Narikimalli and Galla
Future Journal of Pharmaceutical Sciences (2023) 9:55
https://doi.org/10.1186/s43094-023-00503-2

Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

A stability indicating UPLC method development and validation for the simultaneous estimation of nateglinide and metformin hydrochloride in bulk and tablet dosage form

Ashritha Narikimalli[1] and Rajitha Galla[2*]

Abstract

Background Nateglinide and metformin HCl are used in combination for the treatment of type 2 diabetes. A simple, sensitive and reliable UPLC method was developed for simultaneous estimation of nateglinide and metformin HCl using Phenomenox C_{18} (50*2.1 mm, 3.5 µm) column at ambient temperature as stationary phase in addition to mobile phase containing 75 volumes of ammonium formate buffer (pH = 3) along with 25 volumes of acetonitrile with a flow rate of 0.2 mL/min with UV detection at 260 nm and a run time of 3 min. The developed method was validated as per ICH Q2(R1) guidelines.

Results The separation of metformin HCl and nateglinide was done at retention times of 1.014 min and 1.435 min, respectively. The mean % recovery for nateglinide and metformin HCl in the accuracy study was observed to be 99.9% and 99.2%, respectively. LOD and LOQ values were determined considering the S/N ratio and were found to be 0.09 µg/mL and 0.3 µg/mL, respectively, for nateglinide and 0.75 µg/mL and 2.5 µg/mL, respectively, for metformin HCl. The method was found to be precise with % RSD values of 0.58 and 0.45, respectively, for repeatability and intermediate precision of nateglinide and 0.43 and 0.43, respectively, for repeatability and intermediate precision of metformin HCl which were within acceptance criteria. The method was found to be linear in the range of 7.5–45 µg/mL and 62.5–375 µg/mL for nateglinide and metformin HCl, respectively. The regression equations for nateglinide and metformin HCl were found to be $y = 17377x + 6543.4$ and $y = 18439x + 43{,}537$, respectively. The method was found to be robust by deliberate changes in the method parameters like flow rate and mobile phase composition. Forced degradation studies were performed as per ICH Q1A(R2) and Q1B guidelines, and peak purity was observed in all types of degradation studies for both the drugs.

Conclusion The developed method was found to be satisfactory as it is simple, sensitive, accurate, precise, robust, rapid, economical and yet stability indicating and can be applied successfully in the routine laboratory analysis for the simultaneous estimation of nateglinide and metformin HCl in the bulk and pharmaceutical dosage forms.

Keywords UPLC, Method development, Validation, Forced degradation studies, Nateglinide, Metformin HCl, ICH guidelines

*Correspondence:
Rajitha Galla
gallarajitha1@gmail.com
Full list of author information is available at the end of the article

Background

Type 2 diabetes (T2D) is a chronic disease that results from ineffective insulin use by the body [1]. Nateglinide (NAT) and metformin HCl (MET) are oral antidiabetic agents used for the treatment of T2D. Nateglinide is a meglitinide, with a molecular formula $C_{19}H_{27}NO_3$, and is chemically known as (2R)-3-phenyl-2-[4-propan-2-yl cyclohexane carbonyl) amino] propanoic acid. Metformin HCl is a biguanide, with a molecular formula $C_4H_{11}N_5$, and is chemically called 1,1-dimethyl biguanide hydrochloride. The chemical structures of NAT and MET are shown in Figs. 1 and 2, respectively [2–5].

The literature review revealed that there are different reported methods for both drugs individually [6–12] and in combination with other drugs [13–21]. Limited methods for the simultaneous estimation of NAT and MET include HPTLC [22], MLC [23] and RP-HPLC [24–26] methods. There is only one reported UPLC method [27] for the simultaneous determination of nateglinide and metformin. The present study focuses on the development and validation of a stability-indicating RP-UPLC method for the simultaneous estimation of nateglinide and metformin HCl. Validation of the developed method was performed following ICH Q2 (R1) [28] guidelines. Stability studies were performed following ICH Q1A(R2) and Q1B [29, 30] guidelines.

Methods

Materials

Pure samples

NAT (99.9% w/w) and MET (99.9% w/w) API samples were procured from Zydus Cadila Health Care Ltd, Secunderabad.

Formulation

Trunate Plus Tablets from AAR ESS Remedies Pvt. Ltd. were used in the study.

Chemicals and reagents

Acetonitrile (ACN), ammonium formate (AF), ammonium acetate, orthophosphoric acid (OPA) (HPLC-grade) and hydrochloric acid, sodium hydroxide, hydrogen peroxide (AR-grade) chemicals were acquired from Rankem Chemicals, Haryana, India.

Instrumentation

Waters Alliance UPLC with 2695 pump, UV detector, auto-injector and Empower 2 software were used to perform the study. Shimadzu UV–visible spectrophotometer, Ohus Electronic balance, Eutech pH Meter, Phoenix 4.5 L digital ultrasonic cleaner, Bionics Scientific hot air oven, LG refrigerator and Millipore BM2EA9672R were used in the study.

Composition of solutions

Buffer solution

6.3 g of ammonium formate was accurately weighed and dissolved in HPLC-grade water. After dissolving, its pH was adjusted to 3 with OPA.

Mobile phase

Acetonitrile and buffer solution were mixed in a ratio of 25:75. The solution is filtered through 0.45-µm membrane filter paper and sonicated to degas.

Diluent

Mobile phase was used as diluent.

Standard stock solution

30 mg of NAT and 250 mg of MET were accurately weighed and transferred to a 100-mL clean, dry volumetric flask and 3/4th of diluent was added to the flask and sonicated to dissolve it completely. Volume was made up of diluent and labeled as standard stock solution. *NAT (300 µg/mL) and MET (2500 µg/mL).*

Working standard solution

5 mL from stock solution was pipetted and transferred into a 50-mL volumetric flask and made up to the mark with diluent. *NAT (30 µg/mL) and MET (250 µg/mL).*

Sample solution

Twenty tablets were taken and weighed. Individual tablet weight and the equivalent weight of NAT and MET

