Pharmaceutical Analysis Laboratory[1], Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; National Institute of Hydrology[2], Roorkee, India

Enantiomeric separation of glutethimide derivatives using a Ceramospher RU-2 column

H. Y. Aboul-Enein[1], I. Ali[2]

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Professor Hassan Y. Aboul-Enein, Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC 03–65), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh – 11211, Saudi Arabia
enein@kfshrc.edu.sa

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The enantiomeric resolution of *p*-acetylaminoglutethimide and *p*-nitroglutethimide was achieved on a Ceramospher RU-2 column using methanol as the mobile phase. The flow rates of the mobile phase were 1.0 and 0.5 mL/min for *p*-acetylaminoglutethimide and *p*-nitroglutethimide, respectively, with UV detection at 254 nm. The values of α of the resolved enantiomers of *p*-acetylaminoglutethimide and *p*-nitroglutethimide were 1.63 and 1.24 while the values of Rs were 1.44 and 0.86 respectively. The possible chiral mechanism was the formation of transient diastereomeric intermediates between the enantiomers and the chiral selector (1,10-phenanthroline) ruthenium II complex which was stabilized by π-π interactions.

1. Introduction

Aminoglutethimide (AG) [3-(4-aminophenyl)-3-ethyl-2,6-piperidinedione] was initially used as an anticonvulsant drug for the treatment of epilepsy but later was withdrawn because of its inhibitory affects on adrenal functions (Stefaneanu et al. 1991; Vanek et al. 1990). But currently this compound is used for the treatment of hormone-dependent metastatic breast cancer (Lake and Hudis 2002; Visvanathan and Davidson 2003). AG contains a chiral center and exists in two enantiomers as is the case with its several derivatives. It is a well-known fact that enantiomers may differ in their pharmacological and toxicological activities, moreover, one of the enantiomers may be inactive or toxic (Aboul-Enein and Abou Basha 1997). These properties of the enantiomers have created an interest to study the pharmacological and toxicological properties of the individual enantiomers of pharmaceuticals, agrochemicals etc. (Ward 1994). The US Food and Drug Administration have issued certain guidelines to pharmaceutical and agrochemical industries to specify the enantiomeric purity of the optically active compounds prior to their marketing (FDA 1992). It was reported that the (+)-R-enantiomer of AG had the most steroidogenesis inhibitory activity (two or three times more potent than the racemate) while the (−)-S-enantiomer had very little activity (Finch et al. 1975). Therefore, enantiomeric resolution of AG derivatives is of great interest to the pharmaceutical and pharmacological studies for possible racemic switch. Some papers on the chiral resolution of AG and its derivatives using polyssacharide chiral stationary phases (CSP) (Aboul-Enein and Islam 1991; Aboul-Enein and Serignese 1994), protein based CSP (Aboul-Enein and Islam 1988), vancomycin CSP (Aboul-Enein and Serignese, 1998) and cyclodextrin based chiral stationary Chirose C-1 phase (Aboul-Enein and Al-Durabi, 1999) have been published. Recently, a reversed phase chiral column named Ceramospher RU-2 was developed which has a very good resolving power with high separation factor (MacFarlane et al. 2002). The structure of this phase is shown in Fig. 1. Little work has been done on this chiral phase, and, hence attempts have been made to resolve the enantiomers of AG derivatives using this newly developed column and the results of this research are presented herein.

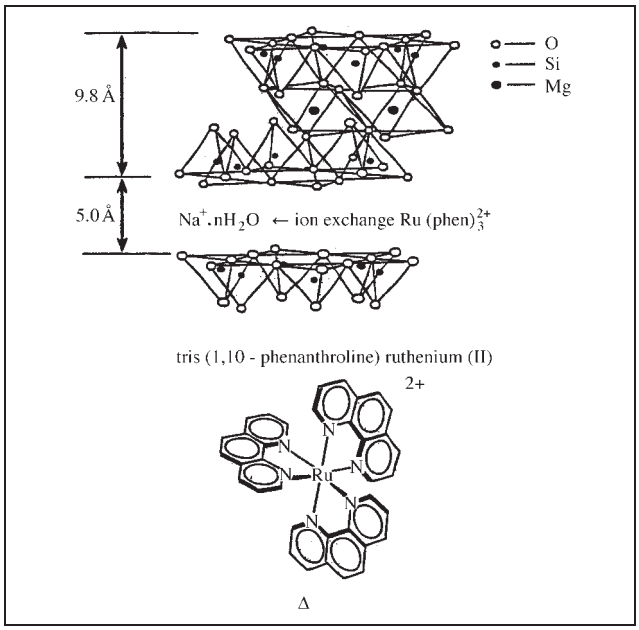

Fig. 1: Structure of Ceramosper RU-2 chiral stationary phase based on a sodium magnesium silicate structure

Table: Capacity (k), separation (α) and resolution (R_s) factors for the enantiomeric resolution of *p*-acetylaminoglutethimide and p-nitroglutethimide

	k_1 (−)	k_2 (+)	α	Rs
p-Acetylaminoglutethimide	1.95	3.18	1.63	1.44
p-Nitroglutethimide	1.69	2.10	1.24	0.86

For details see experimental section.

