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Determination of Carbofuran on Hydrophilic Interaction Liquid Chromatography using TSKgel®Amide-80 as a Stationary Phase

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Cover Page Footnote

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Abstract

Hydrophilic interaction liquid chromatography (HILIC) equipped with an environmentally friendly capillary column was employed to investigate the retention behavior of carbofuran; a polar stationary phase was used as well. Several conditions were conducted to investigate the retention behavior of carbofuran, such as a comparison study TSKgel®Amide-80 with another polar column, a comparison study retention behavior of carbofuran on various wavelengths, the water content effect on HILIC mode, the effect of buffer concentration on HILIC mode, and the analytical performance of carbofuran. The results showed that TSKgel®Amide-80 exhibited a better performance than other polar stationary phases in carbofuran determination, and observations at wavelengths of 251 and 254 nm showed higher absorbance for carbofuran than others. In addition, the increase of water content and salt buffer concentration in the mobile phase led to a shorter retention time. The recovery of this method was 101 ± 10.1%, while the limit of detection and the limit of quantification were 0.66 ppm and 2.22 ppm, respectively. Consequently, TSKgel®Amide-80 offers a good performance in carbofuran determination, even with the application of 10 cm length column capillary liquid chromatography.

Abstrak

Determinasi Karbofuran pada Kondisi Kromatografi Cair Interaksi Hidrofilik menggunakan TSKgel®Amide-80 sebagai Fase Diam. Kromatografi cair interaksi hidrofilik (KCIH) digunakan dengan memanfaatkan kolom kapiler yang ramah lingkungan dalam rangka mempelajari perilaku retensi karbofuran. Perilaku retensi karbofuran diidentifikasi menggunakan beberapa jenis fase diam yang bersifat polar. Beberapa kondisi telah dilakukan untuk menyelidiki perilaku retensi karbofuran seperti studi perbandingan kolom TSKgel®Amide-80 dengan kolom polar lainnya, perbandingan perilaku retensi studi karbofuran pada berbagai panjang gelombang, efek air dalam mode KCIH, pengaruh konsentrasi buffer pada mode KCIH, dan kinerja analitik. Hasil penelitian menunjukkan bahwa TSKgel®Amide-80 lebih baik dibandingkan dengan fase diam polar lain dalam penentuan karbofuran. Selain itu panjang gelombang 251 dan 254 nm menghasilkan absorbansi lebih tinggi untuk karbofuran daripada yang lain. Sebagai tambahan, peningkatan kadar air dan konsentrasi garam penyangga pada fase gerak menyebabkan waktu retensi lebih cepat. Nilai perolehan kembali metode ini adalah 101 ± 10,1, sedangkan limit deteksi dan limit quantifikasi berturut-turut diperoleh sebesar 0,66 ppm dan 2,22 ppm. Disimpulkan bahwa TSKgel®Amide-80 memberikan hasil yang baik dalam penetapan karbofuran meskipun digunakan kromatografi cair kapiler dengan panjang kolom 10 cm.

Introduction

Inappropriate application of pesticide in agricultural activities induces a negative effect on surface water bodies and soil. One of the main causes of pesticide contamination in the water bodies and other areas of the environment is the pollutant release due to intentional or accidental spills, or improper disposal of wastewater from pesticide applications. Carbofuran (2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate) is one of the most toxic pesticide belonging to the carbamate group, which has been used worldwide since the beginning of the 1960s (Figure 1a). Carbofuran serves as both an insecticide and a nematicide. However, using carbofuran as a pesticide could potentially cause environmental damage [1].

Reverse phase chromatography is the most applicable method for pesticide determination [2-11]. The gradient

elution technique combines with binary solvent systems, i.e. with water and acetonitrile or methanol combine with acetate or formate buffer. On the one hand, reverse phase chromatography signifies the use of a nonpolar compound as a stationary phase and a polar compound as a mobile phase. On the other hand, the nonpolar stationary phase in reverse phase mode has disadvantages, such as low compatibility with high aqueous eluent and insufficient selectivity towards polar solutes [12-14].

