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STABILITY INDICATING METHOD EVALUATION AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND SITAGLIPTIN IN ORAL DOSAGE FORM

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ABSTRACT

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of Metformin + Sitagliptin in bulk drug and market dosage form. The method employed, with Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) in a gradient mode, with mobile phase of Octane sulphonic acid buffer: acetonitrile 35:65 %v/v. The flow rate was 1.0 ml/min and effluent was monitored at 260 nm. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100 µg/ml respectively. The LOD and LOQ values for were found to be 2.098(µg/ml) and 6.3597(µg/ml) respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of Metformin + Sitagliptin in market dosage form.

KEYWORDS: Metformin, Sitagliptin, oral dosage form, UPLC method.

INTRODUCTION

Metformin is used with a proper diet and exercise program and possibly with other medications to control high blood sugar. It is used in patients with type 2 diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems.

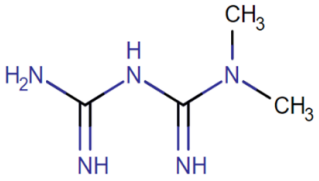


Fig 1: Molecular Structure of Metformin, 1-carbamimidamido-N,N-dimethylmethanimidamide.

Therapeutic category	Antidiabetic drug
CAS Registry number	657-24-9
Chemical name	1-carbamimidamido-N,N-dimethylmethanimidamide
Molecular formula	$C_4H_{11}N_5$
Molecular Weight	129.163
Solubility	Soluble in 10mL of water
pka	12.4
λ_{max}	230 nm
Pharmacology	Metformin is indicated as an adjunct to diet and exercise to increase glycemic control in adults and pediatric patients 10 years of age and older diagnosed with type 2 diabetes mellitus

Sitagliptin is a diabetes drug that works by increasing levels of natural substances called incretins. Incretins help to control blood sugar by increasing insulin release, especially after a meal. They also decrease the amount of sugar your liver makes.

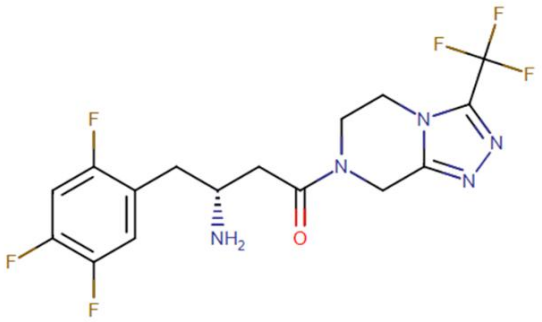


Fig 2: Molecular Structure of Sitagliptin, (3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4] triazolo [4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one.

Therapeutic category	Antidiabetic drug
CAS Registry number	486460-32-6
Chemical name	(3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one
Molecular formula	$C_{16}H_{15}F_6N_5O$
Molecular Weight	407.3136
Solubility	"0.034 mg/mL
pka	8.78
λ_{max}	230 nm
Pharmacology	"Sitagliptin is indicated for the management of glycemic control in type 2 diabetes mellitus along with diet and exercise

Validation of Analytical Methods (USP/ICH)

Method validation, according to the United States Pharmacopeia (USP), is performed to ensure that an analytical methodology is accurate, specific, reproducible, and rugged over the specified range that an analyte will be analyzed. Regulated laboratories must perform method validation in order to be in compliance with FDA regulations. In a 1987 guideline (Guideline for Submitting Samples and Analytical Data for Methods Validation), the FDA designated the specifications in the current edition of the USP as those legally recognized when determining compliance with the Federal Food, Drug and Cosmetic Act can be referred to as the "eight steps of method validation"

EXPERIMENTAL

MATERIALS

EQUIPMENTS	SOURCE
Ultra Pressure Liquid Chromatography (UPLC)	Acquity UPLC Systems, Waters Laboratories
Electrospray ionization and MS-MS	Mass Spectrometer PE Sciex Model: API 3000
Chromatographic data software	Empower
Column	C18 column (250 ×4.6 mm id)—ACE Generix
Detector	PDA
Injector	Automated
Electronic Balance	Eagle
Sonicator	Band Line Sonerex
p^H Meter	Lab India p^H meter

METHODOLOGY

Method Validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

- **Forced degradation studies of our selected pharmaceutical drugs.**

In order to establish the analytical method for a stability indicating method, the drugs are subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid/base hydrolysis, oxidation, reduction, in accordance with ICH Q1A (R2). Several trials with different severity

of each stressed condition are to be conducted, so that upto 10-30% degradation is to be achieved.

