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A REVISED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF VILDAGLIPTIN AND PIOGLITAZONE HCl – APPLICATION TO COMMERCIALLY AVAILABLE DRUG PRODUCTS

Hitesh P. Inamdar *[1], Ashok A. Mhaske [2], Shirish P. Sahastrabudhe [2]

Getz Pharma Research Pvt. Ltd. [1], Mumbai, Maharashtra, India
Shri Jagdishprasad Jhabarmal Tiberewala University [2], Churu-Bishau Road, Chudela, Dist. Jhunjhunu – 333 001, Rajasthan, India

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Correspondence to Author:

Hitesh P Inamdar
Research Officer, Getz Pharma Research Pvt. Ltd., , Mumbai, Maharashtra, India

E-mail: hitesh.inamdar@gmail.com

ABSTRACT

A simple, precise and stability-indicating HPLC method was developed and validated for the simultaneous determination of anti-diabetic drugs. The separation was achieved on ACE 3 150mm*4.6mm, 3.5µm column with gradient flow. The mobile phase at a flow rate of 1.5 mL min^{-1} consisted of 10mM sodium hexane sulphonate monohydrate and 10mM Potassium dihydrogen phosphate buffer with acetonitrile and methanol in gradient ratio. The UV detection was carried out at 210 nm. The method was successfully validated in accordance to ICH guidelines. Further, the validated method was applied for commercially available pharmaceutical dosage form.

INTRODUCTION: An extensive literature survey was conducted on the analytical method for the simultaneous estimation of Vildagliptin and Pioglitazone HCl by HPLC. The absence of literature provides the need for developing a new method. The available 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 [1-11].

Recently, a bio-analytical method has been reported for Pioglitazone [12]. However, so far, no method has been reported for the simultaneous estimation of Vildagliptin and Pioglitazone HCl 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 Vildagliptin and Pioglitazone HCl in there drug product. This method can further be used for the simultaneous estimation of Metformin HCl, Rosiglitazone Maleate and Sitagliptin Phosphate in tablet formulation.

The earlier method proposed to estimate Metformin HCl, Rosiglitazone Maleate and Sitagliptin Phosphate [13]. The same method is further extended for the estimation of Vildagliptin and Pioglitazone HCl in their 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.

EXPERIMENTAL:

Chemicals and Reagents: Drug substances were provided by Getz Pharma Research Pvt. Ltd, India. All the chemicals and reagents Ammonium hydroxide, hydrochloric acid, potassium dihydrogen phosphate, hydrogen peroxide (50 %) were used of Analytical grade. While, Acetonitrile and Methanol was procure 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 Jalara (Vildagliptin tablets, 50mg, Novartis Pharma) and Pioz* 30 (Pioglitazone HCl Tablets, 30mg, USV Limited).

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 volume of the sample solution injected was 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)	Buffer % (10 mM Potassium dihydrogen Phosphate and 10 mM Sodium Hexane sulphonate monohydrate)	% 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 Vildagliptin and Pioglitazone HCl, reference standard in 100 ml volumetric flask. Added to it 70 ml diluents (Water:Aetonitrile::70:30, pH 3.0) and sonicated to dissolve. Diluted this solution up to volume with diluents. Pipette out 5.0 ml of this solution into 50 ml volumetric flask and diluted to volume with diluent. (50 µg/mL each of Vildagliptin and Pioglitazone HCl). Similarly, the test solutions were prepared at same concentration using same diluents. (50 µg/mL of each).

Method Development: A variety of mobile phases were investigated in the development of a stability-indicating LC method for the analysis of Vildagliptin and Pioglitazone HCl drug substances. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay, stability studies 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 for Pioglitazone HCl is (222.8 and 269.0) and for Vildagliptin no as such maxima was found. However, detection was carried out at 210 nm on basis of higher response (**Fig. 1 and Table 2**).

