CHAPTER III

EXPERIMENTAL

3.1 Instrument and apparatus

3.1.1 Liquid chromatography (LC)

Micro-LC system in this research was altered from conventional LC system by connected T-splitter between an injector and a capillary column as shown in Figure 3.1. This component was put in for reduce the volume of mobile phase to suit a capillary column. Any part of LC system is still the same which consist of pump (Jasco PU-980 Intelligent HPLC pump, Japan), injector (Rheodyne 7125 injector, Germany), UV-Visible Detector (Jasco UV-2075 plus intelligent UV-Visible Detector, Japan) and Data acquisition (Power Chrom 280 Data acquisition).

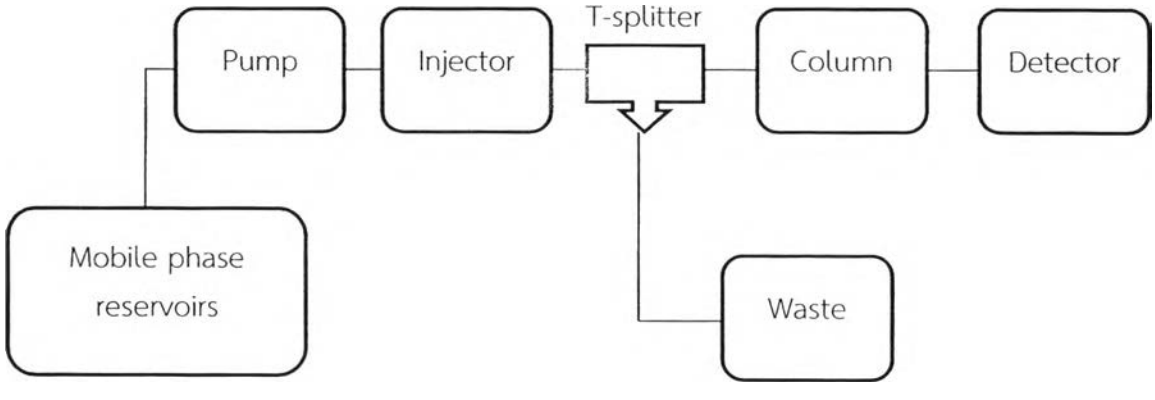

Figure 3.1 The schematic diagram of capillary liquid chromatography instrument.

3.1.2 Milli-Q (Merck, Germany)

3.1.3 pH meter (Model 744, Metrohm, Herisau, Switzerland)

3.1.4 Micropipettes 10-100, 100-1000 µm and tips,

3.1.5 Volumetric flask 25.00, 50.00, 100.00, 250.00, 500.00 and 1000.00 mL

3.1.6 Beakers 5, 10, 50, 100, 250, 1000 mL.

3.2 Chemicals

3.2.1 Preparation of a C_{18}-AP silica monolithic capillary column

Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMS), poly-(ethylene glycol) (all of reagent from Merck, Germany) and urea (Sigma-Aldrich, Germany) were mixed together to synthesis a bared monolithic silica capillary column. Then column was modified with octadecyltrimethoxysilane (ODS) and aminopropyl- trimethoxysilane (APTMS) (Acros Organics, Belgium).

3.2.2 Preparation of a C_{14}-amine embedded silica monolithic capillary column

Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMS), poly-(ethylene glycol) (all of reagent from Merck, Germany) and urea (Sigma-Aldrich, Germany) were mixed together to synthesis a bared monolithic silica capillary column. Then column was modified with glycidoxytrimethoxysilane (GPTMS) and tetradecylamine (Acros Organics, Belgium).

3.2.3 Chromatographic characterization by Tanaka's procedure

Reagents of Tanaka's test are butylbenzene, pentylbenzene, triphenylene, o-terphenyl, benzylamine, phenol and caffeine (Fluka, Sigma-Aldrich and Merck). All reagents at a concentration of 50 mgL^{1} were prepared in methanol.

3.2.4 Separation performance for modified monolithic silica capillary column

There are five groups of test analytes. Mixture 1 was an alkylbenzenes mixture which composed of ethylbenzene ($-C_2H_5$), propylbenzene ($-C_3H_7$), butylbenzene ($-C_4H_9$) and pentylbenzene ($-C_5H_{11}$) (Fluka, Germany), Mixture 2 was a benzoic acids mixture which contained 4-hydroxy benzoic acid, 3,4-dihydroxybenzoic acid and 3,4,5-trihydroxybenzoic acid (Sigma-Aldrich, Germany). Mixture 3 was a benzoate anions mixture which were 3-fluorobenzoic acid, 3-chlorobenzoic acid and

3,4-dichlorobenzoic acid (Sigma-Aldrich, Germany). Mixture 4 was polycyclic aromatic hydrocarbons (PAHs) which were anthracene, triphenylene, fluoranthene and benzo[k]fluoranthene (Sigma-Aldrich, Germany). Mixture 5 was a basic analytes mixture which compposed of diphenylamine, aniline, N-methylaniline and N,N-dimethylaniline (Fluka, Germany). All of these test analytes were prepared at a concentration of 50 mgL^{-1} in methanol. Mixture 1 and 2 were evaluated the separation performance based on hydrophobicity. The separation based on anion exchange behavior was tested by mixture 3. The column selectivity was evaluated by mixture 4 and the improvement of peak symmetry for basic analytes was examined by mixture 5.

