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Analysis Of Vildagliptin And Nateglinide For Simultaneous Estimation Using Spectro-Chromatographic Methods

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ABSTRACT: Background: The combination therapy of Vildagliptin and Nateglinide was found to be an effective and safe in type 2 diabetes mellitus.Objectives:Study a new, simple, precise, accurate and specific stability indicating RP-HPLC and First-order derivative UV spectrophotometric method for the simultaneous estimation of Vildagliptin and Nateglinide in combination. Materials and Methods:The chromatographic separation was achieved isocratically. The mobile phase, Acetonitrile: Phosphate buffer (70:30; % v/v; pH 3.2) was selected as it was found to resolve the peaks with better intensity. For UV spectrophotometric method, methanol was used as a solvent, the spectrum was recorded between 200-400 nm wavelengths, and all the zero-order spectrum (D_0) were converted to first-order derivative spectrum (D_1) using delta lambda 2.0 and scaling factor 4. 253 nm (zero crossing point of Nateglinide) and 270 nm (zero crossing point of Vildagliptin) were used for determination of Vildagliptin and Nateglinide respectively. The methods were proved linearin the concentration range of 5-25 µg/ml for Vildagliptin and9-45 µg/ml for Nateglinide. Both drugs were subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Results: The proposed methods were validated in terms of Linearity, Range, Accuracy, Precision, LOD, LOQ, Specificity, Robustness, System suitability tests and stability studies and itsresults were proved within limits. The results showed that Vildagliptin and Nateglinide were more sensitive towards thermal and acidic degradation, respectively. Conclusion:Both methods were highly sensitive, precise, accurate and applicable for the reliable quantitation in their combination.

Keywords: Vildagliptin, Nateglinide, RP-HPLC method, UV method.

1. INTRODUCTION

In current scenario, death ratio of hyperglycemic patient havingcardiovascular and micro vascular complication are more compared to type 2 diabetes mellitus alone patients. Number of clinical trials reported Meglitinides (Nateglinide) class of drugs produces synergistic effect withDPP 4 enzyme inhibitors (Vildagliptin) in type 2 diabetes mellitus patientsmight be reduced the risk of said complications and improves the quality of life**.** Vildagliptin (Figure 1a),(2S)-1-{2-[(3-hydroxyadamantan-1-yl) amino] acetyl} pyrrolidine-2-carbonitrile.**(Maryadele, 2006)**,is dipeptidyl peptidase 4 (DPP-4) enzyme inhibitor. DPP-4 enzyme breaks the incretins Glyco Lipoprotein -1 (GLP-1), which is gastrointestinal hormones released in response to a meal. It improves pancreatic islet cell sensitivity to

glucose**(Tripathi, 2008)**.Nateglinide (Figure 1b), (2R)-3-phenyl-2-{[4-(propan-2yl) cyclohexyl] formamido} propionic acid**(Maryadele, 2006)**,is an oral anti-hyperglycaemic agent which stimulate insulin release by binding to β cells of the pancreas**(Rang& Dale, 2012)**. Individually, Vildagliptin and Nateglinide are available in different dosage forms in market.This combination therapy is effective and safe in Japanese type 2 diabetes patients and the augmentation of Nateglinide-induced early phase insulin secretion with Vildagliptin which improved glycemic control**(Kudo-Fujimaki et al., 2014)**.From the Exhaustive literature survey, Analysis of Vildagliptin and Nateglinide by various methods like Spectroscopic methods viz. UV and Mass Spectroscopy; and Chromatographic methods viz. HighPerformance Liquid Chromatography (HPLC); High Performance Thin Layer Chromatography (HPTLC) has been reported individually**(Patil et al. 2020; Snyder et al. 1997)**and also, in different class of combination like Vildagliptinalone**(Waghulde&Naik 2018; Boovizhikannan&Palanirajan, 2013; Barden et al., 2012)**, Nateglinidealone**(Pathareet al., 2007; Raghavendraet al., 2019; Sankalia et al., 2007; Bauer et al., 2003; Jain et al., 2009)**, Vildagliptin and Metformin **(Abdel-Ghany et al., 2014; Satheeshkumar et al., 2014)**, Nateglinideand Metformin **(Thomas et al., 2011; Thomas et al., 2012)**and many more. Since no method has been develop and validated for simultaneous estimation of Vildagliptin and Nateglinide in combination. Hence, the objectives of the present work were to develop and validate Stability indicating RP-HPLC and first order derivative UV Spectrophotometric method for simultaneous estimation of Vildagliptin and Nateglinide in combination.

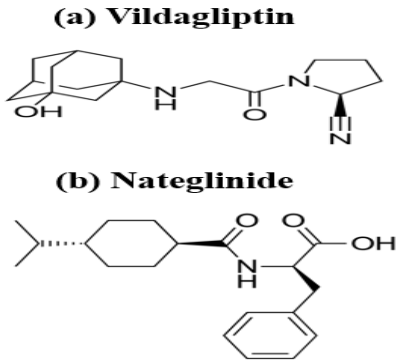

Figure 1: Chemical structures of (a) Vildagliptin and (b) Nateglinide

2. MATERIALS AND METHODS

Chemicals and Reagents

The bulk drug, Vildagliptin and Nateglinidewere procured as gift sample from Cadila Pharmaceuticals Ltd., Ahmedabad. Methanol, Acetone and Water used of HPLC grade were procured from Finar chemicals, Ahmedabad. Potassium dihydrogen phosphate and ortho phosphoric acid, 75 % (AR Grade) were purchased from Astron Chemicals Ltd., India. All solutions were prepared fresh on daily basis.