RESULTS

Preparation of Standard Stock Solution

Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 1.0 mL min-1 shows good resolution. The PDA detector response of Metformin + Sitagliptin was studied and the best wavelength was found to be 230 nm showing highest sensitivity.

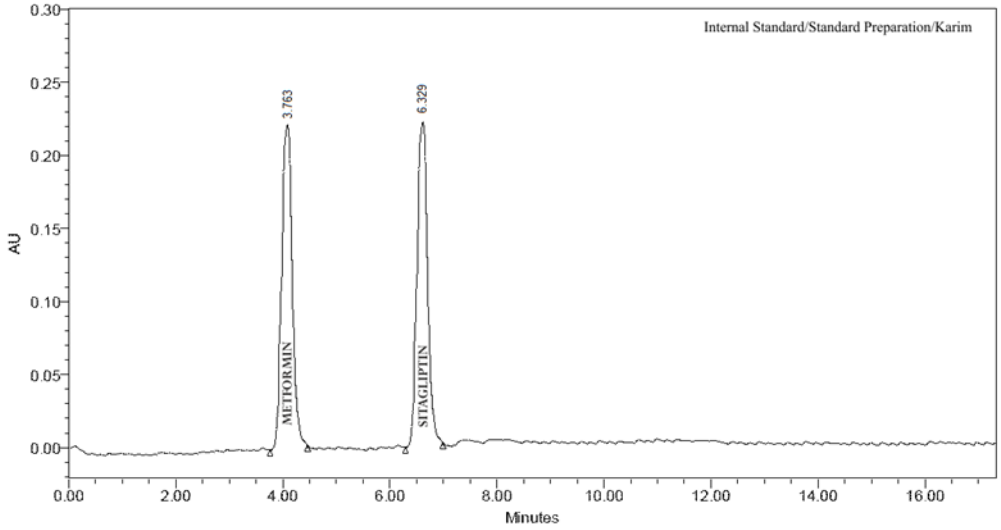
The mixture of two solutions Methanol: acetonitrile 40:60 %v/v". The buffer used is 100 mg of anhydrous octane sulphonic acid sodium salt was weighed and transferred to 100 ml of water and sonicated well. The pH of the solution was adjusted to 3 with orthophosphoric acid solution. Gradient programming was employed to mobile phase at 1.0 mL/min flow rate was found to be an appropriate mobile phase for separation of Metformin + Sitagliptin. The column was maintained at 25°C temperature.

Preparation of internal standard solution

Weighed accurately about 10 mg of papaverine into a clean and dry 100 mL volumetric flask, dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

Preparation of Metformin + Sitagliptin standard solution

Transfer accurately about 10 mg of Metformin + Sitagliptin into 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get the concentration of 100 µg/mL of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.



Chromatogram of standard preparation of Metformin + Sitagliptin (Octane sulphonic acid buffer : acetonitrile 35:65 %v/v)

Accuracy study

Metformin						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std. Dev**	**% RSD**
50	07.81	07.64	97.82	98.93	0.9634	0.97%
100	15.62	15.55	99.55			
150	23.43	22.30	99.42			

Sitagliptin						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	07.51	07.34	97.73	98.69	0.9615	0.98%
100	15.33	15.21	99.21			
150	23.12	22.92	99.13			

System Precision

Procedure

"The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated at a concentration of 20 µg/mL (Metformin + Sitagliptin)."

