WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 13, 1148-1167. Research Article ISSN 2277– 7105

# DEVELOPMENT AND VALIDATION OF SOME PHARMACEUTICAL DRUGS BY STABILITY ASSAY METHOD

**Vishwas Mohini[1], Sharma Ranu[*2], Garg Ayush[3], Kachawa Singh Vijay[4], Dr. Dwivedi Jayesh[5] and Khathuriya Rajesh[6]**

[*1]Research Scholar, Department of Quality Assurance, Pacific College of Pharmacy, Udaipur, Rajasthan, India.
[2]Associate Professor, Department of Pharmaceutical chemistry, Pacific College of Pharmacy, Udaipur, Rajasthan, India.
[3]Associate Professor, Department of Pharmaceutics, Pacific College of Pharmacy, Udaipur, Rajasthan, India.
[4]Assistant Professor, Department of Quality Assurance, Pacific College of Pharmacy, Udaipur, Rajasthan, India.
[5]Professor, Department of Pharmaceutics, Pacific College of Pharmacy, Udaipur, Rajasthan, India.
[6]Associate Professor, Department of Pharmacognosy, Pacific College of Pharmacy, Udaipur, Rajasthan, India.

Article Received on
24 July 2022,
Revised on 14 August 2022,
Accepted on 04 Sept. 2022
DOI: 10. 20959/wjpr202213-25461

***Corresponding Author**
**Sharma Ranu**
Associate Professor, Department of Pharmaceutical chemistry, Pacific College of Pharmacy, Udaipur, Rajasthan, India.

## ABSTRACT

A stability-indicating HPLC, UV and HPTLC assay method has been developed and validated for Metformin Hydrochloride and Benfotiamine in bulk drug and pharmaceutical dosage forms. An isocratic HPLC was achieved on Waters 2695 using Symmetry C18 (250mm × 4.6mm × 5µ) column with the mobile phase consisting of 0.02 mM sodium dihydrogen ortho-phosphate, pH adjusted to 2.5 using ortho-phosphoric acid (solvent A), and acetonitrile (solvent B) in the ratio of 58:42 %v/v. The stress testing of Metformin Hydrochloride and Benfotiamine was carried out under acidic, alkaline, oxidative, thermal, and photolytic conditions. Metformin Hydrochloride and Benfotiamine was well resolved from its degradation products. The proposed method was validated as per ICH guidelines. The method was found to be suitable for the quality control of Metformin Hydrochloride and Benfotiamine in bulk and pharmaceutical dosage forms as well as the stability-indicating studies.

**KEYWORDS:** Stability assay (HPLC, UV and HPTLC), Metformin and Benfotiamine.

## 1. INTRODUCTION

Analytical chemistry involves the techniques of separation, quantifications and chemical additives identification of herbal and synthetic materials having one or more than one compounds. Analytical chemistry is separated into two classes, a qualitative evaluation that is to say the identification with regard to the chemical additives exists in the sample, whereas quantitative evaluation estimates the amount of positive detail or compound within the substance, i.e. the sample.[1,2] Analytical methods are generally classified into two categories:1) Instrumental & 2) Non-Instrumental.

In the Instrumental method certain physical property is measured to determinethe contents of compositions of a substance, while in non-instrumental; theconventional physicochemical properties are used to analyse the sample.[7,8]

### 1.2 High Performance Thin Layer Chromatography (HPTLC)

HPTLC is the simplest separation technique today available to the analyst. It can be considered as time machine that can speed your work and allows you to domany things at a time usually not possible with other analytical techniques. It canconcurrently analyse several samples even of divergent nature and compositionsupporting several analysts at a given time. A major advantage of HPTLC is its abilityto analyse a number of samples simultaneously using a small quantity of mobilephase; this reduces the time and cost of analysis. In addition, it also minimizesexposure risks and disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with the same or different parameters.[9,10] Furthermore, in the case of HPTLC, there are no limits for the choice of solvents and mobile phases; drug and lipophilic excipients can be dissolved in a suitable solvent and that was evaporated during spotting on HPTLC plate leaving behind analyte as a thin band. Therefore, for such methods, extraction procedure is not required always and could be developed for analyzing drug without any interference from excipients.[11,12]

### 1.3 High performance liquid chromatography (HPLC)

Chromatography is essentially a group of techniques for separation of thecompounds from the mixtures by their continuous distribution between two phases, one of which is moving over the other. The systems associated with this definitionare.

- **Adsorption chromatography**
- **Partition chromatography**
- **Ion exchange chromatography**
- **Gel chromatography**

The basis of separation of the component from a mixture may be defined in terms of one of these four modes of separation or by a combination. The technique of HPLCis developed from advances made in column chromatography. The technique is basedon the same modes of separation mentioned above. It is different than conventionalcolumnchromatography as the mobile phase is pumped through the packed columnunder high pressure. Liquid chromatography though more troublesome than gas chromatographyhas the main advantage of operating at low temperatures and can be used withadvantage for separation of substances as proteins, nucleosides which arethermolabile.[13,14] In conventional liquid chromatography, a dilute solution of the sample ispassed through a column packed with solid particles. Thus, the liquid is passedthrough vertical columns under gravitational flow. This is passed with slow speed andespecially if the packing granules were small enough to give efficient separation, thenthe delivery under gravity decreases even up to a few drops per minute.[15,16] The obvious way to increase the flow rate and efficient separation is to force theliquid by a positive displacement pump or by gas pressure. This versatility can beachieved by making certain modifications in columns and by using smaller diameterand smaller surface area of column particles and by using other suitable packingstructure. This is HPLC i.e., High Pressure/Performance Liquid Chromatography.[17,18]

## 2. MATERIAL AND METHODS

Drug (Metformin Hydrochloride & Benfotiamine) are received as gift sample from Alkem Pharmaceuticals Mumbai. All other chemical is analytical grade.

### 2.1 Development and validation of Stability Indicating HPTLC, UV and HPTLC method for simultaneous estimation of metformin (MET) and Benfotiamine (BENT) in combined soliddosage form.

#### 2.1.1 Solvent

MET and BENT both are soluble in methanol, methanol was selected as a solvent.

### 2.1.2 Selection of analytical wavelength

Standard stock solutions (10µg/ml) of drugs were prepared in methanol and theirisosbestic point is observed at 249 nm on UV-spectrophotometer.

### 2.1.3 Preparation of standard stock solution

Standard stock solution of MET was prepared by dissolving 50 mg of drug in 10ml of methanol to get concentration of 5000 µg/ml from which 1 ml was further dilutedwith methanol to get the final concentration 500 ng/µl. Standard stock solution of BENT was prepared by dissolving 7.5 mg of drug in 10ml of methanol to get concentration of 750 µg/ml from which 1 ml was further dilutedwith methanol to get the final concentration 75 ng/µl.

### 2.1.4 Selection of mobile phase and chromatographic conditions

Chromatographic separation studies were carried out initially by using differentsolvents like Chloroform, Methanol, Toluene, Ethyl Acetate, Benzene and Triethylaminein different proportions. Finally, the combination Benzene: Methanol: Triethylamine(8.5:1:0.5, v/v/v) offered good resolution. This mobile phase system was observed togive compact spots for both MET and BENT and the Rf values were 0.26±0.04 and 0.72± 0.03 for MET and BENT respectively. The chamber saturationtime for mobile phase was 20 min and activation of plate was done at $110^0$ C for five minto obtain distinct spots.