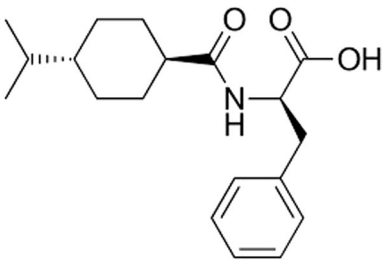


Fig. 1 Chemical structure of nateglinide

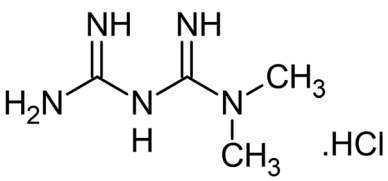


Fig. 2 Chemical structure of metformin HCl

were calculated. Tablet powder containing an equivalent weight of 30 mg of NAT and 250 mg of MET was weighed accurately and transferred to a 100-mL clean, dry volumetric flask, 3/4th of diluent was added and sonicated up to 30 min to dissolve, and volume was made up to the mark with the diluent. *(NAT (300 µg/mL) and MET (2500 µg/mL)).*

5 mL of the above solution was pipetted into a 50-mL volumetric flask and made up to the mark with diluent. *NAT (30 µg/mL) and MET (250 µg/mL).*

Placebo solution

10 mg of placebo (HPMC) was weighed accurately and transferred to a 10-mL clean, dry volumetric flask, 3/4th of diluent was added and sonicated up to 30 min to dissolve, and volume was made up to the mark with the diluent.

1 mL of the above solution was pipetted into a 10-mL volumetric flask and made up to the mark with diluent *(100 µg/mL placebo solution).*

Determination of working wavelength

Working standard solutions of NAT and MET were scanned in the UV region (200–400 nm) separately, and the resultant overlay spectrum obtained was used to determine the suitable working wavelength. The UV spectrum is shown in Fig. 3. 260 nm was selected as the suitable wavelength for the simultaneous determination of NAT and MET.

Optimization of UPLC method

Trials were performed by varying chromatographic conditions like stationary phase, mobile phase composition and buffer pH. Observations were carefully scrutinized for optimizing the method for low retention time, better resolution, theoretical plates and peak symmetry. Some of the trials and their observations are listed in Table 1. Mobile phase containing acetonitrile and ammonium formate (25:75 v/v, pH = 3), Phenomenox C_{18} column (50 × 2.1 mm, 3.5 µ), 0.2 mL/min flow rate, UV detection at 260 nm with 5 µL injection volume and 3 min runtime resulted in better resolution, theoretical plates and tailing factor with 1.014 min and 1.435 min retention times for MET and NAT, respectively. The optimized chromatogram is shown in Fig. 4.

Validation

Validation of the proposed method was performed following ICH Q2(R1) guidelines which include system suitability, specificity, linearity, precision, accuracy, LOD, LOQ and robustness. Forced degradation studies were performed following ICH Q1A (R2) and Q1B guidelines which include acid hydrolysis, alkali hydrolysis, peroxide degradation, thermal degradation, hydrolytic degradation and photolysis.

System suitability test

A system suitability test was performed to validate measurement system and analytical operations related to the

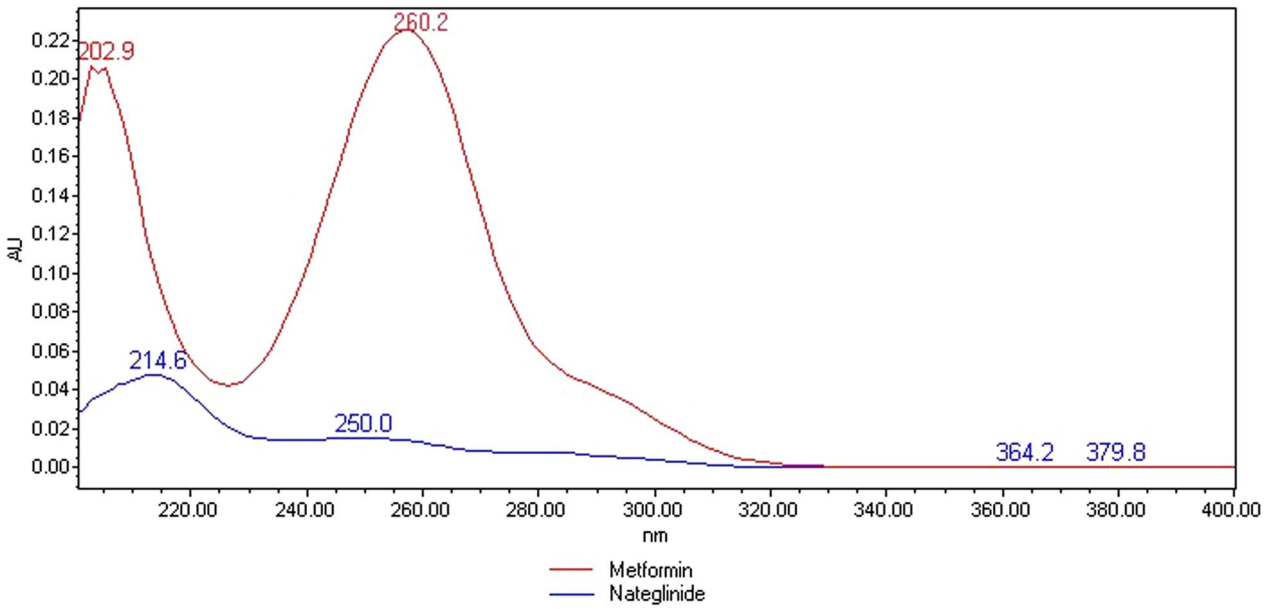
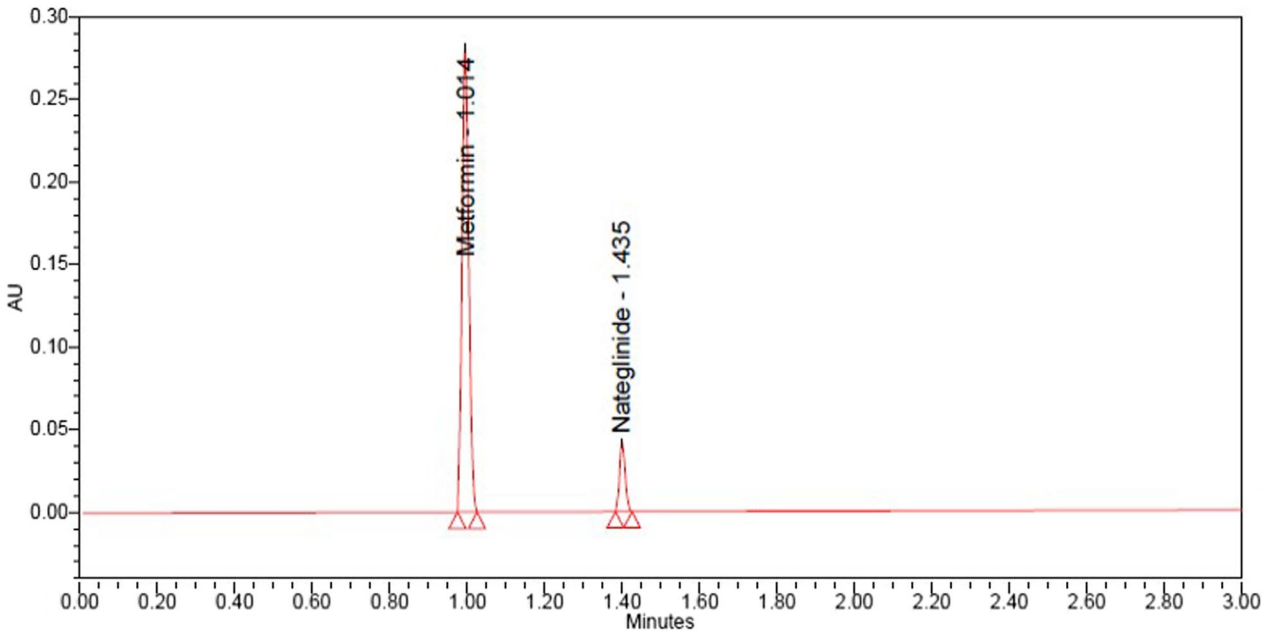


