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Original scientific paper

Simultaneous stability-indicating analysis of nateglinide and pioglitazone using RP-HPLC

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Abstract

A simple, rapid and sensitive RP-HPLC has been developed and validated for the simultaneous estimation of anti-diabetic drugs nateglinide (NAT) and pioglitazone (PIO), at a selected wavelength of 210 nm. The method provided linear responses within the concentration range which varies from 50 to 250 µg/mL for both NAT and PIO with LOD values of 0.18 µg/mL and 0.22 µg/mL for NAT and PIO, respectively, and the LOQ value of 0.57 µg/mL & 0.63 µg/mL, respectively. Mean percent recovery varied between 100.06 and 100.31% and mean RSD of 0.08% (repeatability) and 0.26% (intermediate precision). Other characteristics, such as robustness, system suitability and specificity, were also verified for the approach. According to the degradation investigation, NAT and PIO exhibited slight degradation under acid hydrolysis and were stable under oxidative and photocatalytic degradation. PIO remained stable in basic condition, while NAT was degraded by 15.36%. The approach that has been proposed holds great potential for routine quality control analysis involving the qualitative and quantitative determination of NAT and PIO in various pharmaceutical formulations.

Key words: nateglinide, piogliatazone, HPLC, UV detection.

Introduction

Diabetes mellitus (DM) refers to a collection of metabolic conditions defined by persistent hyperglycemia and certain changes of carbohydrate, lipid, and protein metabolism brought on by a deficiency in insulin production, action, or both. Anti-diabetic drugs treat diabetes mellitus by reducing blood glucose levels in diabetic patients to maintain blood glucose levels at or near

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normal levels (Sebaiy et al., 2019).

An oral antihyperglycemic drug named nateglinide (NAT), an amino acid derivative, chemically known as N-(trans-4-isopropylcyclohexylcarbonyl)-D phenylalanine, is used for the treatment of non-insulin-dependent diabetic mellitus (NIDDM). This member of fast-acting insulin secretagogues of meglitinide class, stimulate insulin secretion by attaching to pancreatic β cells. NAT induces an early insulin response to meals, lowering blood sugar levels after meals. It enhances extracellular glucose's effects on ATP-sensitive potassium channels while having minimal impact on insulin levels during the day and nighttime. Therefore, NAT is more successful in lowering postprandial blood sugar levels compared to blood sugar levels during fasting and needs to be taken for a longer period before fasting blood glucose levels are reduced (Hacıoğlu et al., 2015). With a $t_{1/2}$ of 1.4 hours, it gets eliminated after being metabolized by the cytochrome P-450 enzymes (Dey et al., 2019).

A thiazolidinedione derivative, Pioglitazone (PIO), is a nuclear receptor, peroxisome proliferator-activated receptor gamma (PPAR-γ) selective agonist as well as an insulin sensitizer, chemically referred to as [(±)-5-[[4-[2-[5-ethyl -2- pyridinyl) ethoxy] phenyl] methyl]-2,4-]thiazolidinedionemonohydro-chloride also works by decreasing insulin resistance in hepatic and peripheral tissues of patients, thus used in the treatment of non-insulin-dependent diabetic mellitus (NIDDM). It undergoes substantial hydroxylation and oxidation-based metabolism (Karthik et al., 2008).

It has been evident from the literature survey that numerous chromatographic and spectrophotometric methods have been reported till date for the determination of NAT and PIO, either alone or in combination with other antidiabetic drugs (Sayed et al., 2009; Swapna et al., 2012). Several UV spectrophotometric techniques are documented for the analysis of NAT in pharmaceutical formulations and bulk. For the purpose of estimating the quantity of NAT in human plasma, HPLC analysis with UV detection and pre-column derivatization was used (Madhavi et al., 2008; Pani et al., 2012). NAT, cilostazol, and its active metabolite, 3,4-dehydro-cilostazol, were also studied for their pharmacokinetics in plasma using the LC/MS assay. In addition, HPTLC/TLC techniques and fluorescence detection of the coumarin type were also described (Han et al., 2010; Varanasi et al., 2008). Spectrophotometric techniques have also been published for determining NAT in tablets and bulk drugs (Bauer et al., 2003; Jain et al., 2009; Malli et al., 2007; Thomas et al., 2012). Various analytical methods, including HPLC, have been mostly used for the estimation of PIO in pharmaceutical formulations and biological samples (Satheeshkumar et al., 2014; Venkatesh et al., 2006; Xue et al., 2003; Yao et al., 2007).

