IMPROVING CHROMATOGRAPHIC ANALYSIS OF PHENOLIC COMPOUNDS

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น.ส.ธาริกา ล้อมเจริญวงษ์

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สารประกอบฟีนอลเป็นกลุ่มสารที่สำคัญและมีการประยุกต์ใช้ในหลายด้าน ส่งผลให้พบ
เป็นสารปนเปื้อนในสิ่งแวดล้อม งานวิจัยนี้จึงมุ่งที่จะปรับปรุงการวิเคราะห์สารประกอบฟีนอลด้วย
เทคนิคทางโครมาโทกราฟี โดยแบ่งงานเป็น 2 ส่วน ส่วนแรกเน้นที่การปรับปรุงการแยกสารผสม
ของสารประกอบฟีนอล 18 ชนิดด้วยแก๊สโครมาโทกราฟีและใช้อนุพันธ์ไซโคลเดกซ์ทรินเป็นเฟส
คงที่ ผลการทดลองพบว่าเฟสคงที่ของอนุพันธ์ไซโคลเดกซ์ทรินทั้ง 3 ชนิด ที่มีขนาดวงแตกต่างกัน
สามารถแยกสารผสมทั้ง 18 ชนิดได้ดีกว่าเฟสคงที่ชนิดพอลิไซลอกเซน OV-1701 อนุพันธ์ขนาด
เล็กของแอลฟาไซโคลเดกซ์ทรินสามารถแยกสารผสมได้ดีที่สุด โดยใช้คอลัมน์ยาว 15 เมตรและ
โปรแกรมอุณหภูมิช่วง 130-220 °C การแยกสมบูรณ์ภายในเวลา 9.4 นาที ส่วนเฟสคงที่ของ
อนุพันธ์บีตาและแกมมาไซโคลเดกซ์ทรินพบว่าบางพีกยังแยกไม่สมบูรณ์

งานในส่วนที่สอง เป็นการพัฒนาการวิเคราะห์บิสฟีนอลเอด้วยทินเลเยอร์โครมาโทกราฟี
ร่วมกับแมสสเปกโทรเมตรีชนิด MALDI-TOF พบว่า ภาวะที่เหมาะสมคือ ใช้กราฟีนเป็นเมทริกซ์,
ความเข้มข้นของไนโตรเจนเลเซอร์ คือ 50%, และอัตราส่วนของเมทริกซ์ต่อบิสฟีนอลเอ คือ
1:1 การวิเคราะห์เชิงปริมาณของบิสฟีนอลเอ พบว่าช่วงความเข้มข้นที่เหมาะสม คือ 80-160 มิลลิ
โมลาร์ (หรือ 18.3-36.5 ไมโครกรัม) โดยมีค่าการถดถอยเชิงเส้นที่ยอมรับได้ (R^2 = 0.9634)

สาขาวิชา	ปิโตรเคมีและวิทยาศาสตร์พอลิ เมอร์	ลายมือชื่อนิสิต ....
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KEYWORD: gas chromatography, cyclodextrin, phenols, phenolic compounds, bisphenol A, MALDI-TOF MS

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Phenolic compounds are important chemicals and are applied in many fields. Moreover, they are found as contaminants in the environment. This research aimed to improve the chromatographic analysis of phenolic compounds and was divided into two parts. The first part focused on improving the separation of a mixture containing eighteen phenols using gas chromatography with derivatized cyclodextrin stationary phases. Results showed that all three cyclodextrin-based stationary phases, with different ring sizes, improved the separation of the mixture in comparison to polysiloxane OV-1701 stationary phase. The best separation was obtained from the small-size alpha-cyclodextrin with a capillary column of 15-meter long and a temperature program from 130 to 220 °C. Complete separation of all 18 phenols was obtained in 9.4 minutes. Some incomplete separations were observed when beta- and gamma-cyclodextrin stationary phases were used. The second part investigated optimum conditions for bisphenol A analysis using TLC combined with MALDI-TOF MS. These conditions included the use of graphene as a matrix, laser concentrations of 50% nitrogen, and a graphene-to-analyte ratio of 1:1. To validate the quantitative method, a suitable concentration range for BPA analysis using TLC combined with MALDI-TOF MS was determined to be 80 to 160 mM (or 18.3 to 36.5 μg), with an acceptable linear regression value (R^2 = 0.9634).

Field of Study:	Petrochemistry and Polymer Science	Student's Signature ....
Academic Year:	2022	Advisor's Signature ....
		Co-advisor's Signature ....

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CHAPTER 1

INTRODUCTION

The petrochemical industry is a very important industry because it is an upstream industry that transforms various chemicals into important products used in daily life, such as plastics, textiles, and medicines [1]. The petrochemical industry has grown rapidly, including the process of synthesizing, and treating waste before releasing it into the environment. It is necessary to analyze the appropriate amount of chemicals of interest. However, the petrochemical industry often encounters problems in analyses, such as low concentration of hazardous substances, the sample contains many components and the difficulty of separating mixed substances. Moreover, when the sample substances are dirty, additional steps are required to prepare the sample before analysis can be performed.

Phenols or phenolic compounds are a group of organic chemicals that contain one or more hydroxyl (-OH) groups attached to the aromatic ring. Phenols have a wide range of applications, including their use as disinfectants, antioxidants, and as intermediates in the production of plastics, drugs, and other chemicals [2]. However, they can also pose a potential health and environmental hazard due to their toxic and carcinogenic properties. Therefore, accurate analysis and separation of phenolic compounds are important in various fields, including environmental monitoring, food safety, and pharmaceutical development.

For analyses of phenolic compounds, gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) using polysiloxanes as stationary phases are commonly used. However, previous results have shown that coelution and/or incomplete separation were still observed for several phenolic compounds, especially isomers and compounds with similar properties [3-6]. In this work,

derivatized cyclodextrins mixed with polysiloxane will be used as GC stationary phases to improve the separation of a mixture of phenolic compounds. Cyclodextrins (CDs) are chiral molecules with unique structure. Several types of CD derivatives were used as stationary phases in chromatographic techniques to separate enantiomers and isomers. In addition, previous studies have shown that GC stationary phases containing CD derivatives can successfully separate isomers and enantiomers [7-9].

Thin layer chromatography (TLC) is well-suited for analyzing mixtures of relatively simple components. It is often used for preliminary analysis or reaction monitoring. It can also be utilized for samples with some degree of impurities. Common methods for detecting substances on TLC plates include staining or UV light, which require a relatively high concentration of the substance [10]. However, the use of matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) allows for direct detection of substances on TLC plates, providing qualitative analysis with high sensitivity and low detection limits. Thus, TLC combined with MALDI-MS can be applied to analyze phenols such as bisphenol A (BPA).

This research was divided into two parts. First, the single-run separation of several phenolic compounds was investigated by gas chromatography using derivatized cyclodextrins as stationary phases in order to improve resolution and analysis time. Second, TLC analysis combined with MALDI-MS detection of some selected phenol(s) was developed as a model to improve the detection limit of TLC.

CHAPTER 2

THEORY

Part 1 GC

2.1 Phenolic compounds

Phenolic compounds or phenols are benzene derivatives with one or more hydroxyl (-OH) groups directly bonded to an aromatic carbon. Phenol is the smallest phenolic compound with no other substitution. Other phenolic compounds contain functional groups such as alkyl-, chloro- or nitro-, which can be substituted at *ortho-*, *meta-* or *para*-position(s) on the aromatic ring. Phenolic compounds can be found in various natural sources such as plants, but they can also be produced synthetically.
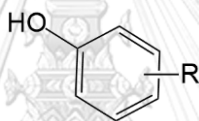
Figure 2.1 Structure of simple phenol

Phenolic compounds or phenols have significant importance in agriculture and industries, where they are used in the production of insecticides, fungicides, herbicides, plastics, drugs, textiles, dyes, and petrochemicals. Phenols are hazardous chemicals as they are toxic to health and environment. They can enter the body through inhalation, ingestion, and dermal contact, causing respiratory and eye irritation, skin burns, headaches, and fainting. Phenols are also known to be potential carcinogens [11]. Due to their widespread applications, phenols are often found as contaminants in various environmental matrices, including groundwater, surface waters, industrial wastewater, sewage water, soil, and sediments [12].

Due to their toxicity and persistence in the environment, the US Environmental Protection Agency (EPA) and the European Union (EU) have classified many phenols as priority pollutants. The US EPA [13] listed 11 phenols as priority pollutants: they are phenol; 2-chlorophenol; 2,4-dichlorophenol; 2,4,6-trichlorophenol; pentachlorophenol; 2-nitrophenol; 4-nitrophenol; 2,4-dinitrophenol; 2-methyl-4,6-dinitrophenol; 2,4-dimethylphenol; and 4-chloro-3-methylphenol. Their structures are shown in Figure 2.2.
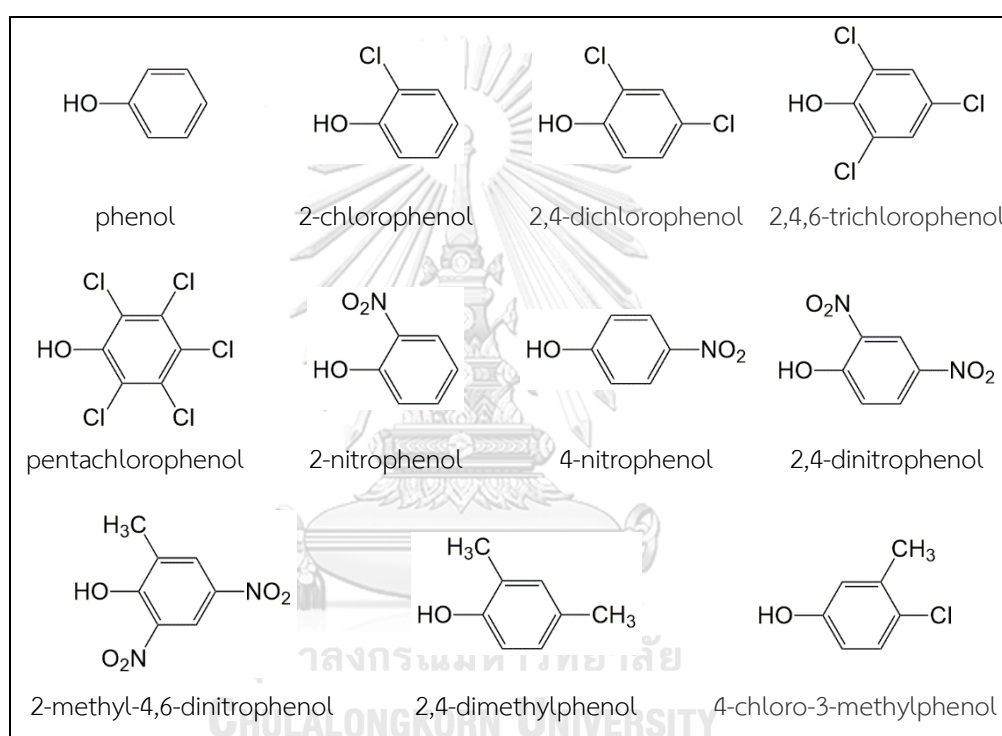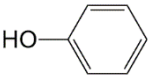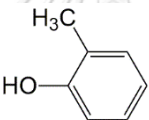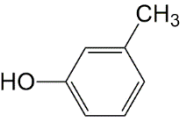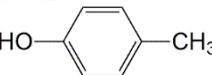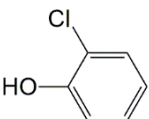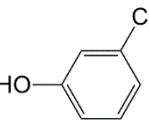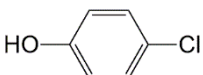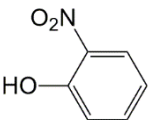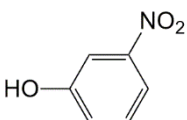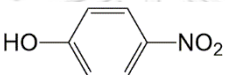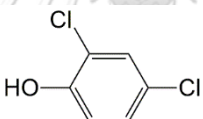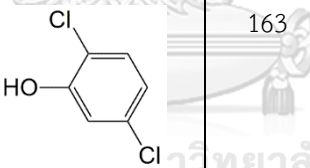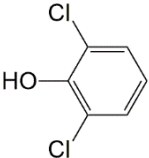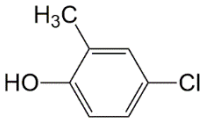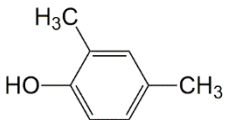
Figure 2.2 Structures of 11 phenols classified as priority pollutants by the US EPA.
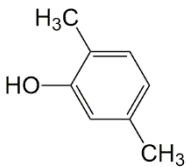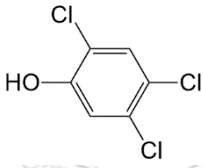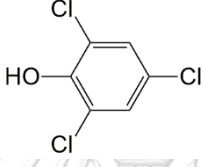
The 80/778/EC directive of the EU permits maximum total and individual phenols concentrations in drinking water should be lower than 0.5 and 0.1 µg/L, respectively [14]. In Thailand, the Ministry of Industry requires that the maximum concentration of phenols content in industrial effluents not exceed 1 mg/L [15]. In addition, some chlorophenols have been classified as potential carcinogens by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) [16].

In this work, the mixture of eighteen phenols of interested were investigated by GC. Their structures and physical properties are shown in Table 2.1.

Table 2.1 Structure and physical properties of phenols used in this study [17, 18]

compound	abbreviation	structure	molecular weight (g/mol)	melting point (°C)	boiling point (°C)	water solubility @25 °C (g/L)
phenol	P		94.11	42.8	181.7	82.8
2-methylphenol (*o*-cresol)	2MP		108.14	31.1	191.1	25.9
3-methylphenol (*m*-cresol)	3MP		108.14	11.5	202.8	22.7
4-methylphenol (*p*-cresol)	4MP		108.14	34.8	201.8	21.5
2-chlorophenol	2Cl		128.56	9	175.0-176.1	28.5 @20
3-chlorophenol	3Cl		128.56	32.8	213.9	26.0 @20
4-chlorophenol	4Cl		128.56	43.2	220	27.0 @20

compound	abbreviation	structure	molecular weight (g/mol)	melting point (°C)	boiling point (°C)	water solubility @25 °C (g/L)
2-nitrophenol	2NP		139.11	45.0-46.1	213.9-216.1	25.0
3-nitrophenol	3NP		139.11	96.1-97.8	193.9	13.55
4-nitrophenol	4NP		139.11	112.8-115.0	278.9	15.6
2,4-dichlorophenol	2,4DCl		163	45.0	210.0	4.5 @20
2,5-dichlorophenol	2,5DCl		163	56.1-57.8	211.1	2.0
2,6-dichlorophenol	2,6DCl		163	67.8-68.9	217.8-220.0	1.9
4-chloro-2-methylphenol	4Cl-2MP		142.58	47.8-51.1	222.2-225.0	2.3
2,4-dimethylphenol	2,4DMP		122.16	22.2-22.8	212.2	7.87

compound	abbreviation	structure	molecular weight (g/mol)	melting point (°C)	boiling point (°C)	water solubility @25 °C (g/L)
2,5-dimethylphenol	2,5DMP	H₃C ... HO ... CH₃	122.16	71.1-72.8	212.2	3.54
2,4,5-trichlorophenol	2,4,5TCl	Cl ... HO ... Cl ... Cl	197.4	67.8	252.8	1.2
2,4,6-trichlorophenol	2,4,6TCl	Cl ... HO ... Cl ... Cl	197.4	69.5	246.1	0.5

2.2 Gas chromatography [19, 20]

Gas chromatography (GC) is a chromatographic technique used to separate a mixture of volatile and thermally stable compounds. The components of a mixture are vaporized and carried into a column by mobile phase. The separation is accomplished based on the difference in their distributions between the stationary phase and mobile phase (GC mobile phase is a gas, commonly called carrier gas). Furthermore, the difference of boiling point, chemical properties, and molecular weight affected the separation. The components that pass through the column are carried by the carrier gas and detected by a detector, which generates a signal that is displayed in the form of a "chromatogram". GC is widely used in industrial, research, and laboratory because it provides high resolution, sensitivity, simplicity, and fast analysis. GC chromatogram provides both qualitative and quantitative information.

2.2.1 Oven temperature [21-23]

Temperature program: is the process of adjusting the column temperature during a run. It is good for screening new samples. The advantage is that it can be

used to separate a wide range of compounds with different properties and obtain more symmetrical peaks than isothermal temperature. However, column temperature must be cooled down to the initial temperature before starting the next run. The elution temperature of each peak can be calculated from:

$$\text{elution temperature} = T_i + (\text{rate} \times t_R)$$

When T_i = initial column temperature

       rate   = temperature program rate (°C/minute)

Isothermal condition: is the process of using a constant column temperature throughout a run. The oven is always ready to analyze because there is no need to change the temperature. Isothermal condition is used for components of sample with similar properties and/or boiling point. The disadvantage is the separation of a sample with several components with different properties. The analysis time will be long and may lead to broad peaks which are difficult to detect.

2.2.2 Gas chromatographic parameters [24]

column efficiency, N/m: Column efficiency is a critical parameter that determines the separation capability and performance of the column. It is often measured by the number of theoretical plates (N) per unit length of the column. A higher column efficiency results in improved separation and sharper peak profiles.

$$N = 5.54 \left(\frac{t_R}{w_h}\right)^2$$

when   N   = number of theoretical plates

       t_R = retention time

       w_h = width at half height

retention factor or capacity factor (k′): Retention factor is a measure of how long a compound can be retained in the stationary phase of the column relative to its time in mobile phase. Retention factor does not depend on column length or on mobile phase velocity.

$$k' = \frac{t_R - t_M}{t_M}$$

when t_M = time of unretained compound

separation factor or selectivity (α): Separation factor is a measure of the degree of separation between two compounds. It refers to the ability of the GC system to separate different compounds in a mixture based on their properties.

$$\alpha = \left(\frac{k'_2}{k'_1}\right) = \left(\frac{t_{R,2} - t_M}{t_{R,1} - t_M}\right) \quad , \alpha \geq 1$$

when $t_{R,1}, t_{R,2}$ = retention time of compounds ($t_{R,2} \geq t_{R,1}$)
k'_1, k'_2 = retention factor

Resolution (Rs): Resolution indicates the relative distance between two bands in relation to their widths. It is a quantitative measure that provides information about the ability of the column to separate two peaks of analytes. A higher value of resolution indicates better separation. A baseline resolution greater than 1.5 indicates an essentially complete separation.

$$Rs = 1.177 \times \left(\frac{t_{R,2} - t_{R,1}}{w_{h,2} + w_{h,1}}\right)$$

2.3 Separation of phenolic compounds by GC using polysiloxane stationary phases

In 2005, Vermeulen et al. [3] analyzed 35 phenolic compounds (phenol, alkylphenols and chlorophenols) in drinking water and groundwater. All phenols were derivatized into phenyl acetate esters prior to analysis by GC-MS using a nonpolar DB-XLB capillary column (30 m, 0.25 mm i.d., 0.25 μm film). The separation of mixture used a temperature program from 40-320 °C and was finished in 35 minutes. From the chromatogram, coelutions were observed for *m*-ethylphenol/2-isopropylphenol and 2,3-dimethylphenol/3,5-dimethylphenol/*p*-ethylphenol. Incomplete separations were observed for 2,5-dichlorophenol/2,4-dichlorophenol and 3,5-dichlorophenol/2,3,5-trimethylphenol.

In 2007, Pino et al. [4] determined the alkyl- and methoxy-phenolic content in wood extractives. The extraction was performed by solid-phase microextraction prior to analysis by GC-MS using a nonpolar VF-5ms column (30 m, 0.25 mm i.d.) and temperature program from 60-280 °C in 19 minutes. Separations of 3-ethylphenol/3,5-dimethylphenol/2,3-dimethylphenol were incomplete and coelutions were observed for 3-methylphenol/4-methylphenol; 2,4-dichlorophenol/2,5-dichlorophenol and 3-ethylphenol/3,5-dimethylphenol.

In 2008, Kovacs et al. [5] used GC-MS to detect 6 phenols (phenol, *o*-, *m*-, *p*-cresols, catechol and resorcinol) and 19 chlorophenols (all mono-, di-, tri-, and tetrachlorophenol isomers and pentachlorophenol) in aqueous samples. All phenols were derivatized with trimethylsilyl-*N,N*-dimethylcarbamate (TMSDMC). They used a nonpolar HP-5ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film) and a temperature program from 60-260 °C to separate the trimethylsilyl derivatives of phenols. However, incomplete separations were observed for 2,5-dichlorophenol/2,6-dichlorophenol/3,5-dichlorophenol.

In 2010, Bernardo et al. [6] determined 11 alkylphenols in eluates of chars produced in the co-pyrolysis of different wastes. It was extracted via dispersive liquid-liquid microextraction prior to GC-MS analysis using TR-5MS capillary column (30 m, 0.25 mm i.d., 0.25 µm film). The separation of the mixture was done using temperature programming from 35-220 °C and was completed in 23 minutes. However, they observed incomplete separation for *m*-methylphenol/*p*-methylphenol and coelution for 2,4-dimethylphenol/2,5-dimethylphenol.