Fig. 3 UV spectrum of nateglinide and metformin HCl

Table 1 Observations of various chromatographic trails

Trail No	Buffer	Mobile phase	Stationary phase	Observation
1	0.1% OPA pH—2.2	ACN: Buffer (50:50 v/v)	Kinetex column 100*4.6 mm, 2.6 µm	First peak plate count is not within the limit
2	0.1% OPA pH—2.2	ACN: Buffer (60:40 v/v)	Kinetex column 100*4.6 mm, 2.6 µm	Second peak is broad
3	0.1% OPA pH—2.2	ACN: Buffer (40:60 v/v)	Luna C_{18} column, 100*2.6 mm, 1.6 µm	First peak plate count is not within the limit
4	Ammonium acetate pH—4.75	ACN: Buffer (30:70 v/v)	Luna C_{18} column, 100*2.6 mm, 1.6 µm	Tailing is not within the limit
5	Ammonium acetate pH—4.75	ACN: Buffer (40:60 v/v)	Luna C_{18} column, 100*2.6 mm, 1.6 µm	First peak plate count is not within the limit
6	Ammonium acetate pH—4.75	ACN: Buffer (50:50 v/v)	Luna C_{18} column, 100*2.6 mm, 1.6 µm	Plate count is not within the limit



Peak Results

	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Metformin	1.014	2856393	7584	1.05	
2	Nateglinide	1.435	523952	5625	1.06	5.64

Fig. 4 Optimized chromatogram for MET and NAT

analytical method are suitable for the proposed analysis. Six identical samples were determined.

Specificity

It is the ability to assess the analyte in the presence of expected impurities and degradants by any interference noticed at the retention times of the drugs NAT and MET. It was determined by comparing the results of sample, blank and placebo solutions.

Linearity

It is the capacity of an analytical method to give test results that are directly proportional to the amount of analyte in the sample within a given range. The linearity was determined by calculating the regression equation from the calibration curve constructed from six standard concentrations.

Precision

Precision denotes the closeness of agreement among a series of measurements obtained from multiple sampling of the same homogeneous sample under set conditions. Precision is considered at two levels: repeatability and intermediate precision. It is expressed in terms of relative standard deviation.

Repeatability It is the precision under the same operating conditions over a short interval of time. It was assessed by 6 determinations of sample solution.

Intermediate precision It expresses within-laboratories variations: different days, different analysts, different equipment, etc. It is also known as method precision. It was assessed by 6 determinations of 6 different sample solutions on 6 different days.

Accuracy

Accuracy, also known as trueness, expresses the closeness of agreement between the true value and the value found. It is expressed as % recovery, which was determined by spiking known quantities of standards to pre-analyzed samples at three different levels.

Limit of detection and limit of quantitation

The limit of detection and limit of quantitation were determined for NAT and MET at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentrations.

Robustness

It is the reliability of the method concerning deliberate variations in method parameters. It was performed by changing the flow rate and mobile phase.

Assay

5 µL of standard solution and sample solution were injected separately into the UPLC to record the chromatogram. The amounts of the drugs present were determined based on the peak area of recorded chromatograms.

Forced degradation studies

ICH guidelines recommended stress testing to know the intrinsic stability of drug substances. In these studies, the solution of the standard was subjected to acid degradation (2 N HCl, refluxed at 60 °C for 30 min), alkali degradation (2 N NaOH, refluxed at 60 °C for 30 min), peroxide degradation (20% hydrogen peroxide heated at 60 °C for 30 min), thermal degradation (samples arranged in a hot air oven at 105 °C for 1 h), photolytic degradation (sample in UV chamber for 1 day), hydrolysis (water, refluxed at 60 °C for 1 h). The so-stressed samples were observed for the peak areas and compared with peak areas of the standard.

Results

System suitability test

Six identical samples were determined, and the results are shown in Table 2. System suitability test results were within the limit as the % RSD values for retention times, plate count, tailing factor and resolution were less than 2.

Specificity

The proposed method was found to be specific as there were no interfering peaks in the blank, placebo and the sample at the retention times of NAT and MET. The chromatograms of blank, placebo and sample are shown in Figs. 5, 6 and 7.

