A simple and convenient method for the simultaneous *in vitro* study of metformin and glimepiride tablets

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Abstract: A simple and convenient method was developed for the simultaneous determination of metformin HCl and glimepiride in tablet dosage form of different pharmaceuticals companies. This method was validated and proved to be applicable for assay determination in intermediate and finished staged. More over a single medium dissolution of metformin HCl and glimepiride was established and the media was evaluated for comparative studies for different formulations. Reverse phase HPLC equipped with UV detector was used for the determination of metformin HCl and glimepiride. A mixture of acetonitrile and ammonium acetate buffer 0.05M pH 3.0 was used as mobile phase at flow rate of 1.0ml/min. Promocil C18 5µ 100A° 4.6 x 100mm C18 silica column was used and detection was carried out at 270nm. Method was found to be linear over the range of 4ppm to 16ppm for glimepiride and 170ppm to 680ppm for metformin HCl. Regression co-efficient were found to be 0.9949 and 0.9864 for glimepiride and metformin HCl respectively. Dissolution was performed in 500ml 0.2% sodium lauryl sulfate at 37ºC for 45min using paddle apparatus. Dissolution of glimepiride was found to be 98.60% and 101.08% in Orinase Met1 tablet and Amaryl M tablet respectively whereas metformin was found 99.41% and 98.59% in Orinase Met 1 tablet and Amaryl M tablet. RSD for all the dissolutions was less than 2.0% after completion.

Keywords: Metformin HCl, glimepiride dissolution simultaneous determination and HPLC.

INTRODUCTION

Metformin HCl is an antidiabetic agent belongs to biguanide class, chemically it is known as *NN*, Dimethylimidodicarbonimidediamide. It is a first choice anti-diabetic for the treatment of type 2 diabetic especially in case of overweight patients. Whereas chemically, glimepiride is identified as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1carboxamido) ethyl]phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl)urea ($C_{24}H_{34}N_4O_5S$) with a molecular weight of 490.62. Glimepiride is a white to yellowish-white, crystalline, odorless to practically odorless powder and is practically insoluble in water. The primary mechanism of action of Glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells.

Metformin-glimepiride combination can be considered as the best combination in patients with increased lipid parameters as compare to other combination of Metformin in diabetic patients.

Combination of Metformin and glimepiride reduce the Glycosylated Hemoglobin level, Fasting and post-prandial plasma glucose significantly. While the significant reduction in the total cholesterol, serum triglyceride and LDL cholesterol has also been observed in the Metformin-glimepiride combination Absorption of a drug substance from a solid oral dosage form depends upon the release of that substance from the drug, solubility of that substance under certain physiological condition and the permeability across the gastrointestinal tract. The first two factors lead us to use *in vitro* study to predict the behavior of API *in vivo* conditions. Metformin HCl have high solubility and low permeability where as glimipride has low solubility and high permeability and both are placed in class 3 and class 4 respectively according to biopharmaceuticals classification system. So for the study of glimipride *in vivo* study is quiet suitable because the solubility and dissolution of API is the rate determining step in absorption.

In this study Model Independent approach using similarity factor is adopted due to the availability of sufficient time points.

Purpose

Metformin and glimepiride are both used in different combination with pioglitazon, glibenclamide and sitaglipton phosphate. In these types of combinations qualitative determination of each ingredient without the interference of other active or inactive is quiet important. Most of cases these compounds are detected on HPLC equipped with a UV detector. As the amounts of these actives are different especially when they are compared to the concentration of Metformin HCl which is may times greater than all others. For the combination of Metformin HCl and glimepiride this difference is quiet significant as glimepiride is present 1mg or 2mg on the other hand Metformin HCl is 500mg. Furthermore the wavelength for maximum absorbance for both compounds is almost same as 228nm for glimepiride and 232 for Metformin HC l. This difference in concentration and same λ_{max}

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make it quiet difficult to get comparable peaks of Metformin HCl and glimepiride in same chromatogram.

The purpose of this study is the development of a method for the simultaneous determination of glimepiride and Metformin HCl, which must be sensitive in order to determine very low quantities faced in dissolution profile studies. Main part of this study is to use this method selection of media for simultaneous dissolution study of glimepiride and Metformin and then perform comparative study of dissolution of different pharmaceutical brands available in the market.

MATERIAL AND METHOD

Method development and optimization of conditions

For method development; literature survey was conducted which showed that Metformin HCl and glimepiride are mostly determined by separated methods and rarely a method for simultaneous determination was found. Here are the few conditions applied in method development based upon the study of chemical and physical properties of both Metformin HCl and glimepiride.

