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REVIEW ON METHODS FOR ANALYSIS /ESTIMATION OF PESTICIDE RESIDUE IN FOOD

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Abstract

Pesticide residue analysis of fruits and vegetables is of great importance not only towards protection of human health but also for international trade and regulatory control. In the last years, pesticide residue analysis, based on high-resolution accurate mass spectrometry (HRAMS), has gained great acceptance in this field due to the improvements in sensitivity and resolution incorporated in modern HRAMS instruments. The current work deals with the process and general procedure to estimate a pesticide residue in food which includes the sampling procedures for different food commodities, packaging, trans- mission of laboratory samples, sub-sampling, homogenizing the sample for analysis, re- serve storage of sample, solvent selection, extraction, clean-up and then estimating the pesticide residues using few standard estimation procedures (micro bio assay, enzyme inhibition method, spectrophotometric method, chromatography, etc.). This also includes a case study by Asi. et.al.[9]. The conclusion made out from the study is that the use of pesticide is necessary in certain limits which comes under the Good Agricultural Practice (GAP). But uncontrolled application of these leaves residues which cause adverse health effect. The study also provides farmers with adequate information for safe practices for use of pesticides, side effects caused by handling of the harmful chemicals. Our study revealed that chemical control is the principle pest control method followed by farmers in the study area and this usage could be reduced by providing them with training and information about the bio pesticides and helping them know the seriousness that is being posed by excessive use of chemical pesticides and their residue.

Keywords: Pesticide, residue, GAP, GLP, HAACCP, HRAMS, chemical substances.

1 Introduction

Pesticides are chemical substances that are meant to kill pests. In general, a pesticide is a chemical or a biological agent such as a virus, bacterium, antimicrobial, or disinfectant that deters, incapacitates, kills, pests. Thus, use of pesticides is so common that the term pesticide is often treated as synonymous with plant protection product. It is commonly used to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity. The most commonly applied pesticides are insecticides to kill insects, herbicides to kill weeds, rodenticides to kill rodents, and fungicides

to control fungi, mold, and mildew. The Food and Agriculture Organization(FAO) has defined pesticide as any substance or mixture of substances intended for preventing, de- stroying or controlling any pest including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the produc- tion, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feed stuff or substances that may be administered to animals for the control of insects arachnids or other pests in or on their body. Pesticides are used worldwide to protect crops before and after harvest in agriculture and gardening. Variety of pesticides are used in current agricultural practice to manage pests and infections that spoil crops [1]. Pesticides have potentially adverse effects on vegetables, fruits, animal resources and human health [3]. Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. The pesticides must undergo extensive efficacy, environmental, and toxicological testing to be registered by governments for legal use in specified applications. The applied chemicals or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure. Selected sampling programs can be used to investigate residual levels of pesticide in the environment, their movement and their relative rates of degradation. The maximum residue levels (MRLs) or tolerances in the United States) limit the types and amounts of residues that can be legally present on foods are set by regulatory bodies worldwide. Pesticide residue analysis is tremendously an important process in determining the safety of using certain pesticides. Pesticides polluting the earth and causing problems in human beings and wildlife, the quantity of pesticide being consumed becomes necessary knowledge. Analytical quality requirements like precision, sensitivity and selectivity have been met to suit the need for any particular analysis.

GENERAL PROCEDURE TO ESTIMATE A PESTICIDE RESIDUE IN FOOD

Residue of fungicide are present in micro quantities in the matrix. Hence, involve a complicated procedure involving many steps for their analysis. Analysis does not depend on high cost equipment like GC (gas liquid chromatography) and HPLC (high performance liquid chromatography). But based on series of cumulative operation like: -

1. Sampling
2. Extraction
3. Clean-up
4. Estimation

SAMPLING

Sampling can be defined as the procedure or step adopted to obtain a representative quantity from the large consignment, so that selected representative quantity can be handled conveniently.

