Stability-Indicating HPLC Determination of Benfotiamine in Bulk Drug and Pharmaceutical Dosage Forms

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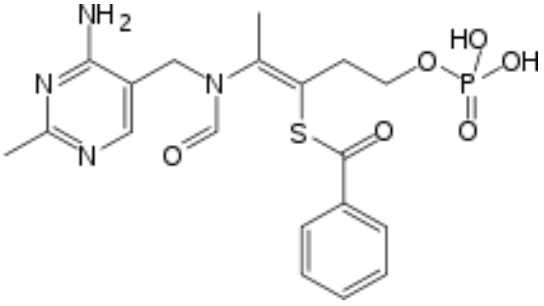
Abstract :

Drug profile:

Benfotiamine[1-5] S-[(2Z)-2-{[(4-amino-2-methylpyrimidin-5-yl) methyl] (formyl) amino}-5-(phosphonooxy) pent-2-en-3-yl] benzene carbothioate, is a synthetic S-acyl derivative of thiamine (vitamin B1) [**Fig.1.01**] therapeutic role in pain reduction and diabetic complications (neuropathies and nephropathies).

FIG.1.01. STRUCTURE OF BENFOTIAMINE



It increases the transketolase activity, an important enzyme in glucose metabolism which results into blockage of three major molecular pathways leading to hyperglycemic damage. It prevents the increase in UDP-N-acetyl glucosamine (UDPGlc- NAc) and increases hexosamine pathway activity that decreases the buildup of detrimental glucose metabolites leading to advanced glycation end products (AGE). It also normalizes protein kinase C (PKC) activity and prevents nuclear factor –kappa (NF- Kb) activation in the retina of diabetics. It can also be used to correct the imbalance in the polyol pathways by decreasing aldose reductase activity, sorbitol concentrations and intracellular glucose thereby protecting endothelial cells from glucose induced damage by normalizing cell replication rates and decreasing apoptosis. Benfotiamine enhancement in transketolase activity in erythrocytes and renal glomeruli protects the kidneys from glucose induced damage and prevents the development of diabetic neuropathy.

It is available as commercial formulation in the brand name of **BENALGIS** manufactured by Franco Indian Remedies (Containing 100mg of benfotiamine) rendered for the treatment of neuropathies and nephropathies

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So far, to our knowledge, very few analytical methods [6-10] have been reported for the determination of benfotiamine in pharmaceutical dosage forms either in single or in combined forms. As per discussion in the literature review reported, so far to our present knowledge, a validated stability indicating RP- HPLC method of benfotiamine in pharmaceutical dosage forms was not yet reported and this enabled the author to make an attempt in developing a new RP-HPLC method for the assay of benfotiamine in pharmaceuticals dosage forms under stress condition like acid hydrolysis, base hydrolysis, oxidation, thermal and photolytic stress.

The present part describes the development and validation of a stability-indicating liquid chromatographic analytical method for assay of benfotiamine in pure and in tablet formulation.

Experimental:

i. INSTRUMENTATION: The present analysis was performed using HPLC system (Waters Alliance 2695 separations module) equipped with 600e controller pump, 776 auto sampler, 2487 dual variable wavelength UV detector equipped with Empower software on Dell computer. A stainless steel ODS, C_{18} RP-Column (4.6mmx250mm) purchased from Waters Corporation (Bedford, MA, USA) was used in the present assay. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. Dona analytical balance was used for weighing the materials.

ii. CHEMICALS AND REAGENTS: Benfotiamine (99.9% pure) was obtained as a gift sample from MSN Labs. Dosage formulation in the brand name of **BENALGIS** tablets (each 100 mg tablet contains benfotiamine equivalent to 100 mg of benfotiamine) was purchased from Local pharmacy Ammonium dihydrogen phosphate (AR grade),orthophosphoric acid (AR grade), Acetonitrile (HPLC grade) and Milli-Q water was used for preparing buffer and other reagents for this assay.

iii. PREPARTION OF BUFFER SOLUTION: 2.3gms of Ammonium dihydrogen phosphate was weighed and dissolved in 1000mL HPLC grade water and then adjusted to pH 3.8 with orthophosphoric acid. This buffer solution was filtered and degassed prior to the assay.

iv. PREPARATION OF MOBILE PHASE: The mobile phase in the present assay is prepared by dissolving 0.02M Ammonium dihydrogen phosphate buffer (pH-4.0) and Acetonitrile in the ratio of 60:40 v/v. This mobile phase is filtered and degassed prior to the assay.

v. Preparation Of Diluent: Mobile phase is used as diluent in the present assay.

A. STANDARD STOCK SOLUTION: An accurately weighted sample of 10mg of benfotiamine was dissolved in methanol to give standard stock solution of 100µg/mL. A series of working standard solutions (2.5µg/mL – 12.5µg/mL) were obtained by diluting the aliquots of stock solution with the same diluent. All the above volumetric flasks of working standard solutions were wrapped with aluminium foil and stored in the dark.

RESULTS AND DISCUSSIONS:

i. Method Development (Method Optimization Studies): In the present study for developing the new RP-HPLC method a systematic study of the effect of various factors [i.e, the influence of column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters] was carried out by varying one parameter at a time and keeping all other conditions constant.

From these studies it was revealed that in the current study ODS, C_{18} RP-Column (4.6mmx250mm) column having 5µm particle size was used among the other columns because of its advantages of high degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing. A good symmetrical peak for benfotiamine was obtained, when water was replaced by phosphate buffer (adjusted to acidic pH by orthophosphoric acid) as aqueous phase in mobile phase. Preliminary trials on mobile phase proportion were carried to provide good resolution for benfotiamine using different compositions of mobile phase. From these trails the proportion of phosphate buffer (pH-3.8) and Acetonitrile in the ratio of 60:40 v/v was finalized as it gave good symmetrical peak for benfotiamine.

The appropriate wavelength for determination of benfotiamine was scanned by UV-visible spectrophotometer and was observed that the maximum absorbance (λ_{max}) was obtained at 245nm. At this wavelength benfotiamine offered high response with good linearity. The best separation with adequate resolution and symmetric peak of benfotiamine was obtained when the injection volume was fixed to 20µL with a flow rate was set to 1.0mL/min for the mobile phase respectively.On this finalized chromatographic conditions, obtained chromatogram of benfotiamine exhibited good peak symmetry with higher theoretical plates and elution time of 4.013 minutes respectively. The representative chromatogram of benfotiamine is shown in **Fig.1.02**.

ii. METHOD VALIDATION: After fixing the optimization studies the developed method was validated as per ICH guidelines which include system suitability, specificity, linearity, accuracy, precision, robustness, ruggedness, sensitivity, limit of detection and quantification.

a. SYSTEM SUITABILITY: The present HPLC system was equilibrated initially with the above said mobile phase, followed by six injections of the same standard **Fig.1.02**. These six consecutive injections were used to evaluate the system suitability. Parameters of system suitability studies include the peak symmetry (symmetry factor), no. of theoretical plates of the column, resolution, mass distribution ratio (capacity factor) and relative retention and the results of these studies were summarized in **Table.1.01**. The number of theoretical plates was higher than 2000, making the proposed method acceptable for the assay of benfotiamine in dosage forms as reported in **Table.1.01**.

b. FORCED DEGRADATION STUDIES: In the present study above said drug was submitted to various stress degradation studies as per the ICH recommended guidelines. As benfotiamine is soluble in methanol all solutions of benfotiamine for use in forced degradation studies were prepared in methanol. This is done by subjecting benfotiamine powder to acidic (0.1N HCl), basic (0.1N NaOH) and oxidizing (3% H_2O_2) stress conditions.

The chromatograms of benfotiamine under acidic, basic and oxidativestress conditions revealed benfotiamine (Rt = 4.013min) was not fully degraded and its degradation products were eluted separately at different retention times respectively.

