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# Development and Validation of a RP-HPLC-PDA Method for the Simultaneous Determination of Metformin and Benfotiamine in Active Pharmaceutical Ingredient and Pharmaceutical Dosage Form

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**Original Research Article**

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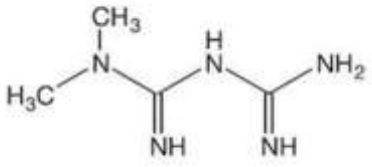
**Abstract:** A new simple, sensitive, accurate and economical analytical method was developed for the simultaneous estimation of Benfotiamine and Metformin in pure and tablet dosage form by RP-HPLC. The method was performed with. Phenomenex Gemini C18 (4.6×250mm) 5µ with mobile phase containing TEA buffer (pH 4.0): Methanol in proportion 65:35 v/v respectively; at a flow rate of 1ml/min with a run time of 6 minutes; detection was done at 230 nm. The retention time of MET and BEN is found to be 3.643 and 2.121 respectively for the standard and for the sample the retention time was found to be 3.649and 2.142 respectively. The MET and BEN followed linearity in the concentration range of 20-100 µg/ml and 10-50 µg/ml with $r^2$ = 0.99 respectively. The amount of the drugs estimated by proposed method was found to be in agreement with the label claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The method precision for the determination of assay was below 2% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

**Keywords**: Benfotiamine, Metformin, RP-HPLC, Validation, Method development.

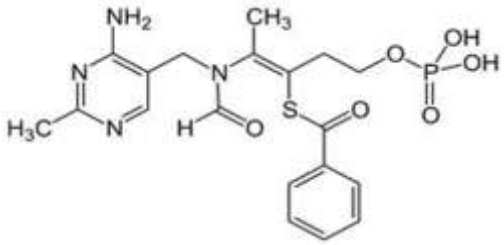
## INTRODUCTION

Metformin (MET) is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). Chemically it is N, N-Dimethyl imido dicarbonimidiamide.

It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. When used alone, metformin does not cause hypoglycemia; however, it may potentiate the hypoglycemic effects of sulfonylureas and insulin. Metformin's mechanisms of action differ from other classes of oral antihyperglycemic agents. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization [1]. A literature survey revealed spectrophotometry [2, 3], HPLC [4-8] and LC-MS/MS [9] methods for estimation of MET in pharmaceutical formulation.




Metformin



**Benfotiamine**

Benfotiamine (BEN) is a synthetic derivative of thiamine (vitamin-1) and is chemically S-[2-{[(4-Amino-2-methylpyrimidin-5-yl) methyl] (formyl) amino}-5-(phosphonooxy) pent-2-en-3-yl] benzene carbothioate. Benfotiamine is an allothiamine that boosts Advanced Glycation Endproduct (AGE)-inhibiting thiamine pyrophosphate and cell-shielding transketolase activity [10-13].

Extensive literature survey was done in this subject. It was found out that individual estimation of the drugs as well as simultaneous estimation of both metformin and benfotiamine was done using HPLC, RP-HPLC. But here we have used different columns and mobile phase [14-15].

## MATERIALS AND METHODS

### Instruments

The chromatographic analysis was performed using Waters Alliance 2695 HPLC with PDA Detector 486 model. The output signal was checked and processed by Empower 2, Alliance Software. The pH of the solutions was measured by a Digital pH meter (Lab India).

### Chemicals and reagents

Benfotiamine, Metformin were purchased by college, HPLC grade acetonitrile, methanol, Water (HPLC grade), Triethylamine, ortho-phosphoric acid and all other chemicals were obtained from Labchem Chemicals.

### Chromatographic conditions

The method was developed by using a Phenomenex Gemini C18 (4.6×250mm) 5µcolumn with a mobile phase comprising TEA Buffer (pH 4): Methanol in the volume ratio of (35:65% v/v). Flow rate of the mobile phase was 1.0 ml/min and the eluted compounds were monitored at the wavelength of 230 nm.

### Preparation of Triethylamine Buffer (pH4.0)

Take 6.0 ml of Triethylamine in 750 ml of HPLC water in 1000 ml volumetric flask and mix well. Make up the volume up to mark with water and adjust pH to 4.0 by using ortho-Phosphoric acid, filter and sonicate.

### Preparation of mobile phase

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultra sonicator for 10 minutes and then filtered through 0.45µ filtered under vacuum filtration.

### Preparation of Diluent

The mobile phase was used as the diluent.

### Preparation of Standard Stock Solution

Accurately weigh and transfer 10 mg of BEN and MET working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3 ml of BEN and 0.6 ml of MET from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol. The samples were filtered using 0.45 µm nylon filters.

