การแยกแอลคิลฟีนอลด้วยแก๊สโครมาโทกราฟีที่ใช้อนุพันธ์ไซโคลเดกซ์ทรินเป็นเฟสคงที่

นางสาวเอมอร พ้นภัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

SEPARATION OF ALKYLPHENOLS BY GAS CHROMATOGRAPHY USING DERIVATIZED CYCLODEXTRINS AS STATIONARY PHASES

Miss Aimon Phonphai

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2015

Thesis Title	SEPARATION OF ALKYLPHENOLS BY GAS CHROMATOGRAPHY USING DERIVATIZED CYCLODEXTRINS AS STATIONARY PHASES
By	Miss Aimon Phonphai
Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Assistant Professor Aroonsiri Shitangkoon, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

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เอมอร พ้นภัย : การแยกแอลคิลฟีนอลด้วยแก๊สโครมาโทกราฟีที่ใช้อนุพันธ์ไซโคลเดกซ์ทรินเป็นเฟสคงที่ (SEPARATION OF ALKYLPHENOLS BY GAS CHROMATOGRAPHY USING DERIVATIZED CYCLODEXTRINS AS STATIONARY PHASES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.อรุณศิริ ชิตางกูร, 57 หน้า.

ศึกษาการแยกสารผสมของแอลคิลฟีนอล 22 ชนิดในการวิเคราะห์ครั้งเดียวด้วยแคพิลลารีแก๊สโครมาโทกราฟีโดยใช้อนุพันธ์ไซโคลเดกซ์ทริน 3 ชนิดผสมในพอลิไซลอกเซนเป็นเฟสคงที่ อนุพันธ์ไซโคลเดกซ์ทริน ได้แก่ เฮกซะคิส(2,3-ได-*โอ*-แอซีทิล-6-*โอ-เทอร์ต*-บิวทิลไดเมทิลไซลิล)-แอลฟาไซโคลเดกซ์ทริน (ASiAc), เฮปตะคิส(2,3-ได-*โอ*-แอซีทิล-6-*โอ-เทอร์ต*-บิวทิลไดเมทิลไซลิล)-บีตาไซโคลเดกซ์ทริน (BSiAc) และออกตะคิส(2,3-ได-*โอ*-แอซีทิล-6-*โอ-เทอร์ต*-บิวทิลไดเมทิลไซลิล)-แกมมาไซโคลเดกซ์ทริน (GSiAc) พบว่าทั้ง 3 คอลัมน์สามารถใช้แยกสารผสมแอลคิลฟีนอลออกจากกันได้ แต่จะเห็นว่าขนาดของวงไซโคลเดกซ์ทรินมีผลต่อการแยก โดยอนุพันธ์ขนาดกลางของ BSiAc ให้ผลการวิเคราะห์ในครั้งเดียวดีที่สุด โดยมีไอโซเมอร์เพียงคู่เดียวที่ไม่สามารถแยกออกจากกันได้ การแยกใช้เวลาา 8.8 นาที ใช้คอลัมน์ยาว 15 เมตร เส้นผ่านศูนย์กลาง 0.25 มิลลิเมตร และใช้โปรแกรมอุณหภูมิจาก 70-128 °C ด้วยอัตรา 10 °C/นาที; คงที่เป็นเวลา 2 นาที; จากนั้นเพิ่มอุณหภูมิถึง 200 °C ด้วยอัตรา 30 °C/นาที นอกจากนี้พบว่าพีกที่ได้จากการวิเคราะห์สารผสมทั้ง 3 คอลัมน์ค่อนข้างสมมาตรแม้จะวิเคราะห์สารโดยตรงโดยไม่ได้เปลี่ยนสารให้อยู่ในรูปอนุพันธ์ก่อนการวิเคราะห์

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5672152523 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORDS:

AIMON PHONPHAI: SEPARATION OF ALKYLPHENOLS BY GAS CHROMATOGRAPHY USING DERIVATIZED CYCLODEXTRINS AS STATIONARY PHASES. ADVISOR: ASST. PROF. AROONSIRI SHITANGKOON, Ph.D., 57 pp.

The single-run separation of twenty-two underivatized C_0-C_3 alkylphenols was studied by capillary gas chromatography using three derivatized cyclodextrins mixed in polysiloxane as stationary phases. These derivatized cyclodextrins were hexakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-alpha-cyclodextrin (ASiAc), heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-beta-cyclodextrin (BSiAc) and octakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-gamma-cyclodextrin (GSiAc). The results showed that three cyclodextrin-based stationary phases could be used to separate the 22 alkylphenol mixture. However, it could be suggested that the size of cyclodextrin ring affect the separation. Among three stationary phases, the medium-size BSiAc gave the best single-run separation with only two co-eluted compounds. The mixture could be separated in 8.8 minutes using a 15 m long, 0.25 mm i.d. column and a temperature program from 70-128 °C at 10 °C/min, held for 2 min; then to 220 °C at 30 °C/min. Moreover, direct analyses of the mixture without prior derivatization gave acceptable peak shapes on these three columns.

Field of Study: Petrochemistry and Polymer Science Student's Signature .....
Advisor's Signature .....

Academic Year: 2015

ACKNOWLEDGEMENTS

First of all, I would like to express sincere gratitude to my advisor, Assistant Professor Dr. Aroonsiri Shitangkoon, who has provided me with valuable support and guidance throughout my time as both an undergraduate and a graduate student. This thesis would not have been possible without her kindness. I would also like to thank my committee members Professor Dr. Pattarapan Prasassarakich, Assistant Professor Dr. Amarawan Intasiri and Assistant Professor Dr. Jongkolnee Jongaramruong for their valuable comments and suggestions.

Special recognition is given to Professor Gyula Vigh for his kindness of providing the cyclodextrin derivatives used in this research.

I am grateful to the funding support from Petrochemistry and Polymer Science Program, Faculty of Science, Chulalongkorn University. My appreciation also extends to the staffs at the program for their helpfulness.

My friends and all members in chiral separation group who have made the lab a convivial place to work. They all had encouraged me in research and life during the period of the lab.

Lastly, I would like to extend my deepest thanks to my beloved family for their continuous support throughout my entire education.

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CHAPTER I

INTRODUCTION

Presently, petrochemical products have had a considerable effect on our food, clothing, housing and health. They are crucial parts of the production in pharmaceutical, agricultural and petrochemical industries. Accordingly, the use of mass production has led to a growth of numerous environmental problems such as soil, water and air pollution. Among the most important pollution sources are phenolic compounds.

Phenol and low molecular weight alkylphenols (C_0-C_3 APs) are essential chemicals for petrochemical industries. They are served as starting materials for various products such as drugs, plastics, dyes, pesticides, herbicides, wood extractives, antioxidants, textiles, and detergents, etc. As a result of their relatively good water solubility, they are generally found as contaminants in wastewater from industries [1-5].

The AP contamination into the environment can cause toxicity and is hazardous to the health of animals and humans. APs are rapidly absorbed following inhalation, ingestion, and through the skin. Breathing APs into the body for a short time is irritating to the respiratory tract, headaches and burning eyes. Long term exposure to high concentration of APs can cause weakness, muscle pain, anorexia, weight loss and also effects on the heart. Ingestion of APs for a short period may cause burning to the mouth and throat, wounds to the mouth, oesophagus and stomach, abdominal pain, vomiting and diarrhoea, whereas ingestion of a large amount of phenols is usually fatal. Dermal contact with significant amounts of phenol may lead human to get liver damage, diarrhoea, dark urine, damage to the red blood cells, and in severe cases death may occur [6].

Owing to their harmful effects, both the US Environmental Protection Agency (EPA) and the European Union (EU) have classified a number of APs as priority pollutants [7]. Moreover, the 80/778/EC directive limits 0.5 μg/L as maximum

concentration for total phenols in drinking water, while individual concentration of phenols should be under 0.1 μg/L [8]. In Thailand, the Ministry of Industry restricts the maximum concentration of total phenolic compounds in wastewater not to exceed 1 mg/L [9]. Consequently, there is currently a need for an accurate, sensitive, efficient and rapid method to identify and quantify each isomer of APs.

Many analytical techniques have been used for analysis of APs, including high performance liquid chromatography (HPLC) [10], capillary electrophoresis (CE) [11] and gas chromatography (GC) [10, 12, 13]. Among them, GC is the most generally used technique for the separation of low molecular weight APs due to their high volatility. Previously, there were reports on the separation of derivatized and underivatized APs by GC using different types of polysiloxanes as stationary phases [10, 12, 13]. However, coelution and incomplete separation were observed for several compounds on polysiloxane columns.

Cyclodextrins (CDs) and their derivatives were introduced as chiral selectors for GC, HPLC, and CE. Several CD derivatives have proven to be good chiral selectors for GC separation of enantiomers and isomers for various classes of compounds [14-16]. The uses of different alkylated β-CDs as GC stationary phases for successful isomer separations of APs were demonstrated for cresols only and dimethylphenols [17-19]. Later, the single-run GC separations of a mixture of underivatized C_0-C_3 APs were studied using (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-α-, β- and γ-CDs as stationary phases [20]. It was shown that the derivatized β-CD provided the best results among three different sized of CDs. However, some incomplete separations were still observed.

This research therefore aims to determine the optimum GC condition for the mixture of twenty-two C_0-C_3 AP isomers in a single-run using (2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-α-, β- and γ-CDs as stationary phases. Based on good enantiomer separation properties for various classes of compounds of these three CD derivatives, we expect that they will provide different and good selectivity for the separation of C_0-C_3 AP isomers.

CHAPTER II

THEORY

2.1 C_0-C_3 alkylphenols

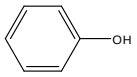
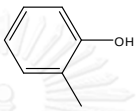
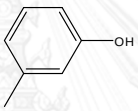
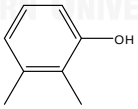
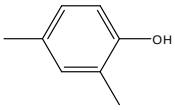
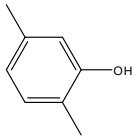
Phenol (P) is the aromatic alcohol consisting of a hydroxyl group (-OH) attached to the carbon atom of a benzene ring.

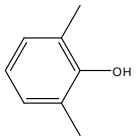
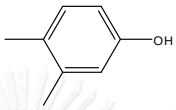
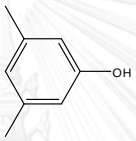
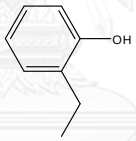
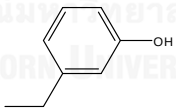
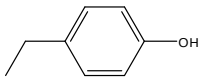
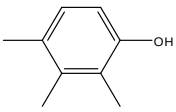
Alkylphenols (APs) are a family of organic compounds manufactured by the alkylation of phenols. The low molecular weight of APs can be categorized into four groups according to the number of carbon atom of alkyl substituted on the phenolic ring.

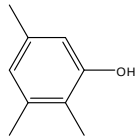
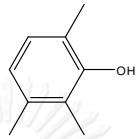
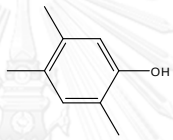
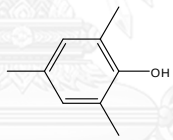
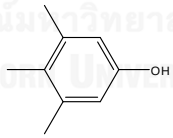
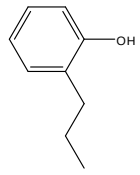
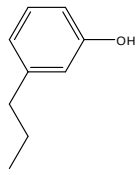
- C_0 AP is an unsubstituted phenol.
- C_1 APs consist of three isomers of methylphenols (MPs).
- C_2 APs have nine isomers including six dimethylphenols (DMPs) and three ethylphenols (EPs).
- C_3 APs have twelve isomers including six trimethylphenols (TMPs), three propylphenols (PPs) and three isopropylphenols (IPPs).

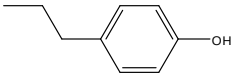
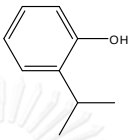
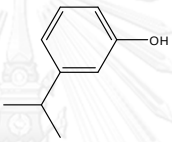
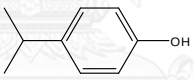
The physical properties of APs are most strongly influenced by the type of alkyl substituent, its branching, their number and position of substituent on the aromatic ring. Most APs in pure form are either colorless solid or liquid at room temperature with smelling of medicine. Structure and some physical properties of C_0-C_3 APs are shown in Table 2.1.

