FORCED DEGRADATION STUDIES OF NATEGLINIDE BY THE FIRST-ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD AND THE DENSITY FUNCTIONAL THEORY OF THE NATEGLINIDE MOLECULE

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Nateglinide (NAT) is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus. We evaluated the NAT stability under various forced degradation tests (acidic, basic) and predicted the degradation mechanism of the NAT molecule in the gaseous phase and aqueous media. A first-order derivative spectrophotometric method was used for the identification of NAT and the products of its degradation. NAT appeared to be stable in acidic but not in basic media. A probable reaction path of the NAT molecule with OH radicals was analyzed. The optimized geometry was calculated with Gauss View 5. Subsequently, the lowest energy status was determined through geometric optimization using Gaussian 09 software. Aiming to determine the intermediates in the photocatalytic degradation mechanism, the geometric optimization of the molecule was realized using the density functional theory method. The activation energy for the probable reaction path was calculated, and their most stable state from the thermodynamic perspective determined for the gaseous phase and aqueous media. The predicted mechanism was confirmed by comparison with the experimental results on simple structures reported in the literature.

Keywords: *nateglinide, forced degradation study, density functional theory, first order derivative spectrophotometric method.*

ИССЛЕДОВАНИЕ ПРИНУДИТЕЛЬНОЙ ДЕГРАДАЦИИ НАТЕГЛИНИДА МЕТОДОМ СПЕКТРОФОТОМЕТРИИ НА ОСНОВЕ ПЕРВОЙ ПРОИЗВОДНОЙ И ТЕОРИИ ФУНКЦИОНАЛА ПЛОТНОСТИ

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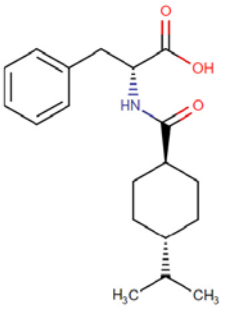
Оценена стабильность натеглинида (NAT) — перорального антигипергликемического препарата для лечения инсулинозависимого сахарного диабета — в различных условиях принудительной деградации (кислотной, щелочной) и предсказан механизм деградации молекулы NAT в газе и водной среде. Для идентификации NAT и продуктов его распада использован метод спектрофотометрии на основе первой производной. Обнаружена устойчивость NAT к кислой среде, но не к щелочной. Проанализирован вероятный путь взаимодействия молекулы NAT с радикалами OH. Оптимизированная геометрия рассчитана с помощью программы Gauss View 5. Для данной геометрии с помощью программы Gaussian 09 определено самое низкое энергетическое состояние. Для определения промежуточных звеньев фотокаталитического разложения с помощью теории функционала плотности рассчитаны оптимизированная геометрия молекулы и энергия активации реакции. Найдено наиболее стабильное с термодинамической точки зрения состояние продуктов распада для газовой фазы и водной среды. Предсказанный механизм подтвержден сравнением с экспериментальными результатами для простых структур.

Ключевые слова: *нателинид, принудительная деградация, теория функционала плотности, метод спектрофотометрии на основе первой производной.*

Introduction. Nateglinide (NAT) is the short name of *N*-(trans-4-isopropylcyclohexyl-carbonyl)-D-phenylalanine [1]



NAT is a phenylalanine derivative that is chemically and pharmacologically different from other antidiabetics [2]. Diabetes mellitus, commonly known as diabetes, is a metabolic disease that causes high blood sugar. The hormone insulin moves sugar from the blood into body cells to be stored or used for energy. The most common types of diabetes are type 1, type 2, and gestational diabetes. Diabetes causes heart disease, stroke, kidney disease, eye problems, dental disease, nerve damage, and foot problems [3].

A few analytical methods have been used for the determination of NAT in pharmaceutical preparations. Among them one should note high performance thin layer chromatography (HPTLC), UV spectrophotometry, and liquid chromatography (LC-ESI-MS) [4–7]. The derivative spectrophotometric methods are simple, fast, and economical methods of great utility for extracting both qualitative and quantitative information from the spectra. Derivative spectrophotometry consists of calculating and plotting one of the mathematical derivatives of a spectral curve. There are several studies relevant to derivatization in the literature [8–13]. Force degradation studies have shown the stability-indicating nature of the method. Forced degradation studies are used for determining impurities and degradation pathways in pharmaceutical preparations and performed in accordance with the established ICH guidelines [14, 15]. Derivative spectrophotometric methods offer the advantage of higher sensitivity over the zero-order spectrophotometric methods and the possibility for application in the drug analysis in presence of degradation products and other impurities.