Every impurity in drug substances and completed pharmaceutical products must be identified, qualified, and regulated according to current regulatory criteria (ICH Q3B R2, 2006). This is because each impurity has the potential to compromise the effectiveness, safety, and quality of the finished pharmaceutical product. As advised by International Conference on Harmonization, ICH Q2 (R1) recommendations (ICH, 2005), the content of related chemicals in final pharmaceutical products must be ascertained using appropriate stability-indicating HPLC procedures in order to comply with the requirements.

Thus, the goal of our work was to optimize and validate a straightforward and trustworthy HPLC approach that indicates stability for the simultaneous detection of NAT and PIO, as well as to analyse the degradation of these compounds in bulk and synthetic mixtures.

Material and methods

Chemicals and reagents

NAT and PIO were sent as gift samples by Dr. Reddy's Lab. Ltd. Hyderabad and Lupin Ltd. Goa, respectively, to develop a liquid chromatography method for the estimation of NAT and PIO in both bulk and synthetic dosage form. HPLC grade solvents such as: methanol, acetonitrile (ACN) & water were supplied by Merck Pvt.Ltd., Mumbai. Other reagents, including perchloric acid and orthophosphoric acid (85%) of analytical grade were purchased from Merck Pvt.Ltd. Mumbai.

Instrumentation and chromatographic conditions

Chromatographic separation was carried out using a Waters HPLC system solvent delivery module that was outfitted with a Waters 2489 UV-Vis detector, a manual injector with a fixed loop (20 µL), and a pump (model number-515). Analysis and separation were carried out by using a commercially available waters Nova-Pak® C8G column with the specifications of 150 mm column length, 3.9 mm internal diameter, and 4 µm particle size respectively at room temperature. Empower 2 Software Build 2154® (Waters Corporation) was employed for the data acquisition. The mobile phase consisting of acetonitrile and 10 mM potassium dihydrogen phosphate in the ratio of 60:40 *v/v* has been adjusted to pH 3.5 using 10% orthophosphoric acid and it was delivered at a flow rate of 1.5 mL/min through the column with detection at 210 nm. The mobile phase was degassed prior using and filtered through a 0.45 µm membrane filter.

Preparation of standard stock solutions

For the preparation of NAT and PIO standard solutions, methanol was utilized as a solvent. To achieve a concentration of 1000 µg/mL, 25 mg of NAT was precisely measured and transferred into a 25 mL volumetric flask. 15 mL of previously prepared mobile phase was subsequently incorporated into NAT, and after 10 minutes of sonication, the final volume was brought up to 25 mL using diluents.

In another separate volumetric flask, the same procedure was followed to dissolve 25 mg PIO.

Preparation of working standard solutions

An aliquot of 2.5 mL from two corresponding flasks containing stock solutions has been transferred into 25 mL volumetric flask for each solutionand the remaining volume was made up with the mobile phase in order to get working standard solution of NAT (100 µg/mL) and PIO (100 µg/mL) respectively.

Preparation of synthetic mixture

Synthetic mixture of NAT and PIO were combined in a ratio of 4:1. A volumetric flask (100 mL) was filled with a 50 mg of the powdered synthetic mixture, to which the 2% magnesium stearate was added as diluents. The solution was sonicated for 5 mins. The final concentrations of NAT and PIO were adjusted to 240 µg/mL for NAT and 60 µg/mL for PIO.

Validation of developed method

The optimized method has been validated in accordance with International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines Q2 (R1) for assessing linearity, precision, accuracy, specificity, system suitability, robustness, limit of detection (LOD), limit of quantitation (LOQ), and force degradation studies. A coefficient of variation of less than 2% and relative errors of less than 2% were deemed admissible.

