CHAPTER 1

INTRODUCTION

1.1 General Introduction

Pesticides are widely used to protect the crops from a variety of pests. The use of pesticides benefits in increasing agricultural production but the repeated and indiscriminate uses of certain pesticides have led to their accumulation in plants**,** animals, solid and sediments, thus effecting widespread contamination of the environment [1]. Such application of pesticides has the drawback of pesticide residues which remain on fruits and vegetables, constituting a potential risk to consumers [2]. Fruits and vegetables are the foods that receive the highest doses of pesticides [3].

There are 850 pesticides used globally [4]. Currently, Thailand is one of the biggest users of pesticides in the south Asia region [5]. In 1987, the amount of imported pesticides was about 14, 625 tonnes and insecticides were 8, 115 tonnes and of which carbamates such as methomyl, and carbofuran ranked in 5^{th}, and 9^{th} respectively [7]. In Thailand, the usage of pesticides is increasing year by year and imported quantities of pesticides from 1981 to 2004 are mentioned in Table 1.1 [8].

Consumption of fruits and vegetables has gradually increased over the past few years and has become important foods in the Thai diet. Fruits and vegetables are good for public health but the publicity regarding the excess use of pesticides in agriculture have created a certain apprehension and fear of having pesticide residues in their daily food. The public is confused and alarmed about their food safety.

Consumption of vegetables with excess in amount of pesticides beyond acceptable daily intake (ADI) limit may cause health hazard and may create the environment pollution [9]. Determination of pesticides residues in food is one of the most important approaches to minimize the potential hazards to human health [10]. More studies are needed to be done on the occurrence of pesticides residues in food in order to estimate the daily exposure of the human population.

Table 1.1 Imported Quantities of Pesticides in Thailand (1981- 2004) [8]

Year	Insecticides		Fungicides		Herbicides		Others		Total	
	Quantity (Tonnes)	Value ($)x1000	Quantity (Tonnes)	Value ($)x1000	Quantity (Tonnes)	Value ($)x1000	Quantity (Tonnes)	Value ($)x1000	Quantity (Tonnes)	Value ($)x1000
1981	3575	782	2048	149	3627	460	53	10	9301	1401
1986	5799	876	2512	214	4262	388	204	36	12777	1514
1991	5560	1257	2087	371	7071	1228	311	171	15029	3045
1996	6479	1711	4446	616	14041	2445	579	152	25542	4924
2000	6875	2001	4931	1120	17507	3841	2140	333	31454	7294
2001	8356	2553	5384	1265	20957	4502	2341	440	37038	8760
2002	9046	2930	5680	1443	22670	4348	2238	395	39634	9116
2003	9790	3136	6732	1678	31879	6101	8549	471	56950	11386
2004	8372	2835	6429	1719	35572	6080	2676	542	53049	11176

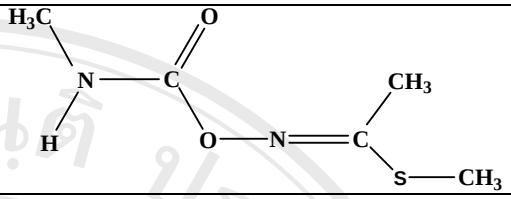
1.2 Methomyl, Carbofuran and Carboxin

Methomyl and carbofuran constitute a family of carbamate pesticides which cover a wide range of uses in the treatment of seed, soil and crops [11]. Carboxin is anilide fungicide and intensively applied at various stages of cultivation and during post harvest storage to provide protection against rotting. Although it has low mammalian toxicity, fungicide residues levels in foodstaffs are generally legislated to minimize the exposure of consumers to the harmful or unnecessary intake of pesticides [12].

1.3 Methomyl in the Environment

Methomyl was introduced in 1966 as a broad spectrum carbamate insecticide. It is used for foliar treatment of vegetable, fruit and field crops, cotton, commercial ornamentals, and in and around poultry houses and dairies. It is also used as fly bait. Methomyl is effective in two ways: (a) as a contact insecticide, because it kills target insects upon direct contact and (b) as a systemic insecticide because of its capability to cause overall systemic poisoning in target insects, after it is absorbed and transported throughout the pests that feed on treated plants. It is capable of being absorbed by plants without being "phytotoxic" or harmful, to the plant. Methomyl is a highly toxic compound which comes under the class I of the classification of EPA toxicity as shown in Appendix A. It is classified as Restricted Use Pesticide (RUP) by EPA because of its high acute toxicity to humans. Acceptable Daily Intake (ADI) is 0.03 mg/kg/day and Reference Dose (RfD) is 0.025 mg/kg/day [13].

