

Quantitative Calculation of Metformin in Diabetic Patients Serum by RP-HPLC

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Abstract:

Metformin is an oral medication that helps to control blood glucose (sugar) for treating type 2 diabetes. Most of diabetes patients are used this medicine as per suggestions of their doctors. After using this medicine, some amount of that only released in to the body and remains in the blood. This article proposes quantitative estimation of remaining drug in the body by RP-HPLC. In the latest study of serum analysis, this metoformin in the blood was high amount in old people comparing with the young ones because of their metabolic activities.

Keywords: Metformin, metabolic, serum, RP-HPLC, diabetes.

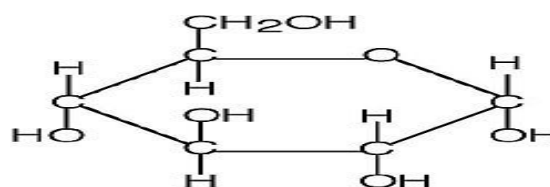
I. INTRODUCTION

The metformin normally suggested for patients who suffered by diabetes mellitus type 2, particularly overweight people. This metformin helps to reduce diabetes complications with the long time treatment. In the first four years this medicine helps to reduce overall mortality by about 30% when compared with other medicines like insulin and sulfonylureas (glibenclamide and chlorpropamide) and by about 40% when compared with the group only given dietary advice. This difference held in the patients who were followed for 5–10 years after the study.

Since intensive glucose control with metformin appears to reduce the risk of diabetes-related endpoints in obesity diabetic patients, and is associated with less weight gain and fewer hypoglycaemic attacks than are insulin and sulphonylureas, it may be the starting level pharmacological therapy of choice in these patients. In addition, metformin will not help to reduce the body weight: Over the 10-year treatment period, the metformin group gained about 1 kg, the same as the dietary advice group, while the sulfonylureas group gained 3 kg, and the insulin group, 6 kg. As metformin affords a similar level of blood sugar control to insulin and sulfonylureas, it appears to diminish mortality primarily through decreasing cardiac arrest, strokes and other cardiovascular complications.

Metformin has a less risk of hypoglycemia than the sulfonylureas, although it has exceptionally

occurred during intense exercise, calorie deficit, or when used with other agents that lower blood glucose. Metformin is also not associated with weight gain, and modestly reduces LDL and triglyceride levels. Metformin formula is $C_4H_{11}N_5$ IUPAC Name is N,N-dimethylimidodicarbonimidicdiamide. Molecular weight is 129.164 g/mol.



Structure of Glucose Figure 1.1

II. EXPERIMENTAL

Chemicals and Reagents HPLC grade methanol and water were purchased from Merck Specialties Pvt. Ltd.

A. Instrumentation and Analytical Conditions

The analysis of drug was carried out on a PEAK HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5 μ m in particle size), a LC-P7000 isocratic pump, a 20 μ l injection loop and a LC-UV7000 absorbance detector and running on PEAK Chromatographic Software version 1.06. Isocratic elution with methanol: water 70:30 (V/V) PH- 4.6 adjusted with ortho phosphoric acid was used at a flow rate of 1.4ml/min UV detection at 232nm. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

B. Collection of Samples

Blood samples are collected from Mahatma Gandhi Government hospital of diabetic patients those people are used metformin according to their prescription.

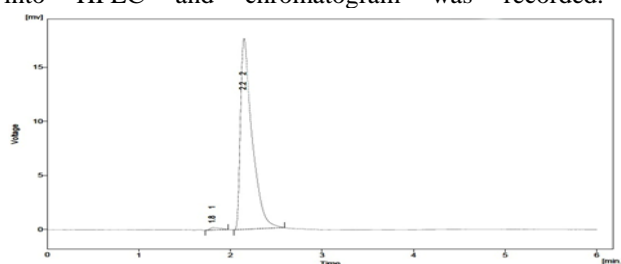
C. Preparation of Samples

From the blood serum was separated. 0.5ml of this serum was taken in a test tube and added 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000

rpm for 10min. From this centrifuged solution 4ml of organic layer was separated and evaporated to dryness to get residue. To this residue 100µl of 1M acetic acid and 3ml of n-Hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded.

D. Preparation of Standard Metformin

10mg of metformin was accurately weighed and it transferred into a 10ml volumetric flask and dissolved in mobile phase and make up the volume up to the mark with mobile phase (stock solution) and it sonicated for 5min. Finally 5µg/ml sample was prepared from stock solution. The sample was injected into HPLC and chromatogram was recorded.



Typical Chromatogram of Metformin Fig 1.2

III. RESULT AND DISCUSSION

From the experiment in different age group people the amount of metformin rest in the blood was different. Generally when the drug enter in to the body some amount of that consumed by the body the rest of drug is remaining in the blood. So that we come to know, the excessive metformin medicine deposited in blood. Consuming of this medicine continuously will not create any significant side effects to our body. Also this will not help to reduce weight. The proper diet and exercise will help to control the insulin in our body.

S.No	Gender and Age	Amount of metformin found
Patient 1	Female 49 years	0.67µg/ml
Patient 2	Male 54 years	0.34µg/ml
Patient 3	Male 58 Years	0.29µg/ml
Patient 4	Female 45 years	0.44µg/ml
Patient 5	Male 55 years	0.69µg/ml

IV. CONCLUSION

The drug is remains high amount in blood along with the age increment after absorption into the tissues. Though this medicine remains in blood, this will not produce any side effects to the patients. After certain period of time, this metformin drug will be automatically pushed out from the blood serum. Also this will not help to reduce weight of the patient. Being in follow of balanced food habits and physical exercise will be the only solution to come out from long time patients suffered by diabetic. Meanwhile, taking medicine will not create any important issues in patients' health condition as per this research.

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