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Enantioseparation of 2-(1*H*-imidazol-1-yl)-1-(naphthalen-2-yl)ethan-1-ol which is active metabolite of anticonvulsant nafimidone

Esma KARACA KARADOGAN[1], Zeynep OZDEMIR[1*], Yaren Nur ZENNI[1], Arzu KARAKURT[1]

[1*] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Inonu University, Malatya, Türkiye

ABSTRACT: Nafimidone and nafimidone alcohol, which were synthesized in 1981 and whose anticonvulsant activities were determined, are important anticonvulsant compounds in the structure of (arylalkyl)azole, which reached the stage of clinical human studies but could not pass this stage. In this study, the commercially available chiral stationary phase amylose tris(3,5-dimethyphenylcarbamate) (Chiralpak AD) was used to establish direct enantiomeric separations of nafimidone alcohol which is the metabolite of nafimidone in the normal phase HPLC mode. Investigations were also done into the compositional influences of the mobile phase. When the mobile phase was switched from methanol to n-hexane, the retention times were shortened. The mobile phase of methanol/n-hexane (70:30 v/v) at a flow rate of 0.2 mL/min produced the best results, with an enantiomer resolution of 0.83. Consequently, further chemical and pharmacological research on nafimidone alcohol and its enantiomers can be facilitated by the proposed HPLC approach.

Key Words: Amylose tris(3,5-dimethyphenylcarbamate), Chiralpak AD, arylalkylazole, nafimidone alcohol.

1 INTRODUCTION

The (arylalkyl)azole structure is shared by the anticonvulsant drugs nafimidone, denzimol, and loreclezole, which lack the ureide structure (Figure 1). One of the most well-known examples of this category is loreclezole, which has a triazole ring as part of its structure. The group's other two members, nafimidone and denzimol, also include an azole group that includes an imidazole ring [1,2].

*Corresponding Author: Zeynep ÖZDEMİR
E-mail: zeynep.bulut@inonu.edu.tr
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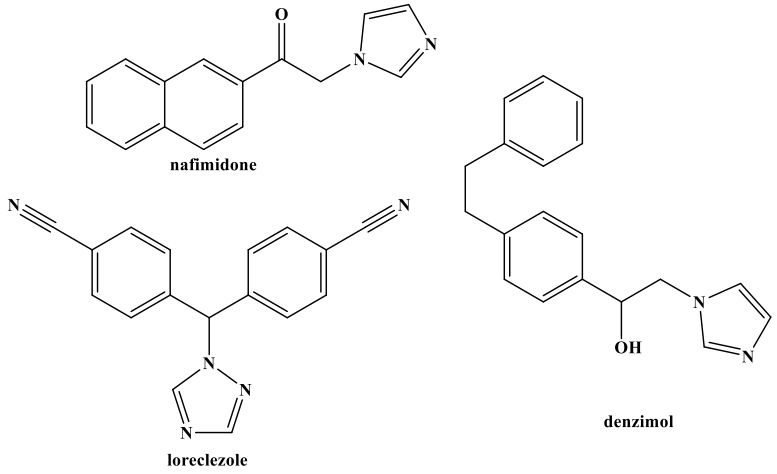

Figure 1. Structure of nafimidone, denzimol and loreclezole.

In contrast to barbiturate and valproic acid, nafimidone and denzimol have an acting profile that is comparable to that of phenytoin and carbamazepine. The main metabolite of nafimidone, nafimidone alcohol, likewise functions as an anticonvulsant (Figure 2) [3,4].

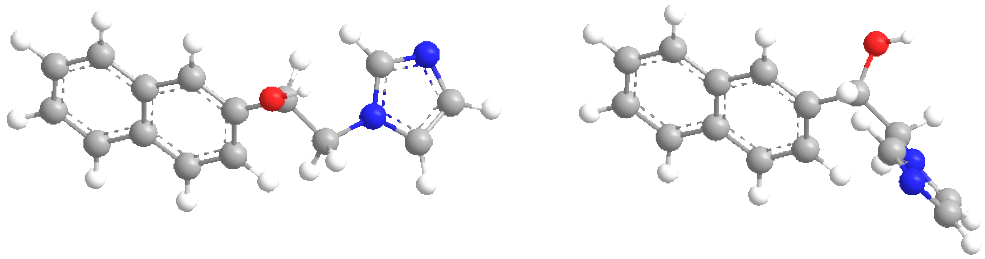

Figure 2. Structure of nafimidone alcohol.