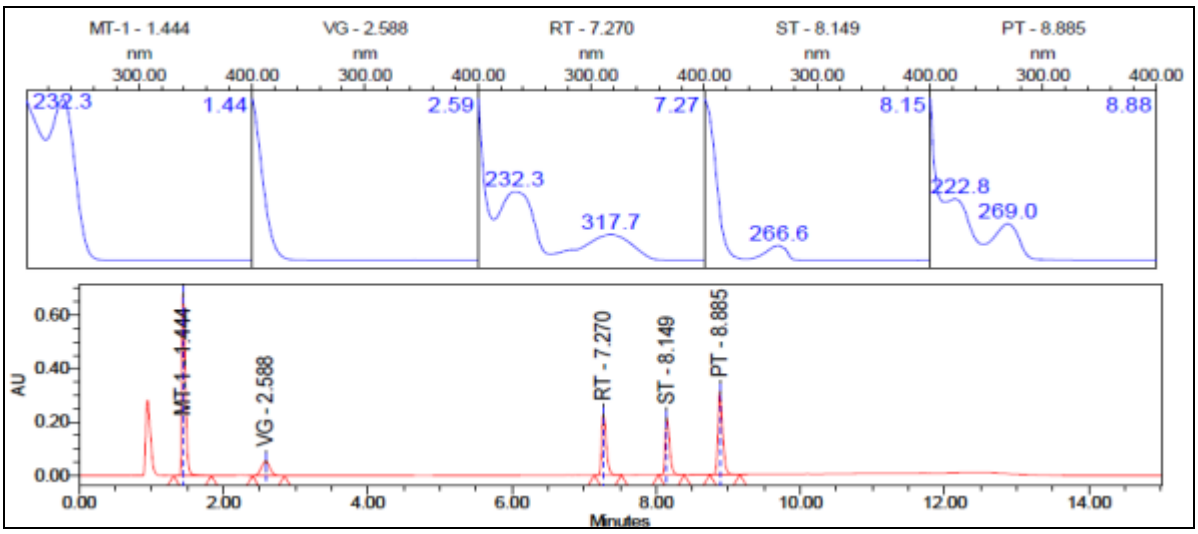


FIG 1: UV SPECTRA FOR ALL DRUG SUBSTANCES

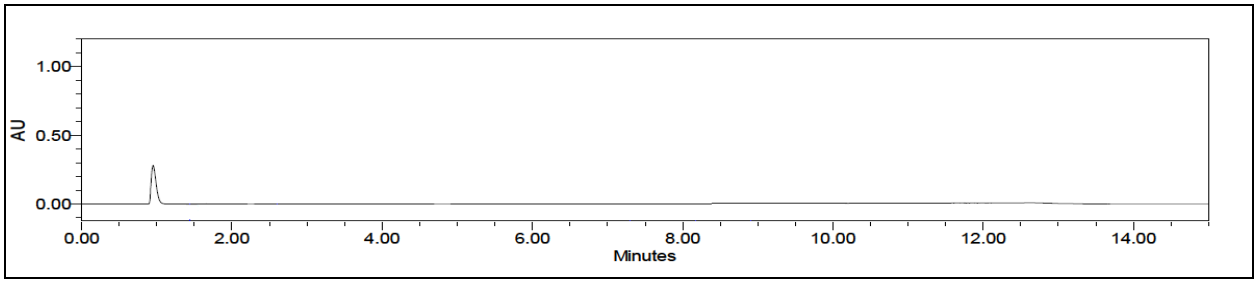
TABLE 2: AREA RESPONSE OF PEAKS AT DIFFERENT WAVELENGTHS

Wavelength (nm)	VG	PT
208	**550213**	**1519137**
210	441133	1400643
220	90580	1413982
230	11735	1221186
240	1722	527725
250	ND	384811
260	ND	644848

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].

Specificity- Forced Degradation study:

1. **Acid and Base Hydrolysis:** Forced degradation study was conducted on 50 mg/mL of drug solution. The solution mixture was prepared by mixing 50mg of each drug in 100 mL flask. This was considered as a stock solution. To 5mL of stock solution 5mL of 1N hydrochloric acid was exposed at 60ºC for 30 minutes and same was followed in case of Ammonium hydroxide and was exposed at 60ºC for 10 minutes. Then, neutralized with acid or base (when necessary) and dilute up to 50 ml with diluent.
2. **Oxidation:** Forced degradation study was conducted on drug substances by exposing with 50% H_2O_2 and dilute up to 50 mL with diluent.
3. **Thermal Degradation:** 5mL stock solution was kept in dry oven at 105°C for 24 hours.
4. **Photolysis:** Photolysis studies were carried out on stock solution in 50mL volumetric flask. The sample was exposed to light in a photo-stability chamber. The study was carried out on transparent and Amber color flask. The method's analytical data were collected at a single wavelength of 210 nm. Additional PDA detector data were collected for the peak purity evaluation.