### 2.1.5 Analysis of marketed formulation

Twenty tablets were weighed accurately and finely powdered. A quantity ofpowder equivalent to 75 mg of BENT and 500 mg of MET was weighed and transferredto a 100 ml volumetric flask containing approximately 50 ml methanol. The mixture wassonicated for 15 min and diluted to volume with methanol. The solution was filteredusing Whatman paper no. 41. One milliliter of the above solution was further diluted withmethanol to obtain the concentration 75 ng /band for BENT and 500 ng/b and for MET. Two microlitre volume of this solution was applied on TLC plate to furnish concentration150 ng /band for BENT and 1000 ng/band for MET. After chromatographic developmentthe peak areas of the bands were measured. The same analytical procedure was repeatedfor six times. MET and BENT produced distinct peak at Rf 0.24±0.04, 0.70±0.03respectively when scanning was done at 249 nm. Theresult also shows that there is no interference between the drug and the excipientspresents in tablet formulation.

### 2.1.6 Validation of analytical method

#### 2.1.6.1 Linearity

Calibration was done by automatic sample applicator Linomat 5 on TLC plate togive concentration 500, 1000, 1500, 2000, 2500, 3000 ng/b and of MET and 75, 150, 225,300, 375, 450 ng/band of BENT. The plates were developed in mobile phase. The graphfor calibration was plotted as peak areas versus concentration.

#### 2.1.6.2 Precision

The precision study was performed by intra-day and inter-day variation study. In the intraday study, three replicates of three different concentrations of MET (1500,2000, 2500 ng/band) and of BENT (225, 300, 375 ng/band) were analysed in a day andpercentage RSD was calculated. The % RSD was found to be in the range of 0.56-0.64 for MET and 0.69-1.07 for BENT. For the inter day variation study, three replicates ofthree different concentrations were analysed on three consecutive days and percentageRSD was calculated. The % RSD was found to be in the range of 0.70-0.84 for MET and1.14-1.43 for BENT.

#### 2.1.6.3 Accuracy

The consistency and accuracy of technique was ensured by recovery study. Thisstudy was carried out by spotting a mixture of standard drug solution to pre-analysedsample solution at three different levels 80, 100 and 120 %. Basic concentration ofsample chosen was 1000 ng/band for MET and 150 ng/band for BENT.

#### 2.1.6.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of Metformin Hydrochloride and Benfotiamine are calculatedby mathematical equation:

LOD= 3.3 X Standard deviation / Slope

LOQ= 10 X Standard deviation / Slope

#### 2.1.6.5 Specificity

Specificity of the developed method was ascertained by analysing standard METand BENT and MET and BENT extracted from tablets. The band for MET and BENT inthe sample was confirmed by comparing the Rf and spectra of the band with thoseobtained from the standard.

**2.1.6.6 Robustness**

As per the ICH, method robustness expresses its capacity to remain unalteredthrough small, deliberate variations in parameters of method. The parameters alteredwere change in mobile phase composition (±1%) and wavelength (±1 nm). The effect onresult is observed by applying 3000 ng/band for MET and 450 ng/band for BENT.

**2.1.7 Forced degradation studies**

Forced degradation studies were carried under condition of acid, base, neutralhydrolysis, oxidation, photolytic and dry heat in order to evaluate the ability of theproposed method to separate both the drugs from their degradation products. Dry heatand photo degradation study was carried out in solid state.

**2.1.7.1 Acid degradation**

From the standard solution of MET (5000 µg/ml) 1 ml solution was mixed with1ml of 0.1N HCl and 8 ml of methanol. The solution was refluxed at 80°C for 4 hrs. 3µl volume was applied on TLC plate to get concentration 1500 ng/band. Similarly, from the standard stock solution of BENT (750 µg/ml) 1 ml solution was mixed with 1ml of 0.1N HCl and 8 ml of methanol. The solution was refluxed at80°C for 4 hrs. 3 µl volume was applied on TLC plate to get concentration 225 ng/band. After acid treatment, MET showed 11.47 % of degradation with additional degradationpeak at Rf 0.44 and BENT showed 23.36% of degradation with two additionaldegradation peak at Rf 0.54,0.59.

**2.1.7.2 Alkaline degradation**

From the standard solution of MET (5000 µg/ml) 1 ml solution was mixed with1ml of 0.1N NaOH and 8 ml of methanol. The solution was refluxed at 80°C for 4 hrs. 3µl volume was applied on TLC plate to get concentration 1500 ng/band. Similarly, from the standard stock solution of BENT (750 µg/ml) 1ml solution wasmixed with 1ml of 0.1N NaOH and 8 ml of methanol. The solution was refluxed at 80°Cfor 4 hrs. 3 µl volume was applied on TLC plate to get concentration 225 ng/band. 13.48% of degradation was observed for MET with degradation product at Rf 0.51 and 12.95% of degradation was observed for BENT with degradation peak at Rf 0.82.

**2.1.7.3 Oxidative degradation**

From the standard solution of MET (5000 µg/ml) 1 ml solution was mixed with 1ml of 3% solution of H2O2 and 8 ml of methanol. The solution was refluxed at 80°C for 4hrs. 3 µl

volume was applied on TLC plate to get concentration 1500 ng/band. Similarly, from the standard stock solution of BENT (750 µg/ml) 1ml solution was mixed with 1 mlof 3% solution of H2O2 and 8 ml of methanol. The solution was refluxed at 80°C for 4hrs. 3 µl volume was applied on TLC plate to get concentration 225 ng/band. In theoxidative condition, 21.77% degradation was observed for MET with two additionalpeaks of degradation at Rf 0.40, 0.45 and 26.29 % of degradation was observed for BENTwith peak of degradation at Rf 0.58.

**2.1.7.4 Neutral Hydrolytic Degradation**

From the standard solution of MET (5000 µg/ml) 1 ml solution was mixed with1ml of water and 8 ml of methanol. The solution was refluxed at 80°C for 4 hrs. 3 µlvolume was applied on TLC plate to get concentration 1500 ng/band.Similarly, from the standard stock solution of BENT (750 µg/ml) 1ml solution was mixed with 1ml of water and 8 ml of methanol. The solution was refluxed at 80°C for 4hrs. 3 µl volume was applied on TLC plate to get concentration 225ng/band. On neutralhydrolysis, 8.11% of MET was degraded without appearance of degradation product and 14.74% of BENT was degraded with degradation product at Rf 0.61.

**2.1.7.5 Degradation under dry heat**

Dry heat studies were performed by keeping drug samples seperately in oven ($80^0$C) for a period of 2 hour. Samples were withdrawn after 2hr, dissolved in methanol anddiluted appropriately to get concentration of 500 µg/ml for MET and 75 µg/ml for BENT.3 µl volume was applied on TLC plate to get concentration 1500 ng/band for MET and225 ng/band for BENT. Under dry heat degradation condition, MET showed 14.67% ofdegradation with additional product at Rf 0.47 and 27.94 % of degradation observed forBENT with degradation products at Rf 0.83 and 0.88.

**2.1.8 Photo-degradation studies**

Photolytic study was carried out by exposure of drug individually to UV light upto 200-watt hours/square meter for period of 4 hrs. Sample was weighed, dissolved anddiluted to get 1500 ng/band and 225 ng/band concentration for MET and BENT resp. After exposing to UV light, 14.04% of degradation was observed for MET withdegradation product at Rf 0.14 and 13.55% of degradation was observed for BENTwithout appearance of degradation product.

## 3. RESULT AND DISCUSSION

### 3.1 Development and validation of Stability Indicating HPTLC, HPLC and UV methodfor simultaneous estimation of Metformine Hydrochloride and Benfotiamine in combinedsolid dosage form.

