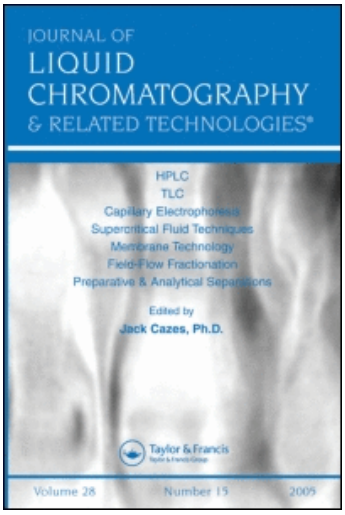

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Hassan Y. Aboul-Enein[a]; Suhair Abu-Zaid[a]
[a] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC 03), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

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Hassan Y. Aboul-Enein* and Suhair Abu-Zaid

Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC 03), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh-11211, Saudi Arabia

ABSTRACT

A comparison of the enantiomeric resolution of several tetralone derivatives, which are 17α-hydroxylase/17,20-lyase (P450 17α) inhibitors has been achieved on different polysaccharides based chiral stationary phases, namely, Chiralcel OB, Chiralcel OD, Chiralpak AD, Chiralpak AS, and Chiralcel OF. The mobile phase used was hexane : ethanol : methanol (90 : 7 : 3 v/v/v) containing 0.5% triethylamine. Most of the compounds were completely separated on the polysaccharide based chiral stationary phases used in this study, with the exception of Chiralcel CA.

*Corresponding author. E-mail: enein@kfshrc.edu.sa

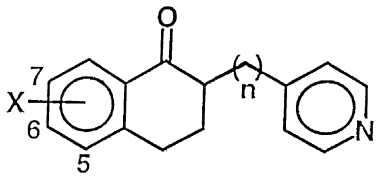
The chromatographic parameters: the capacity factor (k), separation factor (α), and resolution factor (Rs) were calculated. The chiral recognition mechanisms between these analytes and chiral selectors are discussed.

INTRODUCTION

P450 17-α-Hydroxylase is the key enzyme of androgen biosynthesis in human (1,2) which is located in the gonads. This enzyme depends on monooxygenase 17-α-hydroxylase/17,20-lyase (3). The lyase activity of human CYP 17 (17-alpha-hydroxylase-17,20-lyase also P-450 C 17 or P-450 17 alpha) is greatly dependent on the presence of cytochrome b5, and this effect has an important regulatory role in the biochemical processes (4).

Many theories reported that inhibitors of P450 17-α-hydroxylase are of increasing interest for the treatment of prostatic carcinoma, because most of these malignancies are androgen dependent (2,3,5) and most of these inhibitor compounds showed moderate to excellent activity against one enzyme (2).

Hartmann et al. (1) had synthesized several racemic tetralone derivatives, Figure 1, which showed inhibitory activity towards P450 17-α-hydroxylase enzyme. It was of interest, therefore, to determine the pharmacological activities of the individual enantiomers of these derivatives.



Compound	X	n
1	H	1
2	5-CH3	2
3	6-OCH3	1
4	5-OH	1
5	7-OH	1

Figure 1. The chemical structure of substituted 2-(4-Pyridylalkyl)-1-tetralone derivatives used in this study.

This paper describes the enantiomeric separation of these racemic tetralone analogs on several polysaccharides stationary phases (CSPs), namely, Chiralcel OB, Chiralcel OD, Chiralcel CA, Chiralcel OJ, Chiralcel OF, Chiralcel OK, and Chiralpak AS, under normal phase mode.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Waters 501 solvent delivery pump, Waters-Millipore automated Gradient Controller, a Rheodyne model 7125 injector, and a Lambda max model 481 LC spectrophotometer UV detector, operated at 254 nm. The normal phase chiral stationary phases used were: Chiralcel OB [cellulose tribenzoate], Chiralcel OJ [cellulose *tris* (4-methyl benzoate)], Chiralcel OD [cellulose *tris* (3,5-dimethyl phenyl carbamate)], Chiralcel OF [cellulose *tris* (4-chlorophenyl carbamate)], Chiralpak AD [Amylose *tris* (3,5-dimethylphenyl carbamate)], Chiralpak AS [Amylose *tris* (S-α-methylphenyl carbamate)], and Chiralcel OK [cellulose *tris* cinnamate]. All the columns used in this study were 25 cm × 0.46 cm, i.d. and particle size 10 μm and were obtained from Daicel Chemical Industry, Tokyo, Japan. The structures of these phases are shown in Figure 2. The mobile phase used was hexane : ethanol : methanol (90 : 7 : 3 v/v/v).

