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Effect of the Mobile Phase Acid Additives on Enantioselectivity of Amino Acid Derivative Using Quinine Carbamate Based Chiral Stationary Phase

R. FEGAS*†‡, A. BENSALEM, Z. BETTACHE†, F. OUAHBA and M. RIGHEZZA‡
Department of Chemistry, Ecole Normale Supérieure, Vieux Kouba, Alger, Algeria
E-mail: fegasrachid@yahoo.fr

Quinine carbamate-type chiral stationary phase has been used for the direct liquid chromatographic enantiomer separation of a wide range of chiral acids. In the present work, we demonstrate that this the chiral stationary phase can also be extended to chiral discrimination of amino acid derivative phthalylvalin using methanol containing different organic acids in mobile phases. The influence of mobile phase composition and enantioselectivity was systematically investigated to gain insight into the overall chiral recognition mechanism.

Key Words: Enantiomer separation, Chiral stationary phase, liquid chromatography, Mobile phase composition, Phthalylvalin.

INTRODUCTION

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

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 SAs, *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, π-π interactions, dipole-dipole, van der Waals and steric interactions, resulting in enantioseparation of different magnitude for racemic anionic SAs[9-12].

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

†Laboratoire de la police scientifique, Alger, Algeria.
‡UMR 6263 Institut des Sciences Moléculaires de Marseille, Université de Paul Cézanne, Marseille, France.

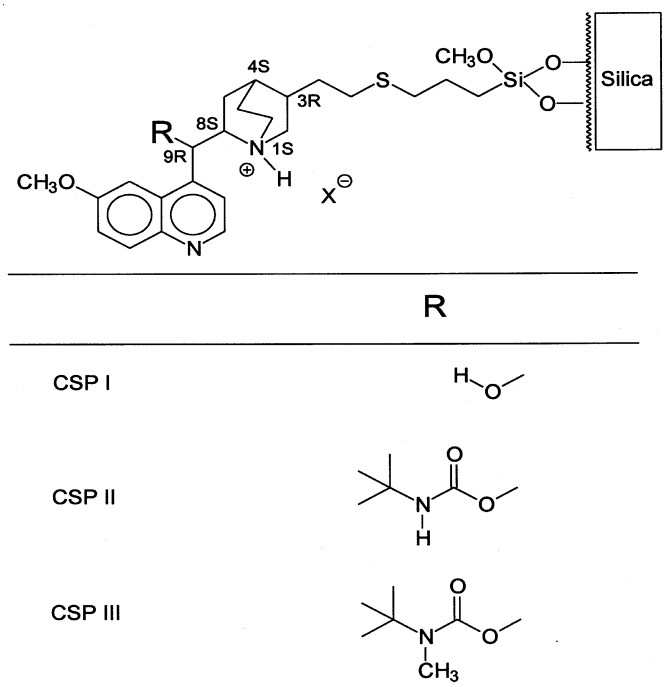

Fig. 1. Structures of chiral stationary phases: CSP I with quinine as selector, CSP II with *tert*-butyl carbamoylated quinine as selector, CSP III with N-methyl-*tert*-butyl carbamoylated quinine as selector

EXPERIMENTAL

HPLC measurements were performed with Waters systems consisted of an M-600 low-pressure gradient pump, an M-996 photodiode array detector.

The mobile phases were prepared with methanoic, ethanoic, propanoic, butanoic acids, *etc.* (polyscience analytical standards USA) and methanol for HPLC (Fluka, Germany).

Synthesis of chiral stationary phase based on t-BuCQN: The following protocol[6] 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 azobis(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 phthalylvalin with a polar mobile phase a mixture of methanol and acetic acid with flow rate 1mL/min[12].

