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Research Article

EXPLORATION OF STABILITY-INDICATING ASSAY UHPLC METHOD FOR SIMULTANEOUS ANALYSIS OF SITAGLIPTIN AND METFORMIN IN PURE MATERIAL AND PHARMACEUTICAL DOSAGE FORMS

Rahul P. Umbarkar*[1], Abhilasha Mittal[1], Manoj S. Charde[3]

[1]*Institute of Pharmacy, NIMS University, Jaipur, Rajasthan,India*

[2]*Government College of Pharmacy, Karad, Maharashtra, India*

**Corresponding author: rpumbarkar@gmail.com*

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ABSTRACT

The scientific novelty of designed work was to develop a specific and precise stability-indicating ultra high performance liquid chromatography (UHPLC) assay method for simultaneous quantification of Sitagliptin and Metforminin extended-release fixed dose combinations (FDCs). The reversed-phase UHPLC resolution was analyzed with the assistance of UPLC BEH C_{18} (150 mm × 2.1 mm) with 1.7 µm particle size column at ambient temperature using a solvent system in a proportion of (90:10 % *v/v*) acetonitrile: potassium dihydrogen orthophosphate buffer; pH 3.0±0.2 was adjusted with 0.1 % ortho phosphoric acid (OPA), with flow rate of 0.4 mL/min of the solvent system. The analytes were supervised at 267 nm by employing photodiode array recognition. The retention times of Sitagliptin and Metformin were found to be1.903±0.01 and 1.301±0.022, respectively. The Sitagliptin and Metformin have confirmed the linearity ranges of 5-30µg/mL, and 100-600 µg/mL respectively, with 0.9996 and 0.9996 determination coefficients. The UHPLC method was effectually validated concerning the accuracy, precision, sensitivity, robustness, ruggedness, selectivity, and specificity. Moreover, the anticipated UHPLC method's capability to analyze the Sitagliptin and Metformin with no obstruction from degradation products.

Keywords: UHPLC, Sitagliptin, Metformin, Stability-indicating assay, Fixed-dose combinations.

1. INTRODUCTION

Sitagliptin chemically is (3R)-3-amino-1-[3-(trifluoro-methyl)-6,8-dihydro-5H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one. It is a dipeptidyl peptidase-4 blocking agent. The molecular formula is $C_{16}H_{15}F_6N_5O$ and molecular weight is 407.31 g/moL. It is white to off powder with a melting point between 216-218°C. It is sparingly soluble in water and slightly soluble in methanol. It has a pKa value of 8.78 [1].

Metformin Hydrochloride (MET) chemically is 1-carbamimidamido-N,N-dimethylmethanimidamide, from the division of biguanide of antidiabetic and antihyperglycemic drugs. It is used in patients with type 2 diabetes. The molecular formula is $C_4H_{12}ClN_5$, and molecular weight is 165.62 gm/moL. Metformin is a white crystalline powder with a melting point between 218-221°C. It is soluble in water. It has a pKa value of 12.4 [1-3].

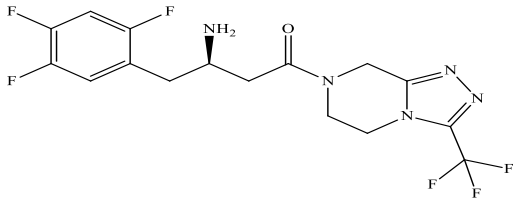

Fig. 1: Chemical structure of Sitagliptin

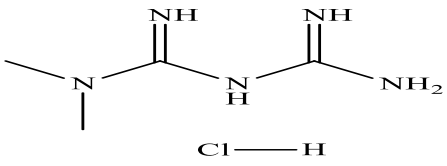

Fig. 2: Chemical structure of Metformin Hydro-chloride

Numerous analytical literature search reports have been addressed for the analysis of cited drugs alone or in combined FDCs with other therapeutic agents by exploring UV-HPLC [4-12], HPTLC [13-18] and LC-

