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HPTLC Estimation of Nateglinide in Bulk Drug and Tablet Dosage Form

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A simple, accurate and precise HPTLC method has been developed and validated for estimation of nateglinide in bulk drug and tablet formulation. Nateglinide from the formulation was separated on silica gel 60 F_{254} HPTLC plates with *n*-hexane, methanol and 2-propanol in the proportion of 7.5:1.5:1 (v/v) as the mobile phase. Densitometric quantification was performed at 210 nm. Band obtained with R_f value 0.56 ± 0.02 for nateglinide. The method was validated in accordance with the requirements of ICH guidelines. The calibration curve found to be linear in the range of 300-1000 ng/band both by area and height with r = 0.9994, 0.9998, slope = 0.121, 3.344 and intercept = 22.039, 250.629, respectively. The limit of detection and limit of quantification both by area and height were 78.95, 33.55 and 236.84, 100.64 ng per band, respectively. The result of estimation was found to be 98.49 ± 0.32 in Glinate tablet. The proposed method can be used successfully for routine analysis of nateglinide from bulk and tablet formulations.

Key Words: Nateglinide, HPTLC, Validation.

INTRODUCTION

Chemically, nateglinide (NAT) is N-(*trans*-4-isopropyl cyclohexyl carbonyl)-D-phenylalanine (Fig. 1). It is nonsulfonyl urea derivative used for the treatment of type II diabetes mellitus[1,2]. It is not official in any Pharmacopoeia. Literature survey reveals that UV spectrophotometric[3,4], visible spectrophotometric[5,6], stereoselective HPLC[7], HPLC in plasma[8,9] liquid chromatography/tandem mass spectroscopic method[10], ESI-MS[11], micellar electrokinetic chromatography[12], stability indicating HPLC[13] and HPLC with pre column derivatisation[14] has been reported for its determination in single and in combination[15] with other drugs. However, there is no high performance thin layer chromatographic method reported for determination of nateglinide. In present study, an attempt has been made to develop HPTLC method for the determination of nateglinide in bulk and marketed formulations using *n*-hexane, methanol and 2-propanol in the proportion of 7.5:1.5:1 (v/v) as the mobile phase on silica gel 60 F_{254} HPTLC plates. The developed method was found to be simple, sensitive and reproducible.

EXPERIMENTAL

Nateglinide pure standard was procured from Glenmark Pharmaceuticals, Mumbai, India. The tablet formulation, containing nateglinide 60 mg is available in market by brand name Glinate; *n*-hexane, methanol and 2-propanol were of analytical

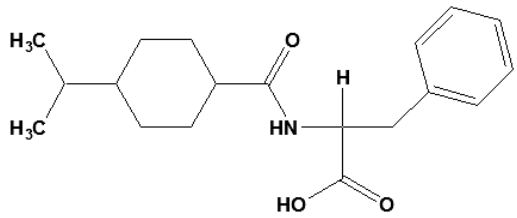

Fig. 1. Chemical structure of nateglinide

grade from Qualigens; pre-coated silica gel 60 F_{254} HPTLC plates (Merck # 5548) of E. Merck. All dilutions were performed in standard volumetric flasks. Double distilled water and Whatmann filter paper Grade I, 0.45 µm filter paper was used throughout the experimental work.

The HPTLC system employed in the experiment was Camag Linomat IV sample applicator (Muttenz, Switzerland), a Camag twin trough chamber of 10 cm × 10 cm size, Camag TLC scanner III, winCATS 4.0 version software as data integrator and a Hamilton syringe of 100 µL capacity. Chromatography was performed on pre-coated silica gel 60 F_{254} HPTLC plates (Merck #5548). The chromatographic plates were pre-washed with methanol and dried in an oven at 105 ºC for 1 h before use. Five µL of sample was spotted 10 mm from the edge of the plates by means of sample applicator. The plates were developed to a distance of 80 mm in a Camag twin-trough chamber previously equilibrated for 10 min with mobile phase *i.e.*, *n*-hexane, methanol and 2-propanol

[7.5:1.5:1 (v/v)]. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. Plates were evaluated by densitometry at 210 nm with a Camag Scanner III, in conjunction with winCATS software for quantitation. The typical chromatogram of sample solution is shown in Fig. 2.

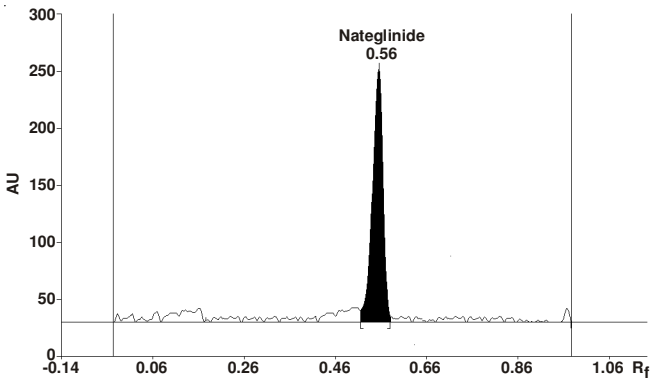

Fig. 2. Densitogram of sample solution

Preparation of standard stock solution of nateglinide: An accurately weighed 50.0 mg pure standard of nateglinide and transferred to 50 mL volumetric flask. The drug was dissolved in methanol, diluted up to the mark with methanol and mixed well. This gave a standard stock solution of strength 1000 µg/mL of nateglinide.