2.4 Cyclodextrin [25, 26]

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of glucopyranose units connected via α-1,4-glycosidic bonds. Natural occurrence of CDs can be categorized into three types: α-, β-, and γ-CDs, which consist of 6, 7, and 8 glucose units, respectively as shown in Figure 2.3. Their physical properties are shown in Table 2.2. The structure of CDs is unique, with hollow torus shapes, a hydrophobic interior, and a hydrophilic exterior. CDs (host molecule) can form inclusion complex with analyte (guest molecule) through host-guest interactions. The forces that drive inclusion complex formation include hydrophobic interactions, hydrogen bonds, van der Waals interactions, dipole-dipole interactions, electrostatic, high-energy water, and solvent effects.
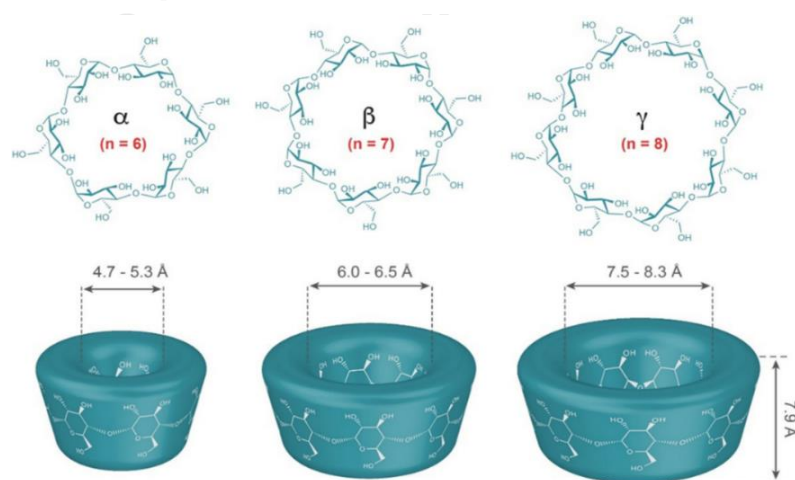
Figure 2.3 Structure and shape of α-, β-, and γ-cyclodextrins [27]

Table 2.2 Physical properties of α-, β-, γ-cyclodextrin [28]

properties	α-CD	β-CD	γ-CD
number of glucopyranose units	6	7	8
molecular weight (g/mol)	972	1135	1297
solubility in water (% w/v) at 25 °C	14.5	1.85	23.2
outer diameter (Å)	14.6	15.4	17.5
cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
height of torus (Å)	7.9	7.9	7.9
cavity volume (Å^3)	174	262	427

The hydroxyl groups at position C-2, C-3, and C-6 of glucopyranose can be derivatized to produce CD derivatives with enhanced properties (solubility, complexation ability and selectivity) compared to the native CDs. CD derivatives have been obtained through reactions such as alkylation, acylation, methylation, or esterification. Due to its beneficial properties and cost-effective industrial production of CDs, it led to their widespread use and practical application in various industrial sectors such as medicine, pharmacy, chromatography, foods, catalysis, biotechnology, and nanotechnology.

Cyclodextrin has been widely researched as chiral stationary phases in GC for the separation of chiral compounds. Previous research has shown that CD derivatives can effectively separate enantiomers and isomers.

In 1996, Jing et al. [7] used three different peralkylated β-CDs as stationary phases for separating achiral and chiral compounds by GC-FID. The compounds tested included xylenes, dichlorobenzenes, dibromobenzenes, nitrobromobenzenes, cresols, chlorotoluenes, nitrotoluenes, bromotoluenes, nitrochlorobenzenes, and dinitrotoluenes. Three derivatized CDs were heptakis(2,3,6-tri-O-ethyl)-β-CD; heptakis(2,3,6-tri-O-butyl)-β-CD and heptakis(2,3,6-tri-O-octyl)-β-CD. The results

demonstrate that all three columns were able to separate isomers (*o-,m-,p-*) of most compounds. Cresol isomers were separated at column temperature of 130 °C, with the same elution order (*o-*, *p*, and *m-*, respectively) on all three columns.

In 2000, Yi and Tang [29] studied the synthesis and characterization of a β-cyclodextrin polymer and used as a stationary phase for capillary GC. The polymer was coated onto three capillary columns using different methods, and chromatographic properties of each column were evaluated. The column that exhibited good chromatographic properties in separating enantiomers and positional isomers was prepared by loading β-CD polymer treated by ultrasonication with OV-17. Two kinds of disubstituted benzene isomers and eight pairs of enantiomers were separated on all three capillary columns. Separations of isomers and enantiomers on all three columns were complete (Rs > 1.5).
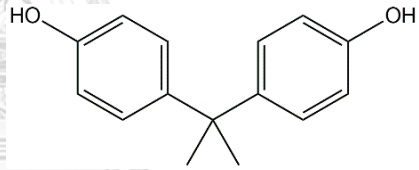
In 2018, Menestrina et al. [8] used GC to separate enantiomers of pesticides. As stationary phases, they used permethyl-β-cyclodextrin (PM-β-CD) combined with three different polymer solvents. These polymers were (14%-cyanopropylphenyl)(1%-vinyl)-86%-methylpolysiloxane (OV-1701-vi); (5%-phenyl)(1%-vinyl)-95%-methylpolysiloxane (SE-54) and polyethyleneglycol (Carbowax 20M). The polarity of the polymer solvent had an effect on the efficiency of the column and the separation of enantiomers of pesticides. The results show that a single column based on PM-β-CD/SE-54 has a lot of potential for separation of enantiomers of polar pesticides and their derivatives, including mecoprop, dichlorprop, fenoprop, metalaxyl, hydroprop, haloxyfop, and fenoxaprop esters.

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Part 2 TLC-MALDI-MS

2.5 Bisphenol A

Bisphenol A (BPA) is an organic compound with two phenol moieties. Structure and physical properties of BPA are shown in Table 2.3. It's a synthetic compound that is used as an intermediate in the production of polycarbonate plastics and epoxy resin. It's commonly found in products of daily life such as plastic bottles, food packaging, flame retardants, adhesive and building material. The global production of BPA, a high-volume chemical, reached 4 billion kilograms in 2006. In the United States, production quantities grew from 736 million kg in 1995 to an estimated 1 billion kg in 2007 [30]. The increasing demand and production capacity of BPA have resulted in rapid growth, which could potentially lead to higher levels of BPA contamination in the environment [31].

Table 2.3 Structure and physical properties of bisphenols A [32]

structure			
name	bisphenol A
additional name	2,2-bis-4-hydroxyphenylpropane
	2,2-(4,4′-dihydroxydiphenyl) propane
	4,4'-dihydroxy-2,2-diphenylpropane
molecular formular	$C_{15}H_{16}O_2$
cas number	80-05-7
molecular weight	228.29 g/mol
melting point	153-156 °C
boiling point	360 °C (760 mmHg)
water solubility	120-200 mg/L (20-25 °C)

BPA has been a topic of concern due to its potential toxicity. According to studies, BPA may act like estrogen in the body, resulting in potential endocrine-disrupting effects. It has been associated with a variety of health hazards, including reproductive disorders, developmental abnormalities, and possibly being associated with certain cancers [33].

In addition, environmental toxicity of BPA has been a major concern. BPA can reach the environment via a variety of pathways, including industrial discharges, improper waste disposal, and leaching from consumer products. Once discharged, it may stay in the environment for a long period and has the ability to bioaccumulate in organisms. Because of these concerns, regulatory procedures and standards have been established to control the use and release of BPA into the environment [31, 34]. BPA can cause harmful effects at low concentrations of 1.5, 0.175 and 1.6 µg/L, according to risk evaluations carried out by the European Union, Canada, and Japan. These effects include impacts on reproduction, development, and multiple modes of action for aquatic organisms [35].

2.6 Thin layer chromatography [10, 36, 37]

Thin layer chromatography (TLC) is a chromatographic technique for separating and purifying a mixture compound into their components. It is a simple and inexpensive method that is commonly used in chemical analysis and research. TLC separates components of a mixture based on their affinity to the stationary phase, which is a thin layer of material (often silica gel or alumina) on inert plate surface, typically glass, plastic, or aluminum. The components are separated as they move up the plate using a solvent (mobile phase), and are then visualized by various techniques, such as UV light or staining. It can be used to determine the purity of a compound, identify unknown compounds, and monitor the progress of a chemical reaction.

2.7 Matrix assisted laser desorption/ionization time-of-flight mass spectrometry

Matrix-assisted laser desorption/ionization (MALDI) is an ionization technique using a laser energy-absorbing matrix to create ions from large molecules with minimal fragmentation. MALDI-TOF MS is used for analyzing and identifying different chemical compounds in a sample. It is based on the laser-induced desorption and ionization of molecules. On the surface of a solid sample containing the target analyte mixed with a matrix compound, a laser beam is focused. The analyte undergoes desorption and ionization because the matrix absorbs laser energy and transfers it to the analyte, as shown in Figure 2.4. Then, following their passing through the vacuum tube, these ions will be indicated by a time-of-flight (TOF) mass analyzer. The speed of ion movement depends on the mass-to-charge ratio (m/z), which enables them to reach the detector at different times. The results will be presented as a mass spectrum, which represents the relative abundance of ions at different m/z. MALDI-TOF MS has numerous applications, including the analysis of drug, metabolite, and lipid. For the analysis and identification of samples, it is commonly used in combination with other techniques like TLC or HPLC. MALDI-TOF MS can be used in combination with TLC to directly detect target analytes on TLC plates, allowing for qualitative analysis and providing high sensitivity and low detection limits.
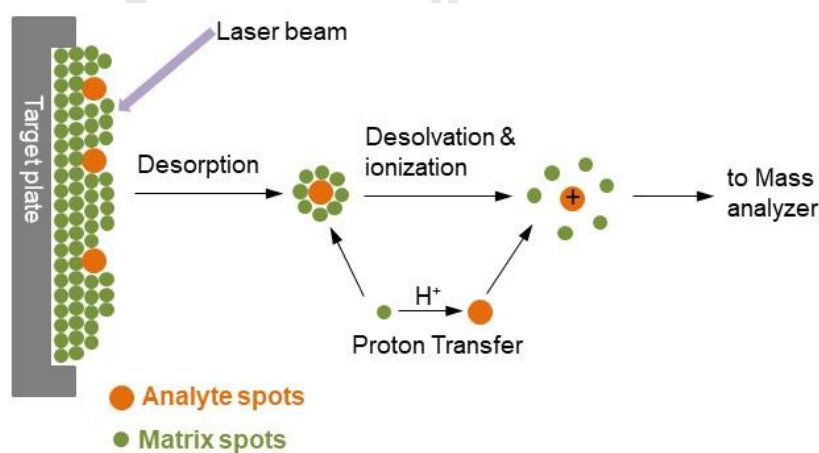
Figure 2.4 Ionization of analytes by MALDI-TOF MS [38]

The combination of TLC with MALDI-TOF MS is a powerful analytical method used for the separation and subsequent detection of the target analytes in mixtures. In this method, the analytes are separated by TLC and then ionized by laser desorption/ionization, followed by detection using mass spectrometry. This method has been used for the analysis of a wide range of analytes, including natural and synthetic organic compounds. Other desorption/ionization approaches have also been developed and applied in conjunction with planar chromatography, competing with MALDI-TOF MS.

TLC combined with MALDI-TOF MS offers several advantages. It may be used to analyze a broad variety of substances, including proteins, lipids, small molecules, peptides, and other biomolecules. The minimum sample preparation needed for TLC MALDI-TOF MS also makes it a reasonably rapid and efficient method. [39]

In general, MALDI-MS requires the traditional organic matrices leading to high fragmentation and interference at low mass region (m/z ≤ 500). Graphene oxide (GO), a derivative of graphene (G) with high oxygen functionalities possesses various interactions including π–π, hydrogen bonding, electrostatic force, hydrophobic interaction and covalently binding with different molecules. Recently, GO has been used to enhances the ionization efficiency in MALDI-MS analysis of small molecules (m/z ≤ 500), including bisphenol-A and its deriviatives [40].

2.8 Literature review

TLC combined with MALDI-TOF MS has been used to analyze a wide range of compounds, which is a method known for its high sensitivity and requirement of small sample volumes.

In 2004, Santo et al. [41] investigated the suitability of an ionic liquid matrix for TLC and direct on-spot positive ion MALDI-TOF MS for low-molecular-weight compound screening. The results discovered that using a UV-absorbing proton donor ionic liquid as the matrix resulted in essentially matrix-free mass spectra with low

matrix background ions. This study successfully identified three arborescidine alkaloids, the anesthetics levobupivacaine and mepivacaine, and the antibiotic tetracycline primarily by detecting their protonated molecules using MS. The method is rapid, highly sensitive, and involves minimal sample preparation and handling, making it well-suited for efficient screening using TLC separation and MS identification of low-molecular-weight substances.

In 2013, Chen et al. [42] investigated a novel technique that involved the combination of TLC and MALDI-MS for the direct monitoring of chemical transformations. The technique was employed to observe the acylation process catalyzed by 4-dimethylaminopyridine (DMAP) in the synthesis of bifunctional sulfonamides. Additionally, it was utilized in the exploration of natural products for the discovery of anti-inflammatory flavonoids derived from Helminthostachys zeylanica, a traditional Chinese herb.

The MALDI-TOF MS study of low-mass molecules uses many types of inorganic materials as the matrix, including graphene, graphene oxide and a derivative of graphene.

In 2010, Dong et al. [43] use of graphene as a novel matrix for the first time in MALDI-TOF MS analysis of small-molecule compounds with molecular weight in range 150-430 Da. It offered advantages like simple sample preparation, high efficiency in analyte desorption/ionization, and improved reproducibility. Graphene demonstrated excellent performance in analyzing amino acids, polyamines, steroids, anticancer drugs, and nucleosides, showing potential for complicated sample matrices and nonpolar compounds. It enhanced the limit of detection through solid-phase extraction and provided a lower laser power threshold and higher signal compared to organic matrices. Graphene simplifies sample preparation, improves reproducibility, and holds promise for rapid analysis in metabolism research and natural product characterization.

In 2018, Liang et al. [44] studied the aggregated graphene oxide (AGO) LDI-MS approach, which provides a rapid, non-toxic, and sensitive method for measuring circulating triacylglycerols (TAGs) in small sample volumes. By utilizing efficient extraction materials and specific MS matrices, AGOLDI-MS demonstrates high selectivity and sensitivity in detecting TAGs in complex samples. The elimination of complex liquid-liquid extraction and chromatographic procedures improves the analysis throughput and accessibility, making it suitable for clinical applications with large-scale sample sizes. This advancement is particularly beneficial for developing TAG biomarkers related to metabolic syndromes and other diseases.

In 2014, Min et al. [45] synthesized and used gas-phase N-doped graphene (gNG) as an efficient matrix for negative ion MALDI-TOF MS analysis of small molecules. The composition and performance of gNG were compared to other graphene-based materials, highlighting its highly efficient desorption/ionization process. This efficiency was attributed to its well-preserved π-conjugated network for laser absorption and energy transfer, as well as the presence of pyridinic nitrogen species that facilitated deprotonation of analytes. The gNG matrix exhibited clear background signals, good salt tolerance, wide applicability, and higher detection sensitivity compared to other materials. The analysis of nilotinib in a real sample demonstrated the potential for clinical monitoring of drug levels in patient serum.
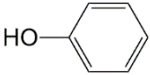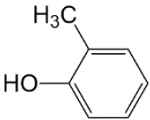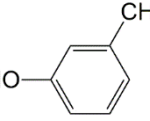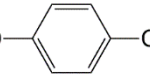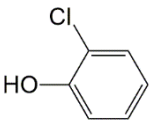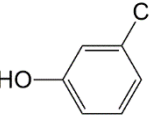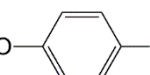
CHAPTER 3

EXPERIMENTAL
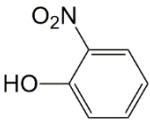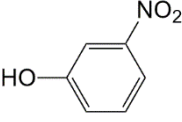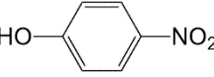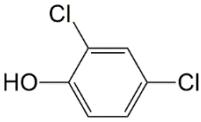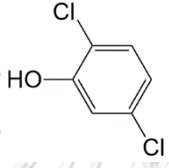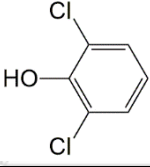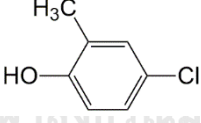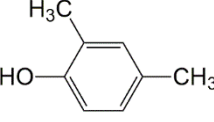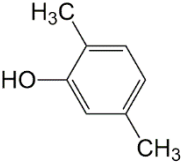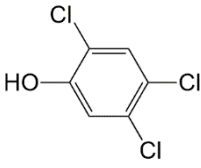
Part 1 GC
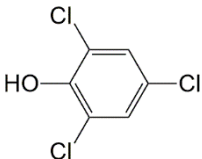
3.1 Analytes

All phenols and solvents were purchased from various vendors and were used without further purification. Grade and vendor of each analyte are shown in Table 3.1. Dichloromethane (analytical reagent grade, Fisher chemical) was used as a solvent. A mixture of 18 phenols was diluted with dichloromethane to the final concentration of ~10 mg/mL of each analyte.

Table 3.1 Phenolic compounds used in this research

no.	compound	structure	abbreviation	cas	grade	vendor
1	phenol	HO—⟨⟩	P	108-95-2	>99%	Carlo
2	2-methylphenol	H₃C ⟨⟩ HO	2MP	95-48-7	>99.5% (GC)	Fluka
3	3-methylphenol	CH₃ ⟨⟩ HO	3MP	108-39-4	99%	Sigma-Aldrich
4	4-methylphenol	HO—⟨⟩—CH₃	4MP	106-44-5	>98% (GC)	Fluka
5	2-chlorophenol	Cl ⟨⟩ HO	2Cl	95-57-8	>98% (HPLC)	Fluka
6	3-chlorophenol	Cl ⟨⟩ HO	3Cl	108-43-0	>98% (GC)	TCI
7	4-chlorophenol	HO—⟨⟩—Cl	4Cl	106-48-9	>98% (HPLC)	Fluka

no.	compound	structure	abbreviation	cas	grade	vendor
8	2-nitrophenol		2NP	88-75-5	99%	BHD
9	3-nitrophenol		3NP	554-84-7	98%	Fluka
10	4-nitrophenol		4NP	100-02-7	98%	Aldrich
11	2,4-dichlorophenol		2,4DCl	120-83-2	95%	Sigma-Aldrich
12	2,5-dichlorophenol		2,5DCl	583-78-8	>98%(GC)	TCI
13	2,6-dichlorophenol		2,6DCl	87-65-0	95%	Sigma-Aldrich
14	4-chloro-2-methylphenol		4Cl-2MP	1570-64-5	>90% (GC)	TCI
15	2,4-dimethylphenol		2,4DMP	105-67-9	>97% (GC)	Fluka
16	2,5-dimethylphenol		2,5DMP	95-87-4	>98% (GC)	TCI
17	2,4,5-trichlorophenol		2,4,5TCl	95-95-4	>99% (GC)	Fluka

no.	compound	structure	abbreviation	cas	grade	vendor
18	2,4,6-trichlorophenol		2,4,6TCl	88-06-2	>97% (GC)	TCl

3.2 Gas chromatographic analyses

All GC analyses were performed on an Agilent 7890 series gas chromatograph using the following condition:

carrier gas   : hydrogen, 50 cm/sec

injector   : split injector, split ratio 100

injector temperature   : 250 °C

detector   : flame ionization detector (FID)

detector temperature   : 250 °C

    hydrogen flow rate   : 40 mL/min

    nitrogen flow rate   : 40 mL/min

    air zero flow rate   : 400 mL/min

Each capillary column of ~15 m long with 0.25 mm i.d. was coated with 0.25 μm thick film of stationary phase. Four types of stationary phases used in this work are:

- **polysiloxane OV-1701**: poly(14%-cyanopropylphenyl-86%-dimethylsiloxane) (15.41 m)
- **ACD**: hexakis(2,3-di-*O*-methyl-6-*O-tert*-butyldimethylsilyl)-α-cyclodextrin mixed in OV-1701 (15.52 m)
- **BCD**: heptakis(2,3-di-*O*-methyl-6-*O-tert*-butyldimethylsilyl)-β-cyclodextrin mixed in OV-1701 (14.91 m)
- **GCD**: octakis(2,3-di-*O*-methyl-6-*O-tert*-butyldimethylsilyl)-γ-cyclodextrin mixed in OV-1701 (15.17 m)
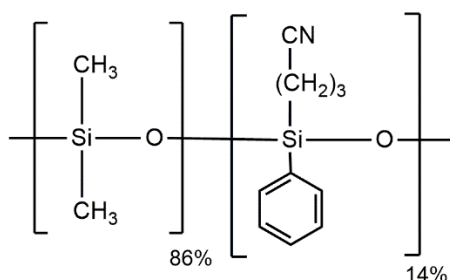
Figure 3.1 Structure of polysiloxane OV-1701
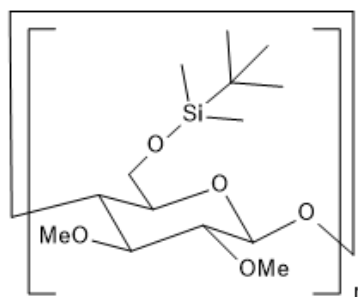
Figure 3.2 Structures of 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-CDs (n = 6, 7, and 8 referred to α-, β-, and γ-CDs, respectively)

Column condition and efficiency testing

Each column was conditioned at 220 °C until the baseline was steady. The column efficiency was tested with *n*-alkane at each working temperature ranged from 60-220 °C. The retention time (t_R) and peak width at half height (w_h) were used to calculate the number of theoretical plate (N). All columns showed good efficiency with N/m values greater than 3,000 plates/m.

