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RAPID HPLC METHOD FOR IMPURITY PROFILE OF METFORMIN HYDROCHLORIDE IN PRESENCE OFITS COMBINATION DRUGS

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ABSTRACT

A fast and selective method using HPLC was developed for determination of impurity profile of Metformin hydrochloride in the presence of its usual combination of drugs. Antidiabetic combination of drugs like Glipizide, Gliclazide, Glibenclamide, Glimepiride along with Metformin Hydrochloride are available for treatment of diabetic patients in India. A single method of analysis for Impurity profile of Metformin along with each of the four drug combination with Metformin was developed. The method was validated and proved to be specific, precise and robust. Area response was found to be linear in the impurity level concentration range of 0.5 to 5ppm for Metformin HCl. The correlation coefficient was found to be 0.999 for Metformin HCl.

Key Words: *Metformin, Glipizide, Gliclazide, Glibenclamide, Glimepiride.*

I. INTRODUCTION

Metformin hydrochloride is an anti-hyperglycemic drug from biguanide class used in management of Type 2 diabetes. Its molecular formula is $C_4H_{11}N_5$.HCl and molecular weight is 165.62g/mol [1] (Figure 1-a). Glipizide is an anti-hyperglycemic drug of the sulfonylurea class. It has molecular formula $C_{21}H_{27}N_5O_4S$ and molecular weight 445.54g/mol(Figure 1-b). Gliclazide is an oral anti-hyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus. It has molecular formula $C_{15}H_{21}N_3O_3S$ and molecular weight 323.411 g/mol (Figure 1-c).Glibenclamide (Glyburide) is an anti-hyperglycemic drug of the sulfonylurea class.Glibenclamide has molecular formula $C_{23}H_{28}ClN_3O_5S$ and molecular weight 494.0 (Figure 1-d). Glimepiride belongs to sulfonylureas class of drugs. It lowers blood sugar by causing the release of body's natural insulin. It has molecular formula $C_{24}H_{34}N_4O_5S$ and molecular weight 490.62g/mol (Figure 1-e).

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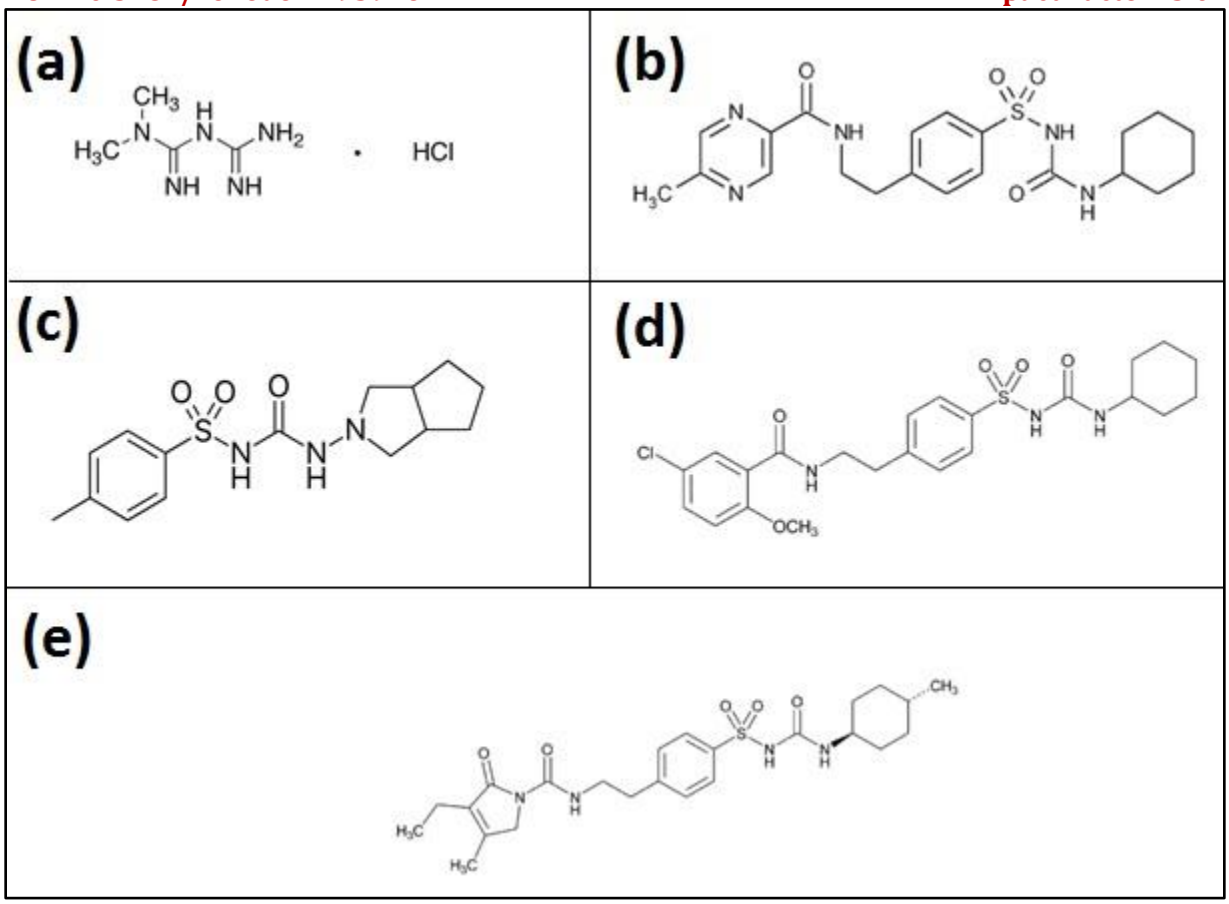