#### 3.1.1 Analysis of MET and BENT tablet formulation

**Table 1: Tablet formulation.**

| Label claim (mg) | Amount taken (ng/band) | Amount found (ng/band) | % Drug content | S. D. | % R.S. D |
|---|---|---|---|---|---|
| 500 MET | 1000 | 999.89 | 99.98 | 1.06 | 1.06 |
| 75 BENT | 150 | 149.25 | 99.41 | 1.58 | 1.59 |

#### 3.1.2 Linearity studies of MET and BENT

**Table 2: Linearity studies.**

| Sr.No | Conc. of MET (ng/band) | Mean Peak Aera | Conc. of BENT (ng/band) | Mean peak aera |
|---|---|---|---|---|
| 1 | 500 | 3613 | 75 | 1278 |
| 2 | 1000 | 4333 | 150 | 1923 |
| 3 | 1500 | 4981 | 225 | 2620 |
| 4 | 2000 | 5601 | 300 | 3312 |
| 5 | 2500 | 6229 | 375 | 3947 |
| 6 | 3000 | 6828 | 450 | 4611 |

#### 3.1.3 Precision Studies of MET and BENT

**Table 3: Precision Studies.**

| | Amount taken (ng/band) | Amount found (%) | % RSD | Amount taken (ng/band) | Amount found (%) | % RSD |
|---|---|---|---|---|---|---|
| Intra day [n=3] | 1500 | 99.44 | 0.56 | 225 | 101.93 | 1.07 |
| | 2000 | 99.86 | 0.64 | 300 | 100.48 | 0.82 |
| | 2500 | 99.86 | 0.64 | 300 | 100.48 | 0.82 |
| Inter day [n=3] | 1500 | 99.59 | 0.84 | 225 | 99.05 | 1.43 |
| | 2000 | 99.56 | 0.76 | 300 | 99.69 | 1.43 |
| | 2500 | 100.29 | 0.70 | 375 | 100.0 | 1.14 |

#### 3.1.4 % Recovery Studies of MET and BENT

**Table 4: % Recovery studies.**

| Drug | Amount taken(ng/band) | Amount standard drug added (ng/band) | Amount recovered (ng/band) | % Amount recovered | % RSD |
|---|---|---|---|---|---|
| MET | 1000 | 800 | 1796.97 | 99.85 | 0.63 |
| | 1000 | 1000 | 2001.25 | 100.58 | 0.62 |
| | 1000 | 1200 | 2205.89 | 100.26 | 0.85 |
| BENT | 150 | 120 | 270.74 | 100.36 | 1.36 |
| | 150 | 150 | 299.65 | 99.88 | 0.63 |
| | 150 | 180 | 331.25 | 100.35 | 0.65 |

### 3.1.5 LOD & LOQ Studies of MET and BENT

**Table 5: LOD & LOQ Studies.**

| Drug | LOD | LOQ |
|---|---|---|
| MET | 140.74 ng/band | 426.48 ng/band |
| BENT | 19.60 ng/band | 59.39 ng/band |

### 3.1.6 Robustness studies of MET and BENT

**Table 6: Robustness studies.**

| S.no | Parameter | Drug | % RSD |
|---|---|---|---|
| 1 | **Mobile phase composition** Methanol (+1%) | MET | 0.53 |
| | | BENT | 1.19 |
| | Methanol (-1%) | MET | 1.83 |
| | | BENT | 1.40 |
| 2 | **Wavelength** (+ 1 nm) | MET | 0.89 |
| | | BENT | 049 |
| | (-1 nm) | MET | 0.92 |
| | | BENT | 0.52 |

### 3.1.7 Forced degradation studies of MET and BENT.

**Table 7: Forced degradation studies.**

| Agent | Time(4h) | Number of degradation products (Rf value) | | % Drug remaining after degradation | |
|---|---|---|---|---|---|
| | | MET | BENT | MET | BENT |
| HCL (0.1N) | 4 | 1(0.44) | 2 (0.54,0.59) | 88.53 76.64 | 2 (0.54,0.59) |
| NaOH(0.1N) | 4 | 1(0.51) | 1 (0.82) | 86.52 87.05 | 1 (0.82) |
| Water | 4 | No degradation | 1 (0.61) | 91.89 85.26 | 1 (0.61) |
| $H_2O_2$ (3%) | 4 | 2(0.40,0.45) | 1 (0.58) | 78.23 73.71 | 1 (0.58) |
| Dry heat | 2 | 1(0.47) | 2 (0.83, 0.88) | 85.32 72.06 | 2 (0.83, 0.88) |
| Photo degradation | 4 | 1(0.14) | No degradation | 85.95 | 86.45 |