The first step in any sampling procedure is to clearly define the population that is going to be sampled. The population may vary in size from a production lot, a day's production, to the contents of a warehouse. Information obtained from a sample of a particular production lot in a warehouse must be used strictly to make inferences about that

particular lot, but conclusions cannot be extended to other lots in the warehouse. Once sampling is conducted, a series of step wise procedures — from sample preparation, lab- oratory analysis, data processing, and interpretation — is needed to obtain data from the samples. In each step, there is a potential for error that would compromise the certainty, or reliability, of the final result. This final result depends on the cumulative errors at each stage that are usually described by the variance (2,3). Variance is an estimate of the un- certainty. The total variance of the whole testing procedure is equal to the sum of the variances associated with each step of the sampling procedure and represents the precision of the process. Precision is a measure of the reproducibility of the data. In contrast, accuracy is a measure of how close the data are to the true value. The most efficient way to improve accuracy is to improve the reliability of the step with the greatest variance that is frequently the initial sampling step. The reliability of sampling is dependent more on the sample size than on the population size. The larger the sample size the more reliable the sampling. However, sample size is limited by time, cost, sampling methods, and the logistics of sample handling, analysis, and data processing.

TABLE 1
FACTORS TO BE CONSIDERED FOR SAMPLING FACTORS TO BE

CONSIDERED	QUESTIONS
Purpose of inspections	• Is it to accept or reject the lot? • Is it to measure the average quality of the lot? • Is it to determine the variability of the product?
Nature of the product	• Is it homogenous or heterogeneous? • What is the unit size? • How consistently have past populations met specifications • What is the cost of the material being sampled?
• Nature of the test method	• Is the test critical or minor? • Will someone become sick or die if the population fail to pass the test? • Is the test destructive or non-destructive? • How much does the test cost to complete?
• Nature of population being investigated	• Is the lot large but uniform? • Does the lot consist of smaller, easily identifiable sub lots? • What is the distribution of the units within the population?

Sampling procedure for different food commodities

Collect a total sample approximating 10 kg by sampling a minimum of 10/ 1 kg sub samples

selected at random from the lot. Small retail units may necessitate the collection of several units to total 1 kg per subdivision. For large individual items (1 kg or more) such as fish, melons, cabbage heads, cauliflower, pineapple etc. collect a total composite of 10 subs taking only one unit from each of 10 different shipping containers or locations in the lot. Collect a total composite sample of 10 kg by collecting 1 kg portions from each of 10 different bulk containers in the lot. The minimum amount of material to be submitted to the laboratory, is as follows

COMMODITY	EXAMPLES	MINIMUM QUANTITY REQUIRED
Small or light products, unit barries weight upto about 25 g	Peas, olives parsley	1 kg
Medium sized products unit weight usually between	apples, oranges, carrots potatoes	1 kg (at least 10 units)
Large sized products unit weight over 250 g	cabbage, melons, cucumber	2 kg (at least 5 units)
Dairy products dairy products	Whole milk, cheese buffer, cream	0.5 kg
Egg (10 unit if whole) meat	Poultry, fat, fish and other fish and animal products	
Oils and fats	cottonseed oil margarine	0.5 kg
Cereals and cereal products		0.5kg- 1 kg
Spices	Chilies, cumin, coriander	0.25kg

FIGURE 1: SAMPLE REQUIRED FOR DIFFERENT COMMODITIES

Packaging and Transportation of laboratory samples

The laboratory sample must be placed in a clean non-reactive container which offer adequate protection from external contamination and protection against damage to the sample in transit. The container must then be sealed in such a manner that unauthorized opening is detectable, and sent to the laboratory as soon as possible taking any necessary precautions against leakage or spoilage, e.g. frozen foods, should be kept frozen, perish- able samples should be kept cooled or frozen. Fruits /vegetables or any other commodities are advised to ship in dry ice while transporting from field to lab for analysis.

