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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CHIRAL SEPARATION OF *D,L*-PHENYLALANINE AND *D,L*-TRYPTOPHAN WITH QUATERNARY MOBILE PHASE MIXTURE BY COPPER MIXED CHELATE COMPLEXATION

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abstract: A simple and rapid HPLC enatioseparation of D,L-phenylalanine (Phe) and D,L-tryptophan (DL-Trp) was developed. The resolution of enantiomers couples was based in the formation of mixed chelating complexes between copper (II) and L-histidine with quaternary mobile phase. The mobile phase was constituted of buffer ammonium acetate (*p*H=7), acetonitrile, isopentyl alcohol and isopropyl alcohol. An excellent separation of D, L couples was obtained with good selectivity and in no more than 15 min.

key words: HPLC; Chiral separation; D,L-amino acids; quaternary mobile phase; copper mixed chelate complexation.

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1. Introduction

It is widely accepted that the D-enantiomers of amino acids have biological properties different from those of the L-enantiomers. Due to the great interest in the biochemistry of D-amino acids, the number of papers dealing with their analysis has considerably increased [1,2] and some reviews have treated this subject [3,4]. Chiral separations can be achieved: by the interactions of the two enantiomers with a chiral ligand or surface and by derivatization reactions of the two enantiomers with chiral reagents. The diastereoisomers couples formed are separated under achiral conditions.

Several Enantioseparations methods of D,L-amino acids have been described using high performance liquid chromatography (HPLC) and some reviews were presented [5,6]. Thus, chiral stationary phases (CSPs) were successfully used within the chiral selector was

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chemically bonded [7÷12]. In the last years, macrocyclic compounds coating of apolair chromatographic surfaces such as teicoplanin were also used [13÷15].

The capillary electrophoresis (CE) with new spectroscopic reagent and micellar electrokinetic chromatography (MEKC) with additive chiral selector (β-Cyclodextrin) were investigated [16÷18]. The enantioseparation of amino acids can occur after chiral derivatization with several reagents such as N-acetyl-L-cycteine in presence of *o*-phthaldialdhyde (OPA) (19), Activated carbamate [20], triazine spectroscopic reagent [21] and with 7-fluoro-4-nitrobenzoxadiazole [22].

However, the simplest and least expensive way of separation of D, L-amino acids was the utilization of mixed chelate complexation in the mobile phase. This technique had the advantage to use a non polar reversed phase column with a mobile phase containing chiral metal complexes. Thus, free and derivatized D, L-amino-acids were resolved using different chelating agents [23÷28]. The study of mechanism of the enantiomeric resolution and computational chemical analysis were also described [29÷31]. In the previous articles, Lam and Co-workers [32÷35] utilized three systems of complexes Cu(II)-L-proline, Cu(II)-L-arginine and Cu(II)-L-histidine to separate dansyl-D, L-amino acids. The binary mobile phases were constituted of aqueous solution and acetonitrile in different percentage. With these systems, a number of D, L-amino acids were resolved. However with Cu(II)-L-Histidine system, no separation of D,L-Phenylalanine and D,L-Tryptophan enantiomers was obtained. We present in here an isocratic reverse-phase liquid chromatography with quaternary mobile phase. These phases contain Cu(II)-L-Histidine system as chelating complexe agent.

The quaternary mixture was performed by introducing two alcohols respectively as organic modifiers with the aqueous solution and acetonitrile. The role of these alcohols is to decrease the polarity of the mobile phase without increasing the percentage of acetonitrile. Indeed, Vollmer and co-workers used the isocratic quaternary mobile phase containing sodium acetate buffer, acetonitrile, isobutyl alcohol, and isopropyl alcohol screening of amino-acid in metabolic disorders in three amino acidopathies [36].

2 Experimental

2.1. Instruments

Chromatography was performed with HPLC system with a Waters 600 pump, a 7625 Rheodyne manual injector with 20µL sample loop, a Waters AC 440 fluorometer detector (excitation wavelength, 340 nm; emission wavelength, 450 nm) and LC-1022 nelson integrator (Perkin Elmer).

