IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
July 2016 Vol.:4, Issue:1

Development and Validation of Stability Indicating Area under Curve Method for Simultaneous Estimation of Metformin HCl and Benfotiamine in Bulk and Pharmaceutical Dosage Form

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Submission: 5 July 2016
Accepted: 10 July 2016
Published: 25 July 2016

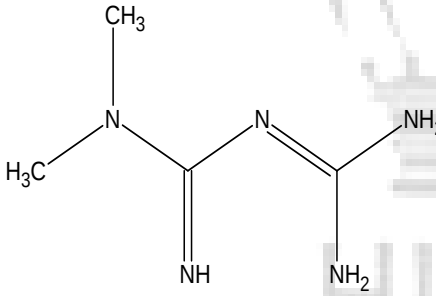
Keywords: Metformin Hydrochloride, Benfotiamine, Area under Curve Method, UV Spectrophotometry

ABSTRACT

Objective: A simple, accurate, precise, and specific area under curve method has been developed for simultaneous determination of Metformin Hydrochloride and Benfotiamine in its combined tablet dosage form by using distilled water as a solvent. Method: The proposed area under curve method involves measurements of area at selected wavelength ranges were selected 220-240nm and 260-280nm for estimation of Metformin Hydrochloride and Benfotiamine respectively. Result and Discussion: The linearity was found to be 10-60µg/ml and 1.5-9 µg/ml for Metformin Hydrochloride and Benfotiamine respectively. The mean % recoveries were found to be 0.999 for Metformin Hydrochloride and 0.998 for Benfotiamine. For repeatability, Intraday precision, Interday precision% RSD were found to be 0.9954, 0.0870, 0.02311 for Metformin Hydrochloride and 0.5866, 0.3847, 0.01157 for Benfotiamine respectively. Limit of Detection and Quantification was found to be 0.0596 µg/ml and 0.1808 µg/ml for Metformin Hydrochloride and 0.0089 µg/ml for and 0.0272 µg/ml for Benfotiamine respectively. Assay results for marketed formulation were found to be 100.83% and 101.02% for Metformin Hydrochloride and Benfotiamine respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of Metformin Hydrochloride and Benfotiamine in their combined dosage form.

INTRODUCTION

Metformin Hydrochloride is an Oral Anti-diabetic drug and is chemically N,N dimethylimidodicarbonimidic diamide hydrochloride (1,1-dimethylbiguanide Hydrochloride)[1] which acts by suppressing excessive hepatic glucose production and improving glucose clearance, its predominant effect is to decrease fasting plasma glucose[2]. It is official in Indian Pharmacopoeia[3], British Pharmacopoeia[4], European Pharmacopoeia[5] and United state Pharmacopoeia[6]. A literature survey revealed that Spectrophotometry[7-9], HPLC [10-14], LC-MS/MS[15] and ion pairing HPLC[16] methods for estimation of Metformin Hydrochloride in Pharmaceutical formulation. Benfotiamine is a synthetic derivative of thiamine (Vitamin- B1) and is chemically N-((4-amino-2-methyl- 5-pyrimidinyl) methyl)-N-(4-hydroxy-2- mercapto-1-methyl-1butenyl) formamide- benzoate dihydrogen phosphate which shows beneficial effects on nerve health, neuropathy, retinopathy, general aging [17-21]. A literature survey revealed that RP-HPLC [22] method for the estimation of benfotiamine in Pharmaceutical formulation. The chemical structures of both drugs are shown in given figures.



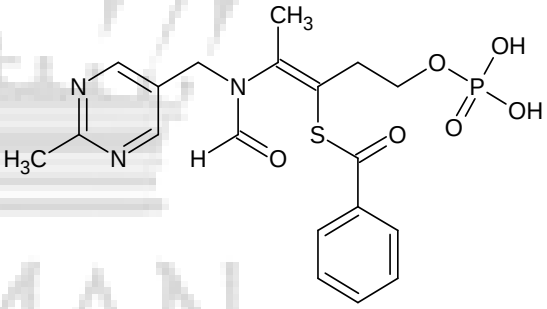


Fig.: 1 Chemical structure of Metformin HCl **Fig.: 2 Chemical structure of Benfotiamine**

