# Chapter 2

# Experimental

## 2.1 Chemical materials and instrumentations

### 2.1.1 Chemical and materials

10 μg/mL standard of 2,3,7, 8 – Tetrachlorinated dibenzo-*p*-dioxin in toluene, Supelco, USA

Toluene, acetone, methanol and hexane, AR grade, Merck, Germany

Amberlite XAD-2 resin, Supelco ,USA

Glass wool , Merck , Germany

### 2.1.2 Instrumentation apparatus and glasswares

**Instrumentation**

Hewlett Packard 6890 Gas Chromatograph – 5973 Mass Selective Detector equipped with a HP-5MS fused silica capillary column ( 30 m X 0.25 mm i.d. , film thickness 0.25 μm )

**Apparatus**

Solid State/Ultrasonic FS–28 ( bath type ) Fisher Scientific ( Pittsburgh, PA, USA )

Glass column ( 30.5 cm length, 1.8 cm i.d. ) with silicone rubber cap

Rotary evaporator vacuum source and recirculating water pump-Buchi, Switzerland equipped with a variable temperature water bath

**Glasswares**

Round – bottom flask- 250 ml, Pyrex, USA

2 mL amber screw top vial, Agilent, USA

100 mL test tubes, Pyrex, USA

1000 microlitre, 250 microlitre autopipette, Gilson, USA

250 mL separatory funnel, Pyrex, USA

50 cm., Buchner funnel and 250 mL filter flask Pyrex, USA

50 mm filter paper diameter Whatman, Germany

## 2.2 Analysis Method

### 2.2.1 Optimization of gas chromatographic conditions

#### 2.2.1.1 Carrier gas flow rate

The optimization of carrier gas flow rate was studied by using 100 μg/L 2, 3, 7, 8,- tetrachlorodibenzo-p-dioxin standard solution. A 1.0 microlitre of standards solution was analyzed by Gas Chromatograph – Mass selective detector ( GC-MS ) equipped with a HP-5MS fused silica capillary column ( 30 m X 0.25 mm i.d., film thickness 0.25 μm ) under the conditions specified in the Agilent Technologies Catalog, 2000. The GC – MS operating conditions were inlet temperature 250 $^{\circ}$ C, temperature program was started from 80 $^{\circ}$C hold for 1.5 minutes, ramped to 220$^{\circ}$C at 25$^{\circ}$C/min, ramped to 240$^{\circ}$C at 5$^{\circ}$C/ min, then ramped to a final temperature of 300 $^{\circ}$ C, with an ion source temperature of 230 $^{\circ}$ C. The carrier gas flow rate was varied from 0.7 to 1.5 ml/min with an incremented of 0.2 ml/ min. Retention time of the peak and the width at half of the peak ( $W_{1/2}$ ) were used to calculate the number of theoretical plates ( n ) and Height Equivalent of a Theoretical Plate( HETP ). The van Demter Plot was plotted between HETP and carrier gas flow rate. The optimum flow rate of carrier gas was obtained at the minimum HETP.

#### 2.2.1.2 Temperature programming

Optimization of temperature programming was investigated by using 1.0 microlitre of 100 μg / L 2, 3, 7, 8,- tetrachlorodibenzo-p-dioxin standard solution. The standard solution was analyzed by GC-MS with the inlet temperature of 300 $^{\circ}$ C, ion source temperature 230 $^{\circ}$ C, and 1 mL / min of carrier gas flow rate (optimum flow rate from 2.2.1.1 ). Nine programs were set for the optimization of the nine parameters i.e. 1) initial temperature, 2) hold time of initial temperature, 3) ramp rate of initial temperature, 4) the second stage temperature, 5) hold time of the second stage temperature, 6) ramp rate to the third stage temperature, 7) the third stage temperature, 8) hold time of the third stage temperature, and 9) ramp rate to the fourth stage temperature. The optimum program was obtained by considering the best resolution and the high response.

Step 1

Different initial temperatures, 80°C, 90°C, 95°C, 100°C, 110 °C and 120°C hold for 3 minutes, were investigated. The temperature was ramped to 200 °C ( hold 2 minutes ) at 30 ° C / min, to 260°C ( hold 5 minutes ) at 5° C / min, and ramped to final temperature 300 ° C ( hold 10 minutes ) at 30 ° C / min.

