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Analytical Method Development and Validation of Metformin Hydrochloride and Benfotiamine in Bulk and Marketed Formulations

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Original Research Article

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Abstract: In the present work, two simple and sensitive UV spectrophotometric methods have been developed for the quantitative estimation of Metformin Hydrochloride and Benfotiamine in combination using bulk and pharmaceutical dosage forms. Method A is Simultaneous equation method which involves measurement of absorbance at selected wavelength and solving the simultaneous equation to calculate the amount of drug present. Distilled water was used as the solvent for the analysis of both drugs. Two wavelengths 230 and 246 nm were selected for the estimation of Metformin Hydrochloride and Benfotiamine. LOD for Metformin Hydrochloride and Benfotiamine were found to be 0.03µg/ml and 0.27µg/ml respectively. Method validation was done as per ICH guidelines. Method B is Absorbance ratio method which involves, formation of Q-Absorbance equation at 239 nm (isoabsorptive point) and 246 nm (λmax of Benfotiamine) in distilled water. The Linearity lies between 2-16 µg/ml for Metformin Hydrochloride and 2-18 µg/ml for Benfotiamine with r2=0.999 and 0.998 respectively. LOD for both drugs were found to be 0.16 µg/ml and 0.3 µg/ml respectively. Method validation was done as per ICH guidelines.

Keywords: Metformin Hydrochloride, Benfotiamine, Distilled water, UV Spectroscopy, Simultaneous equations, Q – Absorbance.

INTRODUCTION

Benfotiamine: (S-benzoylthiamine O-monophosphate) is a synthetic S-acyl derivative of thiamine (vitamin B1) which belongs to a family of compounds known as allithiamines, a member of the class of lipophilic thiamine derivatives first identified in heated garlic in 1950.

Later it was confirmed that similar chemical compounds could be formed by making use of other allium vegetables. A study in rabbits showed that allithiamines can be formed in situ in the intestine, when garlic and thiamine is present. When thiamine reacts with allicin and other sulphur compounds in allium vegetables, it opens thiamine's thiazole ring, which leads to the formation of lipophilic molecule, which diffuses across cell membranes readily [1,2]. This lipophilic molecule contains an open thiazole ring that raises levels of thiamine in blood and tissues to a much higher degree compared to water soluble salts. Benfotiamine shows inhibiting effect on three major biochemical pathways involved in diabetes. It is also known to show beneficial effect in the end stage of renal disease and alcoholic neuropathy [3]. It shows significant decreases in pro-inflammatory mediators [4]. A literature survey revealed that several analytical methods have been reported for Benfotiamine in single form and in combination with other drugs including HPLC [5], HPTLC [6], RP-HPLC [7], UV Area under the curve [8] methods.

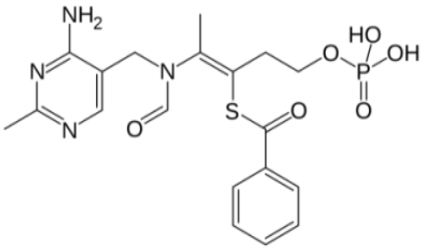

Fig-1: Structure of Benfotiamine

Metformin Hydrochloride is an oral Anti-diabetic drug, which is chemically N, N-dimethylimidodicarbonimidicdiamide Hydrochloride (1, 1-dimethylbiguanide Hydrochloride). It suppresses excessive hepatic glucose production and improves glucose clearance. It also shows predominant effect in decreasing fasting plasma glucose. It is official in Indian Pharmacopoeia, British Pharmacopoeia, European Pharmacopoeia and United state Pharmacopoeia. Metformin is the first-line drug used to treat type 2 diabetes, mostly in overweight and obese people and ones with normal kidney function.

