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

Gandla. Kumara Swamy*[1], Repudi. Lalitha[1], Bathula. Annu[2], M. Lakshi Surekha[3]

[1*]Department of Pharmaceutical Analysis, Care College of Pharmacy, Oglapur (Vill), Damera, (Mdl), Warangal-Rural (Dist)-506001, Telangana, India.
[2]Chaitanya College of Pharmacy Education and Research, Hanmakonda, Warangal, Urban-Telangana, India.
[3]AM Reddy Memorial College of Pharmacy, Narasaraopet, Andhra Pradesh, India.

ABSTRACT

The current research work is validated RP-HPLC method developed and validated for simultaneous determination of Metformin and Sitagliptin in pure and combined tablet dosage forms. An accurate, precise and reproducible high performance liquid chromatographic method was developed for quantitative estimation of Metformin and Sitagliptin simultaneously in tablet dosage forms. Shimadz SPD 10A (S.K.) gradient System UV Detector and C_{18} (Primesil) column with 250mm x4.6mm i.d. and 5µm particle size. Distilled water: Methanol 0.05% Ortho phosphoric acid (50:50) composition of solvent system used as the mobile phase for the method. The detection wavelength was 241nm and flow rate was 1.0ml/min. In the developed method, the retention time of Metformin and Sitagliptin were found to be 2.1min and 7.6min. The proposed research method was validated according to the ICH guidelines. The range, calibration curve, accuracy and precision, robustness was within the limits as specified by the ICH guidelines. So the proposed methods can be used for the routine quality control analysis of Metformin and Sitagliptin simultaneously in tablet dosage forms.

KEYWORDS

RP-HPLC, Metformin and Sitagliptin, Method development, Validation and Tablet dosage forms.

Author for Correspondence:
Gandla. Kumara Swamy
Department of Pharmaceutical Analysis,
Care College of Pharmacy, Oglapur (Vill),
Damera, (Mdl), Warangal, Telangana, India.
Email: kumaraswamy.gandla@gmail.com

INTRODUCTION

Sitagliptin is chemically described as {(2S, 4S)-4-[4-(3- methyl-1phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1, 3- thiazolidin-3-yl) methanone hemipentahydrobromide hydrate is a dipeptidyl peptidase inhibitor. Sitagliptin increasing bloodstream concentrations and reducing fasting

and postprandial glucose concentrations in a glucose-dependant manner in patients with type 2 diabetes mellitus. The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control[1-2].

Metformin hydrochloride (MET) is 1, 1-dimethylbiguanide hydrochloride, a biguanide antidiabetic. It is given orally in the treatment of type 2 diabetes mellitus and is the drug of choice in overweight patients. They do not stimulate insulin release but require that some insulin be present in order to exert their antidiabetic effect. Possible mechanism of action includes the delay in the absorption of glucose from the GIT and increase in insulin sensitivity and glucose uptake in to cells and inhibition of hepatic gluconeogenesis[3-6]. For effective control of blood sugar in diabetic patients more than one medication is required. SITAGLIPTIN shows effective control of blood sugar when combined with METFORMIN. Literature survey reveals various analytical methods for the estimation of SITAGLIPTIN and METFORMIN individually using UV spectrophotometry[7-9], HPLC[10-12], HPTLC[13-15] and LC-MS/MS[16]. Moreover, many methods were reported for the estimation of MET along with other drugs in combined formulation[17-20]. However, the development of simultaneous estimation of SITAGLIPTIN and METFORMIN (Figure No.1) in combined dosage form has not yet been reported by any method. Hence, this manuscript is the first to describe the development and validation of some simpler, sensitive, precise, accurate and cost effective UV spectroscopic methods for the simultaneous determination of SITAGLIPTIN and METFORMIN in combined tablet formulation. Proposed methods possess several advantages which are as follows; methods describe very simple standard and sample preparation procedure, wide concentration range with high sensitivity and all the developed methods were validated as per ICH guidelines.

MATERIAL AND METHODS

Materials and Reagents

The analysis of the drug was carried out on Shimadz SPD 10A Gradient System UV Detector. Equipped with Reverse Phase C_{18} (Primesil) (4.6mm x 250 mm; 5µm), Model no SPD, 20µl injection loop and absorbance detector and Chem station- 3000 software. The API of both drugs Metformin and Sitagliptin procured from Dr. Reddy's Pharma. Pvt. Ltd. Hyderabad. Orthophopsphoric acid (OPA), methanol, acetonitrile, water (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), 0.45µm filter (Millipore, Bangalore). A combination of MET (500mg) and SITAGLIPTIN (20mg) in tablet formulation was procured from local pharmacy (SITAGLIPTIN 20-500M, Centaur Pharmaceutical Pvt. Ltd. Mumbai).

