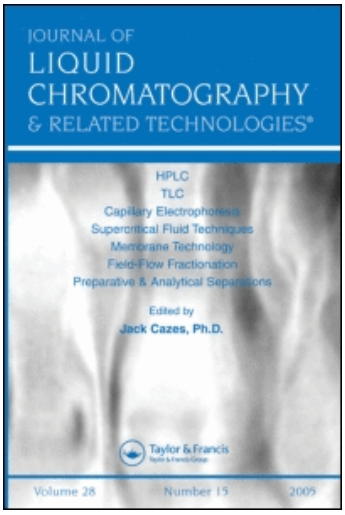

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ENANTIOMERIC SEPARATION OF SOME CLINICALLY USED RACEMIC DRUGS ON PIRKLE-1J CHIRAL STATIONARY PHASE

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ABSTRACT

The enantiomeric separation of several clinically used racemic drugs has been achieved on N-(3,5-dinitrobenzoyl)-3-amino-4-phenylazetidin-2-one bound to silica chiral stationary phase known as Pirkle-1J. The chiral recognition mechanisms involved between the analytes and chiral stationary phase were explained using molecular modelling semi-empirical AM1 method.

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The results indicate that this phase is successful in resolution of β-adrenergic blockers.

INTRODUCTION

It is generally accepted that enantiomers of a compound may exhibit completely different pharmacological activity.(1) It may consider not only the profile of the main activity (pharmacokinetics and/or pharmacodynamics) but also some side effects, as well as the interaction with other drugs. For instance, only the specifically β-blocking (S)-enantiomers of atenolol and propanolol decrease the nocturnal production of melatonin (what might be the reason for sleep disturbances – a well known side effect of β-blockers).(2) Co-administration with warfarin, such drugs as amiodarone(3) or metronidazole(4) leads to the differences in the rate of metabolism between particular warfarin enantiomers; the metabolism of more active (S)-enantiomer(5,6) being inhibited more strongly. The inhibition of warfarin plasma clearance time is supposed to be responsible for its enhanced anticoagulant effect when co-administered with some drugs.(3,4) Therefore, the determination of the enantiomeric ratio might sometimes be necessary, even in the case of drugs marketed as racemic mixtures. Chromatography on chiral stationary phases seems to be one of the most accurate and precise methods for that purpose. In general, one can classify the most popular chiral stationary phases into two groups. One of them consists of a defined monomolecular chiral selectors bound to a stationary phase. The other one is polymeric in nature and includes proteins, polysaccharides, and aromatic helical phases.

In the present paper, we describe the separation of enantiomers of some clinically used racemic drugs on Pirkle 1J column. The column is recommended for the direct separation of underivatized β-blocker, as well as for arylpropionic acid non-steroidal anti-inflammatory drugs (NSAIDs) enantiomers.(7) It consists of dinitrobenzoylated β-lactam (N-(3,5-dinitrobenzoyl)-3-amino-4-phenylazetidin-2-one, Fig. 1) chemically bonded to silica and possesses π-acceptor properties. One could expect, that both aromatic rings would be kept perpendicular to each other, since the stationary phase resembles in a way the Whelk-O1 column in which N-(3,5-dinitrobenzoyl)-4-amino-1,2,3,4-tetrahydrophenantrene is chemically bound to silica (Fig. 2). The Whelk-O1 column was designed to possess a cleft in which face-to-face and face-to-edge π-π interactions could occur, constructed from π-acidic and π-basic aromatic systems held more or less perpendicular to each other. Naproxen was shown to bind within the cleft forming face-to-face π-π interactions with the dinitrobenzene moiety and face-to-edge with the dihydrophenanthrene ring of the CSP.(8)

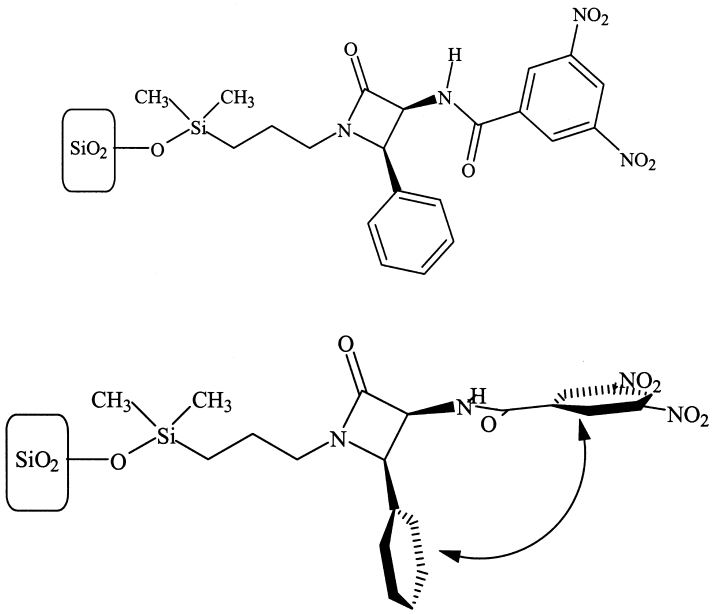


Figure 1. The chemical structure of Pirkle 1-J chiral stationary phase.

