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Chromatographic Separation of Enantiomers Acids Using Quinine as Chiral Counter-Ion in Mobile Phase

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In present studies, the chromatographic behaviour of quinine as chiral counter ion and the separation of 10-camphor sulfonic acid and mandelic acid, using porous graphitic carbon Hypercarb, as stationary phase is described. The 10-camphorsulfonic acid and mandelic acid enantiomers were separated in mobile phase system consisting of dichloromethane-cyclohexane (50:50 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH, λ = 257 nm for mandelic acid, λ = 337 nm for 10-camphorsulfonic acid and 5 µL was injected into the column.

Key Words: 10-Camphorsulfonic acid, Quinine, Mendelic acid, Chiral ion-pair, Porous graphitic carbon, HPLC.

INTRODUCTION

In our previous work[1], the chromatographic separation of quinine and quinidine has been examined. The enantiomers or isomers may exhibit quite distinct pharmacological activities, as in the case of ketamine where the S-enantiomers is largely responsable for the anaesthetics effects and the R-enantiomers induces unwanted psychotics effect[2].

The difference in pharmacological effect of isomers can also be illustrated by quinine and quinidine, the major cinchona alkaloids four chiral carbon atoms (quinine: 3R, 4S, 8S, 9R quinidine: 3R, 4S, 8R, 9S) configurations are used as antimalarial and antiarrythmic agents respectively.

As alternative procedures to indirect methods, methods for the direct resolution of enantiomers without prior derivatization have become very popular. In HPLC where most of the interest lies these methods involve the use of either achiral selector chemically bonded to the stationary phase or is added to the mobile phase[3-9].

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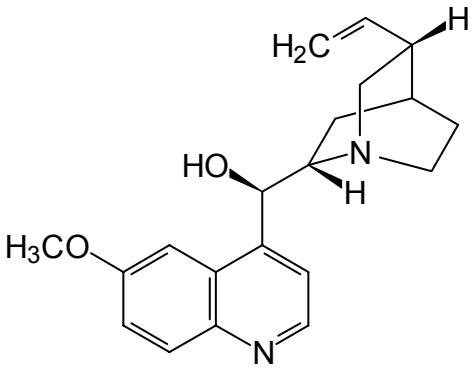
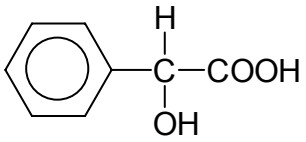
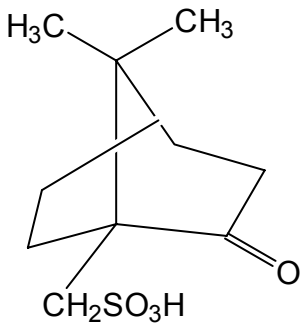


Structure of quinine (1)



Structure of mandelic acid (2)



Structure of camphorsulfonic acid (3)

Fig. 1

In both cases, there is a formation of reversible diastereomeric complexes with the solute enantiomers.

The separation resulted from the differences in stability between the complexes and therefore leads to a difference in retention time.

In addition to these optically active counter-ion dissolved in mobile phase can be used to separate enantiomers of acids and amines. The basis for resolution is the formation of diastereoisomeric complexes (ion-pairs) with different stabilities or distribution properties between the mobile phase and stationary phase. The technique has successfully been applied to the separation of enantiomers β-amino alcohols[10] with 10-camphorsulfonic acid and N-benzoxycarbonyl-glycyl-L-proline (ZGP)[11] as the chiral counter ion cinchona alkaloids, which are alcohols, quinine and quinidine are also effective counter-ion for resolution of enantiomers with carboxylic and sulfonic acids groups and with hydrogen-bonding function on convention achiral supports[12].

These enantiomers have polar function that give a hydrogen bonding property to the hydroxy group in amino alcohols. Simultaneous ion pair formation and hydrogen bonding effect beteween the ions are possible and bulky and rigid groups in molecules may give steric interactions.

The stereoselectivity decreases if the amino alcohols contain bulky substituents in the vicinity of the amine and hydroxyl groups. One disadvandage of this procedure is that the chiral counter ions are generally used with organic mobile phases of low polarity to reduce the possibility of the hydrogen bonding interaction sites being taken up by the mobile phase (*e.g.*, dichloromethane). This is the case in the resolution (RS = 2.5) of metoprolol enantiomers with ZGP added as counter ion to the mobile phase and on organic based mobile phase as carried out by Pettersson[13]. Nevertheless, it has been shown that trace levels of water are beneficial in the separations with ZGP. It has also been concluded that the enantioselectivity is achieved only when there are two carbon chains between the amino group and hydroxyl group in the amino alcohols examined. This article concerns the separation of 10-camphorsulfonic acid and mandelic acid using quinine a chiral ion-pair in mobile phase.