MS/MS [19-25], in pharmaceutical matrices as well in bioanalytical samples. Consequently, none of these approaches have been deemed highly acceptable due to higher retention times of analytes, excessive consumption of polar organic solvents, more generation of waste, higher rate of flows, and unproductive analysis due to an operational cost. However, in order to overcome disadvantage associated with these analytical reports, the ultra-high-performance liquid chromatography (UHPLC) technique have been deemed extremely useful for enabling rapid determination of analytes, requiring lower process cycle time, ensures end-product efficiency by reducing operating costs and shortening run times, faster-resolving power, making it more selective and sensitive. Moreover, it uses a novel column material with a minimum particle size to improve sensitivity and reduce polar organic solvent's excessive consumption.

Therefore, the present work was accordingly undertaken by employing the merits mentioned earlier to design a cost-effective, rapid, and precise UHPLC assay method for quantifying Sitagliptin and Metformin in the pharmaceutical FDCs. Moreover, the application of proposed work to assessed intrinsic stability behavior of the Sitagliptin and Metformin under distinct conditions of stressors.

2. MATERIAL AND METHODS

2.1. Pharmaceutical standards and FDCs

Sitagliptin and Metformin pure drug samples were generously gifted by Amegh Pharma Pvt. Ltd., India. Janumet (label claim Sitagliptin- 50 mg and Metformin-1000 mg and Sitagliptin 50mg and Metformin 500mg) tablets manufactured by MSD Pharmaceuticals Pvt.Ltd., India, were procured from local market.

2.2. Chemicals and reagents

Methanol and Acetonirtrile (HPLC grade) were purchased from Merck, LTD., India, and potassium dihydrogen phosphate buffer & orthophosphoric acid (OPA) analytical grade were supplied from Loba Chemie Pvt. Ltd., India.

2.3. Instrumentation

Agilent UPLC System monitored by Empower 3 Software and fitted with UPLC BEH C_{18} column (150 mm × 2.1 mm, i.d., 1.7 µm particle size) and ACQ-PDA detector was used for the present analysis.

2.4. Selection of solvent

The solubility of Sitagliptin and Metformin were tested in various specified solvents; methanol was selected to be the best solvent for both analytes.

2.5. Preparation of stock standard solution

Standard stock solutions were prepared with the precise amount of 50 mg of Sitagliptin and 1000 mg of Metformin, dissolved in two different 100 mL flasks, consisting of 50 mL of methanol, stirred manually for 10 min. Finally, the volume was diluted to the point of the calibrated flask to obtained 500µg/mL and 10000µg /mL of Sitagliptin and Metformin concentrations.

2.6. Preparation of working standard solution

A working solution of Sitagliptin and Metformin was prepared by moving accurate volume of 0.1 mL into 10 mL of the calibrated flask from standard stock solutions, and to conclude, volume was diluted to the mark with the same to get the 5µg/mL and 100 µg/mL concentrations of Sitagliptin and Metformin respectively.

2.7. Selection of solvent system

The solvent system in a proportion of (90:10 % *v/v*) acetonitrile: potassium dihydrogen orthophosphate buffer; pH 3.0±0.2, adjusted with 0.1 % OPA, was ideally selected and before application, sonicated for 20 min and filtered through Ultipor® N_{66}® Nylon 6, 6 membrane 0.2 µm filter paper.

2.8. Optimization of chromatographic conditions

The Sitagliptin and Metformin from degradation products and, later, from tablet matrices are often meticulously resolved and quantified based on many variables such as polarities of Sitagliptin and Metformin, solubility of drugs into specific and combinations of solvents, and also on published findings. As a result, various solvent system compositions were used to determine the best solvent system composition for resolution and quantification of Sitagliptin and Metformin. Finally, according to appropriate system suitability checks, the solvent system comprising acetonitrile: potassium dihydrogen orthophosphate buffer (90:10 % *v/v*, 3.0±0.2 modified with 0.1 % OPA) demonstrated appropriate symmetrical peaks form and adequate resolution of both eluents. The overall analysis time for Sitagliptin and Metformin quantification was less than 4 minutes. A 10 µL of fixed volume (working solution) was injected, and the chromatogram was studied at a detection wavelength of 267 nm.

