IJPSR (2021), Volume 12, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 July 2020; received in revised form, 02 August 2021; accepted, 07 August 2021; published 01 September 2021

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF REVERSE PHASE ULTRA-FAST LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN PHOSPHATE AND METFORMIN HCL IN BULK AND TABLET DOSAGE FORM

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Keywords:

Sitagliptin phosphate, Metformin HCl, RP-UFLC, Validation, Force degradation

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ABSTRACT: A RP-UFLC method was developed &validated for simultaneous estimation of Sitagliptin Phosphate and Metformin HCl in bulk and tablet dosage form. Separation was achieved by using Phenomenex Luna C18 (150 × 4.6mm, 5µm) column. The mobile phase comprises MeOH: Phosphate Buffer of pH 7 using Triethylamine in the ratio (60:40v/v). The flow rate was 0.6ml/min at temperature 300C. The instrument used for UFLC was Shimadzu autosampler separation model LC 20AD. Quantification was achieved with UV detection using a photodiode array detector at 258nm wavelength. The injection volume was 20µl. The retention times of Sitagliptin Phosphate & Metformin HCl were found to be 7.027 & 2.802, respectively. The linearity range of Sitagliptin Phosphate was 50-100µg/ml & Metformin HCl was 10-60µg/ml. The correlation coefficient was found to be within limits, *i.e.,* 0.999. The % purity of Sitagliptin Phosphate & Metformin HCl was found to be 101.99% and 101.73%. LOD values were found to be 2.21437 for Sitagliptin Phosphate & 1.99084 for Metformin HCl and LOQ values were found to be 6.71024 for Sitagliptin Phosphate & 6.03286 for Metformin HCl. % RSD of both the drugs were found within the acceptance criteria, *i.e.,* <2. Forced degradation studies were also performed to check the stability of the drugs under acidic, basic, oxidation, thermal, photolytic conditions.

INTRODUCTION:

UFLC: (Ultra-Fast Liquid Chromatography): Ultrafast liquid chromatography (UFLC) is a new revolution in chromatography. UFLC is ten times faster & three times better separation than other conventional liquid chromategraphy. UFLC is a derivative of UPLC.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(9).4870-76

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(9).4870-76

UFLC gives speed that is ten times faster and separation that is three times better than UPLC. So that's why the state of being the importance of UFLC is called prominence UFLC, which provides excellent speed and low system backpressure. It is based on high analysis precision & reliability.

Drug Description:

Sitagliptin Phosphate: Sitagliptin increases insulin production and decreases hepatic glucose overproduction. Sitagliptin prolongs the action of GLP-1 and GIP. By enhancing active incretin levels, sitagliptin increases insulin production and lowers glucagon secretion from alpha cells, which decreases hepatic glucose overproduction.

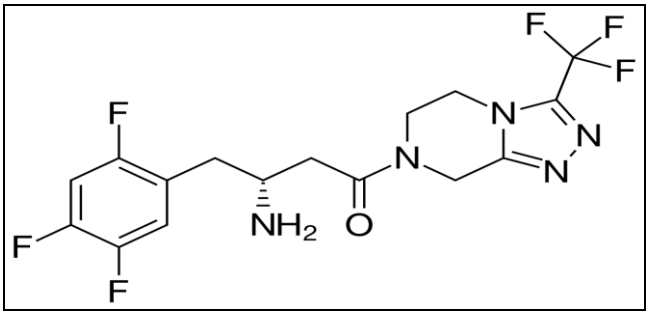

FIG. 1: CHEMICALLY IT IS CALLED AS (3R)-3-AMINO - 1 - [3 - (TRIFLUOROMETHYL) - 6, 8-DIHYDRO-5H [1, 2, 4] TRIAZOLO [4,3-A]PYRAZIN-7-YL]-4-(2,4,5-TRIFLUOROPHENYL)BUTAN-1-ONE.

Metformin HCl: Metformin is a biguanide derivative, it decreases the blood glucose levels by decreasing the hepatic glucose production ,decreases the intestinal absorption of glucose and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization.