Table 2.1 Structure and physical properties of C_0-C_3 APs [21-25].

compound	abbreviation	structure	MW (g/mol)	mp/bp (°C)	water solubility at 25 °C (g/L)
phenol	P		94.11	mp: 40.8 bp: 181.8	82.8
2-methylphenol (*o*-cresol)	2-MP		108.14	mp: 29-31 bp: 191	25.9
3-methylphenol (*m*-cresol)	3-MP		108.14	mp: 8-10 bp: 203	22.7
4-methylphenol (*p*-cresol)	4-MP		108.14	mp: 32-34 bp: 202	21.4
2,3-dimethylphenol	2,3-DMP		122.16	mp: 70-73 bp: 217	4.57
2,4-dimethylphenol	2,4-DMP		122.16	mp: 22-23 bp: 211-212	7.87
2,5-dimethylphenol	2,5-DMP		122.16	mp: 75-77 bp: 212	3.54

compound	abbreviation	structure	MW (g/mol)	mp/bp (°C)	water solubility at 25 °C (g/L)
2,6-dimethylphenol	2,6-DMP		122.16	mp: 43-45 bp: 203	6.05
3,4-dimethylphenol	3,4-DMP		122.16	mp: 63-67 bp: 227	4.76
3,5-dimethylphenol	3,5-DMP		122.16	mp: 61-64 bp: 222	4.88
2-ethylphenol	2-EP		122.16	mp: -18 bp: 195-197	5.34
3-ethylphenol	3-EP		122.16	mp: -4 bp: 218	3.34
4-ethylphenol	4-EP		122.16	mp: -4 bp: 218	3.34
2,3,4-trimethylphenol	2,3,4-TMP		136.19	mp: 81 bp: 236	0.67

compound	abbreviation	structure	MW (g/mol)	mp/bp (°C)	water solubility at 25 °C (g/L)
2,3,5-trimethylphenol	2,3,5-TMP		136.19	mp: 92-95 bp: 230-231	0.76
2,3,6-trimethylphenol	2,3,6-TMP		136.19	mp: 62 bp: 226	1.58
2,4,5-trimethylphenol	2,4,5-TMP		136.19	mp: 72 bp: 232	0.67
2,4,6-trimethylphenol	2,4,6-TMP		136.19	mp: 70-73 bp: 220	1.01
3,4,5-trimethylphenol	3,4,5-TMP		136.19	mp: 108 bp: 248.5	0.67
2-propylphenol	2-PP		136.19	mp: 7 bp: 224-226	1.04
3-propylphenol	3-PP		136.19	mp: 26 bp: 228	0.83

compound	abbreviation	structure	MW (g/mol)	mp/bp (°C)	water solubility at 25 °C (g/L)
4-propylphenol	4-PP		136.19	mp: 22 bp: 232	1.28
2-isopropylphenol	2-IPP		136.19	mp: 12-16 bp: 212-213	1.15
3-isopropylphenol	3-IPP		136.19	mp: 25 bp: 228	0.96
4-isopropylphenol	4-IPP		136.19	mp: 59-62 bp: 212	1.10

APs are widely used in industrial processes. They were classified as aromatic intermediate petrochemicals, and used as feedstock for the synthesis. Phenolic residues therefore are usually found in the environmental pollution. According to their leaching behavior, they are commonly presented as groundwater contaminants.

Exposures to APs cause harmful effects to living organisms even at low concentrations. They are considered to be quite toxic to humans by any route of exposure including skin. Inhalation and dermal exposure to APs is highly irritating to the skin, eyes and mucous membranes. Ingestion of highly concentrated APs can cause burning to the mouth and throat, wounds to mouth, oesophagus and stomach, abdominal pain, vomiting and diarrhoea. The effects on the central nervous system, heart, blood vessels, lung and kidneys usually lead to severe toxicity and death. From many adverse effects of APs on humans, EPA has determined cancer

risks of them and classified three cresol isomers as Group C, possible human carcinogens. Some applications and toxicity of C_0-C_3 APs were shown in Table 2.2.

Table 2.2 Some applications and toxicity of C_0-C_3 APs [26-29].

chemical	application	toxicity
P	- phenolic resins - caprolactam - bisphenol A - slimicides - disinfectants - medical products - abrasives - adhesives and sealant chemicals - flame retardants - fuels and fuel additives - intermediates - ion exchange agents - laboratory chemicals - odor agents - solvents - paints and coatings - plastic and rubber products - toys, playground, and sporting equipment	- $LD_{50\ (oral,\ rat)}$ 317 mg/kg - irritation eyes, nose, throat; anorexia, weight loss; lassitude (weakness, exhaustion), muscle ache, pain; dark urine; cyanosis; liver, kidney damage; skin burns; dermatitis; ochronosis; tremor, convulsions, twitching
2-MP	- adhesives and sealant chemicals - fuels and fuel additives - intermediates - solvents (for cleansing or degreasing) - solvents (which become part of product	- $LD_{50\ (oral,\ rat)}$ 121 mg/kg - irritation eyes, skin, mucous membrane; central nervous system effects: confusion, depression, respiratory failure; dyspnea (breathing difficulty), irregular rapid respiratory, weak

chemical	application	toxicity
	formulation or mixture) - paints and coatings	pulse; eye, skin burns; dermatitis; lung, liver, kidney, pancreas damage
3-MP	- adhesives and sealant chemicals - dyes - intermediates - plasticizers - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - electrical and electronic products - fabric, textile, and leather products - paints and coatings	- $LD_{50\ (oral,\ rat)}$ 242 mg/kg - irritation eyes, skin, mucous membrane; central nervous system effects: confusion, depression, respiratory failure; dyspnea (breathing difficulty), irregular rapid respiratory, weak pulse; eye, skin burns; dermatitis; lung, liver, kidney, pancreas damage
4-MP	- adhesives and sealant chemicals - dyes - intermediates - odor agents - plasticizers - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture)	- $LD_{50\ (oral,\ rat)}$ 207 mg/kg - irritation eyes, skin, mucous membrane; central nervous system effects: confusion, depression, respiratory failure; dyspnea (breathing difficulty), irregular rapid respiratory, weak pulse; eye, skin burns; dermatitis; lung, liver, kidney, pancreas damage

chemical	application	toxicity
	- electrical and electronic products - fabric, textile, and leather products - paints and coatings	
2,3-DMP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings	- $LD_{50\ (intravenous,\ mouse)}$ 56 mg/kg - burning sensation, coughing, wheezing, laryngitis, and shortness of breath. Other symptoms may include severe irritation or burning of the eyes and skin; irritation of the respiratory system; dizziness, stomach pain, exhaustion, and coma. It can cause headaches, nausea, and vomiting. It can also cause corrosion of the mucous membranes, upper respiratory tract, skin, and eyes. Inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi; chemical pneumonitis; and pulmonary edema. Chronic exposure may cause liver or kidney damage.
2,4-DMP	- adhesives and sealant chemicals - intermediates - plating agents and surface	- $LD_{50\ (oral,\ rat)}$ 2,300 mg/kg - severe irritation of the skin and eyes, dizziness, stomach pain,

chemical	application	toxicity
	treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings	exhaustion and damage to the liver and kidneys - headache, nausea and vomiting - severe burns of the eyes and skin, irritation of the respiratory tract and coma - corrosion of tissue of the mucous membranes and upper respiratory tract, eyes and skin - inhalation may result in burning sensation, coughing, wheezing, laryngitis and short ness of breath - inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema - symptoms of exposure to this class of compounds include profuse sweating, skin sensitization, painless blanching or erythema of the skin, intense thirst diarrhea, cyanosis from methemoglobinemia, hyperactivity, stupor, fall in blood pressure, hyperpnea, abdominal pain, hemolysis and convulsions. If death from respiratory failure is not

chemical	application	toxicity
		immediate, jaundice and oliguria or anuria may occur.
2,5-DMP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings	- $LD_{50\ (oral,\ rat)}$ 444 mg/kg - severe irritation and burns of the skin and eyes; irritation of the respiratory tract, dizziness, stomach pain, exhaustion, coma and damage to the liver or kidney - headache, nausea and vomiting - corrosion of the tissues of the mucous membranes and upper respiratory tract, eyes and skin - inhalation may result in burning sensation, coughing, wheezing, laryngitis and shortness of breath - symptoms of exposure to this class of compounds include skin sensitization, profuse sweating, painless blanching or erythema of the skin, intense thirst, diarrhea, cyanosis from methemoglobinemia, hyperactivity, stupor, blood pressure fall, hyperpnea, abdominal pain, hemolysis and convulsions. If death from respiratory failure is not

chemical	application	toxicity
		immediate, jaundice and oliguria or anuria may occur.
2,6-DMP	- adhesives and sealant chemicals - intermediates - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings	- $LD_{50\ (oral,\ rat)}$ 296 mg/kg - burning sensation, coughing, wheezing, laryngitis, and shortness of breath - severe irritation or burning of the eyes and skin - irritation of the respiratory system; dizziness, stomach pain, exhaustion, and coma - headaches, nausea, and vomiting; corrosion of the mucous membranes, upper respiratory tract, skin, and eyes - inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi; chemical pneumonitis; and pulmonary edema - chronic exposure may cause liver or kidney damage
3,4-DMP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture)	- $LD_{50\ (oral,\ mouse)}$ 400 mg/kg - burning sensation, coughing, wheezing, laryngitis, and shortness of breath - severe irritation or burning of the eyes and skin - irritation of the respiratory

chemical	application	toxicity
	- paints and coatings	system - dizziness, stomach pain, exhaustion, and coma - headaches, nausea, and vomiting - corrosion of the mucous membranes, upper respiratory tract, skin, and eyes
3,5-DMP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings	- $LD_{50\ (oral,\ rat)}$ 608 mg/kg - corrosion of the mucous membranes, upper respiratory tract, skin, and eyes; burning sensation, coughing, wheezing, laryngitis, and shortness of breath - severe irritation or burning of the eyes and skin - irritation of the respiratory system - dizziness, stomach pain, exhaustion, and coma - headaches, nausea, and vomiting - dermatitis, muscular weakness, dimness of vision, irregular and rapid breathing, ringing in the ears, weak pulse, dyspnea, and unconsciousness - damage the kidneys, liver,

chemical	application	toxicity
		pancreas, and spleen - edema of the lungs
2-EP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - paints and coatings	- $LD_{50\ (oral,\ mouse)}$ 600 mg/kg - skin irritation, serious eye damage, may cause respiratory irritation
3-EP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings	- irritating to eyes, respiratory system, and skin - severe skin burns and eye damage
4-EP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings - castoreum - food flavor	- $LD_{50\ (oral,\ rat)}$ > 2000 mg/kg - irritating to eyes, respiratory system, and skin - severe skin burns and eye damage
2,3,4-TMP	- raw material	- $LD_{50\ (oral,\ rat)}$ = 318 mg/kg - irritation to respiratory tract, eyes, skin and digestion system

chemical	application	toxicity
2,3,5-TMP	- laboratory chemicals - manufacture of substances	- severe skin burns and eye damage - cough, shortness of breath, headache, nausea, vomiting
2,3,6-TMP	- intermediate for synthetic vitamin E - intermediate for antioxidants and plastics	- $LD_{50\ (oral,\ rat)}$ > 2,000 mg/kg - irritating to eyes, respiratory system, and skin
2,4,5-TMP	- raw material	- $LD_{50\ (intraperitoneal,\ mouse)}$ > 500 mg/kg - severe skin burns and eye damage
2,4,6-TMP	- adhesives and sealant chemicals - plating agents and surface treating agents - solvents (for cleansing or degreasing) - solvents (which become part of product formulation or mixture) - paints and coatings	- $LD_{50\ (oral,\ mouse)}$ 10,000 mg/kg - extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin - spasm, inflammation and edema of the larynx and bronchi - pneumonitis, pulmonary edema, burning sensation - cough, wheezing, laryngitis, shortness of breath, headache, nausea
3,4,5-TMP	- laboratory chemicals - synthesis of substances	- severe skin burns and eye damage - cough, shortness of breath, headache, nausea, vomiting
2-PP	- medical	- $LD_{50\ (oral,\ rat)}$ 500 mg/kg

chemical	application	toxicity
	- fish flavor - coffee fragrance	- irritating to eyes, respiratory system, and skin
3-PP	- laboratory chemicals - manufacture of substances	- severe skin burns and eye damage
4-PP	- laboratory chemicals - manufacture of substances	- $LD_{50\ (oral,\ rat)}$ 500 mg/kg - irritating to eyes, respiratory system, and skin
2-IPP	- intermediates	- $LD_{50\ (intravenous,\ mouse)}$ 100 mg/kg - severe skin burns and eye damage
3-IPP	- intermediates	- $LD_{50\ (oral,\ mouse)}$ 1,630 mg/kg - severe skin burns and eye damage - extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin - cough, shortness of breath, headache, nausea
4-IPP	- intermediates - spice flavor	- $LD_{50\ (oral,\ mouse)}$ 875 mg/kg - severe skin burns and eye damage - allergy or asthma symptoms or breathing difficulties if inhaled - cough, shortness of breath, headache, nausea, vomiting

2.2 Gas chromatographic separation of C_0-C_3 alkylphenols

GC is one of the most important analytical separation techniques used to analyze volatile and thermally stable organic compounds. Capillary GC provides a significant improvement in separation efficiency, sensitivity and speed of separation. Owing to many advantages of capillary GC, a various kind of compounds can be separated without prior derivatization. Thus, the use of capillary GC has become very popular and has been used in the analysis of APs.

