

Determination of Nateglinide in Tablet Formulation by HPLC Using a Pentafluorophenyl Core-Shell Column

Çağrı ÖZKURT¹, Alper ÜNAL¹, Orhan KILIÇ¹, Deniz ÇIKLA YILMAZ^{2*}

ABSTRACT: Nateglinide is an insulin secretagogue which has been used for the treatment of Type 2 Diabetes mellitus. A simple analytical methodology for determination of nateglinide in tablet formulation is described. An isocratic reversed phase high performance liquid chromatographic (HPLC) method was developed using Kinetex pentafluorophenyl (PFP) (5µm particle size and 4.6 x 150 mm id) core-shell column as stationary phase and 0.05 M Na₂HPO₄ (pH=2.0) / methanol (30:70 v/v) mixture as mobile phase with the flow rate of 1.0 mL min⁻¹ and diode-array detector at 215 nm. Column was thermostated at 22°C. Under these conditions nateglinide was eluted with a symmetrical peak shape and the retention time was 4.779 minutes. The system suitability parameters such as tailing factor, capacity factor and theoretical plate were evaluated. The method was validated as per International Council on Harmonisation (ICH) guidelines. Linearity was obtained in the 2.54 - 40.70 µg mL⁻¹ concentration range with equation $y = 10.818x + 4.7048$ ($R^2 = 0.998$). Limit of detection and limit of quantification were 1.07 and 2.54 µg mL⁻¹. Accuracy of the method was tested by recovery studies which were in the range of 98.78 - 100.76 %. The intra-day and inter-day precision studies were carried out and the relative standard deviation values of peak areas were within ICH limits. The proposed method was successfully applied to the determination of nateglinide in tablet dosage form.

Keywords: validation, HPLC, nateglinide, pentafluorophenyl, core-shell column

Tablet Formülasyonundaki Nateglinid'in Pentaflorofenil Core-Shell (Çekirdek-Kabuk) Kolon Kullanılarak HPLC ile Tayini

ÖZET: Nateglinid Tip 2 Diabetes mellitus tedavisinde kullanılan bir insülin salgılatıcıdır. Bu çalışmada tablet formülasyonundaki nateglinidin tayini için basit bir analitik yöntem geliştirilmiştir. İzokratik ve ters faz yüksek performanslı sıvı kromatografi (HPLC) yöntemi, sabit faz olarak Kinetex pentaflorofenil (PFP) (5µm partikül boyutu ve 4.6 x 150 mm kolon boyutu) core-shell kolon, hareketli faz olarak 0.05 M Na₂HPO₄ (pH=2.0) / metanol (30:70 v/v) karışımı 1.0 mL dk⁻¹ akış ve 215 nm'de diod dizi dedektör kullanılarak geliştirilmiştir. Termostat kontrollü kolon sıcaklığı 22°C'dir. Bu koşullar altında nateglinid 4.779 dakika alıkonma zamanı ve simetrik bir pik şekliyle elüe olmuştur. Kuyruklanma faktörü, kapasite faktörü ve teorik plaka sayısı gibi sistem uygunluk parametreleri hesaplanmıştır. Analitik yöntem validasyonunun gerektirdiği testler Uluslararası Uyum Konseyi (ICH) yönergelerine göre yapılmıştır. $y = 10.818x + 4.7048$ ($R^2 = 0.998$) doğru denklemi ile 2.54 – 40.70µg mL⁻¹ konsantrasyon aralığında doğrusallık bulundu. Teşhis sınırı 1.07 ve tayin sınırı 2.54 µg mL⁻¹'dir. Yöntemin doğruluğu geri kazanım çalışması ile belirlendi ve yüzde geri kazanım 98.78 – 100.76 aralığında hesaplandı. Gün içi ve günler arası tekrarlanabilirlik çalışmalarıyla elde edilen pik alanlarının bağıl standart sapma değerleri ICH limitleri içerisinde bulunmuştur. Önerilen yöntem nateglinidin tablet formülasyonundan tayin edilmesinde başarıyla uygulanmıştır.