The literature survey stated that there are many analytical methods available for Sitagliptin Phosphate & Metformin HCl alone and in combination with each other and other combinations. But only RP-HPLC and other methods have been reported for simultaneous estimation of Sitagliptin & Metformin HCl. The objective of the work is to develop an RP-UFLC method for the simultaneous estimation of Sitagliptin Phosphate & Metformin HCl.

To validate the RP-UFLC method according to ICH guidelines like specificity, system suitability, precision, linearity, accuracy, assay, robustness, ruggedness, *etc*.

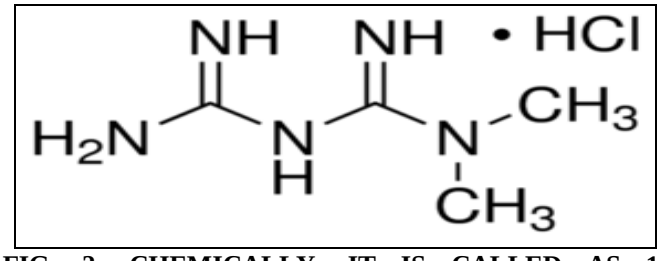

FIG. 2: CHEMICALLY, IT IS CALLED AS 1-CARBAMIMIDAMIDO - N, N-DI-METHYL METHAN IMIDAMIDE HYDROCHLORIDE

MATERIALS & METHODS:

Chemicals and Reagents: Potassium Dihydrogen Orthophosphate (AR Grade), Milliporewater, Triethylamine, Ortho Phosphoric Acid, Aceto-nitrile, Methanol (HPLC Grade).

Drug Samples: Sitagliptin phosphate has a potency of 99.98%, was procured from Dr. Reddy's laboratory, and Metformin hydrochloride having a potency 99.97% was procure from FDC LMT Verna Goa.

Instruments used: A UFLC- Shimadzu instrument & Lab solution software was used. Separation was achieved on column Luna C18 (150 × 4.6mm, 5µ). The autosampler Prominent-LC 20AD injector with reciprocating pump.

PDA detector was used. The weighing balance was used was unibloc & Ultrasonicator was branson-1800. The UV-Spectrophotometer used were UV-1800 Shimadzu with 1mm matched quartz cells integrated with UV probe 2.3.2 software was used for measuring the absorbance of Sitagliptin Phosphate & Metformin HCl respectively.

Methodology:

Diluent: By performing solubility and literature survey, Sitagliptin phosphate and Metformin hydrochloride are both soluble in water, methanol, and acetonitrile. So that water and mobile phase was used as a binary diluent.

Preparation of Buffer: 2.873 grams of Potassiumdihydrogenortho phosphate was weighed and transferred to 1000ml Millipore water in 1000ml of beaker. The pH was adjusted using triethylamine and filter by using 0.2 µ nylon filter and degassing for 10min.

Preparation of Mobile Phase: Phosphate buffer (pH 7) +MeOH in a ratio 40:60and filter through 0.2µ nylon filter and degassing for 10 min.

Preparation of Standard Solution of Sitagliptin Phosphate & Metformin HCl: Accurately weighed 10mg of pure drug in 10ml of volumetric flask add 10ml of Millipore water dissolve and sonicate for 5min to get 1000µg/ml stock solution of Sitagliptin Phosphate and Metformin HCl.

Preparation of Working Standard Solution: From standard solution 0.5ml of Sitagliptin Phos-phate and 0.1ml of Metformin HCl solution was pipetted out into 10ml volumetric flask & made up with the diluent to get the concentration of 50µg/ml of Sitagliptin & 10µg/ml Metformin solution.

Preparation of Sample Stock Solution: Twenty tablets were weighed and crushed to a fine powder. Tablet powder equivalent to 50mg sitagliptin phosphate and 500mg of metformin hydrochloride was taken and dissolve in 50ml Millipore water sonicated for 30 min to get 1000µg/ml of Sitagliptin Phosphate and Metformin HCl.