Step 2

Initial temperature was fixed at 100°C (optimum in Program 1) and the hold time was varied i.e. 0, 2, 3, 4, 6 minutes. The rest of the program was the same as in Program 1.

Step 3

Initial temperature was set at 100 ° C and hold for 3 minutes ( optimum in Program 2 ). The ramp rate to increase the temperature to 200 °C was varied i.e. 20, 25, 30, and 35 °C/min. Other conditions were the same as in program 2.

Step 4

Set initial temperature at 100 ° C hold for 3 minutes then ramped to the second stage temperature at 30° C / min ( optimum in Program 3 ). This second stage temperature was studied at 200, 210, 215, 220, 225°C and hold for 2 minutes. Other conditions were the same as in program 3.

Step 5

The program started with the initial temperatures of 100 ° C, hold for 3 minutes then ramped to 220 ° C ( optimum in Program 4 ) with a ramped rate of 30° C / min. The hold time of this second stage temperature was investigated at 0, 2, 4, 6, 8, 10 minutes. Other conditions were the same as program 4.

Step 6

The first half of the temperature program was set as in Program 5. The second stage temperature was 220°C hold 2 minutes ( optimum in Program 5 ). The ramp rate to the third stage temperature was studied at 2, 4, 5, 6, 8°C/ min. Other conditions were the third stage temperature 260°C ( hold 5 minutes) ramped to a final temperature of 300 ° C ( hold 5 minutes ) at 30 ° C / min.

Step 7

All the conditions that optimized by program 1-6 were set i.e. initial temperatures 100 $^{\circ}$C hold 3 minutes, ramped to 220$^{\circ}$C hold 2 minutes at 30$^{\circ}$ C / min ramped to the third stage temperature at 5$^{\circ}$ C / min ( optimum in Program 6 ). The third stage temperature was studied at 230, 240, 250, 260, 270, 280 $^{\circ}$C hold 5 minutes, and ramped to the final temperature of 300 $^{\circ}$C ( hold 5 minutes) at 30 $^{\circ}$C/min.

Step 8

The initial temperature was set at 100 $^{\circ}$C hold for 3 minutes. Then ramped to the second stage temperature of 220$^{\circ}$C at 30$^{\circ}$ C / min and hold for 2 minutes. The temperature was increased to 240$^{\circ}$C (optimum in Program 7) at 5$^{\circ}$ C / min. The hold time of this temperature was varied i.e. 0, 1, 3, 5, 7, 9 minutes, and ramped to the final the temperature 300 $^{\circ}$C ( hold 5 minutes ) at 30 $^{\circ}$C / min.

Step 9

In this final program the ramp rate to the final temperature was investigate. The program started with an initial temperature of 100 $^{\circ}$C, hold for 3 minutes then ramped to 220$^{\circ}$ C at 30$^{\circ}$ C/min. This second stage temperature was hold for 2 min then ramped to 240$^{\circ}$ C with a rate of 5$^{\circ}$ C/min and hold for 5 min ( optimum in program 8 ). This was then ramped to 300$^{\circ}$ C and hold for 5 min. This final ramped rate was studied at 10, 15, 20, 25, 30, 35 $^{\circ}$ C/min.

### 2.2.1.3 Inlet temperature

The optimization of inlet temperature was investigated by using 1.0 microlitre of 100 µg / L 2, 3, 7, 8,- tetrachlorodibenzo-p-dioxin standard solution. The standard was injected into GC-MS operating at the optimum flow rate of 1 mL / min ( 2.2.1.1 ) with an ion source temperature of 230 $^{\circ}$ C and the optimized temperature program ( optimum temperature programming in 2.3.1.2 ). The inlet temperature was investigated at 260, 270, 280, 290, 300, and 310 $^{\circ}$ C. The optimum inlet temperature was selected by considering the best resolution, the high response. of peak of TCDD, and less analysis time.

