Chapter 2

EXPERIMENT

2.1 Chemicals and materials

2.1.1 Standard chemical

- Acetaldehyde (purity, 99 %, AR grade, Merck, Germany)

2.1.2 General chemicals and solvents

- Porapak Q, 80/100 mesh (Water, USA)
- Glass wool

2.2 Instruments and Apparatus

2.2.1 Gas Chromatography-Flame Ionization Detector (GC-FID)

- Agilent 6890N series Gas Chromatograph equipped with Flame Ionization Detector (Agilent, USA)
- DB-1 capillary column, 15.0 m × 530 μm ID. × 3.00 μm film thickness (Agilent, USA)
- High purity helium carrier gas (purity 99.995%: TIG, Thailand)
- Air zero grade (purity 99.995%: TIG, Thailand)
- High purity nitrogen gas (purity 99.99%: TIG, Thailand)
- Ultra high purity hydrogen gas (purity 99.999%: TIG, Thailand)

2.2.2 Apparatus for inject standard solution and standard gas

- Gas-tight syringe 1.0, 5.0, 10.0 mL Series A-2
  (VICI Precision Sampling, Inc., Louisiana, USA)
- Syringe 10 μl (Hamilton, Switzerland)
- Syringe cleaner (Hamilton, Switzerland)

- Crimper/ Decrimper (Supelco, USA)
- Flow meter (Cole Parmer, USA)

2.2.3 Apparatus for Airspace technique

- Gas sampling bulb, 500 mL with Thermogreen septa (Supelco, USA)

2.2.4 Apparatus for purge and trap technique

- Adsorbent tube (60 mm × 4 mm O.D.)
- Lab-built thermal desorption unit
- Analytical Balance (Denver Industrial Company, USA)
- Vacuum pump (GAST manufacturing, Inc., USA)
- Thermocouple Module 80TK with sensor (Fluke, USA)
- Heating mantle (Electrothermal, UK)
- 3-necked glass round bottom flask 500 mL (Pyrex, USA)

2.3 Analysis system

Figure 5 shows an overview of the analysis system. It consists of two parts: sample preparation and analysis. The sample preparation part consists of a lab-built heating box, temperature control unit, and a vacuum pump. The analysis part consists of a gas chromatograph (Agilent 6890N series) equipped with flame ionization detector (GC-FID). A six-port valve was integrated to the injector part of the gas chromatograph. This was to modify the injector to be used as a gas sampling valve system. A DB-1 (15 m × 0.53 mm i.d. × 0.32 μm) capillary column was used to separate the sample.

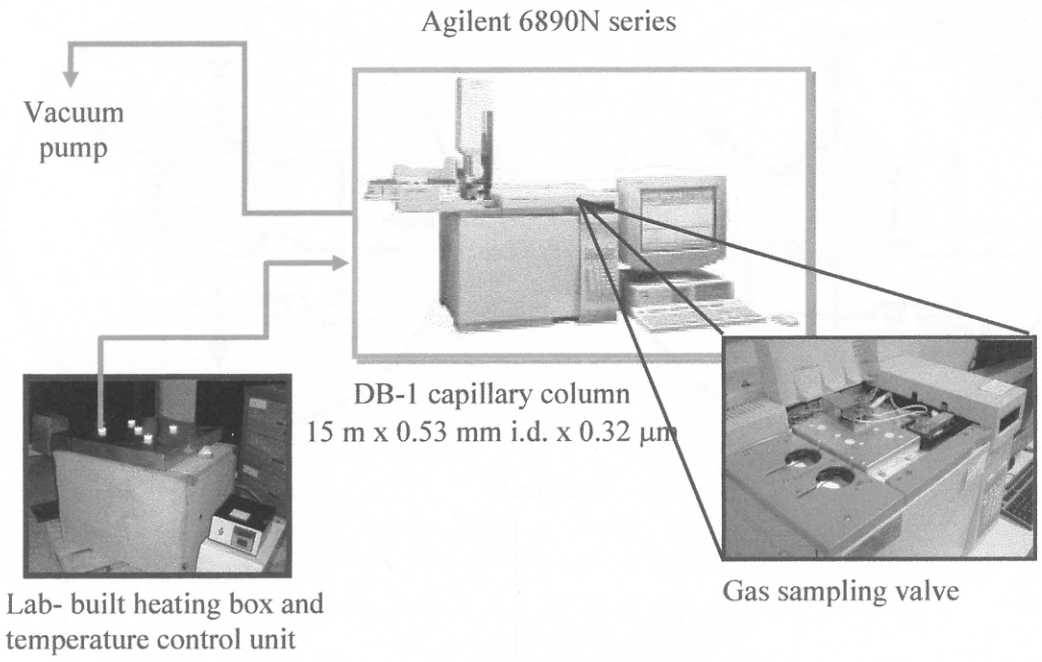

Figure 5 Instrumentation of acetaldehyde analysis

During sample preparation, five bottles were incubated in the lab- built heating box at appropriate temperature for an optimum time. Gas sample in the bottle was then introduced to the analysis part through the sampling valve using a vacuum pump. For the analysis part, while the gas sample from the bottle was being purged into a 0.25 mL heated gas sampling loop in the sampling valve, a carrier gas was purged through the column directly to obtain a baseline (Figure 6(a)). The valve was then switched to the injection position, gas sample was purged to be separated in the column and detected with flame ionization detector providing the signal (Figure 6(b)).

