

**Research Article****Development and Validation of RP-HPLC method for Simultaneous Estimation of Metformin and Pioglitazone**

Uttam kumar agrawal*, Jeyabalan Govindasamy

Department of Pharmacy, Sun Rise University, Alwar, Rajasthan, India

ARTICLE INFO:**Article history:**

Received: 12 January 2017

Received in revised form:

20 January 2017

Accepted: 22 January 2017

Available online: 30 March 2017

Keywords:

Metformin, Pioglitazone, RP-HPLC

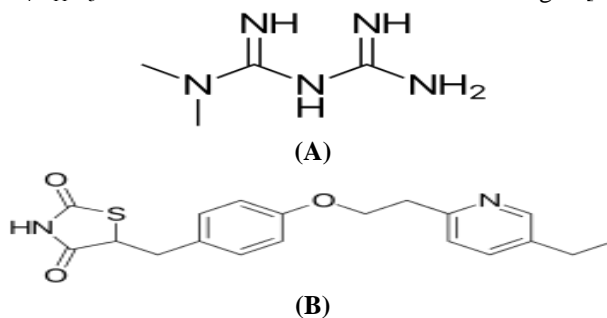
ABSTRACT

A new simple, accurate, precise and reproducible RP-HPLC method has been developed for simultaneous estimation of metformin and pioglitazone in bulk drug form using proper HPLC system. The mobile phase consists of phosphate buffer and acetonitrile in the ratio of 35:65 at pH 3.4. The detection wavelength was carried out at 228 nm. The method was linear over the concentration range for metformin 50-100 µg/mL and for pioglitazone 20-180 µg/mL. The recoveries of metformin and pioglitazone were found to be 100.5 and 98.7% respectively. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully for the analysis of pharmaceutical formulations containing dosage form.

Introduction

Metformin is chemically *N,N*-Dimethylimidodicarbonimidic diamide drug with chemical formula $C_4H_{11}N_5$ and molecular

weight 129.16364 g/mol. Metformin and pioglitazone are used as anti diabetic agent [1-3]. The structural formula is:

**Fig 1: structure of metformin(A) and pioglitazone (B)**

It has been used in conjunction with exercise and diet to improve diabetic condition and normal glucose level.

Pioglitazone is chemically (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione with molecular formula $C_{19}H_{20}N_2O_3S$ and molecular weight is 356.44 g/mol. It is used for the treatment of diabetic patient. The method is developed by rp hplc⁴ and different validation parameter [5,6] are developed with the help of HPLC [7-12] and UV visible instrument.

Experimental**Apparatus and reagent**

The liquid chromatographic system consists of Shimadzu with uv-vis detector, pump and injector valve with 20 micro litre fixed loop. The analytes were monitored at 228 nm. All chemicals and reagents were used AR grade. The metformin and pioglitazone were obtained as gift sample from Cipla.

Selection of detection wavelength

Selection of drug were scanned over the range of 200- 400 nm. It was observed that the drugs showed considerable absorbance at λ_{max} at 205 nm and 270 nm for metformin and pioglitazone respectively. The isobetic point of both drugs was found to be 228 nm.

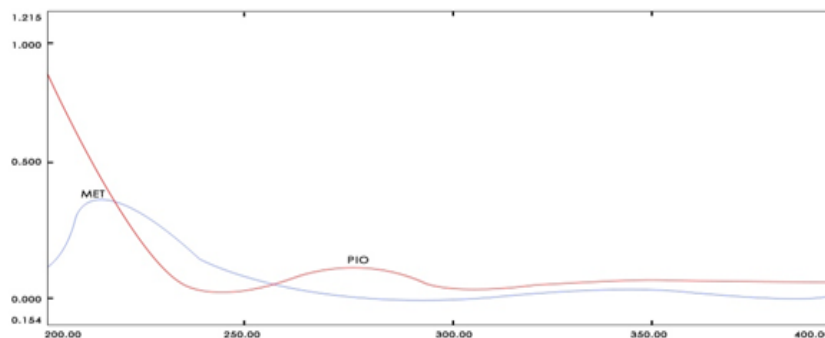


Fig 2: Isobestic point of metformin and pioglitazone

Chromatographic condition

The column is calibrated and equilibrated with mobile phase acetonitrile and phosphate buffer 65:35 at pH 3.4. The flow rate was maintained at 1.5 ml/min, detection wavelength 228 nm and the injection volume was 20 micro litre and temperature 25°C.