Some studies concerning the direct analyses of APs using polysiloxane as stationary phases were concluded as follow:

Ioppolo and coworkers [12] determined 19 C_0-C_3 APs in crude oils, including phenol, 12 methylphenols, 3 ethylphenols and 3 isopropylphenols. They were analyzed by capillary gas chromatography-flame ionization detection (GC-FID) using capillary columns (60 m long, 0.22 mm i.d.) with three stationary phases of different polarities: nonpolar BP1 (dimethyl siloxane), BP5 (5% diphenyl-95% dimethyl polysiloxane) and medium polarity DB1701 (14% cyanopropylphenyl siloxane). The GC oven was programmed from 40-300 °C for nonpolar BP1 and BP5 capillary columns. Both columns separated the mixture in 20 minutes and gave the same elution order with the exception of 2,4,6-TMP and 2-IPP. The 3-EP and 3,5-DMP were co-eluted and incomplete separations between 4-MP/3-MP and 2,4-DMP/2,5-DMP were observed on two stationary phases. The DB1701 capillary column with a temperature program from 50-280 °C was used to separate the mixture in 20 minutes. The 2,4-DMP/2,5-DMP and 3,5-DMP/2,3-DMP were co-eluted and incomplete separations were observed for 4-MP/3-MP; 4-EP/3-EP; 2-IPP/2,3,6-TMP and 4-IPP/3-IPP. The results showed that the DB1701 column was less effective than BP1 and BP5 columns as several compounds co-eluted and incomplete separations were observed.

Pino and coworkers [30] analyzed 14 phenols in wood extractives, including phenol, 13 methylphenols, 2 ethylphenols, 3-methoxyphenol, 2,6-dimethoxyphenol, 4-chloro-3-methylphenol, eugenol and vanillin. The determination was followed by

solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS). A nonpolar VF-5ms (5% phenyl-95% dimethyl polysiloxane) stationary phase was used to coat on a 30 m × 0.25 mm i.d. capillary column. The separation of phenols was carried out with a temperature program from 60-280 °C in 17 minutes. From the chromatogram, the 3-MP/4-MP; 2,4-DMP/2,5-DMP and 3-EP/3,5-DMP were co-eluted and incomplete separation between 3-EP/3,5-DMP and 2,3-DMP was observed.

Bernardo and coworkers [31] used dispersive liquid–liquid microextraction (DLLME) coupled with GC-MS for determination of 11 alkylphenols in eluates from pyrolysis of solid residues. All phenols were separated on a 30 m × 0.25 mm i.d. capillary column coated with a nonpolar TR-5MS (5% phenyl polysilphenylene-siloxane) stationary phase. The separation of all APs used a temperature program from 35-220 °C and was finished in 23 minutes. The 3-MP and 4-MP were co-eluted and incomplete separation was observed for 2,4-DMP and 2,5-DMP.

To improve efficiency for the separation of polar compounds, the derivatization before GC analysis is performed. Previous studies related to the separation of derivatized APs, such as silylated or acylated APs, by GC using polysiloxane as stationary phases are summarized below:

Bennett and coworkers [10] analyzed for 19 C_0-C_3 APs (including phenol, 13 methylphenols, 2 ethylphenols, propylphenol and 2 isopropylphenols) in crude oils and waters. Alkylphenols were derivatized by silylation and analyzed with two capillary columns of different stationary phase: DB-5 (5% phenyl-95% dimethyl polysiloxane, 30 m × 0.32 mm i.d.) and HP-1 (dimethyl siloxane, 25 m × 0.25 mm i.d.). They used a GC-FID with DB-5 column and a GC-MS with HP-1 column with the same temperature program from 35-300 °C. The separations on both columns were completed in 35 minutes. On DB-5 column, incomplete separation were observed for 2,4-DMP/3,5-DMP and 2,4,6-TMP/2,3,5-TMP; while on HP-1 column, incomplete separation were observed for 2,4-DMP/2,6-DMP/3,5-DMP.

Llompart and coworkers [13] studied the separation of phenol, 11 methylphenols and 18 chlorophenols in water samples using solid-phase microextraction (SPME) coupled with GC-MS. Phenols were analyzed as acetyl derivatives using a 30 m × 0.25 mm i.d. column coated with nonpolar VA-5MS (5% phenyl-95% dimethyl polysiloxane) stationary phase. Chromatographic separation was performed with a temperature program from 60-250 °C and tailing peak shapes were observed. The separation of cresol isomers was finished in 6 minutes but the separation of *m*-cresol from *p*-cresol was not achieved. The analysis of five dimethylphenol isomers (except 3,5-DMP) was completed within 14 minutes and incomplete separation was observed for 2,3-DMP/2,6-DMP.

Vermeulen and coworkers [4] reported the analysis of 35 phenols in water, including phenol, 11 methylphenols, 3 ethylphenols, 2-isopropylphenol and 19 chlorophenols. All phenols were derivatized with acetic anhydride and analyzed by liquid-liquid extraction coupled with GC-MS. The separations were achieved on a 30 m × 0.25 mm i.d. capillary column coated with low polarity DB-XLB (arylene/methyl modified polysiloxane) stationary phase using a temperature program from 40-320 °C within 36 minutes. However, incomplete separations of acetyl derivatives of 3-EP/2-IPP and 2,3-DMP/3,5-DMP/4-EP were found on this column.

Josson and coworkers [32] used the solid-phase analytical derivatization with bis(trimethylsilyl)trifluoroacetamide (BSTFA) for determination of 21 C_1-C_9 APs in fish bile. The separation was performed by GC-MS using a 50 m × 0.25 mm i.d. capillary column coated with nonpolar CP-SIL 8 CB-MS (5% diphenyl-95% dimethyl polysiloxane) stationary phase. The column temperature was programmed from 50-300 °C with the analysis time of 30 minutes. From the chromatogram, the symmetric peak shapes were observed but incomplete separation of 3,5-DMP/2,4-DMP was found.

Although the derivatization prior to GC analysis has been used to improve the separation efficiency of APs, coelution and incomplete separation were still observed for several compounds on polysiloxane columns. In chiral GC, the chiral selectors

were used as stationary phases. Among several chiral selectors, cyclodextrin (CD) and their derivatives are the most commonly used selectors to investigate various chiral compounds.

2.3 Cyclodextrins

CDs are cyclic oligosaccharides consisting of several D-glucose units connected by α-1,4-linkages. They are produced from degradation of starch by the enzyme cyclodextrin glucosyl transferase. The three well-known CDs are α-, β-, and γ-CDs which comprise of 6, 7, and 8 glucose units, respectively. The important characteristics of them are given in Table 2.3.

Table 2.3 Characteristics of α-, β-, and γ-CDs [15, 33].

CD	α	β	γ
number of glucose units	6	7	8
anhydrous molecular weight (g/mol)	972.85	1134.99	1297.14
solubility in water (g/100 mL, 25 °C)	14.5	1.85	23.2
outer diameter (Å)	14.6	15.4	17.5
internal diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
cavity depth (Å)	7.9	7.9	7.9
cavity volume ($Å^3$)	174	262	427
decomposition temperature (°C)	278	299	267

Considering the structures of CD, they can be characterized as a doughnut or hollow truncated cone according to chair conformation of glucose units. Each unit of glucose on the CD ring contains three free hydroxyl groups (-OH), which two of them are secondary hydroxyls and the other is primary hydroxyl. The secondary hydroxyls at C2 and C3 atoms of the glucose unit are at the larger edge, while the primary hydroxyls at C6 atoms are located at the narrower rim (Figure 2.1). Consequently, CDs are relatively hydrophobic inside and hydrophilic outside. As a result of

characteristic structure of CDs, they can provide the inclusion complex formation between the analyte (guest molecule) and CD (host molecule).

Many chiral carbon atoms in CD molecules provide their chiral property which are useful as chiral selector. In addition, CDs can be modified with a variety of functional groups on the primary and/or the secondary hydroxyls to improve their selectivity for the separation. The physical properties of modified CDs such as solubility, polarity, and thermal stability could be changed. Hence, different CD derivatives are applied to chiral separation of different kinds of compounds [15].

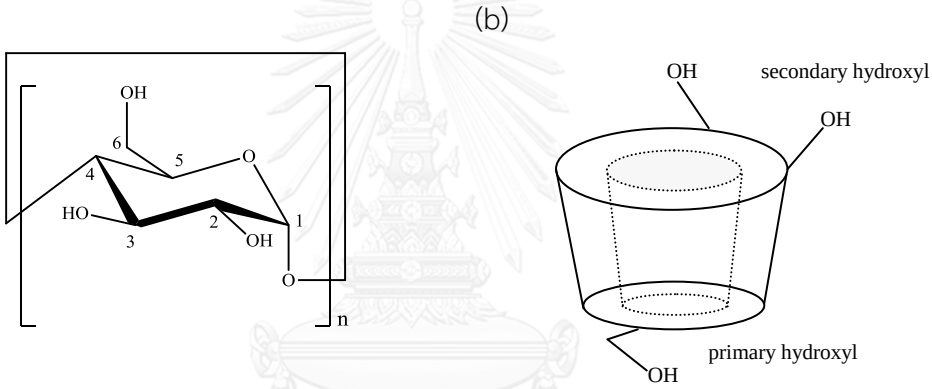


Figure 2.1 (a) A repeating unit of CD and (b) the side view of CD.

2.4 Gas chromatographic separation of C_0-C_3 APs using derivatized CDs

Modified CDs are widely applied as chiral selector for enantiomeric and isomeric separation in chromatographic and electrophoretic methods. They are used in many different fields of research such as pharmaceutical compounds, fragrances, essential oils and herbicides [15, 16]. Isomeric separations of APs have been performed by GC using various types of CD derivatives as chiral stationary phases. Researches related to GC separation of C_0-C_3 APs using CD derivatives are listed below.

Zhang and coworkers [17] synthesized a new gas chromatographic stationary phase, bikis(2,6-di-*O*-pentyl-3-*O*-hex-6-enyl)-pentakis(2,6-di-*O*-pentyl-3-*O*-methyl)-β-CD linked to the polysiloxane, and applied for the GC separation of chiral compounds and isomers. The new 30 m × 0.25 mm i.d. capillary column has high efficiency for the separation of phenol isomers and disubstituted benzenes. Cresol and dimethylphenol isomers were completely separated in 15 minutes. For the separation of cresols (methylphenols), they were separated at 150 ℃ with the elution order of 2-MP, 4-MP and 3-MP, respectively. Dimethylphenols were also successfully separated at 160 ℃ with the elution order of 2,6-DMP/2,4-DMP/2,5-DMP/2,3-DMP/3,5-DMP and 3,4-DMP, respectively. Moreover, this new stationary phase also showed good separation of other isomers including xylenes, chlorotoluenes, bromotoluenes, dichlorobenzenes and dibromobenzenes.

Shi and coworkers [18] synthesized four β-cyclodextrin derivatives including 2,6-di-*O*-pentyl-3-*O*-allyl-β-CD; 2,3-di-*O*-pentyl-6-*O*-allyl-β-CD; 2,6-di-*O*-pentyl-3-*O*-propyl-β-CD and 2,3-di-*O*-pentyl-6-*O*-propyl-β-CD. The four cyclodextrin derivatives were coated on 20 m × 0.25 mm i.d. capillary columns and could completely resolve the three isomers of cresols with the same elution order (2-MP, 4-MP, 3-MP) at 130 ℃ in 15 minutes. Furthermore, some enantiomers such as enantiomers of allethrone acetate, propargyllone acetate, and 2-bromopropionic acid methyl ester were well separated on these stationary phases.

Qi and coworkers [19] prepared a mixed stationary phase containing permethylated-β-CD and perpentylated-β-CD in order to combine different separation properties of both CDs. The mixed stationary phase and each single CD derivative phases were used for the GC separation of aromatic positional isomers and chiral compounds. From the results, cresol isomers were better separated on the mixed stationary phase than on the other two single CD stationary phases. On the mixed stationary phase, cresols were separated at 140 ℃ with the elution order of 2-MP, 4-MP and 3-MP, respectively.

Petcharawuttikri [20] studied the separation of 22 C_0-C_3 alkylphenols by GC-FID using 15 m × 0.25 mm i.d. coated with (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl) derivative of α-, β- and γ-CDs mixed in OV-1701 as stationary phases. On OV-1701 capillary column, the separation of a mixture was finished within 22 minutes with the co-elution of 2,4-DMP and 2,5-DMP and incomplete separations of several compounds. On the other hand, the complete separation of a mixture was found on the derivatized cyclodextrin column. The best separation was achieved with derivatized β-CD with a temperature program from 120-180 °C. The analysis of all APs was completed in 13 minutes on this column. However, α- and γ-CDs did not give satisfying results. The separation on α-CD column was done in 24 minutes with the co-elution of 3-MP/2-EP and 4-IPP/3,4-DMP. While the 3,5-DMP/4-EP and 3-EP/2-IPP were co-eluted on the γ-CD column with the analysis time of 29 minutes. Moreover, chromatograms of all columns showed acceptable peak shapes without any prior derivatization of APs.

