HS 1004 Pesticide Residues Test

1. Purpose

The Pesticide Residues Test is to determine the residues of pesticides in the sample. The test is devised for the major types of organophosphate and organochlorine pesticides that are commonly used in agriculture.

2. Terminology

2.1 Adsorbent

A finely sieved solid material, usually of prescribed mesh size, to which dissolved substances will preferentially attach and thus be removed from solution.

2.2 Column

Either an empty glass tube intended to hold an adsorbent or that tube filled with adsorbent with various column length and diameter. Residue analytical methods often require columns with fritted glass discs at the bottom to hold the adsorbent in place, and Teflon stopcocks to control the rate at which eluant passes through the adsorbent. Columns without stopcocks are suitable only for drying extracts through anhydrous sodium sulphate. If a column has no fritted glass disc, a plug of glass wool is placed in the bottom to retain the adsorbent. Solvent reservoirs incorporated at the top of columns are an optional convenience.

2.3 Eluant

Solvent or mixture of solvents that is passed through the column to remove (elute) adsorbed residues; also known as eluting solvent.

2.4 Eluate

Solvent or mixture of solvents that has passed through the column. The eluate is the cleaned up extract.

3. Apparatus

3.1 Büchner porcelain funnel (Büchner), 12 cm diameter, with vacuum filtration flask

3.2 Filter paper, to fit Büchner, which is sufficiently washed by solvent.

3.3 Long-stemmed glass funnel, 10 cm diameter.

3.4 Kuderna-Danish concentrator (K-D), 250mL and 500 mL, with Snyder column, two-ball micro-Snyder column, graduated or volumetric receiving flask.

3.5 Separatory funnels, of capacity 500 mL and 1000 mL with ground stopper.

3.6 Chromatographic column, 10 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc.

3.7 Gas chromatographic instrument, with flame photometric detector and

electroconductivity detector or other appropriate detectors, for detection of organophosphate pesticide and organochlorine pesticide respectively. The Chromatographic instrument should also be equipped with a Wide Bore Capillary Column (DB-1 or equivalents), 30 m × 0.53 mm id, coated with 100% methyl substituted polysiloxane liquid phase and the film thickness is 1-1.5 μm, bonded and cross-linked.

4. Reagents

4.1 Acetone, distilled from all-glass apparatus.

4.2 Boiling chips, 20-30 mesh carborundum (optional).

4.3 Glass wool, Pyrex, rinse with solvent and air-dry or heat 1 hour at 400 °C.

4.4 Methylene chloride, distilled from all-glass apparatus.

4.5 Petroleum ether, distilled from all-glass apparatus.

4.6 Sodium chloride, reagent grade.

4.7 Sodium sulphate, in anhydrous granular form, reagent grade. Before use, heat 4 hours in muffle furnace at 600 °C and store in glass containers.

4.8 40 % ethanol.

4.9 Acetonitrile, distilled from glass apparatus.

To make use of reagent grade acetonitrile, test by moistened litmus paper over the mouth of storage container. If the litmus paper turns blue, purify 4 L acetonitrile by adding 1 mL 85 % phosphoric acid, 30 g phosphorus pentoxide and boiling chips, then allowing to stand overnight. Distill from all glass apparatus at 81-82 °C, discarding first and last 10 % distillate.

4.10 Florisil, PR grade; see Annex A for handling and testing directions and calculation of lauric acid value.

4.11 Hexane, distilled from all-glass apparatus.

4.12 Eluant, 50 % methylene chloride/1.5 % acetonitrile/48.5 % hexane (v/v/v).

Pipette 15 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust to 1 litre with hexane.

4.13 Pesticides standard stock solutions, prepare the standard stock solutions listed below. Keep all the stock solution in refrigerator and dilute the stock solution with acetone. The concentration to be selected will depend on the instrument used and the expected concentrations in the sample.

