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Simultaneous determination of Glimepiride and Metformin hydrochloride impurities in sustained release pharmaceutical drug product by HPLC

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Abstract

A gradient method is developed for the quantitative simultaneous determination of impurities of glimepiride and metformin hydrochloride in the combined –pharmaceutical dosage form. The method is based on high- performance liquid chromatography (HPLC) on a reverse phase column of Waters Symmetry -C8,5µ 4.6 x 250mm thermo stated at 50^{o} C, using a mobile phase of Pentane sulfonic acid sodium salt buffer pH 3.5 and acetonitrile and evaluated for its ability to simultaneously establish the level of known impurities of glimepiride and metformin Hydrochloride active as well as unknown impurities in glimepiride and metformin hydrochloride tablets. The method shows good resolution between glimepiride sulfonamide (GS), glimepiride urethane (GU), glimepiride 3- isomer (GI), metformin related compound A (MA), glimeiride (G),metformin hydrochloride (M) , unknown impurities and formulation excipients of tablets. A gradient program with UV detection at 230nm is used to quantitate all components. The developed method is validated in term of specificity, linearity and range for GS,GU,GI, MA , M and G , accuracy using spiked levels of impurities (80% -120% of the specified limit) , precision and ruggedness. Limit of quantitation is found to be 1.50 µg mL^{-1} for M , 0.10 µg mL^{-1} for G , 0.30 µg mL^{-1} for MA , 0.24µg mL^{-1} for GS ,0.10 µg mL^{-1} for GU and 0.22 µg mL^{-1} for GI. The proposed method is successfully applied to the pharmaceutical dosage form for the determination and quantitation of known and unknown impurities in M and G tablets without any interference from the excipients.

Key words: Glimepiride, Metformin hydrochloride, impurities, correction factor, validation, HPLC

INTRODUCTION

Diabetes is one of the costliest health problems in the world. Globally, diabetes is likely to be the fourth leading cause of death [1]. Approximately 90% of people with diabetes have type 2 diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises; the pancreas gradually loses its ability to produce insulin. Type II diabetes is associated with older age, obesity, family history of gestational diabetes, impaired glucose metabolism, physical inactivity and race/ ethnicity [2]. If the glycemic target level is not achieved with one oral agent alone, combination oral and/or insulin therapy is recommended [3, 4]. Combination oral therapy becomes an obvious choice when glycemic control is not achieved with conventional monotherapy [5]. The advantages of oral dose combinations as compared to their components which are taken alone are lower cost and better patient compliance [6, 7].

Combination therapy has been shown to achieve greater blood glucose lowering than mono-therapy because different classes have different and complimentary mechanisms of action. Therefore, it is more logical to add another drug than replace the existing drug. The rapid introduction of combination therapy with two or three complementary oral anti diabetics help in targeting the dual effect and also reduced adverse effects [8].

Glimepiride, 1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl]sulphonyl]-3-trans-(4-methylcyclohexyl)urea (Fig -1) is an third generation sulphonyl urea used to reduce blood glucose levels by stimulating insulin secretions from the beta cells of pancreas and also known to increase peripheral insulin sensitivity thereby decreasing insulin resistance. Metformin Hydrochloride (M),1,1-dimethylbiguanidine monohydrochloride (Fig 1b), is an anti-diabetic drug from the biguanide class of oral hypoglycaemic agents, given orally in the treatment of non –insulin-dependent diabetes mellitus [9].The combination of sulfonylurea and Metformin is largely used because both the drugs are ancient and large number of studies have demonstrated their synergistic effects. An improvement in blood glucose level and $HbA1_c$ was solely observed with the association of both drugs.

Drug products contain both drug substance and excipients. The resultant biological, chemical and physical properties of the drug product are directly dependant on the excipients chosen, their concentration and interactions with the drug substances [10].

The safety of a drug is dependent not only on the toxicological properties of the active substance itself, but also on its pharmaceutical impurities, which consist of reaction by-products, generated during synthesis of drug substances and degradation products formed during the formulation manufacturing process and / or storage of drug substances or formulated products.

Pharmaceutical impurities are the unwanted chemical that remains with the APIs or develop during formulation, or upon degradation of both API and formulated APIs to medicines .The presence of these unwanted chemicals even in small amounts might influence the efficacy and safety of pharmaceutical products.

