

DEVELOPMENT OF NOVEL COMPREHENSIVE MULTIDIMENSIONAL GAS  
CHROMATOGRAPHY FOR IMPROVING SEPARATION AND ANALYSIS OF PROPYLENE  
OXIDE SAMPLE



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การพัฒนาแก๊สโครมาโทกราฟีหลายมิติแบบทั่วถึงชนิดใหม่สำหรับปรับปรุงการแยกและการวิเคราะห์  
ตัวอย่างโปรตีนออกไซด์



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OXIDE SAMPLE  
By                                      Miss Pannipa Janta  
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พรรณนิภา จันทา : การพัฒนาแก๊สโครมาโทกราฟีหลายมิติแบบทั่วถึงชนิดใหม่สำหรับปรับปรุงการแยกและการวิเคราะห์ตัวอย่างโพรพิลีนออกไซด์. ( DEVELOPMENT OF NOVEL COMPREHENSIVE MULTIDIMENSIONAL GAS CHROMATOGRAPHY FOR IMPROVING SEPARATION AND ANALYSIS OF PROPYLENE OXIDE SAMPLE) อ.ที่ปรึกษาหลัก : ผศ. ดร.ชฎิล กุลสิงห์

วิธีการทดลอง และการวิเคราะห์ข้อมูลชนิดใหม่ของเทคนิคแก๊สโครมาโทกราฟีหลายมิติแบบทั่วถึง (MDGC) ประกอบด้วย 2 เทคนิคย่อย คือ 1) การเชื่อมต่อแบบสองมิติอย่างทั่วถึง (GCxGC) และ 2) การตัดแบบทั่วถึง (comprehensive heart-cut) โดยเทคนิคการตัดแบบทั่วถึงถูกพัฒนาเพื่อนำไปวิเคราะห์ตัวอย่างโพรพิลีนออกไซด์ เทคนิคการเชื่อมต่อแบบสองมิติอย่างทั่วถึงใช้ชุดคอลัมน์ คือคอลัมน์แรกเป็นแบบไม่มีหัวที่มีความยาว 60 เมตร และคอลัมน์สองเป็นแบบมีหัวที่มีความยาวสั้น 5 เมตร ทั้งสองคอลัมน์เชื่อมต่อเข้ากับตัวควบคุมการไหลของแก๊ส คือ flow modulator และ Deans switch (DS) ในขณะที่เทคนิคการตัดแบบทั่วถึงใช้ชุดคอลัมน์คือ คอลัมน์แรกเป็นแบบไม่มีหัวยาว 60 เมตร และคอลัมน์สองเป็นแบบมีหัวที่มีความยาว 60 เมตร ทั้งสองคอลัมน์เชื่อมต่อเข้ากับตัวควบคุมการไหลของแก๊สเพียงชนิดเดียวคือ DS โดยไม่มีอุปกรณ์การจับสารด้วยความเย็นเข้ามาเกี่ยวข้อง ภายใต้สภาวะของโปรแกรมอุณหภูมิแก๊สโครมาโทกราฟีเดียวกัน ปัจจัยของเวลาในการฉีดสาร และการไหลของแก๊สในคอลัมน์ที่สองในเทคนิคการเชื่อมต่อแบบสองมิติอย่างทั่วถึงนั้นมีผลต่อประสิทธิภาพในการแยกสาร ดังนั้นจึงต้องหาสภาวะที่เหมาะสมของสองปัจจัยนี้ ส่วนการวิเคราะห์ของเทคนิคการตัดแบบทั่วถึง ปัจจัยที่มีผลต่อประสิทธิภาพการแยกคือ ความกว้างของช่องการตัด และระยะเวลาในการวิเคราะห์ทั้งหมด ดังนั้นสองปัจจัยนี้จึงถูกนำมาพิจารณาเพื่อหาสภาวะที่เหมาะสม ประสิทธิภาพในการวิเคราะห์ของเทคนิคการตัดแบบทั่วถึง และการเชื่อมต่อแบบสองมิติอย่างทั่วถึงถูกประเมินด้วยความสามารถในการบรรจุฟิค และจำนวนสารประกอบที่แยกได้ โดยสภาวะที่เหมาะสมของเทคนิคการเชื่อมต่อแบบสองมิติอย่างทั่วถึงคือ เวลาในการฉีดสาร ด้วย 0.60 วินาที และการไหลของแก๊สในคอลัมน์ที่สองด้วย 14 มิลลิลิตรต่อนาที สำหรับเทคนิคการตัดแบบทั่วถึงความกว้างของช่องการตัด 0.20 นาทีด้วยระยะเวลาในการวิเคราะห์ 25 ชั่วโมง เป็นสภาวะที่ดีที่สุด ดังนั้นภายใต้สภาวะการทดลองที่เหมาะสมของสองเทคนิคนี้จะนำไปใช้วิเคราะห์สารระเหยง่ายในตัวอย่างโพรพิลีนออกไซด์ โดยสารระเหยง่ายจะถูกระบุตามการเปรียบเทียบแมสสเปกตรัมของสารกับระบบสืบค้น NIST2014 พร้อมด้วยคาร์เท็นชันอินเด็กซ์จากการทดลองในคอลัมน์ที่หนึ่งและค่าอ้างอิง เทคนิคการเชื่อมต่อแบบสองมิติอย่างทั่วถึงใช้ระยะเวลาในการวิเคราะห์ 1 ชั่วโมง ผลการทดลองได้ความสามารถในการบรรจุฟิค คือ 798 และจำนวนสารประกอบที่แยกได้ คือ 61 สารระเหยที่ระบุได้คือ 27 ชนิดพร้อมกับค่าเฉลี่ยความสอดคล้องกันของแมสสเปกตรัม คือ  $887 \pm 35$  ในขณะที่ค่าที่สอดคล้องกันนี้ถูกปรับปรุงให้เป็น 9198, 107, 38 และ  $898 \pm 24$  ตามลำดับ ด้วยเวลาในการวิเคราะห์ 25 ชั่วโมงด้วยการวิเคราะห์แบบเทคนิคการตัดแบบทั่วถึง

สาขาวิชา เคมี

ปีการศึกษา 2563

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Pannipa Janta : DEVELOPMENT OF NOVEL COMPREHENSIVE MULTIDIMENSIONAL GAS CHROMATOGRAPHY FOR IMPROVING SEPARATION AND ANALYSIS OF PROPYLENE OXIDE SAMPLE. Advisor: Asst. Prof. CHADIN KULSING, Ph.D.

New experimental and data analysis approaches in multidimensional gas chromatography (MDGC) comprising 1) comprehensive two dimensional GC (GCxGC) and 2) comprehensive multiple heart-cut were developed with an example application illustrated for analysis of propylene oxide sample. GCxGC system employed column set of long first dimensional (<sup>1</sup>D) nonpolar (60 m) and short second dimensional (<sup>2</sup>D) polar (5 m) columns with a flow modulator and a Deans switch (DS) as a splitter; whilst, the comprehensive heart-cut system applied solely a DS located between long <sup>1</sup>D nonpolar (60 m) and <sup>2</sup>D polar (60 m) columns without use of cryogenic trapping devices. Under the same oven temperature program, the effects of different experimental conditions on the separation performances in GCxGC (injection time of the flow modulator and <sup>2</sup>D column flow) and in comprehensive heart-cut (heart-cut window and number of injections) were investigated. The analysis performance for each condition was evaluated according to peak capacity and number of separated compounds. The continuum between the two techniques was then established based on the analysis time vs analysis performance relationship. The separation performances were improved with longer analysis time so that the suitable condition can be selected within this compromise. The injection time of 0.60 s and <sup>2</sup>D column flow of 14.0 mL/min were proposed as the best condition in GCxGC. For comprehensive heart-cut, the heart-cut window of 0.20 min with analysis time of 25 h was the selected condition. Under these conditions, volatile compounds in propylene oxide sample were identified according to matches between the experimental mass spectrometry (MS) spectra and first dimensional retention indices (<sup>1</sup>I) with that from NIST2014 database and the literatures. An hour analysis with GCxGC resulted in total peak capacity of 798, number of separated peaks of 61, number of identified compounds of 27 and average MS match score of 887±35. The corresponding numbers were improved to 9198, 107, 38 and 898±24, respectively, with the 25 h comprehensive heart-cut analysis.

Field of Study: Chemistry

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# CHAPTER I

## INTRODUCTION

### 1.1 Problem definition

Gas chromatography hyphenated mass spectrometry (GC-MS) is a widely used technique for analysis of semi-volatile and volatile compounds in both qualitative and quantitative analysis of targeted and untargeted compounds in several areas such as forensic, environment, petroleum, food, flavor and natural products [1]. The components of conventional one dimensional (1D) GC-MS consist of three main parts. The first part is sample introduction for injection of sample into GC column at GC injection port. The second part consists of GC column located inside a GC oven. This is the critical component to separate volatile compounds inside samples. The third is detection and identification part. Separated compounds eluting from the GC column are delivered into a detector such as MS to be identified by matching their MS spectrum patterns with that from NIST library, calculation of retention index and injection of authentic standard compounds. However, 1DGC has a problem with complex samples because the separation performance is insufficient to separate all of compounds inside multi-component samples with the problem of peak co-elution which may lead to incorrect compound identification [2].

To solve this problem, multidimensional gas chromatography (or MDGC) technique is applied to improve separation efficiency, reduce background interference and provide higher peak capacity than 1DGC [3]. The conventional system of MDGC may consist of (1) at least two columns with different stationary phases, (2) a modulator or a heart cutting device. There can be two types of MDGC which perform heart-cut and comprehensive analyses which are different in terms of principle and instrumentation. Heart-cut instrument is presented in **Figure 1** composing of first dimensional (<sup>1</sup>D) column (conventionally 30 m), switching valve or

Deans switch (DS) as a modulator and a long second dimensional (<sup>2</sup>D) column (such as 30-60 m). DS is an interface which is used to switch separated compounds from <sup>1</sup>D column into <sup>2</sup>D column. Heart-cut comprises of two sub techniques which are single and multiple heart-cut. Heart-cut approach cuts (collects) some unresolved fractions of interest eluted from <sup>1</sup>D column and transfers to further <sup>2</sup>D separation. A cryogenic device can be applied which offers trapping of compounds into a narrow pulse before sending it onto <sup>2</sup>D column [4]. Single heart-cut focuses on cutting only one position; whilst, multiple heart-cut samples several regions of interests. Another type of MDGC is comprehensive which consists of two sub techniques which are comprehensive heart-cut two dimensional GC and comprehensive 2D GC (GCxGC). Both comprehensive heart-cut and GCxGC analyze all compounds eluting from <sup>1</sup>D column. To this end, comprehensive mode is a widely used strategy to study full volatile compound profile in a complex sample (untargeted analysis). GCxGC instrument is shown in **Figure 2** comprising of a <sup>1</sup>D column, a modulator as an interface which is used to trap and release compounds and a short <sup>2</sup>D column. The concept of GCxGC is all peaks separated from <sup>1</sup>D column are collected and released (modulated) onto a short <sup>2</sup>D column through a modulator [5]. GCxGC provides fast comprehensive analysis time within solely one injection because of using a short <sup>2</sup>D column. Meanwhile, comprehensive heart-cut is a novel technique which combines the two approaches of heart-cut and GCxGC. Total effluents eluted from <sup>1</sup>D column are cut (collected) and delivered by a DS onto a long <sup>2</sup>D column. Heart cut window ( $t_{H/C}$ ) is a critical parameter affecting <sup>2</sup>D separation which can be improved using a small  $t_{H/C}$  and longer analysis time to complete a comprehensive analysis. Because of a long <sup>2</sup>D column with multiple injections, the separation performance can be much greater [6]. Nevertheless, comprehensive heart-cut analysis has not been widely applied due to the complicated experimental setup, e.g., use of both a DS and a cryogenic trapping device (or cryogenic modulator) providing high cost of instrument and maintenance and the data processing problem [6-8].

Therefore, in this study, simple and low cost comprehensive heart-cut without the use of cryogenic device will be developed in this thesis order to improve peak capacity and number of separated peaks in industrial samples. Data analysis

approach to enhance compound identification based on MS and  $^1H$  data was also established. The results will be compared with the conventional GCxGC using a flow modulator.

## 1.2 Literature Review

Multidimensional gas chromatography (MDGC) is a high performance separation technique enhancing peak capacity for analysis of 'complex' samples such as petroleum, food, beverages, pharmaceutical and illicit drugs, and the environment [9-12]. Hyphenation with mass spectrometry provides molecular-based information of volatile analytes enabling high confidence identification of several hundred compounds within a single analysis [9-11, 13, 14].

In general, MDGC employs two columns with different (ideally orthogonal) selectivities [15] which are connected sequentially via a device offering an effective heart-cut or modulation process.[16, 17] Compared with one dimensional (1D) GC, MDGC provides improved separation resolution and analyte peak capacity, reduced chemical background, improved detection limit and reduction of sample clean-up steps such as liquid or solid phase extraction [13]. A sub class of MDGC which popularly analyzes untargeted analysis in complex sample is comprehensive heart-cut MDGC and GCxGC.

Conventional heart-cut technique is generally suitable for target analysis using single heart-cut or multiple heart-cut such as analysis of monoaromatic hydrocarbons in complex groundwater [18], o,p'-DDT in environmental samples [19] and complex mixtures of organic pollutants in environmental samples [2]. In the system of heart-cut,  $^1D$  and  $^2D$  columns are connected with a sampling device with a cryogenic trap to enhance separation efficiency by reducing band broadening during the sampling process and trapping compounds followed by release of small fractions onto the  $^2D$  column. The sampling device can be divided into three types: (1) in-line valve, (2)



out-line valve and (3) valveless (often called the Deans switch) systems connecting between <sup>1</sup>D and <sup>2</sup>D columns with high thermal stability, leak free and chemical inertness [4, 20]. Comprehensive heart-cut can be applied for untargeted analysis enhancing separation performance with a long <sup>2</sup>D column. However, it has limitations in terms of instrument setup, time consuming and the requirement of multiple injections by re-injection of the same sample vial to complete comprehensive results of volatile profiles together with complicated data analysis [2, 6]. Therefore, this approach is not commonly applied.

GCxGC approach is similar to comprehensive heart-cut with total fractions eluted from <sup>1</sup>D column (30-60 m) albeit with application of a short <sup>2</sup>D column (1-5 m) and a modulator [2]. GCxGC provides fast analysis time and it is a widely used comprehensive technique in several areas such as characterization of odorant patterns in food [21], separation of triacylglycerols in olive oil [6] or analysis of volatile compounds in frankincense [22]. It requires only one injection of sample to obtain comprehensive volatile profiles in complex samples [3]. The important device of GCxGC is a modulator the function of which is to quickly transfer fraction of gas from <sup>1</sup>D column onto a short <sup>2</sup>D column. There can be two main categories of modulators: (1) thermal modulators (cryogen-free thermal modulators and cryogen-based thermal modulators such as using liquid CO<sub>2</sub>) trapping compounds at lower temperature relative to the oven temperature and releasing compounds into <sup>2</sup>D column with a rapid heating system. (2) non-thermal modulators which use gas flow to control movement of compounds from <sup>1</sup>D column for introduction to <sup>2</sup>D column, such as differential flow and flow diversion or valve based modulation [5, 23].

After finishing separation process, identification of separated compounds is an important process. The common detector such as MS is popularly employed with GC because it achieves efficiency to identify the unknown compounds depending on their mass-to-charge ratio ( $m/z$ ) and to compare their mass spectrum with library [24]. In addition, retention index ( $I$ ) calculation is also used for identification of

compounds to confirm the data obtained from MS library. The mixture of *n*-alkanes is conventionally reference standards used in experiment. Calculation of *I* value of MDGC is similar to <sup>1</sup>D retention index (<sup>1</sup>*I*). This is simple process since its calculation can be performed according to elution time of analytes following GC oven temperature and compared with retention times of the alkane references [25].

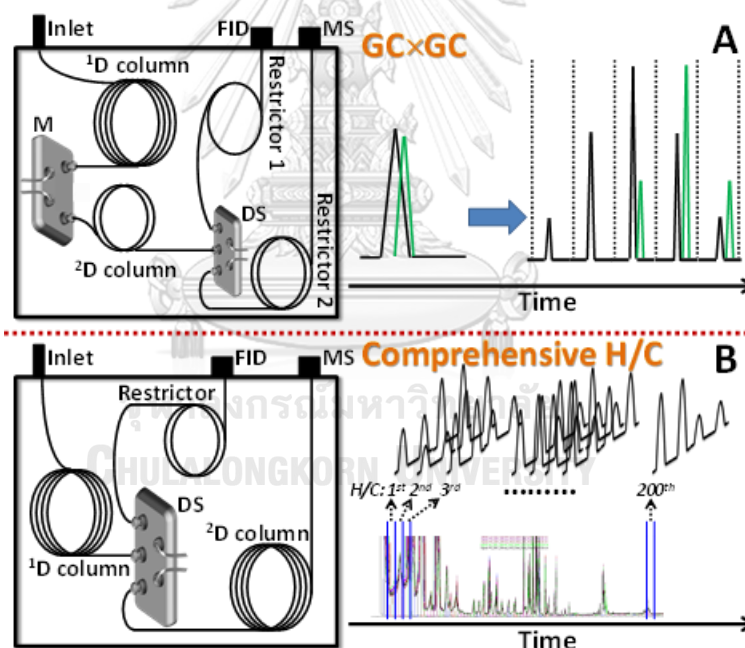
### 1.3 Aim, scope and expected benefits of this work

The instrumental setup for a conventional heart-cut technique normally used a cryogenic device with its critical function allowing analyte pulse trapping with the subsequent release onto a <sup>2</sup>D column within a narrow band of the pulse enhancing <sup>2</sup>D separation. However, cryogenic approach involves additional devices, such as trapping channel and switching valve for automated on/off operation, cryogen consumption and system maintenance.

In order to transfer a narrow heart-cut band without cryogenic device, heart-cut window should be significantly narrower than <sup>2</sup>D separation time [26]. To this end, simulation approach showing the compromise between separation time (heart-cut window) and analysis performance (number of separated peaks) has been previously proposed providing high peak capacity analysis of the petrochemical sample within a reasonable analysis time. However, experimental investigation of the concept as well as approach to generate a contour plot has not been reported. In addition, investigation on its advantages over the conventional GCXGC and further application of this technique are still a challenge.

In this study, approaches in comprehensive heart-cut technique using solely DS without a cryogenic trapping device were developed and compared with the conventional GCXGC approach with flow modulation for analysis of a propylene oxide sample using the system configuration as shown in **Figure 1**. Firstly, the continuum between the two techniques was experimentally investigated in detail.

Useful data presentation method was proposed. Secondly, effects of experimental conditions were investigated, and the selected comprehensive heart-cut and GCxGC results were evaluated, compared and discussed in terms of peak capacity and number of separated compounds. Thirdly, relationship between the analysis time and separation performance was also established. Fourthly, separated compounds of the propylene oxide sample obtained from the two separation approaches were tentatively identified according to a comparison of their mass spectra with those from NIST library with match scores of  $>650$ , and their difference  $^1I$  value from the literature of  $\pm 30$ . The chemical components in the sample were also reported and compared with the conventional GCxGC.