CHAPTER III

EXPERIMENTAL

3.1 Preparation of analyte solutions

High purity grade alkylphenols and solvent were purchased from commercial vendors (Fluka, Acros, Merck, Hopkin & Williams and Tokyo Chemical Industries) and were used without further purification. Some APs are not commercially available and are not used in this research: 2,3,4-trimethylphenol; 2,4,5-trimethylphenol and 3-propylphenol. All analytes used in this work are listed as follows:

- phenol [108-95-2], ≥99.5% (Merck)
- 2-methylphenol [95-48-7], ≥98.0% (Fluka)
- 3-methylphenol [108-39-4], ≥98.0% (Hopkin & Williams)
- 4-methylphenol [106-44-5], 99+% (Acros)
- 2,3-dimethylphenol [526-75-0], ≥98.0% (Fluka)
- 2,4-dimethylphenol [105-67-9], ≥97.0% (Fluka)
- 2,5-dimethylphenol [95-87-4], ≥97.0% (Fluka)
- 2,6-dimethylphenol [527-26-1], ≥98.0% (Fluka)
- 3,4-dimethylphenol [95-65-8], ≥98.0% (Fluka)
- 3,5-dimethylphenol [108-68-9], ≥98.0% (Fluka)
- 2-ethylphenol [90-00-6], ≥97.0% (Fluka)
- 3-ethylphenol [620-17-7], >95.0% (TCI)
- 4-ethylphenol [123-07-9], ≥97.0% (Fluka)
- 2,3,5-trimethylphenol [697-82-5], 98+% (Acros)
- 2,3,6-trimethylphenol [2416-94-6], >95.0% (TCI)
- 2,4,6-trimethylphenol [527-60-6], 99% (Acros)
- 3,4,5-trimethylphenol [527-54-8], ≥95.0% (Fluka)

- 2-propylphenol [644-35-9], 98% (Acros)
- 4-propylphenol [645-56-7], >99.0% (TCI)
- 2-isopropylphenol [88-69-7], ≥98.0% (Fluka)
- 3-isopropylphenol [618-45-1], >98.0% (TCI)
- 4-isopropylphenol [99-89-8], 98% (Acros)

Analyte solutions:

- Individual analyte solutions: Twenty two individual analyte solutions were diluted in dichloromethane to the final concentration of ~1 mg/mL.
- Solutions of AP mixture: Four solutions of AP mixture (C_0-C_1; C_2; C_3 and C_0-C_3) were prepared in dichloromethane to the final concentration of ~1 mg/mL for each analyte.

3.2 Gas chromatographic analyses

All analyses were carried out on an Agilent 6890 series gas chromatograph, equipped with a split injector and a flame ionization detector (FID). The injector and detector temperatures were maintained at 250 °C. A split ratio was adjusted to 100:1. The carrier gas was hydrogen with an average linear velocity of 50 cm/s. Three capillary columns of 15 m long, 0.25 mm i.d., were coated with 0.25 μm thick film of stationary phases. Each stationary phase was a mixture of cyclodextrin derivative in polysiloxane OV-1701. Three types of stationary phases used in this research are:

- **ASiAc**: 30.2% hexakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltohexaose mixed in OV-1701
- **BSiAc**: 33.5% heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose mixed in OV-1701
- **GSiAc**: 36.6% octakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltooctaose mixed in OV-1701

All three columns were conditioned at 220 °C until the baseline was stable. Approximately 0.2 µL of solutions were injected at least in duplicate on each condition. Starting from high isothermal temperature, solutions of AP mixture were analyzed. Individual AP solutions were also injected at the same temperature to identify the elution order. From the chromatograms, retention time and peak width were recorded. Retention factor (k′) for each peak and resolution (Rs) between two closest peaks were calculated. The column temperature was further decreased by 10 °C until complete separations of AP mixtures (with the Rs of two closest peaks $\geq$ 1.5) were obtained. The column programmed temperature will be utilized and optimized when isothermal temperature could not provide complete separations of AP mixtures. The best condition for the single-run analysis of AP mixture with the shortest analysis time will be determined.

CHAPTER IV

RESULTS AND DISCUSSION

In GC analysis, the two parameters influencing the separation selectivity are the type of stationary phase and the column temperature. To determine the optimum conditions for the separation of C_0-C_3 APs mixture by GC, the influence of column temperature towards retention (k′) of all analytes was also studied. Plots of ln k′ versus 1/T of all APs for each column were constructed and they all gave linear relationship with good correlation coefficient (R^2 greater than 0.9985). The ln k′ versus 1/T plots were utilized in adjusting the separation temperature of closest peaks. For example, considering the separation between 2,3,6-TMP and 3-EP, their ln k′ versus 1/T plots were shown in Figure 4.1. Both lines merge at high temperature and begin to separate at temperature lower than 140 °C ($1/T = 2.42 \times 10^{-3}$ K^{-1}). This suggested that the separation between 2,3,6-TMP and 3-EP could be achieved at temperature below 140 °C. Nonetheless, the lower the separation temperature, the longer the analysis time. The optimum condition for the separation of 22 APs mixture on each column was the temperature that provided the best single-run with shortest analysis time, which could be found from the highest temperature that provide complete separation of any two closest peaks.

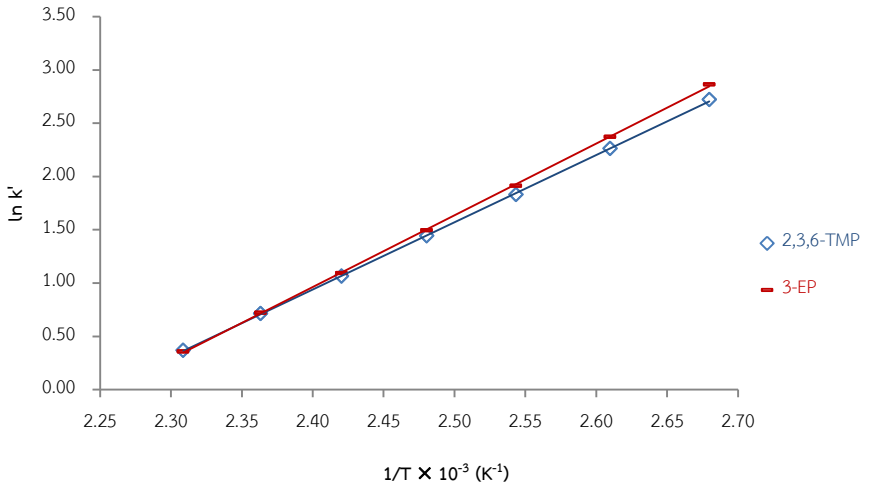


Figure 4.1 Relationship of ln k′ versus 1/T of 2,3,6-TMP and 3-EP on ASiAc column.

4.1 ASiAc column

The influence of column temperature on analyte retention on ASiAc column, a small-size CD, was shown as a plot of ln k′ versus 1/T in Figure 4.2. To determine the optimum condition for the separation of the mixture of all twenty two C_0-C_3 APs, the conditions for the separation of each group of AP isomers (C_0-C_1, C_2, and C_3 APs) were studied.

Firstly, the separation of the mixture of four C_0-C_1 APs was studied. P and 2-MP were well separated from the other two compounds even at high temperature. However, 3-MP and 4-MP closely eluted at all studied temperatures as seen from the ln k′ versus 1/T lines of 4-MP and 3-MP (Figure 4.2). The elution order of C_0-C_1 APs was agreed with their boiling points. P, with the lowest boiling point (182 ℃) among the four compounds, eluted first followed by 2-MP (191 ℃). 4-MP (202 ℃) and 3-MP (203 ℃), having similar boiling points, coeluted at high temperature and was the last eluted peak. When the column temperature was decreased, the separation was improved. The column temperature were decreased to 70 ℃ to achieve a baseline separation between 4-MP and 3-MP (Rs = 1.57); however, the analysis time was quite long (29.4 minutes). Thus, the column temperature of 80 ℃ was selected for the separation of four C_0-C_1 APs with the total analysis time of 16.6 minutes and the resolution between 4-MP and 3-MP of 1.38. The elution order of P > 2-MP > 4-MP > 3-MP was observed (Figure 4.3 a).

Next, the separation of the mixture of nine C_2 AP isomers was studied in a similar fashion. At all temperatures studied, the separation of 2,4-DMP/2,5-DMP and 4-EP/3-EP pairs could not be achieved, even though they showed slightly different retention times when they were individually injected. Again, compounds with similar boiling points were difficult to separate. Therefore, the optimum column temperature of 95 ℃ was chosen for the separation of nine C_2 AP isomers. The mixture of C_2 AP isomers could be separated into seven peaks within 15.7 minutes with the elution order (and boiling point) of 2,6-DMP (203 ℃) > 2-EP (195-197 ℃) > 2,4-DMP (211-212 ℃)/2,5-DMP (212 ℃) > 2,3-DMP (217 ℃) > 3,5-DMP (222 ℃) > 4-EP (218 ℃)/3-EP (218 ℃) > 3,4-DMP (227 ℃) (Figure 4.3 b). Considering the elution order

of C_2 APs, some APs did not elute according to their boiling points. This suggested that there are additional interactions between analytes and CD which affect the elution order. The resolutions between closest peaks were 1.61 and 1.56 for 2-EP and 2,4-DMP/2,5-DMP and for 3,5-DMP and 4-EP/3-EP peak pairs, respectively.

Next, the separation of nine isomers of C_3 APs was investigated. At 130 °C, the chromatogram showed that 3-IPP (228 °C) and 4-IPP (212 °C) coeluted when other seven compounds could be completely separated within 5.8 minutes (Figure 4.3 c). Interestingly, these two compounds have quite different boiling point values but the separation could not be achieved. When the column temperature was decreased, the separation was improved. Partial separation between 3-IPP and 4-IPP was observed at 90 °C together with a long analysis time of 43.3 minutes. However, 3-IPP with higher boiling point eluted before 4-IPP. The elution order was 2,4,6-TMP (220 °C) > 2,3,6-TMP (226 °C) > 2-IPP (212-213 °C) > 2-PP (224-226 °C) > 3-IPP (228 °C) > 4-IPP (212 °C) > 2,3,5-TMP (230-231 °C) > 4-PP (232 °C) > 3,4,5-TMP (248.5 °C) (Figure 4.3 d). Again, the influence of analyte-CD interaction towards the elution order was realized. An attempt to improve the resolution between 3-IPP and 4-IPP peak pair was made by decreasing the column temperature to 80 °C. Unfortunately, the separation of both IPPs slightly improved and the total analysis time was about 1.8 times longer than the analysis time at 90 °C. Therefore, the optimum column temperature of 90 °C was selected for the separation of nine C_3 AP isomers.

Finally, the optimum condition for the separation of twenty two APs on ASiAc column was investigated. Since the mixture contained twenty two APs with varied polarity and boiling point, they could not be analyzed under isothermal condition. Several temperature programs with different initial column temperature and program rate were examined. The optimum condition was the temperature program from 50-110 °C at 5 °C/min, held for 5 min; then to 220 °C at 5 °C/min and mixture of C_0-C_3 APs could be analyzed within 20 minutes (Figure 4.4). The elution order and retention times of all analytes are shown in Table 4.1. From the chromatogram, the separation of 2,4-DMP from 2,5-DMP and 3-EP from 4-EP were still not achieved which similar to the result from the separation of C_2 isomers. In addition, the peak of

(3-EP/4-EP) became to merge with 2-IPP and the peak of 3,4-DMP became to merge with 2-PP. Overall, the separation of twenty two APs on this small-size α-CD column could be separated into eighteen peaks with 3 coeluted peaks of 2,5-DMP/2,4-DMP; 3-EP/4-EP/2-IPP; and 3,4-DMP/2-PP. Moreover, four incomplete separations (Rs < 1.5) were observed for 4-MP and 3-MP; (2,5-DMP/2,4-DMP) and 2,4,6-TMP; 3,5-DMP and (3-EP/4-EP/2-IPP); and 3-IPP and 4-IPP.

