CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

Insecticide use in agriculture has progressively increased after World War II leading to increased world food production.[1] They are widely used in fruit and vegetables because of their susceptibility to insect and disease attacks. Therefore, residues of insecticide could affect the ultimate consumers especially when these commodities are freshly consumed. The total dietary intake of insecticide residues that remain on agricultural commodities are known as carcinogens and/or toxins, and therefore, it is desirable to reduce these residues. The levels of pesticide residues are controlled by the maximum residue limits (MRLs), which are established by each country and sometimes cause conflicts because residue levels acceptable in one country could be unacceptable in another. The required rates of application may very, under different agricultural and climatic conditions, from country to country, and between regions of the same country.[2] Insecticides can be classified into four chemical groups: organophosphorus, organochlorine, carbamate and synthetic pyrethroids.[3] In Thailand, organochlorine insecticides are now rarely used. They have been replaced by synthetic pyrethroids such as λ-cyhalothrin, cyfluthrin, deltamethrin, cypermethrin and fenvalerate for the treatment of vegetable crops. Nowadays, synthetic pyrethroids are increasingly being used for insect control on field crops because of their advantageous environmental properties such as short field life and relatively low mammalian toxicity. Nevertheless, pyrethroid residues in vegetables after application to the crops still pose risks to human health and other species.[4]

The principal sources of insecticides in crops, soil, water and food commodities, are

1. Carry-over from insecticide application to soil or to growing crops
2. Leaching of pesticides, herbicides or insecticides into ground water
3. Drift of the insecticides from adjacent field
4. Translocation of soil applied insecticide into growing crops
5. Disposal of insecticides in streams, rivers and lakes
6. Effluents of insecticide industry in rivers and streams and into soil which may be translocated in crops.[5]

In the Thailand countryside where arable lands are leased to farmers, the overuse of insecticides and random mixture of insecticides are serious problems in vegetable production. Furthermore, food poisoning accidents have been reported among migrant workers and farmers from province to province because of insecticide residues in vegetables.[6]

1.2 Cabbage [7,8]

Scientific classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Brassicales
Family	:	Brassicaceae
Genus	:	*Brassica*
Species	:	***B. oleracea***
Scientific name	:	*Brassica oleracea* L. var. *capitata* L.
Common name	:	Cabbage

Cabbage can be divided two groups

1. Common or white cabbage
2. Red cabbage (gets its color from a pigment called anthocyanin as do all red, blue, and purple plants.)

Cabbage is one of the most important vegetable crops in Thailand. The family of cabbage includes broccoli, brussels sprouts, cauliflower, kohlrabi, kale and collards. Cabbage is a hardy vegetable that grows especially well in fertile soils. There are various shades of green available, as well as red or purple types. Head shape varies from the standard round to flattened or pointed. Most varieties have smooth leaves, but the Savoy types have crinkly textured leaves. They is easy to grow if you select

suitable varieties and practice proper culture and insect management. Always regarded as a good source of vitamins, cabbage recently has been shown to have disease-preventive properties as well. Cabbage contain very high levels of antioxidant and anticancer compounds. Dithioltiones and glucosinolates enhance antioxidant and detoxification effects in the body. Isothiocyanates inhibit tumor growth. Coumarins block cancer causing compounds. Various phenols in cabbage prevent the formation of carcinogens and enhance detoxification enzymes. Other research has suggested that the compounds in cabbage and other crucifers can protect the eyes against macular degeneration.

1.3 Previous analytical method for pyrethroid

Most determination of pyrethroid compounds have been developed using chromatographic techniques because of their high resolution capacity and the availability of selective detectors, mainly gas chromatography (GC) with electron capture detector (ECD) because of the halogen atoms in their chemical structure.[9] GC is a separation technique widely used in the analysis of insecticide residues because of its high separation power and the variety of sensitive and selective detectors, such as, ECD, nitrogen–phosphorus detector (NPD), and mass spectrometry (MS) that can be used.[10]

The basic units of insecticide residues analysis are:

1. Sampling.
2. Extraction of insecticide from the sample.
3. Clean-up/ derivatisation of residues from the sample.

4. Indentification and quantitative determination of the insecticide residue.[11]

Sample preparation is an important step in insecticide residue analyses, which is time consuming and requires the used of large amounts of organic solvents. The insecticides are extracted by different organic solvents such as acetone, acetonitrile, or ethyl acetate[12] which usually provide high recoveries of insecticides over a wide range of polarity, followed by partitioning by ethyl acetate–cyclohexane or dichloromethane–petroleum ether.[13] Many laboratories implement multiresidue screening methods using solvent extraction of the insecticides from fresh vegetables followed by GC with various detectors. Acetone, acetonitrile, dichloromethane, ethyl acetate and *n*-hexane are commonly used for insecticide residues extraction, followed by filtration, evaporation to near-dryness and redissolution in few microliters of organic solvent.[9] In most of the methods the commodity is spiked with the insecticide prior to extraction and the percentage recoveries tested, such an approach may prove the validity of the clean-up method but it does not evaluate satisfactorily the effectiveness of the extraction proceduce.[11]

