CHAPTER I

INTRODUCTION

Problem Definetion.

Volatile organic solvents was played an important role in pharmaceutical indrustry i.e., active ingredient , binder , flavorants, sweeteners and colorants(1). From these steps the organic volatile impurities and other residual solvents may be introduced into the product in high level. In many countries, excessively the United State of America, have the most interested in quality control and analysis organic volatile impurities and other residual solvents.

In Thailand, there were 188 drug manufacturers which reported by Business Informations & Research Company Limited and Department of Industrial Works in 1993. These were included tablet , suspension , solution , parental and suppositories preparations (2). Generally, the several manufacturers have bought the active ingredient from Asia in low cost, and as a result their quality was not sufficient control. A fact, the volatile organic and other residual solvents impurities have never been benifited to the customer, also their will be dangerous if these compounds are retain in drug.

An automated headspace GC method was developed to analyse some organic volatile impurities (OVI) and other residual solvents i.e., methylene chloride,

chloroform, benzene , trichloroethylene and 1,4-dioxane in drug. These organic compounds are rather small molecular weight and low boiling point so many industries extensively used as a solvent or diluent for penicillin drug , rubber, oil, fat, wax, varnish, paint, plastic, and raw material in the manufacturers of other chemical products especially fluorocarbons (3). These organic compound are a group of volatile compounds that has been classified as priority pollutant by EPA (4,5). The toxicity and metabolism of these organic have been studied by many investigators and shown to be mutagenic substances (3,4,6). These organic compounds are relatively unreactive and biologically non-degradable. Hence, the human receive drugs which were contained these compounds for a long time, its may remain in the body and it cause dangerously. The primary health effect is central nervous system depression with anesthesia, inebriation, narcosis and subsequently health effect is heptatotoxicity, nephrotoxicity and carcinogenicity (5). Volatile compounds have been rapid adsorbed on oral and inhalation. They can affect the membrane of mitochondria and endoplasmic recticulum an inhibit enzymes in Krebs'cycle. The results of performing lead to fatty liver, kidney damage and contribular necrosis. Hence, the concentration of these organic in drug are determined to protect harmful effects so the United States Pharmacopoeia (USP) recommend that methylene chloride, benzene, trichloroethylene and 1,4-dioxane should not exceed 100 ppm (μg/mL) and chloroform should not exceed 50 ppm in drug (8).

Analytical methods for determination of trace amounts of these organic compounds in drug generally require a preconcentration step prior to gas chromatographic (GC) analysis. From 1990, there have many country that interested in the preconcentration techniques and there are a lot of papers to developed a several preconcentration techniques for the optimum method i.e., solvent extraction,

direct aqueous injection, purge and trap, headspace technique (8),etc. Each method has advantages and disadvantages which relate to equipment requirements, detection limits, sample matrix, sample volume, analysis speed and complexity. The method that the United States Environmental Protection Agency (EPA) recommended for the determination of trace volatile organic priority pollutant in aqueous solution was purge and trap technique (9) . This method can detect volatile organic compounds in low concentration level of part per billion (ppb) or part per million (ppm) for the suitable condition (9-11) but it necessary required complex equipment to separate the organic from water and bring about serious problem drawback to the qualitative and quantitative analyses i.e., the loss of the organic constituent, the interference of impurities in adsorbent trap or stripping gas, poor resolution and peak tailing caused by a too long stripping time, the large of water passing the adsorbent and consumption (10,12).

Literature Reviews

The headspace technique used in the analytical work was accomplished more than 30 years ago and the first application of the headspace analysis for the quantitative determination of organic substances was the investigation on the enzymatic generation of the volatile components of raspberries(13). This technique was a method in many methods that the United States Pharmacopoeia (USP) used for determination of Organic Volatile Impurities (OVI) in several pharmaceuticals. The USP listed a limit test by as chromatography for five residual volatile compounds in active ingredients, exicipient materials and pharmaceutical products that can cause irreversible toxic effects. These five compounds were methylene chloride, chloroform, benzene, trichloroethyene and 1,4-dioxane, and the proposed limited were 100, 50. 100, 100 and 100, respectively (8).

The first successful application of headspace analysis to the determination of some volatile poisons such as ether, acetone, ethanol, paraldehyde and chloroform in blood was described by Curry and co-worker (14). A 2 mL of blood sample was equilibrated in the closed apparatus that had a total volume of approximate 7 mL and 1 mL of the equilibrium gas phase was analysed by gas chromatography. This success contributed to the widening acceptance of this method and promoted the development of the appropriate instrumentation.