Drug metabolism, also known as biotransformation, is the process by which medications undergo molecular changes within the body after being delivered [5, 6]. Variations in the drug's physicochemical qualities, pharmacological activity, duration of action, and toxicity may arise as a result of chemical changes that metabolic reactions make to the drug's structure. In terms of medication development, metabolism investigations are crucial since they offer crucial information for creating molecules that are more efficient, less toxic, and highly safe. Metabolites are the substances created as a result of metabolic processes. Drug toxicity generally lowers due to metabolism (detoxification), and the compound's removal from the body in water-soluble form is facilitated. Certain medications' biological activity can also be

observed in their metabolites. They are referred to as active metabolites. Occasionally, active metabolites can transform into a structure that has distinct effects [7, 8].

According to reports, the pharmacological properties of several medications' metabolites determine their effects. More efficient molecules were created by synthesizing metabolites after metabolic studies were used to determine the impacts of metabolites [6].

Hepatic microsomes from rats that had previously received phenytoin were used to examine the effects of nafimidone and nafimidone alcohol on the p-hydroxylation of phenytoin. The hydroxylation of phenytoin was inhibited by nafimidone and nafimidone alcohol in a concentration-dependent way at both high-affinity and low-affinity metabolic sites. In doses below micromolar, both substances worked as inhibitors. Both metabolic areas had an inhibitory pattern that was consistent with "mixed-type" inhibition. For nafimidone alcohol, the computed inhibition constants Ki were roughly 0.2 M in both locations. Nafimidone and nafimidone alcohol both prevented carbamazepine epoxidation at submicromolar doses. As a result, it was discovered that nafimidone and nafimidone alcohol are powerful inhibitors of two crucial biotransformation routes [9].

Due to the chiral carbon atom that the nafimidone alcohol molecule bears, it has two enantiomers. The biological, pharmacokinetic, pharmacodynamic, and toxicological characteristics of chiral pharmacological enantiomers can vary [10, 11]. The enantiomers of chiral substances create diastereomeric drug-receptor complexes with chiral receptors, and because the proteins in the organism are likewise chiral, stereoselective activity is seen as a result. It's possible that one of the enantiomers has a therapeutic impact while the other has unfavorable side effects or toxic effects, that one is active while the other is inactive, or that both have various therapeutic effects [12]. Enantiomers can be absorbed, interact with receptors, bind to plasma proteins, undergo biotransformation, and be excreted in stereospecific ways. To examine the pharmacological, pharmacokinetic, pharmacodynamic, and toxicological characteristics of the enantiomers of new biologically active chiral compounds separately, it has become more important in recent years to obtain pure enantiomers and determine the enantiomeric composition of chiral drugs in pharmaceutical analysis [13].

HPLC is frequently used to analyze or prepare enantiomers for separation and to determine the optical purity of each enantiomer. By utilizing chiral mobile phase on non-chiral stationary phase or chiral stationary phase using non-chiral mobile phase, direct enantiomer separation is achieved via chromatographic methods. The separation of racemic substances into their enantiomers has been effectively accomplished in recent years thanks to the development of numerous chiral stationary phases and the utilization of cellulose and amylose derivatives adsorbed on macroporous silica gel [14-17].

The enantiomeric separation studies of 2-(1H-imidazole-1-yl)-1-naphthalene-2-yl)ethanol esters, 1-(naphthalene-2-yl)ethanol esters, and 1-(1-hydroxynaphthalene-2-yl)-2- (1H-imidazole-1-yl)ethanol molecules with anticonvulsant effects have been previously published [18]. The racemic mixture nafimidone alcohol used in this investigation, was analytically separated into its enantiomers by HPLC using various ratios of n-hexane:MeOH solutions as the mobile phase and amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) as stationary phase. Chromatographic information was used to characterize each enantiomer.