Preparation of standard and sample solution

The standard stock solution of metformin was prepared by dissolving 200 mg metformin in 100 ml of diluent to get a solution containing 2mg/ml of metformin. The standard stock solution of pioglitazone was prepared by dissolving 10 mg pioglitazone in 100 ml of diluent to get a solution containing 100 µg /ml of pioglitazone. Working standard solution of metformin was prepared by diluting appropriate aliquot of stock solution with diluent to get a solution containing 50 µg/ml metformin. Working standard solution of pioglitazone

was prepared by diluting appropriate aliquot of standard stock solution of pioglitazone with diluents to get a solution containing 200µg/ml of pioglitazone. Twenty tablets (each tablet containing metformin 500 mg and pioglitazone 15 mg were accurately weighed, their mean weight was determined and the tablets were powdered in a glass mortar. An amount of powder equivalent to two tablets was dissolved in 50 mL of diluent and was sonicated for 20 min. The resulting mixture was filtered through 0.45µ membrane filter (SY25TG, mdi Membrane Technologies, California USA). The filtrate, thus obtained containing metformin equivalent to 50 µg/mL and pioglitazone equivalent to 200 µg/mL was used for analysis.

Method Development

Lots of mobile phase and there different conc. We tried and finally selected acetonitrile and phosphate buffer in ratio 65:35 at pH 3.4 approx. Mobile phase which gave good resolution and acceptable system suitability parameter. The chromatogram of working std. soln. is shown in fig 3.

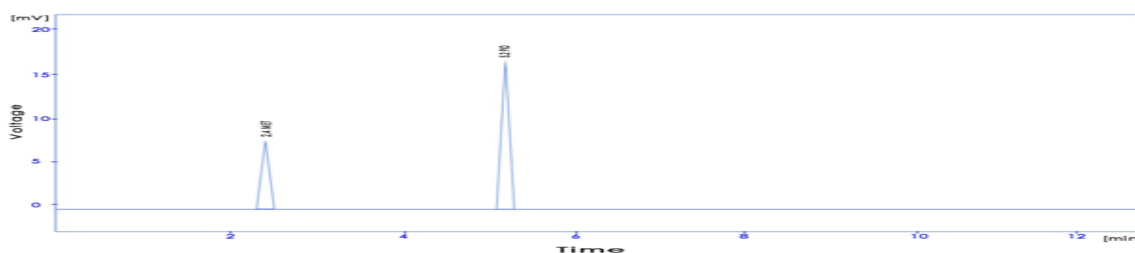


Fig 3: HPLC chromatogram of metformin and pioglitazone

Analytical validation parameter

Linearity: A good linear relationship between concentration and peak areas over a concentration range of 50-100µg/ml for metformin and 20-180 µg/ml for pioglitazone. The correlation coefficient was found to be 0.999 for metformin and 0.999 for pioglitazone which are greater than 0.999.

Accuracy: Percent recoveries were obtained from the difference between the areas of spiked and unspiked samples. The mean recovery of the added standard drug was 100.5 and 98.70% for metformin and pioglitazone respectively. These mean recovery values are well within the 98-102% indicating the method is accurate.

Precision: RSD of mean assay values was found to be 0.28%

for metformin and 1.02% for pioglitazone. These %RSD values which are well below 2% indicate that the repeatability of this method is satisfactory.

LOD AND LOQ: The LOD was found to be 1.52 µg/mL for metformin and 20.2 µg/mL for pioglitazone. LOQ was found to be 12.4 µg/mL for metformin and 13.5 µg/mL for pioglitazone. These values indicate that the method is sensitive.