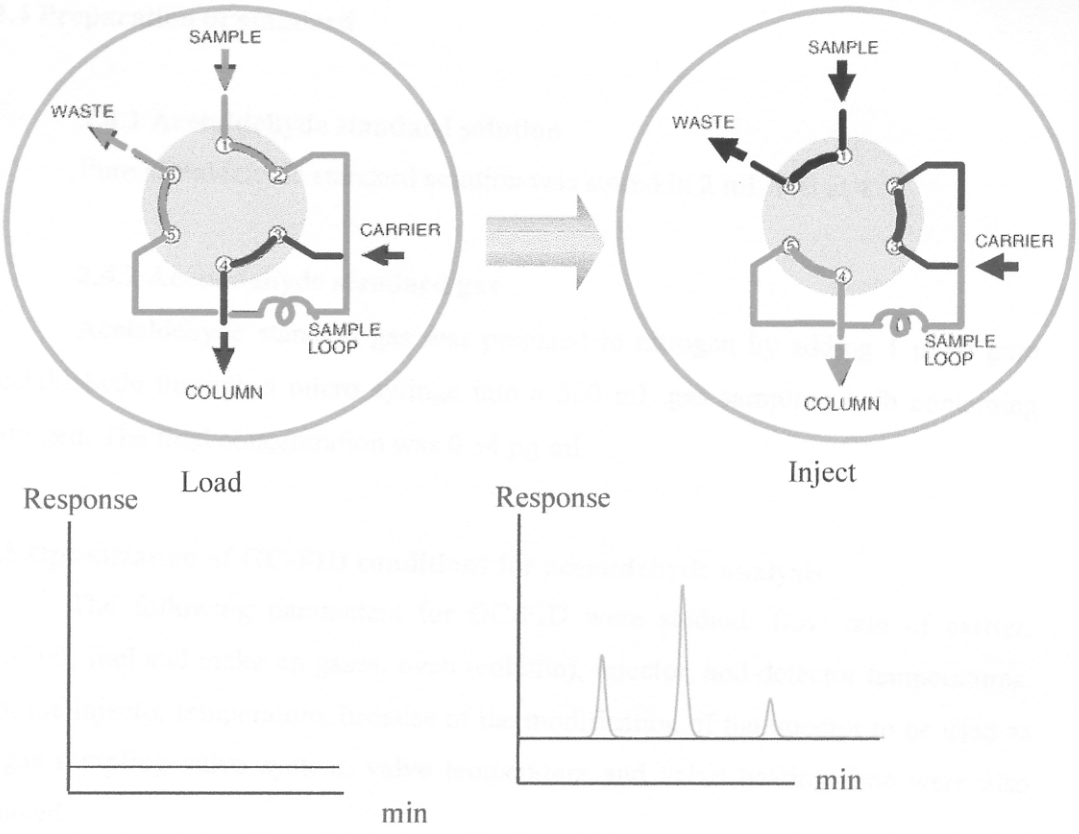

Figure 6 Gas sampling valve system (a) loading step to obtain a baseline (b) injection step to obtain a chromatogram

To obtain the highest efficiency, parameters affecting the performance of the analysis system were optimized and details are given in later sections.

2.4 Preparation of standard

2.4.1 Acetaldehyde standard solution

Pure acetaldehyde standard solution was stored in 2 mL vial at 4 °C.

2.4.2 Acetaldehyde standard gas

Acetaldehyde standard gas was prepared in nitrogen by adding 1 μl of pure acetaldehyde through a micro syringe into a 500 mL gas sampling bulb containing nitrogen. The final concentration was 0.34 μg mL^{-1}.

2.5 Optimization of GC-FID conditions for acetaldehyde analysis

The following parameters for GC-FID were studied: flow rate of carrier, oxidant, fuel and make up gases, oven (column), injector, and detector temperatures. For the injector temperature, because of the modification of the injector to be used as a gas sampling valve system, valve temperature and valve heating time were also studied.

Optimization was done by changing one parameter and keeping other parameters to be constant. When an optimum was obtained it was used to optimize the next parameter. For all optimizations carried out in this section, analysis was done using 0.34 μg mL^{-1} of acetaldehyde standard gas. It was filled into 0.25 mL gas sample loop by vacuum pump. Then the valve was switched to the injection position and the analyte was purged to the column (DB-1 capillary column 15 m × 0.53 mm ID × 0.27 μm thickness) of the gas chromatograph (Agilent 6890N Series) and detected with flame ionization detector.

