SIMULTANEOUS ESTIMATION OF REPAGLINIDE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

ABSTRACT

A highly sensitive isocratic reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Repaglinide and Metformin hydrochloride in Bulk drug and Pharmaceutical dosage forms. Separation of Repaglinide and Metformin hydrochloride successfully achieved on C_{18}, 250x4.6mm,5μ SS column or equivalent utilizing Acetonitrile: phosphate buffer (58:42)v/v as mobile phase at a flow rate of 1mL/min and the eluates was monitored at 230 nm. Chromatogram showed peak at a retention time of 2.053 ± 1 min and 5.537 ± 1 min. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ. Recovery of Repaglinide and Metformin hydrochloride were found to be in the range of 99.80% and 99.70% and showing linearity in the range of 0.1-0.6 μg / ml and 25-150 μg / ml. Proposed method can be successfully applied for the quantitative determination of Repaglinide and Metformin hydrochloride in Bulk drug and Pharmaceutical dosage form.

KEYWORDS: Repaglinide ,Metformin hydrochloride, RP-HPLC, Validation, Acetonitrile, and phosphate buffer.

INTRODUCTION

The combined use of metformin and repaglinide for type 2 diabetes mellitus was shown improved patient compliance by controlling the post prandial glucose levels and reaches normal glycemic levels1. Monotherapy with metformin, an oral anti diabetic agent is not sufficient to reach the target glycemic goals and multiple drugs may be necessary to achieve the basal glycemia. As per the biopharmaceutical classification system (BCS), metformin was belonged to class III, in terms it has high solubility in water and lower permeability to across the biological membranes, while the repaglinide belongs to class II. It has low solubility and higher permeability. The solubility profiles of both drugs can easily influence the chromatographic separations. Metformin showed single pKa value2 at 11.5 and repaglinide3 showed two pKa values at 4.19 and 5.78 due to the zwitterionic crew. Until this decade, this combination for liquid chromatographic separation was not published. Metformin is the good therapeutic agent for type II diabetes mellitus and HPLC techniques for it were reported alone and combination with sulfonylureas3, improvement of patient's compliance is more for combination of metformin and repaglinide rather than with sulfonylureas4, these combinations are commercially available as tablet dosage forms. The HPLC estimation method for metformin in human plasma5, ion-pair6, and in microspheres and tablet dosage forms7 were previously reported. Spectrophotometric study of metformin and repaglinide8and combination9of rosiglitazone and metformin were reported. The combination of oral anti-diabetic agents depends on patient's clinical manifestations. Most of the doctors will choose metformin as the first choice of drug for the treatment of type II diabetes mellitus. Depend on clinical characteristics of the patients; failure monotherapy can switch to a combination of various anti diabetic agents. Adding of such agents to metformin, adequate controls the basal glycemia and post prandial glucose levels.
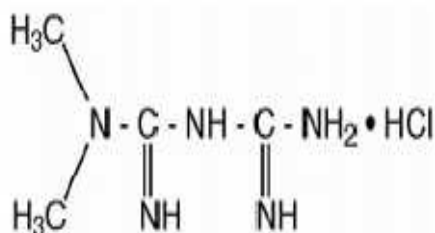
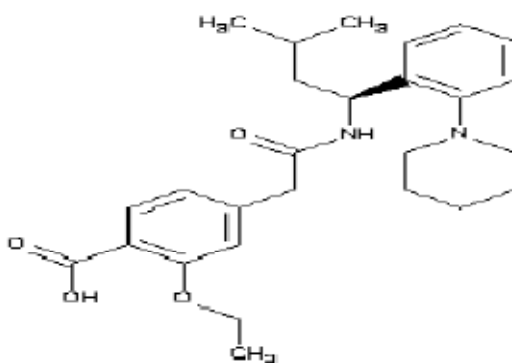**Fig 1: Chemical structure of Metformin**

Fig 2: Chemical structure of Repaglinide

EXPERIMENTAL:
Chemicals and solvents:

HPLC grade Acetonitrile (merck), HPLC grade sodium dihydrogen phosphate (merck) was used for mobile phase preparation. Pure samples of Metformin and repaglinide was a gift sample from a local pharmaceutical industry. Commercial samples of tablets containing the drugs Metformin and Repaglinide were purchased from the local pharmacy.

Instrument/Equipment details:

LC-2010 High Performance Liquid Chromatography with auto sampler and UV detector and Auto injector mode was used with shimadzu software, Analytical Balance (unibloc), UV Spectrophotometer (UV2550), Ph meter (eutech).

