Methods of Pesticides analysis

M.Sc. 2nd semester

Paper – 201

ICA/EC

PESTICIDES

- A pesticide is a substance or mixture of substance intended for preventing, destroying, repelling or lessening the damage caused by the pest.
- A pesticide can be a insect, plant pathogen, weed, bacteria, bird etc. That compete with the human for food, destroy property, spread disease.
- A pesticide can be a chemical, biological agent, antimicrobial, disinfectant etc.
- Many chemical pesticides are poisonous to human and animals.

Classification of pesticides

1. **Herbicide-**These are the chemicals used to kill weeds (i.e., unwanted plants) e.g. Borax, Nitrofen.
2. **Insecticide-**These are used to kill insect. E.g. DDT, BHC.
3. **Rodenticide-**These are used to kill rodents. e.g. Warfarin, Zinc phosphide.
4. **Nematicide-**These are used to kill namatodes e.g. DBCP, Phorate
5. **Molluscicide-**These ar used to kill molluscs e.g Sodium pentachloridephenate.

6.Fungicides-These are used to kill fungus e.g.
Bordeaux mixture

7.Algaecides-These are used to kill algae e.g.
Copper sulphate, Endothal

8.Bactericide-These are used to kill bacteria e.g.
Dichlorophen,Oxolinic acid

9.Piscicides-These are used to kill fishes e.g.
Trifloro methyl nitrophenol(TFM)

Chemical or Synthetic Pesticides

- **Organochlorenes-**These are non-biodegradable and persist in soil for long time e.g., DDT, BHC, Endosulfan, Aldrin.
- **Organophosphate-**These are esters of alcohols with phosphoric acid or with some other acids. These are very toxic acetyl-cholinesterase inhibitors as a result of which the breakdown of acetyl choline stops.The accumulation of acetyl choline resulting in convulsion paralysis and death e.g.,Malathion.
- **Carbamates-**They are derived from carbamic acid. Mode of action of carbamates is almost similar to organo- phosphates e.g.,Carbaryl, Dimetilan.

Working of Pesticides

Pesticides work in the following ways-

- **By blocking the cellular processes of the target organisms in a purely mechanical way e.g., Spray oils, petroleum oils.**
- **By destroying or altering the pest's metabolism e.g., Rotenone and cyanide which disrupt respiratory function in pests.**
- **By disrupting enzyme processes or denature proteins e.g. Inorganic Copper compounds**
- **By simulating or interfering with hormones e.g., Phenoxy herbicides.**
- **By disrupting photosynthesis and preventing the weed plant from producing or storing energy e.g., Triazine.**

Benefits of pesticides

- They are used in public health programmes to control vector born diseases
- They are used to protect the stored food grains.
- They protect the standing crop in the field. They do not increase the crop yield like fertilizer but by protecting the crop from pests.
- They can be used to control household pests.

Types of analytical methods for pesticide residues

There are several approaches which vary in their degree of complexity; in the time, effort and analytical instrumentation required to complete them.

- **Multiresidue** methods(MRMs):

It has been designed to **detect band** measure a **multiplicity of residues** in a range of foods.

multistep contains: sample preparation ➡ extraction ➡ clean up ➡ chromatographic separation

Out of **10 MRMs** currently used by FDA and USDA, ***8 based on gas chromatography** and the ***2 based on HPLC**.

However**, none of these** MRMs procedures can detect all the residues on all crop types.

In practice, they represent a **compromise** among the **number of residues** that can be detected, the **range of food types** that can be handled, and the **levels of residues** that can be measured.

The **principal advantage** resides in the number of **different residues** that they can detected and determined.

Types of analytical methods for pesticide residues.

- **Single** residue methods(MRMs):

It has been designed to **measure** a **single analyte** and, often, its principal metabolites and transformation products of toxicological importance.

multistep contains: sample preparation ➡ extraction ➡ clean up ➡ chromatographic separation

Each step is optimized for the analyte of interest.

Generally, they are **less time consuming** to perform and often provide **lower limits of detection** than MRMs.

However, they do vary in the level of validation to which they have been subjected **Volume 2 of the Pesticides Analytical Manual(PAM 2)** consists solely of SRMs.

