IJPSR (2018), Volume 9, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 May, 2017; received in revised form, 17 July, 2017; accepted, 25 July, 2017; published 01 January, 2018

RP-HPLC METHOD FOR THE SIMULTANEOUS ASSAY OF METFORMIN AND BENFOTIAMINE: DEVELOPMENT AND VALIDATION

K. Lakshmi Prameela [1, 3], P. Rama Krishna Veni [2], P. V.V. Satyanarayana [3] and B. Hari Babu [*3]

Department of Chemistry [1], SGK Government Degree College, Vinukonda - 522647, Andhra Pradesh, India.
Department of Applied Sciences and Humanities [2], Sasi Institute of Technology and Engineering, Tadepalligudem - 534101, Andhra Pradesh, India.
Department of Chemistry [3], Acharya Nagarjuna University, Nagarjuna Nagar, Guntur - 522510, Andhra Pradesh, India.

Keywords:

Metformin, Benfotiamine, Benforce M, RP-HPLC, Analysis

Correspondence to Author:
B. Hari Babu

Assistant Professor,
Department of Chemistry,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur - 522510,
Andhra Pradesh, India.

E-mail: drharibabuanu2015@gmail.com

ABSTRACT: A sensitive, precise and accurate reverse phase high performance liquid chromatographic method (RP-HPLC) using an isocratic elution of 0.1 M NaH_2PO_4 (pH 3.0) and acetonitrile (80:20 *v/v*) with a flow rate of 1.0 mL/min, a column temperature of 30 °C and UV detection at 254 nm was developed for the estimation of metformin and benfotiamine simultaneously. The method was validated following guidelines given by International Conference Harmonization. The method allowed the determination of metformin and benfotiamine in the concentration range of 100-300 µg/mL and 15-45 µg/mL, respectively. The limit of detection & limit of quantification were 0.290 µg/mL & 0.968 µg/mL for metformin and 0.047 µg/mL & 0.156 µg/mL for benfotiamine, respectively. The percent relative standard deviation (%RSD) value was 0.067 % for metformin and 0.047 % for benfotiamine. The accuracies were 99.510 - 99.797% and 9.652 - 99.890% for metformin and benfotiamine, respectively. The developed and validated RP-HPLC method was applied successfully for the quantification of metformin and benfotiamine simultaneously in tablet dosage form. Common excipients in the tablet dosage form did not interfere with the assay of metformin and benfotiamine. Hence, the proposed RP-HPLC method is suggested for routine analysis of metformin and benfotiamine in quality control laboratories.

INTRODUCTION: Benfotiamine, chemically called as S-[(Z)-2-[(4-amino-2-methylpyrimidin-5-yl) methyl-formylamino]-5-phosphonooxypent - 2-en-3-yl] benzenecarbothioate (**Fig. 1**), is a synthetic fat soluble form of Vitamin B1 (thiamine) [1]. Benfotiamine belongs to allithiamines family of compounds.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.9(1).264-70

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(1).264-70

Benfotiamine may be used as a medicine or dietary supplement and is prescribed for the treatment of sciatica and other painful nerve conditions [2]. By reducing tissue advanced glycation end products, it prevents complications such as blood vessel damage due to diabetes and atherosclerosis [3, 4].

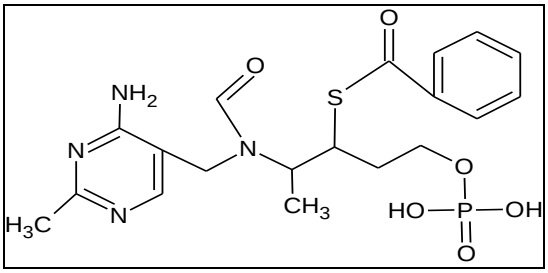


FIG. 1: CHEMICAL STRUCTURE OF BENFOTIAMINE

Metformin, chemically described as 3-(diamino methylidene)-1,1-dimethylguanidine **(Fig. 2)**, is an oral hypoglycemic agent belonging to the biguanides class of compounds. Metformin is prescribed for the management of non insulin dependent diabetes mellitus [5, 6]. Metformin exerts hypoglycemic activity by decreasing hepatic production & intestinal absorption of glucose and improving insulin sensitivity. All the effects are mediated by the activation of enzyme adenosine monophosphate (AMP)-activated protein kinase by metformin[7 - 9].

