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RP- HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND REPAGLINIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A highly sensitive isocratic reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Metformin and Repaglinide in Bulk and tablet dosage forms. Separation of metformin and Repaglinide successfully achieved on Hypersil, C18, v size 250mm 4.6mm column or equivalent utilizing methanol buffer pH 6.0 with OPA (40:60)v/v as mobile phase at a flow rate of 1.0ml/min and the eluates was monitored at 242 nm. Chromatogram showed peak at a retention time of 3.60 min and 2.60 min. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ. Recovery of metformin and Repaglinide were found to be in the range of 99.76% and 100.2833% and showing linearity in the range of 100-1000µg, ml and 0.5-2 µg, ml. Proposed method can be successfully applied for the quantitative determination of metformin and Repaglinide in Bulk and tablet dosage form.

KEYWORDS

Metformin, Repaglinide, RP-HPLC, Validation, Methanol and Buffer pH 6.0 with OPA.

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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 levels[1]. Mono therapy 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 value[2] at 11.5 and repaglinide[3] 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 sulfonylureas[3], improvement of patient's compliance is more for combination of metformin and Repaglinide rather than with sulfonylureas[4], these combinations are commercially available as tablet dosage forms. The HPLC estimation method for metformin in human plasma[5], ion-pair[6], and in microspheres and tablet dosage forms[7] were previously reported. Spectrophotometric study of metformin and repaglinide[8] and combination[9] of 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 mono therapy 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.

Experimental

Chemicals and solvents: A.R. grade methanol loba chem., Mumbai, L.R. Grade sodium dihydrogen SD fine-chem. ltd, Mumbai, HPLC grade Acetonitrile, ortho Phosphoric Acid (OPA) buffer was used for mobile phase preparation. Pure samples of Metformin and Repaglinide was a gift sample from Hipo Labs Private Limited India. Commercial samples of tablets containing the drugs Metformin and Repaglinide were purchased from the local pharmacy.

Instrument/Equipment details

High Performance Liquid Chromatography (1575) Hitachi, UV-Visible double beam spectrophotometer (UV1800) Elico, Ultra sonicator Entrech electronics limited, melting point apparatus.

Chromatographic conditions

Chromatographic separations were achieved by Hypersil, C18, v size 250mm* 4.6mmϕ column, or equivalent utilizing methanol : buffer pH 6.0 with OPA (40:60) v/v as mobile phase at a flow rate of 1.0 ml/ min and the eluates was monitored at 242 nm, run time 10 min.

Preparation of mobile phase

Preparation of Buffer: 0.01mole of sodium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-6.0 with using diluted O-phosphoric acid (OPA).

Preparation of mobile phase

Filtered and degassed mixture of methanol: Buffer PH 6.0 with OPA (40:60) mobile phase was filter through 0.45µm membrane filter.

Preparation of standard stock solution

An accurately weighed quantity of 10 mg of Metformin and 10 mg of Repaglinide was transferred into 100 ml volumetric flask, about 10 ml of HPLC grade methanol was added and sonicated to dissolve. Until all the content has been dissolved, then the volume was made up to the mark with mobile phase. The concentrations of Metformin and Repaglinide were found to be 5000 µg/ml and1µg/ml.

Preparation of sample solution

Accurately weighed 10 mg of Metformin and 10 mg of Repaglinide were transferred to two different10 ml volumetric flask. About 4 ml of mobile phase was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 5 ml of metformin and 0.01 ml of Repaglinide were diluted to 10 ml with the solvent system. The resultant solution was filtered through a 0.45 µm membrane filter by app lying vacuum. Made the volume up to the mark with the mobile phase.

Linearity

Adequate dilutions were made from stock solution to get concentration ranging from 100-1000 µg/ml for Metformin and 0.5-2 µg/ml for Repaglinide. Evaluation was performed with UV detector at 242 nm and Peak area was recorded for all the peaks and a Calibration graph was obtained by plotting peak area

versus concentration of Metformin (Figure No.4), and Repaglinide (Figure No.5). The plot of peak area of each sample against respective concentration was found to be linear in the range of 100-1000µg/ml for Metformin 0.5-2 µg/ml for Repaglinide with correlation coefficient of 0.999 for Metformin and 0.999 for Repaglinide.