Chromatographic Conditions

C_{18} (Primesil) (250 mm× 4.6mm); particle size packing 5µm; detection wavelength 245nm; flow rate 1.0ml/min; temperature ambient; sample size 20µl; mobile phase methanol: water (OPA 0.05%) (50: 50); run time 10min.

Preparation of standard stock solutions

20mg of Metformin and 10mg of Sitagliptin was weighed accurately and transferred to separate 10ml volumetric flask dissolved in methanol and diluted to 10ml with the solvent (Methanol: Water, 50: 50v/v) to give a stock solution of 5000µgm/ml. Metformin and 200µgm/ml Sitagliptin [Figure No.2 and 3 and Table No.1].

Assay preparation for commercial formulation

20 tablets were weighed individually and their average weight was determined after that they were crushed to fine powders and power equivalent to mg was taken and transferred to 10ml volumetric flask and diluted with methanol (Stock I) from the above solution 0.2ml was taken and diluted to 10ml. The solutions were shaken vigorously for 10min and filtered through 0.45µg nylon membrane filters. Then volume was made up to mark with methanol: water the amounts of Metformin and Sitagliptin per tablet were calculated by extrapolating the value of area from the calibration curve. The procedure was

repeated 5 times with tablet formulation. Result is shown in Result is shown in [Table No.2, 3].

Method development and validation

The standard stock solutions of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Linearity and Range

Linearity of MET was observed in the range of 20-100µg/ml and 10- 60µg/ml SITAGLIPTIN was observed in the range of 10-60µg/ml.

Precision

The precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Accuracy

At each concentration, sample was injected thrice to check repeatability and from the %RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.25-100.90% for the Metformin and 99.59-101.05% for Sitagliptin at three different concentrations 50%, 100%, 150%. The results are given in Table No.4 and 5.

Limit of Detection

The LOD (Limit of detection) is an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Metformin

LOD =3.3 × 1760.8/78322 =0.17µg/ml

Sitagliptin

LOD =3.3 × 61155/11150 =0.20µg/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

Metformin: LOQ =10×1760.8/78322 = 0.79µg/ml

Sitagliptin: LOQ= 10 × 61155/11150 = 1.7µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Metformin and Sitagliptin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Metformin and Sitagliptin were injected by changing the conditions of chromatography (Table No.6, 7 and 8 and Figure No.4).

Summary and Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 255nm and the peak purity was excellent. The column used for study was Zorbax C_{18} because it was giving good peak. 35ºC temperatures was found to be suitable for the nature of drug solution. Mobile phase is Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 8min because analyze gave peak around 2.061, 2.462 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. The analytical method was found linearity over the range 20-100µg/ml of Metformin and 5-30µg/ml of Sitagliptin of the target concentration. The analytical passed both robustness and ruggedness tests.

Table No.1: Calibration data of Metformin and Sitagliptin

S.No	Sitagliptin		Metformin	
	Concentration (µg/mL)	Peak Area	Concentration (µg/mL)	Peak Area
1	10	87486	25	672241
2	20	188325	50	79530
3	30	288316	75	101482
4	40	379872	100	119245
5	50	467992	125	141405
6	60	521031	150	161419
r^2	=	0.995	0.997	

Table No.2: Data of Metformin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	1.95	1033686	12079	5343	1.0
2	Metformin	1.90	1034805	12068	5473	1.2
3	Metformin	1.95	1024496	11949	5473	1.1
4	Metformin	1.97	1021822	11811	5389	1.1
5	Metformin	1.99	1011432	11735	5180	1.0
Mean			249082.6			
Std. Dev			1543.964			
% RSD			0.61986			

Table No.3: Precession Data of Sitagliptin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sitagliptin	2.41	324442	59095	6654	1.2
2	Sitagliptin	2.41	318225	57552	6524	1.3
3	Sitagliptin	2.41	317839	57213	6440	1.3
4	Sitagliptin	2.41	316139	57096	6411	1.4
5	Sitagliptin	2.41	316232	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Table No.4: The accuracy results for Metformin

S.No	%Concentration (at specification Level)	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
1	50%	1184204	50	49.9	99.8%
2	100%	2121872	100	99.8	
3	150%	3525766	150	151.0	

Table No.5: The accuracy results for Sitagliptin

S.No	%Concentration (at specification Level)	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
1	50%	52228	50	49.9	99.8%
2	100%	979319	100	99.8	99.4%
3	150%	1576651	150	150.1	99.2%

