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Effect of Different Acids Addition on Chiral Separation of Phthalylvaline by Quinine Carbamate Based Chiral Stationary

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The quinine carbamate type chiral stationary phase used for direct enantiomer separation of amino acid was studied. The influence of mobile phase composition, methanol and different acids were systematically investigated to gain an insight into the overall chiral recognition mechanism.

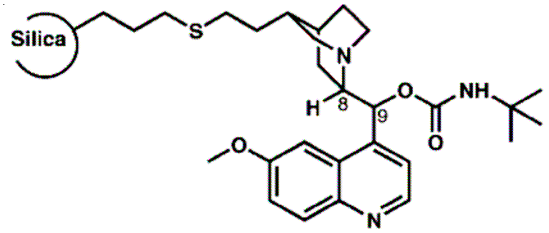
Key Words: Enantiomer separation, Chiral stationary phase, LC mobile phase composition, Phthalylvaline.

INTRODUCTION

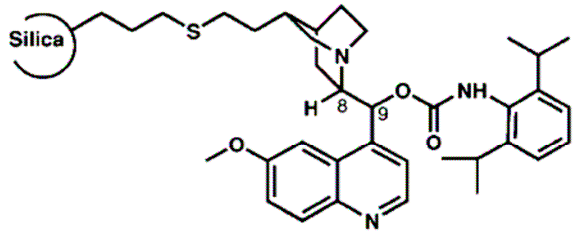
The difference in pharmacological effect of isomers can be illustrated by quinine and quinidine, the major cinchona alkaloids four chiral carbon atoms (quinine: 3R, 4S, 8S, 9R quinidine: 3R, 4S, 8R, 9S) configurations are used as antimalarial and antiarrythmic agents respectively.

Chiral stationary phases (CSPs) with quinine (QN) carbamate derivatives (Fig. 1), depicting *tert*-butyl carbamoylated quinine as chiral template and named CSP II in this contribution, have proved to successfully facilitate the direct high-performance liquid chromatographic enantioseparation of chiral acids (selectands, SAs).

Advantageously, these chiral stationary phases are operated with buffered hydro-organic mobile phases in the anion-exchange mode where the tertiary amine moiety in the quinuclidine ring is positively charged. As shown in earlier publications, these chiral stationary phases exhibit high enantioselectivity for the resolution of a broad range of chiral acidic selectands, such Accordas, *e.g.*, N-derivatized amino acids[1-8]. These chiral stationary phases can be classified as weak chiral anion exchangers. These intermolecular electrostatic interactions are accompanied by additional attractive and/or repulsive forces, such as hydrogen bonding, *p-p* interactions, dipole-dipole, van der Waals and steric interactions, resulting in enantioseparation of different magnitude for racemic anionic selectands[9-13].



CSP 1: ProntoSIL Chiral AX QN-1 (8S,9R)
CSP 1: ProntoSIL Chiral AX QD-1 (8R,9S)



CSP 3: ProntoSIL Chiral AX QN-2 (8S,9R)

Fig. 1. Chiral stationary phases (CSPs) based on quinine carbamate. CSP 1 with *tert*-butyl carbamoyl quinine; CSP 2 with *tert*-butyl carbamoyl quinidine; CSP 3 with diisopropyl phenyl carbamoyl quinine

In this work, a chiral stationary phase (CSP) based on *tert*-butyl carbamoyl quinine (tBuCQN) was used to separate the enantiomer of amino acid derivative phthalylvalin and the influence of different acids in mobile phases (acetic acid, propionic acid, butanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid and dodecanoic acid). Overall enantioselectivity was evaluated to gain more of an insight into the chromatographic mechanism.

The work of Lindner *et al.*[6,14-23] have shown the interest that could represent quinine carbamate based stationary phase[9-11]. We are interested in making a grafting of quinine carbamate *in situ* in a column filled with pure silica stationary phase. The grafting method has been developed to be directly adaptable to the grafting of quinine carbamate on monoliths silica-based capillary for chromatography and electrochro-matography.

In this work, a chiral stationary phase (CSP) based on *tert*-butyl carbamoyl quinine (tBuCQN) was used to separate the enantiomer of amino acid derivative phthalylvaline and the influence of different acids in mobile phases. Overall enantioselectivity was evaluated to gain more of an insight into the chromatographic mechanism.

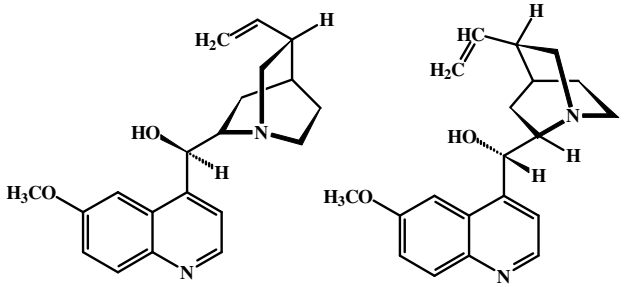


Fig. 2. Structure of quinine and quinidine

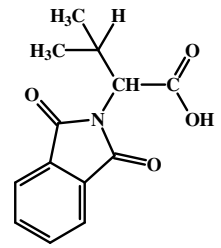


Fig. 3. Structure of phthalylvalin

EXPERIMENTAL

Synthesis of *o*-(*t*-butylcarbamoyl)quinine: The synthesis of carbamate strcture is prepared *via* isocyanate reaction: 3 g of quinine, as free base, were dissolved in dry toluene and 1.2 mL of *t*-butylisocyanate and 1 drop of dibutyl tin dilaurate as catalyst were added. The mixture was refluxed for 4 h, the solvent evaporated and the remaining raw material was washed with *n*-hexane. The white solid was crystallized with cyclohexane resulting *o*-(*t*-butylcarbamoyl) quinine in 80 % yield.