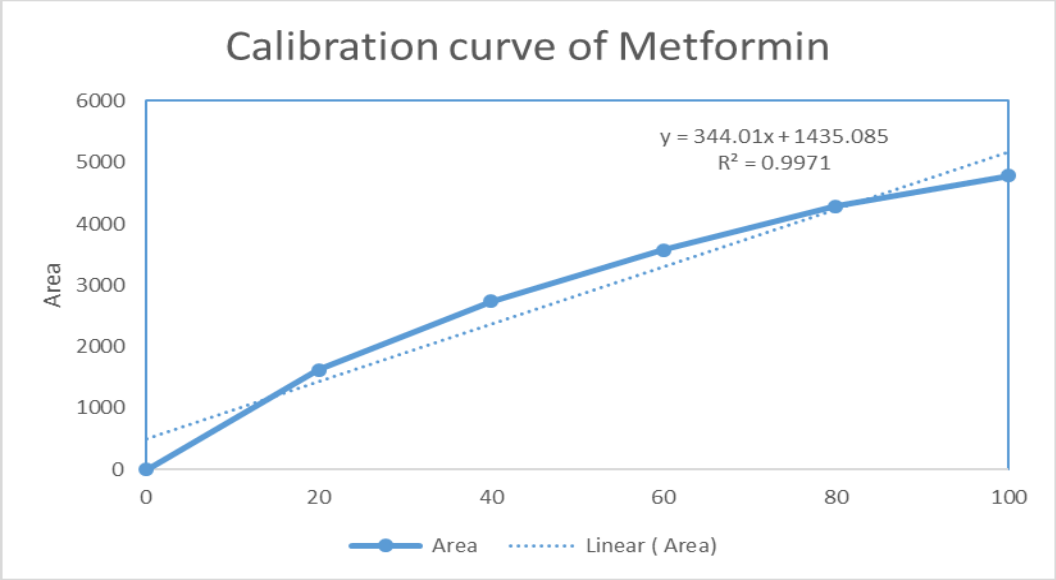
Parameters	**Metformin**	**Sitagliptin**
Retention time (min) ± % RSD	3.821± 0.05	6.385 ± 0.05
Theoretical plates ± % RSD	4227.84 ± 0.50	4354.91 ± 0.50
Asymmetry ± % RSD	1.04 ± 0.05	1.03 ± 0.05
Repeatability (% RSD)	0.05	0.05

