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Design and Validation of Stability Indicating Assay of Glibenclamide Using RP-HPLC Technique in Both Bulk, Pharmaceutical Formulations and Human Plasma

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Abstract

A simple and sensitive high performance liquid chromatography method was developed for determination of glibenclamide in presence of its impurities in pharmaceutical dosage form and human plasma. Instrumentation of the method was very simple and used the mixture of acetonitrile: water (60:40, v/v) as the mobile phase. Separation was carried out on a BDS. Hypersil C_8 (5 um, 250 × 4.6 mm) column. Effects of composition of mobile phase in addition to flow rate and detection wave length were studied. Calibration was obeyed in the range of 20-100 µg/ml of glibenclamide. The method was validated according to ICH parameters.

Keywords: Glibenclamide; Glimepiride; Stability indicating assay; Plasma; HPLC

Introduction

Diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Diabetes symptoms such as being very thirsty, urinating often, feeling very hungry and feelings of pins needles in your feet. Diabetes can take a toll on nearly every organ in your body, including heart, blood vessels, eyes, kidneys, nerves, gums and teeth. Diabetes medication lowers blood sugar levels, and there are a number of different types which work in different ways.

There are several different 'families' (or types) of diabetes medication like biguanide, sulphonylureas, alpha glucosidase inhibitor, Thiazolidinediones and etc. Sulphonylureas medications help your body secrete more insulin. Examples of medications in this class include glibenclamide (glyburide), glipizide and glimepiride. Possible side effects include low blood sugar and weight gain.

Glibenclamide is anti-diabetic drug and it is belong to sulfonylureas group. Glibenclamide chemically is 1-[4-[2-(chloro-2-methoxybenzamido)ethyl]-benzene sulphonyl]-3cyclo hexyl urea, 5-chloro-N-[2-[4-[[[(cyclohexyl(amino)carbonyl]-amino]sulphonyl]phenyl]ethyl-2-methoxy-benzamide (Figure 1). It is used to assist to control mild to moderately severe type II diabetes mellitus that does not require insulin that can be adequately controlled by diet alone [1].

Few analytical methods have been reported as a stability indicating method for simultaneous determination glibenclamide in presence of its degradation products. The present method aim to develop a simple, selective and precise RP-HPLC method for the simultaneous estimation of glibenclamide and its impurities in bulk drug samples and in combined dosage formulation in presence of glimepiride as the internal standard.

Several and different techniques were developed for glibenclamide determination. Spectrophotometric methods [2,3], chromatographic methods [3-12], spectrofluorometric 36 method [13] and electrochemical methods [14,15].

Materials and Experimental Work

Instrumentation

The used apparatus was High performance liquid chromatography apparatus from Agileng technologies (BDS Hypersil C_8 (5 um, 250 × 4.6 mm) column). The system was an isocractic HPLC with isocractic liquid pump. Data was transferred and computerized. The details of chromatographic conditions were showed in Table 1.

Parameters	Conditions
sColumn	BDS Hypersil C_8 (5 um, 250 × 4.6 mm) column
Mobile phase	Isocratic binary mobile phase of acetonitrile: water (60:40 v/v), filtered and degassed using 0.45 µm membrane filter
UV detection	230 nm
Flow rate	1 ml/min
Injected volume	10 µl
Pressure	11 psig
Temperature	Ambient (25 ± 5°C)

Table 1: Chromatographic conditions for stability indicating assay of Glibenclamide.

Reagents and materials

Acetonitrile and water were with HPLC grade. Authentic powder of glibenclamide and glimpiride were supplied by Sanofi Aventis

Company in Egypt and were used directly. Stock standard solutions containing 0.5 mg/ml of glibenclamide and 0.5 mg/ml of glimepiride were prepared by dissolving 50 mg of each drug in the least amount of 48 methanols in 100 ml volumetric flask. Sonication for 10 min and the final volume of 49 solutions was completed to 100 ml with mobile phase to get stock standard solutions.

Chromatographic conditions

10 µl from the sample solutions of the drugs were taken and detected at lambda 230 nm. Liquid chromatography was performed on a BDS Hypersil C_8 (5 um, 250 × 4.6 mm) column. The used mobile phase was (acetonitrile: water (60:40 v/v). Mobile phase pumped at a flow rate equals to 1 ml/min at ambient temperature. Before all, the mobile phase was filtered by a 0.45 µl Nylon membrane filter (USA, MA) under vacuum and degassed by ultrasonication (USA, Vernon Hills).