Anahtar Kelimeler: validasyon, HPLC, nateglinid, pentaflorofenil, core-shell kolon

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INTRODUCTION

Nateglinide is an amino acid derivative with the chemical name (-)-N-[(trans-4-isoproppropylcyclohexane carbonyl)-d-phenylalanine, it is a member of the drug class known as meglitinide antidiabetics. Meglitinides are short-acting antidiabetic agents which lower blood glucose levels by stimulating insulin secretion in pancreatic beta cells. Nateglinide is only taken about ten minutes before meals and was approved by the FDA in December 2000 for the treatment of type 2 diabetes mellitus (Maggi et al., 2013; Upadhyay et al., 2018). It appears as a white powder. It is insoluble in water; partially soluble in acetonitrile and octanol; soluble in chloroform, ether, methanol and ethanol (Frenkel et al., 2009).

Nateglinide has been determined alone in pharmaceutical formulations by spectrophotometry (Jain et al., 2009), HPLC (Hacıoğlu et al., 2015) and UPLC (Xavier et al., 2012). It has also been determined simultaneously with its degradation products and impurities (Madhavi et al., 2008), with metformin and meglitinides class of antidiabetics (El-Zaher et al., 2018), fourteen synthetic antidiabetics (Cui et al., 2010) by UV detector and HPLC. Estimation of nateglinide in rabbit plasma (Sankalia et al., 2007), rat plasma (Dey et al., 2020) and urine (Lam et al., 2020) has been described. Among these studies, significant peak tailing of nateglinide on stationary phases was reported. To overcome this problem, Cui et al. reported the addition of sodium dodecyl sulfate and triethylamine in the mobile phase on a Diamonsil C18 column (Cui et al., 2010), El-Zaher et al. presented the use of Lichrospher NH2 column and the tailing factor was given as 1.422 (El-Zaher et al., 2018), Madhavi et al. used Zorbax C8 column and the tailing factor was given as 1.2 (Madhavi et al., 2008).

In recent years, new generation stationary phases containing core-shell particles have gained increasing attention in order to reduce analysis time and improve separation power. Different vendors commercialized stationary phases with a variety of chemistries, that is, C18, C8, HILIC (hydrophilic interaction liquid chromatography, phenylhexyl and pentafluorophenyl (Ali et al., 2012). Core-shell columns packed with pentafluorophenyl silica were applied with success for the analysis of tocopherols (Bakir et al., 2020), phenolic compounds (Serni et al., 2020) and fluoroquinolones (Yıldırım et al., 2020).

This study delineates the development and validation of an isocratic reversed phase HPLC method using a pentafluorophenyl core-shell column for the determination of nateglinide in pharmaceutical oral dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used in this study were Nateglinide which was bought from Sigma-Aldrich, gradient grade methanol (LiChrosolv®), H₃PO₄ and extra pure NaH₂PO₄ which were bought from Merck. Teglix® film coated tablets, which were produced by Biofarma in Turkey and contained 120 mg of nateglinide according to its package information, were bought from a nearby pharmacy.

Instrument and Chromatographic Conditions

Agilent 1260 Infinity HPLC system was used in chromatographic analysis. The components of the HPLC system included Agilent 1260 Infinity Standard Autosampler (G1329B), Agilent 1260 Infinity Quaternary Pump (G1311B), Agilent 1260 Thermostatted Column Component (G1316A), Agilent 1260 Infinity Diode Array Detector (G1315D), and Kinetex PFP 100-5 (4.6 x 150 mm 5µm) column. The mobile phase used in this study was 0.1 M NaH₂PO₄ solution, with its pH adjusted to 2.0 using 0.1 M H₃PO₄. It was then diluted and mixed with methanol in a 30:70 (v/v) ratio. The detection wavelength was 215 nm and the separation was performed with the temperature kept at 22 °C. The flow rate was 1.0

ml min⁻¹ and the injection volume was 10 µL. Agilent ChemStation software was used in data evaluation. A hot air oven, an ultrasonic bath, a Mettler Toledo MP220 pH meter, and a Hettich Universal 32R centrifuge were also used in various stages of the study.

Standard and Sample Solutions Preparations

Standard solution

15.9 mg of nateglinide was weighed and dissolved in methanol in a 25 ml volumetric flask in order to prepare the standard nateglinide stock solution. The working standard solutions were prepared by dilution of the standard solution with the mobile phase and were used for the construction of the calibration curve.