Table 1: Optimized chromatographic conditions

Chromatographic Mode	Chromatographic Conditions
UHPLC System	Agilent UPLC System
Detector	ACQ-PDA
Column	UPLC BEH C_{18} column (150 mm × 2.1 mm, i.d., 1.7 µm particle size)
Mobile phase	Acetonitrile: potassium dihydrogen orthophosphate buffer (90:10 % *v/v*, pH.3.0 adjusted with 0.1 % OPA)
Detection wavelength	267 nm
Flow rate	0.4 mL/min
Injection Volume	10 µL
Data analysis	Empower 3 Software

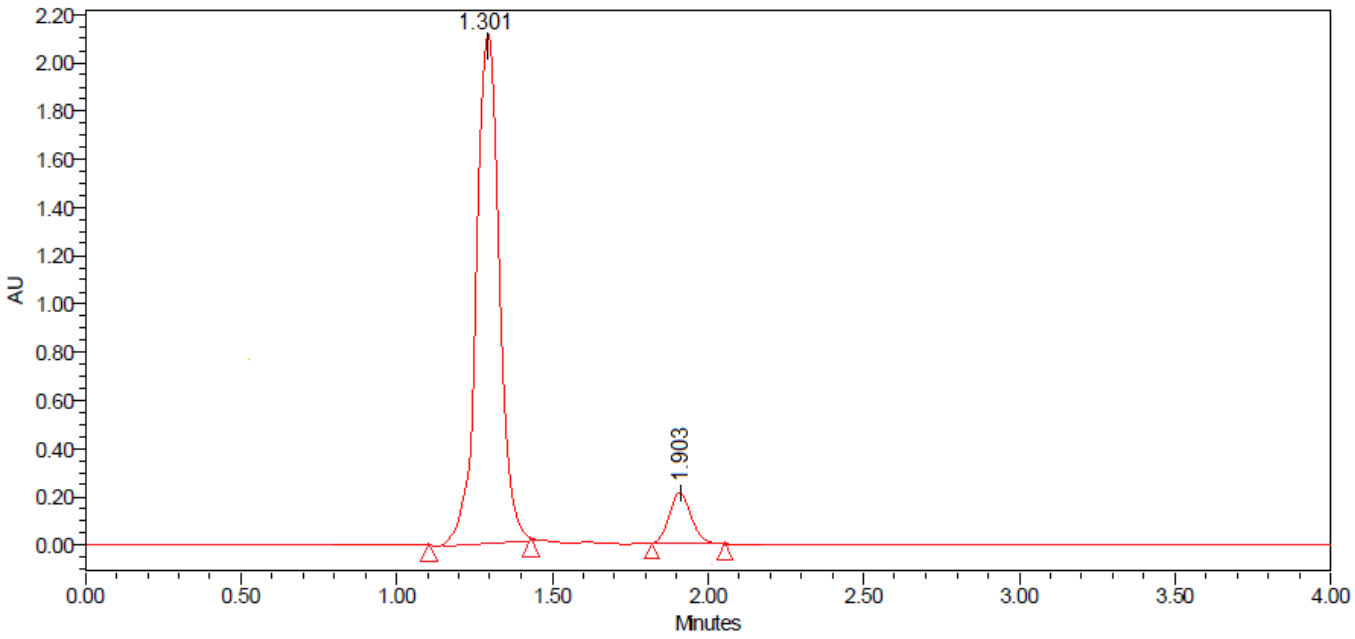

Fig. 3: Optimized chromatogram of Sitagliptin and Metformin

3. RESULTS AND DISCUSSION

3.1. System suitability test

The system suitability parameters were investigated using the concentrations (six determinations). Standard deviation (SD) and relative standard deviation (RSD) % were estimated for responses (peak area and R*t*). The RSD % values of responses were within a 2% range, suggesting that the system development was suitable. The tailing factor and the number of USP plates were both found to be within reasonable limits.