The review of literature stated that various analytical methods involving Spectrophotometry, HPLC, and HPTLC have been reported for Metformin Hydrochloride in single form and in combination with other drugs. Several analytical methods have been reported for Benfotiamine in single form and in combination with other drugs including HPLC, HPTLC methods. However, no references have been found for simultaneous estimation of Metformin hydrochloride and Benfotiamine in combined tablet dosage form. The developed method was validated as per ICH guidelines and successfully applied for the assay of Metformin hydrochloride and Benfotiamine in their combined tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents: Metformin hydrochloride was kindly gifted by Alkem Pharmaceuticals Pvt. Ltd. Mumbai, Maharashtra, India and Benfotiamine was kindly provided by Aquatic Remedies Pvt. Ltd. Hyderabad, Andhra Pradesh, India. Tablet of Metformin Hydrochloride (500mg) and Benfotiamine (75mg) in combined dosage form (Benforce-M) by Shield Health Care was obtained from local market.

Instruments: Double beam UV- visible spectrophotometer (Shimadzu, Model UV-1800) having two matched quartz cells with 1cm light path and loaded with UV probe software. Electronic analytical balance (Anamed). Ultrasonicator (HMG India).

Method Development:

Metformin Hydrochloride standard Stock Solution:

Accurately weighed reference standard of Metformin Hydrochloride (10mg) was transferred to 100 ml volumetric flask and dissolved in 100 ml solvent, to obtain standard stock solution (100µg/ml) of drug. For the preparation of working standard, suitable aliquots of stock solution were pipetted out and volumes were made up to the mark with solvent (Dist. Water) to get required concentrations.

Benfotiamine standard stock solution:

Accurately weighed reference standard of Benfotiamine (10mg) was transferred to 100 ml volumetric flask and dissolved in 50 ml solvent and sonicated for 10 min then volume was made up to the mark with the same solvent, to obtain standard stock solution (100µg/ml) of drug. For the preparation of working standard, suitable aliquots of stock solution were pipetted out and volumes were made up to the mark with Dist. Water to get required concentrations.

Area under Curve Method:

For the selection of analytical wavelength solution of Metformin Hydrochloride (10µg/ml) and Benfotiamine (1.5µg/ml) were prepared separately by appropriate dissolution from standard stock solutions and scanned between 200 to 400 nm using Distilled water as blank. From the

overlain spectra of both drugs the area under curve (AUC) is determined at both the selected analytical wavelength ranges. Wavelength range selected were 220 - 240 nm for determination of AUC of Metformin Hydrochloride and 260 - 280 nm for determination of AUC of Benfotiamine. The Calibration curve was prepared in the concentration range of 10-60 µg/ml for Metformin Hydrochloride at 220 to 240 nm. The Calibration curve was prepared in the concentration range of 1.5-9 µg/ml for Benfotiamine at 260 to 280 nm. The "X „value is the ratio of AUC at selected wavelength ranges (220-240 nm and 260-280nm) with concentration of component in µg/ml. The concentration of each drug was calculated using following "Crammers and Matrix rule" equation:

$$C_{BEN} = \frac{X_{MET}\lambda\ (220\text{-}240)*AUC\ (260\text{-}280) - X_{MET}\lambda\ (260\text{-}280)*AUC\ (220\text{-}240)}{X_{MET}\ \lambda\ (220\text{-}240)*X_{BEN}\lambda\ (260\text{-}280) - X_{MET}\lambda\ (260\text{-}280)*X_{BEN}\lambda\ (220\text{-}240)}$$

$$C_{MET} = \frac{X_{BEN}\lambda\ (260\text{-}280)*AUC\ (220\text{-}240) - X_{BEN}\lambda\ (220\text{-}240)*AUC\ (260\text{-}280)}{X_{BEN}\ \lambda\ (260\text{-}280)*X_{BEN}\lambda\ (220\text{-}240) - X_{MET}\lambda\ (220\text{-}240)*X_{BEN}\ (260\text{-}280)}$$

Where, C_{MET} = Concentration of Metformin HCl in gm/l

C_{BEN} = Concentration of Benfotiamine in gm/l

X_{BEN} (260-280) = AUC BEN (260-280)/Conc. In gm/l

X_{BEN} (260-280) = AUC_{BEN} (260-280) /Conc. In gm/l

$X_{MET}\lambda$ (220-240) = AUC $_{MET}$ (220-240) /Conc. In gm/l

X_{MET} (220-240) AUC_{MET} (220-240)/Conc. In gm/l

Method Validation:

The proposed method has been extensively validated according to ICH guidelines.