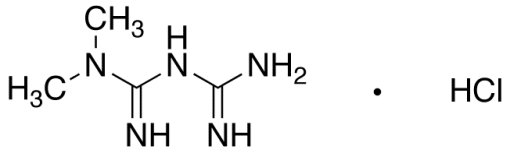

Fig-2: Structure of Metformin Hydrochloride

Combination of these two drugs is also used in case of infertility as it shows prevention of glycation of products which causes aging and hence helps in normalising cycles and conception. Combination of Metformin Hydrochloride and Benfotiamine is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of these two drugs in their combined dosage forms.

A literature survey revealed that several analytical methods have been reported for Benfotiamine in single form and in combination with other drugs including HPLC, HPTLC, RP-HPLC, UV spectroscopy methods. Simultaneous equations and Absorbance ratio methods have been proposed for this combination, which are not reported till date.

MATERIALS AND METHODS

Instrument

Absorption spectral measurements were carried out using a double beam UV- Visible spectrophotometer (JASCO V- 630, JAPAN). Matched quartz cells of length 1 cm were used. Weighing of all the chemicals was carried out on the Axpert weighing balance. Samples were sonicated using PCI Analytics sonicator.

Reagents and chemicals

Standard sample of Metformin HCl was purchased from Yarrow Chem. Mumbai, India. The reference sample of Benfotiamine was procured from Essarkay Chemicals & Equipment Centre, Mangalore, Karnataka, India. Benforce M tablets were purchased from local market. Water was purified by glass distillation apparatus in laboratory.

Preparation of stock solution and selection of wavelength for analysis

Standard stock solutions of Metformin Hydrochloride (MET) and Benfotiamine (BEN) were prepared separately. MET and BEN were accurately weighed and transferred to two separate clean and dry 100 ml volumetric flask. Each drug was dissolved in sufficient volume of distilled water.1 ml of conc. HCl was added to the flask containing Benfotiamine to dissolve the drug completely. Both the flasks were sonicated for 15 mins and volume was made up to the mark with distilled water to obtain final concentration of 100 µg/ml each. The stock solutions of Metformin Hydrochloride and Benfotiamine were further diluted with distilled water to obtain the concentration of 10µg/ml. The resulting solutions were then scanned in UV spectrophotometer from 400 to 200 nm. From the resulting spectra λmax for Metformin Hydrochloride and Benfotiamine were calculated separately. Spectra of Metformin Hydrochloride and Benfotiamine is presented in fig 3 and 4. Both the spectra of Metformin hydrochloride and Benfotiamine were overlayed. From overlay of both the spectra, isoabsorptive point of Metformin Hydrochloride and Benfotiamine was calculated. Overlay of Metformin Hydrochloride and Benfotiamine is presented in fig 5. λmax of MET was found to be 230 nm whereas λmax of BEN was found to be 246 nm. Isoabsorptive point of Metformin Hydrochloride and Benfotiamine was found to be 239 nm.

Method I (Simultaneous equation method)

From the stock solution, working standard solution of drugs was prepared by performing appropriate dilutions and was scanned in the entire U.V. region. Two wavelengths selected for the method are 230 nm and 246 nm that are absorption maximas of MET and BEN respectively in distilled water. A series of dilutions were prepared from standard solutions of MET and BEN 2-16 µg/ml and 2-18 µg/ml respectively. The absorptivities at 230 nm and 246nm were found to be 98.66 and 51.02 for Metformin Hydrochloride, 37.26 and 58.11 for Benfotiamine respectively. For the estimation of drugs in the formulation, ten tablets containing 500 mg of MET and 75 mg of BEN were weighed and average weight was calculated. These tablets were finely powdered and powder equivalent to 500 mg of Metformin hydrochloride and 75 mg of Benfotiamine was weighed and transferred to 100 ml volumetric flask. For the analysis of drugs, a standard addition method was used. To the weighed tablet powder, 25 mg of standard Benfotiamine powder was added to achieve ratio. To this about 50ml of distilled water and 1ml of conc. HCl was added and sonicated for 15 minutes. The volume was made up to the mark with distilled water. The resulting solution was filtered through Whatman filter paper no. 1. Further dilutions were made from this stock solution to get required concentration. Absorbances of these solutions were measured at appropriate wavelengths, and values were substituted in the respective formula to obtain their respective concentrations. Results of tablet analysis are shown in Table No.1. The analysis procedure was repeated six times (n=6).

