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Q-ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE INTABLET DOSAGE FORM

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ABSTRACT

A new, simple, accurate, precise and reproducible UV-Spectrophotometric method is being developed for the simultaneous estimation of Metformin Hydrochloride and Pioglitazone in tablet dosage form. The stock solutions were prepared in methanol. The λmax for Metformin Hydrochloride and Pioglitazone were found to be231 nm and 269nm respectively. The Metformin Hydrochloride and Pioglitazone obeyed Beer's law in concentration range of 5-30µg/ml and 2-12µg/ml respectively. Results of analysis of absorbance ratio method were analysed and validated for various parameters according to ICH guidelines for accuracy, precision, linearity, robustness, LOD and LOQ. The proposed method is highly sensitive, precise and accurate, therefore can be used for intended purpose.

KEYWORDS: Metformin Hydrochloride, Pioglitazone, Absorbance Ratio Method, Validation, ICH.

INTRODUCTION

Metformin HCL is an Antidiabetic drug. It is used to reduce the blood glucose level. A drug used to treat diabetes mellitus. Metformin hydrochloride decreases the amount of glucose (a type of sugar) released into the bloodstream. It is a type of anti diabetic agent. Another well-known benefit of this drug is modest weight loss. Metformin is the drug of choice for obese type II diabetes patients.

Chemical formula: C4H11N5, Molecular weight: 129.1636 g/mol.

IUPAC Name: 1-carbamimidamido-N, N-dimethylmethanimidam. Structure of Metformin Hydrochloride is given in figure 1.

Pioglitazone hydrochloride (PIO) is chemically [(±)-5-[[4-[2-(5-ethyl-2- pyridinyl) ethoxy] phenyl] methyl] -2, 4-] thiazolidinedione monohydrochloride. It is a potent agonist for peroxisome proliferator- activated receptor-gamma (PPARγ), activation of which modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipidmetabolism.

Structure of Pioglitazone is given in figure 2.

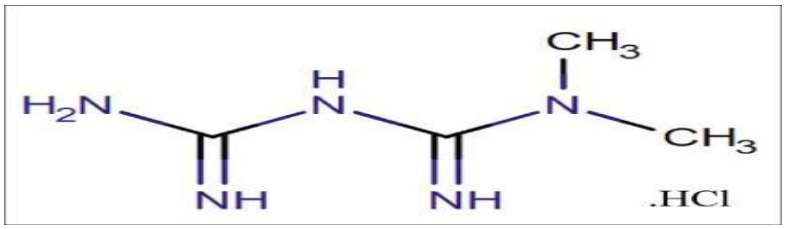

Figure 1

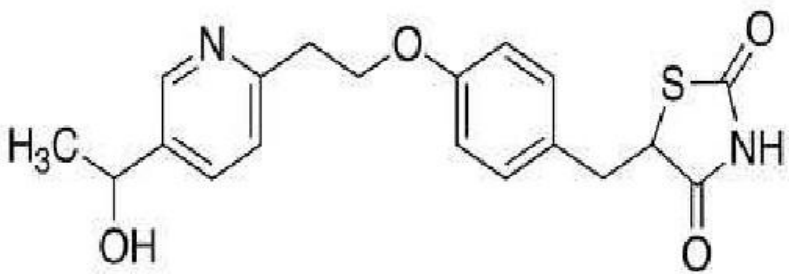

Figure 2

MATERIALS AND METHODS

Chemicals pharmaceutically pure sample of Metformin HCL was obtained from Wanbury Ltd. Vashi Navi Mumbai India and Pioglitazone was obtained from Nanoceut Therapeutics Pvt. Ltd. Puducherry Strides Shasun Ltd. (Puducherry) as gift samples. The commercial tablet Pioz MF-15 (Metformin HCL 500 mg and Pioglitazone 15mg) was procured from the local drug market. All the chemicals and reagentswere of analytical grade.

Instrument

Double beam UV-visible spectrophotometer model Jasco V-530 using spectra manger software. The spectra were recorded over range 200-400nm against solvent in 1 cm quarts cells.

Selection of solvents

On the basis of solubility study methanol was selected as the solvent for dissolving Metformin Hydrochloride and Pioglitazone.

Standard solution preparations

Accurately weighed 10mg of Metformin Hydrochloride and Pioglitazone were transferred into volumetric flasks separately and then volume was made up to 10ml with methanol to get a concentration of 1000µg/ml for all two drugs. Standard stock solution (1000µg/ml) was further diluted with methanol to obtain 5-30µg/ml and 2- 12µg/ml for Metformin HCL and Pioglitazone.

