1. INTRODUCTION

1.1 General Introduction

Carbaryl or 1-naphthyl N-methyl carbamate is a broad-spectrum contact carbamate insecticide that is still widely applied to fruit and vegetable crops [1]. As shown in Table 1.1, carbaryl ranked seventh among the top ten pesticides imported to Thailand in 1987 [2] and it is widely used in the northern part of Thailand. Carbaryl is often used to control major pests because of its low acute mammalian toxicity and its effectiveness against many insects which have become resistant to chlorinated hydrocarbons and/or organophosphates [3]. However, research findings during the last few years have indicated that carbaryl has possible chronic toxic effects. In the United States, the status of carbaryl has recently been changed to a higher risk category. Because of possible chronic toxic effect and because of its wide distribution [4], there is a need to find out if and to what extent carbaryl is present in particular vegetables [4].

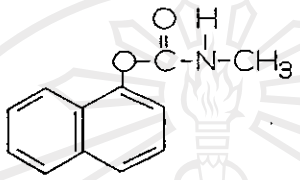
Carbaryl has the empirical formula $C_{12}H_{11}NO_2$ and a molecular weight of 201.20. The material is a white to light tan solid with a mild phenolic odor. It has a melting point of 142 °C and a vapor pressure of less than 4×10^{-5} mm Hg at 26 °C. The solubility of carbaryl in water is 40 ppm at 30 °C [5]. It is moderately soluble in most polar organic solvents, such as dimethylformamide, dimethyl sulfoxide and acetone; it is slightly soluble in hexane, benzene and methanol and about 5% soluble in petroleum oils. Carbaryl is stable under normal storage conditions but is hydrolyzed rapidly at pH 10 or above. It was first synthesized in 1953 and

introduced in 1958 as a broad spectrum contact insecticide with systemic properties [6]. It is used for control of over 150 major pests on more than 120 crops, including field crops, forage, vegetables, fruits, nuts, shade trees, ornamentals, forests, lawns, turf, and range land, as well as control of pests of domestic animals. Carbaryl formulations include baits, dusts, wettable powders, granules, oil, molasses, and aqueous dispersions and suspensions [6]. The structural formula, empirical formula and other names of carbaryl are given in Table 1.2.

Table 1.1 The top ten pesticides imported to Thailand in 1987 [2].

Rank	Pesticide	Quantity(kg)	Value (Baht)
1	Methyl parathion	1,538,599	83,927,568
2	Monocrotophos	951,043	96,042,709
3	Methamidophos	417,452	45,273,444
4	Dimethoate	415,650	34,131,532
5	Methomyl	358,296	130,693,483
6	Malathion	309,354	17,965,900
7	Carbaryl	242,150	25,006,268
8	Mevinphos	240,691	18,803,315
9	Carbofuran	204,661	71,077,271
10	Ethyl parathion	174,038	34,131,532

Table 1.2 Nomenclature system and relevant information for carbaryl [7].

Common name	Carbaryl
Proprietary name	Sevin, Hexavin, Karbaspray Ravyon, UC.
Structural formula	
Chemical name	1- naphthyl N- methylcarbamate
Empirical formula	$C_{12}H_{11}NO_2$

The acute toxicity of carbaryl is summarized in Table 1.3. The symptoms of acute intoxication are typical of acetylcholinesterase inhibition. In rabbits, carbaryl does not irritate the skin and produces only transient conjunctival irritation. Carbaryl does not cause a skin sensitization reaction in guinea pigs. Carbaryl is toxic to fish; the 8-day LC_{50}s for rainbow trout and bluegill reported range from 1.3 to 10 mg/L, respectively. Smaller fish are more susceptible to carbaryl than larger ones of the same species.

The mammalian toxicity of this kind of pesticide is summarized in Table 1.3 [6]. It should be noted, however, that the 1973 FAO/WHO joint meeting on pesticide residues estimated a human ADI (Acceptable Daily Intake) for carbaryl to be 0.01 mg/kg body weight.

Table 1.3 Acute Oral Toxicity of Carbaryl [6].