2 MATERIAL AND METHOD

Following the procedures described in the literature, the nafimidone alcohol molecule that was isolated in this study was created from 2-acetylnaphthalene [18-21]. In an ice bath, acetic acid was dissolved in 0.05 mol of 2-acetylnaphthalene. A few drops of hydrobromic acid were added to the reaction medium in a three-necked flask, and then, while the mixture was being constantly stirred, 0.05 mol of bromine that had been diluted with acetic acid was added dropwise using a dropping funnel attached to the reaction flask after the bromine was added, the reaction was continued for a further three hours at room temperature while being stirred. At the conclusion of the reaction, the mixed was poured into ice water to solidify it. The precipitate was filtered, then dried in the dark after being cleaned with sodium bicarbonate solution. Crystallization from a methanol/water solution is used to purify the compound (naphthacyl bromide). A solution of 0.03 mol imidazole in 2.5 ml of dimethylformamide that had cooled in an ice bath was gradually mixed with a solution of 0.01 mol naphthacyl bromide in 2.5 ml of dimethylformamide. It was stirred for two hours in an ice bath, then left at room temperature overnight. Ice water was added to the reaction media. The precipitated was filtered and dried. By

crystallization from methanol, it was made pure and nafimidone was occured. 25 ml of methanol and 1.5 mmol of nafimidone were combined, and the mixture was then cooled in an ice bath to 5ºC. The reaction vessel was then filled with 4.5 mmol of sodium borohydride, and it was stirred in an ice bath for an hour. When the methanol was removed from the process, the residual residue was made solid by adding ice water, and it was then refined by crystallization with the aid of the proper solvents to get final compound nafimidone alcohol.

2.1 Enantiomer Separation Studies by HPLC

The chiral separation of the nafimidone alcohol was accomplished using an Agilent 1100 Series High Pressure Liquid Chromatography apparatus, an Agilent Quaternary Pump, and an Agilent Multiple Wavelength Detector. The analyses were conducted at room temperature and at the wavelengths where the compound exhibited the greatest absorption.

For the separation, a chiral column covered with silica gel and made of amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD; 25 cm x 4.6 mm inner diameter; particle size 10 m) was utilized by Daicel Chemical Industries, Ltd.

High pressure liquid chromatography solvents were used to create solvent systems with methanol:n-hexane combined in various ratios (100, 90:10, 80:20, and 70:30) as the mobile phase (HPLC purity Merck). In methanol, each chemical was dissolved at a level of roughly 0.5 mg/mL. In accordance with the normal phase mode, analyses were carried out using a Chiralpak AD column at room temperature (22°C). Using an isocratic method, a combination of methanol and n-hexane in varying concentrations was used for the mobile phase elution. Every solvent is HPLC pure. The UV detection wavelength was 254 nm, and the flow rate was adjusted at 0.2 mL min-1. Gallic acid was used to calculate the unretained peak's retention time (t_0). It was employed in subsequent computations and remained constant for each chromatographic run at a fixed flow rate. Average values of duplicate determinations are retention times.

3 RESULTS

On the chiral stationary phase, the molecules amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) were split into their enantiomers (a mixture of methanol and hexane was used as the mobile phase in the ratios specified below) (Table 1).

Table 1. Separation of nafimidone alcohol into its enantiomers on the chiral stationary phase of amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD)

Hex (%)	k_1	k_2	α	R_S	N_1	N_2	t_1	t_2
0	0.12	0.18	1.50	0.69	6527	1499	10.10	10.65
10	0.23	0.28	1.23	0.54	7872	1527	12.20	12.70
20	0.39	0.47	1.21	0.74	3660	2166	12.10	12.80
30	0.36	0,44	1.23	0.83	4860	2218	12.20	12.95

Analyzes were performed at λ max, with a flow rate of 0.2 ml/min. n The compound is not separated into its enantiomers. Plots of the retention factors (k_1 and k_2), selectivity (α), resolution factors (R_S) and number of layers (N_1, N_2).

The synthesized chemicals were created as racemic mixes because they had an asymmetric center in their structures. By using a chiral column made of amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) coated on silica gel, enantiomers of the substances were separated by HPLC.

The polysaccharide phenylcarbamate derivative CSP group, which forms the basis of separation using the Chiralpak AD column, is based on the production of the diastereomeric solute-CSP complex. The polar carbamate groups in the CSP structure make hydrogen bonds with the compounds' O- and N-H groups and engage in - interactions via aromatic rings to produce the solute-CSP complex. The solute-CSP complex is stabilized by the presence of aromatic rings in the compounds' structures, and the enantiomers' steric compatibility with this space varies as a result (Figure 3).