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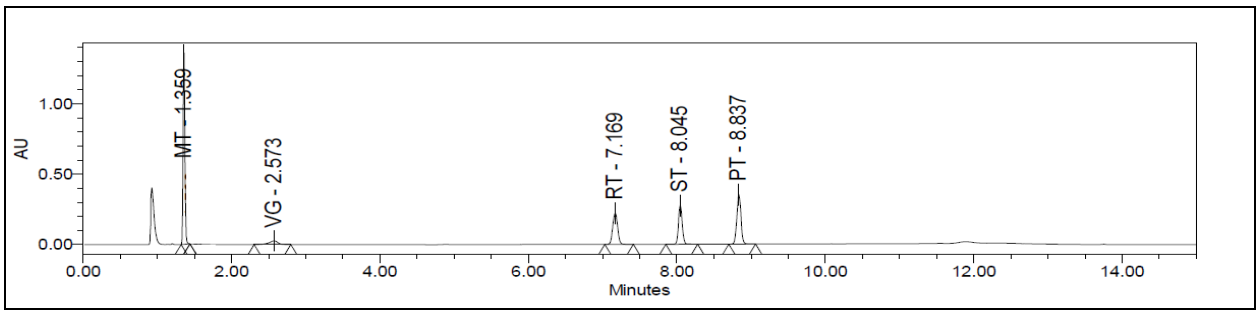
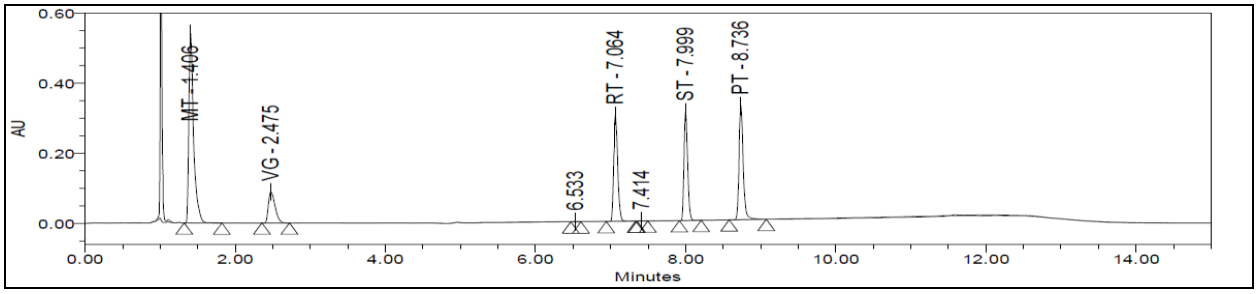
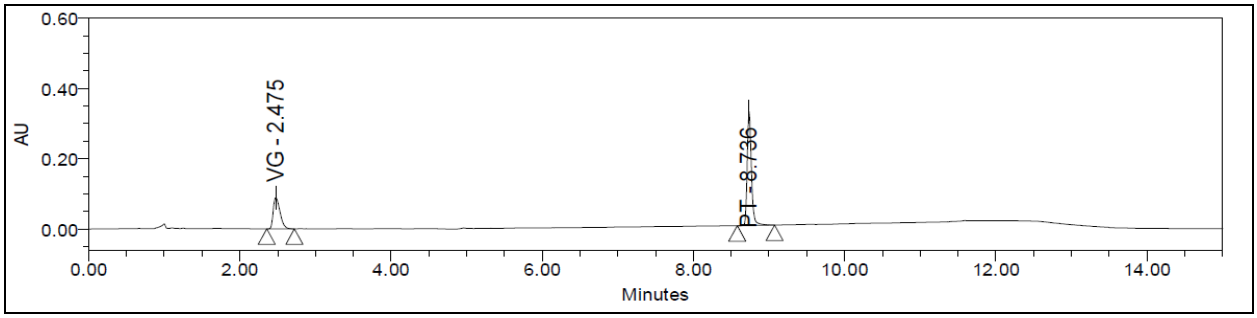
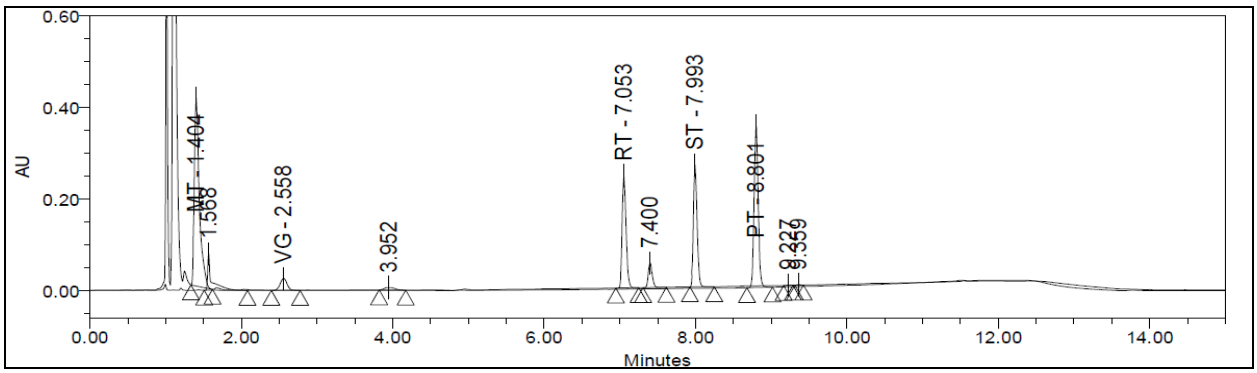