Degradation in acidic condition

For acid-induced degradation study, 0.1 N hydrochloric acid (HCl) was added to a 10 mL volumetric flask that contained 5 mL of the PIO standard stock solution (1000 µg/mL), and the volume was then brought to the necessary level using mobile phase. The volumetric flask was maintained at room temperature for the following 24 hours. 1 mL of the solution was withdrawn from this flask after 24 hours, neutralized with 0.1 N NaOH, and diluted with mobile phase up to 10 mL. After that, the sample solution was introduced to the HPLC. Similarly, for NAT, a 10 mL volumetric flask was filled with 2 mL of standard stock solution (1000 µg/mL), 2 mL of 0.1 N hydrochloric acid (HCl), and the volume was then adjusted with the mobile phase to obtain 200 µg/mL concentration. The volumetric flask was then let to rest for 24 hours at room temperature. After a 24-hour period, the sample solution was injected into the HPLC.

Degradation in basic condition

Base induced degradation study was carried out by taking 5 mL and 2 mL of 1N sodium hydroxide (NaOH) with 5 mL and 2 mL of stock solution (1000 µg/mL) of PIO and NAT respectively and kept for 24 hours in room temperature and the procedure was repeated as per the acid hydrolysis. From the retention time and peaks in the standard chromatogram and chromatogram obtained after degradation, the percentage of degradation was calculated.

Oxidative degradation

Oxidative degradation frequently exhibits various drug degradation patterns such assensitivity to minute levels of free radicals and fast development of contaminants during an induction period etc. Generally, hydrogen peroxide (H_2O_2) is used as oxidizing agent. Oxidative degradation was carried out by taking 5mL standard stock solution (1000 µg/mL) of PIO, 5 mL 5% H_2O_2 as an oxidizing agent in a volumetric flask (10 mL) where the required volume of mobile phase was added to make up the volume. Afterwards, the volumetric flask was left at the room temperature for 24 hours. Following a 24-hour period, 1 mL of the solution was diluted with mobile phase to 10 mL to achieve 50µg/mL concentration. Similarly, for NAT, in order to reach a 200 µg/mL concentration, along with the 2 mL standard stock solution (1000 µg/mL) of NAT, 2 mL of 5% H_2O_2 were added to a 10 mL volumetric flask, and afterwards that volume was brought up to the required level with the mobile phase. The volumetric flask was then allowed to keep at room temperature for 24 hours. The prepared sample was fed into the HPLC after a 24-hour delay. From the peak area and retention time the % degradation was calculated.

Photolytic degradation

Exposure to sunlight or other types of light can also cause degradation of the active pharmaceutical ingredients. Absorption of photons from infrared, visible, ultraviolet, and other types of electromagnetic radiation start the process of photo degradation. To perform photolytic degradation study, sample of the drugs were exposed in UV light for 4 hours. Sample which was prepared from 100 mg of drug sample, dissolved in 50 mL of mobile phase in a 100 mL volumetric flask and diluted up to the mark to prepare a 1000 µg/mL concentration stock solution. From the above stock solution, required amount was taken and diluted to achieve 30 µg/mL concentration. Then the sample was injected in HPLC for analysis.

Results and discussion

Method development and optimization

For a successful chromatographic separation, a number of crucial factors were examined, including the mobile phase's pH, the acid or buffer solution's concentration, the amount and type of organic modifier, etc. Experiments revealed that the reversed-phase waters Nova-Pak® C8G column produces symmetric, crisp peaks when an acidic mobile phase is used. Since ACN dissolves medications well, it was selected as the organic modifier.

At a flow rate of 1.5 mL/min, the mobile phase composition of acetonitrile and 10 mM potassium dihydrogen phosphate in the ratio of 60:40 *v/v* (adjusted to pH 3.5 using 10% orthophosphoric acid) has demonstrated satisfactory resolution. Resolution between NAT and PIO was significantly improved at pH 3.5 with a reduction in peak tailing when o-phosphoric acid was utilized as a modifier. For NAT and PIO, the retention durations of the medications acquired under these circumstances were 3.562 and 5.920 minutes, respectively. The wavelength for the quantitative analytical purposes was fixed at 210 nm.