Previous analytical methods used for determination of pytethroid insecticide upon the level of sensitivity required and examples of methods for pyrethroid residue determinations in different samples are shown in Table 1.1

Table 1.1 Reported procedures for pyrethroid residues determination in different samples

Sample matrix	Preparation	Analytical method	Detection limit	Recovery (%)	Reference
Fruits and vegetables	Solvent extraction (acetone-dichloromethane 4:3)	GC-ECD	-	80-118	[14]
Fruits and vegetables	Solvent extraction (acetone)	GC-ECD	-	-	[15]
Gherkin, eggplant, cabbage and garden peas	Matrix solid phase extraction (MSPD)	GC-ECD	5.1-91.5 ng/g	92-113	[16]
Grape, orange, tomato carrot and green mustard	Strong anion exchanger/primary secondary amine (SAX/PSA) clean-up	GC-ECD and GC-MS	-	70-120	[2]
Apples, oranges, pears, cabbage and tomatoes	Solvent extraction (methanol)	GC	-	70.4-110.0	[17]

Table 1.1 (continued)

Sample matrix	Preparation	Analytical method	Detection limit	Recovery (%)	Reference
Fruits and vegetables	Solvent extraction (acetonitrile)	GC-ECD	0.004-0.028 mg/kg	83.8-112.8	[18]
Grains	Solvent extraction (acetonitrile-water 2:1)	GC-ECD	0.01-0.08 mg/kg	83.8-112.8	[18]
Fruits and vegetables	Gel permeation chromatography (GPC)	GC-ECD and GC-MS	< 0.01 mg/kg	70-108	[19]
Soil	Microwave-assisted extraction (MAE)	GC-ECD	-	89.4-97.8	[20]
Beans and green tea leaves	Solvent extraction and cleaned up on a Florisil column	GC-ECD and GC-MS	-	60.0-103.5	[21]

Current pyrethroid residue screening methods usually require extraction with a polar solvent, followed by liquid-liquid partitioning. The sample is concentrated by evaporation, often with a solvent exchange, and subjected to clean-up before the final determination. Extraction solvents such as acetonitrile, acetone, *n*-hexane-acetone (9:1), acetone-methanol, cyclohexane-chloroform (4:1) and ethanol have been used for pyrethroid analysis.[16]

1.4 Classification of insecticide

Insecticides can be classified based on functional groups in their molecular structure (e.g. inorganic, organonitrogen, organohalogen or organosulfur compounds) or their specific biological activity on target species (e.g. insecticides, fungicides, herbicides, acaricides, etc.).[22]

1.4.1 Organophosphorus

Organophosphorus insecticides are synthetic in origin and are normally esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids. They are used to control insect vectors which are found in food and commercial crops, and infestations in domestic and commercial buildings, and in man or domestic animals. The majority of organophosphorus insecticides are liquid and have different vapour pressures at room temperature. The compounds used for agricultural purposes are available mainly as emulsifiable concentrates or wettable powder formulations for reconstitution as liquid sprays, but also as granules for soil applications. A limited number are also available as fogging formulations, smokes, impregnated resin strips for use indoors, and as animal or

human pharmaceutical preparations. Dispersion of spray droplets by wind is possible, but in general, only small amounts are likely to be dispersed in this way.[23]

All organophosphorus insecticides are subject to degradation by hydrolysis, yielding water-soluble products that are believed to be non-toxic at all practical concentrations. The toxic hazard is therefore essentially short-term in contrast to that of the persistent organochlorine insecticides, although the half-life at neutral pH may vary from a few hours for dichlorvos to several weeks for parathion. At the pH of slightly acidic soils (pH 4 to 5), these half-lifes will be extended many times. However, constituents of soil and of river water may themselves catalyse degradation.