These standard solutions were injected into HPLC column and calibration curves were plotted by taking drug peak area Vs concentrations.

### Preperation of Sample solution

To determine the content of MET and BEN in tablets (Brand Name: Gocyst, Label claim, MET-500mg, BEN-100mg), twenty tablets were weighed, there mean weight determined and finally powdered. 10 mg equivalent weight of BEN and MET sample into a 10mL clean dry volumetric flask and add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.3 ml of BEN and 0.6ml of MET from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

### Validation procedure

Method validation was achieved as per ICH guidelines.

### System Suitability

The standard solution was injected for five times and measured the area for all five injections in

HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of Components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

**Linearity**

Standard solutions at five different concentration levels ranging from (10-50) ppm BEN and MET (20-100) ppm were prepared for and analyzed in order to demonstrate the linearity. The regression curve was obtained by plotting peak area versus concentration. The regression equation was obtained by using the regression analysis.

**Precision**

The precision of the analytical method was studied by analysis of multiple sampling of homogenous sample. Precision was determined by repeatability and repeatability and the repeatability was assessed by analyzing five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The value of precision of repeatability with inter-day for six times for two consecutive days was also performed. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Accuracy**

The standard addition and recovery experiments were conducted to demonstrate the accuracy of the method. The accuracy of the method evaluated in triplicate at three concentration levels, i.e., 50%, 100% and 150% of target concentration and the percentages of recoveries were calculated.

**Robustness**

The robustness of a method was demonstrated by altering experimental conditions and chromatographic resolution to evaluate robustness. The deliberate changes were made in the chromatographic conditions, viz. change in flow rate by± 0.1 ml/min and change in the mobile organic phase ratio.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

Limit of detection and limit of quantification were estimated from signal to noise ratio. LOD is the lowest concentration resulting in a peak area of three times the baseline noise and the equation is LOD= 3.3x ASD/S. LOQ is the lowest concentration that provides signal to noise ratio more than 10 and the equation is LOQ= 10xASD/S, where ASD is the average standard deviation and 'S' is the slope of the line.

## RESULTS AND DISCUSSIONS

**Selection of Chromatographic conditions and optimization of Mobile Phase**

Mobile phase was optimized to separate MET and BEN using Symmetry C18 (4.6×250mm) 5µ, Xterra C18 (4.6×250mm) 5µ, ODS C18 (4.6×250mm) 5µ using different mobile phase compositions like methanol: water, Methanol: Phosphate buffer, ACN: Water (70:30), ACN: Water (55:45). But the results showed more tailing and less plate count in the chromatogram. Optimized chromatogram for both the standard and sample was obtained using mobile phase as Methanol: TEA Buffer (65:35 v/v), Phenomenex Gemini C18 (4.6×250mm) 5µ column.

**Table-1**: **Optimized Chromatogram (Standard)**

| | |
|---|---|
| Mobile phase ratio | Methanol: TEA Buffer (65:35 v/v) |
| Column | Phenomenex Gemini C18 (4.6×250mm) 5µ |
| Column temperature | 40ºC |
| Wavelength | 230 nm |
| Flow rate | 1ml/min |
| Injection volume | 10µl |
| Run time | 6minutes |