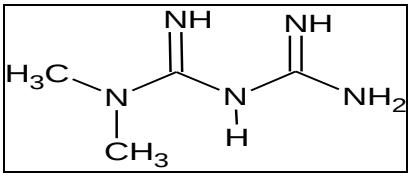


FIG. 2: CHEMICAL STRUCTURE OF METFORMIN

The combination of benfotiamine and metformin is used in the treatment and control of Wernicke-korsakoff syndrome, diabetic neuropathy, maturity onset diabetes, type 2 diabetes mellitus and polycystic ovary syndrome. The simultaneously analysis of these two drugs is not reported in any pharmacopeia. Therefore, it is essential to develop an effective method for the simultaneous determination of benfotiamine and metformin. Few HPLC methods have been proposed for the assay of benfotiamine and metformin simultaneously in pharmaceutical dosage forms[10 - 12].

Adithya *et al.*, have reported an RP-HPLC method using a Phenomenex Luna C18 analytical column (250 mm x 4.6 mm, 5 µm) and a mobile phase consisting of methanol, acetonitrile, water & 0.1 % orthophosphoric (20:40:35:5 *v/v/v/v*) at a flow rate of 1.0 mL/min. The UV detector was set at 249 nm[10]. In the second HPLC method reported by Mihirkumar *et al*., the separation and assay was carried on Waters column C18 (250 mm x 4.6 mm, 5 µm) analytical column using a mixture of water and acetonitrile (75:25 *v/v*) as mobile phase with a flow rate of 0.8 mL/min. The pH of the mobile phase was set to 3.2 with orthophosphoric acid. The effluents were detected with detector set at 254 nm[11]. In the RP-HPLC method proposed by Deepali *et al*.,[12] the chromatographic separation was done with Thermo Hypersil BDS C18 (250 mm × 4.6 mm, 5 µm) analytical column. The mobile phase used was a mixture of methanol and 10 mM phosphate buffer (ortho phosphoric acid was used to adjust the pH to 3.2) in the ratio of 80:20 (*v/v*). The flow rate was maintained at 1 mL/min and effluents were detected at 239 nm.

In the present investigation, a novel RP-HPLC method was developed for determination of benfotiamine and metformin simultaneously in bulk and tablet dosage form. Further, the method validation has been carried out as stated by the International Conference on Harmonization guidelines [13]. The validated and developed RP-HPLC method was successfully applied to tablet dosage form.

The details of the proposed and reported RP-HPLC methods were shown in **Table 1**. The data given in the **Table 1** indicated that the proposed has the advantages of being more sensitive [10, 11], more precise [10 - 12] and more accurate [10 - 12] than the reported methods.

TABLE 1: SUMMARY OF PROPOSED AND REPORTED RP-HPLC METHODS

Method	Drug	Retention time (min)	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	RSD (%)	Recovery (%)	Reference
RP-HPLC	Ben	3.840	5-35	0.159	0.483	0.32-0.69	99.10-100.17	Adithya *et al*., [10]
	Met	2.297	50-200	0.949	2.870	0.28-0.88	99.31-100.44	
RP-HPLC	Ben	3.881	30-90	0.65	1.60	0.67-1.30	99.65-100.15	Mihirkumar *et al*.,[11]
	Met	2.125	200-600	0.19	0.49	0.11-0.31	99.94-100.20	
RP-HPLC	Ben	2.583	1-6	0.01	0.1	0.96-1.22	98.87-111.26	Deepali *et al*.,[12]
	Met	3.233	0.1-5	0.005	0.01	0.78-1.76	98.11-110.01	
RP-HPLC	Ben	3.567	15-45	0.047	0.156	0.067	99.65-99.89	Proposed method
	Met	2.059	100-300	0.290	0.968	0.047	99.51-99.79	

Ben – Benfotiamine: Met – Metformin

The retention times of benfotiamine and metformin in the proposed method were less when compared with the reported methods [10 - 12]. Though the Deepali *et al*.,[12] method is sensitive, it has very narrow range of linearity and system suitability data is not reported. Unlike the method of Adithya

et al.,[10], the proposed method does not use four solvents as mobile phase. The utilization of more solvents as mobile phase may decrease the accuracy of the method and increase the cost of analysis.