Column temperature control

Temperature program: The analysis of eighteen phenols mixture was performed under temperature program. The column temperature was initially programmed from 40 °C at rate of ~ 3.2 - 3.3 °C/min based on Grob method [46]. Approximately 0.2-0.4 µL of solution was injected. Retention time (t_R) and width at half height (w_h) of each peak were noted. Resolution (Rs) between peak pairs and

elution temperature of each peak were calculated. Initial temperature, program rate and hold time were adjusted until all peaks were observed and/or completely separated.

Isothermal condition: Each group of isomers was run under isothermal condition. The column temperature was set to be close to the elution temperature determined from temperature program. Approximately 0.2-0.4 µL of solution was injected. Column temperature was adjusted until all peaks were observed and/or completely separated. Resolution (Rs), retention factor (k´) and selectivity (α) were calculated.

Part 2 TLC-MALDI-MS
3.3 Analytes and solvents

Bisphenol A (Analytical standard) was purchased from Aldrich, ethanol and formic acid (98%) were purchased from Merck, hexane (ACS reagent grade) was purchased from J.T.Baker, ethyl acetate was purchased from CARLO ERBA Reagents, graphene was purchased from XFNANO, graphene oxide was purchased from Graphene Supermarket, Nitrogen-doped graphene was purchased from ACS Material, TLC aluminium sheets were purchased from Merck.

Mass spectrometry: Laser desorption/ionization and matrix assisted laser desorption/ionization mass spectra was performed on a Bruker Autoflex III MALDI-TOF mass spectrometer with 50% nitrogen laser at 337 nm 50 shots.

3.4 Optimization parameters

A stock solution of BPA in ethanol was prepared to a concentration of 1000 mM. Following that, a standard solution was prepared by diluting the stock solution with ethanol to achieve the concentration of 0.1 mol/L. BPA solution was accurately dispensed onto a TLC plate using a micropipette, utilizing a volume of 1 µL. Subsequently, 1 µL of the optimal matrix was carefully deposited onto the surface of

the analyte on the TLC plate. TLC plate with analyte and matrix was deposited on LDI target plate using conductive carbon tape. The MALDI-TOF MS instrument was equipped with a 50% nitrogen laser operating at a wavelength of 337 nm, A total of 50 shots was performed.

3.4.1 Types of matrices

The matrix was varied, including 0.1 mg/mL of graphene, graphene oxide, and nitrogen-doped graphene for the analysis of BPA at a concentration of 0.1 mol/L. The analysis was performed using a TLC plate coupled with MALDI-TOF MS, with a 50% nitrogen laser at 337 nm for 50 shots.

3.4.2 Matrix-to-analyte ratio

After carefully selecting the optimal matrix at a concentration of 0.1 mg/mL in the initial stage, the matrix-to-analyte ratio (v/v) was systematically modified across a range of different ratios, specifically 1:1, 1:2, 2:1, 1:5, and 5:1. BPA at a concentration of 0.1 mol/L was analyzed using a TLC plate coupled with MALDI-TOF MS with a 50% nitrogen laser at 337 nm for 50 shots.

3.4.3 Laser concentrations

The nitrogen laser concentration at 337 nm of the MALDI-TOF MS instrument was carefully adjusted to five different levels: 20%, 30%, 50%, 70%, and 90%. These adjustments were made while employing 0.1 mol/L BPA as the analyte and 0.1 mg/mL of the selected matrix with an optimal matrix-to-analyte ratio.

3.4.4 Calibration curve

BPA sample on a non-developed TLC plate: Prepare a series of standard solutions by diluting the stock solution with ethanol to achieve concentrations of 20, 40, 60, 80, and 100 mM. Then, each specific concentration of BPA (1 μL) and matrix (1 μL) were accurately dispensed onto a TLC plate using a micropipette before conducting MALDI-TOF MS analysis. The resulting mass spectra obtained from each

concentration were subsequently analyzed to construct a linear calibration curve for BPA. Calibration curve was established by least-square linear regression analysis.

BPA sample on a developed TLC plate: The BPA concentration used ranged from 80-160 mM. Additionally, after preparing the analyte on the TLC plate, it was developed with a mobile phase composed of hexane: ethyl acetate: formic acid in a ratio (v/v) of 8:2:1. The optimal matrix was then spotted onto the surface of the analyte on the TLC plate before conducting MALDI-TOF MS analysis.

3.4.5 Limit of detection (LOD)

LOD can be established through the analysis of samples containing known low concentrations of the analyte and evaluating the ratio between the signal and noise. The signal corresponds to the intensity of the analyte peak, whereas the noise corresponds to the background signal or baseline noise observed in the mass spectrum. Typically, the LOD is defined as a signal to noise ratio of 3:1, indicating that the intensity of the analyte signal is at least three times greater than the noise level.
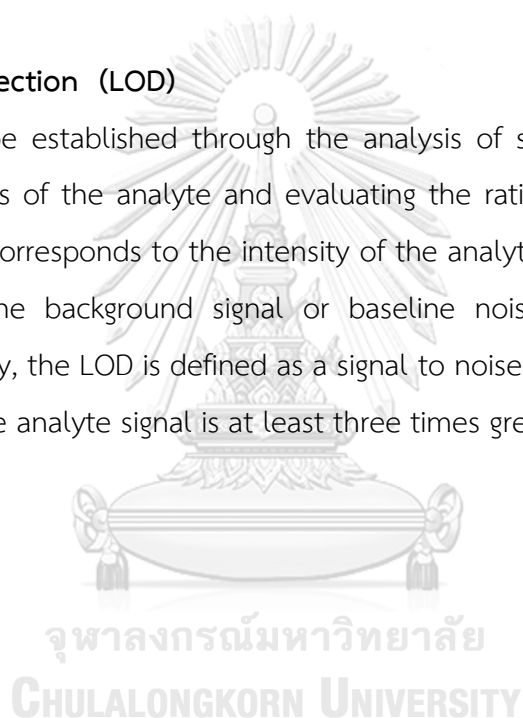
CHAPTER 4

RESULTS AND DISCUSSIONS

Part 1 GC

Phenols or phenolic compounds have been significantly applied in many industries. Nonetheless, they are also known to be potential carcinogens and have been classified as priority pollutants. Due to their wide application, phenolic compounds are often found as contaminants in various environmental matrices. From previous reports, GC-FID or GC-MS using different type of polysiloxanes as stationary phases were commonly used to analyze the mixture. However, coelution and/or incomplete separation were still observed for several phenolic compounds because many compounds have similar properties. In addition, developing a single-run separation of the mixture was a difficult task. In this work, the single-run separation of eighteen phenolic compounds was investigated by gas chromatography using derivatized cyclodextrins as stationary phases.

4.1 Single-run separation of eighteen phenolic compounds

The mixture contains eighteen phenolic compounds (including phenol, chlorophenols, nitrophenols, and methylphenols) which many of them were classified as priority pollutants. Since the mixture contained many phenolic compounds with a wide range of physical properties, a temperature program was chosen over an isothermal condition. Another important parameter for successful separation is the type of stationary phase. In this work, three types of derivatized cyclodextrin (CDs) mixed with polysiloxane OV-1701 were examined to improve the separation. They were (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl) derivative of α-, β-, and γ-CDs of different ring size. The mixture was initially analyzed using a temperature program starting from 40 to 220 °C based on Grob method [46]. Initial temperature, program rate and hold time were adjusted until all peaks were observed and/or completely separated. The optimum conditions obtained from all CD-based columns will be compared with that obtained from OV-1701 column.

4.1.1 OV-1701 column

The single-run separation of 18 phenolic compounds was first performed using polysiloxane OV-1701 column. The separation used temperature program from 40 °C at a program rate of 3.24 °C/min. The total analysis time was 39 minutes and 15 peaks were observed on chromatogram, as shown in Figure 4.1a. Each pure phenolic compound was injected under the same condition in order to determine peak identity. The first eluted peak was 2Cl with retention time of 10.05 minutes and elution temperature of 72.5 °C. Coelution and incomplete separation were found for several peak pairs. The 2,4DMP/2,5DMP; 2,6DCl/2,5DCl; and 4Cl/3Cl were coeluted as single peaks at 18.01, 18.21 and 23.06 minutes, respectively. Incomplete separations were observed for 4MP/3MP and 2,4DMP/2,5DMP with 2,4DCl. The elution order and retention times of all phenolic compounds were listed in Table 4.1.

Base on the first separation, the initial column temperature was increased to 80 °C in order to shorten the analysis time. The total analysis time was 27.2 minutes. However, 15 peaks were still observed with similar elution order. Peak coelutions were still found for 2,4DMP/2,5DMP; 2,6DCl/2,5DCl; and 4Cl/3Cl at 7.65, 7.90 and 11.82 minutes, respectively. The separation between 2,4DCl and 2,4DMP/2,5DMP was improved to Rs of 2.00. When the initial column temperature was increased to 100 °C, the separation was slightly worse as 14 peaks were observed (4MP/3MP coeluted). Therefore, the initial column temperature of 80 °C was selected.

Next, the temperature program rate was adjusted. The optimum condition could be obtained using a program from 80-106 °C at 3.24 °C/min, then to 220 °C at 20 °C/min. The total analysis time was decreased to 13.5 minutes and 15 peaks were observed on chromatogram with no change in elution order, as shown in Figure 4.1b. Although coelution and incomplete separation were still observed, the separation between 2,4DMP/2,5DMP and 2,4DCl was improved. Elution order, elution time and resolution (Rs) of all phenolic compounds obtained from OV-1701 column under initial and optimum conditions were compared in Table 4.1.
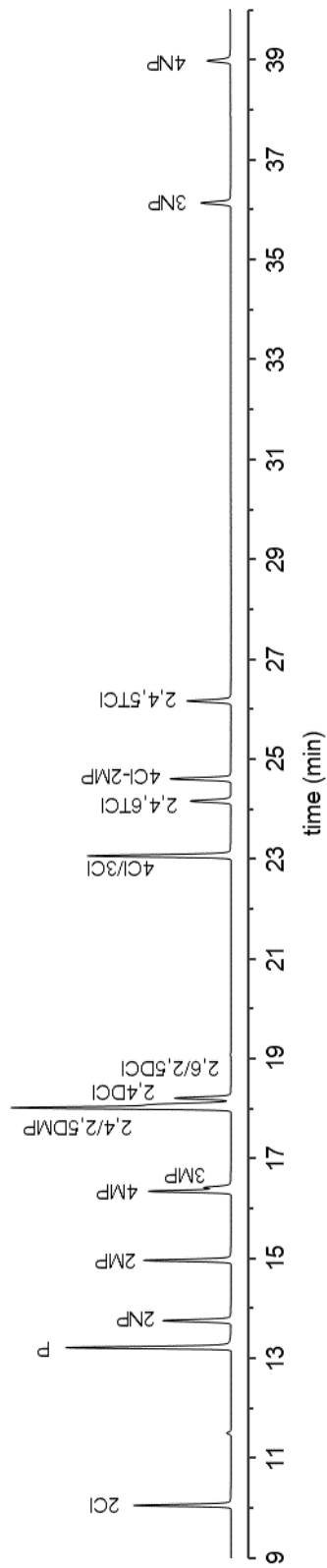
Figure 4.1 Separation of a mixture of 18 phenols on OV-1701 column

a. temperature program 40-220 °C at 3.24 °C/min

b. temperature program 80-106 °C at 3.24 °C/min, then to 220 °C at 20 °C/min

Table 4.1 Elution time and resolution of 18 phenolic compounds from OV-1701
column under initial condition and optimum condition.

		initial condition			optimum condition		
order	analyte	t_R (min)	Rs	elution temp (°C)	t_R (min)	Rs	elution temp (°C)
1	2Cl	10.05	-	72.56	3.17	-	90.27
2	P	13.21	37.58	82.80	4.51	26.91	94.61
3	2NP	13.75	5.97	84.55	5.24	12.37	96.96
4	2MP	14.96	13.34	88.46	5.54	5.00	97.96
5	4MP	16.34	15.72	92.95	6.44	13.52	100.87
6	3MP	16.42	< 0.90	93.18	6.47	< 0.80	100.97
7	2,4DMP/2,5DMP	18.01	17.89	98.37	7.66	16.80	104.81
8	2,4DCl	18.05	< 0.80	98.48	7.80	2.00	105.28
9	2,6DCl/2,5DCl	18.21	1.84	98.99	7.90	1.31	105.60
10	4Cl/3Cl	23.06	52.24	114.70	9.97	38.35	144.85
11	2,4,6TCl	24.16	11.17	118.26	10.35	12.16	152.41
12	4Cl-2MP	24.60	4.53	119.70	10.39	1.58	153.37
13	2,4,5TCl	26.16	16.05	124.76	10.83	14.82	162.03
14	3NP	36.13	97.48	157.06	12.77	71.84	200.85
15	4NP	38.98	26.72	166.29	13.27	19.19	210.83

Next, the single-run separation of the mixture of 18 phenolic compounds
were performed on derivatized CD columns.

4.1.2 ACD column

The separation of 18 phenolic compounds was performed on ACD column, which contained (2,3-di-*O*-methyl-6-*O*-tert-butyldimethylsilyl) derivative of a small-size α-CD. The initial temperature program used was similar to that of OV-1701 column, from 40 °C at a program rate of 3.22 °C/min. The separation was done in 43 minutes. Eighteen peaks were observed and identified as shown on chromatogram, Figure 4.2a. Fortunately, all 18 phenolic compounds were separated on ACD column with Rs values greater than 1.5. The first eluted peak was 2Cl with retention time of 14.87 minutes and elution temperature of 87.87 °C. For all compounds, there were about 10-19 °C increased in elution temperatures compared to those from OV-1701 column. This suggested an increased interaction between phenolic compounds and α-CD derivative. In addition, ACD column provided different interaction for compounds with different structure, as position isomers were well separated. The elution order obtained from ACD column was quite similar to that from OV-1701 column, except for 2,6DCl/2,4DCl and 4Cl-2MP/2,4,5TCl/2,4,6TCl. The elution order and retention times of 18 phenolic compounds under initial condition on ACD column were shown in Table 4.2.

The initial single-run separation of 18 phenolic compounds on ACD column showed complete separation of all peaks. Next, the initial column temperature and temperature program rate will be adjusted to shorten analysis time, considering the Rs of the least separated peak pair. The initial column temperature was then adjusted to 90, 100, 110, 120 and 130 °C. Results showed that all peaks were well separated with Rs values greater than 1.5. Using initial column temperature of 130 °C and temperature program rate of 3.22 °C/min, the least separated peak pair was 2NP/2MP with Rs value of 1.69.

Next, the temperature program rate was adjusted. The optimum condition on ACD column could be obtained using a program from 130-150 °C at 3.22 °C/min, then to 220 °C at 25 °C/min. Complete separation of all 18 peaks could be obtained in a single run within 9.4 minutes, as shown in Figure 4.2b. Elution order, elution time and

resolution (Rs) of all phenolic compounds obtained from ACD column under initial and optimum conditions were compared in Table 4.2.

a.
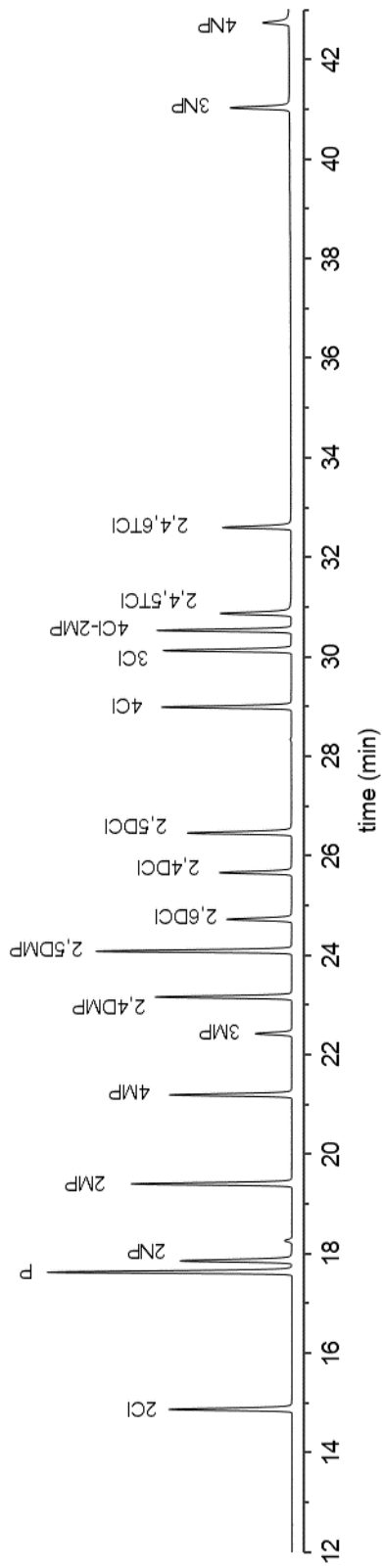
b.
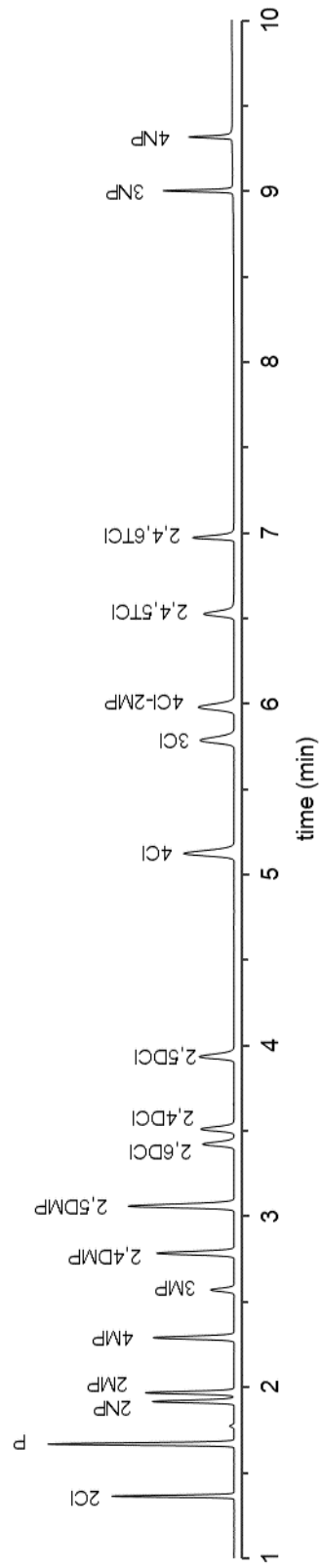
Figure 4.2 Separation of a mixture of 18 phenols on ACD column

a. temperature program 40-220 °C at 3.22 °C/min

b. temperature program 130-150 °C at 3.22 °C/min, then to 220 °C at 25 °C /min

Table 4.2 Elution time and resolution of 18 phenolic compounds from ACD column under initial condition and optimum condition.

order	analyte	initial condition			optimum condition		
		t_R (min)	Rs	elution temp (°C)	t_R (min)	Rs	elution temp (°C)
1	2Cl	14.87	-	87.87	1.36	-	134.38
2	P	17.63	29.73	96.75	1.67	12.51	135.37
3	2NP	17.85	2.37	97.48	1.92	8.88	136.17
4	2MP	19.40	16.14	102.47	1.97	1.69	136.33
5	4MP	21.19	18.86	108.23	2.29	9.84	137.37
6	3MP	22.42	12.97	112.19	2.57	7.74	138.27
7	2,4DMP	23.16	7.91	114.56	2.78	5.54	138.96
8	2,5DMP	24.08	9.99	117.52	3.06	6.79	139.85
9	2,6DCl	24.72	6.73	119.59	3.42	8.22	141.02
10	2,4DCl	25.66	9.80	122.61	3.51	1.86	141.30
11	2,5DCl	26.45	8.42	125.18	3.93	8.32	142.67
12	4Cl	28.98	26.79	133.33	5.12	20.69	146.50
13	3Cl	30.12	12.40	136.99	5.79	10.29	148.63
14	4Cl-2MP	30.52	4.58	138.29	5.98	2.89	149.26
15	2,4,5TCl	30.87	3.50	139.39	6.53	8.65	157.87
16	2,4,6TCl	32.59	16.88	144.95	6.98	9.03	169.17
17	3NP	41.03	83.45	172.11	9.00	61.59	219.80
18	4NP	42.74	14.41	177.62	9.32	12.04	220.00

4.1.3 BCD column:

The mixture of 18 phenolic compounds was analyzed using a medium-size CD derivative as a stationary phase. The BCD column contained (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl) derivative of β-CD. The first run used a temperature program with initial temperature of 40 ℃ and a program rate of 3.35 ℃/min. Only 17 peaks were observed on chromatogram, as shown in Figure 4.3a. The first eluted peak was 2Cl with retention time of 14.91 minutes. At 22.99 minutes, 2,4DCl and 2,6DCl coeluted as a single peak. There was also an incomplete separation of 2,4DMP and 3MP (Rs 1.09). The total analysis time was 42.10 minutes.

