Research Article

RP-HPLC Method for the Quantification and *In-Vitro* Studies of Low Dose Oral Hypoglycemic Tablets

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ABSTRACT

A simple, Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the determination of several low dose hypoglycemic tablets: Glipizide, Glyburide, Glimepiride and Repaglinide for assay, content uniformity and *In-vitro* studies. Analysis was performed on C18 column (250 × 4.6 mm, 5µm) at temperature of 30°C, using mobile phase consisting of phosphate buffer pH 2.8 and acetonitrile (40:60) at a flow rate of 1.0 mL/min. The drugs were monitored at a wavelength of 230 nm for Glipizide, Glyburide and Glimepiride, and 245 nm for Repaglinide using PDA-UV detector. The method was validated according to the International Conference on Harmonization (ICH) guidelines and US Pharmacopeia (USP 35), and the results were found to be within the acceptable range. The developed method was also able to separate the four drugs. The developed method was found to be suitable for the routine analysis of all selected hypoglycemic agents as well as *In-vitro* studies.

Keywords: Glipizide, Glyburide, Glimepiride, Repaglinide, RP-HPLC, *In-vitro* studies.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both[1]. In some individuals with diabetes, adequate glycemic control can be achieved with weight reduction, exercise, and/or oral glucose-lowering agents.[2]

There are many oral hypoglycemic classes including Sulfonylurea, Biguanide, Meglitinide, Dipeptidylpeptidase-4 inhibitors and other classes. Glipizide, Glyburide and Glimepiride (Figures 1-a, b, c) are sulfonylurea hypoglycemics; and Repaglinide (Figures 1-d) is meglitinide hypoglycemic. Sulfonylurea act mainly by increasing endogenous insulin secretion; meglitinide also increase endogenous insulin secretion.[3]

The studied hypoglycemic agents are formulated in low dose tablets, 5 and 10 mg for Glipizide; 1.25, 2.5 and 5 mg for Glyburide; 1, 2 and 4 mg for Glimepiride; 0.5, 1 and 2 mg for Repaglinide. [4-7]

In vitro dissolution testing serves as an important tool for characterizing the quality of a product at different stages in its lifecycle. It is used to guide development of new formulations, to assess batch-to-batch quality and allow batch release[8-10]. It is also commonly used to predict the in vivo performance of a drug, and in certain instances a dissolution test can be used to waive a bioequivalence study.[11-12]

Literature survey reveals that a number of analytical methods have been reported for simultaneous determination of Glipizide, Glyburide, Glimepiride and Repaglinide in pharmaceutical dosage forms and in Human plasma[13-20] and for quantification of dissolved quantities in dissolution studies.[21-23]

The analysis of low-strength solid oral dosage forms poses a number of analytical challenges that can impact potency, purity and dissolution testing of the dosage form, where the low quantity of active ingredient in these dosage forms results in sample solutions with extremely low analyte concentrations that pose difficulties for detection and quantitation. Also the high excipient to drug ratio in low-dose drug products poses additional challenges such as difficulties extracting all the active ingredient, leading to irreproducible assay results, and impacting potency results by interferences from the excipient. In addition, there are two significant challenges in dissolution method development: detection sensitivity of selected analytical method and recovery of trace amounts of drug substance from the formulation matrix and testing system. [24]

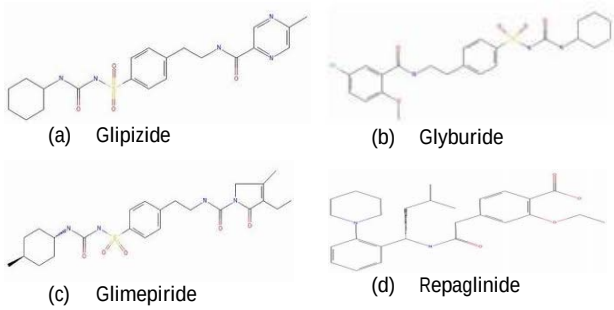

Figure 1: Chemical structure of **(a)** Glipizide, **(b)** Glyburide, **(c)** Glimepiride and **(d)** Repaglinide.

Due to the few number of analytical methods reported for determination of Glipizide, Glyburide, Glimepiride and Repaglinide in *In-vitro* studies, in addition to the importance of using one simple and fast method for the quality control of studied drugs without need to separate methods for the quantification in tablets and in dissolution studies for each drug; the aim of this study

was to develop and validate one precise method for the quantification and *In-vitro* studies of all targeted drugs: Glipizide, Glyburide, Glimepiride and Repaglinide in tablets, has ability to estimate the drugs in presence of high ratio of excipients with adequate sensitivity to determine low quantities faced in quantification and dissolution profile studies.

