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RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN HYDROCHLORIDE, ROSIGLITAZONE AND SITAGLIPTIN – APPLICATION TO COMMERCIALLY AVAILABLE DRUG PRODUCTS

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ABSTRACT

A simple, precise and stability-indicating HPLC method was developed and validated for the simultaneous determination of anti-diabetic drugs Metformin hydrochloride (MT), Rosiglitazone (RT) and Sitagliptin (ST). The separation was achieved on ACE 3 150 mm X 4.6mm, 3.5µm column using gradient method. The mobile phase at a flow rate of 1.5 mL min^{-1} consisted of 10 mM sodium hexane sulphonate monohydrate and 10 mM Potassium dihydrogen phosphate buffer with acetonitrile and methanol in Gradient ratio. The UV detection was carried out at 210 nm. The forced degradation for these drug substances were performed under Acid, Base, Oxidative, Photolytic and Thermal stress conditions. The method was successfully validated in accordance to ICH guidelines. Further, the validated method was applied for commercially available pharmaceutical dosage form.

INTRODUCTION: The parent guideline on drug stability testing Q1A (R2) issued by International Conference on Harmonization (ICH) stipulates stress studies to be carried out on a drug in order to establish the drug's inherent stability characteristics [1, 2].

Literature studies show various analytical methods reported for the estimation of individual, binary or tertiary combination of anti-diabetic drugs or in combination with diuretics [3-11]. Recently A high performance liquid chromatography method for the quantitative determination assay of Sitagliptin in rat plasma has been reported [12]. While, the HPLC method has been developed and validated for quantification of Metformin in human plasma using ion-pairing agent.[13] LC/MS and LC/UV method is also available for the identification of Metformin in plasma sample [14]. Sitagliptin also been quantified in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectrometry [15]. However, so far, no method was reported for the simultaneous determination in combination for Metformin, Rosiglitazone and Sitagliptin and its application to pharmaceutical samples. An attempt was made in this study to develop a rapid, economical, precise and accurate stability-indicating assay method for simultaneous estimation of Metformin, Rosiglitazone and Sitagliptin in tablet formulation.

The proposed method is rapid, simple, accurate, and reproducible, and can be successfully employed in the routine analysis of both these drugs simultaneously, in tablet dosage form.

QUICK RESPONSE CODE

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EXPERIMENTAL:

Chemicals and reagents: Drug substances were provided by Getz Pharmaceuticals Research Pvt. Ltd., India. All the chemicals and reagents sodium hydroxide, hydrochloric acid, potassium dihydrogen phosphate, hydrogen peroxide (50%) were used of Analytical grade. While, Acetonitrile and Methanol was procured from Merck (Germany). A Millipore Milli Q plus water purification system (Milford, USA), was used to prepare distilled water (conductivity >18 µΩ). The commercially available drug products were used as Glycomet (Metformin HCl, 500mg- USV Limited), Januvia (Sitagliptin phosphate tablets- 50mg-MSD Pharmaceuticals Pvt. Ltd.) and Windia (Rosiglitazone Tablets, 2 mg, Glaxo Smith Kline).

Instruments: Integrated HPLC system, manufactured by waters (USA) was used for method development and method validation. This system comprised of a quaternary gradient pump, auto sampler, column oven and a photodiode array detector. PC installed Empower was used to record and integrates the chromatograms. The analysis was carried out at ambient temperature. Photostability studies were performed in a photostability chamber, from Thermolab (India).

Chromatographic conditions: ACE 3 (150 mm × 4.6 mm, 3.5 µm) analytical column was used as a stationary phase. The flow rate was 1.5 mL min^{-1} and the detector was set at 210 nm. The injection volume was set as 20 µL. The gradient mobile phase consisted of 10 mM each of sodium hexane sulphonate monohydrate and Potassium dihydrogen phosphate buffer with acetonitrile and methanol with the gradient as mentioned in **Table 1**. A membrane filter of 0.45 µm porosity was used to filter and degas the mobile phase.