The first optimized parameter was the carrier gas flow rate. The analysis was done by setting the column, detector, and valve (injector) temperatures at 50 (isothermal), 270, and 160 °C, respectively. The valve heating time was 0.2 minute. These values were modified from Airspace Acetaldehyde Test (Airspace Acetaldehyde Test, 1999) that used packed column. Hydrogen, air, and nitrogen were used as the fuel, oxidant, and make up gases at the flow rates of 30, 300, and 20 mL min^{-1}, respectively (following the manual).

2.5.1 Carrier gas (He) flow rate

The optimum carrier gas flow rate was determined by varying the flow rate of the helium carrier gas at 7.5, 8.0, 8.5, 9.0 and 9.5 mL min^{-1}. Four replications were done at each flow rate. The plate number and height equivalent to a theoretical plate (HETP) were calculated using the retention times and the peak heights of the chromatogram. From a van Deemter plot, the optimum flow rate was obtained at the lowest HETP.

2.5.2 Column temperature

An isothermal mode was used for the column temperature. The optimization of the column temperature was investigated by varying the column temperature at 50 °C to 160 °C with an increment of 10 °C. Four replications were analyzed for each temperature. The optimum column temperature was the temperature that increased detectability, sample throughput and reduced the operation time.

2.5.3 Detector temperature

The optimization of detector temperature was determined by varying the detector temperature at 240, 250, 260, and 270 °C. The analysis was performed at each temperature for four times. The temperature that obtained the highest detectable was the optimized detector temperature.

2.5.4 Valve (injector) temperature

The optimization of valve temperature was determined by varying the detector temperature at 120, 130, 140, 150, 160, 170, and 180 °C. The analysis was performed at each temperature for four times. The temperature that obtained the highest detectable was the optimized valve temperature.

2.5.5 Fuel (H_2) gas flow rate

To optimize the flow rate of hydrogen that was used as the fuel gas, its flow rate was varied from 25 mL min^{-1} to 40 mL min^{-1} with an increment of 5 mL min^{-1}. The responses at different hydrogen flow rates were compared and the optimum was selected from the highest response.

2.5.6 Oxidant (air) gas flow rate

The optimum oxidant flow rate was investigated at 250, 300, 350, and 400 mL min^{-1}. The peak heights obtained from all flow rates were compared and the optimum oxidant flow rate was determined from the highest response.

2.5.7 Make up gas (nitrogen) flow rate

The optimum make up flow rate was investigated at 10, 20, 30 and 40 mL min^{-1}. The flow rate that provided the highest peak height was selected.

2.5.8 Valve heating time

The optimum valve heating time was investigated at 0.2, 0.3, 0.4, and 0.5 minute. By comparing the peak heights the optimum valve heating time was selected from the time that gave the highest response.

2.6 Optimization of the airspace conditions for the preparation of acetaldehyde standard gas

The sensitivity of airspace technique or the equilibrium concentration of acetaldehyde in the airspace depends on equilibration temperature and time.

2.6.1 Equilibration temperature

For acetaldehyde residue in PET bottle analysis, the conventional method uses room temperature (22 °C ± 1.5 °C) (Airspace Acetaldehyde Test, 1999) as the equilibration temperature. Thus, equilibration temperature was set to 24 °C ± 2.0 °C which is the laboratory room temperature.

2.6.2 Equilibration time

Acetaldehyde standard gas (0.34 μg mL^{-1}, 0.25 mL) in the gas sampling bulb was equilibrated at 24 °C for different periods of time ,*i.e.*, 1, 2, 5, 10, 20, and 30 minutes. After each equilibration time was reached, 0.25 mL of gas phase from the bulb was filled into the gas sampling loop, heated and allowed the column to separate the analyte and detected with flame ionization detector. The resulted peak heights

were plotted against the equilibration time. The time needed to reach the equilibrium was obtained as the time that the analytical signal was constant.

2.7 Limit of detection

The limit of detection (IUPAC definition), is expressed as a concentration, c_L (or amount, q_L), derived from the smallest measure, x_L, that can be detected with reasonable certainly for a given analytical procedure (Long and Winefordner, 1983). In another word, the limit of detection is the lowest concentration of an analyte that an analytical process can reliably detect (ACS definition). The determination of a limit of detection was determined by measuring blank response (x_B) for 20 times (n_B).

2.8 Linear dynamic range (Linearity)

Concentrations in the range of 0.30 ng mL^{-1} to 0.34 μg mL^{-1} were obtained by diluting acetaldehyde standard gas, 0.34 μg mL^{-1}, with nitrogen. A 0.25 mL aliquot of each standard gas was filled into the gas sampling loop in the GC system that operated at optimum conditions obtained from 2. 5.

Linearity of the response was determined by plotting a calibration curve and considering of the correlation coefficient value.

2.9 Sample preparation

Sample preparation procedure was based on airspace technique (headspace technique).

2.9.1 Conventional method

Trace acetaldehyde residue in PET bottle was analyzed by the following steps; freshly blown PET bottles were purge with nitrogen for 20 seconds, cap with septum and stored the bottles at 24 ± 2 °C for 24 ± 1 hours. A 0.25 mL gas sample was injected to a gas chromatograph at optimum conditions.