The initial column temperature and temperature program rate were adjusted. The optimum condition was achieved with temperature program from 100-142 ℃ at 3.35 ℃/min, then to 220 ℃ at 25 ℃/min. The mixture could be separated in 16 minutes. All 18 peaks were observed on chromatogram, as shown in Figure 4.3b. Coeluted peaks between 2,4DCl and 2,6DCl were separated with Rs value of 1.04. However, peaks of 2,4DMP and 3MP tended to merge and showed lower resolution and a change in elution order compared to the initial condition. Elution order, elution time and resolution of all phenolic compounds obtained from BCD column under initial and optimum conditions were compared in Table 4.3.
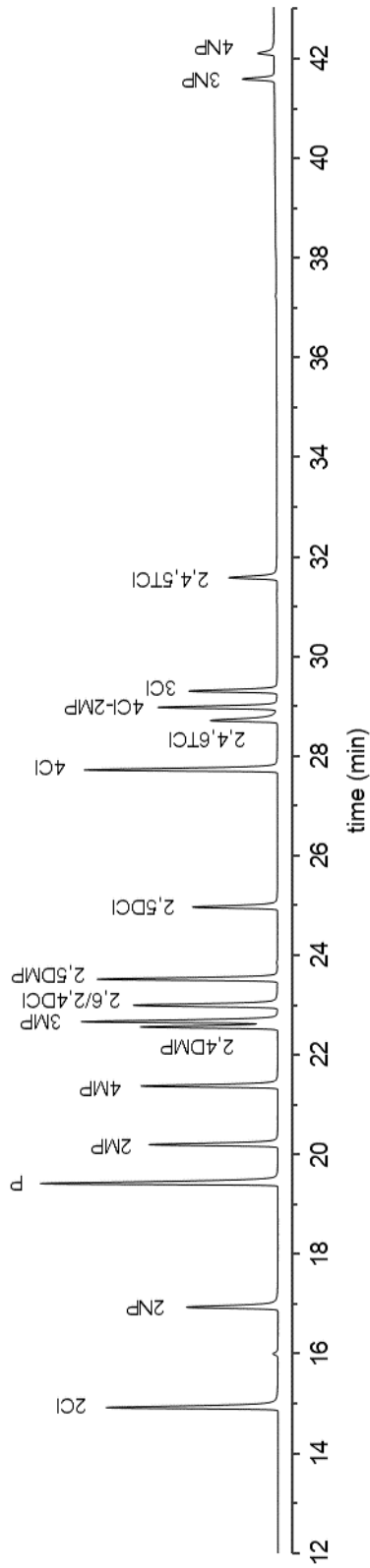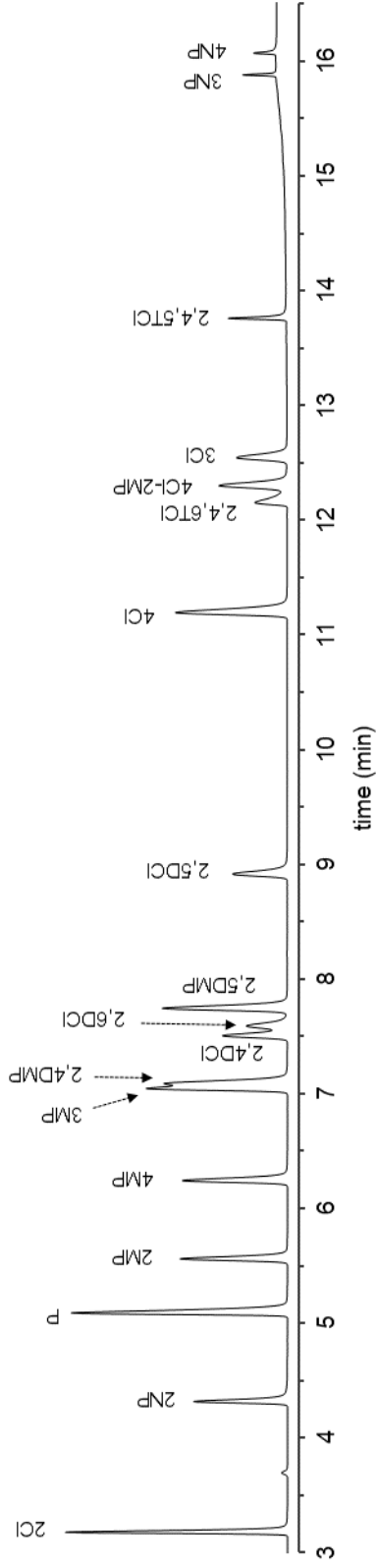
Figure 4.3   Separation of a mixture of 18 phenols on BCD column

a. temperature program 40-220 °C at 3.35 °C/min

b. temperature program 100-142 °C at 3.35 °C/min, then to 220 °C at 25 °C /min

Table 4.3 Elution time and resolution of 18 phenolic compounds from BCD column under initial condition and optimum condition.

order	initial condition				optimum condition			
	analyte	t_R (min)	Rs	elution temp (°C)	analyte	t_R (min)	Rs	elution temp (°C)
1	2Cl	14.91	-	89.96	2Cl	3.18	-	110.64
2	2NP	16.93	18.51	96.72	2NP	4.32	20.69	114.46
3	P	19.41	23.18	105.03	P	5.09	11.94	117.04
4	2MP	20.19	7.73	107.65	2MP	5.56	7.00	118.62
5	4MP	21.27	10.90	111.25	4MP	6.24	9.75	120.91
6	2,4DMP	22.56	13.57	115.56	3MP	7.04	11.81	123.60
7	3MP	22.66	1.09	115.91	2,4DMP	7.09	< 0.80	123.74
8	2,4DCl/ 2,6DCl	22.99	3.16	117.02	2,4DCl	7.50	6.76	125.14
9	2,5DMP	23.51	5.11	118.77	2,6DCl	7.59	1.04	125.42
10	2,5DCl	24.96	14.64	123.62	2,5DMP	7.74	1.79	125.94
11	4Cl	27.72	27.41	132.85	2,5DCl	8.91	13.70	129.86
12	2,4,6TCl	28.71	9.28	136.18	4Cl	11.19	24.95	137.49
13	4Cl-2MP	28.97	2.45	137.06	2,4,6TCl	12.15	10.11	140.71
14	3Cl	29.30	3.28	138.15	4Cl-2MP	12.30	1.56	141.20
15	2,4,5TCl	31.58	21.93	145.78	3Cl	12.54	2.56	142.13
16	3NP	41.59	98.03	179.32	2,4,5TCl	13.76	17.46	172.50
17	4NP	42.10	4.84	181.05	3NP	15.88	58.91	220.00
18					4NP	16.07	5.82	220.00

4.1.4 GCD column:

The mixture of 18 phenolic compounds was analyzed using GCD column. The GCD column contained (2,3-di-*O*-methyl-6-*O-tert*-butyldimethylsilyl) derivative of a large-size γ-CD. The first separation was achieved with temperature program from 40 °C and a program rate of 3.30 °C/min. The total analysis time was 42 minutes. Only 15 peaks were observed on chromatogram, as shown in Figure 4.4a. Results showed that there were two coeluted peaks: 2,4DCl/2,6DCl/2,5DMP at 21.83 minutes and 3Cl/2,4,6TCl at 27.59 minutes. In addition, an incomplete separation was observed for 2,4DMP and 2,4DCl/2,6DCl/2,5DMP (Rs 1.35).

After adjusting initial column temperature and temperature program rate, the optimum condition on GCD column was achieved with temperature program from 130-140 °C at 3.30 °C/min, then to 220 °C at 25 °C /min. All 18 peaks were observed on chromatogram within 7 minutes, as shown in Figure 4.4b. Coelution of 3Cl/2,4,6TCl peaks was well resolved. Two incomplete separations were still observed for 2,4DMP/2,5DMP (Rs 1.04) and 2,4DCl/2,6DCl (Rs 1.13). Elution order, elution time and resolution of all phenolic compounds obtained from GCD column under initial and optimum conditions were compared in Table 4.4.
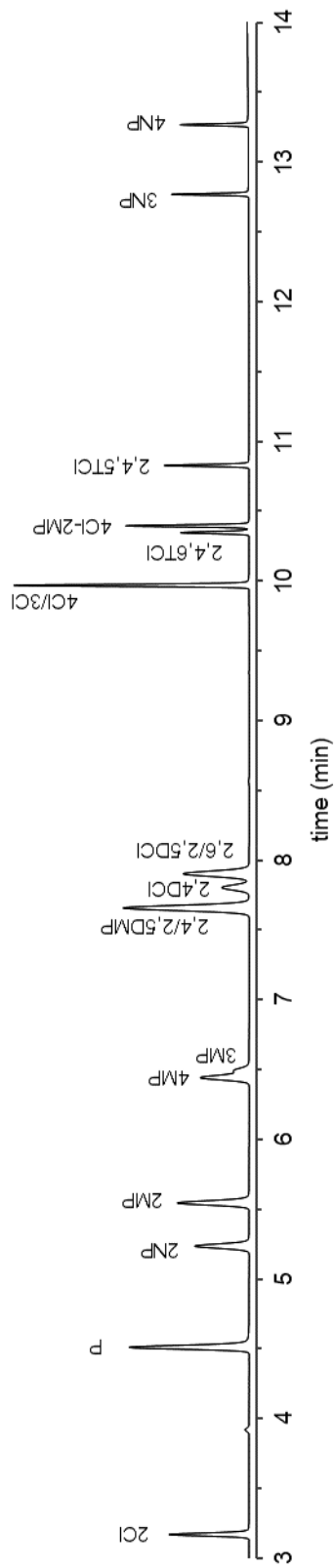
a.

b.
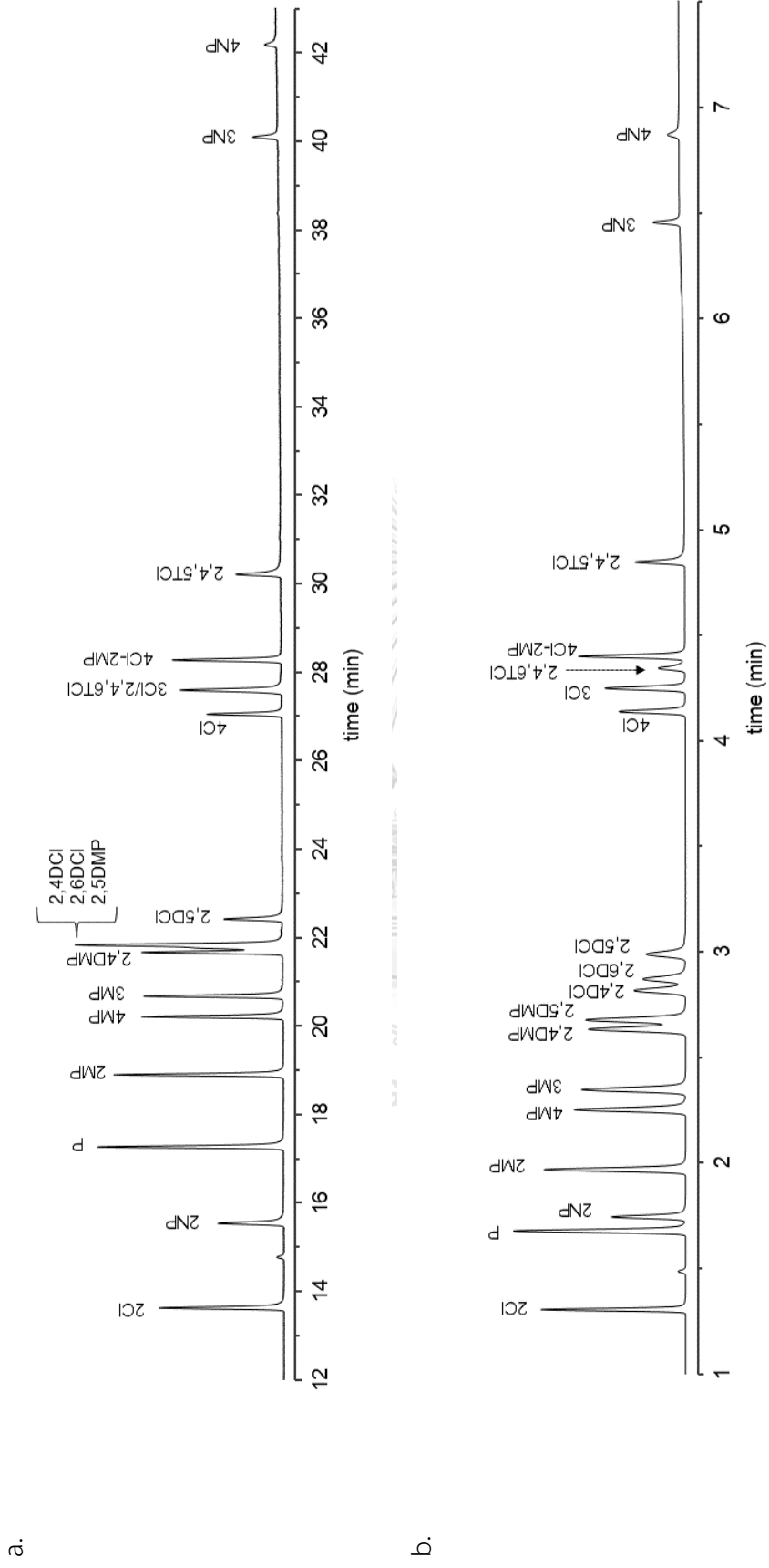
Figure 4.4 Separation of a mixture of 18 phenols on GCD column

a. temperature program 40-220 °C at 3.30 °C/min

b. temperature program 130-140 °C at 3.30 °C/min, then to 220 °C at 25 °C /min

Table 4.4 Elution time and resolution of 18 phenolic compounds from GCD column
under initial condition and optimum condition.

	initial condition			optimum condition			
order	analyte	t_R (min)	Rs	elution temp (°C)	analyte	t_R (min)	Rs	elution temp (°C)
1	2Cl	13.62	-	84.94	2Cl	1.30	-	134.30
2	2NP	15.53	16.83	91.25	P	1.68	13.64	135.53
3	P	17.26	15.20	96.95	2NP	1.74	2.18	135.75
4	2MP	18.89	15.52	102.34	2MP	1.97	6.81	136.49
5	4MP	20.21	12.40	106.68	4MP	2.25	8.00	137.43
6	3MP	20.67	4.30	108.20	3MP	2.35	2.45	137.74
7	2,4DMP	21.66	9.45	111.48	2,4DMP	2.63	6.98	138.69
8	2,4DCl/ 2,6DCl/ 2,5DMP	21.83	1.35	112.03	2,5DMP	2.68	1.04	138.83
9	2,5DCl	22.41	4.35	113.95	2,4DCl	2.82	3.13	139.29
10	4Cl	27.04	39.04	129.25	2,6DCl	2.87	1.13	139.47
11	3Cl/ 2,4,6TCl	27.59	4.62	131.04	2,5DCl	2.99	2.33	139.86
12	4Cl-2MP	28.27	5.85	133.29	4Cl	4.14	27.07	167.69
13	2,4,5TCl	30.21	16.11	139.69	3Cl	4.25	3.33	170.44
14	3NP	40.10	77.40	172.31	2,4,6TCl	4.34	2.74	172.82
15	4NP	42.18	16.16	179.19	4Cl-2MP	4.40	1.69	174.22
16					2,4,5TCl	4.85	13.59	185.39
17					3NP	6.46	46.45	225.64
18					4NP	6.87	9.97	236.02

The single-run separations of 18 phenolic compounds were investigated using four types of GC stationary phases: polysiloxane OV-1701 and three derivatized CDs mixed in OV-1701. Results showed that using derivatized-CDs as stationary phases can improve the single-run separation of the mixture. On OV-1701 column, several isomers showed coelutions and/or incomplete separations. All three derivatized CDs (ACD, BCD and GCD columns) demonstrated enhanced separation of 4MP/3MP; 2,6DCl/2,5DCl; 2,4DMP/2,5DMP; and 4Cl/3Cl peak pairs. This is possibly due to additional interaction between phenolic compounds and functional groups of CDs as well as their inclusion complex formation. CD can contain guest molecules (phenolic compounds) due to their hollow torus shape, which leads to the formation of inclusion complexes.

The effect of CD ring-size on separation was studied. The (2,3-di-O-methyl-6-O-$tert$-butyldimethylsilyl) derivative of α-, β-, and γ-CDs were used. The size of CD derivative increased in the order of ACD < BCD < GCD. Results from optimum conditions showed that all 18 phenolic compounds could be observed on three columns (Table 4.5). GCD column provided the shortest analysis time of 6.87 minutes. However, two incomplete separations were still observed on both BCD and GCD columns. Interestingly, ACD column could provide complete separation of all 18 phenolic compounds in a single run in 9.32 minutes. Although, most commercial columns used derivatized β-CDs as stationary phases due to their broad applications, their selectivities were hard to predict. The separation of isomers or enantiomers using CD-based GC stationary phases depended on several factors, such as size of CD, type of derivatization on CD, and number of functional groups on CD [47].

Elution order of 18 phenolic compounds on four columns were compared in Table 4.5. Elution order of compounds in GC depends on analyte boiling point, separation temperature and the interaction between stationary phase and analyte. The interactions involved with CD derivative and phenolic compounds included van der Waals interaction, hydrogen bonding, changes in conformation, inclusion complex formation, cavity size of CD, and steric interactions [48]. Therefore, it is difficult to

predict the elution order. Among 18 phenolic compounds, 2Cl and 4NP have the lowest and highest boiling points, respectively. 2Cl and 4NP eluted as the first peak and the last peak on all four columns. Elution order of other compounds were quite different. To specify their names, each pure compound must be injected to confirm their identities when the separation condition changed.

Table 4.5 Comparison of elution order, analysis time, and coeluted peaks from 4 columns under optimum conditions.

elution order	OV-1701	ACD	BCD	GCD
1	2Cl	2Cl	2Cl	2Cl
2	P	P	2NP	P
3	2NP	2NP	P	2NP
4	2MP	2MP	2MP	2MP
5	4MP	4MP	4MP	4MP
6	3MP	3MP	3MP	3MP
7	2,4DMP/2,5DMP	2,4DMP	2,4DMP	2,4DMP
8	-	2,5DMP	2,4DCl	2,5DMP
9	2,4DCl	2,6DCl	2,6DCl	2,4DCl
10	2,6DCl/2,5DCl	2,4DCl	2,5DMP	2,6DCl
11	-	2,5DCl	2,5DCl	2,5DCl
12	4Cl/3Cl	4Cl	4Cl	4Cl
13	-	3Cl	2,4,6TCl	3Cl
14	2,4,6TCl	4Cl-2MP	4Cl-2MP	2,4,6TCl
15	4Cl-2MP	2,4,5TCl	3Cl	4Cl-2MP
16	2,4,5TCl	2,4,6TCl	2,4,5TCl	2,4,5TCl
17	3NP	3NP	3NP	3NP
18	4NP	4NP	4NP	4NP
total time (min)	13.27	9.32	16.07	6.87
# peaks observed	15	18	18	18
# coeluted peaks	3	-	-	-
# incompletely separated peak pair(s)	2	-	2	2

4.2 Separation of isomers of phenolic compounds

According to results from temperature program runs, coelutions and/or incomplete separations were still observed on OV-1701, BCD, and GCD columns. Most compounds that showed coelutions and/or incomplete separations were isomers. They were 3 methylphenols, 3 chlorophenols, 3 nitrophenols, 3 dichlorophenols, 2 dimethylphenols and 2 trichlorophenols. Therefore, each group of isomers were further investigated on all four columns. Isothermal condition, instead of temperature program, was selected as it is suitable for separating a few compounds with similar properties. Elution temperature of isomers obtained from initial temperature program run was used as the starting isothermal condition for the analysis of each group of isomers. To determine the optimum condition, the column temperature was increased or decreased until all isomer peaks were completely separated with Rs value ≥ 1.5 with reasonable retention factor (k′ not more than 20).
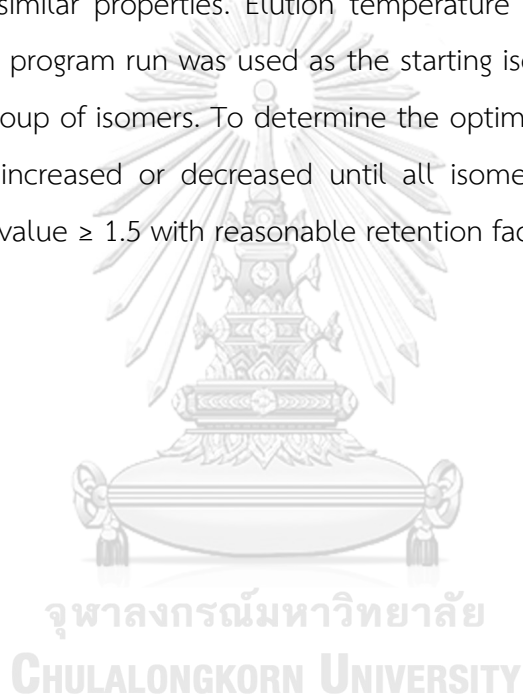
4.2.1 OV-1701 column

Six groups of isomers were separately analyzed on OV-1701 column using isothermal conditions. First, separation of 3 methylphenols (2MP, 3MP and 4MP) was attempted. Based on results obtained from initial temperature program run (Table 4.1), 3 methylphenols eluted in the order of 2MP → 4MP → 3MP with elution temperatures from 88-93 °C. Peaks of 2MP and 4MP were well separated, but peaks of 4MP and 3MP were barely separated. An isothermal temperature of 90 °C was selected for the analysis. The separation of 4MP/3MP was incomplete and the analysis time (t_R) was 7.05 minutes (k' 12.50). Column temperature was then decreased to 80 °C, the separation of 4MP/3MP was slightly improved but still incomplete and the analysis time (t_R) was 11.65 minutes (k' 21.32). Further decrease in column temperature was not attempted because retention factor of last peak (k'_{3MP}) was quite high. Long retained compounds ($k' > 20$) usually lead to broad peak and/or asymmetrical peak shape. Therefore, the best condition for the separation of 3 methylphenols on OV-1701 column was at 80 °C and complete separation of 4MP/3MP isomers could not be achieved using this type of stationary phase.