Table 1.2 Properties of Methomyl [13]

Structural formula	
Appearance	white, crystalline solid with a slight sulfurous odor
Chemical Name	S-methyl N-(methylcarbamoyloxy) thioacetimidate
Molecular Weight	162.21
Water Solubility	57.9 g/l @ 25 ^{o}C
Solubility in Other Solvents	methanol, acetone, ethanol, and isopropanol
Melting Point	79 ^{o}C
Vapor Pressure	6.65 mPa @ 25 ^{o}C
Adsorption Coefficient	72

1.3.1 Toxicological effects

1.3.1.1 Acute toxicity

Methomyl is highly toxic via the oral route, with reported oral LD_{50} values of 17 to 24 mg/kg in rats, 10 mg/kg in mice, and 15 mg/kg in guinea pigs. Symptoms of methomyl exposure are similar to those caused by other carbamate and cholinesterase inhibitors. These may include weakness, blurred vision, headache, nausea, abdominal cramps, chest discomfort, and constriction of pupils, sweating, muscle tremors, and decreased pulse. Death can result from discontinued breathing, paralysis of muscles of the respiratory system, intense constriction of the openings of the lung, or all three.

Other systemic symptoms of cholinesterase inhibition may appear within a few minutes to several hours of exposure. It is slightly toxic via the dermal route, with a reported dermal LD_{50} of 5880 mg/kg in rabbits, and is absorbed only slowly through the skin [13].

1.3.1.2 Chronic toxicity

Prolonged or repeated exposure to methomyl may cause symptoms similar to the pesticide's acute effects. Repeated exposure to small amounts of methomyl may cause an unsuspected inhibition of cholinesterase, resulting in flu-like symptoms, such as weakness, lack of appetite, and muscle aches. Cholinesterase-inhibition may persist for two to six weeks. This condition is reversible if exposure is discontinued. Since cholinesterase is increasingly inhibited with each exposure, severe cholinesterase-inhibition symptoms may be produced in a person who has had previous methomyl exposure, while a person without previous exposure may not experience any symptoms at all. In a 2-year feeding study with dogs, 5 mg/kg/day caused no observed adverse effects. It is not likely that chronic effects would be seen in humans unless exposures were unexpectedly high, as with chronic misuse [13].

1.3.2 Fate in humans and animals

Methomyl is quickly absorbed through the skin, lungs, and gastrointestinal tract and are broken down in the liver. Breakdown products are readily excreted via respiration and urine. Although they do not appear to accumulate in any particular body tissue, they may alter many other enzymes besides the cholinesterase [13].

1.3.3 Environmental fate

1.3.3.1 Breakdown in soil and water

Methomyl has low persistence in the soil environment, with a reported half-life of approximately 14 days. It is very mobile in sandy loam and silty clay loam soils, but only slight leaching was observed in a silt loam and in a sandy soil. Methomyl is rapidly degraded by soil microbes. Methomyl residues are not expected to be found in treated soil after the growing season in which it is applied [13].

Aqueous solutions of methomyl have been reported to decompose more rapidly on aeration, in sunlight, or in alkaline media. The estimated aqueous half-life for the insecticide is 6 days in surface water and over 25 weeks in groundwater. Because of its high solubility in water and low affinity for soil binding, methomyl may have potential for groundwater contamination. In one experiment, the hydrolysis half-lives of methomyl in solutions at pHs of 6.0, 7.0 and 8.0 were 54, 38, and 20 weeks respectively. In pure water, the hydrolysis half-life has been estimated to be 262 days [13].

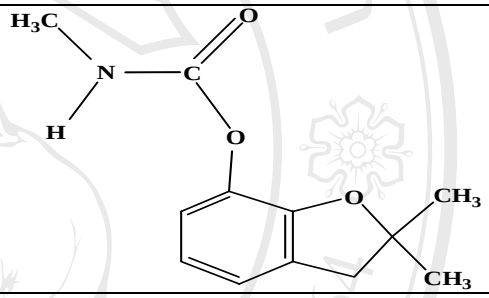
1.3.3.2 Breakdown in vegetation

Following soil treatment, plants take up methomyl through their roots and move it throughout the plant by a process called "translocation." When methomyl is applied to plants, its residues are short-lived. After it is applied to leaves, it has a 3 to 5 day half-life. Less than 3% methomyl remained in cabbage plants 1 week after they were given foliar treatment with the insecticide [13].

1.4 Carbofuran in the Environment

Carbofuran is a broad spectrum carbamate pesticide that kills insects, mites, and nematodes on contact or after ingestion. It is used against soil and foliar pests of field, fruit, vegetable, and forest crops. ADI is 0.01 mg/kg/day and RfD is 0.005 mg/kg/day [13].