Figure 1: Structure of drugs used in this study

Metformin is available along with many fixed dose anti-diabetic drug combinations;Metformin Hydrochloride and Glipizide tablets, Metformin Hydrochloride and Gliclazide tablets, Metformin Hydrochloride and Glyburide tablets, Metformin Hydrochloride and Glimepiride tablets are few of them. A single method of analysis having short run time, for Impurity profile of Metformin, considering these types of combination tablet would be helpful to analytical scientist. The present research work focusses on development of method using sub-two micron particle size HPLC method for impurity profile of Metformin HCl with combination drug.This method will be helpful to analyze multiple combinations of tablets having Metformin HCl as a common drug in them.

Few methods by HPLC are available for estimation of Metformin HCl along with combination drug and also for individual Metformin [3-9]. United States Pharmacopoeia has a monograph for Glipizide and Metformin hydrochloride tablets consisting of separate HPLC methods for Assay and Impurities of each drug [1]. Also monograph for Glyburide and Metformin Hydrochloride tablet is present; this monograph too has separate methods for Metformin HCl and Glyburide [1]. However focus of this study was to develop a single fast and accurate method using modern instruments like rapid resolution liquid chromatography for different combinations of drug. A fast and cost effective method of analysis can be developed thereby saving precious analytical time. Easily available and cheap chemicals are been used for this developmental work.

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II. MATERIALS AND METHODS

Chemicals and Reagents:
Metformin,Glipizide, Gliclazide, Glibenclamide and Glimepiridewere purchased from API vendors. These API were used as working standard. Methanol and Acetonitrile used were of AR grade solvents of Rankem Ltd. Purified water used was from Millipore water purification system. Following combination tablets used for this activity were purchased from local medical stores:

1) Metformin 500mg and Glipizide 5mg tablet
2) Metformin 500mg and Glibenclamide 5mg tablet
3) Metformin 500mg and Gliclazide 80mg tablet
4) Metformin 500mg and Glimepiride2mg tablet

Instruments and Equipment:
HPLC of the make –Agilent 1200 series, was used in the experiment. Ultrasound Sonicator of local make was used during sample preparation.

III. METHOD OF ANALYSIS

A single method of analysis is developed for four different combination of drug along with Metformin, as a common drug. Hence, Metformin standard wasprepared and sample solutions of formulation wereprepared from combination tablets. Users can select their combination drugs as per requirement and prepare sample solutions accordingly.

Mobile phase preparation:
Mix Buffer: Acetonitrile in proportion of (65:35).

Buffer preparation:
Add 1ml of Triethylamine and 1ml of Orthophosphoric acid to 1000ml of water. Measure the pH and filter with 0.2µ filter paper.

Preparation of diluent:
Methanol and mobile phase were used as diluent, wherever applicable

Preparation of standard:
25mg of Metformin HCl was dissolved in methanol and diluted to 250ml in a volumetric flask. Dilute 1ml of stock solution to 100ml with mobile phase.

Preparation of sample solution for Metforminfrom tablet dosage form
(Sample solution for Metformin from all four combination drug tablet dosage form can be prepared as per procedure mentioned below)

Crush five intact tablets to fine powder. Transfer powder equivalent to 1 tablet into a dry 100ml volumetric flask. Add 50ml of methanol to this flask. Sonicate for 15min with intermittent shaking. Cool flask to room temperature and dilute to volume upto the mark with methanol. From this solution, dilute 1ml of solution to 50ml with diluent.Filter with 0.2µ porosity membrane before use.

HPLC chromatographic setup:
Inject 5µL of diluent, Standard and Sample solution on HPLC system setup with 0.3ml/min flow rate. A chromatographic column of Acquity UPLC BEH C8, 2.1 x 50mm, 1.7µ particle size was used. Column temperature was 35°C and Wavelength of detection was 225nm.

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IV. RESULTS AND DISCUSSION

Method development and optimization:

For method development of combination tablet dosage form various analytical aspects of each API, sample preparation, chromatography and pharmaceutical formulation needs to be assessed. Most important factor is interference occurring due to absorption of each drug in UV region and also those due to the soluble excipient from sample matrix need to be considered. For developing a selective method for estimation of impurities of Metformin, the basic aim was to eliminate the interference that may occur due to each of combination drug and its possible impurities. Due to resources limitation, known impurities of Metformin and other drugs could not be used in this research work. However separation of each drug peak was critically achieved, so as to propose a selective method. Since Metformin is a combination drug along with other API in tablet, it was obvious that the sample solution will contain second drug along with Metformin in tablet. This also creates possibility of impurities arising due to each drug. Hence a detailed study of the characteristics of each API was carried out. This includes the structure, solubility, absorbance in UV region and compatibility of the two API with each other. Actual analytical challenge was that all API exhibit absorbance in the range of 200-400nm. Hence solutions of each drug were prepared separately and scanned. Wavelength of estimation was selected such that minimal interference of other API would occur. However since HPLC chromatographic separation is superior technique for resolving complex mixtures of sample component, an optimum separation is achieved using 1.7µ particle size column. Here separation of 5 API is achieved successfully in a HPLC analysis time of 10minutes. The other method parameters like injection volume, column oven temperature, sample temperature and mobile phase parameters were optimized on basis of trial and error method. The effectiveness and practical workability of method was checked by performing Method validation activity.

