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Enantioseparation of Amino Acid Derivative on Quinine Carbamate Based Chiral Stationary Phase

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A *tert*-butylcarbamoyl quinine based chiral stationary phase 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: *tert*-Butylcarbamoyl selector, Chiral stationary phase, Amino acid derivative.

INTRODUCTION

Enantioseparation using chiral stationary phases (CSPs) in high performance liquid chromatography is the subject of continuing interest.

Cinchona alkaloid carbamate type chiral selectors and there of derived chiral stationary phases have been previously developed for HPLC enantiomer separation of chiral, utilizing anionic exchange properties[1]. These chiral stationary phases (Fig. 1) offer several sites for stereoselective intermolecular interactions in the course of complex formation, *i.e.* the aliphatic basic nitrogen of protonated quinuclidine group for ion-pairing, the planar π-basic quinoline skeleton for π-π interactions, the hydrogen acceptor-donor site of carbamate functionality for dipole-dipole interaction, the hydrogen-bond formation and the hydrophobic as well as steric interactions with the substituents of carbamate group or the large quinuclidine ring.

These chiral selectors provide excellent chiral discrimination capability for a broad spectrum of chiral acids, as it has been reported earlier[2-8]. It can be used in an indirect way[9], as mobile phase component or in a direct way, grafted onto the stationary phase.

The work of lindner[1,10-19] has shown the interest that could represent quinine carbamate based stationary phase[20-23], 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 electrochromatography.

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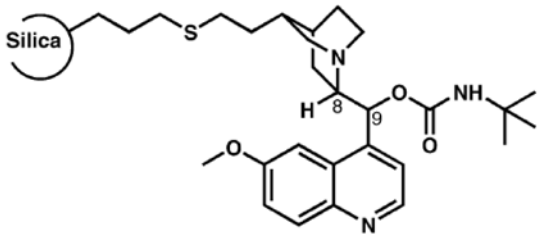
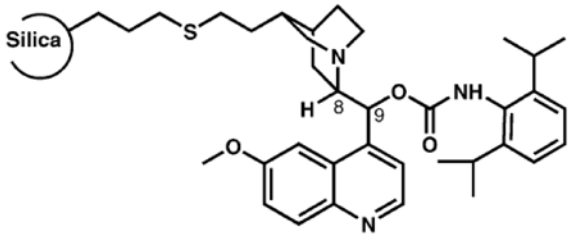


CSP 1: ProntoSIL Chiral AX QN-1 (8S,9R)
CSP 2: ProntoSIL Chiral AX QD-1 (8R,9S)



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 phthalylvaline and the influence of different acids in mobile phases. Overall enantioselectivity was evaluated to gain more of an insight into the chromatographic mechanism.

EXPERIMENTAL

The mobile phases were prepared with water for HPLC (VWR, France), acetic acid 99.9 % or propanoic acid 99.9 % or formic acid 99.9 % (Carlo Erba, France), sodium acetate or ammonium acetate (Fluka Germany) and methanol for HPLC (Fluka, Germany). The solvents used for the synthesis toluene, *n*-hexane and cyclohexane were obtained from Carlo Erba, (France). Quinine (98 %) was obtained from Sigma-Aldrich (France).

HPLC experiments were performed with a Hitachi-Merck HPLC system which consisted of L-6200 intelligent pump, L-4200 UV-Vis detector, D-2500 Integrator from WVR (Germany).