Preparation of Sample Working Standard Solution: From the above sample stock solution 0.5ml of Sitagliptin Phosphate and 0.1ml of Metformin HCl were pipetted out into 10ml volumetric flask & made up with a diluent to get 50µg/ml of Sitagliptin Phosphate& 10µg/ml Metformin HCl.

Method Development: The mobile phase was chosen after several trials to reach the optimum stationary/mobile phase matching. The flow rate is 0.6ml / min. The retention times under the conditions described were 2.803 min, 7.027 min for Metformin HCl and Sitagliptin Phosphate, respectively **Table 1** and **Fig. 3.**

TABLE 1: OPTIMISE METHOD

Mobile phase	Phosphate buffer (pH 7) : MeOH (40:60)
Diluent	Binary diluent -Water and mobile phase
Column	Luna C18 (150 × 4.6mm, 5µm)
Pressure	115kgf/c
Temperature	30 ^{O}C
Flow rate	0.6ml/min
Injection volume	20µl
UV Wavelength	258nm

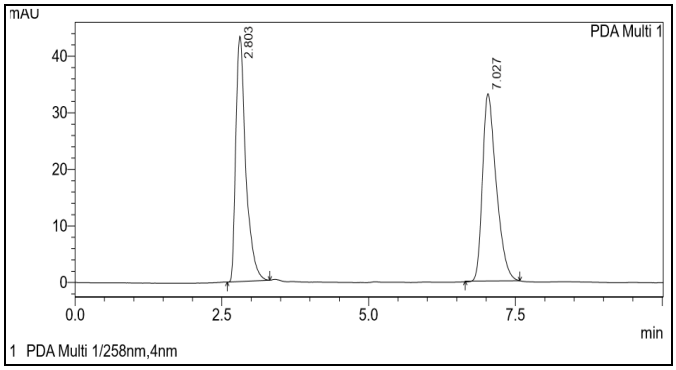

FIG. 3: OPTIMISE CHROMATOGRAM

RESULTS AND DISCUSSIONS:

Method Validation:

Linearity: The linearity was determined by preparing serial dilutions 6 concentrations of standard stock solutions each in triplicate. Average area was calculated and graph was plotted by taking average peak area vs concentration (µg/ml). The linearity range was found to be 50-100µg/mL for Sitagliptin Phosphate and 10-60µg/mL for Metformin HCl. The correlation coefficient was found to be 0.999 for both drugs.

Specificity: The specificity of the UFLC method was demonstrated by interference check by injecting the diluent blank and placebo solution to determine whether any peak in diluent and placebo solution are co-eluting with the peaks of Metformin HCl and Sitagliptin Phosphate.

Sensitivity: Limit of detection and quantification was calculated by using statistical calculations using the formula:

$$LOD= 3.3\times\sigma/S \ \&$$

$$LOQ= 10\times\sigma/S$$

Where, σ = standard deviation. S = slope of the calibration curve.

System Suitability: In order to check the suitability of instrument system suitability was performed by injecting 100 µg/ml & 60 µg/ml of Sitagliptin Phosphate and Metformin HCl solution in six replicates. Chromatograms were obtained and % RSD was calculated (< 2).

Precision: Developed method was found to be precise as the results of intraday and interday was found to be within the acceptance [6]. replicates of 100µg/ml and 60µg/ml of Sitagliptin Phosphate and Metformin HCl respectively were prepared.

Ruggedness: The ruggedness was performed by a change in instrument and by a change in analyst [6]. replicates of 100µg/ml and 60µg/ml of Sitagliptin Phosphate and Metformin HCl respectively were prepared and injected. %RSD was found within limits.

Robustness: It was performed by a slight change in conditions such as flow rate, wavelength, temperature, pH. % RSD was calculated it was less than 2.

Assay: Assay was carried out using the following formula.

Area of Sample × Std Weight × Dilution Factor × Percentage Purity × 100 % Area of Std × Dilution Factor × Sample Weight × 100

Average Weight of 20 Tablets (Janumet 50/500) = 709.427mg. Equivalent Weight = 50mg of Sitagliptin Phosphate & = 500mg of Metformin HCl.