In PAM 2, those methods that have received EPA review are listed with Romain numerals, whereas methods that have not been reviewed are lettered.

Types of analytical methods for pesticide residues.

- **Semiquantitative and Qualitative** methods:

Semiquantitative and qualitative methods **range widely** in their abilities to estimate the level of a particular pesticides residues in a sample.

In general, they are capable of **detecting a limited number** of somewhat similar pesticides.

Also called as **Screening methods** as they are capable of assaying a **large number of samples** for the presence of limited number of pesticides residues in relatively **short time**

Additionally, they are generally robust in character(i.e. less sensitive to small changes in the purity of reagents, quantities of reagent, time, temperature and environmental conditions).

Semiquantitative methods provide an **estimate of the concentration ranges** for detected residues, Qualitative methods will detect the pesticides if present above some predetermined level.

The principal benefit of these methods are their low cost, relative speed, and simplicity.

These methods use TLC, Enzyme inhibition, and Immunoassay.

Types of analytical methods for pesticide residues.

- **Quantitative** methods:

The basic steps of a quantitative analytical method for pesticides residues include the following:

1)**Sample preparation:** the plant parts are separated into **edible** and **non edible** fractions followed by **chopping , grinding, or macerating** of the sample.

2)**Extraction:** Pesticides residues are **removed** from most of the samples other constituents by **solubilizing them in a suitable solvent**. This steps often involves **blending** the chopped sample with solvent in a **homogenizer**, followed by a **filtration.**

3)**Clean up:** The crude extract is purifies further by **removing those co-extractives** that can be interfere in the subsequent determination steps.

4)**Separation:** The components of the purifies extract are further separated by a **differential partitioning** between a mobile phase (liquid or gas) and a stationary phase.

5)**Detection and quantitation:** A physical parameter of the separated components in the mobile phase is **measured as they passthrough a detector**, this signal is then related to the **quantity of analyte via a quantitation step.**

Examination of pesticides residues and heavy metals

The vast number of pesticides, and the fact that it is usually not known whether, which and in what quantity the plant grower has used pesticides, make it expedient to test for typical representatives of major groups of pesticides

1)Examination for **Chlorinated Hydrocarbon** and **Organo-Phosphoric Acid Esters.**

2)Determination of **Total Chlorine and Phosphorus.**

3)Determination of **chlorides.**

4)Determination of **phosphates.**

Examination for Chlorinated Hydrocarbon and Organo-Phosphoric Acid Esters.

A)Preparation of sample:

50g of sample is taken in when the sample contain fat

↓

Extracted 3 times with acetonitrile each time for 15mins

↓

Extract is filtered through a filter paper over calcined sodium sulphate

↓

Flask and filter are rinsed with 50ml acetonitrile.

↓

The extract is evaporated to dryness in a rotary evaporator at 30°C

↓

Acetonitrile free residue is dissolved in mixture of 10 parts dichloromethane and 90 parts petrol

↓

Put on a prepared florisil column(diameter 2.5cm,length 30-35cm).