Species	Sex	LD_{50} (mg/kg)
Rat	Both	233-850
Mouse	Both	108-650
Guinea pig	Female	280
Gerbil	Female	491
Rabbit	Female	710
Dog	Female	250-795
Cat	Female	125-250
Monkey	Female	>1000
Swine	Female	1500-2000

1.2 Application Technique

Knapsack or backpack sprayers are often used in the application of carbaryl to fruit and vegetable crops. This apparatus has a simple air pump to build up spray pressure in the sealed tank. Spray liquid is forced from the bottom of the tank up the tube and by way of the hose and valve to the nozzle. Spray pressure drops as liquid is removed and necessitates pumping this machine up at regular intervals [8]. One type of the knapsack sprayer is shown in Fig 1.1.

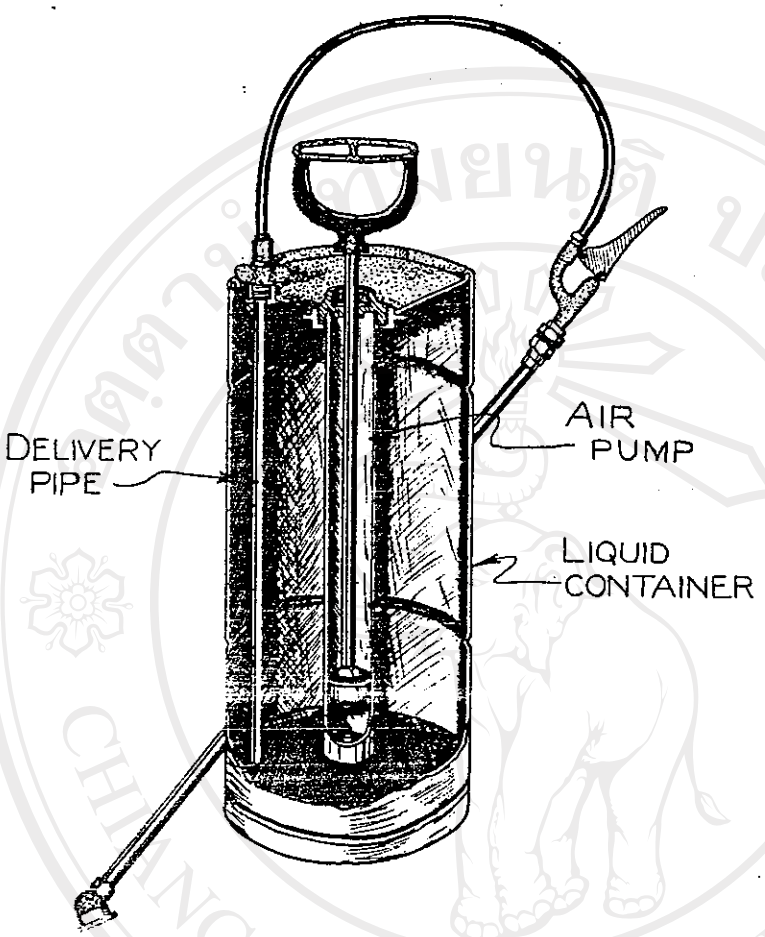


Fig 1.1 Knapsack Sprayer.

1.3 Solid Phase Extraction for Sample Preparation

Solid phase extraction (SPE) has recently emerged as a powerful tool for chemical isolation and purification. From trace level sample preparation to industrial scale chemical isolation, it plays an increasingly important role in a broad range of applications: pharmaceutical, biomedical, environmental, and many others [9]. Traditionally, liquid/liquid extraction has been used for these purposes.

However, this method using separatory funnels is inefficient, tedious, and costly [10].

Solid phase extraction 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 as shown in Fig 1.2. The columns are first conditioned with an appropriate solvent, such as methanol, hexane or chloroform. After that they are further conditioned with a typical sample matrix solvent; the sample is forced through by aspiration or pressure. Subsequently, the column is washed to elute the impurities, leaving the analyte on the column. Finally, the purified analyte is eluted with a solvent strong enough to displace it from the sorbent [11]. Solid phase extraction steps are shown in Fig. 1.3.

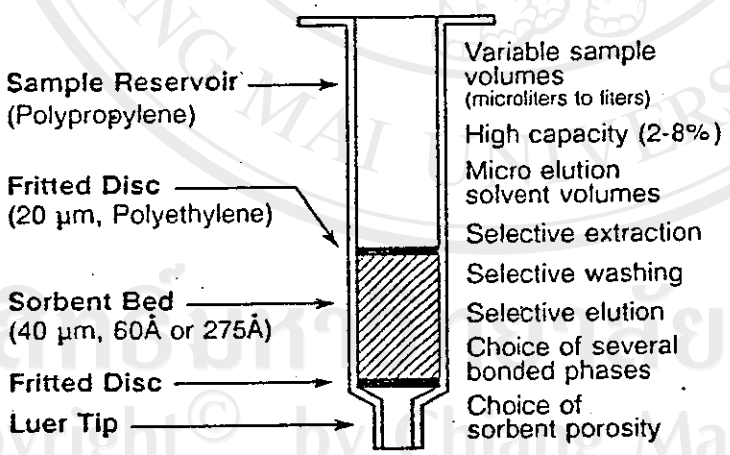


Fig 1.2 Disposable extraction column.