Sample preparations

There is a significant difference in the physical properties of Metformin HCl and glimepiride. Solubility of glimepiride is very low in aqueous solution as compared to Metformin HCl. In HPLC sample preparation it is convenient to use mobile phase as solvent to avoid extra peaks. As the concentration of active ingredients is quiet different in sample dilution and in mobile phase when acquisition is carried out. To get high concentration varied composition of mobile phase component were used as glimepiride is soluble in non-polar media. For this reason component of mobile phase in a high concentration of organic solvent were used in initial step. In optimization step as acetonitrile and ammonium acetate buffer were selected as mobile phase so their different concentrations were applied and finally 80% acetonitrile and 20% buffer was selected.

Column applied in initial studies

Following column were subjected to experiments in order to get desired theoretical plates, retention time, tailing factor and peak symmetry.

Promocil CN 10µ 100A° 4.6 x 250 mm Cat#PM502505-0 (Agela)

Hibar 250-4,6 RP 18e 5µ-100A°4.6x250mm Cat #947643 (Merck)

Mediterranea sea 18 5µ-100A°4.6x250mm Cat#TR-010006 (Teknokroma)

Venusil XBP C_{18} 5µ 100A°4.6x150mm Cat #VX951505-0 (Agela)

Promocil C_{18} 5µ-100A°4.6x150mm Cat #PM951505-0 (Agela)

Finally Promocil C_{18} 5µ-100A°4.6x150mm Cat #PM951505-0 (Agela) was selected due to its better performance and suitability.

Mobile phases applied for initial studies

Following components were used as mobile phase in different concentration to get desired retention time and separation.

Methanol: Buffer pH 6.8 (Potassium Dihydrogen phosphate 0.05M)

Acetonitrile: Buffer pH 6.8 (Potassium Dihydrogen phosphate 0.05M)

Methanol: Acetonitrile: Buffer pH 6.8 (Potassium Dihydrogen phosphate 0.05M)

Methanol: Acetonitrile: Buffer pH 3.0 (Potassium Dihydrogen phosphate 0.05M)

Acetonitrile: Buffer pH 3.0 (Potassium Dihydrogen phosphate 0.05M)

Acetonitrile: Buffer pH 3.0 (Potassium Dihydrogen phosphate 0.01M)

Methanol: Acetonitrile: Buffer pH 6.8 (Potassium Dihydrogen phosphate 0.05M)

Methanol: Acetonitrile: Buffer pH 3.5 (Ammonium Acetate 0.05M)

Methanol: Ammonium acetate Buffer pH 3.0 (0.05M)

Acetonitrile: Ammonium acetate Buffer pH 3.0 (0.05M)

Combination of Acetonitrile and ammonium acetate buffer pH 3.0 gave best result as mobile phase so it was subjected to optimization and was found suitable in the ratio of 55 and 45 respectively.

Flow rate

Different flow rates of different mobile phases were applied in initial step depending upon the dimensions and nature of column. Once the mobile phase and column was selected in optimization step then the flow rate was adjusted according to this column and solvent combination. It was selected 1.5ml/min to achieve minimum injection time with best separation and performance.

UV Detection

Selection of wavelength is very important factor in this case. Metformin absorb at 232nm and glimepiride at 228nm. As the concentration of Metformin HCl is extremely high as compared to glimepiride and its molar absorptivity is also very high so the response of both are difficult to adjust in same scale. To avoid this situation separate dilutions of glimepiride and Metformin HCl were prepared in all mobile phases applied in initial step and a specific wavelength was found in each case where the response of glimepiride is high as compare to Metformin HCl. In case of Acetonitrile and ammonium acetate buffer pH 3.0 it was found 270nm so it was used in the optimization step and satisfactory results were found.

Selection of media for dissolution

Dissolution is performed as an alternate parameter to *in vivo* availability. Methods for dissolution of Metformin HCl and glimepiride available in literature are quite different from each other due to the difference in the

solubility. In this study the main objective it to find out a single media for combined dissolution study of Metformin HCl and Glimepiride in a tablet.

For this purpose different dissolution mediums were applied. Most of them were suitable for Metformin HCl but they were unable to dissolve glimepiride. Due to low solubility of glimepiride solubility enhancer such as Tween 80, PEG 6000, Poloxamer 407, Cremophor RH 40 and Sodium lauryl sulfate have to be used to make it soluble in dissolution medium. Solubility of glimepiride is low in Tween 80, PEG 6000, Poloxamer 407 and Cremophor RH 40 as compared to Sodium lauryl sulfate solution in water as it increases with increase in pH. That is why Sodium lauryl sulfate was selected for this study as solubility enhancer. Moreover Tween 80 shows interference on UV and HPLC in UV range and to avoid this interference it was rejected.

The concentration of Sodium lauryl sulfate (0.2%) was optimized and found to be the lowest concentration at which glimepiride can be dissolved in standard with ease. Below this concentration the preparation of standard solution in dissolution media as well as release of drug in dissolution media was not satisfactory. Higher concentrations of sodium lauryl sulfate were not considered for this study, as they are not required.