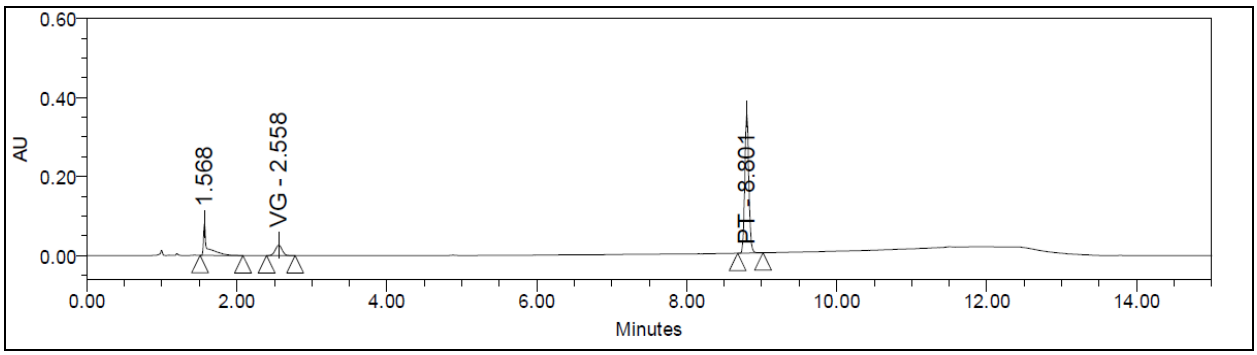
STANDARDS SOLUTION



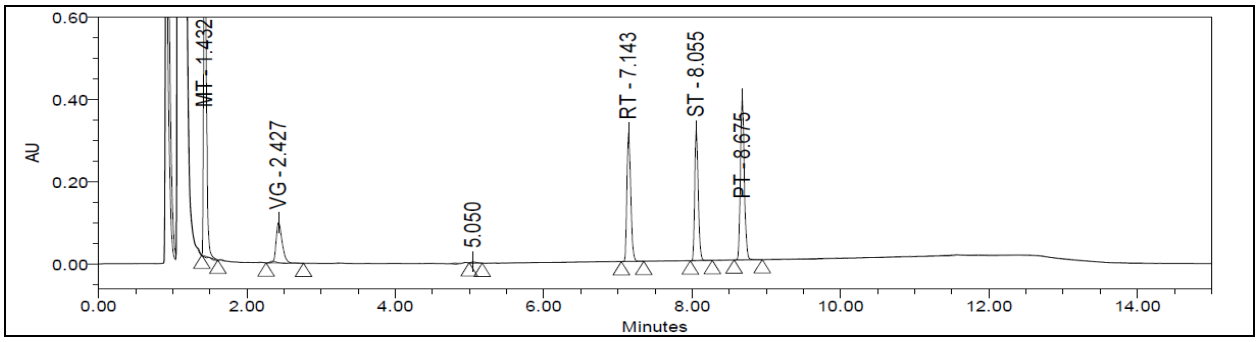
SPECIFICITY- ACID HYDROLYSIS