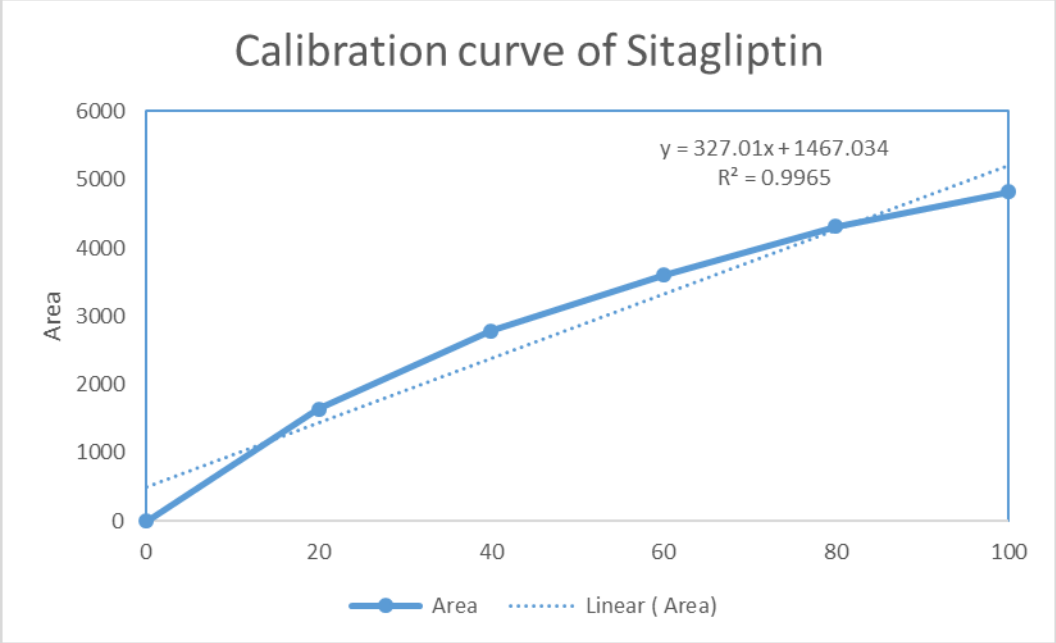
Precision

Replicate		***Metformin + Sitagliptin***		
S.No.	**Concentration Taken (µg/ml)**	**Area *Metformin***	**Area *Sitagliptin***	**%LC**
1	20	2118.211	2121.437	99.93%
2		2119.821	2135.146	100.08%
3		2118.332	2113.252	99.93%
4		2119.241	2145.439	99.98%
5		2118.899	2189.355	99.96%
6		2118.947	2117.433	99.96%
Average				99.97%
Std.Dev				0.0557
% RSD				0.06%
Standard weight				20 mcg
Standard potency				99.50 %

Linearity

Metformin + Sitagliptin			
Linearity level	**Concentration in µg/mL**	**Area Metformin**	**Area Sitagliptin**
1	20 µg/mL	1619.645	1640.432
2	40 µg/mL	2737.159	2784.125
3	60 µg/mL	3569.198	3605.231
4	80 µg/mL	4282.409	4314.104
5	100 µg/mL	4787.021	4823.128
Correlation co-efficient		0.9971	0.9965
Slope		344.01	327.01
Intercept		1435.085	1467.034





Robustness

Robustness Studies				
Parameter	**Value**	**Peak Area Metformin**	**Peak Area Sitagliptin**	**% RSD**
Flow Rate	Low	2118.621	2140.212	0.05%
	Actual	2120.427	2145.439	
	Plus	2120.638	2148.648	
Temperature	Low	2118.932	2141.140	0.04%
	Actual	2119.484	2145.468	
	Plus	2120.691	2147.280	
Wavelength	Low	2118.883	2143.225	0.02%
	Actual	2119.476	2145.338	
	Plus	2119.862	2149.446	

Ruggedness

Metformin + Sitagliptin				
Ruggedness				
Parameter	**Peak Area Metformin**	**Peak Area Sitagliptin**	**% RSD**	**%LC**
Intraday precision	2118.833	2145.127	0.05%	99.96%
	2120.440	2146.658		100.03%
	2120.657	2143.324		100.04%
Inter day precision	2118.738	2147.932	0.02%	99.95%
	2119.431	2148.105		99.98%
	2119.649	2143.137		100.01%
Instrument:1 Acquity UPLC Waters,2695H	2119.233	2146.265	0.05%	99.99%
	2120.849	2142.388		100.05%
	2121.023	2144.345		100.06%
Instrument:2 Agilent Technologies,1290	2119.258	2151.423	0.04%	99.98%
	2119.836	2152.497		100.09%
	2121.019	2154.423		100.06%
Average				100.01
Std.Dev				0.0447
%RSD				0.04%

LOD and LOQ

Procedure

"The limit of detection and limit of quantification were evaluated by serial dilutions of Metformin + Sitagliptin stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ as per ICH guidelines."

Calculations of LOD and LOQ

Slope = a; Intercept = b; The number of tests = N

Standard Error (SE) of Intercept = EXCEL function data analysis → Regression → Table

SD of Intercept = SE of Intercept / Square root of N

LOD

LOD=3.3(SD of intercept/Slope)

Total numbers: 5

SE of Intercept: 487.8871

SD of Intercept: 218.783

LOD= 3.3*(218.783/ 344.01)

LOD= 3.3*(0.63597)

LOD= 2.098(µg/ml)