Table 2 Results for system suitability test for NAT and MET

Sample	Metformin HCL			Nateglinide			
	t_R	N	A_s	t_R	N	A_s	R_s
1	1.014	7584	1.05	1.435	5625	1.03	5.14
2	1.018	7541	1.02	1.436	5636	1.01	5.17
3	1.011	7517	1.03	1.439	5631	1.02	5.28
4	1.017	7529	1.02	1.451	5823	1.03	5.34
5	1.014	7563	1.04	1.447	5817	1.00	5.24
6	1.012	7889	1.06	1.436	5687	1.01	5.17
Mean	1.014	7603.8	1.037	1.441	5703	1.017	5.223
SD	0.00	141.75	0.016	0.007	93.18	0.012	0.077
% RSD	**0.27**	**1.86**	**1.58**	**0.47**	**1.63**	**1.19**	**1.48**

t_R—Retention time, N—No. of theoretical plates, A_s—Peak asymmetry, R_s—Resolution

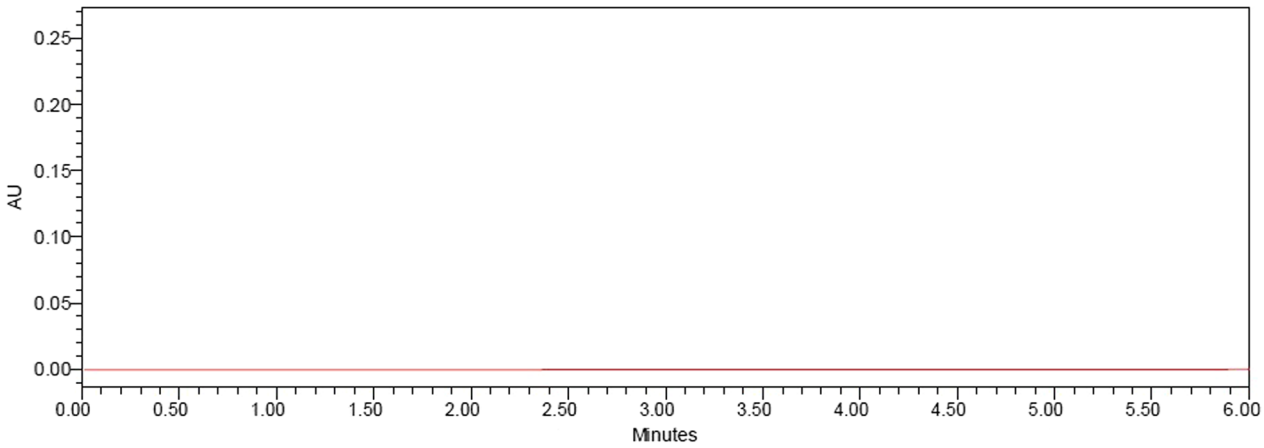


Fig. 5 Chromatogram of Blank

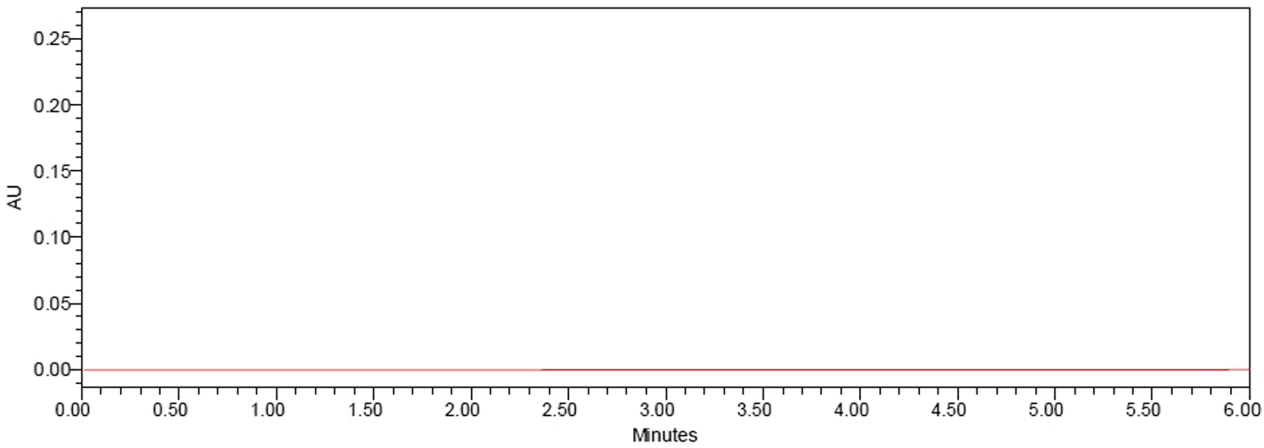


Fig. 6 Chromatogram of Placebo

Linearity

Linearity of the method was determined by preparing aliquots at 7.50, 15.00, 22.50, 30.00, 37.50, 45.00 µg/mL for NAT and 62.5, 125, 187.5, 250, 312.5, 375 µg/mL for MET. The solutions were analyzed in triplicate. The proposed method was found to be linear in the concentration range of 7.5—45 µg/mL and 62.5-375 µg/mL for NAT and MET, respectively. The regression equations for NAT and MET were found to be $y = 17377x + 6543.4$ and $y = 18439x + 43{,}537$, respectively. Table 3 shows the linearity data of NAT and MET. The method is said to be linear as the R^2 value of both drugs was found to be more than 0.999. The calibration curves of NAT and MET are shown in Figs. 8 and 9.

Precision

Repeatability and intermediate precision were assessed by 6 determinations of sample solution *(NAT (30 µg/mL) and MET (250 µg/mL)).* The results are shown in Table 4.

The %RSD values of peak areas for precision were found to be within limits as it is less than 2.

Accuracy

The % recovery was determined by spiking known quantities of standards (NAT and MET) to pre-analyzed samples at 50%, 100% and 150% levels in triplicate. The results are shown in Tables 5 and 6. The mean % recovery was found to be 99.9 for NAT and 99.2 for MET.

Limit of detection and limit of quantitation

The LOD and LOQ were determined by considering the S/N ratio. The limit of detection values for NAT and MET were found to be 0.09 µg/mL and 0.75 µg/mL,

