Asian Journal of Chemistry; *Vol. 24, No. 11 (2012), 4917-4922*

ASIAN JOURNAL OF CHEMISTRY

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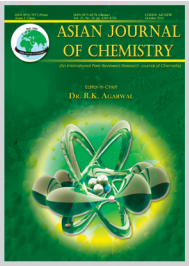

Enantioseparation of Typical Pesticides Using Cellulose Carbamate Stationary Phases by Capillary Liquid Chromatography

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(*Received*: 30 July 2011; *Accepted*: 30 May 2012) AJC-11527

Cellulose-*tris*(3,5-dimethylphenylcarbamate) was initially synthesized as the chiral selector, then stable coated and bonded chiral stationary phases were prepared, respectively, using aminopropyl-functionalized silica gel as the support media. The prepared stationary phases were used for micro-column chiral separation by self-installed capillary high performance liquid chromatography system. Eighteen kind of chiral compounds including some typical pesticides were tested on both prepared chiral stationary phases and different chromatographic parameters such as resolution and retention time were comparatively investigated.

Key Words: Enantioseparation, Chiral pesticide, Cellulose *tris*(3,5-dimethylphenylcarbamate), Capillary liquid chromatography.

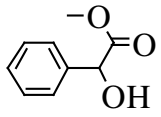
INTRODUCTION

Enantioseparation has received considerable attention in the parmaceutical and pesticidal industries[1-3]. In general, it can be realized in the direct and indirect modes by high performance liquid chromatography (HPLC), capillary-HPLC, capillary electrophoresis (CE) and capillary electrochromatography (CEC)[4-8]. Among them, HPLC can provide outstanding achievements in the resolution of the enantiomers, because in HPLC there are a variety of chiral stationary phases (CSPs) that fit for the enantioseparation of the analytes. Compared with LC, CE features mainly low sensitivity and uncertainty in quantitative analysis, but is perfect for its high-resolution capacity, low reagent consumption and versatility. Capillary electrochromatography has reached increasing interest and showed extensive applications in recent years because of its high efficiency, but the difficulties similar to capillary HPLC involving capillary packing and frit preparation need to be overcome in the application of CEC. In the above techniques, the crucial factor for chiral separations is the choice of the proper chiral selector or chiral stationary phases, which always needs to be properly carried out according to the structure of the analytes to be analyzed. The polysaccharide-based chiral stationary phases (CSPs) like cellulose-based and their derivatives-based chiral stationary phases have been approved to be a very broad applicability to different chiral analytes[9-12].

In this paper, two typical approaches were attempted to fabricate chiral stationary phases through bonding or coating the chiral selector (cellulose *tris*(3,5-dimethylphenylcarbamate) (CDMPC)) onto a 5 μm spherical porous silica, respectively. Then the chiral stationary phases bonded and coated with cellulose *tris*(3,5-dimethylphenylcarbamate) were packed in the capillaries, respectively, using the sintered steel powders as the inlet frit and the packed stationary phases themselves as the outlet frit. Eighteen enantiomers (Fig. 1) including common pesticides and some drugs were tested by a simple chiral separation system of capillary HPLC self-installed in our laboratory. On the optimization condition, some compounds could be separated by using two chiral stationary phases, respectively, which effectively showed the recognition capability of the polysaccharide derivatives.

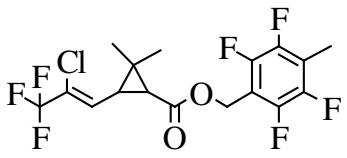
EXPERIMENTAL

Chiral separation experiments were carried out by using a self-assembled capillary HPLC system[13]. It includes a HP1100 Series HPLC pump system (Agilent Technologies, Inc., Walbronn, Germany), equipped with an injector with a 20 mL quantitative tube (Rheodyne 7725i) and an on-capillary column detector with a changeable ultraviolet-visible wavelength in the range of 190-700 nm (Beijing Cailu Scientific Instrument Ltd., China). A T union was used to split delivering appropriate amounts of the mobile phase. The prepared separation column inlet was installed at one outlet of the T union using a PEEK sleeve (0.5 mm i.d.,1.6 mm o.d.) and a screwed joint. Another capillary (4 m × 100 μm i.d) for splitting the injection sample and mobile phase was contacted to the other