Instrumentation and Analytical condition

The RP-HPLCmethod was performed on Systronic RP-HPLC (LC-138), UV Detector SPD-20 A, Rheodyne injector fitted with a 20 µl loop and used Clarify® software for determination. The method was conducted using Reverse phase techniques. Both drugs were eluted isocratically using Acetonitrile: Phosphate buffer (pH 3.2 adjusted with 10% Ortho Phosphoric Acid) (70:30; %v/v) with flow rate 1 ml/min. The detection wavelength of UV-

vis Detector was set to 222 nm. All solutions with mobile phase were prepared daily, which were filtered by 0.45 µm membrane filter (Millipore) and sonicated with Sonicator (Equitron, India) before use. A Kromstar® C_{18} (250 × 4.6 mm, 5 µm) Column and Systronics® pH meter were used. The RP-HPLC system was operated at room temperature (25 ± 1°C).
UV Spectrophotometric method was performed on Shimadzu UV Visible double beam spectrophotometer (Model-1900) and using 1.0 cm quartz cells. UV Probe software was used for all absorbance measurements. All weighing were done on Digital Analytical balance (Wensar Dab 13-220).

Preparation of Standard Solution
Accurately weighed 10 mg each of Vildagliptinand Nateglinidestandard were transferred to 100 ml volumetric flask and dissolved in 100 ml methanol. The flasks were shaken and volume was made up to the mark with Methanol to give solution containing 100 µg/ml of Vildagliptin and Nateglinide.

Preparation of sample Solution
Accurately weighed equivalently weight of Vildagliptin (50 mg) and Nateglinide (90 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was sonicated till the drug dissolves and was made upto mark with methanol. This solution was filtered through Whatmann filter paper. The concentration of Vildagliptin was 500 µg/ml and Nateglinide was 900 µg/ml. From above mixture solutions, 0.2 ml transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with mobile phase to make final concentration of Vildagliptin 10 µg/ml and Nateglinide 18 µg/ml.

Selection of detection wavelength
Vildagliptin (10 µg/ml) and Nateglinide (18 µg/ml) were used for the detection of wavelength. These solutions were scanned and their spectra were recorded in the range of 200-400 nm against Methanol as a reagent blank.

RP-HPLC Method
The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. Vildagliptin and Nateglinide wereobserved good peak height resolution and shape at 222 nm. Hence, wavelength of 222 nm was selected for further study. The overlain UV spectra of Vildagliptin (10 µg/ml) and Nateglinide (18 µg/ml) in Methanol (Zero order D_0) represented in figure 2.

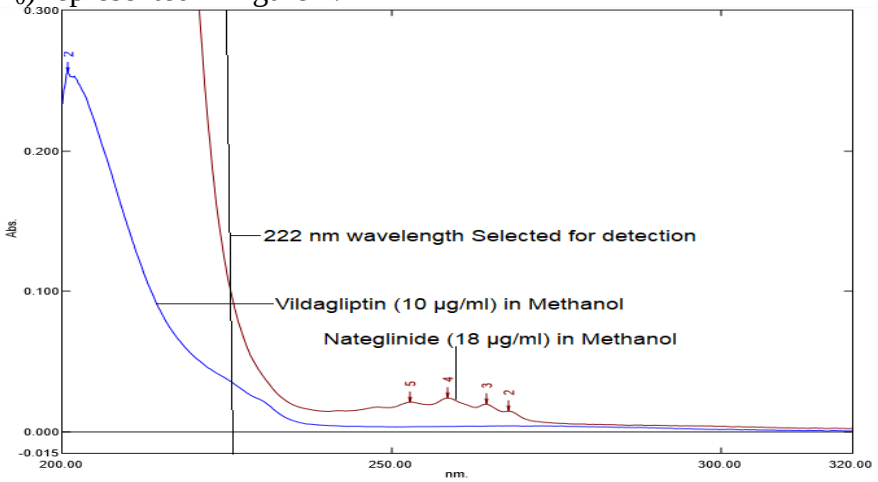

Figure 2: Overlain UV Spectrum of Vildagliptin(10 µg/ml)and Nateglinide (18 µg/ml)in Methanol (Zero order D_0) selection of wavelength

UV (First order derivative) method

All zero-order spectrum (D_0) were converted to first derivative spectrum (D_1) using delta lambda 2.0 and scaling factor 4. The overlain first derivative spectrums of Vildagliptin and Nateglinide at different concentrations were recorded.The zero-crossing point (ZCP) of Nateglinide and Vildagliptin were found to be 253 nm and 270 nm, respectively. The overlain UV spectra of Vildagliptin (10 µg/ml) and Nateglinide (18 µg/ml) in Methanol (First order D_1) represented in figure 3.

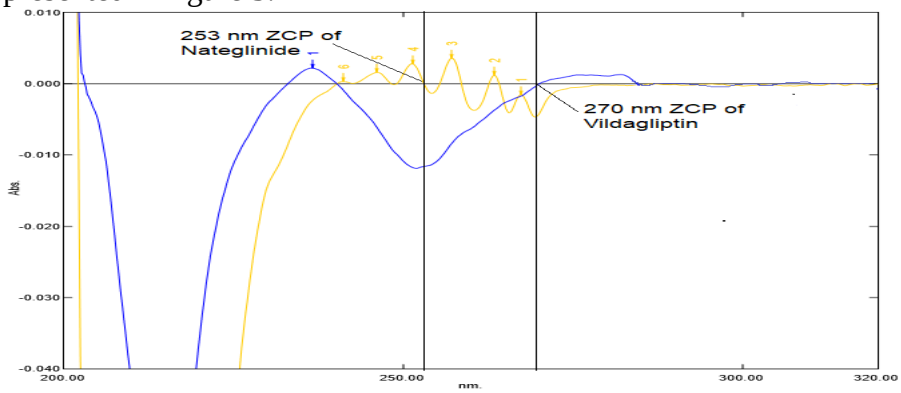

Figure 3: Overlain UV Spectrum of Vildagliptin(10 µg/ml)and Nateglinide (18 µg/ml)in Methanol (First order D_1) showing zero crossing point of Nateglinide(253 nm) and zero crossing point of Vildagliptin (270 nm)

Method Validation

The Methods were validated as per ICH guideline Q2(R1)**(International Conference on Harmonization: Validation of Analytical Procedures: Text and Methodology, 2007)**.The method has been extensively validated in terms of Specificity, Linearity and range, Accuracy, Precision, Detection limit, Quantification limit, Robustness and System suitability tests.