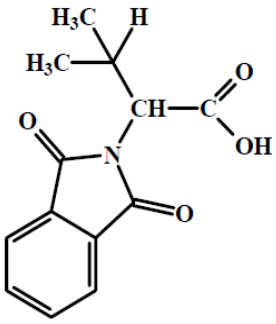

Fig. 2. Structure of phthalylvalin

RESULTS AND DISCUSSION

The study of the retention on quinine carbamate stationary phase was much more interesting. The mobile phase consist in mixture of alcohol, acids.

The concentration of acids was systematically modified in order to highlight its influence on the retention temperatures and the selectivity.

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
INFLUENCE OF THE CHAIN ALKYL ON THE SEPARATION OF PHTHALYLVALINE ENANTIOMERS

Acids	tr-1	tr-2	α
Formic acid (0.5 %)	4.793	4.793	1.0000
Formic acid (1.0 %)	4.444	4.444	1.0000
Acetic acid (0.5 %)	5.576	5.995	1.0751
Acetic acid (1.0 %)	4.686	4.924	1.0507
Propenoic acid (0.5 %)	6.492	7.088	1.0918
Propenoic acid (1.0 %)	4.840	5.119	1.0576
Butanoic acid (0.5 %)	5.672	6.116	1.0782
Butanoic acid (1.0 %)	4.910	5.207	1.0604
Pentanoic acid (0.5 %)	5.983	6.480	1.0830
Pentanoic acid (1.0 %)	5.320	5.695	1.1212
Hexanoic acid (0.5 %)	6.366	6.932	1.0889
Hexanoic acid (1.0 %)	5.650	6.084	1.0768
Heptanoic acid (0.5 %)	6.630	7.244	1.0926
Heptanoic acid (1.0 %)	5.981	6.472	1.0820
Octanoic acid (0.5 %)	6.123	6.642	1.0847
Octanoic acid (1.0 %)	6.842	7.495	1.0954
Nonanoic acid (0.5 %)	7.623	8.423	1.1049
Nonanoic acid (1.0 %)	6.696	7.327	1.0942
Dodecanoic acid (1.0 %)	9.658	10.840	1.1223

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.

The change in the percentage of the acetic acid varies from 1 % to 0.01 % and observed that more the concentration of acid in the mobile phase more the selectivity is good of 1.05 until 1.11.