3.3 The preparation of mixed-mode monolithic silica capillary column

3.3.1 The preparation of bared monolithic silica capillary column

A bared silica monolithic capillary column was prepared follow Kobayashi's method [42]. To clean the surface of capillary column (300 cm × 200.9 μm I.D. × 353.0 μm O.D.), the column was pretreated with 1 M NaOH at 40 °C for 3 hours and washed with water, 1 M HCl at 40 °C for 2 hours and washed with water and acetone, respectively. The preparation condition was as follow: TMOS 6.75 ml, MTMS 2.25 ml (ratio 3:1), PEG 0.9 g, urea 2.025 g and 20 mL of 0.01 M acetic acid were mixed together and stirred. Then a mixture was introduced into the pretreated capillary column and aged at 40 °C for 24 hours. The mesopores were tailored through the hydrolysis of urea by heating at 120 °C. Finally, the bared silica capillary column was washed with methanol for 7 days, and heated at 330 °C for 24 hours.

3.3.2 The modification method of C_{18}-AP silica monolithic capillary column

A pretreated monolithic silica capillary column was functionalized with ODS and APTMS as method of Ye et al. [9]. The first step, dried toluene was flowed through the monolithic silica capillary column. Then a mixture of ODS (9%) and APTMS (1%) in dried toluene was flowed through the capillary column by

pressurized with nitrogen gas for 1 hour and reacted at 110 °C for 1 hour, this step was repeated four times. Finally, the modified monolithic silica capillary column was washed the unreacted chemicals with methanol for 2 hours.

3.3.3 The modification method of C_{14}-amine embedded monolithic silica capillary column

A pretreated monolithic silica capillary column was functionalized with GPTMS and tetradecylamine as method of Ye et al. [30]. The first step, dried toluene was flowed through the monolithic silica capillary column. Then, 12% GPTMS in dehydrated toluene was flowed through the capillary column by pressurized with nitrogen gas for 1 hour and reacted at 110 °C for 2 hours, this step was repeated four times. The column was washed with toluene, methanol and acetone for 3 hours and then purged with nitrogen for 2 hours at 50 °C. The second step, 8% tetradecylamine in dehydrated toluene was introduced through the capillary column by pressurized with nitrogen gas for 1 hour and reacted at 90 °C for 2 hours, this step was repeated for four times. Finally, toluene and methanol was introduce sequentially to flush the column.

3.4 Chromatographic characterization by Tanaka's procedure

In order to characterize the basic chromatographic of these stationary phase, Tanaka's test [2] was used to evaluated hydrophobicity, shape selectivity, ion exchange and silanol activity at low pH and silanol activity at neutral pH.

3.4.1 Hydrophobicity (α_{CH2})

There are two probe analytes (butylbenzene and pentylbenzene) to test hydrophobicity of the column. This is based upon hydrophobic interaction between analytes and long alkyl chain of column and was determined from retention factor (k') and selectivity (α_{CH2}) of the test probes in a mobile phase of 80% methanol.

3.4.2 Shape selectivity ($\alpha_{T/O}$)

We defined this value as the ability of a phase to separate two compounds with different structures (o-terphenyl and triphenylene) in a mobile phase of 80% methanol.

3.4.3 Ion exchange at low pH ($\alpha_{B/P}$)

At low pH, the residual silanol groups is protonated (-SiOH) while the amine groups and analyte are a positive charge ($-NH_3^+$). Benzylamine and phenol were used to determine an ion exchange capacity of the column in a mobile phase of 30% methanol /70% 0.02 M phosphate buffer, pH 2.7.

3.4.4 Ion exchange at neutral pH ($\alpha_{B/P}$)

Benzylamine and phenol were used to determine an ion exchange capacity of the column at neutral pH. At this condition, the residual silanol groups are deprotonated (-SiO-) and some of amine groups on the modified columns and benzylamine are protonated ($-NH_3^+$). The separation was performed in a mobile phase of 30% Methanol /70% 0.02 M phosphate buffer, pH 7.

3.4.5 Silanolphilic Interaction ($\alpha_{C/P}$)

The hydrogen bonding from silanol groups was determined from the selectivity (α) between caffeine and phenol in a mobile phase of 30% methanol.

3.5 Separation performance of mixed-mode monolithic silica capillary column by LC

The separation performance of the C_{18}-AP and C_{14}-amine embedded monolithic silica capillary column were compared at the linear velocity of 1 mms^{-1}.

3.5.1 Reversed phase mechanism

3.5.1.1 Alkylbenzene separation

The mixture contained 50 mg L^{-1} of alkylbenzenes (ethylbenzene, propylbenzene, butylbenzene and pentylbenzene) was separated in a mobile phase of 80% methanol. The retentions of these compounds on the columns were compared and evaluated the separation based on reversed-phase mechanism.

3.5.1.2 Benzoic acid derivatives separation

The mixture contained 50 mgL^{-1} of benzoic acids (4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid and 3,4,5-trihydroxybenzoic acid) was separated in a mobile phase of 25% methanol/75% 0.02 M phosphate buffer, pH 2.5. We expected that the analytes were separated via hydrophobic mechanism.

3.5.2 Anion exchange mechanism

3.5.2.1 Benzoate anions separation

The anion exchange activity under a condition in which some of amine groups are protonated ($-NH_3^+$) was determined by separating benzoate anions mixture at a concentration of 50 mgL^{-1} (2-fluorobenzoic acid, 3-chlorobenzoic acid and 3,4-dichlorobenzoic acid) in a mobile phase of 30% methanol/70% 0.02 M phosphate buffer, pH 6.7.

3.5.3 Basic compound separation

The separation of basic compounds (aniline, *N*-methylaniline, *N,N*-benzylamine and diphenylamine) was performed in a mobile phase of 55% methanol/45% 0.02 M phosphate buffer, pH 7.

3.5.4 Polycyclic aromatic hydrocarbons (PAHs) separation

The separation of PAHs (Anthracene, Fluoranthene, triphenylene and benzo[k]fluoranthene) was performed in a mobile phase of 60% methanol.