Formula used for calculation of concentration of drug,

$$Cx = \frac{\text{A2 ay1} - \text{A1 ay2}}{ax2ay1 - ax1ay2} \quad , \quad Cy = \frac{\text{A1 ax2} - \text{A2 ax1}}{\text{ax2ay1} - \text{ax1ay2}}$$

Where,

C_x= Concentration of Metformin Hydrochloridein µg/ml,

C_y= Concentration of Benfotiaminein µg/ml,

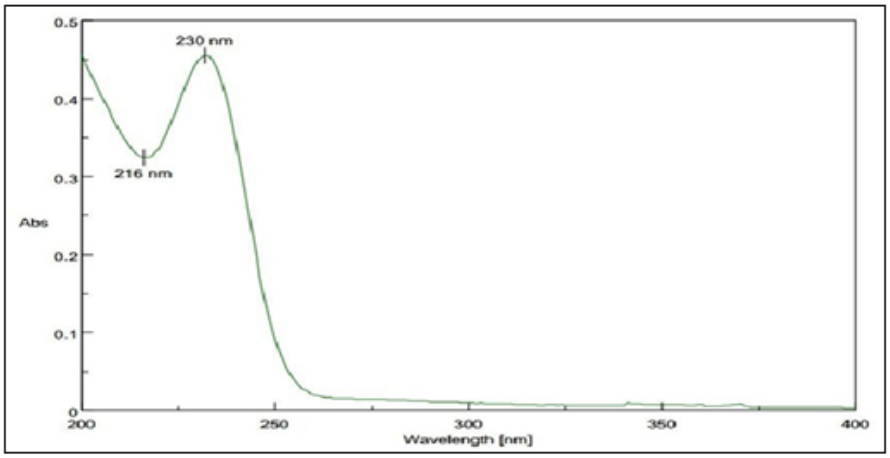

Fig-3: The UV-Spectra of Metformin Hydrochloride

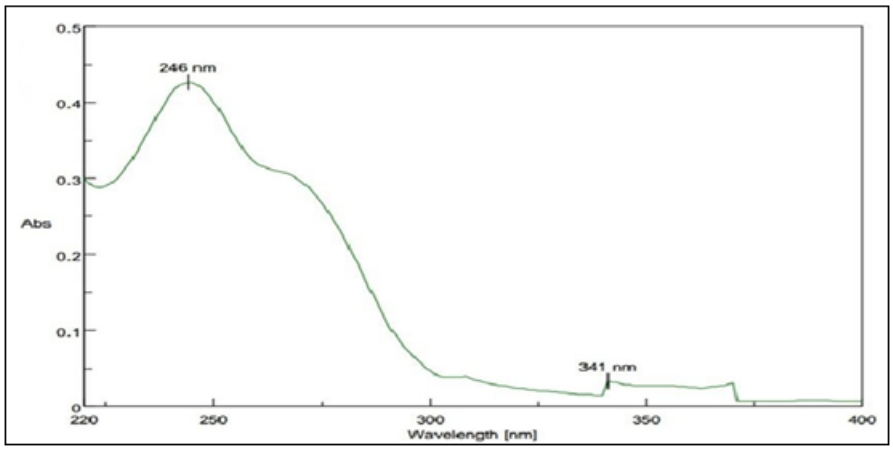

Fig-4: The UV-Spectra of Benfotiamine

Method II (Absorbance ratio method)