MATERIALS AND METHODS:

Materials: Metformin and benfotiamine were supplied by Lara Drugs Private Limited (Telangana, India). The tablet dosage form, Benforce M, strength 500 mg metformin and 75 mg benfotiamine, manufactured by Shield Healthcare Pvt. Ltd., Valencia was purchased from local pharmacy market. The acetonitrile (HPLC grade) was obtained from Merck India Ltd., Mumbai, India. Analytical reagent orthophosphoric acid and sodium dihydrogen orthophosphate were from Sd. Fine Chemicals Ltd., Mumbai, India. Water was obtained from Milli-Q system (Millipore, USA).

Apparatus and HPLC conditions: Waters Alliance 2695 Module HPLC system with a 2998 PDA detector and Empower 2 software was used for the analysis of metformin and benfotiamine. The Hypersil C18 (250 x 4.6 mm; 5 µm particle size) analytical column was used. Isocratic mobile phase was consisted of 0.1 M NaH_2PO_4 and acetonitrile in 80:20 (*v/v*) ratio with pH 3.0 (adjusted with orthophosphoric acid). The prepared mobile phase was also used as diluent. Flow rate was maintained at 1.0 mL/min. The eluted compounds were detected at 254 nm. The column temperature was set at 30±1 ^oC. An injection volume of 10 µL was used.

Standard solutions: A stock standard solution (metformin - 5 mg/mL & benfotiamine - 0.75 mg/mL) was prepared by dissolving 500 mg of metformin and 75 mg of benfotiamine in a final volume of 100 mL mobile phase. Working standard solutions (100 to 300 µg/mL- metformin & 15 to 45 µg/mL - benfotiamine) was prepared from the above stock solution by appropriate dilution with mobile phase.

Tablet sample solution: Ten tablets were weighed, transferred to a clean dry mortar and ground into fine powder. A quantity of tablet powder equivalent to 500 mg of metformin and 75 mg of benfotiamine was taken in a 100 mL volumetric flask containing 30 mL of mobile phase. The flask was sonicated for 10 min to dissolve the drugs completely. The resulting solution was diluted to volume with the mobile phase to give a solution containing 5 mg/mL metformin and 0.75 mg/mL benfotiamine. A 0.45 µm pore size membrane filter was used to filter the solution. Appropriate dilution (200 mg/mL- metformin & 30 mg/mL-benfotiamine) was prepared in mobile phase for analysis.

Calibration graph: Ten µL aliquot of each working standard solution was injected thrice into the column. The chromatograms and peak area response were recorded. Calibration graphs were constructed for metformin and benfotiamine by plotting the concentration of drug versus peak area response. From these calibration graphs, the concentration of metformin and benfotiamine was calculated. Alternatively, the concentration of the selected drugs can be determined from the regression equation obtained from the peak area and concentration data.

Assay of metformin and benfotiamine in combined tablet: Ten µL of the tablet sample solution was injected thrice into the HPLC system. The chromatograms and peak area response were recorded. The amount of metformin and benfotiamine in the combined tablet dosage form was determined from the corresponding calibration graph or corresponding regression equation.

RESULTS AND DISCUSSION:

Method development: The present investigation was aimed to establish a robust, reliable and sensitive RP-HPLC method for the simultaneous estimation of metformin and benfotiamine in combined tablet dosage form. During method development, two different columns like the Inertsil ODS-3V analytical column (250 mm x 4.6 mm, particle size 5 µm) and Hypersil BDS C18 analytical column (250 mm x 4.6 mm, particle size 5 µm) were tried. Better results (good symmetrical sharp peak, acceptable tailing factor and resolution) were obtained with Hypersil BDS C18 analytical column (250 mm x 4.6 mm, particle size 5 µm) maintained at a temperature of 30±1 ^oC. Hence, the same column and temperature was chosen for analysis.