Kojima (15) used the headspace technique to determine ethanol in drug which various tinctures samples. The sample was dissolved in n-propanol (as the internal standard) at a concentration of 1.0 to 5.0% (V/V). A portion was equilibrated at 50°C and 2mL of the headspace gas manually injected onto a column

of either 5% polyethyleneglycol 20m on chanelite CS 60-80 mesh or 5% Reoplex 400 on Chromosorb W 60-80 mesh with a flame ionization detector. He showed that five tinctures had the ethanol concentration range from 65 to 90% and the results were good agreement with those obtained by conventional methods to then he expanded the headspace method for the determination of ethanol in a variety of liquid and solid drug forms where the content ranged from 2 to 73%.

Litchman and Upton (16) reported the determination of triethylamine in streptomycin sulfate and in methacycline hydrochloride to levels as low as 0.05%. A sample was treated with 1M sodium hydroxide solution at 60 °C for 1 hour. A headspace sample was manually withdrawn and analyzed on a polystyrene column at 160 °C using a flame ionization detector. The level of triethylamine was found in the ranged 0.15 to 0.36% for streptomycin sulfate and 0.06 to 0.13% for methacycline hydrochloride. Recoveries were better than 94% and the precision of the determination, based on five replicate weightings of sample that was 2% for streptomycin sulfate and 5% for methacycline hydrochloride.

Romano and co-worker (17) developed a headspace method for the analysis of residual ethylene oxide in sterilized materials. A weighed portion of sample was heated at 100 °C for 15 minutes. Duplicate 5.0 μL headspace samples were removed with a gas-tight syringe and injected onto a column Porapak R. A flame-ionization detector was used a specific and sensitive method for determined of ethylene oxide at a ppm level in sterilized materials. The method was simpler than other reported gaseous method since no elaborate gas extraction apparatus was required. It offers significant advantages over liquid extraction of the sample since no interfering solvent peaks were observed, the method was more rapid (50

samples may be analyzed per 8 hours using automatic injection), and the sensitivity was greater (detection limit of 50 ppb). The method was applicable to a variety of materials.

Application of an automated headspace procedure for trace analysis by gas chromatography was described by Kolb (18). Headspace gas chromatography (HSGC) was a useful method for determined trace compounds in samples that cannot be handled with a syringe or involve too many difficulties. But HSGC requires expensive calibration, an automated instrument was essential for routine work and also eliminates problems related to contamination and sample carry-over. Practical examples were given for the sensitivities that can be achieved with flame-ionization, electron-capture and nitrogen-specific detector. This techniques can quantitative analysis of liquid and solid samples and emphasis was placed on the quantitative determination of monomers in polymer. A new method called "discontinuous gas extraction" which was useful for the quantitative of volatile compounds in solid matter.

The determination of trace levels of dimethylnitrosamine (DMNA) in pharmaceuticals containing aminophenazone has been reported by Perkin Elmer (19). A tablet was pulverized and suspended in a headspace vial in a solution of 2M H_2SO_4 (to remove volatile amines) to which had been added solid potassium sulfate (for salting-out effect). The vial was heated at 120°C for 1 hour. Headspace gases were injected onto a 5% Carbowax 20M on Chromosorb G , AW-DMCS column and detected using a nitrogen phosphorous detector. Calibration was carried out by the method of additions. The detection limit using this method was 20 to 40 ppb. A typical level of DMNA found was 75 ppb.

Bicchi and Bertolino (20) analysed a variety of pharmaceuticals for residual solvents. Samples were equilibrated directly or dissolved in a suitable solvent to be determined. Equilibration condition were 90 or 100 °C for 20 minutes. The chromatographic phase chosen was a column packed with Carbopack coated with 0.1% SP1000. Residual ethanol in phenobarbital sodium was determined by a direct desorption method. An internal standard, t-butanol , was used. Typically, 0.44% of ethanol was detected (compared to a detection limit of 0.02 ppm). The standard deviation of six determination was 0.026. Pharmaceutical preparations which were analyzed by the solution method included lidocaine hydrochloride, calcium pantothenate, methyl nicotinate, sodium ascorbate, nicotinamide, and phenylbutazone. Acetone, ethanol and isopropanol were determined with typical concentrations ranging from 14 ppm for ethanol to 0.27% for acetone. Detection limits were as low as 0.03 ppm (methanol in methylnicotimate).

