


## SIMULTANEOUS ESTIMATION OF ISONIAZID, METFORMIN HYDROCHLORIDE AND GLIMEPIRIDE IN BULK BY TLC METHOD

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<p><b>*For Correspondence:</b> Padhmashri. Dr.Vitthalrao Vikhe Patil Fondation's. College of Pharmacy, Ahmednagar, Maharashtra.</p>	<p><b>ABSTRACT</b> TLC method was developed for simultaneous estimation of isoniazid, metformin hydrochloride and glimepiride. Analysis was done in synthetic mixture of isoniazid, metformin and glimepiride. Stationary phase silica G. was used. Mobile phase methanol: water at the ratio 60:40 v/v was used. Detection was carried out in iodine chamber. Rf value of the isoniazid, metformin hydrochloride and glimepiride was found to be 0.1875, 0.212, 0.53 and for synthetic mixture 0.17, 0.21 and 0.52 respectively.</p> <p><b>KEY WORDS:</b> Isoniazid, Metformin HCL, Glimepiride, Methanol, Distilled water and TLC</p>
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### INTRODUCTION

Isoniazid, metformin hydrochloride and glimepiride plays vital role in the treatment of patient suffering individual diseases diabetes and tuberculosis. Hence there is no method developed for the simultaneous estimation of isoniazid (antituberculosis) and metformin hydrochloride, glimepiride (antidiabetic) drugs in combination. Simultaneous estimation of isoniazid, metformin hydrochloride and glimepiride done by separation using chromatographic method like HPLC, which simpler, economic and effective method for simultaneous estimation of isoniazid (antituberculosis) and metformin hydrochloride, glimepiride (antidiabetic) drugs in synthetic mixture. Isoniazid (pyridine-4-carbohydrazid) heterocyclic compound which first line drug used in tuberculosis. It is effective both in acidic and alkaline medium. Isoniazid is tuberculocidal for rapidly multiplying bacilli but static for increasing resting bacilli. INH inhibits the synthesis of mycolic acid which are important components of the mycobacterial cell wall by which it shows tuberculocidal action. Metformin hydrochloride (MET) (N,N-dimethylimidodicarbonimidic diamide hydrochloride) is a biguanide antihyperglycemic agent used for treating non-insulin dependent diabetes mellitus. It improves glycemic production, decreasing glucose absorption and increasing insulin mediated glucose uptake. Metformin is an oral antihyperglycemic agent that improves glucose tolerance in patient with NIDDM lowering both basal and postprandial plasma glucose. Glimepiride (GLP) 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrrolinocarboxamide)ethyl]-phenyl]-sulfonyl]-3-(trans-4-methyl cyclohexyl) urea. It is used to

treat type II diabetes in which body does not use insulin normally and therefore, cannot control the amount of sugar in the blood. GLP lowers the body sugar by causing the pancreas to produce insulin.

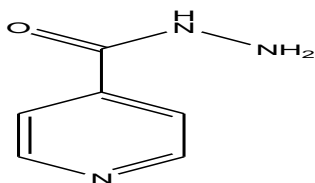


Fig 1a: Structure of isoniazid

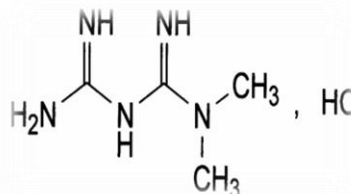


Fig 1b: Structure of metformin hydrochloride

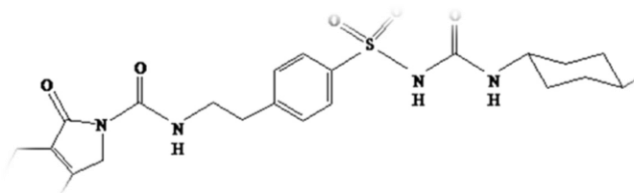


Fig 1c: Structure of glimepiride

## MATERIALS AND METHODS

### EXPERIMENTAL:

#### Materials:

HPLC grade methanol, double distilled water was used which is self-generated from triple distillation glass assembly. Silica G, Isoniazid, metformin hydrochloride and glimepiride bulk drugs sample.

#### Equipment:

**Instrument:** Iodine chamber, TLC plates.

**Preparation of TLC plates:** They were prepared by mixing the adsorbent i.e. silica gel with water. This mixture was spread as a thick slurry on a glass plate. The plate is dried and activated by heating in an oven for 30 min at 101°C. The thickness of the adsorbent layer is typically around 0.1-0.25mm.