Next, separation of 2 trichlorophenols (2,4,5TCl and 2,4,6TCl) was studied. Results from initial temperature program run showed elution order of 2,4,6TCl → 2,4,5TCl and elution temperatures from 118-124 °C with excellent separation. High isothermal temperature of 170 °C was selected. The separation of 2,4,6TCl/2,4,5TCl was well separated with Rs of 8.13. Column temperature was then increased until Rs of 1.5 was obtained. Complete separation of 2,4,5TCl and 2,4,6TCl was observed at 239 °C in less than 1 minute (0.71 minute). Separation of 2,4,5TCl and 2,4,6TCl with Rs of 2.0 could be achieved at 230 °C in 1.75 minutes.

Separation of 3 dichlorophenols (2,4DCl; 2,5DCl and 2,6DCl) was studied. Results from initial temperature program run showed elution order of 2,4DCl → 2,5DCl /2,6DCl and elution temperatures of 98.5-99.0 °C. Two isomers of 2,5DCl and 2,6DCl coeluted as one peak and incompletely separated from 2,4DCl. Effect of column temperature on retention factors of each analyte was studied. A plot of ln k'

versus 1/T of 3 dichlorophenols was shown in Figure 4.5. Their retention behavior was quite interesting. At temperatures higher than 100 °C (1/T = 2.68×10^{-3} K^{-1}), the elution order was 2,4DCl → 2,5DCl → 2,6DCl. While at temperatures lower than 100 °C, the elution order was 2,4DCl → 2,6DCl → 2,5DCl. In addition, 2,4DCl and 2,6DCl coeluted 80 °C and elution order changed at temperatures lower than 80 °C. At 90 °C, 2,4DCl and 2,5DCl tended to merge but the separation continuously improved at temperatures above 110 °C. However, too high temperature resulted in lower peak resolution. Therefore, the best condition for the separation of 3 dichlorophenols on OV-1701 column was at 120 °C.

Isothermal separations of other groups of isomers were studied in a similar fashion and their results were shown in Table 4.6. Results from temperature program and isothermal condition showed that isomers separated with good resolution from temperature program could be completely separated by isothermal condition in a shorter analysis time (3 nitrophenols and 2 trichlorophenols).
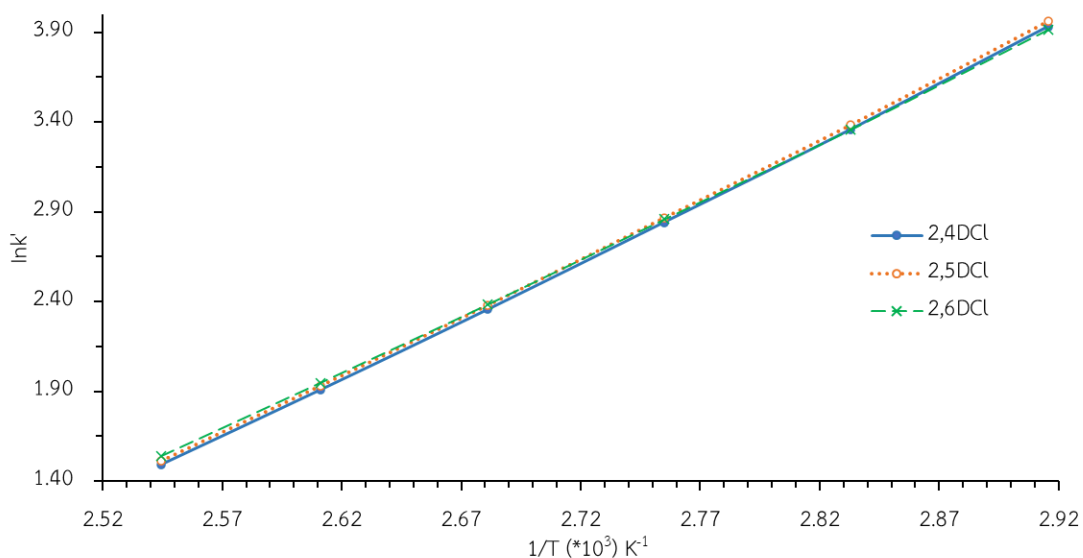
Figure 4.5 Plot of ln k′ versus 1/T of 2,4DCl; 2,5DCl and 2,6DCl on OV-1701 column

Table 4.6 Isothermal separations for 6 groups of isomers on OV-1701 column

#	analyte	temperature program			isothermal condition			
		t_R (min)	elution temp (°C)	Rs	temp (°C)	t_R (min)	k′	Rs
1	2MP	14.96	88.46			8.90	16.05	
	4MP	16.34	92.95	15.72	80	11.49	21.02	16.37
	3MP	16.42	93.18	< 0.90		11.65	21.32	< 0.90
2	2Cl	10.05	72.56			1.51	1.89	
	4Cl	23.06	114.70	147.49	110	8.32	14.93	83.25
	3Cl	23.06	114.70	0		8.32	14.93	0
3	2NP	13.75	84.55			0.60	0.14	
	3NP	36.13	157.06	224.96	240	0.94	0.81	19.09
	4NP	38.98	166.29	26.72		1.09	1.08	6.40
4	2,4DCl	18.05	98.48			2.86	4.48	
	2,5DCl	18.21	98.99	1.84	120	2.91	4.57	0.94
	2,6DCl	18.21	98.99	0		2.97	4.70	1.42
5	2,4DMP	18.01	98.37		100	5.85	10.20	
	2,5DMP	18.01	98.37	0		5.85	10.20	0
6	2,4,6TCl	24.16	118.26		239	0.68	0.30	
	2,4,5TCl	26.16	124.76	19.97		0.71	0.35	1.50

4.2.2 ACD column

Six groups of isomers were separately analyzed on ACD column using isothermal conditions with similar procedure to those on OV-1701 column. Based on results obtained from initial temperature program run, all 6 groups of isomers were well separated on ACD column. High isothermal conditions could be used as a starting condition. For the separation of 3 nitrophenols (2NP, 3NP and 4NP), elution temperatures were 97-177 °C with the elution order of 2NP → 3NP → 4NP. An isothermal temperature of 200 °C was first selected, the analysis showed excellent separation (Rs = 11.67) and analysis time of 2.419 minutes. Column temperature was then increased to 220, 230 and 240 °C. Resolution and retention factor decreased to Rs 9.12 (k′ 2.17), 7.49 (k′ 1.84), and 6.22 (k′ 1.34), respectively. The column temperature was not increased further due to the limiting temperature of the stationary phase. Therefore, the best condition for the separation of 3 nitrophenols on ACD column was at 240 °C. Results showed that ACD column provided complete separation for all groups of isomers. The optimum isothermal conditions for each group were shown in Table 4.7.

Table 4.7 Isothermal separations for 6 groups of isomers on ACD column

#	analyte	temperature program			isothermal condition			
		t_R (min)	elution temp (°C)	Rs	temp (°C)	t_R (min)	k′	Rs
1	2MP	19.40	102.47			0.79	0.47	
	4MP	21.19	108.23	18.86	182	0.83	0.56	2.61
	3MP	22.42	112.19	12.97		0.86	0.60	1.49
2	2Cl	14.87	87.87			0.63	0.19	
	4Cl	28.98	133.33	157.71	204	0.90	0.68	27.68
	3Cl	30.12	136.99	12.40		0.92	0.73	1.50
3	2NP	17.85	97.48			0.61	0.14	
	3NP	41.03	172.11	226.20	240	1.09	1.03	23.88
	4NP	42.74	177.62	14.41		1.25	1.34	6.22
4	2,6DCl	24.72	119.59			3.17	4.93	
	2,4DCl	25.66	122.61	9.80	138	3.25	5.09	1.54
	2,5DCl	26.45	125.18	8.42		3.76	6.04	8.29
5	2,4DMP	23.16	114.56			0.90	0.69	
	2,5DMP	24.08	117.52	9.99	182	0.93	0.74	1.51
6	2,4,5TCl	30.87	139.39			2.36	3.43	
	2,4,6TCl	32.59	144.95	16.88	170	2.43	3.55	1.59

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4.2.3 BCD column

Separations of isomers were studied on BCD column using isothermal conditions. For the separation of 3 dichlorophenols (2,4DCl; 2,5DCl and 2,6DCl), effect of column temperature on retention factors of each analyte was studied. A plot of In k′ versus 1/T of 3 dichlorophenols was shown in Figure 4.6. At all temperature, the elution order was 2,4DCl → 2,6DCl → 2,5DCl. Retention factors of 2,4DCl and 2,6DCl were almost similar as their trend lines for In k′ versus 1/T looked to be parallel and they were difficult to separate. In this case, their trend lines became closer at 120 °C. Interestingly, both compounds showed better resolution at higher temperature (160 °C). The optimum temperature for the separation of 3 dichlorophenols was at 153 °C.

Results showed that BCD column provided complete separation for all groups of isomers. The optimum isothermal conditions for 6 groups of isomers were shown in Table 4.8.
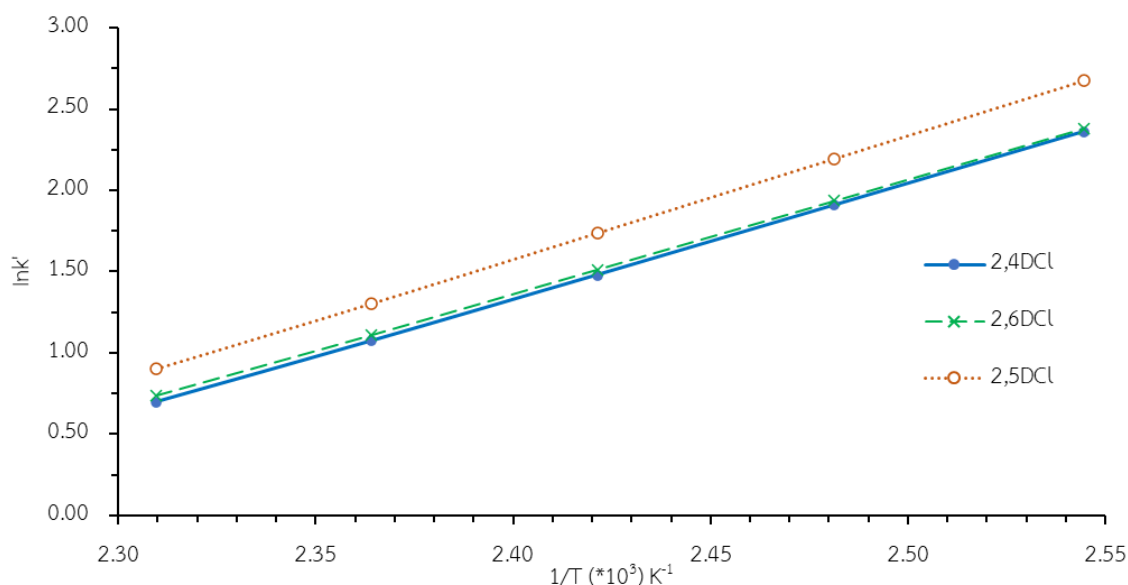
Figure 4.6 Plot of In k′ versus 1/T of 2,4DCl; 2,5DCl and 2,6DCl on BCD column

Table 4.8 Isothermal separations for 6 groups of isomers on BCD column

#	analyte	temperature program			isothermal condition			
		t_R (min)	elution temp (°C)	Rs	temp (°C)	t_R (min)	k′	Rs
1	2MP	20.19	107.65			0.80	0.59	
	4MP	21.27	111.25	10.90	181	0.84	0.67	2.06
	3MP	22.66	115.91	14.00		0.87	0.73	1.57
2	2Cl	14.91	89.96			0.59	0.18	
	4Cl	27.72	132.85	124.56	210	0.82	0.62	15.11
	3Cl	29.30	138.15	15.89		0.84	0.67	1.50
3	2NP	16.93	96.72			0.58	0.15	
	3NP	41.59	179.32	236.91	240	1.11	1.20	20.92
	4NP	42.10	181.05	4.84		1.25	1.49	4.14
4	2,4DCl	22.99	117.02			1.81	2.60	
	2,6DCl	22.99	117.02	0	153	1.86	2.70	1.50
	2,5DCl	24.96	123.62	18.67		2.14	3.25	7.51
5	2,4DMP	22.56	115.56		185	0.86	0.71	
	2,5DMP	23.51	118.77	10.24		0.89	0.76	1.55
6	2,4,6TCl	28.71	136.18		240	0.68	0.36	
	2,4,5TCl	31.58	145.78	25.77		0.73	0.44	2.20

4.2.4 GCD column

Separations of isomers were studied on GCD column using isothermal conditions. Interesting results were found for the separation of 3 dichlorophenols (2,4DCl; 2,5DCl and 2,6DCl). A plot of ln k′ versus 1/T of 3 dichlorophenols were shown in Figure 4.7. At all temperature, the elution order was 2,4DCl → 2,6DCl → 2,5DCl. Retention factors of 2,4DCl and 2,6DCl were almost similar as their trend lines for ln k′ versus 1/T looked to be parallel and they were difficult to separate. In this case, their trend lines seemed to separate at temperatures higher than 100 °C. However, their retention factors at high temperature became closer. Resolution values between two peak pairs were investigated as shown in Table 4.9. The best condition for the separation of 3 dichlorophenols was at 150 °C, where the least separated peak pair gave the highest resolution.
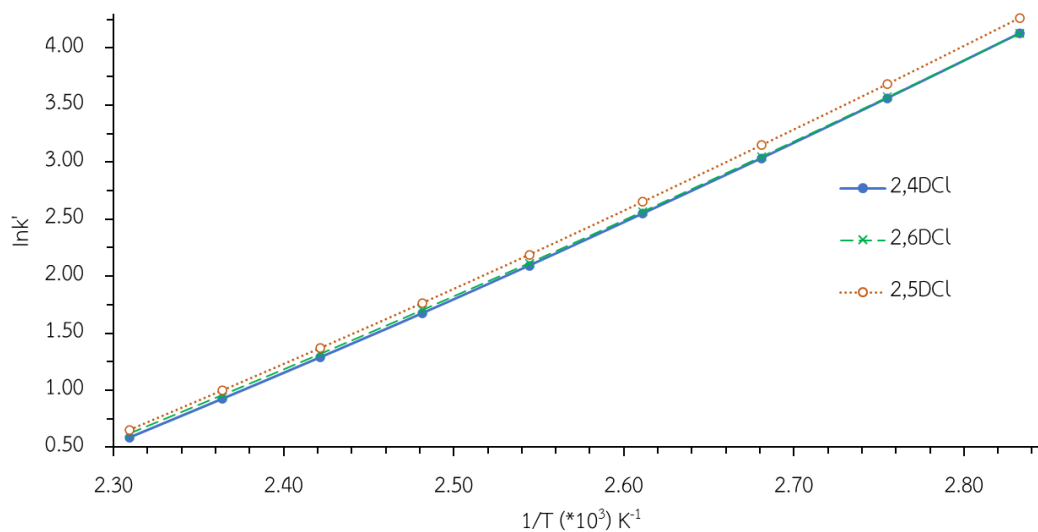
Figure 4.7 Plot of ln k′ versus 1/T of 2,4DCl; 2,5DCl and 2,6DCl on GCD column

Table 4.9 Resolution of 3 dichlorophenols on GCD column at various temperature

peak pair	Rs at various column temperature (°C)								
	80	90	100	110	120	130	140	150	160
2,4DCl/2,6DCl	-	-	< 0.90	0.90	1.03	1.18	1.20	1.23	1.19
2,6DCl/2,5DCl	9.11	6.51	5.47	4.24	3.41	2.81	2.13	1.61	1.14

GCD column provided complete separation for 5 groups of isomers, except for dichlorophenols. The optimum isothermal conditions for 6 groups of isomers were shown in Table 4.10.
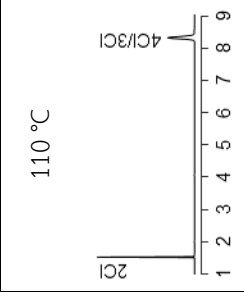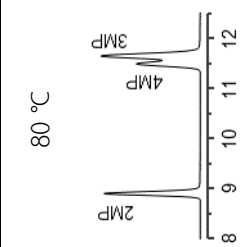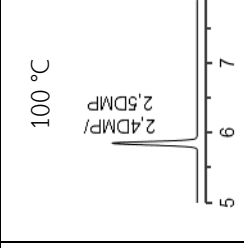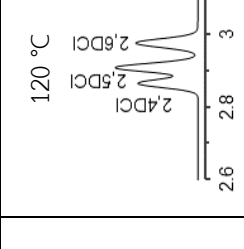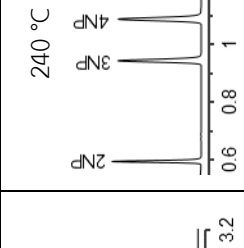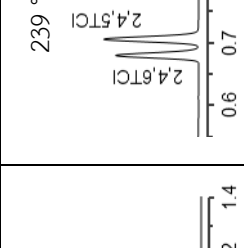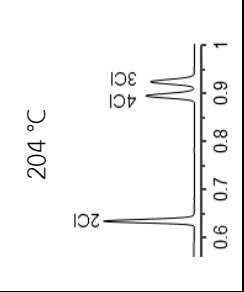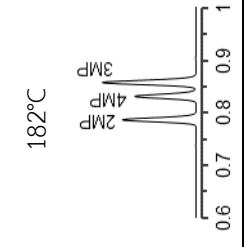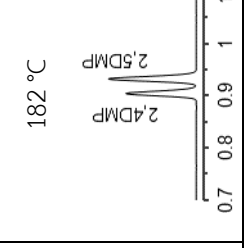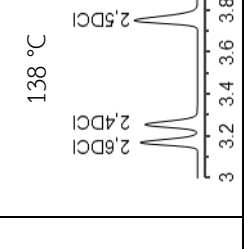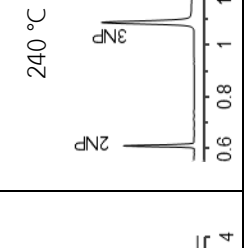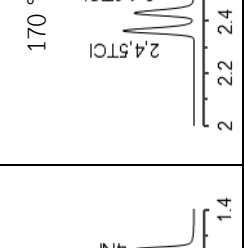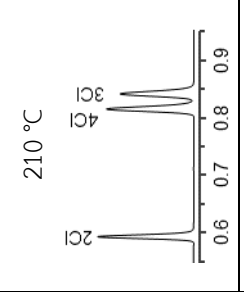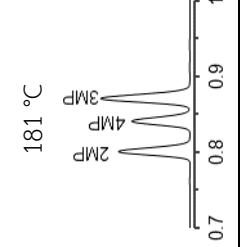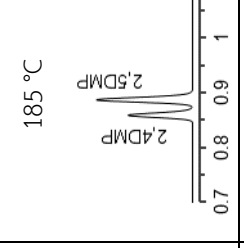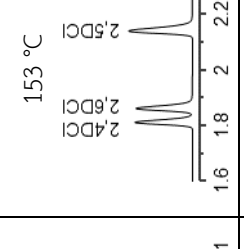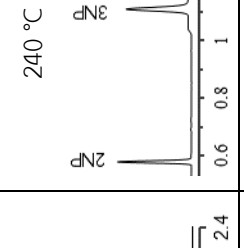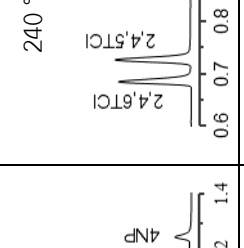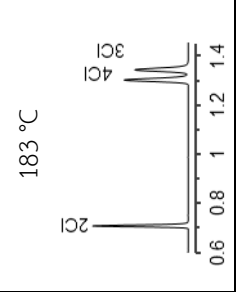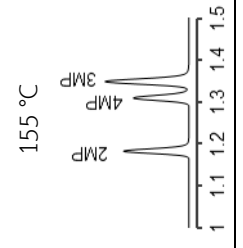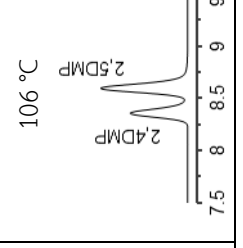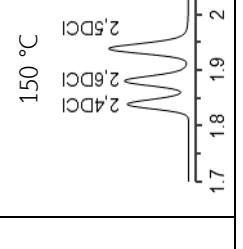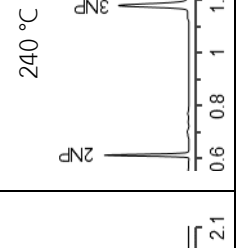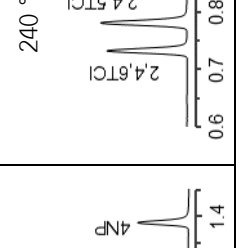
Table 4.10 Isothermal separations for 6 groups of isomers on GCD column

#	analyte	temperature program			isothermal condition			
		t_R (min)	elution temp (°C)	Rs	temp (°C)	t_R (min)	k'	Rs
1	2MP	18.90	102.38			1.18	1.26	
	4MP	20.21	106.71	12.44	155	1.31	1.51	5.17
	3MP	20.68	108.23	4.41		1.35	1.58	1.54
2	2Cl	13.64	85.00			0.71	0.35	
	4Cl	27.05	129.27	126.72	183	1.30	1.49	27.01
	3Cl	27.54	130.87	4.27		1.34	1.57	1.54
3	2NP	15.53	91.26			0.61	0.17	
	3NP	40.09	172.30	220.49	240	1.18	1.26	25.59
	4NP	42.16	179.12	17.17		1.38	1.64	6.39
4	2,4DCl	21.84	112.07			1.84	2.52	
	2,6DCl	21.84	112.07	0	150	1.88	2.60	1.23
	2,5DCl	22.41	113.96	4.50		1.94	2.71	1.61
5	2,4DMP	21.67	111.51			8.35	15.00	
	2,5DMP	21.84	112.07	1.36	106	8.59	15.46	1.54
6	2,4,6TCl	27.60	131.07			0.73	0.40	
	2,4,5TCl	30.20	139.67	22.48	240	0.78	0.50	2.78

Separations of 6 groups of isomers on OV-1701, ACD, BCD and GCD column were performed. Clearly, all CD-based columns showed better separation for all categories of isomers or provided shorter analysis time, compared with OV-1701 column. The results demonstrated that CD derivatives could enhance the separation of isomers and analytes with similar structures. Chromatograms for the separation of 6 groups of isomers under optimum conditions were compared in Table 4.11. Different elution orders may be observed for some analytes due to different selectivities of CD derivatives. For example, elution order of 3 dichlorophenols on ACD column was different from elution orders on BCD and GCD columns.

