# ANALYSIS OF PESTICIDE RESIDUES IN THE FRESH FRUITS BY USING GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTOR (GC-ECD)

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## ABSTRACT

Recently, pesticides are extensively used to ensure high crop yields which is used during production and post-harvest treatment. However, the increased use of the pesticides has resulted in pollution of the environment and also has caused many associated short-term and long-term effects on human health. Thus, in the present study, an effort has been made to analyze the pesticide residues in the fresh fruits purchased from the fruit markets in Gambang, Kuantan. Gas chromatography with selective electron capture detector (GC-ECD) was used to determine the amount of Chlorpyrifos pesticide residues in the apple and pear samples that is firstly being washed by distilled water and fruits detergent, respectively. From the both fruit samples, it shows that both apple and pear samples were positive with the Chlorpyrifos residues within the range of 0.02mg $kg^{-1}$ to 0.06 mg $kg^{-1}$, respectively. From the results, it also shows that by using fruits detergent, it is effectively used in reducing the amount of the pesticide residues in the fruits by range of 35% to 40%. However, from the results of the pesticide residues of both samples, the finding shows that no pesticides detected exceeded the Maximum Residue Limits (MRLs) established by World Heatlh Organization (WHO) which is 1.0 mg $kg^{-1}$ for both fruits. Thus, it shows that despite a high occurrence of pesticide residues in the fruit samples, it could not be considered as a serious public health problem. Nevertheless, an investigation and research into continuous monitoring and more strict regulation of pesticide residues in the food commodities is recommended.

## ABSTRAK

Kebelakangan ini, racun perosak telah digunakan secara meluas untuk memastikan hasil tanaman yang tinggi di mana ia biasanya digunakan semasa pengeluaran dan rawatan selepas hasil tanaman. Walau bagaimanapun, peningkatan penggunaan racun perosak telah mengakibatkan pencemaran alam sekitar dan juga telah menyebabkan orang awam mendapat kesan jangka pendek dan kesan jangka panjang ke atas kesihatan manusia. Oleh itu, dalam kajian ini, usaha telah dibuat untuk menganalisis sisa-sisa racun perosak dalam buah-buahan segar yang dibeli dari pasaran buah-buahan di Gambang, Kuantan. Kromatografi gas dengan pengesan menangkap electron terpilihpengesan terpilih (GC-ECD) telah digunakan untuk menentukan jumlah sisa racun perosak chlorpyrifos dalam epal dan pir sampel yang pertama sekali dicuci terlebih dahulu dengan air suling dan pencuci buah-buahan setiap satu. Daripada kedua-dua sampel buah-buahan, ia menunjukkan bahawa kedua-dua sampel epal dan pir positif dengan sisa-sisa chlorpyrifos dalam julat 0.02mg $kg^{-1}$ hingga 0.06 mg $kg^{-1}$. Daripada keputusan, ia juga menunjukkan bahawa dengan menggunakan pencuci buah-buahan, ia digunakan dengan berkesan dalam mengurangkan jumlah sisa-sisa racun perosak dalam buah-buahan dalam julat sebanyak 35% hingga 40%. Walau bagaimanapun, daripada hasil sisa-sisa racun perosak kedua-dua sampel, kajian menunjukkan bahawa tiada racun perosak dikesan melebihi Maksimum Residu (MRL) yang ditubuhkan oleh World Heatlh Organization (WHO) iaitu 1.0 mg kg-1 untuk kedua-dua buah-buahan. Oleh itu, ia menunjukkan bahawa walaupun keputusan menunjukkan sisa-sisa racun perosak yang tinggi dalam sampel buah-buahan, ia tidak boleh dianggap sebagai masalah kesihatan awam yang serius. Walau bagaimanapun, penyiasatan dan penyelidikan dalam pemantauan berterusan dan peraturan yang lebih ketat terhadap sisa-sisa racun perosak dalam komoditi makanan adalah disyorkan.

