



Development of a RP-HPLC Method for Simultaneous Determination of Some Antidiabetic Sulfonylurea Drugs in Bulk and Pharmaceutical Dosage Forms

Abeer Koul Ali^{1*}, Ahmad Ali Hassan²

¹*License in Pharmacy, Stage of preparing master degree in Pharmaceutical Chemistry and Quality control, Faculty of Pharmacy, Damascus University, Syria.

²Professor at the Department of Pharmaceutical Chemistry and Quality control, Faculty of Pharmacy, Damascus University, Syria.

*Corresponding author's E-mail: abeerkoulali@yahoo.com

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ABSTRACT

A simple, rapid, and precise reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed for the separation and simultaneous determination of Gliclazide, Glibenclamide, Glipizide and Glimepiride in bulk and pharmaceutical dosage forms. The elution was performed using a mobile phase mixture of methanol, acetonitrile and phosphate potassium buffer (pH= 3.5) in the ratio of 60:10:30 at a flow rate of 1.5 ml /min on a reverse phase C₁₈ column (150 × 4.6 mm, 5 μm) at 35 °C. The drugs were monitored at a wavelength of 230 nm and were separated within 8 min. the method was validated as per ICH guidelines and the results were found to be within the acceptable range. Hence, the proposed method can be used for the routine quality control of the drugs.

Keywords: Bulk and pharmaceutical dosage forms, RP-HPLC, Sulfonylureas, Validation.

INTRODUCTION

Sulfonylureas were the first approved class of oral antidiabetics in the United States¹, they were discovered accidentally in 1942.² They are classified as first-generation and second-generation agents. Second generation compounds, which include Gliclazide, glipizide, Glibenclamide and Glimepiride (Figure 1), are more potency and widely used in the treatment of type 2 diabetes.³ Sulfonylureas stimulate insulin release from pancreatic β cells by binding to sulfonylurea receptors (SURs) on the β cell plasma membrane, which closes an adenosine triphosphate-dependent potassium ion (K⁺) channels, leading to depolarization of the membrane and opening of Voltage-dependent calcium ion (Ca⁺²) channels, allowing influx of calcium ions and subsequent secretion of preformed insulin granules.^{4,7} On the other hand, sulfonylureas act by reducing hepatic glucose production⁵⁻⁷ and increasing peripheral insulin sensitivity^{6,7} They are useful in the treatment of type 2 diabetes patient who can't be managed by diet and increasing physical activity alone.^{8,9}

Literature review assembled that a number of analytical methods have been reported for the determination of some antidiabetic sulfonylurea agents alone or with other antidiabetic drugs in human serum using HPLC-UV¹⁰ or LC-MS¹¹ techniques, dosage forms using HPLC-UV^{12,13}, or both of them using HPLC-UV.¹⁴⁻¹⁶ One method has been developed for screening and determination of sulfonylurea agents in adulterated health food.¹⁷ Although HPLC-UV was the most common technique used in these developed methods, HPLC methods using the most commonly available columns and inexpensive solvents were few.

The aim of this study is to develop a rapid, sensitive, accurate and precise reverse phase HPLC method for the

separation and simultaneous determination of Gliclazide (GLC), Glibenclamide (GLB), glipizide (GLP) and Glimepiride (GLM) in bulk and pharmaceutical dosage forms. The results of analysis were treated statically, as per International Conference on Harmonization (ICH) guidelines, for validation of analytical procedure.¹⁸ The developed method would help in assay of drugs in a single run which reduces the time of analysis and doesn't require separate method for each drug. In addition, all used solvents are inexpensive, so the developed method was economical and suitable for undeveloped areas.

MATERIALS AND METHODS

Instrumentation

The analysis of drug was carried out on a SHIMADZU HPLC system equipped with a reverse phase C18 column (150x4.6mm, 5μm in particle size), a 20 μl injection loop and SPD-20A prominence UV/VIS detector, Degasser DGU-20A3.

Reagents and solutions

The reference standards of GLC, GLB, GLP and GLM were obtained from National Institute for the Control of Pharmaceutical and Biological Products in Syria

HPLC grade water, methanol and acetonitrile were purchased from Merck Ltd., Mumbai (India).

Potassium dihydrogen phosphate and ortho phosphoric acid were obtained from BDH Laboratory Supplies (Poole, UK).

Chromatographic conditions

Chromatographic separation of GLC, GLB, GLP and GLM was achieved on a reverse phase C18 column (150x4.6mm, 5μm in particle size) at 35 °C, in a mobile

phase consisting of methanol: acetonitrile: phosphate potassium buffer (pH=3.5) in the ratio of 60:10:30 v/v. The mobile phase was pumped at a flow rate of 1.5 ml/min and the eluents were monitored at 230 nm.

Stock solution

Stock solution of GLC, GLB, GLP and GLM were prepared at a concentration of 100 µg/ml separately by dissolving the appropriate amounts of the reference standards in methanol. These solutions were stable for 1 week when stored at 2–8 °C.¹¹

The standard solutions of 50 µg/ml were prepared from these stocks by the appropriate dilution with mobile phase.