Table 1.3 Properties of Carbofuran [13]

Structural formula	
Appearance	odorless, white crystalline solid
Chemical Name	2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate
Molecular Weight	221.25
Water Solubility	320 mg/L @ 25 ^{o}C
Solubility in Other Solvents	acetonitrile, benzene, cyclohexone
Melting Point	153-154 ^{o}C
Vapor Pressure	2.7 mPa @ 33 ^{o}C
Adsorption Coefficient	22

1.4.1 Toxicological effects

1.4.1.1 Acute toxicity

Carbofuran is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. As other carbamate compounds, Carbofuran cholinesterase-inhibiting effect is short-term and reversible. Symptoms of carbofuran poisoning include nausea, vomiting, abdominal cramps, sweating, diarrhea, excessive salivation, weakness, imbalance, blurring of vision, breathing difficulty, increased blood pressure, and incontinence. Death may result at high doses from respiratory system failure associated with carbofuran exposure. Complete recovery from an acute poisoning by carbofuran, with no long-term health effects, is possible if exposure ceases and the victim has time to regain their normal level of cholinesterase and to recover from symptoms. The oral LD_{50} is 5 to 13 mg/kg in rats, 2 mg/kg in mice, and 19 mg/kg in dogs. The dermal LD_{50} is >1000 mg/kg in rabbits [13].

1.4.1.2 Chronic toxicity

Rats given very high doses (5 mg/kg/day) for two years showed decreases in weight. Similar tests with mice gave the same results. Prolonged or repeated exposure to carbofuran may cause the same effects as an acute exposure [13].

1.4.2 Fate in humans and animals

Carbofuran is poorly absorbed through the skin. It is metabolized in the liver and eventually excreted in the urine. The half-life in the body is from 6 to 12 hours.

Less than 1% of a dose will be excreted in a mother's milk. It does not accumulate in tissue [13].

1.4.3 Environmental fate

1.4.3.1 Breakdown in soil and water

Carbofuran is moderately persistent in soil. Its half-life is 30 to 120 days. In soil, carbofuran is degraded by chemical hydrolysis and microbial processes. Hydrolysis occurs more rapidly in alkaline soils. Carbofuran breaks down in sunlight and is mobile to very mobile in sandy loam, silty clay, and silty loam soils. Moderately mobile in silty clay loam soils and only slightly mobile in muck soils [13].

Carbofuran is soluble in water and has a high potential for groundwater contamination. It is subjected to degradation by chemical hydrolysis under alkaline conditions. Photodegradation and aquatic microbes may also contribute to degradation. The hydrolysis half-lives of carbofuran in water at 25 ^{o}C are 690, 8.2, and 1.0 weeks at pH values of 6.0, 7.0, and 8.0, respectively. Carbofuran does not volatilize from water, nor does it adsorb to sediment or suspended particles [13].

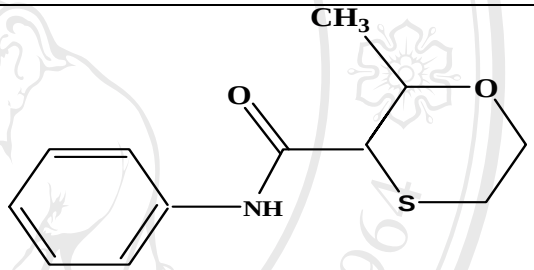
1.4.3.2 Breakdown in vegetation

The half-life of carbofuran on crops is about 4 days when applied to roots, and longer than 4 days if applied to the leaves [13].

1.5 Carboxin in the Environment

Carboxin is a systemic anilide fungicide. It is used as a seed treatment for control of smut, rot, and blight on barley, oats, rice, cotton, vegetables, corn, and wheat. It is also used to control fairy rings on turf grass. Carboxin may be used to prevent the formation of these diseases or may be used to cure existing plant diseases. RfD is 0.1 mg/kg/day [13].

Table 1.4 Properties of Carboxin [13]

Structural Formula	
Appearance	a colorless crystal
Chemical Name	A 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide
Molecular Weight	235.31
Water Solubility	170 mg/L @ 25 ^{o}C
Solubility in Other Solvents	acetone; benzene; methanol
Melting Point	Two crystal structures: 91.5-92.5 ^{o}C and 98-100 ^{o}C
Vapor Pressure	<0.025 mPa @ 25 ^{o}C
Partition Coefficient	2.1703
Adsorption Coefficient	260

1.5.1 Toxicological effects

1.5.1.1 Acute toxicity

Carboxin is slightly toxic. Symptoms of poisoning can include vomiting and headache. Recovery is very rapid if the exposed individual is treated quickly. The oral LD_{50} is 3820 mg/kg in rats and 3550 mg/kg in mice. The compound produces very little skin irritation and can seriously irritate the eyes. Acute dermal exposure results in an LD_{50} of greater than 8000 mg/kg in rabbits [13].

1.5.1.2 Chronic toxicity

Rats fed doses up to 311 mg/kg/day for 28 days showed some fluid accumulation in the liver, even at low doses. Another rat study showed kidney changes at somewhat higher doses (1000 mg/kg) fed for 90 days. Male and female mice also showed liver effects after being fed high doses (912 mg/kg) of carboxin for 1 1/2 years [13].

1.5.2 Fate in humans and animals

Rats excreted almost all of a carboxin dose in 24 hours, with most excreted in urine and some in feces. Rabbits showed a similar excretion pattern. The compound does not accumulate in animal tissues. In milk cows fed up to 5 ppm for 10 days, less than 2% of the administered dose was found in tissues. However, significant levels were found in milk a few days after exposure. The main breakdown product is carboxin sulfoxide, for which the rat oral LD50 is 2000 mg/kg [13].