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| | |
|---|---|
| ECD | Electron Capture Detector |
| FIFRA | Federal Insecticides, Fungicide, and Rodenticide Act |
| FPD | Flame-photometric Detection |
| GAP | Good Agricultural Practices |
| GC | Gas Chromatography |
| GC-ECD | Gas Chromatography with Electron Capture Detector |
| MRLs | Maximum Residue Limits |
| NPD | Nitrogen-Phosphorus Detector |
| WHO | World Health Organization |

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND OF STUDY

Fresh fruit is one of the essential elements of a healthy diet as they are a significant source of vitamins and minerals. In order to fulfil their needs, an increase in the yield crops of fresh fruits is necessary. Thus, the use of pesticides plays an important function in the increment of agricultural production. Pesticides are widely used in agriculture to increase the yield, improve the quality and extend the storage life of food crops (Fernandez-Alba, 2008). However, the increased use of the chemical pesticides has resulted in the pollution of the environment and also caused many related long-term effects on human health (Bhanti and Taneja, 2007). In this concern, monitoring residues of pesticides in fresh fruits wastewater is a necessary aim in pesticide research in order to evaluate the quality of the fresh fruits to avoid the potential harmful risk to human's health.

This research study investigates the pesticides residue in the fresh fruits as it was firstly washed by using distilled water and pesticides detergent. By using analytical procedure, gas-chromatography with selective electron capture detector (GC-ECD) is the suitable technique used for this study as it is most widely used in pesticide analysis. Besides, it is the major analytical technique used for determination of pesticides residues in agricultural commodities; because of its high separation power and its capabilities in determine the pesticides in one single analysis.

In addition, the maximum residue levels (MRLs) limit the types and amounts of residues that can be legally present on foods that are established and set by the regulatory bodies worldwide. Therefore, pesticide residue analysis is tremendously an important tool in determining the safety of using certain pesticides. Pesticides contaminating the environment and effects community and wildlife, hence the amount of pesticide residues being consumed becomes an important knowledge. Analytical quality requirements like precision, sensitivity and selectivity have been met to suit the need for any particular analysis.

Thus, a study is to be conducted in order to monitor the residue level in the fresh fruits as this problem is a major concern for the human health. The proposed method by using GC-ECD analysis was applied for compliance monitoring of fresh fruits pesticide residues. Besides, in monitoring the pesticides residue in fruits, Maximum Residue Limits (MRLs) analysis also being analyzed in this study as to control the use of pesticides residue in food community with good agricultural practice (GAP).

## 1.2 PROBLEM OF STATEMENT

Recently, pesticides continue to be an important input in modern agriculture and are widely used for controlling the pest in agriculture that is destructive and noxious organisms. Pesticides are a chemical compound that is widely used during production and post-harvest treatment of agricultural commodities. Pesticides may be defined as any substance that is used for controlling, preventing, destroying, repelling or mitigating any pest (Oudejans, 1994).

However, in recent research and development has proves that pesticides are the potential source of environmental pollution and exposure to pesticides could have negative consequences for human health. The problem is especially serious when the food commodities are consumed fresh. According to Berrada et al. (2010) studied, it was said that pesticides have been associated with a wide range of possible risk to human health, ranging from short-term effects to chronic effects like cancer, endocrine disruption disease and others. .

Pesticide residues in food commodities are a fast growing global problem with serious consequences on human health (Astamullah, 1996). Besides, unnecessary and irrational use of pesticide has created new pest that have never been a problem before (Huque, 1990). During recent years, there has been an increasing public concern and analytical analysis related to the presence and control of pesticide residues to control and avoid potential health to the human health more thoroughly.

Fruits and vegetables recently have been given a lot of attention in monitoring programs of pesticides since most of them are important in daily dietary and usually are eaten raw, it is expected that they contain higher pesticide residue levels compared to other food commodities. Therefore, assessing the risk of pesticide residues in the fruits and vegetables intended for human consumption is extremely necessary.