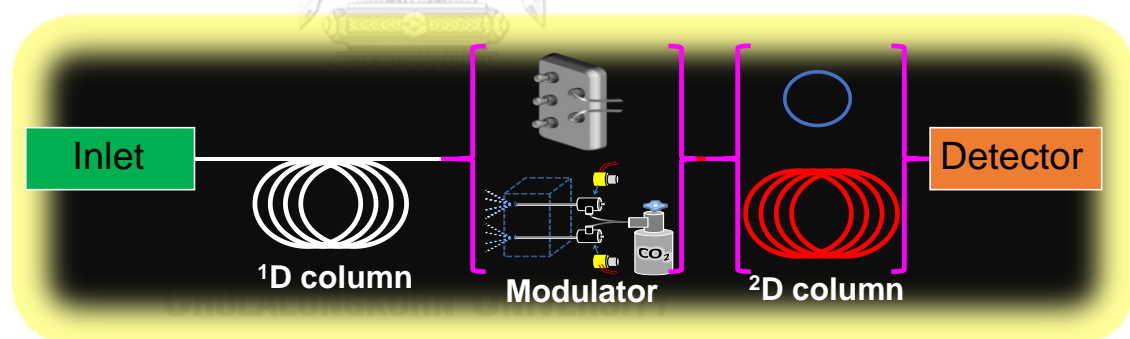
**Figure 1.** Comprehensive MDGC systems used in this thesis: (A) GCxGC and (B) comprehensive heart-cut MDGC. M = modulator and DS = Deans switch.

## CHAPTER II

### THEORY

#### 2.1 MDGC technique

MDGC can consist of two sub classes which are heart-cut and GCxGC. Heart-cut was firstly described by Simmons and Snyder in 1958 for analysis of a stabilized platformate sample [27]. However, GCxGC was later invented in 1991 by Liu and Phillips to quickly solve the peak overlaps in cases of complex mixtures that contain over 30-50 compounds [28, 29]. The overall system of MDGC system mainly includes 1) a <sup>1</sup>D column 2) a modulator and 3) a <sup>2</sup>D column as shown a schematic diagram in **Figure 2**. These two techniques differ in principle and instrument.



**Figure 2.** The schematic diagram of MDGC techniques. Adapted from [30]

#### 2.2 Comprehensive heart-cut

Comprehensive heart-cut approach comprises of four main devices as shown in **Figure 3**.

##### 2.2.1 <sup>1</sup>D column

The normal combination of column set uses <sup>1</sup>D column containing a nonpolar phase which separates compounds based on their boiling points. Thus,

several polar compounds can co-elute in <sup>1</sup>D separation. <sup>2</sup>D polar column is thus employed to enhance separation efficiency of these coeluting polar components. In reverse column configuration, <sup>1</sup>D polar/<sup>2</sup>D nonpolar columns are applied. However, <sup>2</sup>D nonpolar column could provide less separation performance for polar compounds since only dispersive force cannot be performed effective separation. It should be noted that selection of column set depends on application and trial and error [30].

### 2.2.2 <sup>2</sup>D column

The concept of choosing <sup>2</sup>D column can be that with higher selectivity than <sup>1</sup>D column to compensate the lower efficiency of the shorter <sup>2</sup>D separation. The length of <sup>2</sup>D column is also critical point of comprehensive heart-cut technique since if it is increased, separation time and separated peaks will also rise as mentioned in the theoretical trend simulation in the previous study albeit with the compensation of a longer analysis time [27]. Conventional heart-cut approach normally utilized the length of <sup>2</sup>D column of 30 m to reduce analysis time [18, 31].

### 2.2.3 Restrictor

A restrictor column is an uncoated deactivated fused silica column with a small diameter and is usually connected to a detector as shown in **Figure 3**. The function of a restrictor is to monitor separated compounds eluting from <sup>1</sup>D column into a detector. Because the suitable restrictor should provide the same flow resistance as the <sup>2</sup>D column in order to balance flow between <sup>1</sup>D and <sup>2</sup>D columns [4].

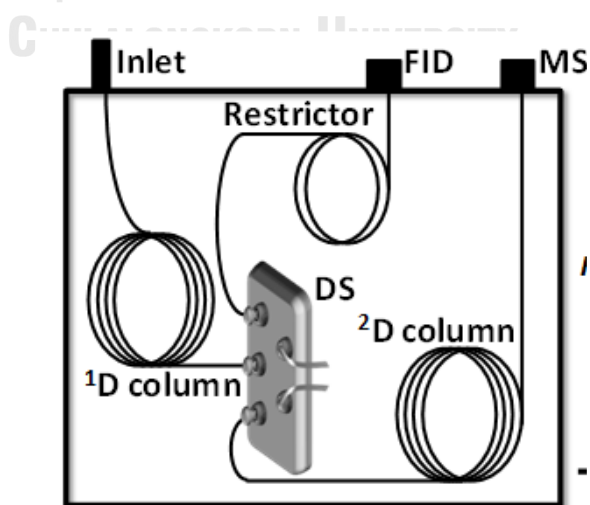
### 2.2.4 Deans switch (DS)

DS is a microfluidic flow switching device comprising of an electronic three-port solenoid valve following **Figure 4** and firstly invented by David Deans in the 1960s. DS is usually an interface applied in heart-cut technique. This type of modulators is thermally stable, leak free, chemically inert without moving parts and low dead volume [4]. The important concept of DS is to deliver effluent from <sup>1</sup>D to the <sup>2</sup>D column [20, 32]. The three columns of <sup>1</sup>D, <sup>2</sup>D and restrictor were connected

through DS as shown in **Figure 3**. DS consists of two operations which are off and on as mentioned in **Figure 5**. For off mode, excess pressure from pressure control module (PCM) is directly supplied to <sup>2</sup>D column diverging <sup>1</sup>D column flow onto the restrictor towards flame ionization detector (FID, detector 1) as shown by the red line in **Figure 5A**). On the other hand, when pressure from PCM was directly switched up to restrictor (red line in **Figure 5B**), <sup>1</sup>D column flow was diverged onto <sup>2</sup>D column towards MS (detector 2). This operation is called on mode.

DS calculator is a program that helps to calculate suitable pressure at inlet of <sup>1</sup>D column and PCM with optimum restrictor length. The outcome of these values depends on column set that is used in the experiment by filling information about flows, column lengths, column i.d. of both <sup>1</sup>D and <sup>2</sup>D columns and restrictor i.d. Then, the calculated numbers obtained from DS calculator were further applied in the actual experiment. The figure of DS calculator is shown in **Figure 5C**.

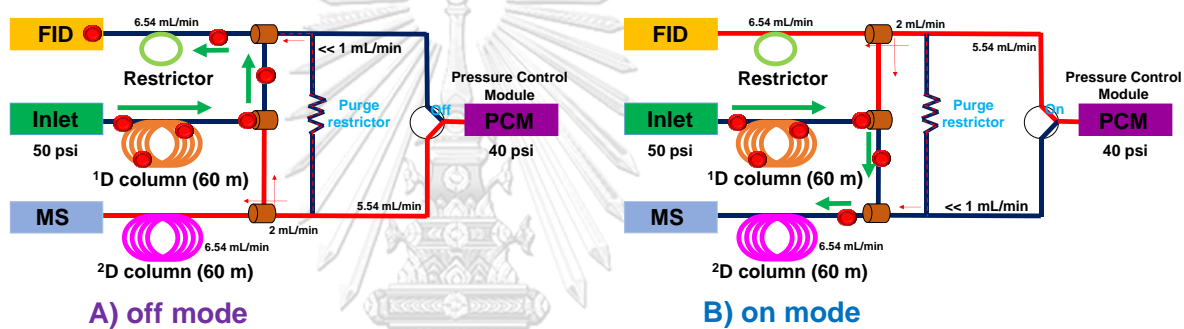
DS is suitable for trace analysis since DS requires small portion to cut (collect) interesting zone from <sup>1</sup>D separation onto <sup>2</sup>D column with decreasing interference signal [5]. Recently, DS can also be applied in GCxGC approach as a splitter to split effluent into different ways such as splitting to a short and a long <sup>2</sup>D column within in the same period in a GC oven [33].



**Figure 3.** The schematic diagram of comprehensive heart-cut approach.



Figure 4. A three-port solenoid valve of DS.



C)

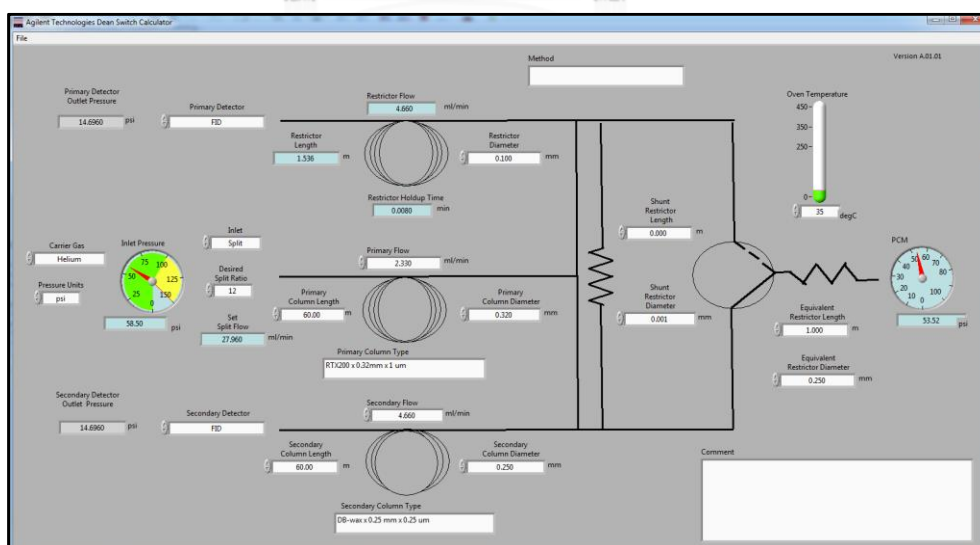


Figure 5. The two operation modes of DS: (A) off mode (B) on mode adapted from <https://www.agilent.com/cs/library/brochures/5989-9384EN.pdf> and (C) DS calculator.

### 2.3 The periodic comprehensive heart-cut

The 2 critical factors of comprehensive heart-cut are

- 1)  ${}^2t_{R,max}$  is maximum time of analyte that eludes in  ${}^2D$  column or  ${}^2D$  separation time
- 2) Sampling time ( $t_{samp}$ ) is width of heart-cut window

Figure 6 showed comprehensive heart-cut procedure which  ${}^2t_{R,max}$  and  $t_{samp}$  is equal to 6 and 0.5 min, respectively. The heart-cut position was shifted to the next 0.5 min until covering  ${}^1D$  separation space but the process cannot be performed continuously within one injection, and difference between each heart-cut position was  ${}^2t_{R,max}$  (6 min). Therefore, the number of injection to complete comprehensive analysis was calculated from  ${}^2t_{R,max}$  divided by  $t_{samp}$  which in this example is equal to 12 injections as shown in Figure 6 [11].

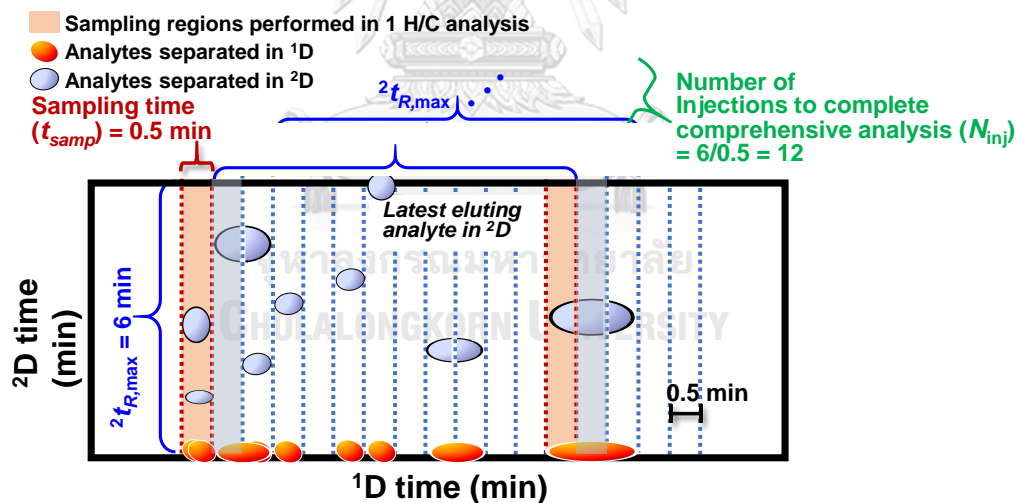


Figure 6. The periodic heart-cut procedure with  ${}^2D$  separation time of 6 min and sampling time of 0.5 min. Reproduced from [11].



## 2.4 GCxGC

GCxGC consists of three main parts which are 1) <sup>1</sup>D column 2) <sup>2</sup>D column and 3) modulator as presented in **Figure 7**.

### 2.4.1 <sup>1</sup>D column

A <sup>1</sup>D column of GCxGC is the same as comprehensive heart-cut mentioned in Section 2.2.1.

### 2.4.2 <sup>2</sup>D column

A <sup>2</sup>D column of GCxGC differs from comprehensive heart-cut in term of the length of <sup>2</sup>D column. A short column around 1-5 m was proposed to present fast <sup>2</sup>D analysis time and provided comprehensive analysis within a single injection [11]. The column combination of this technique is the same as heart-cut which depends on application.

### 2.4.3 Modulator

The concept of modulator is to transfer analytes from <sup>1</sup>D column into <sup>2</sup>D column. The first modulator of GCxGC was reported by Liu and Phillips using heater-based type [34]. Recently, modulator applied in GCxGC is classified into two types which are thermal modulator and non-thermal modulator.

#### 2.4.3.1 Thermal modulator

The concept of this modulator employs lower temperature (than the oven temperature) to trap analytes and higher temperature to release them in the modulation device. Therefore, rapid movement of trapped analytes was necessary to generate narrow band for injection onto a short <sup>2</sup>D column. Thermal modulator can be divided into heater designs, cryogenic designs and other designs. Heater designs utilize sorption of analytes by thick coating of liquid stationary phases or adsorbent phases at below ambient temperature and use of rapid desorption by active heating process such as thermal desorption modulator which was the first thermal modulator developed by Phillips and coworkers and rotating thermal

modulator. However, thermal designs are hard to operate and maintain thermal stability of the hardware inside a GC oven. The use of thermal modulator has not been widely used. Cryogen designs were developed to solve the limitations of thermal designs. The design employs cryogens such as liquid CO<sub>2</sub> and N<sub>2</sub> to trap analytes at low temperature. Trapped analyte is then exposed to the high temperature of GC oven and then released onto a short <sup>2</sup>D column. For example, longitudinally modulated cryogenic system (LMCS) is shown in **Figure 8** with the trapping and releasing processes shown in **Figure 8B** and **Figure 8C**, respectively. The other of thermal modulator is dual-stage jet modulator, dual-jet loop modulator and liquid nitrogen jet modulator. The use of cryogens presents better performance with a high cost of instrument. Other designs require development of thermal modulation without the use of cryogens but it still provides good performance and robustness as well as minimizing device sizes such as single-stage modulator, microfabricated thermal modulator and solid-state modulator [4, 35, 36].

#### 2.4.3.2 Non-thermal modulator

The concept of this modulator is using gas flow to control mobilization of analytes. Non-thermal modulator can be divided into two types which are differential flow modulation including flow modulator as shown in **Figure 9**, flow diversion including DS mentioned in Section 2.2.4 and valve-based modulation.

##### 2.4.3.2.1 Flow modulator

Flow modulator consists of two operations (**Figure 10**) which are load and injection. In the load stage, gas flow moves toward a short <sup>2</sup>D column, while effluent from <sup>1</sup>D column transfers into collection channel (**Figure 10A**). Before the collection channel is overfilled, the injection mode starts by switching gas flow to the top of the collection channel and then effluent was flushed into a short <sup>2</sup>D column to be separates and detected MS as shown in **Figure 10B**. To this end, injection time as the period of time to inject analyte fraction into

$^2\text{D}$  column is the critical parameter affecting to peak shape in  $^2\text{D}$  separation and it should be optimized in flow modulator. Both load and injection operations are continuous process until covering the whole  $^1\text{D}$  separation. This modulator provides simple design and shows good performance which has been successfully applied in several samples [4, 35, 36].

## 2.5 Modulation period ( $P_M$ )

$P_M$  is the period modulation in GCxGC process. It plays an important role in modulation performance and  $^2\text{D}$  separation leading to different peak shape of analytes. Normally,  $P_M$  are in a range of 1-10 s and could be as short as 50 ms [36]. Therefore, the effect of  $P_M$  is normally investigated in GCxGC development.

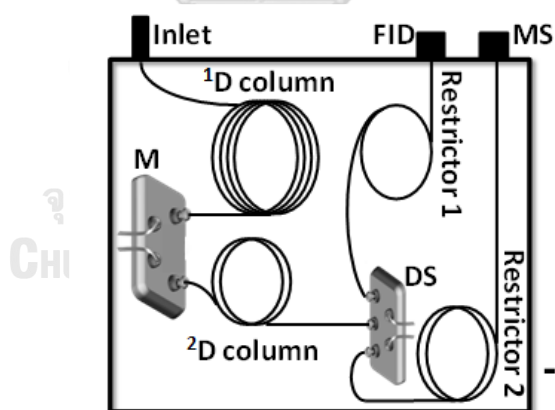


Figure 7. The schematic diagram of GCxGC technique.

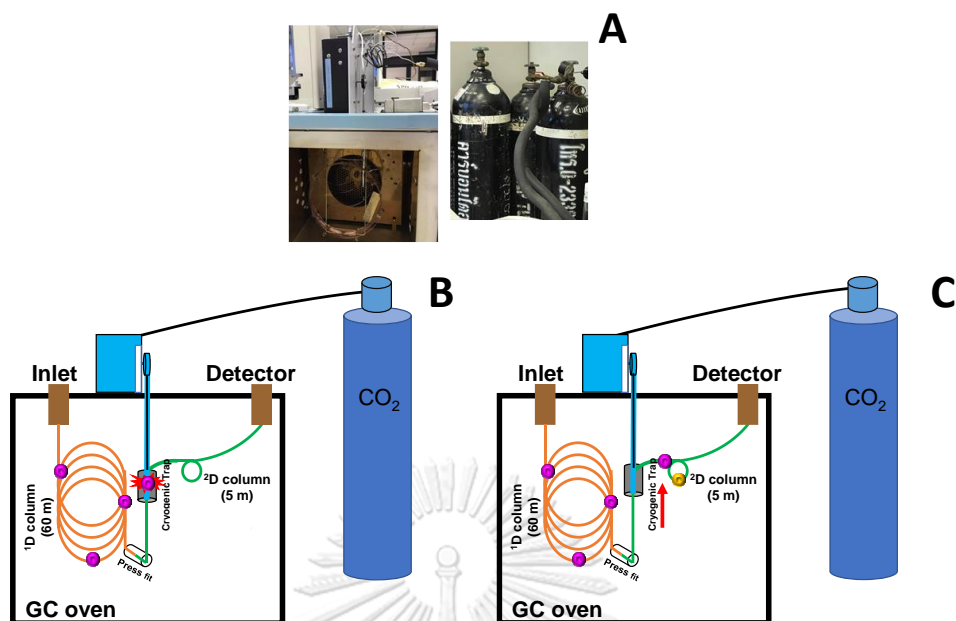


Figure 8. (A)The LMCS with cooled liquid CO<sub>2</sub> (B) Trapping process and (C) Releasing process



Figure 9. A two port valves of a flow modulator.