Table No.6: Robustness data of Metformin

S.No	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	Actual Flow rate of 1.0 mL/min	247392	1.8	7243	1.2
2	Less Flow rate of 0.9 mL/min	69214	1.6	4713	1.3
3	More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
4	Less organic phase	445628	2.165	4709	1.2
5	More organic phase	69404	1.967	5590	1.4

Table No.7: Robustness data of Sitagliptin

S.No	Parameter used for sample analysis	Peak Area	Re Sitagliptintion	Theoretical plates	Tailing factor
1	Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
2	Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
3	More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
4	Less organic phase	3965572	2.590	5550	1.0
5	More organic phase	527708	2.390	6273	1.0

Table No.8: Robustness of Sitagliptin and Metformin

S.No	SITAGLIPTIN				METFORMIN			
	Ret Time	Peak Area	Theoritical Plate	Tailing Factor	Ret Time	Peak Area	Theoritical Plate	Tailing Factor
1	2.4	294724	6299	1.4	1.8	913813	3890	1.5
2	2.4	297194	6635	1.4	1.8	939045	3751	1.4
3	2.4	294660	6402	1.4	1.8	937206	3867	1.4
4	2.4	295944	6166	1.3	1.8	922916	4028	1.5
5	2.4	296844	6413	1.4	1.8	928312	3786	1.4
6	2.4	285850	6416	1.4	1.8	939317	3860	1.4
Mean	2.4	292536	6388.5	1.38	1.8	930101.5	3863.6	1.43
Std. Dev	-	5248.07	-	-	-	10349	-	-
%RSD	-	1.79%	-	-	-	1.11%	-	-