**Preparation of sample solution:** Small amount about 1-2 ml solvent methanol was taken and the drug was slightly mixed with it, till the solution became saturated. Synthetic mixture was prepared by mixing individual sample solution in same proportion.

**Selection of mobile phase-**Different proportion of methanol and water like (80:20, 85:15, 70:30, 60:40v/v) were tried in order to find the best conditions for the separation of metformin hydrochloride, glimepiride and isoniazid. Mobile phase methanol and water of proportion 60:40%v/v was found which gives satisfactory result.

**Preparation of mobile phase-**20ml of methanol and water mobile phase was prepared of 60:40%v/v. The prepared mobile phase was saturated for 30 min.

**Chromatographic conditions:** Method was developed using glass plates. Mobile phase used was methanol: water (glass double grade distilled water) 60:40% v/v. The small spot of sample solution applied to the plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, then plate needs to be dried in a vacuum chamber. The mobile phase run on the spotted plates by transferring small amount of mobile phase in the suitable container covering top with plate to maintain the saturation. Detection was carried out in iodine chamber.

## RESULT AND DISCUSSION

## Method development

### Selection of mobile phase:

Different combinations of methanol and water were tested and the optimum condition at methanol-water (60:40v/v). The obtained chromatograph showed a Rf value for isoniazid 0.1875 (Figure 4 and Table 3), metformin hydrochloride 0.212 (Figure 2 and Table 2) and glimepiride 0.53 (Fig 3 Table 3). In the synthetic mixture of isoniazid, metformin hydrochloride and glimepiride shows rapid separation with Rf value 0.17, 0.21 and 0.52 respectively (Figure 5 and Table 4).

**Selection of HPLC stationary phase:** The best results were obtained by using Silica G.



Fig no 2: Chromatogram of metformin hydrochloride (MET) by using TLC.

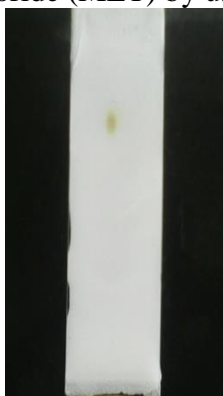


Fig no 3: Chromatogram of Glimepiride (GLP) by using TL



Fig no 4: Chromatogram of isoniazid (INH) by using TLC

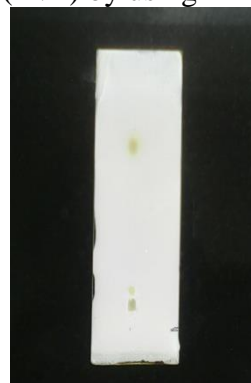


Fig no 5: Chromatogram of synthetic mixture of metformin hydrochloride, glimepiride and isoniazid by using TLC.

**Table no 1: Chromatographic data of metformin hydrochloride**

Name of the drug	Distance travel by drug	Distance travel by solvent	Rf value
Metformin hydrochloride	1.7	8	0.212

**Table no 2: Chromatographic data of Glimepiride.**

Name of the drug	Distance travel by drug	Distance travel by solvent	Rf value
Glimepiride	4.15	8	0.53

**Table no 3: Chromatographic data of Isoniazid.**

Name of the drug	Distance travel by drug	Distance travel by solvent	Rf value
Isoniazid	1.4	8	0.1875

**Table no 4: Chromatographic data of Synthetic mixture of metformin hydrochloride, glimepiride and isoniazid.**

Name	Distance travel by drug	Distance travel by solvent	Rf value
Isoniazid	1.4	8	0.17
Metformin hydrochloride	1.8	8	0.21
Glimepiride	4.2	8	0.52

## CONCLUSION

Method was developed using glass plates. Mobile phase used was methanol: water (glass doubled grade distilled water) 60:40% v/v. The small spot of sample solution applied to the plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, then plate needs to be dried in a vacuum chamber. The mobile phase run on the spot plates by transferring small amount of mobile phase in the suitable container covering top with plate to maintain the saturation. Detection was carried out in iodine chamber. The obtained chromatogram shows Rf's value for metformin hydrochloride 0.212(table no 1), glimepiride 0.53(Table no 2), isoniazid 0.187 (Table no 3) and synthetic mixture for isoniazid 0.17, glimepiride 0.52 and metformin hydrochloride 0.21 (Table no 4). These developed method is new, convenient, economic and rapid.

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