From these respective chromatographs **Figs.1.03-1.05** and it was observed that the degradation products did not interfere in the detection analysis of benfotiamine establishing the high stability of the developed method.

c. LINEARITY: For linearity studies concentration levels corresponding to 50, 75, 100, 125 and 150% of test solution were prepared separately and 20µL of each concentration was injected into the HPLC system and the response was read at 245nm and the corresponding chromatograms were recorded **Figs.1.06(a-e)**.

From these chromatograms a calibration curve was constructed by plotting the peak areas of the drug versus concentration of benfotiamine **Fig.1.06.f**. The linear regression equation for the calibration curve of benfotiamine was found to be **Y= 134.73x-11.853** with a coefficient of regression r^2=0.9998 respectively. The chromatograms of benfotiamine obtained during linearity study were shown in **Figs.1.06 (a-e)** and the calibrated results of benfotiamine were tabulated in **Table.1.02** respectively.

d. LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION: The LOD and LOQ value of benfotiamine by the proposed method was found to be 0.0136µg/mL and 0.0456µg/mL respectively.

e. PRECISION: Precision of the proposed method was determined by repeatability (intra-day precision). It was expressed as % relative standard deviation (%RSD) **Figs.1.07(a-f)** . In this study six replicate standard solutions (10µg/mL) of benfotiamine were injected. The percent relative standard deviation (% RSD) was calculated and it wasfound to be 0.296, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in **Table.1.03**.

f. ACCURACY: The accuracy of this present proposed method was assessed by determination of recovery for three concentrations (corresponding to 50, 100, 150 % of test solution concentration) of benfotiamine covering the within the linearity range of the proposed method. Each concentration, were analyzed in triplicate at each level as per the proposed method and the percent recovery and % RSD was calculated and results are compiled in **Table.1.04**. Chromatograms obtained during this accuracy studies were shown in **Figs.1.08 (a-c)**. The results indicated a high degree of accuracy of the proposed method for determination of benfotiamine.

g. RUGGEDNESS & ROBUSTNESS: The ruggedness of the present RP-HPLC method was determined by carrying out the experiment by different analysts using different columns of similar types. The percentage %RSD of different preparations assay values with two different analysts were 0.296 and 0.113 respectively revealing the proposed method is rugged (**Table.1.05)**.

Robustness of the method was determined by small deliberate changes in flow rate, and

temperature. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust and is represented in **Table.1.06**.

h. ASSAY OF BENFOTIAMINE IN TABLET DOSAGE FORM: The proposed RP-HPLC method has been validated for the assay of benfotiamine in dosage forms as per guidelines of ICH. Contents of 10 tablets of benfotiamine (Benalgis-100mg)procured form local pharmacy were weighed and crushed to fine powder. Then powder equivalent to 25mg of benfotiamine was taken into a 100mL volumetric flask and about 75mL of diluent was added sonicated for 25 min at room temperature with intermediate shaking and filtered through a 0.45µm filter and then made up to volume with the same diluent. From this filtrate, aliquot amountsin the concentration range 2.5 to 12.5µg/ml were pipetted into a series of 10mL graduated test tube and made up to volume with the mobile phase to get concentrations that obey in the beers law limit. 20µL of this sample was injected into the column and the drug content in the formulations was quantified using the regression equation and the results are reported in **Table.1.07** respectively. Chromatograms obtained during this studies were shown in **Figs.1.09**.

