Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (5):168-174
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Method development and validation of sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form

Karimulla S K[1], Vasanth P M*[1], Ramesh T[2], Ramesh M[2]

1. Dept of Pharmaceutical Analysis, UCEV-JNTUK, Vizianagaram, A.P, India.
2. Dept of Biotechnology, UCEV-JNTUK, Vizianagaram, A.P, India.

ABSTRACT

A Simple, accurate and precise method for estimation of reversed phase HPLC method has been developed for the simultaneous determination of sitagliptin and Metformin by using Hypersil BDS C18 (100 x 4.6 mm, 5µm particle size) column and mobile phase of at 215 nm. A mobile phase has a composition of potassium dihydrogen orthophosphate and methanol(50:50v/v),adjusted the pH 8.5 with o-phosphoric acid was used and flow rate 1.0ml/min. Retention times of Sitaglipitin and Metformin were 2.3 min and 4.6 min respectively. The method developed validated as per ICH guideline

Keywords: Sitagliptin, Metformin, reverse phase HPLC, validation

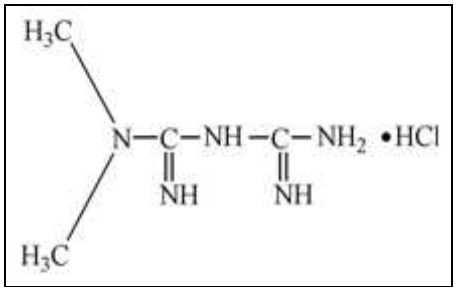
---

INTRODUCTION

Metformin chemically N, N-diethyl imido dicarbonimidic diamide hydrochloride belongs to the bi-guanide class of anti-diabetic drug which is extensively used in the treatment of type II diabetes mellitus [Non-insulin-dependent diabetes mellitus (NIDDM)].The anti hyperglycemic effects 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.Freely soluble in water, slightly soluble in ethanol (95%), practically insoluble in acetone, ether and chloroform.

Sitagliptinchemically7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate. Sitagliptin is a highly selective DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones, thereby increasing the concentration and prolonging the action of these hormones. Soluble in water and N, N-diethyl form amide, slightly soluble in methanol, very slightly soluble in ethanol, acetone and acetonitrile, insoluble in isopropanol and Isopropyl acetate. The present work describes a simple, sensitive and accurate RP-HPLC method for simultaneous estimation of the two drugs in their combined tablet dosage form.

Molecular structure



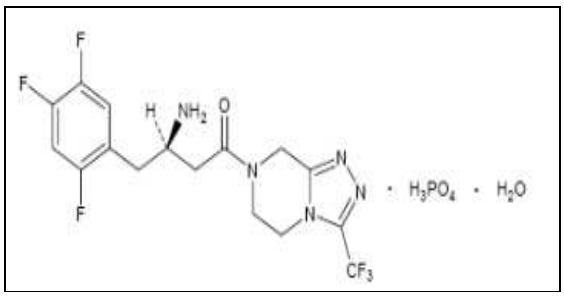


Figure 1 Metformin **Figure 2 Sitagliptin**

MATERIALS AND METHODS

Materials

Sitagliptin and Metformin bulk drugs were obtained from Hetero labs, Hyderabad, Andhra Pradesh, India as gift samples and marketed formulation JANUMET tablets containing 500mg of Metformin and 50mg of Sitagliptin was procured from local market. Methanol of HPLC grade and potassium dihydrogen orthophosphate of AR grade, were purchased from obtained from local mark.

Chromatographic condition

A mobile phase consisted of Methanol and potassium dihydrogen orthophosphate in 50:50 ratios was pumped at a flow rate of 1ml/min. The elution is observed using a PDA detector at 215 nm and the injection volume was 10 µL. The validation of the method was done following the ICH guidelines.

Instrumentation

HPLC device Waters model 2695 with Empower software version 2.0.Detector waters 2996 PDA Detector. Elico Ph meter, Sartorius – Digital balance (0.1 mg – 205 gm).separation was achieved on a Hypesil BDS C18 Column (100 x 4.6 mm,5um particle size)

Preparation of Solutions

Preparation of 0.1M of Potassium dihydrogen orthophosphate Buffer Solution Ph (4.5)

174.18 g of Potassium dihydrogen orthophosphate was dissolved in 1000ml of milliQ water. The solution was adjusted to a PH of 8.5with ortho phosphoric acid (OPA). Then it was degassed in ultra sonicator for 10 minutes and then filtered through 0.45 µ pore size membrane filter.