Various mobile phases [Na_2HPO_4 (0.1M): Methanol, K_2HPO_4(0.1M): Acetonitrile and NaH_2PO_4 (0.1M): Acetonitrile] with different

ratios, flow rate and pH were tried and the responses were recorded. After a series of experiments, highly symmetrical and sharp peaks of metformin and benfotiamine with better resolution were obtained at pH 3.0 by using NaH_2PO_4 (0.1M): acetonitrile (80:20 *v/v*) as mobile phase with a flow rate of 1.0 mL/min. The metformin and benfotiamine in the selected mobile phase have adequate absorption at 254 nm, which was hence chosen for the analysis. **Fig. 3** shows a HPLC chromatogram of metformin and benfotiamine using optimized chromatographic conditions.

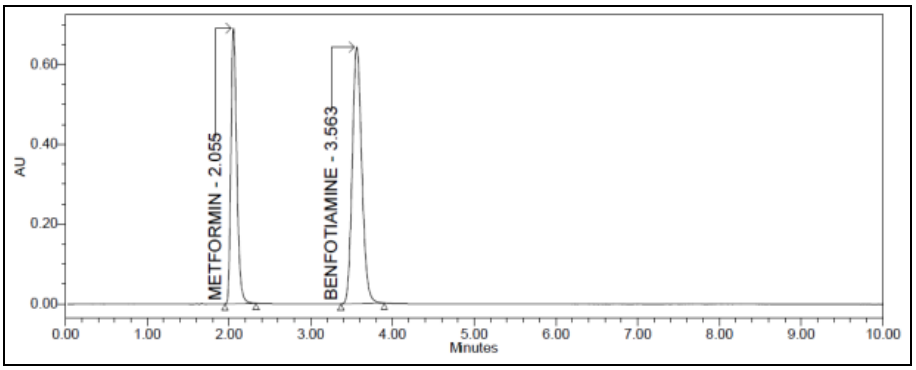


FIG. 3: CHROMATOGRAM OF METFORMIN AND BENFOTIAMINE UNDER OPTIMIZED HPLC CONDITIONS

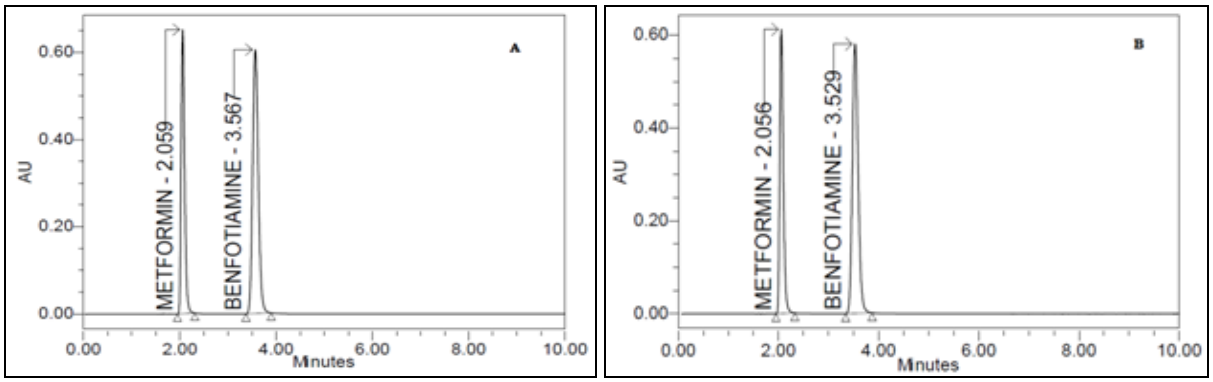
Method validation: Using ICH guidelines[13], the developed method was validated.

System suitability test: In order to evaluate the satisfactory reproducibility and resolution of the proposed method, suitability parameters such as % RSD of retention time, % RSD of peak area, USP tailing factor and USP plate count were investigated. System suitability of the method was evaluated by injecting standard solution (metformin - 200 µg/mL and benfotiamine - 30µg/mL) five times into the HPLC system. The results (**Table 2**) demonstrate the method suitability.