Table 4.11 Chromatograms of 6 groups of isomers under optimum conditions

column	chlorophenols	methylphenols	dimethylphenols	dichlorophenols	nitrophenols	trichlorophenols
OV-1701	110 °C (2Cl, 4Cl/3Cl)	80 °C (2MP, 3MP, 4MP)	100 °C (2,4DMP/2,5DMP)	120 °C (2,6DCl, 2,5DCl, 2,4DCl)	240 °C (2NP, 3NP, 4NP)	239 °C (2,4,6TCl, 2,4,5TCl)
ACD	204 °C (2Cl, 3Cl, 4Cl)	182 °C (2MP, 3MP, 4MP)	182 °C (2,4DMP, 2,5DMP)	138 °C (2,6DCl, 2,4DCl, 2,5DCl)	240 °C (2NP, 3NP, 4NP)	170 °C (2,4,5TCl, 2,4,6TCl)
BCD	210 °C (2Cl, 3Cl, 4Cl)	181 °C (2MP, 3MP, 4MP)	185 °C (2,4DMP, 2,5DMP)	153 °C (2,4DCl, 2,6DCl, 2,5DCl)	240 °C (2NP, 3NP, 4NP)	240 °C (2,4,6TCl, 2,4,5TCl)
GCD	183 °C (2Cl, 3Cl, 4Cl)	155 °C (2MP, 3MP, 4MP)	106 °C (2,4DMP, 2,5DMP)	150 °C (2,4DCl, 2,6DCl, 2,5DCl)	240 °C (2NP, 3NP, 4NP)	240 °C (2,4,6TCl, 2,4,5TCl)

Part 2 TLC-MALDI-MS

In the analysis of bisphenol A (BPA) using TLC combined with MALDI-TOF MS, the determination of optimum conditions involves the consideration of three parameters: the type of matrix, the matrix-to-analyte ratio, and the nitrogen laser concentration. For the purpose of quantitative analysis of BPA, a prepared external calibration curve consisting of five point concentrations was utilized. This calibration curve serves the purpose of establishing linearity within the TLC combined with MALDI-TOF MS method, enabling accurate quantification of BPA.

4.3 Optimization parameters for analysis bisphenol A

4.3.1 Type of matrix

From the experimental results of various matrices using graphene (G), graphene oxide (GO), and nitrogen-doped graphene (NG) using MALDI-TOF MS with 50% nitrogen laser, wavelength of 337 nm. According to the results, m/z of 228 was detected and the ion abundances of BPA with G, GO, and NG matrix, respectively, were 65219, 29219, and 15555 as shown in Figures 4.8. This demonstrates that G is a suitable matrix for the analysis of BPA. Because G has a strong $\pi-\pi$ interaction with aromatic of BPA. It enables for the effective transfer of energy from the matrix to the analyte during laser desorption, resulting in enhanced ionization and detection sensitivity in MALDI-TOF MS. Additionally, G's mechanical and thermal qualities make laser desorption of analytes effective, as its high thermal conductivity reduces analyte degradation and matrix interference during desorption.
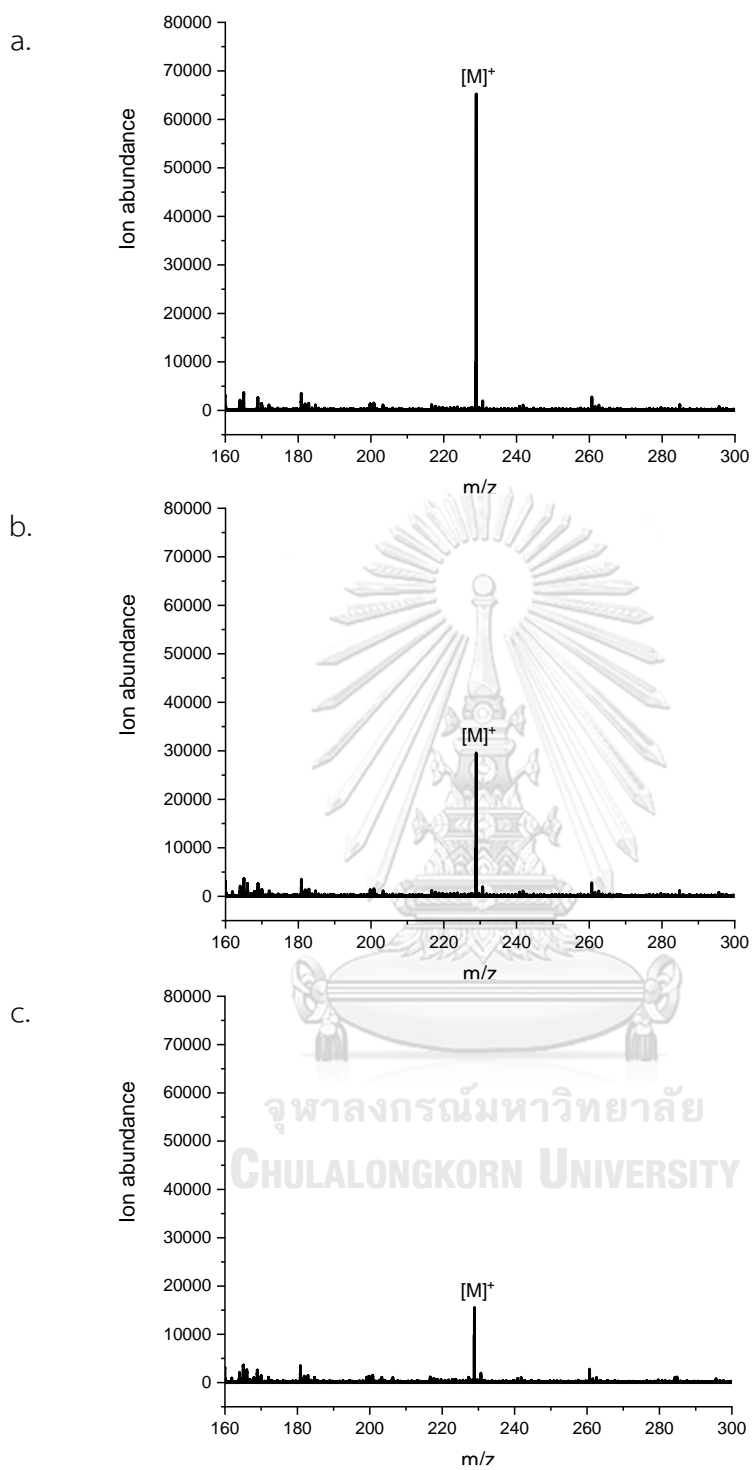
Figure 4.8 Mass spectra of BPA (m/z 228) with; a. graphene, b. graphene oxide and
c. nitrogen-doped graphene matrix

4.3.2 Matrix to analyte ratio

The impact of the matrix to analyte ratio on the obtained mass spectra was investigated using graphene as the matrix. This analysis aimed to determine the appropriate matrix to analyte ratio for measuring BPA using MALDI-TOF MS. Various ratios of matrix to analyte solution (v/v) were tested, including 1:1, 2:1, 1:2, 5:1, and 1:5. Results were shown in Figure 4.9.
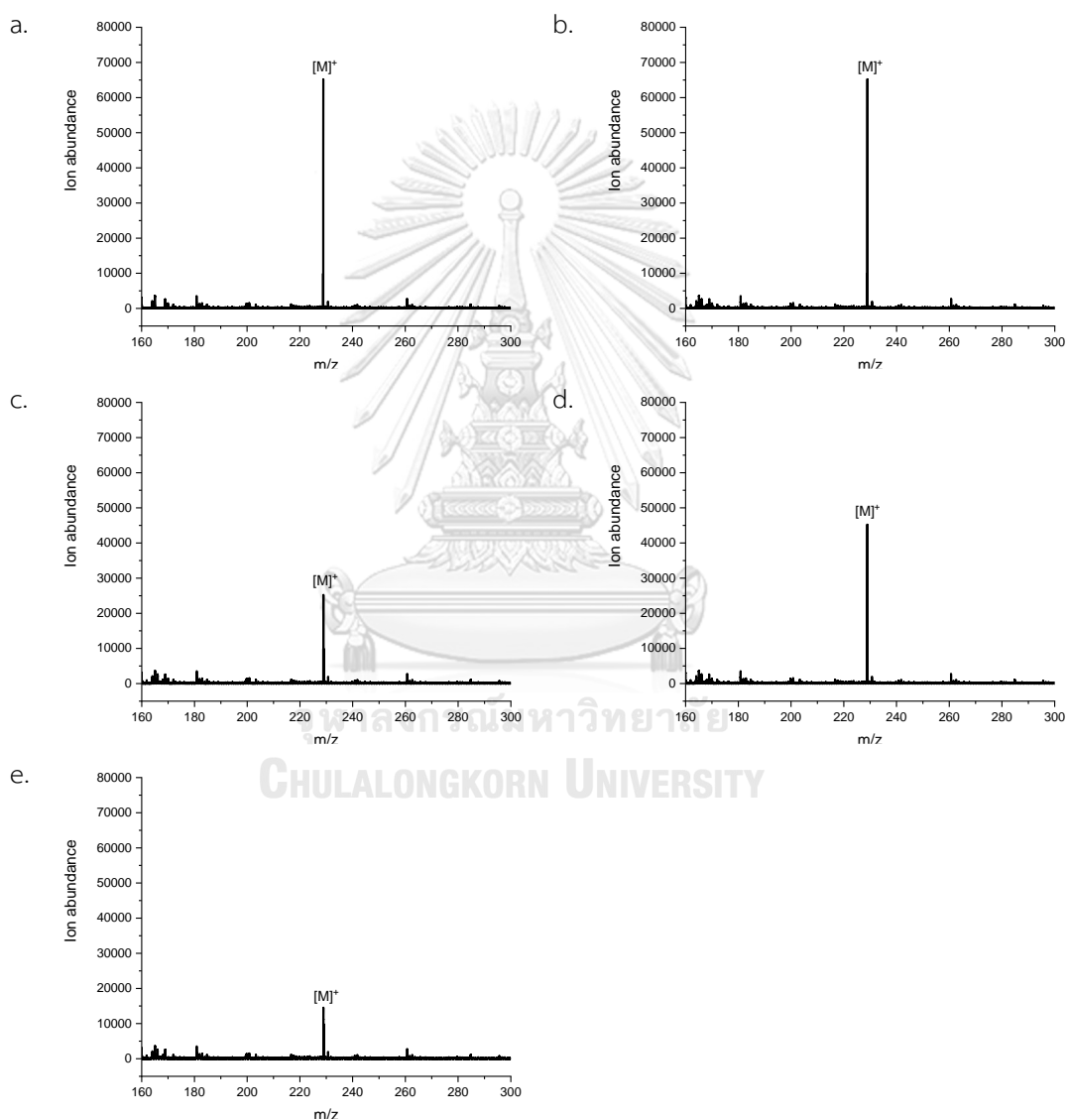
Figure 4.9   Mass spectra of BPA with graphene matrix using different matrix to analyte ratios (v/v); a. 1:1, b. 2:1, c. 1:2, d. 5:1 and e. 1:5

The intensity of individual ion mass peaks in MALDI-TOF MS measurement is strongly influenced by sample preparation, as evidence in the results. This quantitative relationship is illustrated in Figure 4.9 - 4.10. Specifically, for the ion mass peak of BPA, the sensitivity of the MALDI method is found to be highest when using a matrix : analyte ratio of 1:1 (v/v). The results indicated that graphene : BPA ratios of 1:1 and 2:1 were found to be suitable for the matrix : analyte ratio in the analysis. In the MALDI ion source, the production of sample ions relies on the generation of matrix ions. The quantity and quality of the sample ions are dependent on the sufficient presence of matrix ions, which are responsible for ionizing specific molecules of the substance being studied. The collision between matrix ions and BPA molecules gives rise to the formation of BPA ions. In cases where the quantity of matrix ions is limited, only a small fraction of BPA molecules undergo ionization. This can be observed as a small number of BPA ion mass peaks and a weak ion current intensity that is related to ionized BPA.
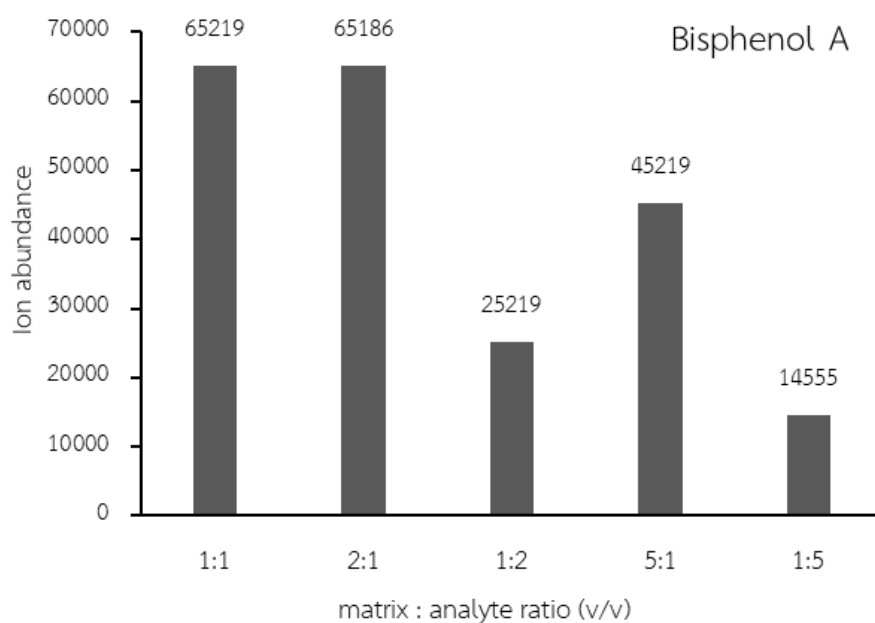
Figure 4.10 The ion abundance of BPA mass peak as a function of the matrix to analyte ratio

4.3.3 Nitrogen laser concentration

According to the experimental findings of measuring the ion abundance of BPA using the MALDI-TOF MS in range of the nitrogen laser concentration from 20% to 90%, as shown in Figure 4.11. The maximum ion abundance of BPA was found to be achieved at a concentration of 50%. Moderate laser concentration is commonly used in many MALDI-TOF MS experiments. It provides a balance between desorption/ionization efficiency and the potential risk of sample degradation. Moderate laser concentration often yields good signal intensities and optimal ionization efficiency for most analytes. Additionally, a high laser concentration delivers more energy to the sample, which facilitates effective desorption and ionization. This can raise mass spectrum signal intensities for low-abundance analytes or samples with limited ionization efficiency. However, high laser concentration increases mass spectrum fragmentation and background noise. At lower laser concentrations, incomplete analyte desorption and ionization can reduce mass spectrum signal intensity.
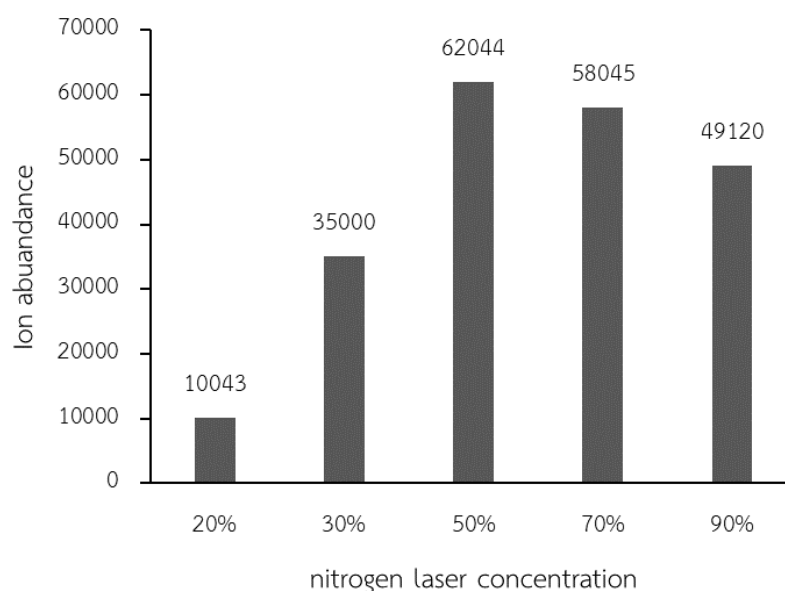
Figure 4.11 The ion abundance of BPA mass peak with various nitrogen laser concentration

4.3.4 Calibration curves

The experiments involved spotting BPA-containing samples ranging from 18.3 to 36.5 µg on TLC plates with graphene as a matrix. These experiments aimed to demonstrate the possibility of quantifying BPA using TLC combined with MALDI-TOF MS and validate the method.