Hydrophilic interaction chromatography (HILIC) becomes an alternative technique for the separation of polar and hydrophilic compounds [15-24]. The term "hydrophilic interaction chromatography" was proposed by Alpert in 1990 as a complementary method for both normal and reverse-phase chromatography, which combines an acetonitrile-rich phase with a low aqueous mobile phase to elute the analytes in order to increase polarity. The partitioning of the solutes from the mobile phase into the hydrophilic layer caused by the water-enriched liquid layer is established in the stationary phase. The selectivity of the separation is influenced by interactions such as ion-exchange, hydrogen bonding, and dipole-dipole interactions. In addition, the use of a high percentage of organic solvents (usually acetonitrile) enhanced the ionization process and increased the sensitivity in mass spectrophotometry [25-27].

Carbofuran is one of the most toxic pesticide belonging to the carbamate group widely used all over the world. Carbofuran carries the polar site on the molecule, which makes it possible to bond and be separated using the polar stationary phase. In an analysis of carbofuran, the most point concerns the extraction method from any sample matrix such as fruit, vegetables, soil, and water. The recent method usually involve using solid phase exctraction, solid phase micro extraction, sol gel immunoaffinity exctraction, and the QuEChERS method [28-31].

TSKgel®Amide-80 involves silica bonded (covalent) carbamoyl groups are usually used in normal or HILIC mode to analyze saccharide, peptides, and polar compounds (Figure 1b). TSKgel®Amide-80 has a superior stability over conventional amide bonded phase columns. The carbamoyl group of TSKgel®Amide-80 shows a good result in determining the organic polar compound. HILIC with the TSKgel®Amide-80 column offers better selectivity, retention time, peak shape, and reproducibility compared to the reverse phase liquid chromatography column [32-35]. In addition, using TSKgel®Amide-80 as the HILIC stationary phase to determine the carbofuran concentration allows an easy process compared to gas chromatography (GC), which requires a derivatization process [36]. The aim of this research is to investigate the retention behavior of carbofuran using TSKgel®Amide-80 as the stationary phase.

Material and Methods

Reagents and materials. This research was conducted using capillary liquid chromatography system [37]. The commercial standard of carbofuran was purchased from Chem Service (USA), and the acetonitrile was of High Performance Liquid Chromatography (HPLC) grade from Merck (Darmstadt, Germany). The ultra-pure water used in all the experiments was from a simplicity UV system (Milipore, Bedford, MA, USA) with an electrical resistivity of 18,2 MΩ cm^{-1}. The stock solutions of carbofuran, as well as a diluted mixture, were prepared in acetonitrile. The ammonium acetate was purchased from Nacalai (Kyoto, Japan), and all solutions were filtered through PTFE 0.45 µm membrane filters (GL chromatodisc). TSKgel®Amide-80 (5 µm size particle) as a stationary phase and DEAE-2SW were purchased from Tosoh Corp. (Tokyo, Japan); polar imidazole and polar pyridine were purchased from Sepax Technologies, Inc. (USA).

Instruments. The capillary LC system used in this work consists of an L.TEX 8301 micro-feeder equipped with an MS-GAN 050 gas-tight syringe (0.5 mL Ito, Fuji, Japan) as a pump, a model Rheodyne-7520 microinjection valve (Cotati, California, USA) with the injection volume of 0.2 µL as an injector, a 0.32 mm i.d x 0.45 mm o.d x 100 mm micro-column, and a model Jasco UV-2075 as the UV detector. The data were acquired by a CDS ver. 5 data processor (LAsoft, Chiba, Japan) (Figure 2).
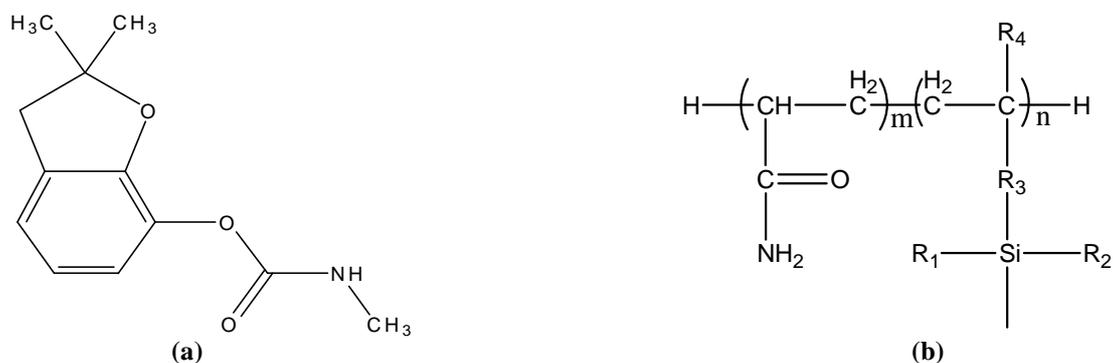