## 1.3 OBJECTIVES OF STUDY

The objectives of this study are:

1. to compare the Chlorpyrifos pesticide residue in the fruit samples that is first being washed by distilled water and fruit detergent.
2. to determine the pesticides residue level of Chlorpyrifos in fresh fruits by using gas chromatography with electron capture detector (GC-ECD) analysis.
3. to monitor the maximum residue limit (MRL) of the apple and pear samples.

## 1.4 SCOPE OF STUDY

The scope of this study is regarding the residue level of specific pesticides in fresh fruits that is firstly washed by using distilled water and pesticide detergent for washing fruits. The pesticide detergent that is being used in this study is commercially used by the public as a liquid cleanser to wash fruits and vegetables. This study is conducted in Gambang, Kuantan where the fruit samples will be purchased from the supplier of fresh fruit or fruit market in Gambang area. There are two types of fruits that will be used for this study which is apple and pear. Apple and pear are being used as two of them is usually being eaten raw and potentially have higher pesticide residues. Besides, the specific pesticides that will be analyzed throughout this study is Chlorpyrifos. This analytical study is using multi level residue method to analyze for determining the pesticides residues in the fruits. The analytical method that is being used in this study is the gas chromatography with selective electron detector (GC-ECD). GC-ECD analysis is being used in determining the pesticide residue level in the fruits. Furthermore, Maximum Residue Limits (MRLs) method also being used in monitoring the pesticide residue level in the fruits as to determine whether the residues exceeded the MRLs established by the World Health Organization (WHO).

## 1.5 SIGNIFICANCE OF STUDY

In compliance of analyze the pesticide residues in the fruits, the fruit is firstly being washed by the distilled water and pesticide detergent. This is due to compare the amount of pesticide residues in the fruits that is firstly being washed by the pesticide detergent and distilled water. Throughout this study, we can see if the pesticide detergent is effectively can remove some of the amount of pesticide residue in the fruits. If it is effective in removing the residues, it is highly recommended for the public to be used in controlling the pesticide residue in the fruits and also can reduce the possible health risk to human.

GC-ECD analysis is being used in this research as they are widely used in monitoring programs and to determine the pesticide residue in the fruits. GC-ECD also is known as the favoured technique for the determination of majority of pesticides. Besides, the analytical procedure by using GC-ECD also can contribute to a research field which is moving toward the development of very wide range of screening methods.

Additionally, to monitor the pesticide residues in the fruits, Maximum Residue Limits (MRLs) analysis also was being conducted in this study. The pesticide residue level in the fruits is being compared to MRLs that was established by the World Health Organization (WHO) as if it exceeded or not. By the MRLs analysis, it hopefully can contribute in monitoring the pesticide residues in the fruits as well as reduce the potential risk to the human health.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 INTRODUCTION

Fresh fruit is one of the important parts of a healthy diet as they are significantly enriched with vitamins and minerals. However, they also can be a source of noxious toxic substances which is pesticides. Pesticides are unavoidably or improperly used for protection against pests during production and post-harvest treatment of agricultural commodities. Besides, pesticides are also widely used in increasing the crop yields and quality of agricultural products. However, the widespread use of pesticides has resulted in pollution to the environment and also caused many potential hazards to human health (Bhanti & Taneja, 2007). These have been a matter of serious concern as the presence of pesticides residues in fruits that are consumed fresh.

## 2.2 TERMS AND DEFINITON OF PESTICIDES

As stated in Oxford dictionary, pesticides are defined as a substance used for destroying insects or other organisms harmful to cultivated plants or to animals. Pesticides are a numerous and diverse group of chemical compounds, which are used to eliminate pests in crop yields and households (Biziuk et. al, 2011). Meanwhile, pesticides are also widely defined by the United States' Federal Insecticides, Fungicide, and Rodenticide Act (FIFRA) as a substance or a mixture intended to prevent, destroy, repel, or mitigate any pest including insects, rodents and weeds (Laws & Hayes, 1991). In general, pesticides also may simply be defined as any substance or compound used for controlling, preventing, destroying, repelling or mitigating any pest. Pesticides significantly used as an input in modern agriculture and are used for management of controlling the pests that are noxious, destructive or troublesome organisms. Pesticides are chemical substances that kill the pests.