The separation was carried out on Ultrasphere® ODS Column (4.6 mm ID, 250 mm length and 5µm dp) from Beckman Coulter (USA).

2.2. Chemicals and Reagents

The dansyl-D,L-amino acids and dansyl-L-amino acids were obtained from Sigma (Switzerland). The acetonitrile, isopropyl alcohol, and isopentyl alcohol were HPLC grade

and obtained from Prolabo (France). The copper sulphate ($CuSO_4.5H_2O$) ammonium acetate and ammoniac were from Merck. (Germany). Ultrapure Water is produced in our laboratory by the MilliQ-Millipore system (USA).

2.3. Mobile phase

The mobile phase was containing a mixture of organic modifiers (acetonitrile, isopropyl alcohol and isopentyl alcohol) and the aqueous solution (**Aq.Sol**). The latter was prepared with 2.5 mM of $CuSO_4$ and 5 mM of L-Histidine with molar ratio 1:2 and buffered by ammonium acetate (2g/L) which adjusted at pH=7 with ammoniac solution 37%. At this value of pH, the copper complexes with L-amino acids are stables [32, 33].

Various mobile phases with different compositions of organic modifiers with the Cu(II)-L-Histidine sysem were tested for D,L-amino acids separation. The mobile phases were degassed and filtered before use. The flow rate was 2 mL/min.

3. Results and discussion

The resolution of the isomers of D,L-phe and D,L-Trp is obtained for the first time with this system (Cu(II)-L-Histidine) with quaternary mobile phase Aq.Sol /acetonitrile/ isopentyl alcohol/isopropyl alcohol **77/19/2/2 v/v.** The analysis time was less than 15 min. The acceptable selectivity is observed for the both amio acids D,L-Trp ($\alpha_{D/L}$=0.75) and D,L-Phe ($\alpha_{D/L}$=0.84) (table I) and the isomer D is eluted before the L form (Fig. 1).

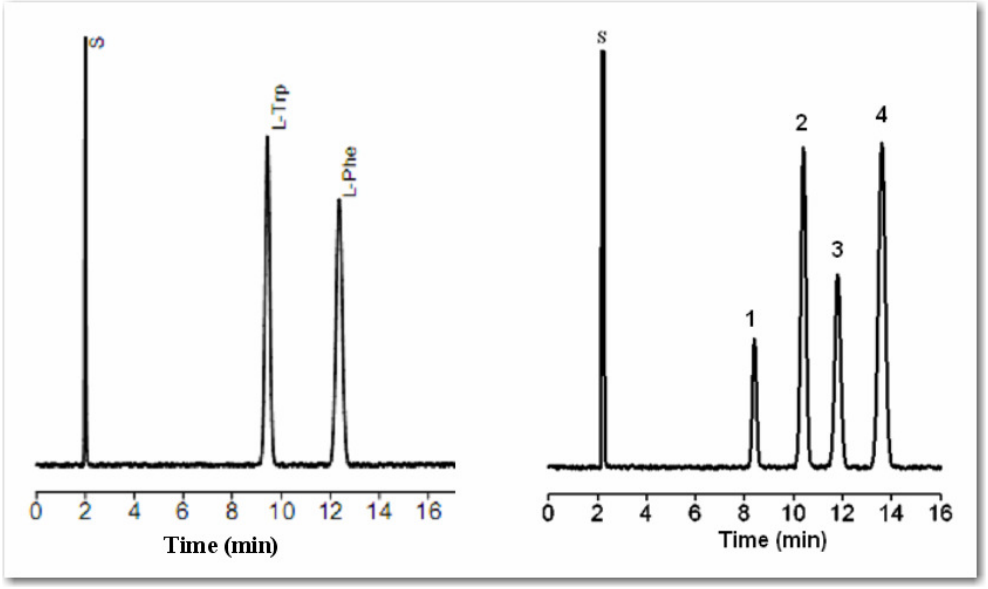

Fig. 1 *Separation of Dns-D,L- Phenylalanine and Dns-D,L-Tryptophan with Cu(II)- L-Histidine system.* Quaternary mobile phase: Aq.Sol (Mixture of Buffer acetate 5mM L-Histidine 2.5mM $CuSO_4$ and 2.0g/L ammonium acetate, *p*H =7.0) Acetonitrile/ isopentylalcohol/ isopropylalcohol with proportion respectively: 77/19/2/2 (v/v). **1**-D-Trp**; 2**-L-Trp**; 3**-D-phe**; 4**-L-phe**.**