Method Validation:

To check the suitability of method for intended use, method validation was carried out as per parameters mentioned in ICH guidelines [2].

Specificity:

Method selectivity nature was proved by injecting separately; diluent, Metformin, Glipizide, Gliclazide, Glibenclamide and Glimepiride. Separate injections of Sample solution from combination tablet were also made to check its conformance with respect to Retention time. It was observed that, no peak was observed from diluent at retention time of Metformin. Also there was no peak due to each of API at the retention time of Metformin and other API. The retention time of Metformin in standard solution and Tablet sample solution was found to be matching. To check the effect of peak shape in presence of each other, mixture of all API was also injected. All peaks were well separated from each other.Hence the method is selective for Metformin at 225nm.

Preparation of Metformin HCl solution for specificity (10ppm):

About 25mg of Metformin HCl was dissolved in methanol and diluted to 250ml in a volumetric flask. Dilute 1ml of stock solution to 10ml with mobile phase.

Preparation of Glipizide solution for specificity (10ppm):

About 25mg of Glipizide was dissolved in methanol and diluted to 250ml in a volumetric flask. Dilute 1ml of stock solution to 10ml with mobile phase.

Preparation of Gliclazide solution for specificity (10ppm):

About 25mg of Gliclazide was dissolved in methanol and diluted to 250ml in a volumetric flask. Dilute 1ml of stock solution to 10ml with mobile phase.

Preparation of Glibenclamide solution for specificity (10ppm):

About 25mg of Glibenclamide was dissolved in methanol and diluted to 250ml in a volumetric flask. Dilute 1ml of stock solution to 10ml with mobile phase.

Preparation of Glimepiride solution for specificity (10ppm):

About 25mg of Glimepiride was dissolved in methanol and diluted to 250ml in a volumetric flask. Dilute 1ml of stock solution to 10ml with mobile phase.

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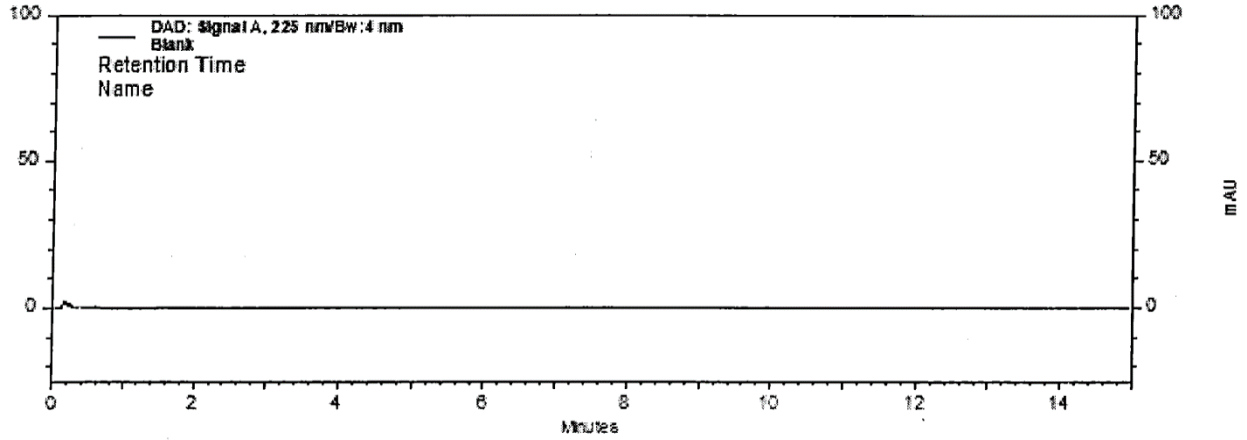