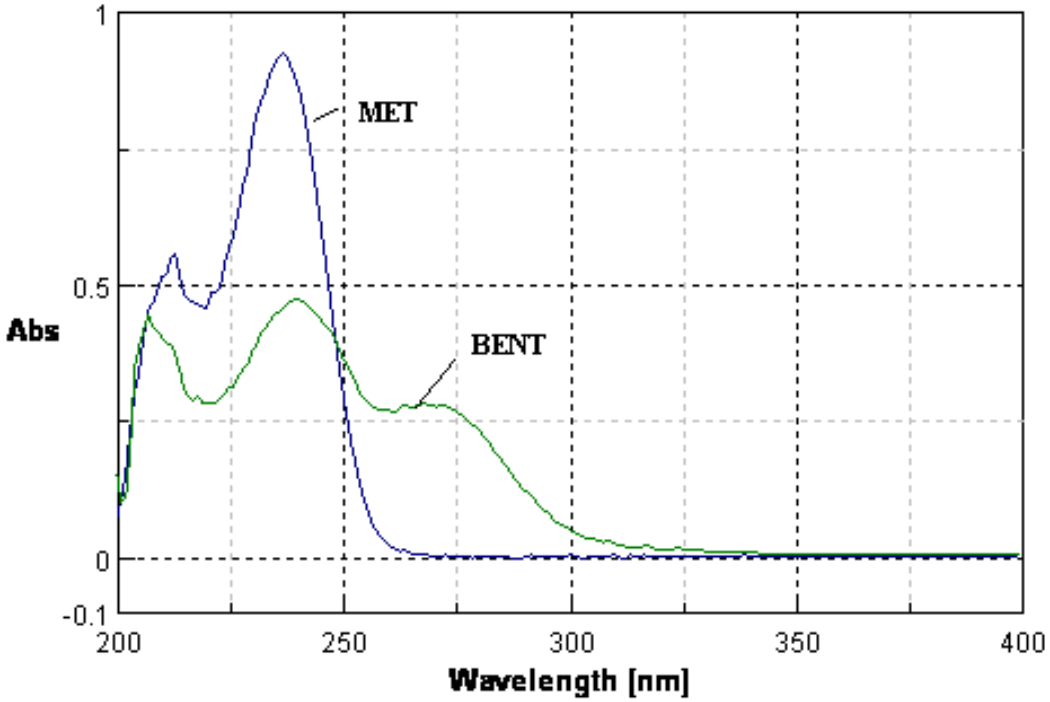


**Figure 1: UV Spectra of MET and BENT.**

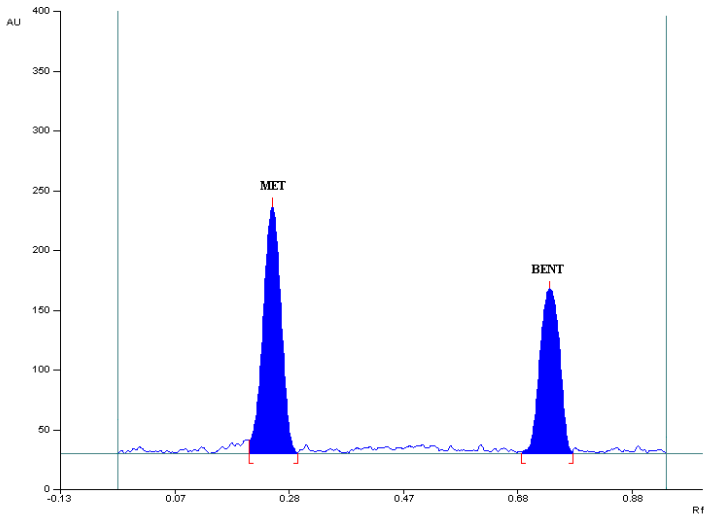


**Figure 2: mixed standard solution of MET and BENT**

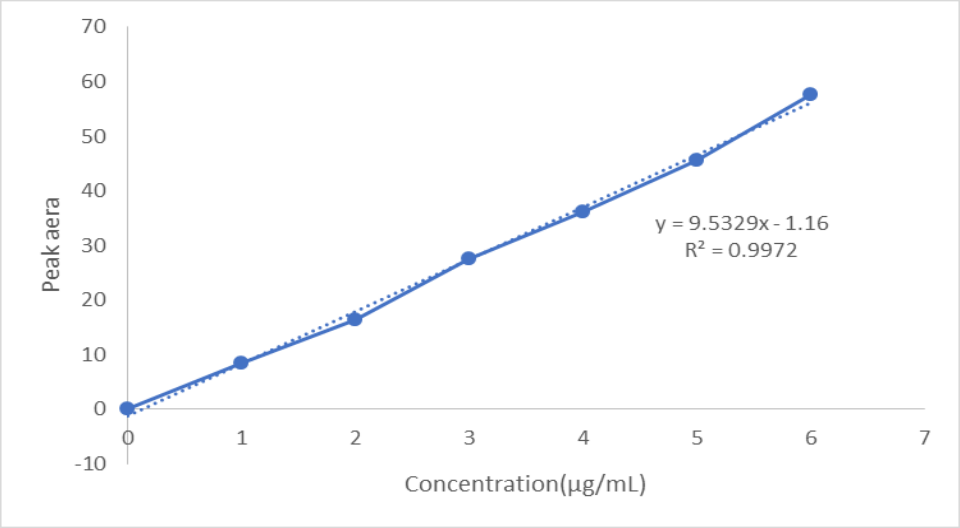


**Figure 3: Calibration curve of Metformin.**

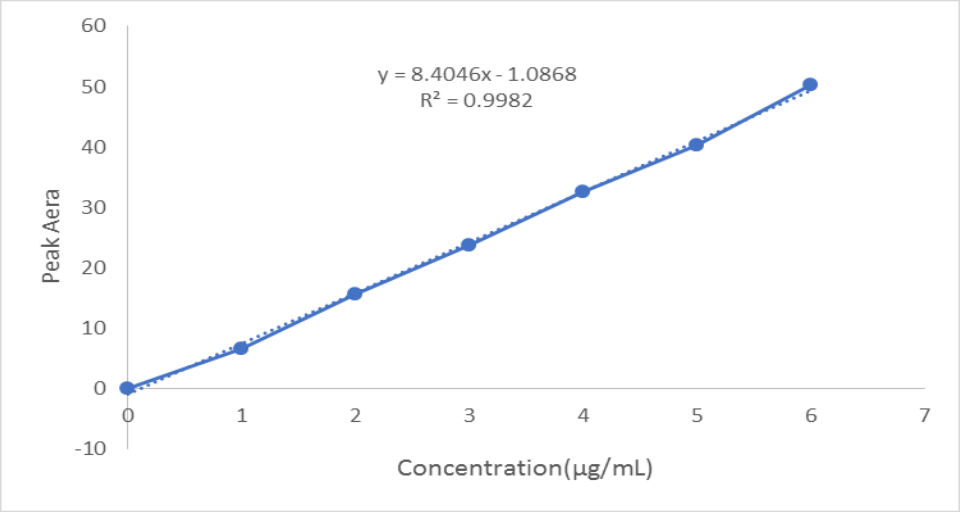


**Figure 4: Calibration curve of Benfotiamine.**

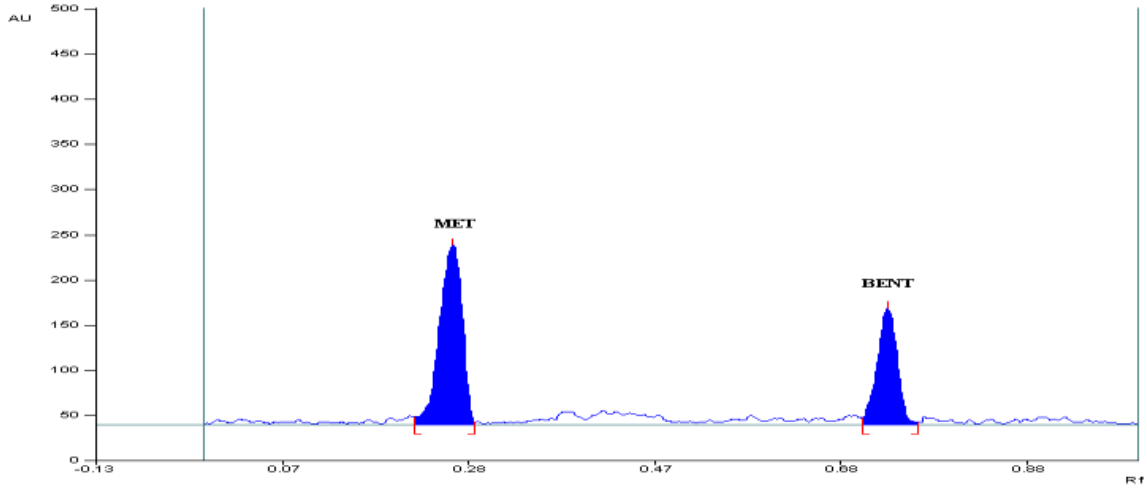


**Figure 5: Sample MET and BENT.**

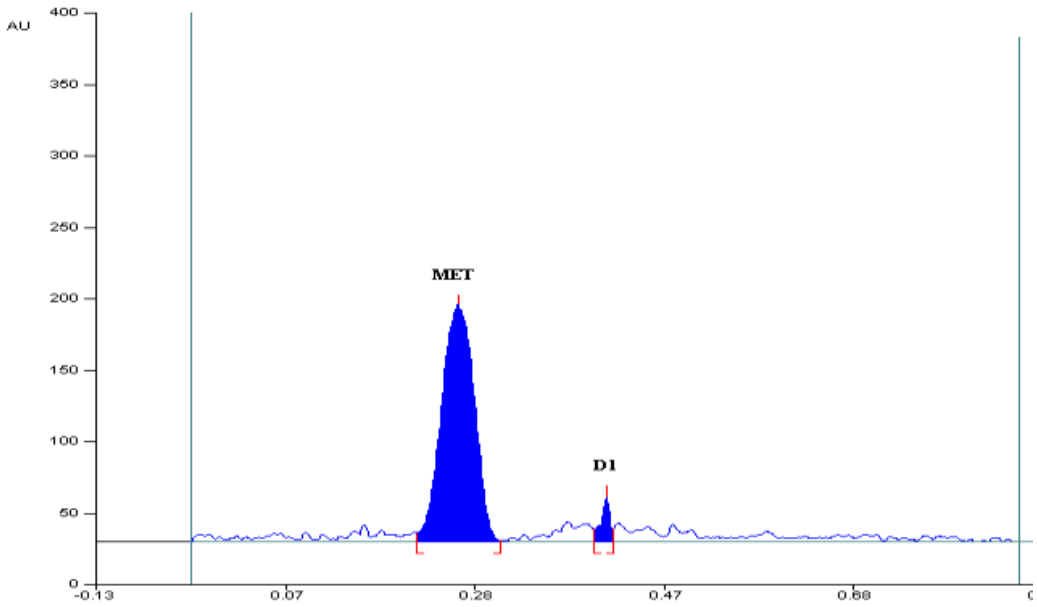


**Figure 6: MET after acid degradation with degradation product.**

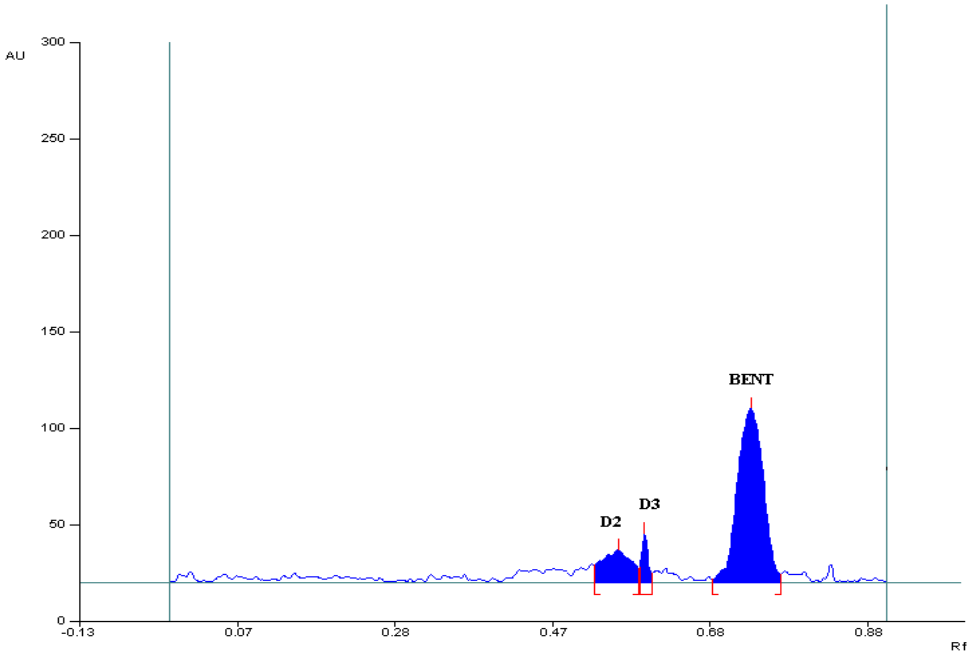


**Figure 7: BENT after acid degradation with degradation product.**

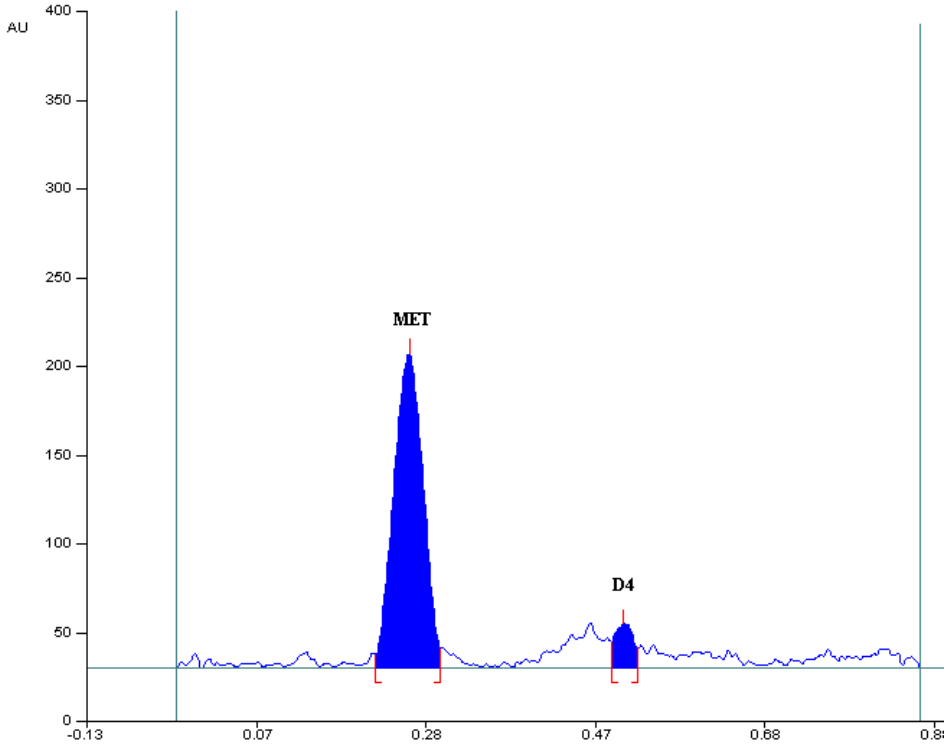


**Figure 8: MET after alkaline degradation with degradation product.**

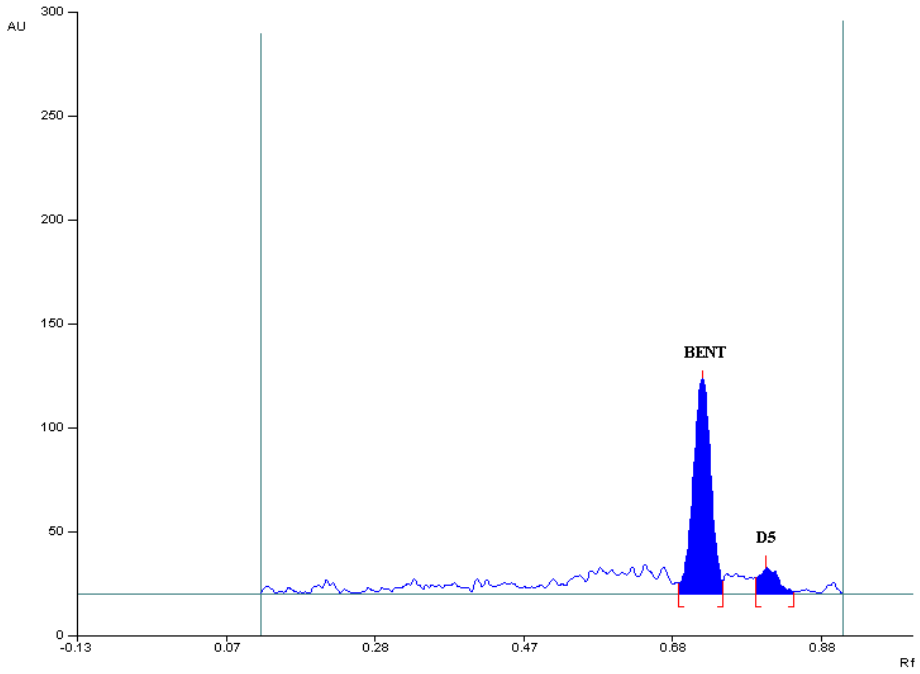


**Figure 9: BENT after alkaline degradation with degradation product.**

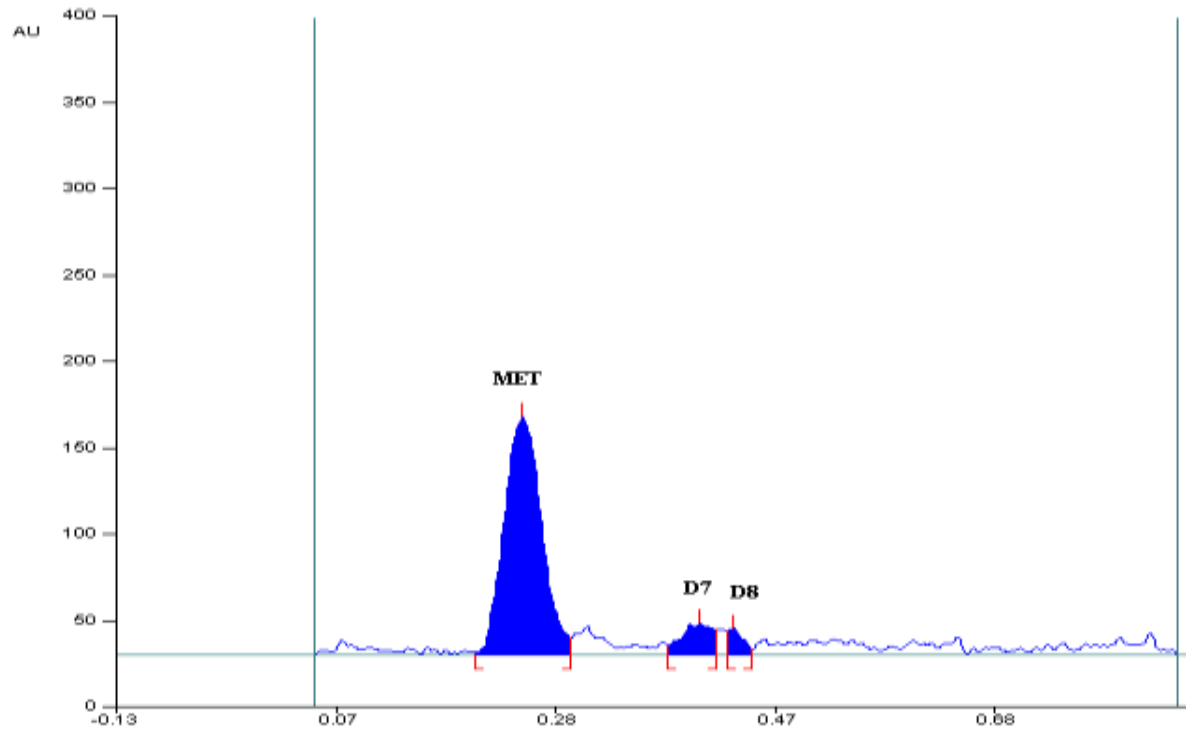


**Figure 10: MET after oxidative degradation with degradation product.**

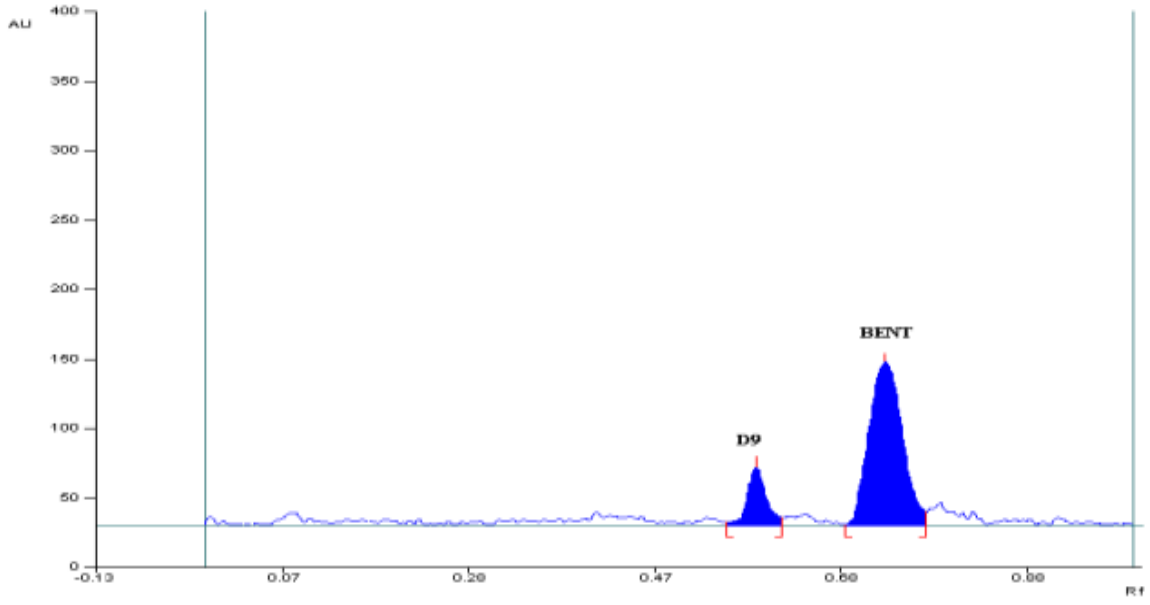


**Figure 11: BENT after oxidative degradation with degradation product.**

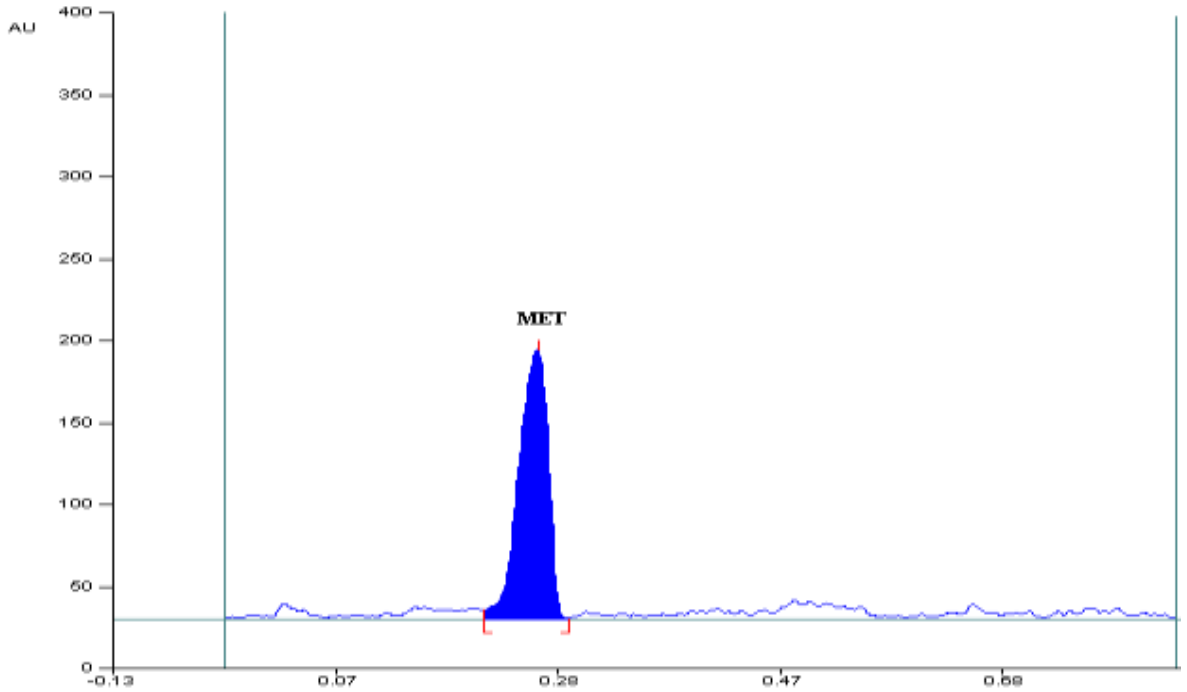


**Figure 12: MET after neutral degradation with degradation product.**

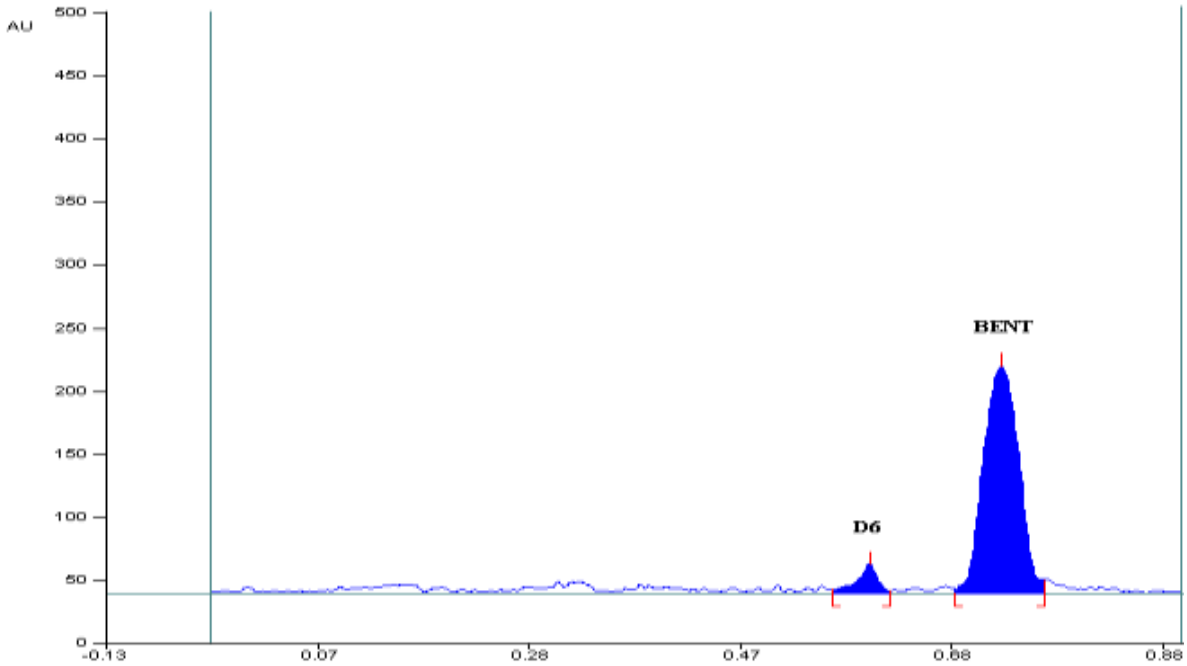


**Figure 13: MET after neutral degradation with degradation product.**

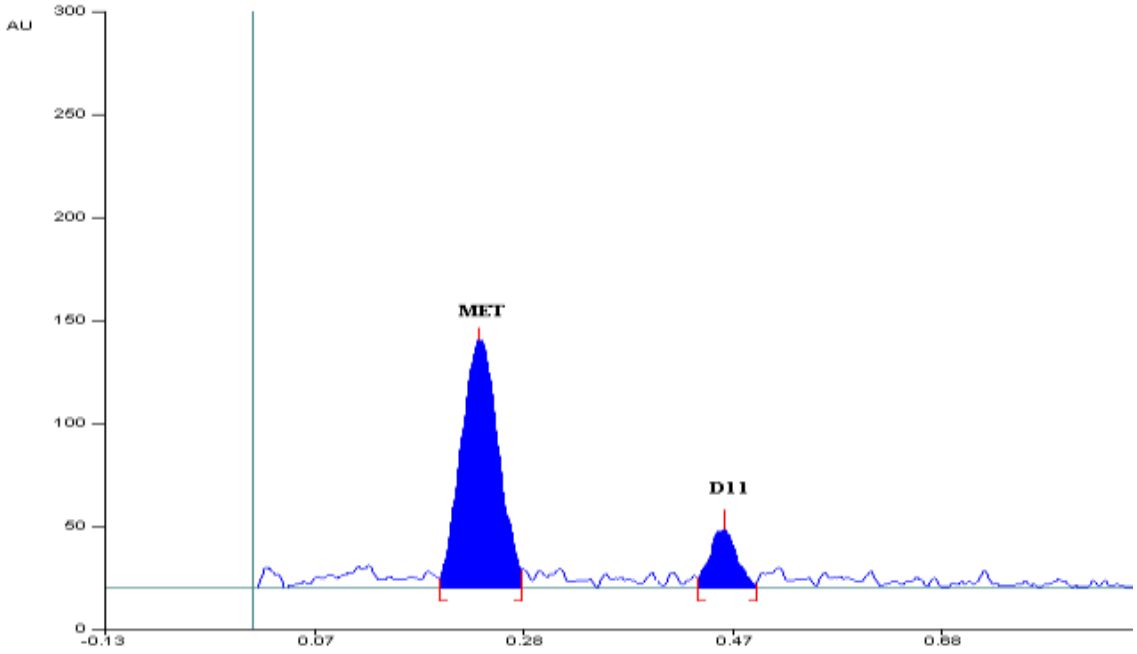