RESULTS AND DISCUSSION

As per the USP-XXVI system suitability tests were carried out on freshly prepared standard stock solution of Metformin and Repaglinide. Parameters that were studied to evaluate the suitability of the system are given in Table No.1, 8. These parameters indicate good sensitivity, more ruggedness and robustness of the method. From the typical chromatogram of Metformin and Repaglinide as shown in figure No.3. It was found that the retention times 3.60mins for metformin and 2.60mins for Repaglinide. Methanol and buffer pH 6.0 with OPA in a ratio 40:60v/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=0.999 and 0.999) was observed for metformin and Repaglinide in the concentration range of 100-1000µg/ml and 0.5-2 µg/ml respectively. The percentage assay was found to be 100.007% for metformin and 99.80% for Repaglinide in tablets. Recovery studies shows good extraction and recovery from 80% to 100% of test concentration. It was found percentage recovery was about 99.76% for metformin and 100.2833 % 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 RP- HPLC method is simple, linear, accurate, sensitive, rugged and reproducible.

Table No.1: Accuracy Readings Accuracy of Metformin

S.No	Sample ID	Concentration (µg/ml)		% Recovery of Pure drug	Statistical Analysis
		Pure drug	Formulation		
1	S_1 : 80 %	400	500	99.13	Mean= 98.94667%
2	S_2 : 80 %	400	500	98.79	S.D. = 0.171561
3	S_3 : 80 %	400	500	98.92	% R.S.D.= 0.1733
4	S_4 : 100 %	500	500	99.72	Mean= 99.76%
5	S_5 : 100 %	500	500	99.81	S.D. = 0.045826
6	S_6 : 100 %	500	500	99.75	% R.S.D.= 0.0459
7	S_7 : 120 %	600	500	99.36	Mean= 99.37667%
8	S_8 : 120 %	600	500	99.28	S.D. = 0.105987
9	S_9 : 120 %	600	500	99.49	% R.S.D. = 0.1066

Table No.2: Data of Recovery Studies of Repaglinide

S.No	Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis
		Pure drug	Formulation	Pure drug	
1	S_1 : 80 %	0.8	1	101.3	Mean= 100.2833%
2	S_2 : 80 %	0.8	1	99.25	S.D. = 1.025004
3	S_3 : 80 %	0.8	1	100.27	% R.S.D.= 1.02221
4	S_4 : 100 %	1	1	99.14	Mean= 99.18%
5	S_5 : 100 %	1	1	99.29	S.D. = 0.096437
6	S_6 : 100 %	1	1	99.11	% R.S.D.= 0.097234
7	S_7 : 120 %	1.20	1	99.21	Mean= 99.46%
8	S_8 : 120 %	1.20	1	99.54	S.D. = 0.221133
9	S_9 : 120 %	1.20	1	99.63	% R.S.D.= 0.222334

Table No.3: Data Showing Repeatability Analysis

S.No	HPLC Injection Replicates	AUC for Metformin	AUC for Repaglinide
1	Replicate – 1	1121057	26472
2	Replicate – 2	1124587	26984
3	Replicate – 3	1145896	26984
4	Replicate – 4	1123574	26478
5	Replicate – 5	1123498	26474
6	Average	1127722	26678.4
7	Standard Deviation	10241.98	278.9817
8	% RSD	0.908201	1.045721

Table No.4: Results of Intra-assay and Inter-assay Repaglinide

S.No	Concentration of Repaglinide (µg/ml)	Observed Concentration Of Repaglinide (µg/ml) by the proposed Method			
		Intra-Day		Inter-Day	
		Mean (n=6)	% RSD	Mean (n=6)	% RSD
1	0.8	0.901	1.05	0.897	0.46
2	1	1.29	0.51	1.25	0.28
3	1.20	1.41	0.19	1.4009	0.15

Table No.5: Results of Intra-assay and Inter-assay for Metformin

S.No	Concentration of Metformin (µg/ml)	Observed Concentration Of Metformin (µg/ml) by the proposed Method			
		Intra-Day		Inter-Day	
		Mean (n=6)	% RSD	Mean (n=6)	% RSD
1	400	400.01	0.86	400.03	0.87
2	500	500.02	0.30	500.03	0.32
3	600	599.97	0.13	599.95	0.11

Table No.6: Data of System Suitability Parameter

S.No	Parameter	Limit	Result
1	Resolution	Rs > 2	3.56
2	Asymmetry	T ≤ 2	Repaglinide =0.9, Metformin =1.2
3	Theoretical plate	N > 2000	Repaglinide =2898, Metformin= 4568