TABLE 1: GRADIENT FOR CHROMATOGRAPHIC METHOD

Time (min)	% 10 mM Potassium dihydrogen Phosphate and 10 mM Sodium Hexane sulphonate monohydrate buffer	% Acetonitrile	% Methanol
0	80	20	0
3	80	20	0
10	50	40	10
11	50	40	10
12	80	20	0
15	80	20	0

Standard and Test solutions: Weighed accurately about 50 mg of each Metformin hydrochloride, Rosiglitazone and Sitagliptin reference standard into 100 ml volumetric flask. Added to it 70 ml diluent i.e. Water:ACN:70:30 %v/v having pH 3.0 and sonicated to dissolve, cool and dilute up to volume using same diluent. Pipette out 5.0 ml of this solution into 50 ml volumetric flask and diluted to volume with diluent. (50 µg/mL each of Metformin hydrochloride, Rosiglitazone and Sitagliptin). Similarly, the test solutions were prepared at same concentration using same diluents. (50 µg/mL of each).

A. **Method Development:** A variety of mobile phases were investigated in the development of a stability-indicating LC method for the analysis of Metformin hydrochloride, Rosiglitazone and Sitagliptin Phosphate drug substances. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the active peak and separation among impurities formed during forced degradation studies.

1. **Wavelength Selection**: The individual drug substance solution at concentration of 50µg mL^{-1} in diluent was scanned on PDA from 200nm to 400nm. The maximum wavelength was observed Metformin HCl (232nm), Rosiglitazone (232nm and 318nm) and Sitagliptin phosphate (267nm). However detection was carried out at 210 nm on basis of optimum response for all of these drug substance(**Fig. 1 and Table 2**).

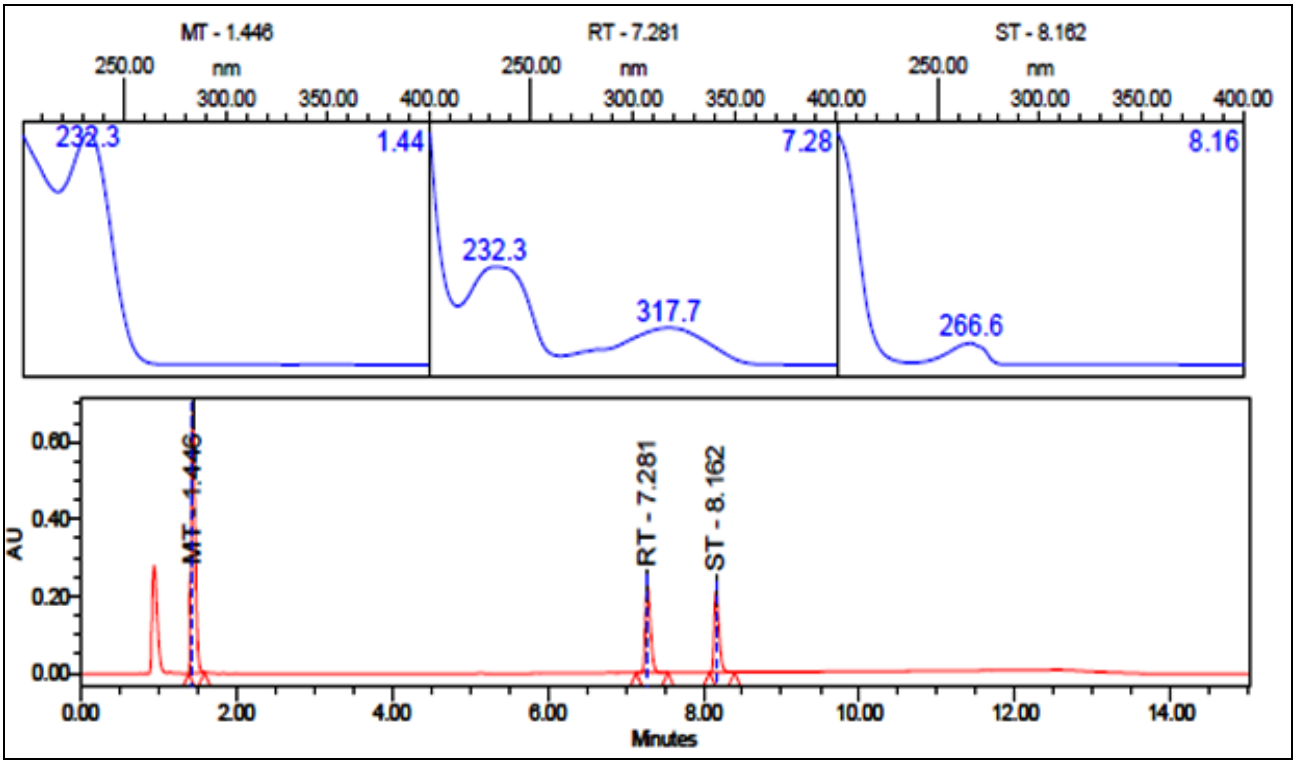


FIG. 1: UV SPECTRA FOR ALL DRUG SUBSTANCES

TABLE 2: AREA RESPONSE OF PEAKS AT DIFFERENT WAVELENGTHS

Wavelength (nm)	MT	RT	ST
208	**2425392**	1227521	**1114220**
210	2326804	1011344	909710
220	2137907	1054194	142122
230	2754904	**1440753**	23850
240	2215073	1405000	14608
250	589147	824912	44531
260	48835	161670	113378

B. **Method Validation:** The optimized chromatographic conditions were validated by evaluating specificity-Forced degradation, linearity, precision, accuracy, robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1) [2].