Furthermore, the calibration curve data for non-developed TLC analysis of BPA ranged from 4.6 to 22.8 µg (or 1 µL solution of 20 mM to 100 mM, with a 3-7% R.S.D., n=3), revealing a high linear regression value (R^2) of 0.9998. The equation for the curve, $y = 2632.7x - 350.3$, established the relationship between the y-axis representing the abundance from MS spectra and the x-axis representing the amount of BPA (µg), shown in Figure 4.12.
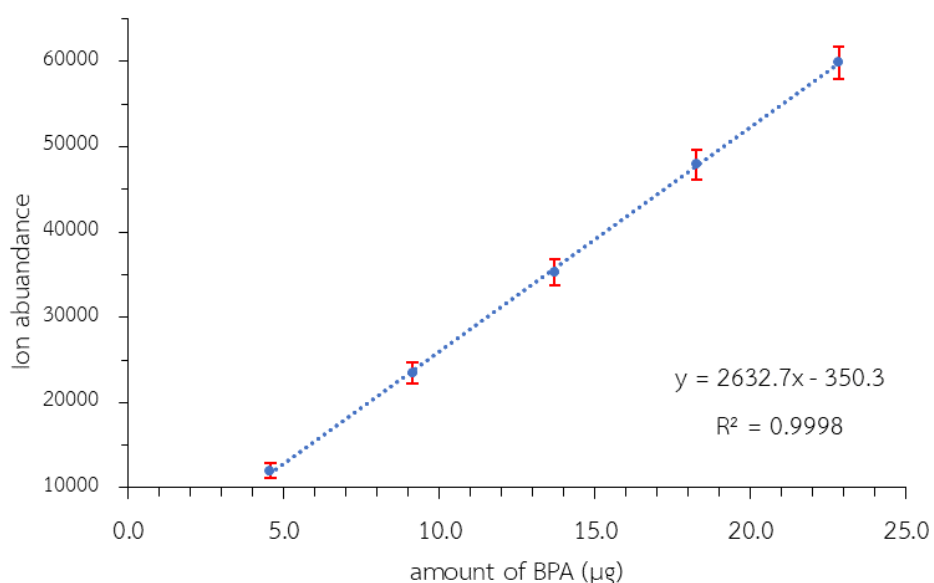
Figure 4.12 The calibration plot between amount of BPA (µg) and ion abundance on non-developed TLC in range 4.6 to 22.8 µg

However, the calibration curve for developed TLC analysis of BPA within the same concentration range 4.6 to 22.8 µg (or 1 µL solution of 20 mM to 100 mM, with a 10-20% R.S.D., n=3). The results displayed a lower linear regression value (R^2 = 0.6516), indicating it is unsuitable for quantitative analysis (Figure 4.13). The ion abundance of BPA from the developed TLC method was dramatically decreased by approximately 82-95% compared to the non-developed TLC method. This reduction can be attributed to the application of analyte spots on a TLC plate, followed by development using a mobile phase. As a result, the analyte spots exhibit a higher degree of dispersion, leading to a broader spread.
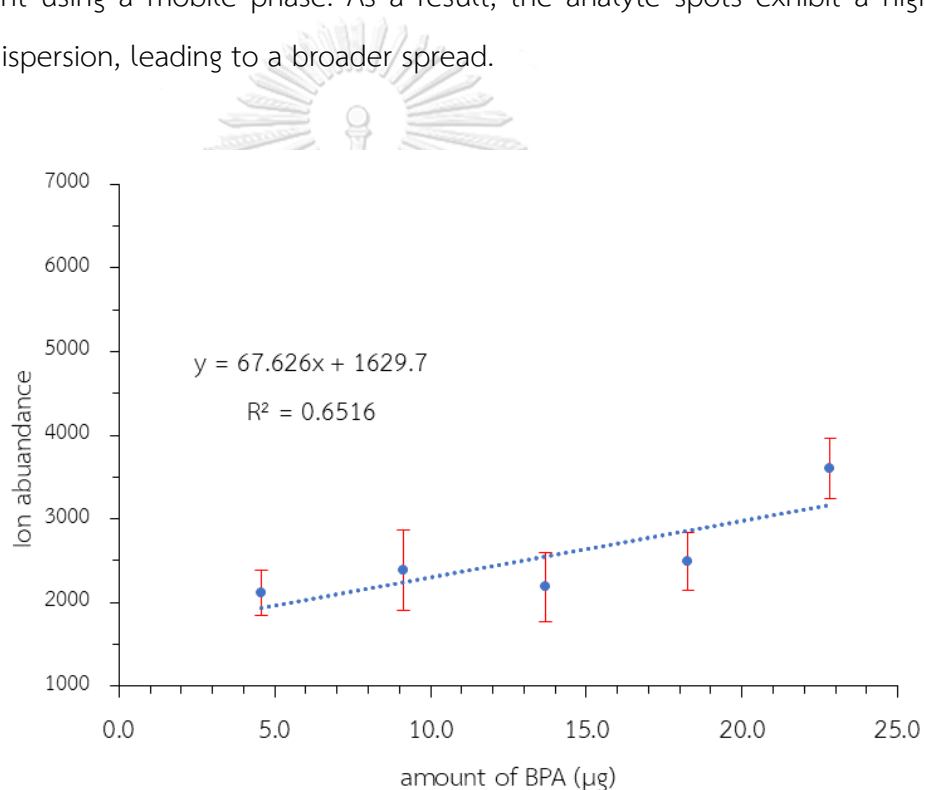
Figure 4.13 The calibration plot between amount of BPA (µg) and ion abundance on developed TLC in range 4.6 to 22.8 µg

Additionally, in the calibration of the developed TLC analysis of BPA, which spanned from 80 to 160 mM (or 18.3 to 36.5 µg, with a 5-14% R.S.D., n=3), the obtained linear regression value was acceptable (R^2 = 0.9634), as showed in Figure 4.14. This value was higher, within the range of 20 to 100 mM in the developed TLC analysis. Consequently, for quantitative analysis purposes, the developed TLC method proved to be acceptable when measuring BPA concentrations above 80 mM.
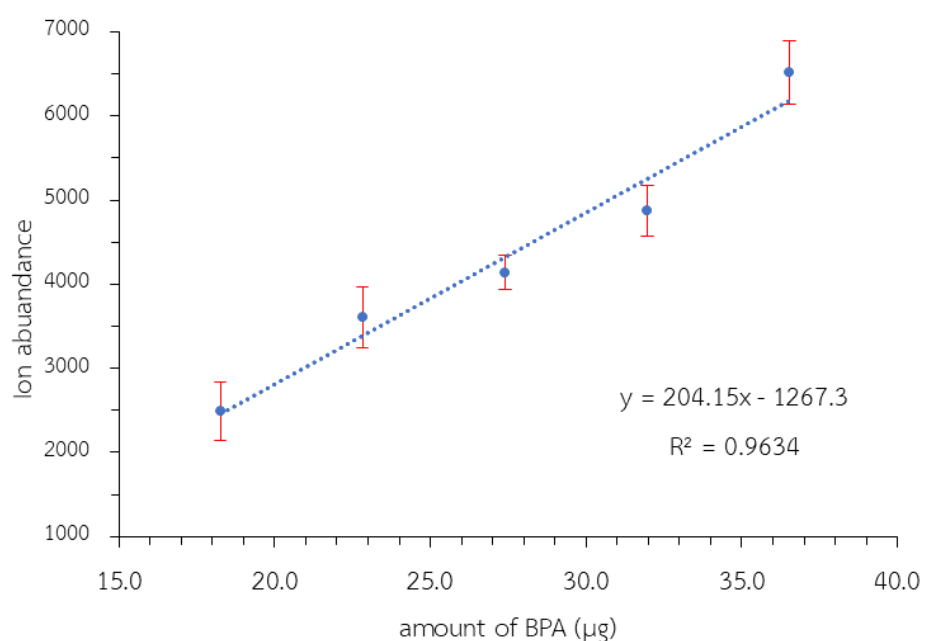
Figure 4.14 The calibration plot between amount of BPA (µg) and ion abundance on developed TLC in range 18.3 to 36.5 µg

Currently, the lowest detectable concentration of BPA on non-developed TLC is 0.23 μg (or 1 μL of 1 mM BPA solution), shown in Figure 4.15. And LOD is defined as a signal-to-noise ratio of 3:1, which will be measured lower than 0.23 μg.
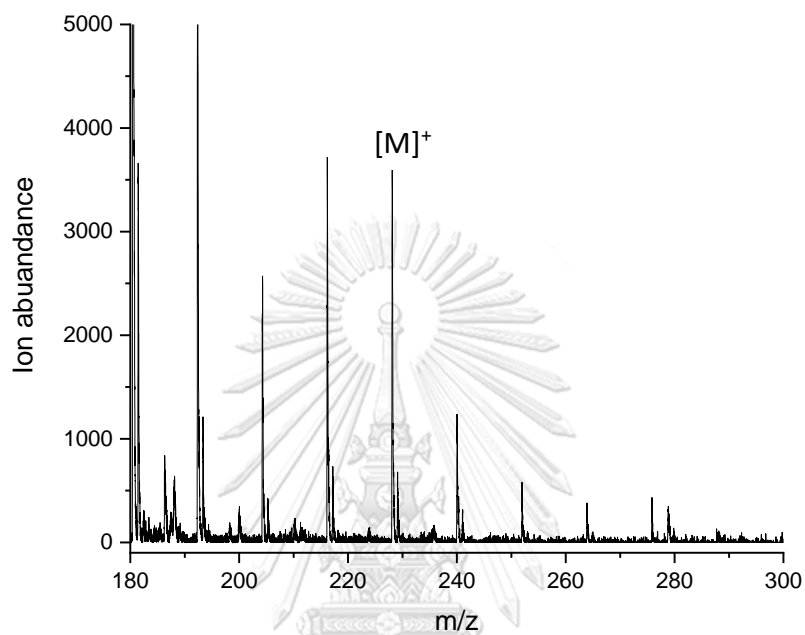
Figure 4.15 Mass spectra of 0.23 μg BPA on non-developed TLC

CHAPTER 5

CONCLUSION

This research aimed to improve the chromatographic analysis of phenolic compounds. The first part was to improve the single-run separation of 18 phenolic compounds using 15 m long column and derivatized CDs as stationary phases. Three derivatized CDs were (2,3-di-*O*-methyl-6-*O-tert*-butyldimethylsilyl) derivative of α-, β-, and γ-CDs, called ACD, BCD, and GCD, respectively. Results showed that all three CD-based stationary phases improved the separation of the mixture in comparison to polysiloxane OV-1701 stationary phase. The mixture of 18 phenolic compounds was analyzed by GC without prior derivatization and chromatograms showed quite symmetrical peak shapes. Interestingly, a small-size CD, ACD column, provided completed separation of all 18 phenolic compounds within 9.4 minutes using a temperature program from 130-150 °C at 3.22 °C/min, then to 220 °C at 25 °C/min. For BCD and GCD columns, they showed incomplete separations for a few compounds. In addition, the separations of isomers of phenolic compounds were studied under isothermal conditions. All three CD-based columns showed improved separation for all groups of isomers compared to OV-1701 column.

In the second part, TLC analysis combined with MALDI-TOF MS detection of bisphenol A (BPA) was developed. The optimum conditions for BPA analysis were using graphene as a matrix, 50% nitrogen laser concentration, and graphene-to-analyte ratio of 1:1. To validate the quantitative method, a suitable concentration range for BPA analysis using TLC combined with MALDI-TOF MS was identified to be 18.3 to 36.5 µg, with a 5-14% R.S.D., n=3), with an acceptable linear regression value (R^2 = 0.9634).

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APPENDIX

APPENDIX

Table A1   Chromatographic data of 18 phenolic compounds from OV-1701 column under initial condition and optimum condition.

order	analyte	initial condition				optimum condition			
		t_R (min)	w_h	Rs	elution temp (°C)	t_R (min)	w_h	Rs	elution temp (°C)
1	2Cl	10.05	0.0478	-	72.56	3.17	0.0258	-	90.27
2	P	13.21	0.0512	37.58	82.80	4.51	0.0328	26.91	94.61
3	2NP	13.75	0.0551	5.97	84.55	5.24	0.0363	12.37	96.96
4	2MP	14.96	0.0514	13.34	88.46	5.54	0.0362	5.00	97.96
5	4MP	16.34	0.0523	15.72	92.95	6.44	0.0420	13.52	100.87
6	3MP	16.42	0.0490	< 0.90	93.18	6.47	0.0428	< 0.80	100.97
7	2,4DMP /2,5DMP	18.01	0.0562	17.89	98.37	7.66	0.0431	16.80	104.81
8	2,4DCl	18.05	0.0488	< 0.80	98.48	7.80	0.0434	2.00	105.28
9	2,6DCl /2,5DCl	18.21	0.0533	1.84	98.99	7.90	0.0448	1.31	105.60
10	4Cl/3Cl	23.06	0.0560	52.24	114.70	9.97	0.0186	38.35	144.85
11	2,4,6TCl	24.16	0.0597	11.17	118.26	10.35	0.0180	12.16	152.41
12	4Cl-2MP	24.60	0.0559	4.53	119.70	10.39	0.0178	1.58	153.37
13	2,4,5TCl	26.16	0.0584	16.05	124.76	10.83	0.0166	14.82	162.03
14	3NP	36.13	0.0620	97.48	157.06	12.77	0.0152	71.84	200.85
15	4NP	38.98	0.0634	26.72	166.29	13.27	0.0154	19.19	210.83

Table A2   Chromatographic data of 18 phenolic compounds from ACD column under initial condition and optimum condition.

order	initial condition				optimum condition			
	analyte	t_R (min)	w_h	Rs	elution temp (°C)	t_R (min)	w_h	Rs	elution temp (°C)
1	2Cl	14.87	0.0540	-	87.87	1.36	0.0128	-	134.38
2	P	17.63	0.0552	29.73	96.75	1.67	0.0160	12.51	135.37
3	2NP	17.85	0.0577	2.37	97.48	1.92	0.0170	8.88	136.17
4	2MP	19.40	0.0552	16.14	102.47	1.97	0.0185	1.69	136.33
5	4MP	21.19	0.0564	18.86	108.23	2.29	0.0200	9.84	137.37
6	3MP	22.42	0.0553	12.97	112.19	2.57	0.0226	7.74	138.27
7	2,4DMP	23.16	0.0543	7.91	114.56	2.78	0.0231	5.54	138.96
8	2,5DMP	24.08	0.0541	9.99	117.52	3.06	0.0246	6.79	139.85
9	2,6DCl	24.72	0.0578	6.73	119.59	3.42	0.0274	8.22	141.02
10	2,4DCl	25.66	0.0550	9.80	122.61	3.51	0.0284	1.86	141.30
11	2,5DCl	26.45	0.0564	8.42	125.18	3.93	0.0316	8.32	142.67
12	4Cl	28.98	0.0548	26.79	133.33	5.12	0.0361	20.69	146.50
13	3Cl	30.12	0.0532	12.40	136.99	5.79	0.0396	10.29	148.63
14	4Cl-2MP	30.52	0.0503	4.58	138.29	5.98	0.0394	2.89	149.26
15	2,4,5TCl	30.87	0.0643	3.50	139.39	6.53	0.0349	8.65	157.87
16	2,4,6TCl	32.59	0.0561	16.88	144.95	6.98	0.0240	9.03	169.17
17	3NP	41.03	0.0629	83.45	172.11	9.00	0.0147	61.59	219.80
18	4NP	42.74	0.0768	14.41	177.62	9.32	0.0161	12.04	220.00

Table A3   Chromatographic data of 18 phenolic compounds from BCD column under initial condition and optimum condition.

order	initial condition					optimum condition				
	analyte	t_R (min)	w_h	Rs	elution temp (°C)	analyte	t_R (min)	w_h	Rs	elution temp (°C)
1	2Cl	14.91	0.0629	-	89.96	2Cl	3.18	0.0288	-	110.64
2	2NP	16.93	0.0654	18.51	96.72	2NP	4.32	0.0360	20.69	114.46
3	P	19.41	0.0606	23.18	105.03	P	5.09	0.0402	11.94	117.04
4	2MP	20.19	0.0585	7.73	107.65	2MP	5.56	0.039	7.00	118.62
5	4MP	21.27	0.0577	10.90	111.25	4MP	6.24	0.0433	9.75	120.91
6	2,4DMP	22.56	0.0539	13.57	115.56	3MP	7.04	0.0367	11.81	123.60
7	3MP	22.66	0.0593	1.09	115.91	2,4DMP	7.09	0.0429	< 0.80	123.74
8	2,4DCl/ 2,5DCl	22.99	0.0640	3.16	117.02	2,4DCl	7.50	0.0434	6.76	125.14
9	2,5DMP	23.51	0.0562	5.11	118.77	2,6DCl	7.59	0.0527	1.04	125.42
10	2,5DCl	24.96	0.0602	14.64	123.62	2,5DMP	7.74	0.0485	1.79	125.94
11	4Cl	27.72	0.0581	27.41	132.85	2,5DCl	8.91	0.0520	13.70	129.86
12	2,4,6TCl	28.71	0.0678	9.28	136.18	4Cl	11.19	0.0555	24.95	137.49
13	4Cl-2MP	28.97	0.0581	2.45	137.06	2,4,6TCl	12.15	0.0561	10.11	140.71
14	3Cl	29.30	0.0591	3.28	138.15	4Cl-2MP	12.30	0.0556	1.56	141.20
15	2,4,5TCl	31.58	0.0631	21.93	145.78	3Cl	12.54	0.0560	2.56	142.13
16	3NP	41.59	0.0571	98.03	179.32	2,4,5TCl	13.76	0.0259	17.46	172.50
17	4NP	42.10	0.0686	4.84	181.05	3NP	15.88	0.0165	58.91	220.00
18						4NP	16.07	0.0219	5.82	220.00

Table A4   Chromatographic data of 18 phenolic compounds from GCD column under initial condition and optimum condition.

order	initial condition				optimum condition				
	analyte	t_R (min)	w_h	Rs	elution temp (°C)	analyte	t_R (min)	w_h	Rs	elution temp (°C)
1	2Cl	13.62	0.0623	-	84.94	2Cl	1.30	0.0146	-	134.30
2	2NP	15.53	0.0714	16.83	91.25	P	1.68	0.0175	13.64	135.53
3	P	17.26	0.0626	15.20	96.95	2NP	1.74	0.0186	2.18	135.75
4	2MP	18.89	0.0612	15.52	102.34	2MP	1.97	0.0201	6.81	136.49
5	4MP	20.21	0.0635	12.40	106.68	4MP	2.25	0.0217	8.00	137.43
6	3MP	20.67	0.0630	4.30	108.20	3MP	2.35	0.0240	2.45	137.74
7	2,4DMP	21.66	0.0607	9.45	111.48	2,4DMP	2.63	0.0242	6.98	138.69
8	2,4DCl/ 2,6DCl/ 2,5DMP	21.83	0.0844	1.35	112.03	2,5DMP	2.68	0.0257	1.04	138.83
9	2,5DCl	22.41	0.0735	4.35	113.95	2,4DCl	2.82	0.0269	3.13	139.29
10	4Cl	27.04	0.0662	39.04	129.25	2,6DCl	2.87	0.0292	1.13	139.47
11	3Cl/ 2,4,6TCl	27.59	0.0727	4.62	131.04	2,5DCl	2.99	0.0304	2.33	139.86
12	4Cl-2MP	28.27	0.0643	5.85	133.29	4Cl	4.14	0.0196	27.07	167.69
13	2,4,5TCl	30.21	0.0771	16.11	139.69	3Cl	4.25	0.0193	3.33	170.44
14	3NP	40.10	0.0733	77.40	172.31	2,4,6TCl	4.34	0.0215	2.74	172.82
15	4NP	42.18	0.0784	16.16	179.19	4Cl-2MP	4.40	0.0174	1.69	174.22
16						2,4,5TCl	4.85	0.0213	13.59	185.39
17						3NP	6.46	0.0195	46.45	225.64
18						4NP	6.87	0.0295	9.97	236.02

Table A5   Chromatographic data for 6 groups of isomers on OV-1701 column using isothermal conditions.

isomer	temp (℃)	analyte	t_R (min)	w_h	k'	α	Rs
methylphenols	100	2MP	3.62	0.0349	5.94	-	-
		4MP	4.46	0.0375	7.54	1.27	13.61
		3MP	4.51	0.0435	7.64	1.01	< 0.80
	90	2MP	5.52	0.0541	9.57	-	-
		4MP	6.96	0.0603	12.34	1.29	14.84
		3MP	7.05	0.0692	12.50	1.01	< 0.80
	80	2MP	8.90	0.0862	16.05	-	-
		4MP	11.49	0.1002	21.02	1.31	16.37
		3MP	11.65	0.1087	21.32	1.01	< 0.90
chlorophenols	130	2Cl	1.03	0.0112	0.97	-	-
		4Cl	3.63	0.0361	5.95	6.11	64.65
		3Cl	3.63	0.0361	5.95	6.11	0
	120	2Cl	1.23	0.0135	1.35	-	-
		4Cl	5.38	0.0537	9.31	6.89	72.74
		3Cl	5.38	0.0537	9.31	6.89	0
	110	2Cl	1.51	0.0152	1.89	-	-
		4Cl	8.32	0.0810	14.93	7.88	83.25
		3Cl	8.32	0.0810	14.93	7.88	0
nitrophenols	170	2NP	0.84	0.0103	0.61	-	-
		3NP	4.76	0.0478	8.13	13.21	79.43
		4NP	6.94	0.0704	12.29	1.51	21.63
	180	2NP	0.73	0.1010	0.40	-	-
		3NP	3.38	0.0357	5.47	13.54	22.78
		4NP	4.76	0.0493	8.11	1.48	19.05
	200	2NP	0.68	0.0094	0.30	-	-
		3NP	1.91	0.0215	2.66	8.75	46.97
		4NP	2.51	0.0267	3.80	1.43	14.50
	230	2NP	0.61	0.0091	0.17	-	-
		3NP	1.07	0.0147	1.06	6.34	23.03
		4NP	1.27	0.0163	1.44	1.36	7.56
	240	2NP	0.60	0.0088	0.14	-	-
		3NP	0.94	0.0127	0.81	5.77	19.09
		4NP	1.09	0.0138	1.08	1.34	6.40
dichlorophenol	120	2,4DCl	2.86	0.0261	4.48	-	-
		2,5DCl	2.91	0.0279	4.57	1.02	0.94
		2,6DCl	2.97	0.0284	4.70	1.03	1.42