Determinations of drug impurity and drug degradation products are very important from both pharmacological and toxicological perspectives. Impurity profiling is very important during the synthesis of drug substances and manufacture of dosage forms, as it can provide crucial data regarding the toxicity, safety, various limits of detection, and limits of quantitation, of several organic and inorganic impurities, usually accompany with bulk drugs and finished products.

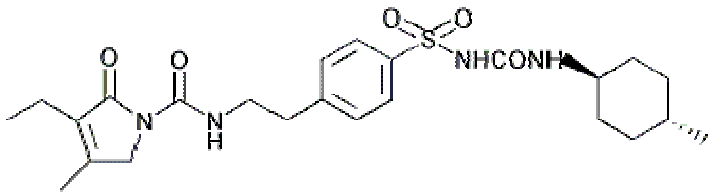
An accurate analytical method development and validation of the procedures make the impurity profiling task easy. Also, Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now receiving critical attention from regulatory authorities.

For analytical method, in addition to precision and accuracy , a good resolution of a target drug from its impurities and identification of impurities is often required for drug development. Identification of impurities can provide a clear picture of impurity profile in drug product; helps identify their origin and improve the quality of the drug product by minimizing or even eliminating the impurities. The present study explains such an analytical method for M and G tablets.

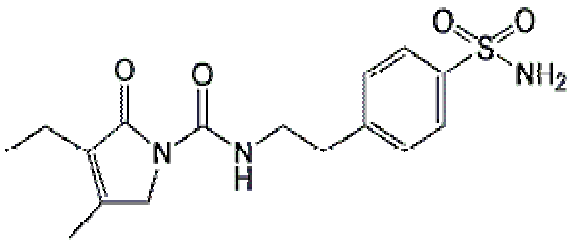
Liquid chromatography with UV detection (LC-UV) has gained a widespread acceptance for quality control of most of the pharmaceutical due to its simplicity , high resolution and satisfactory precision and accuracy .HPLC is an extensively used technique in the pharmaceutical industry due to the availability of fully automated systems, excellent quantitative precision , accuracy , broad linear dynamic range and availability of a wide variety of column stationary phases. As per ICH Q3A(R) and ICH Q3B(R) guidelines, unknown impurities associated with bulk drug and dosage form, greater than the identification threshold should be identified. A need for Analytical methods for consistent quality establishment through out the shelf life of the product arises. Therefore, the aim of the research work was to develop and validate specific, accurate and precise method for simultaneous determination of impurities of G and M in the combination pharmaceutical drug product.

Pharmacopoeial methods have been reported for the determination of G and its related impurities i.e. GS, GU and GI and M and its related impurity MR individually [11, 12]. The structural formula for G, GS, GI, MA and M are as shown in Fig 1. Literature search revealed that several analytical methods are available of determination of assay of M and G separately in formulations, in biological fluids and in presence of other anti-diabetic agents [13-17]. If the reported individual methods are applied for the related substances analysis of tablets containing M and G, it would require double time of analysis, and expensive, whereas simultaneous determination of related substances would save analysis time and also economy. So far, to our present knowledge, there is no method for concomitant determination of impurities of M and G in the combination product using single chromatographic conditions. Recently published method for the determination of M along with its impurities by mixed mode HILIC [18] and by Ion – Pairing liquid chromatography [19] limits its application in M tablets only. All the pharmacopeial impurities of both the actives were considered during the development. In the work, discussed in this paper, we therefore focused on finding optimum HPLC conditions with gradient elution for separation and quantitation of all the potential impurities in M and G dosage form and validation as per ICH guidance documents. The developed and validated method is specific, precise, accurate and stable with improved sensitivity

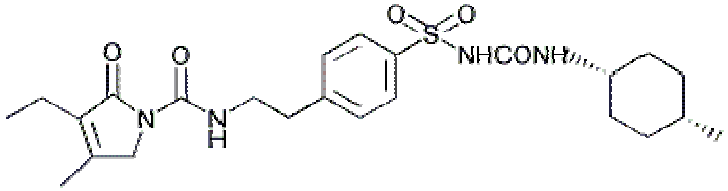
Fig 1: Glimepiride



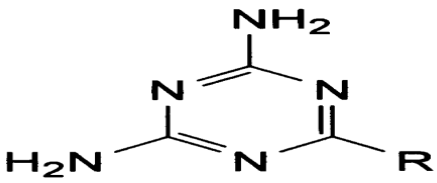
GS: N-[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] benzene sulfonamide