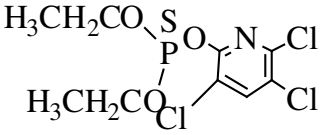


1. methyl-DL-mandlate

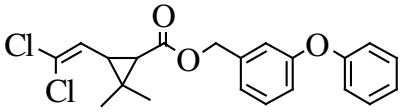
2. 2,2,2-trifluoro-1-(9-Anthyl) ethanol



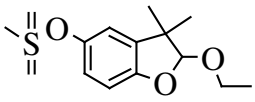
3. tefluthrin



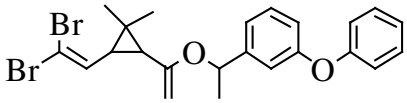
4. chlorpyrifos



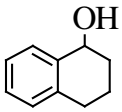
5. permethrin



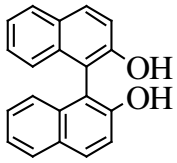
6. ethofumesate



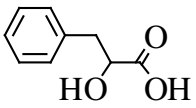
7. decamethrim



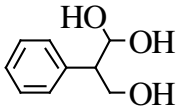
8. 1,2,3,4-tetrahydro-1-naph



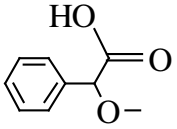
9. R,S-1,1'-bi(2-naphthol)



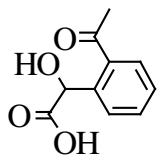
10. DL-3-pheylactic acid



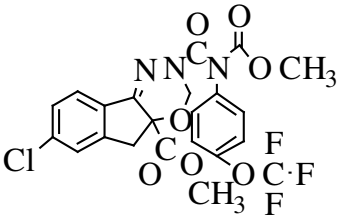
11. tropic acid



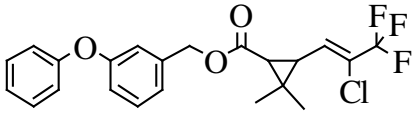
12. R,S-α-methoxyphenylacetic acid



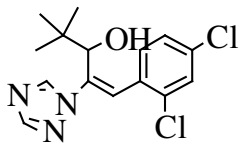
13. R,S-acethylandelic acid



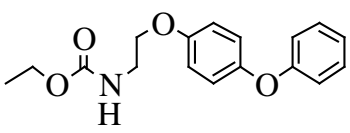
14. indoxacarb



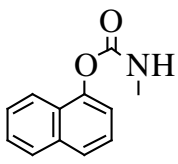
15. cyhalothri



16. diniconazole



17. fenoxycarb



18. Carbaryl

Fig. 1. Molecular structures of the selected chiral compounds

outlet of the T union. Chromatograms were recorded using the computer software N2000 chromatography data system supplied by Zhida Information Engineering Ltd., Zhejiang University, China.

Fused-silica capillaries (100 μm i.d., 375 μm o.d.) were purchased from Yongnian Ruipu Optic Fiber Plant (Yongnian, Hebei Province, China). Stainless steel powders (under 500-mesh) were purchased from Beijing Gelubo Alloy Material Limited Company. Spherical silica (Akzo Nobel, Sweden, particle size 5 μm, average pore size 10 nm), 3-aminopropyltriethoxysilane (Aldrich, USA), microcrystalline cellulose (Aldrich, USA), 3,5-dimethylphenyl isocyanate (TCI, Japan), pyridine (Junsei, Japan). Eighteen chiral compounds including tefluthrin, chlorpyrifos, diniconazole, permethrin, ethofumesate, 2,2,2-trifluoro-1-(9-anthryl) enthanol, *etc.*, were donated from China Agricultural University and Hunan Agricultural University. Acetonitrile (ACN), methanol, isopropanol (IPA), hexane (HEX) and trifluoroacetic acid (TFA) were purchased from Beijing Bailingwei Chemical Reagent Company and Tianjing Chemical Reagent Company, China.

Preparation of chiral stationary phases by bonding the chiral selector onto silica: The chiral stationary phases were prepared by the reaction of cellulose derivatives and 3-aminopropyl-functionalized silica gel with 1,6-diisocyanatohexane[14,15]. Cellulose derivatives were regioselectively bonded to silica gel on their glucose units. Fig. 2 shows the immobilization of cellulose (3,5-dimethylphenyl) carbamates to silica gel at the 6-position of the glucose unit.

Silica gel was treated with an excess of the silanizing reagent 3-aminopropyltriethoxysilane in dry toluene. Cellulose (2.97 g) was allowed to react with a large excess triphenylmethyl chloride (10 g) in dry pyridine (60 mL) at 100 ºC for 24 h under N_2 atmosphere, then an excess of 3,5-dimethylphenyl isocyanate (9 mL) was added to form carbamate residues with the hydroxy groups at the 2- and 3-positions.The 2,3-*bis*(3,5-dimethylphenylcarbamoyl)-6-O-trityl cellulose obtained was suspended in a large excess of methanol containing a small amount of hydrochloric acid so as to remove the trityl group at room temperature.