Density functional theory (DFT) explains the reactions that may occur in organic molecules. For example, OH behaves as an electrophile, whereas O is a nucleophile. Thus, OH readily attaches to unsaturated bonds while O does not. Both forms of the radicals remove H from C–H bonds, and this can result in the formation of different products when the pH is raised to a range where O is the reactant, rather than OH. For example, if an aromatic molecule carries an aliphatic side chain, O attacks there by H removal whilst OH binds preferentially to the aromatic ring [16]. Hydroxyl radicals, which are the most reactive type known in biological systems, react with every biomolecule they may encounter, including water. Potentially, every biomolecule is a hydroxyl radical scavenger but at different speeds [17]. Aromatic compounds are good detectors since they can be hydroxylated. In addition, the position of attack to the ring depends on the electron withdrawal and repulsion of previously present substituents. The attack of an aromatic compound by any hydroxyl radical results in the formation of a hydroxylated product [18].

We aimed to develop methods for the estimation of the percentage degradation of NAT by the zero- and first-order derivative spectrophotometric methods. In addition, we aimed to predict the degradation mechanism of the NAT molecule in the gaseous phase. The probable reaction path of the NAT molecule with OH was analyzed through the DFT method.

Materials and methods. All the chemicals and reagents used were of analytical grade. NAT was kindly provided by Biofarma (Istanbul, Turkey). Hydrochloric acid (HCl, 37% v/v), methanol, sodium hydroxide, and hydrogen peroxide (H_2O_2, 30% v/v) were purchased from Sigma–Aldrich (St. Louis, USA).

The stock solution was prepared in methanol at a concentration of 1 mg/mL NAT. For acidic, basic, and oxidative degradation, the NAT stock solution was prepared at a concentration of 50 mg/50 mL in methanol and further diluted with MeOH to obtain working solutions of 100 μg/mL. Then, 500 μL aliquots were transferred to a 10 mL test tube and mixed with 5 mL of the respective degradation solutions (0.1 M NaOH (basic degradation), 0.1 M HCl (acidic degradation), and 3% H_2O_2 (oxidative degradation).

First-order derivative spectrophotometric method. The distances between two extremum values (peak-to-peak amplitudes), 227.56–250.64 nm ($\Delta\lambda$ = 10 nm), were measured in the first derivative ($dA/d\lambda$). The amounts % of the degraded in all degradation studies were calculated in order to provide indication of the stability.

Forced degradation study. 500 μL of 1 mg/mL of NAT was kept in 5 mL of 0.1 M HCl (acidic degradation) and 5 mL of 0.1 M NaOH (basic degradation) at room temperature for 4 h. The first-order derivative spectrum of the RIL was measured in between two extremum values (peak-to-peak amplitudes), 227.56–250.64 nm ($\Delta\lambda$ = 10 nm) for the first-order derivative spectrophotometric method. To study hydrogen peroxide-induced degradation, initial studies were performed in 3% hydrogen peroxide at room temperature immediately by the first-order derivative spectrophotometric method.

Computational set-up and methodology. The models of the molecules were formed by the use of the mean bond distances and the geometric parameters of the benzene ring. Tetrahedral angles were used for the sp^3-hybridized carbon and oxygen atoms, and 120° angles were used for the sp^2-hybridized carbon atoms in the computational modelling. The aromatic ring was left planar, excluding the position of attack.

Due to the change in the hybridization state of the carbon at the attachment center from sp^2 to sp^3, it was presumed that the attacking OH creates a tetrahedral angle with the C–H bond [19]. Molecular orbital calculations in the photocatalytic degradation reactions of NAT have shown the possibility that more harmful products than those in the original material could be formed.

Therefore, before conducting a photocatalytic degradation reaction experimentally, it is essential to determine the nature of the primary intermediate products. The most reliable and accurate information is gathered through calculations realized with quantum mechanical methods.