Preparation of stock standard solutions

50 mg of both glibenclamide and glimepiride individually weighed and dissolved in the least amount of methanol in 100 ml volumetric flask. Sonicate for 10 min and then make up the volume with mobile phase. The resulted concentrations of solutions were of 0.5 mg/ml of both glibenclamide and glimepiride.

Procedure and calibration plot

General chromatographic procedure: drug sample solutions (10 µl) were monitored at 230 nm for glibenclamide and glimepiride. Liquid chromatography was performed on BDS Hypersil C_8 (5 um, 250 × 4.6 mm) column and the mobile phase consisted of a mixture of acetonitrile: water (60:40, v/v) which pumped at a flow rate equals to 1 ml/min at ambient temperature. A 0.45 µl Nylon membrane filter (Millipore, Milford, MA, USA) was used for mobile phase filtration under vacuum and then mobile phase was degassed by ultrasonication (Cole Palmer, Vernon Hills, USA) before usage.

To construct calibration plots, the stock standard solutions were diluted with mobile phase to prepare working solutions in the concentration ranges 20-100 ug/ml for glibenclamide. Each solution (n=5) was injected in triplicate and chromatographed under the mentioned conditions above. Linear relationships were obtained when average drug standard peak area were plotted against the corresponding concentrations for each drug. Regression equation was computed.

Preparation of Daonil® tablet solutions

Daonil® tablets labeled to contain 5 mg glibenclamide per tablet. Batch No. 6223003990510 (Sanofi Aventis, Egypt). The average weight of one tablet was calculated. Amount of one tablet (equivalent to 5 mg of glibenclamide) was accurately transferred to 10 ml volumetric flasks containing methanol. Sonication for 10 min was done and then the solutions were filtered through 0.45 Mm nylon membrane filters (Millipro, Milforod, MA, USA).

Stock standard solutions were diluted with mobile phase to prepare working solutions in the concentration ranges 20-100 µg/ml for the drug. Each solution (n=5) was injected in triplicate and chromatographed under the mentioned conditions above as in Figure 1.

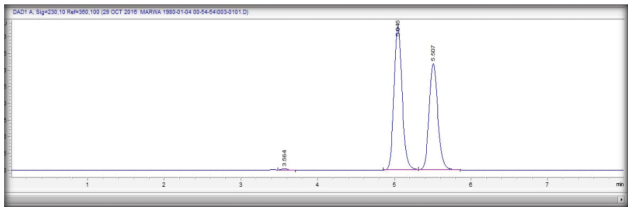

Figure 1: HPLC chromatogram of glibenclamide in Daonil® tablet at 5.1 min and glimepiride at 5.6 min respectively.

Preparation of spiked human plasma sample for glibenclamide

Spiking of working solution of glibenclamide containing the internal standard with human plasma has been done by adding 0.5 ml of human plasma (supplied from blood bank at Zagazig university hospitals). Extraction of GLB and GLI from plasma was done by using methanol as the extracting solvent which was centrifuged at 5000 rpm for 10 min. The supernatant was transferred to a clean 10 ml volumetric flask and diluted to volume with methanol.

Forced degradation for stability indicating assay of GLB

To determine the proposed method as a stability-indicating method for glibenclamide assay in presence of GLI as an internal standard, GLB was stressed under different conditions in forced degradation studies.

Acidic degradation: The drug was subjected to acidic force by adding 1.5 ml of 1M HCl to the stock solution of glibenclamide. 1M of HCl was prepared by adding 9 ml of conc. HCl in 100 ml distilled water. The solution of glibenclamide with the acid was heated at 90°C for 1.5 hr in the dark (to exclude the possible degradative effect of light). The solutions (2 mL) were then transferred to 10 mL volumetric flasks, neutralized by addition of 1.5 ml of 1M NaOH and diluted to final volume with mobile phase and measured [16,17].