1. **Specificity- Forced degradation study:**

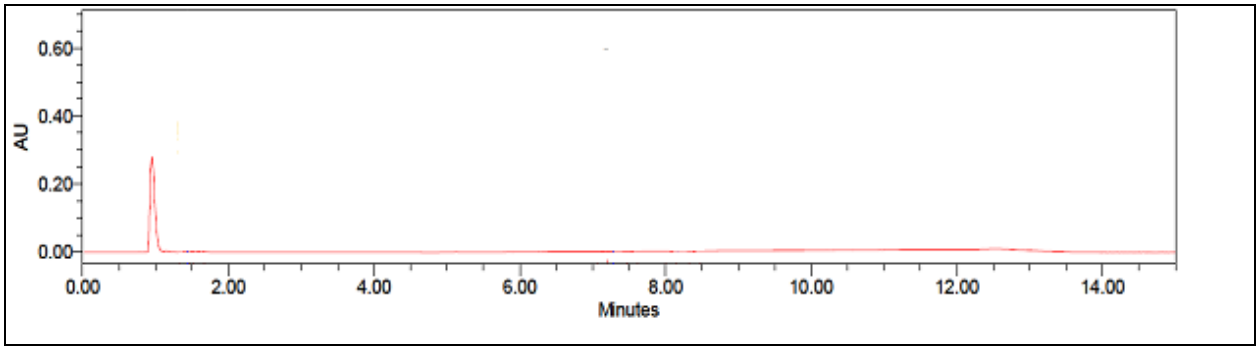
a. **Acid Hydrolysis:** Forced degradation study was conducted on 50 mg/mL of each drug solution. The solution mixture was prepared by mixing 50 mg of each drug in 100 mL flask. This was considered as a stock solution. To 5mL of stock solution 5 mL of 1N hydrochloric acid was added and the solution was exposed at 60°C for 30 minutes. Then neutralized with base, cooled and diluted up to 50 ml with diluent.

b. **Base Hydrolysis:** Forced degradation study was conducted on 50 mg/mL of each drug solution. The solution mixture was prepared by mixing 50 mg of each drug in 100 mL flask. This was considered as a stock solution. To 5mL of stock solution 5 mL of 1N Sodium hydroxide acid was added and the solution was exposed at 60°C for 30 minutes. Then neutralized with base, cooled and diluted up to 50 ml with diluent.

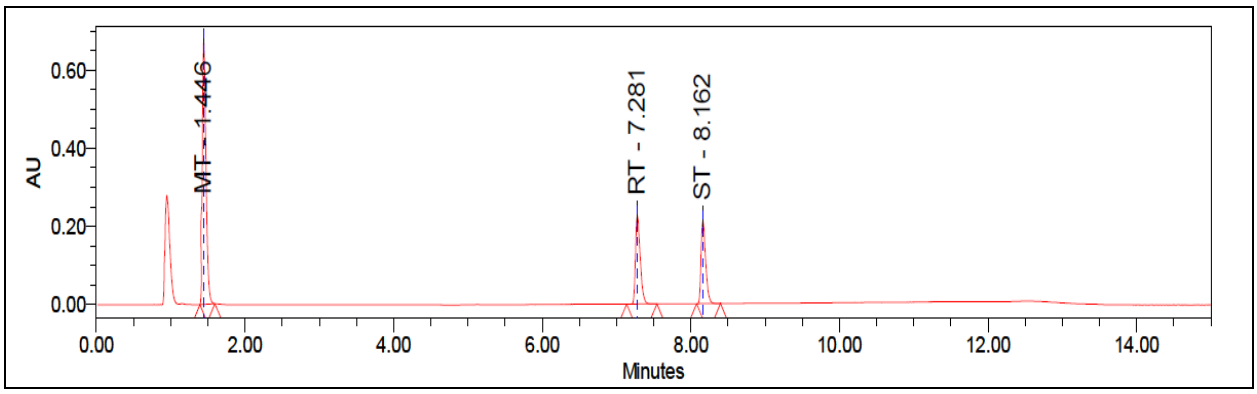
c. **Oxidation:** Forced degradation study was conducted on drug substances by exposing with 50% H_2O_2 for 30 minutes at 60°C.

d. **Thermal degradation**: The thermal degradation performed in wet condition. 5mL stock solution was kept in dry oven at 105°C for 24 hours.