**Figure 14: MET after dry heat degradation with degradation product.**

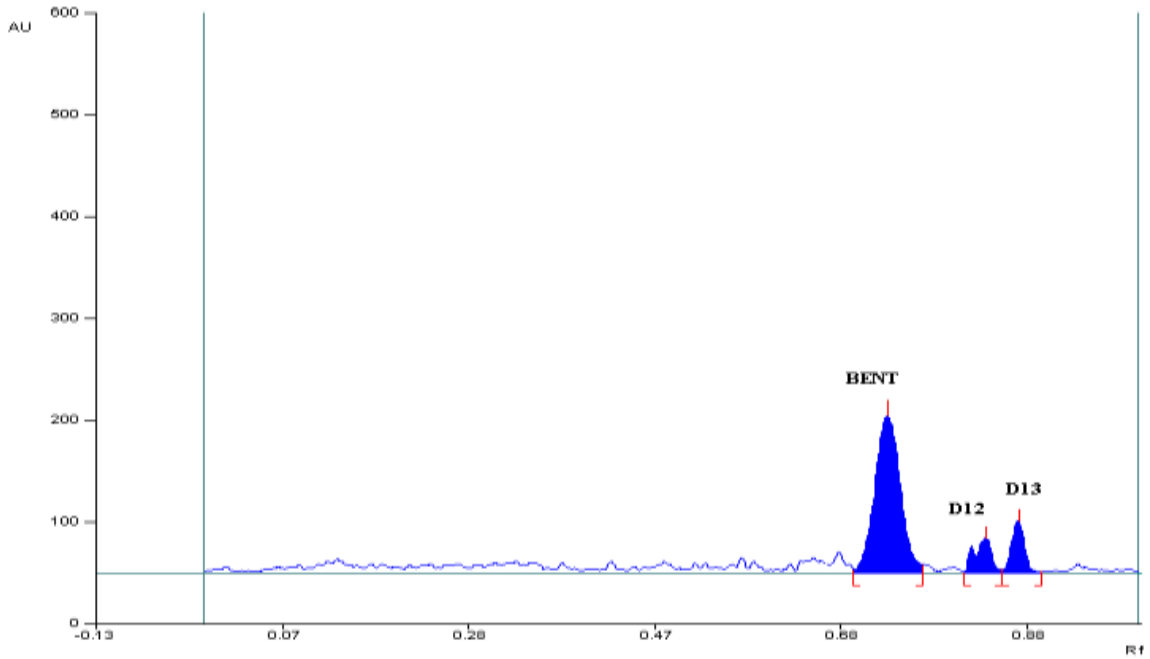


**Figure 15: BENT after dry heat degradation with degradation product.**

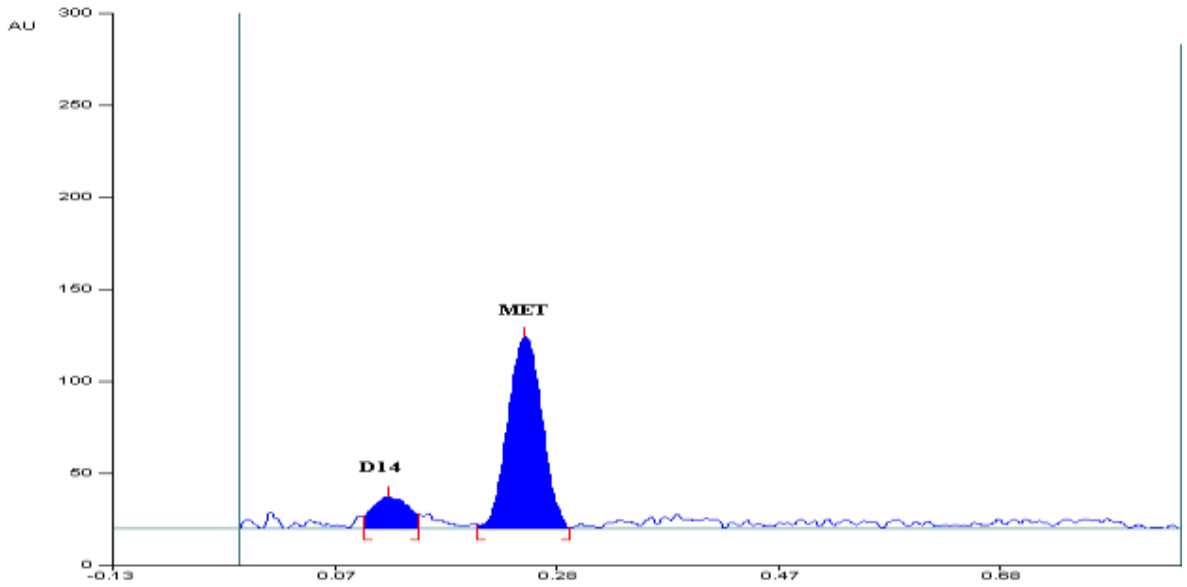


**Figure 16: MET after exposing to UV light with degradation product.**

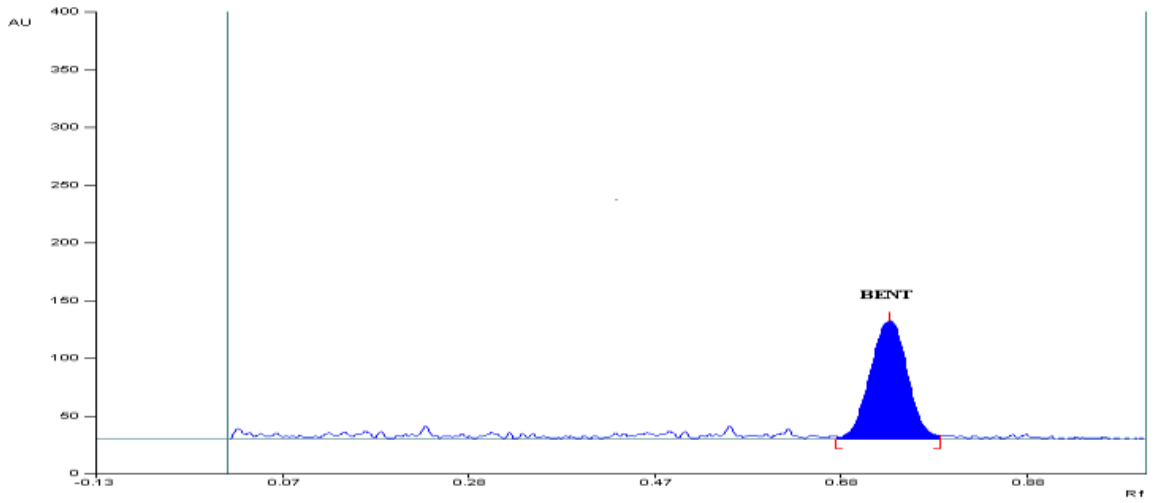


**Figure 17: MET after exposing to UV light with degradation product.**

## 4. SUMMARY AND CONCLUSION

Metformin Hydrochloride and Benfotiamine in combination are used as antihyperglycemic agent. Market survey reveals that Benforce-M combination manufacturedby Shield Health Care Pvt. Ltd. is recently introduced in market containing MetforminHydrochloride (500mg) and Benfotiamine (75mg) as solid dosage form.MET and BENT were separated on silica gel 60 F254 TLC plate using Benzene:Methanol:Triethylamine(8.5: 1: 0.5, v/v/v)as mobile phase. Chamber saturation timewas 15min.The optimum wavelength for detection and quantification used was 249 nm. The retention factors for MET and BENT were found to be

0.26±0.04 and 0.72±0.03respectively. Straight-line calibration graphs were obtained in the concentration range500-3000 ng/band for MET and 75-450 ng/band for BENT with high correlationcoefficient. The commercially available tablet formulation was evaluated and % drugcontent was found to