1.5.3 Environmental fate

1.5.3.1 Breakdown in soil and water

Carboxin is rapidly degraded to carboxin sulfoxide in soil. It has a low persistence, with a half-life of about 3 days in soil. In one study after 7 days, 95% of the parent was gone and the sulfoxide, a breakdown product, represented 31 to 45% of the amount applied. Minor products formed were carboxin sulfone, hydroxy carboxin, and CO_2. Carboxin does not readily adsorb to soil [13].

In water, carboxin oxidizes to the sulfoxide and sulfone within 7 days. This happens both under ultraviolet light and in the dark. Both parent and sulfoxide are very mobile and could possibly leach to groundwater. Blue-green algae like Anabaena degrade the pesticide extensively. Other algae can also break down carboxin, but not to the same extent [13].

1.5.3.2 Breakdown in vegetation

Although the distribution pattern of the parent and sulfoxide metabolite vary, carboxin is found systemically (throughout the plant) in all species of plants studied. Plants grown from treated seed had no carboxin present 6 weeks after emergence. Carboxin sulfoxide found in plants can come either from the soil or through oxidation within the plant [13].

1.6 High Performance Liquid Chromatography (HPLC)

The first commercial introduction of High Performance Liquid Chromatography (HPLC) was in 1969 and since that time it has become one of the

most important and fastest growing techniques in the modern laboratory. HPLC analysis is routinely fast and efficient with detection of as little as 200 pg of material. Modern HPLC is a technique for making precision separations of complex mixtures and offers high resolution separating capability to solve problems faster and better. Opportunities for applying HPLC are almost unlimited, with the result that HPLC instruments have become indispensable tools for a variety of scientist and industries [14].

Most analytical methods for pesticide analysis are based on chromatographic techniques by both Gas Chromatography (GC) and High Performance Liquid Chromatogrphy (HPLC). HPLC is obviously preferred approach for polar, less volatile and thermally labile pesticides such as carbamate [15]. This is an effective method for separating and analyzing the various carbamate pesticides employing different detectors [14]. The methods for the determination of pesticides residues in fruits and vegetables could employ reversed-phase chromatography with C_{18} or C_8 columns and aqueous mobile phase, followed by UV absorption, UV diode array detection, mass spectrometric or florescence detection [2].

1.6.1 Theoretical principle of HPLC

Chromatography is a separation processes in which the sample mixture is distributed between two phase in the chromatographic bed (column or plane). One plane is stationary whilst the other passes through the chromatographic bed. The stationary phase is either a solid, porous, surface active material in small particle form or a thin film of liquid coated on a solid support or column wall. The mobile phase is a gas or liquid. If a gas is used, the process is known as gas chromatography. The

mobile phase is always liquid in all types of liquid chromatography, including the thin layer variety [16]. The components of a simple HPLC instrument are shown in Figure 1.1.

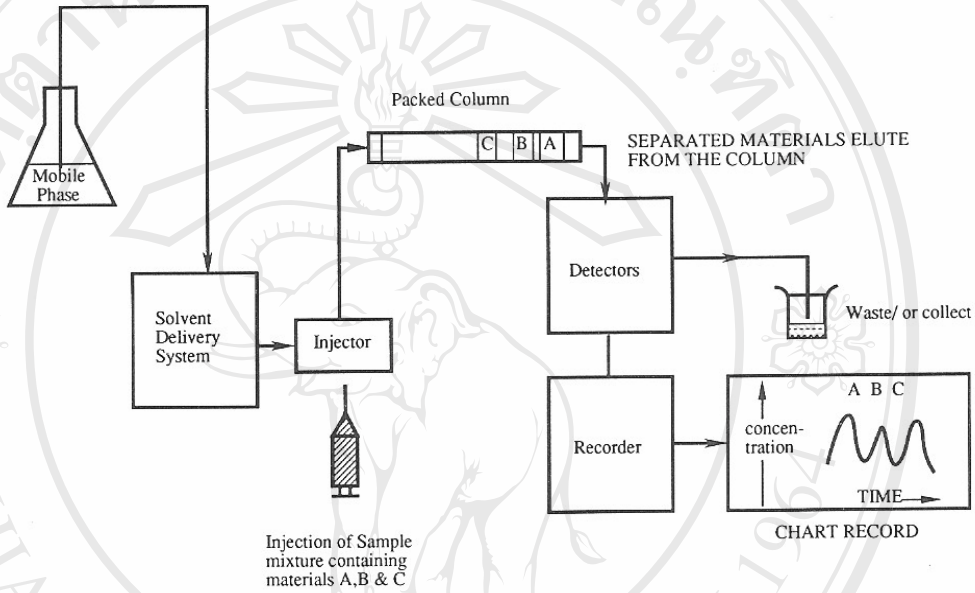


Figure 1.1 Block diagram showing the components of an isocratic HPLC instrument [16].