The absorbance ratio method is a modification of the simultaneous equation procedure. This method depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. This constant is knows as "Hufner's Quotient" or "Q value". In this method, absorbance of the two components in the mixture is measured at two wavelengths, one is the λ_{max} of one component (λ_2) and other wavelength is of equal absorptivities of both the components (λ_1) known as Isoabsorptive point. A series of standard solutions of MET and BEN in concentration range of 2-16 µg/ml and 2-18 µg/ml respectively were prepared in distilled water and the absorbance of these solutions was taken at isoabsorptive point (λ_1) and λ_{max} of BEN (λ_2). Calibration curves were plotted and Beer's law was verified. The absorptivity values were calculated for both the drugsat the respective wavelengths. The absorptivity values are given in Table-2.

The concentration of two drugs in mixture was calculated by using the following equations:

$$C_x = (Q_m - Q_y / Q_x - Q_y) \times (A_1 / a_{x1})$$

$$C_y = (Q_m - Q_x / Q_y - Q_x) \times A_1 / a_{y1})$$

Where

a_{x1} = A (1%, 1cm) of ALG at 239 nm,
a_{y1}= A (1%, 1cm) of MET at 239 nm
a_{x2} = A (1%, 1cm) of ALG at 246 nm,
a_{y2} = A (1%, 1cm) of MET at 246 nm.

A_1 and A_2 are the absorbances of mixture at 239 nm and 246 nm. C_x and C_y are the concentrations of MET and BEN in gm/100 ml respectively in sample solution

$$Q_m = A_2 / A_1,$$

$$Q_x = a_{x2} / a_{x1} \text{ and}$$

$$Q_y = a_{y2} / a_{y1}$$

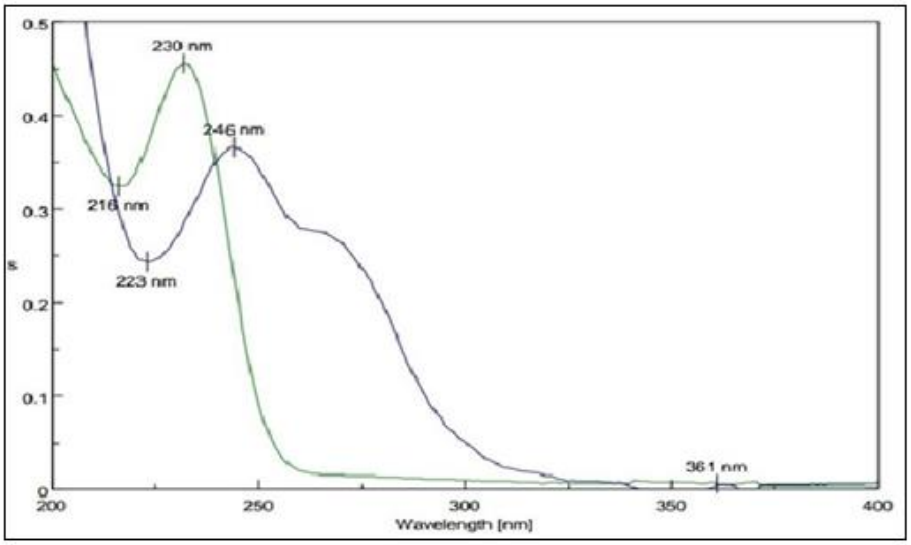

Fig-5: Overlain UV absorption spectra of Metformin Hydrochloride and Benfotiamine

Table-1: Assay of formulation (n=6)

Drug	METHOD A		METHOD B	
	% Assay	% RSD	% Assay	% RSD
MET	97.4 ± 0.015	0.0154	98 ± 0.0132	0.0134
BEN	97.5 ± 0.0089	0.0092	99 ± 0.0145	0.0146

Validation: The UV Spectrophotometric methods were validated as per ICH Q2A guidelines. Parameters like Sensitivity, Linearity, Range, Accuracy, Precision, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness were evaluated.