Validation of method

Following parameter of validations were evaluated for the testing method used to determine dissolution. Limit of detection and limit of quantization were not performed as they are required for cleaning validation.

Linearity

Solutions having concentrations of Metformin HCl and Glimepiride (2000, 3000, 4000, 5000, 6000, 7000 and 8000ppm for Metformin and 4, 6, 8, 10, 12, 14 and 16ppm for Glimepiride) were prepared and subjected to analysis. The relation between response and concentrations was found linear for both Metformin HCl and glimepiride.

Precision

Precision was determined as both repeatability and intermediate precision. For instrument precision or injection repeatability 6 injections of one sample solution is made to test the performance of the chromatographic instrument. Intra-assay precision data are obtained by repeatedly analyzing, in one laboratory on one day, 3 aliquots of a homogeneous sample, each of which has been independently prepared according to the method procedure. 3 injections from each preparation were injected and the data obtained

Accuracy

Accuracy of a method is its ability to give actual results. Accuracy was studied by preparing three sample having concentrations in the range of 80% to 120% of the target dilution for the method. Placebo was prepared and then known amount of Metformin HCL and Glimepiride were added. Three sets of samples having concentrations 80% 100% and 120% were prepared on three different days and analyzed. These samples gave intended results, which showed that the method is accurate.

Robustness

To see the rigidity of method slight changes in different parameter such as pH, flow rate, mobile phase concentration and solvent concentration were made

Reproducibility

The method should be so accurate and simple that it should produce same results all the time. Factor which can be changed are analyst, Instruments and time. To check the effect of all these things same sample was tested by different analyst on different instrument and on different days.

DISSOLUTION

Dissolution medium

Dissolution was performed in 0.2% Sodium lauryl sulfate. 7gm of sodium lauryl sulfate was dissolved in 3.5 liter of distilled water.

Dissolution conditions

Dissolution studies were performed in 500ml medium, using USP apparatus II at a speed of 100 rpm and sample were collected after 5, 10, 15, 30 and 45 minutes.

Sample preparation

Assemble the Dissolution apparatus and fill each vessel with 500ml of the medium. Set the rotation speed at 100rpm. Allow each vessel to equilibrate to 37°C±0.5°C.Put 1 tablet in each vessel and commence dissolution. Withdraw the 10ml sample at desired time point. Filter the solution through 0.2µm filter paper with a syringe. Inject 20µl of each solution.

Standard preparation

Step 1: Weigh accurately about 50mg of the Working Standard of Glimepiride and transfer into a 100ml volumetric flask. Dissolve in 80ml of solvent (Acetonitrile: Buffer pH 3.0 in 80: 20) by sonication and make up the volume up to 100ml with the same solvent. Step 2: Weigh accurately about 250mg of the Working Standard of Metformin HCl and transfer into a 250ml volumetric flask. Add 1ml of Glimepiride standard dilution from step 1 in this flask; make up volume up to 250ml with the dissolution medium and dissolve it by sonication. Filter the solution through 0.2µm filter paper with a syringe. Inject 20µl of each solution.

Chromatographic conditions

Wave length: 270nm

Mobile phase: Acetonitrile 55%, Ammonium acetate buffer pH 3.0 45%
Injection Volume: 20µL
Flow rate: 1.00ml/min
Column: Promocil C_{18} 5µ 100A° 4.6x150mm Cat# PM951505-0 (Agela)

Comparative dissolution profile

Samples

Orinase Met tablet 1.0 Each film coated tablet contains 1mg of Glimepiride and 500mg Metformin HCl. Manufactured by CCL Pharmaceuticals (PVT.) Ltd. 62-Industrial Estate, Kotlakhpat Lahore, Pakistan.

Reference

Amaryl M tablet Each film coated tablet contains 1mg of Glimepiride and 500mg Metformin HCl. Sanofi-aventis Pakistan Limited**,** Registered Office, Plot 23, Sector 22, Korangi Industrial Area Pakistan.

Time points

Samples (10ml) were taken at 5, 10, 15, 30 and 45minutes for all dissolutions.

Comparative dissolution approach

Model independent approach was used to compare the dissolution profile of these two products. As the solubility of glimepiride is low and it takes time to get dissolved so we have sufficient time points to use this approach

Dissolution medium

0.2% Sodium lauryl sulfate, Water and 0.1N HCl were used as dissolution medium to compare the dissolution profile

RESULTS

Analytical method

Method was found linear for both Metformin HCl and glimepiride. Regression coefficient for Metformin HCl and glimepiride was found 0.9949 and 0.9864 respectively which is greater than 0.980.