Tablet sample solution

10 tablets were weighed and pulverized into powder with a mortar and pestle. The arithmetic average of the weights of ten tablets was calculated and the powder, assumed to contain 120 mg of nateglinide, was weighed. It was then put into a volumetric flask and dissolved with 100 ml of methanol. The solution was sonicated in an ultrasonic bath for 15 minutes before being centrifuged at 12000 rpm for 10 minutes. Then a solution containing 10.18 µg ml⁻¹ nateglinide was prepared by dilution with the mobile phase.

RESULTS AND DISCUSSION

At the start of the optimization of the chromatographic conditions, the pH of the buffer as the mobile phase component, was considered first. 0.05 M NaH₂PO₄ at pH 3.0 and pH 2.0 were tried. The tailing factor was calculated as 1.34402 at pH 3.0 and as 1.06151 at pH 2.0. As a result, the buffer pH was chosen as 2.0. The mobile phase ratio using methanol was determined by evaluating system suitability parameters *i.e.*, capacity factor, tailing factor and theoretical plate (CDER, 1998). A symmetrical peak was attained with the mixture of 0.05 M NaH₂PO₄ (pH 2.0)/methanol (70/30, v/v) at a flow rate of 1 mL min⁻¹. In Figure 1, the typical chromatogram obtained under these conditions is shown and system suitability parameters were given in Table 1. The retention time of nateglinide was 4.779 minutes which provided a sufficient analysis time. The detector was set at absorption maxima of nateglinide in methanol solution at a wavelength of 215 nm (Jain et al., 2009). The method was applied to commercial tablets and assay results were given at Table 2.

Table 1. System suitability specifications

Parameter	Values	Required limits
Theoretical plate (N)	9979	N>2000
Capacity factor (k')	2.31	k'>2
Tailing factor (T)	1.06151	T<2

Method Validation

Selectivity

Injection of mobile phase showed no interference at the retention zone of nateglinide peak (Figure 1, 2 and 3). A representative chromatogram of tablet sample solution spiked with standard nateglinide solution demonstrated that no matrix components were found to interfere.

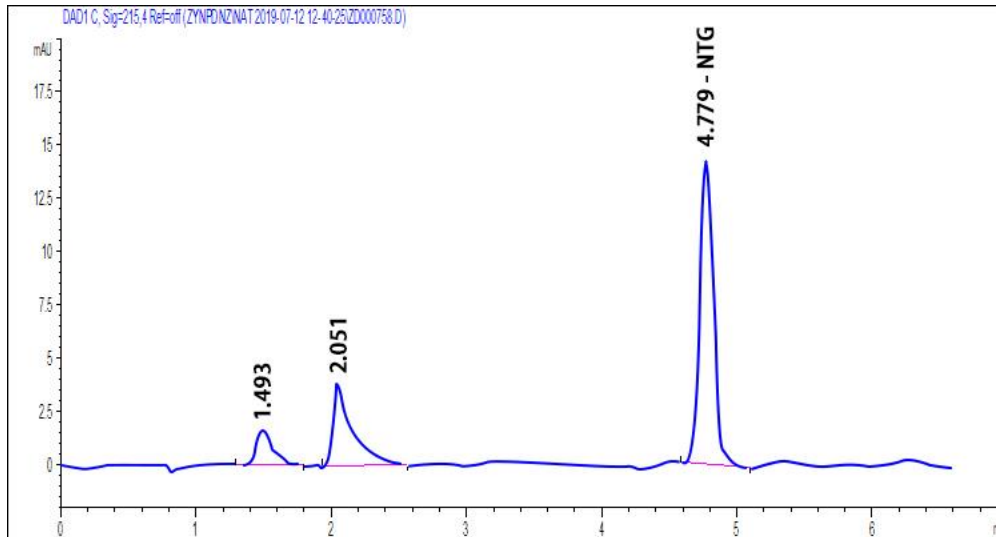


Figure 1. Chromatogram of nateglinide standard solution.