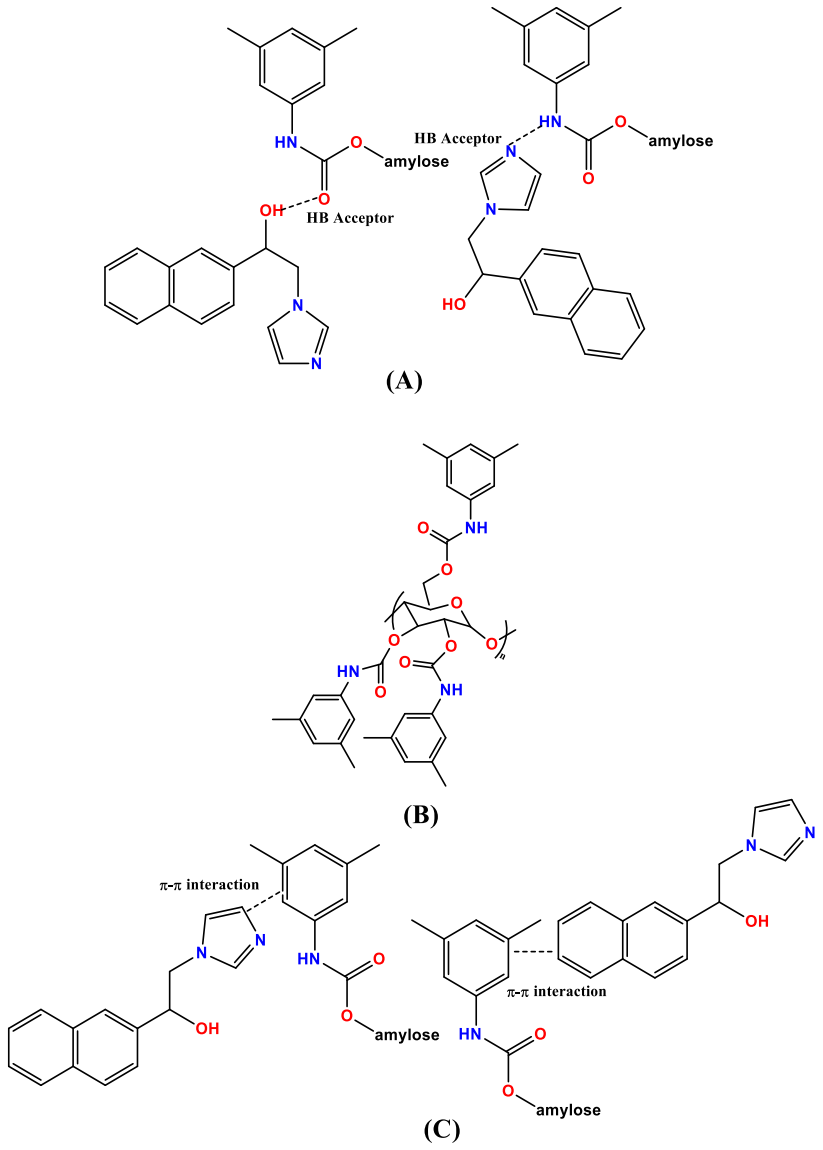

Figure 3. Chemical interactions between synthesized compounds and CSP; (A) proposed interactions of amylose CSP with acceptor hydrogen bonds of compounds, (B) structure of Amylose tris(3,5-dimethylphenylcarbamate, and (C) proposed interactions of π-π-bonds with compounds between amylose CSP [22].

In this research, the chiral separation of the compounds was carried out using methanol:n-hexane mixed mobile phases, which included just methanol and varying concentrations of n-hexane (0–30%). Our chromatographic system is seen within the context of the normal phase mode due to the high methanol content in our mobile phase. The chemical was successfully separated at all ratios, and it was noted that the resolution and selectivity values rose as the hexane ratio rose. Reverse phase HPLC analyses employing C18 columns have also shown the capability of Chiralpak AD columns to perform chiral separation investigations of substances (Figure 4).