Synthesis of O-(*t*-butylcarbamoyl)quinine: The synthesis of carbamate structure is prepared *via* isocyanate reaction: 3 g of quinine as free base, was 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. m.p.: 162-164 °C; $[\alpha]^{23}_{Na589}$ = +62.7°, $[\alpha]^{23}_{Hg546}$ = + 74.5° (c = 1.00; MeOH); IR (KBr): 3200, 1700, 1620, 1580, 1500 cm^{-1}; ^{1}H NMR (TMS, ^{d}MeOD): 8.67 (d, 1H), 7.98 (d, 1H), 7.53 (m, 2H), 7.45 (dd, 1H), 6.50 (d, 1H), 5.82 (m, 1H), 5.20 (d, IH), 5.07 (d, 1H), 4.00 (s, 3H), 3.70 (d, 2H), 3.27 (m, 1H), 3.08 (m, 2H), 2.73 (m, 1H), 2.37 (m, 1H), 2.00-1.40 (m, 6H), 1.28 (m, 2H), 0.85 (t, 3H) ppm.

Synthesis of *t*-BuCQN: Synthesis of tBuCQN were performed according to protocol proposed by Lindner *et al.*[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. m.p. 86 °C: $[\alpha]^{23}_{Na589}$ = +37.5°, $[\alpha]^{23}_{Hg546}$ = 41.4 °(c = 2.04, MeOH); IR (Na-Cl-window): 3340, 2973, 2934, 2880, 1715, 1622, 1593, 1511, 1475, 1435, 1244, 1103, 1079 cm^{-1}. ^{1} H NMR (TMS, ^{d}MeOD): 8.68 (d, IH), 7.97 (d, IH), 7.54 (m, 2H), 7.43 (dd, IH), 6.58 (d, IH), 6.20 (m, 1H), 5.16 (d, IH), 5.09 (s, 1H), 4.00 (s, 3H), 3.77 (q, 6H), 3.40-2.60 (m, 5H), 2.32 (m, IH), 2.10 (m, IH), 1.80-0.80 (m, 17H), 0.58 (m, 2H) ppm.

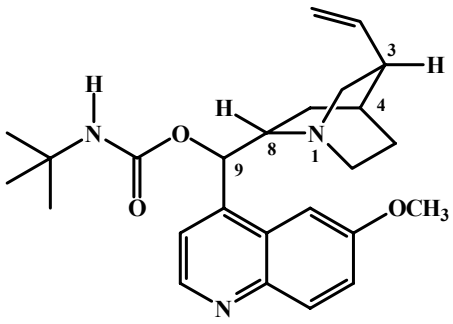


Fig. 2. Structure of *tert*-butyl carbamoylquinine (tBuCQN) (8S, 9R)

Synthesis of chiral stationary phase based on tBuCQN: The following protocol[1] 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 phtalylvaline with a polar mobile phase a mixture of methanol and acetic acid with flow rate 1mL/min and detection was carried at 245 nm.

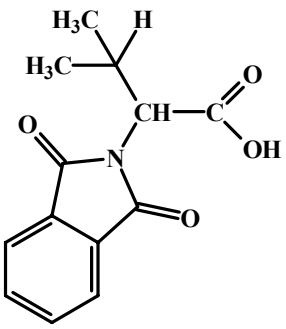


Fig. 3. Structure of phthalylvaline

RESULTS AND DISCUSSION

First series of experiments was performed using the chiral selector, quinine carbamate into the mobile phase. Several stationary phases were investigated such as diol, phenyl and amine silica based. In the indirect mode, these stationary phases act chiral selectors. The quinine carbamate plays only the role of chiral selector. A systematic study using different mobile phases was done but no separation was obtained for derivative amino solutes. The lack of retention of the ion pair formed between the quinine cabamate and the amino acid was probably due to weak hydrophilic character of ion pair.

The study of the retention on quinine carbamate stationary phase was much more interesting. The mobile phase consist in mixture of alcohol, acid and one of its derivatives sodium or ammonium salt. The concentration of acid was systematically modified in order to highlight its influence on the retention temps and the selectivity. The observed selectivity seems to be relatively independent of composition of mobile phase (Table-1 and Fig. 4-6). The selectivity varies between 1.16 and 1.2. Disregarding the weak resolution due to the weak plate number obtained with the large diameter of particles, results 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.