## 2.3 EFFECTS OF USING PESTICIDES

Pesticides enter the environment normally in various forms such as powders, moistened powders, powders for preparing aqueous solutions and sprays (Biziuk, Fenik & Tankiewicz, 2011). The usage of pesticides enables the quantities and the quality of crops and food to be monitored, and help to control many potential human diseases transmitted by insect or rodent vectors. They are also used to :

- control the numbers of pests destroying whole plants ;
- increase the production of animal and plant biomass ;
- combat microorganisms causing farm produce to rot and to decay;
- combat algae, bacteria, fungi and weeds;
- combat animal pests damaging crops
- stimulate or inhibit plant-growth processes
- make possible the action of other substances
- counteract growths on boats and ships; and
- kill harmful organisms in farm building, the home, hospitals, stores and vehicles.

However, besides their benefits to the agriculture, pesticides are some of the most noxious toxic substances in the environment (Biziuk et. al, 2011). The highly usage of pesticides not only pollutes the environment but also causes them to accumulates in yield crops (Fig.1). Pesticides are mainly transported by rain and wind from their point of application to neighbouring crops, where their presence may be harmful or unwanted (Moreno et al., 2006). Their excessive use in crops has a harmful and dangerous effect on human health and in contamination of the environment (Barnett et al., 2001). Besides, pesticides have been related with a wide range of human health hazards, ranging from short-term impacts such as headache and nausea to chronic and long-term impacts like cancer and endocrine disruption (Berrada et al., 2010).

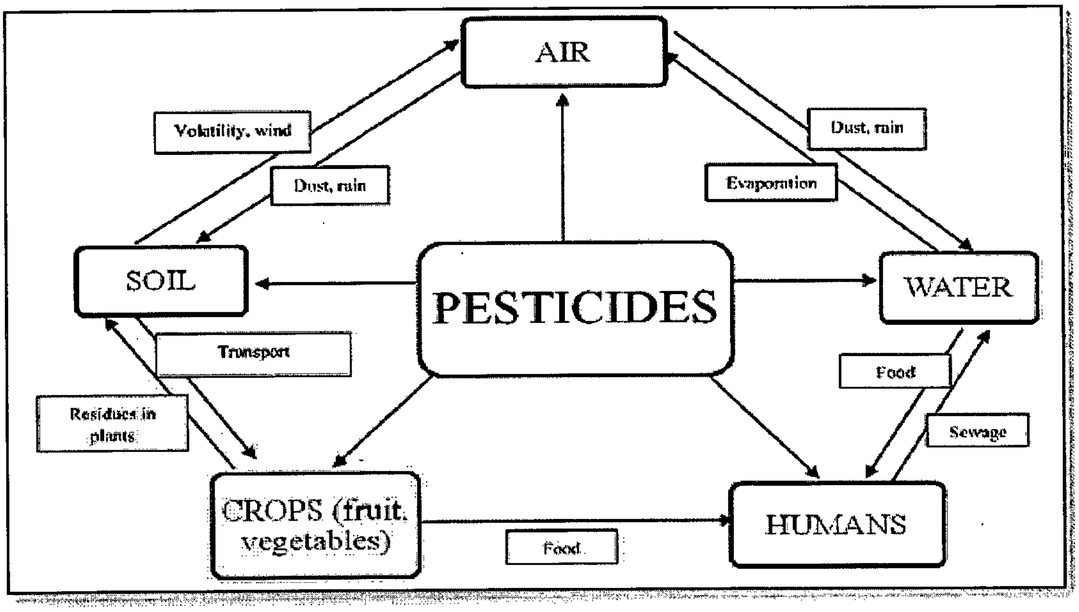


**Figure 2.1** The circulation of pesticides in nature (including crops)

Pesticides have many advantages, but they also have disadvantages that bring much harmful to the environment and human health. Figure 2 lists some of the effects of using pesticides.