Preparation of mobile phase

Mix a mixture of above buffer 500 ml and 500 ml of Methanol HPLC grade and degas in ultrasonic water bath for 10 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of standard stock solution of Sitagliptin and Metformin

50mg of Metformin and 5 mg of sitagliptin working standard were taken in 50ml volumetric flask. It was dissolved in 10ml water and made up to the mark with the water. It was degassed in ultra sonicator and then filtered through membrane filter of 0.45µ pore size

Preparation of sample solution of Sitagliptin and Metformin

10 tablets were crushed and powder equivalent to 708mg was taken into 100ml volumetric flask. It was made to dissolve with water and made up to the mark with water. The solution was degassed and filtered through membrane filter paper of pore size 0.45.Transfer 5ml of above solution into 25ml volumetric flask and make up to the volume with water

Selection of mobile phase for method Optimization and experimental condition

Several trials have been taken for the proper optimization of RP HPLC method by changing different mobile phase with different ratio. And finally the mobile phase for optimized condition was selected and given follows. And the optimized parameters was for MET and SIT was given (Table 1).

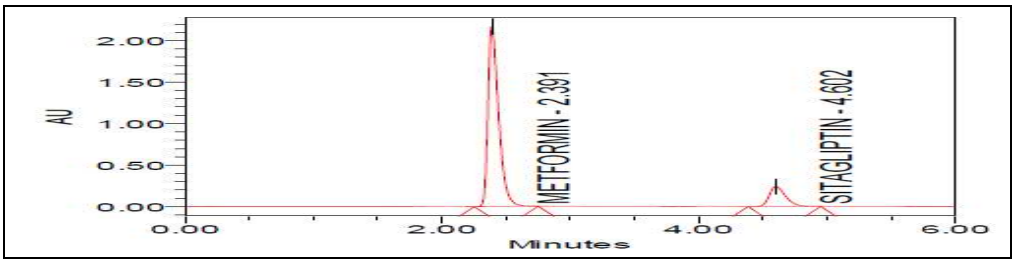


Figure 3 Optimized Chromatogram of Met & Sit

Table 1 Optimized chromatographic condition of Met & Sit

Chromatographic conditions	Mode of separation
Separation	Waters 2695
Column	Hypersil BDS C18(100 x 4.6 mm,5um particle size)
Flow rate	1.0ml/min
Solvent	Water
Column temperature	30˚C
Sample temperature	25^0C
Wavelength selected	215 nm
Injection volume	10µl
Run time	6 minutes
Mobile phase	Methanol: buffer(50:50v/v)

Assay

Assay of marketed tablet formulation containing 500mg of Metformin and 50mg of Sitagliptin Six injections of above prepared sample and standard solutions were injected. The assay of the commercial sample was calculated by comparing the areas of standard and sample peaks. The assay of marketed formulation JANUMET found within limit. And the chromatogram was given.

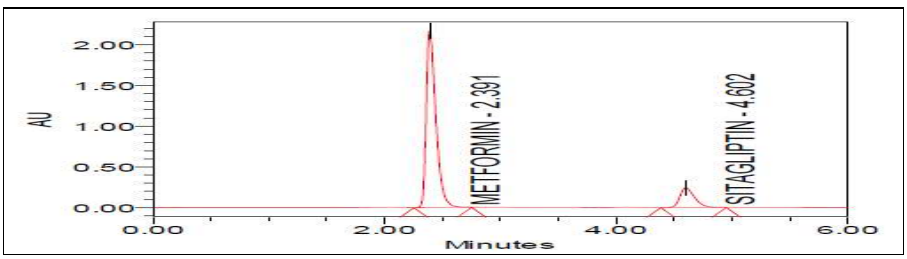


Figure 4 Chromatogram for the Assay of Met & Sit

Method validation

This optimized method was validated in terms of linearity, accuracy, precision, specificity, limit of detection, limit of quantification as per ICH guidelines

Linearity

Calibration curve of Metformin and Sitagliptin were constructed by plotting concentration vs. peak areas, and the regression equations were calculated. The linearity of this method was investigated by using the concentrations 25, 50, 75, 100, 125, 150% of both drugs. These concentrations were prepared by diluting appropriate volume of working standard with mobile phase.