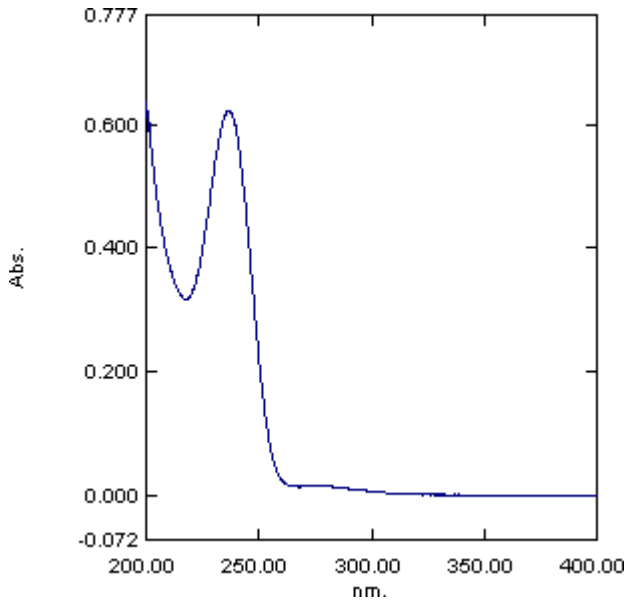

Figure 3 A: UV-Spectra of Metformin HCL at 231nm.

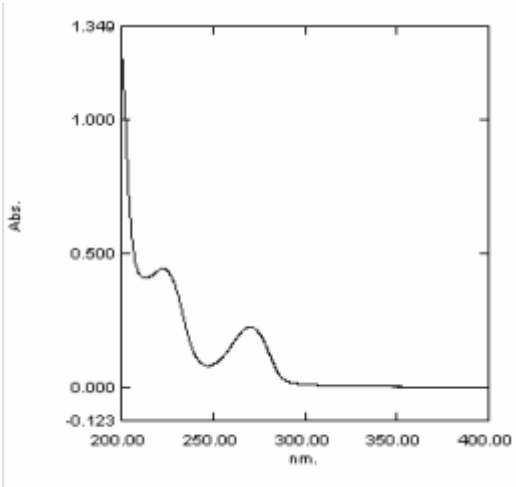

Figure 3 B: UV-Spectra of Pioglitazone. At 269nm.

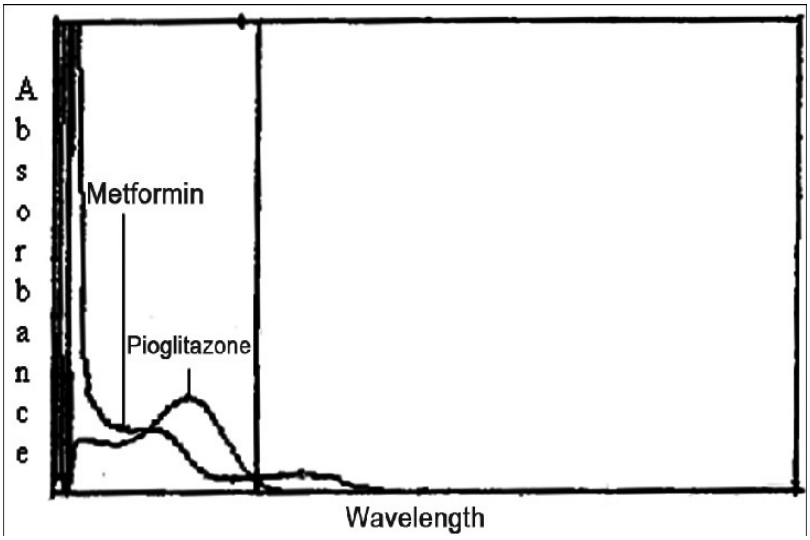

Figure 3 C: Overlain spectra of Metformin HCL and Pioglitazone.

Study of Spectra and Selection of wavelength

From the overlain spectrum of Metformin HCL and Voglibose, two wavelengths were selected one at 231 nm (λmax of Metformin HCL) and other at 247.5nm (Isoabsorptive point). The method employed Q -values, and the concentrations of drugs in sample solutions were determined using the following equations.

$$C = \frac{Q - Q_y}{Q_x - Q_y} \times \frac{A}{ax} \quad \text{.....equation 1.}$$

$$C_2 = \frac{Q - Q_x}{Q_y - Q_x} \times \frac{A_2}{ay} \quad \text{.....equation 2.}$$

Where, A and A_2 are the absorbances of mixture at 231 and 247.5nm, Q = A_2 / A, Q_y = ay_2 / ay and Q x = ax_2 /ax, ax (0.1471), ax_2 (1.1582), ay (0.7102) and ay_2 (2.4281) are absorptivities (1%, 1cm) of Metformin HCL and Pioglitazone at 231 and 247.5nm (Figure 3 A, 3 B and 3 C).