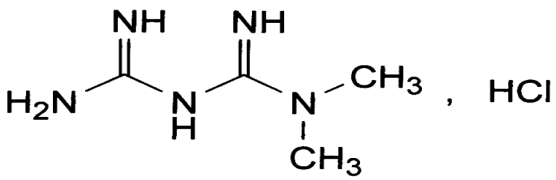
GI: Glimepiride Ortho Isomer



MA: Metformin Related compound A:



Metformin Hydrochloride



MATERIALS AND METHODS

2.1 Chemical and Reagents:

USP reference standard of G, GI, GU and GS , M and its related impurity MA along with the R&D samples containing M and G (500mg M/2 mg G) were supplied by Ipca laboratories Ltd.Mumbai. Acetonitrile, Methanol of HPLC grade, Pentane Sulfonic acid sodium salt , ortho phosphoric acid AR grade (88%) were procured from Merck. Milli-Q water was used. GF/C filter paper was obtained from Whatmann. All dilutions were prepared in standard volumetric flasks.

2.2 Instrumentation and Chromatographic conditions:

Chromatography was performed using HPLC of Waters 2695 Alliance separation module system, Waters 2996 with PDA detector and column oven. Chromatograms and data were recorded by means of Empower software version 2.10. Separation was achieved on Waters Symmetry -C8, (250mm × 4.6mm dimensions) having particle size 5µ , with flow rate as 1.5mL min^{-1} and column oven temperature as 50^{o} C. The mobile phase consists of Pentane Sulfonic acid sodium salt buffer pH adjusted to 3.5 with diluted ortho phosphoric acid and acetonitrile . The gradient program is as follows:

Time (in minutes)	Buffer (% v/v)	Acetonitrile (%v/v)	Comments
0-8	90	10	Isocratic
8-30	0	100	Linear gradient
30-35	90	10	Linear gradient
35-45	90	10	Re- equilibration

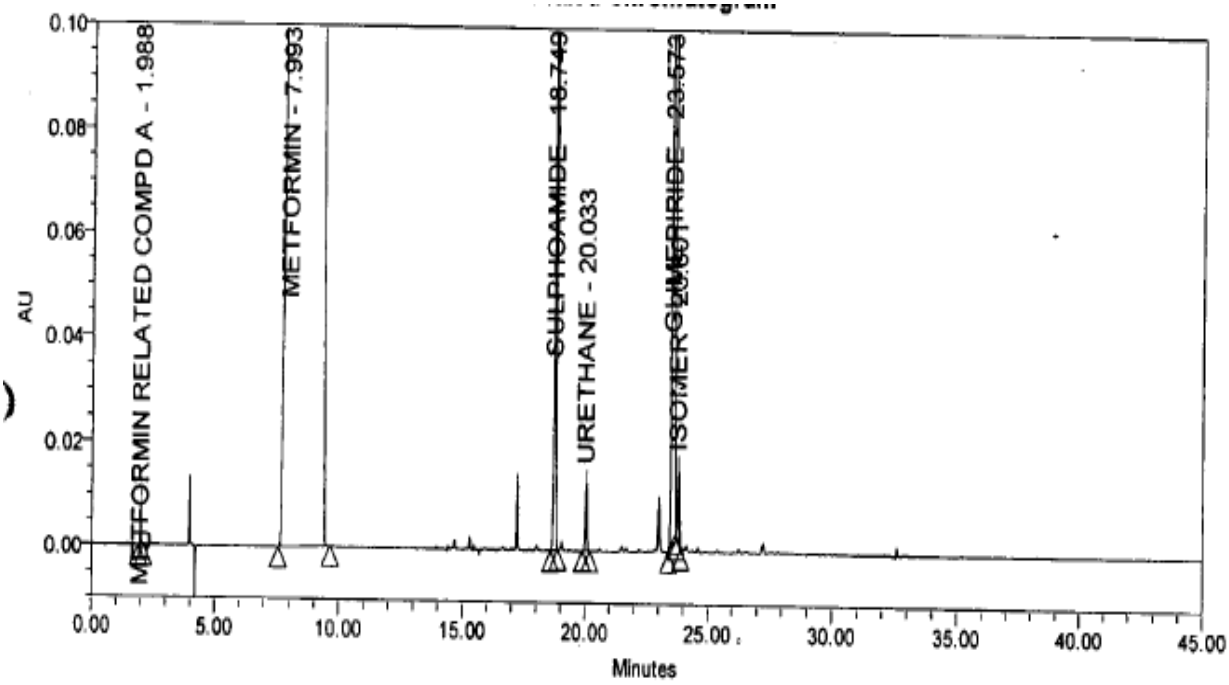
The injection volume for standard and sample for determination of Metformin impurities was 10µl and sample for the determination of glimepiride impurities was 100 µl .The detection wavelength was 230nm. A typical HPLC chromatogram obtained for simultaneous determination of MA, GS, GU, GI along with M and G is shown in Fig. 2.