Alkaline degradation: The drug was subjected to alkaline studies by adding 1.5 ml of 1M NaOH to the stock solution of glibenclamide. 1M of NaOH was prepared by adding 4 g of NaOH in 100 ml distilled water. The solution of glibenclamide with NaOH was heated at 90°C for 1.5 hr in the dark (to exclude the possible degradative effect of light). The solution (2 mL) was then transferred to 10 mL volumetric flasks, neutralized by addition of 1.5 ml of 1M HCl acid. Complete to final volume with mobile phase [16,17].

Oxidative degradation: Hydrogen peroxide (H_2O_2; 10%, v/v, 1.5 mL) was added to the stock solution of glibenclamide. The solution of glibenclamide with H_2O_2 was heated at 90°C for 1.5 hr in the dark (to exclude the possible degradative effect of light). The solution (2 mL) obtained was then transferred to 10 mL volumetric flasks and diluted to final volume with mobile phase [16,17].

Results

Optimization of chromatographic conditions

Spectroscopic analysis of the drugs showed that glibenclamide and glimepiride have maximum UV absorbance (λ_{max}) at 230 nm.

Chromatographic conditions were showed in Table 1. The method used a BDS Hypersil C_8 (5 um, 250 × 4.6 mm) column. To optimize Chromatographic conditions, changing the mobile phase composition, the flow rate and also studying the detection at different wavelengths were carried out.

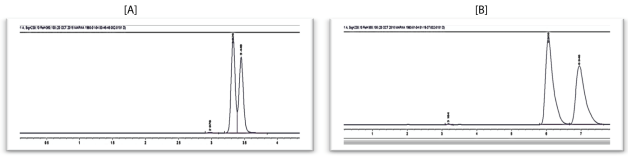

Figure 2: HPLC Chromatogram of glibenclamide for optimization the mobile phase composition, (acetonitrile: water) (A) 40:60; and (B) 50:50.

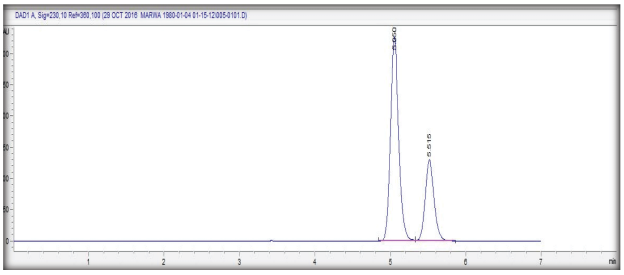

Figure 3: HPLC chromatogram of glibenclamide at 5.1 min and glimepiride at 5.6 min.

Forced degradation products and assay of Glibenclamide

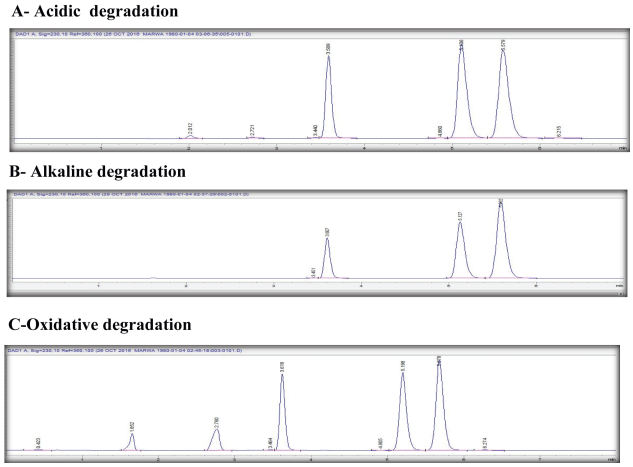

Figure 4: HPLC chromatograms (A, B, C) of glibenclamide after exposure to degradation forces.

For example, altering the composition of mobile phase from (acetonitrile: water v/v) (40:60) and (50:50) to (60:40) as shown in Figure 2. The best resolution and separation was in case of mobile phase with (60:40) while (40:60) not separate the drugs and (50:50) show tailing and broadening. The optimized mobile phase was determined as a mixture of acetonitrile: water (60:40, v/v) at a flow rate of 1 ml/min at 230 nm as shown in Figure 3.

Stress Conditions	Peak Area	% Assay after Degradation
Standard	3550.7	100
Acidic degradation at (1M HCl) 90°C/1.5 hrs	2439.1	70.2
Alkaline degradation at (1M NaOH) 90°C/1.5 hrs	1840.9	51.9
Oxidative degradation at (10% H_2O_2) 90°C/1.5 hrs	1318	37.1

Table 2: Results of degradation percent of Glibenclamide.