2.9.2 Developed method

A sample preparation technique was developed using a lab- built heating box. Five bottles at a time were incubated at an appropriate temperature. Gas sample in the bottles was analyzed using gas chromatography at the optimum conditions obtained from 2.5.

2.10 Lab- built heating box

A lab-built heating box was modified from an old oven. Initially a flat metal plate was modified to fit 5, 1.25 L bottles as shown in Figure 7. This will be called modified lid I. The temperature at each bottle position was checked and compared by using statistical test. After satisfactory results were obtained, *i.e*, no significant difference in temperature between the position, modified lid II (Figure 8) was constructed to accommodate larger bottles.

The picture of the heating box is shown in Figure 9. An old oven (A) was used as a heater that helped to release acetaldehyde from the bottles. The heat was controlled through a heating cord that connected to the heating sensor and the temperature-time control unit (B). Initially the heating of the box was done without the bottles by using the lid of the box. The heating box could be heated from room temperature to the set up temperature with a heating rate of 3-4 $^{o}C\ min^{-1}$. After the temperature reached its set value, the lid of the box was changed to the modified lid where the bottles could be hang (C). The bottles were allowed to incubate for an appropriate time, before the gas sample in the bottles was analyzed by gas chromatography. The temperature of the lab- built heating box was calibrated before operation.