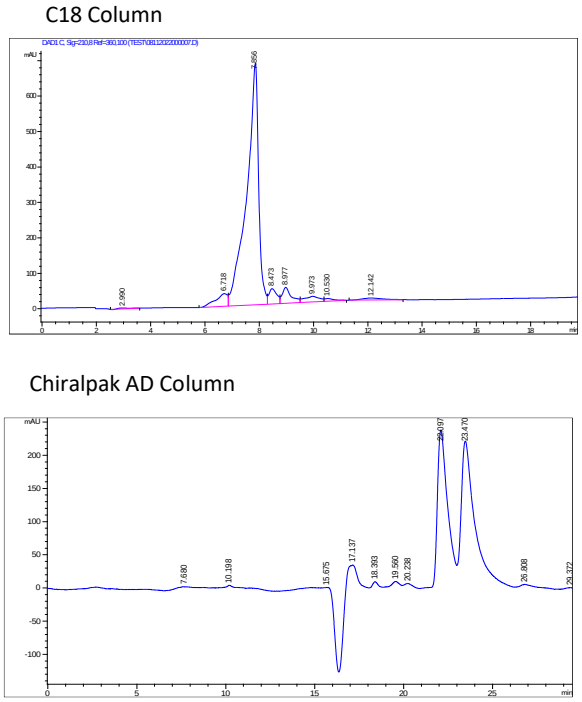

Figure 4. Chromatograms of nafimidone alcohol with C18 column and Chiralpak AD column.

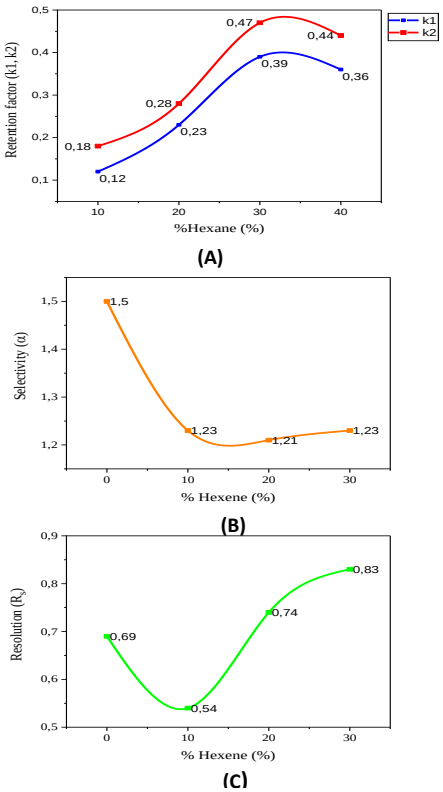

Figure 5. Effect of n-hexane content on retention time (A) k_1: plots of the retention factors for one enantiomer and k_2: plots of the retention factors for one enantiomer, selectivity (B) and resolution (C).

Figure 5's graph illustrates how the amount of n-hexane in the methanol employed as the mobile phase affects retention time, selectivity, and resolution.

4 DISCUSSION

In this study, Chiralpak AD columns were utilized at a flow rate of 0.2 ml/min to analytically separate the enantiomers of the nafimidone alcohol molecule from the arylalkyl azole anticonvulsant drugs using methanol/n-hexane solutions. The composition of the mobile phase had an impact on the chromatographic parameters. The fundamental separations of the compounds under investigation were not visible when methanol and ethanol alone were utilized as the mobile phase, but baseline separations were obtained when methanol/n-hexane combinations were. The methanol/n-hexane (70:30) mobile phase is the most effective for achieving baseline separation in the shortest time. According to the chromatographic results, the analytical HPLC approach created in this study can also be used to prepare the molecule for separation on the Chiralpak AD column in order to determine the pharmacological activity of each enantiomer.

This method development study, which is an early stage of our chiral separation studies, is a step toward the preliminary separations that are planned to take place in the future.

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6 AUTHOR CONTRIBUTIONS

Hypothesis: E.K.K., Z.Ö.; Design: Z.Ö.; Thesis student: E.K.K., Thesis advisor: Z.Ö.; Enantiomer separation studies: E.K.K.; Synthesis of compounds: Y.N.Z., A.K.; Manuscript writing: Z.Ö.

7 CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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