Preparation of working standard solution: 5 mL of standard stock solution was transferred to a 50 mL volumetric flask and then volume was made up to the mark with methanol so as to obtain a concentration of 100 µg/mL working standard.

Preparation of sample solution: Twenty tablets were weighed and the average weight was calculated. The tablets were crushed to furnish a homogeneous powder and a quantity equivalent to 50 mg of nateglinide (127.99 mg) was weighed in a 50 mL standard volumetric flask. The powder was dissolved in 30 mL methanol and the solution was sonicated for 0.5 h. The solution was cooled to room temperature and diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper and the filtrate was used as sample solution. 5 mL of above solution was transferred to a 50 mL volumetric flask and then volume was made up to the mark with methanol to obtain a concentration of 100 µg/mL working sample.

Validation of proposed method: The proposed method was validated for linearity and range, limit of detection and limit of quantitation, precision, accuracy, specificity, robustness and ruggedness. Validation of the proposed method was carried in accordance with the ICH guidelines[16,17].

Linearity: Ten different concentrations of nateglinide were prepared from stock solution in the range of 10-120 µg/mL, respectively, in methanol to obtain desired linearity range. 10 µL of each solution was applied on the plate (*i.e.*, 100-1200 ng/band for nateglinide) by sample applicator and the plate was developed.

The detector response to the different concentrations was measured. The drug peak-area was calculated for each concentration level. The graph of drug concentration against the peak area was plotted. The plot was linear in the concentration range 300-1000 ng/band. This experiment was carried out thrice and the mean peak area response was used for the calculations. The data were analyzed by linear regression least-squares fitting. The statistical data obtained are given in Table-1.

TABLE-1
ANALYTICAL PERFORMANCE DATA

Parameters	Values (at 210 nm)	
	By height	By area
Linear dynamic range (ng/band)	300-1000	300-1000
Slope	0.121	3.344
Y-Intercept	22.039	250.629
Correlation coefficient (r)	0.9994	0.9998
LOD	33.55	78.95
LOQ	100.64	236.84

Linear regression data for calibration curve.

Limit of detection and limit of quantitation: The limit of detection (LOD) by height and area were found to be 33.55 and 78.95 ng/band, respectively. Limit of quantitation (LOQ) was determined experimentally by spotting six replicates of each drug at LOQ concentration. The LOQ by height and area were found to be 100.64 and 236.84 ng/band, respectively.

Precision for assay of the pharmaceutical preparation: 10 µL working standard solution (1000 ng/band) and sample solutions were spotted on the plate and the plate was developed and evaluated as described above. The procedure was repeated five times, individually weighing the tablet powder each time. The densitometric responses from the standard and sample were used to calculate the amounts of the drug in the tablet.

Accuracy: The accuracy of the experiment was established by spiking pre-analyzed sample with known amounts of the corresponding drugs at three different concentration levels *i.e.*, 80, 100 and 120 % of the drug in the tablet (the external standard addition technique). The spiked samples were then analyzed for five times.

Specificity: The specificity of the method was ascertained by how accurately and specifically the analyte of interest are estimated in the presence of other components (*e.g.*, impurities, degradation products, *etc.*) by exposing the sample to different stress conditions such as light, heat, oxidation, acids and alkali and then analyzing them by proposed method.

Robustness: Robustness was checked by analysis of sample solutions after making small changes to mobile phase composition *n*-hexane, methanol and 2-propanol. The low value of % RSD shows the method is robust and slight change in concentration of *n*-hexane and methanol does not vary the results.

Ruggedness: Ruggedness is to measure the reproducibility of the test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst.

System suitability: A system-suitability experiment was performed before determination of nateglinide in unknown samples. The coefficient of variation for peak area and R_f value for both the drugs was less than 2.0 % for six replicates measurement of the same sample. This shows that the method and the system are suitable for determination of nateglinide.

RESULTS AND DISCUSSION

Normal phase HPTLC using mobile phase *n*-hexane, methanol and 2-propanol in the proportion of 7.5:1.5:1 (v/v) gave satisfactory baseline resolution, with reasonably acceptable R_f values for quantitation purpose. The R_f value observed was 0.56 ± 0.02 for the parent drug. The chamber saturation period of 10 min was found to be suitable as higher saturation period has resulted in band broadening. The λ_{max} of nateglinide, 210 nm was sensitive enough for densitometric evaluation of the degradation product as well.