1.4.2 Organochlorine

Organochlorine is an organic compound containing at least one covalently bonded chlorine atom. Their wide structural variety and divergent chemical properties lead to a broad range of uses. The simplest form of organochlorines are chlorinated hydrocarbons. These consist of simple hydrocarbons in which one or more hydrogen atoms have been replaced with chlorine. Many organochlorines have significant biological activities. For example, many powerful and effective insecticides are organochlorines.[24]

There are three major types of organochlorine insecticides:

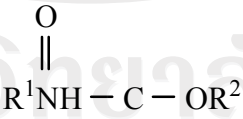
1. Dichlorodiphenylethanes: such as DDT, DDD, TDE, methoxychlor, rhothane, methlochlor, perthane, dicofol.
2. Chlorinated Cyclodienes: such as aldrin, dieldrin, endrin, heptachlor, chlordane, endosulfan.
3. Chlorinated Benzenes and Cyclohexanes: such as lindane, toxaphene,

mirex, HCB, HCH, chlordecone.

Organochlorine insecticides are neurotoxins that have high lipophilicity, are very hydrophobic, and are chemically stable. Metabolic degradation in target and non-target organisms or environmentally by either chemical, photolytic, or microbial processes is slow. As a result, organochlorine insecticides are persistent in the environment and have a long half-life. Many organochlorines have significant toxicity to animals, including humans. Dioxins, produced when organic matter is burned in the presence of chlorine, and some insecticides such as DDT are persistent organic pollutants which pose dangers to the environment. Chlorinated solvents, when not handled and disposed of properly, present problems with groundwater pollution. Some organochlorines such as phosgene have even been used as chemical warfare agents. However, the presence of chlorine in an organic compound does not in any way ensure toxicity.

1.4.3 Carbamate

Carbamates are *N* -substituted esters of carbamic acid. Their general formula is:



where R^2 is an aromatic or aliphatic moiety. Three main classes of carbamate insecticides are known:

(a) Carbamate insecticides; R^1 is a methyl group

(b) Carbamate herbicides; R^1 is an aromatic moiety

(c) Carbamate fungicides; R^1 is a benzimidazole moiety

Carbamate ester derivatives are crystalline solids of low vapour pressure with variable, but usually low, water solubility. They are moderately soluble in solvents such as benzene, toluene, xylene, chloroform, dichloromethane. In general, they are poorly soluble in nonpolar organic solvents such as petroleum hydrocarbons but highly soluble in polar organic solvents such as methanol, ethanol, acetone, dimethylformamide. Carbamates insecticides act by a similar mechanism to organophosphorus insecticides, but have a shorter duration of action. They are widely used, and have varying degrees of toxicity. The toxicity of carbamates for wildlife is low, but exceptions exist. This means that, in order to judge the impact of carbamates on the organisms in the environment, the information on the individual carbamates should be referred to. As a rule, birds are not very sensitive to carbamates while bees are extremely sensitive.[25]

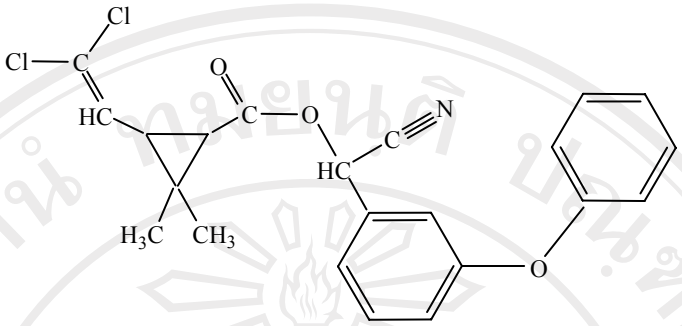
1.4.4 Pyrethroid

Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers (the term “pyrethrum” is often used as a generic term to describe either natural pyrethrins or synthetic pyrethroids). The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids. These acids are strongly lipophilic and rapidly penetrate many insects and aralyze their nervous system. Both pyrethrins and synthetic pyrethroids are sold as commercial insecticides used to control pest insects in agriculture, homes, communities, restaurants, hospitals, schools, and as a topical head lice treatment.

Various formulations of these insecticides are often combined with other chemicals, known as synergists, to increase potency and persistence in the environment.[3]