2.3 Diluent: Mixture of Acetonitrile and water (80:20 v/v).

2.4 Standard preparation: Prepare a standard solution containing G and M of concentration 2µg mL^{-1} and 5µg mL^{-1} respectively in diluent.

2.5 Sample preparation: Weigh 20 tablets and crush into fine powder. Transfer accurately weighed powder equivalent to 5 tablets in to 500ml volumetric flask, add about 300ml of diluent ,sonicate for 15 minutes with intermettant shaking. Further shake for 10mins. After cooling to ambient temperature make up to volume with the diluent, mix and filter the solution through GF/C and inject.

Fig 2



RESULTS AND DISCUSSION

The aim of the method development was to resolve all the known impurities, actives and the formulation excipients simultaneously using the same chromatographic setup. For this purpose, the influence of column type, mobile phase composition, buffer type, buffer pH, column oven temperature and flow rate was systematically investigated [20]. Pharmacopoeial methods were referred for the development. Also references listed in this paper were taken as a base. The rationale for the use of reverse phase chromatography includes simplicity, versatility and the scope .and a quick equilibration of stationary phase with modifications in mobile phase composition , as a result well suited for use with gradient elution .The scope was further extended to ion pair chromatography which provides an important additional selectivity option to improve the band spacing .The objective in selecting a particular ion –pair reagent is to be able to achieve a significant column uptake of the reagent for a reasonable reagent concentration . M is highly polar and strongly basic in nature, also M along with MR can easily ionize in solution but the polarity of these compounds are so strong that they do not retain on C_{18} column and C_8 column, whereas G along with its impurities shows good retention on C_8 column. Also, the preliminary experiments indicated that using different concentration of acetonitrile and even different pHs of the buffers did not produce suitable retention of M. Hence, ion pair reagent (oppositely charged ion) in the mobile phase was used which reacts with them to form neutral ion pair enabling to retain on non –polar stationary phase. Ion exchange is an predominant mode of interaction with basic compounds, where most of the undesirable influence of the column silanols becomes apparent .Silanols acts as a cation exchange groups of intermediate strength which is an function of mobile phase pH. At around pH 3.0 , all except the most acidic silanols

are protonated and therefore do not undergo the ion exchange interaction with positively charged analytes .As the pH is increased, more and more silanols become negatively charged and are free to interact with the analyte resulting in increase in retention and tailing. Involvement of two drugs and their impurities containing multiple functional groups results in a complex retention behaviour.pH plays an important role in optimizing liquid chromatographic method because of the ionization of the charged analytes . The pKa value of the various sample components are in the range of 1-5 based on the compound structure enables the use of mobile phase with low pH. The range of variations as pH (3.2-3.7) and flow (1.3-1.7 mL min^{-1}) have no significant change on the HPLC – UV chromatographic resolution. Hence pH 3.5 was chosen as the optimized pH .The Symmetry reversed phase packing is preferred than the conventional C8 packings due to its deactivated , high purity silica exhibiting less of the silanols effect at pH 3.5 with respect to control of band spacing and resolution . Wavelength was selected by scanning both the drugs and the known impurities over the wide range of wavelength 200nm to 400nm. All the components show reasonably good response at 230nm.

Theoretically as column temperature increases, the viscosity of the mobile phase decreases and consequently decreases the backpressure of the column, which helps in running the experiment at higher flow rate. This supported the column temperature of 50 ^{o} C
Hence, after studying different column make and composition of mobile phase of buffer and acetonitrile, the above method has been finalized to optimize the retention time of P , G along with its related impurities.

4.0 Method Validation

The method validation was performed as per the ICH guidelines for impurities [21-22] taking into consideration the specified limits for GS as 0.4% , GU as 0.1% , GI as 0.2% , MA as 0.02% and for single maximum unknown impurity as 0.1%.