Application on spiked human plasma

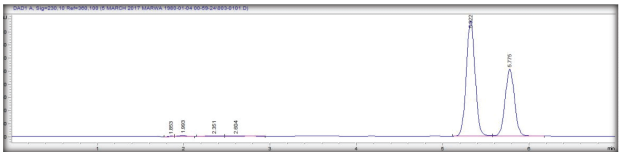

Figure 5: Typical HPLC chromatogram of glibenclamide at 5.1 min and glimepiride at 5.6 min in human plasma.

Method Validation

The developed method was validated according to international conference on harmonization guidelines (Figures 4 and 5; Table 2) [18].

Specificity

Specificity shows the ability to assess unequivocally the analyte in presence of components which may be expected to be present. Typically these might include degrades, impurities, matrix, etc. A bulk of Daonil® tablets (solution containing excipients only like Lactose Monohydrate 79.0 mg, Maize Starch, Pre-gelatinised maize starch, Talc, Colloidal anhydrous silica, Magnesium stearate) has been prepared by mixing its excipients. Known concentration of these excipients was added to the bulk then determined under previous condition. Recovery results indicate that excipients have negligible effect which means that the bulk did not interfere with the developed method.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain results which are directly proportional to the concentration of analyte in the tested sample. For construction of linearity, a minimum of 5 concentrations were recommended [18]. Five Concentrations were chosen in the ranges (20-100 µg/ml) of glibenclamide. The linearity of peak area responses versus concentrations was established by linear least square regression analysis. The linear regression equation was {y=35.611x-29.12 (r=0.9998)}, Table 3 and calibration curve showed in Figure 6. Where Y is the peak area of standard solution and X is the drug concentration.

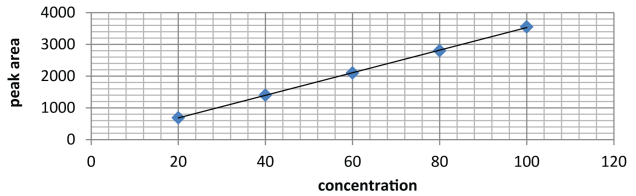

Figure 6: Calibration curve of glibenclamide using the proposed HPLC method for stability indicating assay.

Mean	100.1	**Variance**	0.42
S D	0.65	**Slope**	35.61
RSD	0.65	**LOD**	0.32
SE	0.29	**LOQ**	0.96
*Average of three independent procedures			

Table 3: Results of the calibration curve for HPLC determination of glibenclamide in presence of its impurities.

Limits of detection and Limits of quantitation

According to the ICH guidelines, the Limit of Detection (LOD) and the Limit of Quantitation (LOQ) for the method were calculated using the following equations [18] LOD=3.3 σ/s, LOQ=10 σ/s where σ is the standard deviation of intercept, s is the slope of calibration curve. LOQs and LODs are listed in Table 3. Determination of limits of detection and quantitation was based on the standard deviation of the y-intercepts of regression lines (n=3) and the slope of the calibration plots.

Precision

Precision is a measure of statistical variability. It shows the degree to which repeated measurement under unchanged conditions gives the same results. The precision is related to reproducibility and repeatability [18,19]. Repeatability measures the variation in measurements taken by a single instrument or person under the same conditions, while reproducibility measures whether an entire study or experiment can be reproduced in its entirety.

		AV ± SD %
Repeatability		100.7 ± 0.76%
Intermediate precision		100.4 ± 1.1%
Accuracy and Recovery%	**80%**	100.02 ± 1.2%
	100%	101.1 ± 0.78%
	120%	100.7 ± 0.78%

Table 4: Repeatability, intermediate precision and accuracy (Recovery %) of the proposed HPLC method for stability indicating assay of glibenclamide.

Repeatability: Repeatability (intra-day precision) was investigated by injecting a 100% of the test concentration for 6 determinations and SD values were calculated in Table 4.

Intermediate precision: In the inter-day studies, standard and sample solutions prepared as described above, were analyzed in triplicate on three consecutive days at 100% of the test concentration and percentage SD were calculated (Table 4).