2. Investigations, results and discussion

Chromatographic parameters, capacity factor (k), separation factor (α) and resolution factor (Rs) for the resolved enantiomers of *p*-acetylaminoglutethimide and *p*-nitroglutethimide at 1 and 0.5 mL/min, flow rates, respectively, are given in the Table. It is clear that *p*-acetylaminoglutethimide has been resolved successfully while an only partial resolution of *p*-nitroglutethimide was achieved on this column. The values of separation factor of the resolved enantiomers of *p*-acetylaminoglutethimide and *p*-nitroglutethimide were 1.63 and 1.24 respectively while the values of resolution factor were 1.44 and 0.86 respectively. A typical chromatogram of the resolved enantiomers of *p*-acetylaminoglutethimide on Ceramospher RU-2 column is shown in Fig. 2. The order of the elution was confirmed by using optically active pure (+)-forms of each compounds. It has been observed that the (−)-enantiomer eluted first followed by the (+)-enantiomer of both the studied AG derivatives. A variation in the chromatographic parameters was carried out to obtain the best resolution. Ethanol, acetonitrile, sodium perchlorate and several buffers were tested but good resolution could not be achieved. Methanol containing 0.1% trifluoroacetic acid and triethylamine was also tested as the mobile phase without success. As a result of extensive experimentation the optimized chromatographic conditions were developed and reported herein.

The structure of the used CSP is shown in Fig. 1. It contains a spherical clay (sodium magnesium silicate) as the basic packing material with a ruthenium complex [(1,10-phenanthroline) ruthenium II complex, Δ configuration] as the chiral selector. It has alternative sheets of octahedron of magnesium and tetrahedron of silica sandwiching the chiral selector resulting in chiral cavities. Therefore, the tetrahedral and octahedral cavities having the ruthenium complex serve as chiral baskets and the molecules can be separated once they enter these cavities. The exact chiral recognition mechanism on this phase is not known but it can be rationalized that the enantiomers form diasteromeric intermediate complexes with the ruthenium complex which are stabilized mainly by π-π interactions with the π electron of the phenanthroline ring system. Besides, many oxygen atoms present in the clay form strong hydrogen bondings (achiral in nature) and, hence, mostly the retention time on this CSP is larger.

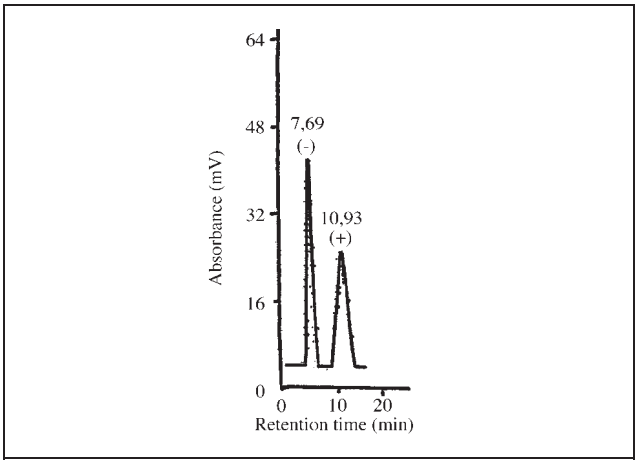

Fig. 2: Chromatograms of the resolved enantiomer of *p*-acetylaminoglutethimide

The structures of the studied AG derivatives indicate the presence of one aromatic ring attached to chiral carbon along with oxygen, nitrogen atoms. Enantiomeric separation of AG derivatives is due to their penetration into the tetrahedron and octahedral cavities of silica and magnesium clay where they form diasteromeric intermediate complexes with the chiral ruthenium complex. The formation of diasteromeric intermediate complexes is stabilized by π-π interactions between the aromatic ring of the AG derivatives and 1,10-phenanthroline. Besides, some steric, van der Waals forces may contribute to the chiral resolution on the reported CSP. Briefly, the aromatic ring of each enantiomer fits stereogenically in the different fashion into the chiral cavities of the stationary phases which is stabilized by the π-π interactions of different magnitude for both (+) and (−) enantiomers and, hence, the resolution of enantiomers occurrs. However, stronger hydrogen bondings are formed between the hydrogen atom, attached to the N–H amide group and oxygen atoms of the clay. It is important to mention here that the nature of this hydrogen bonding is achiral, which binds the enantiomers strongly to the clay and, hence, enantiomers elute at high retention times. However, achiral hydrogen bondings provide sufficient time to the enantiomers to rest onto the ruthenium complex, which may be helpful in the chiral resolution. The partial resolution of *p*-nitroglutethimide supports the fact that π-π interactions are only responsible for chiral separations of these derivatives on the reported CSP. The *p*-nitro group in this molecule makes the phenyl ring electron deficient which decreases the magnitude of π-π interactions, thus, resulting into an only partial resolution of this molecule.

3. Experimental

3.1. Chemicals and reagents

The racemic mixtures and optically active (+)-forms of *p*-acetylaminoglutethimide and *p*-nitroglutethimide were obtained from Ciba-Geigy, Basle, Switzerland. The solutions of these derivatives (1 mg/mL) were prepared in methanol. Methanol, triethylamine and trifluoroacetic acid of HPLC grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA).

3.2. Chromatographic conditions

An aliquot of 20 μL of each solution was injected into a HPLC system consisting of a Waters solvent delivery pump (model 510), a Waters injector (model WISP 710B), a Waters tunable absorbance detector (model 484) and a Waters integrator (model 740). The order of elution of the enantiomers was confirmed by using the optically active (+)-form of each compound. The column used was Ceramosphere RU-2 (25 cm × 0.46 cm, particle size 50 μm) [Sodium magnesium silcate with optically active (1,10-phenanthroline) ruthenium II complex, Δ configuration, Fig. 1] and obtained from Shiseido Co., Ltd., Tokyo, Japan. The mobile phase was methanol, which was filtered and degassed before use. The flow rates of the mobile phase were 1.0 and 0.5 mL/min for *p*-acetylaminoglutethimide and *p*-nitroglutethimide, respectively. The chart speed was kept constant at 0.1 cm/min. All the experiments were carried out at 23 ± 1 °C. The detection was carried out at 254 nm. The chromatographic parameters such as capacity factor (k), separation factor (α) and resolution factor (Rs) were calculated.

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