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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND SITAGLIPTIN IN TABLET DOSAGE FORMS

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ABSTRACT

A new simple and precise reverse phase high performance liquid chromatographic method has been developed and subsequently validated for the simultaneous estimation of Metformin and Sitagliptin in combination. The chromatographic separation was performed in Waters equipment using mobile phase consisting of Potassium dihydrogen orthophosphate: Methanol in the ratio of 50:50 and the pH -4 adjusted by orthophosphoric acid. The column used was Hypersil BDS C 18, 5µ, 150mm x 4.6 mm internal diameter with flow rate of 1 ml/min using PDA detection at 260 nm. The retention time was found to be 1.773 min for Metformin and 3.696 min for Sitagliptin .The described method was found to be linear and correlation coefficient was 0.999. Results of analysis were validated statistically and by recovery studies. Precision were performed as per ICH guidelines with the result shows relative standard deviation not more than 2%. The assay value for Metformin and Sitagliptin were found to be 99.89% and 99.94% respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, reliable, accurate and economical which is useful for the routine determination of Metformin and Sitagliptin bulk drug and in its pharmaceutical dosage form.

Keywords: Metformin, Sitagliptin, High performance liquid chromatography, Validation, Simultaneous estimation.

INTRODUCTION

Metformin belongs to the bi-guanide class of anti-diabetic drug which is extensively used in the treatment of type II diabetes mellitus. Metformin is N, N dimethylimidodicarbonimidic diamide. The oral antihyperglycemic effect of Metformin are not only due to the inhibition of intestinal glucose absorption and the improvement of peripheral and hepatic insulin sensitivity but also the reduction of hepatic glucose production and the enhancement of peripheral glucose utilization. Metformin is white to off-white crystalline powder and hygroscopic. Freely soluble in water, slightly soluble in ethanol (95%), practically insoluble in acetone, ether, chloroform [1].

Sitagliptin is (R)-4-oxo-4-[3-(trifluromethyl)-5,6-dihydro [1,2,4] triazolo[4,3-a] pyrazin -7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine. Sitagliptin blocks dipeptidyl peptidase-4 (DPP-4) activity. Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion. Sitagliptin is white to off-white powder. Soluble in water and N, N –dimethyl formamide, slightly soluble in methanol, insoluble in isopropanol.

Figure 1. Structure of Metformin

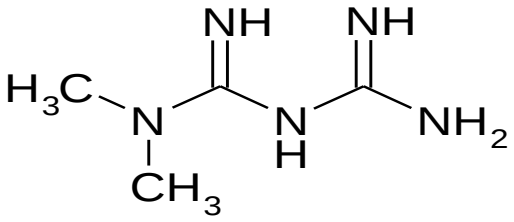

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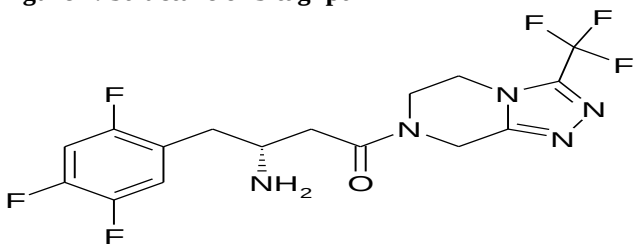
Figure 2. Structure of Sitagliptin



The literature research reveals UV, HPTLC methods for the analysis of Metformin as single and combined dosage forms with other drugs. UV, HPLC methods for analysis of Sitagliptin as single component systems. There are no reported HPLC methods for analysis of both drugs in combination. This paper presents simple, rapid, accurate, reliable and economical methods for simultaneous analysis of Metformin and Sitagliptin in tablet dosage form with very short run time and can be used for routine analysis. This method was validated as per ICH guidelines [2].