Pyrethroids are a relatively newly developed group of insecticide widely used in the control of agricultural, forestry and stored products pests. Considering that they are harmless for mammals under normal circumstances and toxic metabolites are absent, their MRLs are, in general, higher than those of organophosphorous, organochlorine or carbamate insecticides. Notwithstanding their relatively low toxicity, the residue analysis of pyrethroids in crops, foods and environment matrices is of importance in agricultural and environmental sciences.[26] While chemically and toxicologically similar, pyrethrins are extremely sensitive to light, heat and moisture. In direct sunlight, half-life that can be measured in hours. However, the pyrethroids, the synthetic analogues of naturally occurring insecticides, were developed to capture the effective insecticidal activity of this botanical insecticide, with increased stability in light, yielding longer residence times. Pyrethroids are axonic poisons that work by keeping open the sodium channels in the neuronal membranes of insects. The sodium channel is a small hole through which sodium ions are permitted to enter the axon and cause excitation. When left open, nerves cells will produce repetitive discharges and eventually cause paralysis. Pyrethroids are usually combined with piperonyl butoxide, a known inhibitor of key liver enzymes. This prevents the liver enzymes from clearing the pyrethroid from the body of the insect, and assures the pyrethroid will be lethal and not merely a paralyzing agent. Combined, pyrethroids are toxic to most beneficial insects like bees.

1.5 Cypermethrin insecticide



Figture 1.1 Structure of cypermethrin

Cypermethrin [(*R*,*S*)-alpha-cyano-3-phenoxybenzyl (1*RS*)-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] was first synthesized in 1974 and first marketed in 1977 as a highly active synthetic pyrethroid insecticide effective against a wide range of pests in agriculture, public health, and animal husbandry. In agriculture, its main use is against foliage pests and certain surface soil pests, such as cutworms because of its physical and chemical properties, it is not recommended against soil-borne pests below the surface.[27] This insecticide is highly stable to light and at temperatures below 220 °C. It is resistant to acidic rather than alkaline media with an optimum stability at pH 4. Cypermethrin hydrolyses under alkaline conditions in a similar way to simple aliphatic esters. Dilute aqueous solutions are subject to photolysis, which occurs at a moderate rate.[28] The properties of cypermethrin as shown in Table 1.2

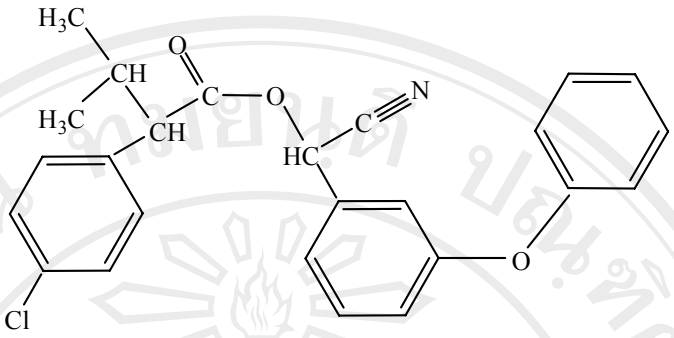
Table 1.2 Properties of cypermethrin

Property	Information
Molecular formula	$C_{22}H_{19}Cl_2NO_3$
Common name	Cypermethrin
Common trade names	Ammo, Barricade, Polytrin, CCN 52
Molecular weigh	416.32
Physical state	Solid
Color	Yellowish brown
Odor	Odorless
Boiling point	220 °C
Melting point	60-80 °C
Solubility in other solvents	- 103 g/L in hexane at 20 °C - soluble in acetone, cyclohexane, ethanol, xylene and chloroform.
Vapor pressure	1.4 x 10^{-9} mmHg at 20 °C
Half-life	- 8 weeks (in soil) - 100 days (in water) - 8 – 16 days (in direct sunlight)

Toxicity of cypermethrin

Cypermethrin is a moderately toxic material by dermal absorption or ingestion. Symptoms of high dermal exposure include numbness, tingling, itching, burning sensation, loss of bladder control, incoordination, seizures, and possible death. Symptoms of high dose ingestion include nausea, prolonged vomiting, stomach pains, and diarrhoea which may progress to convulsions, unconsciousness, and coma. The acute toxicity of cypermethrin for mammals is of a moderate order. The oral LD_{50} for the rat ranged from 200-4000 mg/kg body weight. Short-term and long-term toxicity studies on rats, mice, and dogs have shown effects on growth, the liver and kidneys, the nervous system, and the blood. A no-observed-adverse-effect level of 7.5 mg/kg body weight has been adopted by the Task Group. Cypermethrin is considered to be moderately toxic (oral male rat LD_{50} = 187 to 326 mg/kg, dermal rat LD_{50} = 1600 mg/kg) and like all pyrethroids, affects the central nervous system. In Thailand, cypermethrin is the fourth most common cause of pesticide-related illness in pest control operators. EPA classifies cypermethrin as a class C carcinogen. Studies in laboratory animals have shown exposure to cypermethrin to cause reproductive effects, including abnormal sperm and disruption of sex hormones. Cypermethrin should not be applied near water, because it is very toxic to fish and other aquatic organisms.