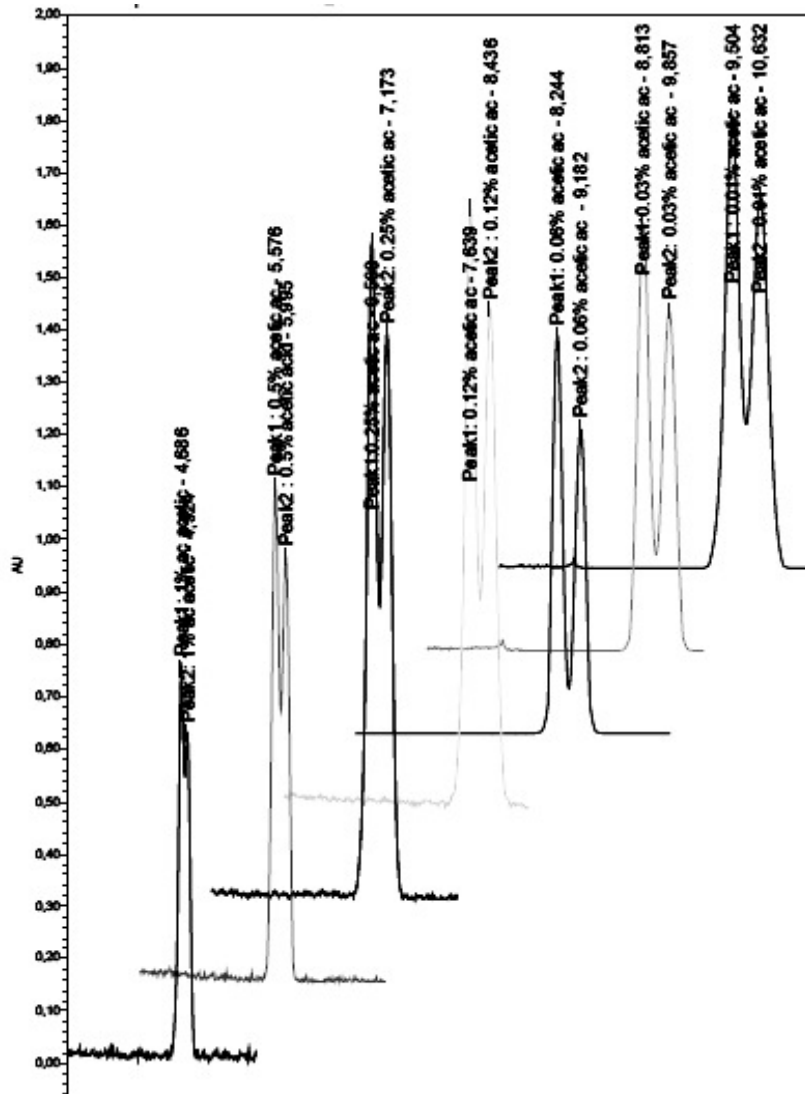

Fig. 3. Influence of percentage of the acetic acid on the separation of phthalylvalin enantiomers

TABLE-2
INFLUENCE OF PERCENTAGE OF THE ACETIC ACID ON THE SEPARATION OF PHTHALYLVALIN ENANTIOMERS

Acids	tr-1	tr-2	α
Acetic acid (1.00) %	4.686	4.924	1.0507
Acetic acid (0.50) %	5.576	5.995	1.0751
Acetic acid (0.25) %	6.568	7.173	1.0921
Acetic acid (0.12) %	7.639	8.436	1.1043
Acetic acid (0.06) %	8.244	9.182	1.1137
Acetic acid (0.03) %	8.813	9.857	1.1184
Acetic acid (0.01) %	9.504	10.632	1.1186

Injection of 5 µL phthalylvalin (1 mg/mL in methanol) on chiral column, with mobile phase methanol and 1 % à 0.01 % acetic acid, FR 1 mL/min

Conclusion

It is noticed that more the chain alkyl increase more the retention temperatures of phthalylvalin enantiomers increase. The two forms of phtalylvalin enantiomers have resolved by HPLC using quinine carbamate as chiral stationary phase. Base line separation was improved with more concentration of acetic acid (0.01 %).

REFERENCES

1. M. Lammerhofer and W. Lindner, *J. Chromatogr. A*, **741**, 33 (1996).
2. M. Lammerhofer and W. Lindner, *GIT Special-Chromatogr. Int.*, **96**, 16 (1996).
3. O.P. Kleidernigg, M. Lammerhofer and W. Lindner, *Enantiomer*, **1**, 387 (1996).
4. M. Lammerhofer, P.D. Eugenio, I. Molnar and W. Lindner, *J. Chromatogr. B*, **689**, 123 (1997).
5. V. Piette, M. Lammerhofer, K. Bischoff and W. Lindner, *Chirality*, **9**, 157 (1997).
6. W. Lindner, M. Lammerhofer and N.M. Maier, PCT/EP97patent application/02888.
7. M. Lammerhofer, N.M. Maier and W. Lindner, *Am. Lab.*, **30**, 71 (1998).
8. N.M. Maier, L. Nicoletti, M. Lammerhofer and W. Lindner, *Chirality*, **11**, 522 (1999).
9. L. Asnin and G. Guiochon, *J. Chromatogr. A*, **1091**, 11 (2005).
10. A. Maximini, H. Chmiel and N.W. Maier, *J. Membrane Sci.*, **276**, 221 (2006).
11. X.H. Zhang, Y. Wang and W.J. Jin, *Talanta*, **73**, 938 (2007).
12. R. Fegas, M. Righezza and A. Hamdi, *Asian J. Chem.*, **21**, 4001 (2009).

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