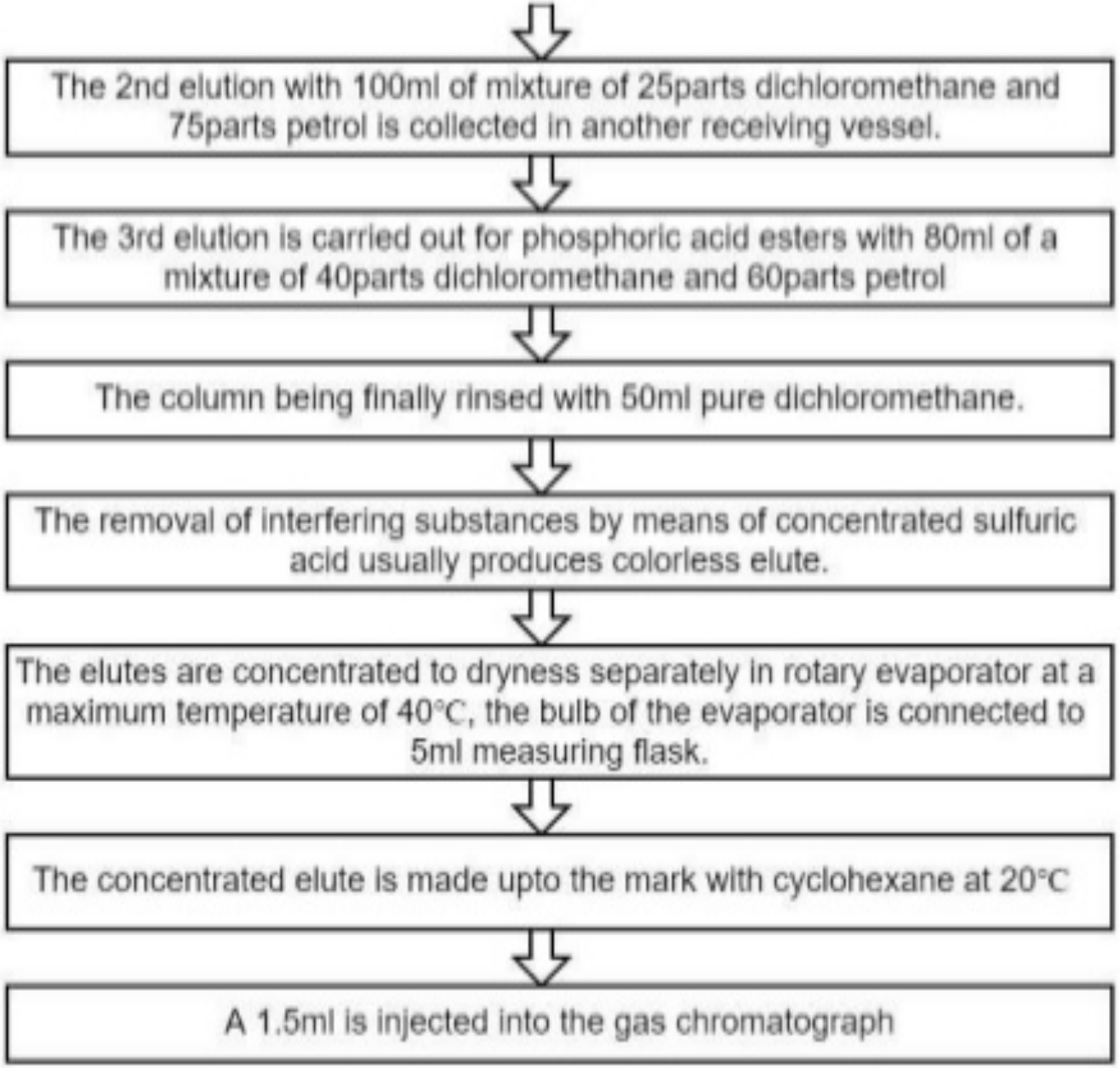
↓

Main fraction of chlorinated hydrocarbon is eluted(1st elution) with 100ml of mixture of 15parts dichloromethane and 85 parts petrol, it is collected Receiving vessel.

↓

Contd..

contd..


The 2nd elution with 100ml of mixture of 25parts dichloromethane and 75parts petrol is collected in another receiving vessel.
The 3rd elution is carried out for phosphoric acid esters with 80ml of a mixture of 40parts dichloromethane and 60parts petrol
The column being finally rinsed with 50ml pure dichloromethane.
The removal of interfering substances by means of concentrated sulfuric acid usually produces colorless elute.
The elutes are concentrated to dryness separately in rotary evaporator at a maximum temperature of 40°C, the bulb of the evaporator is connected to 5ml measuring flask.
The concentrated elute is made upto the mark with cyclohexane at 20°C
A 1.5ml is injected into the gas chromatograph

Examination for Chlorinated Hydrocarbon and Organo-Phosphoric Acid Esters.

B) **Gas Chromatography**

HP 5750 G gas chromatography with ECD-63Ni and FID.

Temperatures: Injector block 240℃, detector 290℃, furnace running isothermally190 ℃, and in program 150-200℃.

Carrier gas: Purified helium, 30ml\min.