3.2. Calibration curve

The calibration curve for Sitagliptin and Metformin were assessed using 5-30µg/mL, and 100-600µg/mL concentrations of Sitagliptin and Metformin, respectively. The calibration curves of peak area against the µg/mL concentrations for Sitagliptin and Metformin were plotted and analyzed using the equation of linear regression in order to develop a relationship as a calibration curve. The determination coefficient (r^2 0.9996 for both drugs) of the calibration curve obtained from the line indicates the excellent connection between the peak area and the Sitagliptin and Metformin concentrations.

Table 2: System suitability test

Parameters	Estimates for Metformin	Estimates for Sitagliptin
Retention time (Rt) (min)	1.301±0.022	1.903±0.01
Theoretical Plates	2871.50±0.56	3544.36±0.11
Tailing factor	1.02±0.01	1.11±0.01
Resolution	2.34	

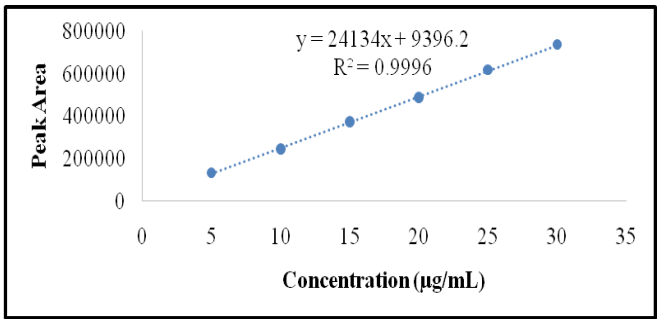

Fig. 4: Calibration curve for Sitagliptin

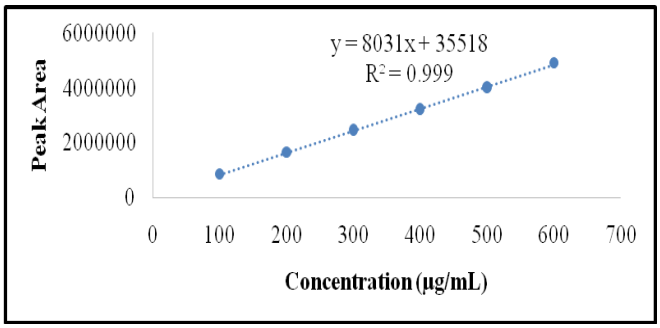

Fig. 5: Calibration curve for Metformin

3.3. Validation

The designed UHPLC method for Sitagliptin and Metformin was confirmed as per ICH recommendations.

3.3.1. Accuracy

The accuracy of the design UHPLC method for Sitagliptin and Metformin was addressed in the context of % recovery and accomplished at three distinct levels, i.e., 80%, 100%, and 120%. The % recovery was exercised by adding a fixed amount of Sitagliptin and Metformin standard to pre-analyzed tablet solution (Sitagliptin- 10 µg/mL and Metformin- 200 µg/mL).

3.3.2. Precision (Repeatability)

The precision analysis of the designed UHPLC method for Sitagliptin and Metformin were investigated for intra and inter-day and repeatability variability. The %RSD value for six replicate injections of concentrations of Sitagliptin and Metformin on intra and interday was within 2% indicating the method is repeatable.

3.3.3. Sensitivity

The method recorded LOD and LOQ values of 0.14µg/mL and 0.43µg/mL for Sitagliptin and 0.49µg/mL and 1.49µg/mL for Metformin, respectively.