Sub sampling

The way in which a sample is taken for analysis is the first of a series of potential sources of error in food analysis. Some liquid foods are reasonably homogeneous, but solid and semi-solid foods are most always heterogeneous. It must be assumed that the attribute for which the food is being examined is unevenly distributed throughout the sample. Liquids are advised to bring back to room temperature before sub sampling. The taking of a representative sample is obviously the most difficult task. A liquid food (e.g. milk) generally need only be well mixed or shaken before sub sampling. Semi-solid foods

are those containing a solid material plus a large portion of free liquid. Examples include many canned foods. In the event that the solid or the liquid are to be analyzed individually, they are separated using a sieve or filter and individually mixed for sub sampling. When both solid and liquid phases are to be analyzed as a unit, it is often advisable to blend or otherwise homogenize the two before sub sampling. Solid samples can be of three general types, namely finely divided (e.g. whole cereal grains or flour), an aggregate (e.g. solid mixtures such as sausage), or a whole unit (e.g. an entire fruit). Finely divided dry products can be mixed for sub sampling using commercial portioning equipment such as a Jones Divider, or by spreading the sample over a large surface, quartering with a straight-edge and mixing opposite quarters. The two mixed halves can be recombined and the process repeated one or more times to make the sub sample portion even more representative. An aggregate solid sample is probably the most difficult as it consists of different food materials usually with different physical properties. The challenge is to take a sub sample having a composition representing an average of the food sampled. This most often requires that the aggregate food be chopped or ground before mixing and sub sampling. The whole unit sample can be most easily sub sampled by taking a representative portion of the food. This could be a quarter of a fruit, a piece of loin from a whole fish or other similar sectioning.

Composting

A composite is defined as an admixture of two or more portions of a substance. A composite is formed by first sub sampling two or more portions of the same food. An example would be sub sampling several individual cans from the same food lot. These sub samples are then combined and mixed so that a portion taken of the composite would be representative of the whole. A composite is simply a physical attempt to average the normal variation between individual sample units or portions, it is most useful when the analytical result must be compared to a standard or requirement involving the entire food product. As the composite is to be representative, the sub samples of the individual sample units must not only be taken correctly, but must all be approximately the same size, weight or volume. Given correct sub sampling, the only remaining problem is to make the composite reasonably uniform and representative. This may involve chopping and grinding as well as physical mixing.

Chopping, Grinding, Mixing (Reducing the size and Increasing the surface area)

The well-equipped food analysis laboratory should have a variety of sample preparation equipment including mechanical choppers, mincers, grinders, blenders and a hammer or similar mill. Use of dry ice is recommended in case of volatile and unstable molecules during extraction. The type of mechanical processing equipment selected will depend on the food product to be treated. The analyst must also keep in mind that mechanical grinders, mills, etc. usually generate heat during the processing. This can possibly change the sample composition, such as for fatty foods where the heat may be sufficient to partially melt the fat. In such cases, hand chopping and mixing may be the best procedure. In other instances, the sample may have to be frozen before grinding. The analyst must judge the best method for himself, depending on the kind of food and the substance for which it is to be analyzed. The moisture content of a food also plays an important role in determining the food processing procedure or equipment to use. Dry foods can

generally be milled, while moist foods can be chopped, minced or ground. Very moist and liquid foods can be blended. The home food processors now available are very useful for many products. If no mechanical processing equipment is available, then of course hand processing must be done. The tools used include knives, granters and choppers. When a sample is processed by hand, it must be sufficiently finely divided to permit proper mixing and later sub-sampling of the mixture. The analyst must always keep in mind that proper sample preparation is not only to gain a representative portion for analysis, but is also to prevent change in the sample which may result in a biased analytical result.

Homogenization of sample composite

Ideally, the entire sample should be homogenized using equipment such as a Hobart food cutter. Before being placed in the chopper, samples may need to be either halved or quartered (e.g. apples, peaches) or cut into small (5-10 cm) pieces (e.g. cantaloupe, carrots, squash). After homogenization, remove a portion for analysis. If equipment such as a Hobart food cutter is not available, the sample may be homogenized in an appropriate blender. In this case, prepare a composite sample consisting of approximately equal parts (weight or number) from each unit. Special attention must be paid to the method of cutting sections of fruit and vegetables. Pesticides may tend to collect in the stem area of fruits and on the top of vegetables. Vertical sections must therefore, be cut through the stem and center of fruits and the top and center of vegetables. Finely cube the composite sample and reduce by mixing and quartering to ca 300 g. Homogenize the 300 g in an appropriate blender. If the homogenized sample is not immediately analyzed, store it in a clean container with a tight closure and freeze. Samples should be refrigerated, if they are expected to be analyzed within four days. Aqueous or semi-aqueous samples should be kept at < -10 $^{\circ}$C or below, before analysis. Freezing is often the only way to prevent a change in food before analysis or for reserve storage. Some foods, like whole fish, may need to be frozen before grinding. The single most important problem in handling frozen food samples is proper thawing before analysis. Thawing must take place in such a manner that the composition of the food remains unchanged. Thawing should be done slowly without heat. Any separated liquid must be mixed back in thawed product before composite preparation.