Figure 7 Modified lid I

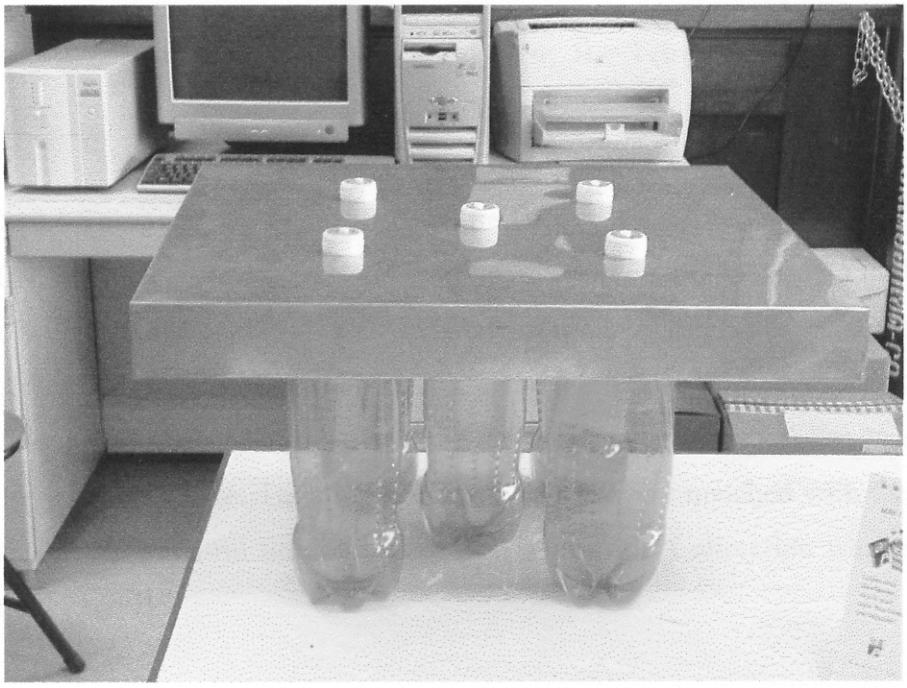

Figure 8 Modified lid II

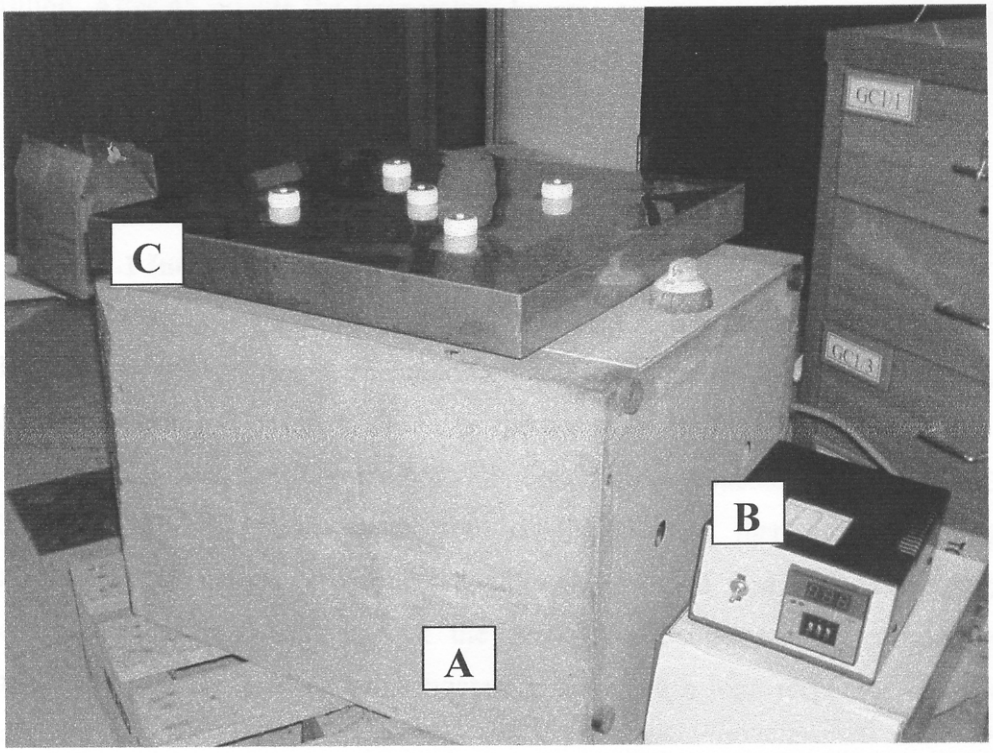

Figure 9 Lab-built heating box A = old oven; B = Temperature control unit; C = A modified lid

2.10.1 Temperature calibration of the lab-built heating box

The temperature of the lab-built heating box was calibrated using a multimeter equipped with a thermocouple. The temperature of the lab-built heating box was set at the temperature-time control unit. To calibrate, the thermocouple was inserted into the box and the temperature was read from the multimeter every 5 minute for 45 minutes of each tested temperature. The obtained temperatures were matched with the values on the temperature control unit to be used in later work. Calibration was done for modified lid I and II.

2.10.2 Temperature at each position in the lab-built heating box

The temperature of the lab-built heating box was set by the temperature-time control unit. The temperatures of each position where the bottles were hung in the lab-built heating box were measured by inserting a thermocouple into the box at each

position. After 45 minute the temperature became constant and the value was read. The temperature at each position was studied for both the modified lid I and II.

2.11 Optimization of incubation

2.11.1 Incubation temperature

The heating box with its original lid was first turn on. Five newly blown 1.25 L PET bottles were screwed on the modified lid I. After the set temperature of the lab-built heating box was constant, the lid of the box was removed and the modified lid with the bottles was placed on the box. The bottles were incubated for an hour at 37, 42, 50, and 60 °C. Gas sample from the bottles was then analyzed by gas chromatographic method at optimum conditions (obtained from 2.5). The resulted peak heights were plotted against the incubation temperature. The optimized temperature was obtained as the temperature that gave a high response with short analysis time.

2.11.2 Incubation time

After the set temperature of the lab- built heating box was constant, modified lid I with 5 newly blown 1.25 L PET bottles was placed on to the heating box. The bottles were incubated at 60 °C (optimum obtained from 2.11.1). Five incubation times were tested, *i.e.*, 1, 3, 5, 7, and 9 hours. Gas sample in the bottles was then analyzed. The optimum time was obtained from the minimum time that gave the same or better response as conventional method.

2.12 Purge and trap technique

Purge and trap technique was a sample preparation procedure based on the use of adsorbent to preconcentrate volatile organic compounds (acetaldehyde) prior to GC analysis.

2.12.1 Adsorbent conditioning

Porapak Q is a porous polymer resin based on polydivinylbenzene with the following physical properties:

- Chemical structure	:	Divinylbenzene/ Ethylvinylbenzene
- Temperature limit	:	250 °C
- Specific surface area:		500 -600 m^2/g
- Polarity	:	slightly polar
- Mesh size	:	80/100 mesh

Porapak Q can be activated or conditioned by heating to an appropriate temperature (200 °C) in the stream of inert gas, N_2. This was done by using the apparatus that was made in the laboratory as shown in Figure 10. Porapak Q was placed in the 3-necked round bottom flask. A thermometer and an inert gas (N_2) flow line were connected to the flask by using the modified connector sealed with parafilm. After the N_2 valve was turned on to let N_2 stream passed through porapak Q in the flask, the heating mantle was turned on to heat Porapak Q at 200 °C. The heat was turned off after 3 hours and the Porapak Q was allowed to cool down in the nitrogen stream. The conditioned adsorbent was then kept in a clean (Nalgene®) bottle and stored in a desiccator.

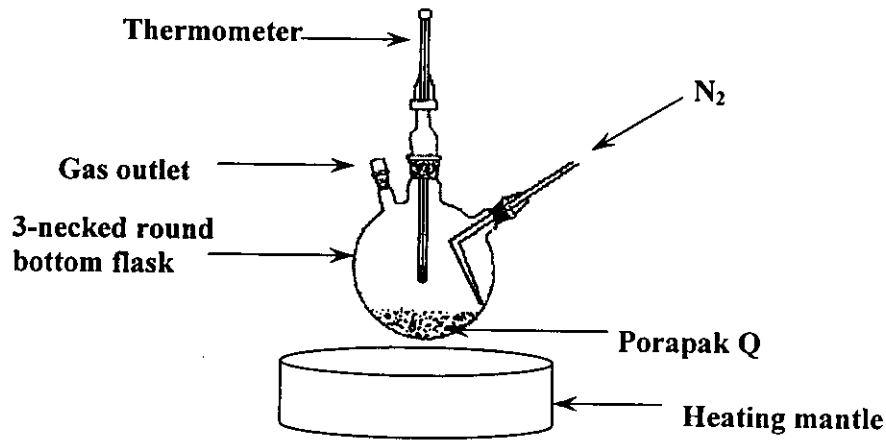

Figure 10 Conditioning of adsorbent, Porapak Q

2.12.2 Preparation of the adsorbent tubes

Glass tubes, 2 mm inner diameter, 4 mm outer diameter and 60 mm in length, with one end sealed were washed thoroughly and sonicated in an ultrasonic bath for 30 minutes. After rinsing with distilled water to make sure that they were cleaned and free from any residue or obstructions inside the tubes, they were baked dry in an oven and kept in a clean box.

