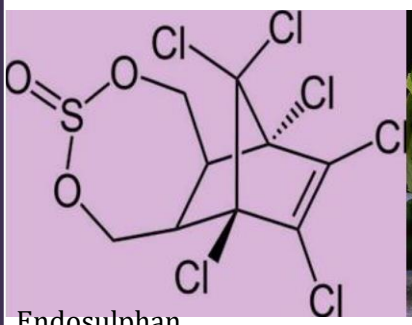
MANUAL OF METHODS OF ANALYSIS OF FOODS

PESTICIDE RESIDUES



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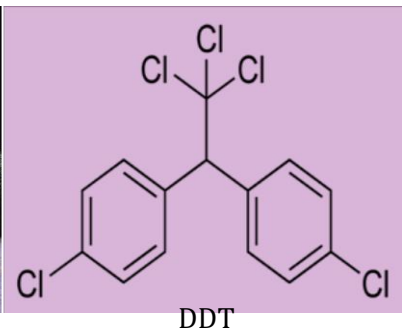

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FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
MINISTRY OF HEALTH AND FAMILY WELFARE
GOVERNMENT OF INDIA
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MANUAL FOR ANALYSIS OF PESTICIDE RESIDUE IN FOODS

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Note: The test methods given in the manuals are validated/ standardized test methods. However, it would be the responsibility of the respective testing laboratory to confirm that the above methods are validated in its laboratory and gives proper result in their laboratory.

MANUAL FOR ANALYSIS OF PESTICIDE RESIDUES

1. CODEX GUIDELINES ON GOOD PRACTICE IN PESTICIDE RESIDUE ANALYSIS

1.1 Introduction:

The Codex document ALINORM 76/24 Appendix IV (Report of the ad hoc Working Group on Methods of Analysis) contained the following statement:

"It was considered that the ultimate goal in fair practice in international trade depended, among other things, on the reliability of analytical results. This in turn, particularly in pesticide residue analysis, depended not only on the availability of reliable analytical methods, but also on the experience of the analyst and on the maintenance of good practice in the analysis of pesticides.

These guidelines define such good analytical practice and may be considered in three inter-related parts:

The Analyst
Basic Resources
The Analysis

1.2 The Analyst:

Residue analysis consists of a chain of procedures, most of which are known, or readily understood, by a trained chemist, but because the analyte concentrations are in the range g/kg to mg/kg and because the analysis can be challenging, attention to detail is essential. The analyst/ in-charge should have an appropriate professional qualification and be experienced and competent in residue analysis. Staff must be fully trained and experienced in the correct use of apparatus and in appropriate laboratory skills. The analyst can use the method within the expected performance parameters established during method validation prior to analysis of samples. They must have an understanding

of the principles of pesticide residue analysis and the requirements of Analytical Quality Assurance (AQA) systems. They must understand the purpose of each stage in the method, the importance of following the methods exactly as described and of nothing any unavoidable deviations. They must also be trained in the evaluation and interpretation of the data they produce. A record of training and experience must be kept for all laboratory staff.

When a laboratory for residue analysis is set up, the staff should spend some of their training period in a well established laboratory where experienced advice and training is available. If the laboratory is to be involved in the analysis for a wide range of pesticide residues, it may be necessary for the staff to gain experience in more than one expert laboratory.

1.3 Basic Resources

1.3.1 The laboratory:

The laboratory and its facilities must be designed to allow tasks to be allocated to well-defined areas where maximum safety and minimum chance of contamination of samples prevail. Laboratories should be constructed of, and utilise materials resistant to chemicals likely to be used within them. Under ideal conditions, separate rooms would be designated for sample receipt and storage, for sample preparation, for extraction and clean-up and for instrumentation used in the determinative step. The area used for extraction and clean-up must meet solvent laboratory specifications and all fume extraction facilities must be of high quality. Simple receipt, storage and preparation should be handled in areas devoted to work at residue levels. Maintenance of sample integrity and adequate provisions for personal safety for priority requirements.

Laboratory safety must also be considered in terms of what is essential and what is preferable, as it must be recognised that the stringent working conditions enforced in residue laboratories in some parts of the world could be totally unrealistic in others. No smoking, eating, drinking or application of cosmetics should be permitted in the working area. Only small volumes of solvents should be held in the working area and the bulk of

the solvents stored separately, away from the main working area. The use of highly toxic solvents and reagents should be minimised whenever possible. All waste solvent should be stored safely and disposed of both safely and in an environmentally friendly manner taking into account specific national regulations where available.

The main working area should be designed and equipped for utilisation of an appropriate range of analytical solvents. All equipment such as macerators and refrigerators should be spark free or explosion proof. Extraction, clean-up and concentration steps should be carried out in a well ventilated area, preferably in fume cupboards.

Safety screens should be used when glassware is used under vacuum or pressure. There should be an ample supply of safety glasses, gloves and other protective clothing, emergency washing facilities and a spillage treatment kit. Adequate fire fighting equipment must be available. Staff must be aware that many pesticides have acutely or chronically toxic properties and therefore, great care is necessary in the handling of standard reference compounds.

1.4 Equipment and Supplies:

The laboratory will require adequate, reliable, supplies of electricity and water. Adequate supplies of reagents, solvents, gas, glassware, chromatographic materials etc. of suitable quality are essential.

Chromatographic equipment, balances, spectrophotometers etc. must be serviced and calibrated regularly and a record of all servicing/repairs must be maintained for every such item of equipment. Calibration is essential for equipment including glassware performing measurements. Calibration curves and comparison with standards may suffice. Regular calbration and re-calibration of measuring equipment must be done where the possible change in nominal value may significantly contribute to the uncertainty of the measurement. Balances and automated pipettes/dispensers and similar equipment must be calibrated regularly. The operating temperatures of refrigerators and freezers should be continually monitored or be checked at specified intervals. All records should be kept up-to-date and retained.

All laboratories require pesticide reference standards of known and acceptably high purity. Analytical standards should be available for all parent compounds for which the laboratory is monitoring samples, as well as those metabolites that are included in MRLs.

All analytical standards, stock solutions and reagents whose integrity could be influenced by degradative processes must be clearly labelled with an expiry date and stored under proper conditions. Pure reference standards must be kept under conditions specified by manufacturer or lower that will minimise the rate of degradation, e.g. low temperature, exclusion of moisture, darkness. Equal care must be taken that standard solutions of pesticides are not decomposed by the effect of light or heat during storage or become concentrated owing to solvent evaporation.

1.5 The Analysis

1.5.1 Avoidance of contamination:

One of the significant areas in which pesticide residue analysis differs significantly from macro-analysis is that of contamination and interference. Trace amounts of contamination in the final samples used for the determination stage of the method can give rise to errors such as false positive or false negative results or to a loss of sensitivity that may prevent the residue from being detected. Contamination may arise from almost anything that is used, for or is associated with, sampling, sample transport and storage, and the analysis. All glassware, reagents, organic solvents and water should be checked for possible interfering contaminations before use, by analysis of a reagent blank.

Polishes, barrier creams, soaps containing germicides, insect sprays, perfumes and cosmetics can given rise to interference problems and are especially significant when an electron-capture detector is being used. There is no real solution to the problem other than to ban their use by staff while in the laboratory.

Lubricants, sealants, plastics, natural and synthetic rubbers, protective gloves, oil from ordinary compressed air lines and manufacturing impurities in thimbles, filter papers and

cotton-wool can also give rise to contamination.

Chemical reagents, adsorbents and general laboratory solvents may contain, adsorb or absorb compounds that interfere in the analysis. It may be necessary to purify reagents and adsorbents and it is generally necessary to use re-distilled solvents. Deionised water is often suspected; re-distilled water is preferable, although in many instances tap water or well water may be satisfactory.

Contamination of glassware, syringes and gas chromatographic columns can arise from contact with previous samples or extracts. All glassware should be cleaned with detergent solution, rinsed thoroughly with distilled (or other clean) water and then rinsed with the solvent to be used. Glassware to be used for trace analysis must be kept separate and must not be used for any other purpose.

Pesticide reference standards should always be stored at a suitable temperature in a room separate from the main residue laboratory. Concentrated analytical standard solutions and extracts should not be kept in the same storage area. Apparatus containing polyvinylchloride (PVC) should be regarded as suspect and, if shown to be a source of contamination, should not be allowed in the residue laboratory. Other materials containing plasticisers should also be regarded as suspect but PTFE (Polytetrafluoroethylene) and silicone rubbers are usually acceptable and others may be acceptable in certain circumstances. Sample storage containers can cause contamination and glass bottles with ground glass stoppers may be required. Analytical instrumentation ideally should be housed in a separate room. The nature and importance of contamination can vary according to the type of determination technique used and the level of pesticide residue to be determined.

Residues and formulation analyses must have completely separated laboratory facilities provided. Samples and sample preparation must be kept separate from the all residue laboratory operations in order to preclude cross contamination.

1.6 Reception and storage of samples:

Every sample received into the laboratory should be accompanied by complete information on the source of the sample, on the analysis required and on potential hazards associated with the handling of that sample.

On receipt of a sample it must immediately be assigned a unique sample identification code which should accompany it through all stages of the analysis to the reporting of the results. If possible, the samples should be subjected to an appropriate disposal review system and records should be kept.

Sample processing and sub-sampling should be carried out using procedures that have been demonstrated to provide a representative analytical portion and to have no effect on the concentration of residues present.

Laboratory samples which are not analysed immediately should be stored under conditions that minimise decay. Fresh produce should be stored in the refrigerator, but typically no longer than 5 days. Dried products may be stored at room temperature, but if storage time is expected to exceed two weeks, they should be sub-sampled and stored in the freeze. If samples cannot be analysed immediately but are to be analysed quickly, they should be stored at (1-5°C) away from direct sunlight, and analysed within a few days. However, samples received deep-frozen must be kept at < -16°C until analysis. In some instances, samples may require storage for a longer period before analysis. In this case, storage temperature should be approximately -20°C, at which temperature enzymic degradation of pesticide residues is usually extremely slow. If prolonged storage is unavoidable, the effects of storage should be checked by analysing fortified samples stored under the same conditions for a similar period.

When samples are to be frozen it is recommended that analytical test portions be taken prior to freezing in order to minimise the possible effect of water separation as ice crystals during storage. Care must still be taken to ensure that the entire test portion is used in the analysis.

The containers must not leak. Neither the containers used for storage nor their caps or stoppers should allow migration of the analyte(s) into the storage compartment.

1.7 Standard Operating Procedures (SOPs):

SOPs should be used for all operations. The SOPs should contain full working instructions as well as information or applicability, expected performance, internal quality control (performance verification) requirements and example calculation of results. It should also contain information on any hazards arising from the method, from standards or from reagents. Any deviations from a SOP must be recorded and authorised by the analyst in charge.

1.8 Validation of methods:

Guidelines have been published for validation of analytical procedures for various purposes. The principles described in this section are considered practical and suitable for validation of pesticide residue analytical methods. The guidance is not normative. The analyst should decide on the degree of validation required to demonstrate that the method is fit for the intended purpose, and should produce the necessary validation data accordingly. For instance, the requirements for testing for compliance with MRLs or providing data for intake estimation may be quite different.

An analytical method is the series of procedures from receipt of a sample to the production of the final result. Validation is the process of verifying that a method is fit for the intended purpose. The method may be developed in-house, taken from the literature or otherwise obtained from a third party. The method may then be adapted or modified to match the requirements and capabilities of the laboratory and/or the purpose for which the method will be used. Typically, validation follows completion of the development of a method and it is assumed that requirements such as calibration, system suitability, analyte stability, etc. have been established satisfactorily. When validating and using a method of analysis, measurements must be made within the calibrated range of the detection system used. In general, validation will precede practical application of the method to the analysis of samples but subsequent performance verifications an important continuing aspect of

the process. Requirements for performance verification data are a sub-set of those required for method validation.

Proficiency testing (or other inter-laboratory testing procedures), where practicable, provides an important means for verifying the general accuracy of results generated by a method, and provides information on the between laboratory variability of the results. However, proficiency testing generally does not address analyte stability or homogeneity and extractability of analyst in the processed sample.

Where uncertainty data are required, this information should incorporate performance verification data and not rely solely on method validation data.

Whenever, a laboratory undertakes method development and/or method modification, the effect of analytical variables should be established, e.g. by using ruggedness tests, prior to validation. Rigorous controls must be exercised with respect to all aspects of the method that may influence the results, such as : sample size; partition volumes, variations in the performance of the clean-up systems used; the stability of reagents or of the derivatives prepared; the effects of light, temperature, solvent and storage of analytes in extracts; the effects of solvent, injector, separation column, mobile phase characteristics (composition and flow-rate), temperature, detection system, co- extractives etc. on the determination system. It is most important that the qualitative and quantitative relationship between the signal measured and the analyte sought are established unequivocally.

Preference should be given to methods having multi-residue and or multi-matrix applicability. The use of representative analytes or matrices is important in validating methods. For this purpose, commodities should be differentiated sufficiently but not unnecessarily. For example, some products are available in a wide range of minor manufactured variants, or cultivated varieties, or breeds, etc. Generally, though not invariably, a single variant of a particular commodity may be considered to represent others of the same commodity but, for example, a single fruit or vegetable species must not be taken to represent all fruit or vegetables. Each case must be considered on its merits but where particular variants within a commodity are known to differ from others

in their effects on method performance, analyses of those variants are required. Considerable differences in the accuracy and precision of methods, especially with respect to the determination step, may occur from species to species.

1.9 Confirmatory Tests:

When analyses are performed for monitoring or enforcement purposes, it is especially important that confirmatory data are generated (As per DG SANTE 11945/2015) before reporting on samples containing residues of pesticides that are not normally associated with that commodity, or where MRLs appear to have been exceeded. Samples may contain interfering chemicals that may be misidentified as pesticides. Examples in gas chromatography include the responses of electron-capture detectors to phthalate esters and of phoshorus-selective detectors to compounds containing sulphur and nitrogen. As a first step, the analysis should be repeated using the same method, if only one portion was analyzed initially. This will provide evidence of the repeatability of the result, if the residue is conformed. It should be noted that the only evidence supporting the absence of detectable residues is provided by the performance verification data.

Confirmatory tests may be quantitative and/or qualitative but, in most cases, both types of information will be required. Particular problems occur when residues must be confirmed at or about the limit of determination but, although it is difficult to quantify at this level, it is essential to provide adequate confirmation of both level and identity (e.g. in case of mass spectrometry Different types and modes of mass spectrometric detectors provide different degrees of selectivity and specificity, which relates to the confidence in identification. The requirements for identification are given in Table 1. They should be regarded as guidance criteria for identification, not as absolute criteria to prove presence or absence of a compound.

Table 1 Identification criteria for different MS techniques

MS mode:	Single-stage MS (unit mass resolution)	Single-stage MS (high resolution/high mass accuracy)	MS/MS
Typical systems (examples):	Quadrupole, ion trap, time-offlight (TOF)	TOF, Orbitrap, FTMS, magnetic sector	Triple quadrupole, ion trap, hybrid MS

			(e.g. Q-TOF, Q-trap)
Acquisition mode:	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Selected/multiple reaction monitoring (SRM/MRM), full scan product-ion spectra
Requirements for identification:	≥ 3 diagnostic ions, preferably including the (quasi) molecular ion	≥ 2 diagnostic ions, preferably including the (quasi) molecular ion; mass accuracy < 5 ppm;at least one fragment ion	≥ 2 product ions
	Ion ratio(s): according to Table 5		

The relative intensities or ratios of selective ions (full-scan MS or SIM) or product ions (MS/MS), expressed as a ratio relative to the most intense (product) ion, should correspond to those of the calibration standard at comparable concentrations and measured under the same conditions. Matrix-matched calibration solutions may need to be used. Table 5 below indicates the recommended maximum tolerances for ion ratios.

Table 2 Recommended maximum (default) tolerances for ion ratios using different MS techniques

Ion ratio (least/most intense ion)	**Maximum tolerance (relative) for GC-EI-MS**	**Maximum tolerance (relative) for LC-MSn, LC-MS, GC-MSn, GC-CI-MS**
0.50-1.00	± 10 %	± 30 %
0.20-0.50	± 15 %	± 30 %
0.10-0.20	± 20 %	± 30 %
<0.10	± 50 %	± 30 %

The need for confirmatory tests may depend upon the type of sample or its known history. In some crops or commodities, certain residues are frequently found. For a series of samples of similar origin, which contain residues of the same pesticides, it may be sufficient to confirm the identity of residues in a small proportion of the samples selected randomly. Similarly, when it is known that a particular pesticide has been applied to the sample material there may be little need for conformation of identity, although a randomly selected results should be conformed. Where blank samples are available, these should be used to check the occurrence of possible interfering substances.

Depending upon the initial technique of determination, an alternative procedure which may be a different detection technique may be necessary for verification of quantity. For qualitative conformation (identity) the use of mass-spectral data or a combination of techniques based on different physico-chemical properties is desirable.

1.10 The concept of lowest Calibrated level (LCL):

The Lowest Calibration Level (LCL) must be equal to or lower than the calibration level corresponding to the Reporting Limit (RL). The RL must not be lower than the LOQ. When the objective of the analysis is to monitor and verify the compliance with Maximum Residue Limits (MRLs), the residue methods must be sufficiently sensitive to reliably determine the residues likely to be a present in a crop or an environmental sample at or around the MRL or AL. However, for this purpose it is not necessary to use methods with sufficient sensitivity to determine residues at levels two or more orders of magnitude lower. Methods developed to measure residues at very low levels usually become very expensive and difficult to apply. The use of LCL would have the advantage of reducing the technical difficulty of obtaining the data and would also reduce costs. The following proposals for LCLs in various samples may be useful in enabling the residue chemist to devise suitable methods.

Residues with agreed MRLs, the LCL can be specified as a fraction of the MRL. For analytical convenience this fraction will vary and could be as follows:

MRL (mg/kg)	**LCL (mg/kg)**
5 or greater	0.5
0.5 upto 5	0.1 increasing to 0.5 for higher MRLs
0.05 upto 0.5	0.02 increasing to 0.1 for MRL

When the MRL is set at the limit of determination of the analytical method, the LCL will also be at this level.

1.11 Expression of results:

For regulatory purposes, only confirmed data should be reported, expressed as defined by the MRL. Null values should be reported as being less than lowest calibrated level, rather than less than a level calculated by extrapolation. Generally, results are not corrected for recovery, and they may only be corrected if the recovery is significantly different from 100%. If results are reported corrected for recovery, then both measured and corrected values should be given. The basis for correction should also be reported. Where positive results obtained by replicate determinations (e.g. on different GC columns, with different detectors or based on different ions of mass spectra) of a single test portion (sub-sample), the lowest valued value obtained should be reported. Where positive results derive from analysis of multiple test portions, the arithmetic mean of the lowest valid values obtained from each test portion should be reported. Taking into account, in general, a 20-30 % relative precision, the results should be expressed only with 2 significant figures (e.g. 0.11, 1.1, 11 and 1.1 × 102). Since at lower concentrations the precision may be in the range of 50 %, the residue values below 0.1 should be expressed with one significant figure only.

1.12 References:

Joint FAO/WHO Food Standard Programme. Codex Alimentarius Commission. Report of the thirty fifth session of the Codex Committee on Pesticide Residues, Rotterdom, The Netherlands. 31st March - 5th April 2003. pp. 46-55.

Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANCO/12571/2013

2. SAMPLE COLLECTION

The present chapter is designed to deal only with the collection of samples for pesticide analysis. To provide a background of problem areas associated with pesticides the following information is repeated from the FAO Food Inspection manual.

Collect a total sample approximating 10 kg by sampling a minimum of 10/1 kg subs 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 gm	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 gm	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

2.1 Packaging and Transmission of laboratory samples:

The laboratory sample must be placed in a clean inert container offering 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, perishable samples should be kept cooled or frozen. Fruits/vegetables/perishable commodities are advised to ship in dry ice while transporting from field to lab.

2.2 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 subsampling. 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 subsampling. When both solid and liquid phases are to be analysed 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 subsampling 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 subsample 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 subsample 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 subsampling. The whole unit sample can be most easily subsampled 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.

In summary, a selective subsample consists only of suspect portions and ignores the remainder of the sample. A representative subsample, however, must as best possible represent an average of the whole sample.

2.3 General Guidelines:

A sample must be statistically representative of the population from which it is taken. Transportation and storage must be adequate to keep residues in their original condition in order to justify the expertise and expense involved in a pesticide residue analysis.

2.4 Composting:

A composite is defined as an admixture of two or more portions of a substance. A composite is formed by first subsampling two or more portions of the same food. An example would be subsampling several individual cans from the same food lot. These subsamples 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 subsamples of the individual sample units must not only be taken correctly, but must all be approximately the same size, weight or volume. Given correct subsampling, the only remaining problem is to make the composite reasonably uniform and representative. This may involve chopping and grinding as well as physical mixing.

2.5 Chopping, Grinding, Mixing:

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 analysed.

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.

2.6 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 or smaller) 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 which helps in homogenization to provide the fine particle size.

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 centre of fruits and the top and center of vegetables. Finely cube the composite sample and reduce by mixing and quartering to ca 300gm. Homogenize the 300gm in an appropriate blender.

If the homogenized sample is not immediately analysed, store it in a clean container with a tight closure and freeze. Samples should be refrigerated, if they are expected to be analysed within four days. Aqueous or semi-aqueous samples should be kept at < -10°C or below, before analysis.

Freezing is often the only way to prevent a change in a 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.

2.7 Sample storage:

Normally the whole sample is stored depending analysis at <-10°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. Homogeneization will increase the rate of enzymatic hydrolysis so frozen homogenized samples should be analysed 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

2.8 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 analysed 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.

2.9 Records:

Each laboratory sample must be correctly identified and should be accompanied by a note giving the nature and origin of the sample and the date and place of sampling, together with any additional information likely to be of assistance to the analyst.

2.10 References:

1. Food and Agriculture Organisation of the United Nations. Manuals of Food Quality Control, introduction to food sampling, food and nutrition. Paper 1419 Section 11, Rome (1988).
2. COMMISSION DIRECTIVE 2002/63/EC establishingCommunity methods of sampling for the official control of pesticide residues in and on products of plant and animal origin and repealing Directive 79/700/EEC.
3. Degradation kinetics and safety evaluation of buprofezin residues in grape (Vitis

vinifera L.) and three different soils of India, Pest Management Science. 65 (2009) 183–188. doi:10.1002/ps.1666.

2.11 Sample pre-processing technique:

Temperature + 2 to - 20°C should be maintained during sampling depending upon the commodity/compound of interest.

3. REPORTING ANALYTICAL RESULTS

It is extremely important in pesticide residue studies that analytical results be reported in a consistent and unambiguous manner. Often, national or international organisations that summarize the analytical results and calculate dietary intakes must evaluate and interpret data obtained from different laboratories, each reporting results in a different format. To facilitate these evaluations, laboratories should report enough details about the detection and quantitation limits of the analytical method to enable correct interpretations of the data to be made. Laboratories should ensure consistent detection and quantitation limits throughout a study. Results of recovery tests for the different contaminants should also be reported. However, analytical findings should be reported as measured, without the use of correction factors that take recovery into account.