MATERIALS AND METHODS

Chemicals and Reagents

Methanol of HPLC grade was purchased from MERCK. Potassium dihydrogen orthophosphate and Trimethylamine were purchased from SD fine chem., Mumbai, India. Standard gift samples of Metformin and Sitagliptin was provided by Spectrum labs, Hyderabad. Marketed formulation Janumet tablet containing Metformin HCL 500mg and Sitagliptin phosphate 50 mg was used as sample, purchased from local pharmacy Hyderabad.

System used

The HPLC system used was Waters 2695 equipped with Photo Diode Array 2996 detector. The chromatograms were recorded and peaks are quantified by using PC based alliance empower software. Other different kinds of equipments like analytical weighing balance, ultrasonicator, pH meter, mobile phase reservoir, glasswares are used throughout the work.

Preparation of buffer

2.87 g of potassium dihydrogen phosphate in 1000 ml beaker and add upto mark with HPLC grade water and make upto pH 4 with orthophosphoric acid.

Diluent Preparation

Water is used as diluent

Mobile phase preparation

500 ml buffer with 500 ml of methanol (50:50). The mobile phase is subjected for sonication by means of ultrasonicator.

Preparation of standard stock solution

An accurately weighed quantity of 100 mg of Metformin and 10 mg of Sitagliptin were transferred into a 50ml volumetric flask. Dissolve with mobile phase and sonicated for 15 min.

Preparation of Standard solution

From the standard stock solution 1 ml was pipetted out into a 20 ml volumetric flask and made upto volume with mobile phase and having the concentration of 0.1mg/ml of Metformin and 0.01mg/ml of Sitagliptin.

Preparation of sample solution

Ten tablets were weighed and ground to a fine powder. Powder equivalent to 100mg of Metformin was weighed and then transferred to a 50 ml volumetric flask and made up with mobile phase, sonicated for about 10 min, filtered. 1 ml of filtrate transferred into 20 ml volumetric flask and made up with mobile phase. The concentration is 100µg/ml and10 µg/ml for Metformin and Sitagliptin respectively.

Chromatographic conditions

Separation was carried using Hypersil BDS C_{18} (150 x 4.6 mm, 5 µ) column at ambient temperature on a reverse phase technique. Mobile phase is phosphate buffer: methanol in the ratio of 50:50. Flow rate is 1 ml/ min and wavelength used is 260nm. The volume of injection is 20 µl. Mode of elution is isocratic and the runtime is 6 min [3,4].

METHOD VALIDATION

The method was validated in terms of the following parameters; linearity, specificity, accuracy, precision, and system suitability parameters as per the ICH guidelines [5-7].

Accuracy

A known amount of pure drug at three different levels i.e. 50%, 100%, and 150% was added to preanalyzed sample solutions and total concentration was determined by the proposed HPLC method. Results of the recovery studies are tabulated in Table no. 1

Precision

Precision was performed with six replicates of standard stock solution and the peak area was considered. The results are tabulated in Table no.2

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability during normal usage. There was no change in system suitability parameters. The result of

robustness studies along with its different parameters are tabulated in Table no.3

Ruggedness

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory, and from analyst to analyst. There was no marked difference obtained in results. The results are tabulated in Table no .4

Linearity

Linearity was performed with five different concentrations of a solution which were prepared and injected. The linearity range for Metformin 50-150 µg/ml and for Sitagliptin 5-15µg/ml. The correlation co-efficient was found to be 0.999 for both Metformin and Sitagliptin. The results are tabulated in Table no.5 and linearity graph were shown in Figure in 4 and 5.

System suitability

The peak resolution, theoretical plates, tailing factor, peak symmetry were calculated for the standard solutions. Results shown in Table no. 7. The results obtained indicate the suitability of the system for the analysis of the drug combination and the system suitability parameters are within the range during method. The system suitability report tabulated in Table no.6

Assay

The assay was performed on the prepared standard and sample which were injected into the HPLC system. Results of assay are tabulated in Table no. 8

RESULTS AND DISCUSSIONS

The proposed method was developed and validated as per the ICH guidelines. An RP-HPLC method for simultaneous estimation of Metformin and Sitagliptin was developed and validated. The results obtained indicate that the proposed method is simple, rapid, accurate, selective, economical and reproducible. Linearity was observed over a concentration range of 50 to 150µg/ml for Metformin and 5 to 15 µg/ml for Sitagliptin. System suitability parameters are satisfactory and resolution was >2. The theoretical plates are > 2000. Tailing factor <2. %RSD not more than 2%. The assay value for Metformin and Sitagliptin were found to be 99.89% and 99.94% respectively. The low RSD value confirms the robustness of the method and there are no marked changes in the results of the ruggedness.