Specificity: Good resolution was obtained between the drugs and excipients showing complete separation of metformin and pioglitazone. No interference from excipients, impurities, or degradation products ensured that the peak response was due to metformin and pioglitazone only.

Analytical parameter

| Parameter | Metformin | Pioglitazone |
|-------------------------------------|-----------|--------------|
| Linearity range($\mu\text{g/ml}$) | 50-100 | 20-180 |
| Retention time | 2.4 | 5.2 |
| Accuracy(% recovery) | 100.5 | 98.7 |
| Precision repeatability(%RSD) | 0.20 | 1.02 |
| LOD ($\mu\text{g/ml}$) | 1.52 | 1.02 |
| LOQ ($\mu\text{g/ml}$) | 1.24 | 1.35 |
| Precision intermediate(%RSD) | 1.71 | 1.82 |

Results and discussion

The present work done on this combination comprises a simple and accurate method by reverse phase hplc. An attempt has been made to estimate metformin and pioglitazone by rp-hplc. Calibration curve depicting the linearity and range for metformin and pioglitazone were determine from mixed std. and were found to be order 50-100 and 20-180 respectively. The retention time for metformin and pioglitazone were 2.4 and 5.2 respectively. The results obtained from hplc method were reproducible and encouraging. The values % was within limit(>2%) and recover close to 100% indicating reproducible and accuracy of method.

Conclusion

Proposed study describes method for the estimation of metformin and pioglitazone combination in mixture. The method was validated and found to be simple, accurate and precise as per ICH guidelines. The method was successful used for determination of drugs in their pharmaceutical formulation.

References

1. Available online at: <https://en.wikipedia.org/wiki/metformin>.
2. Available online at: <https://en.wikipedia.org/wiki/pioglitazone>.
3. Tripathi K.D. Essential of Medical Phamacology, 2003, 7th Edn, Jaypee Brother Medical publisher New Delhi, p-275-277.
4. Beckett, A. H.; Stenlake, J. B. Practical pharmaceutical chemistry. In CBS publishers and distributors, New Delhi: 1997; Vol.2, p -163
5. ICH QIA (R2).” Stability Testing of New Drug Substances and Products”. 2003
6. ICH Q2B, Validation of Analytical Procedures: Methodology, adopted in 1996, Geneva Q2B, in 2005 incorporated in Q2(R1)
7. Kasture AV, Wadodkar SG, Mahadik KR, More HM. “Introduction to Instrumental Techniques”. 12th ed., Vol. II. Pune: Nirali Prakasan; 2005. pg. 1-10.
8. Ranetti M, Ionescu M L, Ionica E, Anuja V, Aurrian E.etal, 2009, Validation od a hplc method for the simultaneous analysis of metformin and pioglitazone, Farmacia. 57(6), 720-735.
9. Lakshmi KS, Rajesh T, Sharma S, Simultaneous determination of metformin and pioglitazone by rphplc, J Chromotogracic, 501-504
10. Kasture A.V., Wadodkar. S.G., Mahadik. K.R., More. H.M., “Introduction to Instrumental Techniques”. 12th ed. Vol. II. Nirali Prakasan, Pune; 2005; Pg. 1-10.
11. Ewing G.W., “Instrumental Methods of Chemical Analysis”. 5th ed.: McGraw-Hill Book Company, New York; 1985. Pg. 1-7
12. Mendham J., Denney R.C., Baraes J.D., Thomas M.J.K. “Vogel’s Textbook of Quantitative Chemical Analysis”. 5th ed. Pears Education, Singapore; 1991; Pg. 5-11.

Cite this article as: **Uttam kumar agrawal, Jeyabalan Govindasamy**. Development and Validation of RP-HPLC method for Simultaneous Estimation of Metformin and Pioglitazone. **Indian J. Pharm. Biol. Res.** 2017; 5(1):48-50.

All © 2017 are reserved by Indian Journal of Pharmaceutical and Biological Research

This Journal is licensed under a **Creative Commons Attribution-Non Commercial -Share Alike 3.0 Unported License**. This article can be downloaded to ANDROID OS based mobile.