Specificity

Sample solutions (Vildagliptin 10 µg/ml and Nateglinide 18 µg/ml) were prepared and injected in injector of HPLC to verify degradation and interferences. Interference from Vildagliptin and Nateglinide were checked for analysis of drugs with blank chromatogram.

Linearity and Range

The Calibration curve was constructed at a concentrations 5-25 µg/ml of Vildagliptin and 9-45 µg/ml of Nateglinide (n=6) for RP-HPLC and UV method.

Aliquots of stock solution of Vildagliptin (100 µg/ml) i.e. 0.5, 1.0, 1.5, 2.0 and 2.5 and for Nateglinide (100 µg/ml) i.e. 0.9, 1.8, 2.7, 3.6, and 4.5, were pipetted in 10 ml of volumetric flask. Further diluted with diluent to obtain the different concentration like 5, 10, 15, 20 and 25 µg/ml for Vildagliptin and 9, 18, 27, 36 and 45 for Nateglinide. Linearity was evaluated by linear regression analysis in terms of slope, intercept and correlation coefficient.

Accuracy

Recovery study of RP-HPLC and UV method were conducted as per ICH guideline to determine accuracy at three different concentration levels i.e. 50 %, 100 % and 150 %. Solutions containing 10 µg/ml of Vildagliptin and 18 µg/ml of Nateglinide were analyzed as 100 %. This performance was done in triplicate. Accuracy was calculated in percentage of recovery by standard addition method.

Precision

The precision study of RP-HPLC and UV method were conducted at three levels like Intermediate (Intraday) precision, Reproducibility (Interday precision) and Repeatability. In Intraday precision, solutions containing 5, 10, 15 µg/ml of Vildagliptin and 9, 18, 27 µg/ml of Nateglinide were analyzed three times on the same day. In Interday precision, solutions containing 5, 10, 15 µg/ml of Vildagliptin and 9, 18, 27 µg/ml of Nateglinide were analyzed on three different successive days and in Repeatability, solutions containing 10 µg/ml of Vildagliptin and 18 µg/ml of Nateglinide were analyzed for six times. All the results were expressed in % R.S.D.

Detection Limit (DL) and Quantification Limit (QL)

Detection limit and Quantification limit of RP-HPLC and UV method were calculated using following equation as per ICH guidelines.

$$\text{Detection limit} = 3.3 \times \left(\frac{\sigma}{S}\right)$$

$$\text{Quantification limit} = 10 \times \left(\frac{\sigma}{S}\right)$$

Where, σ = standard deviation of the Y intercept of calibration curve
S = Mean slope of the corresponding calibration curve.

Robustness

The Robustness of the RP-HPLC method was determined three times by analysis of samples under a variety of conditions as flow rate (1± 0.2 ml/min), detection wavelength (222± 2 nm), and mobile phase ratio (Acetonitrile: Phosphate buffer (pH 3.2), 70:30 ± 2 % v/v).

System suitability tests

A system suitability test (Resolution, Column efficiency, tailing factor and Theoretical plates)were performed to verify resolution and reproducibility of chromatography system.

Forced degradation studies

Selectivity was assessed by performing Forced degradation studies. Combination of Vildagliptin (10 µg/ml) and Nateglinide (18 µg/ml) used as samplewas stressed under various conditions like acid, alkaline, oxidative, photo and thermal to conduct forced degradation studies.**(International Conference on Harmonization (ICH) guideline, Q1A(R2), 2003)**.
Although, Vildagliptin and Nateglinide are practically soluble in Acetonitrile: Phosphate Buffer (pH 3.2) (70:30 % v/v) was used as a solvent throughout studies.

Acid degradation:

Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask. 1 ml of 0.1 N Hydrochloric acid added to each flask and kept for 2 hat 40 ˚C.To neutralize, 1 ml of 0.1 N Sodium hydroxide was added in each flask and dilute upto volume with methanol. Filter the solution through 0.45 micron membrane filters and injected into chromatography and chromatogram has been recorded.

Base degradation:

Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask. 1 ml of 0.1 N Sodium hydroxide added to each flask and kept for 2 hat 40 ˚C.To neutralize, 1 ml of 0.1 N Hydrochloric acid was added in each flask and dilute upto volume with methanol. Filter the solution through 0.45 micron membrane filters and injected into chromatography and chromatogram has been recorded.

Oxidative degradation:
Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask. 1 ml of 3 % Hydrogen peroxide added to each flask and kept for 2 hat 40 ˚C.Filter the solution through 0.45 micron membrane filters and injected into chromatography and chromatogram has been recorded.

Photolytic degradation:
Drugs were placed in a photo stability chamber and exposed to direct UV light for 2 h. At different time intervals the drugs were taken out, dilute appropriately and injected into chromatography to determine the amount of degradation of the drugs.

Thermal degradation:
Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask and exposed under heat at 80 ºC for 2 h. At different time intervals, make volume up to the mark with methanol and injected into chromatography to determine the amount of degradation of the drugs.