Accuracy: Accuracy was performed in three levels 50%, 100%, and 150%. The mean value was calculated. % Recovery was found within limits.

TABLE 2: LINEARITY DATA OF SITAGLIPTIN PHOSPHATE & METFORMIN HCL

Sitagliptin Phosphate		Metformin HCl	
Conc(µg/ml)	**Peak Area**	**Conc(µg/ml)**	**Peak Area**
50	349492	50	349492
60	394322	60	394322
70	436655	70	436655
80	486740	80	486740
90	523553	90	523553
100	573628	100	573628

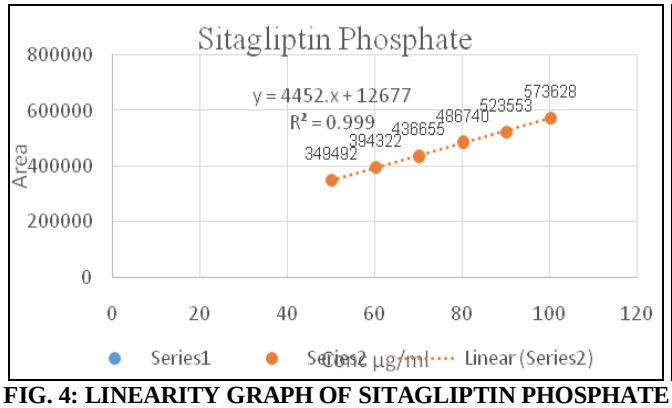

FIG. 4: LINEARITY GRAPH OF SITAGLIPTIN PHOSPHATE

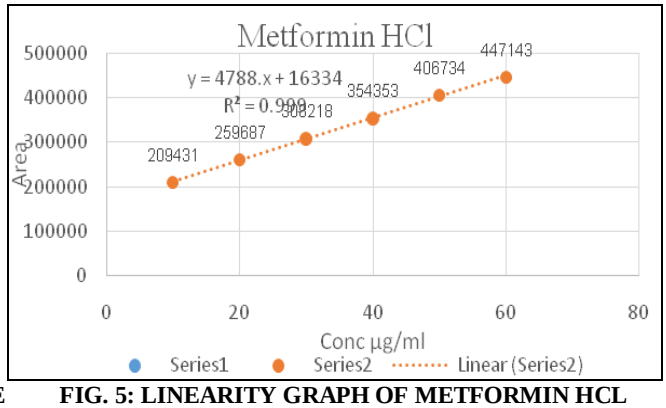

FIG. 5: LINEARITY GRAPH OF METFORMIN HCL

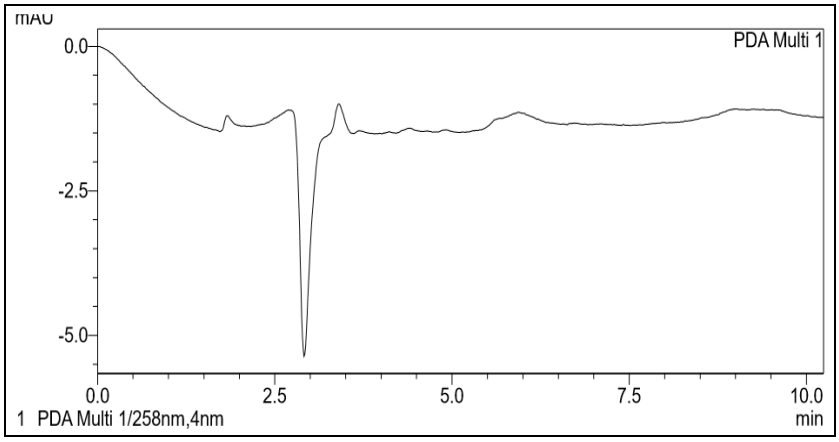

FIG. 6: CHROMATOGRAM OF PLACEBO SOLUTION

TABLE 3: SENSITIVITY DATA OF SITAGLIPTIN PHOSPHATE & METFORMIN HCL

Sample	LOD	LOQ
Sitagliptin Phosphate	2.214379	6.71024
Metformin HCl	1.990846	6.032867