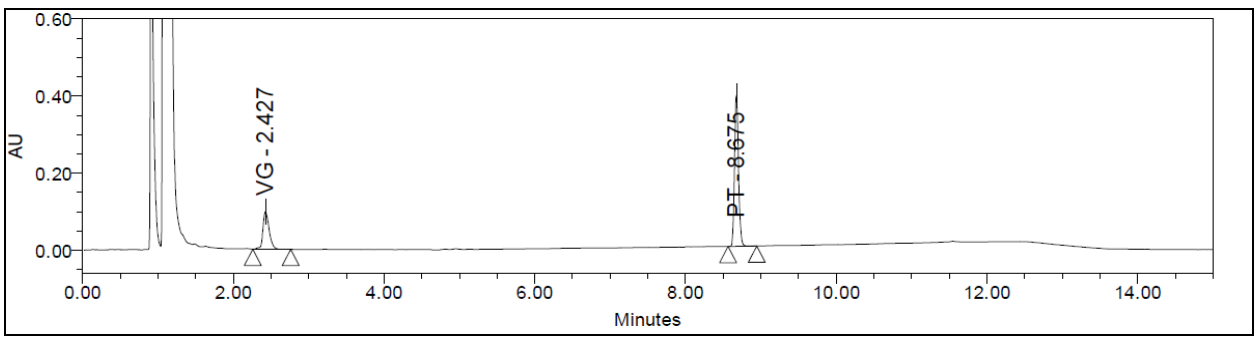




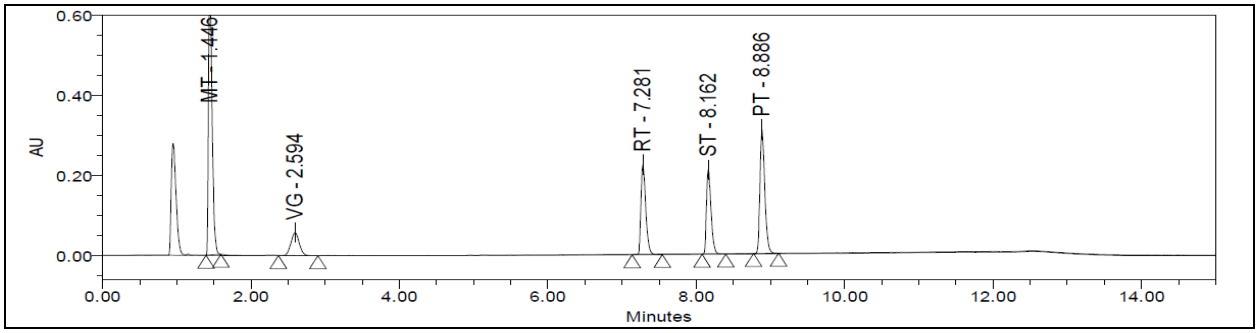


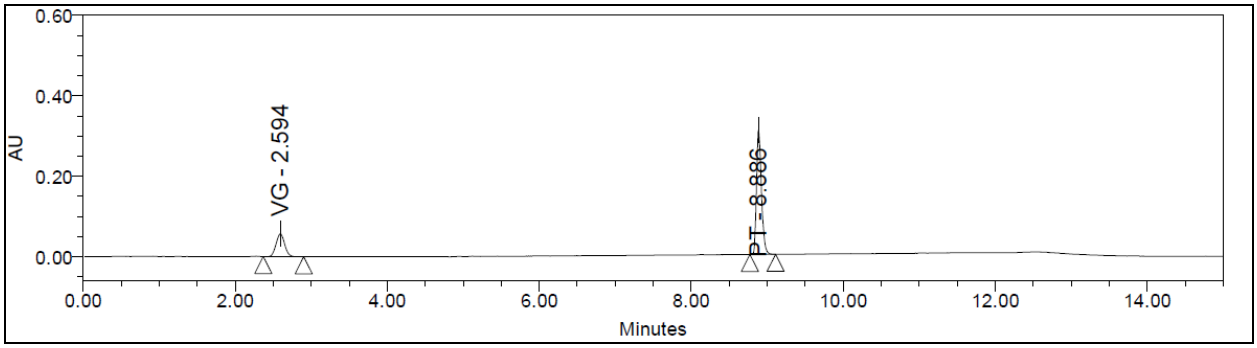
SPECIFICITY-ALKALINE HYDROLYSIS:



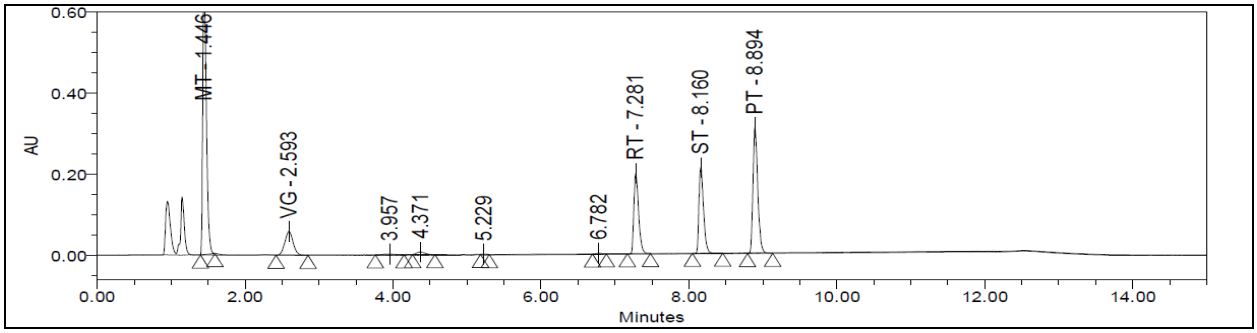


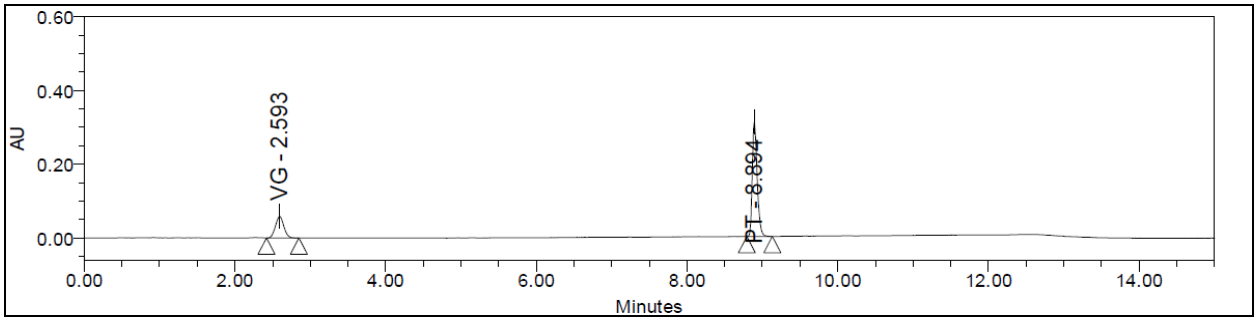
SPECIFICITY-PEROXIDE OXIDATION



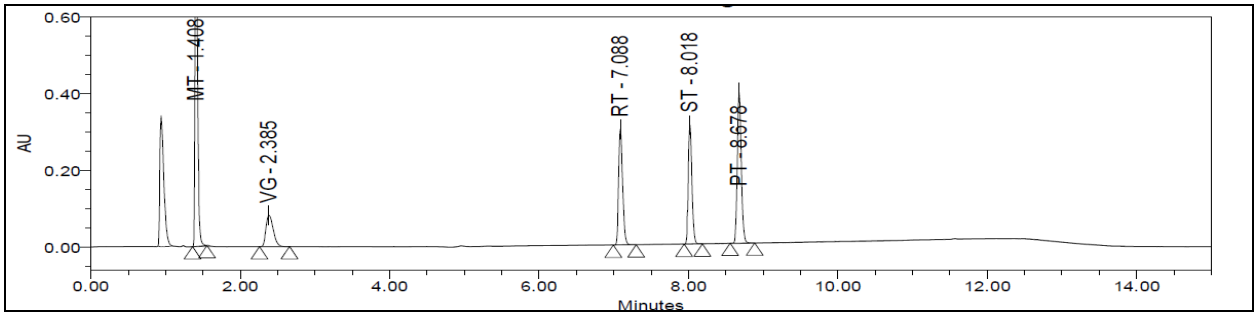


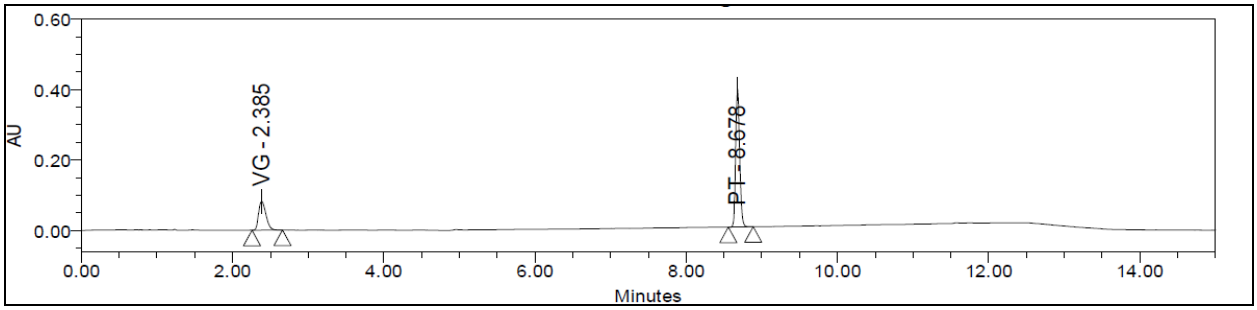
SPECIFICITY PHOTOLYTIC DEGRADATION (AMBER GLASS)





SPECIFICITY PHOTOLYTIC DEGRADATION (TRANSPARENT GLASS)





SPECIFICITY-THERMAL DEGRADATION

FIG 2: OVERLAY AND INDIVIDUAL CHROMATOGRAM FOR STRESSED CONDITION

Force Degradation study was conducted by varying various parameters. No degradation was observed. A peak was observed in alkaline hydrolysis adjacent to metformin peak. The resolution between the respective peaks was found to be greater than 2. i.e. 2.3. The overlay chromatogram specifies each parameter of force degradation study. Peak Purity assessment is given in **Table 3**.

TABLE 3: PEAK PURITY ASSESSMENTS

Component/Test	Purity Angle	Purity Threshold	Peak Purity
Acid Hydrolysis			
Vildagliptin	0.460	4.906	**Passes**
Pioglitazone HCl	0.482	2.051	**Passes**
Base Hydrolysis			