3.1 Detection and quantitation limits of the analytical method:

The analytical methods used in the analysis of food samples must be as sensitive as possible. The sensitivity of the overall analytical procedure is usually defined in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ) or determination.

3.2 Limit of detection:

The Limit of Detection (LOD) as it applies to food contaminants may be defined as the minimum concentration of contaminant in a food sample that can just be qualitatively detected, but not quantitatively determined, under a pre-established set of analysis

conditions. The level of concentration which provide signal to noise ratio 3:1 in matrix by applying the complete analytical method will be considered as LOD. This is necessarily a very broad definition, since it encompasses all classes of contaminants and all detection techniques. LOD defines the minimum amount of contaminant in a food sample below which no finite value can be reported, e.g. not detected at limit of detection of 1 g/kg.
In many instances particularly for the determination of pesticides, it is appropriate to place a special restriction on the term detected. Because of the possibility that a small detectable signal at a particular GLC retention time could result from interference, it is desirable to specify that the identity of detection be confirmed before it is reported. A detection whose identity cannot be confirmed would not be reported as a positive finding.

3.3 Limit of Quantitation:

The Limit of Quantitation (LOQ) is the minimum concentration of a contaminant in a food sample that can be determined quantitatively with an acceptable accuracy and consistency by applying the complete analytical method. The level of concentration which provide signal to noise ratio 10:1 in matrix will be considered as LOQ.

The Codex Committee on Pesticide Residues employs the term limit of determination which is defined as the lowest practical concentration of a pesticide residue on contaminant that can be quantitatively measured and identified in the specified food commodity or animal feed stuff with an acceptable degree of certainty by current regulatory methods of analysis.

3.4 Lower Limit of Detection (LLD):

The smallest amount of sample activity which will yield a net count for which there is confidence at a predetermined level that activity is present.

3.5 Reporting results:

Report the exact portion of food taken for analysis and report results in mg/kg or parts per million (ppm).

3.5.1 Raw Agricultural Commodities: Report the results as mg/kg or ppm on portion examined, except report raw milk on fat basis.

3.5.2 Processed foods: Report the results as mg/kg or ppm on portion examined, except for dairy products and concentrates.

3.5.3 Dairy products: Report results as mg/kg or ppm on a fat basis, except report residues in low fat dairy products (e.g. skim milk, buttermilk, nonfat dried milk, uncreamed cottage cheese) on a whole as is basis.

3.5.4 Concentrated and Dehydrated Products: Report results as mg/kg or ppm on as is basis. Where significant residues are encountered on concentrates which must be reconstituted to the whole product basis before consumption, it is useful to calculate to the whole basis and record both results.

3.6 Analytical limits of quantitation and detection:

When a method is used in a specified way, the lowest concentration in the sample of a residue producing a response of sufficient magnitude (S/N =10:1) for reliable measurement is referred to as the limit of quantitation. Residues causing recognizable GLC peaks (or other type detector response) below the limit of quantitation may still be within the limit of detection though not within the limit of quantitation. These findings are reported as Trace or below limit of quantitation (BLQ). Below that level, residues whose peaks cannot be distinguished from the baseline with confidence are considered not detected.

Analytical limits are defined for each chemical recovered by a given multiresidue method according to the following considerations:

1. Response of the determinative step (GLC detector) to the chemical when the detector has been adjusted to a pre-determined response (sensitivity) to a particular compound. For example, the Food and Drug Administration laboratories

have GLC detectors adjusted to produce 50 % full scale recorder deflection (FSD) for 1 ng heptachlor epoxide (EC detector) or 2 ng parathion (TCD and FPD, phosphorus mode).

2. Weights of sample equivalent routinely injected into the GLC determinative step. In FDA programmes, aliquots of extract equivalent to 20-30mg of nonfatty food (or fatty food calculated on the whole basis) and 3mg fat are injected for routine analysis.

3. Minimum measurable peak of 10 % FSD.

Based on the above considerations, approximate limits of quantitation for residue recovered are:

	Whole basis	**Fat basis**
Heptachlor epoxide	0.01 ppm	0.05 ppm
Parathion	0.02 ppm	0.10 ppm

Limits of quantitation for other chemicals determined by these same methods are in the same relation to the above values as their particular GLC detector reasons are to heptachlor epoxide or parathion.

Modern GLC detectors do not always operate best at the operating conditions which have been recommended for many years. It is still very important, however, that programmes retain consistent limits of quantitation in examining for and reporting residues. Thus, the weight of sample equivalent injected must be adjusted to provide the same limits of quantitation as have been in effect in the past. For example, modern Ni^{63} EC detectors often are most linear and stable at conditions which produce 50 % FSD for 0.1 ng heptachlor epoxide (10 % of the amount formerly required). IN this case, to maintain a limit of quantitation consistent with that used in the past, the weight of equivalent sample injected must be only 10 % of that formerly used i.e. 2-3 mg whole food or 0.3 mg fat.

Similarly, other limits of quantitation directed in specific programmes can be met by appropriate balancing of the weight of equivalent sample injected and sensitivity of the detector.

3.7 Other factors affecting analytical limits:

Beyond the specifications defining limits of quantitation, certain other factors can affect the levels at which residues can be detected or quantitated. In particular

1. Nature of the product and amount of nature of co-extractives. For example, fats and concentrated and dehydrated products often present problems of sample size and cleanup and thus usually can be examined only at a higher level of quantitation than other products.

2. Background signal resulting from detector response to sample coextractives and/or from normal operation of the instrument imposes a requirement for the residue peaks to be larger than otherwise necessary in order that they can be distinguished from the background and accurately measured.

3. Metabolized, weathered or degraded residues. Quantitation of residues which differ from the parent chemical is often complicated, especially when the parent and/or terminal residue is a mixture of chemicals. The accuracy and precision of such quantitation is higher than would be expected based on the detector sensitivity to the particular residue.

Each of these factors may reduce the certainty of quantitation and/or identification of a residue and thereby raise the level at which the residue can be quantitated and/or reported with confidence. In some instances a limiting factor can be overcome (e.g. additional cleanup can be used to remove coextractives, or an interfering residue can be separated or degraded) so that the expected limit of quantitation can be reached.

3.8 General rules:

Within the qualifications already discussed, the following guidelines are applied to routine residue analysis:

Calculate finite values for all residues to the limit of quantitation of the finding were not confirmed. Report calculated values to the limit of quantitation or to the particular residue

by that method. Such levels should be conformable, even if the particular level specified by the programme.

3.9 Reporting not detected:

The term not detected should be used to indicate that food sample(s) was analysed or a particular contaminant or class of contaminants, e.g. organochlorine pesticides, and analytical response was observed. A not detected reporting should be accompanied by a limit of detection for the analytical method used, and a short summary of which compounds or types of compounds were amenable to the method. For example, if a leafy-vegetable composite were analysed using a method capable of recovering several organchlorine pesticides, the report might state organochlorine pesticides; not detected at detection limit of 1 g/kg or heptachlor, 25 g/kg; other organochlorine residues, not detected at detection limit of 1 g/kg. Values of zero should be avoided, or at least qualified by defining the limit of detection.

3.10 Reporting Trace values:

Occasionally, although not a good practice, laboratories will use the term trace to report the detection of a contaminant. This usually refers to an analytical response that is just above the limit of detection but below the limit of quantitation i.e. the compound is detectable (with confirmed identity), but cannot be quantified (detection limit < trace < quantitation limit).

Laboratories should report such low-level detections, but also should clearly state the meaning of trace, and the analytical uncertainties associated with it.

3.11 Rounding of numbers:

To report correct significant digits we must round off numbers. To obtain this approximate number you must do off certain of the lower digits in the number, the process known as rounding off. An error is of course introduced in rounding off, and it is desirable to make this error be as small as possible. By using the proper method of rounding, it is always possible to make the absolute error no greater than half a unit in the last place retained.

This is done by increasing by one the last digit kept if the discarded part is greater than half a unit in that digit position. If the discarded part is exactly one-half a unit the last place kept, it is best to increase the last kept digit by one sometimes, and not other times, so that if several round offs are made during the problem, all will not introduce errors in the same direction. A simple way to do this is always to round the last kept digit to an even number when the discarded part is exactly half unit in the last kept place. E.g. 2.324, 2.316 and 2.315 would take the form 2.32 if rounded off to two places.

3.12 References:

1. U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. I, Section 143, (1977).

2. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANCO/12571/2013.

4. PESTICIDE STANDARDS

4.1 Preparation and storage of reference standard solutions:

The greatest source of quantitative error in GC analysis is undoubtedly inaccurate standard solutions. SHERMA, J. 1981. The pesticides reference standards stock solutions are prepared as per SANCO/12571/2013 guideline.

4.2 Storage of primary standards:

Proper storage of reference standards in order to maximize preservation of chemical integrity is an essential part of the analytical process. Studies have consistently shown that most classes of standards in neat (or essentially pure) form are generally stable for at least one year if kept tightly sealed, protected from light, and either refrigerated or frozen. Many of the organochlorine, triazine and carbamate compounds are included in this group. The organophosphate compounds tend to be more susceptible to decomposition.

Small sample vessels, containing the net standards, are best stored refrigerated within a secondary, air-tight container. A cabinet style desiccator loaded with 0.5 % to 1.0 % (by volume) of indicating silica gel or similar capacity desiccant will generally prove quite satisfactory, providing both isolation and a reasonably moisture-free atmosphere. Laboratories with limited refrigeration space may conveniently substitute large, wide-mouth, resaleable glass jars loaded with 2 to 4 cm of an appropriate desiccant.

Whenever a vessel is removed from refrigeration, it should be allowed to gradually rise to room temperature before opening. Vessel closures should remain removed only as long as is necessary to withdraw samples. After withdrawing aliquots, primary standard vessels should be returned to cold storage immediately.

4.3 Solvents:

The advantages and disadvantages of the following solvents are outlined below: toluene, iso-octane (2,2,4-trimethylpentane), ethyl acetate and/or hexane. All solvents should be pesticide grade and distilled-in glass.

Toluene and iso-octane are suitable solvents for most pesticide standards. Iso- octane does not dissolve as many compounds as toluene but is preferred and used whenever possible because it does not affect Florisil elutions. In addition, iso-octane is much more compatible with electron capture detectors.

Iso-octane and hexane are both suitable for standard dilutions. Iso-octane, while more expensive offers the advantage of a much lower vapour pressure than hexane. Thus, it is much less likely to evaporate through seals during long-term storage and during repeated vessel openings.

Ethyl acetate, dichloromethane, other chlorinated solvents are not recommended as a final dilution solvent for electron capture GLC/ECD analysis but maybe used for preparation of the concentrated stock solutions of the more polar compounds.

4.4 Weight units:

In residue analysis, weights smaller than mg are usually used. They are explained below:

1. 1 microgram (µg) 1 µg = 1^{-6} g or 1 g = 1000000 µg
2. Nanogram (ng) 1 ng = 10^{-9} g or 1 g = 1000,000,000 ng
3. Picogram (pg) 1 pg = 10^{-12} g or 1 g = 1000,000,000,000 pg

4.5 Preparation of standard solution of pesticides:

4.5.1 Preparation of stock solution 1 mg/mL (1000 ppm)

Weigh 10 mg of the pesticide of pesticide reference standard and transfer to 10 mL volumetric flask. Dissolve the pesticide in a suitable solvent and make to volume.

4.5.2 Intermediate solution 10 µg/mL (10 ppm)

Pipet 1 mL of the stock solution into individual 100 mL volumetric flask. Dilute to volume with the suitable solvent.

4.5.3 Working solution 1 µg/mL (1 ppm)

Pipet 10 mL of the intermediate solution into 100 mL volumetric flask. Dilute to volume with the suitable solution.

5. PURIFICATION OF SOLVENTS AND REAGENTS

Before any analysis is attempted it is of utmost importance that the purity of the solvents and all reagents is ensured. If reagent blank analysis does not indicate any contamination, purification of reagents and solvents can be avoided.

5.1 Purification of Florisil:

Florisil, a synthetic magnesium silicate long used as an adsorbent in FDA methodology, is subject to variations in adsorptivity common to most analytical grade adsorbents. Years of experience in using Florisil have lead to establishment of procedures for purchasing, handling, and testing the material to optimize and standardize its application. PR Grade Florisil is specified because other grades available from chemical supply companies may be prepared differently by the manufacturer and may exhibit drastically different adsorptivity from what is required. Handling directions are designed to prevent contamination that may interfere with subsequent analyses and to ensure consistent adsorptivity throughout use of a particular lot of Florisil.

5.2 Observe these procedures for handling Florisil:

Use PR Grade Florisil, 60-100 mesh, calcined (heated) 3 hr at 1250°F (677°C), for all for all the residue analysis methods that require Florisil.

Other grades may not be capable of providing the elution patterns required for successful application of the methods.

Immediately after opening bulk lots of Florisil, transfer to glass containers (preferably amber) that are glass-stoppered or have Teflon-lined or foil-lined screw caps; store in dark. Activate each portion by heating at 130°C for 168 hr (1 wk) before use. Top most rack of the oven.

Store at 130°C in foil-covered bottles in the Florisil may be heated in bulk in pint glass bottles or in individual column amounts in 50 mL Erlenmeyer flasks. 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.

Alternatively, in case PR grade is not available, reflex 1 kg of Florisil with 2.5 mL of distilled water for one hour. After repeating the process two more times, decant the water through Buchner funnel and dry in an oven at 130°C for two hr. Partially dried Florisil should then be transferred to a silica bowl and kept in muffle furnace at 660°C for 3 hr and stored as described below.

5.3 Purification of sodium sulfate:

All methods specify use of anhydrous, granular, reagent grade sodium sulfate. To remove phthalate esters that interfere in determinations using electron capture detectors, heat sodium sulfate 4 hr in muffle furnace at 600 °C. Store in glass containers; if plastic lids are used, separate them from sodium sulfate with layer of foil.

If reagent blank tests indicate that sodium sulfate is contributing interferences to otherdeterminations, rinse several times with acetone and ethanol, then dry.

5.4 Purification of Glass Wool:

All methods specify use of Pyrex glass wool, which can have contaminants that interfere with determination. If reagent blank tests indicate that glass wool is contaminated, rinse it with solvent and air-dry or heat 1 hr at 400°C.

Some methods specify silanized glass wool, which may be purchased. To silanize glass

wool in laboratory, soak 10 min in 10 % dimethyl dichlorosilane, rinse with toluene, and soak another 10 min in methanol. Rinse with methanol and allow air-drying.

5.5 Purification of Celite 545:

Purification of Celite should be done by making a slurry with 6M hydro chloric acid while heating on a steam bath. Then the slurry is washed with distilled water several times until neutral. Again wash with several solvents ranging from high to low polarity and dry.

Impurities interfering with phosphorus selective detectors are removed by heating Celite at 600°C in a muffle furnace for a period of a minimum of 4 hr.

5.6 Purification of charcoal:

Make 200 gm slurry with 500 mL hydrochloric acid; stir magnetically while boiling for one hour. Add 500 mL of water; stir and boil for another 30 min. Recover the charcoal by filtering through a Buchner funnel, wash with distilled water until washings are neutral and dry at 130°C. Store in a tightly stoppered bottle.

5.7 Purification of magnesium oxide:

Make 500 gm slurry with enough distilled water to cover it, in an one litre Erlenmeyer flask. Heat with occasional shaking for 30 min on a steam bath. Filter with suction. Dry for 12-24 hr at 105-130°C and pulverize to pass a No. 60 sieve. About 10 % water is absorbed in this process. Store in a closed jar.

Purification of solvents — n-Hexane, n-Heptane, Chloroform, Toluene, Isopropanol, Methanol, Methylene Chloride, Petroleum Ether, Ethyl Acetate, Isooctane

All these should be of AR grade and should be redistilled using all-glass apparatus.

5.8 Florisil cleanup procedure for the distilled solvents

5.8.1 Purification of acetone:

Reflex acetone (2.5 mL) with Potassium permanganate (0.5 gm) until the violet colour persists. Collect fractions at 55-60°C.

5.8.2 Purification of ethyl ether:

Use all-glass distilled ethyl ether and assume the presence of 2 % ethanol added as a stabilizer to prevent formation of peroxides. Practical shelf life is limited, however, even when alcohol is added peroxides form readily. Test for peroxides using "Peroxide Test" paper.

*n-Heptane is recommended to use in place of n-Hexane due to comparative boiling point and volatability.

5.8.3 Purification of acetonitrile:

All methods specify use of acetonitrile distilled from all-glass apparatus. To make use of reagent grade acetonitrile, test for impurities by holding moistened litmus paper over mouth of storage container. If litmus paper turns blue, purify 5 L acetonitrile by adding 1 mL of 85 % phosphoric acid, 30 gm phosphorus pentoxide, and boiling chips, then allowing to stand overnight. Distill from all glass apparatus at 81-82°C, discarding first and last 10 % of distillate; do not exceed 82°C.

6. EXTRACTION AND CLEAN-UP METHODS IN PESTICIDE RESIDUE ANALYSIS

6.1 Extraction techniques:

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.

6.2 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 solubilised 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.

6.3 Liquid substrates:

6.3.1 Partitioning*:* Samples like water, body fluids, and juices are extracted by partitioning 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.

6.3.2 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:

Solid adsorbents	**Liquid coated or bonded on inert solids**
1. Activated charcoal /graphitised carbon black	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
10. Primary secondary amine (PSA)

6.4 Solid substrates

6.4.1 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.

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

6.5 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 conditions.

- 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.

6.5.1 Choice of solvent depending on type of substrate

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

6.5.1.1 Aqueous substrate: Water immiscible solvent like hexane, petroleum ether, benzene, dichloromethane, chloroform, ethyl acetate, etc.

6.5.1.2 Solid substrates (soil, fodder etc.): Different solvents like acetonitrile, hexane and mixed solvents are used for dry sample with low moisture like grains, samples with high moisture content (green plant samples and substrates with high fat content (grains, oilseeds, egg, meat, fish etc.).

6.5.2 Choice of solvent depending on nature of pesticides

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

6.6 Recent techniques of extraction

6.6.1 Solid Phase Extraction (SPE):

Solid phase extraction technique is based on the concept of selective retention by the device for the analyte, in this case the pesticide. SPE can be made to work on either the batch or column mode. This method has not become popular as there is loss of the pesticide as it tends to adhere to the surface of the beaker and tubes. The method is modified by the use of adsorbents contained in cartridges of various sizes usually made of plastic such as polyethylene or polypropylene of extremely high purity and is termed as column-liquid solid extraction (CLSE), however for simpliciaty it is referred to a SPE cartridges.

6.6.2 Solid Phase Micro-Extraction (SPME):

In this technique a droplet of extractant, or a fiber coated with the extractant is suspended in the solution to be extracted and then transferred to an analytical device.

6.6.3Accelerated Solvent Extraction (ASE):

The extracting solvent is passed under amabient temperature or pressure through the matrix, removing the analyte using a smaller volume of the solvent.

6.6.4 Microwave-Assisted Solvent Extraction (MASE):

The technique employs the use of microwave energy and a suitable solvent to extract the analyte from the matrix, water is commonly preferred solvent in this procedure.

6.6.5 Supercriticial Fluid Extraction (SFE):

In the supercritical fluid extraction (SFE) method carbon dioxide gas is passed under supercritical temperature and pressure (liquefied carbon dioxide) through the matrix to extract the pesticide and then transferred to the analytical device for quantitation.

6.6.6 Stir-Bar Sorptive Extraction (SSE)

SBSE was introduced in 1999 by Pat Sandra's group to overcome some of the limits of the existing techniques, in particular in the recovery of medium-to-high volatility analytes when sampled in liquid phase with polydimethylsiloxane-open tubular traps (PDMS-OTT); further aim was to improve the limited recovery achievable in ultra trace analysis with solid-phase micro extraction (SPME), especially under un favourable phase ratios when working with small volumes of sorptive material (in general PDMS) coating the fused-silica fibre . SBSE was first developed for sampling in liquid phase and is based upon sorption of the investigated analytes or fraction onto a very thick film of PDMS coated onto a glass-coated magnetic stir bar. Sampling is done by directly introducing the SBSE device into the aqueous sample; in the original experiments, the

analytes sampled for a given time were recovered by thermal desorption and then on-line transferred to a gas chromatography (GC) or GC–mass spectrometry (MS) system for analysis. Later, liquid desorption in combination with high performance liquid chromatography (HPLC) also was applied, mainly for analytes not analyzable by GC.

6.7 Basic Concepts of SBSE:

SBSE is based upon sorption, which is a form of partition based upon the analytes dissolution in a liquid-retaining polymer from a liquid or vapour sample, thus, originating a bulk retention. The main advantages of sorption are related to high inertness of PDMS, which gives better performance for labile, polar, or reactive compounds; absence of catalytic degradation reactions; analyte recovery mechanism based upon a well-known chromatographic process; and linearity of sorption isotherms, which is fundamental for quantitative analysis.

References:

Carlo Bicchi, Erica Liberto, Chiara Cordero, Barbara Sgorbini, Patrizia Rubiolo LCGC, May 1, 2009.

6.8 Clean-up Techniques:

Cleanup refers to a step or series of steps in the analytical procledure in which the bulk of the potentially interfering coextractives are removed by physical or chemical methods.

During extraction, the solvent comes in contact with the substrate matrix, to enable extraction of the pesticide along with some of the constituents of the substrate matrix also get solubilized. The extract not only contains pesticide residues but also other constituents, which are called co-extractives. The removal of interfering co- extractives from extract is called clean up. The co-extractive generally extracted along with pesticide from various substrates are moisture, coloured pigment like chlorophyll, xanthophylls and anthocyanins, colourless compounds like oil, fat and waxes etc.

When dry substrate is extracted with water immiscible solvent, it contains traces of

moisture, which can be removed by passing the extract through anhydrous sodium sulfate. High moisture containing substrate are extracted with water miscible solvent, the extract contains lot of water and water soluble compounds, the extract is concentrated to remove organic solvent, the aqueous phase is diluted with saturated sodium chloride solution and then extracted with water immiscible solvent just like water samples. After removal of moisture, the other coextractives are removed by using various separation techniques.

6.9 Liquid-liquid partitioning:

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

6.9.1 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 ofextracts of oil seeds, milk, butter etc.

6.9.2 Partitioning with acid/base treatment: This technique can be used for the pesticides, which are either acidic or basic in nature. This technique can not be used for neutral type of pesticides.

6.10 Chemical Treatment:

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

6.10.1 Saponification: This technique is employed to remove fats and oils from the extract. The fat and oil is saponified or hydrolysed 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 analysed easily.

6.10.2 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.

6.10.3 Oxidation: In this technique, the co-extractives are oxidized with concentrated Sulphuric 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.