The cellulose 2,3-*bis*(3,5-dimethylphenylcarbamate) (0.93 g) thus obtained was dissolved in 25 mL dry THF and the solution was coated on 3-aminopropylsilica gel (3.86 g) as described previously. After THF had been removed *in vacuo*, the cellulose derivative on silica gel was dispersed in a mixture of dry toluene (10 mL) containing 34 μL 1,6-diisocyanatohexane (10 mol % based on the 6-position hydroxy groups of cellulose) and dry pyridine (2 mL) and then the mixture was heated at 100 ºC. After 5 h, an excess of 3,5-dimethylphenyl isocyanate was added and allowed to react with the remaining hydroxy groups at the 6-position at 100 ºC for 24 h. The chiral stationary phases thus obtained was collected by filtration and washed with THF to remove free cellulose derivative.

Preparation of chiral stationary phases by coating the chiral selector onto silica: To begin with, aminopropyl-modified silica gel and synthesis of cellulose *tris*(3,5-dimethylphenylcarbamate) were prepared, which was identical to the above processes and other reports[16,17]. Secondly, chiral selector was coated onto modified silica particles as follows: cellulose *tris*(3-chloro-4-methylphenylcarbamate) (0.020 g) was dissolved in 30 mL THF by ultrosonic bath. Place the aminopropyl-modified silica gel (0.400 g) in a round-bottom flask. Add the solution (10 mL) to silica gel by drops and shake the flask to uniformly coat the polysaccharide derivative (5 %, w/w) on the silica surface. Then dry the silica gel under vaccum at 60 ºC for 8 h.

Slurry packing of chiral stationary phases and home-made chiral separation system: Miniaturization is an attractive trend in HPLC, which possesses some advantages such as economic use of chiral stationary phases and of expensive and/or toxic solvents. Here, a pneumatic pump (RPL-ZD10, Dalian Replete Scientific Instrument Co., Ltd, Dalian, China) were used for slurry packing both chiral stationary phases into capillaries, respectively. The procedure for the inlet and outlet frit prepa-