Table No.7: Assay of Marketed Formulation

S.No	Brand name of tablets	Labelled amount of Drug (mg) Metformin and Repaglinide	Mean (±SD) amount (mg) found by the proposed Method (n=6)	Mean (± SD) Assay (n = 6)
1	Reglide-Plus (Grownbury Pharmaceuticals Pvt. Ltd)	500 and 1	500.05 (±0.05), 0.998 (±0.08)	100.007 (±0.59) 99.8 (±0.81)

Table No.8: Validation Parameters Results

S.No	Parameters	Metformin	Repaglinide
1	Calibration range(mcg/ml)	100-1000	0.5– 2
2	Optimized wavelength	242	242
3	Mobile phase (Acetonitrile: Buffer)	40:60	40:60
4	Column	Hypersil, C-18, V size(250mm*4.6mm (150 × 4.6 mm i.d 5mm)	Hypersil,C-18,V size(250mm*4.6mm) (150 × 4.6 mm i.d 5mm)
5	Retention time	3.60	2.60
6	Regression equation(Y*)	y = 2446x -35666	y = 27536x -899.9
7	Correlation coefficient(r^2)	0.999	0.999
8	Precision (% RSD*)	0.908201	1.045721
9	% Recovery	99.76%	100.2833%
10	LOD(mcg/ml)	2.081	0.377
11	LOQ(mcg/ml)	6.307	1.143

