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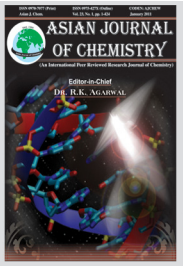

Development and Validation of Type-2 Diabetes Drug in Combination (Metformin and Glibenclamide) by HPLC

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A simple, selective, linear, precise and accurate HPLC method was developed and validated for Type-2 diabetes medicine. In this regard metformin HCl and glibenclamide (in combination) were used.10 mL of sample with isocratic elution at a flow rate of 1mL/min was employed on a C_{18} column (150 × 4.6 mm) at ambient temperature 35 ºC. The mobile phase consisted of acetonitrile and potassium dihydrogen phosphate adjusted to pH 3. The UV-detector at wavelength 332 nm was used. The percentage RSD for precision and accuracy of the method was found to be less than 3 %. The method was validated as per the standard analytical procedures and statistical parameters.

Key Words: HPLC, UV/VIS, Metformin HCl, Glibenclamide, Acetonitrile.

INTRODUCTION

A drug, broadly speaking, is any chemical substance that, when absorbed into the body of a living organism, alters normal body function. There is no single, precise definition, as there are different meanings in medicine, government regulations and colloquial usage.

A medication or medicine is a drug taken to cure and/or ameliorate any symptoms of an illness or medical condition, or may be used as preventive medicine that has future benefits but does not treat any existing or pre-existing diseases or symptoms[1,2]. Diabetes mellitus, often referred to simply as diabetes (Greek: διαβη´της), is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from insufficient levels of the hormone insulin, with or without additional resistance to insulin's effects in many body cells. The characteristic symptoms are excessive urine production (polyuria) due to high blood glucose levels, excessive thirst and increased fluid intake (polydipsia) attempting to compensate for increased urination, blurred vision due to high blood glucose effects on the eye's optics, unexplained weight loss and lethargy. These symptoms are likely to be less apparent if the blood sugar is only mildly elevated[3]. The World Health Organization recognizes three main forms of diabetes mellitus: type 1, type 2 and gestational diabetes (occurring during pregnancy), which have different causes and population distributions. While, ultimately, all forms are due to the β-cells of the pancreas being unable to produce sufficient insulin to prevent hyperglycemia, the causes are different. Type 1 diabetes is usually due to auto immune destruction of the pancreatic β-cells. Type 2 diabetes is characterized by insulin resistance in target tissues. This causes a need for abnormally high amounts of insulin and diabetes develops when the β-cells cannot meet this demand. Gestational diabetes is similar to type 2 diabetes in that it involves insulin resistance; the hormones of pregnancy can cause insulin resistance in women genetically predisposed to developing this condition[4].

Medicines for type 2 diabetes: Type 2 diabetes, once called adult-onset diabetes or non insulin-dependent diabetes, is the most common form of diabetes. It can start when the body doesn't use insulin as it should, a condition called insulin resistance. If the body can't keep up with the need for insulin, you may need diabetes medicines. Many choices are available. Your doctor might prescribe two or more medicines. The ADA recommends that most people start with metformin, a kind of diabetes pill. A metformin-glibenclamide combination tablet may provide type 2 diabetics, which improved glycogenic control than higher doses of either drug alone. While using any medicine which contains glibenclamide and metformin in combination. Certain side effects are observed such as short breath, mild exertion, swelling, rapid weight gain, sneezing, running nose, cough, headache, dizziness, mild nausea, vomiting, diarrhea, stomach pain. And the most threatening problem which can occur while taking medicine with this combination is lactic

acidosis. Most diabetic patients use a combination of medicines containing metformin and glibenclamide individually than taking a single medicine (such as glucovance) containing both of these drugs. The choice ultimately depends upon the doctor's advice that analyses every patient's individual needs.

Glibenclamide: Glibenclamide is a chemical in a class of chemicals known as the sulfourenyls. It is used in treatment of type 2 diabetes, therefore it is an antidiabetic drug. It is sold in doses of 1.25, 2.5 and 5.0 mg, under the trade names of Diabeta, Glynase and Micronase in the United States and Daonil, Semi-daonil and Euglucon in the United Kingdom. It is also sold in combination with metformin under the trade name of glucovance[5].

Medical aspects of glibenclamide: It stimulates the pancreatic cells known as β-cells. It causes the β-cells to produce more insulin. Thus reducing blood sugar in type 2 diabetic patients. Glibenclamide inhibits the ATP-sensitive potassium channels in pancreatic β-cells. The inhibition causes membrane depolarization, opening the voltage dependent calcium channels, thus triggering an increase in insulin.