Figure 1.1 showed that the mixture to be analyzed is dissolved in a suitable solvent, introduced "Injected" at one end of the "column" and carried through the column by a continuous flow of the same solvent "mobile phase" in which the mixture was dissolved. The separation takes place in this column, which contains "sorptive" particles of large surface area. These particles are referred to as the "stationary phase .The device for applying a precise volume of sample onto the column is the injector. Sample components that are injected reversibly interact with

the stationary phase in a continuous manner. The mobile phase also called the elute is pumped through the column bed of the tightly packed chromatographic particles using a solvent delivery system “pump”. With the selection of the proper mobile phase and column packing material, some components of the mixture will travel through the column more slowly than others. As the sample components emerge from the column, a suitable detector is used to monitor and transmit a signal to a recording device. The “chromatogram” is a record of the detector response as a function of time and indicates the presence of the components as “peaks”. Sometimes, the instrumentation also contains a solvent mixing device and another pump for gradient elution [14]. Chromatographic process is described in Figure 1.2.

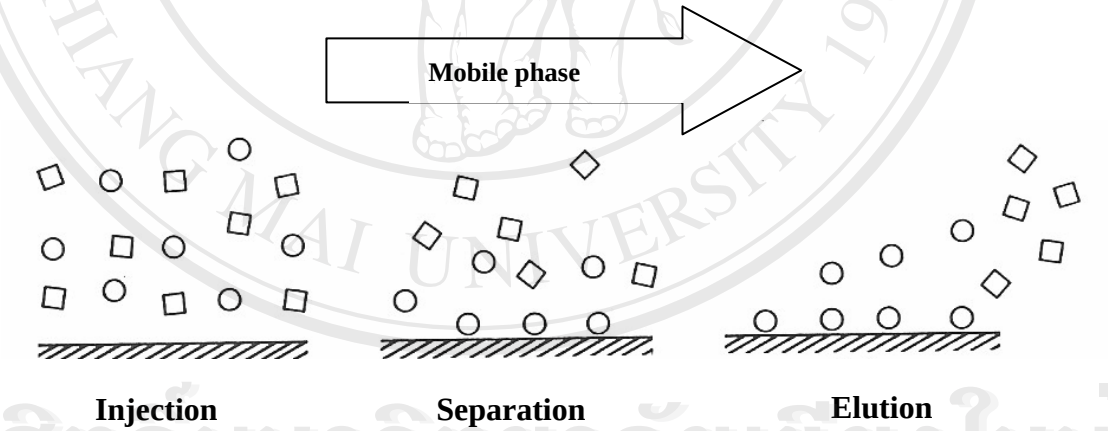


Figure 1.2 Liquid chromatographic processes of segmentation at the surface [16]

The three basic steps shown in Figure 1.2 are injection, separation and elution. During injection, components are “sorbed” onto the stationary phase. Migration of the sample components through the stationary phase is essentially the

result of two forces- movement driven by the mobile phase and retardation resulting from the stationary phase and transported by the mobile phase. These two opposing forces cause an equilibrium distribution between the two phases for each compound. When a sample is injected onto an LC column, it forms a narrow band at the head of the column as shown in part 1 of the Figure 1.3. If the equilibrium distributions for two compounds differ, the chromatography will result in a different rate of migration for each compound. As the mobile phase passes through the column, the initial band separates into individual solute bands as shown in part 2 of the Figure 1.3 each of which migrates at a rate governed by the equilibrium distribution of solute between the mobile phase and the surface of the column packing (stationary phase). Finally, the segmentation of the compound is complete as shown in part 3 of the Figure 1.3 and the separated components continue to elute from the column as zones or bands that pass through a detector. The amount of component present in an eluted zone is sensed by the detector and is proportional to the peak height recorded on the chromatogram Figure 1.1

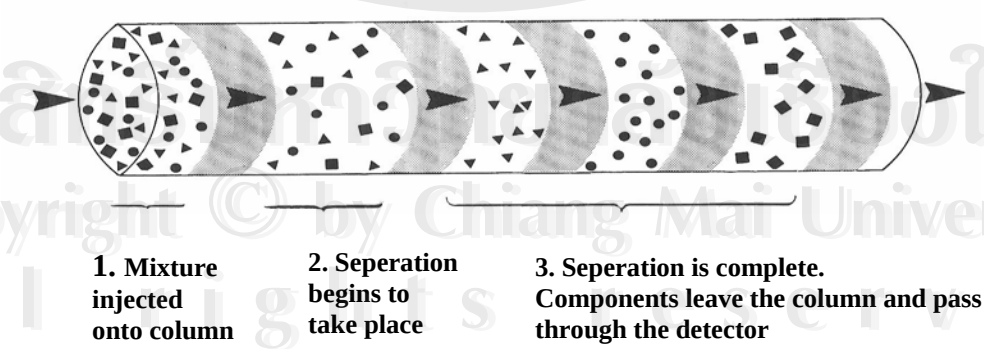


Figure 1.3 Liquid chromatographic processes of migration through the column [16]