be 99.98±1.06 for MET and 99.41±1.58 for BENT. The method was validated as per ICH guidelines for Linearity, accuracy, precision, and robustness. The accuracy of method was studied by recovery study at 80%, 100% and 120 %. The precision of the method was expressed as % RSD and observed less than 2 indicate method is precise. The low value of LOD and LOQ indicates sensitivity of the method. The method robustness was studied by modifying chromatographic conditions and results were concluded in terms of % RSD and found within acceptable limits for each parameter which express method is robust.

MET and BENT were exposed to various stress degradation conditions. Peaks obtained from the samples degraded by acid, alkali, water, hydrogen peroxide, dry heat and Photo treatment showed well separated spots of pure drugs and few degradation products spots at various Rf values. MET showed degradation product peak under acid (0.44), alkali (0.51), oxidation (0.40, 0.45), dry heat (0.47) and Photo (0.14) conditions but did not show any observable peak in neutral condition. BENT showed degradants peaks for acid (0.54, 0.59), alkali (0.82), neutral (0.61), oxidation (0.58) and dry heat (0.83, 0.88) condition but did not show any observable peak in photo stress condition. The degradation peaks developed under various stress condition for both MET and BENT were well separated from the peak of the intact drugs. The peaks of the MET and BENT were not remarkably shifted in the presence of the degradation peaks, which specify the stability-indicating character of the developed method.

## 5. REFERENCES

1. Sharma Shivani, GoyalSwapnil, ChauhanKalindi, A Review on Analytical Method Development and Validation, *International Journal of Applied Pharmaceutics*, 2018; 10(6): 8-15.
2. Ravisankar P, Navya CN, Pravallika D, Sri DN. A review on step-by-step analytical method validation. *IOSR J Pharm,* 2015; 5: 7-19.
3. Christian GD. Analytical Chemistry. John Wiley and Sons, Inc, 6th edn, 2003; 131-132.

4. Kasture AV, Wadodkar SG, Mahadik KR. Pharmaceutical Analysis. Nirali Prakashan; Vol-I. 2001; 1-9, 64-67.
5. Beckett AH and Stanlake JB. Practical Pharmaceutical Chemistry. Part 2, CBS Publisher and Distributers; 4th edn, 2001; 1-7.
6. Chatwal GR and Anand S. Instrumental Method of Chemical Analysis. Himalaya Publishing House; 1st edn, 2002; 585.
7. Sharma BK. Instrumental Method of Chemical Analysis. Goel Publishing House, Meerut; 21st edn. 2002; 6.
8. David GW. Pharmaceutical Analysis A textbook for pharmaceutical students and pharmaceutical chemists. Elsevier Sciences Ltd, 2nd edn; 11-13.
9. Chatwal GR and Anand S. Instrumental Method of Chemical Analysis. Himalaya Publishing House, Mumbai; 5th edn, 2004; 1.1-1.2.