Linearity:

Solutions of MET ranging from10-60µg/ml were prepared by pipetting out 1, 2, 3, 4, 5 and 6 ml stock solution of MET into series of 10ml volumetric flask and diluted up to the mark with

distilled water. Then, solutions of Benfotiamine ranging from 1.5-9 µg/ml were pipetting out by stock solution of BEN into series of 10ml volumetric flasks and diluted up to the mark with distilled water. The absorption spectra of above solutions were recorded in the range of 200 to 400 nm using distilled water as blank. Area determined at both wavelengths ranges 220-240nm and 260-280 nm for MET and BEN respectively.

Precision (Repeatability):

AUC determined at wavelength range between 220-240 nm for MET (10µg/ml) and AUC determined at wavelength range between 260-280 nm for BEN (1.5µgml) were measured six times and %RSD was calculated and it was within limit (less than 2%).

Intermediate Precision:

Intraday precision was determined by analyzing MET (10µg/ml) and BEN (1.5µg/ml) in combined solution for six times in the same day. Interday precision was determined by analyzing MET (10µg/ml) and BEN (1.5µg/ml) in for three days. Intraday and Interday precision was determined in terms of %RSD.

Reproducibility:

Reproducibility expresses the precision between laboratories. It was performed by preparing the standard solution of MET (10µg/ml) and BEN (1.5µg/ml) for six times and analysed as per the proposed method. It was determined in terms of %RSD.

Accuracy:

Accuracy often expressed as % Recovery by the assay of known, added amount of analyte by standard addition method. Known amount of standard solution of MET and BEN were added at 80%, 100% and 120% to pre-quantified sample solutions of MET (10µg/ml) and BEN (1.5µg/ml). The amount of MET and BEN were estimated from straight line equation of calibration curve.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD and LOQ are estimated from the set of 5 calibration curves used to determine method

linearity. The LOD and LOQ may be calculated as:

LOD= 3.3X(SD/Slope)

LOQ= 10 X (SD/Slope)

Where, SD= the standard deviation of Y-intercept of 5 calibration curves

Slope= the mean slope of the 5 calibration curves.

Determination of MET and BEN in their Combined tablet Dosage form:

Twenty tablets were weighed and average weight of content was determined and the contents of tablets were powdered. The powder equivalent to 500mg of MET and 75mg of BEN was transferred to 50ml volumetric flask, dissolved and diluted up to the mark with distilled water. Aliquots of 0.500 ml of this solution was diluted to 10ml with distilled water six times. AUC determined at wavelength between 220-240nm and 260-280nm. The concentration of each drug was calculated using following "Crammers and Matrix rule" equation.

RESULT AND DISCUSSION

Solution of MET (10µg/ml) and BEN (1.5µg/ml) were scanned separately between 200-400nm using distilled water as blank. Maximum absorbance obtained at 232nm for MET and 265nm for BEN. Two wavelength ranges were selected i.e. 220-240 nm and 260-280nm for estimation of MET and BEN respectively. (Fig. 1 and Fig. 2). The linear regression equations for MET and BEN was found to be y=0.233x + 0.279, R^2= 0.999 (Fig. 4) and y=0.037x+0.004, R^2= 0.998 (Fig. 5) (in respectively concentration range of 10-60µg/ml and 1.5-9µg/ml. Which are within specified criteria of ICH guideline, all data prove that method is linear (Table 1). Linearity data was summarized in table 3. For Repeatability (Table 4), intraday precision, Interday precision, Reproducibility (Table 5) %RSD were found to be 0.2494, 0.5104, and 0.3317 for MET and 1.3756, 1.7762, 1.5780 for BEN respectively. %RSD was found to be less than 2% (which is recommended by ICH guideline) for within a day and day to day variation, which proves that method is precise. Mean % Recovery studies was found to be 100.28% for MET and 100.25% for BEN. Recovery studies data was summarized in table 5 and table 6. Recovery greater than 98% with low standard deviation justifies the accuracy of the method. LOD and LOQ values

were found to be 0.0596µg/ml and 0.1808µg/ml for MET and 0.0089µg/ml and 0.0272µg/ml for BEN. LOD and LOQ were summarized in table 7. Assay data was summarized in table 8. The proposed validated method was successfully applied for Simultaneous estimation of MET and BEN.