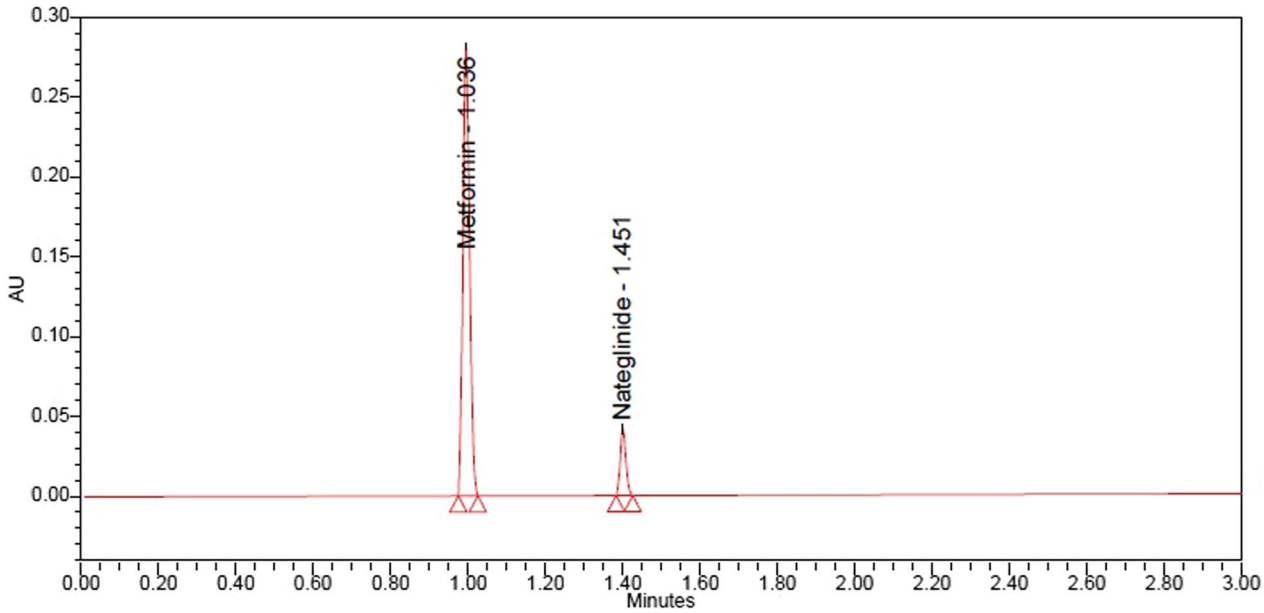


Fig. 7 Chromatogram of Sample

Table 3 Linearity data for nateglinide and metformin HCl

Sample	Nateglinide		Metformin HCL	
	Conc. (µg/mL)	Peak area (Mean ± SD)	Conc. (µg/mL)	Peak Area (Mean ± SD)
1	7.50	136,455 ± 944	62.50	717,923 ± 4927
2	15.00	266,702 ± 1844	125.00	1,463,602 ± 8622
3	22.50	397,289 ± 2240	187.50	2,085,176 ± 9958
4	30.00	532,591 ± 3722	250.00	2,855,164 ± 12,663
5	37.50	653,582 ± 5679	312.50	3,443,907 ± 30,527
6	45.00	789,578 ± 8785	375.00	4,215,888 ± 36,152
Slope	17,377		18,439	
Intercept	6543.4		43,537	
R^2	0.9998		0.999	

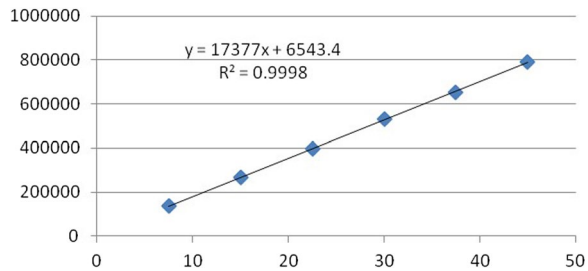


Fig. 8 Calibration curve of nateglinide

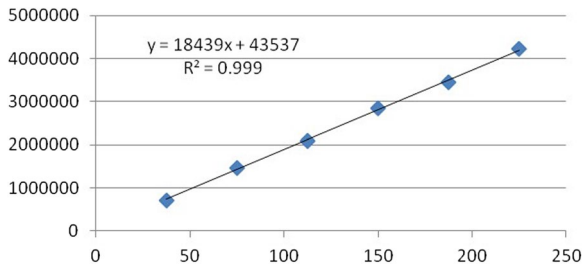


Fig. 9 Calibration curve of metformin HCl

Table 4 Precision data of nateglinide and metformin HCl

Peak area	Repeatability (for sample)		Intermediate precision (method precision)	
	MET	NAT	MET	NAT
1	2,843,598	522,735	2,857,759	524,471
2	2,811,696	523,895	2,841,695	523,722
3	2,832,323	528,878	2,860,724	525,628
4	2,822,357	528,481	2,831,596	524,699
5	2,821,758	521,365	2,841,691	520,816
6	2,812,665	525,815	2,833,213	528,074
Mean	2,824,066.2	525,194.8	2,844,446.3	524,568.3
SD	12,173.121	3071.064	12,236.585	2376.36
% RSD	0.43	0.58	0.43	0.45

respectively. The limit of quantitation values for NAT and MET were found to be 0.3 µg/mL and 2.5 µg/mL, respectively.

Robustness

It was performed by changing flow rate, 0.45 mL/min (−) and 0.55 mL/min (+) and mobile phase, Buffer: ACN 77:23 (−) and 63:37 (+). The results are shown in Table 7. The % RSD values for peak area were found to be within limits as they were less than 2. So the method was considered to be robust.

Assay

Separately 5 µL of standard solution (of pure drugs) and sample solution (extracted from tablets) were injected into the UPLC to record the chromatograms in triplicate. The percentage assay of the sample was calculated by comparing the areas of standard and sample peaks, and the results for assay of NAT and MET are shown in Table 8.

Forced degradation studies

These studies were carried out as per ICH Q1A and Q1B guidelines.

Table 5 Accuracy data for Nateglinide

% Level	Nateglinide				
	API added (mg)	Actual API (mg)	Amount recovered (µg/mL)	% Recovery	Level mean % recovery
50%	15	15.00	14.95	99.7	99.9
	15	15.00	15.12	100.8	
	15	15.00	14.89	99.3	
100%	30	30.00	30.07	100.2	100.1
	30	30.00	29.97	99.9	
	30	30.00	30.04	100.1	
150%	45	45.00	45.34	100.8	99.9
	45	45.00	44.88	99.7	
	45	45.00	44.65	99.2	
	Mean % Recovery				99.9

Table 6 Accuracy data for Metfomin HCl

% Level	Metformin HCL				
	API added (mg)	Actual API (mg)	Amount recovered (µg/mL)	% Recovery	Level mean % recovery
50%	125	125.0	123.35	98.7	98.9
	125	125.0	124.28	99.4	
	125	125.0	123.28	98.6	
100%	250	250.0	248.85	99.5	99.5
	250	250.0	247.79	99.1	
	250	250.0	249.54	99.8	
150%	375	375.0	373.25	99.5	99.2
	375	375.0	373.41	99.6	
	375	375.0	369	98.4	
	Mean % Recovery				99.2