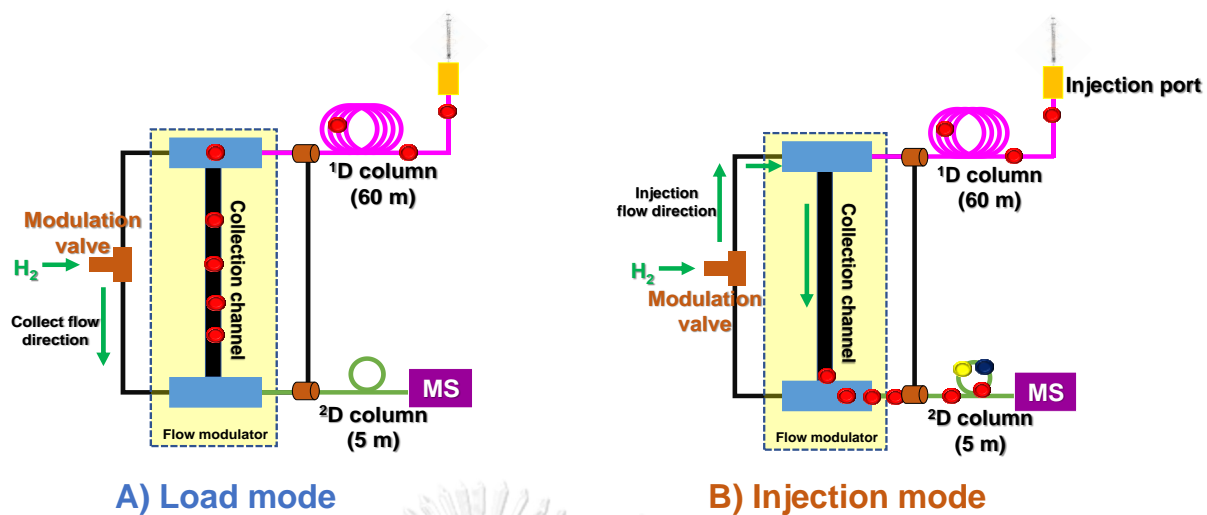


Figure 10. The two stages of a flow modulator: (A) load mode and (B) injection mode. Adapted from [4].



## CHAPTER III

### EXPERIMENTAL

#### 3.1 Instruments and apparatus

3.1.1 Gas chromatograph-mass spectrometer (GC–MS) with GC Model 7890A and 5975C MSD (Agilent Technologies Inc., USA), where GC consists of auto-sampler, column oven, flame ionization detector (FID) and MS consists of single quadrupole mass analyzer, electron ionization (EI) source and GC Chemstation software processing (Agilent MSD Chemstation (version E.02.02.1431)).

3.1.2 A DB-1MS capillary column (60 m × 0.25 mm inner diameter (i.d.) × 0.25 film thickness; J&W Scientific, USA)

3.1.3 A Rtx-200 capillary column (60 m × 0.32 mm i.d. × 1 film thickness; Restex, USA)

3.1.4 A DB-WAX capillary column (60 m × 0.25 mm i.d. × 0.25 film thickness; J&W Scientific, USA)

3.1.5 An HP-INNOWAX capillary column (5 m × 0.25 mm i.d. × 0.15 film thickness; J&W Scientific, USA)

3.1.6 A restrictor capillary column (1.5 m × 0.1 mm i.d.; Agilent Technologies Inc.)

3.1.7 A restrictor capillary column for GC×GC system (2.25 m × 0.18 mm i.d. and 0.75 m × 0.18 mm i.d.; Agilent Technologies Inc.) towards MS and FID, respectively.

3.1.8 A Deans switch device (part no. G2855-80510 and G3183-61500; Agilent Technologies Inc.) with Agilent G2855B software control.

3.1.9 A Deans switch calculator (version A.01.01; Agilent Technologies Inc.)

3.1.10 A flow modulator (part no. G3486-61810; Agilent Technologies Inc.) with Agilent G3486A software control

3.1.11 A inlet liner, split, single taper, glass wool, deactivated, low pressure drop (part no. 5183-4647; Agilent Technologies Inc.)

### 3.2 Chemicals

The authentic standard compounds were purchased from Merck (Darmstadt, Germany); Alfa Aesar, TCI and Sigma-Aldrich (St. Louis, MO). *n*-alkane standards (C6-C22) purchased from Sigma-Aldrich (St. Louis, MO) were used as a reference to calculate the first dimensional retention indices (<sup>1</sup>) of the compounds.

### 3.3 Sample preparation

Due to the conflict of interest issues, details of sample preparation could not be provided in this work.

### 3.4 1D-GC-single MS condition

A nonpolar DB-1MS column (60 m × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific, USA) and a midpolar Rtx-200 column (60 m × 0.32 mm i.d. × 1 μm film thickness; Restex, USA) were applied and their separation performances in the sample were investigated. The sample with injection volume of 1 μL was injected at GC inlet temperature of 250 °C. GC oven temperature program was set at 35 °C for 10 min, increased to 135 °C (4 °C/min), then to 250 °C (10 °C/min) and held at this temperature for 20 min with the constant flow rate of helium (He) of 2.3 mL/min (total run time of 66.5 min) controlled by Agilent MSD Chemstation (version E.02.02.1431). The split ratio of 12:1 was applied. The ion source temperature,

emission current and electron energy were set at 250 °C, 34.6  $\mu\text{A}$  and 70 eV, respectively, with a mass range of 28–550 m/z and scan rate of 2.80 scan/sec.

### 3.5 Optimization of comprehensive heart-cut

The comprehensive heart-cut column set was <sup>1</sup>D nonpolar DB-1MS column (60 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$ ; J&W Scientific, USA) or <sup>1</sup>D midpolar Rtx-200 capillary column (60 m  $\times$  0.32 mm i.d.  $\times$  1  $\mu\text{m}$ ; Restex, USA) and <sup>2</sup>D polar DB-Wax column (60 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$ ; J&W Scientific, US). The restrictor column (1.5 m  $\times$  0.1 mm i.d.; Agilent Technologies Inc.) was used to balance flows between first and second dimension column. The first, second and restrictor columns were connected through a Deans switch (DS, Agilent Technologies Inc.) device towards the FID and MS as shown in the **Figure 3**. The primary <sup>1</sup>D column flow of helium was 2.3 mL/min and <sup>2</sup>D column flow of 3.5 and 4.7 mL/min were applied to prevent leak of effluent. For each heart-cut analysis, GC oven temperature program and MS condition were the same as that applied in 1D-GC-single MS. The total analysis time in comprehensive heart-cut analysis varies depending on the number of injections applied (an injection per run with the analysis time of 1 h per run). In this study, comprehensive multiple heart-cut was performed with different heart-cut windows ( $t_{\text{H/C}}$ , periods to transfer an effluent from <sup>1</sup>D to <sup>2</sup>D column) of 5.00, 2.50, 1.25, 1.00, 0.50 and 0.20 min covering the range from 8.5 to 60 min of <sup>1</sup>D separation time. For each injection, several heart-cut were performed in a periodic strategy [8]. In this thesis, each heart-cut was performed within every 5 min (which is <sup>2</sup>D separation time,  ${}^2t_{\text{R,max}}$  (maximum time of analyte that elutes in <sup>2</sup>D column) and can be considerably the modulation period). The number of injections for each comprehensive analysis was thus calculated as  ${}^2t_{\text{R,max}}/t_{\text{H/C}}$ . The number of runs for comprehensive heart-cut analyses with  $t_{\text{H/C}}$  of 5.00, 2.50, 1.25, 1.00, 0.50 and 0.20 min were 1, 2, 4, 5, 10 and



25 runs, respectively. The corresponding heart-cut events for each comprehensive heart-cut analysis were summarized in **Table S-1** (Appendices).

### 3.6 Optimization of GCxGC

The GCxGC column set was a nonpolar DB-1MS (60 m × 0.25 mm i.d. × 0.25 μm; J&W Scientific, US) and a midpolar Rtx-200 (60 m × 0.32 mm i.d. × 1 μm film thickness; Restex, USA) as <sup>1</sup>D column with a short polar HP-INNOWAX column (5 m × 0.25 mm I.D. × 0.15 μm; J&W Scientific, USA) as <sup>2</sup>D column. The <sup>1</sup>D and <sup>2</sup>D columns were connected through a flow modulator (Agilent Technologies Inc.). As shown in **Figure 7**, the <sup>2</sup>D column end was further connected to the DS which behaved as a splitter via two restrictors to FID and MS. The restrictor dimensions were 2.25 m × 0.18 mm i.d. and 0.75 m × 0.18 mm i.d. towards MS and FID, respectively. This is performed in order to reduce flow to MS. A modulation period ( $P_M$ ) of 6 s was applied. Helium was used as carrier gas with a <sup>1</sup>D column flow rate of 0.8 mL/min. Different injection time of the flow modulator (0.15, 0.30, 0.60, 1.20 and 2.40 s) and <sup>2</sup>D column flow (7, 14 and 21 mL/min) were optimized in order to select the modulation period and separation performance. GC-MS condition was that applied in 1D-GC-single MS.

### 3.7 Data processing

General data acquisition and processing were performed by using GC Chemstation software. Microsoft Excel 2016 and Fortner Transform 3.3 (Fortner, Inc., Savoy, IL) were used for data visualisation.

### 3.7.1 Comprehensive heart-cut

In generally, use of cryogenic trapping benefits data analysis with simple calculation of  ${}^2t_R$  of peaks within each heart-cut. For example with the 2.0 min heart-cut window applying along the period from 10.0 to 12.0 min in  ${}^1D$  separation, all the heart-cut compounds can be cryogenically trapped and then released with the same starting time in  ${}^2D$  separation [7]. However, the sampling process from  ${}^1D$  column without using cryogenic devices can lead to immediate elution onto  ${}^2D$  column without trapping and thus variation of the start time in  ${}^2D$  separation. In the case of a 10.0-12.0 min heart-cut, two compounds with  ${}^1t_R$  of 11.9 min and 11.4 min will be eluting with 0.5 min difference in  ${}^2D$  separation. In this thesis without use of cryogenic trap, all the heart-cut compounds from 10.0-12.0 min will be set to have the same starting time in  ${}^2D$  separation which is in the middle of the heart-cut period (11.0 min). This leads to the maximum error of “half of the heart-cut window” in calculation of both  ${}^1t_R$  and  ${}^2t_R$ , e.g.  $\pm 1.0$  min for the 2 min heart-cut window. Thus, narrower heart-cut window resulted in more accurate calculation of  ${}^2t_R$ .

According to the approximation of starting time in  ${}^2D$  separation above,  ${}^1t_R$  and  ${}^2t_R$  of a peak detected by MS in each multiple heart-cut analysis were approximated as

$${}^1t_{R,approx} = t_{H/Cmid} = \frac{t_{H/C}}{2} = \frac{t_{H/Cstart} + t_{H/Cend}}{2} \quad (1)$$

$${}^2t_{R,approx} = t_{R,observed} - {}^1t_R \quad (2)$$

where  $t_{H/C}$  is heart-cut window.  $t_{H/Cmid}$  and  $t_{H/Cstart}$ ,  $t_{H/Cend}$  are middle time, starting time and ending time of the heart-cut, respectively.  $t_{observed}$  is the peak time observed with MS detector (after elution through both  ${}^1D$  and  ${}^2D$  columns). In case of modulated (or split) peaks into different heart-cut fractions, calculation of  ${}^1t_R$  and  ${}^2t_R$  of a compound was performed by selecting the modulated peak with highest peak area for each compound.

In general, by considering a heart-cut event, when the applied heart-cut window ( $t_{H/C}$ ) is significantly longer than average widths in <sup>1</sup>D separation ( ${}^1w_{b,ave}$ ) of compounds, any compounds separated within the heart-cut interval will undergo under sampling process leading to recombination (e.g. by cryogenic trapping process or being collected into the same loop) of the separated peaks into the same H/C fraction before <sup>2</sup>D separation. This results in  $t_{H/C} = {}^1w_{b,ave}$  as previously reported in [11]. In this study, there was no recombination effect since sample loop or cryogenic trapping approach was not applied. In addition, when  $t_{H/C}$  is closer to or shorter than  ${}^1w_{b,ave}$  of peaks, peak splitting can occur and the apparent  ${}^1w_{b,ave}$  will expressed as

$${}^1w_{b,ave} = t_{H/C} \times \mathbf{Roundup} \left( \frac{w_{b,ave,1DGC}}{t_{H/C}} \right) \quad (3)$$

where  $w_{b,ave,1DGC}$  is average peak width at baseline of all the separated peaks (excluding the solvent peak) obtained from 1DGC analysis (e.g. with the DS off). “Roundup” function resulted in the rounded number of a given value. The average width at baseline in chromatograms obtained in each heart-cut analysis (observed with MS detector after heart-cut) was assumed to be average widths in <sup>2</sup>D separation ( ${}^2w_{b,ave}$ ).

The 2D contour plots in comprehensive heart-cut analysis were obtained by combination of the elution profiles subtracted by  $t_{H/Cmid}$  obtained from all the heart-cut fractions into the same data which was then converted into a contour plot by using Fortner Transform 3.3 (Fortner, Inc., Savoy, IL).

### 3.7.2 GCxGC

Peak identification and estimation of retention time ( ${}^1t_R$  and  ${}^2t_R$ ) and average peak widths at baseline ( ${}^1w_{b,ave}$  and  ${}^2w_{b,ave}$ ) excluding that of the solvent in <sup>1</sup>D and <sup>2</sup>D separations were performed using GC image version 2.7r1 GCxGC. This was performed using pixel based approach for identification of peak from a contour plot (<http://www.gcimage.com/gcxgc/usersguide/statistics.pdf>). The GCxGC contour plots

were generated by transformation of 1D modulated chromatogram data into a matrix which was further transformed into a contour plot. All of these processes were performed using GC image.

### 3.7.3 Peak capacity

Total peak capacity ( $n_{c,total}$ ) is calculated according to

$$n_{c,total} = {}^1n_c \times {}^2n_c \quad (4)$$

$${}^1n_c = 1 + \frac{{}^1t_{R,last} - {}^1t_{R,first}}{{}^1w_{b,ave}} \quad (5)$$

$${}^2n_c = \frac{{}^2D \text{ separation time}}{{}^2w_{b,ave}} \quad (6)$$

where the superscripts 1 or 2 indicate that the parameters are in <sup>1</sup>D or <sup>2</sup>D separation, respectively, with the retention times of the first and the lastest eluting analytes of  $t_{R,first}$  and  $t_{R,last}$ , respectively, and average peak width at baseline of  $w_{b,ave}$ . <sup>2</sup>D separation time is  $P_M$  in GCxGC (6 s) or  ${}^2t_{R,max}$  in comprehensive heart-cut analysis (5 min).

### 3.7.4 A number of separated compounds

All separated compounds excluding solvent peak obtained from comprehensive heart-cut (every heart-cut window) and GCxGC were directly counted from peaks in contour plots.

### 3.7.5 Compound identification

The chromatographic peak and MS data in propylene oxide sample were identified by GC Chemstation software. Compounds in the sample were identified based on comparison with mass spectra and <sup>1</sup>D retention index (<sup>1</sup>*I*) in NIST14 library and literatures. For comparison with MS of authentic standard compounds, each authentic standard compound was firstly injected and followed by propylene oxide sample under the same GC oven temperature program that applied

in 1D-GC-single MS (Section 3.4) on the same day and using same tuning file. The identification followed the criteria of matching between retention time and mass spectra. For the  $^1I$  determination, a mixture of alkanes was injected using the same experimental condition as that of sample analysis except the modulator was turned off.  $^1I$  were calculated using the relationship [37]:

$$^1I = 100n + 100 \left( \frac{^1t_{R(i)} - ^1t_{R(n)}}{^1t_{R(n+1)} - ^1t_{R(n)}} \right) \quad (7)$$

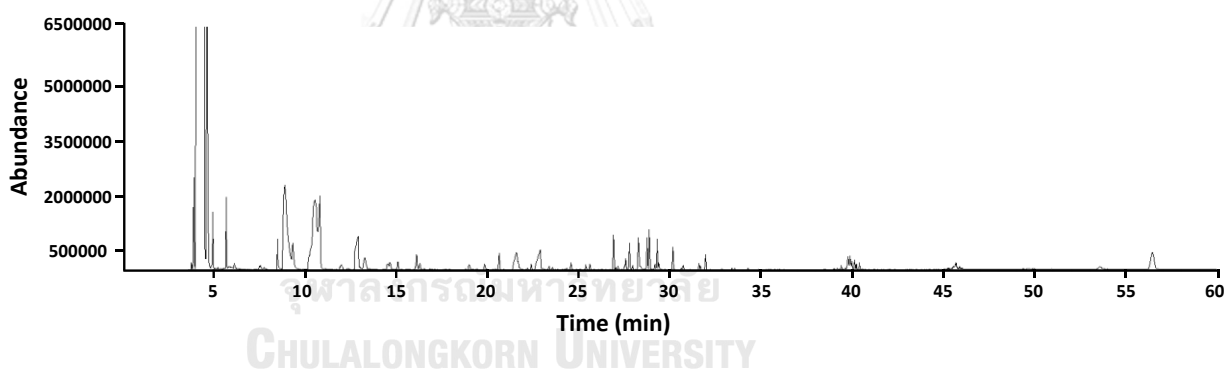
where  $^1t_{R(i)}$  is the retention time of analyte of interest on  $^1D$  column.  $n$  and  $n+1$  are the numbers of carbons of alkane standards eluting in the positions bracketing the analyte  $i$ . Note that  $^2I$  was not calculated in this study. The compounds reported in this work were identified with MS match score of  $>650$  [27] and  $^1I$  difference of  $\pm 30$  unit from the literature values. The number of separated compounds is obtained by counting all the separated peaks in each analysis.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 1D-GC-single MS separation of propylene oxide sample

Propylene oxide sample was separated using DB-1 MS column hyphenated with MS detector with the optimized GC-MS conditions mentioned in Section 3.4. The 1D-GC-single MS chromatogram is shown in **Figure 11**. The result showed that several overlapping and broad peaks were observed in during time of 8.5 – 60 minutes due to over limitations of conventional 1D separation in terms of selectivity and peak capacity. It should be noted that 1D-GC-single MS technique might not be suitable to analyze any complex samples (>100 volatile compounds) or that with target compound coeluting. To this end, the period of 8.5 – 60 minutes was selected for further study with the comprehensive analyses.