**Chromatograms depicting the method development**

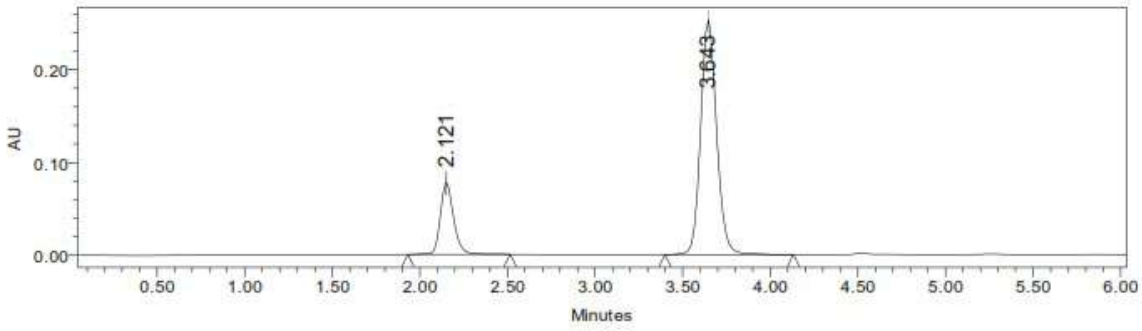



**Fig-1: Optimized Chromatogram (Standard)**

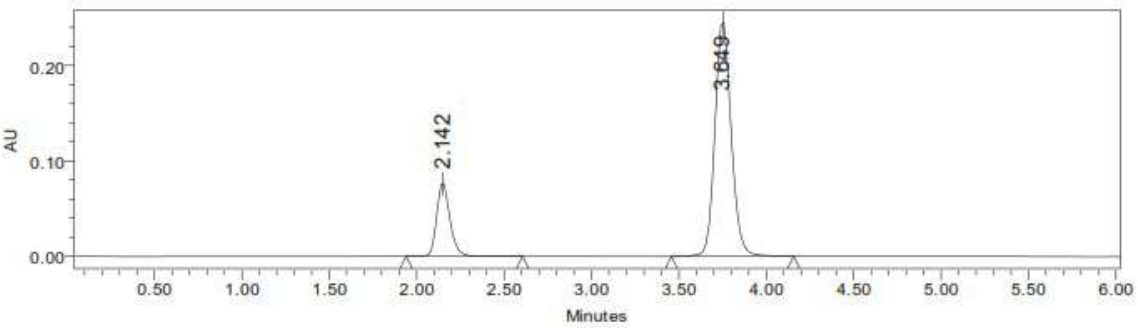



**Fig-2: Optimized Chromatogram (Sample)**

**Linearity**

The linearity of the optimized method was determined for six concentrations (n=6) and the correlation coefficient for BEN and MET was found to be 0.999 and 0.998 respectively. Results are shown in the Table 2.

**Table-2: Linearity data for BEN and MET**

| PARAMETER | BEN | MET |
|---|---|---|
| Linearity(µg/ml) | 10-50 | 20-100 |
| Linearity Equation | Y=13396x + 2467 | Y=27563x - 15679 |
| Slope | 13396 | 27563 |
| Intercept | 2467 | 15679 |
| Co-relation co-efficient | 0.999 | 0.998 |

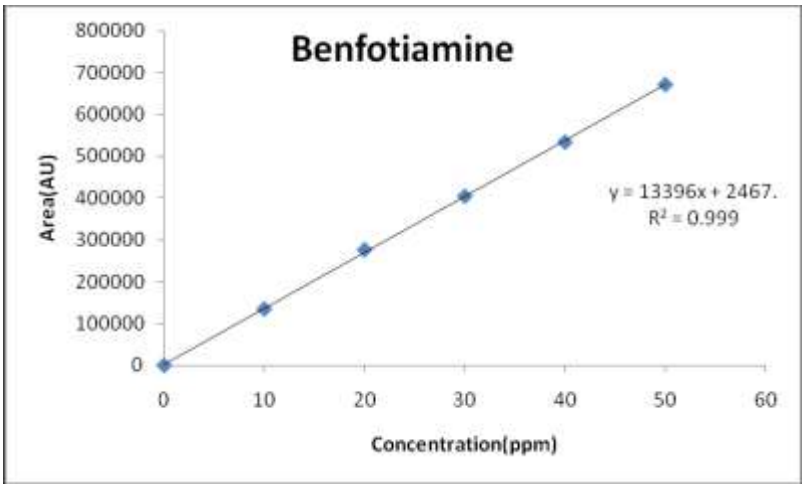



**Fig-3: Linearity plot of Benfotiamine**

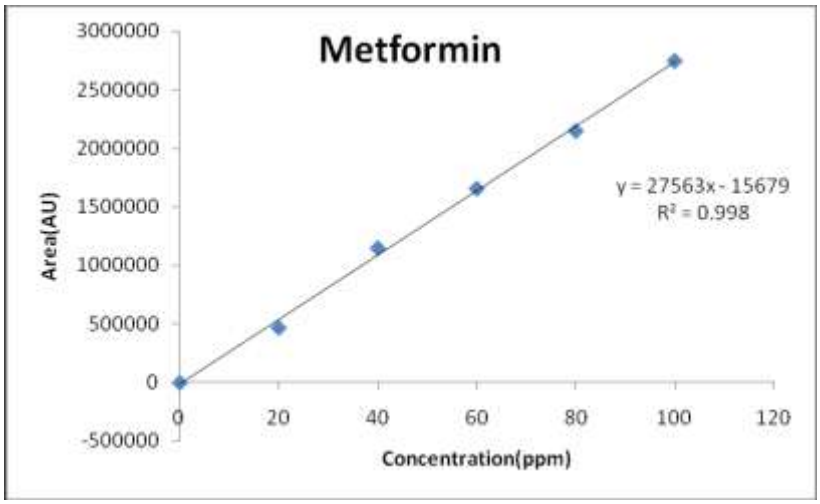



**Fig-4: Linearity plot of Metformin**

**Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Five (5) replicates of 100% accuracy solution as per experimental conditions were obtained. The peak areas were recorded and % RSD was calculated. The % RSD for repeatability of BEN and MET is found to be 0.671 and 0.266 respectively. The RSD value for the inter-day precision of BEN and MET was found to be 0.316 and 0.377 respectively. The acceptance criteria set in the validation was RSD not more than 2%. Results of the precision study are shown in the following Table 3.