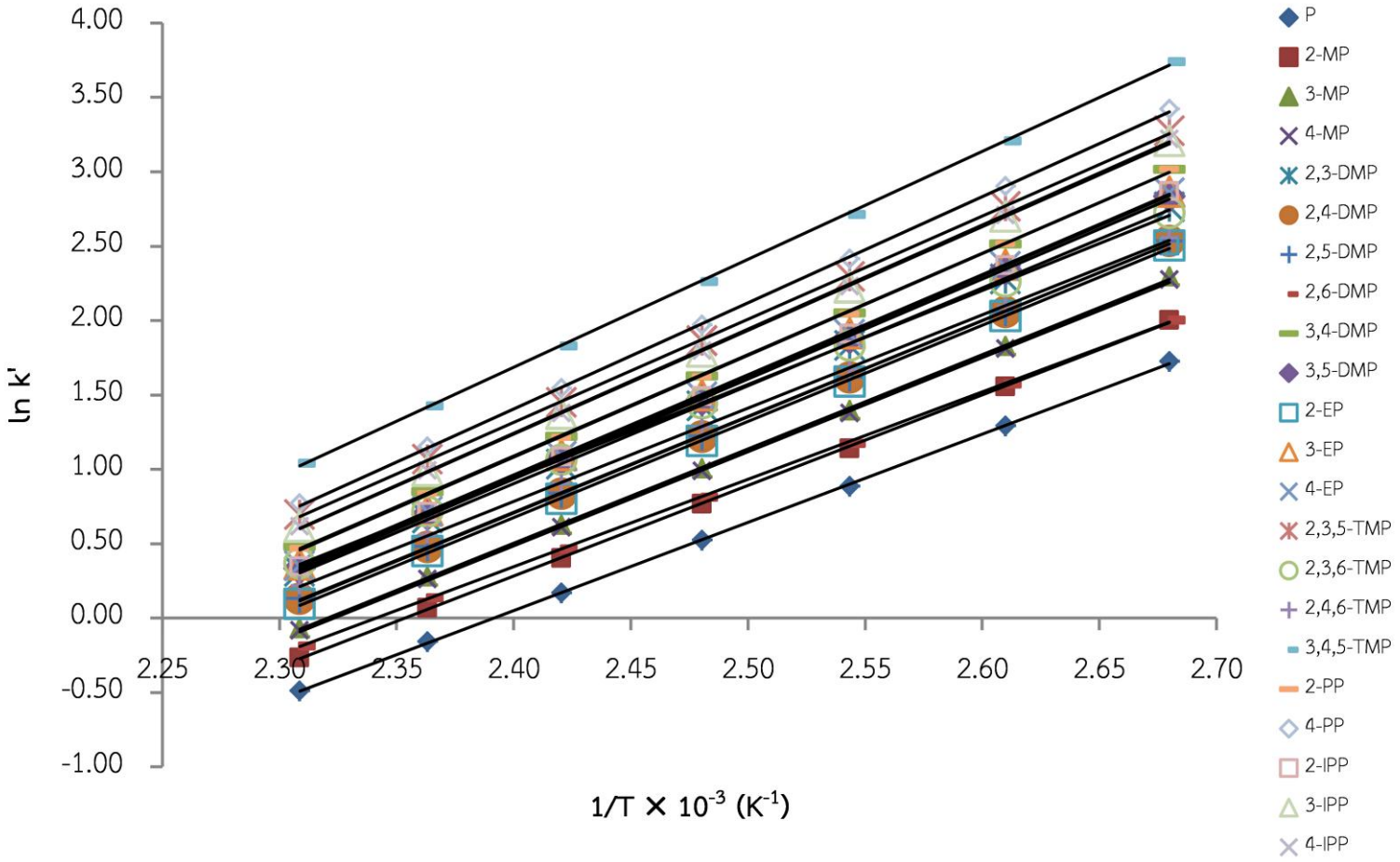


Figure 4.2 Relationship of ln k′ versus 1/T of C_0-C_3 APs on ASiAc column.

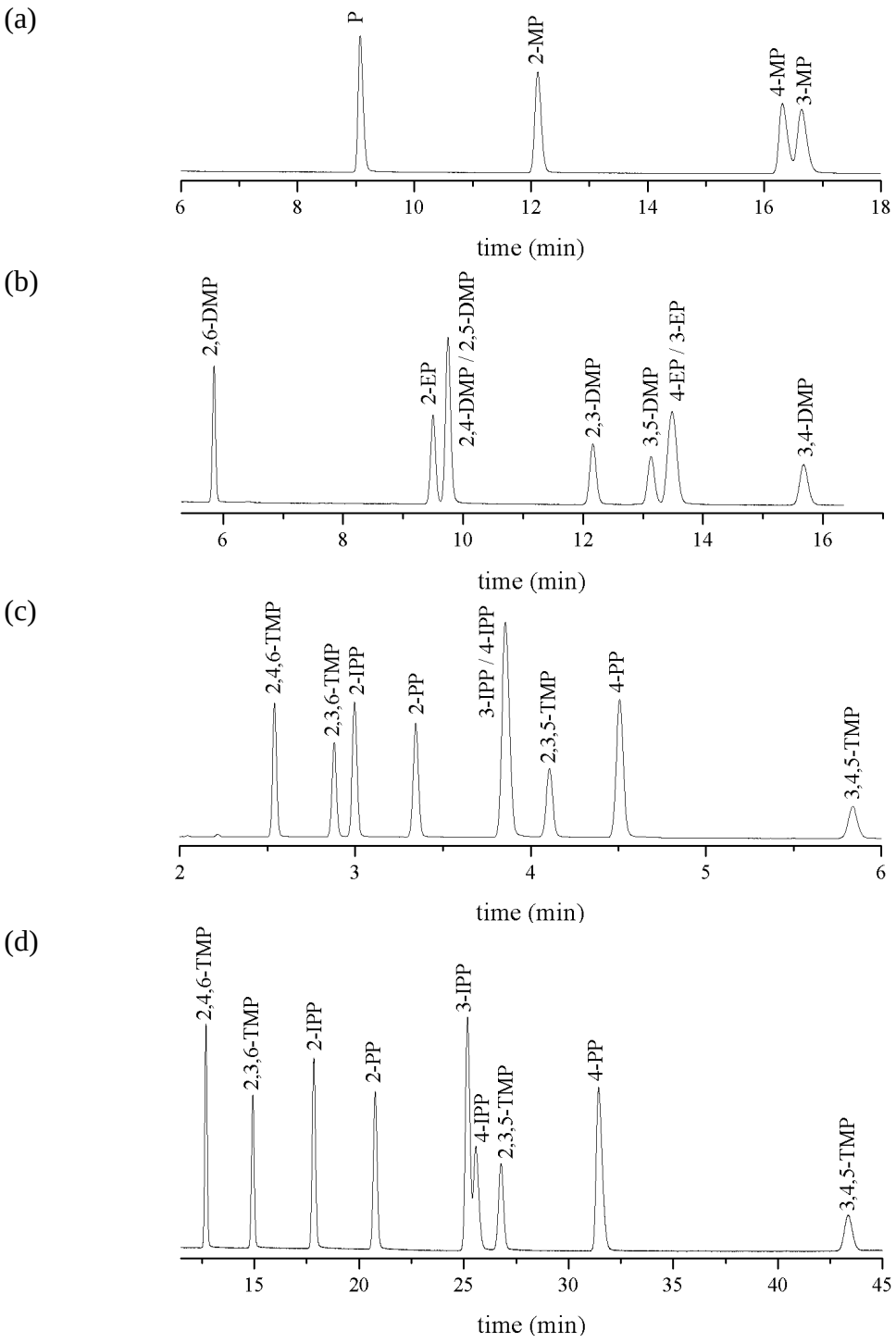


Figure 4.3 Separations of (a) the mixture of four C_0-C_1 AP isomers (condition: 80 °C); (b) the mixture of nine C_2 AP isomers (condition: 95 °C); (c) the mixture of nine C_3 AP isomers (condition: 130 °C); and (d) the mixture of nine C_3 AP isomers (condition: 90 °C) on ASiAc column.

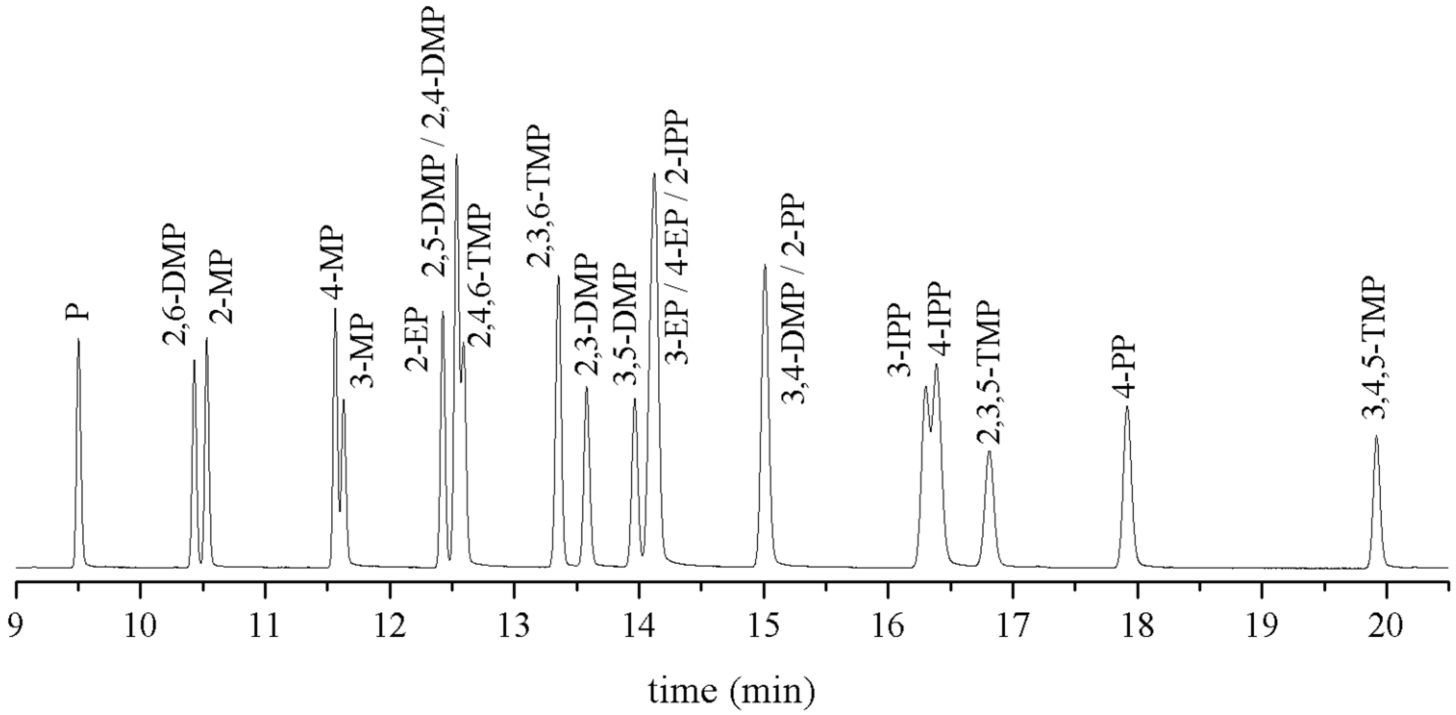


Figure 4.4 Separation of the mixture of C_0-C_3 APs on ASiAc column (condition: from 50-110 °C at 5 °C/min, held for 5 min; then to 220 °C at 5 °C/min).

Table 4.1 Elution order and retention times of C_0-C_3 APs on ASiAc column.

elution order	retention time (min)	compound
1	9.50	P
2	10.43	2,6-DMP
3	10.53	2-MP
4	11.56	4-MP
5	11.63	3-MP
6	12.43	2-EP
7	12.54	2,5-DMP/2,4-DMP
8	12.59	2,4,6-TMP
9	13.35	2,3,6-TMP
10	13.58	2,3-DMP
11	13.97	3,5-DMP
12	14.12	3-EP/4-EP/2-IPP
13	15.01	3,4-DMP/2-PP
14	16.30	3-IPP
15	16.39	4-IPP
16	16.81	2,3,5-TMP
17	17.92	4-PP
18	19.92	3,4,5-TMP

4.2 BSiAc column

The separation of APs was also investigated using β-CD derivative (BSiAc column) as a stationary phase to study the effect of CD ring size on selectivity and elution order. The relationships of ln k′ versus 1/T of C_0-C_3 APs were shown in Figure 4.5. When a medium-size acetylated CD was used, the results were different from a smaller size acetylated α-CD. The study began with the separation condition of each group of AP isomers.

The mixture of C_0-C_1 APs could be completely separated on BSiAc column at 120 °C with the same elution order as on ASiAc column: P > 2-MP > 4-MP > 3-MP (Figure 4.6 a). Fortunately, acetylated β-CD derivative showed excellent selectivity toward P and MP isomers. The baseline separation of these four isomers could be obtained in only 4.1 minutes, about 4 times faster than the separation on ASiAc column. The resolution of 1.54 was observed for the 4-MP and 3-MP peak pair.

Next, the separation of the mixture of nine C_2 AP isomers was studied. BSiAc also showed better selectivity towards C_2 AP isomers than ASiAc. At column temperature of 120 °C, nine peaks were observed with the elution order of 2,6-DMP > 2,4-DMP > 2-EP > 2,5-DMP > 3,5-DMP > 2,3-DMP > 4-EP > 3-EP > 3,4-DMP and the total analysis time of 6.1 minutes (Figure 4.6 b). However, incomplete separation between 2,5-DMP and 3,5-DMP peak pair was observed with the resolution of 0.99. Decreasing the column temperature to 110 °C in an attempt to improve separation led to a longer analysis time (9.8 minutes) and a coelution of 3-EP (218 °C) and 3,4-DMP (227 °C) (Figure 4.6 c). Therefore, the optimum column temperature of 120 °C was chosen for the separation of nine C_2 AP isomers.

Next, the separation of C_3 APs was studied on BSiAc column. At all temperatures studied, the separation of 3-IPP/4-IPP could not be achieved similar to the results obtained from ASiAc column. At 170 °C, the mixture of nine C_3 AP isomers could be separated into eight peaks within 1.7 minutes with the elution order of 2,4,6-TMP > 2-IPP > 2,3,6-TMP > 2-PP > 3-IPP/4-IPP > 2,3,5-TMP > 4-PP > 3,4,5-TMP (Figure 4.6 d). The elution orders for the analytes on ASiAc and BSiAc were very

similar except for 2-IPP and 2,3,6-TMP. The resolution between two closest peaks of 3-IPP/4-IPP and 2,3,5-DMP was 1.77 (Figure 4.6 d).