1.6 Fenvalerate insecticide



Figture 1.2 Structure of fenvalerate

Fenvalerate [(*RS*)-α-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate] is a mixture of four optical isomers which have different insecticidal activities. The 2-S *alpha*-S (or SS) configuration is the most insecticidally active isomer. Fenvalerate consists of about 23% of this isomer.[29] Fenvalerate is registered for use on a wide array of crops including cotton, soybeans, corn, vegetables, apples, peaches, pears and nuts, as well as a termiticide and insect repellent. Fenvalerate was first formulated for agricultural use in 1974, but was approved as a termiticide in 1987, as an alternative to the voluntarily cancelled cyclodiene termiticides. Fenvalerate is a non-leaching, non-volatile readily degradable synthetic pyrethroid insecticide which is similar in behavior to permethrin but remains in the soil for a longer time than permethrin.[30] The stability of fenvalerate in sunlight allows its application against a wide range of pests. Residue levels are minimized by low application rates and poor translocation characteristics in plants and in soil. The properties of fenvalerate as shown in Table 1.3

Table 1.3 Properties of fenvalerate

Property	Information
Molecular formula	$C_{25}H_{22}ClNO_3$
Common name	Fenvalerate
Common trade names	Pydrin, Belmark, Sumicidin, Fenvalethrin and S-5602.
Molecular weigh	419.91
Physical state	Solid
Color	Yellow or brown
Odor	Mild chemical
Boiling point	300 °C
Melting point	59-60 °C
Solubility in other solvents	- 134 g/L in hexane at 20 °C - > 500 g/L in acetone, chloroform, cyclohexanone, ethanol, methanol, and xylene is at 20 °C
Vapor pressure	0.037 mPa at 25 ºC
Half-life	- 14 days (on plants) - 4 to 15 days (in natural water) - 15 days to 3 months (in soil)

Toxicity of fenvalerate

Fenvalerate is a pyrethroid insecticide of moderate mammalian toxicity. In laboratory animals central nervous system toxicity is observed following acute or short-term exposure. Fenvalerate has applications against a wide range of pests. Residue levels are minimized by low application rates. Fenvalerate is most toxic to bees and fish. It is found in some emulsifiable concentrates, ULV, wettable powders, slow release formulations, insecticidal fogs, and granules. It is most commonly used to control insects in food, feed, and cotton products, and for the control of flies and ticks in barns and stables. This is insecticide may irritate the skin and eyes on contact, and is also harmful if swallowed. EPA classifies fenvalerate products as toxicity class II (I = most toxic, IV = least toxic), and include the word WARNING on all product labels. Some formulations are Restricted Use Pesticides, and may only be purchased or applied by certified applicators or persons under the direct supervision of a certified applicator. Fenvalerate is considered to be moderately toxic (oral rat LD_{50} = 486 mg/kg). Symptoms of poisoning through direct contact include dizziness, burning and itching (which is worsened by sweating and washing), blurred vision, tightness in the chest, and convulsions. When ingested by laboratory animals, symptoms of poisoning include muscle incoordination, tremors, convulsions, nerve damage, and weight loss. Fenvalerate is a strong eye irritant and a suspected endocrine disruptor. Sweden has banned the chemical for use in forestry following health related complaints from workers. Studies have found that immediate application of vitamin E to exposed areas can lessen the painful effects. Fenvalerate is extremely toxic to bees and fish, and is slightly toxic to birds.

1.7 Gas chromatography (GC)

GC is still the method of first choice for the analysis of pyrethrin and pyrethroid residues. Although there is a lack of pyrethroid specific detection systems, many pyrethroid insecticides (biphenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, flucythrinate, fluvalinate, permethrin) possess one several halogen atom(s) in the molecule of compound, which are sensitive to ECD. Some derivatization methods have been developed to create a sensitive group in the molecule of those pyrethroid insecticides which have not a halogen atom (allethrin, resmethrin, phenothrin, tetramethrin) or to improve the sensitive and the peak tailing situation in some halogenated pyrethroids. Generally, a detection method should have one to two orders of magnitude of sensitivity higher than the established MRLs of compounds of interest. Due to the relatively low chronic toxicity, the MRLs of most pyrethroids are generally established at several mg/L level, around one order of magnitude higher than that of organophosphate and carbamate insecticides.[3]

1.7.1 Theoretical principles

Chromatography is a separation method in which the components of a sample partition between two phases: one of these phases is a stationary bed with a large surface area, and the other is a gas which percolates through the stationary bed. The sample is vaporized and carried by the mobile gas phase (the carrier gas) through the column. Samples partition into the stationary liquid phase, based on their solubilities at the give temperature. The components of the sample separate from one another based on their relative vapor pressures and affinities for the stationary bed.