Figure2: Chromatograph of diluents

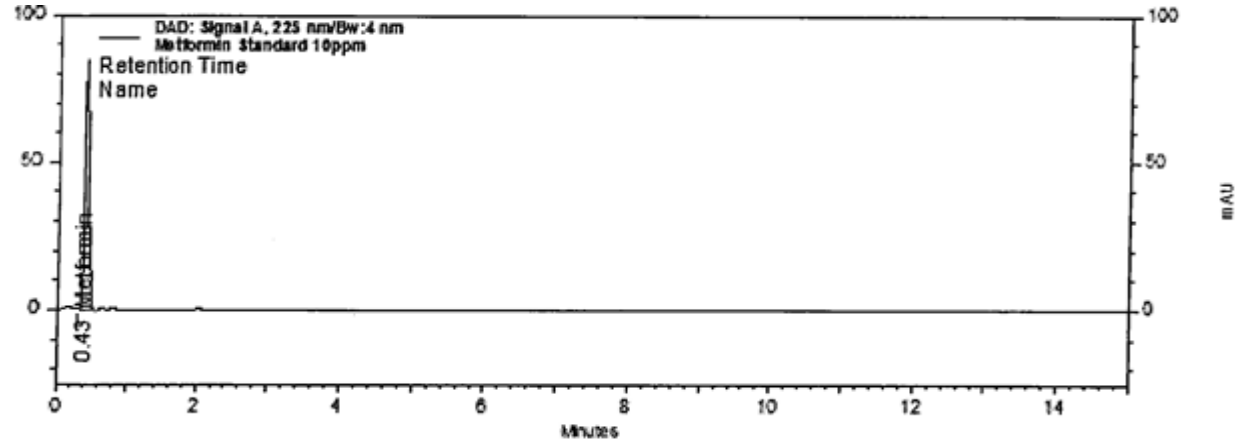

Figure3: Chromatograph of Metformin

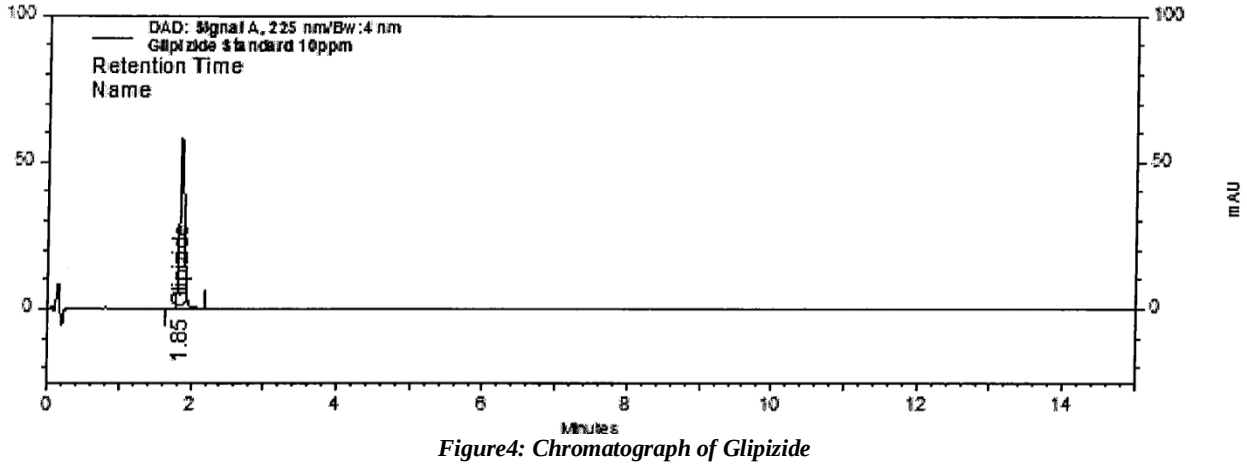

Figure4: Chromatograph of Glipizide

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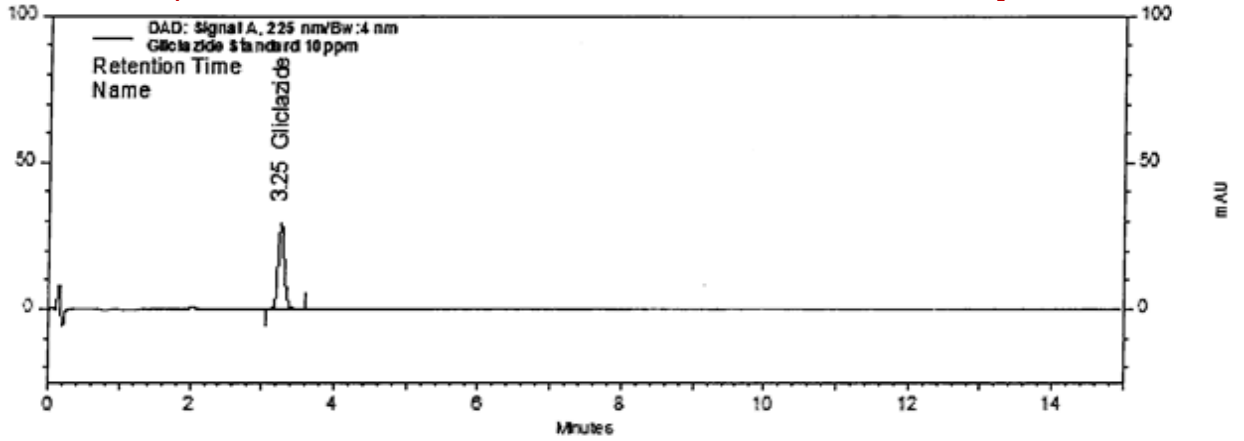