Flushing gas: Argon\methane 90:10, 100-130ml\min.

Advanced speed of paper: 6.35mm\min

Columns: 3.8% SE 30 on chromosorb W AW-DMCS(80-100 mesh), 1.8m glass column.

The quantitative evaluation is made either on the area, of the height of the peak, in comparison with comparative substance and internal or external standards.

Examination for Chlorinated Hydrocarbon and Organo-Phosphoric Acid Esters.

C) Determination by Gas Chromatography

(1) First separation system

Use vitreous silica column, 30m long with an internal diameter of 0.25mm, packed with a chemically bound phase of 5% phenyl, 95% methyl-polysiloxane. Use the following temperature program:

- Heat at 60°C for 0.5 minutes.
- Increase the temperature at a rate of 30°C per minute to 160°C and maintain this temperature for 2 minutes.
- Increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use a "split/split-free" injector to inject the sample solution and maintain the injection port at a temperature of 240°C. Inject a volume of 1 microliter at a rate of 30 seconds("split-free"). The detector temperature should be at 300°C.

(2) Second separation system

Use a vitreous silica column, 15m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 7% cyanopropyl, 7% phenyl, 86% methyl polysiloxane. Use the following temperature program:

- Heat at 60°C for 0.2 minutes;
- Increase the temperature at a rate of 30°C per minute to 180°C and maintain this temperature for 1 minute.

Examination for Chlorinated Hydrocarbon and Organo-Phosphoric Acid Esters.

Determination by Gas Chromatography

- Increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use an on-column injector to inject a volume of 1 micro liter of sample solution. The detector temperature should be 300°C.

Measure the peak height of the pesticides obtained in the chromatograms and calculate the **concentration of the residues in mg\kg** using the following formula:

$$Ht \times 10/W \times Wt/ht$$

Where

Ht= Peak height obtained for the test solution in mm.

W= Quantity of the sample in the purified extract (g).

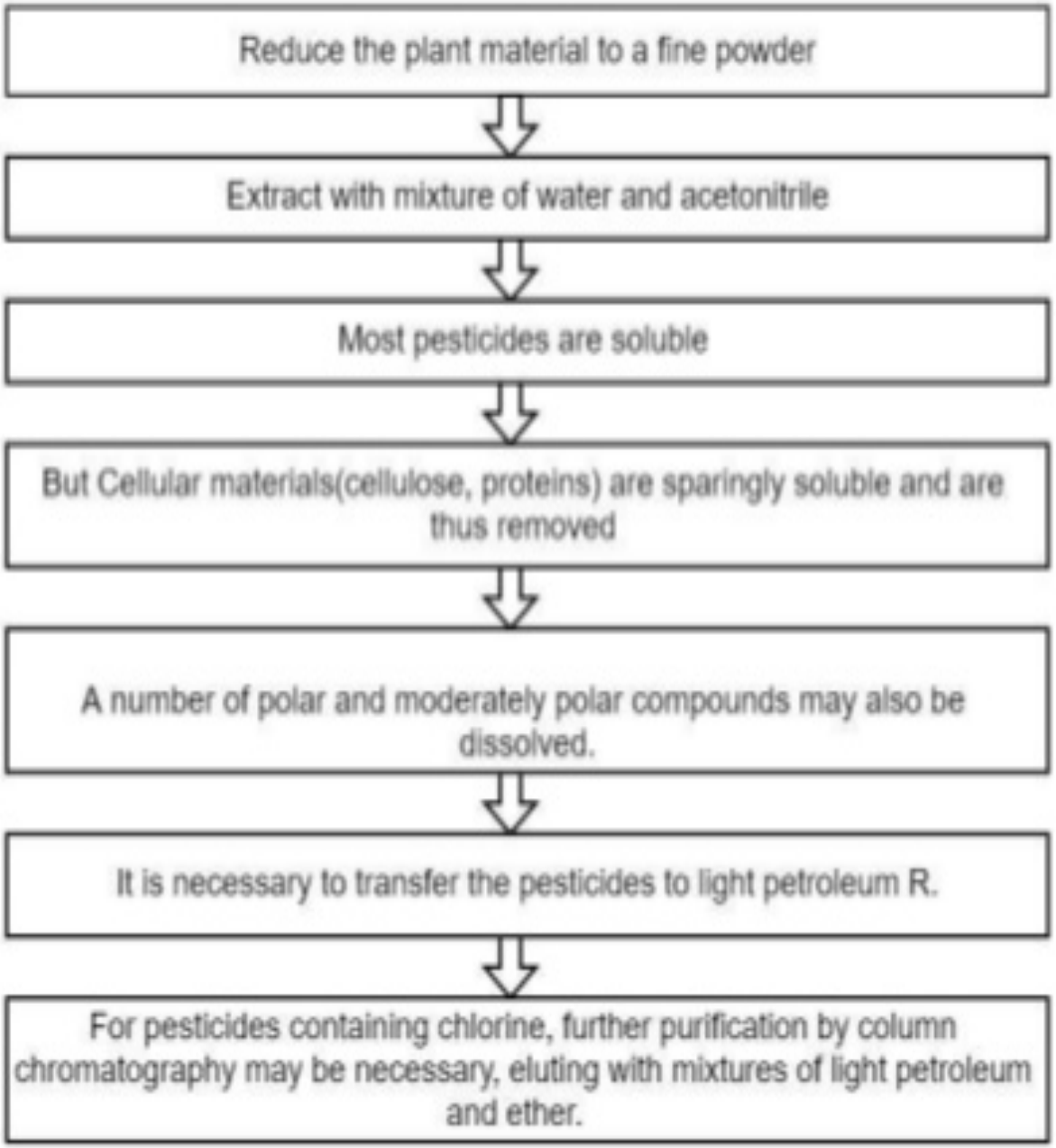
Wt= Quantity of the pesticides in ng in the reference solution injected.

