“ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF BENFOTIAMINE: REVIEW”

NANJESHWAR*, JOSE GNANA BABU C and TAMIZH MANI T.

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

ABSTRACT

Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria and stability of results. Validation should demonstrate that the analytical procedure is suitable for its intended purpose. Design of experiment is a powerful tool for the method characterization and validation. Analytical professionals should be comfortable to use it to characterize and optimize the analytical method. An effective analytical method development and its validation can provide significant improvements in precision and a reduction in bias errors. It can further help to avoid costly and time consuming exercises. Literature survey reveals that few HPLC methods have been reported for the estimation of Benfotiamine. The published methods were validated for various parameters as per ICH guidelines. Statistical analysis provided that the published methods were reproducible and selective for the estimation of the Benfotiamine in pure and pharmaceutical dosage form.

Key words: Benfotiamine, Literature Survey, Validation, Method Development, ICH Guidelines.

*Corresponding address: Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathi Nagar, K.M.Doddi, Maddur Taluk, Mandya District, Karnataka, India – 571 422, Email:nanjeshgowda330@gmail.com

INTRODUCTION

Benfotiamine (S-benzoylthiamine O-monophosphate) is a synthetic S-acyl derivative of thiamine (vitamin B1). It is a lipid-soluble form of the Vitamin B-1. It may ease pain from neuropathy, retinopathy, nephropathy, by blocking AGEs (advanced glycation end products), it prevents some complication due to diabetes. The nomenclature of the Benfotiamine is \{[(4-Amino-2-methylpyrimidin-5-yl)methyl] (formyl) amino}5-(phosphonooxy)pent-2-en-3-yl benzenecarbothioate and its molecular
formula is \( C_{19}H_{23}N_4O_6 \)PS and its molecular weight is 466.448 g/mol. It is a white crystalline powder, soluble in 0.1N HCl, poorly soluble in water.

![Chemical structure of Benfotiamine](image)

**Figure 1: Chemical structure of Benfotiamine.**

**Literature survey**

1. **B. Pavan Adithya** et al., have developed a new simple, precise, sensitive and validated RP-HPLC method for the estimation of Benfotiamine in bulk and pharmaceutical dosage form. The chromatographic conditions used for the separation was Phenomenex Luna C18 (4.6x250mm, 5\( \mu \)) 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 \( \mu \)g/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 quantization (LOQ) were found to be 0.1448 and 0.4388 \( \mu \)g/ml, respectively. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from tablet excipients were found.

2. **Deepali A. Nanaware** et al., have developed a simple, sensitive and rapid reverse phase high performance liquid chromatographic method for the estimation of Benfotamine (BEN) and Metformin Hydrochloride (MET) in pure and in pharmaceutical dosage forms. Thermo Hypersil BDS–C18 Column (250 mm \( \times \) 4.6 mm, 5.0 \( \mu \) Germany) with isocratic conditions was used with a mobile phase containing mixture of Methanol and Aq. Phosphate buffer (10mM of Potassium Dihydrogen Phosphate adjusted to 3.2 with ortho phosphoric acid) in the ratio of 80: 20. The flow rate was 1 ml/min and effluents were monitored at 239nm and eluted at 2.583 min (BEN) and 3.233 min (MET). Calibration curve was plotted with a range of 1-6 \( \mu \)g/ml for BEN and 0.1-5 \( \mu \)g/ml for MET. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination of Benfotiamine and Metformin Hydrochloride in pharmaceutical dosage forms.

3. **S. Poongothai** et al., have developed a simple, precise, rapid and validated selective reverse phase high performance
liquid chromatographic (RP-HPLC) method for the simultaneous determination of Benfotiamine (B1) 100 mg, Pyridoxine hydrochloride (B6) 100 mg, Mecobalamin (B12) 1000 mcg and Alpha-lipoic acid 100 mg in multivitamin capsules. The method uses X-Terra reverse phase (RP-18, 250 x 4.6 mm, 5 μm) column and gradient elution. The aqueous mobile phase contained 0.05 M phosphate buffer adjusted to pH 2.5 and acetonitrile. Separation and quantification was achieved by changing the proportion of the system linearly with a time-schedule programme. Detection was carried out in the range of 200 to 600 nm using photodiode array detector and set at 320 nm and further analysis was carried out using a UV detector. This method has been validated and found to be applicable in routine analysis for multivitamin capsules. The precision is exemplified by relative standard deviations of 1.1% for benfotiamine, 0.9% for pyridoxine hydrochloride, 0.7% for mecobalamin and 1.2% for Alpha-lipoic acid. Good linearity was observed between the concentration of the analytes and peak area with correlation coefficients (R2) of 0.9997, 0.9990, 0.9995, and 0.9998 respectively. Mean recoveries obtained during spiking experiments were in the range of 99.8-100.2%.