TABLE 4: SYSTEM SUITABILITY DATA OF SITAGLIPTIN AND METFORMIN

S. no.	Metformin HCl				Sitagliptin Phosphate			
Injection	**RT (min)**	**USP plate count**	**Tailing**	**Peak area**	**RT (min)**	**USP plate count**	**Tailing**	**Peak area**
1	2.799	2409.4	1.635	602397	7.219	4552.5	1.479	714529
2	2.799	2413.5	1.63	602677	7.198	4579.5	1.472	714478
3	2.807	2419.9	1.631	601656	7.194	4555.2	1.476	716832
4	2.803	2418.8	1.65	602240	7.167	4501.5	1.475	715301
5	2.794	2413.7	1.645	600346	7.17	4517.8	1.477	715961
6	2.804	2425.8	1.636	600262	7.168	4497.9	1.472	711948
%RSD	0.1643	0.2409	0.4875	0.1754	0.2944	0.7304	0.1889	0.2343

TABLE 5: PRECISION DATA OF SITAGLIPTIN AND METFORMIN

Conditions	Intraday Precision			Interday Precision		
	1st H	2nd H	3rd H	1st Day	2nd Day	3rd Day
Area of Metformin	656272.3	577507	578344	561045.8	578798.3	637804
%RSD of Metformin	1.040551	0.200099	0.660399	0.742175	1.296464	1.10949
Area of Sitagliptin	760832.8	768765.2	766314.3	773055.5	730710.7	735145.7
% RSD of Sitagliptin	0.123362	0.524053	0.343098	0.602975	0.142768	0.231653

TABLE 6: RUGGEDNESS DATA OF SITAGLIPTIN AND METFORMIN

Conditions	Peak Area		%RSD	
	Metformin	Sitagliptin	Metformin	Sitagliptin
Change in analyst	563680.5	781241.2	0.114845	0.570576
Change in instrument	601596.3	714841.5	0.175452	0.234322

TABLE 7: ROBUSTNESS DATA OF SITAGLIPTIN AND METFORMIN

Conditions	Peak area	%RSD	Peak area	%RSD
	Metformin HCl		Sitagliptin Phosphate	
pH (7.04)	564123	1.647324	741509.5	0.154173
Wavelength (259nm)	441351.8	0.529722	657200.3	0.167715
Temperature (25 ℃)	577103	1.57225	740701.2	0.05159
Flow rate (0.62ml/min)	544265	0.15896	757827	0.190395

TABLE 8: ASSAY DATA OF SITAGLIPTIN PHOSPHATE & METFORMIN HCL

S. no.	Sitagliptin Phosphate		Metformin HCl	
Injection	Area	RT	Area	RT
1	797703	7.040	599291	2.784
2	797194	7.034	588564	2.790
3	794188	7.037	590219	2.784
Average	796381		592691.3	
% Assay	101.99		592691.3	

TABLE 9: ACCURACY DATA OF SITAGLIPTIN PHOSPHATE & METFORMIN HCL

Accuracy data of Sitagliptin Phosphate					
Levels	Std Conc	Sample Conc	Recovered Amount	Peak Area	% Recovery
50%	50	25	74.81	541222	100.2%
100%	50	50	105.20	568223	105.2%
150%	50	75	134.15	579684	107.32%
Accuracy data of Metformin HCl					
Levels	Std Conc	Sample Conc	Recovered Amount	Peak Area	% Recovery
50%	20	10	29.98	474849	99.95
100%	20	20	41.07	487887	102.69
150%	20	30	52.76	501395	105.54

Stability: Stability of drugs was checked by bench and freeze conditions at 24 and 74 h.