Metformin HCl: Metformin HCl is an oral antidiabetic drug, one of the two drugs in the World Health Organization Model list of essential medicines; it belongs to the class of iguanids. Metformin is the most preferred choice of treatment in type 2 diabetic patients, especially in obese and overweight patients with kidney problems. Biguanide can function as oral ant hyperglycemic drugs used for diabetes mellitus or pre diabetes treatment. They are also used as antimalarial drugs[6]. The disinfectant polyaminopropyl biguanide (PAPB) features biguanide functional groups[7].

Combination of metformin HCl and glibenclamide: In type 2 diabetic patients, the amount of increased insulin preceding diagnosis, causes attendant fasting and postprandial hyperinsulinaemia, compensate to some degree for the deficit in hepatic and peripheral insulin sensitivity. The β-cell eventually fail to function and thus insulin production stops, causing glucose intolerance and hyperglycemia[8]. Various studies have been carried out to find out the effectiveness of glibenclamide-metformin combination in a single tablet, rather than the monotherapy. Some previous studies showed that this treatment in patients with hyperglycemia despite treatment with diet and exercise or oral antidiabetic immunotherapy, have better results as metformin-glibenclamide combination taken than immunotherapy with either one[9].

EXPERIMENTAL

Metformin HCl and glibenclamide (in combination): Method development and validation of metformin HCl & glibenclamide (in combination) by HPLC method is described as follows. The HPLC used for the method development was of Shimadzu; model CTO-10 as VP. The method developed for metformin HCl & glibenclamide therefore works at 218 nm with 1.5 mL flow rate. The mobile phase used is of potassium dihydrogen phosphate and acetonitrile with pH adjusted at 3 with dilute phosphoric acid. The column was C_{18} (150 mm × 4.6 mm) and the injectible sample 10 mL had 0.15 mg/mL of metformin HCl and 0.40 mg/mL of glibenclamide.

Potassium dihydogen phosphate (Merck), acetonitrile (Merck), distilled water, methanol (Merck), phosphoric acid (Merck), glibenclamide (pure form), metformin HCl (pure form). Different preparations like metformin HCl stock solution, glibenclamide stock solution, potassium dihydrogen phosphate and dilute phosphoric acid solution are prepared in the laboratory.

Preparation of mobile phase: 500 mL of potassium dihydrogen was taken in a 1000 mL measuring flask. 443 mL of HPLC grade acetonitrile was added to the buffer. The pH of the mobile phase was adjusted with the diluted phosphoric acid solution to 3, with the help of a pH meter. The mobile phase was constantly stirred during the pH adjustment. Mobile phase was prepared according to the requirement of HPLC.

Method development: After the mobile phase preparation the HPLC was set for simultaneous determination of metformin HCl and glibenclamide (in combination). The flow rate was kept the same for method development throughout. First of all, the analysis was observed at different wavelengths, 218, 230, 226 and 240 nm. Although good peaks for both metformin HCl and glibenclamide were obtained at 226 and 230 nm but very small peaks were obtained at 240 nm. The best peaks (height and areas) were obtained at 218 nm. Therefore the wavelength was adjusted at 218 nm for this method.

Spiking was done with different concentrations of glibenclamide and metformin HCl for obtaining the best results. The concentrations of the metformin HCl and glibenclamide which were analyzed from their respective stocks for the 10 mL of injectible sample were in ratio of 4.0:1.5 after different trial ratio.

The ambient temperature throughout the experiment was kept at 35 ºC. After the analysis at the end of the day, the column of HPLC was washed with methanol (Merck standard) and then with distilled water.

Validation methods: The procedure is validated according to different methods as:

Method precision: The relative standard deviation for metformin HCl within a day ranged from 1.33 to 2.12 and between 3 days was 1.75 and for glibenclamide it was between 1.68 to 2.37 within a day and 2.05 between 3 days. All these values of RSD are below 3 %; thus, this method is precise (Table-1).

Method repeatability: Method repeatability for metformin HCl and glibenclamide (in combination) was evaluated by assaying 10 samples by the same analyst on the same day and the RSD calculated for metformn HCl was 1.12 and for glibenclamide was 0.70. All the RSD values for method repeatablitiy are below 3 % and therefore, the above described method is acceptable in terms of method repeatability. The data is summarized in Table-2.

Method ruggedness: Ruggeddness of the method for determination of metformin HCl and glibenclamide (in combination) was evaluated by assaying the same sample of the product by different analysts. The RSD was 1.40 for metformin HCl and 0.49 for glibenclamide. Method ruggedness has RSD values for metformin HCl and glibenclamide (in combination) below 3 %, therefore the ruggedness for this method is valid and acceptable (Table-3).