Fig.1.02: VALIDATIVE CHROMATOGRAM OF BENFOTIAMINE

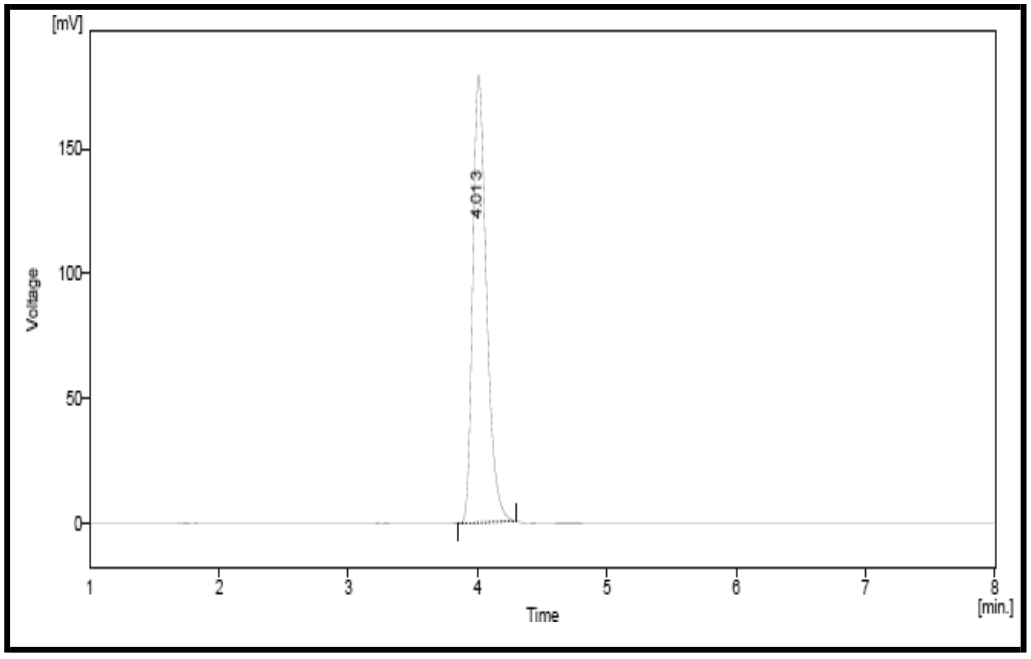

Fig.1.03: VALIDATIVE CHROMATOGRAM OF BENFOTIAMINE IN ACID STRESS

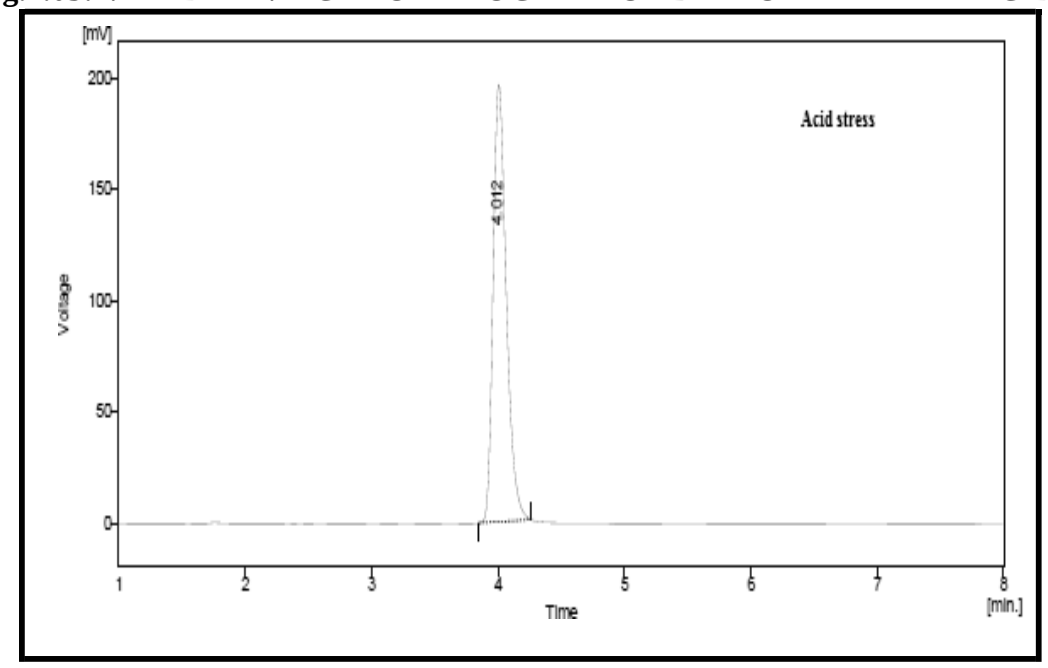

Fig.1.04: VALIDATIVE CHROMATOGRAM OF BENFOTIAMINE IN BASE STRESS

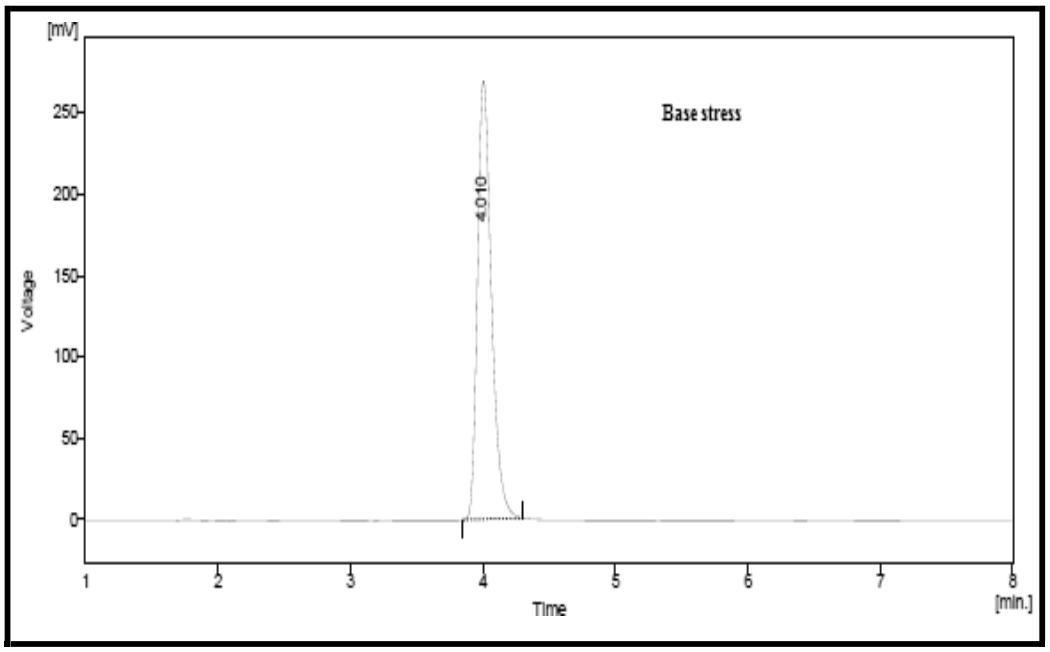

Fig.1.05: VALIDATIVE CHROMATOGRAM OF BENFOTIAMINE IN OXIDATIVE STRESS

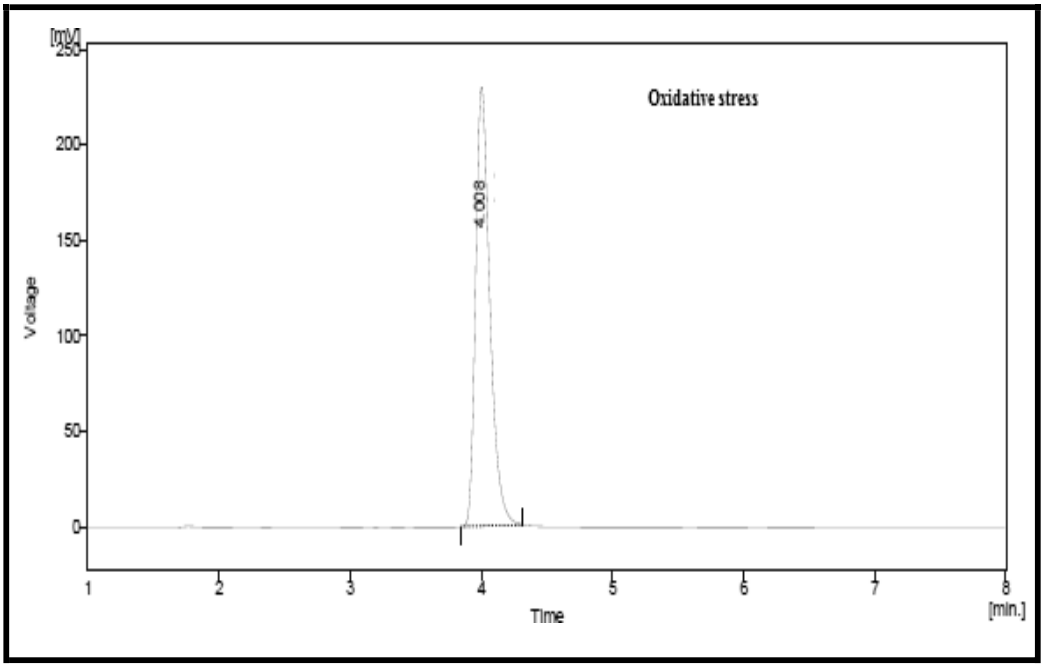