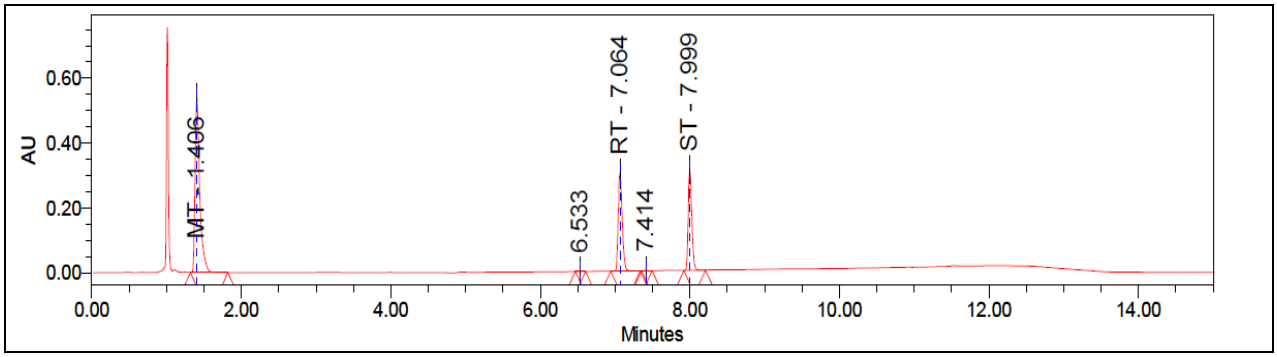
e. **Photolysis:** Photolysis studies were carried out on stock solution in 50 mL volumetric flask. The sample was exposed to light in a photo-stability chamber for 24 hours using transparent as well as Amber colored flask as a control. The analytical data obtained under each of above stressed conditions were collected by PDA detector and used for peak purity evaluation.