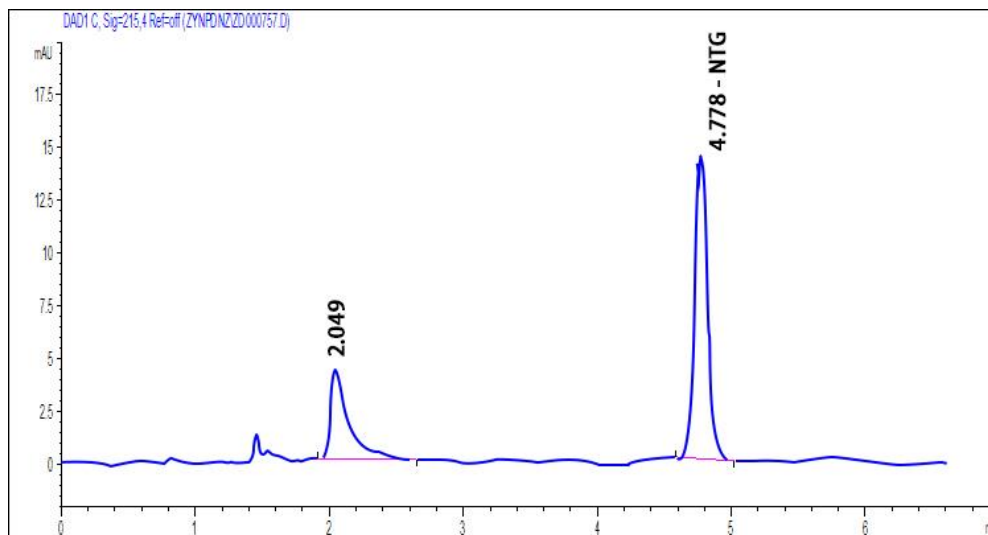


Figure 2. Chromatogram of nateglinide tablet sample solution

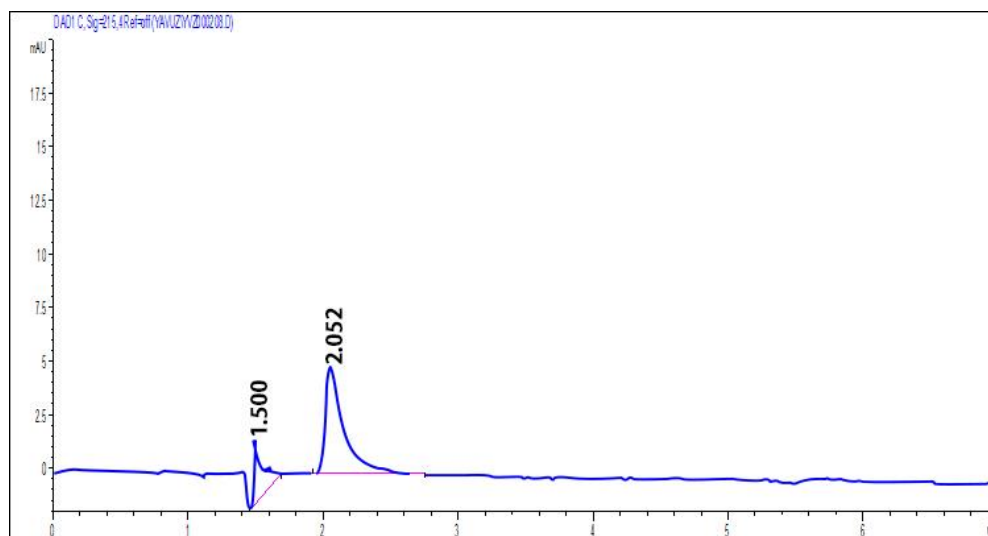


Figure 3. Chromatogram of mobile phase (blank)