Organophosphate Pesticides name	**Concentration (mg of pesticide/mL of acetone)**
Dichlorvos	1 mg/mL
Dimethoate	1 mg/mL

Fenitrothion	1 mg/mL
Fenthion	1 mg/mL
Malathion	1 mg/mL
Parathion	1 mg/mL
Phorate	1 mg/mL
Organochlorine Pesticides name	**Concentration (mg of pesticide/mL of acetone)**
Aldrin	1 mg/mL
BHC	1 mg/mL
DDT	1 mg/mL
Dieldrin	1 mg/mL
Heptachlor	1 mg/mL

5. Preparation of samples

5.1 General

The sample taken for testing should be in its ready-for-use state. Obtain a sample that meets the requirements of the sampling programme and handle it in such a manner to avoid contamination prior to testing. The sample should be put into a container or bag instantly after collection. To avoid contamination, certain precautions are necessary which are common to all sampling procedures:

- Scrupulous care should be taken to avoid accidental contamination of the sample during collection and subsequent handling.
- The sample should be collected in a sufficient amount and tested as soon as possible after collection.

5.2 Preparation of 40 % ethanol extract for articles in container form

5.2.1 Measure a sufficient volume of 40 % ethanol (Section 4.8) by measuring cylinder and fill the test specimen to a level within 0.5 cm of the top of the test specimen. Mark the volume of water/acetone solution that has been used.

5.2.2 Cover the test specimen with an inert material to prevent evaporation, e.g. glass. This part of the operation should be carried out in the minimum time to prevent evaporation of ethanol. The bottom of test specimen should be wrapped by aluminum foil to prevent leakage of solution. Leave this preparation for 2 hours at room temperature.

5.2.3 Decant the solution and wash test specimen twice with 40 % ethanol. Transfer the solution and washings to a 12 cm Büchner funnel fitted with filter paper. Filter paper should be pre-washed with acetone to remove artifacts. Filter the solution with suction and collect extract in a suction flask. Use the content of the flask for further investigation. The total volume of 40 % ethanol for washing should not exceed 5% of the volume used for filling up the container.

Note: Filtration is normally completed in less than 1 minute. Continuation of vacuum for excessive period can reduce volume of extract and cause error in calculation.

5.3 Preparation of 40 % ethanol extract for articles in bag form

5.3.1 Tear or cut sample into pieces of approximately 1 cm^2 to 2 cm^2 in size. Wear protective glove to prevent contamination.

5.3.2 Weigh 10 ± 0.1 g of the test pieces to accuracy of 0.01 g, put them into the conical flask, add 200 mL of 40 % ethanol and stopper the flask. Leave this preparation to stand for 2 hours at room temperature, shake occasionally.

5.3.3 Decant the solution and wash test pieces in the flask twice with a fresh portion of 40 % ethanol. Transfer the extract and washings to a 12 cm Büchner funnel fitted with filter paper. Filter paper should be pre-washed with acetone to remove artifacts. Filter the solution with suction and collect extract in a suction flask. Transfer the filtrate to a marked 250 mL volumetric flask. Use the content of the flask for further investigation.

Note: Filtration is normally completed in less than 1 minute. Continuation of vacuum for excessive period can reduce volume of extract and cause error in calculation.

5.4 Extraction

5.4.1 Place 100 mL of sample solution in 1000 mL separatory funnel containing 30 mL methylene chloride. Add 100 mL petroleum ether and shake vigorously for 2 minutes. Transfer the methylene chloride layer to a clean 250 mL conical flask.

5.4.2 Dry the methylene chloride layer by passing through about 4 cm sodium sulphate supported on washed glass wool in glass funnel (Section 3.3), collecting in K-D concentrator.

5.4.3 To separatory funnel with aqueous phase, add 7 g sodium chloride and shake vigorously 30 seconds until most of the sodium chloride is dissolved.

5.4.4 Add 100 mL methylene chloride and shake for 1 minute, dry the methylene chloride layer by passing through the same sodium sulphate and collect it in K-D concentrator.

