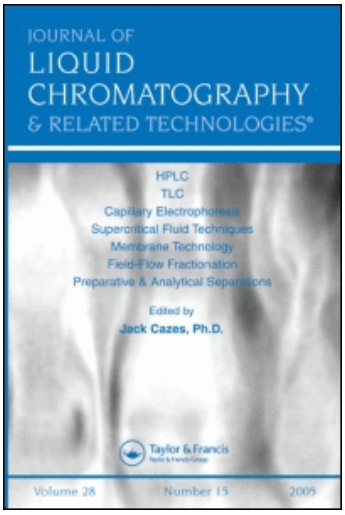

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Direct Optical Resolution of Chiral Pesticides by HPLC on Emamectin CSP under Normal Phase Conditions

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Abstract: A chiral stationary phase (CSP) was prepared by bonding emamectin onto unmodified spherical gel. Normal-phase high performance liquid chromatography (HPLC) methods for the resolution of three chiral triazole pesticides, triadimefon, difenoconazole, and propiconazol, on the CSP were developed. Several operating parameters, such as mobile phase composition, column temperature, and flow rate were studied for the optimization of the resolution. Better separations were achieved using 5% isopropanol for triadimefon, 20% iso-propanol for difenoconazole, and 5% isopropanol for propiconazol as modifiers, in n-hexane at 10°C with the selectivity factors (α) of 1.29, 1.29, and 1.48, respectively.

Keywords: Chiral triazole pesticides, HPLC, Resolution

INTRODUCTION

Along with research in stereochemistry, researchers progressively recognize that chiral compounds have very important effects in medicine, pesticides, and other areas. The enantiomers of chiral compounds resemble reflecting physicochemistry properties, but they have greater differences in bioactivity and metabolic pathways.[1] As for pesticides, presently, most chiral pesticides sold on the market are produced and used in the form of racemic mixtures; the active effect that different enantiomers reflect to a target organism is different. Consequently, it is very important to produce and use chiral pesticides that are

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mostly composed of the highly active enantiomer, which not only can improve the efficiency of a pesticide and decrease the cost, but more important is that it greatly reduces pollution to the environment. Correspondingly, it is very important that we establish an analytical method for chiral pesticides, and accordingly, assess the optical purity of chiral pesticides, thereby monitoring and guiding production.

Many techniques can be used for chiral resolution, such as high performance liquid chromatography (HPLC), capillary electrophoresis (CE), gas chromatography (GC), thin-layer chromatography (TLC), capillary electrochromatography (CEC), and supercritical fluid chromatography (SFC).[2] High performance liquid chromatography is the most used method of separating enantiomers. It is highly developed and widely applied because of its high efficiency and ease of operation. In the 1970s, liquid chromatographic separation of enantiomers had attracted great attention and several useful chiral stationary phases were reported. In 1994, Armstrong et al. reported that the macrocyclic antibiotics, such as vancomycin, rifamycin B, and thiostrepton exhibit a unique ability as chiral stationary phases for HPLC.[3] From then on, many such types of these CSP's were developed.[4] But, all most workers have focused on the antibiotics that are used in human disease therapy, and ignored many other antibiotics. We have been preparing CSPs with antibiotics that are used as pesticides in agriculture and use this CSP to separate triazole pesticide enantiomers directly.

EXPERIMENTAL

Materials

Emamectin (95.6%) was provided by the Institute for Control of Agrichemicals, Ministry of Agriculture. 3-Isocyanatopropyltriethoxysilane (95.0%) was purchased from ABCR GmbH & Co. KG. Unmodified spherical silica was purchased from the Academy of Sciences, Lanzhou, with the following properties: particle size, 5 μm; average pore diameter, 6.0 nm; specific surface area, 390 m^2g^{-1}. The three chiral triazole pesticides, triadimefon (96.7%), difenoconazole (94.3%) and propiconazol (95.0%), see Fig. 1, were provided by the Institute for Control of Agrichemicals Ministry of Agriculture, and the chemical structures were shown by Fig. 1. All the other reagents used were analytical grade.

Apparatus

Chromatography was performed using an Agilent 1100 series HPLC system (Agilent Technologies Palo Alto, CA, USA) equipped with a G1311A

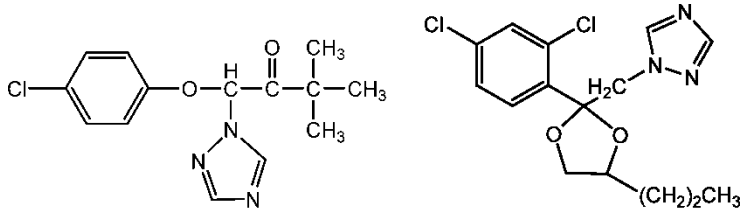

Figure 1. Chemical structures of the samples.

pump, G1322A degasser, G1328A injector, 10 μL sample loop and a G1314A Variable Wavelength Detector. The signal was acquired and processed by an HP1100 workstation.