TABLE-1
ENANTIOMER SEPARATIONS OF PHTHALYLVALINE WITH PHASE Si-QN

Mobile phase	Retention time (min)	Selectivity 'α'
Methanol/acetic acid 99.5/0.5 (v/v)	11.2 and 12.7	1.18
Methanol/propionic acid 99.5/0.5 (v/v)	13.12 and 14.99	1.2
Methanol 0.125 g sodium acetate	5.22 and 5.56	1.16
Methanol/acetic acid 99.75/0.25 (v/v) 0.0625 g ammonium acetate	8.9 and 9.9	1.19
Methanol/acetic acid 99.5/0.5 (v/v) 0.0625 g ammonium acetate	7.62 and 8.52	1.19
Methanol/acetic acid 99.5/0.5 (v/v) 0.125 g sodium acetate	6.5 and 7.2	1.19
Methanol/acetic acid 99.5/0.5 (v/v) 0.125 g ammonium acetate	6.50 and 7.15	1.19
Methanol/acetic acid 99.0/1.0 (v/v) 0.125 g ammonium acetate	7.16 and 6.51	1.2

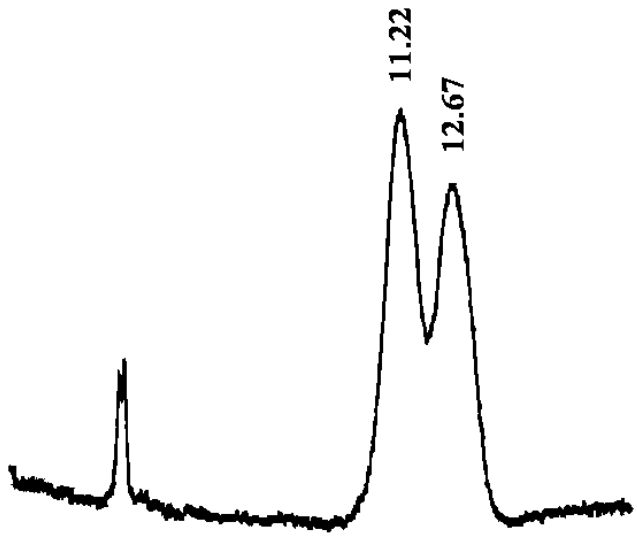


Fig. 4. Separation of the enantiomers of phthalylvaline. Wavelength 245 nm, flow rate 1 mL/min, volume injected 5 µL. Column, Si-QN (250 mm × 6 mm, 12 mm). Mobile phase, methanol/acetic acid (99.5/0.5 v/v)

The influence of the nature of carboxylic acid was noted. With formic acid, a weak retention was observed. The retention was increased by increasing the length of the carbon chain of the acid. Overall, the multiple retention mechanism was linked to the nature and intensity of hydrophobic and electrostatic interactions between solute, stationary and mobile phases.

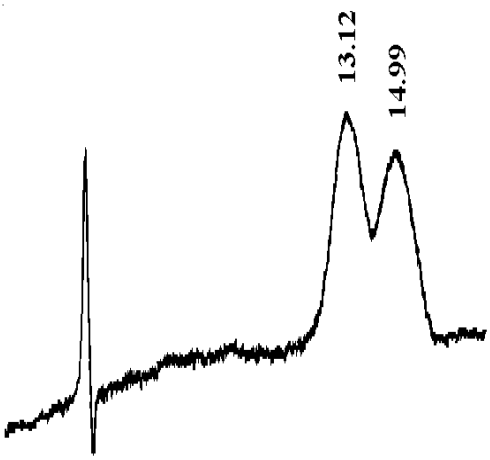


Fig. 5. Separation of the enantiomers of phthalylvaline. Wavelength 245 nm, flow rate 1 mL/min, volume injected 5 µL. Column, Si-QN (250 mm × 6 mm, 12 mm). Mobile phase, Methanol/propanoic acid (99.5/0.5 v/v)