Methylene chloride in a tablet was analyzed by Kolb (21) using the multiple headspace extraction method (three steps). The sample was analyzed as a dry powdered material using a glass capillary column, Marlophen 87, isothermally at 35 °C. A concentration of 35 ppm was found, which was in reasonable agreement with that obtained (40 ppm) when the sample was dissolved in water and analyzed by normal headspace analysis using the method of standard addition for quantitation. The extrapolated total area (four step) was similar to the total area value obtained using the two step MHE process.

Boyer and Probecker (22) determined organic solvents in several pharmaceutical forms using headspace sampler. The samples were heated at 90 °C

for 10 minutes to establish equilibrium. Headspace samples were injected onto a chromosorb 102 column. Ten injections of mixed ethanol-acetone standard using methanol as the internal standard gave better precision than manual injections as measured by the relative standard deviation, 1.63 and 2.48% for ethanol and acetone, respectively, using the sampler as compared to 4.77 and 3.93% by manual injection. Methods were reported for acetone and ethanol in dry forms such as tablets and microgranules, ethanol of crystallization in raw materials, and ethanol in syrups. Denaturants such as n-butanol and isopropanol in ethyl alcohol were determined by using ethyl acetate as the internal standard.

Nakajima and Yasuda (23) have successfully applied headspace gas chromatography to the analysis of 1-methanol, d,l-camphor, and methyl salicylate. Sample portions with ethyl salicylate as internal standard were added in 1 mL measures to 50 mL of 30% ethanol in a 100 mL vial, which was subsequently sealed. After shaking for 30 minutes, the vial was equilibrated in a constant temperature water bath at room temperature for 30 minutes. Headspace gas (1mL) was withdrawn with a gas-tight syringe and injected onto a 1.5 m x 3mm Gaschrom Q (80-100 mesh) column coated with 2% DCQF-1 and 1.5% OV-17. Standard solutions were analyzed in a similar manner. Recoveries were better than 97% in a variety of preparations. This method could not be applied to samples containing castor oil.

Haky and Stickeny (24) used automated gas chromatographic method for the determination of residual solvents in bulk pharmaceuticals. Samples to be determined were prepared by dissolution in benzyl alcohol and analyzed by using autosampler gas chromatograph equipped with a flame ionization detector. A 6 in. x

2 mm I.D. glass column that the first 2 in. of column (injector end) were packed with 3% OV-101 on 100-110 mesh Anachrom Q (analabs, North Haven, CT, U.S.A.) and the rest of the column was packed with 3% SP1500 on 80-120 mesh Carbopack 13 (Supelco, Bellefonte, PA, U.S.A). Haky and Stickney compared the accuracy and precision of the two methods that used for quantitating the amount of a wide variety of residual solvents and other volatile contaminants in bluk pharmaceuticals by using the autosampler gas chromatogrph, between standard addition and external standard. The comparison of results obtained from the standard addition method is not a very efficient technique for analyses of multiple samples. Generally, then, the use of external calibration curves for multiple samples of determination appears to give acceptably accurate results which are comparable to those obtained by standard addition methods.

Wampler (25) used dynamic headspace analysis to determine the presence of three types of volatile materials in pharmaceuticals; naturally occurring volatile in raw materials, processing agents, and decomposition products due either to the chemical instability of the compound or to bacterial action. Thermal desorption was accomplished using a Chemical Data Systems Model 320 sample concentration with Tenax traps. A capillary gas chromatograph equipped with a 50m x 0.25mm fused silica capillary column (SE-54) and a flame-ionization detector was utilized. Aspirin samples which was either past their prime or which have been improperly stored degrade to give acetic acid which produces a vinegary small. One crushed aspirin tablet was subjected to analysis at desorption temperatures ranging from room temperature to 70°C. The most intense peak in the chromatogram was toluene which was used as a solvent in the manufacturing process. The amount of toluene was quantitated using as internal standard. One microlitter of benzene (1% in

methanol) was added to powder sample before analysis. A toluene concentration of 0.0086% was found. Spiked samples showed the recovery of toluene to be 95%. The method gave a relative standard derivation of 1.5%.