1.6.2 UV detector

The function of the detector for HPLC is to sense with a compound leaving the column and to provide a signal that is proportional to the concentration of the compound in the mobile phase [17]. There are a large number of types of detector available. UV detector is the most commonly used type of detector system for the HPLC as it can be rather sensitive, has a wide linear range, is relatively unaffected by temperature fluctuations and is also suitable for gradient elution[16]. UV detector is a photometer and measures changes in the absorbance of ultraviolet UV or visible (VIS) light resulting as the components pass through the detector [14]. When light passed through a liquid (mobile phase), the intensity of absorption is proportional to the concentration of the analyte in the mobile phase and the optical length, L (Lambert-Beer law) [17]. The wavelength of absorbance can be chosen to enhance specificity and/or sensitivity for a specific compound [14].

$$A = \varepsilon bc = \log P_o / P$$

Where; A = an absorbance of solute in cell

ε = a molar absorptivity ($L.cm^{-1}\ mol^{-1}$)

b = an optical path – length through the cell (cm)

c = the molar concentration of the solute

P_o = a light intensity (or power) focused onto the cell

P = a light intensity transmitted

1.6.3 Qualitative and quantitative analysis by chromatography

1.6.3.1 Qualitative analysis

In general chromatography, the common way is to compare the retention time of the sample peaks with the standard peaks separated using the same conditions. A more identification is obtained if the match is again obtained when the interest of sample peak and known compounds are run on a second column containing a different type of stationary phase from that used the first time. Alternatively, chromatographic techniques such as GC or HPLC may be coupled with MS to identify the mass fragmentation pattern of a chromatographic peak [18, 19].

1.6.3.2 Quantitative analysis

Quantitative analysis can be accomplished because of a linear relationship of the concentration of the compound to its peak height or pea area in the chromatogram. A calibration curve was constructed by injecting a standard compound at known concentration into HPLC, measuring the peak height or area response and plotting peak height or area versus amount injected. The concentration of compound in the sample is determined by measuring the peak height or area of the component in the chromatogram of the sample and comparing that value to the calibration curve to determine the amount of the compound present in the sample [20].

1.7 Relevant Application Methods for Methomyl, Carbofuran and Carboxin

Several methods for the determination of methomyl, carbofuran and carboxin generally focused on the use of HPLC and Liquid Chromatography-Mass Spectrometry (LC-MS) have been proposed.

In 1994, Chumni [23] studied the method for determination of carbamate group of insecticide in treated vegetables. The plant co-extractives were removed using SPE clean up procedure. The reverse phase HPLC separation was accomplished with μBondpak C_{18} column and 50 % (v/v) acetonitrile-water as the eluent. The residues were detected by UV detection. Average recoveries were higher than 90% and the level of detected residues in treated vegetables were not harmful to potential health risk in terms of ADI value.

In 2004, Pico *et al*. [12] made an attempt to clean up samples sufficiently to screen for fungicides including carboxin in grape using SPE and stair bar sportive extraction (SBPE). The residue analysis was performed by LC-MS followed by mass selective detector (MSD). Chromatographic separation was performed by μBondpak C_{18} column. SPE provided higher recoveries, best LOQ and was more rapid to carry out compared with SBPE.

Some of the relevant application methods developed for these pesticides analysis is briefly described in the Table 1.4.

Table 1.5 Relevent Application Methods for Methomyl, Carbofuran and Carboxin

Year	Sample matrix	Analytes	Techniques for sample preparation	Analysis method	Authors
1991	Fruits and vegetables	Pesticides including carboxin	Soxhlet extraction	LC-MS	Joseph, D.R [21]
1994	Soil, Water	Carbamate (Carbaryl)	SPE	HPLC-UV	Somporn,C [22]
1994	Kale, edible rape	Carbamate (Carbaryl)	SPE	HPLC-UV	Chumni, S [23]
1996	Fruit and vegetables	Carbofuran Carboxin	-	HPLC – UV HPLC-Thermospray	Torres, C.M [2]
1998	Carrot, Potato	N-methyl carbamates	SPE, Silica, Alumina and Florisil columns	HPLC–UV Detector	Barcelo, D [11]
2000	Soil	Carbamates including	Sonication assisted extraction	HPLC-Fluorescence Detector	Pico, Y. [24]

Table 1.5 Relevent Application Methods or Methomyl, Carbofuran and Carboxin (Continued)

Year	Sample matrix	Analytes	Techniques for sample preparation	Analysis method	Authors
2003	Soil	Carbamates including	Sonicated assisted extraction	HPLC - fluorescence detection	Tadeo J.L [25]
2004	Fruit and vegetables	N- methyl carbamates	Florisil Extraction	RP- HPLC	Brunete C. S [26]
2004	Vegetable	Pesticides including carbofuran	silica gel column chromatography	RP- HPLC UV detector	Afzal, H.M [10]
2004	Grape	Pesticides including carboxin	SPE and SBSE	LC-MS	Pico.Y [12]
2005	Fruits and Vegetables	Carbamates and Organophosphates	SPE	LC – MS	Yuki, H [27]