5.4.5 Extract aqueous phase with additional 100 mL methylene chloride and dry as above. Rinse sodium sulphate with about 50 mL methylene chloride.

5.4.6 Add boiling chips to K-D concentrator and concentrate solvent in K-D concentrator. Start evaporation slowly by placing only receiver tube into steam. After 100-150 mL has evaporated, concentrator may be exposed to more steam. When liquid level

in hot concentrator tube is about 2 mL, add 100 mL petroleum ether through Snyder column and re-concentrate to about 2 mL.

5.4.7 Add 50 mL petroleum ether and repeat concentration step. Add 20 mL ethanol and re-concentrate to about 2 mL. Do not allow solution to go to dryness during any of the concentration steps. Adjust volume of extract to suitable definite volume by acetone.

5.5 Cleanup process

5.5.1 Generally speaking, the extracted solution is suitable for pesticide analysis. The cleanup process should be performed if interference has been detected.

5.5.2 Place activated Florisil (weight of Florisil = 110/lauric acid value × 4 g) in 10 mm Chromatographic column; add about 2 cm sodium sulphate. Completely open stopcock and tap column to settle adsorbent. Pre-wet column with 15 mL hexane. Do not allow column to dry. Place K-D concentrator with volumetric or graduated receiving flask under column to receive value.

5.5.3 Dilute extract with hexane to produce solution of 10 % acetone/hexane. Volumes depend on concentration of extract, volume taken for cleanup; e.g., dilute 1 mL of extract prepared in Section 5.4.7, previously concentrated to 7 mL acetone, to 10 mL with hexane.

5.5.4 Transfer solution to Florisil column, letting it pass through at about 5 mL/min. Rinse container with two 3 mL portions hexane, transfer rinsing to column, and rinse walls of chromatographic tube with additional small portions of hexane. Elute column at about 5 mL/min with eluant.

5.5.5 Add boiling chips to K-D concentrator and concentrate eluate to suitable definite volume. For example, if 1 mL of extract prepared in Section 5.4.7 was cleaned up, concentrate Florisil eluate to 1 mL for same final concentration.

Note: When volume less than 5 mL is needed, use two-ball micro-Snyder or micro-Vigreaux column during evaporation.

5.6 Determination of organophosphate pesticide residues

5.6.1 The quantity of organophosphate pesticide is determined by using Gas-Liquid Chromatography. Detailed instructions depend on the type of equipment used. Follow the instructions provided by the manufacturer of the equipment. The set up of the Gas-Liquid Chromatograph should be based as follows:

Column

- Wide bore capillary, 30 m × 0.53 m id, coated with 100 % methyl substituted polysiloxane liquid phase, 1-1.5 μm film thickness, bonded and cross-linked. For example, DB-1 or equivalents. See operation manual for recommended

operating procedure for wide bore capillary. The column operating conditions are as follows:

Column temperature: 200 °C isothermal;

Carrier gas: Helium;

Injector temperature: 220-250 °C

Detector

- Flame photometric detector, phosphorus mode (FPD-P) is used.
- Set the detector temperature at 220-250 °C.

5.6.2 Inject 3 to 4 μL of extract solution prepared in Section 5.4.7 (or extract solution in Section 5.5.5 if cleanup is required) into the capillary column. Always inject the same volume of sample. Inspect resulting chromatogram for peaks corresponding to pesticides of concern.

5.6.3 Inject standards of organophosphate pesticide frequently to ensure optimum operating conditions can be obtained. If necessary, concentrate or dilute the extract so that peak size of pesticide is very close to the corresponding peaks of the standard.