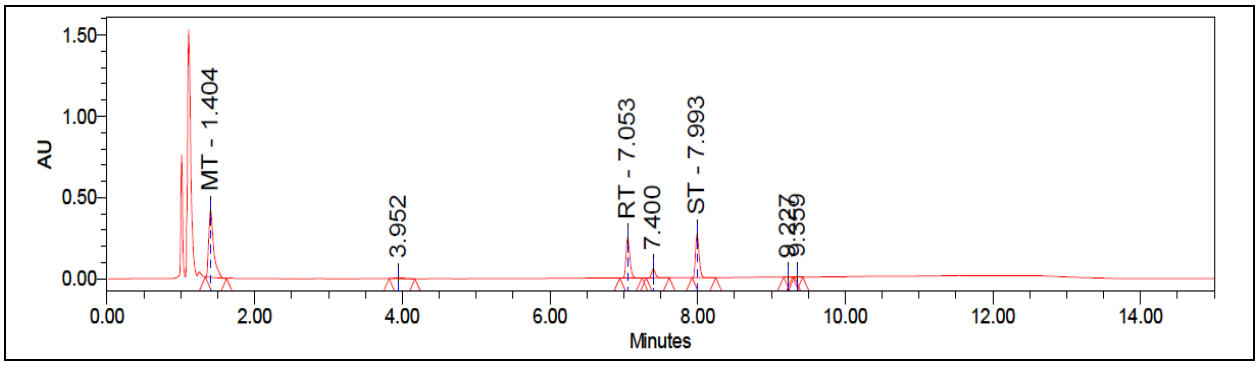
Standards solution



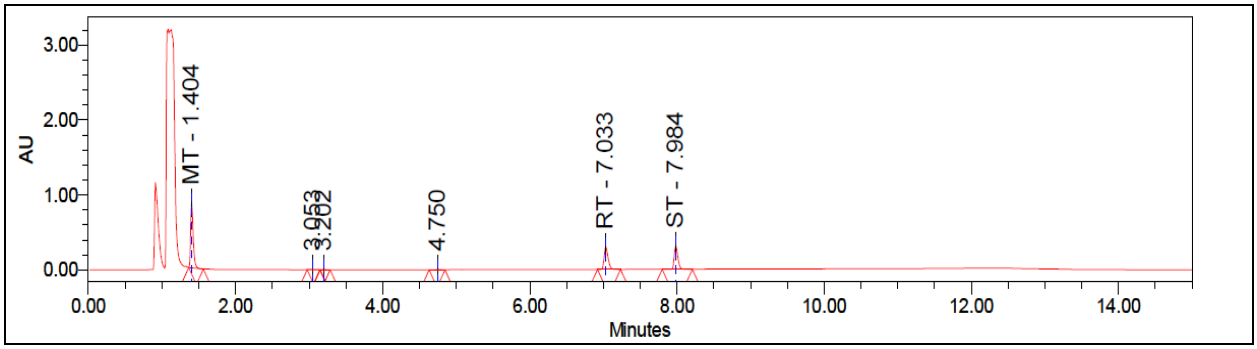
Specificity-Acid hydrolysis



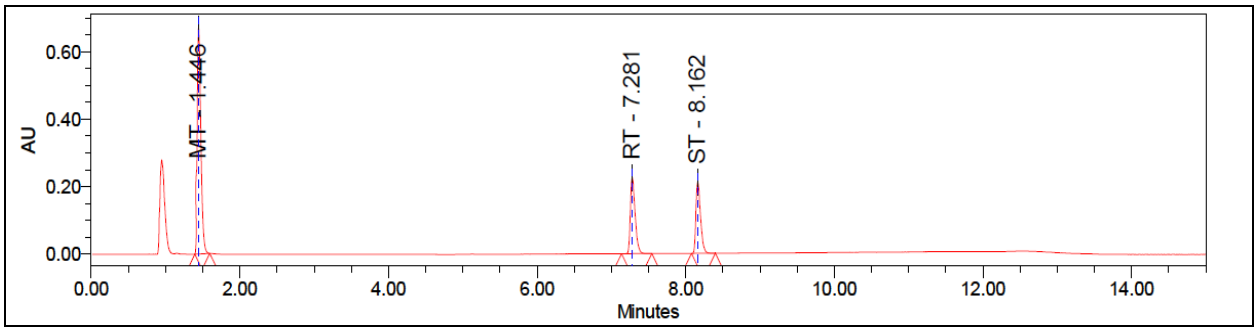
Specificity-Alkaline hydrolysis



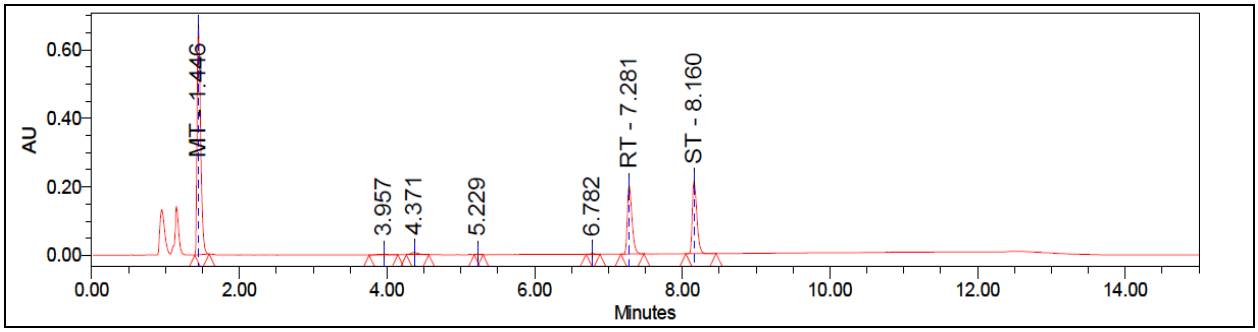
Specificity-Peroxide oxidation



Specificity Photolytic degradation (Amber glass)



Specificity Photolytic degradation (Transparent glass)