Since the produced yield is the same, the photocatalytic degradation reactions of lincomycin and its hydroxy derivatives are based on the direct reaction of these molecules with OH. With this aim, the kinetics of the reactions of NAT with OH were theoretically analyzed. The study was initiated with NAT and then exposed to reaction with OH and the reaction yields were modelled in the gaseous phase. The experimental results in the scientific literature showed that OH detaches a hydrogen atom from saturated hydrocarbons, and OH is attached to unsaturated hydrocarbons and materials with this structure [20]. For this purpose, all possible reaction paths for the analyzed reactions were determined. For every reaction path, molecular orbital calculations of the reactant, yield, and transition state complexes were performed with the DFT, and their geometries were optimized. In order to explore the conformational landscape of the molecules, a potential energy surface scan was performed along the torsional coordinates mentioned above in a relaxed manner (i.e., all other geometrical parameters were optimized at each point) for both conformers. The scan was calculated using the B3LYP/6-31G* method [21].

The DFT method with Gaussian 09 software was used in order to perform the geometry optimization of the reactants, the product radicals, and the pre-reactive and transition state complexes [22]. The DFT method, taking the electron correlation into account, uses the precise electron density to calculate the molecular properties and energies. Spin contamination does not affect them, and hence, for calculations involving open-shell systems, they become favorable.

DFT calculations were made by the hybrid B3LYP functional combining the HF and Becke exchange terms with the Lee–Yang–Parr correlation functional [19].

It is essential in such calculations to choose the correct basis set. Based upon the obtained results, optimization in the current study was carried out at the B3LYP/6-31G(d) level. In the determination of the transition states, the C–O bond forming in the attachment paths and the H–O bond in the detachment paths were chosen as the reaction coordinates. By using frequency analyses at the same level, the ground state and transition state structures were confirmed. For the characterization of transition structures, one imaginary frequency that belonged to the reaction coordinate was determined, which corresponds to a first-order saddle point. The zero-point vibrational energies (ZPEs) were calculated at the B3LYP/6-31G(d) level [19].

Results and discussion. The effect of different extraction solvents on the absorbance of NAT was investigated using methanol, water, and acetonitrile. It was found that methanol was the best solvent for extraction since it gave the highest absorbance ratio, which was used as the extraction solvent henceforward. During the method development, several tests were performed in order to establish the assay parameters.

In order to obtain more sensitive results for the study, the first, second, third, and fourth derivative spectra of NAT were achieved. The results showed that the first derivative was the most suitable for the study. Figure 1 shows the zero- and first-order derivative spectra of 1 mg/mL NAT, respectively. From the first-order derivative spectra of NAT (Fig. 1b) it was determined that the positive peak was at 250.64 nm, while the negative dip was at 227.56 nm.

The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency, and safety. Changes in drug stability can risk patient safety through formation of toxic degradation products. Therefore, it is essential to determine the purity profile and behavior of a drug substance under various environmental conditions.

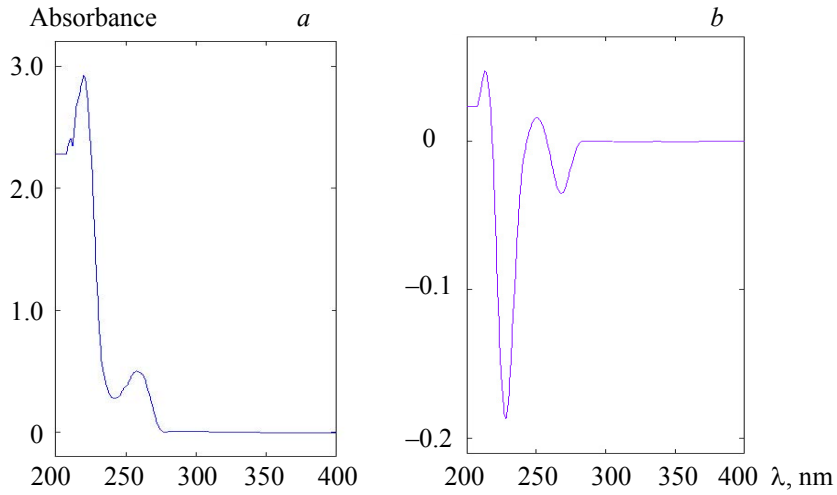


Fig. 1. The zero (a) and first (b) order derivative spectrum of 1 mg/mL of NAT.