Synthesis of *t*-BuCQN: We conducted a synthesis of *t*-BuCQN according to protocol proposed by Lindner and Lammerhofer[1]. 2 g of quinine carbamate were dissolved in dry toluene, 1.5 mL of 3-triethoxysilyl isocyanate and 1 drop of dibutyl tin dilaurate as catalyst were added. The mixture was refluxed for 4 h. The solvent was evaporated and the remaining raw materiel washed with dry diethyl ether. The white solid (quinine derivative) was crystallized (99 %). The resulting product structure was confirmed by ^{1}H NMR.

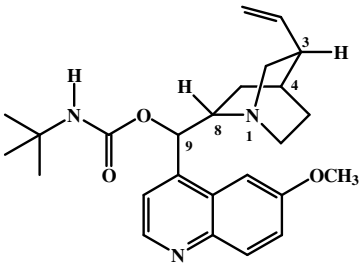


Fig. 4. Structure of *tert*-butyl carbamoylquinine (tBuCQN) (8S, 9R)

Synthesis of chiral stationary phase based on *t*-BuCQN: The following protocol was applied: a column filled with particles of pure silica was dried by circulation of helium. 3 g of 3-mercaptopropyl trimethoxysilane were suspended in chloroform after addition of 3 g of *o*-(*t*-butylcarbamoyl)quinine and 200 mg of radical initiator azo-α,α′-*bis*-isobutyronitrile (AIBN) in 100 mL methanol. The mixture was percolated into the column for 15 h with a flow rate of 1 mL/min. The preparation was ended by washed with different polarities solvents.

The column of pure silica was a column type Lichrospher 60 (250 mm × 6 mm, 12 mm) (VWR, France). The chiral phase obtained (Si-QN) was used to separation of amino acid derivative phthalylvaline with a polar mobile phase a mixture of methanol and acid $C_nH_{(2n+1)}$-COOH, n ranges from 2-18 (Table-1) with flow rate 1 mL/min and detection was carried at 245 nm.

TABLE-1
NAME AND LABEL OF THE ACIDS
USED IN THE MOBILE PHASE

C2	Ethanoic acid	C9	Nonanoic acid
C3	Propanoic acid	C10	Decanoic acid
C4	Butanoic acid	C12	Dodecanoic acid
C5	Pentanoic acid	C14	Tetradecanoic acid
C6	Hexanoic acid	C16	Hexadecanoic acid
C7	Heptanoic acid	C18	Octadecanoic acid
C8	Octanoic acid		

RESULTS AND DISCUSSION

The study of the retention on quinine carbamate stationary phase was much more interesting. The mobile phase consists in mixture of alcohol (methanol) and different acids.

The concentration of acid was systematically modified in order to highlight its influence on the retention temps (tr_1, tr_2), retention factor (k) and the selectivity (α) (Tables 2-4). As expected, the retention times of enantiomers depends on the concentration of acid in the mobile phase. The concentration factor directly influences the retention mechanism involved in electrostatic interactions between the solute and the stationary phase. The selectivity between the enantiomers is influenced both by the nature of the acid and its concentration. The

TABLE-2
RETENTION TIME OF BOTH ENANTIOMERS tr_1 AND tr_2 AND SELECTIVITY (α) FOR SIX DIFFERENT CONCENTRATIONS OF ADDED MODIFIERS IN THE MOBILE PHASE (0.001, 0.03, 0.06, 0.125, 0.25 AND 0.5 %), THE LABEL EXPRESSES THE LENGTH OF THE ALKYL CHAIN