Sample cleanup, sample concentration, and matrix removal are the three most popular modes of solid phase extraction use. However, the distinction may be a bit blurred. In general terms, the distinction is that in the sample cleanup mode, the sorbent retains the analyte from a small volume of sample solution and allows impurities to pass through the column. Sample concentration or trace enrichment is essentially the same technique; however, the concentration of the analyte in the sample solution is generally very dilute that liters rather than 2 or 3 ml of sample solution must be processed. In matrix removal mode, the role of the sorbent is reversed, i.e., the impurities are retained, and the unretained analyte passes through the column with the sample solution [12].

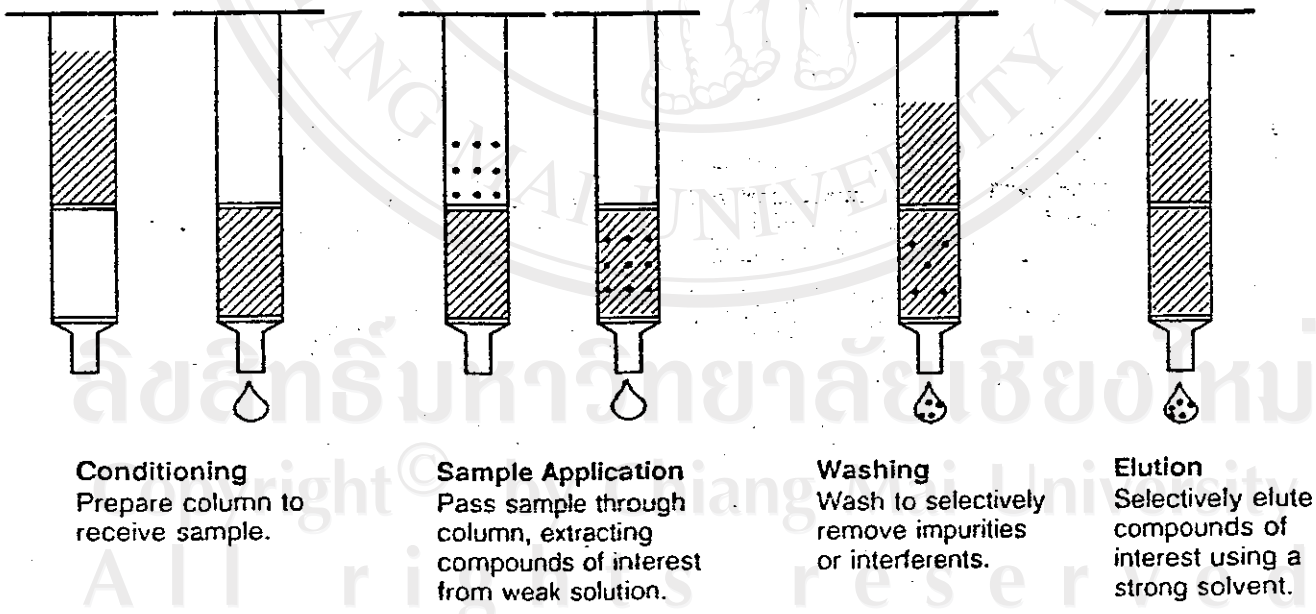


Fig 1.3 Solid phase extraction steps.

Extraction of carbaryl insecticide from the vegetable matrices is achieved by reversed phase solid phase extraction system which refers to any system in which the solvent is less polar than the mobile phase or sample solution. Here, octadecyl substituted siloxanes can be used to extract nonpolar or slightly polar analytes from solvents with ε° (eluting solvent strength) greater than approximately 0.6. The analyte is then eluted from the column with a nonpolar solvent. In such an experiment 50 % acetonitrile/water has been reported to be an effective eluent [13].