### 2.2.1.4 Interface temperature

Interface temperature was optimized by using 1.0 microlitre of 100 μg / L 2, 3, 7, 8,-tetrachlorodibenzo-p-dioxin standard solution. The standard was injected into the GC-MS operated at the optimum conditions i.e. 1 mL / min flow rate ( 2.2.1.1 ), ion source 230° C, optimum temperature programming in 2.2.1.2, and inlet temperature of 280 ° C ( optimum in 2.2.1.3 ). The interface temperature was studied at 280, 290 and 300 ° C. The interface temperature that gave the highest response peak of TCDD was then chosen.

### 2.2.2 Linear dynamic range

The linear dynamic range of 2, 3, 7, 8,- tetrachlorodibenzo-p-dioxin was studied by using 1.0 microlitre of a series of standard solution i.e. 0.5, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 and 100.0 μg / L. They were analyzed by GC-MS at the optimum conditions ( 2.2.1 ). The linear dynamic range was obtained by plotting the detector response ( peak area – Y axis) *versus* the concentration ( X-axis ).

### 2.2.3 Detection limit

The detection limit was the lowest concentration which could be measured ( IUPAC definition ). It was studied by analysing 4 standard concentrations ( 0.5, 1.0, 5.0 and 10.0 μ g / L ) and 30 blanks, $X_B$, ( toluene ) with GC-MS at the optimum conditions ( 2.3.1 ). The calibration curve was plotted and the mean value of the blank responses, $\overline{X}_B$ , and the standard deviation , $S_B$ , were calculated ( Long and Winefordner, 1983).

The probability that the smallest discernible analytical signal, $X_L$ , can be measured is

$$X_L = \overline{X}_B + kS_B \qquad (1)$$

Where k is a numerical factor chosen in accordance with the confidence level desired.

$C_L$, the limit of detection concentration, is a function of $X_L$, i.e.

$$C_L = ( X_L - \overline{X}_B ) / m \qquad (2)$$

Where m is the analytical sensitivity.

Because the average blank reading, $\overline{X}_B$, is not always 0, the signal must be corrected with the background. By substituting Equation 1 into Equation 2.

$$C_L = kS_B / m \qquad (3)$$

Where $C_L$ = Limit of detection concentration value ( IUPAC Definition )

k = 3 allows a confidence level of 99.86 %

$S_B$ = the standard deviation

m = the analytical sensitivity

This definition of $C_L$ can be illustrated as shown in Figure 2. The limit of detection is found by relating $kS_B$ to a concentration value by dividing by the slope of the calibration curve line obtained from the regression analysis.

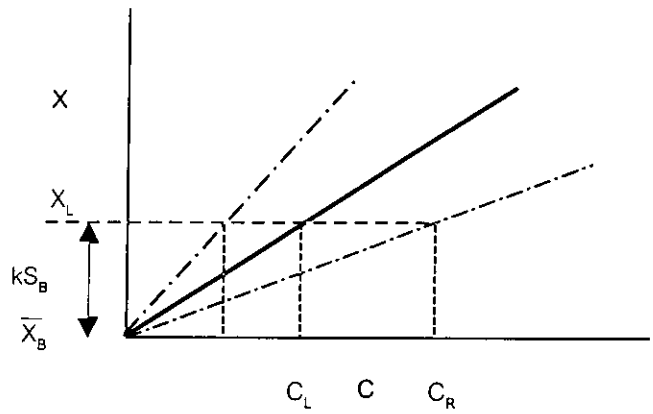



Figure 2 Analytical calibration curve of signal, X, vs. concentration, C, showing the relationship of $kS_B$ to the limit of detection, $C_L$ ( Long and Winefordner, 1983 )

## 2.3 Sample Preparation

### 2.3.1 Preparation of Amberlite XAD-2 glass column

A 25 grams of Amberlite XAD-2 resin was conditioned by adding 300 mL of methanol to cover the resin bed in the 1,000 mL beaker then gently stirred for a few minutes to ensure the complete mixing. The resin slurry was standed for 15 minutes, then the methanol was decanted and the slurry was washed with 4 times of 1000 mL distilled water, it was then allowed to stand for 5-10 minutes( Supelco, 1998 ).