System suitability
Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Accuracy
The accuracy of the method was evaluated by standard addition method. A previously analyzed test solution was spiked with drug standard solutions at 50%, 100% and 150% concentration levels and percent recovery was determined.

Precision
The method precision of test method was done by performing assay on five replicate determination of sample preparation at test concentration level (as per method of analysis) and the relative standard deviation of assay results was obtained.

Specificity: Forced degradation studies
Forced degradation studies were performed on SIT and MET to prove the stability- indicating property of the method. The stress conditions employed for degradation study of SIT and MET include light exposure, heat (105°C), acid hydrolysis (0.1 M HCl), base hydrolysis (0.1M NaOH), water hydrolysis and oxidation (1% H_2O_2). For light studies, the monitoring period was 10 days whereas for heat, acid, base and water hydrolysis it was 48 h. Oxidation was carried out for 24 h. Peak purity of the principal peak in the chromatogram of stressed samples of SIT and MET tablets was checked using PDA detector.

Robustness
Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from rate (0.2 ml/min of set value i.e. 0.8 ml/min and 1.2 ml/min) and variation of temperature.

LOD and LOQ
Calibration curve was repeated five times and the standard deviation of the intercepts of regression equations was calculated. The LOD and LOQ were calculated using equation:

LOD = 3.3 * SD/ S and LOQ = 10 * SD/ S

Where; SD = standard deviation of intercepts
S = mean slope of calibration

RESULTS AND DISCUSSION

System suitability: Theoretical plates, Tailing factor and resolution between MET and SIT were determined for each drug. The results were within acceptable limits and are summarized

Table 2 System suitability Parameters

Parameters	MET	SIT
Retention time (minutes±SD)	2.39±0.2	4.6 ± 0.2
Repeatability (% RSD)	0.18	0.08
Theoretical plates per meter	4020	6366
Tailing factor	1.53	1.35
Resolution	11.32	

Linearity
The calibration peak areas were found to be linear and the Correlation Coefficient obtained was 1 for Metformin and 0.999 for Sitagliptin.

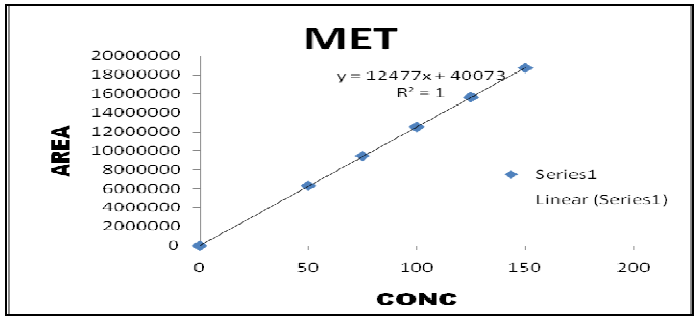


Figure 5 Linearity of Metformin

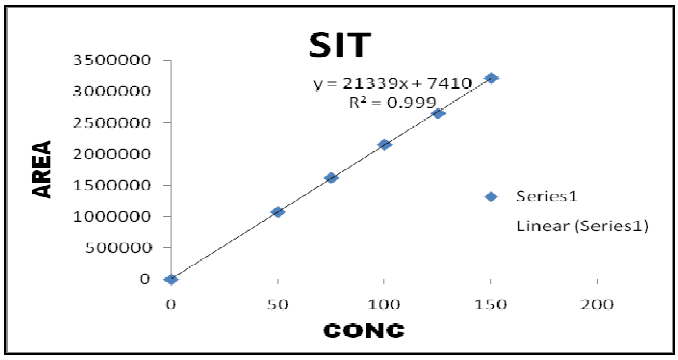


Figure 6 Linearity of Sitagliptin

Table 3 Linearity parameters

Parameters	MET	SIT
Linearity range	50-150%	50-150%
Regression equations	Y=12477x-40073	y = 21339x-7410
Slope	12477	21339
Intercept	40073	7410
Correlation coefficient(R^2)	1	0.999

Accuracy

The Mean percentage recovery of Met is 100.6 and Sit is 100. Very good recoveries were made at each added concentration. Data is presented in table 4.