4. B. Pavan Adithya6 et al., have developed a new precise, reproducible and validated RP-HPLC method for the simultaneous estimation of Benfotiamine and Metformin hydrochloride in tablet dosage form. The chromatographic conditions used for the separation was Phenomenex Luna C18 (4.6x250mm, 5μ) with 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 of benfotiamine and metformin hydrochloride was found to be 3.84 and 2.297 min respectively. The linearity was found to be in the range of 5–35 μg/ml for benfotiamine and for metformin hydrochloride 50–200 μg/ml with correlation coefficients of 0.9999 and 0.9995 respectively. The proposed method is accurate with 99.1%-100.17% recovery for benfotiamine and 99.31% -100.44% recovery for metformin hydrochloride and precise (%RSD of repeatability, intra-day and inter-day variations were 0.33, 0.32–0.51, 0.48–0.69 for benfotiamine and 0.28, 0.43–0.67, 0.53–0.88 for metformin hydrochloride). The limit of detection (LOD) and limit of quantization (LOQ) for benfotiamine 0.159, 0.483μg/ml and for metformin hydrochloride 0.949, 2.87μg/ml respectively. The method can be used for the estimation of dosage form in routine analysis.

5. Mihirkumar G. Patel7 et al., have developed a new simple, precise, accurate, selective and economical RP-HPLC method for simultaneous estimation of Metformin Hydrochloride
(MET) and Benfotiamine (BEN) in tablet dosage form. The method was carried out on a Waters column C-18 (250 mm x 4.6 mm, 5 µm) with a mobile phase consisting of water (pH 3.2 adjusted with orthophosphoric acid) and acetonitrile (75:25 v/v); at a flow rate of 0.8 mL min⁻¹ with run time of 10 min. Detection was carried out at 254 nm. The retention time for MET and BEN was found to be 2.125 and 3.881 min, respectively. The MET and BEN followed linearity in the concentration range of 200-600 µg mL⁻¹ and 30-90 µg mL⁻¹ with r²=0.999, respectively. The amounts of both drugs estimated by proposed method were found to be in good agreement with label claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The LOD and LOQ were found to be 0.49 and 0.19 µg for MET and 1.6 and 0.65 µg for BEN respectively. The developed method can be used for routine analysis of titled drugs in tablet formulation.

6. Hesham Salem⁸ et al., have developed a simple, selective, sensitive, precise, simultaneous liquid chromatographic analysis of capsules containing thioctic acid, Benfotiamine and cyanocobalamin was described. Good chromatographic separation was achieved using a Zorbax C18 (4.6cmx250mm, 5µm) and a mobile phase consisting of acetonitrile-phosphate buffer pH 3.5 (15:85v/v) at a flow rate of 0.9mLmin⁻¹. The ultraviolet detector was set a wavelength at 280nm. Thiocytic acid, Benfotiamine and cyanocobalamin were eluted at 2.869, 3.752, and 13.689 min, respectively. The linear ranges for thioctic acid, Benfotiamine, and cyanocobalamin were 30-180, 4-24, and 0.025-1.50µg/mL, respectively. The recoveries of thioctic acid, Benfotiamine, and cyanocobalamin in pharmaceutical preparation were all greaterthan 98% and their relative standard deviations were less than 2.0%. The limit of detection was 2.57, 0.19, and 0.003µg/mL for thioctic acid, Benfotiamine, and cyanocobalamin, respectively.

CONCLUSION

Literature survey suggested that various HPLC methods were developed and reported. The published methods were validated for various parameters as per ICH guidelines. Statistical analysis proved that the published methods were reproducible and selective. Thus it can be concluded that the reported and published methods can be successfully applied for the estimation of the Benfotiamine in pure and pharmaceutical dosage form.

REFERENCES

3. B. Pavan Adithya, M. Vijayalakshmi. Development and