The treated Amberlite XAD-2 resin was then packed into a glass column, 30.5 cm X 1.8 cm, i.d. plugged with 3 grams of glass wool at the bottom. Deionized water was added to the column, before the slurry treated resin was slowly poured into the column. As the column was filled, the excess water was drained through the bottom of the column. Another 500 mL of deionized water was then added to settle the XAD-2. The column was then ready for sample loading.

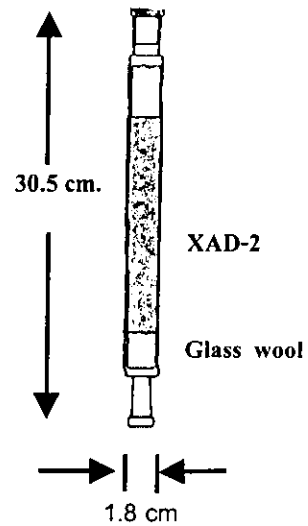



Figure 3 XAD-2 column

### 2.3.2 Ultrasonic extraction

A 4 ng TCDD standard spiked water ( 1000 mL ) was loaded to the column in 2.3.1. The XAD-2 resin was dried by an air pumped for 10 minutes. Then, transferred the dried XAD-2 resin ( with standards ) to a 100 mL test tube, and added 60 mL of mixed acetone - toluene ( 1:1, v / v ) to cover the resin. Then extracted by ultrasonic at the optimum extraction time ( 20 minutes ). The supernatant was transferred into a separatory funnel to remove the water. The residue was twice extracted with the same procedures. The supernatant was then transferred to a round bottom flask and concentrated by a rotary evaporator. The almost dried content in the round bottom flask was made to a final volume of 4 mL with toluene. If 100% recovery was obtained the concentration of the analyte ( TCDD ) would be 1 ng/mL.

### 2.3.3 Optimization of sample preparation steps

#### 2.3.3.1 Sequencing of conditioning solvents series

There are some variables in the sample preparation process which would affect the extraction efficiency. First was the sequencing of the conditioning solvents series used in the preparation of the XAD-2 column ( 2.3.1). Two series of solvents series were studied instead of just using methanol i.e. a methanol-acetone-toluene-acetone-methanol-water system and a methanol-water system. After the XAD-2 column preparation was completed, the column was loaded and extracted ( as described in Procedure of ultrasonic extraction in 2.3.2 ) and analyzed by GC-MS at the optimum conditions. Extraction efficiency was compared between the two series. The system with the highest extraction efficiency and used less time was selected.

#### 2.3.3.2 Extraction solvent

Extraction solvent was another parameter that could affect extraction efficiency. These studies were carried out by using the Amberlite XAD-2 glass column ( as described in preparation of the XAD-2 column 2.3.1 ). The spiked XAD-2 was extracted in ultrasonic bath ( as described in Procedure of ultrasonic extraction 2.3.2 ) with three solvents i.e. toluene, mixed acetone – toluene ( 1:1, v/v ) and mixed acetone – hexane ( 1:1, v/v ). The extractants were pre-concentrated and analyzed by GC-MS at the optimum conditions and chosen the solvent that showed the best extraction efficiency.

#### 2.3.3.3 Extraction time

This study was to find the sufficient extraction time for the complete extraction of TCDD from XAD-2. The studied was carried out by using Amberlite XAD-2 glass column ( as described in Preparation of the XAD-2 column in 2.3.1 ) loaded with 4 ng, 1000 mL spiked water. The spiked XAD-2 was extracted in ultrasonic bath with mixed acetone - toluene ( 1:1, v / v optimum in 2.3.4.2 ). The investigation times were 10, 20, 30 and 40 minutes. The optimum time was the time that provided the highest extraction efficiency.

#### 2.3.3.4 Volume of extraction solvent

The volume of extraction solvent was investigated after obtaining the optimum extraction time. The XAD-2 glass column ( was prepared as described in Preparation of the XAD-2 column in 2.3.1 ) was loaded by 4 ng dioxin, 1000 mL spiked water and the spiked XAD-2 was extracted in ultrasonic bath ( as described in Procedure of ultrasonic extraction in 2.3.2 ) for 20 minutes ( optimum in 2.3.3.3 ). The various solvent volumes i.e. 30, 40, 50, 60, and 70 mL were studied and the optimum volume was obtained as the one that gave the highest extraction efficiency.