The adsorbent tube was packed by, first inserted a small amount of clean glass wool into one end of the tube followed by 0.0800 g (±0.0005g) of Porapak Q and a 12.5 mg glass wool plug at the end of the tube to hold the resin in place. The tube was closed with a plastic cap, sealed with parafilm, and kept in a desicator. The packed adsorbent tube is shown in Figure 11.

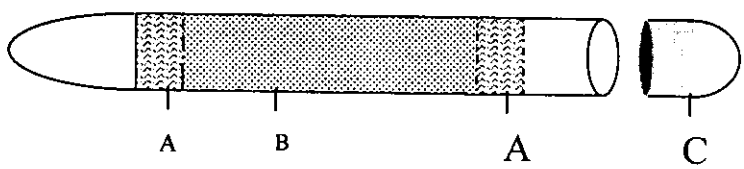

Figure 11 Porapak Q adsorbent tube A = 12.5 mg glass wool plug; B = 0.0800 g of Porapax Q; C = plastic cap

2.12.3 Quantity of adsorbent (Porapak Q)

In this study as well as all the studies on adsorption that follows, 1.5 ng mL^{-1} acetaldehyde standard gas was used by filling into a 1.25 L PET bottle. The system used to study optimization is shown in Figure 12. The bottles were incubated at 60 °C for an hour (results from 2.11), purged with inert gas (nitrogen) for 10 minutes to carry analyte to the adsorbent tube, where the amount of Porapak Q was varied from 40 to 100 mg with an increment of 20 mg. The analyte was then desorbed by thermal desorption and flowed to the gas chromatographic system. The optimized amount of adsorbent was the amount that gave the constant response.

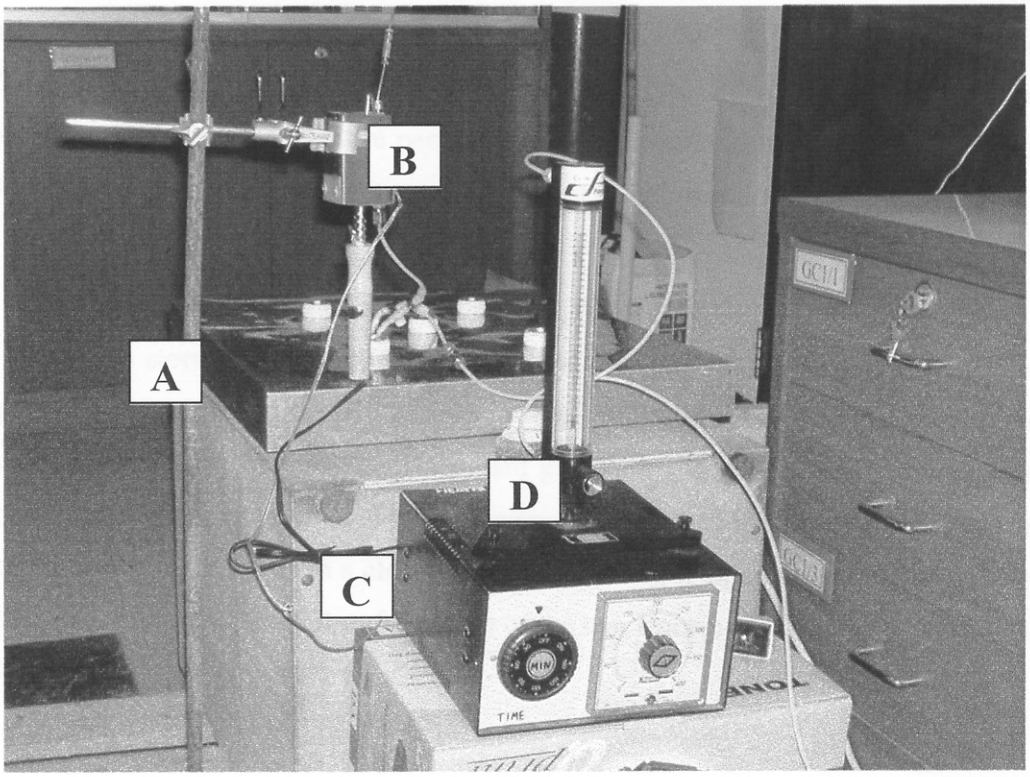

Figure 12 System used to optimize the amount of adsorbent; A = Lab- built heating box; B = Thermal desorption unit; C = Temperature control unit; D = Flowmeter

2.13 Optimization of adsorption conditions

2.13.1 Adsorption flow rate

Optimization of the adsorption flow rate was determined by varying the flow rate at 60, 70, 80, 90, and 100 mL min^{-1}. Five replicates were performed for each flow rate and the optimum adsorption flow rate was chosen as the one that gave the highest response.