Fig. 1.06 (a):LINEARITY CHROMATOGRAM OF BENFOTIAMINE AT 2.5µg/mL

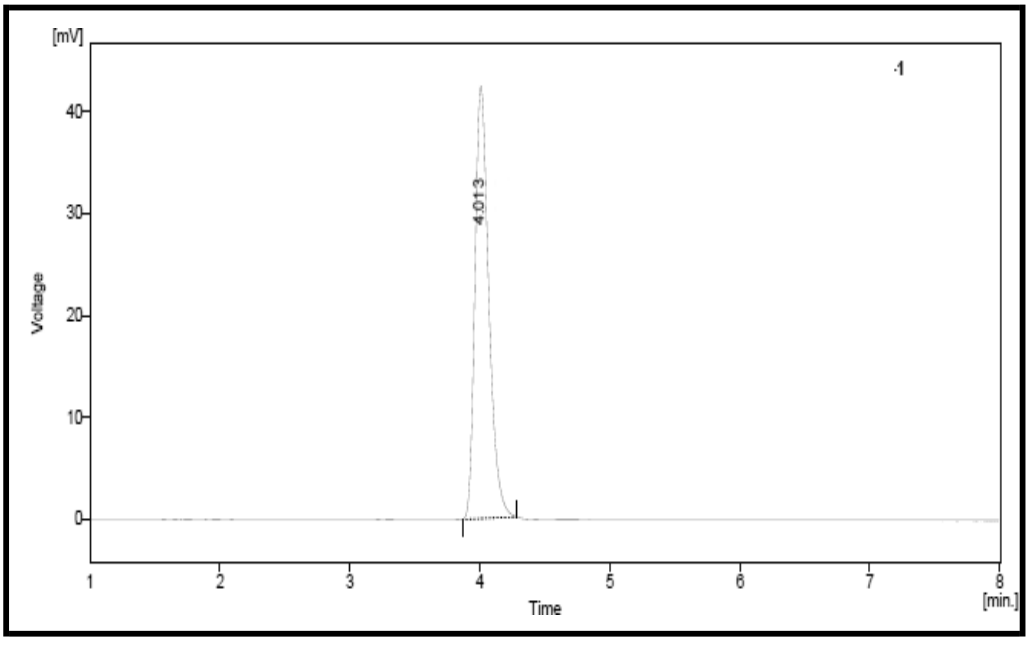

Fig. 1.06 (b):LINEARITY CHROMATOGRAM OF BENFOTIAMINE AT 5.0µg/mL

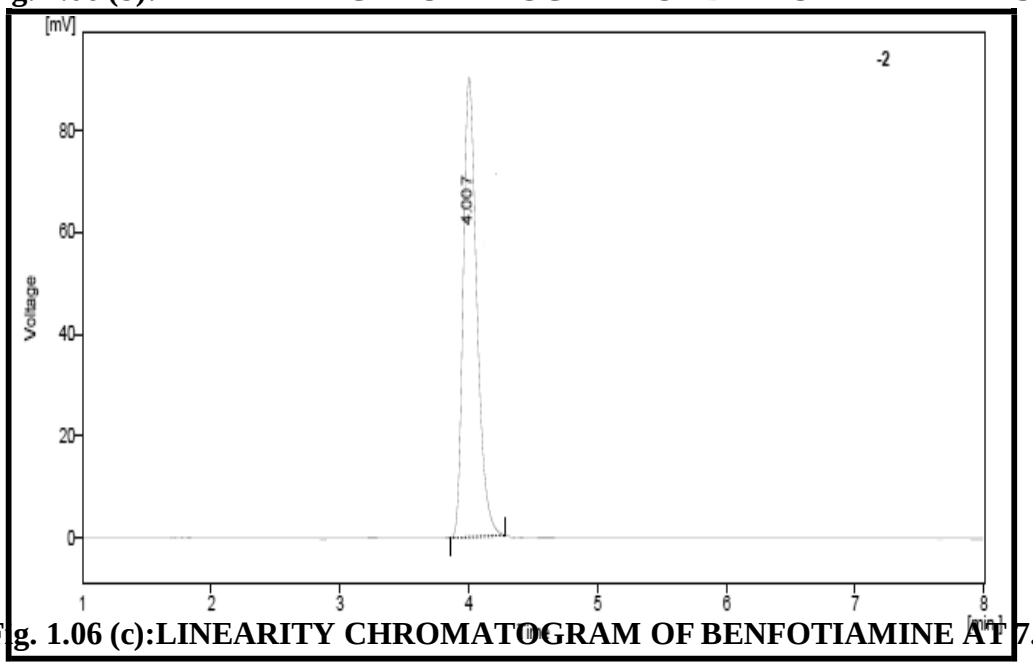

Fig. 1.06 (c):LINEARITY CHROMATOGRAM OF BENFOTIAMINE AT 7.5µg/mL

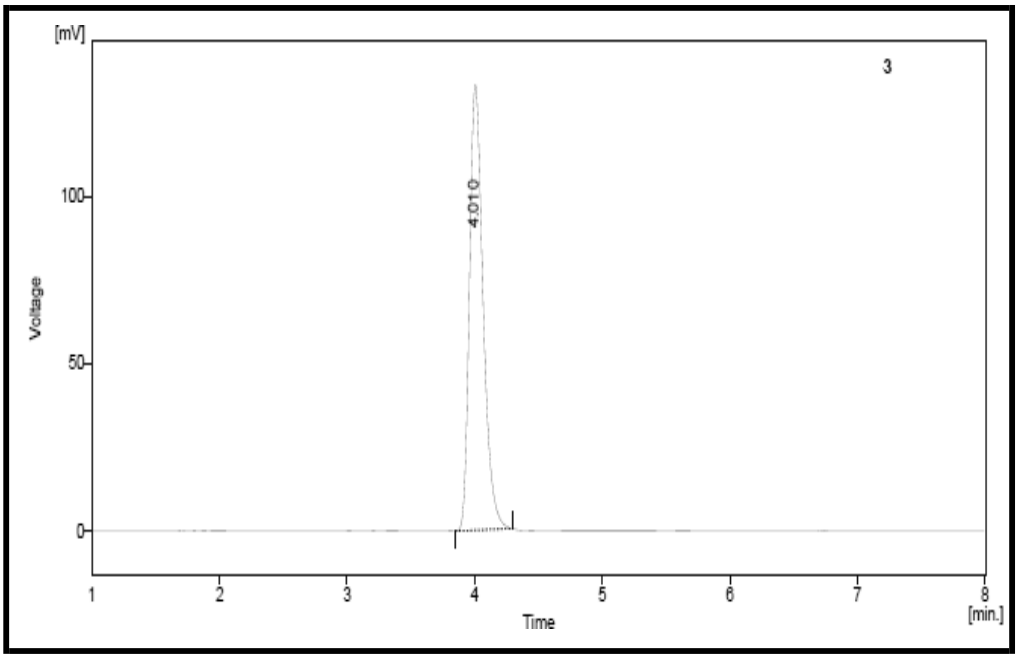

Fig. 1.06 (d):LINEARITY CHROMATOGRAM OF BENFOTIAMINE AT 10.0µg/mL

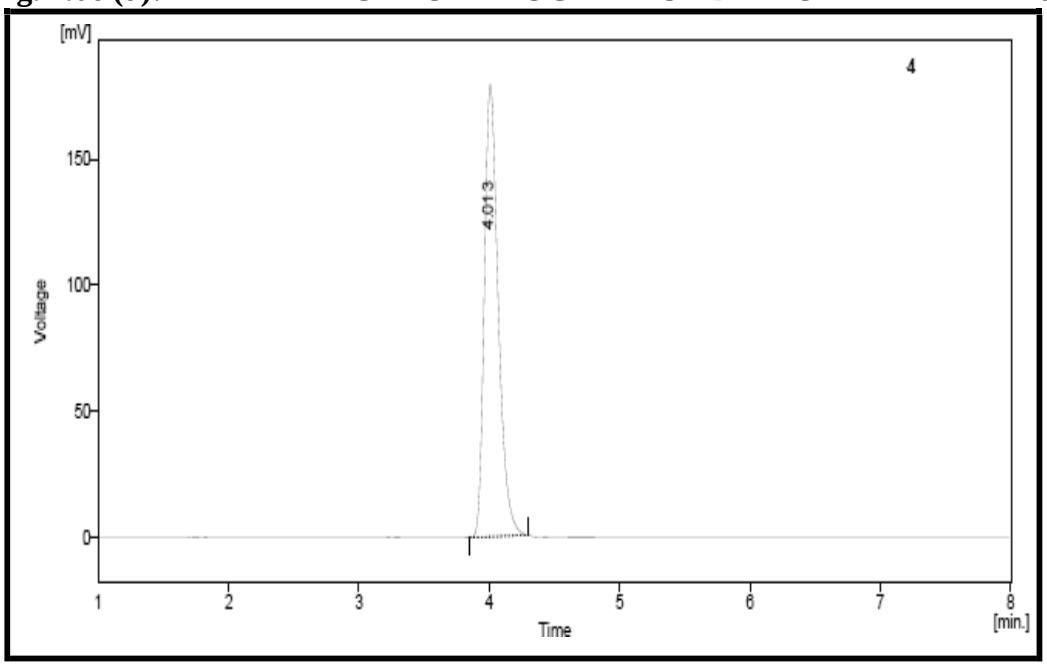