EXPERIMENTAL

Dichloromethane anhydride, glacial acetic acid 99.9 %, (Carlo Erba Reactifs SDS, France), *n*-Hexane 99 % HPLC, methanol 99.9 %, acetonitrile 99.9 %, chromasolv, anhydrous cyclohexane 99.7 %, anhydrous quinine 5 g 98.0 % (Fluka, Germany), D(-)-mandelic acid 99 % (Fluka, Japan), DL-(±)-mandelic acid 99 % (Fluka, Switzerland), (1S)-(+)-10-camphorsulfonic acid 99 % (Adrich, Germany), (±)-10-camphorsulfonic acid 98.0 % (France).

The chromatographic system consisted of an HPLC Merck Hitachi, a variale UV-detector L 4200 UV-Vis, Merck, an L 6200 A Intelligent Pump Merck, the column (100 × 4.6 mm i.d 5 μm packed with porous graphitic carbon, $\lambda = 257$ nm for mandelic acid, $\lambda = 337$ nm for 10-camphorsulfonic acid.

Preparation of mobile phase: A quantity of 0.00973 g was dissolved in 300 mL of mobile phase (dichloromethane-cyclohexane (50:50 % v/v) and 24 μL of glacial acitic acid was added to the final solution of the mobile phase.

RESULTS AND DISCUSSION

Attempts to obtain separation of 10-camphorsulfonic acid and mandelic acid enantiomers were conducted on porous graphitic carbon Hypercarb (100 × 4.6 mm i.d), 5 μm using different systems of mobile phase (Tables 1 and 2).

TABLE-1
SEPARATION OF 10-CAMPHORSULFONIC ACID (CSA), λ = 337 nm

Mobile phase	Retention time (S- CSA)	Retention time (S,R-CSA)
Dichloromethane-cyclohexane (50:50 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH	2.71	2.60-2.80
Dichloromethane-cyclohexane (40:60 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH	2.60	4.63-5.44
Dichloromethane-cyclohexane (40:60 % v/v) + 0.20 mM quinine	4.03	3.62-4.09
Dichloromethane-cyclohexane (50:50 % v/v) + 0.05 mM quinine	–	–
Dichloromethane-cyclohexane (50:50 % v/v)	–	–

TABLE-2
SEPARATION OF MANDELIC ACID (MD), λ = 257 nm

Mobile phase	Retention time (D-MD)	Retention time (D,L-MD)
Dichloromethane-cyclohexane (50:50 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH	5.19	5.56-6.08
Dichloromethane-cyclohexane (40:60 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH	3.89	4.49-5.25
Methanol 100 % 0.10 mM quinine + 0.008 % CH_3COOH	5.16	4.70-5.10
Acetonitrile 100 % 0.10 mM quinine + 0.008 % CH_3COOH	–	–
Methanol:acetonitrile (80-20 % v/v) 0.10 mM quinine + 0.008 % CH_3COOH	4.26	3.76-4.07
Methanol:acetonitrile (10-90 % v/v) 0.10 mM quinine + 0.008 % CH_3COOH	–	–

Two variables were investigated for their nature and concentration of the organic modifier and concentration of the counter-ion quinine. This led to the following optimum mobile phase composition. Fig. 2 shows the enantiomeric separation of 10-camphorsulfonic acid (CSA) using mobile phase

composition dichloromethne-cyclohexane (50:50 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH and a separation was achieved.

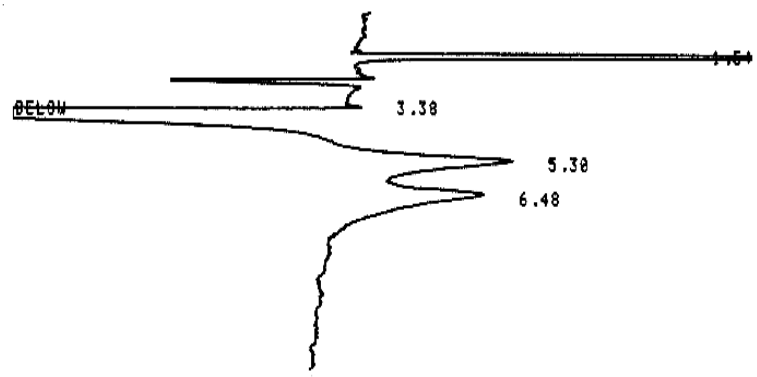


Fig. 2. (RS) CSA; λ = 337 nm, FR = 0.5 mL/min, sample inject 5 µL
column: porous graphite carbon Hypercarb (100 × 4.6 mm, id 5µm)
mobile phase: dichloromethne-cyclohexane (50:50 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH

Influence of dicloromethane on the enantiomeric retention time: The retention of injected solutes decreases with increasing concentration of dichloromethane Table-1 (system 1 and system 2) and also with increasing quinine concentration (counter ion). This may be due to the interaction of the counter ion with the enantiomers to give the corresponding diastereomeric ion-pair, distributed between the organic mobile phase and the hydrophobic stationary phase. The same conclusion can be made for mendelic acid enantiomers.