TABLE 2: RESULTS OF SYSTEM SUITABILITY OF THE PROPOSED METHOD

Parameters	Metformin (200 µg/mL)	Benfotiamine (30 µg/mL)
Retention time of drug	2.059 (% RSD – 0.111)	3.562 (% RSD – 0.188)
Peak area response	3323893 (%RSD – 0.482)	5044426 (%RSD – 0.495)
USP resolution	-	8.424
USP plate count	3797	4345
USP tailing factor	1.328	1.160

Specificity: Specificity was determined by comparing the chromatograms of mobile phase blank, placebo blank, working standard (metformin 500 µg/mL and benfotiamine 30 µg/mL) and tablet sample (metformin 500 µg/mL and benfotiamine 30 µg/mL). It was confirmed that the signal observed was merely by the analytes. For this purpose, solutions of placebo blank, mobile phase blank, working standard and tablet sample were injected into the system. The resulting chromatograms are shown in **Fig. 4**.



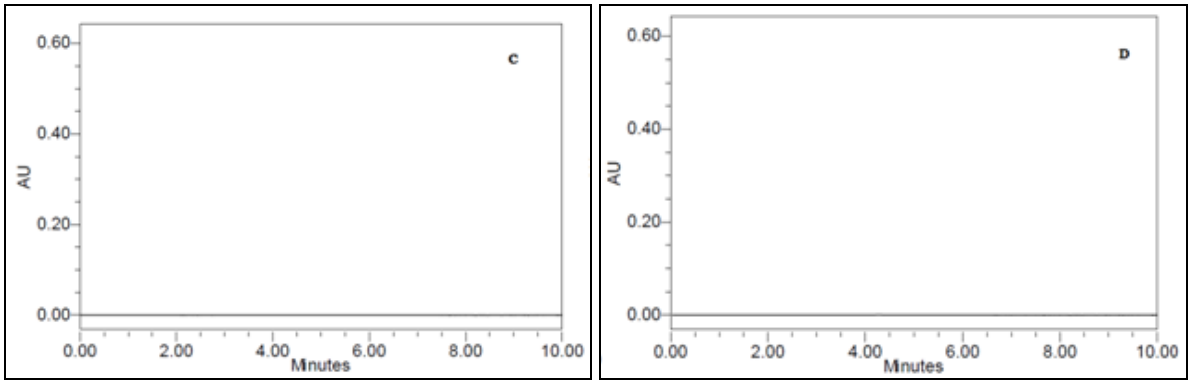


FIG. 4: CHROMATOGRAM OF (a) METFORMIN AND BENFOTIAMINE WORKING STANDARD SOLUTION (b) TABLET SAMPLE SOLUTION (c) PLACEBO BLANK (d) MOBILE PHASE BLANK

The chromatograms of placebo blank and mobile phase did not show any peaks whereas the chromatograms of working standard and tablet sample did not show any peaks other than that of metformin and benfotiamine. This confirmed the method specificity.

Linearity: Plot of the peak area response against concentration gave the linear relationship in the concentration range 100-300 µg/mL for metformin (**Fig. 5**) and 15-37.5 µg/mL for benfotiamine (**Fig. 6**). From the regression analysis, the linear equation was: $y = 16533\,x + 6168$ for metformin; $y = 16766\,x + 6204$ for benfotiamine (where y = peak area and x = concentration of analyte in µg/mL).