Fig. 1.06 (e):LINEARITY CHROMATOGRAM OF BENFOTIAMINE AT 12.5µg/mL

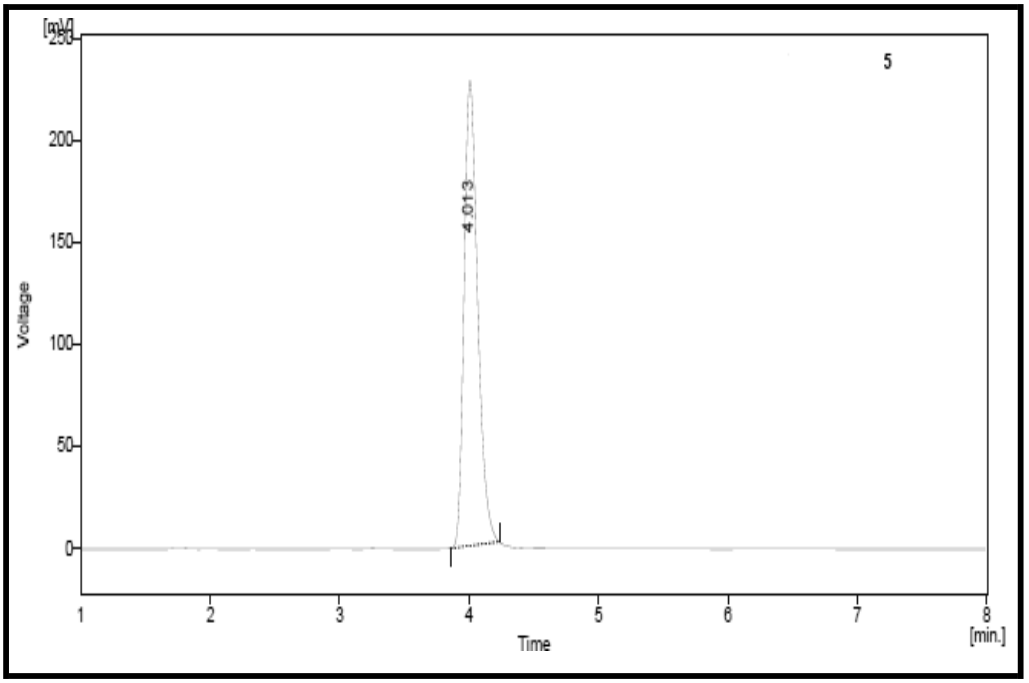

Fig.1.06(f):LINEARITY CURVE OF BENFOTIAMINE

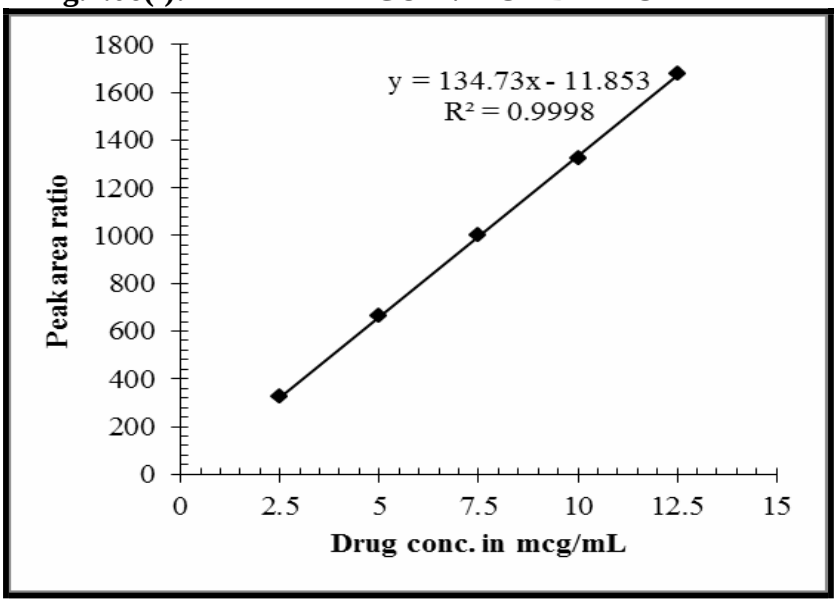

Fig. 1.07 (a):PRECISION CHROMATOGRAM OF BENFOTIAMINE (SET-1)

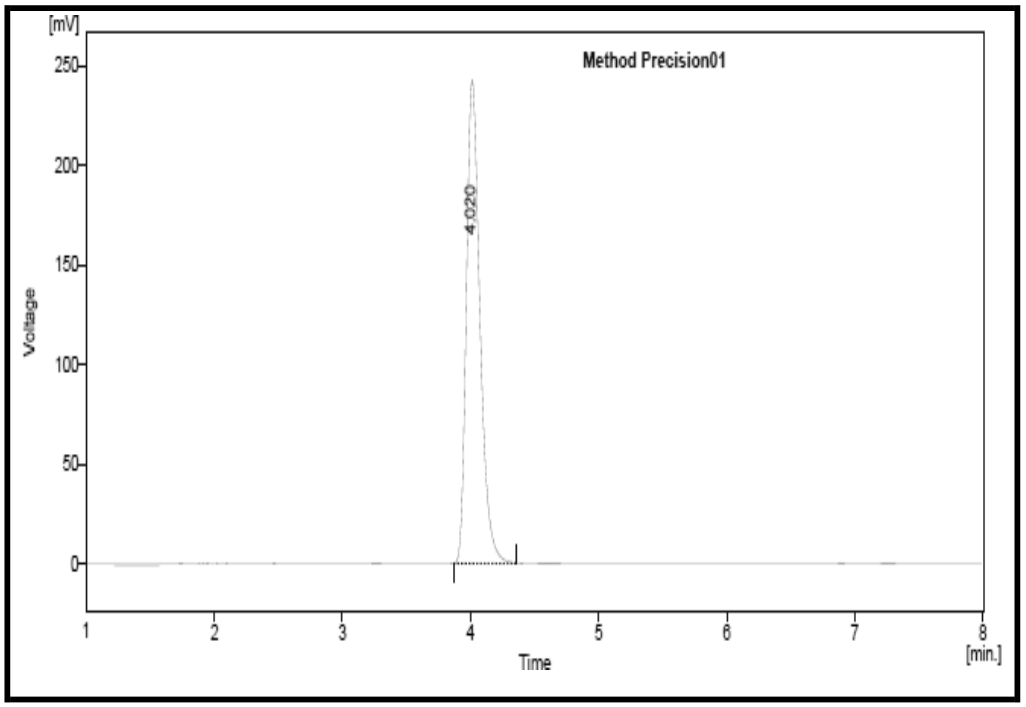

Fig. 1.07 (b):PRECISION CHROMATOGRAM OF BENFOTIAMINE (SET-2)

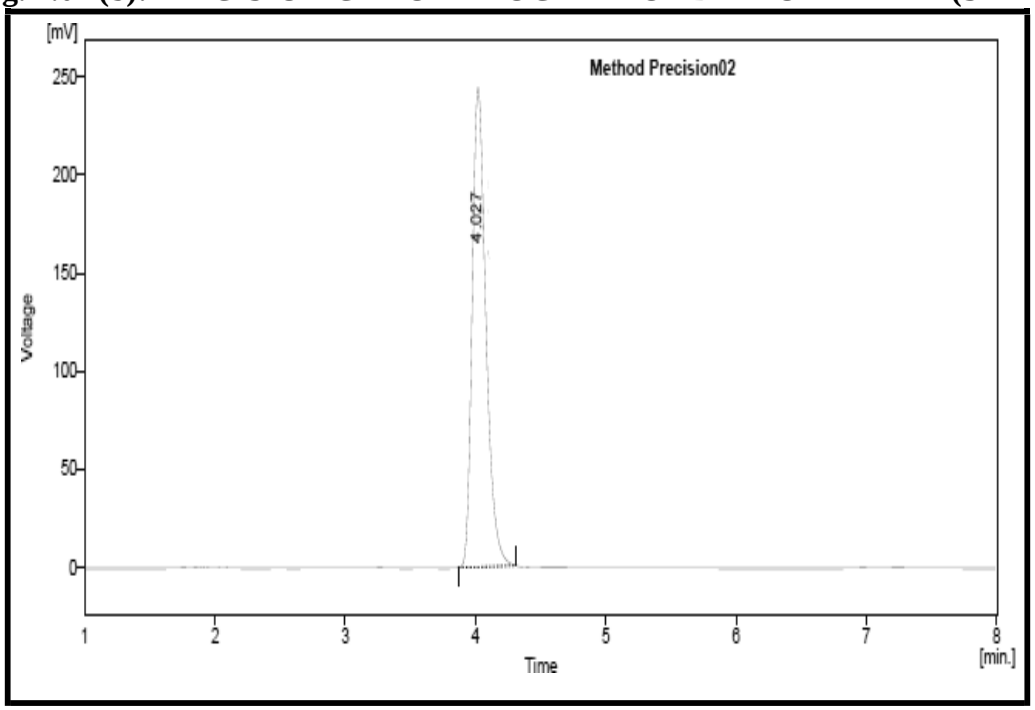

Fig. 1.07 (c):PRECISION CHROMATOGRAM OF BENFOTIAMINE (SET-3)