Table 3: Accuracy studies for Sitagliptin and Metformin

Accuracy	Amount of Sitagliptin	% Recovery	Amount of Metformin	% Recovery
80	18.03±0.04	100.44±0.55	361.52±0.18	100.95±0.11
100	19.94±0.05	99.43±0.57	399.55±0.73	99.77±0.36
120	22.07±0.02	100.59±0.24	441.20±0.48	100.50±0.20

Table 4: Repeatability studies for Sitagliptin and Metformin

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
Sitagliptin	20	19.99	99.97
	20	19.92	99.61
	20	19.91	99.56
	20	19.981	99.90
	20	19.93	99.69
	20	19.93	99.66
	Mean±SD	19.47±0.03	99.73±0.16
	% RSD	0.16	0.16
Metformin	400	397.08	99.27
	400	396.25	99.06
	400	396.39	99.09
	400	396.69	99.17
	400	398.37	99.59
	400	400.10	100.02
	Mean±SD	397.48±1.49	99.37±0.37
	% RSD	0.37	0.37

Table 5: Repeatability studies for Sitagliptin and Metformin

Drug	LOD (µg/mL)	LOQ (µg/mL)
Sitagliptin	0.14	0.43
Metformin	0.49	1.49

3.3.4. Robustness

Robustness analysis of the designed UHPLC method was carried out by attempting to make significant changes in % proportion of acetonitrile in a solvent system, the temperature of the column oven compartment and flow rate.

3.3.5. Specificity and selectivity

The proposed method is quite well selective and specific. It was noticed that there was no other specific intervention was recorded around the R*t* of Sitagliptin and Metformin; neither the baseline exhibits a substantial unavoidable noise.

Table 6: Robustness studies for Sitagliptin and Metformin

Chromatographic Conditions	% RSD of Sitagliptin	% RSD of Metformin
Proportion of ACN (85 - 95 %)	0.30	0.28
Column oven temperature (25 - 35 ℃)	0.35	0.16
Flow rate (0.3- 0.5 mL/min)	0.37	0.45