Table 1 *Capacity Ratio (k') and selectivity (α) of D- and L-Dns-Amino Acids with L-Histidine System*

Amino -acid	Capacity Ratio		Selectivity
	k'_L	k'_D	$\alpha_{D/L}$
Phe	5.18	4.36	0.84
Trp	3.72	2.81	0.75

The separation of these dansyled D,L amino acids can be explained as below:

The two alcohols can link by hydrogen bonding with the carboxylate group of the L-Histidine. Thus, amine and imidazole groups of the L-histidine are bonded in the trans manner and the carboxylate group is weakly coordinated on apical site. The aromatic ring of phenylalanine (Phe) or tryptophan (Trp) is coordinated on the opposite site. Therefore, the mixed complex having both carboxylate and the ring system, which is competing for the same coordination site, is less stable (here is the D-form).

The discrimination between the D form and the L-form can occur and the mixed complex of L-form is more retained. Fig. 2 presents the possible structure of L-Histidine mixed complexes based on the model given by lam and co-workers in ref [33,34]. We have introduced the effect of the alcohols and their contribution to decrease the retention time.

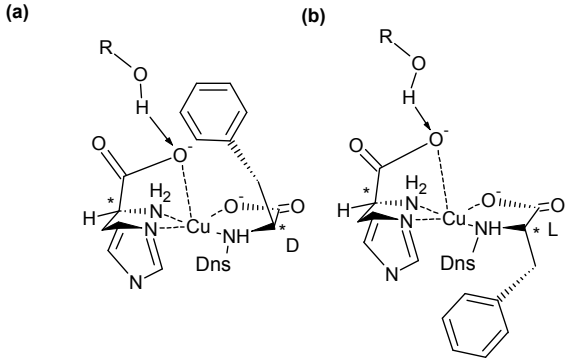

Fig. 2 *Molecular model of mixed complexes of DL-amino acid.*
a) D-Phe-Cu(II)-L-His; b) L-Phe-Cu(II)-L-His

4. Conclusion

We have presented a simple and no expensive enantioseparation method of dansyl-D, L-amino acids. Quaternary mobile phase containing L-Histidine as chelating agent was used to separate the enantiomers of dansyled D,L-Phe and D,L-Trp with good selectivity and in no more than 15 min. We have also observed that the introduction of the two alcohols (isopropyl alcohol and isopentyl alcohol) as organic modifier in the mobile phase can reduce the analysis time without alteration in the selectivity.