Table 7 Robustness data for nateglinide and metformin HCl

Injection	Nateglinide				Metformin HCL			
	FR (−)	FR (+)	MP (−)	MP (+)	FR (−)	FR (+)	MP (−)	MP (+)
1	532,479	504,217	556,479	494,788	3,290,290	2,456,263	3,083,632	2,633,880
2	536,623	503,989	555,422	494,251	3,247,134	2,465,645	3,078,701	2,614,596
3	533,741	502,638	558,759	496,698	3,269,784	2,476,136	3,065,592	2,646,661
Mean	534,281	503,615	556,887	495,246	3,269,069	2,466,015	3,075,975	2,631,712
SD	2124.1	853.5	1705.4	1286.1	21,586.9	9941.7	9323.8	16,142.0
% RSD	0.40	0.17	0.31	0.26	0.66	0.40	0.30	0.61

Table 8 Assay data for nateglinide and metformin HCl

Drug	Label claim (mg)	Amount found (mg ± SD)	% Assay (%w/w ± SD)
Nateglinide	60	60.15 ± 0.05	100.3 ± 0.1
Metformin HCL	500	500.4 ± 0.1	100.08 ± 0.02

Acid degradation 1 mL 2N hydrochloric acid was added to 1 mL standard stock solution of MET and NAT and refluxed for 30 min at 60 °C.

Alkali degradation 1 mL 2N NaOH was added to 1 mL standard stock solution of MET and NAT and refluxed for 30 min at 60 °C.

Peroxide degradation 1 mL 20% H_2O_2 was added to 1 mL standard stock solution of MET and NAT and refluxed for 30 min at 60 °C.

Thermal degradation The standard stock solution was placed in an oven at 105 °C for 1 h to study dry heat degradation.

Photolytic degradation The photochemical stability of the drug was studied by exposing a standard stock solution of MET and NAT to UV light by keeping the beaker in a UV chamber for one day.

Hydrolysis Stress testing under neutral conditions was studied by refluxing the drug in water for 1 h at 60 °C.

The above solutions were diluted to obtain 250 µg/mL MET and 30 µg/mL NAT solutions, and 10 µl of each solution was injected into the system to assess the

Table 9 Degradation data for nateglinide

S.NO	Degradation condition	Peak area	% Recovery	% Drug degradation	Peak purity		
					Purity angle	Purity threshold	Pass /Fail
1	Acid	457,512	87.3	12.7	1.232	8.025	Pass
2	Alkali	455,849	87	13	1.217	8.054	Pass
3	Peroxide	450,125	85.9	14.1	1.249	8.173	Pass
4	Thermal	479,856	91.6	8.4	1.263	8.007	Pass
5	Photolysis	500,478	95.5	4.5	1.248	8.063	Pass
6	Hydrolysis	502,154	95.8	4.2	1.252	8.077	Pass

Table 10 Degradation data for metformin HCl

S.NO	Degradation condition	Peak area	% Recovery	% Drug degradation	Peak purity		
					Purity angle	Purity threshold	Pass/Fail
1	Acid	2,443,017	85.7	14.2	0.124	1.254	Pass
2	Alkali	2,465,075	86.5	13.4	0.131	1.231	Pass
3	Peroxide	2,401,507	84.2	15.7	0.108	1.227	Pass
4	Thermal	2,709,854	95.1	4.8	0.136	1.251	Pass
5	Photolysis	2,685,412	94.2	5.7	0.117	1.232	Pass
6	Hydrolysis	2,720,156	95.4	4.5	0.152	1.209	Pass