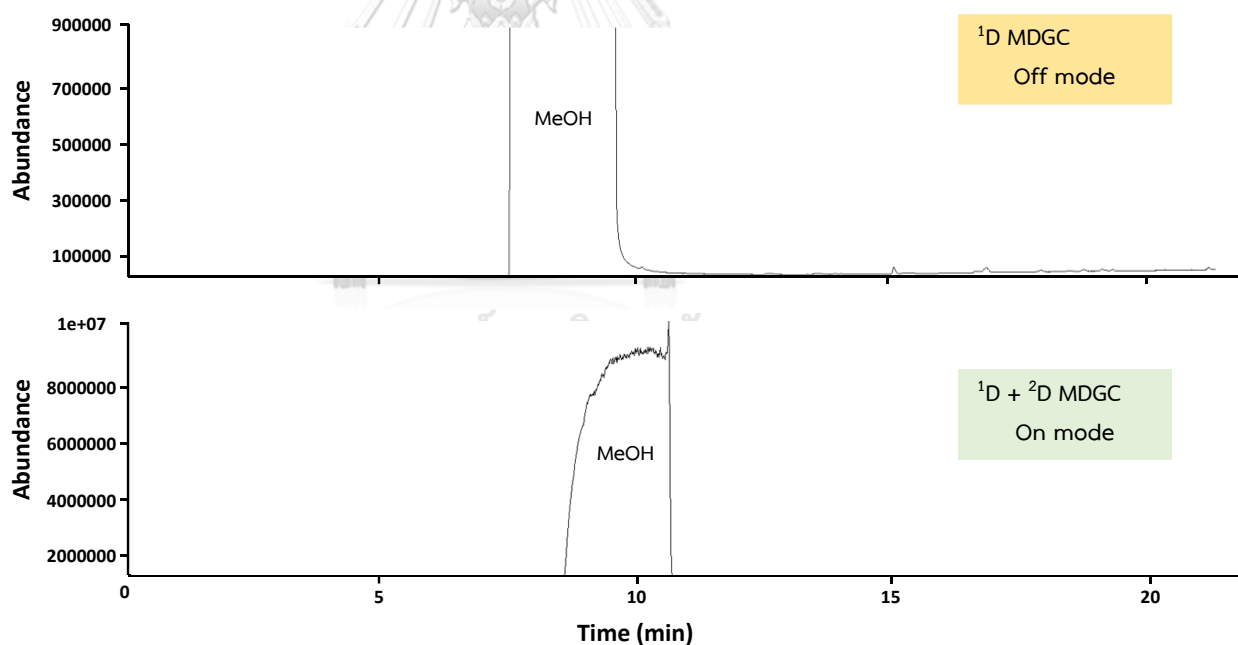
**Figure 11.** The 1D-GC-single MS total ion chromatogram (DB-1MS) of a propylene oxide sample.

#### 4.2 The developed comprehensive heart-cut system with the column set of DB-1MS as <sup>1</sup>D column and DB-WAX as <sup>2</sup>D column

##### 4.2.1 Optimization of <sup>1</sup>D and <sup>2</sup>D column flows

After complete setting up the heart-cut instrument mentioned in Section 3.5, <sup>1</sup>D and <sup>2</sup>D column flows were optimized to prevent leak of effluent. The

first and second experiment were optimized using methanol (MeOH) by applying 1.5 and 2 times of <sup>2</sup>D flow, respectively, with constant <sup>1</sup>D column flow of 2.3 mL/min. MeOH was run all the way in off or on mode. The directions of effluents in each mode will be mentioned in Section 2.2.4. The result of applying 1.5 time of <sup>2</sup>D column flow (3.5 mL/min) is shown in **Figure 12** resulting in leak of MeOH in both off and on modes. This means the insufficient <sup>2</sup>D column flow. This problem is solved by using 2 times of the flow of 4.7 mL/min. The results indicated no effluent leak in both off and on modes as shown in **Figures 13A and 13B**. Therefore, the <sup>1</sup>D and <sup>2</sup>D column flows of 2.3 and 4.7 mL/min showed were selected for further analyses of the propylene oxide sample. The optimized results without compounds leak are shown in **Figures 14A and 14B**, respectively.



**Figure 12.** Optimized results using <sup>1</sup>D column flow of 2.3 mL/min and <sup>2</sup>D column flow of 3.5 mL/min.

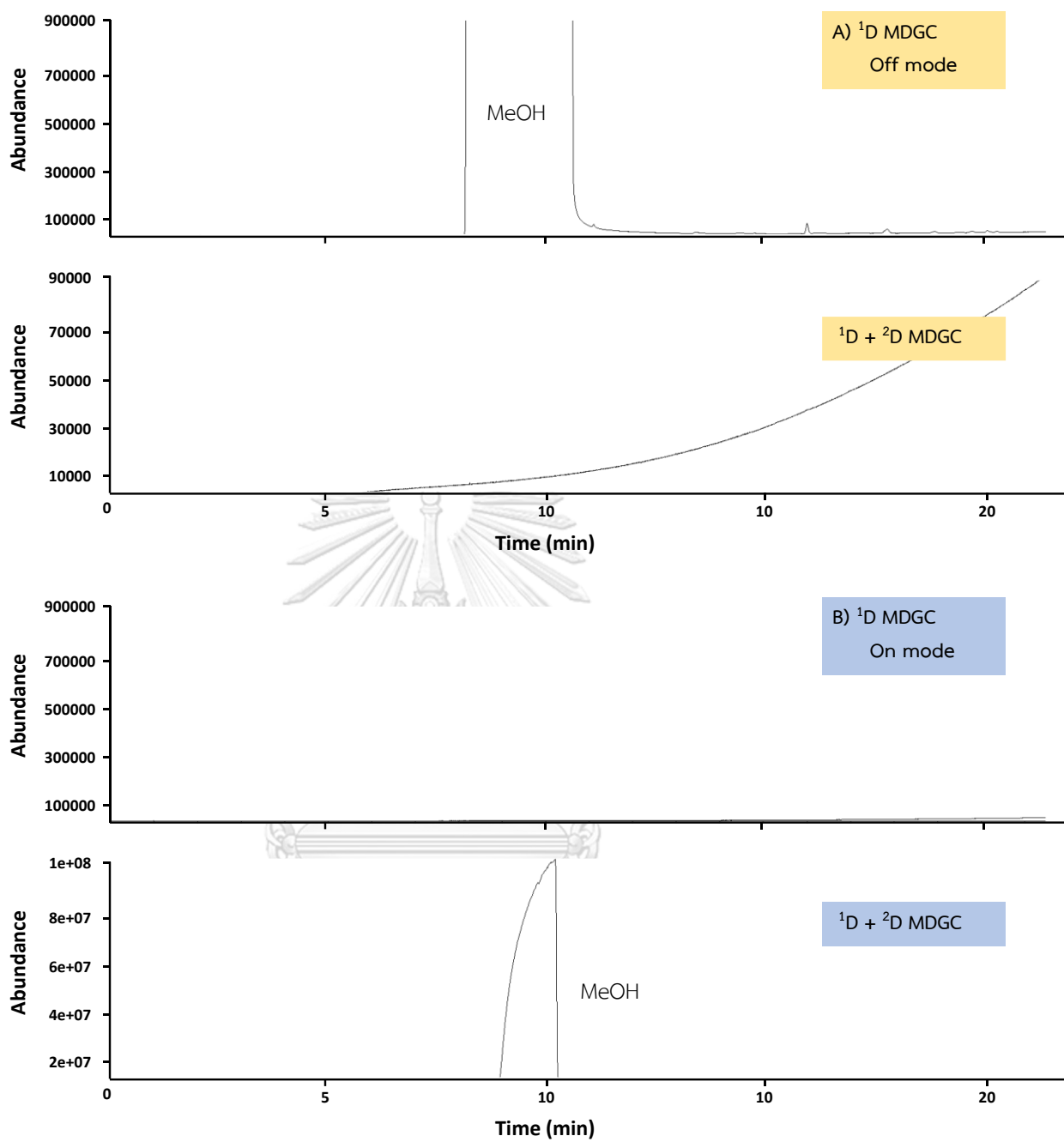


Figure 13. Optimized results using <sup>1</sup>D column flow of 2.3 mL/min and <sup>2</sup>D column flow of 4.7 mL/min: (A) MeOH in off mode and (B) MeOH in on mode.



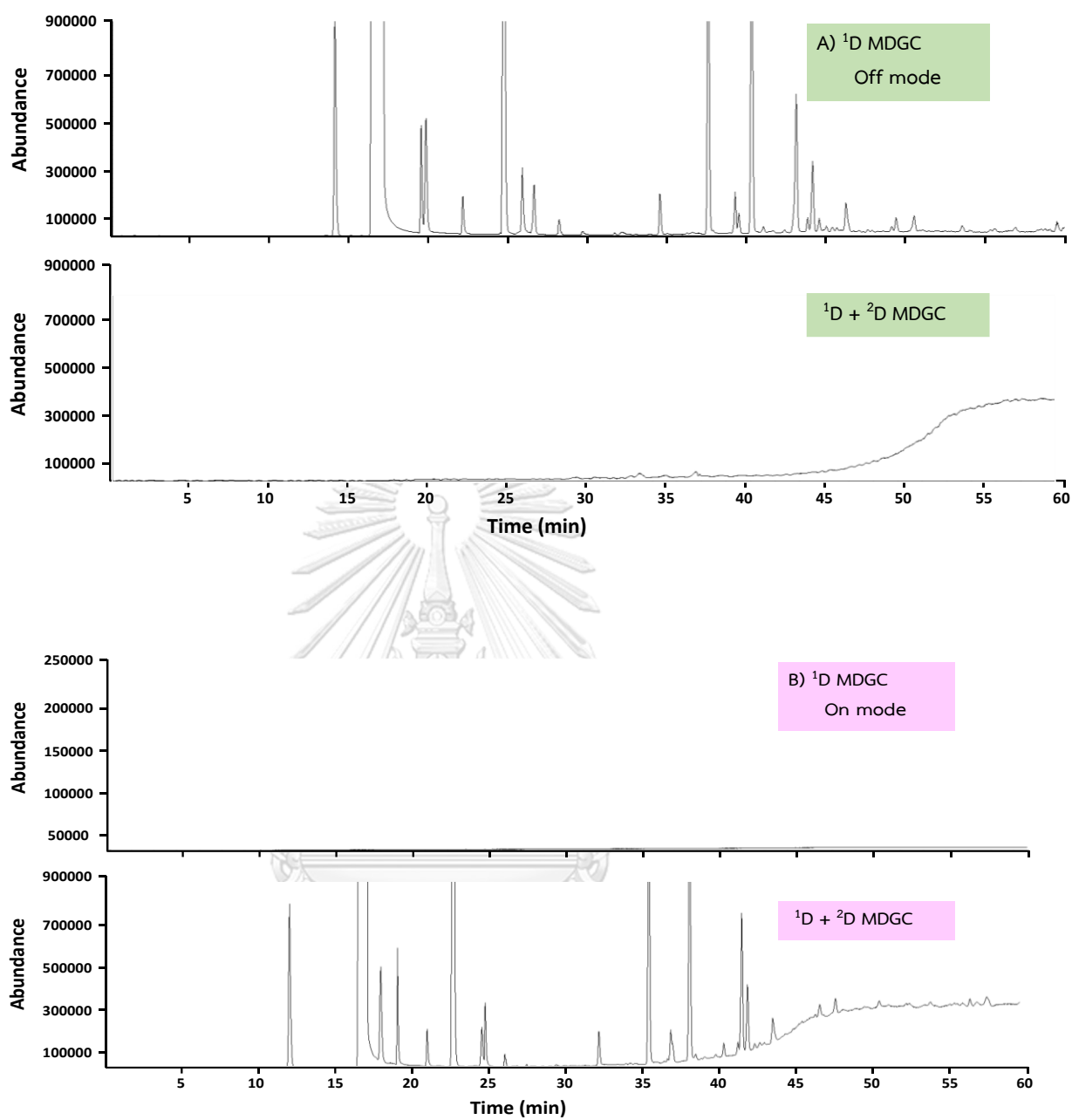
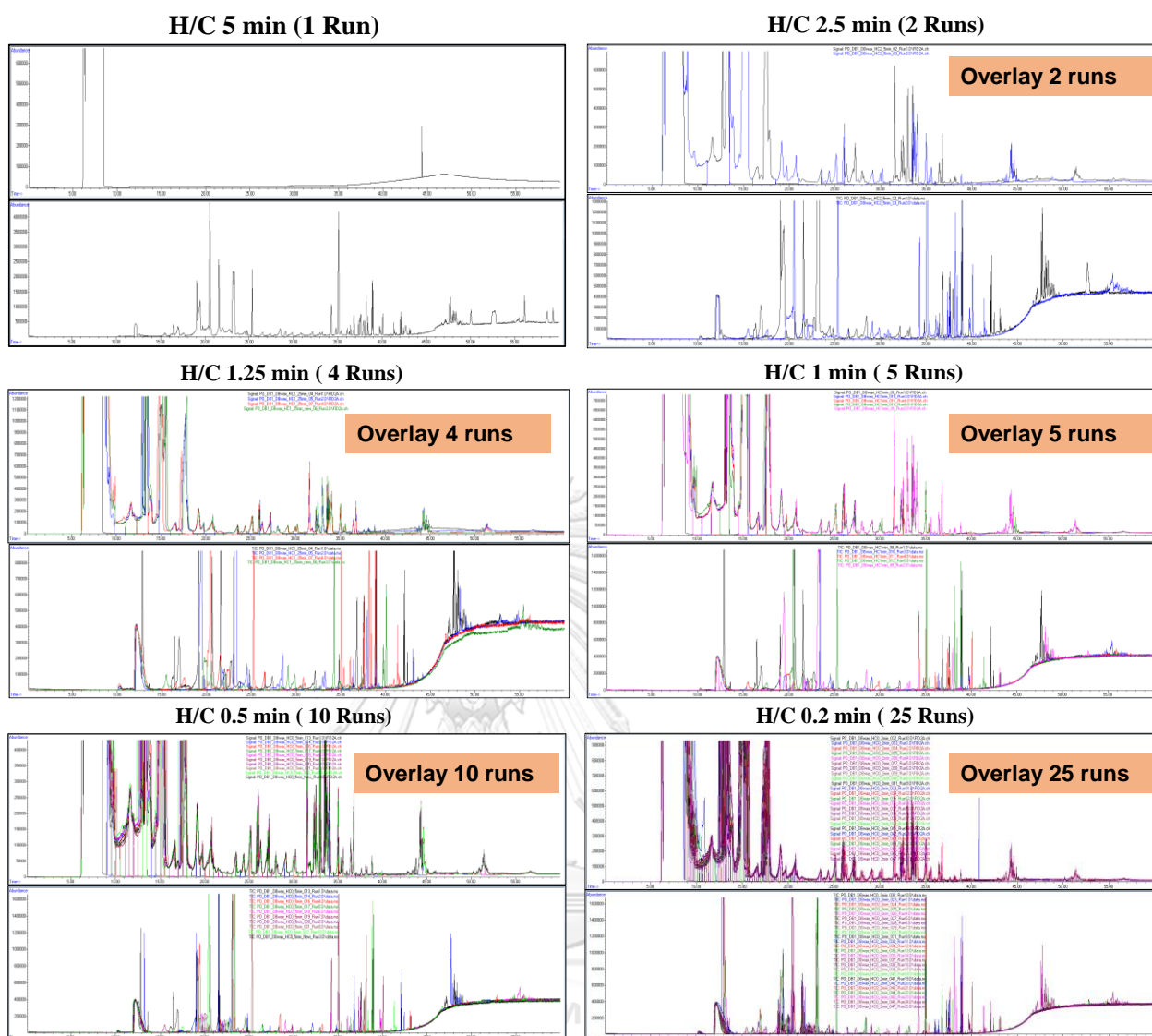


Figure 14. Optimized results using  $^1\text{D}$  column flow of 2.3 mL/min and  $^2\text{D}$  column flow of 3.5 mL/min: (A) propylene oxide sample in off mode and (B) in on mode.

#### 4.2.2 Effects of heart-cut window and analysis time in comprehensive heart-cut analysis

With constant <sup>1</sup>D and <sup>2</sup>D column flows of 2.3 and 4.7 mL/min, comprehensive heart-cut analyses of a propylene oxide sample during <sup>1</sup>D time of 8.5-60 min were performed using different  $t_{H/C}$ . The resulting raw data and contour plots for all the conditions were shown in **Figures 15** and **16**.

The raw data of each heart-cut window indicated the difference of number of experiments in each  $t_{H/C}$  used to qualitative analyze the sample. Then, total raw data using each  $t_{H/C}$  were converted into a contour plot as shown in **Figure 16** by using Fortner Transform 3.3 (Fortner, Inc., Savoy, IL). The contour plot consists of the X and Y-axis which present retention time on <sup>1</sup>D and <sup>2</sup>D columns, respectively. The color code in the graph represents intensities of separated peaks. Ideally, one spot means one separated peak. The contour plots in **Figure 16** showed increasing analysis time from 1 hour to 25 h with reducing  $t_{H/C}$ . Better results were also obtained with the shorter  $t_{H/C}$ , see much greater resolution in the horizontal direction from 5.00 to 0.20 min heart-cut window. Shorter  $t_{H/C}$  directly resulted in smaller  $^1w_{b,ave}$ , thus increasing  $^1n_c$ , see also the trend in **Figure 26**. This also reduced sampling amount (which decreased  $^2w_{b,ave}$ ) as well as reducing coelution in each heart-cut pulse. Thus,  $^2n_c$  slightly increased as shown by the greater resolution in the vertical direction with the narrower heart-cut windows in **Figure 16**. Briefly, use of longer analysis time (shorter  $t_{H/C}$ ) improved total  $n_c$  with the larger number of separated compounds.



**Figure 15.** The raw data obtained from comprehensive heart-cut analysis ( $^1\text{D}$  DB-1MS column as following all top chromatograms and  $^2\text{D}$  DB-WAX column as following all bottom chromatograms) of propylene oxide sample using different  $t_{\text{H/C}}$ .