4.1 System Suitability: System suitability was performed by injecting Relative retention time solution and determining resolution between closely eluting peak of G and GI ,GS and GU . Also the RSD of peak responses of G and M in standard solution in six replicates was calculated (Table 1).

Table 1 : Results of system suitability

Parameter	Value
Resolution between G and GI	1.99
Resolution between GS and GU	11.33
% RSD of G	0.80
% RSD of M	0.43

4.2 Specificity: The specificity of the method was studied by injecting the placebo (containing all the ingredients of the formulation except the analytes) of the tablets as per the procedure applied to sample solution. Individual impurities, actives and the mixture were analyzed. No peak was detected at the retention time of G,M and their related impurities hence proving the specificity of the method (fig 3,4).

Fig 3

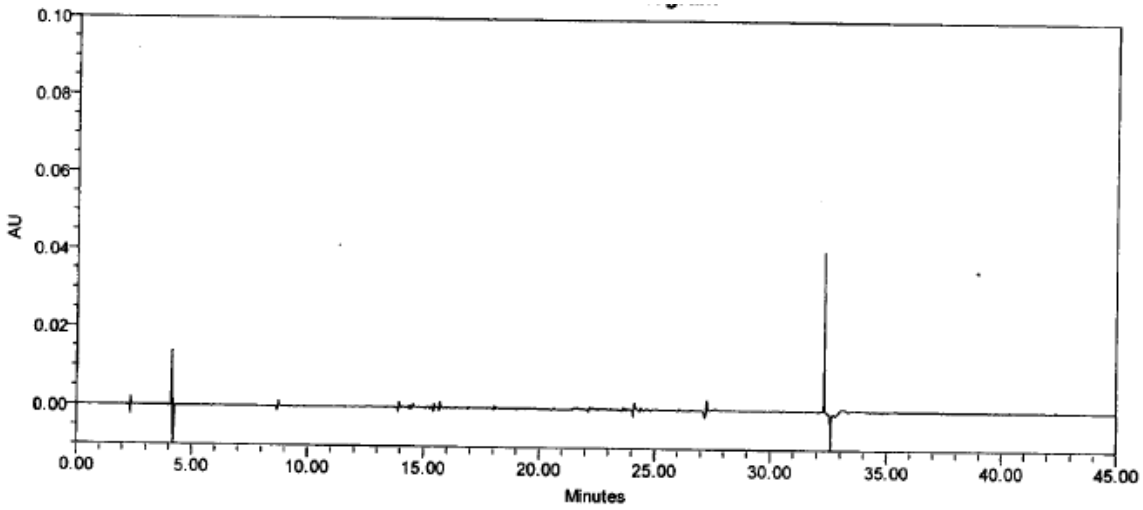
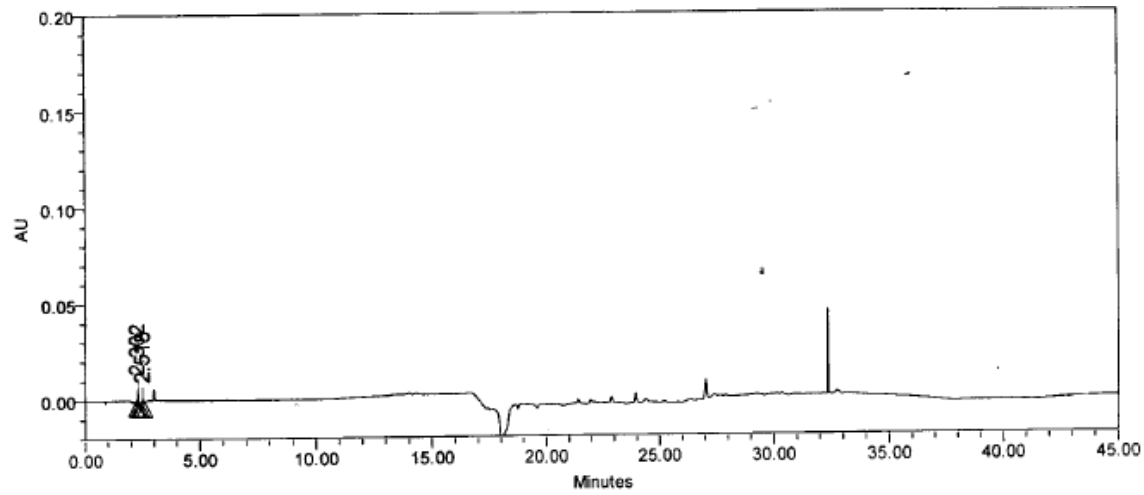


Fig 4



4.3 Correction Factor : Response factor for GI, GS , GU and MA was determined by injecting solution containing mixture of all known impurities and actives at same concentration. Correction factor is a reverse of response factor. The results are listed in Table 2.