(a)   **(b)**

Figure 1. (a) Chemical Structure of Carbofuran, (b) Chemical Structure of TSKgel®Amide-80
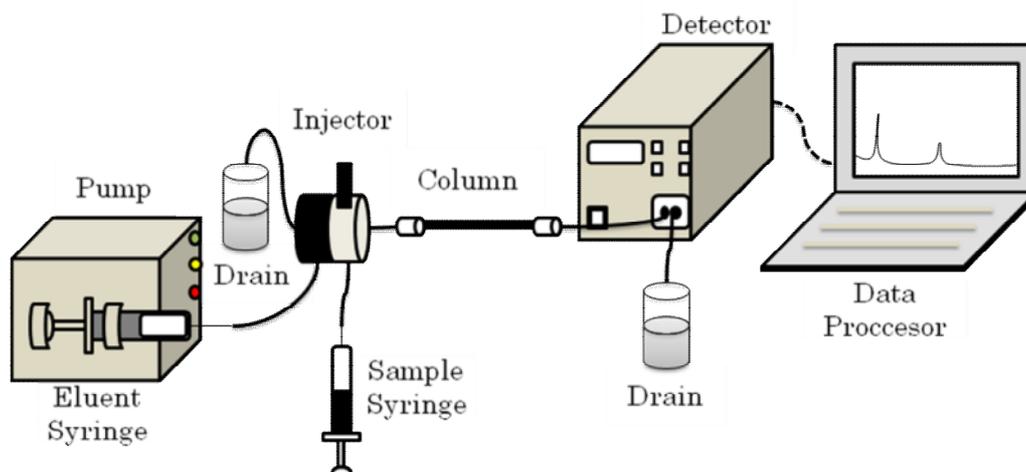
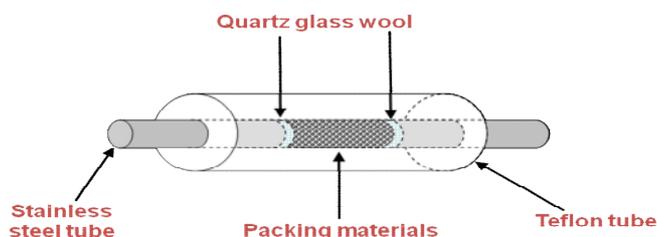**Figure 2. Capillary Liquid Chromatography System**

Figure 3. Capillary Column

Column packing. The TSKgel®Amide-80 capillary column was arranged by a slurry packing method. The column packing conditions were as follows: 0.1 g of TSKgel®Amide-80 was mixed with 1 ml methanol to form a suspension and then slurry-packed into a fuse silica capillary column (0.32 mm i.d x 0.45 mm o.d x 100 mm) using a syringe equipped with a long Teflon tube with 0.2 mL methanol as the propelling solvent (Figure 3).

Chromatographic condition. The chromatographic evaluation, the column temperature was set at room temperature (about 25 °C). The flow rate was 2 µL min^{-1}, and the injection volume was 0.2 µL. All HPLC analytical determinations were carried out at least in duplication: a). A comparison study of TSKgel®Amide-80 as a stationary phase with another polar stationary phase; b). A comparison study of the various wavelengths on carbofuran retention behavior. Observation was performed at wavelengths of 251, 254, 276, 280, and 282 nm; c). The water content effect on HILIC mode: The effects of various concentrations of water in HILIC mode on carbofuran retention behavior were studied. The binary mobile phase system used was acetonitrile and water with the following concentrations of water: 10%, 20%, 30%, and 40%; d). The effect of the buffer concentration on HILIC mode: The effects of various concentrations

of salt buffer on carbofuran retention behavior were studied. The mobile phase was acetonitrile with the following variations of ammonium acetate: 5, 10, 15, and 20 mM; e). Analytical performance such as accuracy, RSD percentage, LOD, and LOQ.