Ht= Peak height obtained for the reference solution in mm.

Examination for Total Chlorine and Phosphorus.

As most of the pesticides contain organically bound chlorine and phosphorus, the WHO has recommended the following procedures for estimation:

A) Preparation of Samples



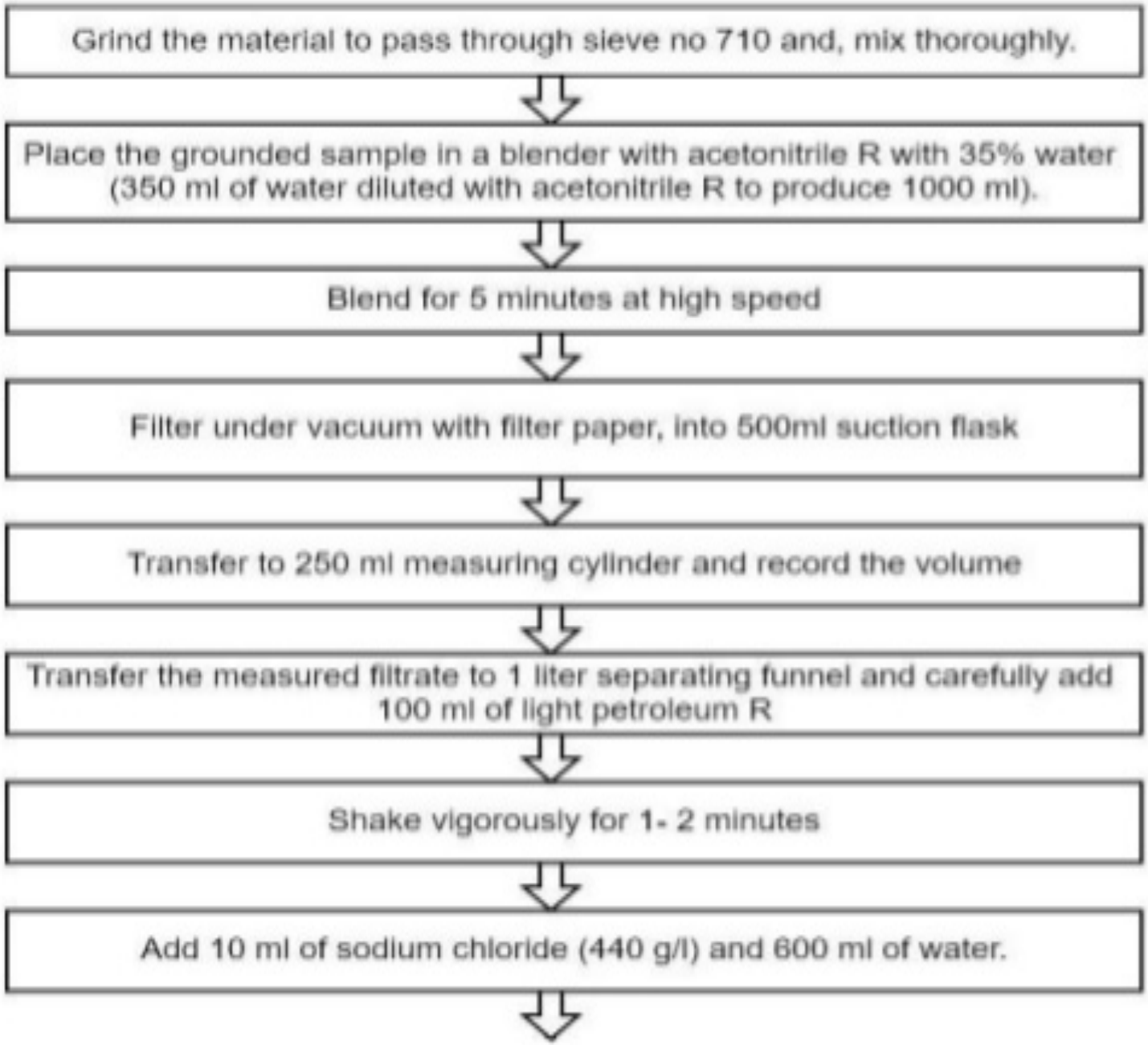
Examination for Total Chlorine and Phosphorus.