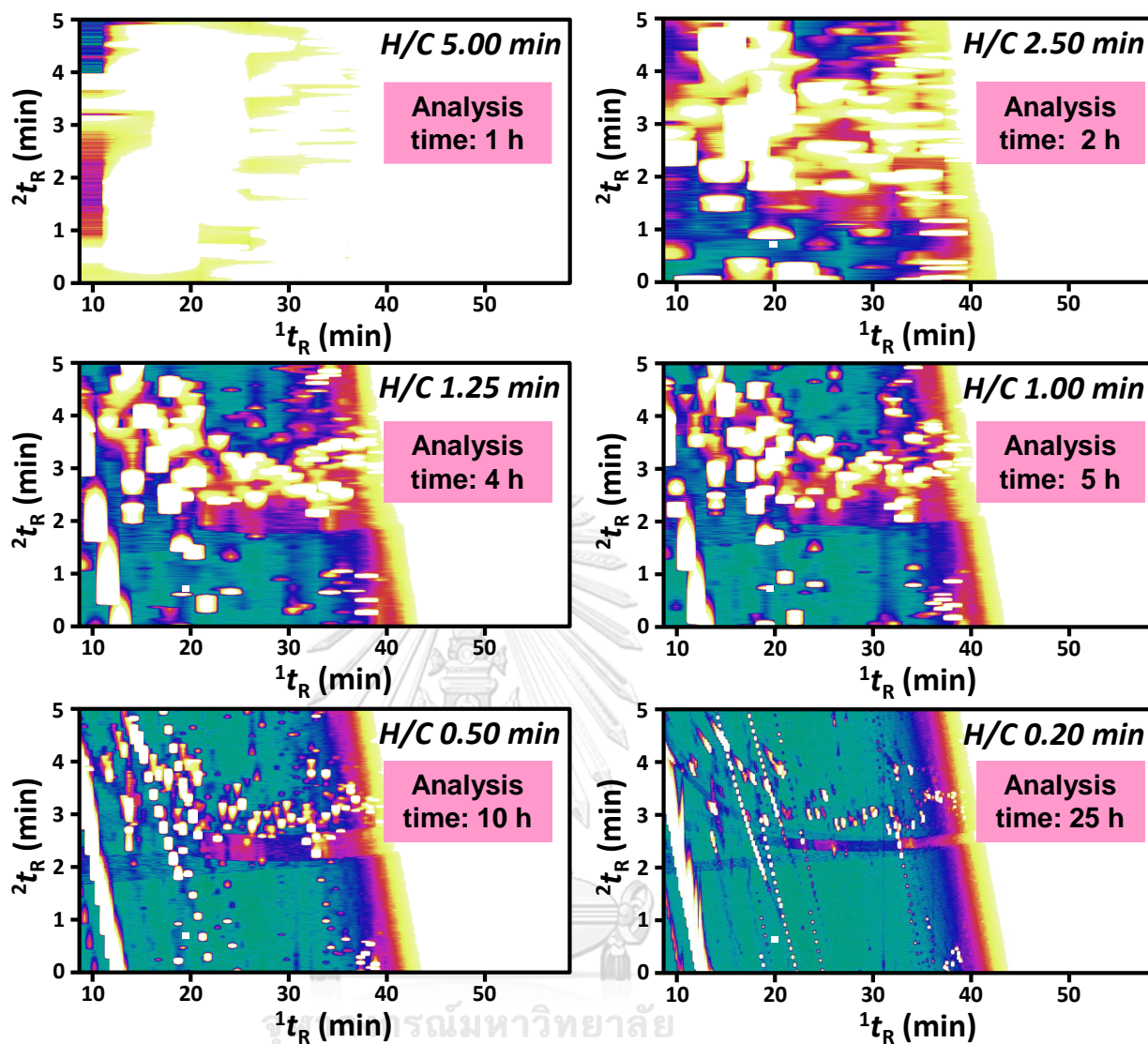


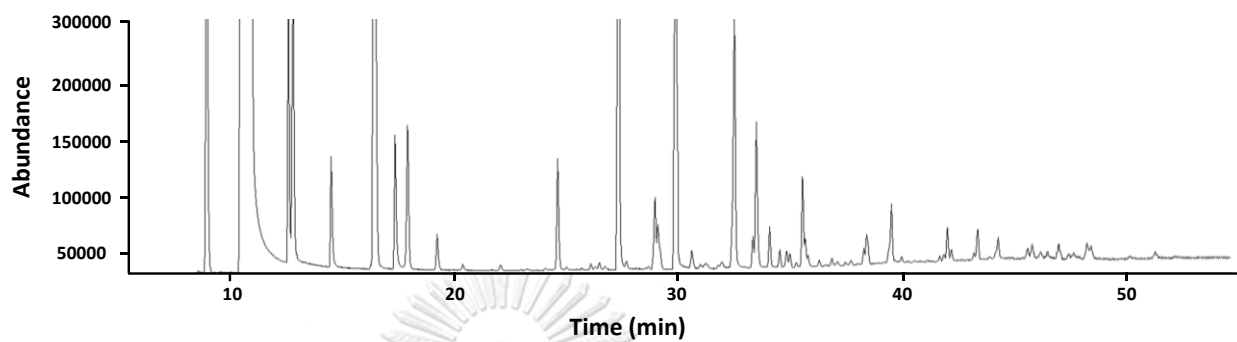
Figure 16. The contour plots obtained from comprehensive heart-cut analysis ( $^1D$  DB-1MS and  $^2D$  DB-WAX columns) of propylene oxide sample using different  $t_{H/C}$ .

#### 4.3 The developed comprehensive heart-cut with the column set of Rtx-200 as <sup>1</sup>D column and DB-WAX as <sup>2</sup>D column

The same optimum condition of comprehensive heart-cut approach was also applied for the column set of a mid-polar as <sup>1</sup>D and polar as <sup>2</sup>D columns, respectively. The same instrumental set up mentioned in **Figure 3** was applied. The 1D-GC-single MS chromatogram of propylene oxide sample separated by Rtx-200 column is shown in **Figure 17**. The results showed that Rtx-200 could separate volatile compounds in the sample better than the DB-1MS system (**Figure 11**) since it may be from the sample mostly consisting of polar compounds. However, several coeluting compounds observed during time of 20-54 minute. Therefore, this range was further studied for comprehensive heart-cut with the same heart-cut window of 5.00, 2.50, 1.50, 1.00, 0.50 and 0.20 min.

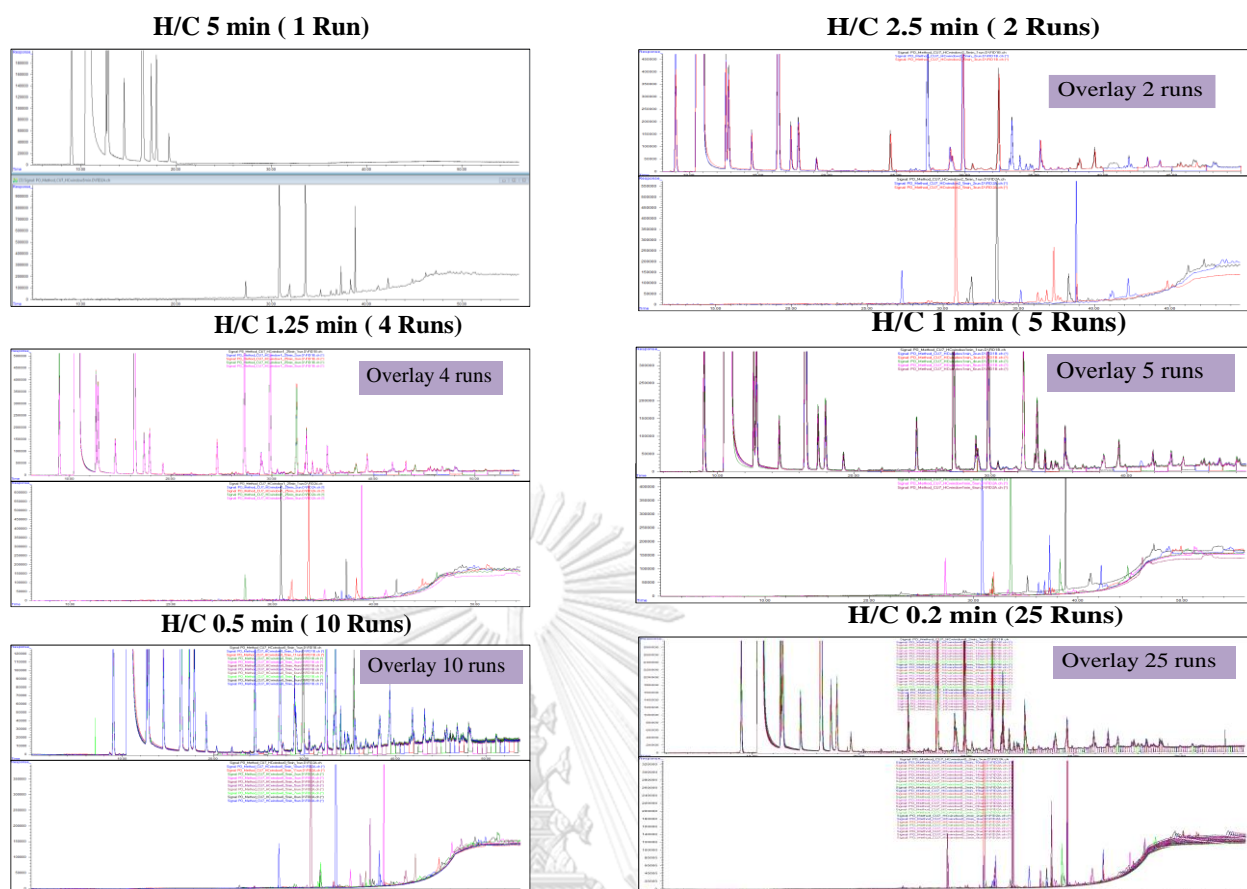
The raw data and contour plots obtained from different heart-cut windows of this column set were presented in **Figure 18** and **Figure 19**, respectively. The narrower heart-cut window of 0.20 min provided the best condition as well as the column set of DB-1MS connected with DB-WAX.

Comparison of the column set of DB-1MS connected with DB-WAX at heart-cut window of 0.20 min is shown in **Figure 19**. Although Rtx-200 provided better <sup>1</sup>D separation (**Figure 20B**), <sup>1</sup>D DB-1MS × <sup>2</sup>D DB-WAX performed greater performance in overall 2D separation and showed larger number of well separated peaks (**Figure 20A**). This can be explained based on the difference in <sup>1</sup>D and <sup>2</sup>D column polarities. Since <sup>1</sup>D non-polar/<sup>2</sup>D polar column polarities were greater difference (higher orthogonality) than that of <sup>1</sup>D midpolar/<sup>2</sup>D polar columns, the former column set performance was thus greater. As a result, the column set of <sup>1</sup>D DB-1MS connected with <sup>2</sup>D DB-WAX applying  $t_{H/C}$  of 0.20 min displayed the proper condition for further untargeted identification in the propylene oxide sample.



**Figure 17.** The 1D-GC-single MS total ion chromatogram (Rtx-200) of propylene oxide sample.





**Figure 18.** The raw data of obtained from comprehensive heart-cut analysis ( $^1\text{D}$  RtX-200 column as following all top chromatograms and  $^2\text{D}$  DB-WAX column as following all bottom chromatograms) of propylene oxide sample using different  $t_{\text{H/C}}$ .

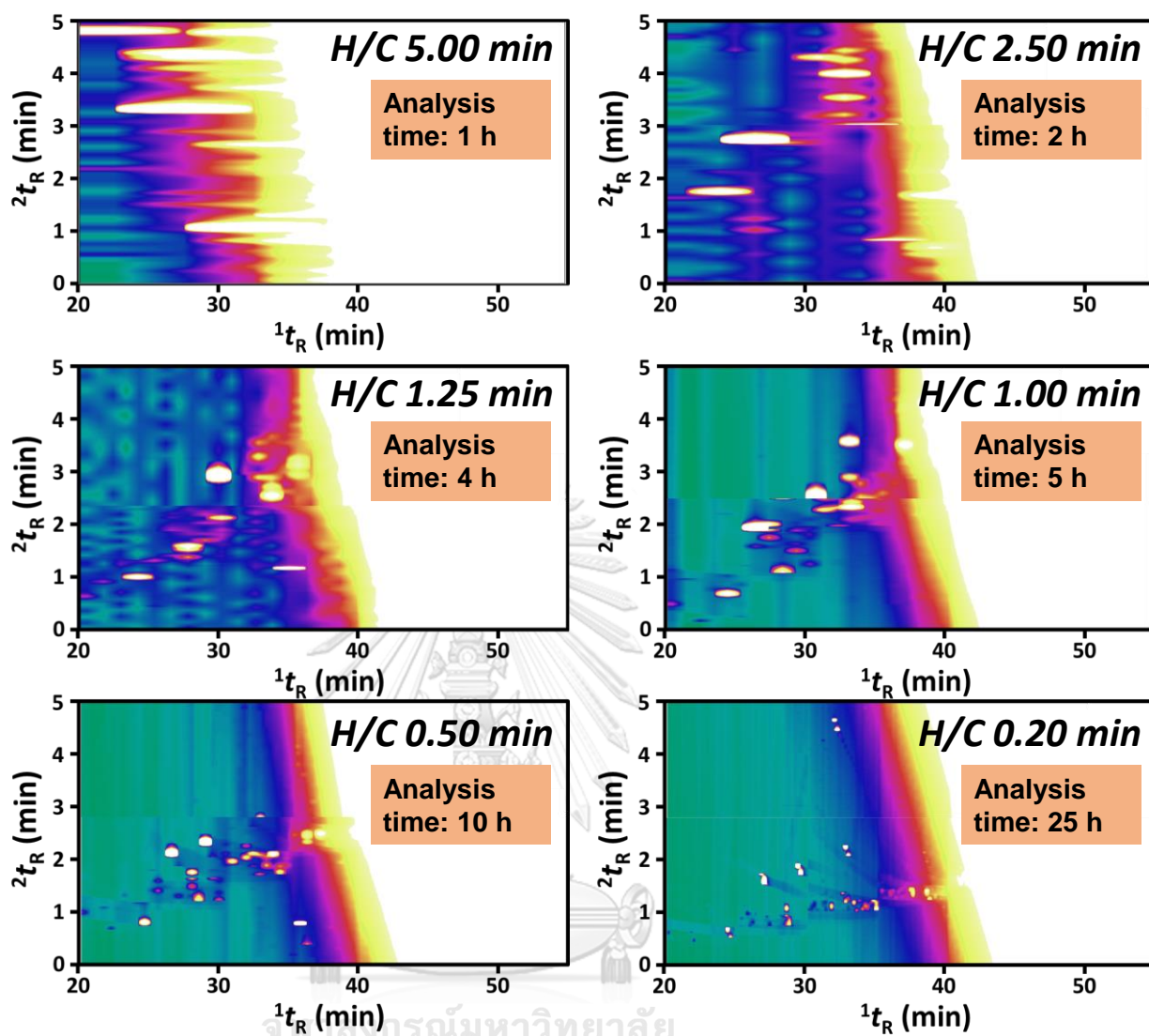
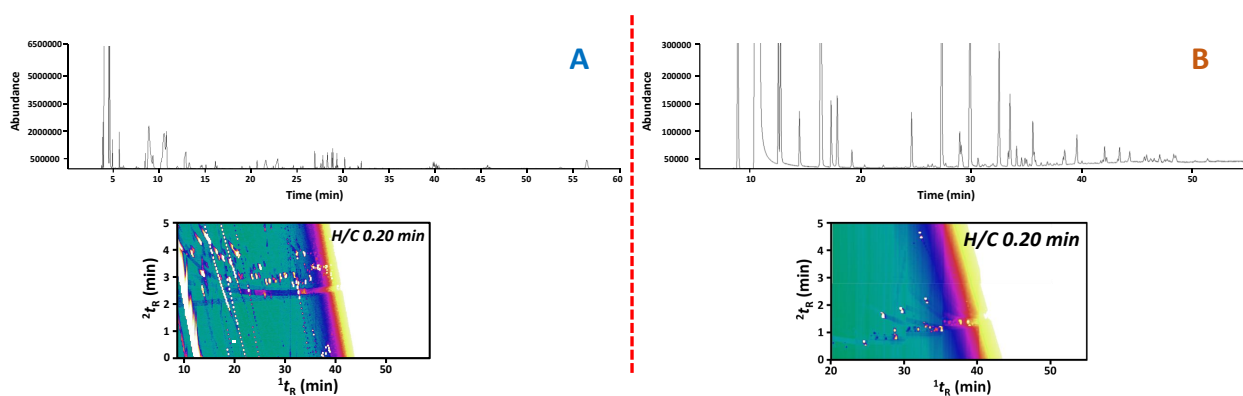


Figure 19. The contour plots obtained from comprehensive heart-cut analysis ( $^1\text{D}$  Rtx-200 and  $^2\text{D}$  DB-WAX columns) of propylene oxide sample using different  $t_{\text{H/C}}$ .





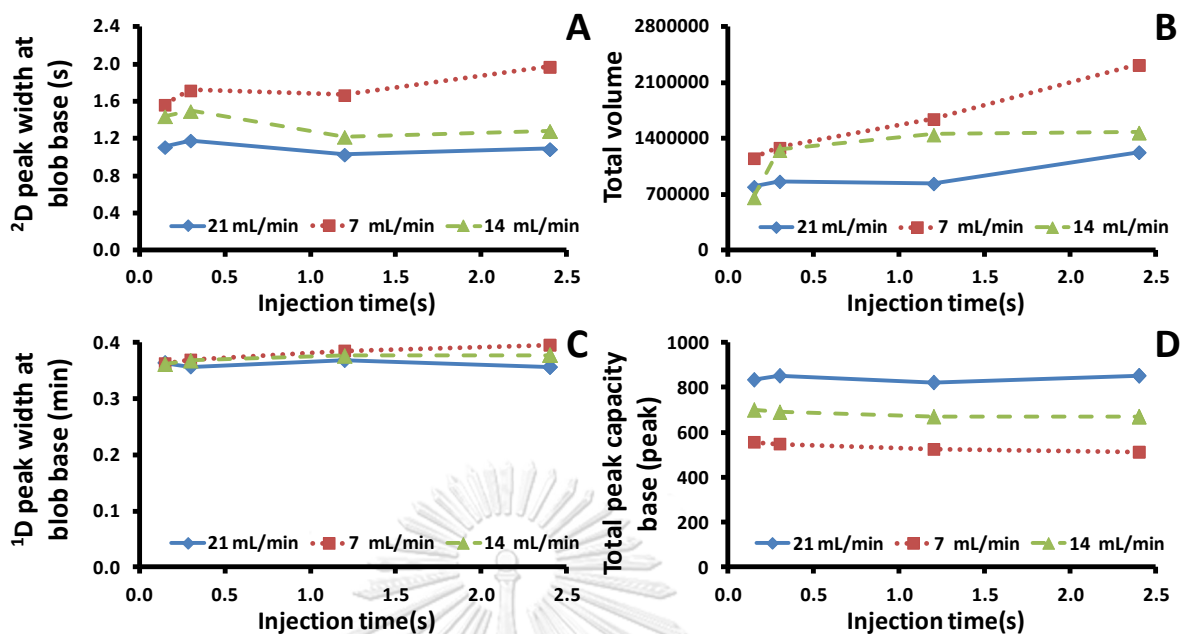
**Figure 20.** Comparison the results obtained from 1D-GC-single MS and comprehensive heart-cut analyses of propylene oxide sample using  $t_{H/C}$  of 0.20 min with different column set: (A) 1D-GC-single MS: DB-1MS as shown in top chromatogram and comprehensive heart-cut:  $^1D$  DB-1MS and  $^2D$  DB-WAX columns as shown in bottom contour plot (B) 1D-GC-single MS: Rtx-200 as shown in top chromatogram and comprehensive heart-cut:  $^1D$  Rtx-200 and  $^2D$  DB-WAX columns. as shown in bottom contour plot.

#### 4.4 The conventional GCxGC approach

In this study, the conventional GCxGC system using a long <sup>1</sup>D nonpolar column (60 m) and a short <sup>2</sup>D polar column (5 m) connected with a flow modulator was optimized. The parameters of <sup>2</sup>D column flow and injection time were studied for investigation the proper condition.

##### 4.4.1 Effect of <sup>2</sup>D column flow

With a constant <sup>1</sup>D column flow of 0.8 mL/min, different injection time and <sup>2</sup>D column flow were investigated in this study. The corresponding GCxGC results were evaluated according to  $n_c$  (related to average peak width), total peak area (indicating greater peak focusing effect during the modulation) and the number of identified compounds as shown in **Figure 21**. Change of modulator injection time and <sup>2</sup>D flow in GCxGC significantly affected  ${}^2w_{b,ave}$  and total intensity (see <sup>2</sup>D width at blob base (pixel based approach for identification of peak from a contour plot (<http://www.gcimage.com/gcxgc/usersguide/statistics.pdf>) and total volume data in **Figure 21A** and **Figure 21B**); whilst,  ${}^1w_{b,ave}$  slightly varied as shown by similar <sup>1</sup>D width at blob base values plotted in **Figure 21C**. The <sup>1</sup>D and <sup>2</sup>D peak widths at blob bases led to values of total peak capacity with the smallest peak width of <sup>1</sup>D and <sup>2</sup>D separation providing the highest value of total peak capacity as as shown in **Figure 21D**. Therefore, the <sup>2</sup>D column flow of 21 mL/min was chosen for optimization of injection time.