Figure5: Chromatograph of Gliclazide

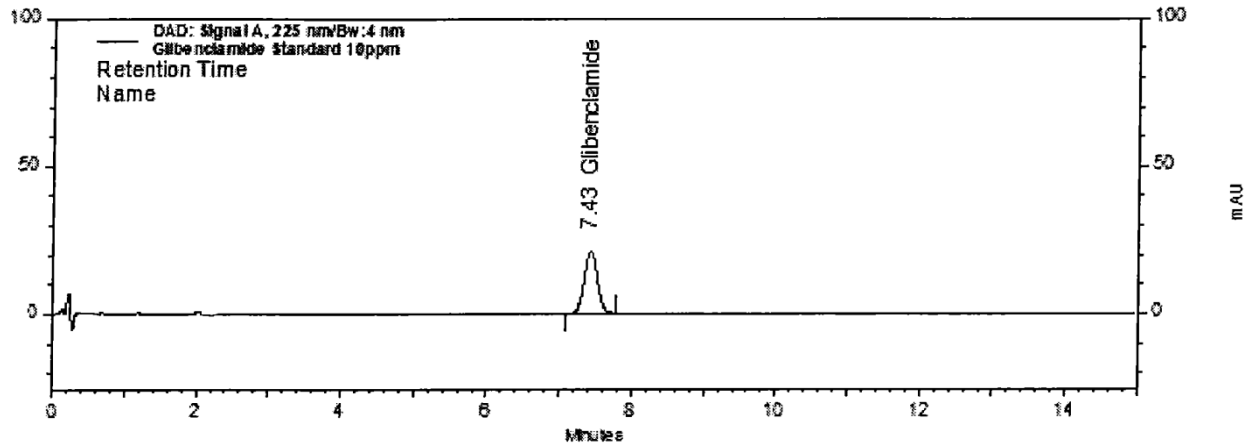

Figure6: Chromatograph of Glibenclamide

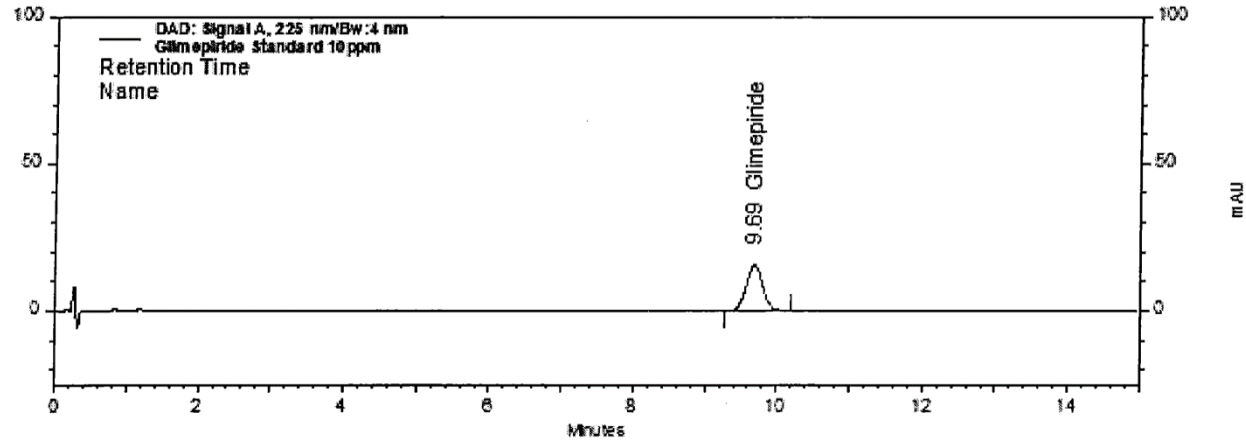

Figure7: Chromatograph of Glimepiride

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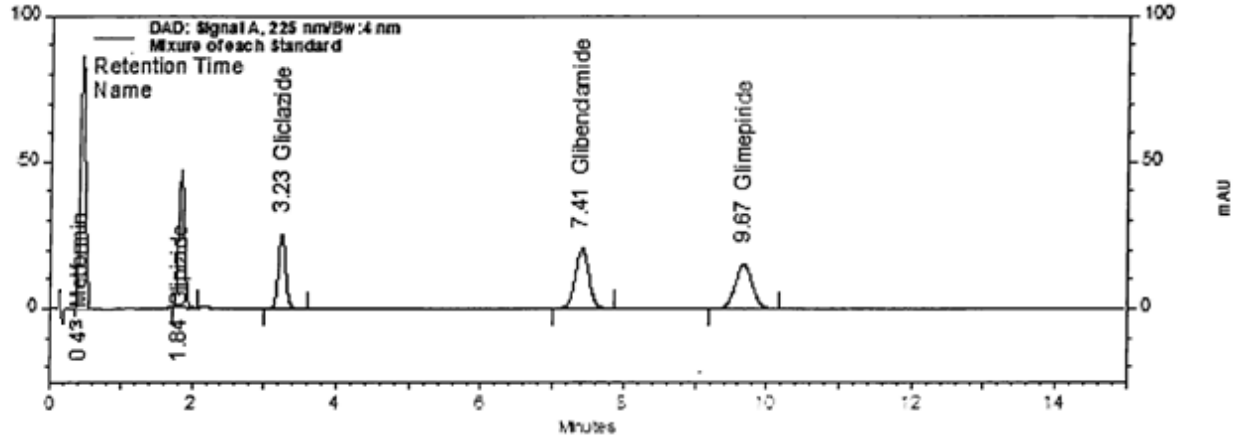

Figure8: Chromatograph of all five API

Precision:

To check precision parameter; System precision, Method precision and Intermediate precision was carried out for Metformin. System precision was carried out on replicate measurements of Metforminstandard solution (Limit: % RSD not more than 5%). Method precision was carried out by preparing sample solution six times and injecting on system. % Impurity value and %RSD of impurity value were calculated (Limit: % RSD Not more than 10%). Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was calculated (Limit: % RSD Not more than 10%). Method precision was carried out on separate set of combination tablet solution.