Letavernier (26) analysed residual solvents from processing operation in film-coated tablets. They also determined solvents which arise from migration from packaging materials into pharmaceutical products. A weighed sample (35 mg to 1 g) was heated and volatile swept with nitrogen gas onto a Tenax trap refrigerated with liquid nitrogen. After a specified time, the Tenax trap was rapidly heated (maximum of 300 °C) to desorb volatile which were swept onto a Porpak Q column. Cyclohexanone could be detected at a level of 0.2 mg/g of sample.

A statistical evaluation of methods using headspace gas chromatagraphy for the determination of ethylene oxide was performed by Kaye and Nevell (27). Two method determinate of levels of ethylene oxide were external standard method using ethylene oxide in are and internal standard method using a dilute aqueous solution with acetone as the internal standard. The external standard method used Carbowax 20m (10%) column at 120 °C and the internal standard method used a chromosorb 101 (80-100 mesh) column at 125 °C. Sealed vial was placed in a heated block at 120 °C for 10 minutes. For the external standard method, each vial containing wiggled plastic sample was placed in an air-circulation oven at 120 °C of 15 minutes. A sample of headspace was taken immediately often removing the vial from the oven. Studies were conducted on hiqh-level (90μg/g) and low level (10 μg/g). The two methods gave similar results. Determinations using either method were reliable to within 3% for residual levels of 90 μg/g or 7% for residual levels of 10μg/g.

Chen and co-worker (28) proposed changes to Method I for Organic Volatile Impurities <467>. The proposed modifications offer several advantages over the draft Method I (direct injection). A single DB-624 fused silica column provides separation of residual solvent in bulk pharmaceutical chemicals with superior efficiency and long-term stability of repeated injection of aqueous samples. The excellent solubilities of a greater spectrum of bluk drug substances in water or DMSO also eliminates the necessity of the standard addition procedure as outlined in draft Method I without incurring the inherent contamination problem with toluene and other oxidation products or the problematic generation of artifactual benzene when using benzyl alcohol for sample preparation. The five years ago they have found produce for the analysis of residue solvents that it to be rugged, liable and free of the many problems associated with using benzyl alcohol and the DB-5 Megabore column as specified in the draft Method I.

Bergern and Foust (29) were suggested the improvements to analysis of Organic Volatile Impurities <467> Method I (direct inject) from USP general chapter. There are five improvements that recommend to make Method I acceptable. (1) Specify instrumental parameters that are appropriate for the solvents. Example, if water is used as the solvent, the injection port at 180 $^{\circ}$C is not appropriate but the temperature at 70 $^{\circ}$C was suitable more than, because of the water vaporizes more slowly at 70 $^{\circ}$C and the data are more reproducible. (2) Provide a choice of suitable solvents for sample preparation, methanol were interfere OVI that interested , benzyl alcohol reacted with the drug so DMSO were suitable solvents. (3) Specify the analytical system more completely i.e., column ,

detector and gas. (4) Modify the requirements for chloroform and (5) Allow for optional mass spectrometric detection.

Kasowski and co-worker (30) studied Organic Volatile Impurities in drug using method I from USP and determined the system suitability by compared four solvent, methanol , benzyl alcohol, water and dimethyl sulfoxide. The optimization of injection port temperature and most of the precision for aqueous solution was 70°C, while nonaqueous solution was 140°C. Moreover they found that methanol and benzyl alcohol were interfere in the region of interest but dimethyl sulfoxide was found to be free of interfering. When using the aqueous solution, a percent relative standard deviation (%RSD) were 1.6-9.1 while using DMSO %RSD were 0.7-12.0 and 5% phenylmethylsiloxane was using for determination.

Dennis and co-worker (31) determined Organic Volatile Impurities <467> and other residual solvent in drug by using automated headspace technique. Automated headspace sampling coupled with capillary gas chromatographic separation provides an ideal approach for routine control of volatile in pharmaceutical products. The sample preparation for method I was using water or 0.1N hydrochloric acid or 0.1M pH 9 ammonium carbonate buffer and add 5 mL of water or other appropriate diluent to a headspace vial containing 1g of anhydrous sodium sulfate. The vial was sealed and heated for 60 mintues at 85°C, then 2 mL of headspace gas were injected into gas chromatograph equipped with a flame ionization detector. Bulk pharmaceutical concentration of this analysis was from 0.2-8.0 ppm and the percent relative standard deviation range 0.6-2.9.