MATERIALS AND METHODS

Instrumentation

The analysis was performed using HPLC (LA Chrom ELITE, VWR Hitachi, Germany) equipped with L-2130 pump, L-2200 auto sampler, L-2300 column oven, and UV photo diode array detector L-2455 was used for analysis. Data acquisition was performed using EZ Chrom ELITE software. The analytical column used was Knauer, C18 (250 × 4.6 mm, i.d., 5 µm).

Materials

Glipizide, Glyburide, Glimepiride and Repaglinide purchased from SIGMA-ALDRICH®. HPLC grade Acetonitrile and Methanol used were purchased from Sham-Lab. Sodium dihydrogen phosphate and phosphoric acid were purchased from Merck Company Ltd. All the other reagents used were of analytical grade. Purified water was used throughout the analysis.

Chromatographic conditions

The column used was C18 column (250 × 4.6 mm, i.d., 5µm). The mobile phase consisted of a mixture of 40 volumes of sodium dihydrogen phosphate buffer (1 g/L, pH 2.8) and 60 volumes of acetonitrile, where the pH of buffer was adjusted with phosphoric acid 10%. The mobile phase was filtered through 0.45 µm filter and degassed in ultrasonic bath prior to use. Chromatography was performed at temperature of 30°C by pumping the mobile phase at a flow rate of 1.0 mL/min. The drugs were monitored at a wavelength of 230 nm for Glipizide, Glyburide and Glimepiride, and 245 nm for Repaglinide. Solutions were filtered with a 0.45 µm nylon syringe filter before injection.

Preparation of solutions

Preparation of standard stock solutions

Quantification in tablets: The standard stock solutions having concentration 800 µg/mL of Glipizide, Glimepiride and Repaglinide; and 1000 µg/mL of Glyburide were prepared separately in methanol.

In-vitro studies: The standard stock solutions 100 µg/ml of Glipizide, Glyburide, Glimepiride and Repaglinide were prepared separately in methanol, further diluted in dissolution media to obtain solutions containing 5 µg/mL of Glipizide and Glyburide, 2 µg/mL of Glimepiride and 1 µg/mL of Repaglinide.

Preparation of standard solutions

Quantification in tablets: The standard solutions were prepared by diluting the standard stock solutions in methanol to obtain solutions containing 80 µg/mL of each of Glipizide, Glimepiride and Repaglinide; and 100 µg/mL of Glyburide

In-vitro studies: The standard solutions were prepared by diluting the standard stock solutions in mobile phase to obtain solutions containing 2.5 µg/mL of Glipizide and Glyburide, 1 µg/mL of Glimepiride and 0.5 µg/mL of Repaglinide.

Preparation of sample solutions

Quantification in tablets: Twenty tablets of each drug were weighed and powdered individually. A suitable amount equivalent to the label claim of each drug was accurately weighed and transferred to volumetric flask. A suitable volume of methanol was added to the volumetric flask. The solution was sonicated for 30 min, made to volume with methanol and filtered.

In-vitro studies: The dissolution samples, resulted from applying dissolution test, were filtered and diluted with mobile phase to concentrations correspond to standard solutions.

Preparation of placebo solutions

Quantification in tablets: 200 mg of placebo containing the common excipients of tablets was accurately weighed and transferred into a 100 mL volumetric flask; methanol was added to the volumetric flask. The resulted solution was sonicated in an ultrasonic bath for 30 min, completed to volume with methanol and filtered.

In-vitro studies: 200 mg of placebo containing the common excipients of tablets was accurately weighed and transferred into a 1000 mL volumetric flask. 900 mL of dissolution media was added to the volumetric flask. The resulted solution was sonicated in an ultrasonic bath for 60 min and filtered.

Method validation

The current method was validated according to relevant ICH guideline and US Pharmacopoeia (USP 35)[25-27]. The validation was carried out for Glipizide, Glyburide, Glimepiride and Repaglinide separately. The following validation characteristics: system suitability, specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness were evaluated.

System Suitability

System suitability test parameters, including relative standard deviation RSD%, tailing factor T and theoretical plates´ number N, from five replicate injections of standard solutions were measured.

Specificity and Selectivity

Selectivity studies were performed by chromatographing the Standard solutions and Placebo solutions and comparing the chromatograms.