6.11 Chromatographic techniques:

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

6.11.1 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.

6.11.2 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 column of ion exchange resin. The exchange resin holds up the pesticide being ionic, whereas non-ionic co- extractives pass out of the column. The held up pesticide is eluted out using suitable electrolyte solution.

6.12.1Gel Permeation Chromatography and Molecular Sieves: The separation in gel permeation Chromatography and molecular sieves is based on the principle of size exclusion. Both gel and molecular sieves have tubular structures with inner diameter (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, enabling 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.

6.12.2 Adsorption Column Chromatography: Adsorption column Chromatography is the most common and widely used technique for clean up. Different type of adsorbent 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.

6.13 Recent Trends in Clean up:

6.13.1 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.

7. MULTIRESIDUE METHOD FOR DETERMINATION OF CHLORINATED PESTICIDES in fatty and non-fatty food

The method is applied for determination in food stuffs of the residue content of the following organochlorine pesticides and some of their isomers and degradation products, p,p'-dichlorodiphenyldichloroethylene, o,p'- dichlorodiphenyltrichloroethane, p,p'-dichlorodiphenyltrichloroethane, Endosulfan-α, Endosulfan-β and Endosulfan Sulphate, α-hexachlorocyclohexane, β-hexachlorocyclohexane, γ-hexachlorocyclohexane, δ-hexachlorocyclohexane, Aldrin, Dieldrin, Chlordane, Heptachlor, Heptachlor Epoxide.

7.1 Principle:

Thoroughly mixed test portion is extracted with Acetonitrile (high -H2O foods) or

aqueous Acetonitrile (low-H_2O or high sugar foods). Fat is extracted from fatty foods and partitioned between pertoleum ether and acetonitrile. Aliquot (non-fatty foods) or entire solution (fatty foods) of Acetonitrile is diluted with H_2O and residues are extracted into petroleum ether. Further purification is done by chromatography on Florisil column, eluting with mixture of petroleum ether and ethyl ether. Residues in concentrated eluates are measured by Gas- Chromatography (GC) and identified by combination of Gas - Chromatography.

Analyst competence in applying method for trace residues should be assured before analysis. Recoveries of added compounds through method should be = 80 %.

7.2 Scope:

The present method describes the determination of the analysis of the following chlorinated pesticides:

OC: 22 items;

1.α- BHC
2.β- BHC
3.γ -BHC
4.δ -BHC
5. Heptachlor
6.Aldrin
7.Heptachlorepoxide
8.Gamma-Chlordane
9.Endosulfan I
10. α-chlordane
11. p,p'-DDE
12. Dieldrin
13. Endrin
14. Endosulfan II
15. p,p'-DDD
16. Endrin aldehyde
17. Endosulfan sulfate
18. p,p-dichlorodiphenyltrichloroethane
19. Endrin ketone
20. Methoxychlor
21. o,p-dichlorodiphenyltrichloroethane
22. Dicofol

7.3 General Reagents:

Solvents must be purified and final distillation conducted in all glass apparatus.

7.3.1Solvent purity test - Electron capture GC requires absence of substances causing detector response as indicated by following test: Place 300 mL solvent in kunderman-danish concentrator fitted with 3-ball Snyder column and calibrated collection vessel or use flash evaporator and concentrate to 5 mL. Inject 5 µL concentrate from 10 µL, syringe into gas chromatograph, using conditions as described. Concentrate must not cause recorder deflection >1 mm from baseline for 2-60 min after injection.

(a) Acetonitrile - Purify technical Acetonitrile as follows: To 4 L Acetonitrile add 1 mL phosphoric acid, 30 g phosphorus pentoxide and boiling chips, and distil in all-glass apparatus at 81-82°C.Do not exceed 82°C.

Some lots of reagent grade Acetonitrile are impure and require distillation. Generally vapors from such lots will turn moistened red litmus paper blue when held over mouth of storage container. Pronounced amine odor is detectable.

(b) Acetonitrile saturated with petroleum ether - Saturate Acetonitrile, (a) with redistilled petroleum ether, (m).

(c) Alcohol-USP, reagent grade, or methanol, oaks

(d) Eluting solvent: Pipet 3.5 mL Acetonitrile into 500 mL dichloromethane and dilute with hexane to 1L.

(e) Florisil: 60/100 PR grade activated at 675°C.
Store at 130°C in glass stoppered bottles or in air tight desiccator at room temperature.

(f) Sulphuric acid: 96 % purity analytical grade.

(g) Sodium chloride

(h) H. Methanol (HPLC grade)

(i) Tri-sodiumcitrate dihydrate

(j) Di-sodium hydrogen citrate sesquihydrate

(k) Formic acid (AR grade)

(l) Sulphuric acid (AR grade)

(m) Primary secondary amine (PSA) sorbent, 40 µm particle size SPE

(n) C18 sorbent, 40 µm particle size SPE, where samples contain >1 % fat

(o) Graphitized carbon black, 120/140 mesh size dispersive SPE

(p) Magnesium sulfate anhydrous

7.4 Preparation of laboratory sample and extraction:

7.4.1 Non-fatty foods:

Pit soft fruits, if necessary. Chop or blend representative laboratory sample of leafy or cole-type vegetables, pitted soft fruits, firm fruits, and roots. Mix thoroughly to obtain homogeneous test sample before taking portions for analysis. Grind dry or low moisture products, e.g., hays, to pass No. 20 sieve and mix thoroughly. Proceed as in (a), (b) or (c).

(a) High moisture (more than 75 % H_2O) products containing less than 5 % sugar

(1) Products other than eggs - Weigh 100 gm chopped or blended test portion into high-speed blender jar, add 200 mL Acetonitrile and ca 10 gm Celite, and blend 2 min. at high speed. Filter with suction through 12 cm Buchner fitted with filter paper into 500 mL suction flask. Transfer filtrate to 250mL graduate and record volume (V). Transfer measured filtrate to 1 L separator, and proceed as in (d)

(2) Whole eggs - Discard shells and blend combined yolks and whites at low speed >5 min or until test sample is homogeneous. Low speed blending will minimize foaming or whipping of sample. Weigh < 25 gm thoroughly mixed yolks and whites into high-speed blender jar, and proceed with addition of Acetonitrile as in (a).

(b) High moisture (more than 75 % H_2O) products containing 5-15 % sugar –Add 200 mL acetonitrile and 50 mL H_2O to 100 gm test portion in blender and proceed as in (a).

(c) Dry or low-moisture products e.g. hays - Add 350 mL H_2O- acetonitrile (7+13) (350 mL H_2O diluted to 1 L with acetonitrile) to 20-50 gm ground test portion in blender (if larger test portion sample is required, and enough additional extraction mixture to wet sample and permit thorough blending). Blend 5 min at high speed and proceed as in (a), beginning "Filter with suction. Transfer < 250 mL filtered extract [record volume (V)] to 1 L separator and proceed as in (d).

(d) Transfer of residues to petroleum ether - Carefully measure 100 mL petroleum ether and pour into separator containing filtrate. Shake vigorously 1- 2 min and add 10 mL saturated Sodium chloride solution and 600 mL H_2O. Hold separator in horizontal position and mix vigorously 30-45^{0}C. Let separate, discard aqueous layer, and gently wash solvent layer with two 100 mL portions H_2O. Discard washings, transfer solvent layer to 100 mL glass-stoppered cylinder, and record volume (V). Add approximately 15 gm anhydrous Sodium sulfate and shake vigorously. Do not let extract remain with Sodium sulfate > 1 hr otherwise this may result in loss of organochlorine pesticides by adsorption. Concentrate the solvent to 5-10 mL in K-D concentrator/ flash evaporator.

7.5 Pesticide standard solutions:

p,p'-dichlorodiphenyltrichloroethane, p,p'-DDE, p,p'-dichlorodiphenyltrichloroethane, aldrin, dieldrin, heptachlor, helptachlor expoxide, α-HCH, β-HCH, γ-HCH, , δ-HCH α-endosulfan, β-endosulfan and endosulfan sulphate.

(A) Stock solution 1 mg/mL. Weigh 10 mg of each pesticide standard reference (as per DG SANTE 11945/2015) and transfer into individual corresponding 10 mL volumetric flask, and accordingly dissolve and make up to 10 mL with n-Hexane/ n-Heptane in the volumetric flask

(B) Intermediate solution 10 µg/mL. Pipet 1 mL of each stock solution into individual 100 mL volumetric flask. Dilute to volume with hexane.

(C) Working solution 0.1 µg /mL. Dilute 1 mL of solution B to 100 mL volumetric flask and make the volume with n-hexane.

7.5.1 Fat Containing Foods:

Milk - To 100 mL fluid milk (dilute evaporated milk 1 + 1 with H_2O) in 500 mL centrifuge bottle, add 100 mL alcohol or methanol and ca 1 gm sodium or potassiumoxalate, and mix. Add 50 mL ether and shake vigorously 1 min; then add 50 mL petroleum ether and shake vigirously 1 min. Centrifuge ca 5 min at ca 1500 rpm. Blow off solvent layer with wash bottle device into 1 L separator containing 500-600 mL H_2O and 30 mL saturated Sodium chloride solution. Re extract aqueous residue twice, shaking vigorously with 50 mL portions ether - petroleum ethe (1+1); centrifuge and blow off solvent layer into separator after each extraction. Mix combined extracts and H_2O cautiously. Drain and discard H_2O. Rewash solvent layer twice with 100 mL portions H_2O.

Animal and Vegetable fats and oils: If solid, warm until liquid and filter through dry filter paper.

Butter: Warm to about 50°C until the fat separates and decant the fat through a dry filter.

Cheese : Place 25-100 gm (sufficient to provide 3 gm fat) of diced sample, about 2 gm of oxalate and 100 mL ethanol in a high-speed blender and blend 2-3 min. (If experience with the product indicates that emulsions will not be broken by centrifuging, add 1 mL water/2 gm sample before blending). Pour into a 500 mL centrifuge bottle. Add 50 mL of ethyl ether and shake vigorously 1 min. Then add 50 mL of petroluem ether and shake vigorously 1 min. Centrifuge about 5 min at 1500 rpm. Siphon off the solvent layer using a solvent delivery tube. Blow ether layer off by gently blowing through the mouthpiece tube into a 1 L separator containing 500-600 mL of water and 30 mL of saturated salt solution. Re-extract the aqueous residue twice, shaking vigorously with 50 mL portions of ethyl ether: petroleum ether (1:1). Centrifuge and siphon off the solvent layer into the separator after each extraction. Mix the combined extracts and water cautiously. Drain

and discard the water layer. Rewash the solvent layer twice with 100 mL portions of water, discarding the water each time. (If emulsions form, add about 5 mL of saturated salt solution to the solvent layer or include it with the water wash). Pass the ether solution through a column of anhydrous sodium sulphate 50 mm high in a tube and collect the eluate in a 400 mL beaker. Wash the column with small portions of petroluem ether and evaporate the solvent from the combined extracts on a water-bath at 100°C with the assistance of a current of air.

Fish: Weigh 25-50 gm thoroughly ground and mixed sample into a high speed blender. (If the fat content is known or can be estimated, adjust the sample size so that a maximum of about 3 gm of fat will be extracted). Add 100 gm of anhydrous sodium sulphate to combine with water present and disintegrate the sample. Alternately blend and mix with a spatula until he sample and sulphate are well mixed. Scrape down the sides of the blender jar and break up caked material with a spatula. Add 150 mL of petroleum ether and blend at high speed for 2 min. Decant the supernatant petroluem ether through a 12 cm Buchner funnel fitted with 2 sharkskin papers into a 500 mL suction flask. Scrape down the sides of the blender jar and break lip the caked material with a spatula. Re-extract the residue in the blender jar with two 100 mL portions of petroleum ether, blending for 2 min each time. (After blending 1 min, stop the blender, scrape down the sides of the jar and break up caked material with a spatula, then continue blending for 1 min). Scrape down the sides of the blender jar and break up caked material between extractions. Decant the supernatant petroleum ether from repeated blendings through the Buchner funnel and combine with the first extract. After the last blending, transfer the residue from the blender jar to the Buchner funnel and rinse the blender jar and material in the Buchner funnel with several portions of petroleum ether. Pour the combined extracts through a 40 mm column of anhydrous sodium sulphate in a tube, and collect the eluate in a 500 mL K-D/vacuum evaporator concentrator/flash evaporator with a plain collection tube. Wash the flask and column with small portions of petroleum ether and evaporate most of the petroleum ether from the combined extracts and rinses. Transfer the concentrated fat extract to a tared beaker using small amounts of petroleum ether. Evaporate the petroleum ether on a water-bath at 100°C under a current of dry air.

Other fatty foods: Weigh into blender jar amount estimated to provide ca 3 gm of fat. In

separate container mix amountof sodium sulphate equal to 2.5 × estimated weight of water in sample with 100 mL petroleum ether, and transfer to blender jar. Mix at medium speed for 3 min. Allow solids to settle and decant petroleum ether through medium porosity filter paper into tared 500 mL Erlenmeyer flask to which a few boiling chips had been added before weighing. Add 100 mL petroleum ether to residue in blender jar, mix at medium speed for 1 min, allow solids to settle and decant petroleum ether through filter to combine with above filtrate. Transfer solid residue in blender jar to filter paper, fold paper over solids, and squeeze paper gently against side of funnel with spatula to recover as much solvent as possible. Evaporate petroleum ether on a water bath. Dry flask and weigh flask plus contents. Determine amount of fat by subtracting tare weight of flask.

7.6 Acetonitrile partitioning:

Weigh 3 gm of fat into a 125 mL separator and add petroleum ether so that the total volume of fat and petroleum ether is 15 mL. Add 30 mL of acetonitrile saturated with petroleum ether. Shake vigorously 1 min, allow the layers to separate and drain the acetonitrile into a 1 L separator containing 650 mL water, 40 mL saturated salt solution and 100 mL of petroleum ether. Extract the remaining petroleum ether in the 125 mL separator with 3 additional 30 mL portions of acetonitrile saturated with petroleum ether, shaking vigorously 1 min each time. Combine all the extracts in the 1 L separator.

Hold the 1 L separator in a horizontal position and mix thoroughly 30-45 s. Let the layers separate and drain the aqueous phase into a second 1 L separator. Add 100 mL of petroleum ether to the second separator shake vigorously 15 s and let the layers separate. Discard the aqueous phase, combine the petroleum ether with that in the first separator and wash with two 100 mL portions of water.

Discard the washings and draw off the petroleum ether layer through a 50 mm column of anhydrous sodium sulphate into a 500 mL K-D concentrator/flash evaporator. Rinse the separator and then the column with three approximately 10 mL portions of petroleum ether. Evaporate the combined extract and washings to about 5 mL.

7.7 Clean up:

Clean up of aldrin, dieldrin, α-chlordane, heptachlor, o,p-dichlorodiphenyltrichloroethane, p,p'-DDE, p,p'- dichlorodiphenyltrichloroethane, α-HCH, β -HCH, γ-HCH and δ-HCH.

Take the concentrated petroleum ether extract into 250 mL separatory funnel. Add 100mL of petroleum ether saturated with acetonitrile, after vigorous shaking, discard the petroleum ether layer.collect the acetonitrile layer and reduce the volume to 10 mL. Add 20 mL of 10 % Sodium chloride solution; extract the residues with 75 mL of n-Hexane two times. Collect the n-hexane layers each time and reduce to small volume.

OR

Take the concentrated petroleum ether extract into 250 mL separatory funnel and drop wise concentrated sulphuric acid a Pasteur pipette till the upper layer of petroleum ether becomes clear, discard the lower phase of spent sulphuric acid and wash the upper layer with three 10 mL portion of distilled water. Dry the petroleum ether layer over sodium sulphate and make to the desired volume. In general 50 gm of samples of cereals pulses, vegetables will require about 20 mL of sulpuric acid.

NOTE: to use Sulphuric acid digestion method, it is advised to keep the temperature of the sample at less than -10°C.

7.8 Column chromatography - Clean up:

Prepare 22 mm i.d. Florisil column containing 10 cm of activated Florosil tapped with ca 1 cm anhydrous sodium sulfate. Wet column with 40-50 mL hexane. Use Kuderna-Danish (K-D) concentrator with volumetric or graduated tube or flash evaporator to collect eluate. Transfer petroleum ether or hexane solution of test sample extract to column, and let it elute at ca 5 mL/min. rinse container (and sodium sulfate. if present) with 2 ca 5 mL portions hexane, transfer rinsings to column, and rinse walls of chromatographic tube with additional small portions of hexane. Elute endosulfan I and II, endulfan sulfate, at ca 5 mL/min with 200 mL eluant I (3.5 mL acetonitrile into 500 dichloromethane and dilute with hexane to 1 L).

Concentrate eluate to suitable definite volume in K-D concentrator or in flash evaporator. For evaporation < 5 mL use 2 ball micro snyder or Vigruex column.

7.9 GLC determination:

Appropriate gas chromatography equipped with electron capture detector (H^3 or Ni^{63}). The suggested operating conditons are:

(1) Column made of borosilicate glass, 1.8 m long, 4 mm i.d. and packed with any of the material given below:

(1) 10 % DC-200 or DV-101 column on 80-100 mesh chromosorb WHP, operating conditions Tempertaure injector 225 °C, column 200°C, electron capture detector < 210 °C, carrier gas flow > 120 mL N 2/min.

(2) For good separation of chlorinated pesticides, a non-polar/fused silicated quartz capillary column and similar are recommended.

GC operating conditions for organochlorine compounds single-column analysis using narrow-bore columns and electron capture detector:

Column 1 - 30 m × 0.25 or 0.32 mm ID fused silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1µ film thickness.

Carrier gas	Helium
Carrier gas pressure	16 psi
Injector temperature	225°C
Detector temperature	300°C
Initial temperature	100°C, hold 2 min
Temperature program	100 °C to 160 °C at 15°C/min followed
Final temperature	by 160°C to 270°C at 5°C/min.

Column 2 - 30 m × 0.25 mm ID fused silica capillary column chemically bonded with 35

% phenyl methylpolysiloxane (DB-608, SPB-608, or equivalent), 25 µm coating thickness.

Carrier gas	Nitrogen
Carrier gas pressure	20 psi
Injector temperature	225 °C
Detector temperature	300 °C
Initial temperature	160 °C, hold 2 min
Temperature program	160 °C to 290 °C at 5 °C/min
Final temperature	290 °C, hold 1 min

Table 1 Gas chromatographic retention times for the organochlorine pesticides using narrow-bore capillary columns single-column method of analysis

Compound	**Retention time (min)**	
	DB608	**DB5**
Aldrin	14.51	14.70
α -BHC	11.43	10.94
β-BHC	12.59	11.51
γ -BHC	13.69	12.20
δ -BHC (Lindane)	12.46	11.71
α -Chlordane	17.34	17.02
4,4'-DDD	21.67	20.11
p,p'-DDE	19.09	18.30
p,p'-Dichlorodiphenyltrichloroethane	23.13	21.84
Dieldrin	19.67	18.74
Endosulfan 1	18.27	17.62
Endosulfan II	22.17	20.11
Endosulfan sulfate	24.45	21.84
Heptachlor	13.41	13.59
Heptachlor epoxide	16.62	16.05

7.10 Calculation: Calculate the residue levels using the following formula:

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract Concentration in (mL)}}{\text{Mass in g of the sample solution}} \times \text{ppm of reference}$$

7.11 Reference:

(i) Official Methods of Analysis. AOAC. 17th Edition, pp. 1-10.

(ii) Indian Standard 14628:1999

(iii) J. AOAC Int. (2005), 88 (2), 630-638

(iv) J. Sep. Sci. (2007), 30, 620-632.

7.12 Quantitative method for organo-chlorine Pesticides by GC-ECD/GC-MS/MS:

7.12.1 Sample preparation technique:

1. Take 2 kg sample
2. Homogenize the sample
3. Extract the samples with 10 mL of ethyl acetate, 10 gm of anhydrous sodium sulphate followed by centrifugation at 2000 rpm for 5 min.
4. Draw/Take 5 mL of an aliquot from the supernatant.
5. Clean up using 125 mg of PSA in Dispersive Solid Phase Extraction.
6. Place the cleaned extract in a 10 mL test tube.
7. Add 200 mL of 10 % diethylene glycol (in methanol) which will act as keeper as well as analyte protector.
8. Mix thoroughly using vortex mixer.
9. Put the mixture for evaporation to near dryness in a low volume concentrator using gentle stream of dry Nitrogen (at 35^{o}C).
10. Dissolve the residues in a mixture of 1mL of methanol and 1mL of 0.1 % acetic acid in water by sonication (1 min) followed by vortexing (30 s).
11. Centrifuge solution at 10,000 rpm for 5 min.

12. Filter the supernatant through 0.2 µm polyvinylidene fluoride (PVDF)/nylon membrane filters and then analyze by LC–MS/MS.
13. GC – Conditions: An ion trap, quadrupole, time-of-flight (TOF), or other GC/MS instrument may be used with electron impact (EI) ionization, an autosampler (AS), and computerized instrument control/data collection and has an AS. However, with reference to the compound of interest on matrix based refers to the method as has been published in Food Chemistry 125 (2011), pp. 803-812.

<u>GC/ECD condition (for organochlorine & pyrethroid)</u>

Column: Ultra2, 5 %Phenyl Methyl Siloxane

(25 m × 0.33µm × 0.2mm)

Carrier: He (Flow rate 0.8 mL/min)

Splitless Inlet 250°C

Oven temperature program

Initial 180°C,

Ramp 5°C /min to 210°C, hold 1 min

Ramp 2°C /min to 245°C, hold 0 min

Ramp 2°C /min to 280°C, hold 2 min

Post run 300°C hold 2 min

Run time 44 min*

Injection volume 2 µL

<u>GC/FPD condition (for Organophosphorus)</u>

Column: HP 5, 5 % Phenyl Methyl Siloxane

(30 m × 0.32 mm × 0.25 µm)

Carrier: He (1.0 mL/min)

Splitless Inlet 210°C

Oven temperature program

Initial 50°C,

Ramp 35 °C /min to 200 °C hold 5 min

Ramp 15 °C /min to 250˚C hold 6 min

Ramp 35 °C /min to 290˚C hold 10 min

Post run 300 °C hold 2 min

Detector: FPD 250 °C

H_2 flow 75.0 mL/min

N_2 Make up gas flow 60.0 mL/min

Air flow 100.0 mL/min

Injection volume 2 µL

NOTE: It is suggested to follow mass spectrometry for the determination of pesticide residues in foods at low level and to adopt the collaborative study for determination of pesticide residues in food as per the method published in Journal of AOAC International Vol.90, No. 2, (2007), pp. 485-520.