1.8 Solid Phase Extraction [14]

Sample preparation is often the most time consuming step in a chemical analysis and the sample matrix frequently interferences with measurement. In gas and liquid chromatography, the life of columns may be drastically shortened by impurities. Successful analysis of samples require a rapid , reliable, precise method for sample preparation and clean up to remove potential interfering components so accuracy is maximized. Traditionally, liquid liquid extraction has remained the preferred technique for the preparation of liquid samples for several years, especially in the environmental field. However, an innovative way to perform a sample clean up that is fast, accurate and easy is the use of solid phase extraction (SPE) columns. Using SPE eliminates many of the drawbacks associated with traditional liquid liquid extraction such as (1) the use of large amounts of expensive organic solvents, (2) low recovery due to solvent emulsion formation, and (3) large requirements for labor, time, and glassware and bench space.

SPE is being increasingly used in food analysis, mainly for sample clean up. SPE columns containing a normal (polar) - phase or reversed (non polar) phase support not only offer the potential of simplying the purification of the initial extract but also reducing the amount of solvent consumed. SPE clean up is inserted as a part of the chromatographic system, mostly using HPLC because of the compatibility of mobile phase [28]. SPE is similar to low pressure liquid chromatography. It involves the use of small, disposable extraction columns, filled with one of a wide variety of sorbents.

Typical SPE tube and disk are shown in Figure 1.4 and the basic steps involved in SPE are shown in Figure 1.5. It involves conditioning, loading the sample, eluting the undesirable material and finally isolating the desired material.

1. Conditioning

Conditioning address two purposes. First, any contamination that could be present in the cartridge owing to the packaging and handling process is washed off. Removing contaminants is essential since they have the potential to elute with and contaminate the sample. Second, conditioning "wets" the packing material and leaves it in a state that is compatible with the initial mobile phase and sample. The first solvent for conditioning should be of equal or stronger eluting strength than the strongest mobile phase to be used. This ensures that all possible contamination that might elute with the analytes is removed.

2. Loading the sample

The loading step involves placing the sample onto the packing and pumping through the packing, whereupon the analytes of interest are sorbed onto the packing.

3. Washing undesirable material

After loading the sample, the packing is usually rinsed with a mobile phase to wash off any undesirable sample components. A mobile phase, that is slightly stronger or the same strength as the initial mobile phase In this way, unwanted sample components that are early elutes are removed. Washing step also ensures that the entire sample comes in contact with the packing and small droplets of the loading

solvent adhering to the walls of the tube and remaining sample will be washed onto cartridge bed.

4. Elution of analyte

After removing the unretained and weakly retained components, it is time to elute the desired components from the cartridge using the optimal mobile phase (solvent) strength and amount of solvent to be affected the release of analytes. Using too strong a mobile phase will result in the elution of unnecessary sample components that are more strongly retained than the analytes. The use of optimal mobile phase strength would keep these sample components retained on the packing instead of being co eluted with compounds of interest. Using too weak an eluting strength will result in too broad an elution volume, which negates the concentrating advantage of SPE.

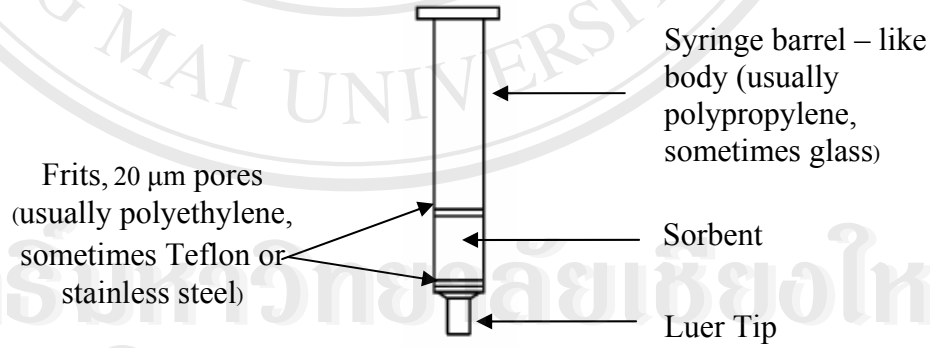


Figure 1.4 Typical SPE Tube and Disk [29]