Specificity-Thermal degradation

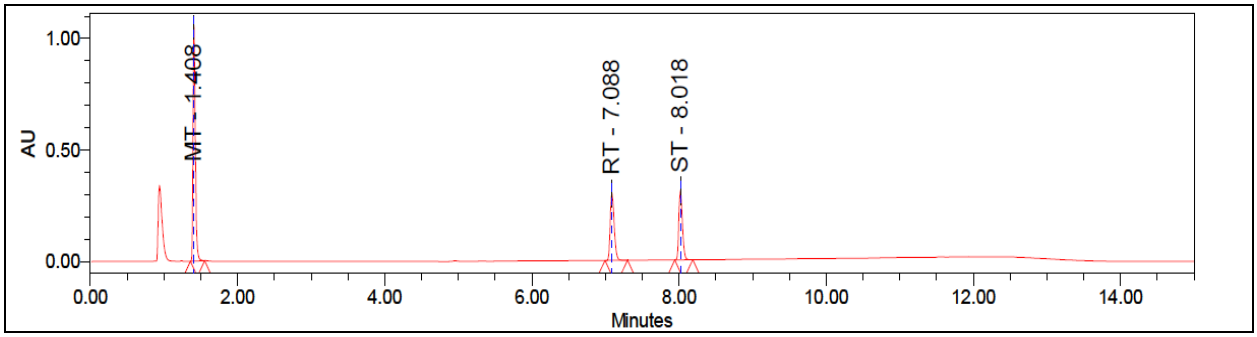


FIG. 2: OVERLAY- BLANK, COMBINED CHROMATOGRAM FOR STRESSED CONDITION BLANK SOLUTION

TABLE 3: RESULTS FOR FORCED DEGRADATION STUDY

Degradation Condition	% Degradation		
	MT	RT	ST
Acid hydrolysis(1 N HCl, 30 minutes at 60^{0}C)	0.0	0.20	0.0
Alkaline hydrolysis(1 N NaOH, 30 minutes at 60^{0}C)	0.0	18.3	5.4
Peroxide Oxidation(50 % v/v, 30 minutes at 60^{0}C)	0.0	1.3	0.15
Photolytic degradation(Amber vial, 24 hours)	0.0	0.0	0.0
Photolytic degradation (Transparent vial, 24 hrs)	0.0	6.0	1.0
Thermal degradation(24 hours at 105^{0}C)	0.0	0.0	0.0

TABLE 4: PEAK PURITY ASSESSMENT

Component/Test	Purity Angle	Purity Threshold	Peak Purity
Acid Hydrolysis			
Metformin	0.117	1.730	**Passes**
Rosiglitazone	0.368	1.617	**Passes**
Sitagliptin	0.301	2.545	**Passes**
Base Hydrolysis			
Metformin	0.679	1.736	**Passes**
Rosiglitazone	0.265	1.686	**Passes**
Sitagliptin	0.310	3.928	**Passes**
Peroxide Oxidation			
Metformin	0.315	1.298	**Passes**
Rosiglitazone	0.385	1.513	**Passes**
Sitagliptin	0.192	2.458	**Passes**
Thermal Degradation			
Metformin	0.098	1.327	**Passes**
Rosiglitazone	0.330	1.538	**Passes**
Sitagliptin	0.119	2.469	**Passes**
Photo Degradation (Amber Glass)			
Metformin	0.370	1.115	**Passes**
Rosiglitazone	0.494	1.310	**Passes**
Sitagliptin	0.225	1.779	**Passes**
Photo Degradation (Transparent Glass)			
Metformin	0.639	2.293	**Passes**
Rosiglitazone	0.398	2.245	**Passes**
Sitagliptin	0.519	8.250	**Passes**

2. **Linearity:** Standard stock solution of the drug was diluted to prepare linearity standard solutions in the concentration range of 12-100 µg mL^{-1} for all Metformin HCl, Rosiglitazone and Sitagliptin Phosphate. Three sets of such solutions were prepared. Each set was analyzed to plot a calibration curve. Slope, intercept and coefficient of determination (r^2) of the calibration curves were calculated to ascertain linearity of the method.