7.12.2 References;

1. J. Chromatogr. A, (2007), 1173, 98–109.
2. J. AOAC Int., (2008), 91(6), 1435–1445.
3. J. AOAC Int. (2010), 93 (2): 368-379.
4. J. AOAC Int. (2007), 90(2), 485-520.
5. J. Chromatogr. A, (2012),1270, 283-295
6. J. Chromatogr. A, (2010), 1217 (24), 3881-3889

8. MULTI RESIDUE METHOD FOR DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN FOOD AND AGRICULTURAL SAMPLES

8.1 Scope:

The method describes the simultaneous determination of Acephate, Chlorpyriphos, Chlorpyriphos-methyl, Demeton O, Diazinon, Dimethoate, Ethion, Fenitrothion, Malaoxon, Malathion, Methamidophos, Monocrotophos, Omethoate Paraoxon, Paraoxon-methyl, Parathion, parathion-methyl Phosalone, Pirimiphos-methyl residues in Food and Agricultural samples.

8.2 Apparatus and Reagents:

(a) A Gas chromatographs equipped with: flame photometric detector operated in phosphorus mode (FPD- P), 526 nm filter. Operating conditions: injector 220°C, detector 250°C; establish stable flame at electrometer setting that will produce 40 % full-scale deflection for 1ng parathion-methyl; baseline noise should be <2%. Columns (a) Suitable, please see references, 30 m × 0.53 mm id; hold at 140°C for 2 min, increase to 240°C at 5°C/min and hold 2 min; N_2 crrier gas, 15 mL/min; N_2 makeup, 15 mL/min (b) Glass, 2 m × 3 mm id, packed with 5 % OV-101 on 80-100 mesh (suitable, please see references)170 °C, increase to 250 °C at 5 °C/min and hold 2 min; N_2 carrier gas, 45 mL/min (c) Glass, 2 m × 3 mm id, packed with 1.5 % SP 2250 + 1.95 % SP 2401 on 100-120 mesh (suitable, please see references) , hold at 175°C for 2 min and increase to 240°C at 5°C/min and hold 10 min; N_2 carrier gas, 45 mL/ min (2) Gas chromatograph with NPD/TID detector operated with 30 m × 0.53 mm column (1.50 µm film thickness) and DL 210 (1.0 µm film thickness) or equivalent. Both connected to press fit Y-shaped inlet splitter. Temperature programme 120°C (3 min hold) to 270°C (10 min hold at 50°C/min), injector temperature 250°C, detector temperature 300°C, head temperature 400°C, bias voltage 4.0 hydrogen gas pressure 20 psi, helium carrier gas 20 mL/min is also recommended. (b) Chromatographic cleanup columns: (1) Ready to use extrelut-3, fix needle at column end as flow regulator. (2) Glass column 30 cm x 20 mm id with glass septum (Carbon-celite cleanup).

(b) Chromatographic cleanup columns: (1) Ready to use extrelut-3, fix needle at column end as flow regulator. (2) Glass column 30 cm × 20 mm id with glass septum (Carbon-celite cleanup).

(c) Chopper grinder

(d) A high speed blender

(e) Rotary vacuum evaporator

8.3 Reagents:

(1) Reference standard solutions

Prepare individual standard solutions in heptane (or n-hexane), adding benzene (ca 2 %) if solubilization is difficult, then prepare suitable dilution with heptane. Prepare standard cumulative solutions by mixing suitable volumes of individual standard solutions and diluting with heptane.

(2) Solvents—acetone, acetonitrile, benzene, dichloromethane, methanol, n- hexane, all HPLC grade.

(3) Silanized glass wool.

(4) Celite 545 - 0.020-0.045 mm

(5) Active carbon -

(6) Sodium chloride AR

(7) Cotton wool - washed with acetone and n-hexane

8.3.1 Pesticide standard solutions:

(A)Stock solutions 1 mg/mL

Weigh 10 mg of each organophosphorus insecticide reference standard and transfer into 10 mL individual volumetric flasks. Dissolve in ethyl acetate and fill the volume to 10 mL.

(B) Intermediate solution 10 µg/mL

Pipet 1 mL of each stock solution into individual 100 mL volumetric flasks. Dilute to volume with ethyl acetate.

(C) Working solution 0.5 µg/mL

Pipet 5 mL of each intermediate solution into a 100 mL volumetric flask and dilute to volume with ethyl acetate.

8.4 Extraction:

Foods are divided into 3 main groups according to moisture and fat content:

Group I Vegetables and fruits: Weigh 50 gm of chopped sample into high speed blender jar, add 100 mL acetone, blend 2 min at high speed. Filter with suction though Buchner funnel with glass septum waste residue with Ca 50 mL acetone. Bring extract to an exact volume (150-200 mL) with acetone-water (2+1).

Group II Milk: Fresh milk (500 mL) is placed in a 250 mL separatory funnel. Acetone (150 mL) is immediately added to the milk and the flask is manually shaken for 10 min. The entire contents were filtered through a Buchner funnel with filter paper in a 500 mL Erlanmyer flask. An additional 25 mL acetone is used to wash the filter cake in the Buchner tunnel and this is added to the filterate. The filterate is extracted first with 100mL methylene chloride and then with 50 mL of methylene chloride. The extract is dried over anhydrous sodium sulfate at 50-60°C, under reduced pressure.

Group III Grains (wheat, rice): Weigh 50 gm chopped sample into high speed blender jar and 50 mL distilled water and proceed for group I.

8.5 Partition:

For all groups place half the volume of food extract equivalent to 25 gm of sample (reserve the 2nd half) in a separatory funnel and add 100 mL of dichloromethane and 100 mL acetone and 10 gm of sodium chloride. Shake vigorously 1 min until most sodium chloride is dissolved. Allow layers to separate, transfer the aqueous layer to a second separatory funnel. Dry organic layer through sodium sulfate. Add 200 mL portion of dichloromethane to second seperatory funnel and shake vigorously 1 min each time and dry organic layer above. Rinse sodium sulfate with Ca 50 mL dichloromethane. Collect organic layers and washings and concentrate just to dryness in rotary evaporatory (40- 45°C) water bath reduced pressure.

8.6 Chromatographic column Clean up:

Group I and III

Fill glass column (2 cm id glass column) with 2 gm celite followed by 4 gm of carbon celite (1+4) and top with glass wool plug. Wash column with 20 mL benzene. Transfer sample quantitatively to column with small portions of benzene (Ca 2 mL) and elute pesticide with 60 mL of acetonitrile-benzene (1+1). Concentrate just to dryness in rotary evaporator (45- 50°C) water bath, reduced pressure). Add suitable benzene and analyze by GC.

Group II

Transfer sample quantitatively to disposable extrelut 3 mini column with Ca 3 mL n-hexane. Allow solution to drain into filling material. Wait 10 min to obtain even distribution then elute 3 times with 5 mL acetonitrile equilibrated with n-hexane. Add 4mL methanol to elute and concentrate to dryness in rotary evaporator of 50-55°C reduced pressure. Add suitable volume (1 mL) benzene and analyse by GC.

8.7 Determination:

Quantity residues by height or area measurement from solution of known concentration of the authentic compounds. Table 2 shows the retention time and table 3 shows the limit of detection of organophosphorus insecticides.

Table 2 GC retention times (min) or organophosphorus insecticides relative to parathion-methyl

COMPOUNDS	COLUMN		
	SPB-5[a]	OV-101[b]	SP2250-SP2401[C]
Acephate	0.26	----	0.97
Chlorpyriphos	1.18	1.30	0.82
Chlorpyriphos-methyl	0.99	1.00	0.60
Demeton-O	0.48	0.45	0.55
Diazinon	0.84	0.81	0.89
Dimethoate	0.69	0.64	1.69
Ethion	1.73	2.18	1.09
Fenitrothion	1.11	1.16	1.19
Malaoxon	1.03	1.04	1.09
Malathion	1.16	1.22	---
Methamidophos	0.09	---	1.12
Monocrotophos	0.64	0.66	0.9

Omethoate	0.48	0.52	1.21
Paraoxon	1.06	0.82	1.08
Paraoxon-methyl	0.85	0.84	1.16
Parathion	1.19	1.30	19
parathion-methyl	12.20 [e]	1[f]	2.45
Phosalone		2.90	0.92
Pirimiphos-methyl	1.11	1.58	0.97

[a]Fused silica, (suitable, please see references) 30 m × 0.53 mm id; hold at 140°C for 2 min, increase to 240 °C at 5 °C/min and hold 2 min; N_2 carrier gas, 15 mL/min or any other alternative columns can also be used provided standardization is done.

[b]Glass, 2m × 3 mm id, packed with 4 % OV-101 on 80-100 mesh Supelcoport; 170°C increase to 250°C at 5°C/min and hold to 2 min; N_2 carrier gas, 45 mL/min. [c] Glass, 2 m × 3 mm id, packed with 1.5 % SP2250 + 1.95 % SP2401 on 100-120 mesh Supelcoport; hold at 175 °C for 2 min, increase to 240°C at 5°C/min and hold 10 min; N_2 carrier gas, 45 mL/min. [d]compound is not revealed at these operating conditions.

NOTE: Capillary column in place of packed column may be used, provided the method is standardized using the changed column/s.

Table 3 Detection limits for organophosphorus insecticides

Compound	**Detection limit, mg**
Azinphos-ethyl	0.21
Chlorpyriphos	0.13
Chlorpyriphos-methyl	0.10
Demeton-O	0.07
Diazinon	0.13
Dimethoate	0.21
Ethion	0.14
Fenitrothion	0.18
Malaoxon	0.29
Malathion	0.18
Methidathion	0.17
Monocrotophos	0.55

Omethoate	0.10
Paraoxon	0.15
Paraoxon-methyl	0.21
Parathion	0.26
Parathion-methyl	0.12
Phosalone	0.31
Pirimiphos-methyl	0.11

8.8 Calculation:

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract Concentration in (mL)}}{\text{Mass in g of the sample solution}} \times \text{Concentration in ppm of reference standard}$$

8.9 References:

1. JAOAC, Vol. 75, No. 3, 511-518, 1992
2. JAOAC, Vol. 78, No. 3, 813-815, 1995
3. J. Chromatogr. A, (2007), 1173, 98–109
4. J. AOAC Int., (2008), 91(6), 1435–1445
5. J. AOAC Int. (2010), 93 (2): 368-379
6. J. AOAC Int. (2007), 90(2), 485-520
7. J. Chromatogr. A, (2012),1270, 283-295
8. J. Chromatogr. A, (2010), 1217 (24), 3881-3889
9. J. AOAC Int. (2005), 88 (2), 630-638

9. MULTI RESIDUE GAS CHROMATOGRAPHY METHOD FOR SYNTHETIC PYRETHROID IN FOOD COMMODITIES

The analytical method describes the determination by GLC of bifenthrin, fenpropathrin, cyhalothrin, permethrin, cypermethrin, fluvalinate, fenvalerate, and deltamethrin in food commodities.

9.1 Principle:

Fruits and vegetables are extracted with acetone, and grains are extracted with acetonitrile-water. Analytes are partitioned into hexane, evaporated to dryness, and dissolved in hexane. The extract is partitioned with acetonitrile and cleaned up on a deactivated Florisil column with 6 % ethyl ether in hexane. Analyte concentrations are determined by gas chromatography with electron capture detection (GC-ECD) and comparison with calibration standards.

9.2 Apparatus and Reagents:

Gas chromatograph - Fitted with autosampler and operating conditions : injection port temperature, 180°C; ECD temperature; 300°C; He carrier gas, 29 cm/s; make-up gas 30mL/min; column temperature programme 50 °C for 1 min, to 205°C at 30°C/min, to 240°C at 1°C/min, hold 2 min at 240°C; splitless injection, opening plitter 0.8 min after injection; split vent flow, 22 mL/min; purge flow, 9 mL/min. Difference in detector response between 2 successive injections of the same standard solution should not exceed 6 %.

GC column: 30 m × 0.25 mm id, 0.10 µm film thickness (DB-5, 5 % phenylmethylpolysiloxane, J&W scientific; USA or equivalent provided standardization is done).

Solvents - Acetone, heptane or n-hexane, acetonitrile, and ethyl ether; HPLC grade, or redistill in all-glass apparatus and check for interferences by GC-ECD (a).

Sodium suflfate, anhydrous - Heat at 650°C for 4 hr. Cool in desiccator. Eluting solvent - 6 % ethyl ether in hexane. Mix 60 mL ethyl ether with 940 mL hexane.

(i) Insecticide standards - Purity, > 90 %. Prepare individual standard stock solutions in hexane at 10.0 mg/100 mL (deltamethrin, cypermethrin, 250 µg/mL) and 50 mg/mL (fenpropathrin, permethrin, fenvalerate, and fluvalinate, 500 µg /mL. Prepare a mixed standard solution for determining the Florisil elution pattern by pipetting 5 mL of each of bifenthrin and cyhalothrin, 10mL of each deltamethrin, cypermethrin, fenopropathrin,

permethrin, fenvalerate, and fluvalinate individual standard stock solutions into 100 mL volumetric flask and diluting to volume with hexane.

(ii) Deactivated Florisil - 60-100 mesh, pesticide grade. Activate at 650°C 4hr in muffle furnace. Transfer to oven at 130°C and let stand 5 hr. Store in glass-stoppered bottles or in air-tight desiccator and let cool overnight. Deactivate Florisil by carefully adding 5 % (w/w) distilled water. Shake 1 hr on mechanical shaker and let stand overnight. Store in sealed container at room temperature. Deactivated Florisil is stable for up to 7 days.

(iii) Acetonitrile saturated with hexane - Add 300 mL acetonitrile and 100 mL hexane to 500 mL separatory funnel. Shake vigorously, with frequent venting, 2 min. Let layers separate. Drain acetonitrile layer into a storage bottle.

9.3 Determination:

(1) Florisil elution pattern - Place small plug of glass wool at bottom of 400 x 22 mm id glass column. Add 1 cm layer of anhydrous Sodium sulfate. Add ca 50 mL hexane to column, then add10 gm deactivated Florisil, and tap sides of column for even packing. Top with l cm layer of anhydrous Sodium sulfate.

Prewash column with ca 50 mL hexane. Do not let column dry until elution is complete. Place 1.0 mL of the mixed standard solution, on the Florisil column and elute the synthetic pyrethroids with 6 % eluting solvent. Inject 1 µL aliquots onto GC, and determine the recovery of each insecticide. A recovery of ca 95 % of fluvalinate is expected which depends upon the activity of the Florisil and volume of eluting solvent, which may range from 130 to 200 mL. Adjust volume to achieve ca 95 % fluvalinate recovery.

(2) For extraction from high-moisture (> 75 %) products, weigh 50.0 gm (to nearest 0.1 gm) chopped product into homogenizer jar; add 120 mL acetone; and homogenize 3 min at 18,000 rpm. Suction filter through 12 cm Buchner funnel with filter paper into 500 mL suction flask. Rinse homogenizer with two 25 mL portions acetone and use rinses to wash residues in Buchner funnel. Transfer filtrate to 500 mL separatory funnel. Wash suction flask with two 10 mL portions of acetone, adding washes to separatory funnel.

For dry or low-moisture products such as grains, weigh 20.0 gm (to nearest 0.1 gm) into homogenizer jar, add 150 mL acetonitrile: water (2:1), in place of acetone.

(3) Add 60 mL hexane to separatory funnel containing extract. Shake vigorously, with frequent venting, 5 min. Add 200 mL 4.0 % aqueous sodium chloride (w/v) and mix vigorously ca 30s. Let layers separate and discard aqueous layer. Pass hexane layer through glass funnel containing glass wool plug and ca 15 gm sodium sulfate, collecting extract in 250 mL round-bottom flask. Rinse separatory funnel with two 20 mL portions of hexane and pass rinses through glass wool into round-bottom flask

(4) Evaporate contents of round-bottom flask to dryness on rotary evaporator, at 40°C. Redissolve residue in 10 mL hexane and transfer to 125 mL separatory funnel, rinse round-bottom flask with two 5 mL portions hexane and add rinse to separatory funnel. Add 30 mL acetonitrile-saturated hexane, and shake vigorously, with frequent venting, 5min. Let layers separate, and drain acetonitrile phase into 250 mL round-bottom flask. Add 30 mL acetonitrile saturated hexane to hexane phase in separatory funnel and shake vigorously with fequent venting, 5 min. Let phases separate and drain acetonitrile layer into same 250 mL round-bottom flask. Repeat 30 mL acetonitrile extraction and collect acetonitrile (Note Accurately measure volumes of acetronitrile and hexane for optimal results). Evaporate acetonitrile extract to dryness on rotary evaporator at 60°C. Dissolve residue in 5 mL hexane. (Note: Ensure acetonitrile is evaporated completely to dryness).

(5) Transfer extracts to column and let level fall until just above Florisil packing, rinse the 250 mL round-bottom flask with two 10 mL portions of hexane, add each rinse to column, and let run through column. Elute pyrethroid residues with same volume of 6 % eluting solvent, used in Florisil elution standardization C (l), collecting eluate at 3 mL/min in 250 mL round-bottom flask. Evaporate eluate to less than <50 mL on rotary evaporator at 40°C and transfer to 50 mL volumetric flask. Dilute to volume with hexane so that the final concentration is 1.0 g/mL for fruits and vegetables or 0.4 g/mL for grains.

(6) Tentatively identify residue peaks by comparing retention times from extract to those of standards solution. According Ca concentration of pyrethroids in extracts, select the standard solution with peak heights similar to those of the extracts. Inject a standard solution with peak height ± 25 % of analyte peak height, dilute and reanalyze if analyte concentration exceeds 500 g/mL.

Calculate amounts of each pyrethrin in test extract with the following equation:

$$\text{Residue mg/kg} = C_{std} \times (H_{Ex}/H_{std}) \times (V_{std} \times V_{Ex}) \times (D/W)$$

Where C_{std} = standard concentration (µg/mL), H_{Ex} = peak height in extract, H_{std} = peak height in standard, V_{std} = standard volume injected (µL), V_{Ex} = extract volume injected (µL), D = dilution volume (mL), and W = test portion weight (gm).

9.4 References:

1. Official Methods of Analysis.17th Edition.998.0, pp. 65-67 (2000)
2. GC-MS and LC/MS-MS (collaborative study) may be used for synthetic pyrethroids. Ref: JAOAC International vol. 90 No.2, (2007), pp.485-520
3. J. AOAC Int. (2010), 93 (2), 368-379
4. J. Chromatogr. A (2008), 1190 (1), 350-357
5. J. Chromatogr. A, (2012),1270, 283-295
6. J. Chromatogr. A, (2010), 1217 (24), 3881-3889
7. J. Agric. Food Chem., (2015), 63 (18), 4449–4456

10. MULTI RESIDUE METHOD FOR THE DETERMINATION OF N-METHYLCARBAMATE INSECTICIDES IN FOOD COMMODITIES

The analytical method prescribes gas liquid chromatographic method for determination of carbaryl and carbofuron in food commodities.

10.1 Principle:

Residue is extracted from crop with dichloromethane, and extract is purified by partitioning with petroleum ether and coagulating with phosphoric acid-Ammonium chloride solution. Phenolic impurities are largely eliminated by partitioning dichloromethane extract with potassium hrydroxide solution. Carbamate residues are treated with 1-fluoro-2, 4-dinitrobenzene to form ether derivative. Residues may be determined at levels > 0.05 ppm (µg/g). Recoveries range from 90 to 110 %.

10.2 Reagents:

(a) Borax – 5 % aqueous solution (b) Diatomaceous earth - Wash thoroughly with acetone and dry 2 hr at 110°C.

(c) Coagulating solution - (1) Stock solution - Dissolve 20 gm NH_4CI and 40 mL phosphoric acid in 360 mL H_2O. (2) Working solution - Dilute 100 mL stock solution to 1 L for coagulation.

(d) 1-fluoro-2,4-dinitrobenzene solution - Redistill at 128°C and 1 mm pressure. Dissolve 1.5 mL in 25 mL acetone.

(e) Pesticides - Best quality obtainable from manufacturer; analytical grades when available.

(f) potassium hrydroxide solution - 0.5 M aqueous solution.

(g) Sodium chloride solution – 20 % aqueous solution.

(h) Solvents - Acetone dichloromethane, isooctane, and acetonitrile and petroleum ether (distilled in glass; see statement regarding solvents; acetophenone and methanol (analytical grade).

10.3 Gas Chromatographic Apparatus:

A gas chromatograph equiped with electron capture detector and 46 × 0.643 (o.d.) cm (18 x 1/4 in)glass column containing 10 % DC 200 (12,500 cst) on 60-70 mesh. Porous Teflon end plugs for 1/4 in (0.64 mm) o.d. glass tubing, but glass wool can be used at

outlet and omitted at inlet if necessary. (Glass wool at inlet tends to absorb derivatives gradually and to release them later, giving rise to ghost images of compounds).

Equilibrate column 2 days at 250°C and 2 weeks at 212°C. Operating conditions: temperatures – column 212 °C detector 218 °C, standby temperatures 190 °C and 200°C, respectively; N_2 carrier gas 60mL/min; sensitivity 1 x 10 A full 29 scale; and detector potential either 25 or 50 V depending on response level needed (1/3 to 2/3 full scale peak height with injections of 4 ng carbamate)

Alernatively, use instrument with ^{63}Ni detector and 1.8 m (6 ft × 4 mm id glass column containing 10 % DC-200 on 60-70 mesh. Do not use glass wool at beginning of column. Operating conditions temperatures -column 232°C, detector 250°C N_2 carrier gas 80mL/min, sensitivityl x $1Q^{-9}$ A full scale, and detector potential 50 or 75 V.

Note: Other alternative equivalent capillary columns can also be used, provided standardization is done.

10.4 Extraction of pesticides:

Place 100 gm test portion and 200 mL acetonitrile (add 50 mL dry ice/ H_2O/ with fruit or other test samples containing 5-15 % sugar) in square screw-top jar, and macerate in blender operated 2 min at moderate speed. Filter solution with suction into 500 mL round-bottom flask through rapid paper in 11 cm Buchner. Transfer aliquot equivalent to 40 gm crop (mL aliquot = mL H_2O intest portion + mL acetonitrile added + mL H_2O added - 5 mL volume contraction) x 40/100) to 250mL separator. Shake 10 s with 25 mL Sodium chloride solution B (g). Drain and discard aqueous phase. Repeat with fresh Sodium chloride solution B (g). Add 100 mL petroleum ether, and shake 30s. Drain acetonitrile into 1 L separator. Stripe petroleum ether by shaking 20 s with 50 and 10mL portions acetonitrile draining each into the 1 L separator. Add 300 mL H_2O, 25 mL Sodium chloride solution, B (g) and 50 mL methanol. Extract mixture with 100 mL and two 25 mL portions dichloromethane, shaking each 20 s, and drain lower layer into 500mL round-bottom flask. Add 2 drops acetophenone, and evaporate in rotary evaporator connected to aspirator pump. Druing evaporation, keep H_2O bath within 40-50°C range and remove flask from H_2O bath when extract volume has been reduced to 2 mL, so that final evaporation to dryness takes place at low temperature.