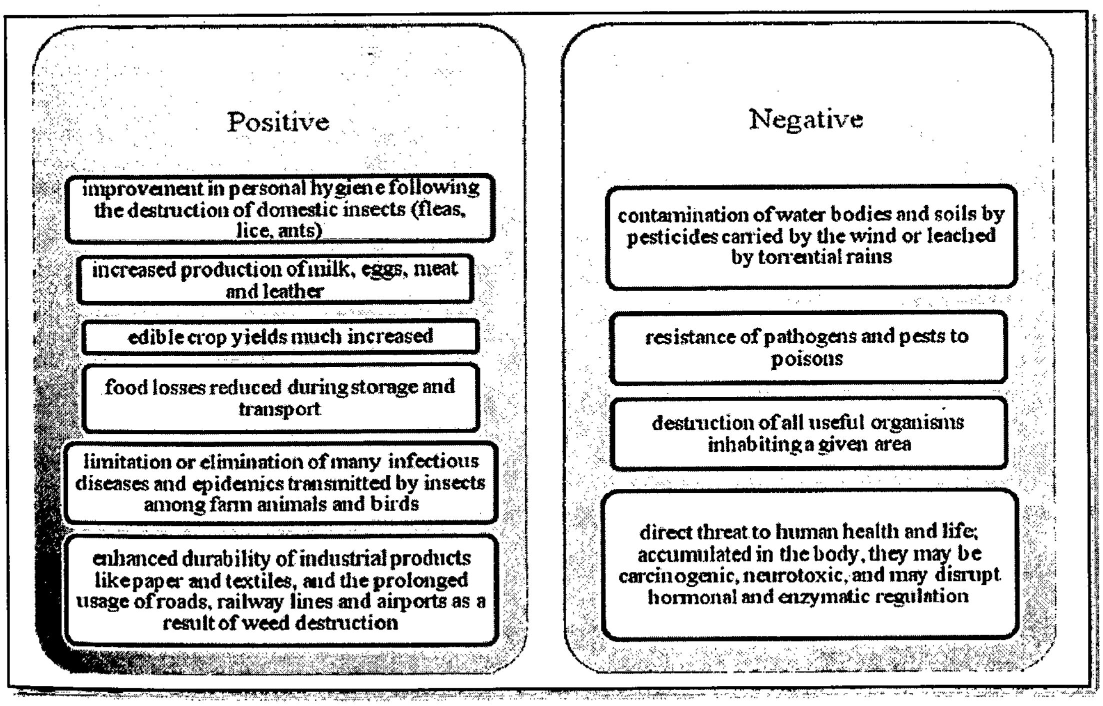


**Figure 2.2** The effects of using pesticides

## 2.4 MONITORING PESTICIDE RESIDUES IN FRUITS

Nowadays, pesticides are extensively used to ensure high crop yields and commonly used during production and after harvest treatment of agricultural commodities (FAO & WHO, 2004). However, the widely used of pesticides in food commodities have brought to a serious concern for the public safety and health especially when this products are consumed fresh (Solecki et al., 2005). The high usage of pesticides have brought to possible human health hazards as the chemical properties in pesticides will brought numerous type of diseases to human such as respiratory and cutaneous irritations and also can brought to carcinogenic diseases that can cause death (Ascherio et al., 2006). Furthermore, it also additionally can cause changes in the ecosystem with harmful effect for the environment, due to wide spread used of using pesticides (Costa et al., 2008).

Hence, a risk assessment is needed to control the health effects that caused by the intakes of pesticides residue in food commodities. Monitoring pesticides residue in the food is the only techniques to control the amount of pesticides in food. Besides, according to Blasco et al. (2001), pesticide residue monitoring is recognized as an important aspect of effort to reduce potential hazard to human health due to the increase use of pesticides in food. Furthermore, in monitoring programs of the pesticides residue in food, fruits and vegetables have been given a lot of attention since most of them are eaten raw and they are expected to contain higher pesticide residue levels compared to other food groups (Chen at al., 2011). Therefore, monitoring the pesticides residue in the fruits for human consumption is necessary.