EXPERIMENTAL

Apparatus

A Varian 9000 liquid chromatograph, equipped with a UV-VIS variable wavelength detector and a gradient pump (Varian Associates, Palo Alto, CA, USA) was used. A Valco valve injector (10 μL loop) was employed. The HPLC system was operated under a Star Workstation 4.0 program.

Chemicals

All racemic compounds were purchased from Sigma (St. Louis, MO, USA) with exceptions of carvedilol, which was obtained in Pharmaceutical Research Institute (Warsaw, Poland) and benzoin, which was obtained from POCH (Gliwice, Poland). HPLC grade hexane, propan-2-ol, methanol and acetic acid, ammonium acetate were purchased from J.T. Baker (Deventer, Holland).

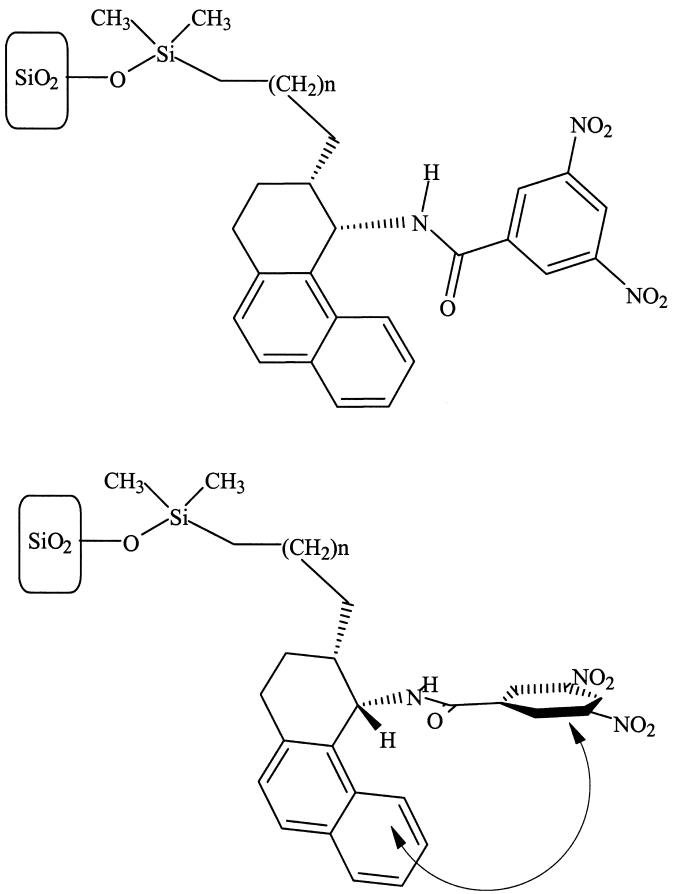


Figure 2. The chemical structure of Whelk-O1 chiral stationary phase.

Dichloromethane for HPLC was obtained from Lab-Scan (Dublin, Ireland). Ethanol was purchased from POCH (Gliwice, Poland).

Chromatographic Conditions

Mobile Phase

A: Dichloromethane-Ethanol (4:1) + 2.68g/L Ammonium Acetate
B: Dichloromethane-Ethanol (9:1) + 1 g/L Ammonium Acetate

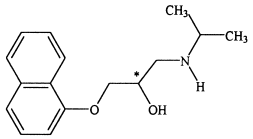
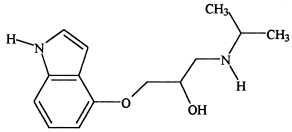
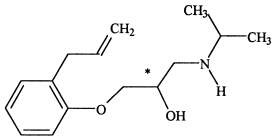
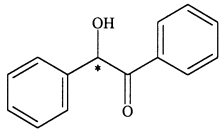
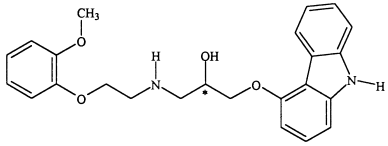
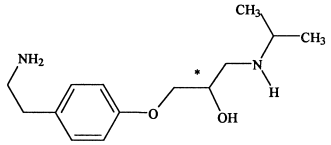
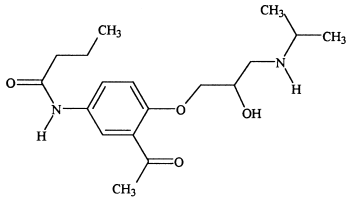
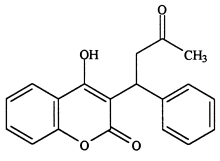
C: Hexane-Propan-2-ol (98:2).
D: Dichloromethane-Methanol (95:5) + 1g/L Ammonium Acetate
E: Hexane-Propan-2-ol-Acetic Acid (90:10:0.1)
The temperature of analyses was 21°C with detection at 254 nm.