5.7 Determination of organochlorine pesticide residues

5.7.1 The quantity of organochlorine pesticide is determined by using Gas-Liquid Chromatography. Detailed instructions depend on the type of equipment used. Follow the instructions provided by the manufacturer of the equipment. The set up of the Gas-Liquid Chromatograph should be based as follows:

Column

- Wide bore capillary, 30 m × 0.53 m id, coated with 100 % methyl substituted polysiloxane liquid phase, 1-1.5 μm film thickness, bonded and cross-linked. For example, DB-1 or equivalents. See operation manual for recommended operating procedure for wide bore capillary. The column operating conditions are as follows:

Column temperature: 200 °C isothermal;

Carrier gas: Helium;

Injector temperature: 220-250 °C

Detector

- Electroconductivity detector, halogen mode (ELCD-X) is used. The detector

operating conditions are as follows:

Base temperature:	250 °C
Furnace temperature:	900 °C (or as specified in operating manual)
Reactor gas:	Hydrogen, flow as required by specific detector model (see operating manual)

5.7.2 Inject 3 to 4 μL of extract solution prepared in Section 5.4.7 (or extract solution in Section 5.5.5 if cleanup is required) into the capillary column. Always inject the same volume of sample. Inspect resulting chromatogram for peaks corresponding to pesticides of concern.

5.7.3 Inject standards of organochlorine pesticide frequently to ensure optimum operating conditions can be obtained. If necessary, concentrate or dilute the extract so that peak size of pesticide is very close to the corresponding peaks of the standard.

6 Expression of results

Determine pesticides concentrations by direct comparison with single standard manually or electronically when injection volume and response are within 10 % of those of the sample pesticide of interest. Calculate the pesticide concentration using the following formula and express the results in microgram per litre (μg/L) of the extract:

$$Concentration = \frac{A \times B \times C \times D}{E \times F \times G} \quad (1)$$

where:

A is the mass of standard pesticide in nanograms (ng);

B is the peak height of sample, mm, or peak area, mm^2;

C is the extract volume in Section 5.4.7 (or in Section 5.5.5 after cleanup process), μL;

D is the dilution factor, if there was no cleanup, then the dilution factor is 1; if a portion of the extract solution was concentrated after cleanup process in Section 5.5.5, then the dilution factor is a decimal; if it was diluted, the dilution factor exceeds 1;

E is the peak height of standard, mm, or peak area, mm^2;

F is the volume of extract injected, μL;

G is the volume of sample extracted, mL (which is 100 mL).

7 References

1. Pesticide Analytical Manual, Volume 1: Multi-residues methods, 3rd edition, 1994, U.S. Health and Human Services, Public Health Service, Food and Drug Administration, U.S.A.

Annex A – Handling and Testing of Florisil

A.1 Handling

- Use PR Grade Florisil, 60 – 100 mesh, heat 3 hours at 677 °C.
- Immediately after opening bulk lots of Florisil, transfer to glass containers that are glass-stoppered or have Teflon-lined or foil-lined screw caps; store in dark. Activate each portion by heating at 130 °C in foil-covered bottles. Florisil may be heated in bulk in pint glass bottles or in individual column amounts in 50 mL conical flask. Cover containers with foil to prevent contamination, and use in rotation to avoid lengthy storage time. Alternatively, store stoppered container of activated Florisil in desiccator at room temperature and reheat at 130 °C after 2 days.
- If entire lot of Florisil is purchased, perform tests below on composite of four-five sub-samples taken from each drum with grain trier. Combine sub-samples, mix well, and activate mix at 130 °C for 168 hours before testing.

A.2 Testing

Each lot of activated Florisil must be tested before use to determine whether adjustments in column size are needed to ensure proper elution and quantitative recovery of pesticides. Florisil column size is decreased or increased to adjust for over-retentive or under-retentive Florisil. Two tests should be performed: Lauric Acid Test measures the general adsorptivity, and Elution Test confirms the appropriate elution of pesticides.

