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# DEVELOPMENT AND VALIDATION OF RP-HPLC AND UV DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF METFORMIN HCL AND PIOGLITAZONE IN PHARMACEUTICAL FORMULATIONS

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## ABSTRACT

A UV derivative spectrophotometric method and high-performance liquid chromatographic method for the simultaneous determination of metformin (MET) and pioglitazone (PIO) in tablets were developed in the present work. First and second order derivatization was carried out in Agilent Cary 60 UV/Vis double beam spectrophotometer. The first and second order derivative spectra obtained for Metformin and Pioglitazone were at 210 nm, 226 nm and 230 nm, 240 nm respectively. HPLC method was carried out by using Agilent 1220 Infinity LC equipped with Eclipse XDB plus C18 Column (4.6 × 150 mm, 5µm) with a mobile phase consisting of Methanol and Tetrahydrofuran in the ratio of 70:30%v/v at a flow rate of 1ml/min. The drugs were monitored at 230 nm. The retention time of Metformin HCl and Pioglitazone were found to be 1.02 and 2.51min respectively. The proposed methods were validated as per ICH guidelines in terms of accuracy, precision, linearity, LOD and LOQ. The proposed methods were found to be suitable for simultaneous determination of Metformin HCl and Pioglitazone in bulk and in pharmaceutical dosage forms.

**KEYWORDS:** UV derivative spectroscopy, RP-HPLC, Metformin HCl, Pioglitazone, Validation, Optimization, Zero crossing point.

## INTRODUCTION

Chemically Metformin (MET), 1, 1-dimethyl biguanide hydrochloride (Fig. 1.), is a first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese

people.[1] It decreases hepatic glucose production and intestinal absorption of glucose, improves insulin sensitivity by increasing peripheral glucose uptake. It has no significant effect on the secretion of glucagon, cortisol, growth hormone, or somatostatin.

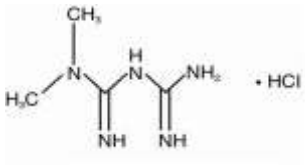


**Fig. 1: Chemical structure of Metformin HCl**

Pioglitazone (PIO), (RS)-5-{4-[2-(5-ethyl-2-pyridinyl} ethoxy] benzyl} thiazolidine-2, 4-dione (Fig. 2.) is a thiazolidinedione a potent and highly selective agonist for the nuclear receptor and peroxisome proliferator-activated receptor gamma (PPAR-γ).[2] PPARs are found in tissues like adipose tissue, skeletal muscle and liver, which are critical to insulin action. Activation of PPAR-γ modulates the transcription of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism.

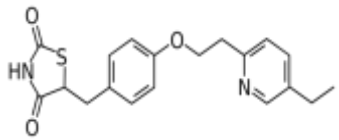


**Fig. 2: Chemical structure of Pioglitazone**

The literature survey revealed few analytical methods for determination of the MET and PIO individually in pharmaceutical dosage forms, in biological samples and in combined dosage forms.[3,11] The present paper describes both HPLC and UV derivative spectroscopic (UVDS) methods for the determination of Metformin HCl and Pioglitazone in combined dosage forms.[12-18]

## MATERIALS AND METHODS

### Chemicals

Authentic drug sample of Metformin HCl and Pioglitazone were provided by Yarrow Chem Products (Mumbai, India). Commercial tablets (Pioglit MF 15) containing MET and PIO were obtained from the local market. Analytical grade Methanol (Fisher scientific, Mumbai) was used as solvent for the UVDS method. HPLC grade Methanol and Acetonitrile were obtained from Fischer Scientific, Mumbai.

**Instruments**

Derivatization was performed on Agilent Cary 60 UV-Vis double beam Spectrophotometer with 1 cm matched quartzcells. Other equipments used in the study were Shimadzu (BL 220H) analytical balance and ultrasonic bath (Amkette industries-ANM USC). HPLC method was performed on Agilent prominence1220 infinity LC series equipped with Eclipse XDB plus C18 column.

**Method development**

Solvent selection: For optimization of the method, various solvents was tested and it was found that both MET and PIO were soluble in Methanol hence for the present investigation Methanol was used as solvent for both the drugs in HPLC and UVDS methods

**Preparation of the Standard Solutions**

**For HPLC method:** Accurately weighed 25 mg of each of MET and PIO standards were transferred to separate 25 ml volumetric flasks and dissolved in HPLC grade methanol. The solutions were made upto the mark to get 1 mg/ml stock solutions of each of MET and PIO. From these stock solutions, working standard solutions ranging from 10-50µg/ml of MET and PIO were prepared separately.

**For UVDS method:** Accurately weighed 25 mg of reference standards of MET and PIO were transferred to separate 25 ml volumetric flasks and dissolved in methanol AR grade to get a final concentration of 1 mg/ml. From these stock solutions, different working standards were prepared in the concentration range of 5-30µg/ml for each of MET and PIO.