Table 1. Results for Accuracy

Levels of recovery	Amount present (µg/ml)		Added conc. (µg/ml)		Total amount recovered (µg/ml)		% recovery	
DRUG	MET	SIT	MET	SIT	MET	SIT	MET	SIT
50	100	10	50	5	149.91	14.89	99.94	99.26
100	100	10	100	10	200.18	19.96	100.09	99.80
150	100	10	150	15	249.69	25.09	99.87	100.36

Table 2. Results for Precision

	Metformin		Sitagliptin	
S.No	Retention time	Peak area	Retention time	Peak area
1	1.771	360396	3.691	221286
2	1.771	362197	3.690	222649
3	1.766	358848	3.682	223784
4	1.773	360411	3.696	224337
5	1.768	360368	3.686	222996
6	1.766	358884	3.681	223783
Average	1.769	360184	3.687	223783
%RSD	0.1654	0.3435	0.15625	0.489

Table 3. Results for Robustness

Effect	Retention time of Metformin	Retention time of Sitagliptin
Temp (23^0c)	1.792	3.798
Temp plus (30^0c)	1.753	3.349
Flow plus(1.1ml/min)	1.759	3.675
Flow minus(0.9ml/min)	2.204	4.758

Table 4. Results for Ruggedness

Analyst	Area of Metformin	Area of Sitagliptin
Analyst 1	361588	221878
Analyst 2	359015	221152

Table 5. Results for Linearity

Concentration of Metformin (µg/ml)	Peak area of Metformin	Concentration of Sitagliptin (µg/ml)	Peak area of Sitagliptin
50	176988	5	109514
70	256255	7	156625
100	365796	10	219268
120	435902	12	262543
150	536678	15	321935