Due to the wide variety using of pesticides, multi-residue methods are important tools to be applied to monitor and control the quantity of pesticides on food. This method allows monitoring a great number of multi-class pesticides by a single run (Fernandez et al., 2006). The monitoring of pesticides residues also is recognized as an important element of initiatives to reduce potential harmful risk to human health ([Blasco et al., 2005] and [Dogheim et al., 2002]). Therefore, multi-residue analysis methods are necessarily plays an important role in monitoring the pesticides residue level in fresh fruits.

In the determination of pesticides residues in fruits, there are many methods that is being used and continually being revised and improved with new and conventional techniques. Gas chromatography is the technique most widely used in the analysis of pesticides residue because of its high resolution capacity and the availability of selective detectors in monitoring the pesticides (Fernandez, Pico & Manes, 2001). This technique has the ability to determine a significant number of pesticides and their compatibility in a wide range of food and environmental samples (Frost, 1996). Additionally, gas chromatography is also used because its capability for sensitive and specific detection to determine several multiclass pesticide in one single analysis ([Fernandez et al., 2001] and [Sannino et al., 1996]).

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases one stationary phase and one mobile. The sample is carried in the mobile phase through the stationary phase and one mobile. The sample is carried in the mobile phase through the stationary phase in a column. The components of the sample interact with stationary phase and separate into bands. These emerge from the end of the column in order of their increasing interaction with the stationary phase. Those compounds that interact least emerge first. The mobile phase may be either gas or liquid, and the stationary phase either liquid or solid. Gas chromatography has a gaseous mobile phase and a solid or liquid stationary phase. It is extensively used for thermally stable and volatile organic and inorganic compounds.

Chromatographic methods are the most suitable method in analysis of pesticides residue in particular gas chromatography by using long, narrow-bone capillary columns equipped with selective and sensitive detection methods such as electron capture detection (ECD), nitrogen-phosphorus detection (NPD) and flame-photometric detection (FPD) according to different types of pesticides. The choice of chromatographic column is necessary for separating analytes and for their qualitative and quantitative determination. The chromatographic column should be highly efficient and resistant to changes in the parameters of the separation process of the analytes.

## 2.5 ANALYTICAL PROCEDURES BY USING GC-ECD

Recently, with the advanced technologies emerged, many laboratories have developed their own multi residue methods for monitoring pesticides in food, especially in pesticide residue. However, only a limited range of pesticides is determined by varieties of the proposed screening methods. Thus, this has led to the development in the modern multi residue methodology in producing reliable procedures in determining as many pesticides as possible in the most rapid and accurate technique. Gas chromatography with selective electron capture detector (GC-ECD) is the favoured technique in determine majority of pesticides in foods. GC-ECD is highly sensitive in relation to compounds containing electronegative atoms and generally used in determination of pesticides residue (Jolanta, Maciej & Marek, 2011).

Besides, the electron capture detector (ECD) is one of the most widely used ionisation detector and it owes much of its popularity to its high sensitivity to a wide range of toxic and biologically-active compounds (Aue and Kapila, 1973). Consequently, it is widely used in trace analysis for the determination of pesticides, herbicides, industrial chemical in the environment, drugs and other biologically active compounds and for the determination of the fate of the volatile organic compounds in the upper atmosphere. These highly selective and sensitive detector have enables the development of gas chromatography (GC) methods to the determination of pesticide residues in fruits and vegetables.