**Method optimization**

**HPLC:** HPLC separation was carried out by Agilent prominence 1220 infinity LC C18 column with mobile phase consisting of Methanol and Tetrahydrofuran in the ratio of 70:30 %v/v at a flow rate of 1 ml/min. The sample injection volume was 20 µl and the UV detection was carried out at 230 nm for the determination of both drugs. A typical chromatogram of PIO and MET is shown in fig. 3. The retention times for PIO and MET was found to be 1.02 min and 2.51 min, respectively. The calibration curves for MET and PIO showed good linearity in the concentration range of 10-50 µg/ml as shown in Table 1.

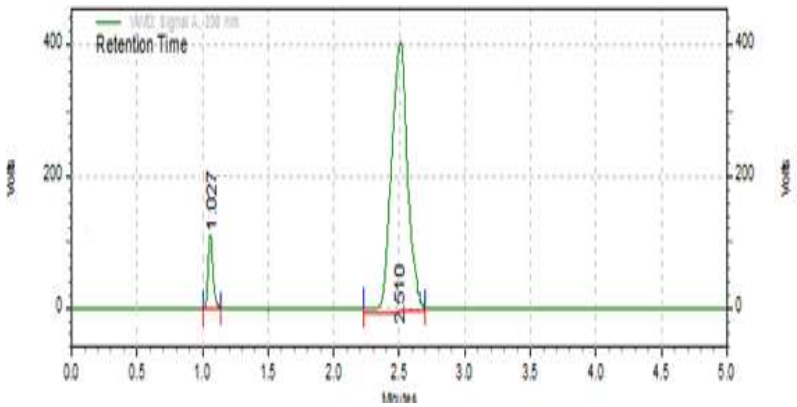


**Fig. 3: Chromatogram of Metformin HCl and Pioglitazone**

**UVDS:** The working standard solutions containing 20 µg/ml of MET and PIO were scanned in the wavelength range of 200-400 nm using methanol as reference in Agilent Cary 60 UV/Vis spectrophotometer (version 5. 0.0.999) in derivative mode and the corresponding overlain zero order spectrum was recorded which was converted to first (Fig 4) and second (Fig 5) order derivative spectra. Each spectrum was recorded in triplicate. For each replicate measurement the cell was refilled with fresh solution.

One particular wavelength was selected for each drug at which the absorbance of the other was found zero. From the examination of first and second order overlay derivative spectra, the working wavelengths were selected as 210 nm, 226 nm for MET where PIO exhibited zero absorbance and 230nm, 240 nm for PIO where MET exhibited zero absorbance.

The regression equations for the first and second order derivative spectra were obtained as y=0.00175x+0.00007, y=0.00010x+0.00002 for MET and y=0.00098x+0.00547, y=0.00011x+0.00099 for PIO.

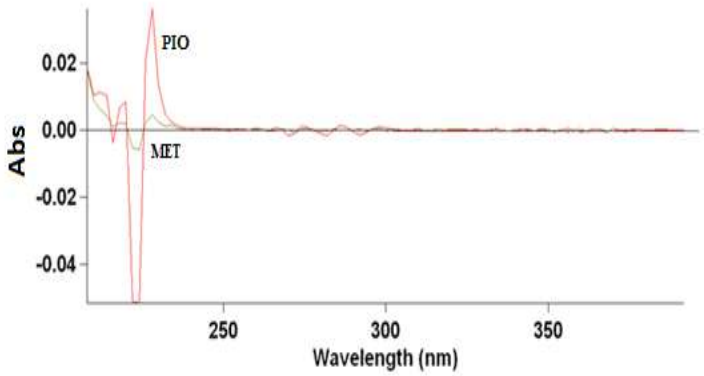


**Fig. 4: First order derivative spectrum of MET and PIO**

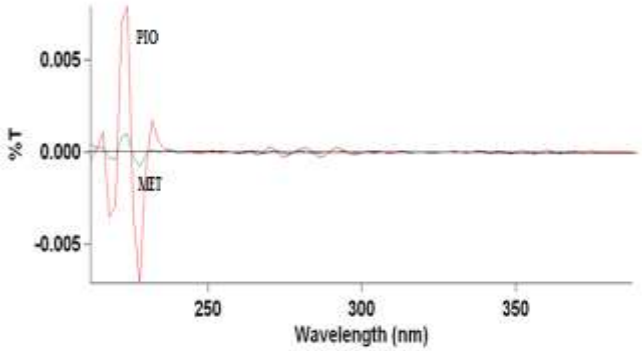


**Fig. 5: Second order derivative spectrum of MET and PIO**

**Assay of commercial Tablets**

Twenty tablets (Pioglit MF15) were weighed and made into a fine powder. The amount of powder equivalent to labeled claim of the drugs was placed in a volumetric flask. To it around 20ml of solvent (methanol) was added and the flask was placed in an ultrasonic bath for 15 min. The solution was then cooled and made upto volume with the same solvent. The solution was filtered through a 0.45 µm filter and then the filtrate was used to prepare sample solutions of different concentrations for both HPLC and UVDS.