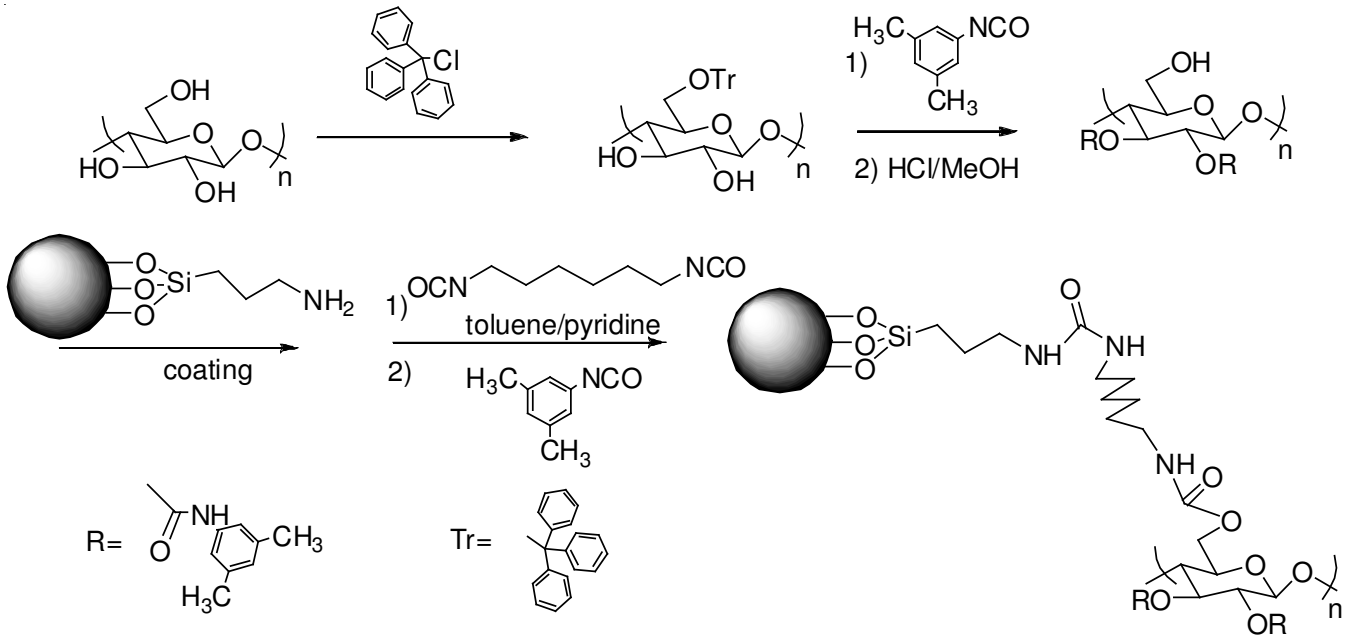


Fig. 2. Regioselective bonding of cellulose (3,5-dimethylphenyl)carbamates at the 6-position

ration was similar to our previous reports[13]. A window was created by burning the polyimide coating of the capillary at the desired position. After that, the chromatographic performance of the packed chiral stationary phases was evaluated with using our self-installed capillary chiral liquid chromatography system[15]. In our experiment, eluent flow through the capillary column was controlled by a custom built adjustable flow splitter based upon a T-piece connector with a capillary. The backpressure enforced on the separation column could be adjusted by the change of another capillary inner diameter and length. The splitting capillary was linked to the waste container under the atmosphere. Splitting ratio was calculated by weighing the eluate from the capillary outlets, which was collected in a sealed vial. The usage of on-column detector could effectively improve the sensitivity and avoid the peak broadening due to the dead volume in the conventional flow cell of UV detector.

RESULTS AND DISCUSSION

Characterization of both chiral capillary columns: An FEI QUANTA 200 scanning electron microscope (Philips-FEI Corporation, Netherlands) was used to study the morphology of chiral stationary phases. After two kinds of chiral stationary phases were packed into the capillaries and evaluated, respectively, both capillaries were sectioned into about 1 cm segments without sputtering with gold prior to SEM analysis. Fig. 3 showed that the chiral stationary phases microspheres with a clean smooth surface were packed tightly and with some tight contact points between sphere-sphere and sphere-capillary wall. The sample preparation process for SEM resulted in the beads being scattered on the capillary surface in Fig. 3b. Furthermore, the bonded chiral stationary phases looked like more even than that coated chiral stationary phases with different sizes, it was probably attributed the uneven coating process might affect the surface thickness.

Enantioseparation of chiral compounds using bonded chiral stationary phases: Similar polysaccharide derivative chiral stationary phases have been widely evaluated in normal phase mode with binary solvent (*n*-hexane/alcohol) as the mobile phase and they are also useful in combination with aqueous-organic (reversed phase mode) and purely organic (polar organic mode) mobile phases. Here, the enantiomeric separation using the synthesized cellulose-based chiral stationary phase was just examined in former mode. In order to obtain a broad perspective on the performance of the bonded chiral stationary phase, the resulting chiral stationary phases were evaluated in the chiral separation of different kinds of compounds. 1 % TFA (w/v) was to be weighed and added to a desired volume of mobile phase for the analytes 1-14. Table-1 gives the separation parameters of the selected analytes eluted with *n*-hexane/alcohol mixtures from a capillary column packed with the bonded stationary phases about 20 cm length. Among the randomly chosen racemic analytes, three analytes including methyl-DL-mandlate, 2,2,2-trifluoro-1-(9-anthyl)ethanol and chlorpyrifos were baseline separated using the prepared materials. Seven analytes could be partly separated and others could not be separated on this column. As it is known that resolution between the pair of enantiomers depends on both separation factor and column efficiency, higher column efficiency would allow reduction of column lengths and thus