Vildagliptin	0.490	8.980	**Passes**
Pioglitazone HCl	0.247	1.341	**Passes**
Peroxide Oxidation			
Vildagliptin	0.630	4.317	**Passes**
Pioglitazone HCl	0.621	1.598	**Passes**
Thermal Degradation			
Vildagliptin	0.280	5.094	**Passes**
Pioglitazone HCl	0.582	1.675	**Passes**
Photo Degradation (Amber Glass)			
Vildagliptin	0.222	3.453	**Passes**
Pioglitazone HCl	0.602	1.283	**Passes**
Photo Degradation (Transparent Glass)			
Vildagliptin	0.211	3.064	**Passes**
Pioglitazone HCl	0.587	1.266	**Passes**

Note: Peak Purity: Purity angle should be less than purity threshold

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 Vildagliptin and Pioglitazone HCl. 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.

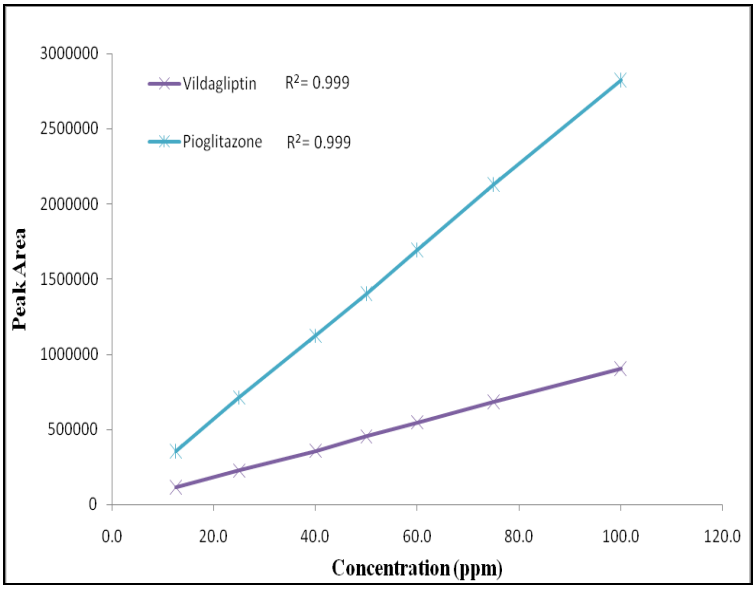


FIG. 3: LINEARITY CURVE WITH CORRELATION CO-EFFICIENT

3. **Recovery:** Recovery of the method was determined by analyzed the drug products and synthetic mixture of drug products 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 4**).