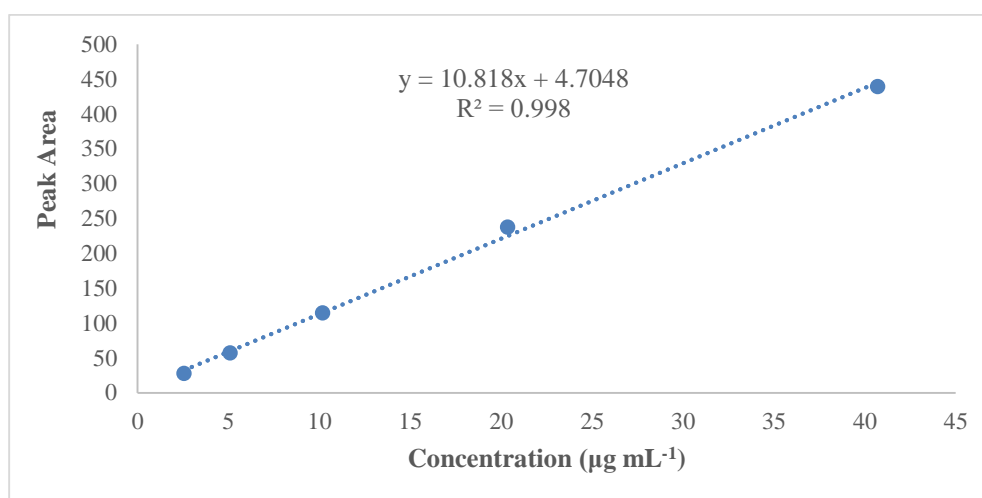
Table 2. Assay results for the commercial tablets

Labeled claim (mg)	Amount found (mg)	RSD%
120	119.74	1.22

*n=3 (mean value)

Linearity and range

For the evaluation of linearity, replicates (n = 3) of five concentrations, which ranged from 2.54 to 40.70 $\mu\text{g mL}^{-1}$, were analyzed. Linear correlation was obtained between concentrations and the average peak areas. The equation of the linear regression line was $y = 10.818x + 4.7048$ with a correlation coefficient of $R^2 = 0.998$. The linearity curve of nateglinide is shown in Figure 4.

**Figure 4.** Linearity plot of nateglinide

Limit of detection and quantification

The limit of detection and quantification was determined to be $1.07\mu\text{g mL}^{-1}$ and $2.54\mu\text{g mL}^{-1}$ with a single to noise ratio of 3:1 and 10:1 respectively.

Accuracy

Accuracy was determined by spiking standard nateglinide solution into the analyzed tablet sample solution at three different levels (80, 100 and 120). As can be seen from Table 3, the percentage of recoveries were in the range of 98.68 - 100.76 and relative standard deviation (RSD%) values <2 confirms the accuracy of the method (ICH, 2005).

Table 3. Results of the accuracy study.

Accuracy level	Amount of drug taken (mg)	Amount of drug spiked (mg)	Recovery* %	RSD %
80	120	96	100.76	0.68
100	120	120	99.21	0.52
120	120	144	98.68	1.18

*n=3 (mean value)

Precision

The precision of the method was evaluated through interday and intraday repeatability experiments. To assess the intraday repeatability, six consecutive injections of 20.35 µg ml⁻¹ nateglinide solution were performed in one day. RSD value of nateglinide peak areas was found to be 0.38. To assess the interday repeatability, injections of the aforementioned solution were performed in three separate days. RSD value of nateglinide peak areas was found to be 0.84 %, which is within the acceptance criterion (ICH, 2005).

Robustness

By intentionally changing the conditions including temperature and flow rate, the robustness test was evaluated. Our data at Table 4 shows that, while the results remained unaffected by changes in the temperature, there were small variations arising from changes in the flow rate.

Table 4. Results of the robustness study

Parameter		T _R *	Calculated Conc. % *
Flow rate (1mL min ⁻¹)	0.95	5.068	104.39
	1.00	4.851	100.00
	1.05	4.565	95.31
T (22°C)	21	4.853	99.09
	22	4.851	100.00
	23	4.800	99.18

T_R*: Retention time, *n= 3 (mean value)

CONCLUSION

This study is a typical example of the development of a quantification method for drug substance in tablet formulation and the establishment of the validation of the analytical procedure by ICH instructions. The method described here using new generation core-shell column with a pentafluorophenyl stationary phase provided a symmetrical nategline peak. The fluorinated phases can provide solutions for the peak tailing problem on conventional C18 bonded silica columns in reversed phase liquid chromatography.

Based on the above results, the developed method is simple with a short analysis time and validation studies proved the reliability of the method which can be useful for quality control in industrial laboratories. However further investigations can be performed concerning to the related impurities and degradation products of nateglinide.

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