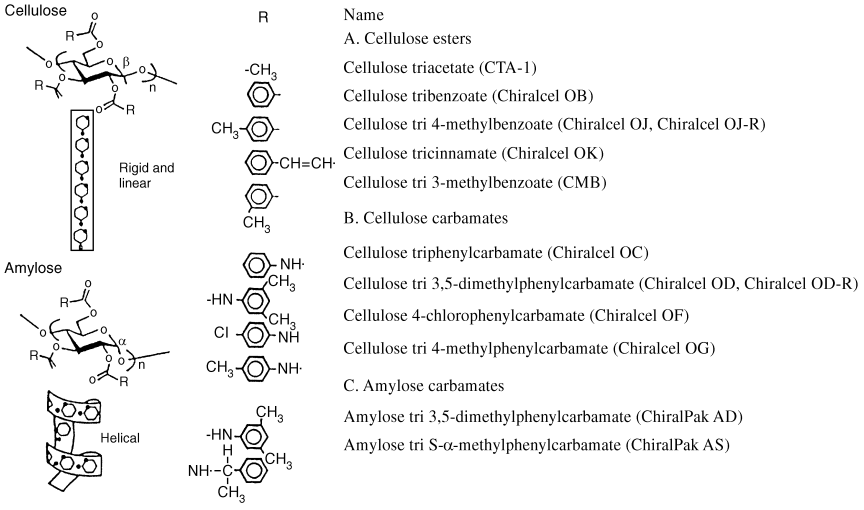


Figure 2. Structure of polysaccharide chiral stationary phases.

Triethylamine was used as a mobile phase additive in concentration not exceeding 0.1%. The mobile phase was filtered and degassed before use. The flow rate was 0.7 mL/min. The chart speed was kept constant at 0.3 cm/minute, and all the experiments were performed at room temperature. The chromatographic parameters namely capacity factor (k), separation factor (α) and resolution factor (Rs) were calculated.

Chemicals

Hexane, methanol, and triethylamine of HPLC grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Ethanol was obtained from E. Merck (Darmstadt, Germany). 2-(4-pyridylalkyl)-1-tetralone derivatives were provided as a gift from Prof. Dr. R.W. Hartmann (University of Saarlandes, Saarbrücken, Germany). All compounds were dissolved in pure ethanol in concentration of 0.1 mg/mL.

RESULTS AND DISCUSSION

An extensive experiment was carried out to optimize the chromatographic conditions, which are reported. The chromatographic parameters, i.e., capacity, separation, and resolution factors of the resolved enantiomers are shown in Table 1.

Table 1 shows that the Chiralpak AS column did achieve complete base line separation, Figure 3, for the tetralone derivatives under study with separation factor (α) ranging from 1.1 to 1.3 and resolution factor (Rs) from 2.28 to 10.23. All other cellulose and amylose chiral stationary phases used in this study did not achieve baseline resolution, with the exception being compound 3 on Chiralcel OB, OJ, OD, and Chiralpak AD. Compound 4 did resolve on Chiralcel OB, while compound 5 was separated on Chiralpak AD. Accordingly, the best resolution for these tetralone derivatives was on the amylose-*tris*-(S-alpha-methylphenyl-carbamate) chiral stationary phase.

The chiral recognition mechanisms on the polysaccharides chiral stationary phase are still not precisely known. However, it has been reported that π–π interaction and hydrogen bonds are playing an important role. Steric effect is also crucial for the chiral discrimination on these phases (5,6). It is clear from the results, that the amylose phases resolved these tetralone derivatives more efficiently than the cellulose phases. This could be due to the following reasons; the amylose phases are more helical than the cellulose phases, which

Table 1. The Chromatographic Parameters [Capacity Factor (k), Separation Factor (α) and Resolution Factor (Rs)] of the Tested Compounds

Cpd/CSP	k_1	k_2	α	Rs
*Chiralcel OB				
1	3.16	3.6	1.13	0.9
2	5	5.8	1.16	0.5
3	6.7	9	1.3	8.84
4	5	7	1.4	7.72
5	NS	NS	NS	NS
*Chiralcel OJ				
1	3.6	3.8	1.05	0.3
2	NS	NS	NS	NS
3	7.5	8.9	1.18	8.2
4	NS	NS	NS	NS
5	NS	NS	NS	NS
**Chiralcel OD				
1	NS	NS	NS	NS
2	NS	NS	NS	NS
3	5.4	5.43	1	8.2
4	7.9	10.4	1.3	5
5	7.9	9.1	1.15	0.5
**Chiralpak AD				
1	6.4	6.88	1.07	0.84
2	NS	NS	NS	NS
3	6	7.5	1.25	4
4	NS	NS	NS	NS
5	12	14.2	1.18	7.4
**Chiralpak AS				
1	2.25	2.7	1.2	3.65
2	3	3.31	1.1	2.28
3	4.8	6	1.25	6.45
4	3.8	5	1.3	10.23
5	4.75	5.87	1.23	7.02
**Chiralcel OF				
1	8.46	9.9	1.17	0.83
2	16.20	18.8	1.16	0.83
3	18	22	1.2	0.9
4	14.3	15.3	1	0.4
5	NS	NS	NS	NS