B)Preparation of column

- Use Florisil grade 60/100 PR, activated at 650°C as support.
- If this material is obtained in bulk, It is transfer to the glass jar with glass stopper and Stored in dark.
- Before use, heat at not less than 130°C, cool in a desiccator to room temperature
- Heat once again to 130°C after 2 days
- Prepare the column (external diameter, 22 mm) which contains, after setting.
- 10cm of activated Florisil topped with about 1cm of anhydrous sodium sulfate R.
- Pre-wet the column with 40-50 ml of light petroleum, place a graduated flask under the column to receive the elute.

Examination for Total Chlorine and Phosphorus.

C) Method



Examination for Total Chlorine and Phosphorus.

Method Cont....

Hold the separating funnel in a horizontal position and mix vigorously for 30-45 sec.

↓

Allow to separate, discard the aqueous layer and transfer the solvent layer to a 100 ml glass-stoppered cylinder and record the volume.

↓

Add 15 g of anhydrous sodium sulfate and shake vigorously

↓

The extract must not remain in contact with this reagent for longer than 1 hour.

↓

Transfer the extract directly to a Florisil column at a rate of not more than 5 ml per minute

↓

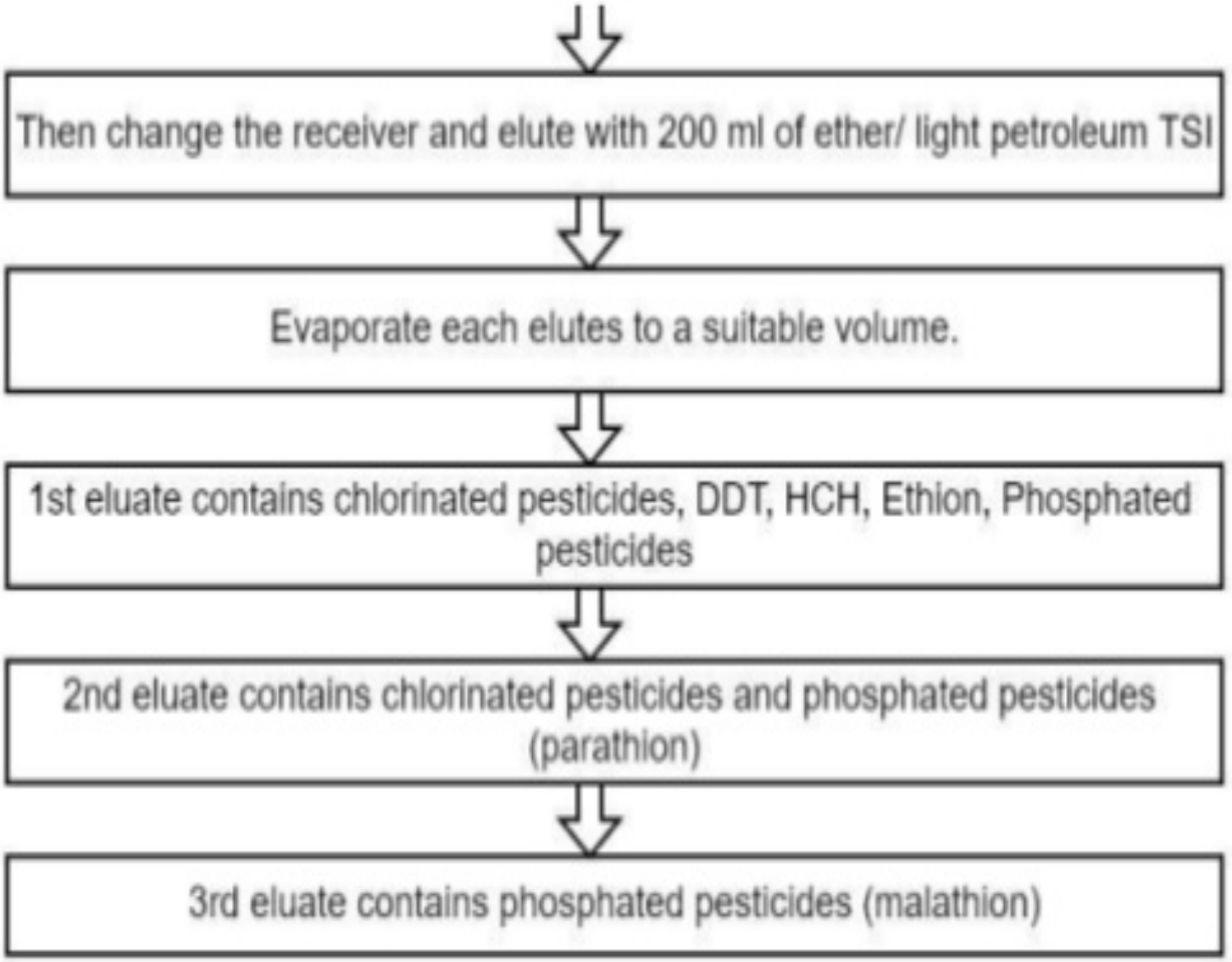
Carefully rinse the cylinder with two portions each of 5 ml, of light petroleum transfer them to the column, rinse with further small portions of light petroleum if necessary,

↓

Then elute with 200 ml of ether / light petroleum TSI

Examination for Total Chlorine and Phosphorus.

Method Cont....