Accuracy: Accuracy is a measure of statistical variability. It is the proximity of mean results to the reference values [18,19]. It was assessed using 9 determinations over 3 concentration levels covering the specified range (80, 100 and 120%) as present in linearity range. Accuracy was reported as percent recovery by the assay of known added amount of analyte in the sample (Table 4).

Robustness

Robustness of an analytical procedure is a measure of its capacity to still unaffected by small variations in method parameters and gives an indication of its reliability during normal usage [18]. Robustness was tested by evaluating the effect of changing the percentage of organic solvent (acetonitrile) in the mobile phase, wavelengths and flow rate where there was no significant effect on the chromatographic resolution of the method (Table 5).

Flow rate 0.9	100.03 ± 1.21	Mobile phase 61:39	100.16 ± 1.07
Flow rate 1.1	100.43 ± 1.12	Wave length 229 nm	99.86 ± 0.89
Mobile phase 59:41	100.16 ± 1.07	Wave length 231 nm	99.71 ± 1.01

Table 5: Results for test the robustness of the proposed HPLC method for stability indicating assay of glibenclamide.

Stability of analytical solution: Also as part of assaying of robustness, solution stability was evaluated by monitoring the peak area response. Standard stock solutions were assayed right after its preparation 1, 2 and 3 days after at room temperature. The change in standard solution peak area response over 3 days had negliable effect. Their solutions were found to be three days stable at room temperature at least.

System suitability

System suitability was checked for the conformance of suitability and reproducibility of chromatographic system for analysis. System suitability was determined before sample analysis from three replicate injections of the standard solution for the two drugs. The acceptance criteria were less than 2% Relative Standard Deviation (RSD) for peak areas, USP tailing factor (T) less than 2.0, USP plate count (N) more than 2000, capacity factor $K=t^R-t^0/t^0$ more than 0.5, and resolution $R=t_{r2}-t_{r1}/0.5^*(w_1+w_2)$ more than 1.5 for peaks from standard solution. All critical parameters tested met the acceptance criteria (Table 6) [20].

Resolution factor (R): Resolution is a measure of the extent degree of the resolution between the resulted peaks.

Selectivity factor (s): Selectivity parameter is a measure of resolution of two drugs in the sample under certain conditions.

Capacity factor (k): Capacity factor (retention factor) is a measure of the retention time of a drug in the sample with a given combination of mobile phase and column.

Tailing factor (T): Tailing factor refers to peak asymmetry (Gaussian distribution). A tailing factor of one refers to a symmetric peak.

Drugs	Theoretical Plates (N)	Resolution (R)	Capacity Factor (K)	Tailing Factor (T)	Selectivity (s)
Glibenclamide	13132	-	3.04	0.96	-
Glimepiride	13649	2.35	3.38	0.98	1.11

Table 6: System suitability parameters for stability indicating assay of glibenclamide.

Comparison between the Proposed Method and the Reference Method

The method was applied on the pharmaceutical form of glibenclamide Daonil® tablets and on the spiked human plasma with GLB. The results were compared to those obtained by applying reported reference one [21,22] and where Student's T-test and F-ratio were used for comparison. Results are shown in Tables 7 and 8. There is no significant difference between proposed method and reference ones relative to accuracy and precision as the calculated T and F values were less than tabulated values.

Drug name	Recovery ± SD			
	Proposed method in spiked plasma	Reference method	Calculated T-values	Calculated F-ratios
Glibenclamide [21]	99.86 ± 0.89	100.42 ± 1.12	0.89	1.6
Where the Tabulated T values and F-ratios at p=0.05 are 2.57 and 6.256 respectively				

Table 7: Statistical comparison of the proposed and reference method [21] for determination of glibenclamide in spiked human plasma.

Drug name	Recovery ± SD			
	Proposed method in Daonil® tablet	Reference method	Calculated T-values	Calculated F-ratios
Glibenclamide [22]	100.17 ± 0.898	100.02 ± 1.2	0.22	0.55
Where the Tabulated T values and F-ratios at p=0.05 are 2.57 and 6.256 respectively				

Table 8: Statistical comparison of the proposed and reference method [22] for determination of glibenclamide in Daonil® tablets.

Conclusion

Estimation and stability indicating assay for glibenclamide in pharmaceutical dosage form and in human plasma where the results compared with reference one and indicates that there was no significant difference between the proposed methods and reference one. The method was validated according to ICH guidelines and it was very simple [23].

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