The constructed calibration plots were found to be linear over the concentration range 300-1200 ng/band both by area and height with correlation coefficient 0.9998 by area and 0.9994 by height. The limit of quantitation (LOQ) that produced the requisite precision and accuracy were 100.64 ng per band by height and 236.84 ng per band by area. The limit of detection (LOD) values were found to be 33.55 and 78.95 ng per band by height and area, respectively. Precision of the method was determined by analyzing the marketed formulations. Replicate estimations of nateglinide in the tablet analyzed by proposed method have yielded quite concurrent results (Table-2), which reports about repeatability of the method. Accuracy was ascertained by carrying out recovery studies on marketed formulations with standard addition method over the range of 80-120 % of labeled claim. The results of recovery being close to 100 % are indicative of the accuracy of the method and shows that the method is free from interference of excipients present in the formulation (Table-3). The specificity studies were carried out by attempting deliberate degradation of the tablet sample with exposure to stress conditions like acidic (0.1N HCl), alkaline (0.1N NaOH), oxidizing (3 % H_2O_2), heat (60 ºC) and UV. Results of degradation products under these conditions are also studiued. No degradation was observed except under acidic and basic hydrolysis. Results showed that degradation product was formed in acidic and basic degradation. Precision studies were carried out for different parameters *i.e.*, different elapsed times (intraday and interday) and different analysts. This study also signifies the ruggedness of method under varying condition of its performance (Table-4). The method was found to be robust from the studies carried for parameters like small changes in wavelength and temperature. Estimation of nateglinide in, H_2O_2, UV light and heat exposed samples shows no significant difference with assay results, but remarkable decrease in assay result in acid and alkali indicating nateglinide is degradable in acid and base. Results are shown in Table-5.

TABLE-2
RESULTS OF HPTLC ASSAY STUDIES (FROM PHARMACEUTICAL PREPARATIONS) (n = 5)

Glinate tablet (avg. wt. 153.58 mg for 60 mg of NAT)					
S. No.	Wt. of tablet powder taken (mg)	Amount of NAT estimated in applied 5 µL volume (ng)		Percentage of labeled claim	
		By height	By area	By height	By area
1	25.597	493.53	492.11	99.04	98.80
2	25.599	485.31	488.03	97.78	98.24
3	25.589	490.42	492.19	98.40	98.70
4	25.592	489.29	490.56	98.45	98.67
5	25.595	486.76	488.39	97.91	98.06
Mean				98.31	98.49
± Standard deviation				0.5003	0.3240
Relative standard deviation (%)				0.5089	0.3290

TABLE-3
RESULTS FROM RECOVERY STUDY FOR NAT (N = 5)

Glinate tablet (avg. wt. 153.58 mg for 60 mg of NAT)						
S. No.	Spiking level (%)	Wt. of tablet powder taken (mg)	Amount of NAT estimated in applied 5 µL vol. (ng)		Recovery (%)	
			By height	By area	By height	By area
1	80	153.48	399.78	410.09	98.51	99.89
		153.60	400.41	416.01	98.50	100.83
2	100	153.54	487.03	492.84	97.67	99.61
		153.60	490.62	489.96	99.35	98.65
3	120	153.60	608.40	612.71	98.66	99.86
		153.54	611.17	610.27	99.21	98.96
Mean					98.65	99.63
± Standard deviation					0.6010	0.7707
Relative standard deviation (%)					0.6092	0.7735

TABLE-4
RESULTS OF SYSTEM, METHOD AND INTERMEDIATE PRECISION

Formulation	Parameter		System precision	Method precision	Intermediate precision		
					Interday	Intraday	Different analysts
Glinate	Height	Mean ± SD	98.61 ± 0.459	97.98 ± 0.960	98.43 ± 1.084	99.01 ± 0.795	98.13 ± 1.021
		RSD (%)	0.466	0.980	1.102	0.803	1.041
	Area	Mean ± SD	99.13 ± 0.390	99.04 ± 1.162	98.82 ± 0.874	99.94 ± 0.933	98.61 ± 0.792
		RSD (%)	0.393	1.173	0.885	0.933	0.803

Each value is a mean of five determinations (n = 5). n= Number of samples. SD = Standard deviation, RSD = Relative standard deviation.

TABLE-5
RESULTS OF SPECIFICITY

Formulation		Normal	Acid	Alkali	Oxide	Heat	UV
Glinate	By area	99.73	57.02	45.72	100.02	98.91	99.96
	By height	99.07	59.17	46.37	99. 01	99.37	101.13

Conclusion

The developed and validated HPTLC method reported here is rapid, simple, accurate, sensitive and specific. The method was also successful for quantitative estimation and analysis of nateglinide from formulation. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of nateglinide from bulk and formulations.

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