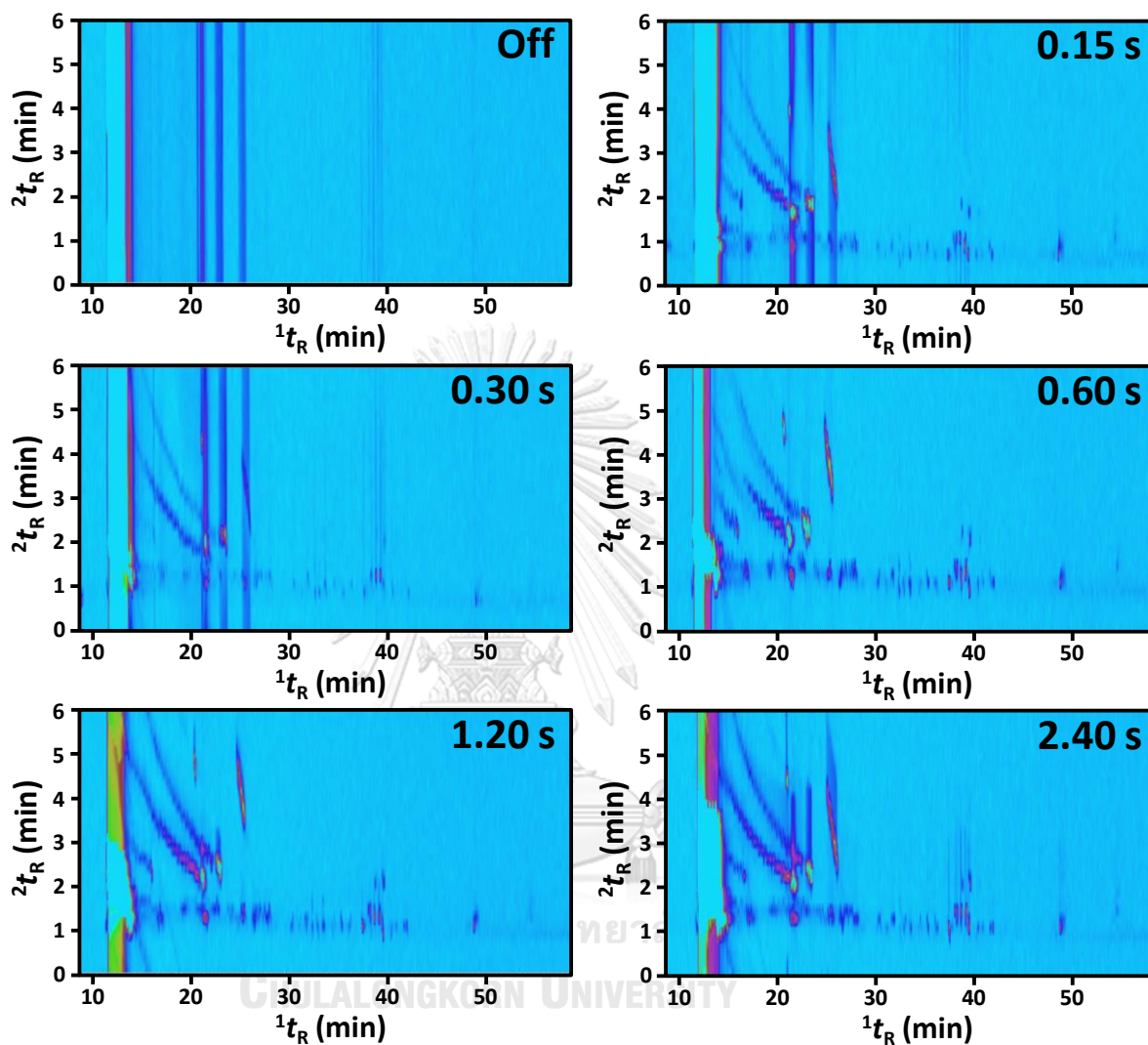


**Figure 21.** Effects of modulator injection time on separation performance using different  $^2\text{D}$  column flows: 21, 14 and 7 mL/min ( $\blacklozenge$ ,  $\blacktriangle$  and  $\blacksquare$ , respectively): (A)  $^2\text{D}$  width at blob base (B) total volume (C)  $^1\text{D}$  width at blob base (D) Total peak capacity base.

#### 4.4.2 Effect of injection time

With a constant  $^2\text{D}$  flow of 21 mL/min, different modulator injection time was applied. The results are shown in **Figure 22**. Injection time is period that a pulse from the end of  $^1\text{D}$  column is filled into the channel inside the modulator prior to injection onto  $^2\text{D}$  column. This is a critical parameter in GCXGC which could cause peak dispersion or breakthrough during the modulation process. With the studied conditions, too short injection time (0.15 s) could cause peak fronting as shown by the downward plateau of the peaks located between 20-30 min  $^1t_R$  in **Figure 22**; whilst, too long injection time (2.40 s) led to peak tailing (e.g. see the upward plateau of the peaks located between 20-30 min  $^1t_R$  in **Figure 22**). An effective injection time was selected to be 0.60 s due to reducing effects of peak broadening as shown by

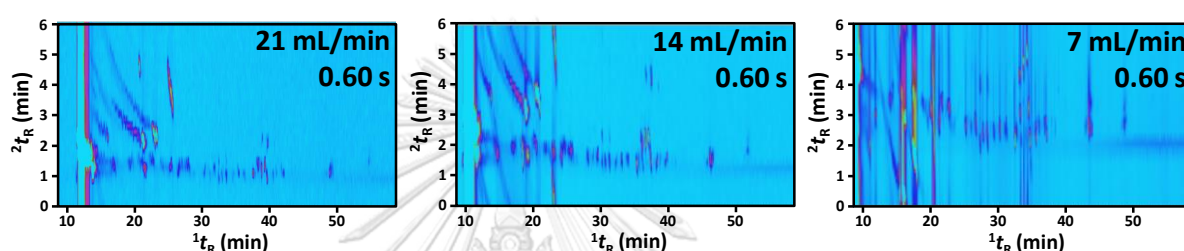
the minimum <sup>2</sup>D width at blob base ( ${}^2w_{b,ave}$ ) with the <sup>2</sup>D flows of 21 and 14 mL/min in Figure 21A.



**Figure 22.** GCXGC results obtained by using different injection time (0.15-2.40 s) using a constant <sup>1</sup>D and <sup>2</sup>D column flows of 0.8 and 21 mL/min, respectively.

It should be noted that a suitable condition cannot be only that resulting in the best performance, e.g., with the highest  $n_c$  at 21 mL/min of <sup>2</sup>D flow and 0.60 s injection time (Figure 21D) or highest total volume (total intensity) at 7 mL/min of <sup>2</sup>D flow and 0.60 s injection time (Figure 21D). Other factors need to be taken into account. Use of high pressure at the modulator is required for effective modulation process (e.g., well focused peaks or prevention of leakage), which resulted in high <sup>2</sup>D

flow. However, low flow is required to preserve lifetime of MS vacuum pump and improved sensitivity, as well as providing effective flow of 20-40 cm/s with He as carrier gas. The  $^2D$  flow should thus be decreased. However, too low  $^2D$  flow also causes ineffective modulation process, *e.g.*, further resulting in weak focusing effect or peak splitting, and peak broadening. With a constant injection time of 0.60 s, different  $^2D$  flow was applied with the results shown in **Figure 23**.



**Figure 23.** GCXGC results obtained by using different  $^2D$  column flow (21, 14 and 7 mL/min) using a constant  $^1D$  column flow and injection time of 0.8 mL/min and 0.60 s, respectively.

The result showed improved separation (also with broader peaks) at lower flow due to the increasing void time. However, modulation performance decreased at the lower  $^2D$  column flow as can be seen with the significantly broader peak width in  $^2D$  separation, see the larger  $^2D$  width at blob base ( $^2w_{b,ave}$ ) by using  $^2D$  flow of 7 mL/min in **Figure 21A**, as well as the split peaks (*e.g.* that after 40 min) by this flow in **Figure 23**. Based on the improved separation performance with significantly high intensity and low  $^2D$  flow, 14 mL/min of  $^2D$  flow and 0.60 s of injection time were suggested.

#### 4.5 The conventional GCxGC approach with the column set of Rtx-200 and HP-INNOWAX

The suitable condition of GCxGC technique was also applied to analyze volatile compounds in the propylene oxide sample using the column set of <sup>1</sup>D mid-polar and <sup>2</sup>D polar column. The result presented in contour plot as following **Figure 24**. Several tailing peaks were observed since the efficiency of injection time (in this case 0.60 s) might not enough to flush total effluents inside collection channel going to the <sup>2</sup>D column because of overfilled of effluent. Therefore, some compounds still remain inside collection channel and it led to tailing peak shape.

Comparison the results of separated compounds with the column set of <sup>1</sup>D DB-1MS and <sup>2</sup>D HP-INNOWAX presented in **Figure 25**. The results showed that the <sup>2</sup>D separation (vertical direction) of this column set provided better separated compounds (**Figure 25A**) than the column set of <sup>1</sup>D Rtx-200 and <sup>2</sup>D HP-INNOWAX (**Figure 25B**) as well as the results obtained from comprehensive heart-cut approach as shown in **Figure 20A**. Therefore, the column set of <sup>1</sup>D DB-1MS/<sup>2</sup>D HP-INNOWAX with low <sup>2</sup>D flow of 14 mL/min and 0.60 s of injection time were selected for further analysis with compound identification.

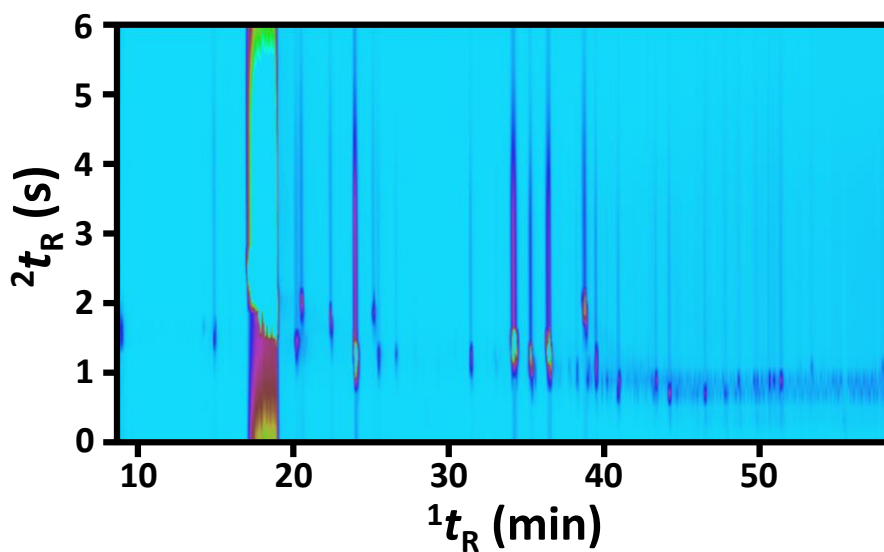


Figure 24. The contour plot of propylene oxide sample obtained from GCxGC approach with the column set of  $^1D$  Rtx-200  $\times$   $^2D$  HP-INNOWAX.

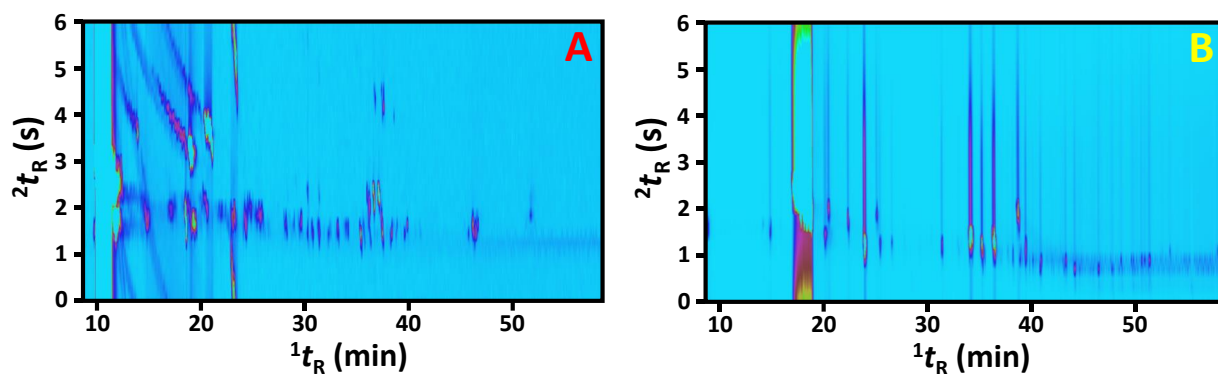


Figure 25. Comparison of the results of GCxGC approach in propylene oxide sample: (A) the column set of  $^1D$  DB-1MS  $\times$   $^2D$  HP-INNOWAX (B) the column set of Rtx-200 column connected with HP-INNOWAX).

#### 4.6 Comparison of analysis performances in developed comprehensive heart-cut and conventional GCxGC

The separation performances of the developed comprehensive heart-cut of the column set of DB-1MS and DB-WAX and conventional GCxGC with the column set of DB-1MS and HP-INNOWAX were chosen to evaluate peak capacity and number of separated peaks in the propylene oxide sample. The results were plotted as shown in **Figure 26**. The plots (●) presented the performance of comprehensive heart-cut analysis with different heart-cut windows. The corresponding performance of the selected GCxGC analysis is also shown by the data (×).

According to **Figure 26A**, total peak capacity obtained from comprehensive heart-cut and GCxGC approach was compared. For comprehensive heart-cut analysis, reducing heart-cut window gradually increased total peak capacity. The highest value was performed by using heart-cut window of 0.20 min around 9198. Compared with GCxGC system, it presented total peak capacity of 798. Its value was lower than comprehensive heart-cut with heart-cut window of 1.25, 1.00, 0.50 and 0.20 min. It should be noted that comprehensive heart-cut analysis with the smallest heart-cut window (0.20 min) can improve total peak capacity around 12 times improvement using heart-cut window of 0.20 min since a narrower H/C window decreased  $^1w_b$  and  $^2w_b$  of peaks as shown in **Figures 26B** and **26C** and it also increased total peak capacity value.

In **Figure 26B**, the  $^1n_c$  was calculated from  $^1w_b$  of separated peaks in the horizontal direction. The widest heart-cut window of 5.00 min showed the lowest value ( $^1n_c$  of 11) because it affected the widest heart-cut window. However, the value of  $^1n_c$  was continuously improved by applying the narrower heart-cut window which the highest  $^1n_c$  of comprehensive heart-cut presented at the shortest heart-cut window of 0.20 min. However, GCxGC analysis provided the highest value ( $^1n_c$  of 133) since GCxGC applied  $P_M$  of 0.10 min with  $P_M < t_{H/C}$ . To this end,  $^1w_b$  of GCxGC can perform lower than comprehensive heart-cut. Nevertheless, the  $^1n_c$  of



comprehensive heart-cut could be further improved to be greater than GCxGC by use of  $t_{H/C} < P_M$ .

The  ${}^2n_c$  was calculated from  ${}^2w_b$  of separated peaks in the vertical direction. Although the  ${}^1w_b$  value of GCxGC provided better than comprehensive heart-cut, a short  ${}^2D$  column (in this case only 5 m) led to loss of separation space. Therefore,  ${}^2n_c$  slightly increased as following **Figure 26C** in comprehensive heart-cut due to applying a longer  ${}^2D$  separation space (60 m).

Numbers of separated compounds obtained from these 2 techniques are shown in **Figure 26D**. The results showed that reducing heart-cut window can improve number of separated compounds. The shortest  $t_{H/C}$  of 0.20 min presented the greatest value of 107 peaks compared with 61 peaks for GCxGC with  $\sim 2$  times improvement.

The effect of the shortest  $t_{H/C}$  and a long  ${}^2D$  column in comprehensive heart-cut led to increasing analysis time from 1 h ( $t_{H/C}$  of 5.00 min) to 25 h ( $t_{H/C}$  of 0.20 min), **Figure 26E**. Furthermore, increasing analysis time also increased number of separated compounds as shown by the trend in **Figure 26F** corresponding to the theoretical trend simulated in the previous study [27]. To this end, the shortest  $t_{H/C}$  of 0.20 min provided the best condition in comprehensive heart-cut for further identification of untargeted compounds in the propylene oxide sample since it showed the highest number of separated compounds and total peak capacity with long analysis time of 25 h.

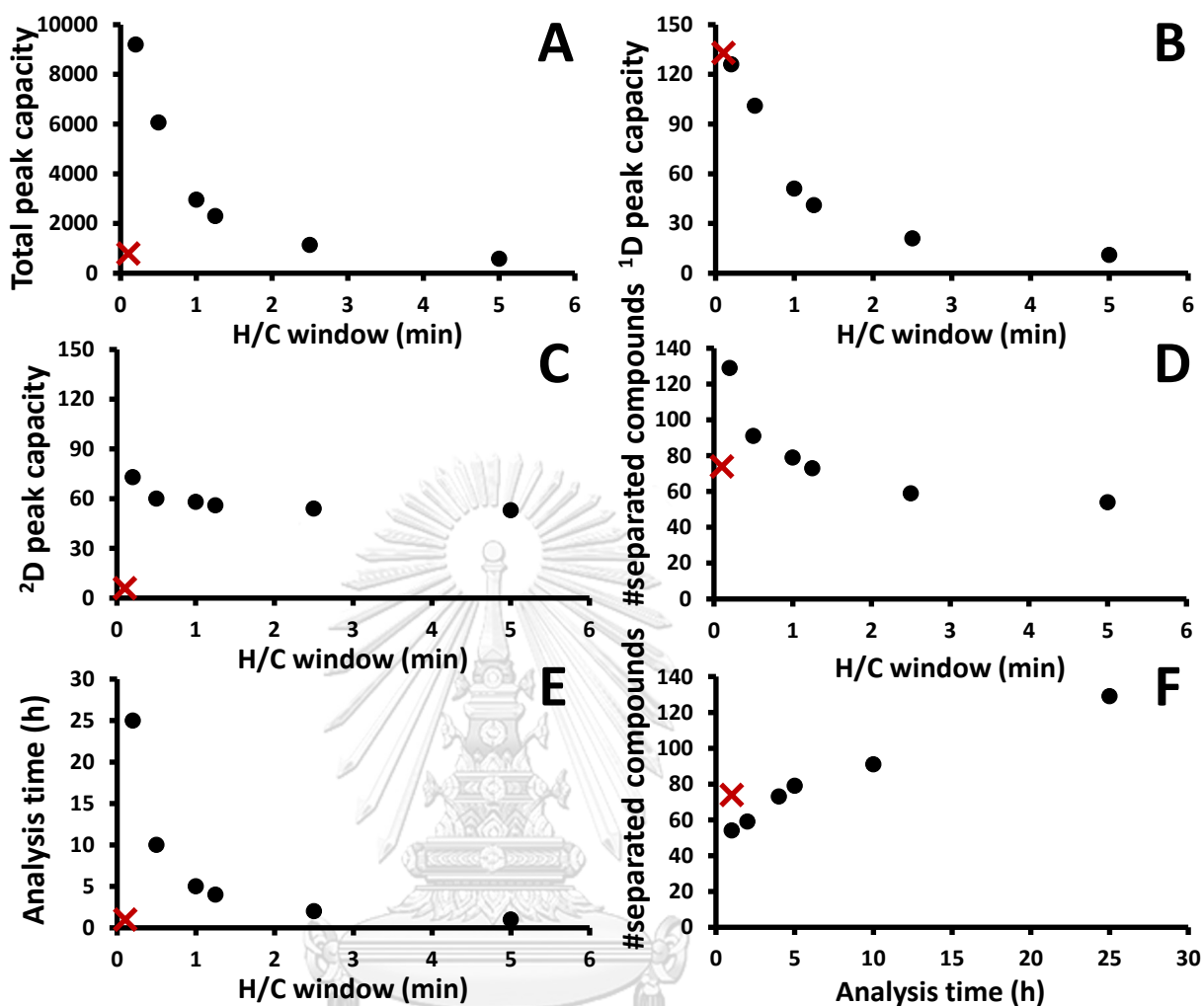


Figure 26. Effects of H/C window ( $t_{H/C}$ ) on separation performance in comprehensive heart-cut analyses (●):  $^1n_C$ ,  $^2n_C$ , total  $n_C$ , number of identified compounds and analysis time (A-E, respectively), using the same  $^1D$  and  $^2D$  column flows of 2.3 and 4.7 mL/min, respectively. The number of tentatively identified compounds vs analysis time plot is also shown in F. The corresponding data for GCXGC analysis are shown by X, and it can also be said that the H/C window in GCXGC is  $P_M$ .