Calibration curve

The calibration curves exhibited linear relationship of peak area to concentration in the 50-250 µg/mL for both the drugs. The R^2 value, slope, and intercept were 0.999, 31.294 and 1.9888, respectively, for NAT, and 0.999, 44.84, and 1.098, respectively, for PIO (Table 1). The retention time of NAT was 3.562 minutes and PIO was 5.920 minute respectively (Fig. 1). Therefore, the curve can be used for estimation of NAT and PIO in synthetic mixture.

Linearity

The correlation coefficient (R^2) value for both NAT and PIO were found to be 0.999, which shows that the analytical calibration curves developed for both NAT and PIO were found to be linear in the defined ranges. The linear regression equations for NAT and PIO are y=31.29x+1.988 (R^2=0.999) and y=44.89x+1.098 (R^2=0.999).

Specificity

Specificity was studied for the examination of the presence of interfering components. Nateglinide and pioglitazone standard solution of 10 mg/mL was injected and none of the impurities were interfering in its assay. The retention time obtained by HPLC for NAT and PIO is about 3.562 min and 5.920 min respectively. The specificity results were summarized in Table 2.

Precision

Both, NAT and PIO's relative standard deviations were determined to be less than 2.0%, indicating a high degree of precision. The results of the precision analysis for NAT and PIO, respectively, are summarized in Table 3.

Accuracy

The mean recoveries of NAT and PIO were determined to be in the range 100.06–100.10% and 100.06–100.31%, respectively, and it was within the acceptable limits at 80%, 100 and 120%. The data are represented in Table 4.

Table 1. Linear regression equation generated from validation of NAT and PIO: Slope, Intercept and Coefficient of determination.

NAT			PIO		
Conc (µg/mL)	Peak area (mV.s)		Conc (µg/mL)	Peak area (mV.s)	
50	1604.66	Slope: 31.294	50	2203.17	Slope 44.84
100	3067.30		100	4368.87	
150	4596.70	Intercept: 1.9888	150	6813.31	Intercept 1.098
200	6335.76	R^2: 0.9991	200	8928.43	R^2: 0.9995
250	7843.43		250	11274.39	

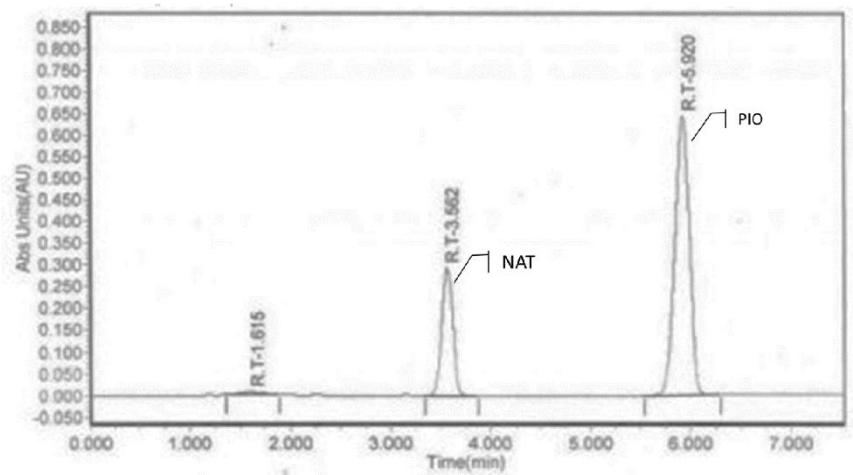

Fig. 1. Chromatogram of NAT and PIO.