**Table 1: Analysis of marketed formulation**

| Formulation | | Amount present (mg) | HPLC | | UVDS | |
|---|---|---|---|---|---|---|
| | | | Amount found (mg) | %recovery | Amount found (mg) | %recovery |
| PIOGLIT MF 15 | MET | 500 | 498 | 99.6 | 496 | 99.2 |
| | PIO | 15 | 14.91 | 99.4 | 14.87 | 99.13 |

**RESULTS AND DISCUSSION**

Both UVDS and HPLC methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

**Linearity**

The calibration curves constructed for both the drugs showed good linearity in the concentration range of 10-50 µg/ml for MET and PIO in HPLC method and 5-30µg/ml for MET and PIO in UVDS method respectively. The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis and the values are shown in Table 2.

**Table 2: Optical characteristics of Metformin HCl and Pioglitazone**

| Statistical parameters | HPLC | | First order | | Second order | |
|---|---|---|---|---|---|---|
| | MET | PIO | MET | PIO | MET | PIO |
| Linearity (µg/ml) | 10-50 | 10-50 | 5-30 | 5-30 | 5-30 | 5-30 |
| Correlation coefficient ($R^2$) | 0.998 | 0.999 | 0.992 | 0.998 | 0.996 | 0.998 |
| Regression equation y=mx+c | Y=234242 x+70014 | Y=33066x +1148.4 | Y=0.00175x +0.00007 | Y=0.00098x +0.00547 | Y=0.0010x +0.00002 | Y=0.00011 x+0.00099 |
| Slope (m) | 234242 | 33066 | 0.00175 | 0.00098 | 0.0010 | 0.00011 |
| Intercept(c) | 70014 | 1148.4 | 0.00007 | 0.00547 | 0.00002 | 0.00099 |

**Accuracy**

To check the accuracy of the proposed method, recovery studies were carried out by applying standard addition method. A known amount of standard MET and PIO corresponding to 80, 100 and 120% of the label claim was added to pre-analyzed sample of the tablet. The recovery studies were carried out in triplicate at each level and are shown in Table 3.

**Table 3: Accuracy of the proposed methods**

| Sample | Amount of tablet powder (mg) | Amount of pure drug added (mg) | Amount recovered± SD | | % Recovery | | % RSD | |
|---|---|---|---|---|---|---|---|---|
| | | | UVDS | HPLC | UVDS | HPLC | UVDS | HPLC |
| MET | 50 | 40 | 89.33±0.577 | 89.40±1.15 | 99.22 | 99.33 | 0.63 | 1.2 |
| | | 50 | 99.58±0.504 | 99.36±0.40 | 99.58 | 99.36 | 0.56 | 0.48 |
| | | 60 | 109.94±0.09 | 109.63±0.50 | 99.90 | 99.66 | 0.087 | 0.45 |
| PIO | 5 | 5.4 | 10.39±0.02 | 10.38±0.03 | 99.9 | 99.8 | 0.190 | 0.28 |
| | | 6.0 | 10.80±0.18 | 10.86±0.10 | 98.1 | 98.7 | 1.6 | 0.97 |
| | | 6.6 | 11.45±0.20 | 11.57±0.03 | 98.7 | 99.7 | 1.7 | 0.25 |

**PRECISION**

Precision is defined as "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample". The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Three sample solutions were prepared and analyzed. The values of intra-day and inter-day precision were shown in Table 4.

**Limit of Detection and Limit of Quantitation**

LOD and LOQ were calculated based on the standard deviation of the analytical response and the slope of the calibration curve using the equations LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the SD of the response and S is the slope of calibration curve as shown in Table 4.

**Table 4: Sensitivity and Precision of the proposed methods**

| Parameter | HPLC | | 1st order | | 2nd order | |
|---|---|---|---|---|---|---|
| | MET | PIO | MET | PIO | MET | PIO |
| LOD (µg/ml) | 0.019 | 0.010 | 0.132 | 0.09 | 0.525 | 0.3 |
| LOQ (µg/ml) | 0.059 | 0.030 | 0.4 | 0.3 | 0.9 | 0.9 |
| Precision (n=3) %RSD<br>Intra-day | 0.36<br>0.16<br>0.34 | 0.45<br>0.43<br>0.42 | 0.38<br>0.41<br>0.49 | 0.53<br>0.58<br>0.59 | 0.42<br>0.56<br>0.51 | 0.63<br>0.59<br>0.61 |
| Inter-day | 0.24<br>0.16<br>0.40 | 0.56<br>0.62<br>0.73 | 0.33<br>0.42<br>0.42 | 0.50<br>0.69<br>0.80 | 0.39<br>0.51<br>0.49 | 0.52<br>0.65<br>0.83 |

## CONCLUSION

The present article describes application of first order and second order derivative spectroscopy and RP-HPLC for the simultaneous estimation of Metformin HCl and Pioglitazone in tablet dosage forms. The developed methods were found to be simple, accurate, precise and rapid for estimation of both the drugs. The proposed UVDS exploits the zero crossing points for first and second order derivative spectra indicating the simplicity of the method.The elution times were found to be less than 5minutes for both the drugs which shows that the proposed HPLC method is quite rapid. Hence both the methods can effectively be applied for the routine analysis of Metformin HCl and Pioglitazone in bulk and in pharmaceutical dosage forms.

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