Observation from Precision study of Glipizide:

For system precision, it was found that %RSD for replicates injections of standard solution were 1.2%.

For method precision using Metformin and Glipizide tablets %RSD of unknown impurity at RT 0.80minute, from six preparations was found to be 3.9% Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was found to be 5.2%.

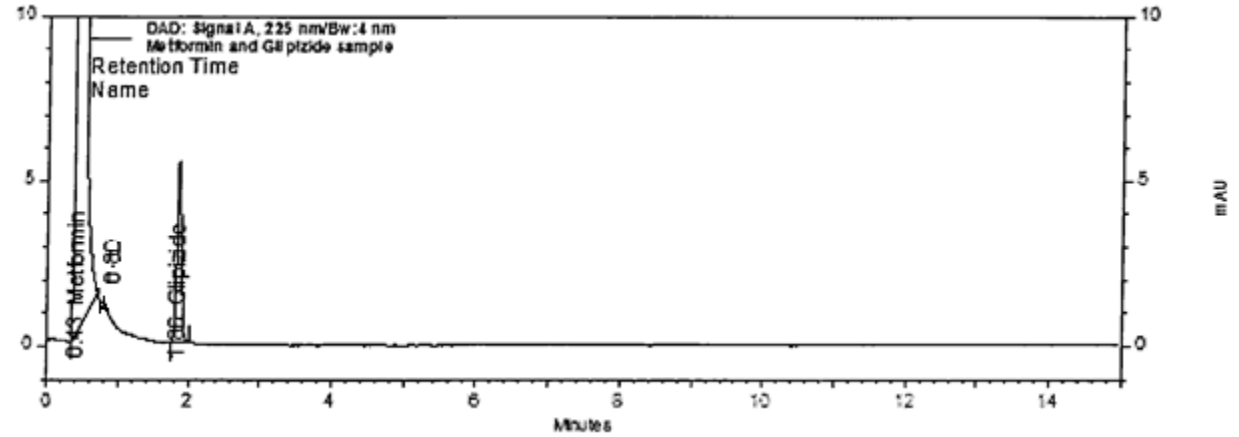

Figure9: Typical chromatograph of Tablet sample for Metformin and Glipizide

For method precision using Metformin and Gliclazide tablets %RSD of unknown impurity at RT 1.05minute, from six preparations was found to be 4.8% Intermediate precision was carried out by carrying out the precision

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experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was found to be 6.0%.

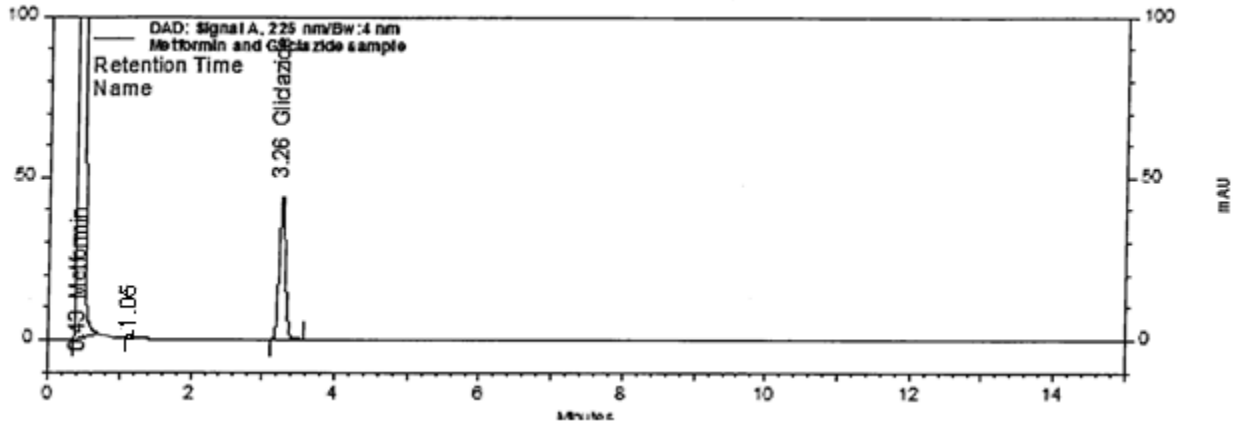

Figure10: Typical chromatograph of Tablet sample for Metformin and Gliclazide

For method precision using Metformin and Glibenclamide tablets %RSD of unknown impurity at RT 2.06minute, from six preparations was found to be 3.4% Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was found to be 4.9%.