10. Dong MW. Modern HPLC for Practicing Scientists. Wiley Interscience A John Wiley & Sons, Inc, 2006; 135-142.
11. Ahuja S, Scypinski S. Handbook of pharmaceutical analysis, Separation science and technology. Academic press, USA, Vol.3. 2001; 16-17, 374, 95-118, 445-448, 340-342.
12. Kasture AV, Wadodkar SG, Mahadik KR, More HM. Pharmaceutical analysis. Vol. I, 17th edn. 2007; 1-3.
13. Elke H, Leach RG. Applied Thin-Layer Chromatography best practice and avoidence of mistakes. Wiley-VCH Verlag GmbH and co. KGaA; 2nd edn, 2007; 1-200.
14. Braithwaite A, Smith FJ. Chromatographic Methods. Kluwer Academic publishers; 5th edn, 1999; 44-81.
15. Ravisankar P, Lokapavani CH. HPTLC: A versatile method for rapid analysis of pharmaceutical formulations and comparison with other chromatographic techniques and its applications. Indian Journal of Research in Pharmacy and Biotechnology, 2014; 1208-1217.
16. Andola HC, Purohit VK. High Performance Thin Layer Chromatography (HPTLC): A Modern Analytical tool for Biological Analysis, Nature and Science, 2010; Vol-8; 58-61.
17. Patel R, Patel M. HPTLC Method Development and Validation: Strategy to Minimize Methodological Failures. Journal of Food and Drug Analysis, 2012; 20(4): 794-804.

18. Remington. The Science and Practice of Pharmacy. 20th edn., Volume I, Lippincott Williams and Wilkins Publication, Philadelphia, 2004; 603-605.