Chromatographic conditions:

Chromatographic separations were achieved by C_{18}, 250x4.6mm,5μ SS column or equivalent utilizing Acetonitrile: phosphate buffer (58:42)v/v as mobile phase at a flow rate of 1mL/min and the eluates was monitored at 230 nm.

Preparation of mobile phase:

Preparation of Buffer:

0.01mole of sodium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

Preparation of mobile phase:

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 58:42 and filter through 0.45 micron membrane filter.

Preparation of standard stock solution

An accurately weighed quantity of 500 mg of Metformin HCl and 2 mg of Repaglinide was transferred into 100 ml volumetric flask, dissolve in about 15 ml of mobile phase sonicate about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase. The concentrations of Metformin HCl and Repaglinide were found to be 5000 μg/ml and 20 μg/ml.

Preparation of sample solution

Weigh about 20 tablets and powdered. From that an equivalent amount of 500 mg of Metformin HCl and 1 mg of Propranolol hydrochloride was taken into 100 ml volumetric flask. Add about 10 ml of mobile phase and sonicate until the content was dissolved. Filter the content by using 0.45μ membrane filter by applying vacuum. Made the volume up to the mark with the mobile phase.
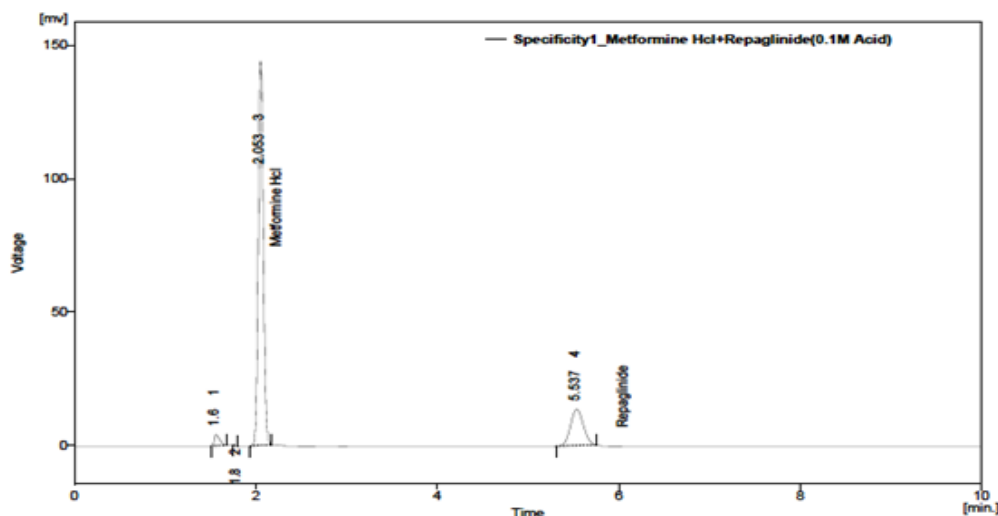

Figure: 3 Typical Chromatogram of Metformin (2.053min) and Repaglinide (5.537min)

Linearity:

Adequate dilutions were made from stock solution to get concentration ranging from 25-250 μg/ml for Metformin hydrochloride and 0.1- 0.6 μg/ml for Repaglinide. Evaluation was performed with UV detector at 230 nm and Peak area was recorded for all the peaks and a Calibration graph was obtained by plotting peak area versus concentration of Metformin hydrochloride (Fig 4 A), and Repaglinide (Fig 4 B). The plot of peak area of each sample against respective concentration was found to be linear in the range of 25-150 μg/ml for Metformin hydro chloride and 0.1-0.6 μg/ml for Repaglinide with correlation coefficient of 0.9995 for Metformin hydro chloride and 0.999 for Repaglinide.
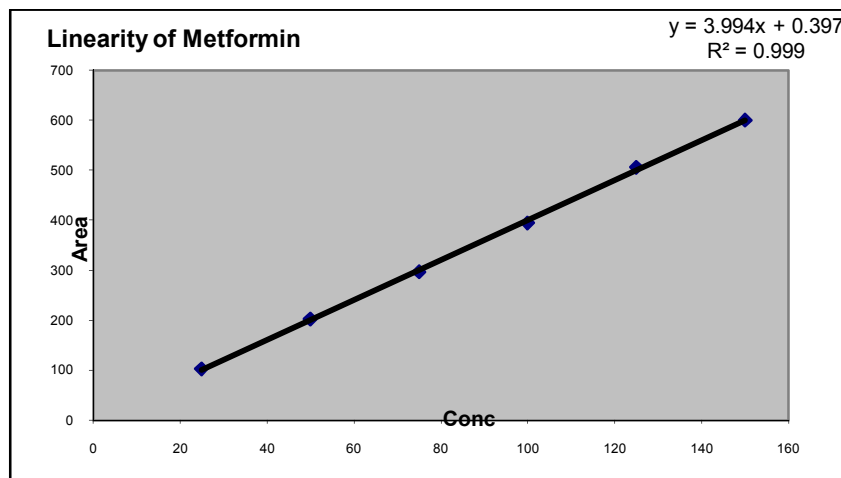