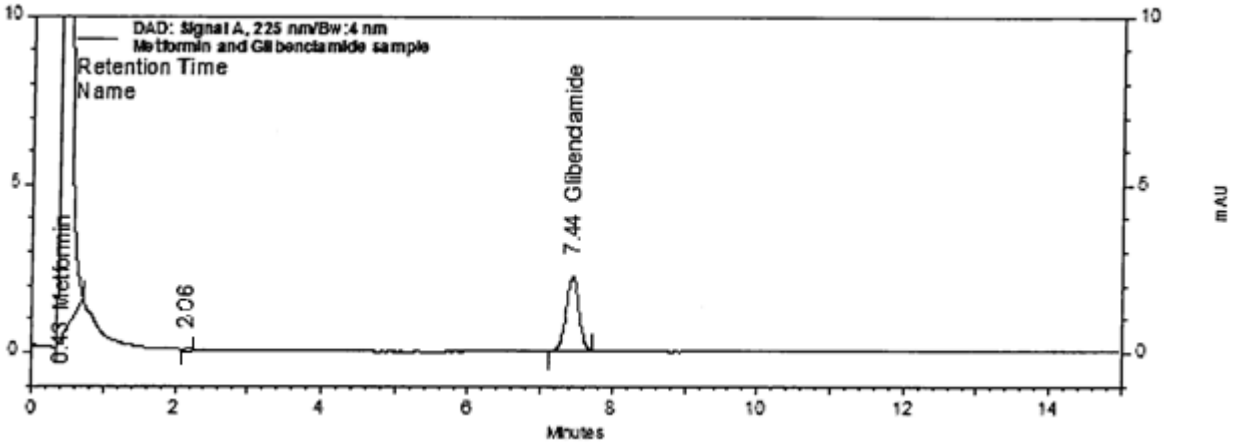

Figure11: Typical chromatograph of Tablet sample for Metformin and Glibenclamide

For method precision using Metformin and Glimepiride tablets %RSD of unknown impurity at RT 1.25minute, from six preparations was found to be 4.8% Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was found to be 5.8%, hence it is concluded that the method was precise.

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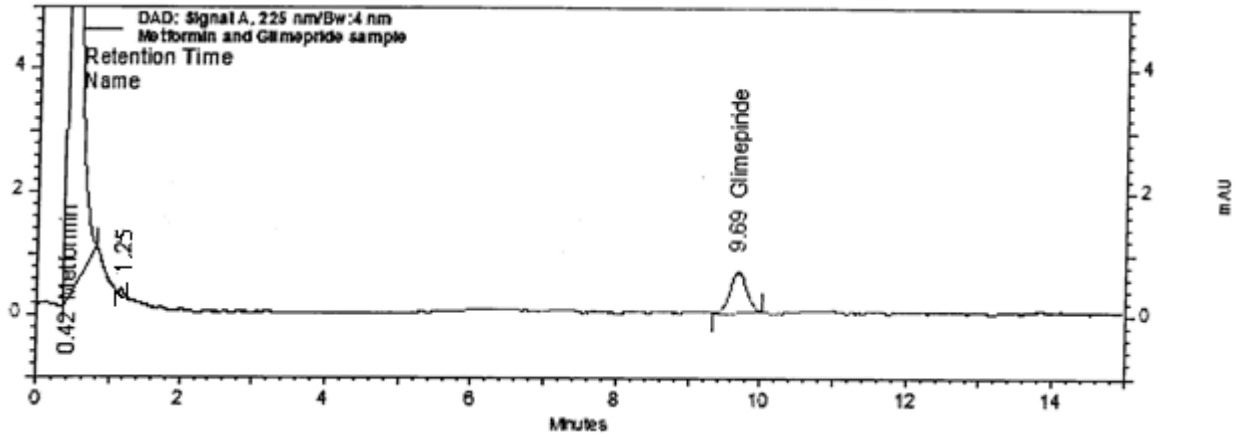

Figure12: Typical chromatograph of Tablet sample for Metformin and Glimepiride

Linearity and Range:

In Linearity and Range parameter of the method, five solutions of different concentration from LOQ to 500% for the impurity level of Metformin HCl were prepared. Injections of these levels were made and responses were recorded(Figure 13). Correlation coefficient, Slope and Intercept was determined by statistical calculations. For a method to be linear within the workable range, the Correlation coefficient should be more than 0.99