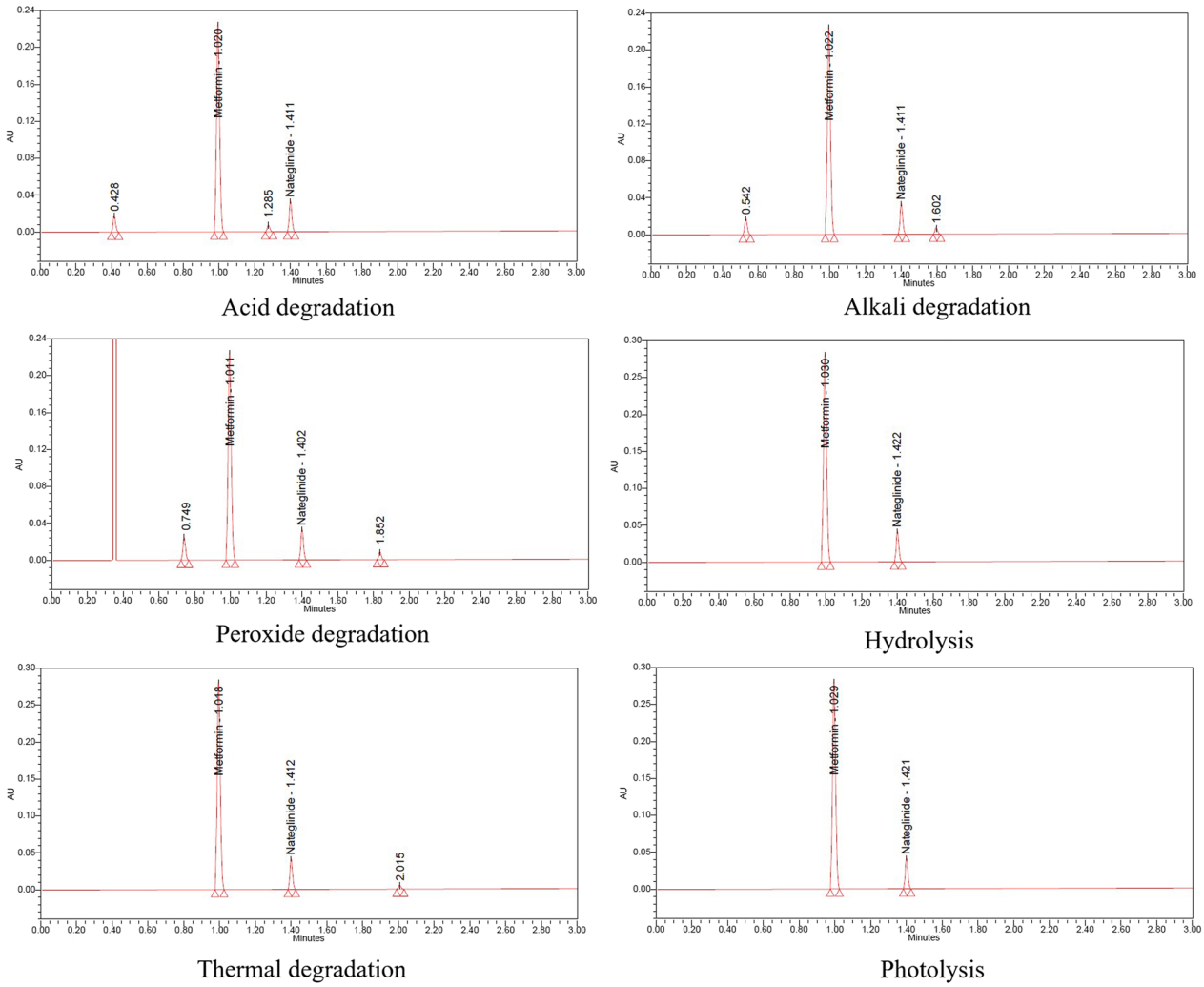


Fig. 10 Forced degradation chromatograms for NAT and MET

stability of the sample. The results for NAT and MET obtained are shown in Tables 9 and 10. The chromatograms are shown in Fig. 10.

Discussion

Preceding to development of optimized method, different trials were performed by varying chromatographic conditions like buffer pH (2.2, 3 and 4.75), mobile phase composition (ACN and buffer, MEOH and buffer) and stationary phase (Kinetex column 100*4.6 mm, 2.6 µm and Luna C_{18} column 100*2.6 mm, 1.6 µm). Observations were scrutinized for optimizing the method for low retention time, better tailing factor, resolution and theoretical plates. Mobile phase containing acetonitrile and ammonium formate (25:75 v/v, pH = 3), Phenomenox C_{18} column (50 × 2.1 mm, 3.5µ), 0.2 mL/min flow rate, UV detection at 260 nm with 5 µL injection volume and 3 min run time resulted in better tailing factor (1.05, 1.06), resolution (5.64), theoretical plates (7584, 5625) and with 1.014 min and 1.435 min retention times for MET and NAT, respectively. The optimized chromatogram is shown in Fig. 4.

During normal usage, the performance of the system may vary; therefore system suitability tests were performed to check the reproducibility in terms of injection repeatability, retention time, plate count, peak asymmetry and resolution which were found to be satisfactory. It certifies that the proposed method was satisfied and can progress for the study of validation parameters. Interfering peaks from excipients and solvent did not co-elute at the retention times of NAT and MET, representing the optimized condition was specific which is understood from Figs. 5, 6 and 7. Regression lines were linear in the concentration range of 7.5—45 µg/mL for NAT, 62.5–375 µg/mL for MET. The regression coefficients were found to be 0.999 and 0.9999 for NAT and

MET, respectively, indicating the method was linear. The method was considered to be accurate as the % mean recovery values were found to be 99.9 and 99.2, respectively, for NAT and MET. The %RSD values of peak area for repeatability were found to be 0.43 and 0.58 for MET and NAT, and the %RSD values of peak area for intermediate precision were found to be 0.43 and 0.45 for MET and NAT which were less than 2 which indicates method was repeatable, reproducible and rugged. LOD and LOQ values were determined using the S/N ratio and they were found to be 0.09 µg/mL and 0.3 µg/mL, respectively, for nateglinide and 0.75 µg/mL and 2.5 µg/mL, respectively, for metformin HCl, which indicates the sensitivity of the method. Results due to variation in the conditions were compared with that of optimized chromatographic conditions. The %RSD values were within the limits indicating the optimized method is robust.

In forced degradation studies, due to the induced stress conditions, the recorded peak areas of NAT and MET were not as much of as the peak areas of standard which indicate the degradation of drug components to some degree in all the stress conditions. All the responses were within the limits of the assay without any major changes in the drugs retention time and peak area. From forced degradation studies, we can infer that the combination of NAT and MET was more prone to acid, alkali, peroxide degradation and to some extent thermal degradation. The results imply that the method developed is stability indicating.

The retention times of drugs in the present study were found to be less compared to the retention times 1.28 min for MET and 1.71 min for NAT in the previous method. The mobile phase in the present study consists of greater proportions of the aqueous phase than the organic phase as compared to the previous method, which comprises 50:50 v/v of 0.01% potassium dihydrogen phosphate buffer pH 5.8 and ACN as mobile phase. The flow rate of the previous method was 0.3 mL/min as compared to 0.2 mL/min in the present method. It indicates that the developed method is rapid and more economical than the previously reported UPLC method [27].

Conclusions

The developed UPLC method was simple, rapid, economical, specific and reliable for the estimation of nateglinide and metformin HCl. The retention times of metformin HCl and nateglinide were found to be 1.014 min and 1.435 min, respectively, which are less compared to the previous reported method. The mobile phase consists of more proportion of the aqueous phase than the organic phase compared to previous literature [27]. The statistical evaluation of the proposed method showed good linearity and all validation parameters were performed as per ICH guidelines and were found to be in approval with the acceptance criteria. Forced degradation studies were performed by applying various stress conditions to the sample to evaluate the stability-indicating nature and robustness of the developed method. The method was found to be satisfactory and can be used successfully in the routine laboratory analysis for the simultaneous estimation of nateglinide and metformin HCl in the bulk and pharmaceutical dosage forms.

Abbreviations

UPLC	Ultra-performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
S/N	Signal/ noise ratio
RSD	Relative standard deviation
NAT	Nateglinide
MET	Metformin hydrochloride
HPTLC	High-performance thin layer chromatography
MLC	Micellar liquid chromatography
t_R	Retention time
N	No. of theoretical plates
A_s	Peak asymmetry
R_s	Resolution
R^2	Coefficient of determination

Acknowledgements

We are thankful to Zydus Cadila Health Care Ltd., Secunderabad, for providing the API. The authors are acknowledged for their contributions.

Author contributions

AN has performed experimentation as well as prepared draft manuscript. RG was the mentor. All authors read and approved the final manuscript.

Funding

No funding was received for this research work.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors declare no conflict of interest.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences and Technologies, Jawaharlal Nehru Technological University Kakinada, Kakinada, Andhra Pradesh 533003, India. [2]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh 517503, India.