Figure 3. Linearity for Metformin

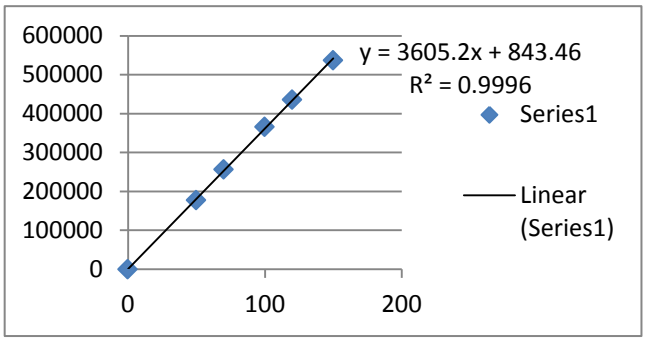


Figure 4. Linearity for Sitagliptin

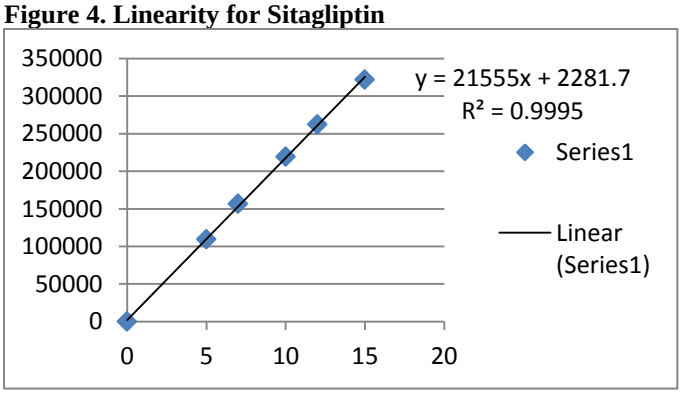


Figure 5. Chromatogram of Standard

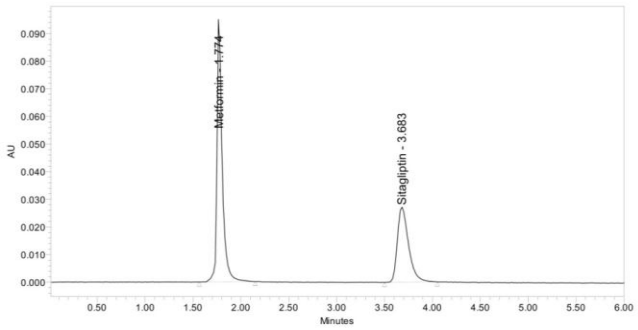


Figure 6. Chromatogram of Sample

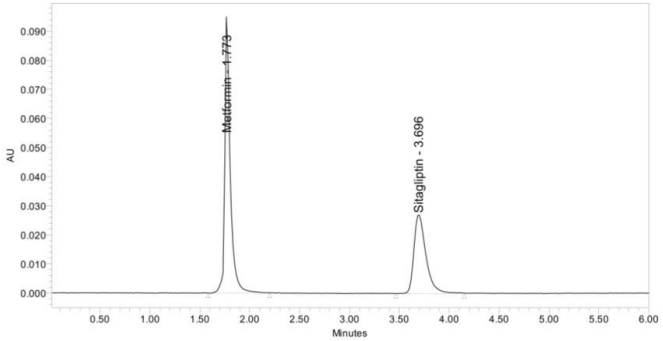


Table 6. System suitability report

	Metformin		Sitagliptin	
S.No	Retention time	Peak area	Retention time	Peak area
1	1.774	360415	3.691	221268
2	1.771	362719	3.683	224347
3	1.769	365488	3.689	222969
4	1.772	360369	3.686	223874
5	1.765	360444	3.693	224337
Avg	1.770	361887	3.688	223359
%RSD	0.1936	0.6211	0.1084	0.3737

Table 7. System suitability parameters

Parameters	Metformin	Sitagliptin
Linearity range	50-150 µg/ml	5-15µg/ml
Correlation coefficient	0.999	0.999
Slope(m)	3605.0	21555
Intercept	843.4	2281
Theoretical plates	5357	4972
Tailing factor	1.33	1.42
Retention time	1.771	3.691

Table 8. Results for Assay

Drug	Label claim	% assay	Amount present
Metformin	500 mg	99.89	501.4
Sitagliptin	50 mg	99.94	50.35

CONCLUSION

The developed RP-HPLC method for simultaneous determination of Metformin and Sitagliptin can be used for the routine analysis of both the components in single or in combined dosage forms.

REFERENCES

1. Anonymous. www.drugs.com
2. Gurudeep Chatwal and Sham Anand. Instrumental methods of chemical analysis. Himalaya publishers, 7th edition, 1992, Pg. no 2.624-2.639.
3. Skoog et al. Principles of Instrumental Analysis. Barkhanath publishers, 8th edition, Pg. no. 973-995.
4. Hobart H.Willard et al. Instrumental methods of analysis, CBS Publications and Distributors, New Delhi, 1st edition ,1986, Pg. no 529-563.
5. Malleswararao SN et al. Simultaneous determination of sitagliptin phosphate monohydrate and metformin hydrochloride in tablets by a validated UPLC method. *Sci Pharm*, 80, 2012, 139–152.
6. Sheetal Sharma, Ankur Kothari, Harsoliya MS. Simultaneous UV spectrophotometric method for estimation of sitagliptin phosphate and simvastatin in tablet dosage form. *Journal of Pharmacy Research,* 2012, 5(1), 2012, 444-446.
7. Jain Pritam, Chaudhari Amar, Desai Bhargav, Patel Shani, Patel Santsaran, Shimpi Hiren. Validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in Formulation. *Int. J. Drug Dev. & Res*, 3 (4), 2011, 194-199.