Accuracy

For quantification in tablets, Accuracy of the method was performed using the standard addition method, where specified aliquots of Standard stock solutions, corresponding to 80%, 100% and 120% of standard solution concentration of each drug, were added to pre-assayed sample solution. Solutions were prepared in triplicate, analyzed, and the differences between the results of the assays, before and after adding, were compared with the expected values. For *In-vitro* studies, Accuracy of the method was performed using Spiked – placebo recovery method, where aliquots of dissolution studies placebo solution were spiked with specified aliquots of stock standard solutions corresponding to 10%, 50% and 100% of standard solution concentration of each drug. Solutions were prepared in triplicate, analyzed, and the results obtained were compared with the expected results. The accuracy was calculated as the percentage of each drug recovered.

Precision

For quantification in tablets, the precision of the method was determined by preparing and assaying aliquots of a homogeneous sample of powdered tablets corresponding to 50%, 100% and 200% of standard solution concentration of each drug, in triplicate. The repeatability (Intra-day) was performed in same day. The Intermediate precision (Inter-day) was performed over 3 days on solutions prepared freshly on each day.

For *In-vitro* studies, the precision was determined by 6 replicate injections of the standard solution of each drug at 100% concentration in one day for the repeatability and on 3 days for the intermediate precision. The precision was measured by calculating the relative standard deviation (coefficient of variation) of resulted peaks areas.

Linearity and Range

The linearity of the method was demonstrated by measuring peak areas at five concentrations ranging from 50% to 200 % of standard solution concentration of each drug for quantification in tablets (50%, 80%, 100%, 120% and 200%) and from 10% to 100 % of standard solution concentration of each drug for dissolution studies (10%, 30%, 50%, 80% and 100%). The linearity solutions were prepared by appropriate dilution of standard stock solutions with the diluent (as mentioned in preparation of standard solutions). The calibration curves were constructed by plotting peak areas against concentrations of each drug. Linearity was described by the correlation coefficients.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were estimated by determining the signal to noise ratio from samples with known low concentrations of each drug.

LOD is the concentration that can be established at a signal to noise ratio of 3.

LOQ is the concentration that can be established at a signal to noise ratio of 10. LOQ of each drug was verified by six injections of each drug at its LOQ concentration.

Robustness

The robustness of the method was evaluated by making small changes in some method parameters including flow rate (0.8 and 1.2 mL/min), pH of buffer (2.6 and 3.0) and organic percentage of mobile phase (55% and 65% of acetonitrile); and measuring system suitability parameters from five replicate injections of standard solution of each drug under each condition.[28, 29]

RESULTS AND DISCUSSION

The developed method with the mentioned chromatographic conditions was able to estimate the content of the low dose drugs, Glipizide, Glyburide, Glimepiride and Repaglinide, in tablets and in dissolution tests of tablets in many dissolution media (1.2, 4.5, 6.8), that used in In-vitro bioequivalence studies. All studied drugs could be measured in short time, 10 min. In addition to that, the developed method was able to separate the above drugs with good resolution values, 16.60, 3.60 and 2.56 between consecutive peaks.

A chromatogram of standard mixture of Glipizide, Glyburide, Glimepiride and Repaglinide is shown in Figure 2.

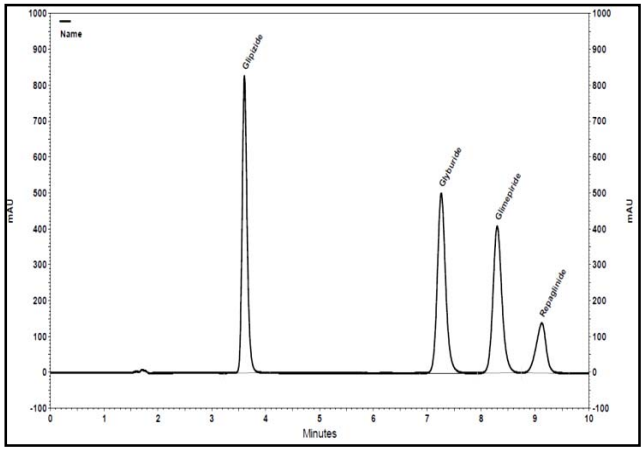

Figure 2: Chromatogram of standard mixture of Glipizide, Glyburide, Glimepiride and Repaglinide

Method validation

System suitability

The results of system suitability test were found to be within the acceptable limits[30] as presented in Table 1.

Specificity and Selectivity

The chromatograms of standard solutions and placebo solution of quantification in tablets are demonstrated in Figure 3, and the chromatograms of standard solutions and placebo solution of *In-vitro* studies are demonstrated in Figure 4.

Figure 3 and Figure 4 show the absence of interfering peaks at the retention time of each drug in the placebo chromatograms, which demonstrate selectivity.