Linearity: Linearity of the drugs was studied by preparing serial dilutions of standard stock solution of Metformin Hydrochloride and Benfotiamine. Concentrations of the working solutions of Metformin Hydrochloride and Benfotiamine were 2-16 µg/ml and 2-18 µg/ml respectively (n=3). Graph of concentration verses absorbance was plotted at their respective wavelengths. Regression analysis was applied to the data obtained using the least square method. The standard calibration curves for Metformin Hydrochloride and Benfotiamine are shown in (Fig-6 & 7) respectively and data is presented in Table-3.

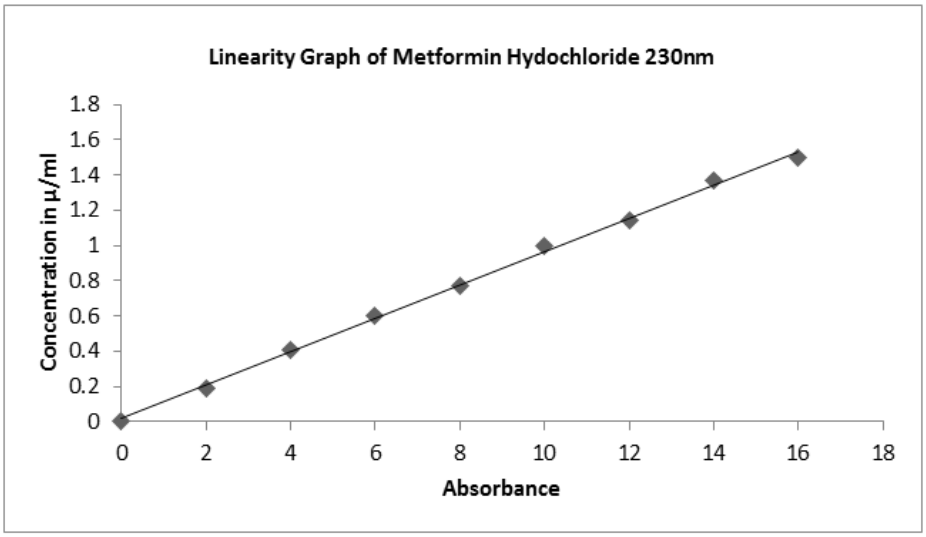

Fig-6: Linearity Range Graph of Metformin Hydrochloride at 230 nm

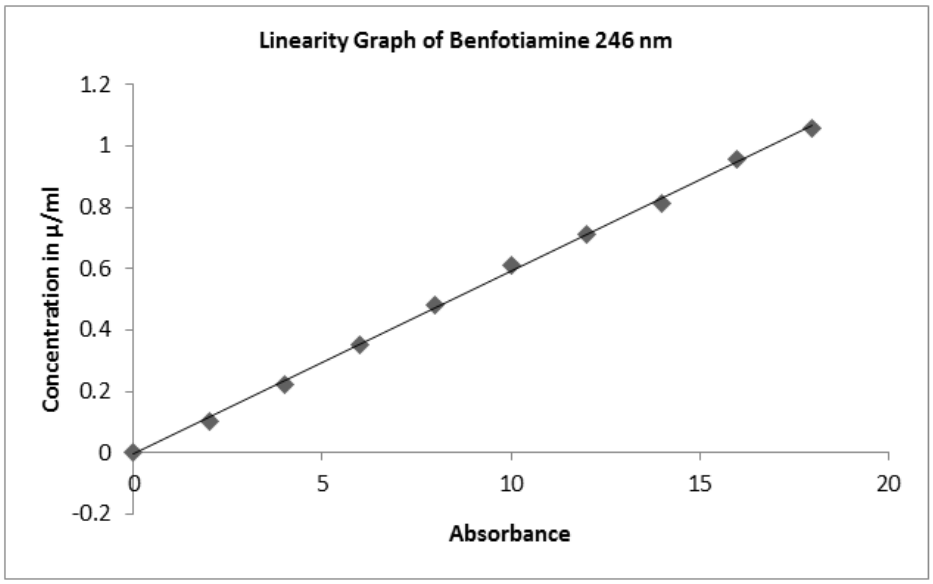

Fig-7: Linearity Range Graph of Benfotiamine 246 nm

Table-2: Absoptivity values (A1%, 1cm) of Metformin Hydrochloride (MET) and Benfotiamine (BEN) for method A and B.