Chromatographic Conditions

The column was 250 mm × 4.6 mm i.d. The mobile phases were mixtures of different concentrations of isopropanol and n-hexane; the flow rate was 1.0 mL/min except during the flow rate experiment. Chromatographic resolutions were performed at 10°C, except for the experiment investigating the influence of temperature, which was performed over a range of 0–40°C with 10%, 20%, and 20% isopropanol in n-hexane, for triadimefon, difenoconazole, and propiconazol, respectively. Detection wavelength was 230 nm.

Preparation of the CSP

The chiral CSP was prepared according the method described by D'Acquarica.[5] Emamectin (1.0 g) was dried at 60°C for 12 h under vacuum, and was then dissolved in dry pyridine (100 mL), and stirred for 5 min. 3-Isocyanatopropyltriethoxysilane (0.2 mL) was slowly added, under a nitrogen atmosphere, and the mixture was heated to 70°C for 2 h with

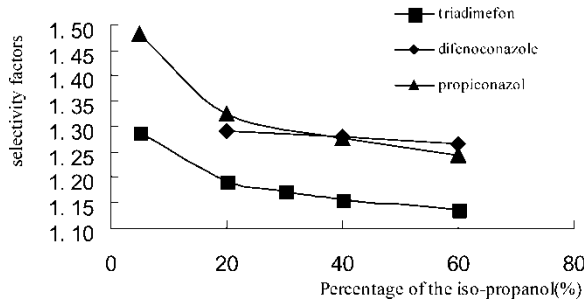

Figure 2. Influence of the isopropanol percentage on the selectivity factors; temperature: 10°C; detection wavelengh: 230 nm; flow rate: 1.0 mL/min.

continuous stirring. After cooling to room temperature, 3.5 g acid-treated and dried unmodified spherical silica was added and the mixture was heated to 70°C for 12 h with continuous stirring, under a nitrogen atmosphere. The mixture was cooled to room temperature, filtered, and the residue was washed several times with pyridine, DMF, water, methanol, acetonitrile, and acetone, and then dried under vacuum at 60°C for 12 h. The emamectin-modified silica gel (3.0 g) was packed into a stainless steel HPLC column (250 × 4.6 mm i.d.) by the flow of absolute alcohol at 5.0×10^7 Pa pressure. This emamectin column then was washed with methanol and stored.

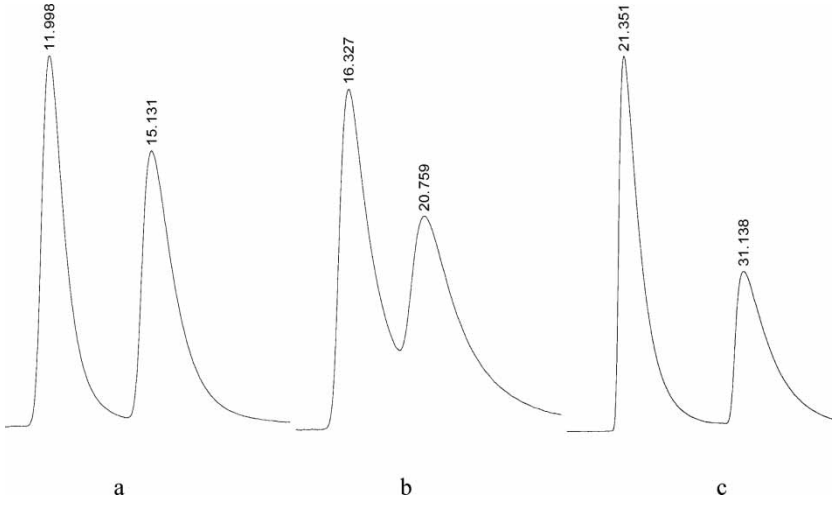

Figure 3. Chromatograms of the chiral separations of the samples. A) Triadimefon, 5% isopropanol; b) Difenoconazole, 20% iso-propanol; c) Propiconazol, 5% isopropanol, at 10°C, 230 nm, 1.0 mL/min.

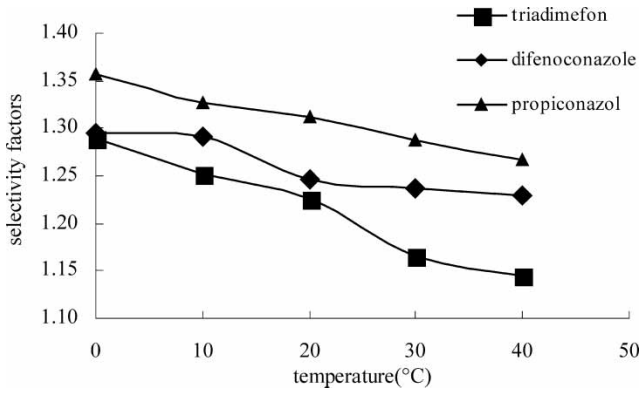

Figure 4. Effect of the temperature on the selectivity factors. Triadimefon, 10% isopropanol; difenoconazole, 20% isopropanol; propiconazol, 20% isopropanol, at 230 nm, 1.0 mL/min.