2.13.2 Adsorption time

After the bottles with standard gas were incubated at 60 °C for an hour, nitrogen gas was then purged through the bottle at 70 mL min^{-1} (the result from 2.13.1). The purging time was varied from 10 minutes to 25 minutes with a 5 minutes increment. Five replicates were performed for each time and the optimum adsorption time was chosen as the one that gave the highest response and short analysis time.

2.13.3 Storage lifetime of the adsorbent tube

The storage lifetime of adsorption tube for acetaldehyde was investigated by preparing 45 adsorbent tubes. The fused end of each tube was broken off and spiked with 1.5 ng mL^{-1} of acetaldehyde standard gas. Four adsorbent tubes were analyzed on the day of preparation. Twenty tubes were stored in the refrigerator at 4 °C and the other twenty were stored in a closed drawer at ambient temperature (24 °C). At 3-day intervals, four adsorbent tubes from each of the two storage places were analyzed and their responses were compared.

2.14 Thermal desorption

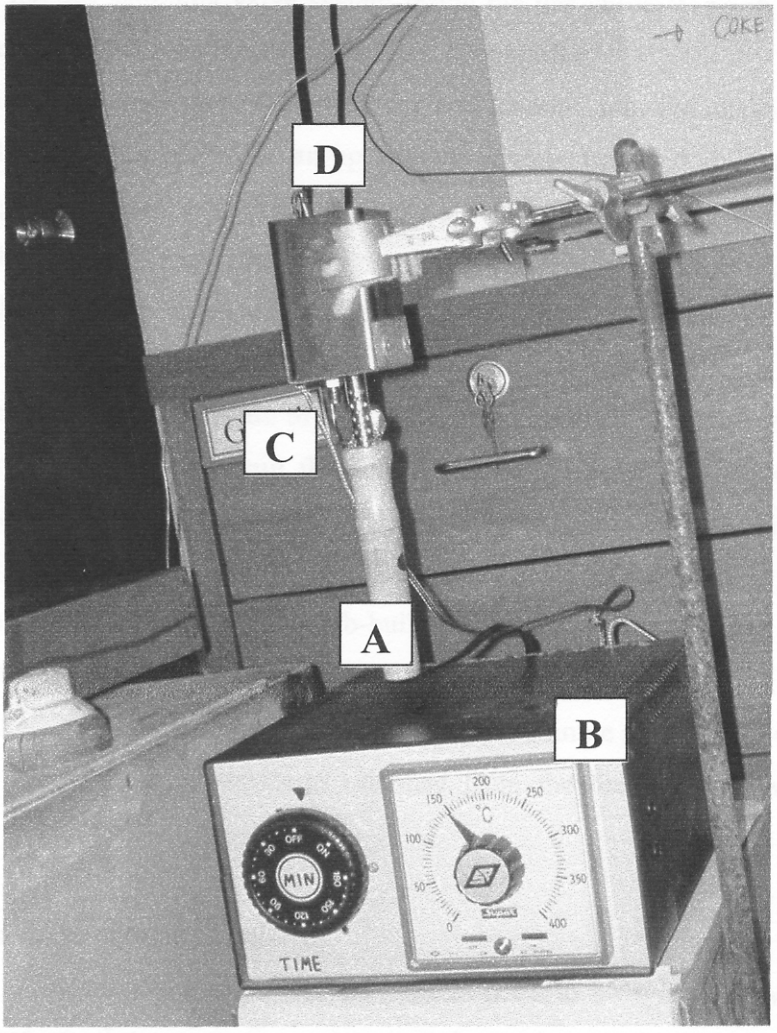

Figure 13 Lab-built thermal desorption unit; A = small heater; B = Temperature control unit; C = lower end; D = upper end

A lab-built thermal desorption unit (Figure 13) was designed to desorp acetaldehyde from the adsorbent tube. A small heater (A) was constructed using a heating element connected to an aluminium block. A channel was drilled through the length of the block with a diameter large enough to tightly fit the outer diameter of the adsorbent tube. With the aid of the heating cord that was connected to the heating sensor and the temperature-time control unit (B), the adsorbent tube could be heated to a set temperature with a heating rate of 10 - 15 $^{o}C\ min^{-1}$. The lower end (C) of the

adsorbent tube was connected to a flow meter and a nitrogen source and the upper end (D) was connected to a small tube that led the desorbed components to the gas sampling loop in the gas sampling valve system inside the gas chromatograph. The temperature of the desorption unit was calibrated before operation.