Conc. (µg/ml)	Method A				Method B			
	MET		BEN		MET		BEN	
	230	246	230	246	239	246	239	246
2	96.2	49.25	34.65	51.45	82.1	49.25	51.25	51.45
4	101.65	48.9	39.95	59.02	78.8	48.9	52.2	59.02
6	99.98	50.7	36.51	58.71	82.81	50.73	56.4	58.71
8	96.07	52.81	37.92	60.1	81.48	52.81	53.91	50.1
10	99.43	53.37	37.27	61.29	80.82	53.37	55.8	61.29
12	98.56	51.05	37.25	58.20	81.46	51.02	53.9	58.2
MEAN	**98.66**	**51.012**	**37.26**	**58.114**	**81.45**	**51.01**	**53.92**	**58.11**

Table-3: Regression analysis of calibration curves and summary of validation parameter for method A and B

Sr. No.	Parameter	Drug	Method A		Method B	
			230	246	239	246
1	**Beer's law limit (µg/ml)**	MET	2-16 µg/ml			
		BEN	2-18 µg/ml			
2	**Sandell's Sensitivity (µg/cm2/0.001)**	MET	1.0200	1.3932	1.2310	1.3932
		BEN	1.9823	1.7376	1.8459	1.7376
3	**Intercept**	MET	0.015	0.002	0.004	0.002
		BEN	0.005	0.005	0.005	0.005
4	**Slope**	MET	0.094	0.052	0.080	0.052
		BEN	0.036	0.059	0.053	0.059
5	**Correlation cofficient**	MET	0.998	0.998	0.999	0.998
		BEN	0.999	0.998	0.998	0.998

Accuracy: The accuracy of the developed methods was tested by performing recovery studies at three different concentration levels i.e. 80 %, 100 %, 120 %. Standard solution of MET and BEN was added to the pre analysed tablet solutions. These solutions were then re-analysed by using developed methods; the results of the same are shown in Table 4.

Table-4: Recovery study data for MET and BEN by method A and B (n=3)

Drug	Pre-analyzed conc.	Drug added	Method A		Method B	
			% Recovery	% RSD	% Recovery	% RSD
MET	10 µg/ml	8 µg/ml	97.7±0.015	0.015	97.8 ± 0.0125	0.0127
		10 µg/ml	98.5±0.020	0.020	98.6 ± 0.0136	0.0137
		12 µg/ml	98.6±0.009	0.009	98.7 ± 0.0113	0.0114
BEN	2 µg/ml	1.6 µg/ml	97.5±0.018	0.001	98.1 ± 0.0132	0.0134
		2 µg/ml	98.5±0.016	0.016	98 ± 0.0116	0.0118
		2.4 µg/ml	98.3±0.018	0.018	98.7 ± 0.0128	0.0129

Precision

Repeatability: A mixture of drugs was prepared, containing 10 µg/ml of Metformin Hydrochloride and 2 µg/ml of Benfotiamine and analyzed by method A and B (n=6). The data of is represented in Table 5.

Table-5: Repeatability study data for mixture of MET and BEN (n=6)

Drug	Conc. Taken	Method A		Method B	
		% Found	%RSD	% Found	%RSD
MET	10 µg/ml	98.28 ± 0.0098	0.009	9.86 ± 0.0012	0.0012
BEN	2 µg/ml	98.13 ± 0.0012	0.001	1.4865 ± 0.0008	0.0053

Intermediate precision: Intermediate precision is studied in terms of intra-day and inter-day precision. Three different concentrations of Metformin Hydrochloride and Benfotiamine were taken in a mixture and analyzed by method A and B (n=3). In case of intra-day studies, the analysis was carried out at different intervals on the same day and for inter-day, the analysis was carried out on different days. Results for intraday and inter-day studies are given in Table 6 and 7 respectively.