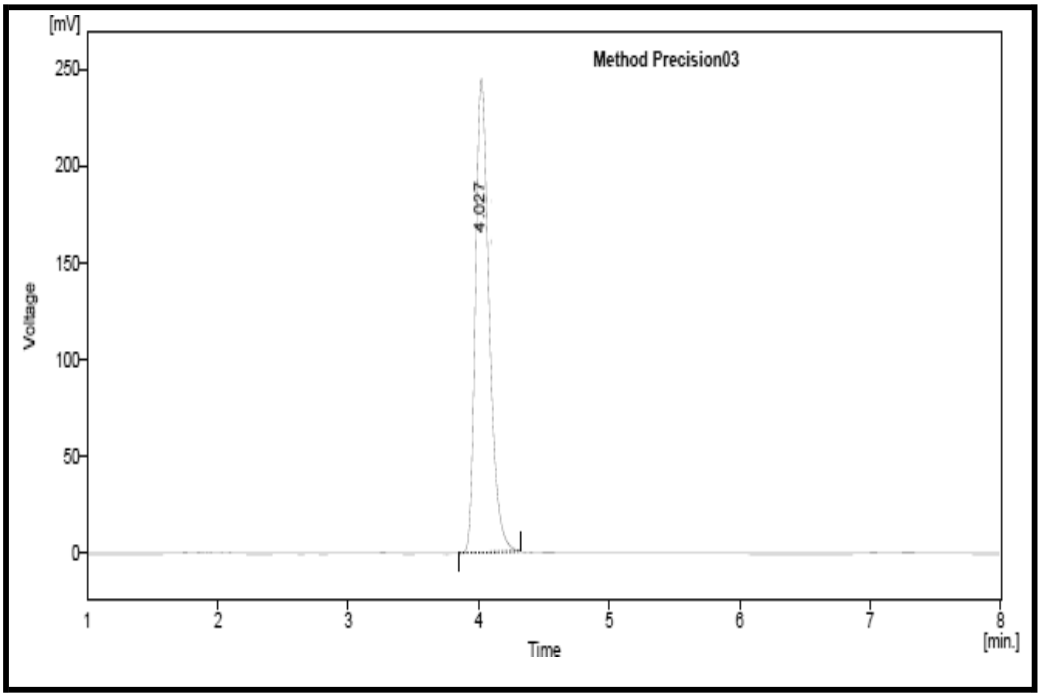

Fig. 1.07 (d):PRECISION CHROMATOGRAM OF BENFOTIAMINE (SET-4)

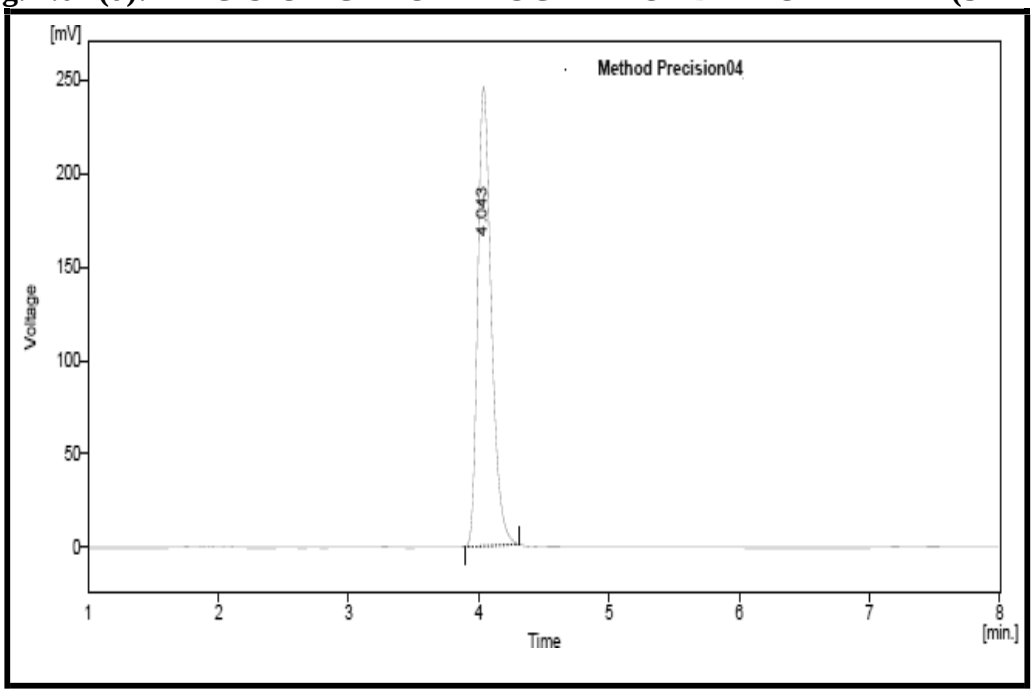

Fig. 1.07 (e):PRECISION CHROMATOGRAM OF BENFOTIAMINE (SET-5)

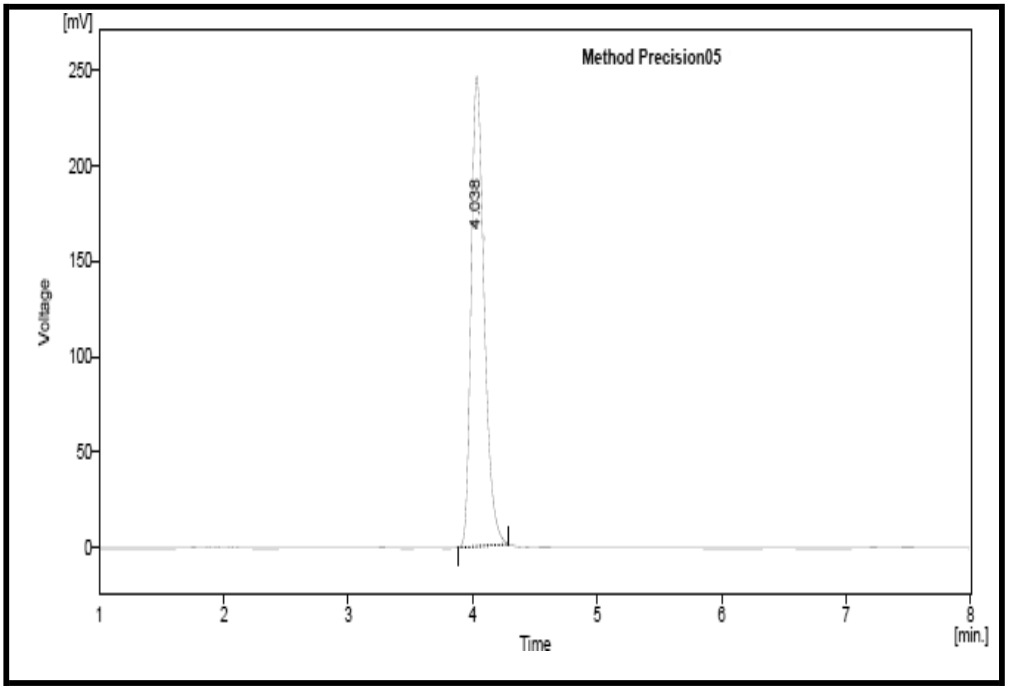

Fig.1.07(f):PRECISION CHROMATOGRAM OF BENFOTIAMINE (SET-6)