Accuracy

The results of the recoveries are summarized in Table 2, which indicate that the method is accurate.

Precision

All RSD values were found to be less than 2%, indicating that this method is precise. The results of precision studies are summarized in Table 2.

Table 1: System suitability parameters (n=5) in Standard condition and in Robustness study

Set Name	Drug	RSD% of Area	RSD% of R.t.	Tailing factor	Theoretical plates
Acceptable limits		≤ 1	≤ 1	≤ 2	> 2000
Standard condition	**Glipizide**	0.07	0.11	1.17	8113
	Glyburide	0.08	0.20	1.10	10793
	Glimepiride	0.40	0.13	1.10	11794
	Repaglinide	0.24	0.32	1.01	9460
Flow rate 0.8 mL/min	**Glipizide**	0.18	0.11	1.20	9256
	Glyburide	0.22	0.37	1.10	11752
	Glimepiride	0.13	0.12	1.11	12935
	Repaglinide	0.12	0.38	0.83	10704
Flow rate 1.2 mL/min	**Glipizide**	0.37	0.21	1.16	7251
	Glyburide	0.11	0.10	1.09	9836
	Glimepiride	0.13	0.18	1.12	10286
	Repaglinide	0.18	0.15	0.87	8789
pH 2.6	**Glipizide**	0.94	0.08	1.18	8431
	Glyburide	0.23	0.06	1.09	11067
	Glimepiride	0.33	0.26	1.10	12168
	Repaglinide	0.20	0.97	0.92	9880
pH 3.0	**Glipizide**	0.14	0.13	1.19	8213
	Glyburide	0.10	0.21	1.11	10832
	Glimepiride	0.23	0.32	1.09	12082
	Repaglinide	0.38	0.30	0.89	12257
Acetonitrile 55% in Mobile phase	**Glipizide**	0.22	0.44	1.18	8962
	Glyburide	0.17	0.03	1.08	12125
	Glimepiride	0.58	0.27	1.10	13285
	Repaglinide	0.23	0.34	0.89	10251
Acetonitrile 65% in Mobile phase	**Glipizide**	0.12	0.19	1.23	7368
	Glyburide	0.16	0.58	1.12	9543
	Glimepiride	0.04	0.04	1.14	11298
	Repaglinide	0.05	0.32	0.85	9403

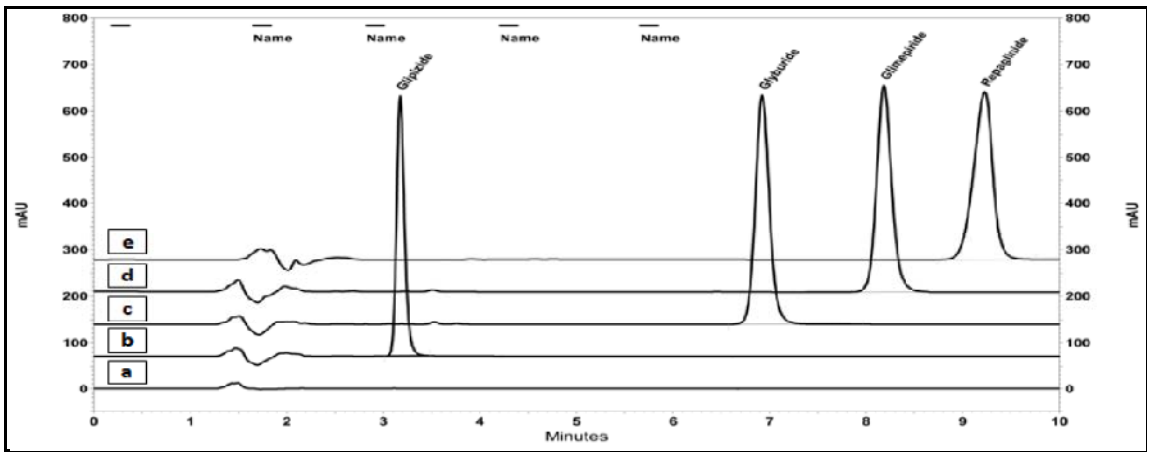

Figure 3: Chromatograms of quantification in tablets (a) placebo solution (b) Glipizide standard solution (c) Glyburide standard solution (d) Glimepiride standard solution (e) Repaglinide standard solution