Results and Discussion

Comparison study of TSKgel®Amide-80 as a stationary phase with another polar stationary phase. The purpose of this experiment is to investigate the retention behavior of carbofuran in different polar stationary phases. The stationary phases used were polar pyridine, polar imidazole, and DEAE-2SW. Investigation of the retention behavior of carbofuran was conducted using a 10 ppm concentration of carbofuran and an 85% concentration of acetonitrile. Figure 4 shows that when TSKgel®Amide-80 was employed, the peak was two times higher than the other stationary phases, and that TSKgel®Amide-80 was more sensitive than others. TSKgel®Amide-80 holds a superior stability over a conventional amide bonded phase column. The carbamoyl group of TSKgel®Amide-80 generated a better analytical performance in determining an organic polar compound. The result indicated that a pesticide compound was able to be detected by HILIC due to its hydrophilic property and polarity. Carbofuran carries an amine functional group as a secondary amine, which has a very

active nitrogen species as a proton donor (i.e. a positive charge) on the surface of the amide functional group of TSKgel®Amide-80. This compound possesses a carbonyl group as well, which can be double bonded with oxygen and actively interact with the surface of the amide functional group of TSKgel®Amide-80 (Figure 5).

Comparison study of various wavelengths. The wavelengths of 251, 254, 276, 280, and 282 nm were applied in this experiment, as in other similar studies. Figure 6 shows that the wavelengths of 251 and 254 nm generated higher peaks than others. It showed that wavelength of 251 and 254 nm were *the* optimum wavelength for carbofuran.

Water content effect in the mobile phase on HILIC mode. The mobile phase composition greatly affects the retention of carbofuran in HILIC mode, and the large amount of organic solvent is more advantageous to the

compound with low aqueous solubility. In HILIC, retention of the solution in the column is influenced by many factors such as water content, pH, salt concentration and the temperature of the buffer column. In HILIC, a water-rich layer on the surface of the stationary phase formed by the mobile phase versus the water-deficient mobile phase, creates a liquid-liquid extraction system where the analyte can be distributed between these two layers.

The concentrations of water varied between 10% and 40%; Figure 7 shows the retention behavior of carbofuran at various concentrations of water in the mobile phase. The retention time decreased upon the increase of water content in the mobile phase, indicated by a typical HILIC retention mechanism. The stationary phase TSKgel®Amide-80 exhibited retention for carbofuran in HILIC mode.
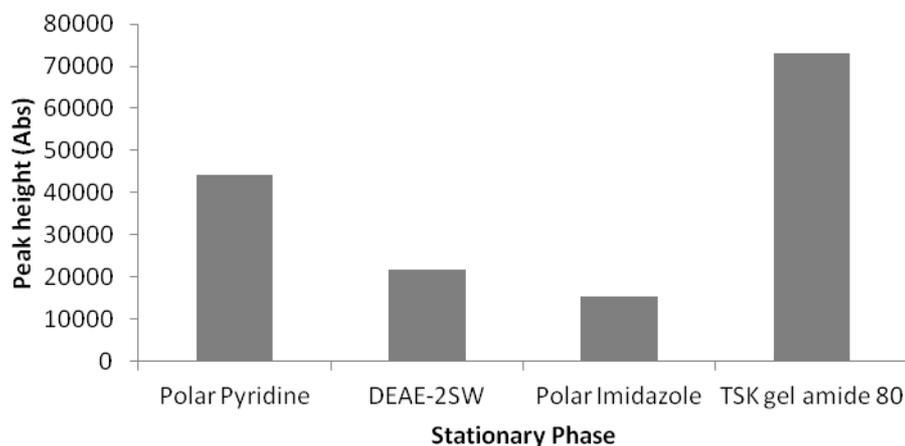