Table 2. Specificity data of the developed method

Sample no.	excipient conc. (mg)	NAT input (mg)	PIO input (mg)	NAT recovered (mg)	NAT recovered %	PIO recovered (mg)	PIO recovered %
1	5	10	10	10.1	101	9.99	99.9
2	10	10	10i	9.99	99.9	9.98	99.8
3	15	10	10	9.98	99.8	10	100
				Mean recovery	100.2		99.9
				St. deviation	0.67		0.10
				%RSD	0.66		0.10

Table 3. Summary of precision study

Intra Day Precision (n =3)									
NAT					PIO				
Conc (µg/mL)	Recovery	Average	S.D.	%RSD	Conc (µg/mL)	Conc found (µg/mL)	Average	S.D.	%RSD
50	49.80	49.56	0.49	0.99	50	49.96	49.85	0.13	0.27
50	49.89				50	49.70			
50	49.00				50	49.88			
100	99.89	99.58	0.51	0.51	100	99.78	99.90	0.10	0.26
100	98.99				100	99.97			
100	99.87				100	99.96			
200	199.88	199.84	0.16	0.08	200	199.40	199.79	0.33	0.26
200	199.99				200	199.98			
200	199.66				200	199.93			
Inter Day Precision (n =3)									
NAT					PIO				
Conc (µg/mL)	Recovery	Average	S.D.	%RSD	Conc (µg/mL)	Conc found (µg/mL)	Average	S.D.	%RSD
50	50.10	50.11	0.18	0.36	50	50.20	50.06	0.11	0.23
50	49.93				50	50.01			
50	50.30				50	49.98			
100	100.01	99.98	0.02	0.02	100	100.01	100.01	0.03	0.03
100	99.97				100	100.05			
100	99.98				100	99.99			
200	200.09	199.92	0.20	0.10	200	199.98	199.99	0.02	0.01
200	199.99				200	199.99			
200	199.70				200	200.02			
Repeatability									
NAT					PIO				
Conc (µg/mL)	Recovery	Avg	S.D.	%RSD	Conc (µg/mL)	Conc. Found (µg/mL)	Avg	S.D.	%RSD
100	99.98	100.01	0.01	0.01	100	100.10	100.03	0.08	0.08
100	100.01				100	100.11			
100	100.01				100	99.98			
100	99.98				100	99.99			
100	100.02				100	100.10			
100	99.99				100	99.90			

Table 4. Accuracy study of NAT and PIO

Analyte	% Added	Constant amount added a	Amount added b	Total conc found c	Amount found d	% Recovery	Average % Recovery	S.D.	R.S.D.
NAT	80	120	96	216.10	96.10	100.10	100.06	0.05	0.05
	80	120	96	215.90	95.99	99.99			
	80	120	96	216.90	96.09	100.09			
	100	120	120	240.40	120.4	100.33	100.10	0.19	0.19
	100	120	120	239.99	119.99	99.99			
	100	120	120	239.98	119.98	99.98			
	120	120	144	264.2	144.20	100.13	100.08	0.04	0.04
	120	120	144	264.08	144.08	100.05			
	120	120	144	264.09	144.09	100.06			
PIO	80	120	96	216.5	96.50	100.52	100.31	0.18	0.17
	80	120	96	216.2	96.20	100.20			
	80	120	96	216.2	96.20	100.20			
	100	120	120	240.4	120.40	100.33	100.15	0.15	0.15
	100	120	120	240.08	120.08	100.06			
	100	120	120	240.09	120.09	100.07			
	120	120	144	264.09	144.09	100.06	100.06	0.04	0.04
	120	120	144	264.08	144.08	100.05			
	120	120	144	264.09	144.09	100.06			

Robustness

The parameters like flow rate, mobile phase ratio, and pH were varied intentionally, to check the robustness of the method. The %RSD was determined along with the mean of the analytes, which remained the same and did not vary significantly and is shown in Table 5.

Assay of synthetic mixture

The assay % was found to be 100.08 and 100.34 for NAT and PIO, respectively. The result is summarized in Table 6.

System suitability

In chromatographic analysis, system suitability is considered to be an important element and is used to verify that the chromatography system's reproducibility and resolution are sufficient for analysis. To examine the different parameters like resolution, theoretical plate, HETP (Height Equivalent to a Theoretical Plate), etc., the chromatograms from the sample solution were subjected to a system suitability test in accordance with USP (Bose, 2014). The results from six replicate injections of the standard solution are shown in the Table 7.

