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Nanjeshgowda *et al.*
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Original Article

Validated RP-HPLC Method for the Quantitation of Benfotiamine in Bulk and Tablet Dosage Form

Nanjeshgowda*, Jose Gnana Babu C, Tamizh Mani T

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathi Nagara, K.M.Doddi, Maddur Taluk, Mandya District, Karnataka, India – 571 422

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ABSTRACT

A simple, novel, accurate, precise, linear, rapid and economical RP-HPLC method was developed for the estimation of Benfotiamine. The chromatographic separation was achieved using a Grace Smart RP 18 (250 x 4.6 mm, 5 µ) column and isocratic elution, a mobile phase comprising of Acetonitrile: 50mM Ammonium acetate buffer at P^H 6.0 was adjusted with O-Phosphoric acid in the ratio of 60:40 (v/v). The flow rate was 1.0 ml/min with detection at 245 nm using a UV detector and drug eluted with retention time of 2.743 min. The calibration curves were linear (r^2=1) in the concentration range of 2-64 µg/ml. The limit of detection and limit of quantitation were found to be 0.1727 and 0.5235 µg/ml respectively. Thus the simple, novel, economical, accurate, precise and rapid RP-HPLC method was developed for estimation of Benfotiamine and validated as per ICH guidelines. Hence the method holds good for routine analysis of Benfotiamine in pure and pharmaceutical dosage form.

Keywords: Benfotiamine, ICH guidelines, RP-HPLC, Validation.

1. 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. It is used for treating sciatica and other painful nerve conditions[1] and also it may ease pain from neuropathy, retinopathy, nephropathy, by blocking AGEs (advanced glycation end products), it prevent some complication due to diabetes, such as blood vessel damage and atherosclerosis[2]. As compared to thiamine, Benfotiamine has unique open thiazole structure which makes it pass directly through cell

Corresponding authors *
Nanjeshgowda
Department of Pharmaceutical Analysis,
Bharathi College of Pharmacy, Bharathi Nagara, K.M.Doddi,
Maddur Taluk,
Mandya District, Karnataka, India – 571 422
E Mail: nanjeshgowda330@gmail.com

membranes, readily crossing the intestinal wall and being taken straight into the cell[3]. Compared to water soluble thiamine, Benfotiamine is absorbed better in the intestine reaching maximum plasma levels of thiamine about 5 times higher[4].

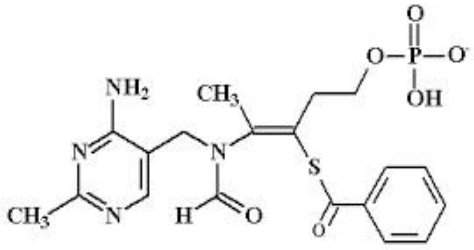

Fig 1: Chemical structure of Benfotiamine

The nomenclature of the Benfotiamine is {[(4-Amino-2-methylpyrimidin-5-yl)methyl] (formyl)amino}-5-(phosphonooxy)pent-2-en-3-yl] benzenecarbothioate and it has a molecular formula of $C_{19}H_{23}N_4O_6PS$ and a molecular weight is 466.448 g/mol. It is a white crystalline powder, soluble in 0.1 M HCl, poorly soluble in water.

To the best of our knowledge few UV-spectrophotometric[5-7] and HPLC[8-13] methods were reported for estimation of Benfotiamine individually and combination with other drugs in bulk and pharmaceutical dosage form. The attempt was to develop simple, novel, economical, accurate, precise and rapid RP-HPLC method for estimation of Benfotiamine in bulk and tablet dosage form. The developed method was validated as per ICH guidelines.

2. MATERIALS AND METHODS

Instrumentation:

Chromatographic separation was performed on a Shimadzu LC-10ATVP HPLC system comprising a Shimadzu SPD-10A UV-Vis detector, Shimadzu LC-10ATVP pump and enable Grace Smart RP18 column (250 x 4.6 mm, 5µ). A manually operating Rheodyne injector 50µl (20 µl injection valve) was used for injecting sample and standard solution. Baseline chromatography Data system N2000 software was used to collect and process the data.

Chemicals and reagents:

Benfotiamine pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form BenFORCE labelled claim 150 mg were purchased from local pharmacy, manufactured by Shield healthcare Pvt. Ltd. Acetonitrile, Water and Ammonium acetate buffer were obtained from Thermo Fischer Scientific India Pvt. Ltd. Mumbai. All the chemicals used in this investigation are HPLC grade.

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: 50mM Ammonium acetate buffer adjusted to P^H 6.0 using O-Phosphoric acid (60:40 v/v) was chosen with detection wavelength 245 nm, since it gave sharp peak with good symmetry within limits.