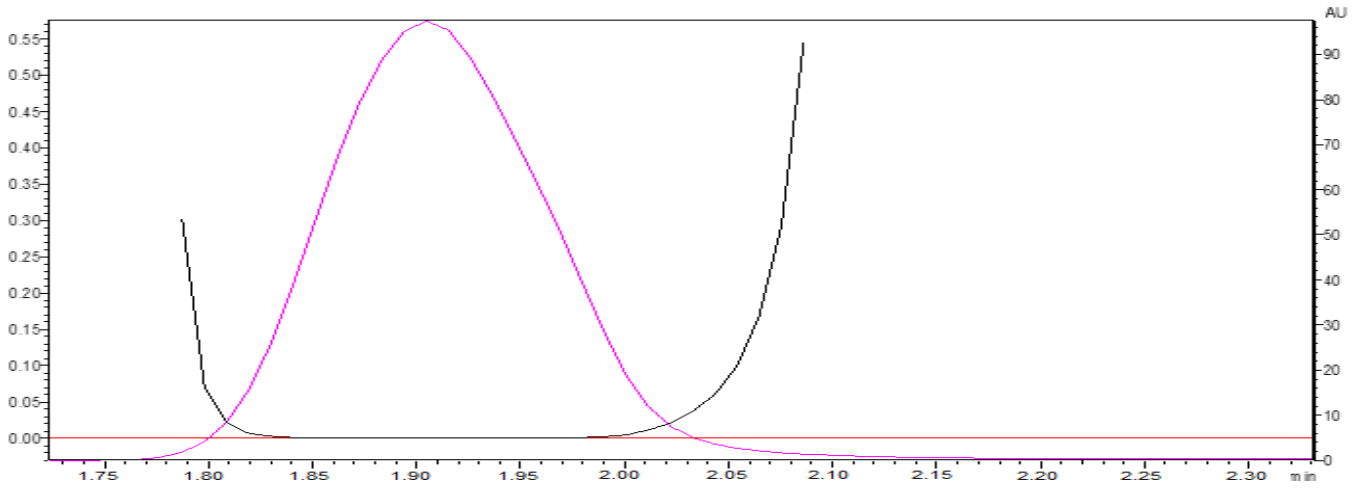

Fig. 6: Peak-purity spectrum for Sitagliptin

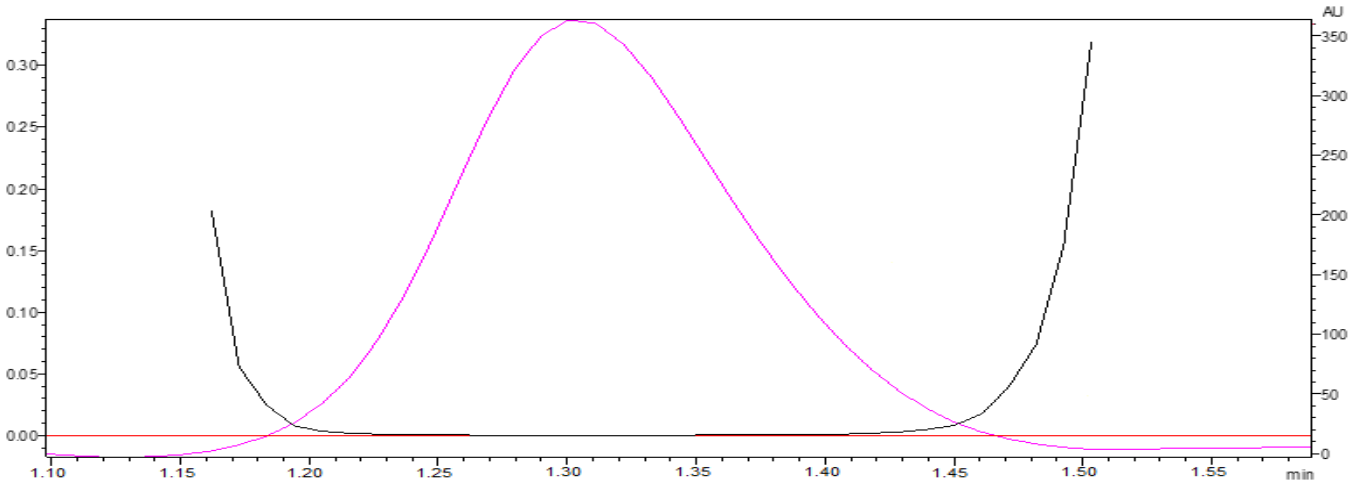

Fig. 7: Peak-purity spectrum for Metformin

3.4. Stress degradation studies

The present UHPLC method was used to address the intrinsic stability behavior of the Sitagliptin and Metformin under distinct conditions of stressors. It was investigated according to the Q1A (R2) guideline of ICH references for hydrolysis, oxidation, thermal (dry

heat and wet heat stress), and photolysis as per the references of Q1B. The slight changes in mobile phase composition and flow rate were made to resolve all the potential degradants.

3.4.1. Acidic hydrolysis

Acidic hydrolytic stress studies for Sitagliptin and Metformin were carried out by precisely solubilizing 10 mg of Sitagliptin and Metformin separately into calibrated flask consisting of 10 mL of 2 M methanolic HCl for Sitagliptin and 1 M methanolic HCl.

3.4.2. Alkaline hydrolysis

Alkaline hydrolytic stress studies for Sitagliptin and Metformin were investigated using precisely solubilizing 10 mg of Sitagliptin and Metformin separately into calibrated flask consisting of 10 mL of 2 M methanolic NaOH.

3.4.3. Neutral hydrolysis

To analyze the hydrolytic influence on Sitagliptin and Metformin in a neutral condition investigated. Subsequently, both analytes were practically insoluble in water. The stress of hydrolytic influence was initiated by precisely solubilizing 10 mg of Sitagliptin and Metformin discretely into a 10 mL calibrated flask with methanol as a stressor.