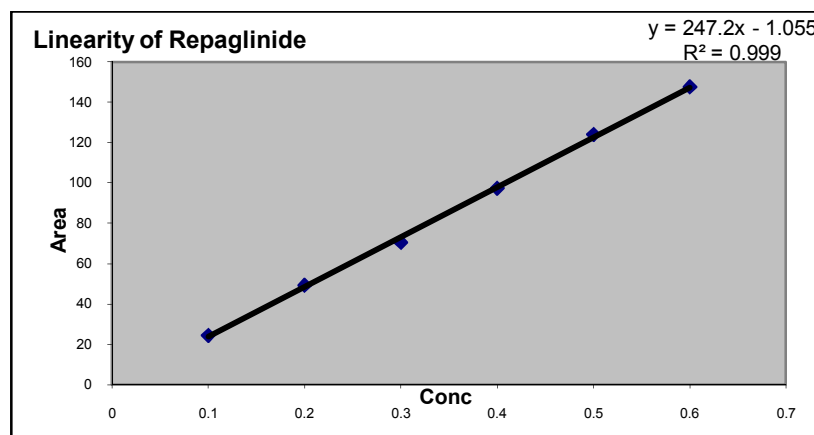**Figure 4 A: Calibration curve of Metformin hydrochloride by HPLC**

Figure 4 B: Calibration curve of Repaglinide by HPLC

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RESULTS AND DISCUSSION:

As per the USP-XXVI system suitability tests were carried out on freshly prepared standard stock solution of Metformin hydrochloride and Repaglinide. Parameters that were studied to evaluate the suitability of the system are given in Table 1. These parameters indicate good sensitivity, more ruggedness and robustness of the method.

From the typical chromatogram of Metformin hydrochloride and Repaglinide as shown in fig 3, it was found that the retention times 2.053mins for metformin hydrochloride and 5.537mins for repaglinide. Acetonitrile and buffer in a ratio 58:42v/v as mobile phase was found to be most suitable mobile phase combination to obtain well defined peaks with sharp peak shapes, high theoretical plates and less tailing. In the present developed HPLC method, the standard and sample preparation involve very simple extraction procedure and required very less time. A good linear relationship (r=0.9995 and 0.999) was observed for metformin hydrochloride and repaglinide in the concentration range of 25-150 μg/ml and 0.1-0.6 μg/ml respectively. The percentage assay was found to be 99.97% for metformin hydrochloride and 99.46 % for repaglinide in tablets. Recovery studies shows good extraction and recovery from 50% to 150% of test concentration. It was found percentage recovery was about 99.80% for metformin hydro chloride and 99.70 % for repaglinide indicates good extraction and good recovery and accuracy of the method. There is no additional peaks in the chromatogram at the main peak Retention times indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive, rugged and reproducible.

CONCLUSION:

A method was developed for the simultaneous estimation of Metformin hyrochloride and Repaglinide in bulk and pharmaceutical dosage forms which is simple, quick, reliable, inexpensive and simple. The results indicate that the described method can be used for quantitative analysis of the compound.

ACKNOWLEDGEMENTS:

S.NO.	PARAMETERS			RESULT		LIMIT
				Metformin Hcl	Repaglinide	
1.	System Precision (n=6)	% RSD		0.88	0.76	NMT-2.0%
2.	Method Precision (n=6)	% RSD		0.49	0.64	NMT-2.0%
3.	Assay	% Mean Assay		99.97%	99.46%	95-105%
4.	Accuracy	% Recovery	50 %	99.33%	99.16%	% Recovery 98-102
			100 %	99.89%%	99.88%	
			150 %	99.89%	101.38%	
5.	Robustness	% RSD	Flow -	1.01	0.86	% RSD NMT 2.0.
			Flow +	1.15	0.96	
			Organic -	0.95	0.66	
			Organic+	0.85	0.86	
6.	Ruggedness	Day-1, Analyst-1		0.811	0.85	% RSD NMT 2.0.
		Day-2, Analyst-2		0.811	0.81	

TABLE: 1 VALIDATION PARAMETERS OF THE PROPOSED METHOD FOR METFORMIN AND REPAGLIIDE

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