Based on the optimum conditions for the separation of each group of APs on BSiAc column and the information from ln k′ versus 1/T plot, the optimum condition for the separation of twenty two APs on this column could be attained. Chromatographic separation was achieved with a temperature program from 70-128 °C at 10 °C/min, held for 2 min; then to 220 °C at 30 °C/min (Figure 4.7). All analytes eluted from the column in only 8.8 minutes. As seen from the chromatogram, most peaks could be resolved except for 3-IPP and 4-IPP (similar to the result from the analysis of C_3 group). Partial resolutions were also observed for 2,3-DMP/2-IPP; 2-IPP/2,3,6-TMP; and 2,3,5-TMP/(3-IPP+4-IPP). The elution order and retention times of all 22 analytes were shown in Table 4.2. It was interesting to observe that 2,6-DMP eluted before P on this column.

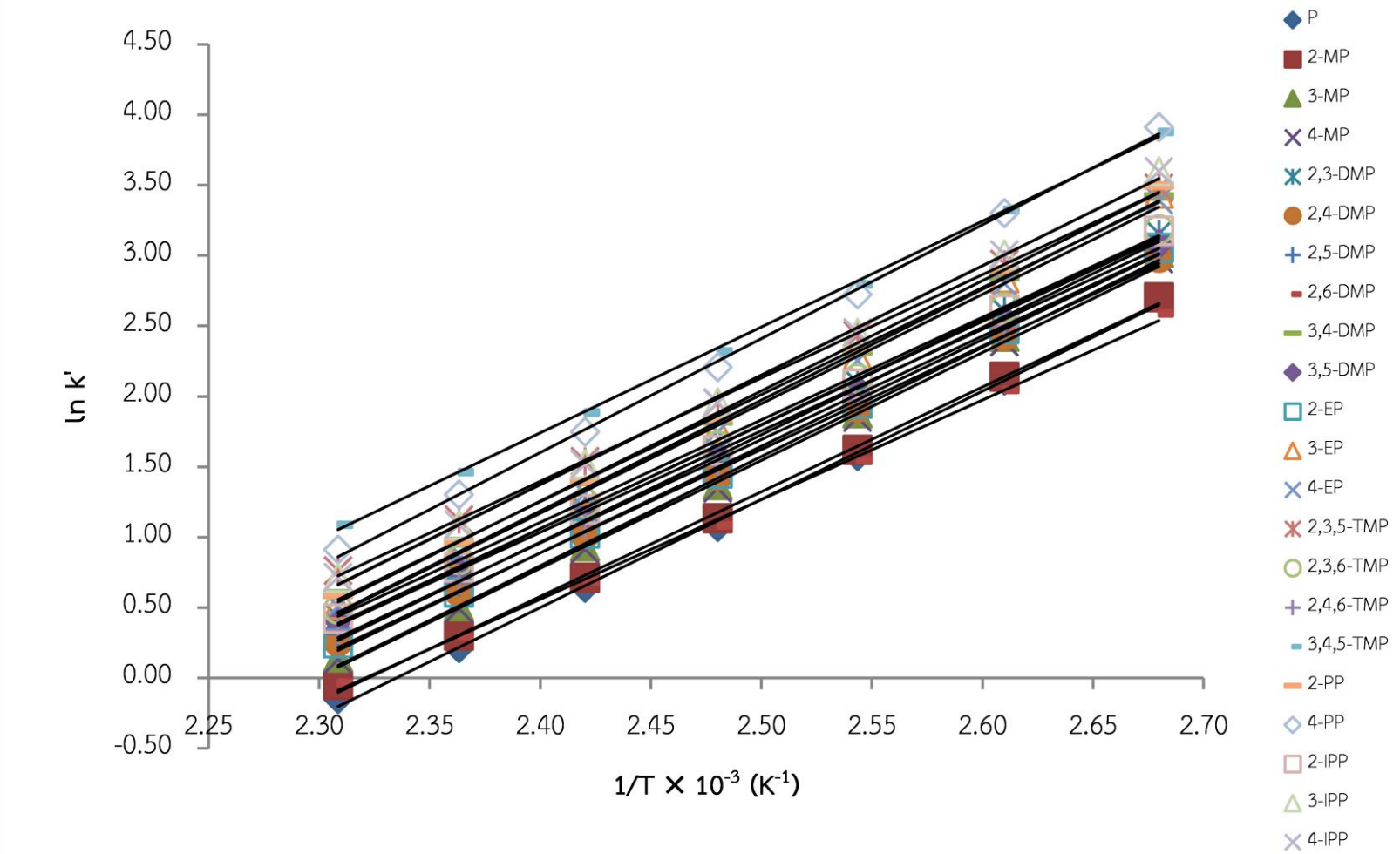


Figure 4.5 Relationship of ln k′ versus 1/T of C_0-C_3 APs on BSiAc

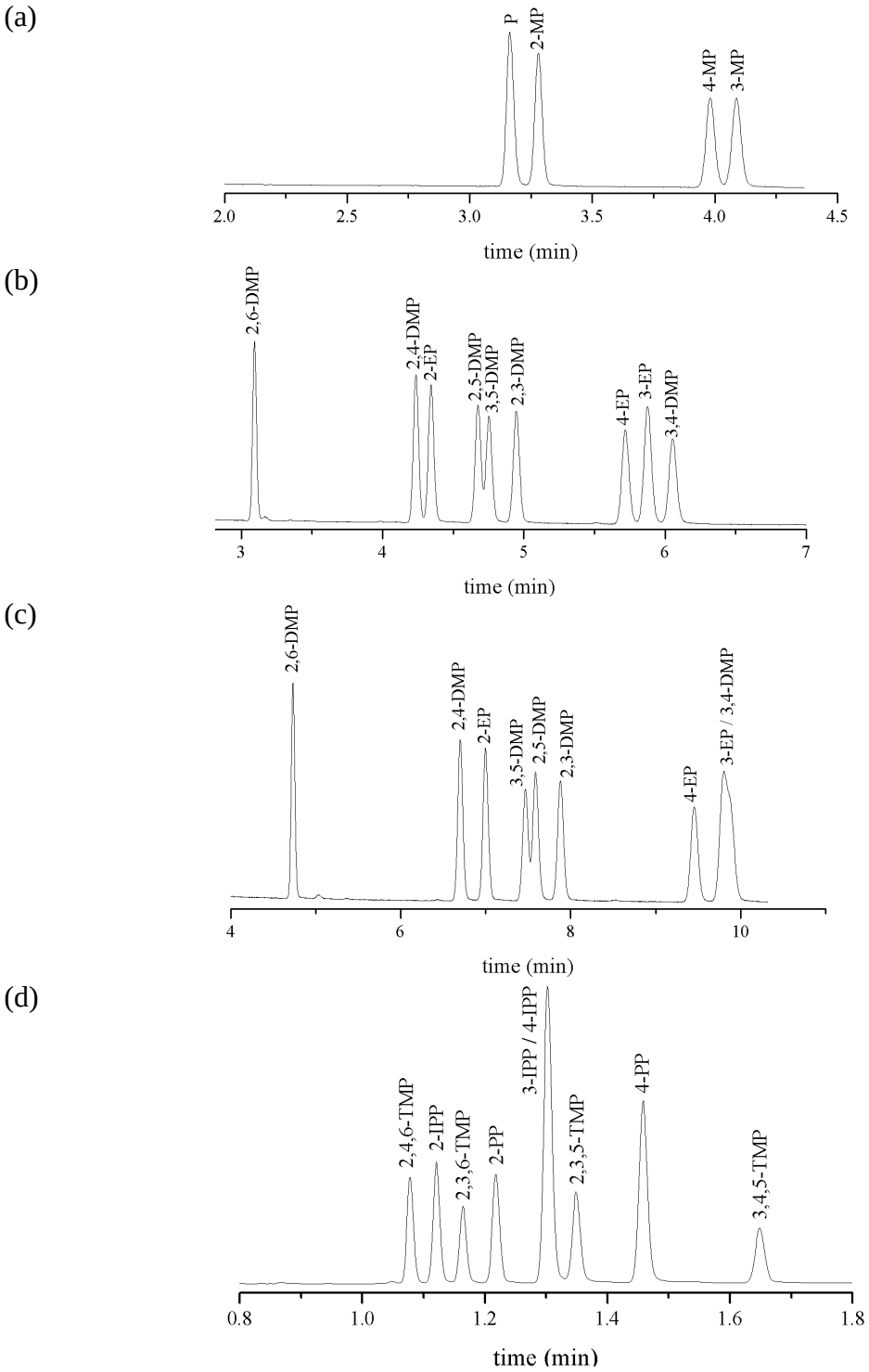


Figure 4.6 Separation of (a) the mixture of four C_0-C_1 AP isomers (condition: 120 °C); (b) the mixture of nine C_2 AP isomers (condition: 120 °C); (c) the mixture of nine C_2 AP isomers (condition: 110 °C); and (d) the mixture of nine C_3 AP isomers (condition: 170 °C) on BSiAc column.

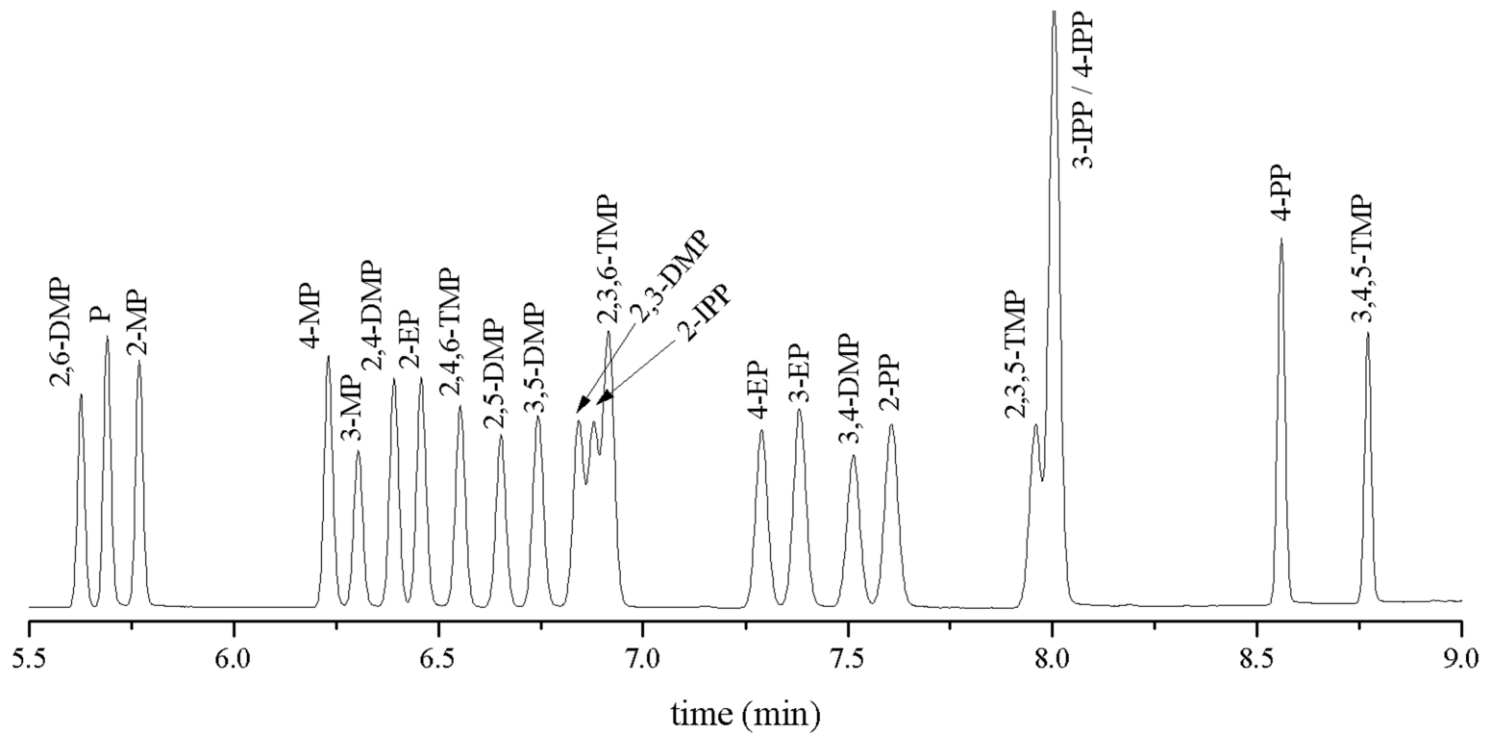


Figure 4.7 Separation of the mixture of C_0-C_3 APs on BSiAc column (condition: from 70-128 °C at 10 °C/min, held for 2 min; then to 220 °C at 30 °C/min).

Table 4.2 Retention times of C_0-C_3 APs on BSiAc column.

elution order	retention time (min)	compound
1	5.63	2,6-DMP
2	5.69	P
3	5.77	2-MP
4	6.23	4-MP
5	6.30	3-MP
6	6.39	2,4-DMP
7	6.46	2-EP
8	6.55	2,4,6-TMP
9	6.65	2,5-DMP
10	6.74	3,5-DMP
11	6.84	2,3-DMP
12	6.88	2-IPP
13	6.92	2,3,6-TMP
14	7.29	4-EP
15	7.38	3-EP
16	7.51	3,4-DMP
17	7.61	2-PP
18	7.96	2,3,5-TMP
19	8.00	3-IPP/4-IPP
20	8.56	4-PP
21	8.77	3,4,5-TMP

4.3 GSiAc column

The ln k′ versus 1/T plot of C_0-C_3 APs obtained from a large-size acetylated CD, GSiAc column, was shown in Figure 4.8. Using a large-size CD, the separation for each group of APs got better. The separation of C_0-C_1 APs on GSiAc column was much better and faster than on both ASiAc and BSiAc columns. At 160 °C, complete separation of four APs was observed in only 1.2 minutes, about 3 times faster than the condition obtained with the BSiAc column. The elution order was P > 2-MP > 4-MP > 3-MP (Figure 4.9 a), the same order as two other columns. Additionally, all isomers were well separated with the resolution of 1.70 for the closest peak pair (4-MP and 3-MP).