#### 4.7 Tentative volatile compounds in propylene oxide sample obtained from developed comprehensive heart-cut and conventional GCxGC.

The suitable conditions of comprehensive heart-cut ( $t_{H/C}$  of 0.20 min) and conventional GCxGC with the flow modulator under the same temperature program applied in the propylene oxide sample. The results as contour plot were shown in **Figure 27**. It should be noted that comprehensive heart-cut analysis clearly showed much greater performance in <sup>2</sup>D separation (**Figure 27A**) with a larger number of well separated compounds compared to that obtained from conventional GCxGC (**Figure 27B**).

Compounds were tentatively identified by MS library (match score > 650), calculation of  $I$  with a difference of 30 units between the calculated retention index ( $I$ ) and the  $I$  data from the literature for the same (or a similar) stationary phase and injection of authentic standard compound. The corresponding compounds were summarized in **Table 1**. Since it is a proprietary sample, only the compounds classes are listed in the table.

The numbers of focused compounds that were successfully identified were 27 and 38 compounds in GCxGC and comprehensive heart-cut, respectively. Volatile compounds in propylene oxide sample mainly consisted of alcohol, aldehyde, ether, ester, ketone, cyclic ether, oxygenate, and glycol classes. All compounds were the impurities in the propylene oxide sample. Total identified compounds listed in **Table 1** were converted into Venn diagram as shown in **Figure 28** and illustrated that all the compounds identified by GCxGC were the subset of that obtained with the developed comprehensive heart-cut. The 11 extra compounds (compound no. 3, 6, 9, 11, 14, 17, 19, 20, 22, 26, and 27) were solely detected and identified by comprehensive heart-cut approach with undetectable signal in GCxGC system.

In addition, higher confidence in the analysis was obtained as illustrated by the improved average MS match scores from  $887\pm 35$  with GCxGC to  $898\pm 24$  with comprehensive heart-cut analysis for the set of compounds identified by both techniques. The greater separation performance was obtained in the latter approach.

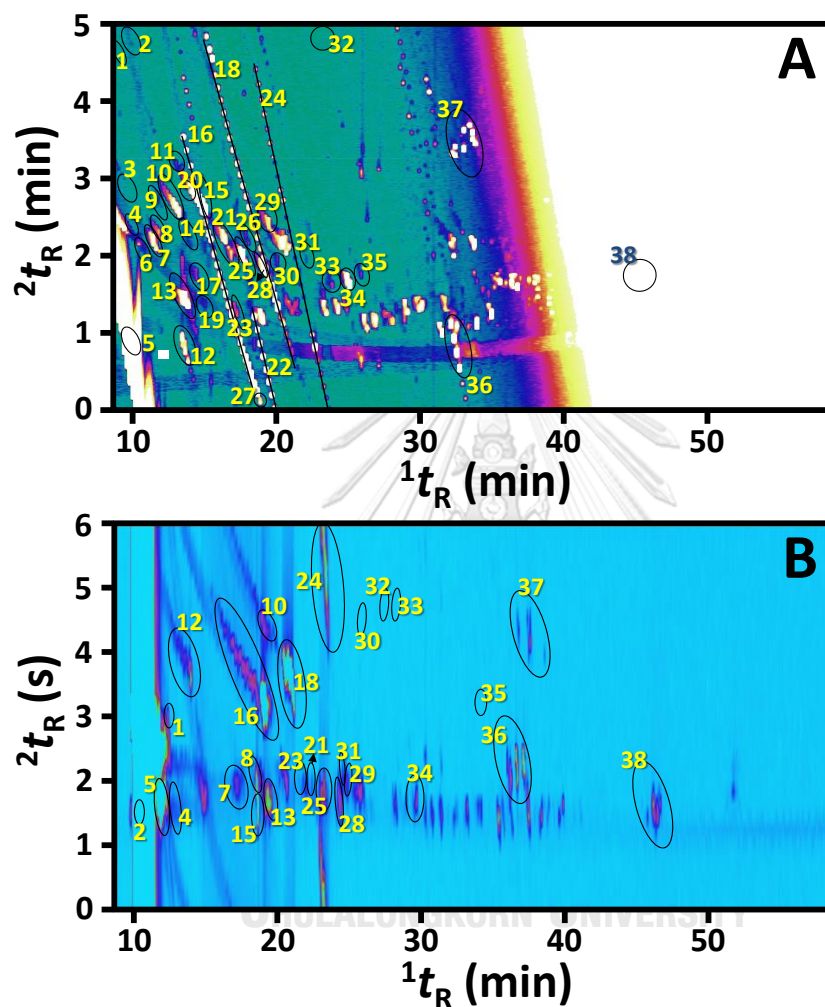


Figure 27. Comparison between the selected results in: (A) comprehensive heart-cut and (B) GCxGC analyses used for compound identification in Table 1.

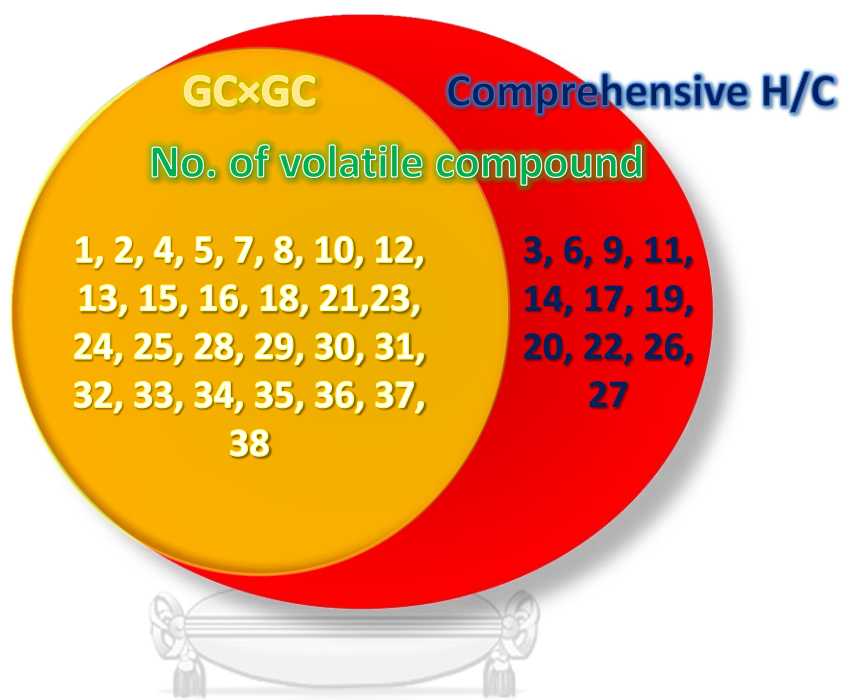
**Table 1.** Volatile compound profile in propylene oxide sample obtained using different methods.

No.	Compound	GCxGC		comprehensive heart-cut		$\Delta I$
		$I_{(Expriment)}$	Match score	$I_{(Expriment)}$	Match score	
1	Alcohol 1*	<600	940	<600	922	Not available
2	Alcohol 2*	<600	879	<600	941	Not available
3	Aldehyde 1*			<600	866	Not available
4	Aldehyde 2*	<600	831	<600	841	Not available
5	Ether 1*	<600	949	<600	934	Not available
6	Ester 1			604	830	$\pm 13$
7	Aldehyde 3	618	937	621	933	$\pm 12$
8	Ketone 1	659	844	616	853	$\pm 25$
9	Cyclic ether 1*			629	748	$\pm 4$
10	Ketone 2	657	926	637	773	$\pm 30$
11	Alcohol 4*			<600	857	Not available
12	Alcohol 3*	<600	914	<600	908	Not available
13	Alkane 1*	670	933	667	922	$\pm 17$
14	Ketone 3			667	819	$\pm 8$
15	Ketone 4*	657	853	646	890	0
16	Alcohol 5*	661	937	663	933	$\pm 8$
17	Ether 2*			680	832	$\pm 30$
18	Alcohol 6*	710	935	688	921	$\pm 19$
19	Oxygenate 1			692	828	$\pm 6$
20	Ester 2			696	705	$\pm 1$
21	Ketone 5	722	924	719	925	$\pm 3$
22	Alcohol 7			754	814	$\pm 13$
23	Alkane 2	732	721	729	884	$\pm 4$
24	Alcohol 8*	742	964	732	899	$\pm 14$
25	Aldehyde 4	741	931	741	930	$\pm 2$
26	Aldehyde 5			744	861	$\pm 25$
27	Alcohol 9			750	813	$\pm 6$
28	Ester 3	761	896	763	912	$\pm 1$
29	Ketone 6*	769	927	763	928	$\pm 5$
30	Ether 3*	778	781	766	845	$\pm 12$
31	Ester 4	809	915	807	760	$\pm 1$
32	Alcohol 10	833	787	830	926	$\pm 10$
33	Ester 5	836	882	833	868	$\pm 4$
34	Ester 6	856	947	852	945	$\pm 1$
35	Ester 7	873	707	869	906	$\pm 3$

36	Glycol 1*	996	939	1006	951	±25
37	Glycol 2*	1046	877	1015	912	±2
38	Glycol 3*	1288	873	1284	877	Not available

$$\Delta I = \pm |I_{(\text{Experiment})} - I_{(\text{Literature})}|$$

\*Compound identification was confirmed by injection of authentic standard.



**Figure 28.** The Venn diagram showing the number of tentatively identified compounds in **Table 1** using GCxGC and the comprehensive heart-cut techniques.

#### 4.8 Previous application of comprehensive heart-cut approach

The comprehensive heart-cut with  $t_{HC}$  of 0.20 min has been applied in this research group to analyze volatile compounds in other samples which were petrochemical products derived from palmitic acid oxidation and perfume samples.

The first application was “Cryogen-free comprehensive heart-cut multidimensional gas chromatography using a Deans switch for improved analysis of petrochemical products derived from palmitic acid” [26] is shown in **Figure 29**. Volatile compounds in the sample were obtained from palmitic acid oxidation which was extracted by solid phase microextraction with fiber 50/30 mm divinylbenzene/Carboxen/poly(dimethylsiloxane) at 25 °C for 5 min. The extracted volatile compounds were separated by the developed comprehensive heart-cut approach with the column set of a <sup>1</sup>D semi-standard nonpolar HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a <sup>2</sup>D polar DB-Wax column (60 m × 0.25 mm, i.d. 0.5 μm film thickness), and the result was compared the results with conventional 1DGC-MS technique with the same GC temperature program within 40 to 200 °C (held for 10 min) at a rate of 6 °C/min

The 1D-GC-MS system provided a total peak capacity of 172 and 92 separated compounds. However, these two parameters can improve by a cyclic multiple heart-cut strategy consisting of 150 heart-cut with  $t_{HC}$  of 0.20 min and total analysis time of 15.3 h. The capacity values of 5840 and 714 separated compounds were observed by using the comprehensive heart-cut approach with 34 and 8 times improvement, respectively. Tentative volatile compounds obtained from these two techniques were identified by MS spectra and calculation of  $^1I$  compared with the NIST library. The results showed that 43 and 235 identified compounds obtained from 1DGC-MS and comprehensive heart-cut, respectively. Furthermore, the values of MS match score obtained from 1D-GC-single MS and comprehensive heart-cut were 769±81 and

836 ±88, respectively. It indicated that comprehensive heart-cut provided much higher confidence in untargeted identification than 1DGC approach. The sample mainly consisted of 2-octanone, 1-methylcyclohexanol, 2,3,6-trimethylphenol, 3-phenylpropanol and 2-nonanone, respectively.

The second work was a “Multi-location peak parking approach for calculation of second dimensional retention indices for improved volatile compound identification with cryogen-free comprehensive heart-cut two-dimensional gas chromatography” [38] as shown in **Figure 30**. The calculation of second retention indices ( $^2I$ ) was applied by construction of alkane isovolatility curves to improve compound identification obtained from MS spectra and  $^1I$  value. Isovolatility curves were established by injection of sixteen sets of automated injections of standard series alkane with the least square curve fitting approach and thus applied for  $^2I$  calculation in perfume samples.

In this work, volatile compounds in perfume samples were separated by the column set of a  $^1D$  nonpolar DB-1MS column (60 m × 0.25 mm i.d. × 0.25 μm) and a  $^2D$  polar DB-Wax column (60 m × 0.25 mm i.d. × 0.25 μm) with the GC temperature program of 60 °C, increased to 250 °C with a rate of 4 °C/min and held at this temperature for 12.5 min (split ratio of 20:1) and total analysis time of 25 h to complete comprehensive analysis.

Separated compounds were tentatively identified by using the libraries of mass spectra,  $^1I$  and  $^2I$  calculation with match score of >700, and  $^1I$  and  $^2I$  differences of ±37 and ±44 units, respectively. Peak capacities ( $n_c$ ) of 9198 and 128 separated peaks were obtained with 71 compounds identified according to MS,  $^1I$  and  $^2I$  library match under the established error approximation criteria. Furthermore, relationship between the analysis time and number of separated peaks was proposed based on the set of 84 identifiable compounds. The major volatile compounds consisted of



benzyl ethanoate (12.41% peak area), linalool (9.84%), diethyl phthalate (9.14%), phenylethyl alcohol (6.56%) and citronellol (5.48%), respectively. It should be noted that the calculation of  $^2I$  can help to eliminate inappropriate compounds with higher confidence in untargeted identification.

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**Cryogen-free comprehensive heartcut multidimensional gas chromatography using a Deans switch for improved analysis of petrochemical products derived from palmitic acid oxidation†**

Nilobon Thongdorn-Ae,<sup>a</sup> Thumnoon Nhujak,<sup>b</sup> Pannipa Janta,<sup>b</sup> Atitarn Rueangthawee,<sup>a</sup> Napida Hinchiranan<sup>\*cde</sup> and Chadin Kulsing<sup>†\*b</sup>

**Figure 29.** Application of comprehensive heart-cut with  $t_{HC}$  of 0.20 min for improved analysis of petrochemical products derived from palmitic acid oxidation.

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**A multi-location peak parking approach for calculation of second dimensional retention indices for improved volatile compound identification with cryogen-free comprehensive heart-cut two-dimensional gas chromatography†**

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**Figure 30.** Application of comprehensive heart-cut with  $t_{HC}$  of 0.20 min for improved volatile compound identification in perfume samples.

## CHAPTER V

### CONCLUSION

The 1D-GC-single MS separation of propylene oxide sample was the first experiment to investigate using the two columns of nonpolar DB-1MS and midpolar Rtx-200 capillary columns. The result obtained from these two columns showed more coeluting peaks at any positions in <sup>1</sup>D separation. Therefore, the comprehensive heart-cut and GCxGC experiments were proposed and investigated with the benefit of improved overall separation performance.

Comprehensive heart-cut using solely DS without the use of cryogenic devices was developed to analyze chemical components in propylene oxide sample. The two critical factors affecting the separation performance of the developed comprehensive heart-cut which are column selection of <sup>1</sup>D: DB-1MS (60 m), <sup>2</sup>D: DB-WAX (60 m) and <sup>1</sup>D: Rtx-200 (60 m) and <sup>2</sup>D: DB-WAX (60 m) and sampling time of 5.00, 2.50, 1.25, 1.00, 0.50 and 0.20 min were studied. The suitable sampling time of the smallest heart-cut window of 0.20 min with analysis time of 25 h and column set of <sup>1</sup>D: DB-1MS (60 m) and <sup>2</sup>D: DB-WAX (60 m) was applied to achieve greater resolutions both in <sup>1</sup>D and <sup>2</sup>D directions.

Conventional GCxGC with the flow modulator was used to compare the separation efficiency obtained from the developed comprehensive heart-cut. The main parameters of <sup>2</sup>D column flow, injection time and column combination were investigated. The optimum <sup>2</sup>D column flow of 14 mL/min, injection time of 0.60 s and column set of <sup>1</sup>D: DB-1MS (60 m) and <sup>2</sup>D: HP-INNOWAX (5 m) were selected to provide the best separation efficiency both in <sup>1</sup>D and <sup>2</sup>D separations within analysis time of 1 h.

The separation performance of the developed comprehensive heart-cut and conventional GCxGC was evaluated in terms of peak capacity and number of

separated compounds. The results clearly showed improved separation performance in the comprehensive analysis by use of a longer <sup>2</sup>D column and longer analysis time (mainly improving <sup>2</sup>*n<sub>c</sub>* and <sup>1</sup>*n<sub>c</sub>*, respectively). Interestingly, the trend in **Figure 26F** is matched with the theoretical trend simulated in the previous study [27]. Comprehensive heart-cut approach provided 12 times improved total peak capacity with 2 times of the number of separated compounds.

The volatile compounds in the sample were tentatively identified by a comparison of their MS spectra with those obtained from the NIST14 library as well as experimental and literature one dimension retention index (<sup>1</sup>*I*) data for the same (or a similar) stationary phase. The comprehensive heart-cut operation with a narrower H/C window allows sampling of significantly sharp band of analytes from <sup>1</sup>D separation prior to <sup>2</sup>D separation. Thus, <sup>1</sup>*t<sub>R</sub>* and <sup>1</sup>*I* calculation can be reliable, and use of cryogenic trapping can be avoided. Thus 38 identified compounds were detected in comprehensive heart-cut. Nevertheless only 27 identified compounds were found in GCxGC with the extra 11 compounds only analyzed by comprehensive heart-cut approach. Moreover, the result of average MS match score of CH/C (898±24) is higher than GCxGC (887±35) which means comprehensive heart-cut provides higher reliability of compound identification.

In application, the developed comprehensive heart-cut using a DS with heart-cut window of 0.20 min was also applied to identify volatile compounds in other samples such as petrochemical products derived from palmitic acid and perfumes. The impressive results were obtained in terms of improved peak capacity, increased number of separated compounds and improved compound identification. Therefore, these two applications were successfully published in journal of Analytical Methods [26, 38].

To this end, the developed comprehensive heart-cut could offer higher performance comprehensive analysis by use of a single DS and a long <sup>2</sup>D column without cryogenic devices. A compromise between time and separation performance

could be made with this technique. With the expense of longer analysis time, improved separation performance could be obtained. The established technique is thus simple, useful and applicable for high resolution separation of any volatile samples in the further.