Sample storage

Normally the whole sample is stored depending on analysis at <-10 $^{\circ}$C. In certain cases where unusually large samples are submitted or where storage space is at a premium, a suitable sub-sample may be taken. The sub-sample must be homogeneous and truly representative of the original sample. Its size will be determined by the analyses required and the methods of analysis employed. Homogenization will increase the rate of enzymatic hydrolysis so frozen homogenized samples should be analyzed within two weeks. In the case of fruits and vegetables for human consumption only the edible portion is analyzed. Soil will be removed from root vegetables, by gentle brushing under a stream of water. Outer leaves of cabbage, cauliflower, etc. will be removed. Various commodities are stored as follows:

(a) Butter, cheese, eggs and ice cream — freeze the whole sample.

(b) Dry feeds — store at room temperature in airtight container.

(c) Feeds for fumigant analysis — seal in plastic bags and freeze.

(d) Fruits and vegetables freeze or refrigerate the whole sample.

(e) Animal fats — freeze the whole sample.

Reserve storage

The reserve portion of a food sample must be maintained in storage so that there is very little or no change from the original analysis. Ideally, the reserve portion analyzed at a future time will give a result equivalent to the original. It should consist of an adequate portion for reanalysis and a second party's analytical challenge. The recommended storage is < -10 °C.

EXTRACTION AND CLEAN-UP METHODS IN PESTICIDE RESIDUE ANALYSIS

Extraction

Extraction means separation of pesticide residues from the matrix by using solvent.

The extraction procedure should be such that it quantitatively removes pesticides form matrix (high efficiency), does not cause chemical change in pesticide and use inexpensive and easily cleaned apparatus. The extraction method and solvent type determine the extraction efficiency from substrates.

Choice of extraction method

The main objective behind employing a particular method for a specific substrate is to bring the solvent to close proximity of the pesticide residues for sufficient period so that pesticide residues get solubilized in the solvent. The choice of method depends on the type of substrate and ageing of residues. The substrates in pesticide residue analysis could be liquids like water, fruit juices, body fluids (urine, blood etc.) and solids like soil, flesh, green plant materials (leaves, fruit etc.), dry fodder, grains etc.

LIQUID SUBSTRATE

(a) Partitioning: Samples like water, body fluids, and juices are extracted by partition- ing with water immiscible solvent. The addition of sodium chloride in aqueous samples improves the extraction efficiency by reducing the solubility of pesticide in water. It also prevents the emulsion formation, which is frequently encountered during partitioning.

(b) Use of absorbent: The pesticide residues from aqueous samples can be extracted by passing the sample through solid adsorbents packed in glass column. The adsorbents have high affinity for pesticide molecules; therefore, they are held up on the absorbent whereas water passes out. The solid adsorbents are then extracted with organic solvent. The solid adsorbents normally used for removal of pesticide from aqueous samples are given below:

Small packed columns of RPC 18 and C-8 with trade name Sepack are commercially available in the market.

SOLID SUBSTRATE

(a) Fresh residues

Dipping, tumbling, shaking: This method is usually employed for solid substrate when pesticide residues are present on the surface as in case of freshly applied pesticide.