The drug was comparatively more resistant to acid hydrolysis than to basic and oxidative degradation. Severe decomposition of the drug on basic and oxidative degradation was determined. NAT exhibited extensive degradation under acidic (0.1 M HCl) conditions, as about 32.4 % degradation occurred after 2 h by the first-order derivative spectrophotometric method in Table 1. The first-order derivative spectrum of the acidically degraded NAT is shown in Fig. 2b. The drug was relatively degraded under basic and oxidative conditions (0.1 M NaOH and 3% v/v H_2O_2 solutions). The 100% degradation takes less than 1 h by the first-order derivative spectrophotometric method. Figure 2 shows the first-order derivative spectra of the acidically, basically, and oxidatively degraded NAT.

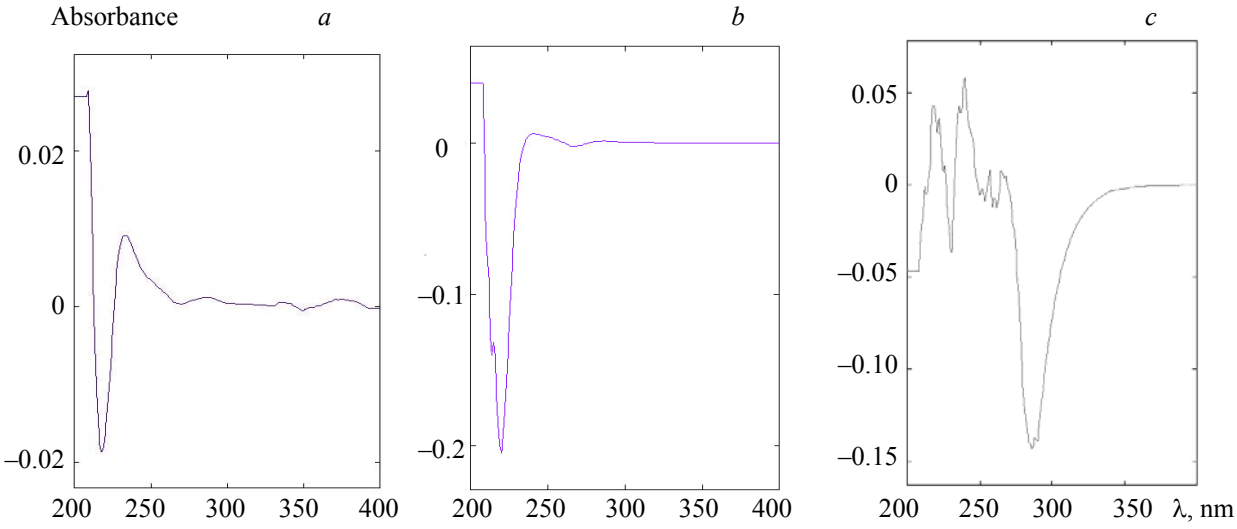


Fig. 2. The first-order derivative spectrum of a) acidically degraded NAT after 2 h, b) basically degraded NAT after 1 h, and c) oxidatively degraded NAT after 1 h (absorbance vs wavelength).

TABLE 1. The Results of Degradation Studies of NAT by the First-Order Derivative Spectrophotometric Method

Time, h	Acidic degradation, %	Basic degradation, %	Oxidative degradation, %
0	11.4	21.9	50.4
1	20.9	Degraded	Degraded
2	32.4		
4	Degraded		

Pathare et al. developed a simple, isocratic, rapid, and accurate reverse phase high-performance liquid chromatography (RP-HPLC) method for the quantitative determination of NAT, which is determined in our study as the percentage recovery of NAT varying from 98.4 to 100.9. The percentage recovery of impurities in the NAT sample varied from 96.8 to 103.5 [23]. Asha et al. worked to determine two anti-diabetic drugs, NAT and metformin hydrochloride in co-formulations. NAT and metformin hydrochloride were also subjected to acid, base, oxidation, wet, heat, and photo-degradation studies. The degradation products obtained were well resolved from the pure drugs with significantly different R_f values. As the method could effectively separate the drugs from its degradation products, it can be used for stability-indicating analysis [24].