Statistical comparison (analysis) between RP-HPLC and UV Method by Student t-test:
The Student's t-test calculated using following formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(S^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right))}}$$

where, t is the t-value, x_1 and x_2 are the means of HPLC and UV respectively, s^2 is the pooled standard error of the two groups, and n_1 and n_2 are the number of observations in each of the groups.

3. RESULTS AND DISCUSSION

HPLC Method: Method development and validation
In order to select mobile phase, various solvents with different proportions as Acetonitrile: Water, Methanol: Water, Acetonitrile: Phosphate buffer were used. Resulting, Acetonitrile: Potassium dihydrogen phosphate Buffer (pH 3.2) (70:30 %v/v) has been selected as optimized mobile phase based on peak parameters which obeyed ideal system suitability parameters like proper migration, separation and resolution at flow rate (1 ml/min) at 222 nm of Nateglinide and Vildagliptin. Specificity has been determined and found none interference with the chromatogram of Vildagliptin, Nateglinide and blank which showed HPLC method is specific (Figure 4).
The Retention time of Vildagliptin and Nateglinide were observed at 3.0 and 6.0 min, allows a rapid determination of the drugs, which was important for routine analysis. The results of system suitability parameters were tabulated in table 1.

Table 1: System suitability parameter

Parameters	**Retention Time**	**Tailing Factor**	**Number of Theoretical plates**	**Resolution**
Nateglinide	3.0	1.206	5599	2.5
Vildagliptin	6.0	1.192	11947	

Nateglinide and Vildagliptin were eluted and forming symmetrical peaks, also well separated from solvent front as indicated in figure 5. The linearity were obtained at a concentrations 9-45 µg/ml for Nateglinide and 5-25 µg/ml for Vildagliptin as showed in figure 6. Calibration

curve were constructed by plotting average Peak area versus Concentration. Straight line equations were obtained from calibration curve. The linear regression equation for Vildagliptinwas y = 134.84x -289.97, with correlation coefficient (r= 0.9983), and y = 26.814x -146.52, with correlation coefficient (r= 0.999) for Nateglinide which showed highly significant for the method (Table 2).

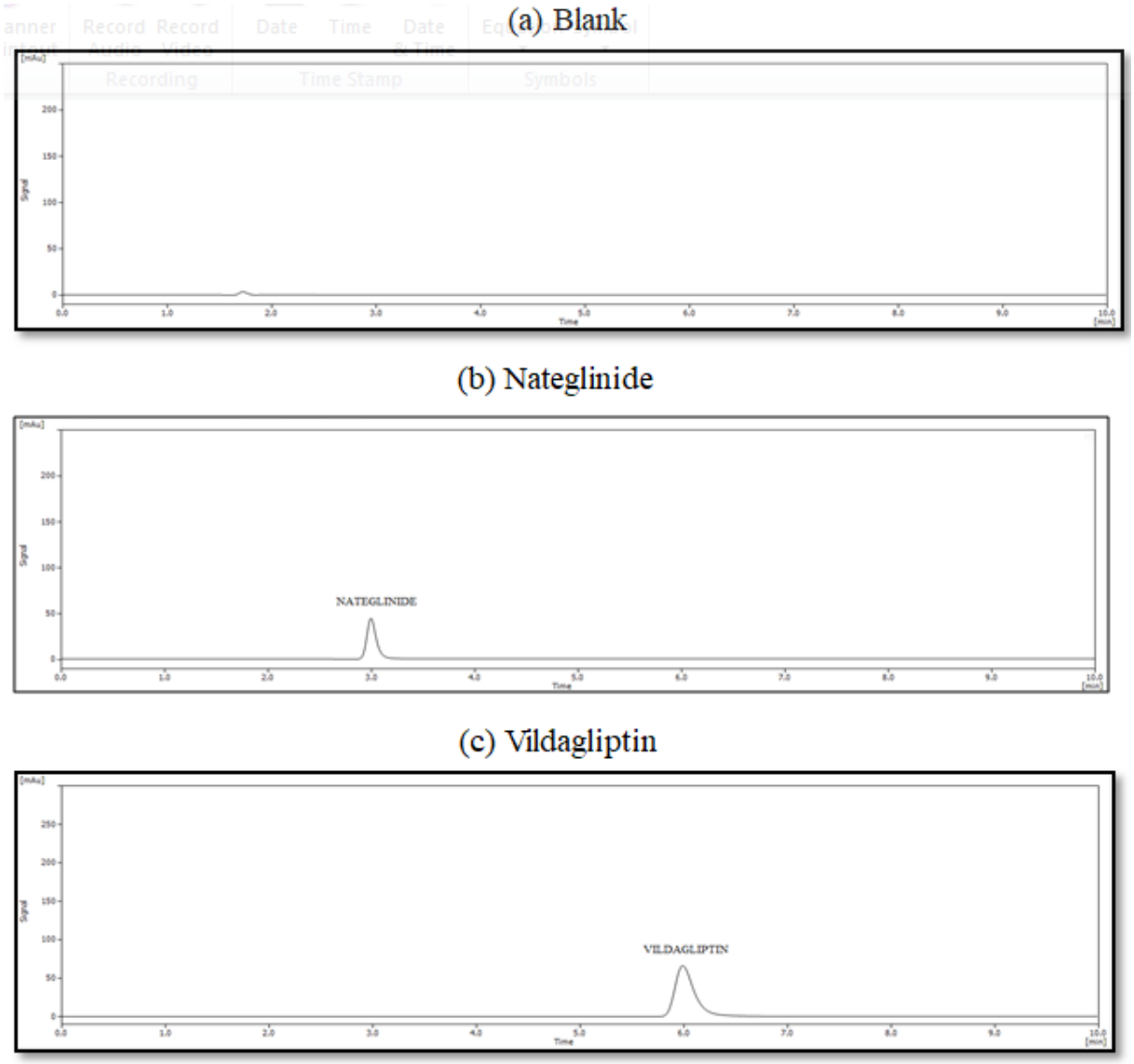

Figure 4: RP-HPLC Chromatogram for (a) Blank, (b) Nateglinide(18 µg/ml) and (c) Vildagliptin(10 µg/ml) in Acetonitrile: Phosphate buffer (pH=3.2) (70: 30 % v/v)at 222 nm {Run time: 10 min, Flow rate: 1ml/min}