RESULTS AND DISCUSSION

Influence of Mobile Phase Composition

The influence of the iso-propanol content in the mobile phase on the chiral separation was investigated. Figure 2 shows the effect on the selectivity factors (α) of the resolutions of the chiral pesticides in n-hexane. The results demonstrated that α increased with the n-hexane content of the mobile phase. The difenoconazole and propiconazol both have two chiral centers, and there are four optical isomers. But our CSP cannot

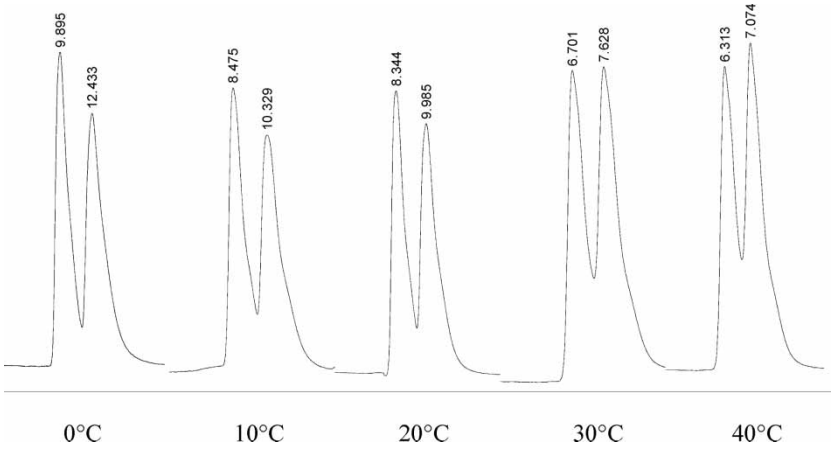

Figure 5. Chromatograms of triadimefon at different column temperatures. 10% isopropanol, 230 nm, 1.0 mL/min.

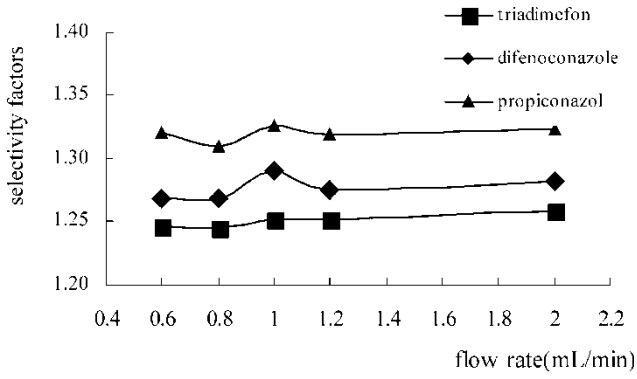

Figure 6. Influence of the flow rate on the selectivity factors. Triadimefon, 10% isopropanol; difenoconazole, 20% isopropanol; propiconazol, 20% isopropanol, at 10°C, 230 nm.

separate them completely, and we were only able to obtain two peaks in the chromatograms. In addition, decreasing the volume percentage of the isopropanol resulted in a longer retention times. This is not what we hoped for. Figure 3 shows the chromatograms of the resolutions at 10°C.

Influence of Temperature on the Separations

The influence of temperatures from 0°C to 40°C on the separation are given in Fig. 4. Temperature affected both resolution and retention of the enantiomers on the CSP. Low temperature gave better separations and longer retentions for the samples. The resolution on the CSP is proposed to be due to the difference of the interactions between the enantiomers and CSP. The influence of temperature on the chiral resolution may be due to the difference of the interactions being changed with temperature. Figure 5 shows the chromatographic separation of triadimefon at different column temperatures, respectively.

Influence of Flow Rate

The influence of flow rate was investigated over a range from 0.6 to 2.0 mL/min. The result is shown in Fig. 6. However, no significant change in retention and resolution was observed.

CONCLUSION

The chiral resolution of triadimefon, difenoconazole, and propiconazol by high-performance liquid chromatography has been investigated and is

reported in this paper. The Emamectin CSP was applicable for the chiral separations of the samples. Chromatographic conditions were optimized. Better separations were achieved using 5% iso-propanol for triadimefon, 20% iso-propanol for difenoconazole, and 5% iso-propanol for propiconazol as modifiers in n-hexane at 10°C with the selectivity factors (α) of 1.29, 1.29, and 1.48. The procedures are relatively simple, rapid, and inexpensive.

ACKNOWLEDGMENTS

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