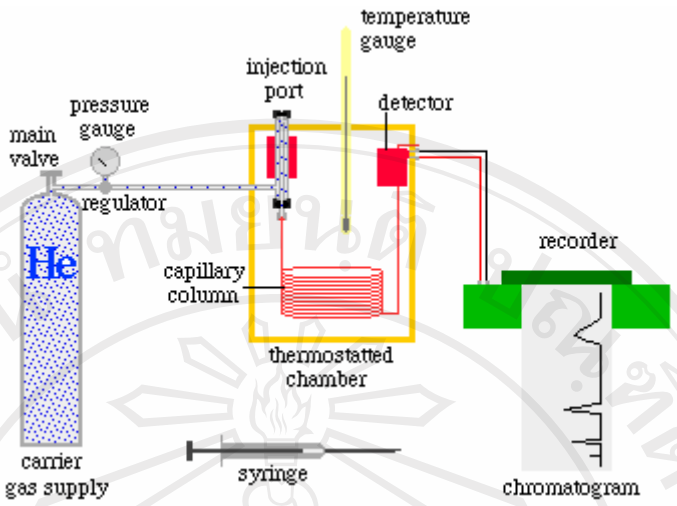
The various chromatographic processes are named according to the physical state of the mobile phase. Thus, in GC the mobile phase is a gas, and in liquid chromatography (LC) the mobile is a liquid. If the stationary phase is a solid, the GC technique is called gas-solid chromatography (GSC), and if it is a liquid, gas-liquid chromatography (GLC).

Advantages of gas chromatography

1. Fast analysis, typically minutes
2. Efficient, providing high resolution
3. Sensitive, easily detecting mg/L and often ppb
4. Nondestructive, making possible on-line coupling; e.g., to mass spectrometer
5. Highly accurate quantitative analysis
6. Requires small samples, typically μL
7. Reliable and relatively simple
8. Inexpensive

Disadvantages of gas chromatography

1. Limited to volatile samples
2. Not suitable for thermally labile samples
3. Fairly difficult for large, preparative samples
4. Requires spectroscopy, usually mass spectroscopy, for confirmation of peak identity



Figture 1.3 Schematic of a typical gas chromatograph [31]

1.7.2 Instrumental [32,33]

1.7.2.1 Carrier gas

The carrier gas acts as the mobile phase and transports the sample components through the column to the detector. The selection of the carrier gas is important because it affects both the column separation processes and the detector performance. Unfortunately the carrier gas that gives the optimum column performance may not be the one most suitable for the detector used. The carrier gas has to be inert to the column materials and sample components; the gas with the smallest diffusion coefficient will give the best column performance, for example, high molecular weight gases, nitrogen, carbon dioxide, argon, give lower flow rate than hydrogen or helium. Viscosity dictates the gas pressure required for a given flow rate. Impurity of carrier gas such as air and water vapour can cause sample reactions, column deterioration and affect detector performance. There are may carrier gases for GC with different properties, thus in practice a compromise is employed to choose a suitable carrier gas

for a detector used. For the TCD, helium is the most popular. While hydrogen is commonly used in some parts of the world (where helium is very expensive), it is not recommended because of the potential for fire and explosions. With the FID, either nitrogen or helium may be used. Nitrogen provides slightly more sensitivity, but a slower analysis than helium. For the electron capture detector, very dry nitrogen, or a mixture of argon with 5% methane is recommended.

1.7.2.2 Sample inlet

The purpose of the sample inlet is introduction of gaseous or vaporized liquid or solid sample into the carrier gas stream before entering the column. The gaseous sample is introduced through a gas sample valve. Most of the liquid and solid samples which can be dissolved in a solvent are injected through the injector. There are many techniques and injectors for handling the sample such as headspace, on column, split and splitless injection.

1.7.2.3 Columns

It is know that in the gas chromatographic system, the separation occurs in the column and everything in column can thus affect the separation efficiency. Normally, there are two types of column:

I. Packed column

Packed column are normally three, six, or twelve feet in length. The outside diameter is usually 1/4" or 1/8". Stainless steel is used most often, primarily because of its strength. Glass column are more inert, and they are often used for trace pesticide and biomedical samples that might react with the more active stainless steel tubing.

Packed columns are easy to make and easy to use. A large variety of liquid phases is available. Because the columns are tightly packed with small particles, lengths over 20 feet are impractical, and only a modest number of plates is usually achieved.