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
	115	2,4DCl	3.43	0.0334	5.56	-	-
		2,5DCl	3.48	0.0340	5.67	1.02	0.98
		2,6DCl	3.55	0.0343	5.80	1.02	1.22
	110	2,4DCl	4.08	0.0388	6.81	-	-
		2,5DCl	4.15	0.0388	6.95	1.02	1.09
		2,6DCl	4.22	0.0389	7.07	1.02	1.01
	100	2,4DCl	6.08	0.0570	10.65	-	-
		2,5DCl	6.22	0.0748	10.91	1.02	1.23
		2,6DCl	6.22	0.0748	10.91	1.02	0
	90	2,4DCl	9.58	0.0875	17.36	-	-
		2,6DCl	9.79	0.1271	17.75	1.02	1.14
		2,5DCl	9.79	0.1271	17.75	1.02	0
	80	2,4DCl	15.77	0.1262	29.20	-	-
		2,6DCl	15.77	0.1262	29.20	1.00	0
		2,5DCl	16.15	0.1404	29.93	1.03	1.69
	70	2,6DCl	27.05	0.1907	50.82	-	-
		2,4DCl	27.38	0.2049	51.45	1.01	0.98
		2,5DCl	28.19	0.2148	52.99	1.03	2.27
dimethylphenols	100	2,4DMP	5.85	0.0569	10.20	-	-
		2,5DMP	5.85	0.0569	10.20	1.00	0
	80	2,4DMP	15.74	0.1275	29.15	-	-
		2,5DMP	15.74	0.1275	29.15	1.00	0
	70	2,4DMP	27.93	0.2079	52.51	-	-
		2,5DMP	27.93	0.2079	52.51	1.00	0
trichlorophenols	170	2,4,6TCl	1.45	0.0153	1.78	-	-
		2,4,5TCl	1.68	0.0177	2.22	1.24	8.13
	190	2,4,6TCl	1.04	0.0114	0.98	-	-
		2,4,5TCl	1.15	0.0121	1.19	1.21	5.51
	210	2,4,6TCl	0.83	0.0106	0.58	-	-
		2,4,5TCl	0.88	0.0111	0.69	1.19	3.15
	230	2,4,6TCl	0.71	0.0095	0.36	-	-
		2,4,5TCl	0.75	0.0097	0.43	1.17	2.02
	239	2,4,6TCl	0.68	0.0102	0.30	-	-
		2,4,5TCl	0.71	0.0102	0.35	1.17	1.50
	240	2,4,6TCl	0.68	0.0102	0.30	-	-
		2,4,5TCl	0.70	0.0103	0.34	1.16	1.44

Table A6   Chromatographic data for 6 groups of isomers on ACD column using isothermal conditions.

isomer	temp (°C)	analyte	t_R (min)	w_h	k′	α	Rs
methylphenols	170	2MP	0.90	0.0108	0.69	-	-
		4MP	0.98	0.0111	0.83	1.19	3.82
		3MP	1.02	0.0115	0.91	1.11	2.45
	175	2MP	0.85	0.0100	0.58	-	-
		4MP	0.90	0.0107	0.69	1.19	3.35
		3MP	0.94	0.0107	0.76	1.10	2.04
	180	2MP	0.80	0.0105	0.50	-	-
		4MP	0.85	0.0104	0.59	1.18	2.70
		3MP	0.88	0.0108	0.65	1.09	1.61
	181	2MP	0.79	0.0098	0.49	-	-
		4MP	0.84	0.0101	0.57	1.18	2.77
		3MP	0.87	0.0103	0.63	1.09	1.56
	182	2MP	0.79	0.0102	0.47	-	-
		4MP	0.83	0.0101	0.56	1.18	2.61
		3MP	0.86	0.0104	0.60	1.09	1.49
chlorophenols	170	2Cl	0.77	0.0097	0.45	-	-
		4Cl	1.71	0.0169	2.20	4.94	41.52
		3Cl	1.88	0.0195	2.53	1.15	5.66
	197	2Cl	0.65	0.0096	0.22	-	-
		4Cl	0.98	0.0116	0.84	3.82	18.29
		3Cl	1.02	0.0131	0.92	1.09	2.00
	200	2Cl	0.64	0.0096	0.20	-	-
		4Cl	0.94	0.0112	0.76	3.74	16.92
		3Cl	0.98	0.0116	0.83	1.09	1.81
	204	2Cl	0.63	0.0095	0.19	-	-
		4Cl	0.90	0.0111	0.68	3.61	14.91
		3Cl	0.92	0.0116	0.73	1.08	1.50
	205	2Cl	0.63	0.0097	0.18	-	-
		4Cl	0.88	0.0122	0.65	3.56	13.51
		3Cl	0.91	0.0133	0.71	1.08	1.29
	210	2Cl	0.62	0.0091	0.16	-	-
		4Cl	0.84	0.0102	0.56	3.46	13.08
		3Cl	0.86	0.0118	0.60	1.07	1.12
nitrophenols	200	2NP	0.72	0.0090	0.34	-	-
		3NP	2.72	0.0301	4.09	12.00	60.30
		4NP	3.42	0.0406	5.40	1.32	11.67

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
	220	2NP	0.65	0.0097	0.22	-	-
		3NP	1.58	0.0182	1.96	8.97	39.32
		4NP	1.91	0.0240	2.58	1.31	9.12
	225	2NP	0.64	0.0086	0.19	-	-
		3NP	1.42	0.0172	1.66	8.59	35.69
		4NP	1.69	0.0205	2.17	1.31	8.52
	230	2NP	0.63	0.0087	0.17	-	-
		3NP	1.29	0.0166	1.41	8.17	30.70
		4NP	1.52	0.0194	1.84	1.30	7.49
	240	2NP	0.61	0.0085	0.14	-	-
		3NP	1.09	0.0149	1.03	7.25	23.88
		4NP	1.25	0.0163	1.34	1.30	6.22
dichlorophenols	130	2,6DCl	4.32	0.0419	7.09	-	-
		2,4DCl	4.53	0.0470	7.49	1.06	2.86
		2,5DCl	5.29	0.0562	8.90	1.19	8.59
	140	2,6DCl	2.95	0.0286	4.51	-	-
		2,4DCl	3.01	0.0311	4.64	1.03	1.32
		2,5DCl	3.47	0.0353	5.49	1.18	8.03
	135	2,6DCl	3.54	0.0350	5.62	-	-
		2,4DCl	3.66	0.0383	5.86	1.04	1.99
		2,5DCl	4.24	0.0427	6.94	1.19	8.44
	134	2,6DCl	3.68	0.0364	5.89	-	-
		2,4DCl	3.82	0.0395	6.15	1.04	2.16
		2,5DCl	4.43	0.0464	7.30	1.19	8.39
	137	2,6DCl	3.29	0.0322	5.15	-	-
		2,4DCl	3.38	0.0340	5.34	1.04	1.72
		2,5DCl	3.91	0.0402	6.32	1.18	8.33
	138	2,6DCl	3.17	0.0318	4.93	-	-
		2,4DCl	3.25	0.0339	5.09	1.03	1.54
		2,5DCl	3.76	0.0375	6.04	1.18	8.29
	139	2,6DCl	3.05	0.0305	4.72	-	-
		2,4DCl	3.13	0.0318	4.86	1.03	1.44
		2,5DCl	3.61	0.0365	5.75	1.18	8.20
dimethylphenols	170	2,4DMP	1.09	0.0121	1.04	-	-
		2,5DMP	1.14	0.0135	1.13	1.09	2.34
	176	2,4DMP	1.00	0.0124	0.87	-	-
		2,5DMP	1.04	0.0116	0.95	1.09	1.96
	180	2,4DMP	0.93	0.0110	0.74	-	-
		2,5DMP	0.96	0.0125	0.80	1.08	1.55

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
	182	2,4DMP	0.90	0.0109	0.69	-	-
		2,5DMP	0.93	0.0109	0.74	1.08	1.51
	183	2,4DMP	0.89	0.0103	0.67	-	-
		2,5DMP	0.92	0.0105	0.72	1.07	1.47
trichlorophenols	150	2,4,5TCl	4.71	0.0487	7.81	-	-
		2,4,6TCl	5.34	0.0541	9.01	1.15	7.29
	160	2,4,5TCl	3.26	0.0332	5.10	-	-
		2,4,6TCl	3.50	0.0358	5.55	1.09	4.15
	168	2,4,5TCl	2.50	0.0250	3.68	-	-
		2,4,6TCl	2.59	0.0267	3.85	1.05	2.03
	170	2,4,5TCl	2.36	0.0240	3.43	-	-
		2,4,6TCl	2.43	0.0263	3.55	1.04	1.59
	171	2,4,5TCl	2.29	0.0237	3.29	-	-
		2,4,6TCl	2.35	0.0254	3.39	1.03	1.37
	172	2,4,5TCl	2.22	0.0226	3.16	-	-
		2,4,6TCl	2.27	0.0245	3.24	1.03	1.17

Table A7 Chromatographic data for 6 groups of isomers on BCD column using isothermal conditions.

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
methylphenols	160	2MP	1.11	0.0136	1.21	-	-
		4MP	1.20	0.0144	1.39	1.15	3.87
		3MP	1.29	0.0154	1.57	1.12	3.44
	170	2MP	0.93	0.0111	0.85	-	-
		4MP	0.99	0.0126	0.97	1.14	2.98
		3MP	1.04	0.0123	1.07	1.11	2.46
	175	2MP	0.86	0.0110	0.72	-	-
		4MP	0.91	0.0113	0.82	1.14	2.64
		3MP	0.95	0.0115	0.90	1.10	2.06
	180	2MP	0.81	0.0099	0.61	-	-
		4MP	0.85	0.0103	0.69	1.13	2.39
		3MP	0.88	0.0105	0.75	1.09	1.81
	181	2MP	0.80	0.0113	0.59	-	-
		4MP	0.84	0.0115	0.67	1.13	2.06
		3MP	0.87	0.0118	0.73	1.04	1.57
	190	2MP	0.72	0.0087	0.44	-	-
		4MP	0.75	0.0084	0.50	1.13	2.00
		3MP	0.77	0.0091	0.54	1.08	1.35
chlorophenols	170	2Cl	0.76	0.0095	0.51	-	-
		4Cl	1.75	0.0188	2.47	4.80	40.91
		3Cl	1.96	0.0205	2.89	1.17	6.35
	190	2Cl	0.65	0.0096	0.29	-	-
		4Cl	1.10	0.0135	1.19	4.04	22.89
		3Cl	1.17	0.0140	1.33	1.12	3.12
	203	2Cl	0.61	0.0090	0.21	-	-
		4Cl	0.89	0.0109	0.77	3.69	16.70
		3Cl	0.93	0.0108	0.84	1.10	2.01
	210	2Cl	0.59	0.0076	0.18	-	-
		4Cl	0.82	0.0098	0.62	3.51	15.11
		3Cl	0.84	0.0107	0.67	1.08	1.50
	220	2Cl	0.58	0.0085	0.14	-	-
		4Cl	0.74	0.0094	0.47	3.25	10.61
		3Cl	0.75	0.0097	0.50	1.07	0.98
nitrophenols	210	2NP	0.65	0.0092	0.29	-	-
		3NP	2.27	0.0267	3.50	12.16	53.08
		4NP	2.62	0.0328	4.21	1.20	7.06

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
	220	2NP	0.62	0.0096	0.23	-	-
		3NP	1.71	0.0213	2.40	10.57	41.54
		4NP	1.97	0.0242	2.91	1.21	6.70
	230	2NP	0.60	0.0082	0.18	-	-
		3NP	1.35	0.0176	1.68	9.16	34.31
		4NP	1.54	0.0204	2.06	1.23	5.95
	240	2NP	0.58	0.0092	0.15	-	-
		3NP	1.11	0.0206	1.20	7.96	20.92
		4NP	1.25	0.0198	1.49	1.23	4.14
dimethylphenols	150	2,4DMP	1.77	0.0186	2.53	-	-
		2,5DMP	1.92	0.0210	2.82	1.12	4.43
	170	2,4DMP	1.10	0.0129	1.18	-	-
		2,5DMP	1.15	0.0132	1.29	1.09	2.53
	177	2,4DMP	0.97	0.0113	0.92	-	-
		2,5DMP	1.01	0.0116	1.00	1.09	2.06
	178	2,4DMP	0.95	0.0122	0.89	-	-
		2,5DMP	0.99	0.0113	0.97	1.09	1.95
	180	2,4DMP	0.92	0.0107	0.83	-	-
		2,5DMP	0.96	0.0123	0.90	1.09	1.84
	182	2,4DMP	0.89	0.0106	0.78	-	-
		2,5DMP	0.93	0.0109	0.84	1.08	1.75
	185	2,4DMP	0.86	0.0103	0.71	-	-
		2,5DMP	0.89	0.0109	0.76	1.08	1.55
	190	2,4DMP	0.81	0.0107	0.60	-	-
		2,5DMP	0.83	0.0109	0.65	1.08	1.25
trichlorophenols	190	2,4,6TCl	1.26	0.0142	1.50	-	-
		2,4,5TCl	1.53	0.0169	2.04	1.36	10.18
	220	2,4,6TCl	0.81	0.0098	0.60	-	-
		2,4,5TCl	0.89	0.0114	0.77	1.28	4.67
	240	2,4,6TCl	0.68	0.0105	0.36	-	-
		2,4,5TCl	0.73	0.0120	0.44	1.23	2.20
dichlorophenols	120	2,4DCl	5.83	0.0582	10.58	-	-
		2,6DCl	5.95	0.0685	10.83	1.02	1.19
		2,5DCl	7.79	0.0870	14.48	1.34	13.88
	130	2,4DCl	3.89	0.0405	6.74	-	-
		2,6DCl	3.99	0.0439	6.94	1.03	1.41
		2,5DCl	5.02	0.0499	8.98	1.29	12.86
	135	2,4DCl	3.22	0.0324	6.41	-	-
		2,6DCl	3.31	0.0361	5.59	1.03	1.53

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
		2,5DCl	4.07	0.0415	7.10	1.27	11.53
		2,4DCl	2.71	0.0278	4.39	-	-
	140	2,6DCl	2.79	0.0300	4.54	1.03	1.57
		2,5DCl	3.36	0.0355	5.68	1.25	10.33
		2,4DCl	2.53	0.0265	4.03	-	-
	142	2,6DCl	2.61	0.0285	4.18	1.04	1.56
		2,5DCl	3.12	0.0329	5.20	1.24	9.85
		2,4DCl	2.29	0.0228	3.56	-	-
	145	2,6DCl	2.36	0.0256	3.69	1.04	1.56
		2,5DCl	2.79	0.0298	4.54	2.43	9.09
		2,4DCl	2.16	0.0223	3.30	-	-
	147	2,6DCl	2.22	0.0247	3.42	1.04	1.55
		2,5DCl	2.61	0.0276	4.19	2.32	8.75
		2,4DCl	1.97	0.0213	2.92	-	-
	150	2,6DCl	2.03	0.0222	3.03	1.69	1.49
		2,5DCl	2.36	0.0258	3.68	1.21	8.02
		2,4DCl	1.87	0.0195	2.71	-	-
	152	2,6DCl	1.92	0.0209	2.81	1.55	1.51
		2,5DCl	2.21	0.0238	3.40	1.21	7.79
		2,4DCl	1.81	0.0190	2.60	-	-
	153	2,6DCl	1.86	0.0203	2.70	1.48	1.50
		2,5DCl	2.14	0.0234	3.25	1.21	7.51
		2,4DCl	1.72	0.0182	2.42	-	-
	155	2,6DCl	1.77	0.0195	2.51	1.35	1.47
		2,5DCl	2.02	0.0216	3.01	1.20	7.16
		2,4DCl	1.51	0.0156	2.01	-	-
	160	2,6DCl	1.55	0.0168	2.09	1.04	1.45
		2,5DCl	1.74	0.0194	2.46	1.18	6.18

Table A8   Chromatographic data for 6 groups of isomers on GCD column using isothermal conditions.

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
methylphenols	140	2MP	1.65	0.0191	2.16	-	-
		4MP	1.89	0.0210	2.62	1.21	7.02
		3MP	1.97	0.0219	2.78	1.06	2.19
	142	2MP	1.57	0.0180	2.01	-	-
		4MP	1.79	0.0205	2.43	1.21	6.70
		3MP	1.86	0.0215	2.57	1.06	2.02
	145	2MP	1.46	0.0165	1.80	-	-
		4MP	1.65	0.0189	2.16	1.20	6.38
		3MP	1.71	0.0198	2.28	1.06	1.92
	150	2MP	1.31	0.0153	1.50	-	-
		4MP	1.46	0.0164	1.80	1.20	5.79
		3MP	1.51	0.0182	1.90	1.05	1.70
	155	2MP	1.18	0.0140	1.26	-	-
		4MP	1.31	0.0149	1.51	1.19	5.17
		3MP	1.35	0.0157	1.58	1.03	1.54
	156	2MP	1.16	0.0129	1.23	-	-
		4MP	1.28	0.0147	1.46	1.19	5.20
		3MP	1.32	0.0154	1.53	1.05	1.45
chlorophenols	160	2Cl	0.86	0.0106	0.64	-	-
		4Cl	2.28	0.0256	3.37	5.23	46.23
		3Cl	2.40	0.0279	3.60	1.07	2.66
	170	2Cl	0.78	0.0102	0.49	-	-
		4Cl	1.74	0.0202	2.33	4.74	37.17
		3Cl	1.81	0.0212	2.48	1.06	2.13
	172	2Cl	0.77	0.0104	0.47	-	-
		4Cl	1.66	0.0193	2.17	4.65	35.27
		3Cl	1.72	0.0203	2.30	1.06	2.02
	180	2Cl	0.72	0.0099	0.38	-	-
		4Cl	1.38	0.0160	1.65	4.31	30.07
		3Cl	1.43	0.0168	1.74	1.05	1.69
	183	2Cl	0.71	0.0098	0.35	-	-
		4Cl	1.30	0.0161	1.49	4.21	27.01
		3Cl	1.34	0.0160	1.57	1.05	1.54
nitrophenols	220	2NP	0.65	0.0099	0.25	-	-
		3NP	1.74	0.0226	2.34	9.32	39.49
		4NP	2.14	0.0294	3.10	1.32	8.94

isomer	temp (°C)	analyte	t_R (min)	w_h	k´	α	Rs
	230	2NP	0.63	0.0096	0.21	-	-
		3NP	1.41	0.0204	1.70	8.17	30.60
		4NP	1.69	0.0228	2.23	1.31	7.52
	240	2NP	0.61	0.0094	0.17	-	-
		3NP	1.18	0.0167	1.26	7.23	25.59
		4NP	1.38	0.0194	1.64	1.30	6.39
dimethylphenols	95	2,4DMP	14.50	0.1271	26.77	-	-
		2,5DMP	14.95	0.1329	27.65	1.03	2.08
	96	2,4DMP	13.75	0.1263	25.34	-	-
		2,5DMP	14.19	0.1240	26.17	1.03	2.04
	90	2,4DMP	19.10	0.1608	35.59	-	-
		2,5DMP	19.75	0.1711	36.84	1.04	2.31
	100	2,4DMP	11.20	0.1171	20.46	-	-
		2,5DMP	11.54	0.1125	21.11	1.03	1.74
	106	2,4DMP	8.35	0.0880	15.00	-	-
		2,5DMP	8.59	0.0941	15.46	1.03	1.54
	107	2,4DMP	7.97	0.0877	14.26	-	-
		2,5DMP	8.19	0.0908	14.69	1.03	1.48
	110	2,4DMP	6.94	0.0758	12.29	-	-
		2,5DMP	7.13	0.0789	12.65	1.03	1.45
	120	2,4DMP	4.52	0.0484	7.65	-	-
		2,5DMP	4.63	0.0513	7.86	1.03	1.29
trichlorophenols	200	2,4,6TCl	1.10	0.0128	1.10	-	-
		2,4,5TCl	1.27	0.0156	1.43	1.31	7.25
	210	2,4,6TCl	0.96	0.0130	0.83	-	-
		2,4,5TCl	1.08	0.0141	1.07	1.29	5.43
	220	2,4,6TCl	0.86	0.0109	0.64	-	-
		2,4,5TCl	0.95	0.0119	0.82	1.27	4.65
	230	2,4,6TCl	0.79	0.0103	0.50	-	-
		2,4,5TCl	0.85	0.0110	0.63	1.25	3.65
	240	2,4,6TCl	0.73	0.0104	0.40	-	-
		2,4,5TCl	0.78	0.0108	0.50	1.24	2.78
dichlorophenols	80	2,4DCl	33.42	0.2853	63.01	-	-
		2,6DCl	33.42	0.2853	63.01	1.00	0
		2,5DCl	38.19	0.3310	72.15	1.15	9.11
	90	2,4DCl	19.21	0.2369	35.80	-	-
		2,6DCl	19.21	0.2369	35.80	1.00	0
		2,5DCl	21.59	0.1931	40.36	1.13	6.51
	100	2,4DCl	11.50	0.1046	21.02	-	-

isomer	temp (°C)	analyte	t_R (min)	w_h	k'	α	Rs
		2,6DCl	11.64	0.1272	21.29	1.01	0.72
		2,5DCl	12.83	0.1289	23.57	1.11	5.47
	110	2,4DCl	7.25	0.0746	13.90	-	-
		2,6DCl	7.38	0.0855	13.13	1.02	0.90
		2,5DCl	8.00	0.0884	14.33	1.09	4.24
	120	2,4DCl	4.80	0.0517	8.19	-	-
		2,6DCl	4.89	0.0560	8.37	1.02	1.03
		2,5DCl	5.23	0.0608	9.02	1.08	3.41
	130	2,4DCl	3.33	0.0357	5.37	-	-
		2,6DCl	3.40	0.0372	5.51	1.03	1.18
		2,5DCl	3.59	0.0408	5.87	1.06	2.81
	140	2,4DCl	2.42	0.0266	3.64	-	-
		2,6DCl	2.48	0.0272	3.74	1.03	1.20
		2,5DCl	2.58	0.0303	3.94	1.05	2.13
	150	2,4DCl	1.84	0.0195	2.52	-	-
		2,6DCl	1.88	0.0208	2.60	1.03	1.23
		2,5DCl	1.94	0.0217	2.71	1.04	1.61
	160	2,4DCl	1.46	0.0156	1.80	-	-
		2,6DCl	1.49	0.0161	1.86	1.03	1.19
		2,5DCl	1.52	0.0169	1.92	1.03	1.14

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