3.4.4. Oxidative degradation

Oxidative stress studies for Sitagliptin and Metformin were carried out by precisely solubilizing 10 mg of Sitagliptin and Metformin separately into a calibrated flask with 6 % $H_2O_2 v/v$.

3.4.5. Photodegradation

The photolysis of Sitagliptin and Metformin were performed using the solid samples (spreading as a thin layer on a petri dish) to the illumination of ≥360Wh/m^2 at 30°C with UV radiation, i.e., for short UV-254 nm and long UV-360 nm for 6 consecutive days.

3.4.6. Thermal degradation

3.4.6.1. Dry heat degradation

By approximately introducing 100 mg of Sitagliptin and 100 mg of Metformin separately into a sealed ampoule and placing it into the digital controlled thermostatic hot air oven at 80°C for 10hrs.

Table 7: Results of force degradation studies for Sitagliptin

Stressor conditions	Number of Degradants	Rt of degradants (min)	% Degradation
Acidic hydrolysis			
2 M HCL reflux for 80°C for 3 hrs	04	0.486	15.29
		1.702	23.14
		0.856	3.95
		2.261	0.86
Alkaline hydrolysis			
2 M NaOH at room temperature for 3 days	03	1.056	12.75
		1.157	29.91
		1.194	14.86
Neutral hydrolysis			
At room temperature for 7 days	0	Stable	
Oxidation			
6 % H_2O_2 at room temperature for 2 days	03	1.121	2.43
		1.651	2.38
		2.198	1.64
Photolysis			
≥360Wh/m2 at 30°C with UV radiation i.e., for short UV-254 nm and long UV-360 nm for 6 consecutive days	03	2.169	11.45
		2.315	9.82
		2.712	34.15
Thermal degradation			
Dry heat Sealed ampoule consisting of 50 mg of Sitagliptin at 80°C for 10 hrs	0	Stable	
Wet heat Digital controlled thermostatic hot air oven at 80°C for 5 hrs	02	1.586	2.53
		2.148	14.08

3.4.6.2. Wet heat degradation

Sitagliptin, and Metformin (1 mg/mL) stock solutions were kept in the digital controlled thermostatic hot air oven at 80°C for 5hrs.

Table 8: Results of force degradation studies for Metformin

Stressor conditions	Number of Degradants	Rt of degradants (min)	% Degradation
Acidic hydrolysis			
1 M HCL reflux for 60°C for 45 min	04	0.267	12.17
		0.368	17.85
		0.698	9.47
		2.334	0.85
Alkaline hydrolysis			
2 M NaOH at room temperature for 3 days	02	2.106	11.35
		2.358	26.14
Neutral hydrolysis			
At room temperature for 7 days	0	Stable	
Oxidation			
6 % H_2O_2 at room temperature for 2 days	01	1.803	13.41
Photolysis			
≥360Wh/m2 at 30°C with UV radiation i.e., for short UV-254 nm and long UV-360 nm for 6 consecutive days	04	0.302	0.87
		0.756	2.86
		0.864	11.12
		1.283	0.91
Thermal degradation			
Dry heat Sealed ampoule consisting of 100 mg of Metformin at 80°C for 10 hrs	03	0.523	1.06
		0.865	2.17
		1.983	4.25
Wet heat Digital controlled thermostatic hot air oven at 80°C for 5 hrs	01	0.613	16.73

4. CONCLUSION

For the simultaneous estimation of Sitagliptin and Metformin in pharmaceutical FDCs, a novel and rapid stability-indicating UHPLC assay method was developed and successfully validated. In all cases, the anticipated UHPLC method's capability to analyze the intact Sitagliptin and Metformin with no obstruction from impurities (degradation products) signifies the stability-indicating potential of the anticipated investigation and, consequently, addresses the specificity of the method. The developed UHPLC method was cost-effective, efficient, and specific, and it can be used for the quality control laboratories for quantification of Sitagliptin and Metformin in FDCs.

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Conflict of interest

None declared

Source of funding

None declared

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