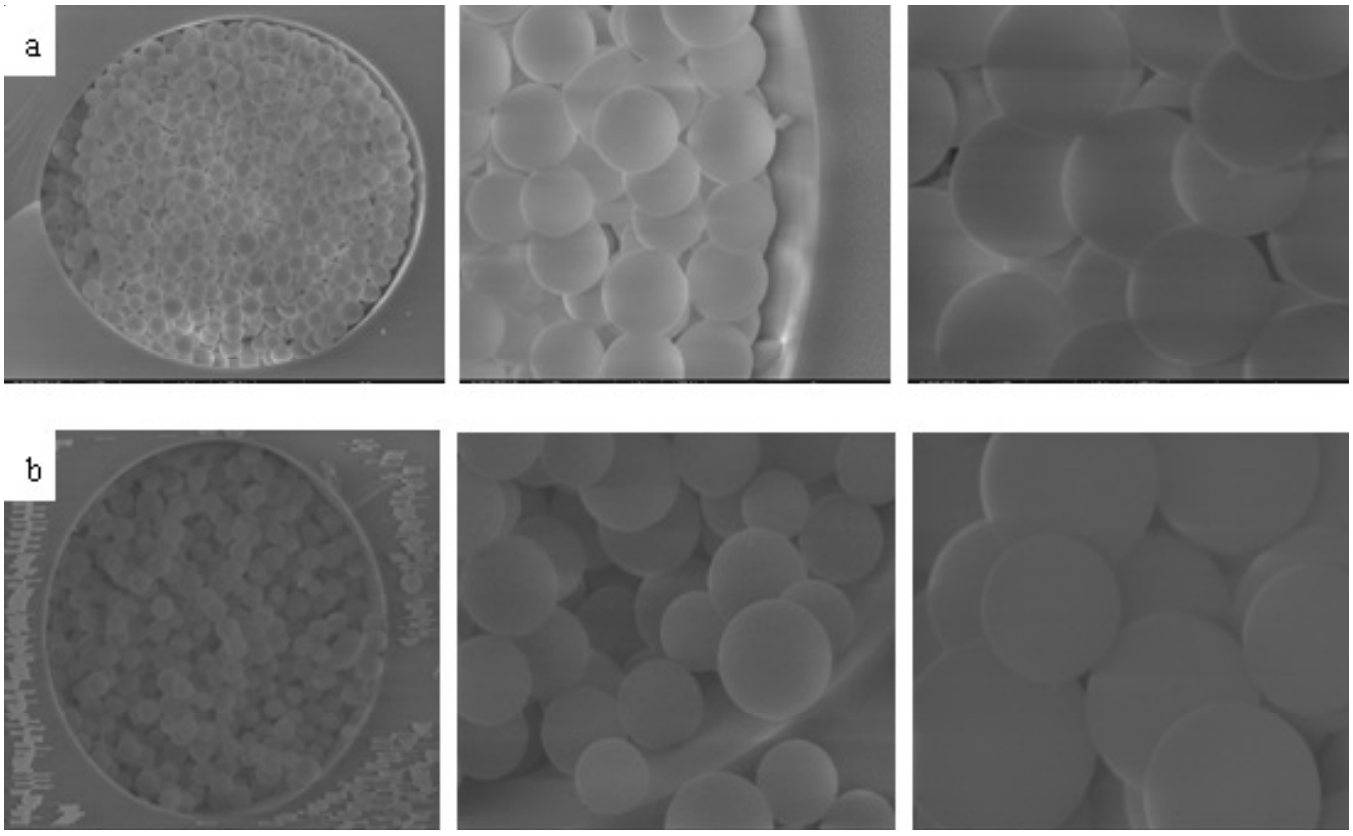


Fig. 3. SEM of both chiral stationary phases

TABLE-1
ENANTIOSEPARATION OF THE ANALYTES USING THE BONDED CELLULOSE-DERIVED CHIRAL STATIONARY PHASES

Samples	U (mL/min)	UV (nm)	HEX/IPA	t_1	t_2	R
1	0.7	254	90/10	2.197	4.707	1.68
2	0.6	254	90/10	5.469	7.806	2.24
4	0.6	246	90/10	3.026	3.874	3.28
6	0.6	230	98/2	7.738	8.743	1.40
8	0.9	254	90/10	2.644	3.118	0.60
9	0.7	254	90/10	7.411	7.938	0.48
10	0.5	200	80/20	2.991	4.173	1.26
11	0.5	200	80/20	3.044	4.184	0.97
12	0.5	200	80/20	3.181	4.116	0.93
13	0.5	200	80/20	3.148	3.982	1.05
17	0.6	230	90/10	3.448	–	0
18	0.6	230	95/5	6.224	–	0

decrease analysis time while adequate resolution is maintained. It is possible to increase the resolution of the above seven analytes by prolonging the packing length of stationary phases.