TABLE 10: STABILITY DATA OF SITAGLIPTIN PHOSPHATE AND METFORMIN HCL

Conditions	Sitagliptin Phosphate			Metformin HCl		
Bench	Average	Std. dev	%RSD	Average	Std. dev	%RSD
24H	620676	658.3102	0.106063	576757	5829.891	1.010806
72H	617559	4942.203	0.800281	575482	9817.605	1.705978
Freeze						
24H	715959	8447.873	1.179938	614132	6347.971	1.033649
72H	722819	2503.407	0.346339	612552	8676.739	1.416491

Force Degradation: Degradation was carried out in 5 different stress conditions like acidic, basic, oxidation, photolytic, thermal conditions at 60-80 ℃ in the water bath & in reflux condition. The limit of forced degradation is 5-20%.

Initial area - Final area % Initial area × 100

TABLE 11: DEGRADATION DATA OF SITAGLIPTIN PHOSPHATE AND METFORMIN HCL

Study	Sitagliptin Phosphate			Metformin HCl		
	Initial	Final	%Degradation	Initial	Final	%Degradation
Acidic	741061	658313	11.16%	562103	501321	10.81%
Basic	733196	628091	14.33%	572131	520066	9.10%
Thermal	739096	600435	18.76%	579311	517916	10.59%
Oxidative	749101	637864	14.84%	559991	507084	9.44%
Photolytic	740866	601004	18.87%	562523	507079	9.85%