(b) Weathered residues

When sufficient time has elapsed after the application (weathering), the residues are not present on the surface but they penetrate the substrate matrix and are in adsorbed form. The substrate matrix needs to be broken down in fine particles before extraction

Solid adsorbents	Liquid coated or bonded on inert solids
1. Activated charcoal	1. Carbowax 4000 coated on chromosorb
2. Polyurethane foam	2. Undecane coated on chromosorb W.
3. Cellulose triacetate	3. RPC-18-HPLC column material
4. Molecular sieves	4. RPC-C-8-HPLC column material
5. Ion exchange resins	
6. Magnsium Sulphate	
7. Silicagel (activated)	
8. Akynuba activated (acidic, basic and neutral)	
9. Florisil and Extrulet	

FIGURE 2: Examples for adsorbents.

with solvent. The methods that employ these techniques are macerating/blending, macerating/blending followed by column extraction, soxhlet extraction, etc.

CHOICE OF SOLVENT

The choice of solvent for extraction depends on the

a) nature of the substrate and

b) the type of pesticide to be extracted. However, the solvent should satisfy the following

- Should have high solubility for the pesticide and least solubility for co-extractives.
- Should not change the pesticide chemically or react with it.
- Economical
- Low boiling.
- Easily separated from the substrate.
- Compatible to the method of final determination.

Choice of solvent depending on type of substrate

The solvent for extraction of pesticide in different substrate is chosen as follows.

1. Aqueous substrate
2. Solid substrates

Choice of solvent depending on nature of pesticides

The pesticide molecules can be broadly divided into two groups' namely non-ionic and ionic type. The non-ionic pesticides also differ in their polarity. For non ionic type of pesticides, organic solvents with varying polarity depending on the polarity of pesticide molecules are employed.

Recent techniques of extraction

1. Solid Phase Extraction
2. Solid Phase Micro-Extraction
3. Accelerated Solvent Extraction
4. Microwave-Assisted Solvent Extraction
5. Supercritical Fluid Extraction
6. Stir-Bar Sorptive Extraction.

Clean-up

Cleanup refers to a step or series of steps in the analytical procedure in which the bulk of the potentially interfering co-extractives are removed by physical or chemical methods. After removal of moisture, the other co-extractives are removed by using various separation techniques.

Liquid-liquid partitioning

In this technique, co-extractives from the extract are removed by partitioning the residues between two immiscible solvents.

1. Acetonitrile-hexane partitioning: Acetonitrile-hexane partitioning is used for the removal of oil and fat from the extract. This technique is used for the cleanup of extracts of oil seeds, milk, butter etc.
2. Partitioning with acid/base treatment: This technique can be used for the pesticides, which are either acidic or basic in nature. This technique cannot be used for neutral type of pesticides.

Chemical Treatment

In these techniques, the co-extractives are either precipitated and separated by filtra- tion or made water-soluble so that pesticide can be partitioned into water miscible organic solvent.

1. Saponification: This technique is employed to remove fats and oils from the extract. The fat and oil are saponified or hydrolyzed by treatment with alkaline aqueous

solution. This method can be employed for the pesticides that are stable to alkali treatment or the pesticides, which give definite product that can be analyzed easily.

2. Precipitation: This technique can be used only for the pesticides having some water solubility. In this technique, the co-extractives are precipitated with a coagulating agent like ammonium chloride.

3. Oxidation: In this technique, the co-extractives are oxidized with concentrated Sul- phuric acid. This technique can be used for pesticides, which are stable to acid. For example, this technique has been used for the clean up of milk extracts containing hexachlorocyclohexane, dichlorodiphenyltrichloroethane, aldrin and dieldrin.

Chromatographic techniques

Chromatography is a technique used for the separation of constituents from the mix- ture. In Chromatography, two phases are involved in separation. The extract from the sample contains mixture of pesticide and co-extractives; various Chromatographic tech- niques can be employed for separating them or removal of the co-extractives from the pesticides.

1. Thin Layer Chromatography (TLC): For clean-up, preparative TLC plates (20 × 20 cm) with thick layer of adsorbent (~ 2 mm) are used. Silica gel plates are normally used but other adsorbents like alumina can also be used.