Preparation for analysis of tablet formulation

Twenty tablets were accurately weighed and crushed to fine powder. The tablet powder equivalent to 100mg of MET was accurately weighed, transferred to 100ml volumetric flask, dissolved in small quantity of methanol and finally make up to mark with methanol. This solution was filtered through Whatmann filter paper No. 41. The filtrate was further diluted

with methanol to get concentration of 30µg/ml of MET. The sample solution was scanned over the range of 325nm to 190nm in multi component mode and concentration of each component was estimated by analysis of spectral data of sample solution with respect to that of mixed standards by the instrument. Results of tablet analysis are reported in Table 1.

Validation of method

As per ICH guideline the method is validated and following parameters were evaluated.

Linearity

Its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The calibration curve was constructed between concentration verses absorbance.

Precision

Precision was determined by repeatability, Interday precision of all two drugs. Repeatability indicates the precision under the same operating condition over short interval time. The Interday precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst.

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

Sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantitation. According to ICH guidelines, the limit of detection is the lowest amount of analyte in a sample that can be detected and the limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Accuracy (% recovery)

For carrying out the accuracy of the proposed method recovery studies were employed by the standard addition method. This was carried out by adding known amounts of standard combination of PIO and MET at three different levels of 80%, 100%, and 120% to the sample.

Robustness

As per ICH norms, small, but deliberate variations by altering the pH and / or concentration of the solvent were made to check themethods capacity to remain unchanged.

RESULTS AND DISCUSSION

The Absorbance ratio method for estimation of Metformin HCL and Pioglitazone tablet dosage form was found to be simple, precise, accurate and reproducible. The solvent used was 100% methanol and do not shows any significant interference in the spectrophotometric assay of all two drugs.

Linearity

The proposed method was found to be linear in the range of 5-30 and 2-12µg/ml with correlation coefficient 0.9997and 0.999 for Metformin HCL and Pioglitazone respectively.

The calibration curve was constructed between concentration verses absorbance. It is shown into the Figure 4 and 5 resp. and result oflinearity study shown in Table 2.

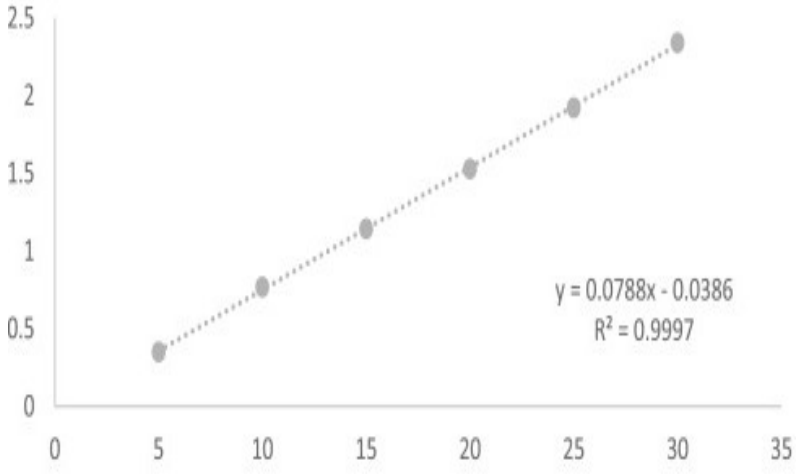

Figure 4: Calibration curve for Metformin HCL at 231nm.

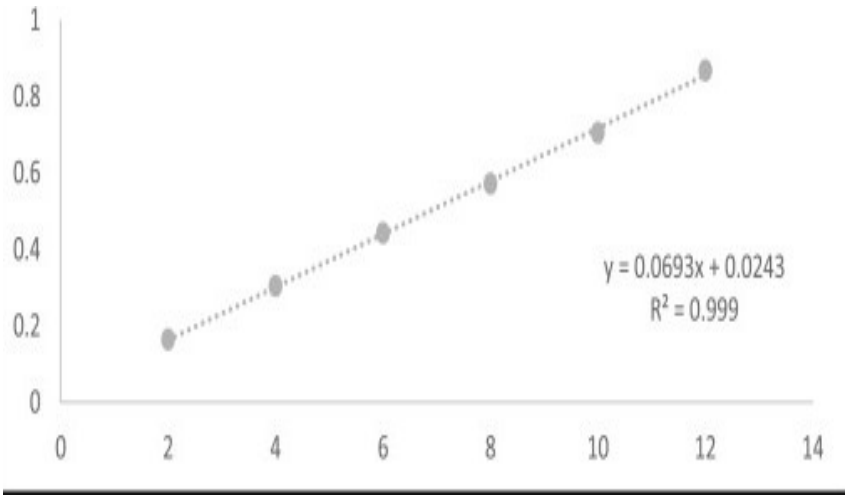

Figure 5: Calibration curve for pioglitazone at 269nm.