Table 2 : Relative retention time and Correction factor

Component	Retention time	Relative retention time	Correction factor
G	23.50	1.0	1.00
M	7.43	1.0	1.00
GS	18.61	0.79	0.70
GU	19.91	0.85	1.00
GI	23.73	1.01	1.35
MA	1.98	0.27 (with respect to M)	1.18 (with respect to M)

4.4 Linearity : Linearity was evaluated by analyzing different concentration levels from 10 - 200% of the specified limit for related impurities and 0.1% limit for both the actives. The regression data obtained are listed in Table 3.

Table 3: Linearity, LOD and LOQ results

Analyte	Linearity range(ppm)	Slope	Intercept	Correlation coefficient	LOD (in µgmL $^{-1}$)	LOQ (in µgmL $^{-1}$)	% RSD at LOQ conc
G	0.02–4.0	22349	433.3	0.9996	0.03	0.10	4.94
M	0.50–10	23579	-1850.25	0.9997	0.50	1.50	0.81
GS	0.08–1.60	31885	545	0.9989	0.08	0.24	2.62
GU	0.02- 0.40	25156	-121.8	0.9973	0.03	0.10	3.10
GI	0.04–0.80	14395	170.99	0.9968	0.07	0.22	3.33
MA	0.10–2.0	15886	-205.89	0.9994	0.10	0.30	5.34

4.5 LOD and LOQ: The limit of detection and limit of quantitation of the known impurities and actives were established from the standard deviation of the response and the slope of the corresponding calibration curve (LOD =3s/m; LOQ = 10s/m) (Table 3).

4.6 Accuracy: The accuracy of the method was checked by recovery study using standard addition method, at three different concentration levels i.e. multilevel recovery study .The pre – analyzed samples were spiked with the GI, GU, GS and MA at the specified limit at 80,100 and 120% level (Fig 5). The mean recoveries of the impurities were found to be in the range of 99 - 101% (Table 4) indicating that the method enables highly accurate estimation of the impurities from the drug product.