LOQ

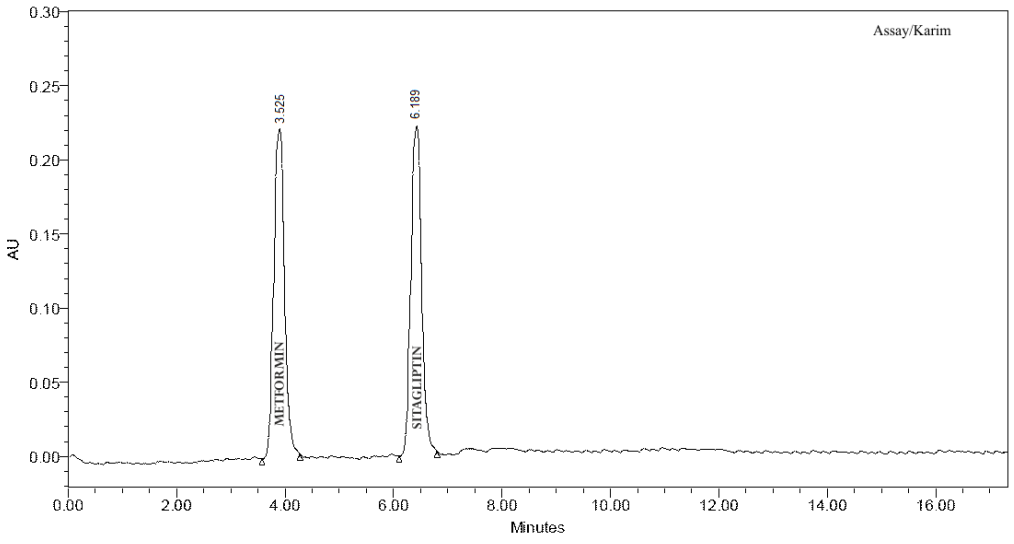
LOQ=10*(SD/S)

LOQ= 10*(218.783/ 344.01)

LOQ= 6.3597(µg/ml)

Forced Degradation Studies

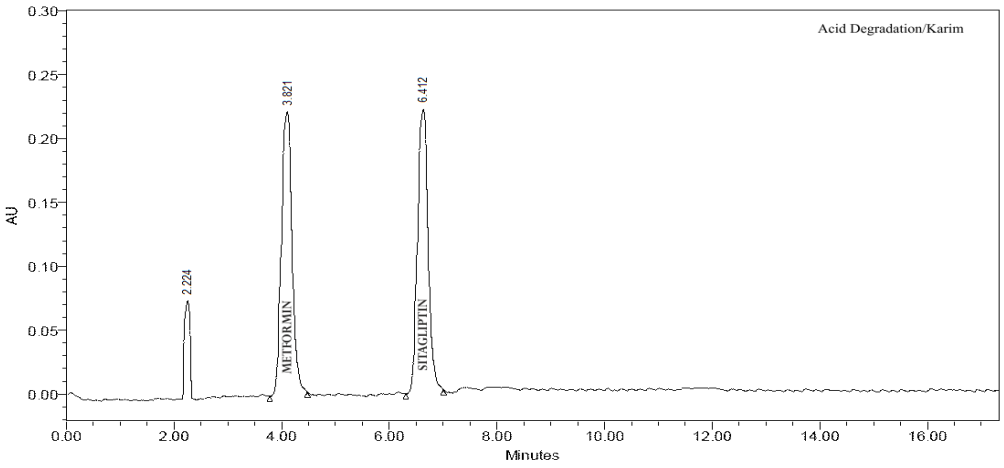
Sample Control: An accurate 10 ml of the prepared pure drug stock solution of working standard was transferred to a clean and dry RBF. The volume of the sample was 100 µg/ml. It was injected into the UPLC system against a blank of Octane sulphonic acid buffer: acetonitrile 35:65 %v/vafter optimizing the mobile phase composition, chromatogram was recorded.