Enantioseparation of chiral compounds using coated chiral stationary phases: Chiral recognition can be affected by the coating amount of cellulose *tris*(3,5-dimethylphenylcarbamate) and degree of homogeneity on silica gel. An insight into how the chromatography is affected when there is a large amount of chiral phase on the outside of the particle was known during previous studies to determine the optimum carbamate loading for a small-pore silica support[14,15]. If the coating is too thin, no enantiomer resolution can be obtained, possibly due to stationary phase overloading. On the other hand, with thicker coatings, several problems are encountered: The high viscosity of the coating solution makes the coating process difficult and slow. By changing the concentration of cellulose *tris*(3,5-dimethylphenylcarbamate) for coating on the aminopropyl-modified silica gel, the thickness of cellulose *tris*(3,5-dimethylphenylcarbamate) film as the chiral stationary phases can be controlled correspondingly in our previous report[14,15]. Here, the silica gel coated with 5 % (w/v) of cellulose *tris*(3,5-dimethylphenylcarbamate) in THF was used for our comparative experiments.

To discern the efficiency of the coated cellulose-based stationary phase comprising a small-pore silica support, similar enantioseparation experiments were carried out in normal phase mode. As listed in Table-2, the chromatographic parameters and resolution of the selected analytes were given using a capillary column packed with the coated stationary phases about 20 cm length. Among the randomly chosen racemic analytes, three analytes including 2,2,2-trifluoro-1-(9-anthyl) ethanol, tefluthrin, chlorpyrifos and ethofumesate were baseline separated using the prepared materials. Four analytes could be partly separated with a resolution in the range of 0.51-1.47, others could not be separated on this capillary column. Fig. 4 showed that resolution of a typical pesticide of ethofumesate were changed by varying the percentage of mobile phase (HEX/IPA v/v) with a constant set flow rate of 0.6 mL/min. With the change of mobile phase percentage in the range of 90:10 to 98:2, resolution of ethofumesate was obviously increased from zero to larger than 1.5. The differences of the interactions including the size and the geometry

TABLE-2
ENANTIOSEPARATION OF THE ANALYTES USING THE COATED CELLULOSE-DERIVED CHIRAL STATIONARY PHASES

Samples	U (mL/min)	UV (nm)	HEX/IPA	t_1	t_2	R
1	0.9	200	90/10	6.912	8.982	1.47
2	0.9	230	90/10	6.106	7.077	1.77
3	0.9	230	90/10	2.620	3.919	2.62
4	0.9	246	90/10	2.310	3.675	3.72
5	0.9	230	90/10	2.299	2.703	1.28
6	0.6	230	98/2	9.247	10.914	3.10
7	0.6	205	98/2	4.721	5.003	0.68
14	0.6	310	98/2	11.084	–	0
15	0.6	230	98/2	4.704	4.881	0.51
16	0.9	230	90/10	3.716	–	0

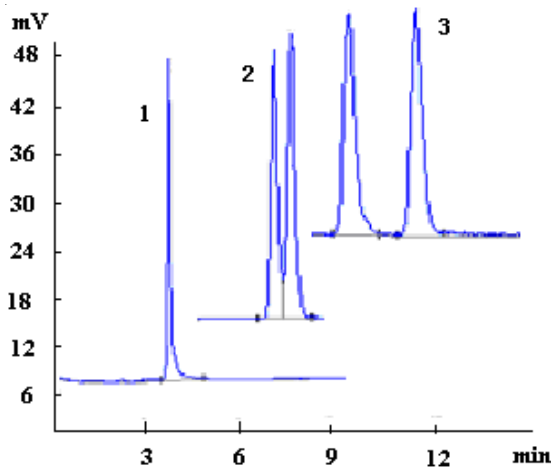


Fig. 4. Effect of different mobile phases on the separation of ethofumesate; Experimental conditions: room temperature, wavelength = 230 nm, flow rate = 0.6 mL/min, the mobile phases for experiments 1-3 are HEX/IPA = 90/10, 95/5 and 98/2, respectivel

configuration of the solutes resulted in differing rate of elution of the enantiomers. Enantiomers 2-4 and 6 possessed the better separation efficiency than other analytes. It is commonly believed that the difference of a combination of attractive forces such as hydrogen bonding, hydrophobic interactions, dipole-dipole interactions and k-k interactions between the two enantiomers and polar carbamate groups of polysaccharide *tris*-phenylcarbamate chiral stationary phase plays an important role in the discrimination.

Conclusion

Cellulose *tris*(3,5-dimethylphenylcarbamate) was bonded and coated on the porous silica gels, respectively and used as the chiral stationary phases for capillary high-performance liquid chromatographic separation of enantiomers. Both typical chiral stationary phases could be packed into the capillary columns and some chiral compounds including ten pesticides were used for the enantioseparation successfully using the self-installed chiral capillary separation system.

ACKNOWLEDGEMENTS

The financial support by the National Natural Science Foundation of China (Nos. 30070506, 30771436) are gratefully acknowledged.

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