Add 5 mL acetone, and swirl flask to dissolve residue. Add 50 mL coagulating solution, swirl to mix, add 1-2 gm diatomaceous earth and swirl again to mix. Pour solution into150 mL suction filter of medium porosity packed with 6 mm (17.4 in) diatomaceous earth, and collect filtrate in 500 mL round-bottom flask. Break vacuum immediately after liquid is drawn into diatomaceous earth layer. Rinse sides of flask with 5 mL acetone, swirl and repeat coagulation. Rinse flask with 20 mL coagulating solution, and add rinse to filter just after liquid of second coagulation is drawn into diatomaceous earth layer. After filtration is complete (ca 5 min), transfer filtrate to 250 mL separator. Extract carbamates by shaking 20s with three 25 mL portions dichloromethane, rinsing filter flask with each portion before adding to separator. Drain dichloromethane (lower) extract into another 250 mL separator. Solution may be held overnight at this point. Add 40 mL H_2O and 10 mL 0.5 M potassium hrydroxide mix briefly by gentle swirling, and shake 20 s. Drain dichloromethane through granular anhydrous Sodium sulfate supported by glass wool in filter funnel and collect filtrate in 250 mL Erlenmeyer. Add 10 mL dichloromethane to separator, swirl gently, and drain organic phase. Repeat once, Rinse filter with two 10 mL portions dichloromethane.

Add 2 drops acetophenone, and evaporate with same technique used in first evaporation in previous paragraph.

10.5 Determination:

Add 100 mL H_2O, 2 mL 0.5 M potassium hrydroxide, and 1 mL 1-fluoro-2, 4-dinitrobenzene 2 solution. Stopper and mix 20 min. at high speed on mechanical agitator. Add 10 mL 5 % borax, swirl to mix and heat on steam bath 20 min. Cool to room temperature by placing flasks in shallow water bath for 10 min. Add 5 mL isooctane, stopper, shake 32 min at high speed, and pour into 250 mL separator. Drain aqueous phase, and rinse twice with H_2O. Drain isooctane solution through funnel contaiing 6 mm glass wool plug into glass-stoppered test tube. Solution may be held overnight at this point. Inject 10 µL extrct into gas chromatograph. If necessary to dilute extract, transfer 1 mL of isooctane extract to another test tube, dilute to exact volume with isooctane, and shake to mix. Chromatograph standard solutions and extracts solutions at approximately same level of response. Methylcarbamates (carbaryl/carbofuron) (µg/g) =

10.6 Reference:

$$\text{Methylcarbmates Carbaryl} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract}}{\text{Mass in g of the sample solution}} \times \frac{\text{Concentration in (mL)}}{\text{Concentration in ppm of reference standard}}$$

Official Methods of Analysis. AOAC, 17th Edition, 985.23 (2000).

Or

LC-MS may also be used for determination of carbamates at low level in foods as per the method published in journal of AOAC International Vol. 90, No. 2, (2007), pp. 485-520.

NOTE: FOR "OC, OP, CARBAMATES" IT IS SUGGESTED TO FOLLOW THE METHOD AS PER SECTION (AOAC 970.52, AOAC 985.23 AND JOURNAL OF AOAC INTERNATIONAL VOLUME 90, NO. 2, 2007; PAGE485-520

1. J. Chromatogr. A, (2007), 1173, 98–109
2. J. AOAC Int., (2008), 91(6), 1435–1445
3. J. Agric. Food Chem., 2015, 63 (18), 4449–4456

11. MULTI RESIDUE METHOD FOR FUMIGANTS

11.1 Principle:

This method is applicable to ethylene dibromide, carbon tetrachloride and methyl bromide. Repeated determinations are advised to average out for the inability of having a good composite. Extra care must be taken to avoid lots of acetone with these impurities present. All these fumigants may be detected by this method with some modification of conditions.

11.2 Apparatus:

Gas chromatograph - With isothermal conditions and electron capture detector. The oven temperature may require the oven door be left open to achieve the lowest possible temperature.

Chart recorder - 1 mV, with a fast chart speed and fast response time. Column - 3 or 4meter ×2 mm glass column packed with varied coating.

Examples: 15 % polypropylene glycol (suitable, please see references).

11.3 Reagents:

Acetone - High grade, checking each lot and bottle or interfering peaks, in window of interest, on the gas chromatography detector combination used.

Standard Fumigants - Weigh amount of fumigant, layered under the surface of acetone into a small volumetric flask. Take the weighed difference. Take care to keep all standards in screw capped containers in freezer or refrigerator between standard use. Dilute final dilutions at time of analysis.

11.4 Procedure:

Store sample at less than 5°C. Quickly weigh 50 gm of sample and immerse in 150 mL acetone-water (5+1), in a 250 mL g/s flask, and stopper. Let stand 48 hr in dark at 20°C - 25°C, swirling at 24 hr. Decant 10 mL supernate into 25 mL g/s graduate, add 2 gm Sodium chloride, stopper and shake vigorously 2 min. Let stand until layers separate. Pour 5 mL clear upper layer into 10 mL g/s graduate; add 1 gm anhydrous Calcium chloride, stopper, and shake 2 min. Let stand 30 min, with occasional shaking.

Withdraw 0.5 µL aliquots from upper layer into 1 µL syringe. Inject into a gas chromatograsph. Inject all solutions in triplicate and average results.

Construct a calibration curve daily of peak heights against ng fumigant.

Reference:

Official Methods of Analysis, AOAC, 15th Edition, 977.18 A, B (1990)

11.5 Phosphine

A GLC method has been described for determining phosphine (hydrogen phosphide) in wheat.

11.5.1 Apparatus:

(a) Gas chromatograph equipped with flame photometric detector and 526 nm phosphorus filter, integrator and pen recorder. Operating conditions temperature (°C)- detector 160, inlet 135, column 70, gas flow (mL/min) carrier gas nitrogen 40, hydrogen 210 air 42, oxygen 20, sensitivity 1x108 amu full scale deflection.

(b) GLC column-6×35 mm id glass tube packed with 30 % OV 101 on 80-100 neck gas chrom Q (Other alternative equivalent capillary columns can also be used, provided standardization is done)

(c) Reaction flask 300mL boiling. 3- Neck, angle type with 24/40 centre joint and 19/38 side joints.

(d) Funnels i) 125 mL separator. Teflon stopcock with 20/40 ground glass inner joint.

(e) Condenser 20 cm jacket with 20/40 ground glass joints.

(f) Gas collection 1 ltr. Flask-volume 1 ltr. Collaborated round bottom, 2- neck with 34/45 ground glass outer joint in centre and 24/40 outer joint on side neck. To centre joint connect straight reducing, brushing-type adopter with34/45 outer and 24/40 inner joint.

(g) Syringe sampling adopter-Straight reducing, bushing type with 21/40 outer and 10/30 inner joints fitted with 9.5mm. Analabs white silicone rubber septa by pressing them one at a time into sleeve with piece of ¼" wood dwelling, leaving 1/8" gap between septa.

(h) Connecting adapter- Glass 24/40, angle-type adapter, with glass stopcock and hose connection arm.

(i) Syringes- (suitable, please see references), gas liquid- tight 50,100 and 1000µL

11.5.2 Reagents:

(a) Sulphuric acid: Reagent grade, concentrated 10 % in water.

(b) Aluminum Phosphide tablets containing 70 % aluminum phosphide 26 % ammonium carbamate, 4 % edible paraffin.

(c) Phosphaphine: Compressed gas 99.5 % hydrogen phosphide.

(d) Preparation of phosphine standards: Working standards for gas chromatography are prepared by nitrogen dilution of 99.5 % phosphine. Bubble phosphine into water filled glass tube fitted with a syringe sampling adapter. Transfer 1000, 100 and 10 µL aliquots of headspace gas to volume calibrated 1 L flask previously flushed with nitrogen and fitted with syringe sampling adapters.

The concentration of phosphine in the standards will be C, pg/µL.

$$C = DT/V$$

Where,

D = density of phosphine (g/L) in sampling tube, connected for temperature, pressure and purity,

T = µL phosphine transferred to 1 L flask;

V = Calibrated volume 1L flask.

11.5.3 Extraction apparatus:

The apparatus is assembled in 2 parts: a reaction unit and a gas collection unit. Apply a thin film of silicone grease (Dow corning) to all joint and stop cocks. Using enlarging adapters, connect separating funnel and condenser to opposite side necks on reaction flask. Stopper center joint and attach connecting type adapter to top joint on condenser. The gas collection flask is close to air by attaching syringe sampling adapter to side neck and connecting type adapter to centre neck. With vacuum line attached to vacuum gauge reads 27" (685mm) mercury below atmospheric pressure, close stopcock and vacuum line.

11.5.4 Grain sampling procedure:

Starting to 100 - 1000 gm wheat, weigh sampling, then divide on grain sample divider to obtain 50-100 gm sub-sample. Accurately weigh resulting sub sample and transfer to reaction flask using powder funnel with joint. Stopper flask and save for extraction. To prevent loss of intact physically bound phosphine, sample must be sub sampled immediately upon receipt.

11.5.5 Extraction:

Assemble reaction unit over heating mental with all stop cocks closed. Connect evacuated gas collection unit to reaction unit by joining balls and socket joints on connecting adapters. Add 100 mL 10 % sulphuric acid to separatory funnel opens stop cocks on connecting adapters to create partial vacuum in reaction unit. Open stop cock on separatory funnel and carefully drop acid into reaction flask; close stop cock just before liquid level enters stop cocks. Turn on condenser water and heating mental and let mixture come to boil. After 20 min from cold, switch off heating mental and remove from under reaction flask. Slowly open stop cock on separator funnel and let air purge fumes from reaction flask into gas collection flask. Purge operation lasts early 10-15 sec during which a hissing noise is heard. When hissing noise stops close all stop cocks and disconnects gas collection unit from reaction unit.

Experiments have shown that gaseous phosphine trapped in gas collection flask for several hr without significant loss of phosphine. Gradual escape of phosphine by diffusion through rubber septum avoidable way, but rate of loss is very slow. However, use of crimp top vials or 2 septa in syringe sampling adapter is suggested which will not affect analytical results.

Samples containing high concentration of phosphine may present explosion hazards. Two precaution should be taken when dealing with samples with strong phosphine odors as guard against accidental explosion in extraction apparatus: Reaction unit should be purge with nitrogen before adding sample, and system should be purge with nitrogen instead of air after refluxing is complete.

11.6.6 Gas Liquid Chromatography:

Using gas tight syringes, inject 10-100 µL gaseous sample into gas chromatograph. Before use, however, prime syringe with water by wetting plunger tip and inserting into

syringe barrel. Expel excess water from syringe before proceeding with sampling. Flush syringe barrel with sample at least 10 times before final fill; then overfill and adjust volume for screening of unknown, inject 50 µL gaseous samples. Proceed from syringe filling to injection with minimum delay. With samples showing positive for phosphine, repeat injections two more tomes. Calculate mean area for triplicate injection and quantitate by comprehension to standard curve. If peak area response for given sample weightily exceeds calibrated linearity range of detector, dilute sample by transferring aliquot to another flask.

Calculation: Phosphine concentratoni in sample is calculated as follows:

Phosphine, ppm (u) = WFD/MS

Phosphine, ppm (c) = ppm (u) [100/Y]

Where (u) singnifies uncorrected recovery; (c) signified corrected recover; W= mean weight of phosphine (pg) in injection aliquot; F= volume of purge collection flask (L); D= dilutin factor; M= injection volume (µL); S= sample weight (g); Y= recovery as extrapolated from recovery curve.

Note: Experiments have shown that gaseous phosphine trapped in gas collection flasks for several hr without any significant loss of phosphine. Gradual escape of phosphine by diffusion using rubber septum /septa is one of the options, wherein the rate of loss is very slow. However, the trapping procedure may further be extended using crimp top vials or 2 septa in syringe sampling adapter using n-heptane as solvent to avoid the risk associated with loss of phosphine and further will not affect analytical results or the personnel doing the experiments.

Reference

J ASSOC OFF. ANAL CHEM. Vol.61 No 4, (1978). pp. 829.836

11.5.7 Headspace Conditions:

1.0-mL sample loop;

90°C sample equilibration temperature;

15-min sample equilibration time;

0.5-min mixing time (at power 10);

110-kPa vial pressurization pressure;

and 0.5-min injection time.

After the sample was introduced into the GC column, split the column flow into two flows before entering the detectors by using a Y-shaped quartz connector. One flow will be introduced into an electron capture detector (ECD) for the detection of compounds of interest and the other into a nitrogen and phosphorus detector (NPD) for the detection of MITC.

HS-GC analysis using an automated headspace sampler,

Increase the temperature (by 5°C) from 70 to 95°C for the soil samples and from 60 to 95°C for the water samples. The temperature that gives the greatest GC response should be selected as the optimum sample equilibration temperature.

Similarly determine the optimum equilibration by varying the sample heating cycle for intervals of 5, 10, 15, 20, 30, and 45 min.

11.5.8 GC conditions:

Capillary column (30 m × 0.25 mm × 1.4 μm);

240°C inlet temperature;

300°C detector temperature for both detectors;

80°C isothermal oven temperature;

and 1.1 mL/min column flow rate.

The salt that produces the greatest GC responses in area will be selected as the best salt type for modifying the matrix. The optimum concentration and volume of the salt solution will be determinde using the selected salt.

The optimum value will be defined as the condition that resulted in the greatest and/or the most reproducible GC responses.

Reference:

J. Agric. Food Chem. 46, (1998), pp. 986-990

http://www.crl-pesticides.eu/userfiles/file/EurlSRM/EurlSRM_meth_Phosphin.pdf

12. THIAMETHOXAM ANALYSIS IN PLANT MATERIAL

12.1 Principle of the method:

The plant material is extracted with a mixture of water/methanol (8+2 vol. + vol.). An aliquot of the filtered extract is diluted with water and cleaned-up on a pheyl solid-phase cartridge and an (suitable, please see references) graphiticed nonporous carbon cartridge. The organic solvent part of the eluate from the clean-up is evaporated and the concentrated solution diluted with water. The analyte is determined on a two-column reversed phase HPLC system with UV-detector at 255 nm.

12.2 Equipment:

- Sample concentrator
- Laboratory wrist action shaker
- High speed homogenizer
- Vacuum manifold for solid phase extraction clean-up

12.3 Reagents:

- Ethanol, analytical grade
- Methanol analytical grade
- Methanol, HPLC grade
- Tetrahydrofurane HPLC grade
- Water; HPLC grade
- Celite 535 filter aid,
- Bond Elute Phenyl cartridge, 500 mg/3 mL

- (suitable, please see references) graphitized nonporous carbon cartridge, 250 mg/3mL
- Reference substance for standardization. Prepare a stock solution of
- Thiomethoxam (200 µg/mL) in ethanol.

12.4 Extraction:

Shake the plant material with 50 mL of a mixture of water/methanol (8+2 vol. + vol) for 1 hr at about 260 rpm or homogenize int he same amount of the same solvent mixture with a high speed blender at about 9000 rpm for 2 min. Filter the homogenized material through a Buchner funnel into an Erlenmeyer flask using Celite filter aid. Due to the high water content of the extracts, the filtrates easily foam if the vacuum used in the filtration apparatus is too strong. Wash the filter cake and extraction jar with the extraction solvent.
Transfer the filtrate into a 100 mL volumetric flask and bring to volume with the solvent mixture.

12.5 Clean up by solid phase extraction:

Take an aliquot of 5 mL (edible parts) or 2 mL (non-edible parts) of the filtered extract and dilute with the same volume of water (5 mL or 2 mL). Condition a phenyl solid-phase extraction cartridge with 3 mL methanol and 3 mL water. Percolate the diluted filtrate through the cartridge and discard the eluate. Wash the cartridge with 2 mL of water and discard the eluate.

Condition a (suitable, please see references) cartridge with 3 mL tetrahydrofurane, 3 mL methanol, and 3 mL water. Attach the phenyl cartridge to the top of the ENVI-Carb cartridge with an adapter. Elute the analyte from the upper onto the lower cartridge with 3 mL water/methanol (1+1 vol + vol). Remove the phenyl cartridge and discard it.

Elute the analyte from the (suitable, please see references) cartridge with 3 mL water/ tetrahydrofurane (8 + 2 vol + vol). Evaporate the elute down to about 2 mL on the sample concentrator and bring to a volume of 2.5 mL with water. Use this solution for HPLC.

12.6 Instrumentation:

12.6.1 High performance Liquid Chromatographic system:

For determination of thiomethoxam use a HPLC two-column switching system with UV-detector, Pump, and autosampler-injector as follows (or with suitable equivalents).

Detector : UV/VIS detector 783 Pumps : Dual piston pump model Autosampler : Automatic sampling system

Recorder: Dual channel recorder SE 120 (suitable, please see references)sensitivity set to 10 mV full scale; chart speed: 0.5 cm/min.

Switching valve: C6W electrically actuated (suitable, please see references)for a scheme of the column switching set up

Column 1: 125 m x 2 mm i.d., packing material Nucleosil C18, particle size 5 m (suitable, please see references)

Column 2: 125 mm x 2 mm i.d. packing material Nucleosil 100 phenyl, particle size 7 m (suitable, please see references)

Mobile phase 1: Water/methanol (8+2 vol + vol) at 0.25 mL/min

Mobile phase 2: Water/methanol (7+3 vol + vol) at 0.25 mL/min

Mobile phase compositions given are approxiamte and can be modified depending on interferences from specific series of specimens.

Detector wave length: 255 nm

Detector sensiivity: 0.003 aufs

Volume injector: 50 µL

Retention time - ~7 min on column 1 and ~7 min on column 2 (depending on conditions chosen).

12.7 Calculation:

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract Concentration in (mL)}}{\text{Mass in g of the sample solution}} \times \text{Concentration in ppm of reference standard}$$

Reference:

1. CIBA Giegy Limited Agrochemical Division.
2. Pesticide Research Journal, 17 (1), (2005). pp 46-50.

12.8 Simultaneous Determination of Fungicides and Herbicides

(viz., Acetamiprid, Atrazine, Cymoxanil, Imidacloprid, Isoprothiolane, Metalaxyl, Propiconazole, Simazine, Thiamethoxam, Thiodicarb, Triadimefon, Triadimenol by using LC-MS/MS in non fatty food matrices).

12.8.1 Scope:

This is a single method which describes simultaneous determination of Acetamiprid, Cymoxanil, Imidacloprid, Isoprothiolane, Metalaxyl, Propiconazole, Atrazine, Simazine, Thiamethoxam, Thiodicarb, Triademifon, and Triadiminol.

12.8.2 Reagents/Chemicals:

- Certified reference standards (>98 % purity)
- HPLC grade solvents.
- Ethyl acetate
- Acetonitrile
- Methanol and water

- Primary secondary amine (suitable, please see references)sorbent
- Anhydrous sodium sulphate (dried)
- Magnesium sulphate (dried)

12.8.3 Apparatus:

- HPLC system Q-Trap mass spectrometer/Triple Quad
- A high-speed homogenizer
- Low-volume concentrator, Non-refrigerated centrifuge

12.8.4 Preparation of Standard Solutions:

Prepare the standard stock solutions by accurately weighing 10 mg (±0.01 mg) of each analyte of interest in 10 mL volumetric flasks and dissolve using 10mL methanol.

Prepare the stock standard mixture of 10 mg/L by mixing the appropriate quantities of the individual stock solutions followed by requisite volume make up.

12.8.5 Sample Preparation Technique:

1. Take 2kg sample
2. Homogenize the sample
3. Extract the samples with 10mL of ethyl acetate, 10gm of anhydrous sodium sulphate followed by centrifugation at 2000 rpm for 5 min.
4. Draw/Take 5mL of an aliquot from the supernatant.
5. Clean up using 125 mg of PSA in Dispersive Solid Phase Extraction.
6. Place the cleaned extract in a 10mL test tube.
7. Add 200mL of 10 % diethylene glycol (in methanol) which will act as keeper as well as analyte

8. Protector. Mix thoroughly using vortex mixer.
9. Put the mixture for evaporation to near dryness in a low volume concentrator using gentle stream of dry Nicogen (at 35°C). Dissolve the residues in a mixture of 1mL of methanol and 1mL of 0.1 % acetic acid in water by sonication (1 min) followed by vortexing (30 s).
10. Centrifuged solution at 10,000 rpm for 5 min.
11. Filter the supernatant through 0.2 µm polyvinylidene fluoride (PVDF) membrane filters and then analyzed by LC–MS/MS.

12.8.5 LC-MS/MS conditions:

- A triple quadrupole, ion trap, or other LC/MS/MS instrument may be used provided it is capable of electrospray ionization (ESI) in the positive mode with computerized instrument control/data collection and has an AS.
- An injection volume (5–10 µL) will be determined for each instrument to achieve S/N > 10 for the quantitation ion for a 10 ng/g equivalent sample concentration

12.8.5.1Suggested LC conditions:

- A 15 cm long, 3.0 mm id, 3 mm particle size C18 column, flow rate of 0.3 mL/min, and gradient elution with an initial condition of 15 % Methanol in 5 mM ammonium formate solution (takes linearly in 15 min to 90 % Methanol in 5 mM ammonium formate solution and hold for 15 min).
- A short C18 guard column must be used to protect the analytical column, and a bypass valve must be used before the MS instrument to avoid introduction of the early and late eluting matrix components into the detector.
- The MS/MS conditions shall be optimized using direct infusion into the ESI source to provide highest S/N for the quantitation ion of each LC-type analyte from a single MS/MS transition. A second transition with reasonably matching relative abundance ratios vs a contemporaneously analyzed reference standard is needed for qualitative purposes.

NOTE: This method can be used as an alternative method to several other individual methods described in the manual wherever it is applicable.

12.8.6 Reference:

1. Journal of Chromatography A, 1173 (2007), pp. 98–109.
2. J. Chromatogr. A, (2007), 1173, 98–109.
3. J. AOAC Int., (2008), 91(6), 1435–1445.
4. J. Agric. Food Chem., 2015, 63 (18), 4449–4456

13. THIODICARB/METHOMYL ANALYSIS IN PLANT MATERIAL

13.1 Principle:

Thiodicarb residues consist of thiodicarb, dimethyl-N,N [thiobis [(methylimino) carbonyloxy]] bis (ethanimidothioate) and its degradation products methomyl and methomyl oxime cumulatively measured as the oxime.

The residues are extracted with a mixture of 9:1 acetone: water. A standard coagulation procedure is used to remove interfering coextractives. Alkaline hydrolysis converts thiodicarb and methomyl to methomyl oxime. The oxime is quantified by gas chromatography utilizing a flame photometric detector selective for sulfur containing compounds.

The method sensitivity is 0.04 ppm for a 25 gm sample. The average recoveries are 89 % for thiodicarb and 93 % for methomyl at several levels over a range of 0.04 to 10 ppm.