For System Suitability test, a standard mixture of the six anti-diabetics at 50 µg/ml was prepared in mobile phase.

Preparation of phosphate buffer (pH 3.5)

0.5 g of KH₂PO₄ was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. PH adjusted to 3.5 with orthophosphoric acid.

Optimization of the chromatographic Conditions

Proper selection of the stationary phase depends upon the nature of the sample molecular weight and solubility. The antidiabetic sulfonylurea compounds are non-polar. Non-polar compounds are preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compounds are very attractive with reverse phase columns. So the elution of the compounds

from the column was influenced by polar mobile phase. Mixture of buffer, acetonitrile and methanol was selected as mobile phase and the effect of composition of mobile phase on the retention time of studied compounds was thoroughly investigated.

The chromatographic conditions were optimized through several trials to achieve good resolution and symmetric peak shapes, as well as a short run time. It was found that a mixture of methanol, acetonitrile and phosphate potassium buffer (pH= 3.5) in the ratio of 60:10:30 v/v was appropriate.

Procedure

A mixture of methanol, acetonitrile and phosphate potassium buffer (pH= 3.5) in the ratio of 60:10:30 v/v was found to be the most suitable mobile phase for ideal separation of GLC, GLB, GLP and GLM. The solvent mixture was filtered through a 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.5 ml/min. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 230 nm. The run time was set at 8 min. Under these optimized chromatographic conditions the retention time obtained for each of GLC, GLB, GLP and GLM was 2.53 min, 3.10 min, 4.96 min and 6.43 min respectively. A typical chromatogram showing the separation of the drugs is given in (Figure 2).

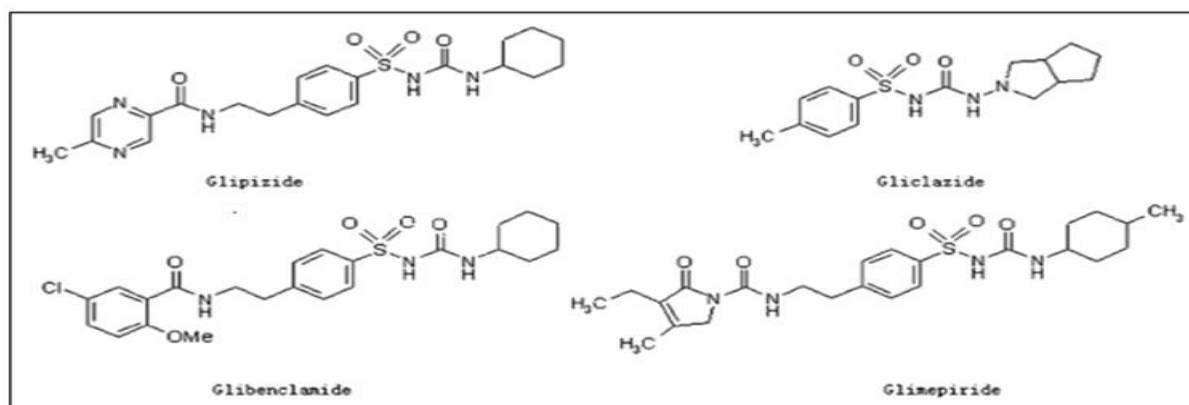


Figure 1: Chemical structures of Gliclazide, Glipizide, Glibenclamide and Glimepiride

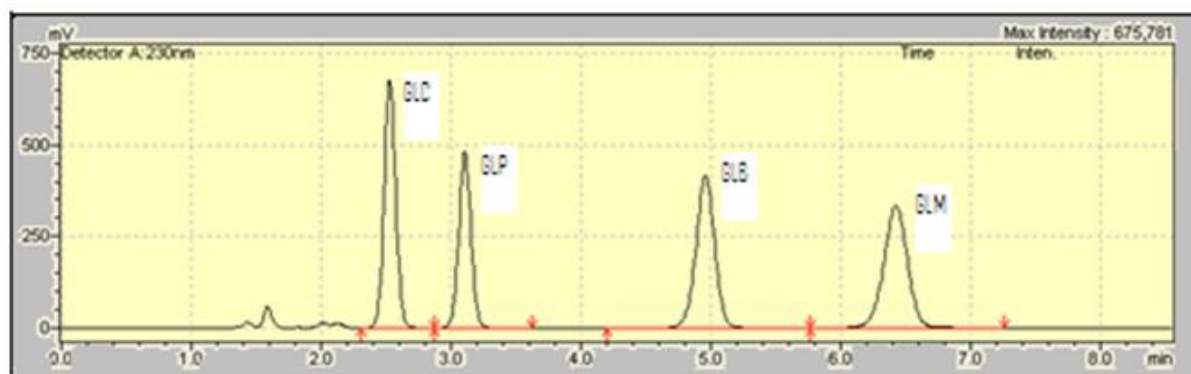


Figure 2: Chromatogram of GLC, GLP, GLB and GLM

Table 1: Results of linearity, LOD and LOQ

Parameter	Result			
	Gliclazide	Gliclazide	Gliclazide	Gliclazide
Correlation factor	0.998	0.999	0.997	0.998
Equation	Y=20944+1E+06	Y=14173+1E+06	Y=12217+2E+06	Y=22840+1E+06
LOD ($\mu\text{g/ml}$)	0.04	0.02	0.23	0.04
LOQ ($\mu\text{g/ml}$)	0.14	0.07	0.75	0.13