Buffer preparation: 50mM Ammonium acetate

0.778 g of Ammonium acetate is dissolved in 200 ml of water and filtered through Millipore 0.4 micron filter; the P^H was adjusted to 6.0 by using O-Phosphoric acid.

Preparation of mobile phase

The composition of mobile phase was prepared from filtered and degassed mixture of Acetonitrile and 50mM Ammonium acetate at P^H 6.0 of the solution was adjusted with O-phosphoric acid in the ratio of 60:40.

Diluent: Mobile phase.

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

Weigh accurately about 100 mg of Benfotiamine pure drug and then transferred into 100ml volumetric flask, a portion of diluent is added and sonicated for 5 min to dissolve it completely. The volume is made up to the mark with diluent (stock solution-1). From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluent (stock solution-2), from this solution pipette out 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 ml into 10 ml individual volumetric flask and add diluent up to the mark, this gives 2, 4, 8, 16, 32 and 64 µg/ml concentrations.

Preparation of sample solution

Ten tablets of Benfotiamine 150 mg were weighed and powdered, the primary stock solution was prepared by dissolving a weight equivalent to 150 mg of Benfotiamine and dissolved in sufficient volume of diluent, the solution was sonicated for 5 minutes and make up the volume up to the mark, and then filtered through Millipore 0.4 micron filter. From this stock solution pipette out 0.66 ml in a 10ml volumetric flask and make up the volume up to the mark with diluent. From this solution pipette out 0.5 ml in 10ml volumetric flask and make up the volume with diluent, this gives 5 µg/ml concentrations.

Flow rate selection

Different flow rates in between 0.50 to 1.50 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time.

3. RESULTS AND DISCUSSION

System suitability

20 µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of system. Parameters such as number of theoretical plates (N), tailing factor (T), retention time (t_R), asymmetry and area were determined. The obtained values indicate good performance of system. The values of system suitability parameters were shown in Table- 2.

Method validation

The method is validated according to the ICH guidelines[14-16]

Specificity

Specificity of the HPLC method was checked for interference of impurities, degradants or excipients in the analysis of sample solution and was determined by injecting a volume of 20µl of sample solution and the chromatogram was recorded. There is no interference of impurities, excipient peak on the peak of Benfotiamine, indicating the high specificity of method.

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig-3 and showed linearity over a concentration range of 2-64 µg/ml shown in Table-3, along with regression parameters in Table-4. Each calibration was injected six times. The calibration curve was performed in six replicates.

Precision

The precision of the analytical method was determined by intraday and interday precision. The sample solution was prepared as per the test method. In intraday precision, the same concentration of sample solution was injected 6 times in the same day and in interday precision, injecting six solutions of same concentration for six different days in a week. The results of precision were tabulated in table-5. The average and standard deviation of mean area were taken and %RSD was calculated and reported. %RSD values were within the limits and the method was found to be precise.

Accuracy

The accuracy of the method was determined by recovery studies by the determination of % mean recovery of the drug at three different levels (80%, 100% and 120%). At each level, three determinations were performed. A known amount of standard pure drug was added to preanalyzed tablet powder and the sample was then analysed by developed method. Results of recovery studies were reported in table-6. The observed data were within the range, which indicates good recovery values.

Robustness

The robustness of analytical method was carried by varying the parameters deliberately from the optimized chromatographic conditions like P^H of mobile phase (variation in ± 0.2 units), flow rate (variation in ± 0.02ml/min.), temperature (variation ± 2.0 °C.).The observed results were within the limit. The results were shown in table-7.

Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was found to be <2, showed ruggedness of developed analytical method. The values were shown in Table-8.

Limit of detection and Limit of quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the response and slope of linearity curve. LOD and LOQ values of Benfotiamine were shown in Table-9.

Resulted diagrams and tabular columns:

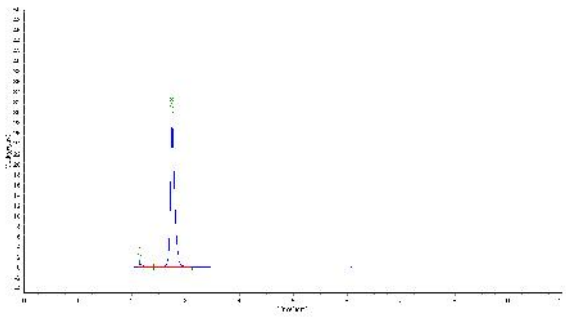

Fig 2: Chromatogram of Benfotiamine

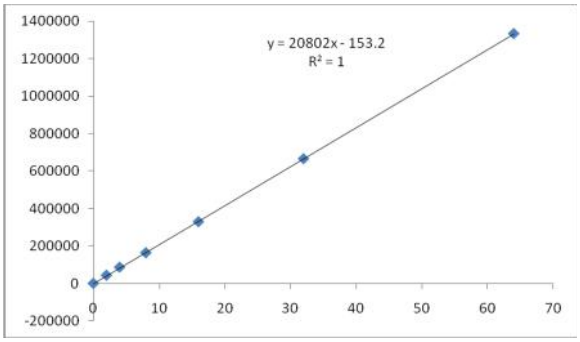

Fig.3: Linearity graph of Benfotiamine

Table 1: Optimized chromatographic conditions

Mobile phase	Acetonitrile: 50mM Ammonium acetate at PH-6 with O-phosphoric acid(60:40 v/v)
Stationary phase	Grace Smart RP-18 column dimension ID : (250x 4.6 mm, 5 µ)
Wavelength	245 nm
Run time	10 min
P^H of mobile phase	6.0
Injector	Rheodyne 50 µl
Flow rate	1.0 ml per min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Isocratic elution

Table 2: Results of System suitability studies

System suitability parameters	Acceptance criteria	Results
Retention time(t_R)	—	2.743
Number of theoretical plates(N)	≥ 2000	6025.749
Asymmetry	—	1.615
Area	—	161537.391
Tailing factor(T)	≤ 2.0	1.292

Table 3: Linearity data

Sl. No.	Concentration (µg/ml)	Retention time (min)	Peak area (mv)
1	2	2.759	43424.4
2	4	2.747	86481.25
3	8	2.747	163562.83
4	16	2.740	329089.71
5	32	2.732	665065.96
6	64	2.733	1332358.2

Table 4: Regression parameters

Regression Parameter	Benfotiamine
Regression Equation	Y=20802x-153.2
Slope (b)	20802
Intercept (a)	-153.2
Correlation Coefficient (r^2)	1.0

Table 5: Results of Precision studies

Concentration (8 µg/ml)				
Precision	Intraday	Interday		
		Day 1	Day 2	Day 3
Mean area*	164909.3	163562.8	164998.3	164600.6
Standard deviation	2402.406	2389.657	1307.391	2663.974
%RSD	1.45	1.46	0.79	1.61

*indicates average of six determinations, RSD indicates relative standard deviation

Table 6: Results of Accuracy studies

Brand name – BenFORCE (150 mg)					
% Spiked levels	Amount of formulated drug added in µg/ml	Amount of pure drug added in µg/ml	Amount found in µg/ml	% recovery	Statistical parameters
80	5	1.4	6.376	99.62	Mean=100.5433
	5	1.4	6.430	100.47	SD=0.962098
	5	1.4	6.499	101.54	%RSD=0.95
100	5	3	7.905	98.82	Mean=100.1733
	5	3	7.974	99.68	SD=1.65606
	5	3	8.162	102.02	%RSD=1.65
120	5	4.6	9.667	100.70	Mean=100.6267
	5	4.6	9.669	100.72	SD=0.144684
	5	4.6	9.644	100.46	%RSD=0.14

SD indicates standard deviation and RSD indicates relative standard deviation.

Table 7: Results of Robustness studies

Concentration (8mcg/ml)				
Parameters	Factor [a]	Level	Mean area* ± Standard deviation	%RSD
P^H	5.8	-0.2	156903.9 ± 1664.898	1.06
	6.0	0	163788.7 ± 2119.257	1.29
	6.2	+0.2	164600.6 ± 2663.974	1.61
Temperature °C	25	-2.0	154104.7 ± 1133.273	0.73
	27	0	163562.8 ± 2389.657	1.46
	29	+2.0	169887.2 ± 2689.584	1.58
Flow rate (ml/min)	0.98	-0.02	154444.4 ± 2032.63	1.31
	1.0	0	163562.8 ± 2389.657	1.46
	1.02	+0.02	161834.3 ± 1542.864	0.95

[a] indicates three factors were slightly changed at three levels, *indicates average of six determinations and RSD indicate relative standard deviation.

Table 8: Results of Ruggedness studies

Analysts	Mean area* ± Standard deviation	%RSD
Analyst 1	163562.8 ± 2389.657	1.46
Analyst 2	164998.3 ± 1307.391	0.79

*indicates average of six determinations, RSD indicates relative standard deviation.

Table 9: Results of LOD and LOQ

Parameters	Results
LOD (µg/ml)	0.17278
LOQ (µg/ml)	0.52358

4. CONCLUSION

Thus it can be concluded that the developed method was found to be simple, novel, accurate, precise, linear, rapid and economical. Hence, the above said method can be successfully applied for the routine estimation of Benfotiamine in pure and tablet dosage form.

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