Method linearity: In method linearity, various dilutions of the sample, 70 to 130 % by weight were prepared and the readings were noted. The concentrations should be such that

TABLE-1
METHOD PRECISION FOR METFORMIN HCl AND GLIBENCLAMIDE

Sample No.	Day 1		Day 2		Day 3	
	Metformin HCl	Glibenclamide	Metformin HCl	Glibenclamide	Metformin HCl	Glibenclamide
1	100.37	103.77	101.63	90.53	97.68	97.36
2	102.24	98.18	100.82	97.93	96.04	101.30
3	106.66	99.03	96.78	93.10	99.75	97.10
Mean (X)	103.09	100.32	99.74	93.85	97.82	98.58
Standard deviation (within a day)	2.28	2.12	1.80	2.23	1.31	1.66
RSD (with a day)	2.12	2.11	1.80	2.37	1.33	1.68
Mean of 3 days (X)	100.21	97.58	100.21	97.58	100.21	97.58
RSD between 3 different days	1.75	2.05	1.75	2.05	1.75	2.05
Limit of RSD	NMT 3 %	NMT 3 %	NMT 3 %	NMT 3 %	NMT 3 %	NMT 3 %

TABLE-2
METHOD REPRODUCIBILITY FOR
METFORMIN HCl AND GLIBENCLAMIDE

Sample	Metformin HCl	Glibenclamide
1	97.25	101.35
2	104.68	99.16
3	103.79	97.02
4	98.70	100.49
5	95.69	99.40
6	97.79	96.07
7	101.02	101.38
8	105.82	96.32
9	101.50	96.83
10	102.43	100.08
Mean (X)	100.86	98.81
Standard deviation (SD)	1.13	0.70
RSD	1.12	0.70

TABLE-3
METHOD RUGGEDNESS FOR
METFORMIN HCl AND GLIBENCLAMIDE

Analyst	Metformin HCl	Glibenclamide
Arfa Altaf	105.87	99.81
Sehrish Sarfraz	102.02	98.46
Abdul Shakoor	102.65	98.79
Mean (X)	103.51	99.02
Standard Deviation	1.45	0.49
RSD	1.40	0.49
Limit of RSD	NMT: 3 %	NMT: 3 %

it represents the lower and higher concentrations of the optimal concentration (optimal concentration is that on which normally the readings are taken). The values were plotted on a graph and the linearity was observed. There was a little curvature for glibenclamide due to its unstability (Fig. 1).

RESULTS AND DISCUSSION

The method for metformin HCl and glibenclamide (in combination) was developed with CTO-10 ASvp Shimadzu high performance liquid chromatography with a C_{18} 150 mm × 4.6 mm) column. The injectable sample was to be made fresh for every third injection because of the low stability of glibenclamide. The analytical method for analysis and determination of metformin HCl and glibenclamide (in combination) was carried out with the mobile phase made from acetonitrile and potassium dihydrogen phosphate (1:1,v/v). The pH was adjusted with diluted phosphoric acid. The mobile phase was then filtered at the pump and kept in ultrasonic bath for removal

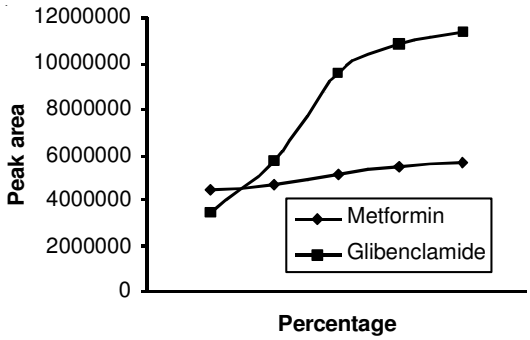

Fig. 1. Method for linearity for metformin HCl and glibenclamide (in combination)

of air bubbles. The stock for metformin HCl and glibenclamide, both were prepared by adding 0.1 g of pure sample to a 100 mL flask and raising upto the level with methanol and distilled water respectively.

The validation was however carried out with water breeze high performance liquid chromatography with a C_{18} column (4.6 mm × 150 mm). The water breeze instrument had a very high sensitivity and responded to even virtually invisible anomalies. In the contrary the Shimadzu HPLC gave a more accurate and précised result. The flow rate for the method was 1.5 mL/min. The method was validated with respect to precision, ruggedness, reproducibility and linearity and the best peaks were shown in figures (not shown).

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