II. Capillary column

Capillary columns are simple chromatographic columns, which are not filled with packing material. Instead, a thin film of liquid phase coats the inside wall of the 0.25 mm fused silica tubing. Such columns are properly called “wall-coated open tubular” or simply WCOT columns. Since the tube is open, its resistance to flow is very low; therefore, long lengths, up to 100 meters, are possible. These long lengths permit very efficient separations of complex sample mixtures. Fused silica capillary columns are the most inter.

1.7.2.4 Detectors

The chromatographic detector measures the concentration of the solutes in the effluent and generates an electrical signal proportional to the sample concentration. The electrical analog output of the detector is amplified and then sent directly to a strip chart recorder or integrator to generate each peak of the chromatogram. At present, microcomputers have become a key part of many of instruments in order to compute electrical digital signals, store them and display the analytical result on a video screen or recorder. There are many types of GC detector that depend on particular applications. The most widely used detectors are the FID, TCD and ECD. Some analytical instruments can also be used as GC detector such as fourier transform infrared spectrometer (FTIR) and mass spectrometer (MS). Under this section only ECD are discussed.

Electron capture detector (ECD)

It is a selective detector that provides very high sensitivity for those compounds that "capture electrons" These compounds include halogenated materials like pesticides and, consequently, one of its primary uses is in pesticide residue analysis. It is an ionization-type detector, but unlike most detectors of this class, samples are detected by causing a decrease in the level of ionization. When no analytes are present, the radioactive ^{63}Ni emits beta particles as shown in equation (1):

$$^{63}Ni \rightarrow \beta^- \qquad (1)$$

These negative charged particles collide with the nitrogen carrier gas and produce more electrons.

$$\beta^- + N_2 \rightarrow 2e^- + N_2^+ \qquad (2)$$

The electrons formed by this combined process result in a high standing current when collected by a positive electrode. When an electronegative analyte is eluted from the column and enters the detector, it captures some of the free electrons and standing current is decreased giving a negative peak.

$$A + e^- \rightarrow A^- \qquad (3)$$

The negative ions formed have slower mobilities than the free electrons and are not collected by the anode. The carrier gas used for the ECD can be very pure nitrogen or a mixture of 5% methane in argon. When used with a capillary column some make-up

gas is usually needed, and it is convenient to use inexpensive as make-up and helium as the carrier gas.

A schematic of a typical ECD is shown in Figure 1.4. ^{63}Ni is shown as the beta emitter although tritium gas also been used; nickel is usually preferred because it can be used at a higher temperature (up to 400 °C) and it has a lower activity.

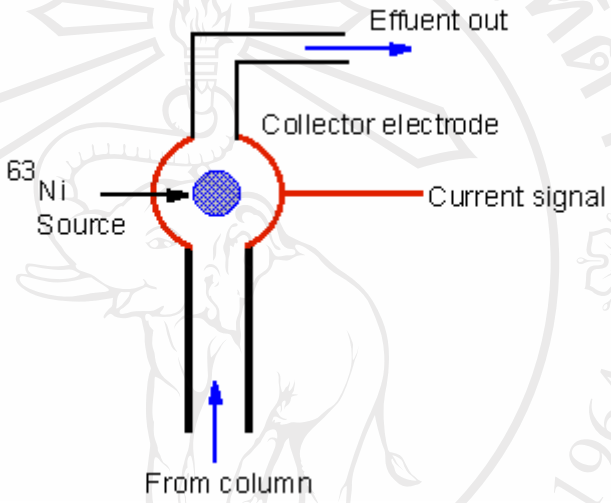


Figure 1.4 Schematic of a typical ECD [34]

The ECD is one of the most easily contaminated detectors and is adversely affected by oxygen and water. Ultrapure, dry gases, freedom from leaks, and clean samples are necessary. Evidence of contamination is usually a noise baseline or peaks that have small negative dips before and after each peak. Cleaning can sometimes be accomplished by operation with hydrogen carrier gas at a high temperature to burn off impurities, but dismantling is often required. ECD is most frequently selected in the pyerthroid residue analysis. It is most suitable for the determination of residue of those pyrethroids which possess the chloro group (biphenthrin, cyfluthrin, cypermethrin, fenvalerate, permethrin) or fluoro group (cyhalothrin, flucythrinate), bromo group

(deltamethrin, tralomethrin) or chloro and fluoro groups (fluvalinate). These pyrethroids show a high response to ECD, the minimum detection limit is range in nanogram to picogram level.

