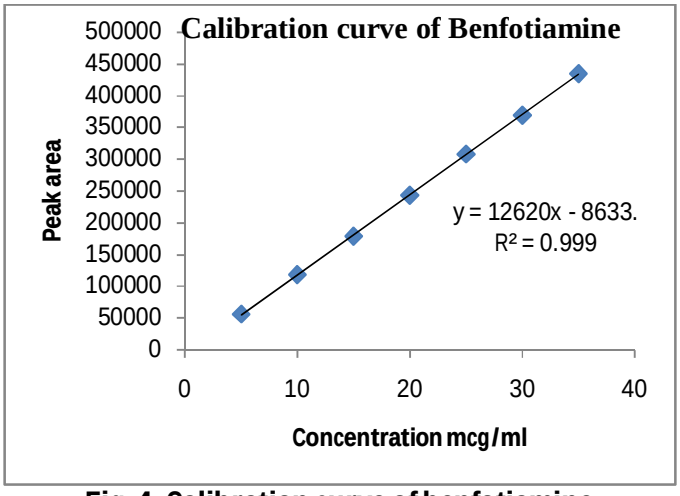


Fig. 4: Calibration curve of benfotiamine

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