RESULTS AND DISCUSSION

We tested the chromatographic behaviour on the new Pirkle-1J column for 31 different drugs, such as acebutolol, alprenolol, aminoglutethimide, atenolol, astif,(9) baclofen, bupivacaine, carvedilol, felodipine, flurbiprofen, ibuprofen, indobufen, ketamine, ketoprofen, mexiletine, mianserin, miconazole, nefopam, nifedipine, nitrendipine, pindolol, phenobarbital, propafenone, propranolol, 1-[4-(pyrimidin-2-yl)piperazin-1-yl]-2-*n*-pentyl-1,3-butanedione, (10) sulpiride, terazosin, terfenadine, verapamil, warfarin. The above drugs consist of therapeutically different (β- and α-blockers, NSAIDs, psychotropics, anesthetics, calcium antagonists, antifungals, analgesics, antihistaminics, anticoagulants) and structurally heterogeneous compounds. Out of those drugs, it was possible to resolve 8 drugs, namely acebutolol, alprenolol, atenolol, benzoin, carvedilol, pindolol, propranolol, and warfarin (Table 1), mostly β-blocking agents. The best selectivity was obtained for propranolol ($\alpha = 2.03$) and the worse for acebutolol ($\alpha = 1.05$). The highest selectivities were obtained for compounds possessing one mono-substituted aromatic centre connected to the chiral carbon atom (propranolol and pindolol). Among the three compounds resolved with the same mobile phase (acebutolol, atenolol, and carvedilol) the highest capacity factors– reflecting the strength of the solute-stationary phase complexes - were obtained for both enantiomers of atenolol and the lowest for the enantiomers of carvedilol. However, for carvedilol the best enantioselectivity was obtained.

Molecular modelling of the stationary phase chiral selector (N-(3,5-dinitrobenzoyl)-3-amino-4-phenylazetidin-2-one, spacer, and stationary phase are mimicked by neoheptyl substitutent, (semiempirical AM1 method implemented in Hyperchem 5.1 program) which showed that, between the two aromatic rings from the sterically less hindered side, an angle of ca. 120° (Fig. 3) is formed. Therefore, it seems that the both aromatic rings may participate in an analyte binding, although there is rather no formal cleft (like in the case of Whelk-O1 column) on that side. The cleft between the two aromatic rings is formed on the side directed towards the azetidinone ring but it, however, looks more hindered than the opposite side. Because of the nature of analytes and the chiral selector π-π, dipole-dipole and steric interactions should be involved in the solute-stationary phase complex stabilisation, as well as in the enantiodifferentiation. Beside, both the stationary phase bound chiral selector and the resolved analytes can

Table 1. Chemical Structures and Chromatographic Parameters for the Compounds Resolved on a Pirkle 1J Column

Compound	Mobile Phase	Flow [mL/min]	Chemical Structure	RS	k'1	k'2	α
Propranolol*	A	1		3.68	0.94	1.91	2.03
Pindolol*	A	1		4.40	2.06	3.64	1.77
Alprenolol*	B	1		2.75	1.74	2.20	1.26
Benzoin*	C	1.5		1.50	8.44	9.71	1.15
Carvedilol	D	1		1.18	5.12	6.26	1.22
Atenolol	D	2		0.79	15.96	17.18	1.08
Acebutolol	D	1		0.98	9.94	10.47	1.05
Warfarin	E	2		1.50	19.20	21.80	1.13

*Compounds included in Regis applications.

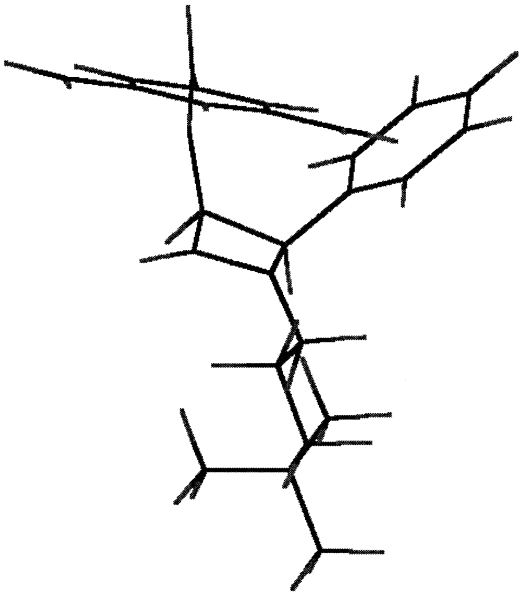


Figure 3. Molecular modeling of Pirkle 1-J chiral stationary phase using semi-empirical AM1 method.

serve as hydrogen bond donors, as well as hydrogen bond acceptors. Therefore, there should be possible several different arrangements of analytes and chiral selector that result in various possibilities for interactions.

The obtained results show that the Pirkle-1J column could be specially recommended for the separation of β-blocking agents.

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