A.2.1 Lauric acid test

Principle:

Adsorptivity capacity of Florisil is measured by exposing weighed amount to excess of lauric acid in hexane solution. Amount of lauric acid not adsorbed is measured by titration with alkali. Weight of lauric acid adsorbed (LA value) is subsequently used to calculate appropriate weight of that lot of Florisil equivalent to standardised Florisil (LA value = 110).

Apparatus:

1. Burette, 25 mL with 0.1 mL graduations.
2. Conical flasks,
3. GLC, equipped with ^{63}Ni electron capture (EC) and flame photometric, phosphorus mode (FPD-P) detectors
4. Pipettes, 10 mL and 20 mL.
5. Volumetric flasks, 500 mL.

Reagents:

1. Ethanol, USP or absolute, neutralised to phenolphthalein.
2. Hexane, distilled from all-glass apparatus.
3. Lauric acid, purified or analytical grade.
4. Phenolphthalein indicator, 1 g/100 mL ethanol.
5. Sodium hydroxide, pellets, reagent grade.
6. Sodium hydroxide solution, 0.05 M. Make 1 N solution (20 g/500 mL water), and dilute 25 mL to 500 mL with water. Standardise by weighing 100-200 mg lauric acid into 125 mL conical flask. Add 50 mL neutralised ethanol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/mL of 0.05 N sodium hydroxide solution (about 10 mg/mL).

Procedures:

Calculate LA value for each Florisil lot by performing the following test in triplicate.

- Transfer 2.000 g Florisil to 25 mL conical flask. Cover loosely with aluminum foil and heat overnight at 130 °C.
- Stopper, cool to room temperature, add 20.0 mL lauric acid solution (400 mg), stopper, and shake occasionally 15 min.
- Let adsorbent settle and pipette 10.0 mL supernatant into 125 mL conical flask. Avoid inclusion of any Florisil.
- Add 50 mL neutral alcohol and 3 drops phenolphthalein indicator solution. Titrate with 0.05 N sodium hydroxide to permanent end point.
- Calculate LA value (mg lauric acid/g Florisil):

$$\text{LA value} = 200 - \frac{\text{mL required for titration} \times \text{mg lauric acid}}{\text{ml 0.05 M of sodium hydroxide}}$$

A.2.2 Elusion Test

Principle:

Solutions of pesticides and butterfat are eluted from Florisil columns, adjusted for LA value by eluants from the testing methods above. Adequate elution pattern and cleanup capacity can be verified by appropriate elution of pesticides and weight of butterfat. Pesticides are chosen to provide indicators of improper elution, poor Florisil, and impure reagents.

Apparatus:

1. Chromatographic column, 22 mm id × 300 mm, Teflon stopcock, coarse porosity fritted disc.
2. K-D concentrator, 500 mL, with Snyder column, two-ball micro-Snyder column, volumetric receiving flask.

Reagents:

1. Acetonitrile, distilled from all-glass apparatus.
2. Ethyl ether, distilled from all-glass apparatus, with 2 % ethanol as preservative, peroxide free.
3. Hexane, distilled from all-glass apparatus.
4. Methylene chloride, distilled from all-glass apparatus.
5. Petroleum ether, distilled from all-glass apparatus.
6. Eluants: 6 % (v/v) ethyl/petroleum ether

   15 % (v/v) ethyl/petroleum ether;

   50 % (v/v) ethyl/petroleum ether;

   Eluant 1 – 20 % methylene chloride/hexane (v/v). Dilute 200 mL methylene chloride with hexane. Allow mixture to reach room temperature, and adjust volume to 1 litre with hexane.

   Eluant 2 – 50 % methylene chloride/0.35 % acetonitrile/49.65 % hexane (v/v/v). Pipette 3.5 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust volume to 1 litre with hexane.