Limit of detection & limit of qualification

Based upon the calibration curve parameters, the limit of detection (LOD) was determined to be 0.18 µg/mL and 0.22 µg/mL for both NAT and PIO and the limit of quantification (LOQ) value for both substances were determined to be 0.57 µg/mL and 0.63 µg/Ml, respectively. The results are reported in Table 8.

Forced degradation study

The degradation studies were carried out as per ICH Q1A (ICH, 2003) suggested stress conditions to assess the stability of NAT and PIO under acid-alkali hydrolysis, oxidative degradation and photolytic degradation. The hydrolytic degradation study is shown in Table 9 for acid degradation and Table 10 for basic degradation for both PIO and NAT. The acid degradation of PIO HCl using 0.1 N HCl was found to be 1.045% after 24 hours and the acid degradation of NAT using 0.1 N HCl was found to be 1.009% after 24 hours (Table 9, Fig. 2a). No degradation was found for PIO HCl under basic conditions after 24 hours and 15.36% degradation was reported for NAT after 24 hours (Table 10) (Fig. 2b). The oxidative degradation study shows no deterioration for both PIO and NAT using 5% H_2O_2 after 24 hours (Fig. 3). Photolytic degradation was carried out according to the ICH guidelines and no major changes were recorded for both the drugs as that suggests the drugs are very much stable in the photolytic conditions.

Table 5. Determination of robustness for NAT and PIO.

Determination of robustness for NAT							
Sample I.D.	Analytical condition	Input (mg)	Recovery	% Recovery	Mean recovery	S.D.	% R.S.D.
Set-1	Flow rate:1.4 mL/min; mobile phase pH: 3.5; mobile phase ratio: 60:40 *v/v*	240	240.5	100.2			
Set-2	Flow rate:1.6 mL/min; mobile phase pH: 3.5; mobile phase ratio: 60:40 *v/v*	240	240.1	100.04			
Set-3	Flow rate:1.5 mL/min; mobile phase pH: 3.4; mobile phase ratio: 60:40 *v/v*	240	239.99	99.99			
Set-4	Flow rate:1.5 mL/min; mobile phase pH: 3.6; mobile phase ratio: 60:40 *v/v*	240	239.93	99.97	100.07	0.08	0.08
Set-5	Flow rate:1.5 mL/min; mobile phase pH: 3.5; mobile phase ratio: 55:45 *v/v*	240	240.2	100.08			
Set-6	Flow rate:1.5 mL/min; mobile phase pH: 3.5; mobile phase ratio: 65:35 *v/v*	240	240.3	100.12			
Determination of robustness for PIO							
Sample I.D.	Analytical condition	Input (mg)	recovery	% Recovery	Mean recovery	S.D.	%R.S.D.
Set-1	Flow rate:1.4 mL/min; mobile phase pH: 3.5; mobile phase ratio: 60:40 *v/v*	60	59.98	99.96			
Set-2	Flow rate:1.6 mL/min; mobile phase pH: 3.5; mobile phase ratio: 60:40 *v/v*	60	59.88	99.8			
Set-3	Flow rate:1.5 mL/min; mobile phase pH: 3.4; mobile phase ratio: 60:40 *v/v*	60	60.8	101.33			
Set-4	Flow rate:1.5 mL/min; mobile phase pH: 3.6; mobile phase ratio: 60:40 *v/v*	60	60.67	101.11	100.52	0.67	0.67
Set-5	Flow rate:1.5 mL/min; mobile phase pH: 3.5; mobile phase ratio: 55:45 *v/v*	60	60.55	100.91			
Set-6	Flow rate:1.5 mL/min; mobile phase pH: 3.5; mobile phase ratio: 65:35 *v/v*	60	59.99	99.99			

Table 6. Determination of percentage assay

Synthetic mix	Drug	Conc taken (µg/mL)	Conc estimated (µg/mL)	Mean conc estimated (mg)	% Assay (*w/w*)	S.D.	%R.S.D.
NAT + PIO	NAT	240	240.30 240.40 239.89 240.50 239.99 239.99	240.17	100.08	0.25	0.10
	PIO	60	60.30 60.50 59.99 59.89 60.60 59.95	60.20	100.34	0.30	0.50