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

The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determinationof detection of limit and quantification limits.

Detection limit= 3.3 σ/s Quantification limit= 10 σ/s

Where, σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) data are given in Table 2.

Accuracy

For carrying out the accuracy of the proposed method recovery studies were employed by the standard addition method. This was carried out by adding known amounts of standard combination of VGB and MET at three different levels of 80%, 100%, and 120% to the sample. Result of recovery study shown in Table 3.

Precision

Precision was determined by repeatability and Interday precision of all two drugs.

i. Repeatability

The repeatability was performed for six concentrations in linearity range 5, 10, 15, 20,25, and 30µg/ml for Metformin HCL and 2, 4, 6,8, 10 and 12µg/ml for Pioglitazone indicates the precision under the same operating condition over short interval time.

ii. Interday precision

Interday precision was also performed within laboratory variation on different days for all two drugs simultaneously in three replicate at three concentrations. Result of precision shown in Table 4.

Robustness

Robustness

Standard stock solution of 1000µg/ml of Metformin HCL and Pioglitazone were prepared using methanol as a solvent. From standard stock solution, sub stock solution of Metformin HCL and Pioglitazone were prepared separately. In this present work the change was made in the ratio of solvent and absorption maxima.

Table 1: Result of tablet formulation.

Sr. No.	Drug Name	Labelled Amount (mg)	S.D.	% COV
1	Metformin HCL	500	0.7102	100%
2	Pioglitazone	15	0.0019	97.55%

(Where, S.D. = Standard Deviation, %COV= % Recovery)

Instead of 100%, 95% methanol was used as solvent. And the absorption maxima were decreased and increased 2 nm and carried out the process. The RSD% was calculated. Results of robustness shown in Table 5.

Table 2: Linear regression parameters for Metformin HCL and Pioglitazone by both proposed methods.

Sr. No.	Parameter	Metformin HCL	Pioglitazone
1.	Wavelength (nm)	231	269
2.	Calibration range(µg/ml)	5-30	2-12
3.	Correlation coefficient (r^2)	0.9997	0.999
4.	Slope(m)	0.0788	0.0693
5.	Intercept(c)	0.0386	0.0243
6.	Limit of detection (µg/ml)	0.167	0.201
7.	Limit of Quantitation (µg/ml)	0.506	0.610

Table 3: Recovery study at three concentration levels for Metformin HCL and Pioglitazone by both proposed meth0ds.

Sr. No.	Drug	Concentration of standard added	S.D.	% RSD	% Recovery
1	Metformin HCL	80%	0.0068	1.73	100.07%
		100%	0.0357	1.84	104.65%
		120%	0.0069	0.30	99.07%
2	Pioglitazone	80%	0.0017	0.19	97.40%
		100%	0.0067	0.78	100.83%
		120%	0.0088	1.10	100.91%

Table 4: Precision study for Metformin HCL and Pioglitazone by both proposed methods.

Sr. No	Parameter	S.D.		% RSD	
1.	Drug	MET	PIO	MET	PIO
2.	Repeatability	0.0069	0.0011	0.38	0.27
3.	Interday Precision	0.0084	0.5976	0.48	0.58

(Where, MET= Metformin HCL, PIO= Pioglitazone)

Table 5: Robustness study at three concentration levels for Metformin HCL and Pioglitazone by both proposed methods.

Sr. No.	Drug	Wavelength (nm)	S.D.	% RSD
1.	Metformin HCL	230	0.0051	0.57
		239	0.0012	0.23
2	Pioglitazone	261	0.0004	0.16
		270	0.0012	0.54

CONCLUSION

In this work, a new UV-Spectrophotometric method has developed and validated for Metformin HCL and Pioglitazone by Absorbance ratio method. The results of present study indicate that the proposed UV Spectrophotometric method is simple, rapid, precise and accurate. The developed UV-Spectrophotometric method was found suitable for determination of Metformin HCL and Pioglitazone in tablet dosage form. They can be easily applied in quality control laboratory tests in the dosage form.

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