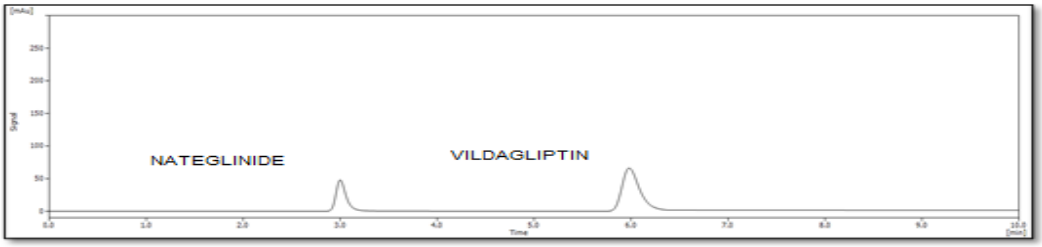

Figure 5: Optimized RP-HPLC Chromatogram ofNateglinide (18 µg/ml) and Vildagliptin(10 µg/ml)in ACN: Buffer (KH_2PO_4) (pH 3.2) (70:30 % v/v); Flow rate: 1 ml/min at 222 nm

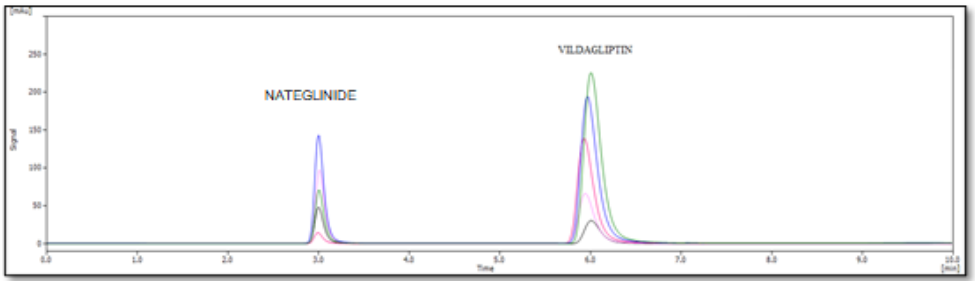

Figure 6: Overlain RP-HPLC Chromatogram of Nateglinide (9-45 µg/ml) and Vildagliptin(5-25 µg/ml) in Acetonitrile: Phosphate Buffer (pH=3.2) (70: 30 % v/v) at 222 nm {Run time: 10 min, Flow rate: 1 ml/min}

Table 2: Regression analysis of data of Vildagliptin and Nateglinide

Statistical parameters	**HPLC Method**		**UV Method**	
Parameters	**Vildagliptin**	**Nateglinide**	**Vildagliptin**	**Nateglinide**
Concentration range (µg/ml)	5-25	9-45	5-25	9-45
Wavelength (nm)	222 nm		253 nm	270 nm
Regression equation (y = mx +c)	y = 134.84x -289.97	y = 26.814x -146.52	y = 0.0006x - 0.0004	y=0.0003x + 0.0003
Correlation coefficient (r)	0.9983	0.999	0.9972	0.9956

The % recovery of Vildagliptin and Nateglinide was found to be 99.87 - 100.28 and 99.71 - 100.34, respectively (Table 3).From the results, good sensitivity has been achieved which reflects the high efficiency of the separation methods.

Table 3: Recovery test for Vildagliptin and Nateglinide

Name of Drug	**% Level of Recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Spiked Std Amount (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Recovery ±S.D. (n=3)**	**Total amount Recovered (µg/ml)**	**% Recovery ±S.D. (n=3)**
					HPLC Method		**UV Method**	
Vildagliptin	50	10	5	15	14.96	99.87±0.0115	14.93	99.57±0.1026
	100	10	10	20	19.06	100.15±0.0208	19.95	99.78±0.1222
	150	10	15	25	25.14	100.28±0.0230	24.97	99.88±0.2136
Nateglinide	50	18	9	27	26.92	99.71±0.0057	26.92	99.72±0.1115
	100	18	18	36	36.09	100.25±0.0115	35.93	99.81±0.1652
	150	18	27	45	45.15	100.34±0.0152	44.92	99.84±0.1965

The intraday, interday and repeatability precision of Vildagliptin and Nateglinidewere expressed in % RSD and indicated in acceptable limits. This result indicates that the method is precise and accurate. The precision study of Vildagliptin and Nateglinideshowed in table 4 and table 5, respectively.