**Table-3: Repeatability data**

| S. No. | Peak area (n=6) | |
|---|---|---|
| | BEN | MET |
| 1 | 400459 | 1617864 |
| 2 | 402118 | 1618493 |
| 3 | 405412 | 1628262 |
| 4 | 406506 | 1615796 |
| 5 | 407673 | 1619626 |
| Mean | 404433.6 | 1620008 |
| SD | 2716.809 | 4310.623 |
| %RSD | 0.671757 | 0.266086 |

**Specificity**

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Benfotiamine and Metformin in drug product. The % purity of Benfotiamine and Metformin in pharmaceutical dosage form was found to be 99.7%.

**Accuracy**

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated for BEN and MET. The results obtained for three different concentration levels showed acceptable % recoveries in the range of 98.6% - 100.2% for BEN and for MET in the range of 101.6% - 98.2%. The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate. Results are depicted in the Table 4

**Table-4: Results of recovery studies**

| Drugs | Amount of standard drug added | Amount of standard drug recovered | % Drug Recovered | Mean Recovery |
|---|---|---|---|---|
| BEN | 15 | 14.8 | 98.6 | 99.7 |
| | 30 | 30.1 | 100.3 | |
| | 45 | 45.1 | 100.2 | |
| MET | 30 | 30.5 | 101.6 | 99.6 |
| | 60 | 59.4 | 99 | |
| | 90 | 88.4 | 98.2 | |

**Limit of detection (LOD) and Limit of Quantification (LOQ)**

LOD for BEN and MET were found to be 1.05µg/ml, 6.9µg/ml respectively. LOQ for BEN and MET were found to be 3.1µg/ml and 20.9µg/ml respectively. The low values of LOD and LOQ indicates high sensitivity of the method.

**Robustness**

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Benfotiamine and Metformin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%.The standard and samples of Benfotiamine and Metformin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. The acceptance criteria is the tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000. Results are given below in the Table 5

**Table-5: Robustness data results**

| Parameters | Method condition | BEN | | | MET | | |
|---|---|---|---|---|---|---|---|
| | | RT | TP | TF | RT | TP | TF |
| Flow rate (ml/min) | Actual Flow rate of 1.0 mL/min | 2.121 | 4009 | 1.2 | 3.643 | 7849 | 1.1 |
| | Less Flow rate of 0.9 ml/min | 2.210 | 3800.8 | 0.9 | 4.498 | 3312.2 | 0.9 |
| | More Flow rate of 1.1 ml /min | 2.184 | 4800.8 | | 3.505 | 4312.2 | 0.8 |
| Mobile phase composition | Less organic phase | 2.200 | 4890.8 | 0.9 | 4.50 | 4392.2 | 0.9 |
| | More Organic phase | 2.172 | 4190.8 | 0.7 | 3.512 | 4292.2 | 0.9 |

RT: Retention Time; TP: Theoretical Plates; TF: Tailing Factor

**System Suitability**

Test according to USP 2009, system suitability test were an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method. System suitability test were used to verify that the resolution and reproducibility were adequate for the performed. The parameter of these tests is column efficiency (number of theoretical plates), tailing factor, resolution, peak asymmetry and capacity factor were calculated foe standard solutions. The results obtained from validation of the methods and system suitability studies are summarized in Table 6.

**Table-6: System Suitability**

| Parameters | BEN | MET |
|---|---|---|
| Retention Time[$t_R$] | 2.166 | 3.629 |
| Theoretical plates [N] | 4009 | 5029 |
| Resolution[$R_S$] | | 10.1 |
| Tailing Factor [T] | 1.2 | 1.1 |

**CONCLUSION**

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Benfotiamine and Metformin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Benfotiamine and Metformin are freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: Triethylamine Buffer was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Benfotiamine and Metformin In bulk drug and in Pharmaceutical dosage forms.

**Abbreviations Used**

MET: Metformin; BEN: Benfotiamine; ICH: International Conference on Harmonisation; SD: Standard deviation; %RSD: % Relative Standard Deviation

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