Albert and Hans-Jurgen (32) compared the static headspace analysis (method IV) and direct liquid injection (method I) for analysis of volatile compounds in excipient materials by gas chromatograph equipped with a flame ionization detector (FID). Standard of five solvents (methylene chloride , chloroform, benzene, trichloroethylene and 1,4-dioxane) prepared in DMSO and diluted with "organic free " water to yield analyse concentrations between 0.5 and 10.0 μg/mL (0.25 and 5.0 μg/mL for chloroform). Samples were prepared by dissolving 20 mg of excipient or active ingredient in 1 mL of organic-free water. For Method I , a sample volume of 2 μL was injected by spitless mode (purge time 1.0 minutes). For Method IV, 5 mL of solution were heated at 85°C for 60 minutes and 1mL of headspace sample was injected by split mode (9:1). Both methods gave acceptable results and precision (RSD range 4.0-10.3 %), but the headspace method more promising for analysis of volatile in pharmaceutical products than because of the reproducibility , sensitivity and the detection limit better than direct liquid injection. For method I, the detection limit of methylene chloride ,chloroform, benzene, trichloroethylene and 1,4-dioxane were 50, 33, 5, 12 and 9 ppm, respectively, while the method IV were 1.3, 2.2, 0.2, 0.6 and 22.2 ppm, respectively.

Firor and Wyliz (33) used headspace gas chromatography for the analysis of five solvents (methylene chloride, chloroform, benzene, trichloroethylene and 1,4-dioxane) in pharmaceutical products. A 100 mg of the sample is accurately weighed into a headspace vial to which 5 mL of water sodium sulfate are added. Replicate analysis of unshaken samples, heated for 60 minutes, gave area count RSDs range 1.5-3.0 %. Heating samples for 10 minutes with shaking resulted in faster equilibration and even better precision (RSDs range 0.5-1.3 %). The detection limit

of methylene chloride, chloroform, benzene, trichloroethylene and 1,4-dioxane were 0.16, 0.35, 0.03, 0.15 and 3.00 ppm, respectively.

Kumar and Gow (34) described an automated headspace GC method for analyses residual solvent in drug. The sample is dissolved in dimethylformamide solvent and the equilibrium headspace gas formed at 60°C is analyzed by using a megabore capillary column. Quantification is performed by the standard addition technique to eliminate any possibility of matrix effects. This method is sensitive , precise and accurate.

Hypothesis

The headspace analysis technique seems to be an attractive alternative for quantitative analysis of organic volatile impurities and other residue solvents in drug because of it is a simple, rapid , sensitive, reliable method and no preconcentration step (11,35-39). This method is based on the sample containg organic compounds in the close system which, equilibrate between the sample phase and the headspace gas phase. The gas phase will be analysed by GC. The distribution of compounds between the two phase depends on temperature, vapor pressure of each compound, sample matrix influencing on compound activity coefficients and phase ratio of the headspace gas of the liquid volume in the vial. The advantage of this technique can be summarized as follow.(10-11)

1. A headspace is an easy to isolate and concentrate many volatile organic compounds in water, soil, drug or sediment for gas chromatographic analysis. The sample has not to be vaporized for the gas chromatographic analysis due to low concentration of the components already exist in the vapor phase.

2. No overloading or contamination of the GC column with high boiling or non-volatile material occurs.

3. There is also no complicated equipment required for sample preparation that indicates and minimum detection limit in ppb level.

4. It is an comfortable method .

The Purpose of the Study

The headspace analysis technique was developed for the determination of organic volatile impurities and other residual solvents in drug i.e., methylene chloride, chloroform, benzene, trichloroethylene and 1,4-dioxane. The various parameters which affect to the sensitivity that would be studied and evaluated for the optimum condition of this technique. The parameter studies were :

1. The temperature for equilibrating sample i.e., 60° ,70 °,80 ° and 90 ° centigrade.
2. The equilibration times of the sample i.e., 10, 20, 30,..., etc. minutes.
3. The liquid to gas phase ratios i.e., 2:8, 4:6, 5:5, 6:4 and 8:2 .
4. The salting out effect i.e., no salt and add anhydrous sodium sulfate.

In addition, the accuracy and precision of this technique were also studied and evaluated prior to use it in the analysis of these compounds in the real drug samples. The gas chromatograph equipped with flame ionization detector (FID) and electron capture detector (ECD) , and interfaced with autoheadspace instrument that was used for the study. The gas chromatograph equipped with mass spectrometer was used for confirm drug samples.