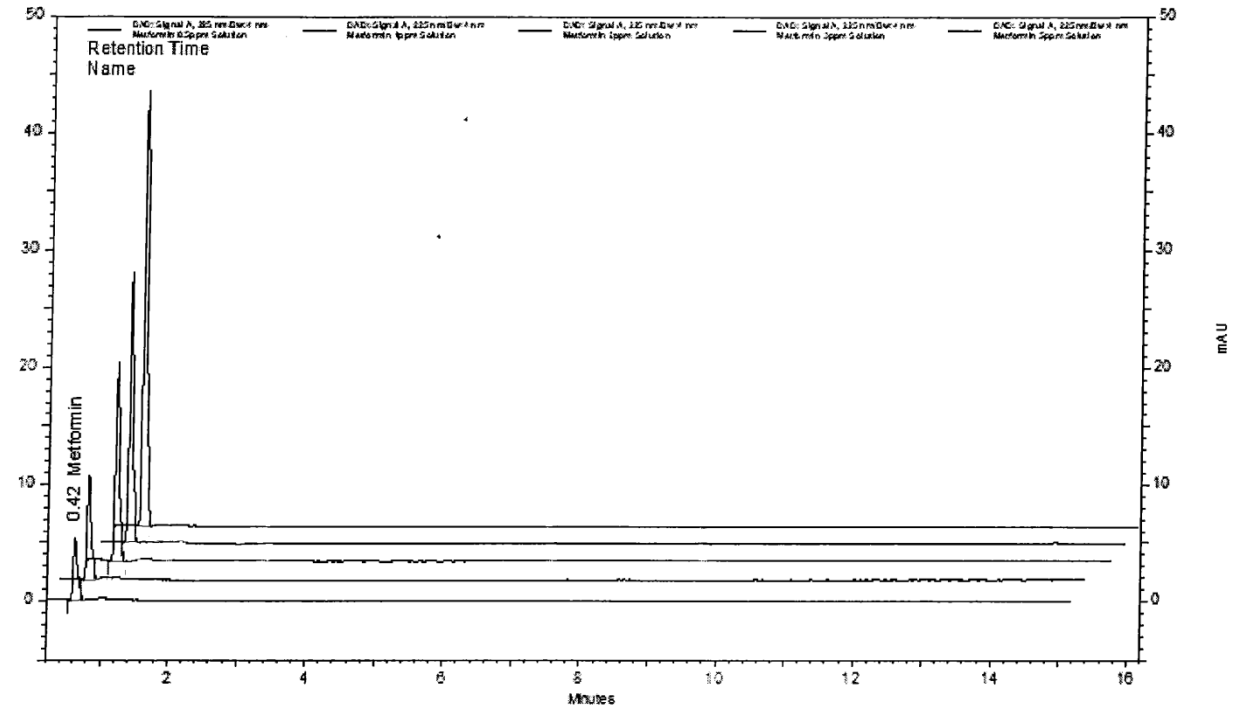

Figure13: Overlay of chromatograph for Metformin HCl Linearity levels

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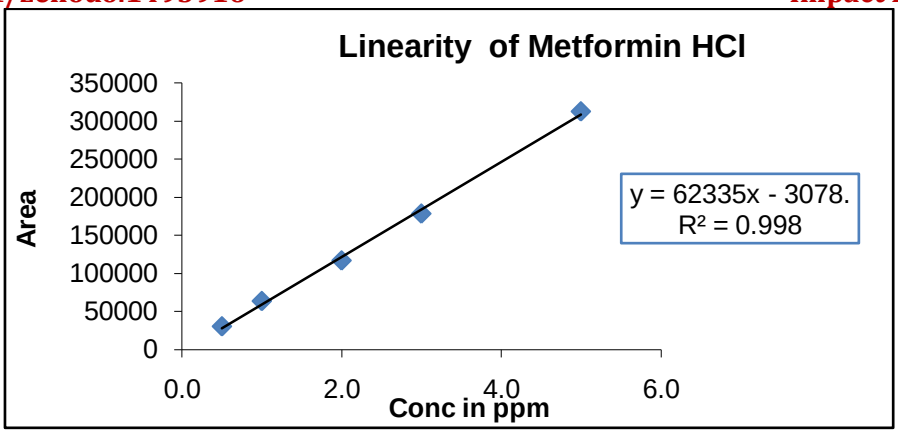

Figure 14: Linearity plot for Metformin HCl- Response vs. concentration

From the linearity data and the subsequent statistical analysis, it was found that the Correlation coefficient (R) was 0.999, Regression coefficient (R^2) was 0.9981, Slope was found to be 62335 and Y- Intercept was -3078(Figure 14) Hence it is concluded that the method is Linear within the working range of 0.5ppm (LOQ) to 5ppm.

LOD and LOQ:

LOD and LOQ were calculated on basis of Signal to noise ratio. LOQ level was found to be 0.5ppm and LOD was found to be 0.3ppm. S/N ratio for LOD was found to be 9 and for LOQ level it was found to be 16.

Accuracy:

Accuracy at impurity level was done by spiking Metformin in blank, at three levels of impurity in the range of LOQ, 100% and 500%. The mean percentage recovery was found to be 98.7%. (Limit 95.0% to 105.0%)

Robustness:

Since a single method of analysis is used for 4 combination drug tablet dosage form along with Metformin HCl, robustness parameter was carried out on basis of worst case approach. Combination tablet of that API which is eluting closest to Metformin peak is considered for robustness parameter experiment. The effect of deliberate changes will result in merging of peaks, if any occurs. Hence the closet eluting peak resolution will be affected. For this study deliberate change in wavelength of detection, flow rate and temperature of column oven were made. The % impurity value from as such condition and deliberate changes condition were calculated. For Impurity level of 0.1%, the Absolute difference should be not more than 0.05.Wavelength of detection was changed by ± 2nm, Flow rate was changed by ± 0.05ml and Column oven temperature was changed by ± 2°C. The results of Robustness study can be found in table below. On basis of these observations, the method was found to be robust.

Table 1: Robustness results for Metformin and Glipizide tablet

	% Single maximum impurity	%Absolute difference
As such condition results	0.042%	-
Change in wavelength (+2nm)	0.038%	0.004
Change in wavelength (-2nm)	0.045%	0.003
Change in Flow rate (+0.05ml)	0.039%	0.003
Change in Flow rate (-0.05ml)	0.046%	0.004
Change in temperature (+2ºC)	0.038%	0.004
Change in temperature (-2ºC)	0.044%	0.002

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V. CONCLUSION

A single analytical method for the determination of impurity of Metformin in combination tablet dosage form for four different combinations with Metformin HCl as common drug, using HPLC were developed and validated. The developed method was found to be specific, accurate, precise, linear and robust for its intended use

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