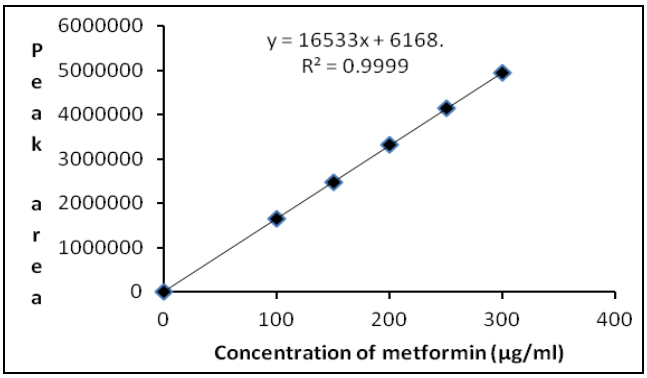


FIG. 5: METFORMIN CALIBRATION CURVE

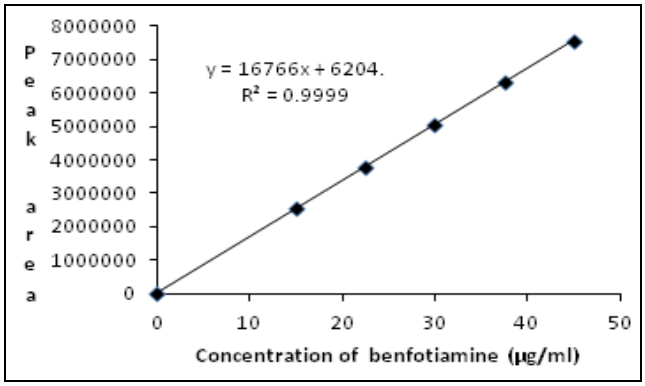


FIG. 6: BENFOTIAMINE CALIBRATION CURVE

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ were calculated according to ICH guidelines. The LOD for metformin and benfotiamine was 0.290 and 0.047 µg/mL, respectively, while LOQ was 0.968 and 0.156 µg/mL, respectively.

Precision and accuracy: The precision & accuracy of the proposed method was assessed by applying the HPLC method for the assay of working standard solution of metformin and benfotiamine at a concentration of 200 and 30µg/mL, respectively for 6 successive times. The precision & accuracy were expressed as % relative standard deviation and % recovery, respectively. The results of the study are shown in **Table 3**. Low values of % RSD & good % recovery values confirm the satisfactory precision & accuracy of this present HPLC method

TABLE 3: PRECISION AND ACCURACY DATA OF THE PROPOSED METHOD

Metformin (200 µg/mL)		Benfotiamine (30 µg/mL)	
Peak area	**Recovery (%)**	**Peak area**	**Recovery (%)**
3322926	99.571	5044307	99.998
3322776	99.567	5039302	99.898
3319201	99.459	5043376	99.979
3324722	99.625	5043516	99.982
3319054	99.455	5039600	99.904
3321506	99.528	5039192	99.896
Average	99.534	**Average**	99.943
RSD (%)	0.067	**RSD (%)**	0.047

Recovery studies: The method accuracy was further checked by means of standard addition method. For this, the pre-analyzed sample solution was spiked with known concentration of metformin and benfotiamine at 3 concentration levels (50 %, 100 % and 150 %). The % recovery data (**Table 4**) shows that the proposed method was accurate.

Common excipients in tablets did not interfere with the assay of metformin and benfotiamine.

Robustness: This was performed by assessing the influence of slight deliberate changes in HPLC conditions on the system suitability parameters of the proposed method. The selected conditions are temperature (± 5 °C) and flow rate (± 0.1 mL/min). The results are summarized in **Table 5**. In all the cases, good separations of both metformin and benfotiamine were achieved and the system suitability parameters are well within the acceptable limits, indicating that the proposed HPLC method remained robust under the optimized conditions.

TABLE 4: RESULTS OF RECOVERY STUDIES OF THE PROPOSED METHOD

Spiked level (%)	Concentration of metformin (µg/mL)		Recovery (%)	Mean (%)	Concentration of benfotiamine (µg/mL)		Recovery (%)	Mean (%)
	Added	Found			Added	Found		
50	99.60	99.193	99.592	999.510	15.00	14.979	99.863	99.890
	99.60	98.772	99.168		15.00	14.988	99.917	
	99.60	99.371	99.770		15.00	14.983	99.889	
100	199.20	199.287	100.043	99.797	30.00	29.940	99.799	99.826
	199.20	197.761	99.278		30.00	29.963	99.877	
	199.20	199.341	100.071		30.00	29.940	99.801	
150	298.80	297.634	99.610	99.749	45.00	44.843	99.651	99.652
	298.80	298.597	99.932		45.00	44.894	99.765	
	298.80	297.923	99.706		45.00	44.794	99.542	