Received: 30 September 2022 Accepted: 17 June 2023
Published online: 28 June 2023

References

1. World health organization (2021) Nateglinide. In: www.who.in. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes. Accessed 14 Apr 2022
2. Nateglinide (2018) In: medlineplus.gov. Available from: https://medlineplus.gov/druginfo/meds/a699057.html#:~:text=Nateglinide%20belongs%20to%20a%20class,the%20pancreas%20to%20release%20insulin. Accessed 14 Apr 2022
3. Nateglinide (2022). In: PubChem Compound Summary for CID 5311309, National Center for Biotechnology Information Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Nateglinide. Accessed 14 Apr 2022
4. Metformin. (2020). In: www.medlineplus.gov: Availablefrom:https://medlineplus.gov/druginfo/meds/a696005.html#:~:text=Metformin%20is%20in%20a%20class,glucose%20made%20by%20your%20liver. Accessed 14 Apr 2022
5. Pernicova I, Korbonits M (2014) Metformin-mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol 10:143–156
6. Patil VP, Angadi SS, Kale SH, Shelke SD, Kawade ST (2015) Stability indicating UV spectroscopic method for the estimation of Metformin Hydrochloride in bulk and tablets. Int J Life Sci Rev 1:27–33
7. Jain S, Bhandari A, Purohit S (2009) Spectrophotometric determination of Nateglinide in bulk and tablet dosage forms. Asian J Pharm 3:218–219
8. Aylin H (2015) Development and validation of an HPLC method for determination of Nateglinide in drug substances. Marmara Pharm J 19:103–108
9. Madathil C, Xavier C, Ramesh J, Basavaiah K (2012) RP-UPLC method development and validation for the determination of Nateglinide in bulk drug and pharmaceutical formulations: a quality by design approach. Malays J Pharm Sci 10:23–44
10. Mansur S, Amina SB, Usman LA (2018) Determination of Infrared, Raman, (1H and13C)-NMR spectra and density of state of Nateglinide oral antidiabetic drug. Int J Nanomed Nanosurg 4:1–5
11. Tuba O O (2018) FT-IR, Laser-Raman, UV–Vis, and NMR spectroscopic studies of antidiabetic molecule Nateglinide. J Spectrosc 1–12
12. Durgawati P, Puspendra K, Swapnil S, Jaya D (2017) Analytical methods for metformin estimation. Crit Rev Anal Chem 47:405–417
13. Geetha SA, Galla R, Ramya Y (2019) Analytical method development and validation of new stability-indicating reverse-phase high-performance liquid chromatography method for simultaneous estimation of metformin hydrochloride and Empagliflozin in tablet dosage form. Asian J Pharm Clin Res 12:241–244
14. Keyur B, EmanualPatelia M (2013) Simultaneous estimation of Metformin hydrochloride and Repaglinide in pharmaceutical formulation by HPTLC-densitometry method. J Chromatogr Sep Tech 4:166–171
15. Mallikarjuna RN, Gowri SD (2015) RP-HPLC method for simultaneous estimation and stability indicating the study of Metformin and Linagliptin in pure and pharmaceutical dosage forms. Int J Pharm Pharm Sci 7:191–197
16. Deepali AN, Vidhya K, Dhaneshwar SB (2013) Validated HPLC method for simultaneous quantitation of Benfotiamine and Metformin hydrochloride in bulk drug and formulation. Int J Pharm Pharm Sci 5:138–142
17. Patel JR, Suhagia BN, Patel BH (2007) Simultaneous spectrophotometric estimation of Metformin and Repaglinide in a synthetic mixture. Indian J Pharm Sci 69:844–846
18. Deepa R, Laxmanbhai J, Madhabhai M (2011) Stability indicating HPLC method for simultaneous determination of Repaglinide and Metformin hydrochloride in pharmaceutical dosage form. Asian J Res Chem 4:500–505
19. Ramanjireddy T, Duraiswamy D, Chandrasekhar BK (2010) Method development and validation of Metformin and Repaglinide in rabbit plasma by RP-HPLC. Fabad J Pharm Sci 35:69–75
20. Sekhar CK (2012) A new simple RP–HPLC method for simultaneous estimation of Metformin HCl and Gliclazide tablet dosage form. Int J Pharm Bio Sci 2:277–283
21. Karimulla SK, Vasanth PM, Ramesh T, Ramesh M (2013) Method development and validation of Sitagliptin and Metformin using reverse phase HPLC method in bulk and tablet dosage form. Der Pharm Lett 5:168–174
22. Asha BT, Shrikrushna DP, Rabindra KN, Lata PK, Shital SB (2011) Stability-indicating HPTLC method for simultaneous determination of Nateglinide and Metformin hydrochloride in pharmaceutical dosage form. Saudi Pharm J 19:221–231
23. Dalia REW (2012) Simultaneous determination of Metformin, Nateglinide and Gliclazide in pharmaceutical preparations using micellar liquid chromatography. Int J Biomed Sci 8:144–151
24. Asha T, Shrikrushna P, Rabindra N, Lata K, Avinash D (2012) Stability-indicating RP-HPLC method for determination of Metformin hydrochloride and Nateglinide in bulk and tablet formulations. Curr Pharm Anal 8:381–388
25. Prasanthi C, Angala PS, Aruna G (2016) Development and validation of RP-HPLC method for Metformin hydrochloride and Nateglinide in bulk and combined dosage form. Int J Pharm Pharm Sci 8:267–271
26. Ismail KM, Lakshmana RA (2021) Validation of a developed analytical method for determination of Nateglinide and Metformin HCl in pure and pharmaceutical dosage form by reversed-phase high-performance liquid chromatography and its degradation studies. Asian J Pharm Clin Res 14:196–202
27. Prameela RA, Madhavi S, Tirumaleswara RB, Sudheer RC (2020) Stability indicating UPLC method for estimation of Metformin Hydrochloride and Nateglinide simultaneously in the presence of stress degradation products. Indian Drugs 57:56–64
28. ICH Harmonized Tripartite (2005) Validation of analytical procedures: text and methodology Q2 (R1). In: International Conference on Harmonization, Geneva Available from: http://www.ich.org/fileadmin/public_web_site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf. Accessed 18 May 2021
29. ICH Harmonized Tripartite (2003) Stability testing of new drug substance and products. Q1A(R2). In: International Conference on Harmonization, Geneva Available from: http://www.ich.org/fileadmin/public_web_site/ICH_Products/Guidelines/Quality/Q1A_R2_Guideline.pdf. Accessed 18 May 2021
30. ICH Harmonized Tripartite (1996) Stability testing: Photostability testing of new drug substances and products Q1B. International Conference on Harmonization, Geneva Available from: https://database.ich.org/sites/default/files/Q1B%20Guideline.pdf. Accessed 18 May 2021

Publisher's Note