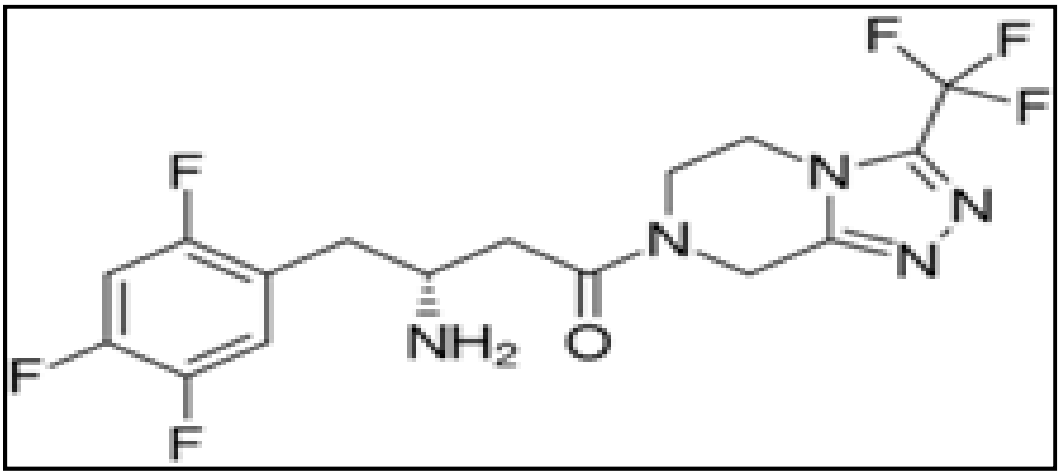

Figure No.1: Chemical structures of a). Sitagliptin and b). Metformin

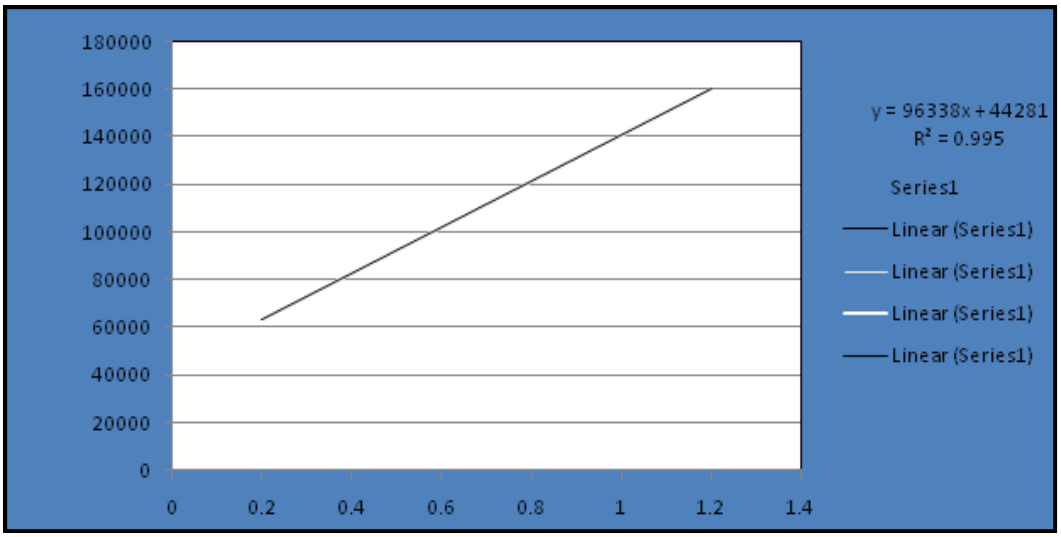

Figure No.2: Calibration curve of Sitagliptin

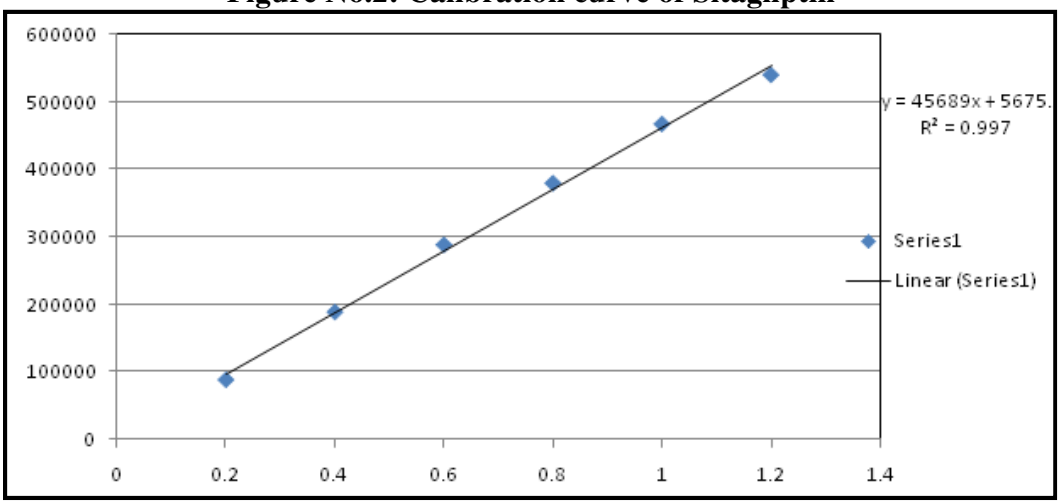

Figure No.3: Calibration curve of Metformin

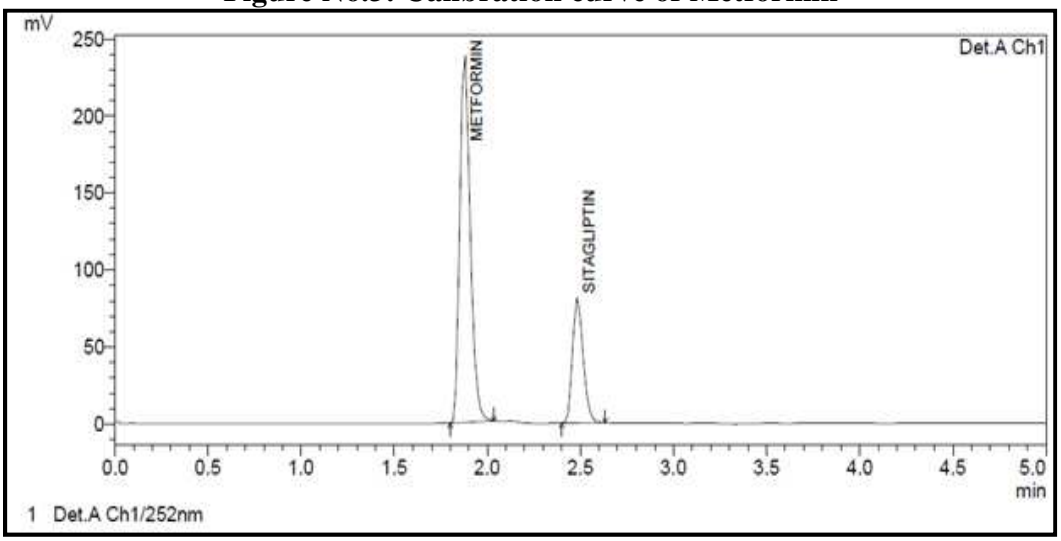

Figure No.4: Chromatogram of standard mixture of Metformin and Sitagliptin

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Metformin and Sitagliptin in bulk drug and pharmaceutical dosage forms. Metformin and Sitagliptin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The percentage of Relative standard deviation values were less than 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Metformin and Sitagliptin in bulk drug and in Pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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