Previous studies on the separation of enantiomeric of amino alcohols with (+)-10-camphorsufonic acid as counter ion have indicated that a simultaneous electrostatic interaction and hydrogen bonding between the ions is vital for the separation.

The enantiomers of 10-camphorsulfonic acid are separated with some overlap and the retention order has been established and (-)-10-camphorsulfonic acid is being eluted first enantiomeric separation mandelic acid has been acheived (Figs. 3 and 4).

Effect of counter ion (quinine) concentration on retention time: From the Tables 1 and 2, it is concluded that increasing concentration of quinine led to decrease retention time.

Conclusion

Chiral ion-pair chromatography on porous graphitic carbon was used to separate enantiomeric acids 10-camphorsulfonic acid and mandelic acid

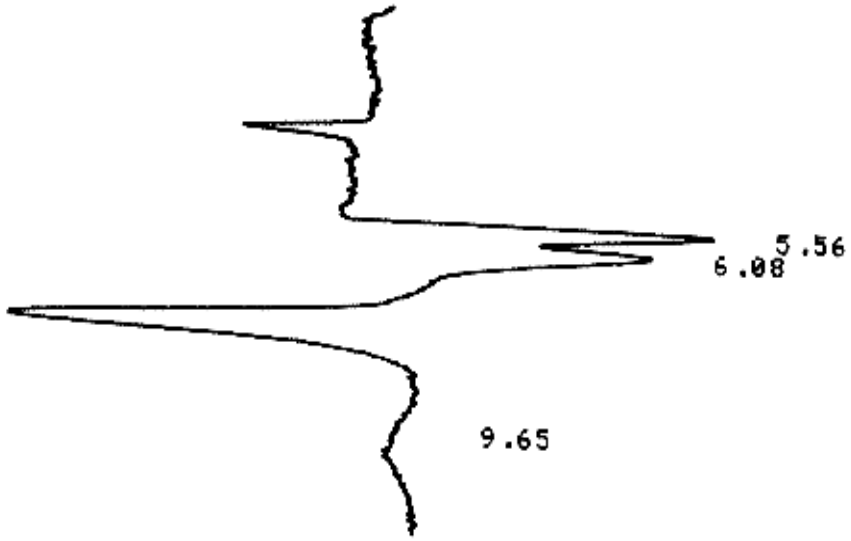


Fig. 3. DL-MD; λ = 257 nm, FR = 1 mL/min, sample inject 5 µL
column: porous graphite carbon Hypercarb (100 × 4.6 mm, id 5µm)
mobile phase: dichloromethne-cyclohexane (50:50 % v/v) + 0.10 mM quinine + 0.008 % CH_3COOH

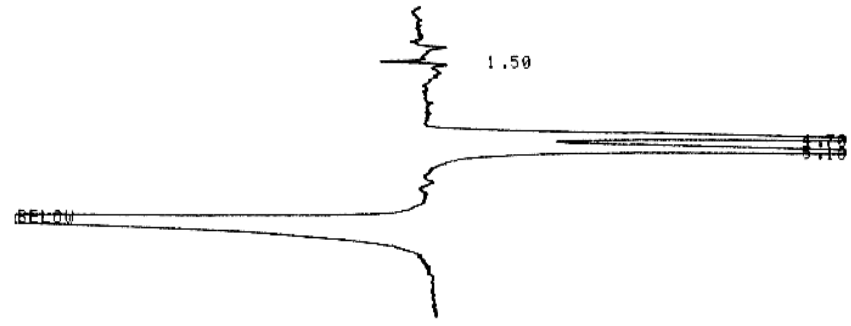


Fig. 4. DL-MD; λ = 257 nm, FR = 1 mL/min, sample inject 5 µL
column: porous graphite carbon Hypercarb (100 × 4.6 mm, id 5 µm)
mobile phase: methanol 100 % + 0.10 mM quinine + 0.008 % CH_3COOH

using quinine as chiral counter-ion. The quantitative analysis for the enantiomers was not possible, because the overlap of the peaks and further study is still required.

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