According to Gelsomino et al. (1997), the selective and highly sensitive detectors in ECD have provided good responses even to the very low concentration of analytes. In ECD, the detector consists of a cavity that contains two electrodes and a radiation source that emits β-radiation. The collision between electrons and the carrier gas produces plasma containing electrons and positive ions. If a compound is present that contain electronegative atoms, those electrons are captured and positive ions formed and rate of electron collection decreases. The detector is extremely selective for compound with atoms of high electron affinity, but has a relatively small linear range. ECD is frequently used in the analysis of pesticide residues in foods.

The sensitivity of ECD is reduced when there is sample that overloading the cell. The induction of too much sample can happen very easily when doing ECD because it is extremely sensitive to certain types of compounds. Since response varies widely, depending on the nature of compound, careful judgement is required of the operator as to the appropriate amount of sample that should be used for any particular analysis.

However, ECD has a small linear range even though it has high sensitivity to electrophilic molecules. Thus, an appropriate preparation of sample is required in order to ensure the sample component concentrations will fall within the range. For a range of compound types, relative response factors have been determined ( Devaux & Guiochon, 1967). Whereas, the choice of carrier gas for use with ECD is only limited to nitrogen and the noble gas (Hartmann, 1973). Pure argon and helium are unsuitable as they can easily transfer excitation energy by collision that produces undesirable ionisation effect (Braithwaite & Smith, 1985).

In addition, the temperature dependent also plays an important role in the maximum response of the ECD towards different organic compounds. For a single compound the change in response of 100 to 1000 fold should occur for 100°C change in detector temperature (Poole, 1976). This high temperature dependence can be derived directly from the kinetic model of the ECD process (Poole & Zlatkis, 1981). The temperature dependence can be used to diminish in order to minimise unwanted interferences during measurement of a particular analytes (Chen & Wentworth, 1967).

Gelsomino et al. (1997) has determined the multi residue analysis of pesticides in fruits and vegetables by using gas chromatography with electron capture detection (ECD). From the analysis, recoveries of majority of pesticides from spiked samples of carrot, melon and tomato at fortification levels of 0.04-0.10 mg/kg ranged from 70 to 108 %. The lowest recovery was for chlormephos (51.5%) Limits of detection were less than 0.01 mg/kg for ECD. The finding has contributed to a research field which is moving toward the development of varieties of screening methods by using GC-ECD.

Besides, Chen et al. (2011) also has used gas chromatography with electron capture detector (GC-ECD) in determining the concentrations of 22 pesticide residues among those recommended for pest treatment for agriculture. Of 1135 samples (37.7%) that contained pesticide residues were most frequently detected, with 17.2%, 18.9% and 17.2% of the samples exceeding the maximum residue limits (MRLs), respectively. Data obtained were used then for estimating the potential health risks associated with the exposures to these pesticides. The results show that despite a high occurrence of pesticide residues in fruits and vegetable, it could not be considered a serious public health problem.

Moreover, Nakamura et al. (1993) also used GC-ECD in determined pyrethroid residues in vegetables and fruits by using a methyl silicone-coated fused silica capillary column. The samples were extracted with acetone filtered after addition of coagulating solution partitioned into n-hexane, and cleaned up on a Florisil column. The coagulation method was not applicable to recoveries. Recoveries for 18 crops at fortification levels of 0.25-1.0 mg/kg were 60 to 103.5 %. No pyrethroid was detected from the non fortified crops tested except green tea leaves in which fluvalinate was detected at 0.89 mg/kg. Thus, it shows that GC-ECD is widely used in determination of wide range of pesticides and other biologically active compounds.

Nevertheless, Anwar et al. (2001) studied also analyzed the pesticide residue n fruits and vegetable samples collected from Islamabad Sunday markets, Pakistan by using GC-ECD. From the analysis, dimethoate was forum in the quantity of 0.032 mg $kg^{-1}$ in banana, 0.004 mg $kg^{-1}$ in brinjal, 1.80 mg $kg^{-1}$ in cauliflower and 0.13 mg $kg^{-1}$ in arvi, fenvalerate 0.010 mg $kg^{-1}$ in apple and chlorpyrifos 0.004 mg $kg^{-1}$ in brinjal.