Table 4: Precision for Vildagliptin

Intraday Precision of Vildagliptin				
	HPLC Method		**UV Method**	
Conc. (µg/ml)	**Mean Peak area ± SD (n=3)**	**% R.S.D.**	**Mean Absorbance ± SD (n=3)**	**% R.S.D.**
	222 nm		**253 nm**	
5	394.362±3.818	0.97	|-0.0030| ± 0.00005	1.66
10	974.261±6.088	0.62	|-0.0060| ± 0.00008	1.33
15	1768.801±4.716	0.27	|-0.0091| ± 0.00011	1.20
Interday Precision of Vildagliptin				
Conc. (µg/ml)	**Mean Peak area ± SD (n=3)**	**% R.S.D.**	**Mean Absorbance ± SD (n=3)**	**% R.S.D.**
	222 nm		**253 nm**	
5	398.364±4.291	1.08	|-0.0031| ± 0.000056	1.80
10	999.266±7.092	0.71	|-0.0060| ± 0.00009	1.50
15	1768.82±5.686	0.33	|-0.0091| ± 0.00012	1.31
Repeatability of Vildagliptin				
Conc. (µg/ml)	**Mean Peak area ± SD (n=6)**	**% R.S.D.**	**Mean Absorbance ± SD (n=6)**	**% R.S.D.**
	222 nm		**253 nm**	
10	998.262±6.184	0.62	|-0.0060| ± 0.00005	0.83

Table 5: Precision for Nateglinide

Intraday Precision of Nateglinide				
	HPLC Method		**UV Method**	
Conc. (µg/ml)	**Mean Peak area ± SD (n=3)**	**% R.S.D.**	**Mean Absorbance ± SD (n=3)**	**% R.S.D.**
	222 nm		**270 nm**	
9	102.096±1.206	1.19	|-0.0030| ± 0.00004	1.33
18	329.484±2.461	0.75	|-0.0050| ±0.00006	1.20
27	566.092±1.692	0.27	|-0.0081| ± 0.00009	1.11
Interday Precision of Nateglinide				
Conc. (µg/ml)	**Mean Peak area ± SD (n=3)**	**% R.S.D.**	**Mean Absorbance ± SD (n=3)**	**% R.S.D.**
	222 nm		**270 nm**	
9	106.092±1.452	1.37	|-0.0030| ±0.00005	1.66
18	333.566±2.431	0.73	|-0.0050| ± 0.00007	1.40
27	568.096±1.437	0.26	|-0.0081| ± 0.00010	1.23

Repeatability of Nateglinide				
Conc. (µg/ml)	**Mean Peak area ± SD (n=6)**	**% R.S.D.**	**Mean Absorbance ± SD (n=6)**	**% R.S.D.**
	222 nm		**270 nm**	
18	332.483±1.103	0.34	|-0.0050| ± 0.00005	1.00

The Detection and Quantitation limit of Vildagliptinwere found to be 0.1809 µg/ml and 0.603 µg/ml, respectively and for Nateglinide, Detection and Quantitation limit were found to be 0.1407 µg/ml and 0.469 µg/ml, respectively at 222 nm which were within the acceptable limits. The % assay of Vildagliptin and Nateglinide were found to be 99.95 and 100.17, respectively.

The Robustness was determined under a variety of conditions as flow rate (± 0.2 ml/min), wavelength (± 2 nm), and mobile phase ratio (± 2 % v/v) and results were expressed in % RSD. The Robustness data showed in table 6.

Table 6: Robustness Study for Vildagliptin and Nateglinide

Sr. No.	**Parameter**	**Variation**	**% Assay ± S.D. (n=3)**		**% R.S.D.**	
			Vildagliptin	**Nateglinide**	**Vildagliptin**	**Nateglinide**
1	Flow rate (1 ml/min) (± 0.2 ml/min)	0.8 ml/min	97.75±1.061	97.37±1.516	1.09	1.56
		1.0 ml/min	99.75±1.717	99.87±1.269	1.73	1.28
		1.2 ml/min	97.25±0.940	98.62±1.477	0.97	1.43
2	Detection wavelength (222 nm) (± 2 nm)	220	97.25±1.069	97.51±1.268	1.10	1.31
		222	100.25±1.505	100.12±1.426	1.51	1.43
		224	98.75±1.376	98.75±1.256	1.39	1.28
3	Mobile phase (70:30 %v/v) (± 2 %v/v)	68:32	97.75±1.116	98.12±1.754	1.15	1.79
		70: 30	99.75±1.552	99.75±1.421	1.56	1.43
		72: 28	98.25±1.245	97.75±1.143	1.27	1.17

UV Method

A reliable, precise and accurate UV spectrophotometric method was developed and validated for simultaneous estimation of Vildagliptin and Nateglinidein combination. Vildagliptin(10 µg/ml) and Nateglinide (18 µg/ml) solutions were scanned between 200-400 nm. The detection wavelength (λ) for Vildagliptin and Nateglinidewere found to be 253 nm and 270 nm, respectively. These wavelengths were used for all measurements. The spectra of Vildagliptin(10 µg/ml) and Nateglinide (18 µg/ml)were constructed and the linearity range were observed (Figure 7 and 8). Calibration curves were constructed and Beer's law was obeyed over the concentration range of 5-25 µg/ml for Vildagliptin and 9-45 µg/ml for Nateglinide. The linear regression equation (correlation coefficient) for Vildagliptinwere y = 0.0006x - 0.0004 at 253 nm (r = 0.9972) and for Nateglinidey = 0.0003x + 0.0003 at 270 nm (r = 0.9956) (Table 2). The % recovery of Vildagliptin and Nateglinidewas found to be 99.57 - 99.88% and 99.72 - 99.84%, respectively (Table 3). Results were obtained lie in acceptable limits. The intraday, interday and repeatability precision of Vildagliptin and Nateglinidewere expressed in % RSD and indicated in acceptable limits. This result indicated that the method

is precise and accurate. The precision data of Vildagliptin and Nateglinideshowed in table 4 and table 5, respectively.

The Detection and Quantitation limit of Vildagliptinwere found to be 0.602 µg/ml and 1.986 µg/ml at 253 nmand for Nateglinide, Detection and Quantitation limit were found to be 0.638 µg/ml and 2.105 µg/ml at 270 nmwhich were within the acceptable limits. The % assay of Vildagliptin and Nateglinidewere found to be 99.60 and 99.77 %, respectively.