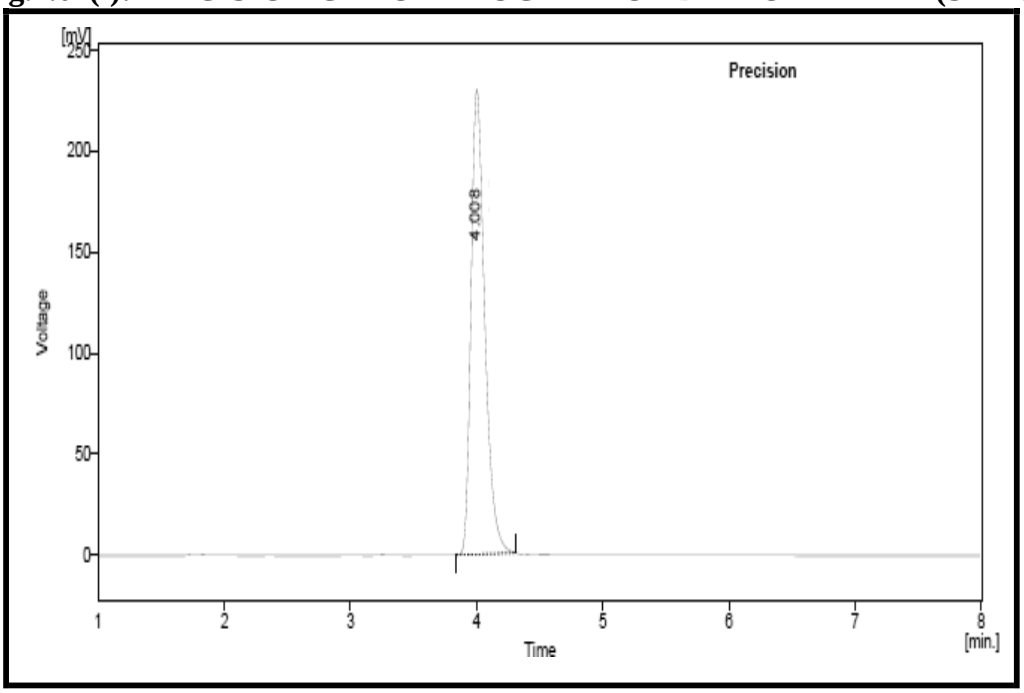

Fig. 1.08 (a):HPLC CHROMATOGRAM OF BENFOTIAMINE AT 50% ACCURACY LEVEL

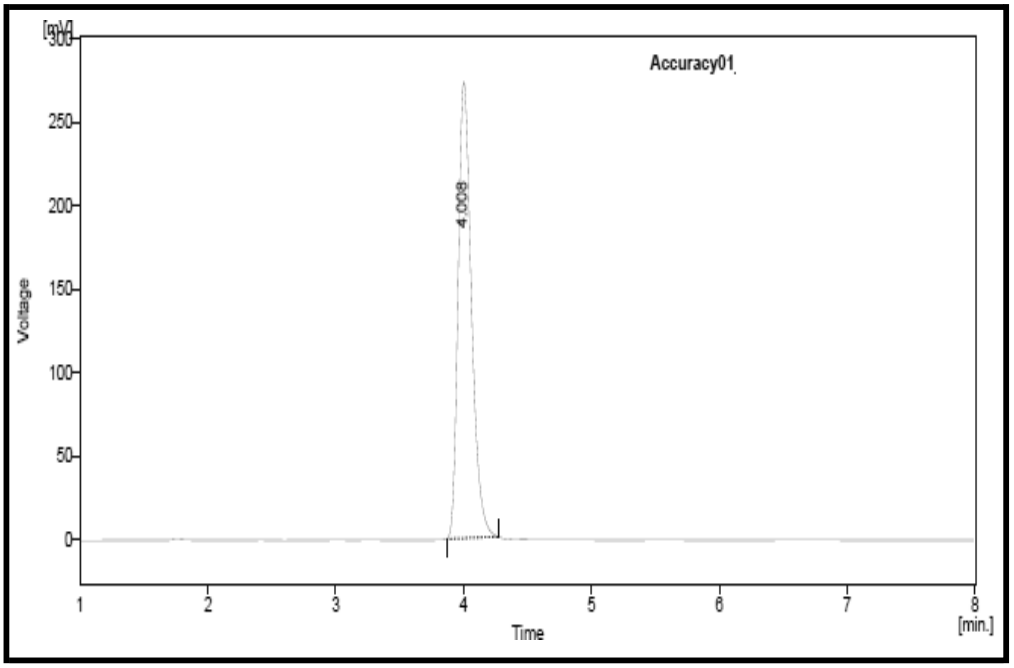

Fig. 1.08 (b):HPLC CHROMATOGRAM OF BENFOTIAMINE AT 100% ACCURACY LEVEL

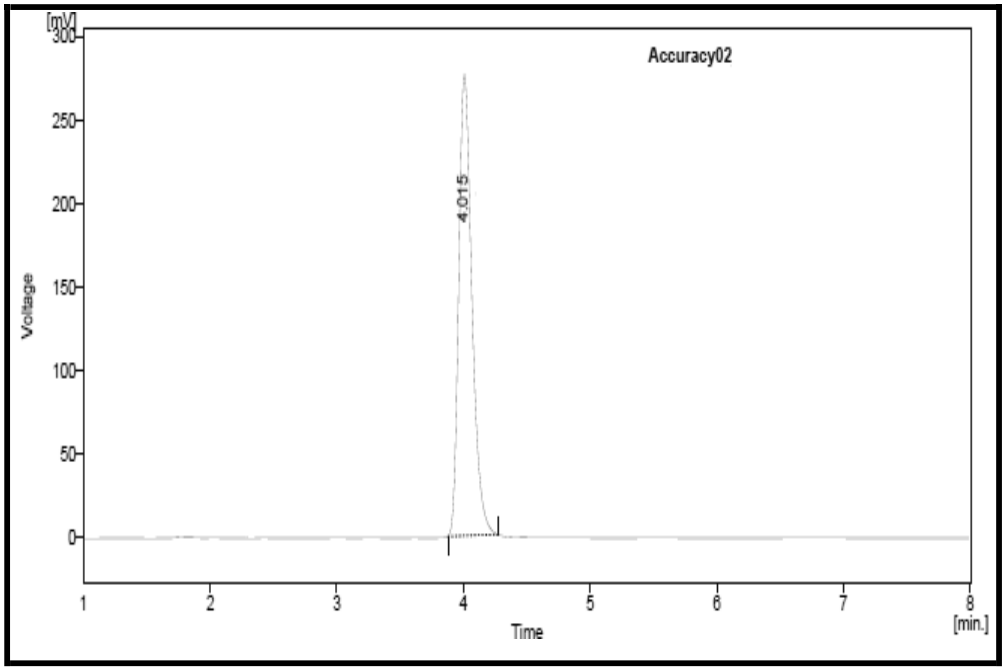

Fig.1.08(c):HPLC CHROMATOGRAM OF BENFOTIAMINE AT 150% ACCURACY LEVEL

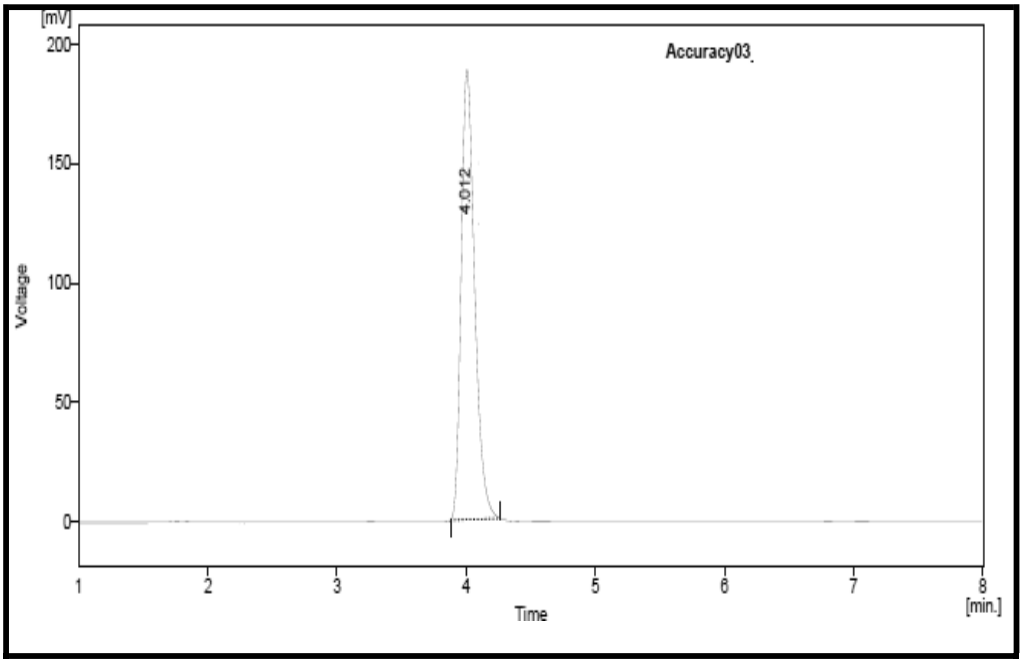

Fig.1.09:VALIDATIVE CHROMATOGRAM OF BENFOTIAMINE IN FORMULATION

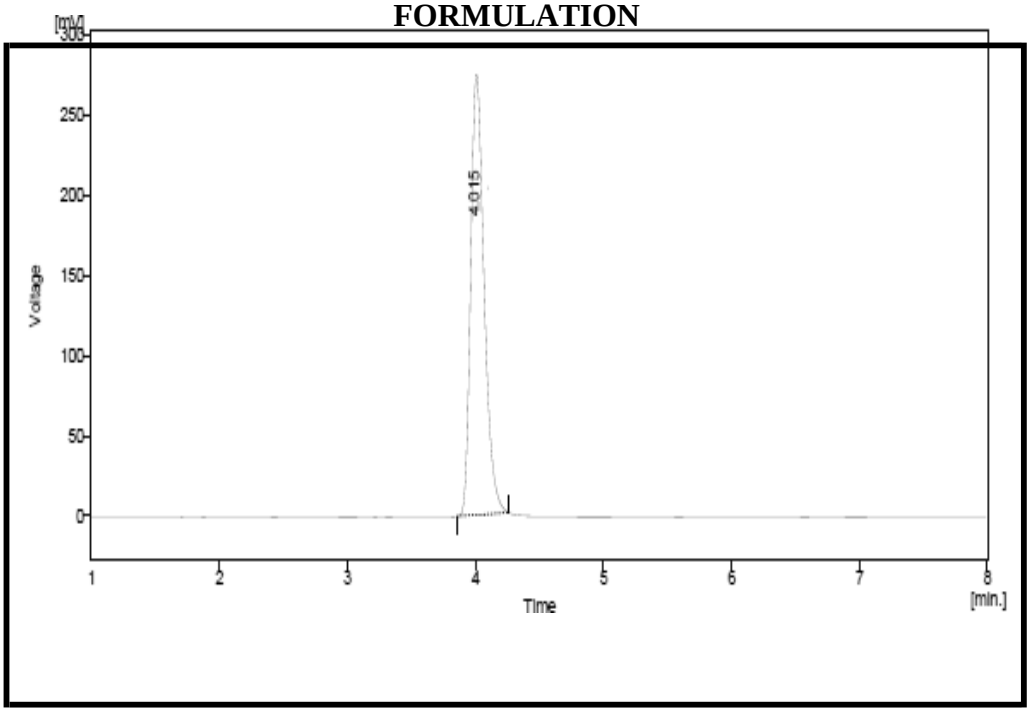

TABLE.1.01: SYSTEM SUITABILITY PARAMETERS

PARAMETERS	BENFOTIAMINE
Retention time	4.013
USP Plate count	6850
USP Tailing	1.129

TABLE.1.02: LINEARITY PERFORMANCE CALCULATIONS FOR BENFOTIAMINE

Concentration (µg.mL)	Area (mAU)
2.5	323.889
5.0	664.794
7.5	1002.264
10.0	1323.282
12.5	1678.714

Regression equation; Intercept (a)	-11.853
Slope (b)	134.73
Correlation coefficient	0.9998
Standard deviation on intercept (Sa)	0.6147
Standard deviation on slope (Sb)	1.065
LOD	0.0136
LOQ	0.0456

TABLE.1.03: RESULTS OF METHOD PRECISION

S No	Name	Area
1	**Injection-1**	1324.282
2	**Injection-2**	1336.249
3	**Injection-3**	1328.652
4	**Injection-4**	1329.312
5	**Injection-5**	1328.176
6	**Injection-6**	1327.532
Avg*		1329.034
Std Dev*		3.945
% RSD*		0.296

TABLE.1.04: RECOVERY STUDIES OF THE PROPOSED RP-HPLC METHOD

S.No	Area ($\mu v^2 sec$)		
	ACETAMINOPHEN		
	50%	**100%**	**150%**
Injection 1	323.889	1002.264	1678.714
Injection 2	324.654	1003.632	1699.645
Injection 3	323.621	1002.215	1656.365
Average*	324.057	1002.704	1678.241
Amt recovered (µg)*	49.98	99.97	149.98
% Recovery	99.96	99.97	99.98

*All the values are the averages of three determinations