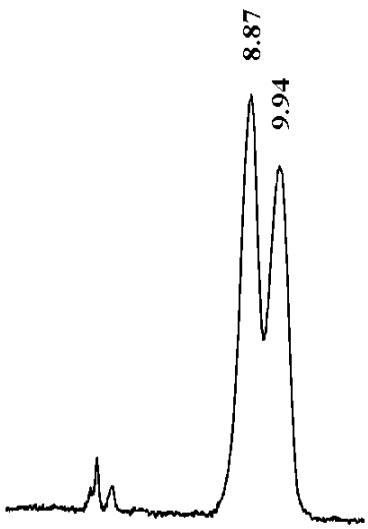


Fig. 6. Separation of the enantiomers of phthalylvaline. Wavelength 245 nm, flow rate 1 mL/min, volume injected 5 µL. Column, Si-QN (250 mm × 6 mm, 12 mm). Mobile phase, methanol/acetic acid (99.5/0.5 v/v), 0.125mg sodium acetate

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 *et al.*[1] 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.

REFERENCES

1. W. Lindner, M. Lämmerhofer and N.M. Maier, PCT/EP 97/02888
2. M. Lämmerhofer and W. Lindner, *J. Chromatogr. A*, **741**, 33 (1996).
3. M. Lämmerhofer, P.E. Di Eugenio, I. Molnar and W. Lindner, *J. Chromatogr. B*, **689**,123 (1997).
4. N.M. Maier, L. Nicoletti, M. Lämmerhofer and W. Lindner, *Chirality*, **11**, 522 (1999).
5. A. Mandl, L. Nicoletti, M. Lämmerhofer and W. Lindner, *J. Chromatogr. A*, **858**, 1 (1999).
6. E. Zarbl, M. Lämmerhofer, F. Hammerschmidt, F. Wuggenig, M. Hanbauer, N.M. Maier, L. Sajovic and W. Lindner, *Anal. Chim. Acta*, **404**, 169 (2000).
7. A. Peter, *J. Chromatogr. A*, **955**, 141 (2002).
8. A. Peter, E. Vekes, A. Arki, D. Tourwe and W. Lindner, *J. Sep. Sci.*, **26**, 1125 (2003).
9. R. Fegas, A. Adoui, M. Righezza and A. Hamdi, *Asian J. Chem.*, **20**, 125 (2008).
10. I.W. Wainer, R.M. Stiffen and T. Shibata, *J. Chromatogr.*, **411**, 139 (1987).
11. A. Mandl, L. Nicoletti and W. Lindner, *J. Chromatogr. A*, **858**, 1 (1999).
12. E. Tobler, M. Lämmerhofer and W. Lindner, *Chromatographia*, **51**, 65 (2000).
13. S. Schfzick, W. Lindner and M. Jalaie, *Chirality*, **12**, 7 (2000).
14. M. Kato and T. Toyooka, *Chromatography*, **22**, 159 (2001).
15. E. Tobler, M. Lämmerhofer and W. Lindner, *Chirality*, **13**, 641 (2001).
16. K. Gymesi-Forràs, J. Kökösi and W. Lindner, *J. Chromatogr. A*, **1047**, 59 (2004).
17. M. Lämmerhofer, O. Gyllenhaal and W. Lindner, *J. Pharm. Bio. Anal.*, **35**, 259 (2004).
18. K. Akasaka, M. Lämmerhofer and W. Lindner, *Chirality*, **17**, 544 (2005).
19. K. Gymesi-Forràs, A. Leiner and W. Lindner, *J. Chromatogr. A*, **1083**, 80 (2005).
20. L. Asnin and G. Guiochon, *J. Chromatogr. A*, **1091**, 11 (2005).
22. A. Maximini, H. Chmiel and N.W. Maier, *J. Membrane Sci.*, **276**, 221 (2006).
23. X.H. Zhang, Y. Wang and W.J. Jin, *Talanta*, **73**, 938 (2007).

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