REFERENCES

1. Fiznar, H.P., Lobbes, J.M. and Kattner, G. (1999) *J. Chromatogr. A* **832**, 123-32.
2. Shulman, Y., Tibbo , P., Grant, S.L., Hampson D.R. and Baker, G.B. (2006) *J. Chromatogr. B* **844**, 278-82.
3. Imai, K., Fukushima, T., Santa, T., Homma, H., Hamase, K. Sakai K. and Kato, M. (1996) *Biomed. Chromatogr*. **10**, 303-12.
4. Hattori, A., Fukushima, T., Hamamura, K., Kato M. and Imai K.A. (2001) *Biomed. Chromatogr*. **15**, 95-9.
5. Davankov, A., Kurganov, A.A. and Bochkov, A.S. (1983) Advances In Chromatography, Vol 22, Marcel Dekker ,New York, 71-103.
6. Heftmann, E. (2004) **Chromatography. Fundamentals and applications of chromatography and related Differential migration methods: Applications**. Journal of Chromatography Library, Vol. 69B, Elsevier B.V, 6th Edition, 600-5.
7. Fujimura, K., Suzuki, S., Hayashi, K. and Masuda, S. (1990) *Anal. Chem*. **62**, 2198-205.
8. Kim, Tae-Y. and Kim, Hie-J. (2001) *J. Chromatogr. A* **933**, 99-106.
9. Lin, C.E., Li, F. K. and Lin, C. H. (1996) *J. Chromatogr. A* **722**, 211-20.
10. Grötzinger, G. J.and Berndt, H. (1987) *J.Chromatogr. A* **397**, 223-32.
11. Toshio, S., Yamaguchi, T., Yanagishita, H., Kitamoto, D., Sakaki, K.and Sugiura, M. (1992) *J. Chromatogr*. **625**, 101-8.
12. Qinghua, M., Shengqing W., Ying, G., Yanshun, M. and Yuande, L. Chin. (2006) *J. Anal. Chem*. **34**(3), 311-5.
13. Janderaa, P., Škavradaa, M., Klemmova, K., Bačkovskà, V. and Guiochon, G. (2001) *J. Chromatogr. A* **917**, 123-33.
14. Masuyer, C., X.Perrin, C.F., Guillaume, Y-C., Truong, T., Millet, J., Thomassin, M., Chaumont, J-P.and Nicod, L. (2002) *Analytica Chimica Acta* **457**, 149-55.
15. Haroun, M., Ravelet, C., Grosset, C., Ravel, A., Villet, A.and Peyrin, E. (2006) *Talanta* **68**,1032-6.
16. Arai, T., Ichinose, M., Kuroda, H., Nimura, N.and Kinoshita, T. (1994) *Analytical Biochemistry* **217**(1), 7-11.
17. Yang, L., Zhang, D. and Yuan, Z. (2001) *Analytica Chimica Acta* **433**, 23-30.
18. Thorsén, G. nd Bergquist, J. (2000) *J. Chromatogr. B* **745**, 389-97.
19. Nimura, N.and Kinoshita, T. (1986) *J. Chromatogr. A* **352**, 169-77.
20. Iwaki, K.,Yoshida, S., Nimura, N., Kinoshita, T., Takeda, K. and Ogura, H. (1987) *Chromatographia* **23**, 899-902.
21. Ma, H.M., Wang, Z.H. and Hsu, M. (2002) *J. Chromatogr. A* **955**, 125-131.
22. Song,Y., Shenwu, M., Zhao, S., Hou, D. and Liu, Y.M. (2005) *J. Chromatogr. A* **1091**, 102-9.
23. Davankov, A., Bochkov, A.S. and Kurganov, A.A. (1980) *Chromatographia* **13**, 677-85.
24. Nimura, N., Toyama, A., Kasahara, Y.and Kinoshita T. (1982) *J. Chromatogr.* **239**, 671-275.
25. Lee, S.H., Sub-Oh, T. and Lee, H.W. (1992) *Bull. Korean Chem. Soc.* **13**, 280-5.
26. Galaverna, G., Corradini, R., De Munari, E., Dossenaa, A.and Marchelli, R. (1993) *J. Chromatogr. A* **657**, 43-54.
27. Tapuhi, Y., Miller, N. and Karger, B.L. (1981) *J. Chromatogr*. **205**, 325-37.
28. Weinstein, S.and Weiner, S. J. (1984) *Chromatogr*. **303**, 244-50.
29. Galaverna, G., Corradini, R., Dallavalle, F., Folesani, G., Dossenaa, A. and Marchelli, R. (2001) *J. Chromatogr. A* **922**, 151-63.

30. Hanai, T. (2003) *Internet Electronic Conference of Molecular Design* November 23-December 6, BioChem Press.

31. Armani, E., Barazzoni, L., Dossena, A.and Marchelli, R. (1988) *J. Chromatogr*. **441**, 287-98.

32. Lam, S., Chow, F. and Karmen, A. (1980) *J. Chromatogr. A* **199**, 295-305.

33. Lam, S. and Karmen, A. (1982) *J. Chromatogr*. **239**, 451-62.

34. Lam, S.and Karmen, A. (1984) *J. Chromatogr*. **289**, 339-45.

35. Lam, S. (1984) *J. Chromatogr. Sci*. **29**(9), 416-23.

36. Vollmer, D.W., Jinks, D.C. and Guthtrie, R. (1990) *Analytical Biochemistry* **189**, 115-21.