Basavaiah K. et al. [25] investigated the determination of NAT in bulk and tablets using UV-spectrophotometry. Their methods were based on the measurement of the absorbance of the drug solution either in 0.1 M NaOH at 210 nm (NaOH method) or in 0.1 M HCl at 270 nm (HCl method). The drug was subjected to forced degradation via acid and alkali hydrolysis, oxidation, thermolysis, and photolysis.

There are a lot of studies related to forced degradation by derivative spectrophotometric methods in the literature. Karasakal and Ozdemir [26] developed zero- and first-order derivative spectrophotometric methods for the analysis of rilmenidine in pharmaceutical preparations. In addition, rilmenidine was exposed to the acidic, basic, and oxidative stress conditions in order to calculate the degradation % by the developed zero- and first-order derivative spectrophotometric methods.

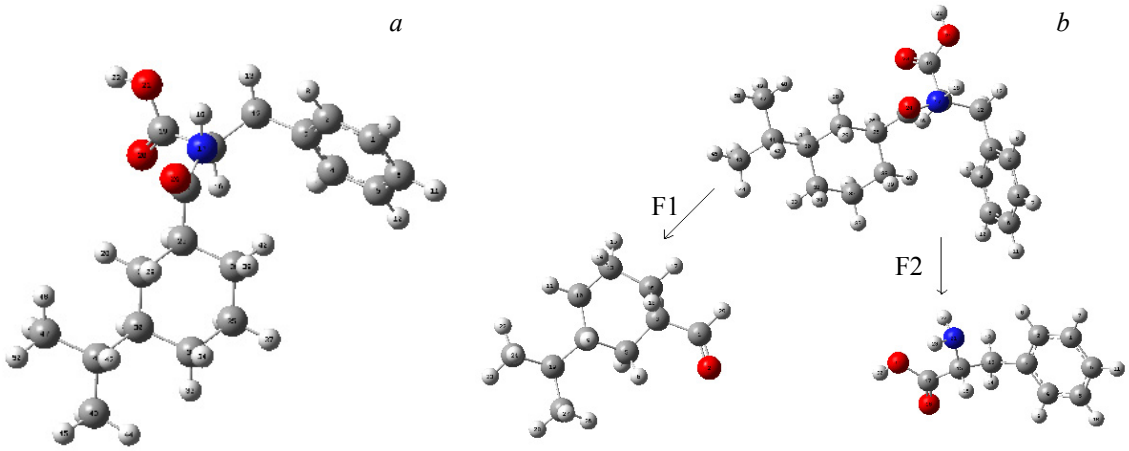


Fig. 3. The optimized structure of NAT and the numbering system (a), possible pathways for the photocatalytic degradation of NAT (b).

In the search for the photocatalytic degradation reaction of NAT, the DFT reactivity descriptors were employed to obtain information about the most susceptible sites for the hydroxyl radical attack. Figure 3a shows the optimized structure of the NAT molecule and the numbering system used throughout the calculations. Three main competing reaction pathways shown in Fig. 3b were determined by selecting the specific sites of the NAT molecule, on the basis of the similarity of softness values to the OH radical. The predicted mechanism was confirmed by comparison with the experimental results on the simple structures reported in the literature. The lowest-energy structure is determined to be the most stable structure. The findings in this fragmentation took place both experimentally and theoretically, which is in good agreement with the Gibbs free energy values of Table 2.

TABLE 2. According to the DFT Method Constant Energy, Enthalpy and Gibbs Free Energy

Molecules	*E*, kcal/mol	Enthalpy, kcal/mol	Gibbs free energy, kcal/mol
NAT	–640241.736	–640241.144	–640292.310
F1	–292951.706	–292951.113	–292983.244
F2	–348006.848	–348006.255	–348038.528

Conclusions. Currently, there is no publication concerning the forced degradation studies of nateglinide by first-order derivative spectrophotometry and DFT studies of the nateglinide molecule in the literature. The first-order derivative method is a sensitive, rapid, accurate, and reliable method. In this study, nate-

glinide was exposed to the stress conditions of hydrolysis (acid, base, and oxidation), and its degradation percent was calculated by the first-order derivative spectrophotometric method. The degradation of nateglinide was predicted to occur through intramolecular F1 and F2 ring cleavages followed by subsequent reactions with OH radicals transforming the fragments into smaller species such as NO_3^- and NH_4^+.

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