1.4 HPLC and Its Relevant Applications

The methods available to analyze carbaryl generally focus on the use of high performance liquid chromatography (HPLC) because of its sensitivity and ability to cope with thermally-labile substances [14]. Numerous HPLC methods have been developed for carbaryl analysis as briefly described in the following paragraphs.

In 1977, Lawrence [15] made an attempt to clean up samples sufficiently to screen for carbamate pesticides including carbaryl in foods using liquid/liquid extraction as an isolation step which was followed by HPLC-UV detection at 254 nm. The detector was a readily available UV filter photometer. Chromatography was carried out on 5 μm silica gel with 5% 2-propanol in iso octane as mobile phase.

In 1980, Krause [16] studied the multiresidue method for determining N-methylcarbamate insecticide including carbaryl in crops. The residues were

extracted from the crops using methanol, and plant coextractives were removed using solvent partitioning and a charcoal-silanized celite column. The carbamate residues were then separated on a RP-HPLC column using an acetonitrile-water gradient mobile phase. The eluted residues were detected using an in-line post-column fluorometric detection technique .

In 1981, Bushway [4] studied the carbaryl analysis in fruit juices. The juice samples were passed through a C_{18} Sep-Pak cartridge and then eluted with 5 ml acetonitrile:water (25:75) and followed by 2 ml acetonitrile:water (75:25). This fraction was injected into the HPLC system .

In 1982, Cabras and others [17] studied the residue determination of some insecticides and fungicides including carbaryl on grapes by RP-HPLC. The residues were extracted from the grapes by liquid/liquid extraction and then separated by a RP-HPLC under isocratic elution conditions.

In 1987, Goewie and others [18] studied HPLC method for the simultaneous determination of N-methylcarbamate pesticides including carbaryl in total diets. The method was extended to a fully automated procedure for a clean-up and analysis by using pre-column switching .

In 1987, DeKok, Hiemstra and Vreeker [19] studied the multiresidue analysis of N-methylcarbamates including carbaryl in grains, fruits and vegetables. Clean-up was simple using a Bond Elut aminopropyl-bonded silica column and extraction required only 1 ml methylene chloride:methanol (99:1) for the double

monochromator fluorometric LC analysis.

In 1988, Ting and others [20] determined N-methylcarbamates including carbaryl in vegetables and fruits using methanol as solvent in liquid extraction prior to HPLC-fluorescence detection.

In 1988, Brayan and others [21] developed a suitable RP-HPLC method for the simultaneous determination of the carbamate pesticides and a group of organophosphate pesticides in methanol extracts from florisil Sep-Pak cartridge.

In 1992, DeKok and Hiemstra [22] studied the determination of N-methylcarbamate pesticides including carbaryl in fruits and vegetables using a solid phase extraction clean up followed by acetonitrile:water extraction before analysis on an on-line liquid chromatography system with the gradient elution .

In 1992, Page and French [23] determined N-methylcarbamate insecticides including carbaryl in vegetables, fruits and feeds accomplished by aspirating through an aminopropyl Bond Elut (NH_2) SPE column. Following aspiration, the sample was eluted with 10 ml 2% methanol-methylene chloride and reconstituted in methanol for fluorometric LC analysis .

1.5 Scope and Aims of the Research Project

Kale (Brassica oleracea L.var.alboglabra Bail) and edible rape (Brassica chinensis L.var oleifera Tsen et Lee.) were chosen as treated vegetables because

they were among the most consuming vegetables in this area. Each of the vegetable samples was collected from the two study sites, namely Ban Sop Pao in Lumphun Province for kale and Ban Pa Sao in Chiang Mai Province for edible rape. In addition, the study areas for these two vegetables were 80 m^2 and 25 m^2, respectively. The quantities of carbaryl before spraying, directly after spraying and up to 8 days after spraying for kale and up to 25 days after spraying for edible rape were investigated using solid phase extraction for isolation steps and followed by the HPLC determinations of carbaryl.

The aims of this research work can be summarized as follows.

1.5.1 To develop an efficient HPLC method for the analysis of carbaryl in vegetables.

1.5.2 To apply the developed HPLC method in determining amounts of carbaryl in chosen vegetable samples.

1.5.3 To study other parameters that influence the quantity of such pesticide in analyzed samples.

1.5.4 To compare the quantities of such pesticide found in vegetables collected from two sampling sites.

1.5.5 To evaluate the residues found in comparison with reported values of the maximum residue limit (MRL) and the acceptable daily intake (ADI) in vegetables.