Table 7. System suitability parameter for NAT and PIO

SL No.	Parameter	NAT	PIO
1	Retention time Rt (min)	5.58	3.58
2	Theoretical Plate (USP)	8153.62	4523.99
3	HETP (mm)	0.04	0.02
4	Resolution (Rs)	10.83	4.20

Table 8. Spectral and statistical data for determination of NAT and PIO by proposed RP-HPLC method

NAT		PIO	
Selected wavelength for HPLC	210 nm	Selected wavelength for HPLC	210 nm
Linearity range	50-250 µg/mL	Linearity range	50-250 µg/mL
Coefficient of determination	0.99	Coefficient of determination	0.99
Regression equation	y = 31.3x + 0.98	Regression equation	y = 44.89x + 1.09
Slope	31.29	Slope	44.89
intercept	1.98	Intercept	1.09
LOD	0.18 µg/mL	LOD	0.22 µg/mL
LOQ	0.57 µg/mL	LOQ	0.63 µg/mL

Table 9. Acid degradation data of NAT and PIO

	Sl no	Time (hrs)	Area	% Degradation
Acid stress of PIO	1	0	1617.72	1.045
	2	24	1600.82	
Acid stress of NAT	1	0	3714.30	1.009
	2	24	3676.83	

Table 10. Alkali degradation data of NAT and PIO

Alkali stress of PIO HCl	Sl no	Time (hrs)	Area	% Degradation
	1	0	1617.72	No degradation
	2	24	1618.82	
Alkali stress of NAT	1	0	3714.30	15.36

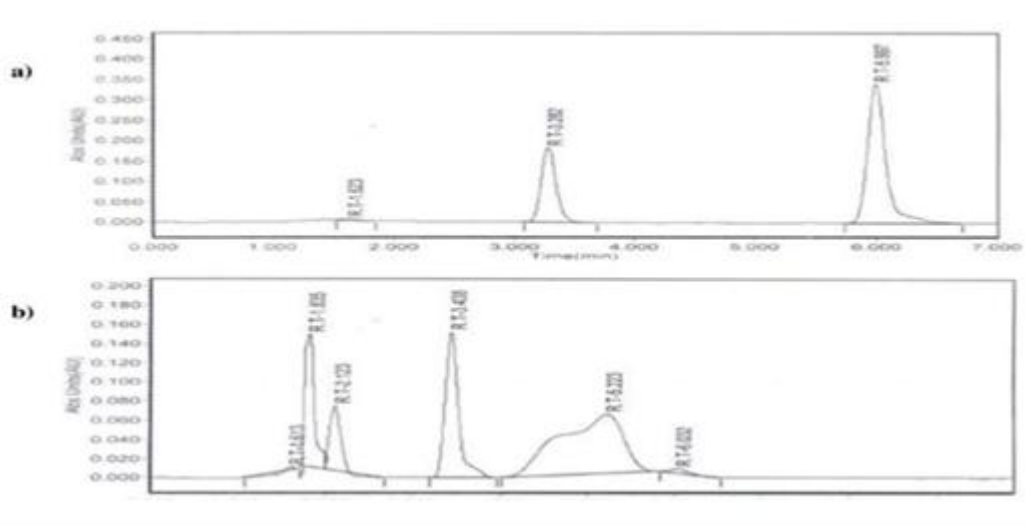

Fig. 2. a) Chromatogram of acid degradation of NAT & PIO; b) Chromatogram of basic degradation of NAT & PIO.