Table-6: Intraday precision data for mixture of MET and BEN. (n=3)

Drug	Conc. Taken	Method A		Method B	
		% Found	%RSD	% Found	%RSD
MET	10 µg/ml	98.3 ± 0.0163	0.016	9.86 ± 0.0116	0.1176
BEN	2 µg/ml	98.13 ± 0.0010	0.001	1.4867 ± 0.0009	0.0605

Table-7: Interday precision data for mixture of MET and BEN. (n=3)

Drug	Conc. Taken	Method A		Method B	
		% Found	%RSD	% Found	%RSD
MET	10 µg/ml	98.31 ± 0.0104	0.010	9.8 ± 0.0051	0.0052
BEN	2 µg/ml	98.33 ± 0.0014	0.001	1.4864 ± 0.018	0.0121

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ of MET and BEN were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ were found to be as in Table no.8 and 9.

Table-8: Limit of Detection

Drug	Method A		Method B	
	At 230	At 246	At 239	At 246
Metformin hydrochloride	0.0336	0.49	0.165	0.4981
Benfotiamine (µg/ml)	0.45	0.27	0.3	0.7573

Table-9: Limit of Quantification

Drug	Method A		Method B	
	At 230	At 246	At 239	At 246
Metformin hydrochloride	0.1020	1.5094	0.5	1.5094
Benfotiamine (µg/ml)	1.38	0.8	0.9090	2.2950

Robustness: Robustness of developed methods was determined by changing certain parameters. These parameters include UV spectrophotometer instrument, analyst, and by repeating the analysis on different days. The data is provided in Table 10, 11 and 12.

Table-10 Robustness: Change of UV instrument

Change of UV instrument	Drug	Method A				Method B			
		At 230nm		At 246nm		At 239nm		At 246	
		Abs	% RSD	Abs	% RSD	Abs	% RSD	Abs	% RSD
Instrument 1	MET	0.5984	0.0001	0.3035	0.0006	0.4954	0.0036	0.3055	0.0068
	BEN	0.2217	0.0004	0.3510	0.0002	0.3375	0.0011	0.3517	0.0014
Instrument 2	MET	0.5975	0.0001	0.3036	0.0003	0.4949	0.0016	0.3037	0.0031
	BEN	0.2252	0.0008	0.3414	0.0008	0.3374	0.0014	0.3415	0.0017

Table-11: Robustness: Change of analyst

Change of analyst	Drug	Method A				Method B			
		At 230nm		At 246nm		At 239nm		At 246 nm	
		Abs	% RSD	Abs	% RSD	Abs	% RSD	Abs	% RSD
Analyst 1	MET	0.5984	0.0001	0.3035	0.0006	0.4944	0.0012	0.3070	0.0016
	BEN	0.2183	0.0229	0.3436	0.0002	0.3373	0.0011	0.3434	0.0017
Analyst 2	MET	0.5975	0.0001	0.3036	0.0003	0.4953	0.0006	0.3033	0.0006
	BEN	0.2223	0.0008	0.3414	0.0008	0.3383	0.0014	0.3433	0.0011

Table-12: Robustness: Analysis on different days

Analysis on different days	Drug	Method A				Method B			
		At 230nm		At 246nm		At 239nm		At 246	
		Abs	% RSD	Abs	% RSD	Abs	% RSD	Abs	% RSD
Day 1	MET	0.5865	0.0008	0.3046	0.0016	0.4972	0.0008	0.3045	0.0016
	BEN	0.2190	0.0054	0.3464	0.0043	0.3385	0.0005	0.3465	0.0008
Day 2	MET	0.5875	0.0004	0.3049	0.0012	0.4986	0.0019	0.3052	0.0018
	BEN	0.2196	0.0006	0.3486	0.0008	0.3376	0.0016	0.3476	0.0013