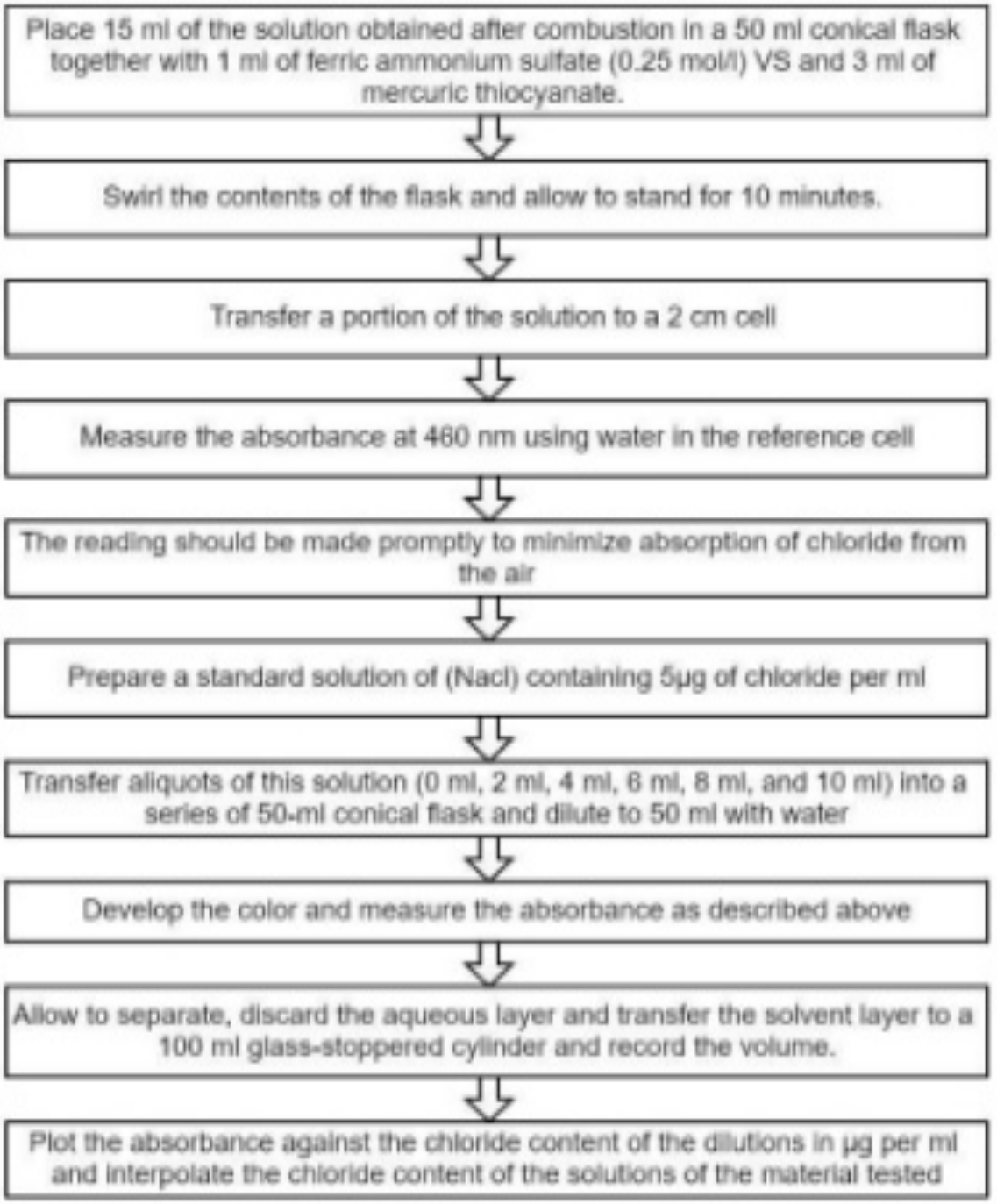


Determination of Chlorides.

Apparatus:

The determination is made with a **spectrophotometer** capable of measuring **absorbance at 460 nm** using absorption cells with **path length of 2cm and 10cm**

Method



Determination of Phosphates.

- The phosphomolybdate method is based on the reaction of the **phosphate ion** with **ammonium molybdate** to form a **molybdophosphate complex.**
- Which is subsequently reduced to form a strongly **blue-colored molybdenum complex,** the **intensity** of the blue color is **measure** spectrophotometrically.
- This method is applicable for the determination of any phosphates that have undergone a **prior separation procedure**.
- Naturally occurring phosphates are **present in most samples** and are often **not removed** during the clean-up procedure.
- In order to obtain background values, therefore, it is necessary to proceed with the determination **for all the samples even those with no phosphate** containing pesticide residues.
- Extracts of most uncontaminated materials contain about **0.05-0.1 mg/kg** of phosphorus.
- Therefore, no contamination with organophosphate pesticides can be assumed for results in this range.

Determination of Phosphates.

Apparatus:

The determination is made with a **spectrophotometer** capable of measuring **absorbance at 820 nm** using absorption cells with **path length of 1cm.**

Method

THANK YOU