(*continued*)

Table 1. Continued

Cpd/CSP	k_1	k_2	α	Rs
*Chiralcel OK				
1	7.43	8.6	1.15	0.97
2	12.2	13.9	1.2	0.88
3	17.7	19	1.07	0.63
4	NS	NS	NS	NS
5	14.7	15.5	1.05	0.3

*Mobile phase used was Hexane : Ethanol : Methanol 90 : 7 : 3 v/v/v.
**Mobile phase used was Hexane : Ethanol : Methanol : Triethylamine 90 : 7 : 3 : 0.1 v/v/v/v.

provide better stereogenic binding with the analytes than the cellulose which is rigid and linear. Furthermore, the interaction between the –NH– group of the carbamate (in the amylose structure) and the ketonic group of the analyte molecule, and the π–π interaction between the aromatic moiety of the analytes and the phenyl carbamate derivatives of polysaccharides, induce efficient chiral discrimination.

In both types of the polysaccharide chiral stationary phases, the racemic analytes have to enter the groove and transiently bind with chiral stationary phases through the various interaction forces. The lack of the stereogenic fit in the groove of the CSP and, consequently, binding of the analyte with the chiral selector results in no enantioseparation, as has been shown with some tetralone derivatives in Table 1.

Partial resolutions occur if only a deficient interaction takes place due to incomplete fitting of the analytes in the polysaccharide grooves. The substituted phenyl moiety in the carbamate residue has an important role in the enantiomeric separation. For example the presence of electron withdrawing atoms, like chlorine atom in para- position of the phenyl moiety (Chiralcel OF), resulted in no enantiomeric separation for all the tetralone compounds studied.

In conclusion, in the case of derivatized cellulose and amylose carbamate chiral stationary phases, the separation and resolution occurred due to increase in the H−bonding between the solute and the −NH− group present in the carbamate moiety.

Furthermore, the helical conformation of the amylose did play an essential role in achieving the resolution of these racemic tetralone analogs beside the steric effect, which plays a crucial role for chiral resolution. Amylose-*tris*-(S-α-methylphenyl carbamate), known as Chiralpak AS, was the most efficient CSP in resolving these racemic tetralone analogs, which can be applied on a semi-

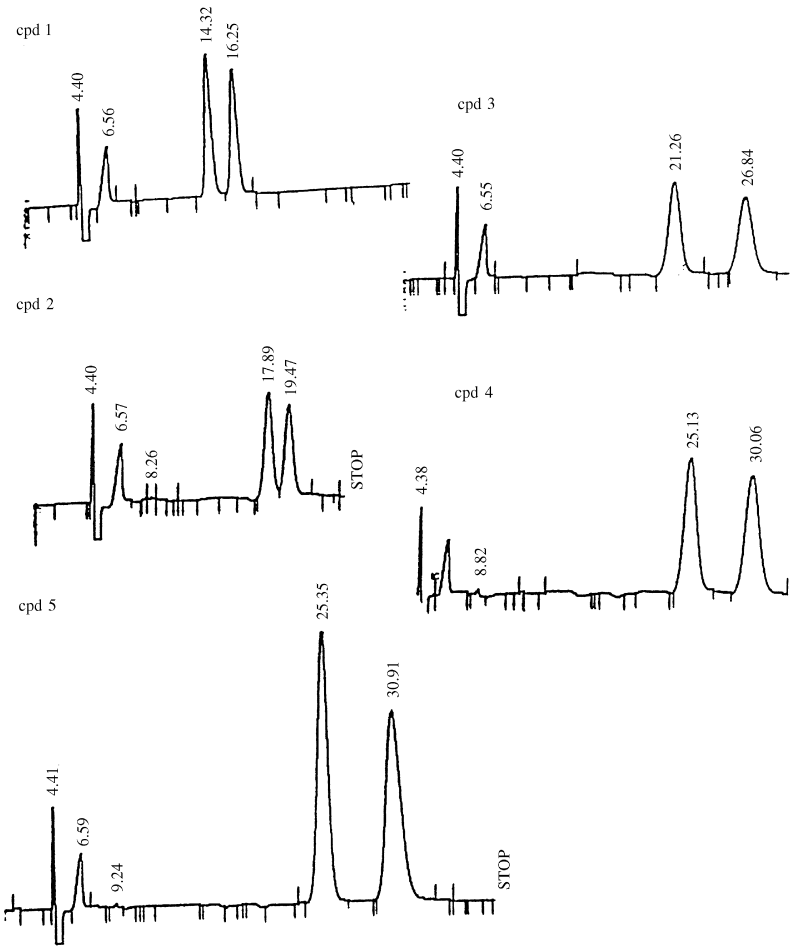


Figure 3. Typical chromatograms of the studied compounds on Chiralpak AS. See experimental section for details.

preparative scale for further pharmacological investigations of the individual enantiomers of these agents.

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