Figure 4. The Peak Height of Carbofuran in Different Concentrations of Carbofuran using Various Stationary Phases
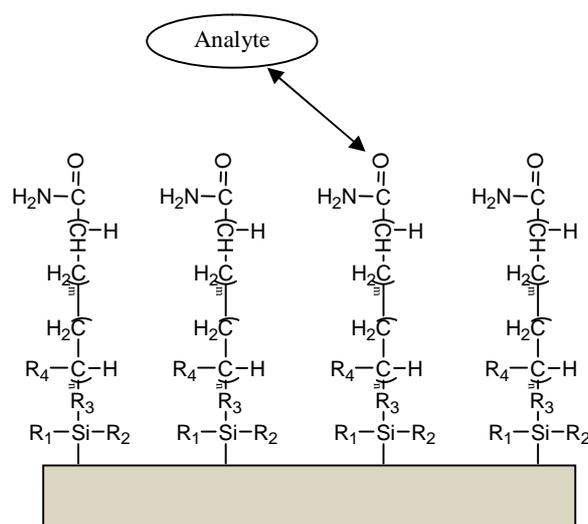

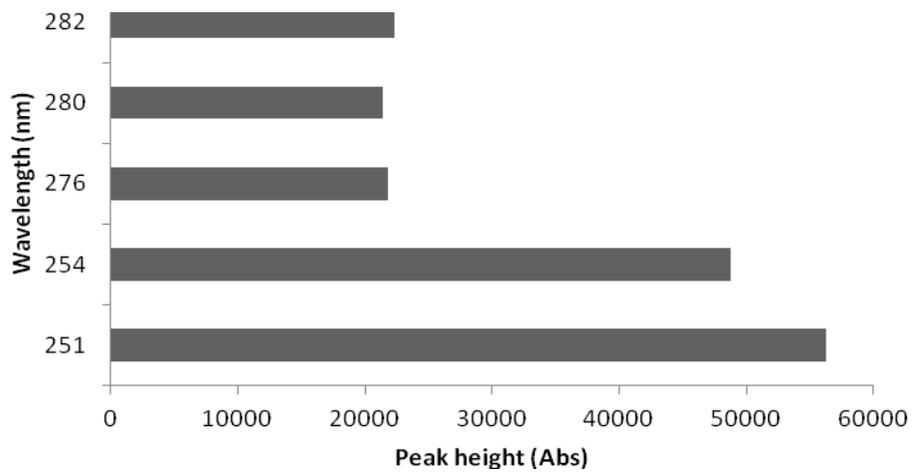**Figure 5. Proposed Interaction between the Stationary Phase and Analytes**

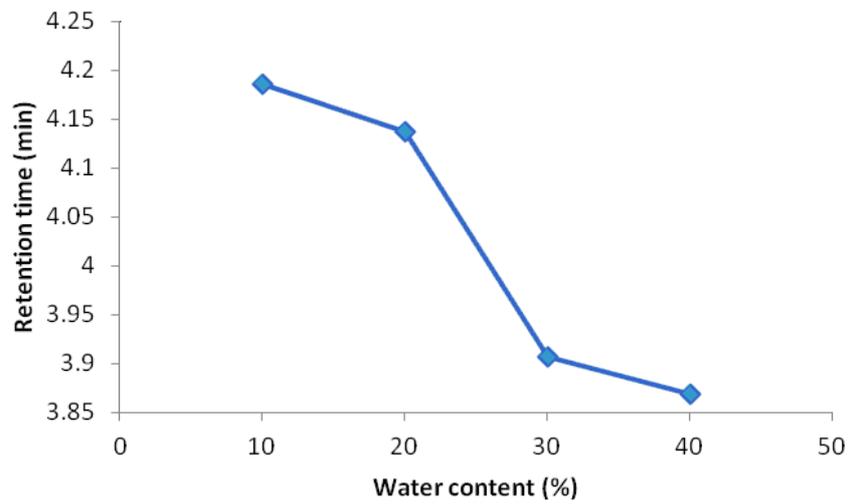**Figure 6. The Peak Height of Carbofuran on Different Wavelength**