2. Ion Exchange Chromatography. Ion exchange resins can also be used for clean-up of ionic pesticide. For cationic pesticides like paraquat and diquat, cation exchange resins (H+) while for anionic pesticide like 2,4-D, anion exchange resins (Ch) are used. The matrix contains fixed charged groups are the counter ions of opposite charge. These counter ions can be exchanged from other ions of similar charge in the mobile phase. The aqueous extract containing pesticide is passed through a col- umn of ion exchange resin. The exchange resin holds up the pesticide being ionic, whereas nonionic co- extractives pass out of the column. The held up pesticide is eluted out using suitable electrolyte solution.

3. Gel Permeation Chromatography and Molecular Sieves: The separation in gel per- meation Chromatography and molecular sieves is based on the principle of size exclusion. Both gel and molecular sieves have tubular structures with inner diam- eter (id) similar to the molecular sizes. The molecules having size greater than the tube id do not pass through it. The molecules having size less than the tube id pass through it. Molecules having greater size moves faster than the smaller ones, en- abling separation of molecules occur depending on their sizes. The co-extractives like chlorophyll, other pigments, etc. have molecular sizes greater than most of the pesticide, therefore, they are easily separated. Also, the co-extractives having molecular size less than pesticide molecule will elute later than pesticide.

4. Adsorption Column Chromatography. Adsorption column Chromatography is the most common and widely used technique for clean-up. Different type of adsor- bent or mixture of adsorbent have been used for clean-up. The adsorbent generally

used for clean-up are Silica gel (80-100 mesh), alumina (acidic, basic, neutral), polyethylene coated alumina, Florisil, Charcoal and mixtures of charcoal + Celite
+ MgO.

Recent Trends in Clean up

1. Solid phase extraction cartridges: Serves the dual purpose of extraction and clean up. Advantages of SPE device over other conventional solvent extraction and clean- up of pesticides includes better reproducibility, reduction in solvent use, high speed, versatility, freedom from interferences and field applications.

ESTIMATION OF RESIDUE

- Both Qualitative and quantitative measurement of small amounts of pesticides in or on any treated surface is known as assay or estimation.

- Techniques of residue estimation -Micro bioassay((a) Direct exposure, (b) Extrac- tion method), Enzyme inhibition method, Spectrophotometric method, Chromatog- raphy(Paper chromatography, Thin Layer chromatography, Gas Chromatography, High pressure liquid chromatography).

Micro bioassay – Bioassay has been defined as measurement of potency of any stimulus, physical, chemical or biological, physiological by means of reactions which it produces in living matter.

(a) Direct exposure

This method consists of macerating and blending the plant tissue and exposing the test insect. No extraction of clean-up is required.

(b) Extraction method

- Dry film method-Test organism is exposed to film of pesticide in solution or de- posited in crystals.

- b) Aqueous Suspension Method-This method involves extraction of pesticide by evaporating solvent, making suspensions of residues in water and testing it.

Enzyme inhibition method

- Inhibition of cholinesterase enzyme system in animals by pesticides particularly OP and carbamates is a basis of this method.

Spectrophotometric method

- Amount of electromagnetic radiations absorbed is a measure of quantity of sample present and provide information regarding chemical identity of sample.

Chromatography

Flow of gas or liquid in a sorptive medium brings about separation of substances by differential migration from narrow zone in a porous sorptive medium.

(a) Paper chromatography-Stationary phase is a sheet of paper containing water or some other polar phase. Two opposing forces are in operation, one is driving which act to move substances in direction of solvent flow and other resistive action which impede movement of substances.

(b) Thin Layer Chromatography-Mixture of silica gel and water in the ratio 1:2 is prepared on carrier plates. Spot of sample is poured on the plates.

(c) Gas Chromatography -Commonly used. Provide both qualitative and quantitative analysis. Mixture of pesticides can be analyzed.

2 Conclusion

It is concluded that these are few procedures that could be used for the analysis of pesticide residues in food commodities, fruits and vegetables, etc that are taken and presented here from the references mentioned in the reference section. Checking for pesticide residues in different samples should be done after regular intervals. This help in maintaining product conformity to the standards established which help in free trade among nations and also used for producing commodities that are less harmful to consumers.

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