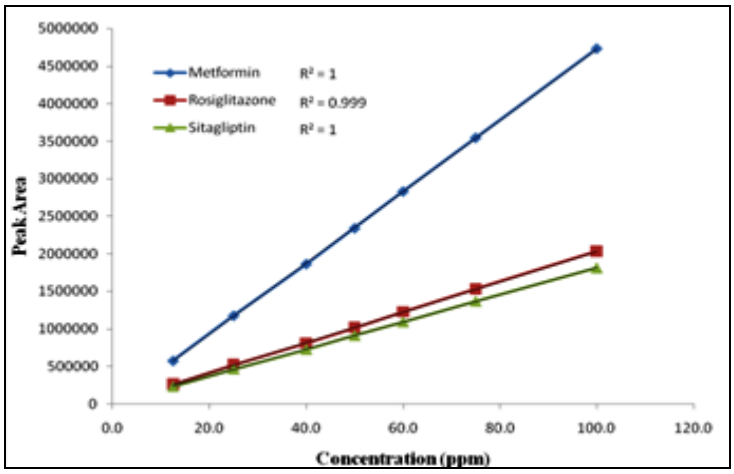


FIG. 3: LINEARITY CURVE WITH CORRELATION CO-EFFICIENT

3. **Recovery:** Recovery of the method was determined by analyzing the synthetic mixture of drug substance with 50%, 100% and 150% levels. These mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (%); RSD (%) were calculated **(Table 5)**. Further, the recovery was determined on the commercially available drug products Glycomet (Metformin HCl, 500mg- USV Limited), Januvia (Sitagliptin phosphate tablets- 50mg-MSD Pharmaceuticals Pvt. Ltd.) and Windia (Rosiglitazone Tablets, 2 mg, Glaxo Smith Kline) in appropriate mixture **(Table 6)**.

4. **Precision and Intermediate Precision:** The precision of the proposed method was evaluated by carrying out six independent assays of test samples. RSD (%) of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument (Table 3).

TABLE 5: RECOVERY FROM COMMERCIALLY AVAILABLE SAMPLES

	% Assay		
Level	**Recovery (%) For MT**	**Recovery (%) For RT**	**Recovery (%) For ST**
50 %	98.6	100.2	100.8
100 %	99.4	99.2	99.7
150 %	100.4	100.6	98.4
Average	**99.5**	**100.0**	**99.6**
RSD	**0.91**	**0.72**	**1.21**

TABLE 6: RECOVERY ON SYNTHETIC MIXTURE OF ALL THREE DRUG SUBSTANCES

% Recovery	**% Assay**		
	MT	**RT**	**ST**
Level – 50%	99.1	99.6	98.9
Level – 100%	99.7	100.3	99.6
Level – 150%	100.5	100.5	99.1
Average	**99.8**	**100.1**	**99.2**
RSD	**0.70**	**0.47**	**0.76**

TABLE 7: PRECISION

	Repeatability			**Intermediate Precision**		
Sr. Nos.	**%MT**	**%RT**	**%ST**	**%MT**	**%RT**	**%ST**
1	99.0	99.7	98.8	100.5	98.2	100.2
2	99.2	98.6	100.5	98.2	99.8	100.9
6	99.3	98.9	99.6	99.5	101.8	98.7
4	100.0	100.2	98.5	101.6	98.1	100.1
5	99.2	98.6	99.8	98.1	99.3	99.1
6	99.5	99.4	100.8	100.1	98.5	98.9
%Average	99.4	99.2	99.7	99.7	99.3	99.7
%RSD	0.35	0.65	0.91	1.36	1.41	0.88

5. **Robustness:** The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.2 mL/min), wavelength (altered by ±0.2 nm), and pH of buffer in mobile phase (altered by ± 0.2). These chromatographic variations were evaluated for resolution between all drug substances.

6. **Solution Stability:** To assess the solution stability, standard and test solutions were kept at 25°C (laboratory temperature) for 24 hrs.

TABLE 8 - CHANGE IN FLOW RATE (1.5 ML/MIN ± 0.2 ML/MIN)

Drug component		**% Assay**	
	1.5 mL/min	**1.3 mL/min**	**1.7 mL/min**
MT		98.7	99.5
		99.6	100.0
%Average	*99.4	99.2	99.8
Absolute Difference	-	0.2	0.4
Drug component		**% Assay**	
	1.5 mL/min	**1.3 mL/min**	**1.7 mL/min**
RT		98.5	98.3
		98.2	99.1
%Average	*99.2	98.4	98.7
Absolute Difference	-	0.8	0.5
Drug component		**% Assay**	
	1.5 mL/min	**1.3 mL/min**	**1.7 mL/min**
ST		100.5	98.7
		100.1	98.3
%Average	*99.7	100.3	98.5
Absolute Difference	-	0.6	1.2

*Avg. Assay Value from Precision study.

TABLE 9: CHANGE IN WAVELENGTH (210NM ± 2NM)

Drug component	% Assay		
	210 nm	208 nm	212 nm
MT		98.4	99.6
		98.2	99.4
Average	99.4	98.3	99.5
Absolute Difference	-	1.1	0.1
Drug component	**% Assay**		
	210 nm	**208 nm**	**212 nm**
RT		99.8	98.7
		99.5	99.2
Average	99.2	99.7	99.5
Absolute Difference	-	0.5	0.3
Drug component	**% Assay**		
	210 nm	**208 nm**	**212 nm**
ST		99.1	98.9
		99.6	99.6
Average	99.7	99.4	99.8
Absolute Difference	-	0.3	0.1

*Avg. Assay Value from Precision study.