To desorb acetaldehyde, the adsorbent tube was inserted into the channel of the aluminium block. It was heated to an appropriate temperature and time while nitrogen was purged through the trap. By this procedure, the adsorbed analyte was desorbed to fill the gas sampling loop in the sampling valve system inside the gas chromatography that was set at optimum conditions.

2.14.1 Temperature calibration of the lab-built thermal desorption unit

The temperature of the lab-built thermal desorption unit was calibrated by using a multimeter equipped with a thermocouple. To calibrate, the thermocouple was inserted into the channel inside the aluminium block of the small heater and was held in place by a clamp. The temperature of the lab-built thermal desorption unit was then set at the temperature-time control unit. The temperature that showed on the multimeter, measured by the thermocouple, was read every 5 minute for 60 minute for each temperature setting. The temperatures obtained from the thermocouple were used in this work.

2.15 Optimization of desorption conditions

In thermal desorption, the important parameters need to be optimized were desorption temperature, desorption flow rate and desorption time. These were investigated by injecting 1.5 ng mL^{-1} of acetaldehyde standard gas into the conditioned adsorbent tube and kept at room temperature (24 °C) for 30 minutes (Barratt, 1981 and Urbach, 1987). The desorption conditioned were then tested as follows.

2.15.1 Desorption flow rate

Acetaldehyde was desorbed by the procedure in 2.14 at 215 °C for 0.3 minute and varied the desorption flow rate at 60, 70, 80, 90 and 100 mL min^{-1}. The relationship between response and the desorption flow rate was obtained. The optimum desorption flow rate was the one that gave the highest response.

2.15.2 Desorption time

Desorption time was investigated by heating the adsorbent at 215 °C with a flow rate of 70 mL min^{-1} (obtained from 2.15.1) and varied the time at 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 minutes. The optimum desorption time was the minimum time that gave the highest response.

2.15.3 Desorption temperature

Acetaldehyde was desorbed using various desorption temperature setting *i.e.*, 150, 160, 170, 180, and 190 °C (the actual temperature are 198, 215, 225, 235, and 245, respectively). The desorption flow rate and desorption time were set at 70 mL min^{-1} and 0.6 minute, respectively (the results obtained from 2.15.1 and 2.15.2). The optimum desorption temperature was the one that gave the highest response.

2.16 Calibration curves

2.16.1 Calibration curve for conventional and developed method

A series of 0.3 ng mL^{-1} to 6.6 μg mL^{-1} acetaldehyde standard gas was prepared in 500 mL gas sampling bulb by diluting acetaldehyde standard gas (2.4) with nitrogen. After the equilibration time was reached (the result from 2.6.1), 0.25 mL of gas phase from the bulb was filled in the gas sampling loop, heated, allowed to be separated by the column and detected with flame ionization detector (using the optimum GC-FID conditions from 2.5). A calibration curve was obtained by plotting the resulted peak heights against the concentrations.

2.16.2 Calibration curve for purge and trap technique

A 0.34 μg mL^{-1} acetaldehyde was prepared by diluting the acetaldehyde standard gas with nitrogen. Then 0, 2.5, 5.0, 7.5, and 10.0 mL of 0.34 μg mL^{-1} were spiked into the incubated bottles (using optimum conditions obtain from 2.11). Nitrogen was then purged through the incubated bottle (using the results from 2.13) carrying the analyte to the adsorbent tube. The analyte was desorbed using optimum conditions from 2.15. A calibration curve was obtained by plotting the subtracted blank (a 0 mL spiking) responses (peak height) *versus* the acetaldehyde content.

2.17 Qualitative and Quantitative Analysis of Real sample

2.17.1 Sampling

Newly blown bottle samples were collected from Haad Thip Co. Ltd., Hat-Yai, Songkhla. There are three types of PET bottle for Coke, Sprite, and Fanta.

2.17.2 Qualitative analysis

Qualitative analysis was carried out by comparing the retention time between the chromatogram of acetaldehyde standard and unknown samples. The retention time, t_R is the time elapsed from injection of the sample component to the recording of the peak maximum.

2.17.3 Quantitative analysis

Quantitative analysis can be determined, from the chromatographic peak that was proportional to the amount of analyte. The analytical technique applied to this work was external standardization. This was carried out by preparing the acetaldehyde standard at concentration levels close to the unknown samples. These standards were then run chromatographically under identical conditions (the optimum conditions) as the samples. A direct relationship between peak height and concentration of components could then be established by a "calibration curve". The concentration of the unknown sample compounds were calculated mathematically from the standards curve (Grob, 1985).

2.17.4 Quality assurance and Quality control

For quality assurance and quality control, the accuracy of a method is the closeness of the measured value to the real value for the sample. In this work, the accuracy was determined by comparing test results from the developed method with the results from existing alternate method (conventional method) that is known to be accurate (Mark, 1996). The precision was measured by making replicate measurement (four replications for GC-FID conditions and five replications for sample preparation technique) and should be less than 4% for purity method and 10 % for impurity method (Mark, 1996).

The contamination that can occur in the laboratory at any stage of sample preparation and analysis was assessed by blank measurement composed of instrument blank, reagent blank and method blank.