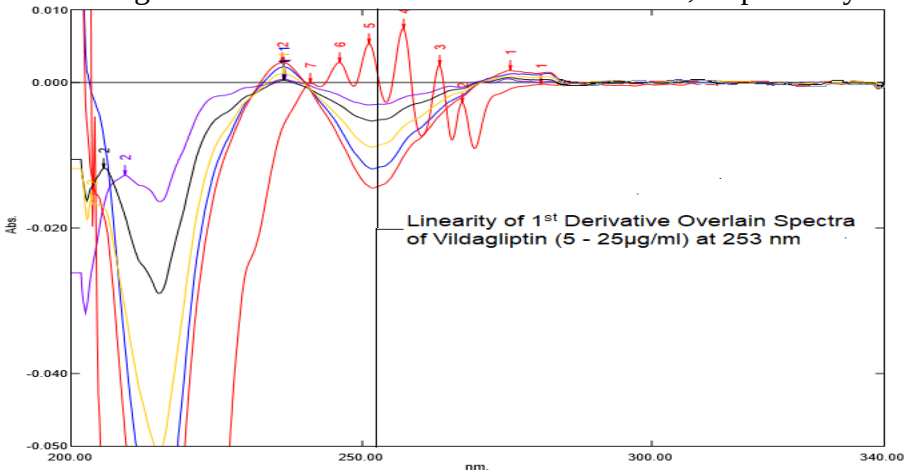

Figure 7: Overlain UV Spectra of Vildagliptin(Linearity)(5-25 µg/ml) at 253 nm

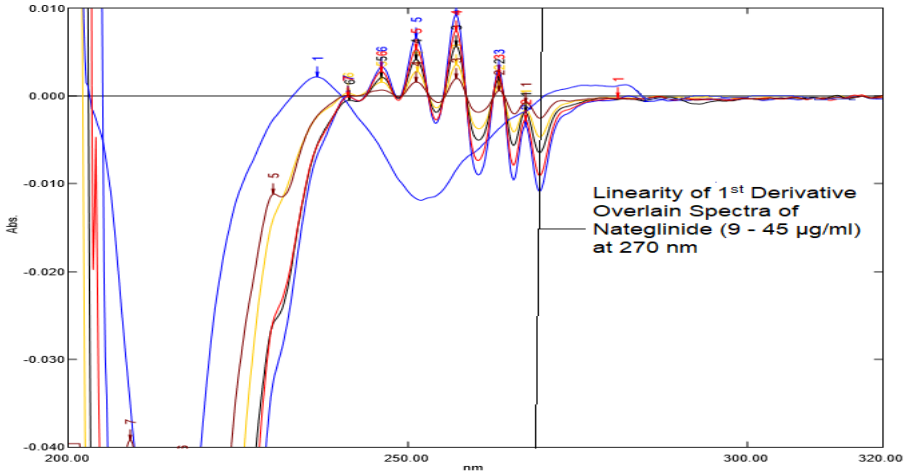

Figure 8: Overlain UV Spectra of of Nateglinide(Linearity) (9-45 µg/ml)at 270 nm

Forced Degradation Studies

Peak area of Vildagliptin and Nateglinidewere found to be 954.262 and 319.484, respectively.% degradation of Vildagliptin and Nateglinidewere calculated using this equation,

$$\%\ degradation = 100 - \left(\frac{Degradation\ area}{Standard\ area}\right) \times 100$$

Acid degradation study

The combination showed sufficient degradation within 2 h with 0.1 N Hydrochloric acid at 40˚C. Vildagliptinshowed 9.83 and 12.67 % degradationat 1 and 2 h, respectively; whereas Nateglinideshowed 9.78 and 19.50 % degradation at 1 and 2 h, respectively (Figure 9).

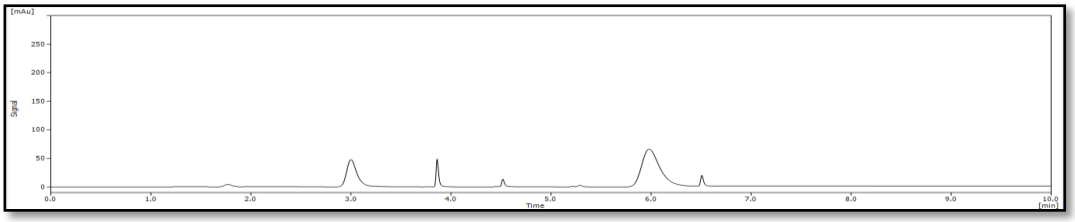

Figure 9: RP-HPLC Chromatogram of Acid Degradation for Nateglinide(18 µg/ml) and Vildagliptin(10 µg/ml) Sample at 2 h at 222 nm {Run time: 10 min, Flow rate: 1ml/min}

Base degradation study

Similar to acid, sufficient degradation was observed within 2 h with 0.1 N Sodium Hydroxide at 40˚C. Vildagliptinshowed5.28 and 9.57 % degradation at 1 and 2 h, respectively; whereas Nateglinideshowed 6.73% and 12.86 % degradation at 1 and 2 h, respectively (Figure 10).