13.2 Apparatus:

a. Gas Chromatograph equipped, with a flame photometric detector utilizing a 94 nm filter selective for sulfur-containing compounds. Operational parameters were:

Injection volume - 4 µL

Attenuation - 2 × 104

Chromatographic column was 100 cm by 6 mm OD (2 mm ID) packed with 5 % SP- 1000 on (suitable, please see references) 100/120 or any other equivalent column provided standardization is done. The column was conditioned for 48 hr at 225°C with a flow rate of 60 mL/min carrier

Temperature		**Gas flows**	
Column	170°	Nitrogen carrier	60 mL/min
Injector	*185°	Hydrogen	100mL/min
detector	180°C	Oxygen	15mL/min.
		Air	100mL/min

b. Water bath

13.3 Reagents:

(a) Acetone, analytical grade
(b) Ammonium chloride, granular, reagent, A.C.S.
(c) Colour phast indicator sticks, pH 0-14 range (suitable, please see references)or equivalent
(d) Dichloromethane, analytical grade (tested for acceptability)
(e) Distilled water
(f) Ethyl acetate, analytical grade
(g) Ethylene glycol, analytical grade (for use as a "keeper"),
(h) Hydrochloric acid, concentrated, reagent ACS
(i) Hyflo supercel (suitable, please see references) diatomaceous filter aid (or equivalent)
(j) Phosphoric acid, reagent 85 % A.C.S.
(k) Potassium phosphate monobasic, analytical grade.
(l) Sodium chloride, analytical grade
(m) Sodium hydroxide, analytical grade

(n) Sodium sulfate, anhydrous granular.

13.4 Sample preparation:

Thiodicarb reidues are unstable at room temparature; therefore, samples must remain frozen until time for analysis. Adequate amounts of dry ice should be added to the sample when grinding. The resulting sample texture should be fine enough to allow for thorough mixing. Store sample in plastic bags in a freezer. Weighed aliquots can be taken for analysis when sublimation of the dry ice is complete.

13.5 Procedure:

1. Weigh 25 gm of thorougly mixed sample into a 400 mL plastic beaker. Add 150mL of 9:1 acetone: water and homogenize for 3 min at moderate speed. Fortification of samples with thiodicarb or methomyl to determine recovery should be done before homogenization.

2. Vacuum filter the contents of the beaker into a 500 mL flask through Whatman No. 1 filter paper covered with a lightly packed 1 cm layer of filter aid. Rinse the beaker with 75 mL 9:1 acetone: water and pour over filter cake.

3. Set the flask in a warm water bath (35-40°C) add one drop of ethylene glycol as a keeper, and evaporate all of the acetone with the aid of a gentle stream of dry air.

4. Remove the flask from the water bath, add 50 mL of coagulation solution (0.5 gm ammoniumchloride + 1.0 mL 85 % phosphoric acid in 400 mL distilled water) and let stand for 45 min, swirling occasionally.

5. Vacuum filter into a 250 mL flask through Whatman No.1 filter paper covered with a lightly packed 1 cm layer of filter aid. Rinse the flask with 50 mL of fresh coagulation solution and pour rinse over filter cake.

6. Partition the extract in a 250 mL separately funnel for 30 s shaking gently) with 50 mL of methylene chloride. Dry the lower methylene chloride layer by draining through 75 gm of sodium sulfate supported in a 10-cm funnel plugged with glass wool. Repeat the partitioning twice more with 50 mL methylene chloride each

time. Collect the extract in a 500 mL Erlenmeyer flask. Rinse the sodium sulfate with 25 mL of methylene chloride.

7. Add one drop of ethylene glycol and evaporate the solvent in a warm water bath (35-40°C) using a gentle stream of dry air until approximately one mL remains. Do not allow the extract to blow completely dry to avoid loss of residues.

8. Add 25 mL of 10 % aqueous sodium hydroxide and 2-inch magnetic stirring bar. Set the flask in a water bath positioned on a Magnetic stirrer/hot plate and stir at 60°C for 45 min.

   Note: Steps 9 through 11 should be completed in the same day.

9. Chill samples in an ice-water bath. Slowly add 4.5 mL concentrated hydrochloric acid. Stir while chilled for one minute. 10.

10. Remove from the ice-water bath. Dissolve 3.5 gm of potassium phosphate monobasic in each sample to buffer the solution. Saturate the solution with 8 gm of sodium chloride. The pH measured by indicator sticks should be between 5 and 6. If not, adjust accordingly with additions of concentrated hydrochloric acid or 10 % aqueous sodium hydroxide.

12. Decant solution into a 250 mL separatory funnel. Partition three times with 50mL of methylene chloride, rinsing the original flask with the first two portions of methylene chloride. Drain the lower methylene chloride layer through 75 gm of sodium sulfate supported in a 10 cm funnel plugged with glass wool into a 250mL Erlenmeyer flask. Rinse the sodium sulfate with an additional 25 mL of methylene chloride.

12. Add one drop of ethylene glycol keeper and evaporate the methylene chloride to about one mL in a warm water bath (35 to 40°C) with the aid of a gentle stream of dry air. Remove the air stream at this point and allow the remaining methylene chloride to evaporate just to dryness by the heat of the bath.

13. Dissolve residue in n-heptane. Dilute to required volume with ethyl acetate and analysis by GLC.

13.6 Calculation:

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract}}{\text{Mass in g of the sample solution}} \times \frac{\text{Concentration in Concentration in (mL)}}{\text{ppm of reference standard}}$$

13.7 References:

1. Edited by Hons Peter Their and Hens Zumer, Manual of Pesticide Residues analysis Vol. I, (1981), pp. 299-308
2. J. Chromatogr. A, (2007), 1173, 98–109
3. J. AOAC Int., (2008), 91(6), 1435–1445
4. J. Agric. Food Chem., 2015, 63 (18), 4449–4456

14. CYMOXANIL

14.1 Outline of method:

Cymoxanil residues are extracted from crop or soil samples with ethyl acetate. The aqueous extract is initially washed with hexane. The aqueous solutionis then extracted with dichloromethane. Water samples are directly extracted with dichloromethane.

In both cases, the dichloromethane phase is rotary-evaporated, the residue is dissolved in ethyl acetate, and the solution is cleaned up on silica gel column. Cymoxanil is determined by gas chromatography using a thermionic detector.

14.2 Apparatus:

- Homogenizer. (suitable, please see references)
- Laboratory centrifuge, type UJ 3 (suitable, please see references)with 340 mL glass tubes
- Round-bottomed flasks, 500 mL and 250 mL with ground joints
- Rotary vacuum evaporator, 40-50°C bath temperature
- Separatory funnel, 250 mL
- Laboratory mechanical shaker
- Chromatographic tube, 15 mm i.d. 30 cm long
- Volumetric flasks, 10 mL
- Gas chromatograph equipped with thermionic nitrogen-specific detector
- Microsyringe, 10 µL

14.3 Reagents:

- Acetone, p.a.
- Dichloromethane
- Ethyl acetate
- n-hexane / heptane
- Eluting mixture 1. ethyl acetate : n hexane (1 : 9 v/v)
- Eluting mixture 2. ethyl acetate : n-hexane (4:6 v/v)
- Cymoxanil standard solutions : 0.5-5 µg /mL acetone
- Filter aid, (suitable, please see references)

14.4 Procedure

14.4.1Extraction:

14.4.1.1 Fruits and vegetables:

Weigh 50 gm of the sample (g) into 340 mL centrifuge tube, and add 100 mL ethyl acetate and 5 gm filter aid. Homogenize the mixture for 5 min, and then centrifuge. Decant the supernatant liquid into a 500 mL round bottomed flask. Extract the residue with 100 mL ethyl acetate for 5 min on a mechanical shaker. Centrifuge and decant the liquid phase. Combine the extracts, add 50 mL water and rotary-evaporate until the water begins to condense, quantitatively transfer the aqeous phase into a 250 mL separatory funnel and wash successively with three 30 mL portions of hexane, with gentle swirling. Discard the organic phases. Extract the aqueous solution successively with three 50 mL portions of dichloromethane for 5 min on a mechanical shaker. Separate the lower organic phases (centrifuge, if necessary) and filter through sodium sulphate into a 250 mL round bottomed flask. Wash the sodium sulphate with dichloromethane. Rotary-evaporate the solution almost to dryness.

14.4.1.2 Water:

Extract 50 to 200 mL water (G) successively with three 50 mL portions of dichloromethane for 5 min on a mechanical shaker. Sepaate the organic phases (centrifuge, if necessary) and filter through sodium sulphate into a 250 mL round bottomed flask. Wash the sodium sulphate with dichloromethane. Rotary-evaporte the solution almost to dryness.

14.4.2 Column chromatography:

Plug the bottom end of the chromatographic tube with glass wool and add about 5 mL hexane. Slurry 10 gm silica gel in 20 mL hexane, and slowly pour the slurry into the column, gently tapping the glass walls. Allow to settle, and add 3 gm sodium sulphate. Drain the hexane to the top of the sodium sulphate.

Dissolve the residue in 1 mL ethyl acetate, and transfer quantitatively to the prepared column, using three 3 mL portions of eluting mixture 1 to complete the transfer. Let the solution percolate into the column packing (flow rate of 1-2 drops per s). Elute the column firstly with 200 mL of eluting mixture 1. Discard this fraction, and then elute cymoxanil with 200 mL of eluting mixture 2. Collect the eluate in a 250 mL flask, and rotary-evaporate almost to dryness.

14.4.3 Gas chromatographic determination:

Quantitatively transfer the residue into a volumetric flask, using acetone to complete the transfer, and dilute the solution with acetone to a given volume, e.g. 10 mL (V End). Inject an alquot of this solution (V.) into the gas chromatograph.

14.4.4 GC Operating conditions:

- Column: Glass, 2 mm i.d. 50 cm long; packed with 2 % OV-101 on chromosorb W-HP, 100-120 mesh.
- Column temperature: Programmed to rise at 30°C/min from 100 to 190°C, then isothermal at 190°C for 4 min.
- Injection port temperature: 190°C.
- Detector: Thermionic nitrogen specific detector; Temperature 320°C
- Gas flow rates: Helium carrier, 25 mL/min; Hydrogen 4.5 mL/min; Air 70 mL/min.
- Attenuation: 4
- Recorder: 1 mV; chart speed 5 mm/min.
- Injection volume: 1 µL
- Linearity range: 0.5-10 ng
- Retention time for cymoxanil: 2 min 24 s

14.5 Calculation of residues:

The residue R, expressed in mg/kg cymoxanil, is calculated from the following equation.

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract in (mL)}}{\text{Mass in g of the sample solution}} \times \frac{\text{Concentration in ppm of reference standard}}{}$$

14.6: Reference:

1. Determination of residues of 1 [2-cyano-2-methoxyminoacetyl]-3-ethylurea by gas liquid chromatography. Holt. Pestic. Sci. 10, (1979), pp. 455-459
2. J. Chromatogr. A, (2007), 1173, 98–109
3. J. AOAC Int., (2008), 91(6), 1435–1445
4. Agric. Food Chem., 2015, 63 (18), 4449–4456

15. MULTIRESIDUE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF ORGANOCHLORINE AND PYRETHROID PESTICIDES IN MILK, FISH AND EGGS

The present method prescribed the analytical method for determination of organochlorine and synthetic pyrethroids in milk, fish and eggs.

15.1 Apparatus:

(a) Refrigerated centrifuge-Able to rotate at 3000 rpm at -15°C.
(b) Rotary evaporator - With vacuum device and cooler
(c) SPE automate - (Optional) or SPE vacuum manifold and vacuum pump.
(d) Gas chromatograph system- with injection device and electron capture detector.
(e) Capillary column- Nonpolar dimethyl-polysiloxane (100 %) phase, thickness 0.25 µm, 50 m × 0.32 mm id.

(f) Pre-column-1.5 m × 0.32 mm id.

(g) Volumetric pipets

15.2 Reagents:

(a) Pesticide standard solution in hexane : Hexachloro-benzene (HCB); endrin; α-HCH; p,p'-TDE; β-HCH; o,p'-dichlorodiphenyltrichloroethane; γ-HCH; p,p'-dichlorodiphenyltrichloroethane; heptachlor; o,p'-dicofol; aldrin; heptachlor; oxychlordane; γ-chlordane; o,p'-DDE; α-endosulfan; α-chlordane; p,p'-DDE; dieldrin; o,p'-TDE; deltamethrin, all at 8ng/mL; permethrin, cypermethrin, fenvalerate, cyfluthrin at 40 ng/mL; and γ-cyalothrin at 10 ng/mL.

(b) IS solution in isooctane: Transnonachlor at 10 µg/mL

(c) Acetone

(d) Diethyl ether

(e) Petroleum ether

(f) n-hexane

(g) Acetontrile

(h) Methanol

(i) Methylene chloride

(j) Dodecane : Used as a keeper

(k) Sodium sulfate-Anhydrous

(l) Filter paper-Whatman No. 110 or equivalent

(m) Nonpolar SPE cartridge C - l g, 6 mL, and particle size 40 fn, pore size 60 A (suitable, see reference)

(n) Polar SPE cartridge Florisil.-l g, 6 mL, particle size 200 m

(o) Mobile phase-Helium 99.99 % purity, filtered for oxygen and water

15.3 General fat extraction:

(a) *For milk* - Shake 50 mL milk with 5 mL methanol and 0.5 gm sodium oxalate for 1 min in 250 mL separating funnel. Add 20 mL diethyl ether and shake again for 1 min. Repeat with 25 mL petroleum ether. After separating the phase (centrifuging for 5 min at 1500 rpm may be required), transfer the organic phase into another separating funnel and extract the aqueous phase twice with 50 mL portions

mixture 1 : 1, (v/v) diethyl ether and petroleum ether. Wash combined solvent extracts over sodium sulfate anhydrous layer and evaporate by using rotary evaporator at ca 35°C.

(b) *For fish* - Add 50 mL n-hexane to 20 gm fish. Mix, and centrifuge for 5 min at 1500rpm, Decant upper phase and repeat extraction with 50 mL n-hexane. Keep the 2 extracts together. Evaporate solvent at ca 35°C to 1 mL, and finish evaporation with gentle stream of nitrogen.

(c) *For eggs* - Use extraction column. In a beaker, carefully mix 15 gm sand, 15 gm sodium sulfate anhydrous, and then 10 gm sample, Stopper a glass column with cotton-wool swab, add 2 cm sodium sulfate anhydrous and pour in the above preparation. Elute with 170 mL n-hexane and acetone (2 +1, v/v).

Evaporate solvent at ca 35°C to 1 mL, and finish evaporation with gentle stream of nitrogen.

15.4 Pesticide Extraction:

For each product pesticides are extracted from fat by cryogenic extraction. Weigh 0.5gm fat extract in centrifuge tube, add 3 mL acetonitrile-methylene chloride (75 +25, v/ v and mix vigourously. Centrifuge 20 min at 3000 rpm and ca -15°C. Keep upper-layer supernatant, and then slowly heat bottom to melt fat and repeat extraction with 3 mL of the same solvent mixture. Evaporate organic phase at ca 35 °C under nitrogen to ca 2 mL (solution A).

15.5 Cleanup:

C_{18} SPE: Process cartridge with 5 mL petroleum ether, 5 mL acetone, and 5 mL methanol twice, eluted to meniscus. Solution A (2 mL) is loaded into cartridge and eluted to meniscus (keep 3 min in contact). Wash solution a container with 10 mL acetonitrile, load it into cartridge, and elute (1 drop/3 s). Elutant is evaporated at about 35°C with 100 µL dodecane; then dilute in n-hexane (solution B).

Florisil SPE- Process cartridge with 10 mL n-hexane eluted to meniscus. Load solution B (3 min contact) and elute with mL petroleum ether-diethyl ether (98 +2, v/v; 1 drop/s); and 12 mL petroleum ether -diethyl ether (85 +15, v/v; 1 drop/3 s). Mix the 2 fractions

and evaporate together with 100 µL dodecane. Dissolve final extract in appropriate volume of n-hexane for GC analysis (solution C).

15.6 Gas chromatographic determination:

(a) Internal control procedure: Before analysis, determine linearity and determination limit of GC system.

(b) Operating conditions : When column (suitable, see reference) and mobile phase helium were used, the following settings were appropriate; helium steam pressure 23 psi; initial oven temperature 100°C, holding time 2 min, rate 7°C/min; temperature 220°C, holding time 10 min, rate 3°C/min; final temperature 285°C, injector intial temperature 50°C, rate 150°C/min; final temperature 250°C; holding time 52 min; detector temperature 320°C; nitrogen make-up detector at 25 mL/min; injection volume 1 µL. data sets were produced by GC-electron capture detector (ECD).

(c) Evaluation: Determine, from calibration chromatogram, masses in ng/g fat of solution injected onto GC column. Calculate mass (M) of unknown substance in ng/g using the equation or programming computer.

15.7 Calculation:

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract}}{\text{Concentration in (mL)}} \times \frac{\text{Concentration in ppm of reference standard}}{\text{Mass in g of the sample solution}}$$

15.8 Reference:

Journal of AOAC International, 85 (6) pp. 1398-1405

Table 4: Retention times, LOQ of pesticides

Compound	Retention time, min	LOQ[a] ng/g
α-HCH	15.200	2.7
β-HCH	15.613	2.3
HCB	16.805	2.8
γ -HCH	16.201	2.7
Heptachlor	19.887	2.7
Aldrin	20.045	3.0
Heptachlor-epoxide	21.246	3.1
Oxychlordane	21.408	3.0
γ -Chlordane	22.116	2.9
o,p'-DDE	22.350	2.5
α -Endosulfan	22.698	3.0
α -Chlordane	23.833	2.9
p,p'- DDE	23.750	2.5
Dieldrin	24.846	2.8
o,p'-TDE	24.981	3.6
Endrin	24.760	2.3
p,p'-TDE	25.642	2.4
o,p'-dichlorodiphenyltrichloroethane	26.173	1.9
p,p'-dichlorodiphenyltrichloroethane	28.316	5.4
o,p-Dicofol	31.708	5.4
Dicofol	32.495	2.2
γ -Cyalothrin	37.099	1.9
Permethrin	40.208	10.3
cyflulhrin	42.044	11.7
Cypermethrin	43.160	7.8
Fenvalerate	46.451	7.2
Deltamethrin	49.099	1.9

Note:

For more details refer AOAC Official Method 970.52 page nos. 485-520 (2007). However, for high fat containing food matrices viz., milk, eggs and seafood samples refer to Food Chemistry 125, (2011). pp. 803-812.

16. ACETAMIPRID

16.1 Principle:

An analytical method has been prescribed for the determination for acetamiprid (E) 1ST- [6-chloro-3- pyridyl] methyl - N-cyano - 1ST- methyl acetamide] (ATP) in crops by gas chromatography. ATP in crops is extracted with methanol, purified by liquid-liquid partition and column chromatography and then determined by electron capture detector.

The limit of detection of ATP for fruits and vegetables in 0.005 ppm and recovery of fortified samples at 0.1 ppm in about 96 % in average (80-104 %).

16.2 Apparatus:

Gas chromatograph: Gas chromatograph with Electron Capture detector, Column: A 5 % PEG HT/ chromosorb WHP column (60-80 mesh, 3.2 m. m i.d. 1m. Length) temperature

- Column oven 260°C
- Injection port 320°C
- Detector 260°C
- Carrier gas 1.5kg/cm
- 2 Microlitre syringe -10µL Capacity
- Waring blender or equivalent
- Rotary vacuum evaporator
- Mechanical shaker
- Chromatographic column: glass 200 cm x 18 mm .I.D.

Capillary Chromatography conditions:

Instrument - A gas chromatograph system connected with Communication

software system equipped with ^{63}Ni Electron capture detector

Detecto - Electron Capture Detector

Column - DB-1 Capillary column or equivalent

(30m length x 0.25mm I.D x 0.1µ film thickness)

Temperature conditions

Oven - 300°C

Injecton - 300°C

Detector - 310°C

Gas flow rate

Nitrogen - 15 mL/min

Makeup - 15 mL/min

Injection mode Split ratio

Split - 3.0 mL

Purge - 1.0 mL

Current - 1 nA

Range - 10

Injection volume - 0.4 µL

Retention time (approximate)

Acetamiprid : 7.5 min

16.3 Reagents:

Methanol -glass redistilled

Florisil

Celite 545

Working standard solution of acetamiprid of 1 µg/ mL.

16.4 Extraction:

Fruits and vegetables: Homogenize the sub sample (20 gm) with 100 mL of methanol using the macerator for 3 min and then shake for 30 min by mechanical shaker. Filter the homogenate through a celite (1-2 in thickness) under reduced pressure. Wash the filter cake and vessel with 25 mL of methanol and combine the filtrates and transfer to a 500mL separatory funnel.

16.5 Liquid-Liquid partition:

Add sodium chloride solution (150 mL) to the filtrate. Wash the aqueous methanol solution with 100 mL hexane for 5 min. Transfer the aqueous methanol to another 500mL separatory funnel. Shake the aqueous methanol twice with each 100 mL of dichloro methane for 5 min. Separate the dichlormethane in another separating funnel and extract again the aqueous methanol with 100 mL dichloromethone. Wash dichloromethane with 100 mL of 0.05 N alkaline solution for 3 min. Discard the alkaline solution. Pass the dichloromethane through filter paper containing sodium sulphate in 300 mL round bottom flask. Add 1 gm of Florisil PR to dichloromethane and evaporate to dryness in a water bath at 40°C under reduced pressure.

16.6 Column chromatography:

Transfer the residue with the aid of n-hexane to a column packed with Florisil PR (9 gm). Rinse the vessel with acetone and hexane (20 + 80, v/v) and transfer to the Florisil column. Wash the column with 130 mL of mixed solvent. Elute the acetamiprid with a mixed solvent of hexane and acetone (50 + 50, v/v 120 mL). Concentrate the solvent to dry ness on water bath ca 40°C under reduced pressure. Dissolve the residue in 5 mL of distilled water and transfer to on the top of sep pack C pretreated with 20 mL of methanol 18 and then with 20 mL distilled water. Wash the vessel with 30 mL of 15 % aceto nitrile and transfer on the top of C18 Cartridge. Elute ATP with 30 mL of 15 % acetonitrilie.

Concentrate the elute to dryness in a water bath at 40-50°C under reduced pressure Transfer the residue in acetone and estimate by GLC.

16.7 Calculation

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract}}{\text{Concentration in (mL)}} \times \frac{\text{Concentration in ppm of reference standard}}{\text{Mass in g of the sample solution}}$$

16.8 Reference:

1. M. Tobiled, K. Tiyoshi, K. Sugioba, and T. Gunayo. Rapid determination method for the insecticide Acetomiprid in crops by gas chromatography, J. pesticide Sci22, (1997). pp. 129-132

2. J.Chromatogr. A, (2007), 1173, 98–109

3. J.AOAC Int., (2008), 91(6), 1435–1445

4. J. Agric. Food Chem., 2015, 63 (18), 4449–4456

5. J. AOAC International Vol. 90, (2), 485-520

17. IMIDACLOPRID

17.1 Scope:

The HPLC method for determination of imidacloprid residues in food commodities, and water is described. The LC-MS/MS method for determination of multiresidue analyte may also be used.