Table 1: Statistical Data of MET and BEN by Area under Curve method

Validation Parameters	MET	BEN
Linearity Range	10-60 µg/ml	1.5-9 µg/ml
Straight line equation	y= 0.233x+0.279	y=0.037x+0.004
Slope	0.233	0.037
Intercept	0.279	0.004
Correlation Coefficient (r^2)	0.999	0.998

Table 2: Summary of Validation Parameters of MET and BEN

Validation Parameters	MET	BEN
Precision (% RSD)	0.1910	0.9221
Repeatability (n=6)	99.46	100.03
Intraday (n=3)	100.09	100.39
Interday (n=3)	100.10	100.26
Mean % Recovery	100.83	101.2
LOD (µg/ml)	0.0596	0.0089
LOQ (µg/ml)	0.1808	0.0272

Table 3: Linearity data for MET (10-60µg/ml) and BEN (1.5-9µg/ml)

MET Concentration (µg/ml)	MET AUC (220-240nm)*	BEN Concentration (µg/ml)	BEN AUC (260-280nm)*
10	2.4443	1.5	0.0600
20	5.0997	3	0.1179
30	7.2687	4.5	0.1772
40	9.7687	6	0.2256
50	11.8005	7.5	0.2926
60	14.237	9	0.3423

*n=6

Table 4: Repeatability data for MET (10 µg/ml) and BEN (1.5µg/ml)

Solutions	MET AUC (220-240nm)	BENAUC (260-280nm)
Mean*	99.46	100.03
S.D.	0.9901	0.5868
%RSD	0.9954	0.5866

Table 5: Accuracy data of MET by Area under curve method

MET	Test (100%) (µg/ml)	Std. Added (µg/ml)	Conc. (µg/ml)	% Recovery	Average	S.D	%RSD
80%	500	400	902.52	100.28	99.99	0.2494	0.2487
	500	400	899.1	99.90			
	500	400	898.26	99.81			
100%	500	500	1002.7	100.27	99.99	0.5104	0.5404
	500	500	999.7	99.97			
	500	500	997.3	99.75			
120%	500	600	1099.12	99.92	99.99	0.3317	0.3738
	500	600	1103.96	100.36			
	500	600	1096.00	99.71			

Table 6: Accuracy data of MET by Area under curve method

MET	Test (100%) (µg/ml)	Std. Added (µg/ml)	Conc. (µg/ml)	% Recovery	Average	S.D	%RSD
80%	75	60	133.31	98.75	100.28	1.3768	1.3756
	75	60	135.00	100.00			
	75	60	137.02	101.05			
100%	75	75	150.46	100.31	100.25	1.7807	1.7762
	75	75	153.00	102.31			
	75	75	147.66	98.44			
120%	75	90	167.92	101.77	100.44	1.5850	1.5780
	75	90	165.59	100.88			
	75	90	162.83	98.69			

Table 7: LOD and LOQ data of MET and BEN

Parameter	MET	BEN
Standard Deviation*	1.3733	0.9071
Slope	0.233	0.037
LOD (µg/ml)	0.0596	0.0089
LOQ (µg/ml)	0.1808	0.0272

Table 8: Assay Results of Marketed Formulation

Tablet	Amount of Drug (µg/ml)		Amount Obtained (µg/ml)		% Assay	
	MET	BEN	MET	BEN	MET	BEN
	500	75	501.16	76.02	100.83	101.2