TABLE.1.05: EVALUATION DATA OF RUGGEDNESS STUDY

No of injections	Ruggedness

	Analyst -1	**Analyst-2**
	Area	**Area**
Injection-1	1324.282	1324.148
Injection-2	1336.249	1323.149
Injection-3	1328.652	1325.687
Injection-4	1329.312	1324.232
Injection-5	1328.176	1327.146
Injection-6	1327.532	1323.524
AVG*	1329.034	1324.648
STDEV*	3.945	1.500
%RSD*	0.296	0.113

*All the values are the averages of three determinations

TABLE.1.06: EVALUATION DATA OF ROBUSTNESS STUDY

ROBUST CONDITIONS		**BENFOTIAMINE**
		RT
Flow rate	**0.8 mL/min**	4.043
	1.2 mL/min	4.007
TEMPERATURE	**35^{O} C**	4.012
	37^{O} C	4.008

TABLE.1.07: RESULTS OF ANALYSIS OF TABLET CONTAINING BENFOTIAMINE

PHARMACEUTICAL FORMULATION	**AMOUNT OF BENFOTIAMINE***		**% RECOVERY**
	LABELLED	**FOUND**	
BENALGIS	100mg	99.95	99. 95%

* Average of three determinations

C.CONCLUSIONS:

A new simple, precise, stability indicating RP-HPLC method was developed for the estimation of benfotiamine in bulk and pharmaceutical dosage form. It has been proved that the method is selective and linear between concentration range of 2.5- 12.5µg/ml for benfotiamine. The LOD was found to be 0.0136µg/ml and LOQ was found to be 0.456µg/ml for benfotiamine. The method was also found to be accurate and precise, as indicated by recovery studies close to 100 and %RSD is not more than 2 Statistical analysis proved that the method is suitable for the analysis of benfotiamine as bulk drug and in pharmaceutical formulation without any interference from the excipients that is recommended for routine and quality-control analysis the investigated drug in formulations.

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