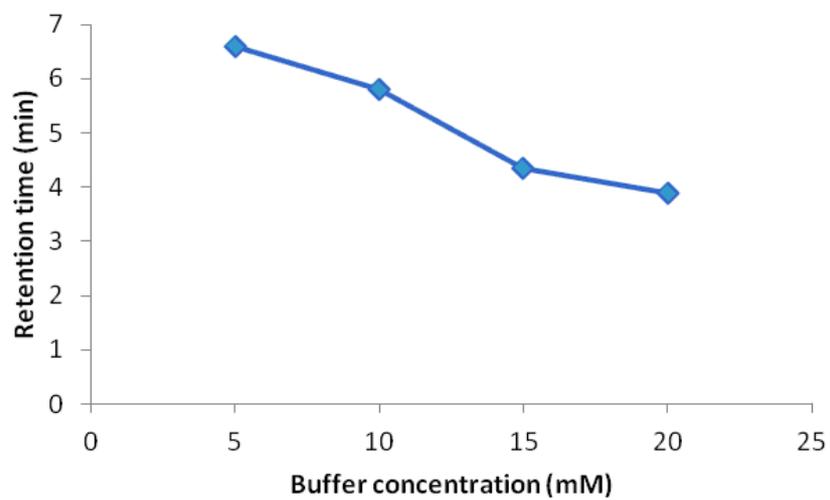**Figure 7. Plot of Retention Time against Water Content in the Mobile Phase for Carbofuran**

Figure 8. Plot of Retention Time and Buffer Concentration in the Mobile Phase for Carbofuran

Column : TSK gel amide 80 (100x0.32 mm)
Eluent : ACN:Amonium Acetate 100 µm (8:2)
Detector : UV 254
Flowrate : 2 µL/min
Injection Vol. : 0.2 µL
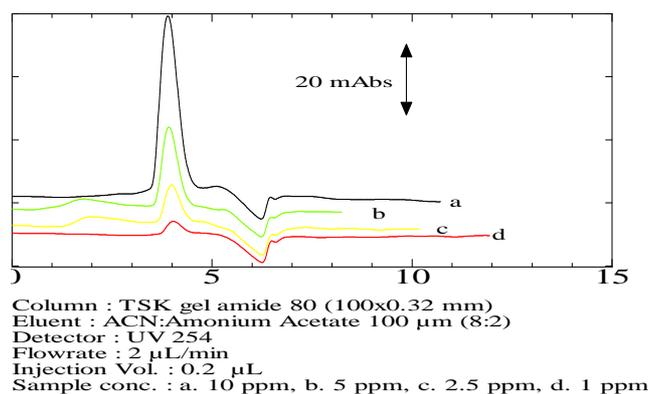Sample conc. : a. 10 ppm, b. 5 ppm, c. 2.5 ppm, d. 1 ppm

Figure 9. Retention Behavior of Carbofuran on Various Concentrations using TSK Gel Amide 80 as the Stationary Phase

Effect of salt buffer concentration. Additives ions are usually added to the mobile phase to control the pH and ion strength. However, they can also affect the polarity of the analyte, resulting in different retention behavior. The effect of salt buffer concentrations was studied by employing different concentrations of ammonium acetate (5, 10, 15, and 20 mM) in the mobile phase. The plot of the retention factor to the concentration of ammonium acetate is shown in Figure 8, which shows that the retention time decreased upon the increase of ammonium acetate concentration. Also, a higher salt concentration resulted in a decrease in the retention time of carbofuran. Hence, repulsive electrostatic interaction could take place between the carbofuran and stationary phases.

Analytical performance. Figure 9 shows the various concentrations of carbofuran were conducted under HILIC mode. According to the chromatogram below, accuracy, RSD percentage, LOD and LOQ value can be calculated. The values are 101±10.1, 10.01%, 0.66 mg/L, and 2.22 mg/L, respectively. Compared to a conventional column, it has a better result on carbofuran determination, since it is still within acceptance value. The maximum residue limit of carbofuran was between 0.05-10 mg/kg on vegetables [38]; so it is useful for determining carbofuran residue on vegetables.

Conclusions

The use of TSKgel®Amide-80 as the stationary phase showed great potential in the determination of carbofuran. The observed retention behavior indicated the existence of an HILIC mechanism in the determination of carbofuran using TSKgel®Amide-80 as the stationary phase. TSKgel®Amide-80 represented a good performance in carbofuran determination, even with the application of 10 cm length column capillary liquid chromatography.

Aknowledgments

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