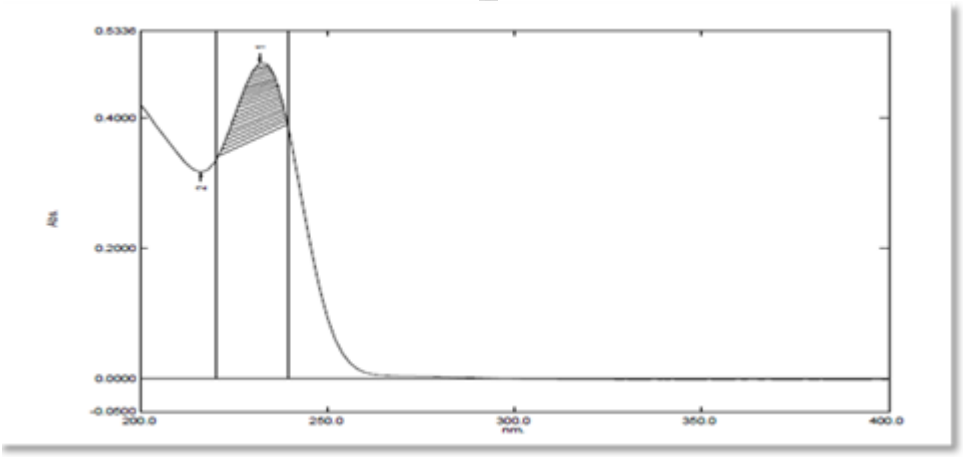


Fig. 1 Spectrum of MET (10µg/ml) AUC at 220-240 nm

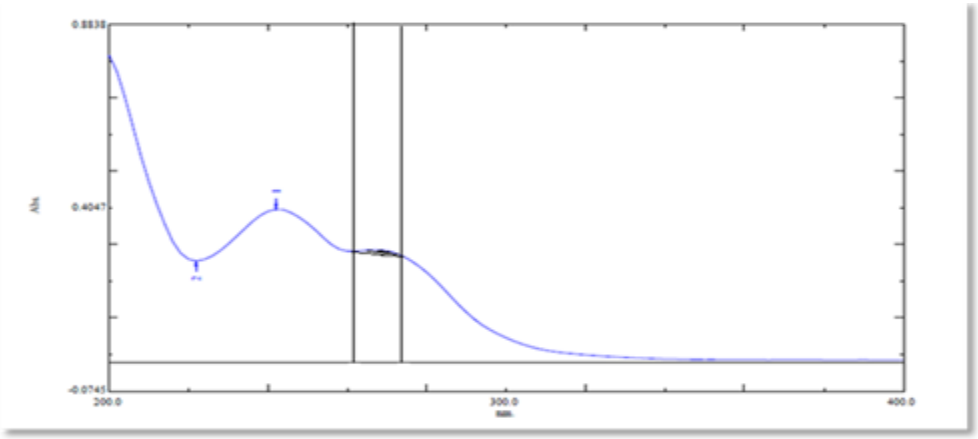


Fig. 2 Spectrum of BEN (1.5µg/ml) AUC at 260-280 nm

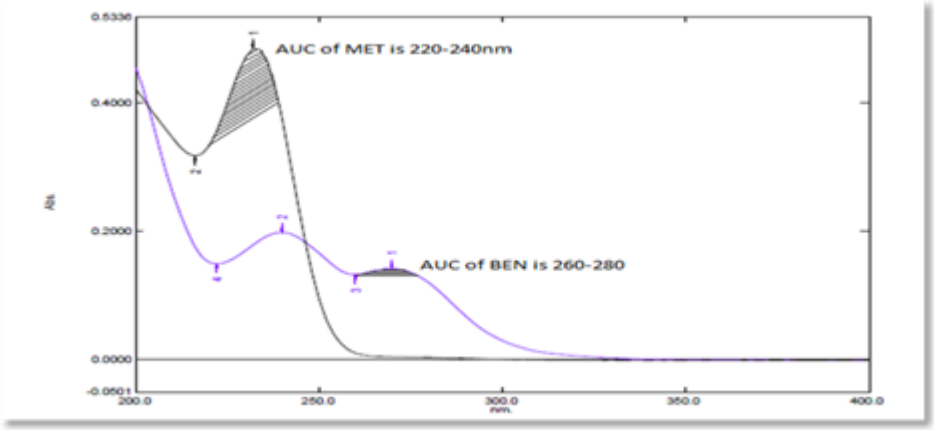


Fig. 3 Overlay Spectrum of MET and BEN

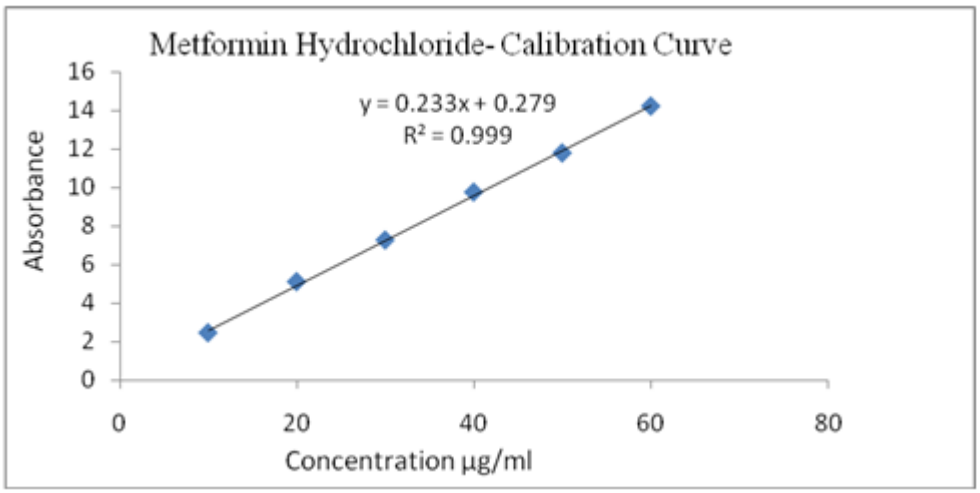


Fig. 4: Calibration curve plot for MET at 220-240 nm in distilled water

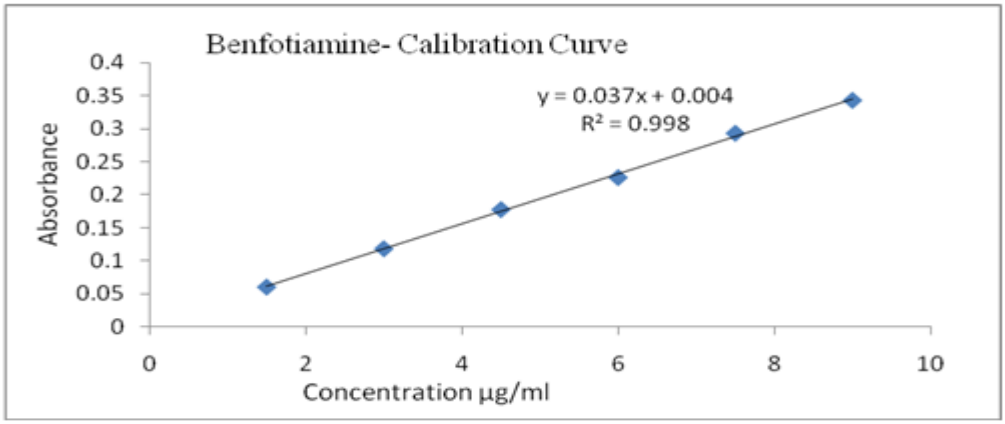


Fig. 5: Calibration curve plot for BEN at 260-280 nm in distilled water

Forced Degradation Study:

The stability indicating assay method is a method that is employed for the analysis of stability samples in pharmaceutical industry.[23] Stress testing of a drug substance can help in turns to establish the degradation pathways and the intrinsic stability of the molecule.[24-25] The quality of the finished product is very important from the point of view of its safety, acceptability and efficacy. Thus stability is considered as one of the most important criteria in pharmaceutical quality control as stable preparations would promise delivery of the drug to the patient.

Forced Degradation Study Data:

Sr. No.	Condition	% Degradation		% Assay	
		MET	BENT	MET	BENT
1.	Acid hydrolysis (0.1N HCl, 60ºC, 4 hr)	14.13%	20.83%	85.87%	79.17%
2.	Base hydrolysis (0.1N NaOH, 60ºC, 4 hrs)	16.81%	15%	83.19%	85%
3.	Neutral hydrolysis (H_2O, 60^0C, 4 hrs)	9.99%	12.5%	90.01%	87.5%
4.	Oxidative degradation (3% H_2O_2, 60^0C, 4hr)	22.72%	30%	77.28%	70%
5.	Photolytic degradation (UV-radiation, 4 hrs)	14.08%	18.66%	85.92%	81.34%
6.	Thermal degradation (80ºC, 2hrs)	12.24%	25%	87.76%	75%
7.	Sunlight degradation (keep under sunlight,4hr)	16.13%	10.66%	83.87%	89.34%

CONCLUSION

The proposed UV method provides simple, accurate and reproducible quantitative analysis for simultaneous estimation of Metformin Hydrochloride and Benfotiamine in tablets. The method was validated as per ICH guidelines.

Acknowledgement

Authors are very much thankful to Director, School of Pharmacy, S. R. T. M. University, Nanded for providing necessary facilities for the research work. The authors are also thankful to

Alkem Pharmaceuticals Mumbai and Aquatic Remedies Hyderabad for providing gift samples of Metformin Hydrochloride and Benfotiamine.

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