		C2	C3	C5	C6	C7	C8	C9	C12	C14	C16	C18
0.5 %	tr_1	7.310	10.342	9.966	11.285	11.425	11.974	12.217	14.081	8.516	8.586	8.217
	tr_2	8.032	11.640	11.110	12.689	12.873	13.500	13.787	15.885	9.572	9.662	9.167
	α	1.0987	1.1255	1.1147	1.1244	1.1267	1.1274	1.1236	1.1281	1.1240	1.1253	1.1156
0.25 %	tr_1	9.028	12.410	11.396	12.652	12.705	13.017	13.478	13.837	8.480	8.261	8.361
	tr_2	10.059	14.062	12.817	14.290	14.345	14.658	15.234	15.777	9.512	9.263	9.303
	α	1.1142	1.1331	1.1246	1.1.1294	1.1290	1.1260	1.1302	1.1402	1.1216	1.1212	1.1126
0.125 %	tr_1	10.958	13.955	14.198	13.709	13.568	13.944	14.604	10.046	8.974	8.414	8.725
	tr_2	12.317	15.861	16.128	15.467	15.343	15.765	16.580	11.568	10.167	9.450	9.788
	α	1.1240	1.1365	1.1359	1.1282	1.1308	1.1305	1.1353	1.1515	1.1632	1.1231	1.1218
0.06 %	tr_1	12.643	15.007	14.941	14.339	14.041	14.255	14.803	9.719	9.382	8.544	8.802
	tr_2	14.271	17.083	16.981	16.285	15.871	16.085	16.737	11.131	10.772	9.610	9.844
	α	1.1287	1.1383	1.1365	1.1357	1.1303	1.1283	1.1306	1.1452	1.1481	1.1247	1.1183
0.03 %	tr_1	13.676	15.516	16.237	14.494	14.273	14.949	14.565	9.507	8.875	8.614	8.807
	tr_2	15.425	17.708	18.520	16.418	16.123	16.368	16.465	10.831	10.000	9.688	9.845
	α	1.1278	1.1412	1.1406	1.1327	1.1296	1.0949	1.1304	1.1392	1.1267	1.1246	1.1178
0.01 %	tr_1	14.504	16.057	16.098	14.443	15.120	15.583	13.620	9.360	8.970	8.624	8.818
	tr_2	16.398	18.312	18.336	16.352	17.103	17.674	15.270	10.624	10.110	9.82	9.868
	α	1.1305	1.1404	1.1390	1.1321	1.1311	1.1341	1.1211	1.1350	1.1270	1.1386	1.098

TABLE-3
RETENTION TIME OF BOTH ENANTIOMERS tr_1 AND tr_2 AND SELECTIVITY (α) FOR FOUR DIFFERENT CONCENTRATIONS OF BUTANOIC ACID (C4) ADDED IN THE MOBILE PHASE (0.05, 0.1, 0.2 AND 0.4)

C4	0.4 %	0.2 %	0.1 %	0.05 %
tr_1; tr_2	11.520; 12.989	12.995; 14.706	15.713; 17.897	15.596; 17.768
α	1.1275	1.1316	1.1389	1.1392

TABLE-4
RETENTION TIME OF BOTH ENANTIOMERS tr_1 AND tr_2 AND SELECTIVITY (α) FOR FOUR DIFFERENT CONCENTRATIONS OF DECANOIC ACID (C10) ADDED IN THE MOBILE PHASE (0.01, 0.02, 0.05 AND 0.1 %)

C10	0.1 %	0.05 %	0.02 %	0.01 %
tr_1tr_2	14.483-16.390	14.316-16.222	14.220-16.088	13.820-15.574
α	1.1316	1.1331	1.1313	1.1269

influence of nature, the length of the carbon chain and the concentration of acid on the retention factor (k) and the resolution factor (R) measured with the Purnell equation:

$$R = \frac{1}{4}\frac{\alpha - 1}{\alpha}\frac{k}{k+1}\sqrt{N}$$

Disregarding the weak resolution due to the weak plate number obtained with the large diameter of particles, N equals 1650 theoretical plates, separations between enantiomers are quite satisfactory. The performance in terms of resolution and efficiency could be easily increased by reducing of the diameter of particles.

The chiral mechanism of separation was mainly based on specific interaction between the solute and the stationary phase. The retention was directly controlled by mobile phase composition but not the selectivity which results of the two mechanisms, electrostatic interactions and partition mechanism.

The retention factor is influenced by the concentration of acid and length of alkyl chain (very clearly visible with acids C2-C9). The retention is higher when the concentration is low and considering the length of the alkyl chain, the factor is low but increases with C2, C3, C4 and C5 and then decreases with lengths over.

The resolution depends on the concentration and length of the alkyl chain of the acid. It is high with low concentrations of acid. C2 resolution is the lowest. It reaches the highest value for C3, C4 and C5 and subsequently decreases with C6 to C9.

Considering acids with length alkyl chain greater than or equal to C10, the effect of the chain on retention (k) or the resolution (R) is most noticeable.

In summary, the mechanisms put into play are sharing induced the ionic interaction between the solute and acid and by the hydrophobic chain of the acid. Sharing is a mechanism that promotes the separation of enantiomers when the alkyl chain of the acid is short (C3-C5). The ionic interaction is controlled by the ionic strength or concentration of acid in the mobile phase. When the concentration of acid augments the retention and separation diminished.

Conclusion

This work was developed for using the quinine chiral selector grafted in stationary phase in HPLC. *In situ* synthesis of a chiral stationary phase based quinine carbamate according to Lindner was performed. The column obtained was enabled to make a separation of enantiomers of phthalylvaline with a good selectivity. The method proposed of synthesis, preparation and activation of pure silica and conditions of grafting, is quite

satisfactory, taking into consideration the possibility of separation of N protected amino acid and its applications to columns of small diameters or capillaries. The results obtained show that the proposed protocol consisting in the *in situ* grafting of quinine carbamate can be extended to more powerful chromatographic systems using stationary phases such as monolithic structure for capillary chromatography or electrochromatography.

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