Accuracy was determined for the samples having different concentrations (80%, 100% and 120%). Result were found satisfactory and according to amount of active ingredients added .RSD was less than 2.0 in all cases confirming the accuracy of method.

All the other parameters such as robustness, repeatability, reproducibility and precision were found to be as per requirements of ICH guideline.

Dissolution

Dissolution was performed in 0.2% sodium lauryl sulfate, water and 0.1NHCl. Metformin is soluble in all three media and hence was determined and compared in both samples. Glimepiride is soluble in 0.2% sodium lauryl sulfate so it was determine in dissolution performed in 0.2% sodium lauryl sulfate. In 0.1N HCl and water glimepiride was detected but peak was very small and was not acceptable for quantification.

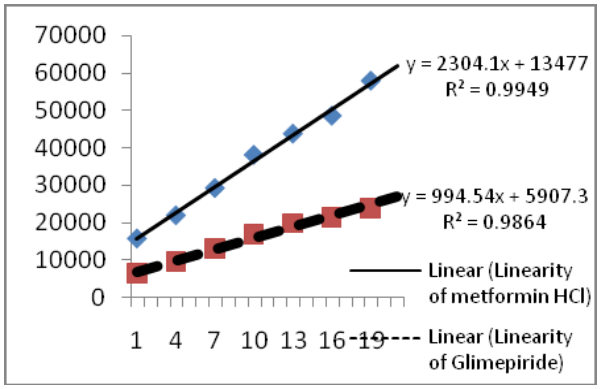

Fig. 1: Plot of linearity study for Metformin and Glimepiride.

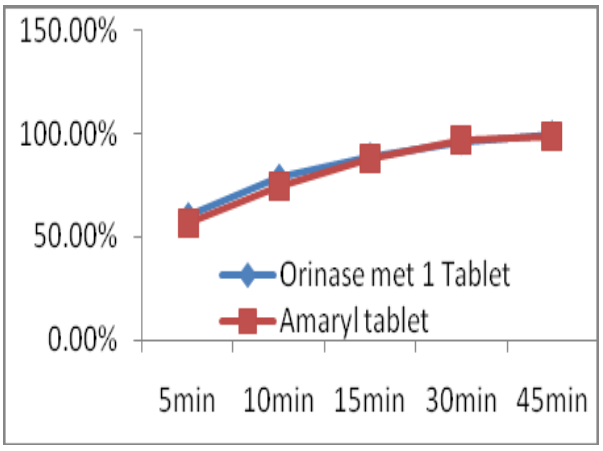

Fig. 2: Plot of dissolution study of Orinase-met 1 and Amaryl M tablet in 0.2% SLS.

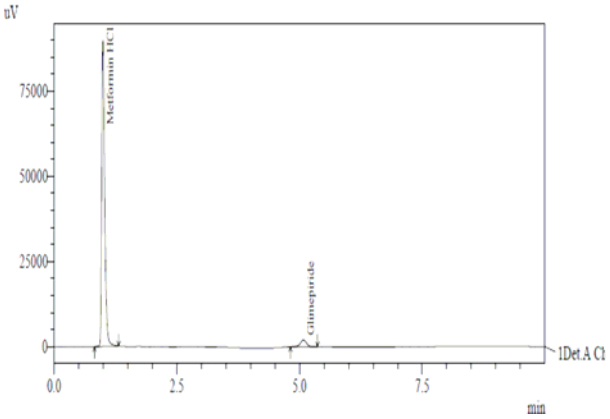

Fig. 3: Chromatogram of dissolution in 0.2% SLS

DISCUSSION

Method was found accurate and gave precised results. No interference of the dissolution media was found in

chromatography. Dissolution rates of both drugs (Metformin and glimepiride) were very high and within first 5 minutes about 60% of each drug was dissolved. In 15 minutes results of Metformin HCl and glimepiride were found to be greater than 85% for both sample and reference so no similarity factor was required. Dissolution of both samples was found to be very much similar.

The chromatogram of a dissolution sample in 0.2% Sodium lauryl sulfate is given below. The retention time of Metformin and glimepiride are 1.1 and 5.2 min respectively. The low area of glimepiride is due to its very low concentration and low molar absorptivity as compared to metformin.

Dissolution of glimepiride was not possible to perform in water and 0.1N HCL due to its extremely low solubility. Concentration of glimepiride in the dissolution samples for both sample and reference was found to be very low which can only be detected and no quantification was possible. Whereas dissolution of Metformin HCl was performed in these media and products were found similar in behavior in these two media. This is mainly due to the excellent solubility of metformin in Water, 0.1N HCl and other water-based media. Moreover the quantity of the metformin in tablet is so high that it form most of the bulk of tablet and hence no issue to become in contact with media and release in dissolution process. This study gives a simple method to perform dissolution study of this combination in day-to-day testing.

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