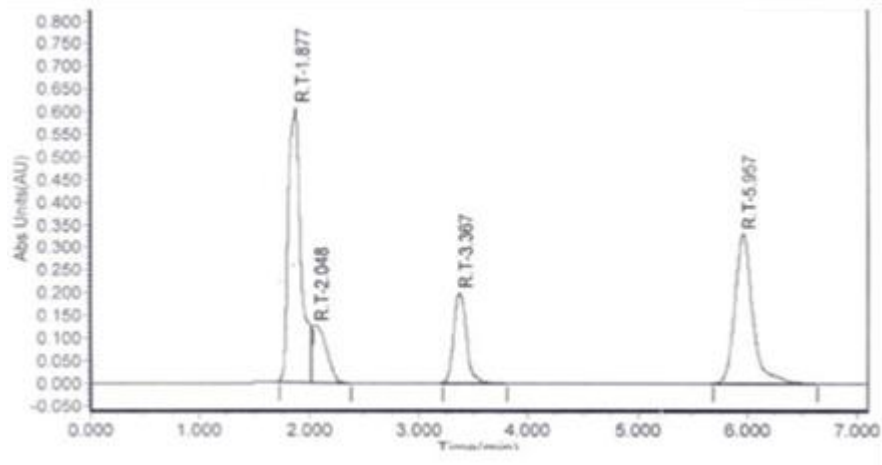

Fig. 3. Chromatogram of oxidative degradation of NAT & PIO.

Therefore, the developed and validated RP-HPLC method is simple, accurate, precise and reliable. The calibration curve's linear regression analysis revealed linear responses for both analytes at the tested concentration range. As a consequence, it may be used to calculate the concentrations of NAT and PIO in a synthetic mixture (40 mg nateglinide + 10 mg pioglitazone + 2% magnesium stearate). There were no additional interfering components eluting at the Rt of NAT and PIO, according to the experiment assessing specificity, which was 5.92 mins and 3.56 min respectively. The permissible limit specified by the regulatory requirements encompassed all the validation criteria evaluated by this methodology. Since there isn't many research on RP-HPLC methods for the study of NAT and PIO in the literature, this method's simplicity, sensitivity, robustness, and use of less expensive chemicals in the mobile phase preparation made it more acceptable. Furthermore, demonstrating the

specificity of the proposed and verified approach is its effective application in the forced degradation research for the quantitative detection of NAT and PIO without interference from degradation products. The degradation study revealed that NAT and PIO were stable under oxidative and photocatalytic degradation and had minimal degradation under acid hydrolysis. PIO was stable under acidic conditions and NAT had 15.36% degradation.

Conclusion

According to the study, we can conclude that the established method was proven to be easy to use, robust and precise to determine both drugs, NAT and PIO as raw material and in synthetic mixture. At the same time, degradation of both substances was carried out and it was found that both drugs were susceptible to acidic condition. Only NAT was susceptible to basic condition compared to PIO, which was found to be stable. NAT and PIO both were found to be very stable when exposed to peroxide and photolytic study. So, both the drugs should be caution stored under hydrolytic conditions.

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Резиме

Симултана анализа на стабилност на натеглинид и пиоглитазон со користење на RP-HPLC

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Клучни зборови: нателинид, пиоглитазон, HPLC, УВ детекција

Развиен е и валидиран едноставен, брз и чувствителен RP-HPLC метод за истовремено проценување на антидијабетичните лекови нателинид (NAT) и пиоглитазон (PIO) на избрана бранова должина од 210 nm. Методот овозможува линеарни одговори во опсег на концентрации од 50 до 250 μg/mL за двата лека, со LOD вредности од 0.18 μg/mL и 0.22 μg/mL за NAT и PIO, соодветно, и LOQ вредности од 0.57 μg/mL и 0.63 μg/mL. Просечното процентуално recovery се движи помеѓу 100.06 и 100.31%, со просечно RSD од 0.08% (повторливост) и 0.26% (прецизност). Дополнителните карактеристики како робустност, соодветност на системот и специфичноста беа исто така потврдени. Според истражувањето за деградација, NAT и PIO покажаа слаба деградација при киселинска хидролиза и беа стабилни при оксидативна и фотокаталитичка деградација. PIO остана стабилен во базна средина, додека NAT се деградираше за 15.36%. Предложениот пристап има голем потенцијал за рутинска контрола на квалитет, вклучувајќи квалитативна и квантитативна анализа на NAT и PIO во различни фармацевтски формулации.

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