For C_2 group, the highest isothermal temperature that provided good resolution for nine analytes was 120 °C. The mixture could be separated within 6.3 minutes with the elution order of 2,6-DMP > 2,4-DMP > 2,5-DMP > 2-EP > 4-EP > 2,3-DMP > 3,5-DMP > 3-EP > 3,4-DMP (Figure 4.9 b). Under the optimum condition, the 2,3-DMP and 3,5-DMP peak pair showed the lowest resolution of 1.45. An attempt to improve the resolution between the 2,3-DMP and 3,5-DMP peak pair was made by decreasing the column to 110 °C. Unfortunately, 2-EP and 4-EP became to merge and the separation between those two peaks was worse with the resolution of 1.05. Therefore, the optimum column temperature of 120 °C was chosen for the separation of nine C_2 AP isomers.

The separation of C_3 APs on GSiAc column could be achieved at 100 °C. The total analysis time was about 32.9 minutes with the elution order of 2,4,6-TMP > 2,3,6-TMP > 2-IPP > 4-IPP > 3-IPP > 4-PP > 2,3,5-TMP > 2-PP > 3,4,5-TMP (Figure 4.9 c). Under the optimum condition, the 2,3,5-TMP and 2-PP peak pair showed incomplete separation with the resolution of 1.00. Interestingly, 3-IPP and 4-IPP were well separated on this column while they coeluted on both ASiAc and BSiAc columns. Unfortunately, the complete separation of all nine peaks could not be attained.

Finally, the optimum condition for the separation of 22 AP isomers was also acquired. With some adjustments, the column temperature was held at 70 °C for 2 min; then to 110 °C at 5 °C/min; then to 220 °C at 40 °C/min (Figure 4.10). The elution order and retention times of all APs were shown in Table 4.3. The separation was finished in 12 minutes. Although, the coelution of APs was not observed in the separation for each group, the chromatographic separation of 22 APs mixture revealed the coelution of 4-PP and 2,3,5-TMP. Many incomplete separations were also observed for 4-MP/2-MP; 2-MP/2,6-DMP; 2,6-DMP/3-MP; 2,4-DMP/2,4,6-TMP; 2-EP/4-EP; 2,3,6-TMP/2,3-DMP; 2,3-DMP/3,5-DMP; 2-IPP/3,4-DMP; and 3-IPP/2-PP.

Considering the results from the three CD-based columns, the separations for each group of AP isomers as well as the single-run separation of 22 C_0-C_3 AP mixture on three columns were investigated. Based on the number of peaks separated, GSiAc gave the best separation results for all three APs single groups among three columns. Unfortunately, when the separation of 22 APs mixture was determined, the results were different. Too small CDs, ASiAc, did not provide enough selectivity of many AP compounds. While both BSiAc and GSiAc columns almost gave a complete separation for all APs mixture with only two different compounds that they could not resolved. However, isomeric separation on β-CD column gave a less number of incomplete peak pair with shorter analysis time than on γ-CD column. Therefore, BSiAc was considered the best acetylated CD derivative for the separation of 22 C_0-C_3 AP mixture.

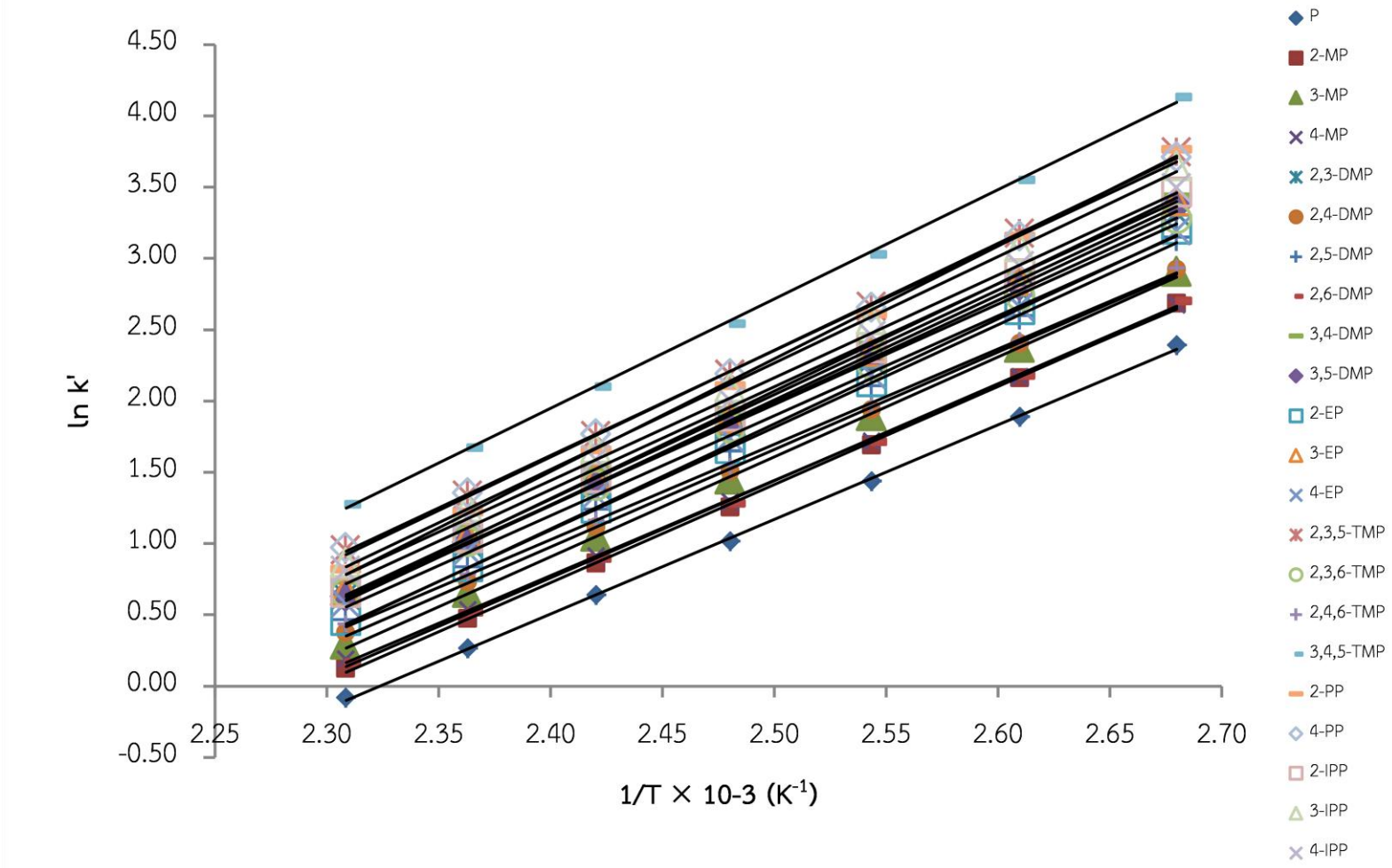


Figure 4.8 Relationship of ln k′ versus 1/T of C_0-C_3 APs on GSiAc column.

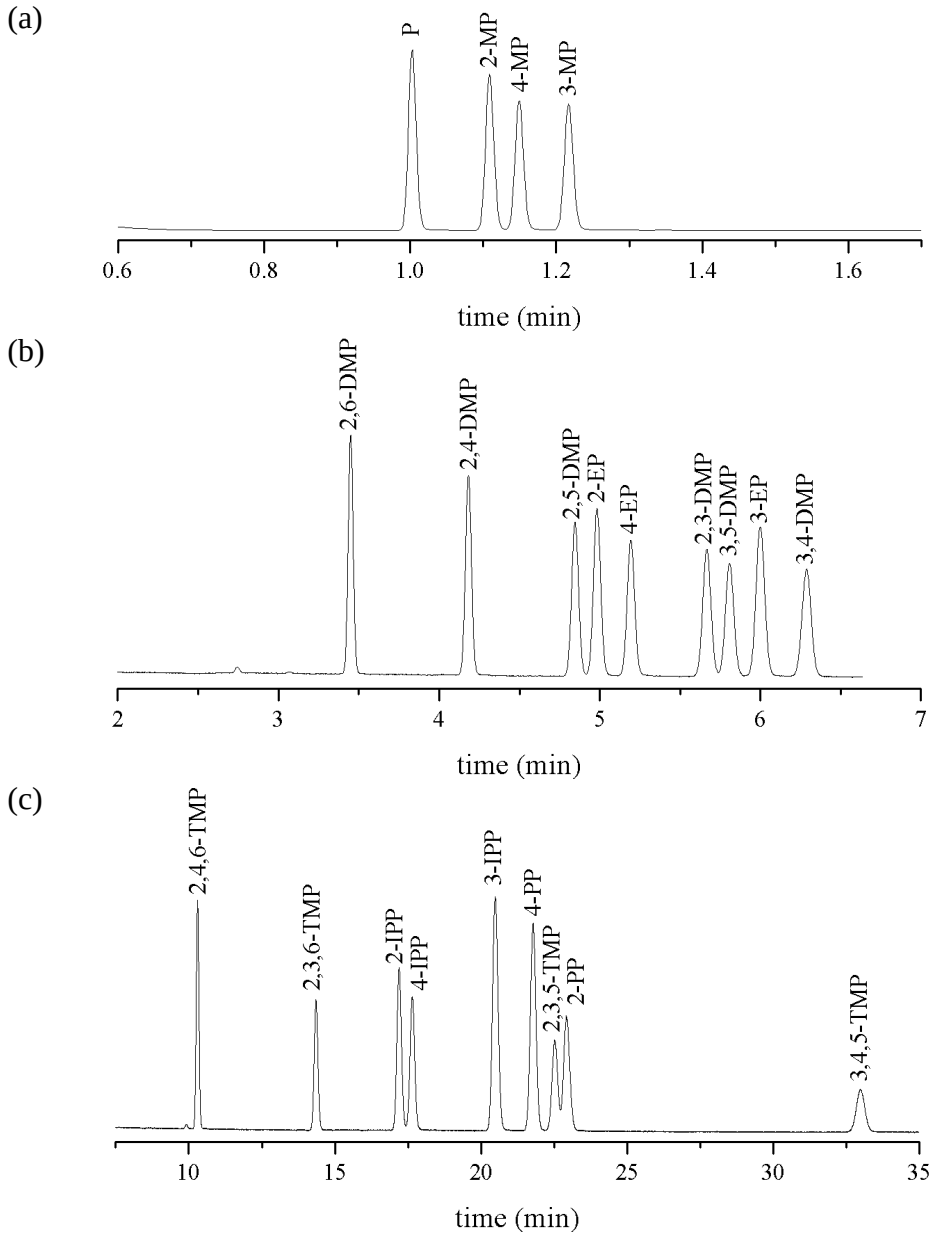


Figure 4.9 Separation of (a) the mixture of four C_0-C_1 AP isomers (condition: 160 °C); (b) the mixture of nine C_2 AP isomers (condition: 120 °C); and (c) the mixture of nine C_3 AP isomers (condition: 100 °C) on GSiAc column.