17.2 Apparatus:

- Warring Blender

- Laboratory Shaker (Rotary Action)
- Chromatographic Glass Column with Teflon cock, 25 cm x 0.8 cm i.d.
- Rotary vacuum evaporator
- Air blower
- Water bath
- High performance liquid chromatograph

  Equipped with UV detector and operating under the following suggestive parameters. These parameters may be varied according to available facilities.

Colum	:	LiChrospher C18, 5µm, length 25 cm × 0.4 cm i.d. or equivalent.
Mobile phase	:	Acetonitrile -water gradient
Flow rate	:	1.0 mL/min
Oven temperature	:	40°C
Injection volume	:	25 µL
Detector UV	:	270 λ max.

Microlitre syringes of 25 µL capacity

Glass apparatus such as storage bottle, columns etc.

17.3 Reagents:

- Acetonitrile HPLC grade or equivalent
- Water HPLC grade or equivalent
- Dichlormethane, finely distilled
- n-hexane, finely distilled

- Ethyl acetate, finely distilled
- Methanol AR grade
- Dilute sulphuric acid 0.1 N
- Sodium sulphate AR grade or equivalent
- Suitable, see reference filter aid
- Florisil 60/100 mesh, deactivated with 5 % water
- Cotton wool, chemically pure
- Imidacloprid reference standard of known purity
- Amberlite XAD-4 resins, 20-50 mesh.

17.4 Extraction:

(I) Plant material High moisture (fruits and vegetables) and Low moisture (dry samples) containing no fat or wax:

Transfer a suitable quantity (about 50 gm of plant material or or dry samples) finely ground, into a 1L storage bottle. Add 300 mL methanol/water (3:1 v/v) and 5 mL dilute sulphuric acid and allow to soak for 30 min. Homogenise for 3 min using a Waring blender. Add 10-15 gm (suitable, see reference)filter aid and filter through a fast suction filter fitted with a fast filter paper and another 10 gm of filter aid. Rinse the bottle with about 100 mL methanol / water mixture (3:1 v/v). Transfer the filtrate into a 500 mL volumetric flask and make up the volume with methanol. Pipette out an aliquot of this solution (100mL of 50 gm of sample, 200 mL for 25 gm of sample etc.) into a 1 L round bottom flask and concentrate to about 20 mL using a rotary vacuum evaporator at temperature not exceeding 60°C. The aqueous residue is further subjected to a clean up method as described below.

(II) Dry samples containing fats and waxes:

Transfer a suitable quantity (about 50 gm of plant material of dry samples) finely ground, into a 1 liter storage bottle. Add 300 mL methanol/water (3/1 v/v) mixture and

5 mL dilute sulphuric acid and allow to soak for 30 min. Homogenise the mixture for 3min. into a waring blender. Add 10-15 gm (suitable, see reference) filter aid and filter through a fast suction filter fitted with a fast filter paper and another 10 gm of filter aid. Rinse the bottle with about 100 mL methanol/ water mixture (3:1 v/v). Transfer the filtrate into a 500 mL volumetric flask and make up the volume with methanol. Pipette out an aliquot of this solution (100 mL for 50 gm of sample, 200 mL for 25 gm of sample etc.) into a 1 L round bottom flask and concentrate to about 20 mL using a rotary vacuum evaporator at temperature not exceeding 60°C. Dilute the aqueous residue with 100 mL of water and transfer to 500 mL separating funnel. Extract 3 times with 100 mL of n-hexane and discard the hexane phase. Transfer the aqueous phase quantitatively into a 1 liter round bottom flask and again concentrate to about 100 mL using a rotary vacuum evaporator at a temperature not exceeding 60°C ensuring complete removal of n-hexane. The aqueous residue is further subjected to a clean up method as described below.

(III) Oils:

Dissolve 10 gm of oil in 100 mL of n-hexane and extract 3 times with 50mL of water in a 250 mL separating funnel. Collect the aqueous phases in a 500 mL round bottom flask. Any foamy intermediate phase developed during extraction is left with hexane phases and discarded. Concentrate the combined aqueous phases on a rotary vacuum evaporator at a temperature not exceeding 60°C to remove traces of n-hexane.

Use 50 mL of aqueous phase for determination of Imidacloprid residue after concentrating to about 40 mL by using rotary vacuum evaporator at a temperature not exceeding 60°C.

17.5 Clean up:

Clean up by column chromatography on XAD-4 resin.

(I) Column with 10 gm Ambertite XAD-4 resin

Suspend 10 gm of Ambertite XAD-4 resin in 30 mL methanol in a 100 mL glass beaker. Allow to settle. Decant the turbid supernatant. Repeat the procedure by resuspending the resin, Fill into a chromatographic column having an inner diameter of 10 mm. Allow

the methanol to percolate upto the top of the column bed which is then plug by cotton wool. Rinse the column further with 50 mL methanol and 100 mL water. Apply the aqueous solution (sample extract) from 3.1 to the top of the column and allow to percolate slowly (Dropping rate about 2 mL /min.) Rinse the flask with 20 mL of water and also apply the rinsing to the column. Flush the column further with 20 mL water at 5 mL/min. Discard all aqueous washings. Elute the bound residues with 100 mL methanol. Use the elute for determination of imidacloprid as described below.

(II) Column with 25 g Ambertite XAD-4 resin

Suspend 25 gm of Amberlite XAD- 4 resin in 50 mL methanol. Allow to settle. Decant the turbid supernatant. Repeat the procedure by resuspending the resin and then fill into a chromatographic column having an inner diameter of 20 mm. Allow the methanol to percolate upto the top of the column bed which is then pluged by cotton wool. Rinse the column further with 125 mL methanol and 250 mL water. Apply the aqueous solution from 3.4 to the top of the column and allow to percolate slowly (flow rate about 5 mL/min.). Rinse the flask with 50 mL of water at 5 mL/ min. flow rate. Discard all the aqueous washings. Elute the residues with 250 mL methanol. Use the elute for determination of imidacloprid as described below.

17.6 Determination of imidacloprid residues:

Concentrate the methanolic residue from clean up step to 5 mL using a rotary vacuum evaporator at temperature not exceeding 60°C. Dissolve further in 20-25 mL water. Apply this solution as well as the aqueous solution to a disposable extraction column containing diatomaceous earth (suitable, see reference)**.** Total volume should not exceed to 50 mL. Wait for 15 minute after a complete percolation to ensure uniform distribution of aqueous phase onto the column. Elute bound residue from the column (4x) with dichloromethane, the first 50 mL dichlormethane being used to rinse the flask. Concentrate the elute just to the point of dryness on a rotary vacuum evaporator at a temperature not exceeding 60°C.

17.7 Column chromatography on Florisil:

Fill the chromatographic column (i.d. 10 mm) with 50 mL of ethyl acetate. Add 10 gm of Florisil (deactivated by adding 5% water) slowly by knocking the column gently.
Cover the column further with 0.5 gm sodium sulphate. Drain the solvent upto the sodium sulphate layer.

Dissolve the residue from above 5.0 in 2 mL of ethyl acetate and apply carefully to column and allow to percolate. Rinse the flask with 2 mL of ethyl acetate and apply the rinsing also to the column. Flush the column with 100 mL ethyl acetate, the first 20 mL being used to rinse the flask again.

Elute imidacloprid with 25 mL acetonitrile. Evaporate the elute to dryness on a rotary vacuum evaporator. Dissolve the residue in 1 mL of acetonitrile: water (1:1 v/v).

Measure the imidacloprid content as described below.

17.8 HPLC:

The solution of the residue extracted from the sample after clean up is estimated using HPLC. HPLC in an instrument equipped with UV detector. The imidacloprid content in the sample is determined by comparing the response with the response of a known standard of similar concentration.

Inject 25 µL of the sample solution and compare with standard solution with a concentration of 1µg/mL. If the concentration is too high, then dilute suitably using acetonitrate and inject again. Identify the peak of imidacloprid in standard as well as sample solution and determine the peak areas.

17.9. Calculation:

$$\text{Residue of imidacloprid (µg/g)} = \frac{A1 \times V2 \times V3 \times D \times C}{A2 \times V1 \times M}$$

Where,

A1 = Peak area of the sample

A2 = Peak area of the standard

V1 = Volume in microlitre of the sample injected

V2 = Volume in microlitre of the standard injected

V3 = Total volume in mL of the sample solution

D = Dilution factor for sample solution

C = Concentration in ppm of the standard solution

M = Mass of the sample taken for analysis

Note: Percent mean recovery is dermined by taking untreated control sample to which a known amount of imidacloprid is added and analysed as described above.

17.10 References:

1. F.J.PleckeandE. Weber. PflanzenschutzNochricktenBayer 46, (1993) pp. 109-182.
2. Pesticide Research Journal, 17 (1), (2005). pp. 46-50
3. J. Chromatogr. A, (2007), 1173, 98–109
4. J. AOAC Int., (2008), 91(6), 1435–1445
5. J. Agric. Food Chem., 2015, 63 (18), 4449–4456
6. J. AOAC International Vol. 90, (2), 485-520
7. J. AOAC International Vol. 88, NO. 2, 2005, 630-638
8. J. Sep. Sci. 2007, 30, 620-632

18. PRETILACHLOR (The LC-MS/MS method for determination of multiresidue analyte may also be used)

18.1 Principle of the method:

Milled grain is extracted with acetonitrile in a high speed homogenizer. Plant material is extracted with methanol in a high speed homogenizer. The extracted grains are cleaned up by acetonitrile, n-hexane partition. The methanol extracts of soil samples and plant materials are diluted with water and cleaned up by water- methanol/n-hexane partition. The hexane phase is evaporated and the residue of the organic phase is cleaned up using alumina column chromatography prior to the final determination by gas chromatography.

18.2 Extraction and cleanup:

18.2.1 Apparatus and reagents:

- Alumina basic, (suitable, see reference)W 200, activity grade V (19 % water added).
- Rotating evaporator (suitable, see reference) or equivalent.
- Soil homogenizing machine: e.g. planetary blender (suitable, see reference) or equivalent.
- Cross beater mill (suitable, see reference) or equivalent. Food cutter (suitable, see reference) or equivalent.
- Food cutter (suitable, see reference) or equivalent.
- High speed homogenizer (suitable, see reference)or equivalent.
- Shaker, mechanical to accommodate 500 mL wide mouth jars (suitable, see reference) or equivalent.
- Fluted filterpaper (suitable, see reference).
- All organic solvents redistilled in glass or analytical grade.

- Sodium chloride solution, saturated.

18.3 Extraction

18.3.1 Grain:

Add 200 mL acetonitrile to 100 gm of milled grain and macerate with the high speed blender (suitable, see reference) or equivalent for 3-4 min in a 500 mL widemouth jar. Shake the tightly closed jar for 2 hr with a mechanical shaker; filter the slurry through a fluted filter paper. Rinse jar and residue two times with 30 mL acetonitrile. Adjust volume to 300 mL and take an aliquot of 100 mL corresponding to 50 gm of crop material.

18.3.2 Other plant materials:

Shear the entire laboratory sample with a food cutter. Weigh a subsample of 100 gm, add 200 mL methanol and macerate in high speed blender (suitable, see reference) for 3-4 min. using a 500 mL wide mouth jar. Shake the tightly closed jar for 2 hr with a mechanical shaker. Filter the slurry through a fluted filter paper. Rinse jar and residue two times with 40 mL methanol. Adjust volume to 300 mL and take an aliquot of 150 mL corresponding to 50 gm.

18.4 Partitioning

18.4.1 Grain:

Transfer the 100 mL sample aliquot to 1000 mL. The active ingredient is re-extracted from the acetonitrile phase after addition of 500 mL water and 25 mL sodium chloride solution with three 100 cm portions of n-hexane by vigorously 3 shaking the separatory funnel for at least 2 min during each extraction. The n- hexane phases are collected, filtered through a plug of cotton and evaporated to dryness using a rotating evaporator, water bath temperature 40°C.

18.4.2 Soil samples and plant materials:

Transfer the corresponding aliquots to a 500 mL sepaatory funnel and add 200 mL water and 20 mL sodium chloride solution. Extract the aqueous solution three times with 70 mL n-hexane each by vigorously shaking the separatory funnel at lest 2 min during each extraction. The n-hexane phases are collected, filtered through a plug of cotton and evaporated to dryness using a rotating evaporator, water bath temperature 40°C.

18.5 Column cleanup:

Fill a chromatographic column 1.8 cm inner diameter, with n-hexane. Add alumina basic, activity grade V, to a height of 7 cm drain the solvent to the top of the alumina. Dissolve the residue to 5 mL n-hexane. Transfer this solution to the column. Rinse the flask twice with 5 mL of n-hexane and transfer each portion to the column. Rinse the column with 100 mL of n-hexane. Discard this eluate. Elute the active ingredient with 100 mL of a mixture of n-hexane/ ethylether (2+1). Collect the eluate and evaporate to dryness in a rotating evaporator at a water bath temperature of 40°C. Transfer the residue with ethyl ether to 10 mL vials and evaporate to drynes in a stream of clean air. Dissolve the residue for GC in 2 mL n-hexane: ethanol (1:1).

18.6 GLC

Capillary Chromatographic Separation Parameters:

Instrument : A GC with communication Module

Detector : Electron Capture Detector

Column used : (suitable, see reference) of 30m × 0.53 mm Internal Diameter and 1.5 µm film thickness) or equivalent

Gas flow rate

Nitrogen (Carrier) - 8 mL/min

Makeup (Nitrogen) - 40 mL/min

Temperature conditions

	Oven	-	200°C
	Injector	-	230°C
	Detector	-	250°C
Volume injected	-	1 µL	
Range	-	1	
Current	-	1 nA	
Injection Mode	-	Split	
Split ratio	-	Purge :	Vent
		1 :	5
Retention time (approximate)			
Pretilachlor		-	6.1 min
Minumum detectable concentration		-	0.004 ppm

Alternatively:

GLC with alkali flame ionization detector (AFID) with Rubidium sulfate pellet (suitable, see reference)

Gases: Hydrogen, flow rate 35 cm^3/min. Air, flow rate 230 cm^3/min.

Temperature: 240°C. Sensitivity: $4.1Q^{-12}$ afs Injection volume: 1 µL

Chromatographic column: Glass, 50 cm length, 2 mm i.d., packed with 10 % OV 101 on Gas Chrom Q, acid washed, DMCS treated, size 150-180 m.

18.7 Calculation:

$$\text{Residue Level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height with the standard}} \times \frac{\mu\text{L of the standard}}{\mu\text{L of the sample injected}} \times \frac{\text{Final volume of the sample extract (mL)}}{\text{Mass in g of the sample solution}} \times \frac{\text{Concentration in ppm of reference standard}}{\text{Concentration in}}$$

18.8 Reference:

1. CIBA Geigy Limited Agrochemical Division. (Complete reference)
2. J. Chromatogr. A, (2012), 1270, 283-295
3. J. Agric. Food Chem., 2015, 63 (18), 4449–4456

19. ISOPROTHIOLANE (The LC-MS/MS method for determination of multiresidue analyte may also be used)

19.1 Principle:

Food samples are extracted with benzene-acetone solvent mixture on a mechanical shaker. The plant material to be analysed is extracted by blending in a high speed blender with benzene-acetone.

Cleanup is achieved by liquid partitioning from acetonitrile to hexane followed by chromatography on a Florisil column. Isoprothiolane residues are determined using GC equipped with an electron capture detector (ECD).

19.2 Experimental

19.2.1 Apparatus:

- Analytical balance with sensitivity of 0.1 mg
- Top loading balance with sensitivity of 1 mg
- Gas chromatography (GC) with Electron capture Detector (ECD)

The GC equipped with ECD is connected to a PC-based data system. The following operating parameters are suggested, which can be changed provided standardisation is done:

- Column : 100 cm × 0.2 cm i.d Pyrex spiral column containing 3 % OV- 1 on chromosorb W(HP)
- Temperaure : Injector 250°C
- Detector : 260°C
- Column oven : 210°C
- Gas flow rates : Nitrogen (Carrier gas): 35 mL/min.
- Detector : Electron capture Detector (ECD)
- Retention time : 6.0 min
- Sensitivity : range - 4, Attenuation - 4
- Microlitre syringe : 10 µL

19.2.2 Capillary Chromatographic Conditions:

Isoprothiolane :

Instrument	-	Gas Chromatograph Model equipped with auto injector coupled with computer and Class GC-10 software.
Detector	-	Electron Capture Detector
Column	-	DB-1, Megabore Column (30 m length x 0.53 m i.d. 1.5µ film thickness).

Gas flow rate

Carrier Gas Helium - 7mL/min

Temperature conditions

Oven Initial - 210°C

Injector - 240°C

Detector - 260°C

Volume injected - 1 µL

Injection Mode - Split

Split ratio - 1 :10

Solvent - Acetone

Retention time (approximate)

Isoprothiolane - 7.4 min

- Other instruments:

Waring Blender or Equivalent

Rotary evaporative concentrator

Sample Grinder

Mechanical shaker

Chromatographic tubes, 18 mm × 450 mm

Buchner funnel

Separatory funnel

19.3 Reagents:

- Reference standard of known purity of isoprothiolane
- Benzene

- Acetone
- Acetonitrile
- n-Hexane
- Diethyl ether
- Sodium chloride
- Sodium sulfate
- Florisil, 60-80 mesh

19.4 Sample preparation:

Finely chop the sample fruits, vegetables, forages, straw, etc. and mix well in a mixer grinder. Using a suitable grinder mill and mill samples of rice grains.

19.5 Standard solution:

Prepare 100 µg/mL stock solution by dissolving 0.01 gm reference isoprothiolone in 100mL benzene. For GC determinations prepare 100 mL working standard solution by taking a suitable aliquot form the above solution into 100 mL volumetric flask and diluting with benzene.

19.6 Extraction

19.6.1Plant material:

Weigh representative 50 gm samples of plant material into a 500 mL wide mouth jar, add 100 mL of benzene-acetone (50:50, v/v) solvent mixture and blend in a high speed waring blender for 2 min. Filter the mixture through a Buchner funnel using Whatman No. 1 filter paper. Re-extract the sample two times with 100 mL benzene-acetone (50:50 v/v) and pool the filtrate together after rinsing the filter cake two times with 20 mL of the same solvent mixture. Combine the extracts and concentrate to about 5 mL in a vacuum rotary evaporator with water bath at 40°C.

19.6.2 Water:

Extract the water sample directly in partitioning step

19.7 Clean up:

Partitioning

19.7.1 Plant material:

Add 20 mL acetonitrile to the extract and transfer the concentrated extract/ filtrate quantitatively into a separatory funnel using another 20 mL of acetonitrile. Add 30 mL n-hexane and shake for 5 min. Allow the layers to separate and draw the acetonitrile layer into another separatory funnel containing 200 mL of 10 % sodium chloride solution. Extract with two 50 mL portions of hexane and combine them. Dry with anhydrous sodium sulfate and evaporate to near dryness on a rotary evaporator with water bath at 40°C.

19.7.2 Water

Use a 250 mL sample of water. Transfer the entire sample into a 500 mL separatory funnel and add 50 mL of saturated sodium chloride solution. Extract the contents by partitioning three times with 100 mL portions of hexane. Collect the upper organic layer (i.e. hexane) each time after passing through anhydrous sodium sulfate and concentrate to near dryness in vacuum rotary evaporator using water both at 40°C and take directly for GC analysis.

19.7.3 Column chromatography:

For clean-up by adsorption column chromatography, place a cotton plug at the bottom of a chromatographic tube and pack it with 2 gm anhydrous sodium sulfate, 15 gm Florisil and 2 gm anhydrous sodium sulfate in tandem using benzene. Drain the excess solvent from the column until the level falls to the top of the packing. Transfer the residue from the above step quantitatively using three 5 mL portions of benzene and wash the column with 100 mL of benzene and discard the eluate. Elute the column with 100 mL of benzene-diethyl ether (33:67, v/v) solvent mixture, collect the eluate and concentrate to dryness at 40°C using rotary evaporator.

19.8 Estimation:

Dissolve the residue derived from the above step in benzene and dilute to suitable volume. Inject 1 µL of this solution into GC operated under the conditions described earlier.

19.9 Calculations:

$$\text{Isoprothiolane residue } \mu g/g \text{ (ppm)} = \frac{A1 \times V2 \times V3 \times C}{A2 \times V1 \times M}$$

Where,

A1 - Peak area of the sample

V2 - Volume in µL of standard isoprothiolane

V3 - Total volume, in mL of sample solution

C - Concentration in µg/g of the standard isoprothiolane solutions

A2 - Peak area of standard Isoprothiolane

V1 - Volume in µL of sample solution injected

M - Mass in g, of sample taken for analysis

19.10 Reference:

1. Zwig, G, Icanouchi, M. and Tamahiro, Hattari. Analytical methods for pesticides and plant growth regulator. Academic Press, New York, (1978). pp. 229-236

2. J. Chromatogr. A, (2012), 1270, 283-295

3. J. Chromatogr. A, (2007), 1173, 98–109

20. FOSETYL ALUMINIUM (The LC-MS/MS method for determination of multiresidue analyte may also be used)

20.1 Principle:

Fosetyl-aluminium and its main metabolite, phosphorus acid, are extracted from plant material with dilute tartaric acid. Water samples are acidified with tartaric acid. An aliquot of the plant extract, or the acidified water samples, is diluted with isopropanol. After methylation with a diazomethane solution in diethyl ether, the resulting o-ethyl o-methyl and o,o-dimethyl phosphonates are determined by GC using a phosphorus-specific flame photometric detector.

20.2 Apparatus:

- Homogenizer
- Laboratory centrifuge with 250 mL glass tubes
- Glass funnel, 9 cm dia.
- Volumetric flasks, 100 mL, 50 mL and 5 mL
- Round-bottomed flask, 50 mL, with ground joint
- Methylation apparatus
- Gas chromatograph equipped with phosphorus specific flame photometric detector
- Microsyringe 10 µL

20.3 Reagents:

- Diethyl ether, high purity, dried over calcium chloride
- 2-propanol (isopropanol),
- Isopropanol : water mixture (9 : 1 v/v)
- Fosetyl standard solutions for recovery experiments: 1, 10, 100 and 1000 µg/mL fosetyl aluminium or phosphorus acid in water.
- Derivative standard solutions: Prepare solutions of 100 µg/mL of fosetyl-aluminium and phosphorus acid, respectively, in isopropanol-water mixture (dissolve fosetyl aluminium in water and dilute with isopropanol to yield a solution containing isopropanol and water in the proportion 9:1 v/v). Transfer 10 mL each of these solutions into volumetric flasks, add 25 µL of tartaric acid, make up to 100 mL with isopropanol-water mixture and shake.