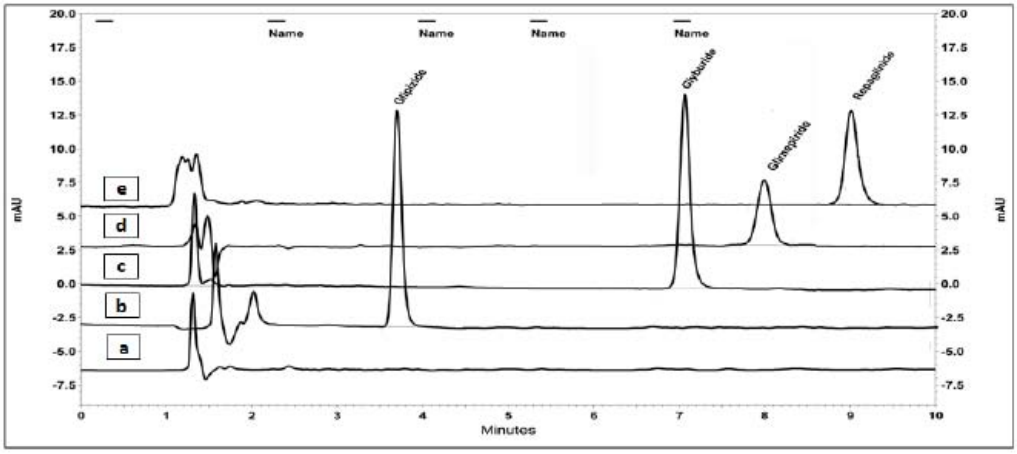

Figure 4: Chromatograms of *In-vitro* studies (a) placebo solution (b) Glipizide standard solution (c) Glyburide standard solution (d) Glimepiride standard solution (e) Repaglinide standard solution

Table 2: Summary of Accuracy and Precision studies result (* n=3, ** n=6)

		Accuracy (Recovery %)			Precision (RSD %)					
					Intra-day			Inter-day		
Quantification In tablets	Con. (%)	80*	100*	120*	50*	100*	200*	50*	100*	200*
	Glipizide	99.48	98.44	98.49	1.71	0.72	0.17	1.41	1.25	0.43
	Glyburide	99.93	99.26	100.02	1.02	1.05	0.96	1.28	1.13	1.24
	Glimepiride	100.67	101.80	101.61	0.52	0.36	0.47	1.22	0.70	0.76
	Repaglinide	100.58	99.46	100.64	0.74	0.78	0.12	0.78	0.71	0.54
In-vitro studies	Con. (%)	10*	50*	100*	100**			100**		
	Glipizide	101.32	101.27	99.82	0.27			0.71		
	Glyburide	100.15	100.87	100.12	0.21			1.60		
	Glimepiride	99.92	98.55	97.16	0.45			1.78		
	Repaglinide	97.86	100.99	97.32	0.16			1.70		

Table 3: Linearity parameters and LOD and LOQ Data

	Drug	Linearity range (µg/mL)	Correlation Coefficient (R2)	LOD (µg/mL)	LOQ (µg/mL)
Quantification Studies	Glipizide	40 – 160	0.9995	0.007	0.024
	Glyburide	50 – 200	0.9992	0.020	0.069
	Glimepiride	40 – 160	0.9995	0.014	0.048
	Repaglinide	40 – 160	0.9992	0.023	0.078
In-vitro studies	Glipizide	0.25-2.5	0.9989	0.017	0.055
	Glyburide	0.25-2.5	0.9993	0.021	0.070
	Glimepiride	0.1 - 1	0.9991	0.016	0.054
	Repaglinide	0.05 – 0.5	0.9990	0.016	0.053

Linearity and Range

Quantification in tablets: the response was found to be linear in the range of 40 – 160 µg/mL for Glipizide, Glimepiride and Repaglinide; and 50 - 200 µg/mL for Glyburide.

In-vitro studies: The response was found to be linear in the range of 0.25 – 2.5 µg/mL for Glipizide and Glyburide, 0.1 – 1 µg/mL for Glimepiride and 0.05 – 0.5 µg/mL for Repaglinide.

Table 3 lists the all linearity parameters of the calibration curves for each drug.

Limit of detection (LOD) and Limit of quantification (LOQ)

Table 3 lists LOD and LOQ values for quantification and *In-vitro* samples for each drug.

Robustness

In all the varied chromatographic conditions applied, the system suitability parameters were found to be within the acceptable limits as presented in Table 1.

CONCLUSION

A simple, accurate, precise, sensitive HPLC method has been developed and validated for the estimation of four oral hypoglycemic drugs, Glipizide, Glyburide, Glimepiride and Repaglinide, in their tablets and for the estimation of dissolution profiles in different media. The method has short chromatographic run time, less than 10 min. Therefore this method can be used as routine analysis in quality control laboratories and in *In-vitro* studies.

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