Table 4 Accuracy

Concentration of Spiked level %	Amount added µg/ml		Total amount found µg/ml		% Recovery µg/ml		Mean	
	MET	SIT	MET	SIT	MET	SIT	MET	SIT
50	49.77	49.77	49.82	49.38	101	99		
100	99.0	99.0	98.16	98.30	100	100	100.6	100
150	148.5	148.5	148.2	1 4 8 . 2	101	101		

Precision

The precision of an analytical method gives information on the random error. It expresses agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The %RSD for Met is 0.21 and for Sit is 0.29.

Table 5 Precision

DRUG	Sample Weight(mg)	Peak Area (n=6)	% Assay (n=6)	STD	%RSD
MET	708	12490616	98	0.20	0.21
SIT	708	2132631	99	0.29	0.29

Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature and buffer pH), all analytes were adequately resolved.

Table 6 Robustness

Flow rate (ml/min)	RT Met(min)	RT Sit(min)	Mean ± SD	
			Met	Sit
0.8ml/min	3.181	6.098	2.551±0.89	4.92±1.65
1.2 ml/min	1.922	3.753		
Temperature				
Temp(25°c)	2.40	4.663	2.395±0.007	4.65±0.014
Temp(30°c)	2.39	4.642		

Forced degradation studies

Table 7 Forced degradation data

Degradation conditions	% Degradation of MET	Peak purity	% Degradation of SIT	Peak purity
0.1M NaOH	10	passes	11	passes
0.1M HCl	7	passes	7	passes
1% H_2O_2	2	passes	2	passes
H_2O	8	passes	9	passes
light	0	passes	0	passes