RESULTS AND DISCUSSIONS

The methods developed and discussed in the present work provide a convenient, precise and accurate way for simultaneous estimation of Metformin Hydrochloride and Benfotiamine in its bulk and pharmaceutical dosage form. Absorbance maxima of MET at 230 nm and BEN at 246 nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 2-16 µg/ml for MET and 2-18 µg/ml for BEN with respective correlation coefficients of 0.998 and 0.999 respectively. The % RSD for repeatability (n=6), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was studied by recovery studies and the results are expressed as % recovery. % recovery for MET was found within the range of 97 % and 99% for both the methods. % recovery for BEN was found within the range of 97 % and 99% for both the methods. Values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of both the methods. Robustness of the analytical procedure was carried out to check whether the method is affected by small, but deliberate variations in method parameters in order to provide an indication of its reliability during normal usage. Robustness of developed method was studied by changing the UV instrument used for analysis, by doing analysis by different analyst, and analyzing the samples on different days. Deliberate changes which were introduced, did not affect the analysis. So UV method developed for Metformin Hydrochloride and Benfotiamine was found to be robust. The assay for MET were found to be 97.4 ± 0.015 and 98 ± 0.0132 for method A and B respectively. The assays for BEN were found to be 97.5 ± 0.0089 and 99 ± 0.0145 for method A and B respectively. The % RSD value for both MET and BEN was found to be less than 2%. In this study the simultaneous estimation of Metformin Hydrochloride and Benfotiamine was carried out by Simultaneous equation and Absorbance ratio methods satisfactorily.

CONCLUSION

Based on the results obtained, it is found that the developed UV- spectrophotometric technique is quite simple, accurate, precise, sensitive, reproducible and economical. They can be used as effective analytical tool for routine quality control of Metformin

Hydrochloride and in Benfotiamine bulk drug combination and its combined pharmaceutical dosage form without any prior separation of components.

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REFERENCES

1. Fujiwara, M., Watanabe, H., & Matsui, K. (1954). "Allithiamine" A Newly Found Derivative Of Vitamin B1. *The Journal of biochemistry*, *41*(1), 29-39.
2. Charrondiere, U. R., & Gott, G. E. N. (2008). Kass. *European food safety journal*, *864*, 1-31.
3. Graves, D. (2007). Latent autoimmune diabetes of adults (LADA): diagnosis & natural approaches to an often-overlooked presentation. *Townsend Letter: The Examiner of Alternative Medicine*, (283), 76-80.
4. Bozic, I., Savic, D., Stevanovic, I., Pekovic, S., Nedeljkovic, N., & Lavrnja, I. (2015). Benfotiamine upregulates antioxidative system in activated BV-2 microglia cells. *Frontiers in cellular neuroscience*, *9*.
5. Nanaware, A. D., Bhusari, K. V., Dhaneshwar, R. S. (2013). Validated HPLC Method For Simultaneous Quantitation Of Benfotiamine And Metformin Hydrochloride In Bulk Drug And Formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(2), 138-142.
6. Sakhare, R. S., Pekamwar, S. S., & Mohkare, D. P. (2017). Development and Validation of Stability Indicating HPTLC Method for the Determination of Metformin Hydrochloride and Benfotiamine in Bulk and Combined Dosage Form. *Indian Journal Of Pharmaceutical Education And Research*, *51*(2), S8-S16.
7. Patel, M. G., Patil, P. O., & Bari, S. B. (2012). 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*, *2*(3), 196-200.
8. Sharma, S., & Sharma, M. C. (2012). Development and validation of new analytical methods for simultaneous estimation of Drotaverine hydrochloride in combination with Omeprazole in a pharmaceutical dosage form. *Arabian Journal of Chemistry*.