Fig 5

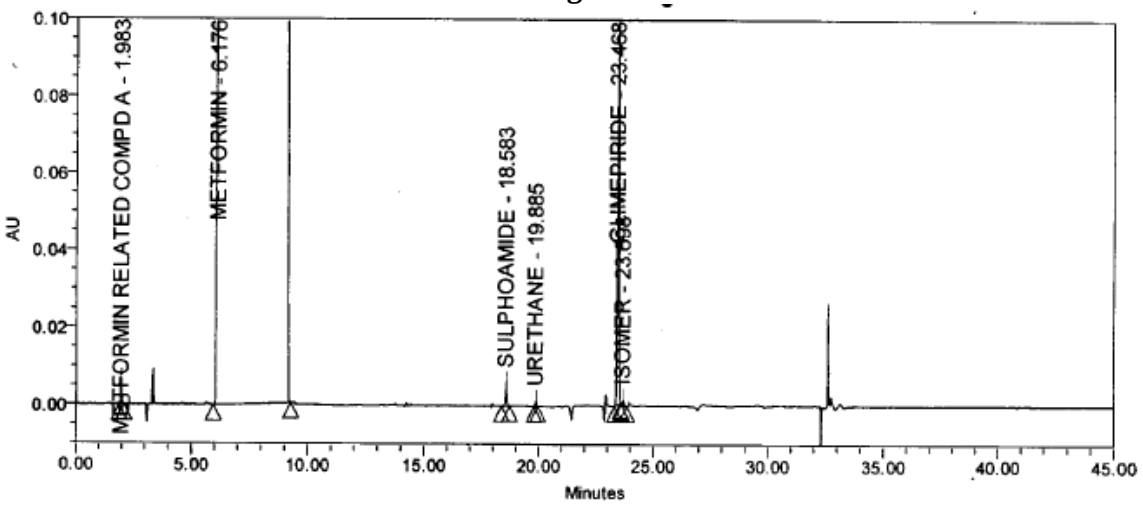


Table 4: Summary of the results of amount added vs. amount recovered

Level	preparation	GS			GU			GI			MA		
		Active added in μgmL^{-1}	Active recovered in μgmL^{-1}	% Recovery	Active Added in μgmL^{-1}	Active recovered in μgmL^{-1})	% Recovery	Active Added in μgmL^{-1}	Active recovered in μgmL^{-1}	% Recovery	Active Added in μgmL^{-1}	Active recovered in μgmL^{-1}	% Recovery
80	1	0.721	0.717	99.4	0.166	0.165	99.4	0.324	0.322	99.4	0.811	0.815	100.5
	2	0.649	0.652	100.5	0.169	0.170	100.6	0.325	0.319	98.2	0.849	0.852	100.4
	3	0.648	0.654	100.9	0.168	0.165	98.2	0.322	0.320	99.4	0.849	0.841	99.1
100	1	0.811	0.806	99.4	0.205	0.202	98.5	0.412	0.416	100.9	0.995	0.987	99.2
	2	0.810	0.811	100.1	0.204	0.201	98.5	0.415	0.412	99.3	0.992	0.987	99.5
	3	0.812	0.815	100.4	0.202	0.199	98.5	0.417	0.415	99.5	0.996	0.991	99.5
120	1	0.973	0.968	99.5	0.241	0.239	99.2	0.485	0.485	100.0	1.216	1.192	98.0
	2	0.998	0.982	98.4	0.247	0.245	99.2	0.482	0.481	99.8	1.222	1.229	100.6
	3	0.985	0.972	98.4	0.245	0.244	99.6	0.479	0.475	99.2	1.213	1.195	98.5

4.7 Precision: Precision study was assessed by injection repeatability and sample repeatability. Injection repeatability was confirmed by performing replicate injection of the standard solution and calculating the % RSD of the peak area responses for both the content (Fig 5). The data show good precision of the system with the RSD ≤ 2.0% (Table I). The sample repeatability was studied by analyzing the same sample for six times and calculating the % impurities and RSD (Fig 6) .Refer Table 5
Fig 6

Table 5: Results of Precision and Ruggedness

	GS	**GU**	**GI**	**MA**	**Unknown Impurity**
RSD (Precision)	9.43	NIL	9.46	0.00	7.88
RSD(Ruggedness)	8.69	NIL	9.41	0.00	8.12

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4.8 Solution stability: The stability of the analytical solutions of the method was studied by analyzing the standard and sample solution immediately as well as till 24 hrs .The stability was assessed by calculating the relative standard deviation of the peak areas for G and M in standard preparation and of GS,GI,GU , MA in sample preparation. The sample and standard solution found to be stable till 19 hrs, but after 19 hrs drastic change in the area response was found proposing an stability indicating method (Table 6).

Table 6: Stability of standard and sample solution

Time	**Area of GS in sample**	**Area of GU in sample**	**Area of GI in sample**	**Area of MA in sample**	**Area of G in standard**	**Area of M in standard**
0 h	38004	NIL	14621	729	42857	105009
11 h	38369	NIL	14298	715	43139	108450
19 h	38361	NIL	14219	733	42997	105383
24 h	50911	NIL	12365	2019	44839	105690
% RSD at 19 h	0.55	Not applicable	1.48	1.30	0.33	1.78
% RSD at 24 h	15.30	Not applicable	7.37	61.65	2.14	1.48

4.9 Ruggedness: The ruggedness study was carried out by analyzing same sample six times by different analyst, on different day using different instrument. % RSD of the results were within the limit of NMT 15.0%. (Table 5).

CONCLUSION

The proposed method for the simultaneous detection and quantitation of GI, GU, GS, MA and unknown impurities in G and M tablets is highly sensitive, accurate and precise. This procedure can be easily adopted for the routine quality control analysis of tablet dosage form without any interference from the excipients or each other. Method was validated for its performance parameters such as Specificity (placebo interference), Linearity and range, Recovery, LOD, LOQ Precision and Ruggedness. The specificity of the method proves that the method is stability indicating. It was concluded that the developed method offers several advantages such as single chromatographic condition for the determination of impurities of two drugs , simple mobile phase and sample preparation steps, improved sensitivity makes it specific and reliable for its intended use.

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