Table 2: Recovery studies

Compound	Labeled (μg)	Amount added			Recovery (%)
		80%	100%	120%	
Gliclazide	50	40	50	60	99.96
Glipizide	50	40	50	60	100.07
Glibenclamide	50	40	50	60	100.06
Glimepiride	50	40	50	60	99.97

Validation of the Developed Method

The proposed method was validated as per the guidelines in ICH for its linearity, accuracy, precision, specificity and selectivity, and robustness.

Linearity, limit of quantitation and limit of detection:

The linearity was tested for the concentration range of 40, 45, 50, 55, 60 $\mu\text{g/ml}$ and the calibration curve was constructed and evaluated by its correlation coefficient. The correlation coefficient (r^2) for all the calibration curves was consistently ≥ 0.997 .

The equations of linear regression were performed using least-squares method.

The limit of quantitation (LOQ) was the lowest concentration assayed where the signal/noise ratio was at least 10:1. The limit of detection (LOD) was defined as a signal/noise ratio of 3:1 (Table 1).

Accuracy and Precision

The accuracy of the proposed method was tested by recovery experiments by adding known amounts of each anti-diabetic drug corresponding to 80, 100 and 120% of the label claim from the respective standard solution. The accuracy was then calculated as the percentage of each anti-diabetic drug recovered by this assay (Table 2).

The precision of the proposed method was assayed by replicate injections of anti-diabetic drugs mixture of three different concentrations (40, 50 and 60 $\mu\text{g/ml}$), three times on three different days. The obtained intra-day and interday precision results are depicted in Table 3. The results indicated sufficient accuracy and precision of the developed method.

Specificity and selectivity

The method specificity was assessed by comparing the chromatograms obtained from a mixture of the drugs and the most commonly used excipients with those obtained

from blank (excipients solution in mobile phase without drug). The excipients such as silica, starch, talc, magnesium stearate, microcrystalline cellulose, polyvidone, carboxymethyl starch sodium, croscarmellose sodium, hypromellose and amylum pregelatinisatum were used to check the interference.

From Figure 2, it can be seen that the method was sufficiently specific to the analytes. The resolution factor for the drug peaks was found to be more than 2 from the nearest resolving peak for all the peaks and no interferences were found in the retention of drugs

The system suitability parameters like tailing factor and number of theoretical plates were also calculated. The system suitability parameters are given in Table 4.

Table 3: Precision Data (n = 3)

Compound	RSD%	
	Intra -day	Inter-day
Gliclazide	0.11	1.08
Glipizide	0.20	1.10
Glibenclamide	0.17	0.97
Glimepiride	0.19	1.02

Table 4: System suitability parameters

Parameter	Result			
	Gliclazide	Glipizide	Glibenclamide	Glimepiride
Retention time (min)	2.53	3.10	4.96	6.43
Theoretical plates (N)	3288	4487	5273	5787
Tailing factor	1.10	1.05	0.99	0.98

Robustness

The robustness was evaluated by deliberate variations of the method parameters. The factors selected to examine were flow rate (ml/min), pH of mobile phase, temperature (°C). One factor was changed at a time to estimate the effect. Flow rate variants: 1.4 and 1.6 ml/min, the pH of buffer variants: 3.4 ± 0.05 and 3.7 ± 0.05 , temperature variants: 34°C and 36°C. The retention time of the compound was evaluated, and the resolution had no significant changes when the parameters were changed.

Applications

The proposed method was applied for determination and assay some anti-diabetic drugs available in Syrian market. Twenty tablets of each drug were weighed and powdered. An accurately weighed portion of the powder equivalent to 5 mg of each drug was transferred into a 100 ml volumetric flask and dissolved in methanol. The content of the flask was sonicated for 15 min and the volume was made up to 100 ml and mixed well, the solution was filtered through a 0.45 µm membrane filter. This solution was injected into the column and the amounts of the drugs presented in the tablet dosage form were calculated. The results were shown in Table 5.

Table 5: Formulation Assay Results

Formulation	Labeled amount(mg)	Amount found (mg)	Assay (%w/w)
A (GLC)	80	79.95	99.94
B (GLP)	10	9.80	98.02
C (GLB)	5	4.97	99.31
D (GLM)	4	3.99	99.72

CONCLUSION

A simple and accurate method was developed for the separation and simultaneous determination of anti-diabetic drugs GLC, GLB, GLP and GLM. The proposed method has been validated by good linearity, precision, accuracy and robustness. The mobile phase was easy to prepare with little or no variation and was economical. The analysis time was found to be less than 10 min. The recovery from formulations was in good agreement and they suggested no interference in the estimation. Hence, this method can be easily and conveniently used for the routine quality control of the drugs in pharmaceutical dosage forms.

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