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

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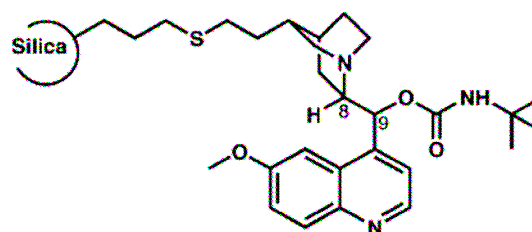
9 The quinine carbamate type chiral stationary phase used for direct enantiomer separation of amino acid was studied. The influence of
10 mobile phase composition, methanol and different acids were systematically investigated to gain an insight into the overall chiral recognition
11 mechanism.

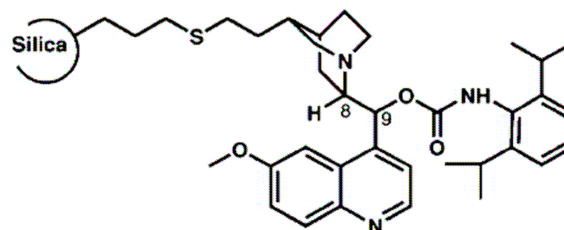
12 **Key Words: Enantiomer separation, Chiral stationary phase, LC mobile phase composition, Phthalylvaline.**

## INTRODUCTION

13 The difference in pharmacological effect of isomers can
14 be illustrated by quinine and quinidine, the major cinchona
15 alkaloids four chiral carbon atoms (quinine: 3R, 4S, 8S, 9R
16 quinidine: 3R, 4S, 8R, 9S) configurations are used as antima-
17 larial and antiarrythmic agents respectively.
18 Chiral stationary phases (CSPs) with quinine (QN) carba-
19 mate derivatives (Fig. 1), depicting *tert*-butyl carbamoylated
20 quinine as chiral template and named CSP II in this contri-
21 bution, have proved to successfully facilitate the direct high-
22 performance liquid chromatographic enantioseparation of
23 chiral acids (selectands, SAs).
24 Advantageously, these chiral stationary phases are
25 operated with buffered hydro-organic mobile phases in the
26 anion-exchange mode where the tertiary amine moiety in the
27 quinuclidine ring is positively charged. As shown in earlier
28 publications, these chiral stationary phases exhibit high
29 enantioselectivity for the resolution of a broad range of chiral
30 acidic selectands, such Accordas, *e.g.*, N-derivatized amino
31 acids[1-8]. These chiral stationary phases can be classified as weak
32 chiral anion exchangers. These intermolecular electrostatic
33 interactions are accompanied by additional attractive and/or
34 repulsive forces, such as hydrogen bonding, *p-p* interactions,
35 dipole-dipole, van der Waals and steric interactions, resulting
36 in enantioseparation of different magnitude for racemic
37 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.

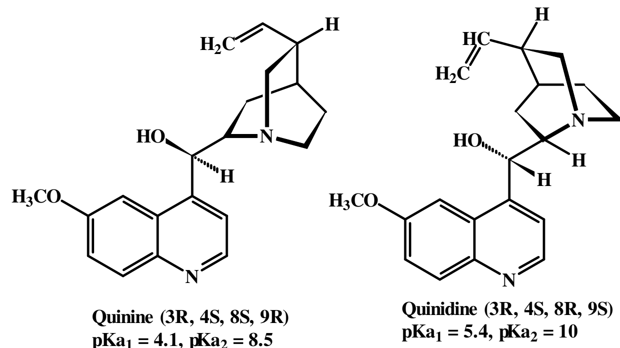



Fig. 2. Structure of quinine and quinidine

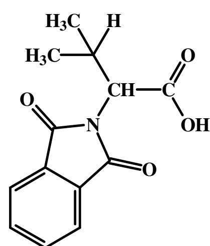


Fig. 3. Structure of phthalylvalin

## 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.

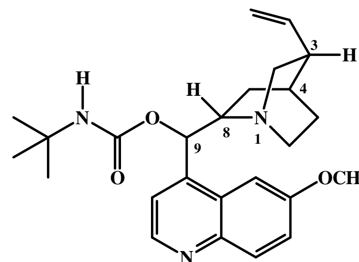


Fig. 4. Structure of *tert*-butyl carbamoylquinine (tBuCQN) (8S, 9R)

**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

148 satisfactory, taking into consideration the possibility of separa-
149 tion of N protected amino acid and its applications to columns
150 of small diameters or capillaries. The results obtained show
151 that the proposed protocol consisting in the *in situ* grafting of
152 quinine carbamate can be extended to more powerful chroma-
153 tographic systems using stationary phases such as monolithic
154 structure for capillary chromatography or electrochroma-
155 tography.
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