TABLE 4: RECOVERY FROM COMMERCIALLY AVAILABLE SAMPLES

Level	Recovery (%) For VG	Recovery (%) For PT
50 %	101.2	101.5
100 %	100.0	101.0
150 %	99.5	99.9
Average	**99.8**	**100.5**
RSD	**0.354**	**0.774**

4. **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 5**).

TABLE 5: PRECESSION

Sr. No.	Repeatability		Intermediate Precision	
	VG	PT	VG	PT
1	99.7	100.8	98.5	98.8
2	99.4	100.7	97.8	101.4
3	100.1	101.0	101.5	99.7
4	100.2	101.6	99.8	100.5
5	100.6	101.0	98.9	98.9
6	100.0	100.8	101.5	101.3
Average	100.0	101.0	99.7	100.1
RSD	0.41	0.32	1.57	1.15

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.

TABLE 6: 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
VG	100.0	98.7	101.2
		99.4	100.7
Average	100.0	99.1	101.0
Absolute Difference	-	0.9	1.0
Drug component	**% Assay**		
	1.5 mL/min	**1.3 mL/min**	**1.7 mL/min**
PT	101.0	100.5	99.5
		101.2	98.5
Average	101.0	100.9	99.7
Absolute Difference	-	0.1	1.3

TABLE 7: CHANGE IN WAVELENGTH (210NM ± 2NM)

Drug component	% Assay		
	210 nm	208 nm	212 nm
VG	100.0	101.2	101.5
		100.4	100.3
Average	100.0	100.8	100.9
Absolute Difference	-	0.8	0.9
Drug component	**% Assay**		
	210 nm	**208 nm**	**212 nm**
PT	101.0	101.8	99.6
		100.8	100.8
Average	101.0	101.3	100.2
Absolute Difference	-	0.3	0.8

TABLE 8: 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
VG	100.0	99.5	100.5
		99.8	99.8
Average	100.0	99.7	100.2
Absolute Difference	-	0.3	0.2
Drug component	**% Assay**		
	pH 3.0	**pH 2.8**	**pH 3.2**
PT	101.0	99.5	100.5
		99.9	100.3
Average	101.0	99.7	100.4
Absolute Difference	-	1.3	0.6

TABLE 9: RESULT OF ROBUSTNESS ON THE RESOLUTION BETWEEN THE DRUGS

Robustness Parameter	Robustness condition	Resolution	
		VG	PT
pH of buffer	pH 2.8	9.16	5.39
	pH 3.2	9.51	11.82
Flow Rate	1.3 mL/min	7.88	5.98
	1.7 mL/min	8.04	6.57
Wavelength	208nm	7.33	6.21
	212nm	7.48	6.19

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

TABLE 10: RESULTS FOR SOLUTION STABILITY (SAMPLE SOLUTION)

Time (Hours)	% Assay	
	VG	PT
Initial	100.0	101.0
5	99.6	101.1
8	99.5	100.8
12	99.6	100.7
18	99.5	100.9
24	99.4	100.6
Average	**99.6**	**100.9**
RSD	**0.211**	**0.186**

7. **System suitability:** The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between peaks of all drug substances were defined.

TABLE 11: CHROMATOGRAPHIC PARAMETERS OF SYSTEM SUITABILITY

Drug substances	RT (min)	Theoretical Plates	Symmetry	Resolution	Purity Angle	Purity Threshold	Peak purity
VG	2.588	89967	1.03	7.33	0.218	3.394	Pass
PT	8.885	2303	1.33	6.21	0.602	1.309	Pass

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 Pioglitazone HCl and Vildagliptin peaks.

An additional peak was also observed at the retention time of about 1.5 minutes, which was supposed to be due to placebo. The resolution between the metformin peak and the placebo peak was more than 2. i.e. 2.30. 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 Vildagliptin and Pioglitazone HCl drug substances and drug products. Hence, this method can be introduced into routine and stability analysis for the assay of Vildagliptin and Pioglitazone HCl drug substances and their product.

CONCLUSION: The stability indicating RP-HPLC assay method was developed and validated for simultaneous determination of Vildagliptin and Pioglitazone HCl and some commercial 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. The earlier method consisting of three compounds that are Metformin HCl, Rosiglitazone Maleate and Sitagliptin Phosphate. This method further extended for the simultaneous estimation of Vildagliptin and Pioglitazone HCl in tablet formulation. So, this method is applicable for the simultaneous estimation of Metformin HCl, Rosiglitazone Maleate, Sitagliptin Phosphate, Vildagliptin and Pioglitazone HCl in the combination as well as in their respective drug product.

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