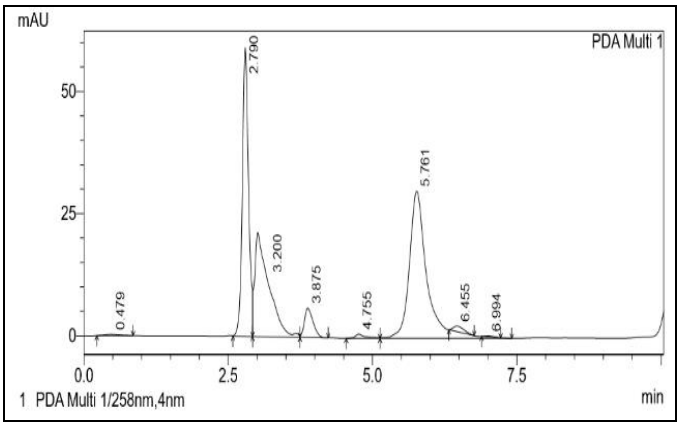

FIG. 7: ACIDIC DEGRADATION

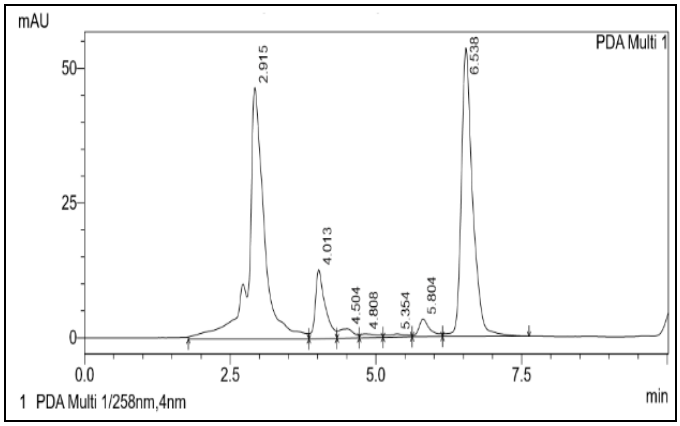

FIG. 8: BASIC DEGRADATION

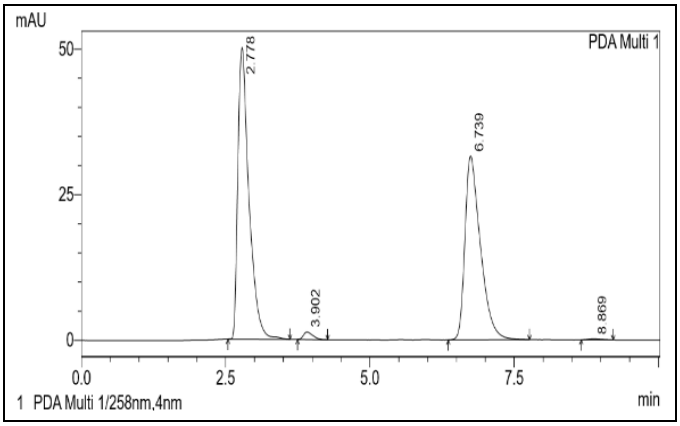

FIG. 9: THERMAL DEGRADATION

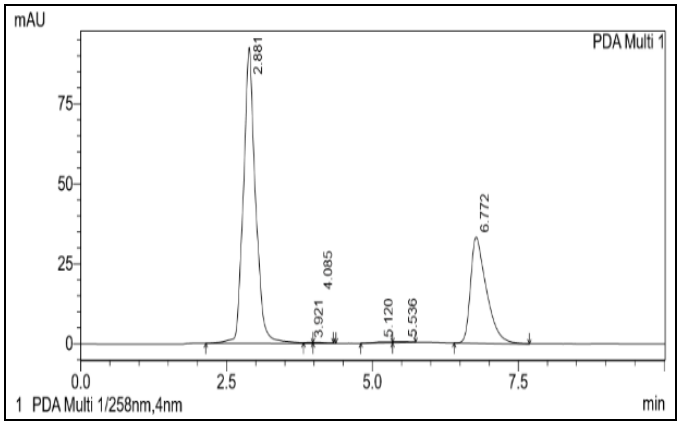

FIG. 10: OXIDATIVE DEGRADATION

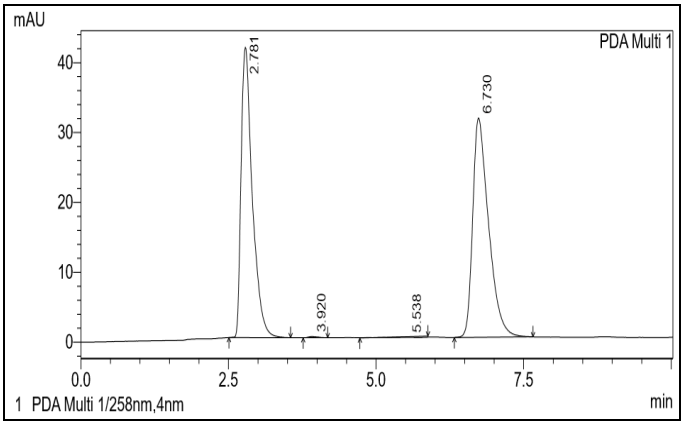

FIG. 11: PHOTOLYTIC DEGRADATION

CONCLUSION: In the present investigation, a simple, sensitive, precise and accurate RP-UFLC method for simultaneous estimation of Sitagliptin Phosphate & Metformin HCl was developed and validated as per ICH guideline & stability study displayed that techniques were helpful in monitoring drug stability & conjointly could also be applied for routine analysis in research

institutions, in quality control division of pharmaceutical industries. Both the drugs were resolved on Luna 5u C18 (150 × 4.6 mm, 5µm) column using MeOH: Phosphate buffer (pH7) using triethylamine. Flow rate 0.6ml/min. UV detection was performed at 258nm. Assay of resulting formulation shows within limits *i. e. 90-*110. Hence, it is concluded that developed method are suitable for simultaneous estimation of Sitagliptin Phosphate & Metformin HCl.

ACKNOWLEDGMENT: The authors are thankful to Principal Dr. S. S. Jalalpure and Former Principal Dr. BM Patil and Vice Principal Dr. MB Patil, Dr. Shankar G. Alegaon, Professor and Head Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Belagavi for providing necessary facility, support and guidance to carry out present research work.

CONFLICT OF INTEREST: There is no conflict of interest in the work presented in the manuscript.

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How to cite this article:
Maruti MS, Shivabasappa PM, Chouhan MK, Uday TC, Sanjay SS and Jalalpure SS: Stability indicating method development and validation of reverse phase Ultra Fast Liquid Chromatography method for simultaneous estimation of sitagliptin phosphate and metformin HCL in bulk and tablet dosage form. Int J Pharm Sci & Res 2021; 12(9): 4870-76. doi: 10.13040/IJPSR.0975-8232.12(9).4870-76.