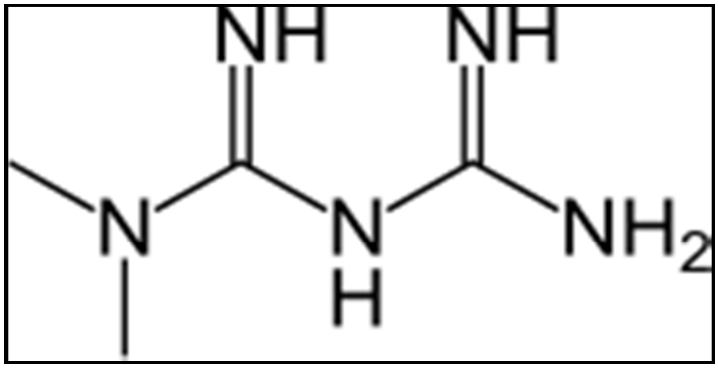

Figure No.1: Structure of Metformin

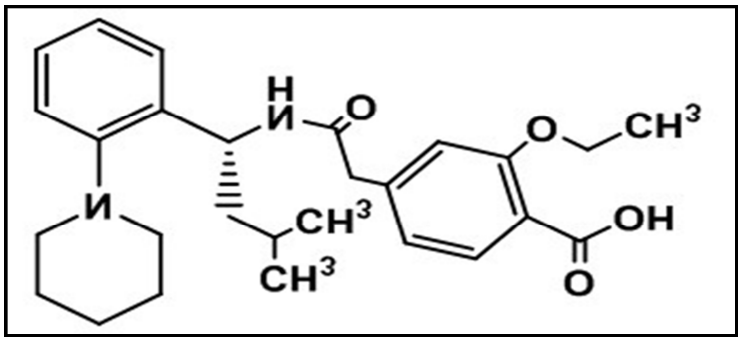

Figure No.2: Structure of Repaglinide

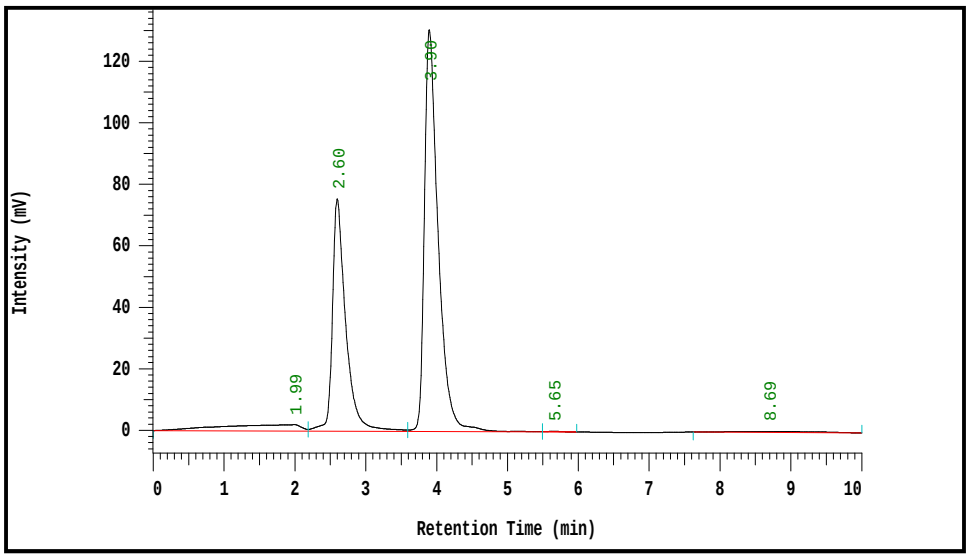

Figure No.3: Chromatogram for Standard Repaglinide (2.60), Metformin (3.60)

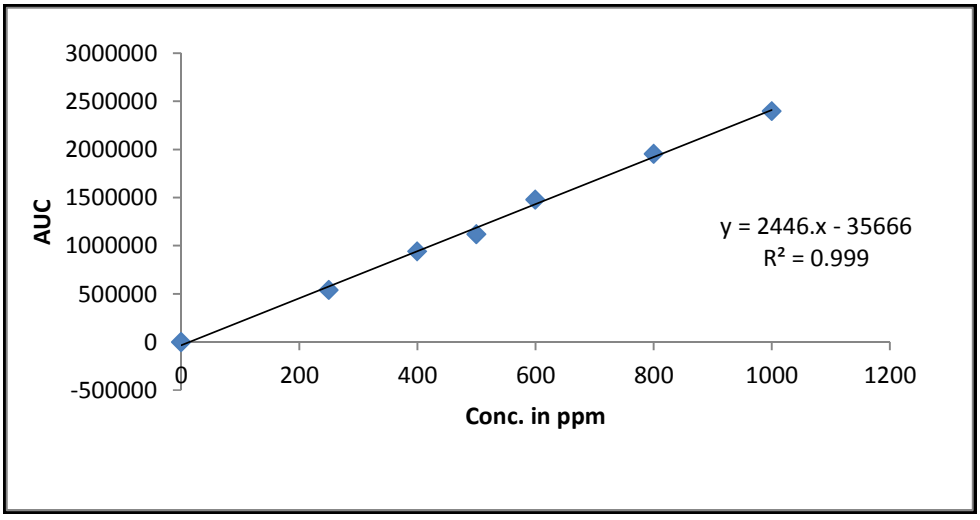

Figure No.4: Calibration Curve for Metformin

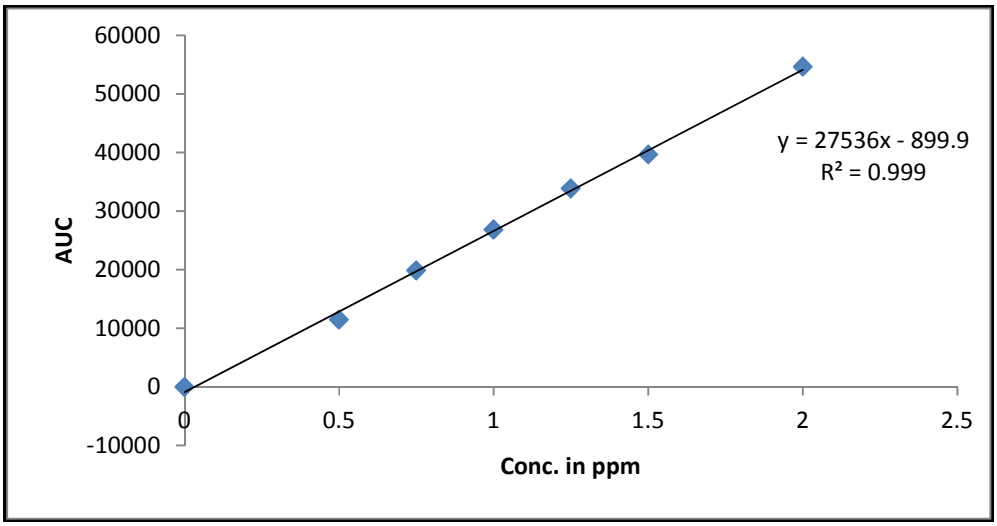

Figure No.5: Calibration Curve for Repaglinide

CONCLUSION

A method was developed for the simultaneous estimation of Metformin and Repaglinide in bulk and tablet 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.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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