TABLE 10: CHANGE IN PH OF BUFFER SOLUTION IN MOBILE PHASE (pH 3.0 ± 0.2)

Drug component	% Assay		
	pH 3.0	pH 2.8	pH 3.2
MT		98.9	99.6
		99.2	99.4
Average	*99.4	99.1	99.5
Absolute Difference	-	0.3	0.1
Drug component	**% Assay**		
	pH 3.0	**pH 2.8**	**pH 3.2**
RT		99.5	99.8
		99.7	99.2
Average	*99.2	99.6	99.5
Absolute Difference	-	0.4	0.3
Drug component	**% Assay**		
	pH 3.0	**pH 2.8**	**pH 3.2**
ST		99.5	99.9
		99.8	99.2
Average	*99.7	99.7	99.6
Absolute Difference	-	0.0	0.1

*Avg. Assay Value from Precision study.

TABLE 11: RESULT OF ROBUSTNESS ON THE RESOLUTION BETWEEN THE DRUGS

Robustness Parameter	Robustness condition	Resolution		
		MT	RT	ST
pH of buffer	pH 2.8	-	33.4	11.5
	pH 3.2	-	32.0	8.3
Flow Rate	1.3 mL/min	-	26.1	6.5
	1.7 mL/min	-	31.2	8.0
Wavelength	208nm	-	27.4	7.5
	212nm	-	27.3	7.5

TABLE 12: RESULTS FOR SOLUTION STABILITY (SAMPLE SOLUTION)

Time (Hours)	% Assay		
	MT	RT	ST
Initial	99.8	100.3	100.2
5	99.6	99.5	100.3
8	99.8	99.3	99.7
12	99.6	99.1	99.5
18	99.5	98.9	98.7
24	99.2	98.5	98.2
Average	**99.6**	**99.3**	**99.4**
RSD	**0.22**	**0.62**	**0.84**

7. **System suitability:** The system suitability were confirmed before each of validation parameter with respect to % RSD, theoretical plates, tailing factor, repeatability and resolution between Rosuvastatin (RT) and Sitagliptin (ST) peak were defined.

TABLE 13: CHROMATOGRAPHIC PARAMETERS OF SYSTEM SUITABILITY OBSERVED FROM PRECISION STUDY)

Drug substances	RT (min)	Theoretical plates	Tailing Factor	Resolution
Metformin hydrochloride(MT)	1.445	4201	1.61	0
Rosuvastatin(RT)	7.270	60942	1.44	27.4
Sitagliptin(ST)	8.154	82958	1.46	7.5

Acceptance Criteria: % RSD for six replicates of each active peak area: NMT 2.0 %. Theoretical Plate for each active Peak: NLT 2000 Resolution in-between RT and ST: NLT 5.0

RESULTS AND DISCUSSION:

HPLC Method Development: The maximum absorption wavelength of the reference drug solution and of the forcefully degraded drug solution was found to be 210 nm. This was observed from the UV absorption spectra (Fig. 1) and was selected as detection wavelength for LC analysis. The main objective of this chromatographic method was separation of degraded impurities from all drugs. Forced degradation study revealed a critical separation of closely eluting impurity formed from the Metformin HCl, Rosiglitazone and Sitagliptin peaks. ACE 3 150mm X 4.6mm, 3.5µm helped in resolving all peaks as the column. This effect was observed by using the mobile phase 10mM sodium hexane sulphonate monohydrate and 10mM potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile and methanol in the gradient ratio.

Summary of Validation Parameters: The assay test method is validated for Specificity, Linearity, Precision, Accuracy (Recovery), Stability of Analytical Solution and Robustness and was found to be meeting the predetermined acceptance criteria. The validated method is Specific, Linear, Precise, Accurate and Robust for determination of assay of Metformin HCl, Rosiglitazone and Sitagliptin drug substances and drug products. Hence this method can be introduced into routine and stability analysis for the assay of Metformin HCl, Rosiglitazone and Sitagliptin drug substances.

CONCLUSION: The stability indicating RP-HPLC assay method was developed and validated for simultaneous determination of Metformin HCl, Rosiglitazone and Sitagliptin drug substances and drug products. The method was found to be simple, specific, Precise and Robust and can be applied for the routine and stability analysis for commercially available formulation.

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