Advantage: The great advantages of ECD are its sensitivity, selectivity and ease to use.

Disadvantage: Because of its sensitivity, it needs the highly pure solvent and extreme pure and dry carrier gas. Normally oxygen-free nitrogen with 99.999% purity is recommended as carrier gas for ECD. ECD response has a very short range of linearity.

1.7.3 Qualitative and quantitative analysis by chromatography

1.7.3.1 Qualitative analysis

The retention data is the most commonly used for the identification of peaks by matching the retention time of the sample component to the standard reference compound in the two chromatograms. However, it can only be used for preliminary identification because there are so many variables in a chromatographic system and the samples may contain unexpected with the same retention time.

The standard addition method is used to confirm the results. The small amount of a known component is added to the sample, which is analyzed first, and this sample is reanalyzed. If a quantitative increase is obtained in the peak of the component corresponding the standard, the component representing the peak is identified.

1.7.3.2 Quantitative analysis

The external standard method can be employed in the quantitative analysis. The standard solutions are then analyzed, and calibration curve are established by plotting the peak area versus concentration for each analyte. The unknown concentrations of analyte in the sample can be determined. In a multilevel calibration, several different amounts of the standard solution are prepared and analyted. A linear least-square regression method is used and it can lead to a more precise value for unknown concentrations.

A quantitative analytical procedure based on chromatographic technique is valid only if each of the following five conditions is satisfied

1. Extraction is complete.
2. Clean-up is effective and the recovery quantitative.
3. Chromatographic resolution is adequate.
4. Detection of the insecticides and measurement of the response of the detector are sensitive, specific and reproducible.
5. Comparison of the unknown calibration standards is reproducible.[11]

1.8 Desorption process [35,36]

Desorption is a phenomenon and process opposite of sorption (that is, adsorption or absorption), whereby some of a sorbed substance is released. This occurs in a system being in the state of sorption equilibrium between bulk (fluid, i.e. gas or liquid solution) phase and an adsorbing surface (solid or boundary separating two fluids). When the concentration of substance in the bulk phase is lowered, some of the sorbed substance changes to the bulk state.

An adsorbed species present on a surface at low temperatures may remain almost indefinitely in that state. As the temperature of the substrate is increased, however, there will come a point at which the thermal energy of the adsorbed species is such that one of several things may occur:

1. A molecular species may decompose to yield either gas phase products or other surface species.
2. An atomic adsorbate may react with the substrate to yield a specific surface compound, or diffuse into the bulk of the underlying solid.
3. The species may desorb from the surface and return into the gas phase.

The last of these options is the desorption process. In the absence of decomposition the desorbing species will generally be the same as that originally adsorbed but this is not necessarily always the case.

Desorption kinetics

The rate of desorption (R_{des}) of an adsorbate from a surface can be expressed in the general form:

$$R_{des} = k_{des} N^{x}$$

Where x - kinetic order of desorption

k - rate constant for the desorption process

N - surface concentration of adsorbed species

The order of desorption can usually be predicted because we are concerned with an elementary step of a reaction: specifically,

1. Atomic or simple molecular desorption

$$A_{(ads)} \rightarrow A_{(g)}$$

$$M_{(ads)} \rightarrow M_{(g)}$$

- will usually be a first order process

2. Recombinative molecular desorption

$$2A_{(ads)} \rightarrow A_{2(g)}$$

- will usually be a second order process

The rate constant for the desorption process may be expressed in an Arrhenius form,

$$K_{des} = A\exp(-E_a^{des} / RT)$$

Where E_a^{des} - the activation energy for desorption

A - the pre-exponential factor; this can also be considered to be the attempt frequency (n) at overcoming the barrier to desorption

Desorption process of insecticides is also important since it determines the release rate and the potential mobility of insecticides in soil.[19] A study of desorption process was done by batch method and mechanical shaker.

1.9 The scope and aims of this research

This research, focuses on the study of cypermethrin and fenvalerate residues in cabbages. Within the Thai agricultural commodity and food standard, MRLs established for cypermethrin and fenvalerate in cabbages are 1 and 3 mg/kg, respectively.[22] The extraction method, according to the Department of Agriculture, Ministry of Agriculture and Cooperatives using acetone and dichloromethane as solvent for extraction has been modified to some other organic solvents to reduce expenses, toxicity and to improve extraction efficiency.

The aims of this research can be summarized as follows:

1.9.1 To determine pyrethroid insecticide residues of cypermethrin and fenvalerate in cabbage using GC-ECD

1.9.2 To study desorption of cypermethrin and fenvalerate insecticide residues from cabbage