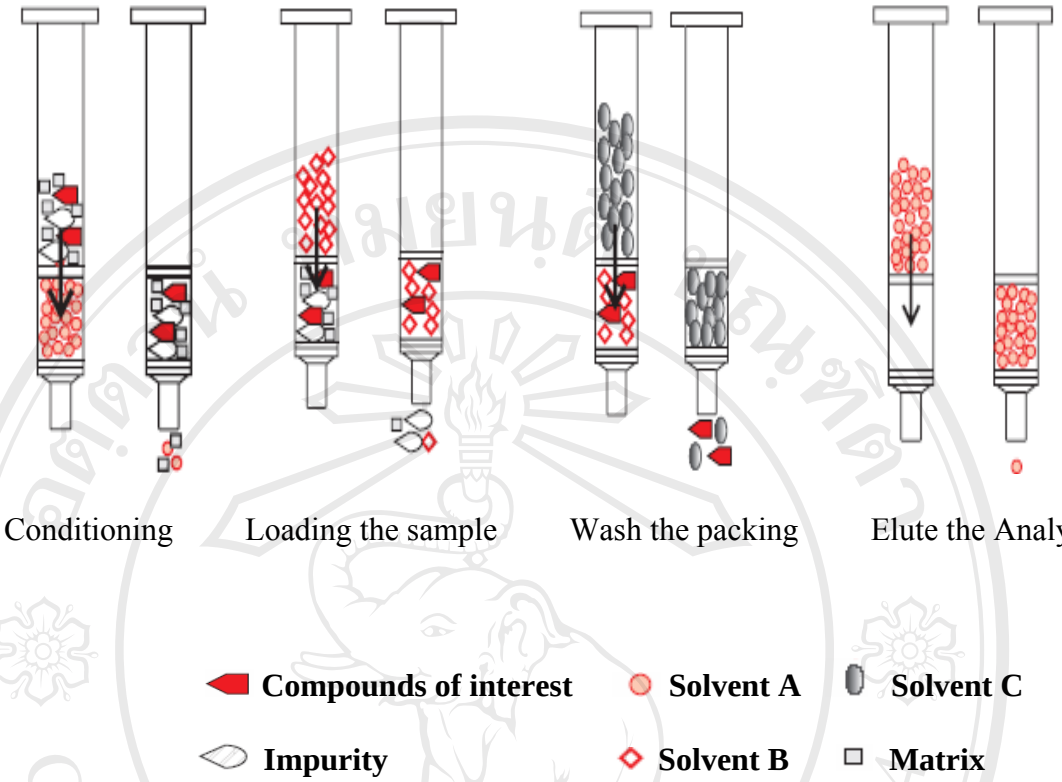


Figure 1.5 SPE Steps in Sample Clean Up [29]

1.8.1 Type of Solid Phase Extraction

1. Normal phase SPE

Normal phase SPE procedures typically involve a polar analyte, a mid- to nonpolar matrix and a polar stationary phase. Polar-functionalized bonded silicas such as cyanopropyl (CN), aminopropyl (NH_2), and polar adsorptionmedia such as silica gel (SiOH), Florisil (Mg_2SiO_3), and Alumina (Al_2O_3) typically are used under normal phase conditions [29].

2. Reversed phase SPE

Reversed phase separations involve a polar moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is typically mid-to nonpolar. Several SPE materials, such as the alkyl- or aryl-bonded silicas octadecyl bonded (C-18), octyl bonded(C-8), phenyl bonded silica (C-Ph) in the reversed phase category [29].

3. Ion exchange SPE

Ion exchange SPE can be used for compounds that are charged when in a solution (usually aqueous, but sometimes organic). Anionic (negatively charged) compounds can be isolated on aminopropyl bonded silica (NH2) bonded silica cartridges. Cationic (positively charged) compounds are isolated by using sulfonic acid bonded silica with Na^+ counterion (SCX) or carboxylic acid bonded silica with Na^+ counterion (WCX) bonded silica cartridges [29].

1.8.2 Solvent properties in SPE

A solvent used to elute the analytes of interest from the sample matrix can be arranged in order of eluting strength measured as elutrophic value. In general, eluting solvent can be useful for the analyst to select solvents that are miscible with each other. The commonly used solvents in SPE are shown in the Table 1.6 [30].

Table 1.6 Characteristics of solvents commonly used in SPE [29]

Polarity			**Solvent**	**Miscible in water**
Non polar	Strong Reverse Phase	Weak Normal Phase		
			n- Hexane	No
			iso-Octane	No
			Carbon tetrachloride	No
			Chloroform	No
			Dichloromethane	No
			Tetrahydrofuran	Yes
			Diethyl ether	No
			Ethyl acetate	Poorly
			Acetonitrile	Yes
			Isopropanol	Yes
			Methanol	Yes
			Water	Yes
Polar	Weak Reverse Phase	Strong Normal Phase	Acetic acid	Yes

1.9 Aims of Research

1. To study and develop a method for methomyl, carbofuran and carboxin residues analysis by HPLC.

2. To determine the level of methomyl, carbofuran and carboxin residues in cabbages (*Brassica oleracea* L. cv. white headed cabbage cruciferae)

3. To assess the human risk from consuming cabbages (*Brassica oleracea* L. cv. white headed cabbage cruciferae) contaminated by methomyl, carbofuran and carboxin.