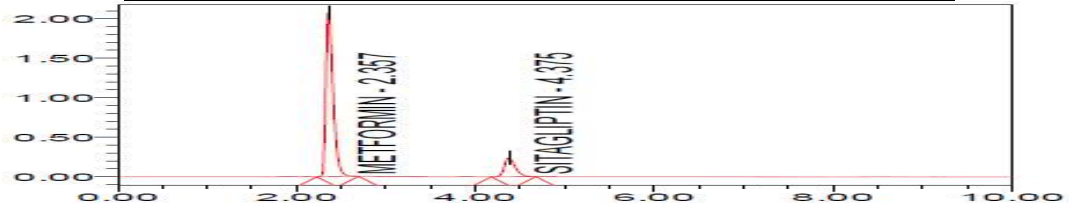


Figure 7 Acid degradation Chromatogram of Metformin & Sitagliptin

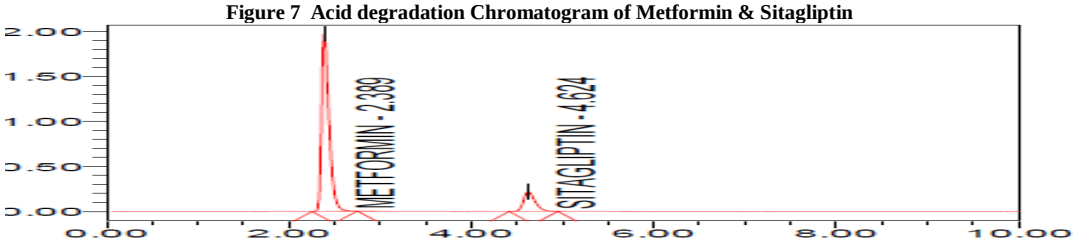


Figure 8 Base degradation Chromatogram of Metformin & Sitagliptin

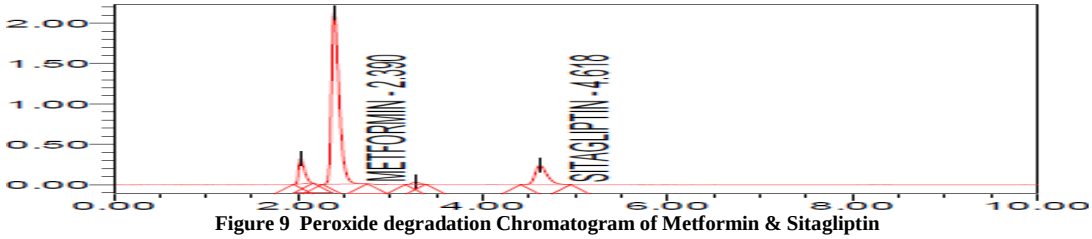


Figure 9 Peroxide degradation Chromatogram of Metformin & Sitagliptin

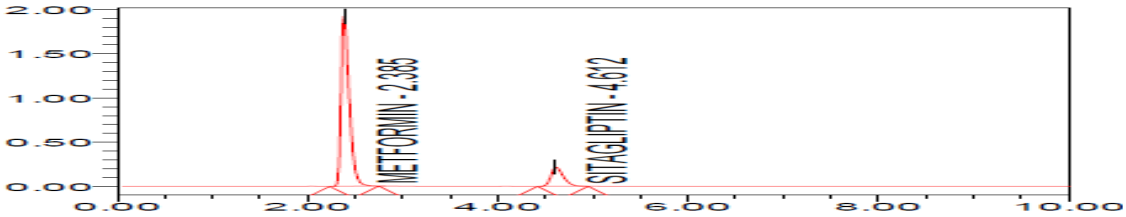


Figure 10 Water degradation Chromatogram of Metformin & Sitagliptin

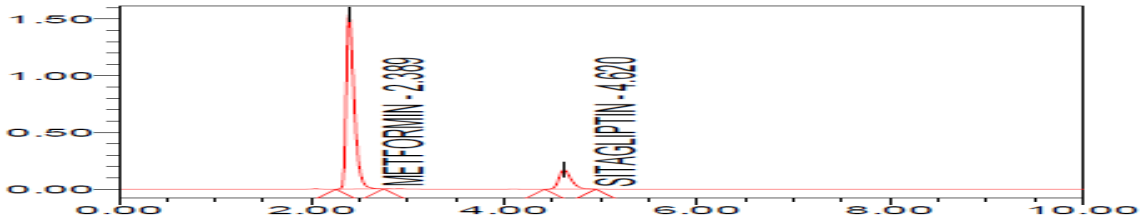


Figure 11 Heat degradation Chromatogram of Metformin & Sitagliptin

LOD & LOQ

The limit of detection was found to be 0.08µg/ml for Metformin and 0.07µg/ml for Sitagliptin. The limit of quantification was found to be 2.6µg/ml for Metformin and 2.3µg/ml for Sitagliptin.

CONCLUSION

A simple specific stability-indicating HPLC method has been developed for the quantification of SIT and MET simultaneously. This method has been validated and found to be specific, precise, accurate, linear, robust, and linear for the detection and quantification of SIT and MET. This method exhibited an excellent performance in terms of sensitivity and speed. The major advantage of this technique is that it is less time consuming and also eco-friendly because of its low consumption of organic solvents as compared to other analytical techniques. It helps in simultaneous estimation of SIT and MET in pharmaceuticals i.e., in combination drugs. This method is suitable for routine analysis and quality control of pharmaceuticals.

REFERENCES

[1] Government of India, Ministry of health and family welfare, Indian Pharmacopoeia, Vol- I & II, *The Controller of Publication*, New Delhi ; **2010**: 1657-60.
[2] h t t p : / / www. m e d i c i n e n e t . c o m /metformin_and_sitagliptin/article.htm
[3] The Internet Drug Index. http://www.rxlist.com/janumet-drug.htm.
[4] Klepser TB, Kelly MW. Metformin hydrochloride: an antihyperglycermic agent. *Am J Health System Pharm.* **1997**; 54:893–903.
[5] Goswami L, Mukhopadyay S and Durgapal S. *Indian Journal of Pharmaceutical Sciences*.**2010**; 72(4):508-510.
[6] Parag P, Imran Md, Vinod B and Yogesh A. *Journal of Pharmacy Research*. **2011**; 4(3):871-873.
[7] Mubeen G, Noor K and Vimala MN. *International Journal of Chem Tech Research*. **2010**; 2(2):1186-1187
[8] ICH Q2A; Guidelines on validation of analytical procedure; definitions and termino- logy, *Federal Register* **1995**; 60: 11260.
[9] Mousumi K, Choudary PK. *Indian J Pharm Sci* **2009**; 2:318-20.