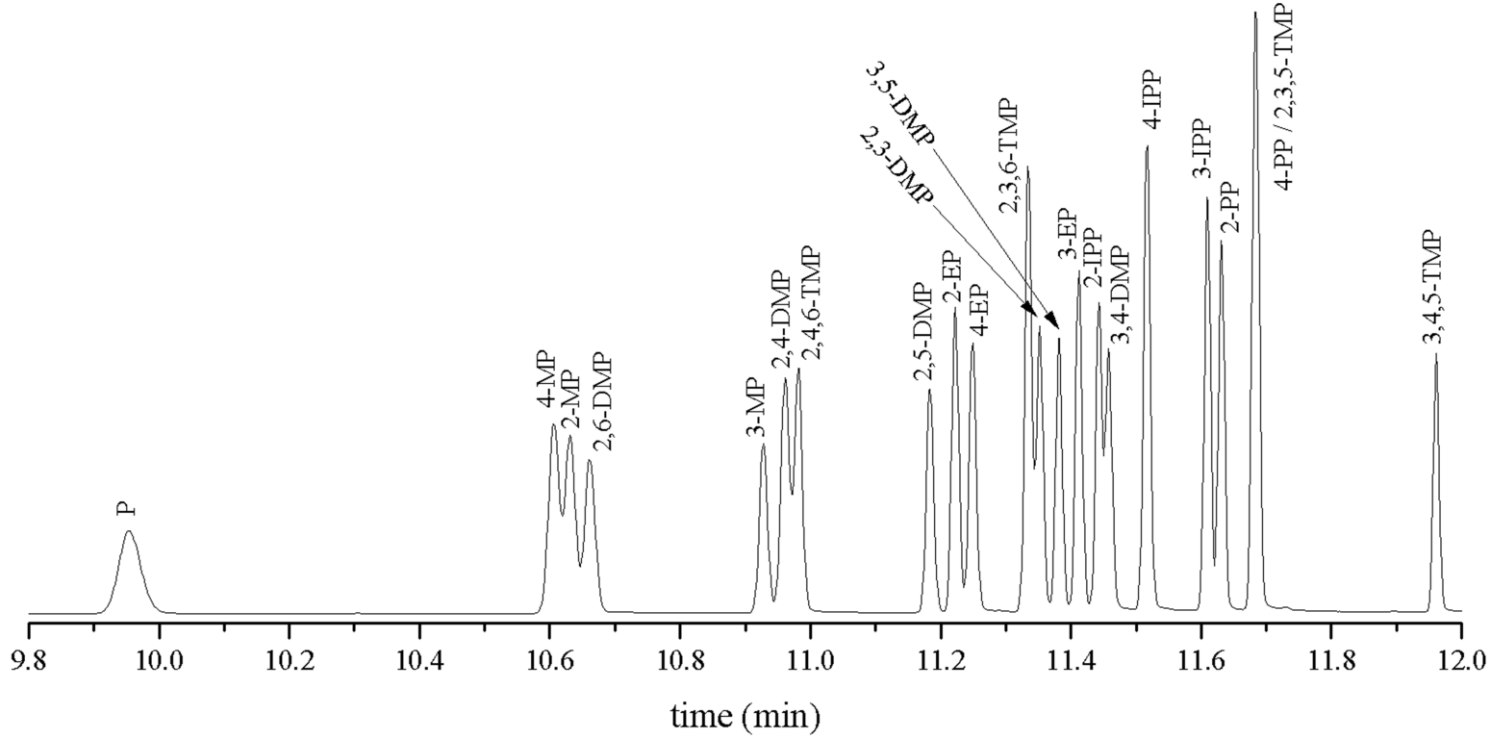


Figure 4.10 Separation of the mixture of C_0-C_3 APs on GSiAc column (condition: held at 70 °C for 2 min; then to 110 °C at 5 °C/min; then to 220 °C at 40 °C/min).

Table 4.3 Retention times of C_0-C_3 APs on GSiAc column.

elution order	retention time (min)	compound
1	9.95	P
2	10.61	4-MP
3	10.63	2-MP
4	10.66	2,6-DMP
5	10.93	3-MP
6	10.96	2,4-DMP
7	10.98	2,4,6-TMP
8	11.18	2,5-DMP
9	11.22	2-EP
10	11.25	4-EP
11	11.33	2,3,6-TMP
12	11.35	2,3-DMP
13	11.38	3,5-DMP
14	11.41	3-EP
15	11.44	2-IPP
16	11.46	3,4-DMP
17	11.52	4-IPP
18	11.61	3-IPP
19	11.63	2-PP
20	11.68	4-PP/2,3,5-TMP
21	11.96	3,4,5-TMP

The elution order and total analysis time for the separation of all APs on three columns were compared and shown in Table 4.4.

Table 4.4 The elution order and total analysis times of C_0-C_3 APs on ASiAc, BSiAc and GSiAc columns.

elution order	ASiAc[(1)]	BSiAc[(2)]	GSiAc[(3)]
1	P	2,6-DMP	P
2	2,6-DMP	P	4-MP
3	2-MP	2-MP	2-MP
4	4-MP	4-MP	2,6-DMP
5	3-MP	3-MP	3-MP
6	2-EP	2,4-DMP	2,4-DMP
7	2,5-/2,4-DMP	2-EP	2,4,6-TMP
8	-	2,4,6-TMP	2,5-DMP
9	2,4,6-TMP	2,5-DMP	2-EP
10	2,3,6-TMP	3,5-DMP	4-EP
11	2,3-DMP	2,3-DMP	2,3,6-TMP
12	3,5-DMP	2-IPP	2,3-DMP
13	3-EP/4-EP/2-IPP	2,3,6-TMP	3,5-DMP
14	-	4-EP	3-EP
15	-	3-EP	2-IPP
16	3,4-DMP/2-PP	3,4-DMP	3,4-DMP
17	-	2-PP	4-IPP
18	3-IPP	2,3,5-TMP	3-IPP
19	4-IPP	3-IPP/4-IPP	2-PP
20	2,3,5-TMP	-	4-PP/2,3,5-TMP
21	4-PP	4-PP	-
22	3,4,5-TMP	3,4,5-TMP	3,4,5-TMP
total analysis time (min)	19.919	8.770	11.961

(1) column temperature: 50-110 ℃ at 5 ℃/min, held for 5 min; then to 220 ℃ at 5 ℃/min

(2) column temperature: 70-128 ℃ at 10 ℃/min, held for 2 min; then to 220 ℃ at 30 ℃/min

(3) column temperature: held at 70 ℃ for 2 min; then 70-110 ℃ at 5 ℃/min; then to 220 ℃ at 40 ℃/min

Table 4.4 summarizes the retention time and elution order of the mixture of C_0-C_3 APs on ASiAc, BSiAc and GSiAc columns. The elution orders of all analytes on three columns were quite different, probably due to the difference in the interaction with CD of different size. The analysis times and elution orders for the separation of C_0-C_1, C_2 and C_3 APs were also compared on three columns as shown in Table 4.5-Table **4.7**, respectively.

Table 4.5 The elution order and total analysis times of C_0-C_1 APs on ASiAc, BSiAc and GSiAc columns.

elution order	ASiAc[(1)]	BSiAc[(2)]	GSiAc[(3)]
1	P	P	P
2	2-MP	2-MP	2-MP
3	4-MP	4-MP	4-MP
4	3-MP	3-MP	3-MP
total analysis time (min)	16.642	4.088	1.217

column temperature: [(1)] 80 ℃, [(2)] 120 ℃, [(3)] 160 ℃.

Table 4.6 The elution order and total analysis times of C_2 APs on ASiAc, BSiAc and GSiAc columns.

elution order	ASiAc[(1)]	BSiAc[(2)]	GSiAc[(3)]
1	2,6-DMP	2,6-DMP	2,6-DMP
2	2-EP	2,4-DMP	2,4-DMP
3	2,4-/2,5-DMP	2-EP	2,5-DMP
4	-	2,5-DMP	2-EP
5	2,3-DMP	3,5-DMP	4-EP
6	3,5-DMP	2,3-DMP	2,3-DMP
7	4-/3-EP	4-EP	3,5-DMP
8	-	3-EP	3-EP
9	3,4-DMP	3,4-DMP	3,4-DMP
total analysis time (min)	15.676	6.054	6.288

column temperature: [(1)] 95 ℃, [(2)] 120 ℃, [(3)] 120 ℃.

Table 4.7 The elution order and total analysis times of C_3 APs on ASiAc, BSiAc and GSiAc columns.

elution order	ASiAc[(1)]	BSiAc[(2)]	GSiAc[(3)]
1	2,4,6-TMP	2,4,6-TMP	2,4,6-TMP
2	2,3,6-TMP	2-IPP	2,3,6-TMP
3	2-IPP	2,3,6-TMP	2-IPP
4	2-PP	2-PP	4-IPP
5	3-IPP	3-/4-IPP	3-IPP
6	4-IPP	-	4-PP
7	2,3,5-TMP	2,3,5-TMP	2,3,5-TMP
8	4-PP	4-PP	2-PP
9	3,4,5-TMP	3,4,5-TMP	3,4,5-TMP
total analysis time (min)	43.347	1.649	32.972

column temperature: [(1)] 90 °C, [(2)] 170 °C, [(3)] 100 °C.

The single-run separations of 22 AP mixture using (2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl) derivatized cyclodextrins as stationary phases in this work were compared to the previous results using (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl) derivatized cyclodextrins [20]. In both cases, it was found that the medium-size β-cyclodextrins showed better selectivity towards the separation of 22 AP mixture than their corresponding α- or γ-derivatives. This would suggest that the cavity size of β-cyclodextrin might be proper for the separation of small compounds with single aromatic ring. BSiMe column provided a better result as it can separate all 22 AP mixture in 13 minutes but five incomplete peak pairs were still observed [20]. The single-run separation for the 22 AP mixture on BSiAc column, on the other hand, provided a coelution of two compounds and three incomplete peak pairs with the analysis time of 8.8 minutes. The results obtained from two derivatized β-cyclodextrins (BSiMe *vs*. BSiAc) of similar ring size suggested that the type of substituent (methyl *vs*. acetyl) also affect the selectivity. The more polar and more steric acetyl groups of BSiAc may affect as well as hinder the interaction between analytes and CD, resulting in different selectivity towards AP mixture.

CHAPTER V

CONCLUSIONS

The separation of the mixture of C_0-C_3 APs was analyzed by GC using (2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-α-, β- and γ-CDs mixed in polysiloxane OV-1701 as stationary phases. The influences of CD ring size on separation selectivity were demonstrated. Each analyte was individually investigated isothermally in the temperature range of 100-160 °C with 10 °C difference. Retention factors (k′) at each temperature were determined to construct a plot of ln k′ versus 1/T for each column.

The results showed that three CD derivatives, having identical substituent but different ring size, could be used to separate these twenty two C_0-C_3 AP isomers. However, they showed different selectivity and provided different elution order. ASiAc column was not appropriate for C_0-C_3 APs as they could not separate many compounds of the mixture. Whereas both BSiAc and GSiAc could separate almost all 22 APs with only two different unresolved compounds. As a result of the smaller number of incomplete peak pairs with shorter analysis time, the best single-run separation was achieved on BSiAc. The separation on this β-CD column was done in 8.8 minutes with a temperature program from 70-128 °C at 10 °C/min, held for 2 min; then to 220 °C at 30 °C/min. In addition, it could be observed that direct analyses of C_0-C_3 APs without prior derivatization gave acceptable peak shapes on these three CD derivative columns.

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APPENDIX

APPENDIX

Table A1 Equations and correlation coefficient of ln k′ vs. 1/T plots on ASiAc, BSiAc and GSiAc columns.

compound	ASiAc			BSiAc			GSiAc		
	ln k′ = m(1/T) + C		R^2	ln k′ = m(1/T) + C		R^2	ln k′ = m(1/T) + C		R^2
	m	c		m	c		m	c	
P	5.9330	-14.188	0.9998	7.6793	-17.930	0.9988	6.6306	-15.408	0.9998
2-MP	6.0830	-14.316	0.9998	7.4383	-17.274	0.9988	6.8806	-15.789	0.9998
3-MP	6.3350	-14.701	0.9998	7.7564	-17.819	0.9985	7.0164	-15.933	0.9998
4-MP	6.3217	-14.684	0.9998	7.6634	-17.615	0.9986	6.6723	-15.238	0.9998
2,3-DMP	6.5681	-14.858	0.9998	7.3512	-16.582	0.9991	7.2059	-16.029	0.9998
2,4-DMP	6.4533	-14.780	0.9998	7.3461	-16.743	0.9990	6.8359	-15.429	0.9998
2,5-DMP	6.4450	-14.756	0.9998	7.6276	-17.344	0.9988	7.2032	-16.194	0.9998
2,6-DMP	5.8725	-13.749	0.9998	7.0731	-16.415	0.9985	6.8046	-15.570	0.9998
3,4-DMP	6.8054	-15.243	0.9998	7.5966	-16.980	0.9990	7.2182	-15.947	0.9998
3,5-DMP	6.7203	-15.192	0.9998	7.2093	-16.270	0.9993	7.3570	-16.384	0.9998
2-EP	6.4633	-14.835	0.9998	7.5739	-17.290	0.9988	7.2085	-16.175	0.9998
3-EP	6.7272	-15.181	0.9998	7.8694	-17.700	0.9987	7.2036	-15.959	0.9998
4-EP	6.7328	-15.203	0.9998	7.7733	-17.488	0.9988	6.8958	-15.338	0.9998
2,3,5-TMP	6.9277	-15.311	0.9998	7.3337	-16.204	0.9994	7.4954	-16.381	0.9998
2,3,6-TMP	6.3115	-14.208	0.9999	7.2787	-16.363	0.9988	7.0488	-15.648	0.9999
2,4,6-TMP	6.2713	-14.267	0.9999	7.3425	-16.663	0.9986	6.6945	-15.041	0.9999
3,4,5-TMP	7.2486	-15.709	0.9998	7.5192	-16.305	0.9994	7.6675	-16.453	0.9998
2-PP	6.8444	-15.344	0.9998	7.8262	-17.527	0.9987	7.9027	-17.461	0.9998
4-PP	7.1281	-15.700	0.9998	8.0868	-17.808	0.9988	7.3491	-16.019	0.9998
2-IPP	6.7652	-15.282	0.9998	7.4122	-16.731	0.9990	7.5463	-16.796	0.9998
3-IPP	6.9705	-15.491	0.9998	7.7748	-17.287	0.9988	7.4677	-16.403	0.9998
4-IPP	6.9944	-15.543	0.9998	7.7627	-17.255	0.9987	7.2060	-15.851	0.9998

VITA

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