## APPENDICES

## APPENDIX A

**Table S-1.** The valve control programs for different heart-cut events applied in each comprehensive heart-cut analysis.

Heart-cut window 5.00 min	
No. of experiment = $5 \div 5 = 1$ Run	
Start (min) 8.5	On
13.5	On
18.5	On
23.5	On
28.5	On
33.5	On
38.5	On
43.5	On
End (min) 48.5	On

Heart-cut window 2.50 min			
No. of experiment = $5 \div 2.5 = 2$ Runs			
Run 1		Run 2	
Start (min) 8.5	On	11	On
11	Off	13.5	Off
13.5	On	16	On
16	Off	18.5	Off
18.5	On	21	On
21	Off	23.5	Off
23.5	On	26	On
26	Off	28.5	Off
28.5	On	31	On
31	Off	33.5	Off
33.5	On	36	On
36	Off	38.5	Off
38.5	On	41	On
41	Off	43.5	Off
43.5	On	46	On
46	Off	48.5	Off
48.5	On	51	On
End (min) 51	Off	53.5	Off

Heart-cut 1.25 min							
No. of experiment = $5 \div 1.25 = 4$ Runs							
Run 1		Run 2		Run 3		Run 4	
Start (min) 8.5	On	9.75	On	11	On	12.25	On
9.75	Off	11	Off	12.25	Off	13.5	Off
13.5	On	14.75	On	16	On	17.25	On
14.75	Off	16	Off	17.25	Off	18.5	Off
18.5	On	19.75	On	21	On	22.25	On
19.75	Off	21	Off	22.25	Off	23.5	Off
23.5	On	24.75	On	26	On	27.25	On
24.75	Off	26	Off	27.25	Off	28.5	Off
28.5	On	29.75	On	31	On	32.25	On
29.75	Off	31	Off	32.25	Off	33.5	Off
33.5	On	34.75	On	36	On	37.25	On
34.75	Off	36	Off	37.25	Off	38.5	Off
38.5	On	39.75	On	41	On	42.25	On
39.75	Off	41	Off	42.25	Off	43.5	Off
43.5	On	44.75	On	46	On	47.25	On
44.75	Off	46	Off	47.25	Off	48.5	Off
48.5	On	49.75	On	51	On	52.25	On
End (min) 49.75	Off	51	Off	52.25	Off	53.5	Off

Heart-cut window 1.00 min									
No. of experiment = 5÷1 = 5 Runs									
Run 1		Run 2		Run 3		Run 4		Run 5	
Start (min) 8.5	On	9.5	On	10.5	On	11.5	On	12.5	On
9.5	Off	10.5	Off	11.5	Off	12.5	Off	13.5	Off
13.5	On	14.5	On	15.5	On	16.5	On	17.5	On
14.5	Off	15.5	Off	16.5	Off	17.5	Off	18.5	Off
18.5	On	19.5	On	20.5	On	21.5	On	22.5	On
19.5	Off	20.5	Off	21.5	Off	22.5	Off	23.5	Off
23.5	On	24.5	On	25.5	On	26.5	On	27.5	On
24.5	Off	25.5	Off	26.5	Off	27.5	Off	28.5	Off
28.5	On	29.5	On	30.5	On	31.5	On	32.5	On
29.5	Off	30.5	Off	31.5	Off	32.5	Off	33.5	Off
33.5	On	34.5	On	35.5	On	36.5	On	37.5	On
34.5	Off	35.5	Off	36.5	Off	37.5	Off	38.5	Off
38.5	On	39.5	On	40.5	On	41.5	On	42.5	On
39.5	Off	40.5	Off	41.5	Off	42.5	Off	43.5	Off
43.5	On	44.5	On	45.5	On	46.5	On	47.5	On
44.5	Off	45.5	Off	46.5	Off	47.5	Off	48.5	Off
48.5	On	49.5	On	50.5	On	51.5	On	52.5	On
End (min) 49.5	Off	50.5	Off	51.5	Off	52.5	Off	53.5	Off



## Heart-cut window 0.50 min

No. of experiment =  $5 \div 0.5 = 10$  Runs

Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10
Start (min) 8.5	On 9	On 9.5	On 10	On 10.5	On 11	On 11.5	On 12	On 12.5	On 13
9	Off 9.5	Off 10	Off 10.5	Off 11	Off 11.5	Off 12	Off 12.5	Off 13	Off 13.5
13.5	On 14	On 14.5	On 15	On 15.5	On 16	On 16.5	On 17	On 17.5	On 18
14	Off 14.5	Off 15	Off 15.5	Off 16	Off 16.5	Off 17	Off 17.5	Off 18	Off 18.5
18.5	On 19	On 19.5	On 20	On 20.5	On 21	On 21.5	On 22	On 22.5	On 23
19	Off 19.5	Off 20	Off 20.5	Off 21	Off 21.5	Off 22	Off 22.5	Off 23	Off 23.5
23.5	On 24	On 24.5	On 25	On 25.5	On 26	On 26.5	On 27	On 27.5	On 28
24	Off 24.5	Off 25	Off 25.5	Off 26	Off 26.5	Off 27	Off 27.5	Off 28	Off 28.5
28.5	On 29	On 29.5	On 30	On 30.5	On 31	On 31.5	On 32	On 32.5	On 33
29	Off 29.5	Off 30	Off 30.5	Off 31	Off 31.5	Off 32	Off 32.5	Off 33	Off 33.5
33.5	On 34	On 34.5	On 35	On 35.5	On 36	On 36.5	On 37	On 37.5	On 38
34	Off 34.5	Off 35	Off 35.5	Off 36	Off 36.5	Off 37	Off 37.5	Off 38	Off 38.5
38.5	On 39	On 39.5	On 40	On 40.5	On 41	On 41.5	On 42	On 42.5	On 43
39	Off 39.5	Off 40	Off 40.5	Off 41	Off 41.5	Off 42	Off 42.5	Off 43	Off 43.5
43.5	On 44	On 44.5	On 45	On 45.5	On 46	On 46.5	On 47	On 47.5	On 48
44	Off 44.5	Off 45	Off 45.5	Off 46	Off 46.5	Off 47	Off 47.5	Off 48	Off 48.5
48.5	On 49	On 49.5	On 50	On 50.5	On 51	On 51.5	On 52	On 52.5	On 53
End (min) 49	Off 49.5	Off 50	Off 50.5	Off 51	Off 51.5	Off 52	Off 52.5	Off 53	Off 53.5

Heart-cut window 0.20 min

No. of experiment =  $5 \div 0.2 = 25$  Runs

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11	Run 12	Run 13												
Start (min) 8.5	On	8.7	On	8.9	On	9.1	On	9.3	On	9.5	On	9.7	On	9.9	On	10.1	On	10.3	On	10.5	On	10.7	On	10.9	On
8.7	Off	8.9	Off	9.1	Off	9.3	Off	9.5	Off	9.7	Off	9.9	Off	10.1	Off	10.3	Off	10.5	Off	10.7	Off	10.9	Off	11.1	Off
13.5	On	13.7	On	13.9	On	14.1	On	14.3	On	14.5	On	14.7	On	14.9	On	15.1	On	15.3	On	15.5	On	15.7	On	15.9	On
13.7	Off	13.9	Off	14.1	Off	14.3	Off	14.5	Off	14.7	Off	14.9	Off	15.1	Off	15.3	Off	15.5	Off	15.7	Off	15.9	Off	16.1	Off
18.5	On	18.7	On	18.9	On	19.1	On	19.3	On	19.5	On	19.7	On	19.9	On	20.1	On	20.3	On	20.5	On	20.7	On	20.9	On
18.7	Off	18.9	Off	19.1	Off	19.3	Off	19.5	Off	19.7	Off	19.9	Off	20.1	Off	20.3	Off	20.5	Off	20.7	Off	20.9	Off	21.1	Off
23.5	On	23.7	On	23.9	On	24.1	On	24.3	On	24.5	On	24.7	On	24.9	On	25.1	On	25.3	On	25.5	On	25.7	On	25.9	On
23.7	Off	23.9	Off	24.1	Off	24.3	Off	24.5	Off	24.7	Off	24.9	Off	25.1	Off	25.3	Off	25.5	Off	25.7	Off	25.9	Off	26.1	Off
28.5	On	28.7	On	28.9	On	29.1	On	29.3	On	29.5	On	29.7	On	29.9	On	30.1	On	30.3	On	30.5	On	30.7	On	30.9	On
28.7	Off	28.9	Off	29.1	Off	29.3	Off	29.5	Off	29.7	Off	29.9	Off	30.1	Off	30.3	Off	30.5	Off	30.7	Off	30.9	Off	31.1	Off
33.5	On	33.7	On	33.9	On	34.1	On	34.3	On	34.5	On	34.7	On	34.9	On	35.1	On	35.3	On	35.5	On	35.7	On	35.9	On
33.7	Off	33.9	Off	34.1	Off	34.3	Off	34.5	Off	34.7	Off	34.9	Off	35.1	Off	35.3	Off	35.5	Off	35.7	Off	35.9	Off	36.1	Off
38.5	On	38.7	On	38.9	On	39.1	On	39.3	On	39.5	On	39.7	On	39.9	On	40.1	On	40.3	On	40.5	On	40.7	On	40.9	On
38.7	Off	38.9	Off	39.1	Off	39.3	Off	39.5	Off	39.7	Off	39.9	Off	40.1	Off	40.3	Off	40.5	Off	40.7	Off	40.9	Off	41.1	Off
43.5	On	43.7	On	43.9	On	44.1	On	44.3	On	44.5	On	44.7	On	44.9	On	45.1	On	45.3	On	45.5	On	45.7	On	45.9	On
43.7	Off	43.9	Off	44.1	Off	44.3	Off	44.5	Off	44.7	Off	44.9	Off	45.1	Off	45.3	Off	45.5	Off	45.7	Off	45.9	Off	46.1	Off
48.5	On	48.7	On	48.9	On	49.1	On	49.3	On	49.5	On	49.7	On	49.9	On	50.1	On	50.3	On	50.5	On	50.7	On	50.9	On
End (min) 48.7	Off	48.9	Off	49.1	Off	49.3	Off	49.5	Off	49.7	Off	49.9	Off	50.1	Off	50.3	Off	50.5	Off	50.7	Off	50.9	Off	51.1	Off

## Heart-cut window 0.20 min (Continued)

No. of experiment =  $5 \div 0.2 = 25$  Run

	Run 14	Run 15	Run 16	Run 17	Run 18	Run 19	Run 20	Run 21	Run 22	Run 23	Run 24	Run 25											
Start (min) 11.1	On	11.3	On	11.5	On	11.7	On	11.9	On	12.1	On	12.3	On	12.5	On	12.7	On	12.9	On	13.1	On	13.3	On
11.3	Off	11.5	Off	11.7	Off	11.9	Off	12.1	Off	12.3	Off	12.5	Off	12.7	Off	12.9	Off	13.1	Off	13.3	Off	13.5	Off
16.1	On	16.3	On	16.5	On	16.7	On	16.9	On	17.1	On	17.3	On	17.5	On	17.7	On	17.9	On	18.1	On	18.3	On
16.3	Off	16.5	Off	16.7	Off	16.9	Off	17.1	Off	17.3	Off	17.5	Off	17.7	Off	17.9	Off	18.1	Off	18.3	Off	18.5	Off
21.1	On	21.3	On	21.5	On	21.7	On	21.9	On	22.1	On	22.3	On	22.5	On	22.7	On	22.9	On	23.1	On	23.3	On
21.3	Off	21.5	Off	21.7	Off	21.9	Off	22.1	Off	22.3	Off	22.5	Off	22.7	Off	22.9	Off	23.1	Off	23.3	Off	23.5	Off
26.1	On	26.3	On	26.5	On	26.7	On	26.9	On	27.1	On	27.3	On	27.5	On	27.7	On	27.9	On	28.1	On	28.3	On
26.3	Off	26.5	Off	26.7	Off	26.9	Off	27.1	Off	27.3	Off	27.5	Off	27.7	Off	27.9	Off	28.1	Off	28.3	Off	28.5	Off
31.1	On	31.3	On	31.5	On	31.7	On	31.9	On	32.1	On	32.3	On	32.5	On	32.7	On	32.9	On	33.1	On	33.3	On
31.3	Off	31.5	Off	31.7	Off	31.9	Off	32.1	Off	32.3	Off	32.5	Off	32.7	Off	32.9	Off	33.1	Off	33.3	Off	33.5	Off
36.1	On	36.3	On	36.5	On	36.7	On	36.9	On	37.1	On	37.3	On	37.5	On	37.7	On	37.9	On	38.1	On	38.3	On
36.3	Off	36.5	Off	36.7	Off	36.9	Off	37.1	Off	37.3	Off	37.5	Off	37.7	Off	37.9	Off	38.1	Off	38.3	Off	38.5	Off
41.1	On	41.3	On	41.5	On	41.7	On	41.9	On	42.1	On	42.3	On	42.5	On	42.7	On	42.9	On	43.1	On	43.3	On
41.3	Off	41.5	Off	41.7	Off	41.9	Off	42.1	Off	42.3	Off	42.5	Off	42.7	Off	42.9	Off	43.1	Off	43.3	Off	43.5	Off
46.1	On	46.3	On	46.5	On	46.7	On	46.9	On	47.1	On	47.3	On	47.5	On	47.7	On	47.9	On	48.1	On	48.3	On
46.3	Off	46.5	Off	46.7	Off	46.9	Off	47.1	Off	47.3	Off	47.5	Off	47.7	Off	47.9	Off	48.1	Off	48.3	Off	48.5	Off
51.1	On	51.3	On	51.5	On	51.7	On	51.9	On	52.1	On	52.3	On	52.5	On	52.7	On	52.9	On	53.1	On	53.3	On
End (min) 51.3	Off	51.5	Off	51.7	Off	51.9	Off	52.1	Off	52.3	Off	52.5	Off	52.7	Off	52.9	Off	53.1	Off	53.3	Off	53.5	Off

## APPENDIX B

The example of periodic comprehensive heart-cut

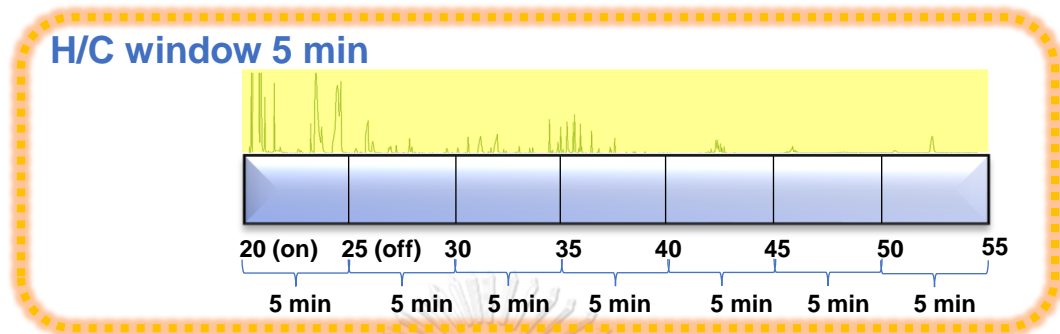


Figure B.1 Heart-cut event of  $t_{H/C}$  5.00 min.

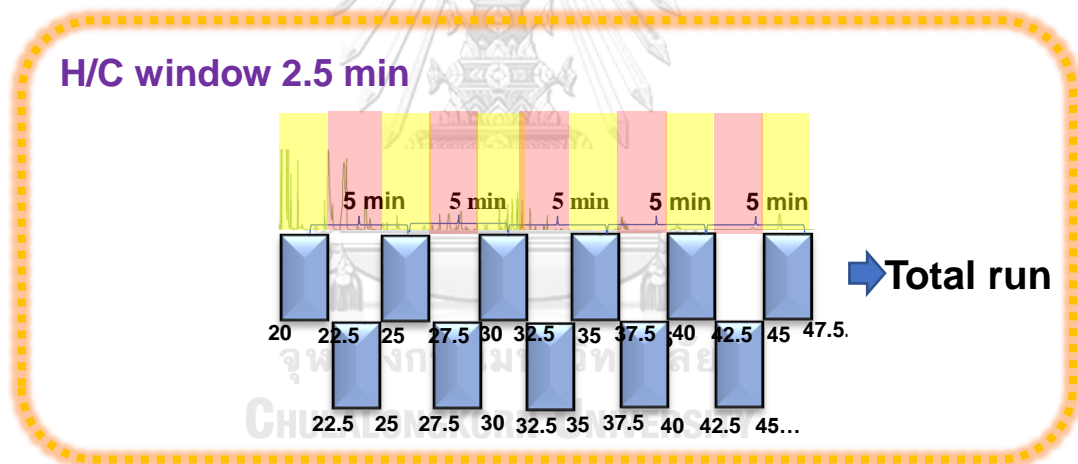


Figure B.2 Heart-cut event of  $t_{H/C}$  2.50 min.

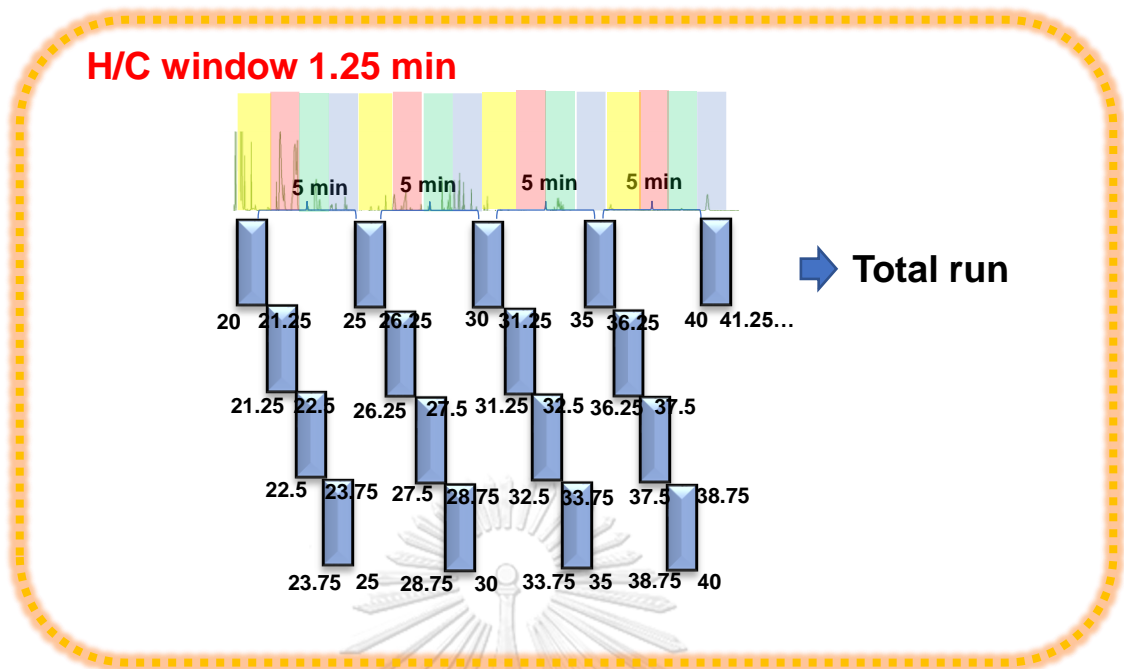


Figure B.3 Heart-cut event of  $t_{HC}$  1.25 min.

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