TABLE 5: ROBUSTNESS DATA OF THE PROPOSED HPLC METHOD

Parameter	Metformin (200 µg/mL)			Benfotiamine (30 µg/mL)		
	USP Tailing	USP plate count	USP resolution	USP Tailing	USP plate count	USP resolution
Flow rate 1.0 + 0.1 mL/min	1.35	3905	-	1.17	4900	8.56
Flow rate 1.0 – 0.1 mL/min	1.33	3553	-	1.15	3993	7.85
Temperature 30 + 5 ^{o}C	1.38	3921	-	1.18	4745	8.48
Temperature 30 - 5 ^{o}C	1.35	3601	-	1.16	3993	7.87

CONCLUSION: An attempt was made in the present study to develop a sensitive, accurate, precise and selective RP-HPLC method for the simultaneous analysis of metformin and benfotiamine in bulk and tablet dosage forms. The developed RP-HPLC method was validated in harmony with ICH guidelines. The main features of the developed RP-HPLC method are economical, low retention time of analytes, selective, robust, sensitive and satisfactory precision and accuracy. Therefore, the developed and validated RP-HPLC method can be recommended for the simultaneous quantification of metformin and benfotiamine in quality control laboratories or industry.

ACKNOWLEDGEMENT: The authors are thankful to Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, India for support and encouragement.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Iva B, Danijela S, Danijela L, Ivana B, Ivan M, Sanja P, Nadezda N and Irena L: Benfotiamine attenuates inflammatory response in LPS stimulated BV-2 microglia. PLoS One 2015; 10: e0118372.
2. Balakumar P, Rohilla A, Krishan P, Solairaj P and Thangathirupathi A: The multifaceted therapeutic potential of benfotiamine. Pharmacological Research 2010; 61: 482–488.
3. Helen V and Jaime U: Advanced glycation end products (AGE) and diabetes: cause, effect, or both?. Current Diabetes Reports 2014; 14: 453.
4. Ferdinando G and Michael B: Oxidative stress and diabetic complications. Circulation Research 2010; 107: 1058–1070.
5. Jahan AA, Nasim K, Sidra Z, Syed SA and Anjum AH: Metformin treatment in type 2 diabetes in pregnancy: an active controlled, parallel-group, randomized, open label study in patients with type 2 diabetes in pregnancy. Journal of Diabetes Research 2015; 2015: 325851.
6. Metformin Hydrochloride. The American Society of Health-System Pharmacists. Retrieved Jan 2016.
7. Rena G, Pearson ER and Sakamoto K: Molecular mechanism of action of metformin: old or new insights?. Diabetologia 2013; 56: 1898–1906.
8. Burcelin R. The antidiabetic gutsy role of metformin uncovered?. Gut 2013; 63: 706–707.

9. Yaeni K and Cheol WP: Adenosine monophosphate–activated protein kinase in diabetic nephropathy. Kidney Research and Clinical Practice 2016; 35: 69–77.
10. Adithya BP, Vijayalakshmi M and Mahesh J: Development and validation of RP-HPLC method for the simultaneous estimation of benfotiamine and metformin hydrochloride in tablet dosage form. Rajiv Gandhi University of Health Sciences Journal of Pharmaceutical Sciences 2012; 2: 87-91.
11. Mihirkumar GP, Pravin OP and Sanjay BB: Validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and benfotiamine in bulk drug and in pharmaceutical dosage form. International Journal of Analytical and Bioanalytical Chemistry 2012; 2: 196-200.
12. Deepali AN, Vidhya KB and Sunil RD: Validated HPLC method for simultaneous quantitation of benfotiamine and metformin hydrochloride in bulk drug and formulation. International Journal of Pharmacy and Pharmaceutical Sciences, International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5 (Suppl 2): 138-142.
13. ICH (Q2B): Note for guidance on validation of analytical procedures: methodology. 1996, Geneva: International conference on Harmonization.

How to cite this article:
Prameela KL, Veni PRK, Satyanarayana PVV and Babu BH: RP-HPLC method for the simultaneous assay of metformin and benfotiamine: development and validation. Int J Pharm Sci & Res 2018; 9(1): 264-70. doi: 10.13040/IJPSR.0975-8232.9(1).264-70.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)