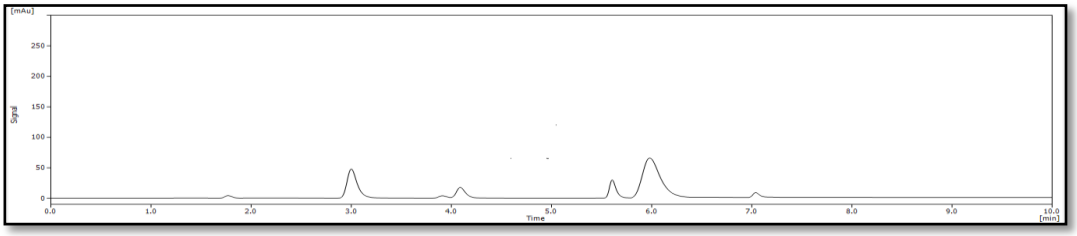

Figure 10: RP-HPLC Chromatogram of Base Degradation for Nateglinide(18 µg/ml) and Vildagliptin(10 µg/ml) Sample at 2 h at 222 nm {Run time: 10 min, Flow rate: 1ml/min}

Oxidative degradation study

Degradation was observed within 2 h after heating with 3 % Hydrogen peroxide at room temperature. Vildagliptinshowed1.56 and 2.96 % degradation at 1 and 2 h, respectively; whereas Nateglinideshowed 3.84and 9.32 % degradation at 1 and 2 h, respectively (Figure 11).

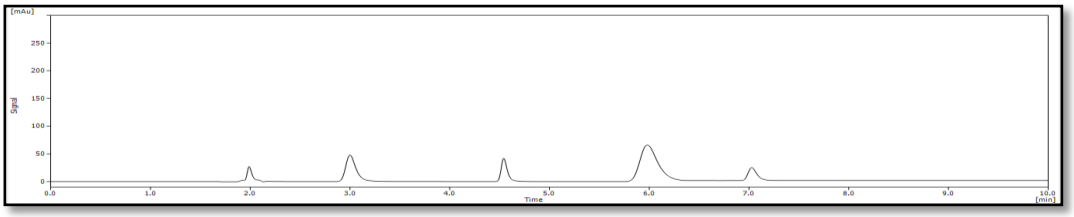

Figure 11: RP-HPLC Chromatogram of Oxidative Degradation for Nateglinide(18 µg/ml) and Vildagliptin(10 µg/ml) Sample at 2 h at 222 nm {Run time: 10 min, Flow **rate:** 1ml/min}

Photolytic degradation study

Drugs were exposed to direct UV light for 2 h. Vildagliptinshowed7.35 and 11.98 % degradation at 1 and 2 h, respectively; whereas Nateglinideshowed 8.94 and 18.97 % degradation at 1 and 2 h, respectively (Figure 12).

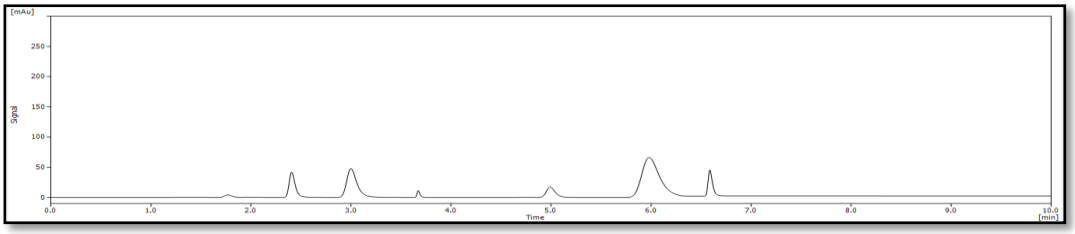

Figure 12: RP-HPLC Chromatogram of Photolytic Degradation for Nateglinide(18 **µg/ml)** and Vildagliptin(10 µg/ml) Sample at 2 h at 222 nm {Run time: 10 min, Flow rate: 1 ml/min}

Thermal degradation study

Drugs were exposed under heat at 80 ºC for 2 h. Vildagliptinshowed9.46 and 13.87 % degradation at 1 and 2 h, respectively; whereas Nateglinideshowed 5.49 and 10.11 % degradation at 1 and 2 h, respectively (Figure 13).

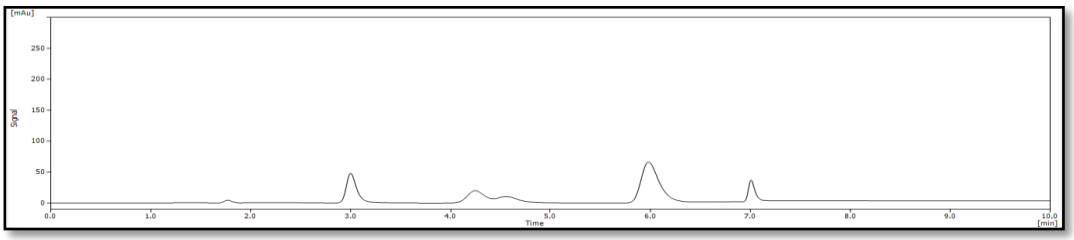

Figure 13: RP-HPLC Chromatogram of Thermal Degradation for Nateglinide(18 µg/ml) and Vildagliptin(10 µg/ml) Sample at 2 h at 222 nm {Run time: 10 min, Flow rate: 1 ml/min}

Statistical comparison of RP-HPLC and UV Method

The proposed analytical methods were compared using Statistical analysis. The student's t-test was applied and did not showed significant difference between experimental values obtained in sample analysis by the two methods. The calculated t-value ($t_{calculated}$) was smaller than critical t-value ($t_{tabulated}$ / $t_{critical}$), at 5 % significance level.

4. CONCLUSION

A Simple, rapid, stability indicating, sensitive, accurate and precise RP-HPLC and UV spectroscopic methods has been developed and validated for routine analysis of Vildagliptin and Nateglinide. These drugs were subjected to forced degradation applying several stress conditions. The proposed methods were suitable and successfully separated for simultaneous estimation without any interference.Validation of proposed methods was also carried out according to ICH guideline Q2 (R1).All results obtained were found within the acceptance criteria. Hence, the proposedmethods can be adaptedto regularquality control analysis.Statistical analysis proved that the proposed methods were repeatable and selective for the analysis of Vildagliptin and Nateglinidein combination.

Conflict Of Interest

Authors have no conflict of interest.

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