Assay of Metformin + Sitagliptin (Sample Control)

a. Acidic Degradation

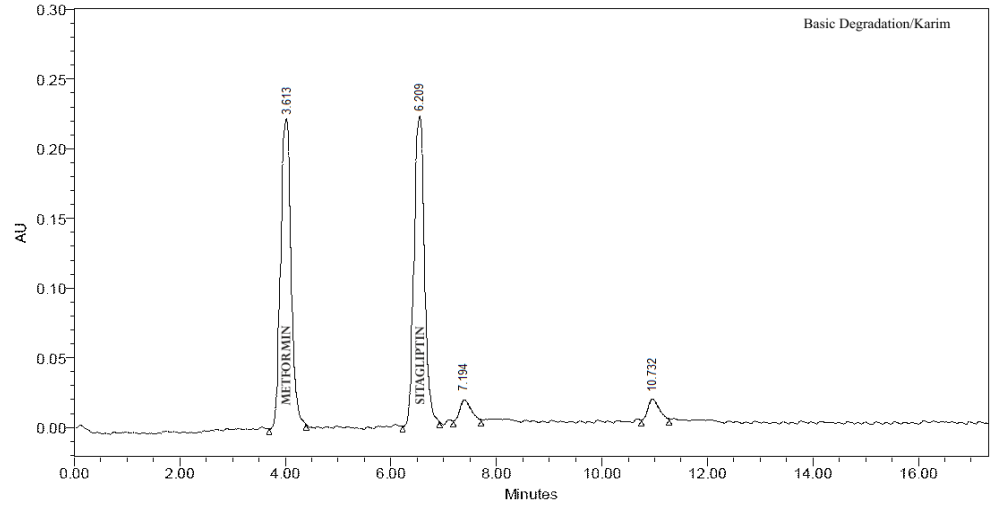
"An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Octane sulphonic acid buffer : acetonitrile 35:65 %v/vafter optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Acidic degradation

b. Basic Degradation

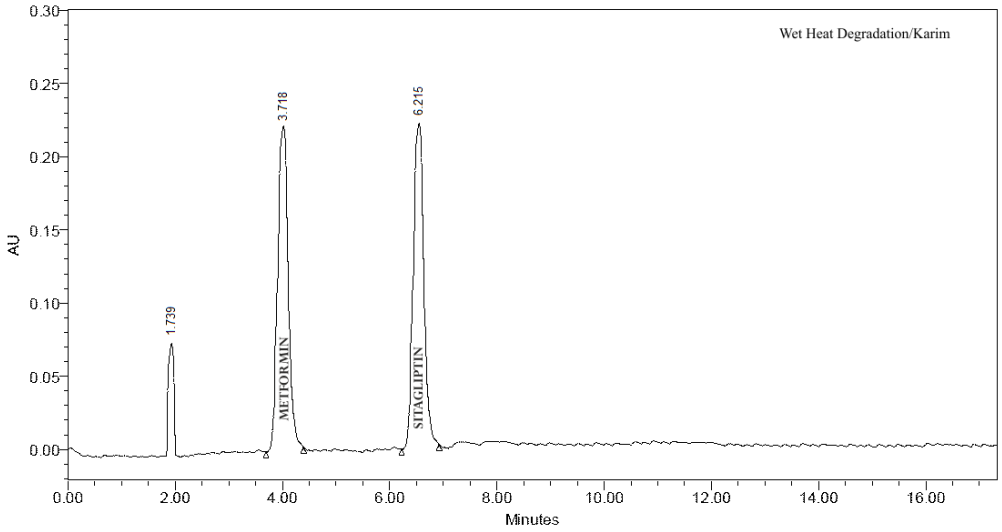
An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Octane sulphonic acid buffer : acetonitrile 35:65 %v/vafter optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Basic degradation

c. Wet heat degradation:

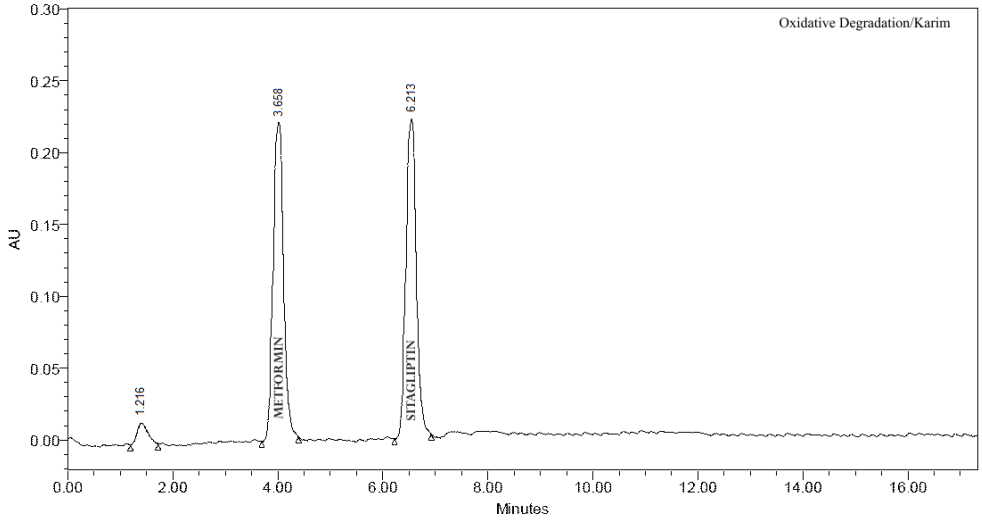
"Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of Octane sulphonic acid buffer : acetonitrile 35:65 %v/vafter optimizing the mobile phase composition, chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Wet heat degradation

d. Oxidation with (3%) H_2O_2

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Chromatogram."



Chromatogram showing the degraded products in Oxidative degradation

EVALUATION OF METHODS

Forced Degradation Studies

- **Analysis of Metformin + Sitagliptin.**

Conditions	Sample Amount (µg/ml)	Peak Area	% claim	% Degradation
Sample Control	04.15	26139	91.67%	-
Acidic Degradation	04.08	24721	*86.33%*	5.34%
Basic Degradation	04.05	23581	*82.42%*	9.25%
Oxidative Degradation	04.03	24357	*85.54%*	6.13%
Wet Heat	04.06	25832	*90.42%*	1.25%

Results of Forced Degradation Assays

Calculation formula for Metformin + Sitagliptin

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

"Whereas,"
"AT = Average area of test preparation, 26139"
"AS = Average area of standard preparation, 28358"
"W1 = Weight taken of reference standard (µg), 04.15"
"W2 = Weight taken of test sample (µg), 04.25"
"AW = Average weight of sample (µg), 3057"
"LC = Label claim (µg), 3000"
"P = Potency of reference standard (%), 99.98%"

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

Sample Control (Metformin + Sitagliptin)

$$\%\ Assay = \frac{26139}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times$$

Error!× 99.98= *91.67%*

Acidic Degradation (Metformin + Sitagliptin)

$$\%\ Assay = \frac{24721}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times$$

Error!× *99.98= 86.33%*

Basic Degradation (Metformin + Sitagliptin)

$$\%\ Assay = \frac{23581}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times$$

Error!× *99.98= 82.42%*

Oxidative Degradation (Metformin + Sitagliptin)

$$\%\ Assay = \frac{24357}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times$$

Error!× *99.98= 85.54%*

Wet Heat (Metformin + Sitagliptin)

$$\%\ Assay = \frac{25832}{28358} \times \frac{04.15}{100} \times \frac{1}{25} \times \frac{100}{04.25} \times \frac{25}{1} \times$$

Error!× *99.98= 90.42%*

CONCLUSION

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of Metformin + Sitagliptin in bulk drug and market dosage form. The method employed, with Hypersil C18 (100 mm x 2.1 mm, 1.7 µm) in a gradient mode, with mobile phase of Octane sulphonic acid buffer : acetonitrile 35:65 %v/v. The flow rate was 1.0 ml/min and effluent was monitored at 260 nm. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 20- 100 µg/ml respectively. The LOD and LOQ values for were found to be 2.098(µg/ml) and 6.3597(µg/ml) respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of Metformin + Sitagliptin in market dosage form.

The method provides selective quantification of Metformin + Sitagliptin without interference from blank affirming precise method. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters.

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