#### 2.3.3.5 Numbers of extraction

The optimum solvent volume, 60 mL ( obtained from 2.3.3.4), was sufficient for extracting dioxin. This study divided the 60 mL solvent into 1 X 60 mL, 2 X 30 mL and 1 X 30 mL plus 2 X 15 mL for single, double and triple extractions, respectively.

Single extraction was performed by extracting the spiked XAD-2 with 60 mL extraction solvent ( optimum in 2.3.3.4 ). The supernatant was collected and analyzed by GC-MS at the optimum conditions.

Double extractions were performed by extracting the spiked XAD-2 with 30 mL extraction solvent. The supernatant was collected, and the residue was extracted again with another 30 mL of solvent using the same procedure. The combine supernatant was analyzed by GC-MS at the optimum conditions.

Triple extractions were performed by extracting the spiked XAD-2 with 30 mL and 2 X 15 mL extraction solvent. All the supernatant was collected and analyzed by GC-MS at the optimum conditions.

#### 2.3.3.6 Volume of spiked water loading

The spike water ( 4 ng, TCDD ) was loaded to the XAD-2 column after conditioned with the solvent system of methanol / water ( optimum in 2.3.3.1 ). The volumes of the spike water were varied, i.e. 100, 200, 400, 600, 800 and 1000 mL. The extraction efficiency of each volume of spike water were compared. The optimum volume of water loading was based on the highest extraction efficiency.

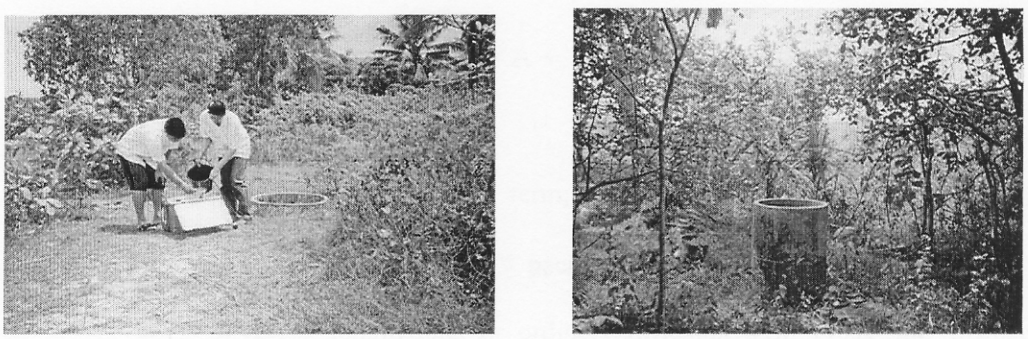
### 2.3.4 Qualitative and Quantitative analysis

This analytical method was validated by analyzing three water samples collected from three water sampling sites as shown in Table 5.

Table 5 Sampling sites

| Water sample no. | Date of sampling | Location |
|---|---|---|
| 1 | June 30, 2002 | Hatyai Regional Water Supply |
| 2 | July 12, 2002 | The first Songkhla Monitoring Municipal Landfill |
| 3 | July 12, 2002 | The second Songkhla Monitoring Municipal Landfill |

Three 10 liters water samples were collected in amber glass bottles and preserved at 0-4 $^{\circ}$C and in the dark during transportation to the laboratory. The water samples were pre-concentrated by the XAD-2 column at the optimum conditions and extracted as 2.3.2. The extractants were analyzed by GC-MS at the optimum conditions. The qualitative analysis was based on the retention data and the mass ratios of $M^+$ and $(M+2)^+$ on the SIM mode ( Hashimoto, *et al.*, 1995 ). The quantitative analysis were calculated from the chromatogram.



**Location A** **Location B**

Figure 4 The Sampling Location of Songkhla Monitoring Municipal Landfill

Location A : The first site , Location B : The second site