   Eluant 3 – 50 % methylene chloride/1.5 % acetonitrile/48.5 % hexane (v/v/v). Pipette 15 mL acetonitrile into 500 mL methylene chloride and dilute with hexane. Allow mixture to reach room temperature and adjust volume to 1 litre with hexane.
7. Pesticide standard solutions, each ml containing these approximate concentrations:

   A: 1.0 μg heptachlor, 3.0 μg chlorpyrifos, 2.0 μg heptachlor epode, 2.0 μg dieldrin, 3.0 μg endosulfan I, 3.0 μg endosulfan II, 10.0 μg endosulfan sulfate.

   B: 4.0 μg malathion, 2.0 μg parathion-methyl, 4.0 μg fonofos, 4.0 μg pirimiphos-methyl.

   C: 20.0 μg Aroclor 1254, 200.0 mg butterfat.

   D: 1.0 μg α- BHC, 3.0 μg chlorpyrifos, 1.0 μg heptachlor, 2.0 μg heptachlor epoxide, 2.0 μg dieldrin, 2.0 μg endrin, 4.0 μg malathion, 2.0 μg parathion-methyl.

Procedures:

- Prepare three Florisil columns to contain, respectively: 110/LA Value × 20 g, 2 g more than that, and 2 g less than that.
- Rinse columns with 50 mL petroleum ether, discarding rinses. Place K-D concentrator with 10 mL volumetric flask under each column.
- Pipette 1.0 mL of each standard solution A and B into each column. Rinse sides of column with two 3 mL portions petroleum ether, then rinse column with 50 mL petroleum ether.
- Elute each column with 200 mL 6 % ethyl ether/petroleum ether. (Collect rinses with eluate.)
- Change receivers; elute each column with 200 mL 15 % ethyl ether/petroleum ether.
- Change receivers; elute each column with 200 mL 50 % ethyl ether/petroleum ether.
- Concentrate each eluate, dilute to volume with hexane, and inject about 5 μL into appropriate

GLC systems to determine recoveries. Dilute 1.0 mL each standard solution A and B to 10 mL and use diluted solution as GLC reference standard.

- Consider Florisil lot acceptable if one of three columns permits complete recovery of test compounds and exhibits proper elution pattern (heptachlor, heptachlor epoxide, chlorpyrifos, and fonofos in 6 % eluate; dieldrin, endosulfan I, parathion-methyl, and pirimiphos-methyl in 15 % eluate; malathion and endosulfan sulfate in 50 % eluate; and endosulfan II in both 15 and 50 % eluates). Acceptable recovery is more than 80 % for all compounds except heptachlor, and 60 – 90 % for heptachlor. In subsequent use of the same lot of Florisil, use same weight as that in column with acceptable elution.
- If none of the three columns exhibits proper elution but a consistent relationship exists between weight and elution, test additional columns of weights 3 g above or 3 g below that calculated using LA Value. If these columns also do not exhibit proper elution, it is best to use a different lot of Florisil.

If acceptable weight of Florisil is determined, test that column size further with following procedures:

- Repeat elution tests above, using 1.0 mL each of standard solution C and D. Elute column with 250 mL petroleum ether, followed by 6, 15, and 50 % ethyl ether/petroleum ether eluants; collect each eluate separately. Determine recoveries of pesticides and verify accuracy of elution pattern using gas chromatographic measurement.
- Transfer each eluate quantitatively to separate tared 20 mL beaker. Evaporate solvent on steam bath or hot plate until constant weight is attained to measure amount of butterfat recovered in each eluate. Acceptable lots of Florisil typically permit about (range 0 – 1.7 mg) butterfat to elute in petroleum ether eluate, 0.1 (0 – 0.4) mg in 6 % ethyl ether / petroleum ether, 82 (40 – 135) mg in 15 %, and 105 (60 – 172) mg in 50 %.
- Repeat elution test above, using 1.0 mL each of standard solution A and B and eluting with Eluants 1, 2, and 3 instead of ethyl ether / petroleum ether eluants.

It is acceptable, once the Florisil lot has been tested and appropriate weight of Florisil determined, to measure and record height of column produced by specified weight; subsequent columns may be prepared by measuring height rather than weight.