  Derivatize 5 mL of the solutions as described under methylation. Concentrate the reaction mixture to 3 mL, transfer to 5 mL volumetric flasks and make up to 5 mL with isopropanol. Dilute these solutions progressively to obtain solutions containing o-ethyl o-methyl phosphonate or o,o-dimethyl phosphonate equivalent to 0.01, 0.02, 0.05, 0.08, 0.1, 0.15 and 0.2 µg/mL of fosetyl aluminium or phosphorus acid.

- Ethanolic potassium hrydroxide solution; Dissolve 7 gm potassium hrydroxide p.a. in 10 mL water and make up to 100 mL with ethanol.
- Diazomethane solution in diethyl ether (for apparatus see Fig. 1 at page 132).
- Dissolve 1.2 gm N-methyl-N-nitroso-p-toluenesulphonamide in 10 mL diethyl ether and transfer to the dropping funnel. Slowly add this solution dropwise to 5 mL ethanolic potassium hrydroxide solution contained in the reaction vessel, and sweep the generated diazomethane into 20 mL diethyl ether, using a gentle stream of nitrogen, while the receiver containing the ether is cooled in an ice - sodium chloride freezing mixture
- Glass wool
- Cotton wool, exhaustively extracted with acetone
- Air, synthetic
- Hydrogen, re-purified
- Nitrogen, re-purified.

20.4 Procedure

20.4.1 Extraction

20.4.1Vegetables and fruits:

Weigh 50 gm of the analytical sample (G) into a centrifuge tube, add 50 mL .1 M tartaric acid (V_{Ex}) and homogenize for 1-2 min. Centrifuge for 15 min at 2500 rpm. Filter the supernatant through glass wool, transfer 5 mL of the filtrate (V_R) into a volumetric flask, and make up to 50 mL (V^) with isopropanol. Shake the solution and filter through cottonwool to remove precipitated material.

20.5 Methylation:

Transfer 5 mL (V_{R3}) of the solution into a 50 mL round bottomed flask and add diazomethane solution (5-10 mL) until produced yellow colour is stable. Stopper the

flask and allow to stand for 15 min with occasional swirling. Remove excess diazomethane and concentrate to approx 3 mL with gentle stream of nitrogen.

Quantitatively transfer the solution into a volumetric flask and make up to an appropriate volume (V_{End}) e.g. 5 mL, with isopropanol. Inject an aliquot of this solution (V.) into the gas chromatograph

20.6 Gas-chromatographic determination

20.6.1 Operating conditions:

- Column : Glass, 2 mm i.d., i.e. 2 m long; packed with 15 % carbowax 20 M on Chromosorb 750, 100-120 mesh
- Column temperature : 130°C Injection
- Port temperature : 200°C
- Detector: Flame photometric detector, (suitable, see reference), equipped with 526 nm phosphorus filter.
- Temperature: 175°C.
- Gas flow rates: Nitrogen carrier, 90 mL/min; Hydrogen 60 mL/min.; Air 200 mL/min.
- Attenuation: 1-32
- Recorder: 10 mV; chart speed 5 mm/min.
- Injection volume: 5 µL
- Retention times for dimethyl phosphonate: 3 min. ethyl methyl phosphonate: 3 min. 36 s.
- Recoveries and limit of determination: The recoveries from untreated control samples, fortified with fosetyl- aluminium and phosphorous acid at levels of 0.1 to 10 mg/kg, ranged from 72 to 120 % for plant material and tap water, and averaged 97 %. Blanks usually were less than 0.1 mg/kg.

Strawberries and grapes occasionally gave blanks corresponding to 0.2 and 0.9 mg/kg phosphorus acid, respectively. The limit of determination was in the range of 0.1 to 1 mg/kg for all materials tested.

20.7 Calculation of residues:

The residue R, expressed in mg/kg fosetyl-aluminium or phosphorus acid, is calculated from the following equations:

$$\text{for plant material } R = \frac{W_A.\ (V_{Ex} + x)\ V_{R2}.\ V_{End}}{V_{R1}.V_{R3}.\ V_i.\ G} \times 0.93$$

$$\text{End for water } R = \frac{W_A.\ V_{R2}.\ V_{End}}{V_{R3}.\ V_i.\ G} \times 0.93$$

Where,

G - Sample weight (in gm)

x = portion of the sample weight (plant material) (in gm)

V_{Ex} - volume of dilute sulphuric acid used for extraction for sample (in mL)

V_{R1} - portion of filtrate (before dilution with isopropanol) used for further processing (in mL)

V_{R2} - volume of solution after dilution with isopropanol

V_{R3} - portion of volume V^ used for methylation

V_{End} - terminal volume of sample solution

$V_{i.}$ - portion of volume

V_{End} injected into gas chromatograph (in IJ)

W_A - amount of fosetyl aluminium of phosphorus acid, respectively for V. read from calibration curve (in mg)

0.93 - factor for conversion of fosetyl aluminium to fosetyl (not required for phoshorus acid residues)

The residues can also be determined using the following LC-MS/MS conditions:

Analysis of Fosetyl Al by LC-MS/MS

LC column **:** C18 (Licrocart * RP 18 150 mm x 4.6 mm, 5 µ) or equivalent.

LC conditions

Mobile phase **:** A- 5 mM Ammonium formate in water

B- 5 mM Ammonium formate in 90 % Methanol + 10 % water

Flow rate **:** 0.8 mL/ min

Gradient program:

Time (min)	Flow Rate (µL/min)	A (%)	B (%)	C (%)	D (%)	TE#1	TE#2
0.0	1000.00	80.0	20.0	0.0	0.0	open	open
0.5	1000.00	80.0	20.0	0.0	0.0	open	open
3.0	1000.00	20.0	80.0	0.0	0.0	open	open
5.0	1000.00	20.0	80.0	0.0	0.0	open	open
7.0	1000.00	80.0	20.0	0.0	0.0	open	open
11.0	1000.00	80.0	20.0	0.0	0.0	open	open

20.8 Mass Parameters for API 2000 mass spectrometer:

Ionization of fosetyl aluminium occurs in negative ionization mode. The negative-ion electrospray full-scan spectrum of fosetyl shows a peak at m/z 109, corresponding to the molecular weight of the fosetyl anion. The MS/MS spectrum shows an important fragment at m/z 81 (Quantifier ion), the product of a McLafferty rearrangement. By

increasing the collision energy a minor fragment, at m/z 63 (Qualifier ion), due to the loss of ethanol was obtained.

Period 1 Experiment 1:

Scan Type: MRM (MRM)

Polarity: Negative

Scan Mode: N/A

Ion Source: Turbo Spray

Resolution Q1: Unit

Resolution Q3: Unit

MR Pause: 5.0070 m/s

Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Param	Start	Stop ID
109.00	81.00	200.00	CE	-12.00	-12.00
			CXP	-4.00	-4.00

Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	Param	Start	StopID
109.00	63.00	200.00	CE	-36.00	-36.00
			CXP	-14.00	-14.00

Parameter Table 5 (Period 1 Experiment 1)

CUR:	20.00
CAD:	6.00
IS:	-4500.00
TEM:	450.00 (Source temperature in ^{0}C)
GS1:	30.00
GS2:	60.00
ihe:	ON
DP	-16.00
FP	-270.00
EP	-12.00
CEP	-10.00

20.9 Reference:

1. A. Bertrandsh. Determination of residues of phosphorus acid and ethyl phosphorate in lettuces. Method No. 22-29, Rhone-Poulene Agrochimia, (1979).
2. IS 14162: 1994. Pesticide determination of Fosetyl-AI residues in agricultural and food commodities. Indian Standard.
3. Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol **Version 8.1.** http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf

21. PROPICONAZOLE (The LC-MS/MS method for determination of multiresidue analyte may also be used)

21.1 Principle:

Propiconazole residues are extracted from cereal green matter and grapes with methanol, and from grains, straw and soil with a mixture of methanol and water. The filtered extract is diluted with water and saturated sodium chloride solution, and propiconazole is partitioned into dichloromethane. Water is diluted with saturated sodium chloride solution, and extracted with dichloromethane. The dichloromethane extracts are rotary-evaporated to dryness. The residue is cleaned up by column chromatography on aluminium oxide.

21.2 Apparatus:

- High speed blender fitted with leak-proof glass jar and explosion proof motor
- Homogenizer
- Wide neck bottle, 500 mL with ground stopper
- Laboratory mechanical shaker
- Buchner procelaen funnel, 9 cm dia.
- Filter paper, 9 cm dia, (suitable, see reference)
- Filtration flask, 1-L

- Separatory funnel 1-L
- Round bottom flasks, 300 mL, 100 mL and 25 mL with ground joints.
- Rotary vacuum evaporator, 40 °C bath temperature
- Test tubes, 10 mL with ground stoppers
- Glass syringe, 10 mL with Luer lock fitting
- Chromatographic tube, 20 mm i.d. 30 cm long
- GC equipped with thermionic nitrogen specific detector
- Microsyringe, 10 µL

21.3 Reagents:

- Cyclohexane,
- Dichloromethane
- Ethanol
- Ethyl acetate
- n-hexane
- Methanol (high purity)
- Toluene
- Cyclohexane : ethyl acetate mixture (1:1 v/v)
- Eluting mixture 1. dichloromethane : n-hexane (4:6 v/v)
- Eluting mixture 2. dichloromethane : n-hexane (6:4 v/v)
- Ethanol : n-hexane mixture (1:1 v/v)
- Methanol : water mixture (8:2 v/v)
- Propiconazole standard solutions; 0.25, 0.5, 1.0 and 10.0 g/mL in ethanol hexane mixture
- Sodium chloride solution saturated Aluminium oxide, activity grade V: To 100 gm Alumina (suitable, see reference) in a 300 mL Erlenmyer flask (with ground joint), add 19 mL water dropwise from a burette, with continuous swirling. Immediately stopper flask with ground stopper, shake vigorously until all lumps have disappeared, and then store in a tightly stoppered container for at least 2 h, 200-400 mesh.
- Dry ice
- Cottonwool

- Compressed air, dried and re-purified
- Hydrogen, re-purified
- Nitrogen, re-purified

21.4 Procedure

21.4.1 Extraction

21.4.1.1Green plant matter, grains, straw grapes:

Weigh 50 gm grapes, 50 gm milled grains, 20 gm milled straw or 10 gm of the other homogenized analytical material (G) into a wide neck bottle. Add 200 mL methanol or 200 mL methanol-water mixture for samples of grains, straw and soil. Tightly stopper the bottle, and shake for 1 hr on a mechanical shaker, suction-filter through a Buchner procelain funnel, and wash the filter cake with two 25 mL portions of methanol. Transfer the filtrates to a separatory funnel. Add 200 mL water and 50 mL sodium chloride solution and extract three times with 75 mL protions of dichloromethane. Filter the dichloromethane phases through a cottonwool plug into a 300 mL round bottomed flask, and rotary-evaporate to dryness. Discard the water phase. Proceed for cleanup of the residue.

21.4.1.2 Water:

Place 500 mL water (G) in a separately funnel, add 50 mL sodium chloride solution, and extract successively with three 75 mL portions of dichloromethane. Filter the dichloromethane phases through a cottonwool plug into a 300 mL round bottomed flask, and rotary-evaporate to dryness.

21.5 Column chromatography:

Pour 15 mL hexane into the chromatographic tube. Slowly add 30 gm Aluminium oxide (free from air bubbles). Allow to settle and then drain the hexane to the top of the column packing. Transfer the residue to column, using three 2 mL portions of toluene to complete the transfer. Drain the toluene to the top of the column packing each time. Elute co-extractives with 50 mL of eluting mixture 1 and then elute propiconazole with 75 mL of eluting mixture 2, using a flow rate of 1 to 2 drops per s. Collect the eluate in a 100 mL round bottomed flask, and rotary evaporate to dryness.

21.6 Gas-chromatogrpahic determination:

Dissolve the residue derived in 2 mL ethanol hexane mixture, and dilute to a suitable volume (V_{End}). Inject an aliquot of this solution (V) into the gas chromatograph.

21.7 Operating conditions:

Gas chromatograph	:	With Nitrogen specific detector (NPD/TID)
Colum	:	Glass, 2 mm i.d. 1.5 m long; packed with 3 % CP Wax 40M on Gas Chrom Q, 80-100 mesh (chrom-pack)
Column temperature:	:	245 °C
Injection port temperature:		250 °C
Temperature	:	250 °C
Gas flow rates	:	Nitrogen carrier, 36 mL/min
		Hydrogen 3 mL/min
		Air 50 mL/min
Attenuation	:	16
Recorder	:	1 mV; chart speed 10 mm/min
Injection volume	:	2 µL
Retention time for propiconazole	:	2 min 20 s

21.8 Reference:

1. Edited by Hens Peter Their, Mannual of Pesticide Residues analyusis Vol.II, (1992). pp. 281-286

2. J. Chromatogr. A, (2007), 1173, 98–109

3. J. AOAC Int., (2008), 91(6), 1435–1445

4. J. Agric. Food Chem., 2015, 63 (18), 4449–4456

5. J. AOAC International Vol. 90, (2), 485-520

22. DITHIOCARBAMATES (The LC-MS/MS method for determination of multiresidue analyte may also be used)

22.1 Spectrophotometric method

22.1.1 Principle:

This analytical method describes the spectrophotometric method for determination of residues of any of the following dithiocarbamate residues in food commodities.

(a) Ferbam;
(b) Ziram;
(c) Thiram;
(d) Maneb;
(e) Zineb;
(f) Mancozeb; and
(g) Nabam

This method has a detection limit of 0.01µg/g (0.01 ppm)

A representative sample of the commodity is blended with deaerated ice-water in a predetermined ratio (normally 1:1, m/v) under nitrogen and an appropriate aliquot of the homogenised material is decomposed with sulphuric acid. The evolved carbon disulphide is absorbed in Vile's reagent. The intensity of the resulting colour complex is measured spectrophotometrically at 380 nm and the absorbance compared by means of a standard curve.

22.1.2 Apparatus :

Absorption train

22.1.3 Reagents:

Vile's Reagent

Dissolve 0.05 gm cupric acetate, monohydrate in 25 mL water in a 1000 mL volumetric flask. Add 800 mL ethanol, 1 mL diethylamine and 20 mL triethanolamine. Make up the volume to the mark with ethanol.

Ethanol

95 % (v/v), alternatively absolute alcohol may be used.

Lead acetate solution

30 % aqueous solution (m/v)

Disodium ethylene dinitrotrichloro tetra acetate (EDTA) Solution

Dissolve 33 gm EDTA in 800 mL water in a 1000 mL volumetric flask and dilute to mark with water.

Sulphuric Acid- ION

Reference standard of the Dithiocarbamate of known purity

Chloroform

Glass re-distilled

22.1.4 Method

i) Preparation of standard solution:

Prepare a solution so as to contain 20gm of the dithiocarbamate per milliliter of chloroform (See notes 1, 2 and 3).

Notes:

1. If the dithiocarbamate is thiram /ferbam/ziram, 0.04gm of the active ingredient shall be dissolved in 100 mL chloroform and dilute the resultant solution to 100 mL with chloroform
2. For maneb, mancozeb and zineb prepare the standard solution as described in Note I but use EDTA solution as solvent.

3. For nabam, prepare the solution as described in Note I/Note 2, but use water as solvent.

ii) Preparation of Blank solution:

Take 12.5 mL Vile's reagent and add 100 mL ethanol.

iii) Preparation of standard curve and calibration:

Transfer 10 mL standard solution of the dithiocarbamate into a 500 mL distillation flask of the decomposition absorption train. Add 10 mL lead acetate solution to the first absorption tower. Add 200 mL water to the distillation flask and assemble the train leaving the vaccum source disconnected. Heat the flask to 85-90°C temperature, leaving the contents just short of boiling. Apply gentle vacuum continuously and add 40 mL boiling sulphuric acid through the dropping funnel and reflux for 30-45 min.

Note:

1. When chloroform is used for preparation of reference standard solution of the dithiocarbamate, remove the solvent by passing a stream of nitrogen at room temperature. This step is not necessary if EDTA or water has been used for preparation of the reference standard solution of the dithiocarbamate.

2. Use 60 mL sulphuric acid for digestion for dithiocarbamate such as maneb, zineb, ad mancozeb.

iv) Drain the contents of the tower containing Vile's reagent to a 25 mL volumetric flask. Wash the tower with several 3-4 mL portions of the ethanol to ensure complete quantitative transfer and collect the washings in the flask. Make up the volume to mark with ethanol.

v) Transfer separately 2.0, 3.0, 5.0 and 8.0 mL portions of the standard solution of the dithiocarbamate into the 500 mL distillation flask and follow the digestion procedure described in e (iii).

vi) Measure the absorbances of the standard solution in 1 cm quartz at 380 nm using blank solution prepared as described in e (ii). Prepare the standard curve by plotting the absorbances in the graph against corresponding dithiocarbamate content.

22.1.5 Estimation:

Select a sample to contain 20-160 gm of the dithiocarbamate. Transfer the sample to the 500 mL distillation flask of the decomposition absorption train. Add 10 mL lead acetate solution to the first absorption tower, 12.5 mL Vile's reagent to the second tower and 200 mL water to distillation flask and digest as described in e (iii).

Prepare the solution of the evolved carbon disulphide according to the procedure described as above and measure the absorbance as described above.

Notes:

1. Water is added to the distillation flask if thiram/ ferban/ziaram/ nabam residues have to be determined.
2. 200 mL EDTA is added to the distillation flask if maneb/zineb/mancozeb residues have to be determined. 60 mL sulphuric acid is added and procedures as described above have to be followed.

Determine the dithiocarbamate residues in the sample using the appropriate calibration curve.

Calculation: Dithiocarbamate content (µg/g)

= µg of dithiocarbamate in the sample / Mass in g of sample taken for test

22.1.6 Determination of recovery factor:

Fortify 50 gm of the fresh commodity (not previously treated with dithiocarbamate) with about 500 g/L of the dithiocarbamate. Blend with water (1:1, m/v) to obtain a homogenous mixture.

Weigh 200 gm of the blended material (containing about 40 µg/g of the dithiocarbamate) in a 500 mL distillation flask. Add 100 mL of EDTA and reflux with 40-60 mL of ION sulphuric acid by following the procedures described in d (iii) measure the absorbance at 380 nm.

22.1.7 Calculation:

$$\text{Recovery factor \%} = \frac{\text{Mass in gm of the dithiocarbamate in treated sample}}{\text{Mass in gm of dithiocarbamate added to material}}$$

Note:

85 – 100 percent recoveries of 0.1 -70 µL /g have been observed in a variety of substrates.

22.1.8 References

1. Indian Standard IS 13832: 1993.
2. Kepple, G.E. J. AOAC 54: 529 (1979). U.S. Food and Drug Administration

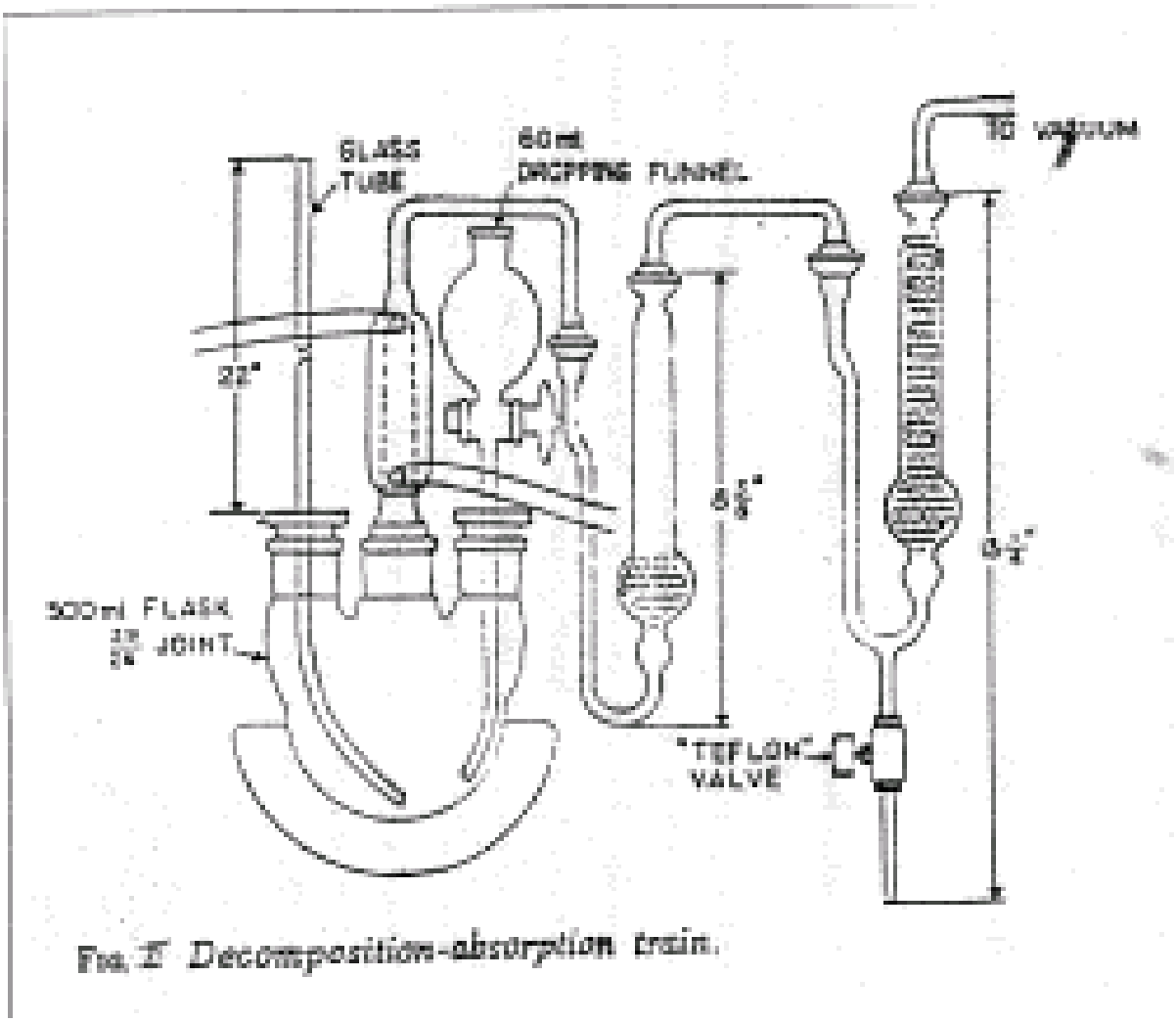

FIG. 1 *Decomposition-absorption train.*

FIG NO: 1

22.2 DITHIOCARBAMATE RESIDUES (The LC-MS/MS method for determination of multiresidue analyte may also be used)

22.2.1 Gas Chromatography

22.2.1.1 Principle:

The procedure describes GC analysis of dithiocarbamate residues in vegetable, fruit and cereal products via their common degradation product carbon disulfide (CS_2) as required by Reg. 396/2005/EC

22.2.1.2 Procedure

22.2.1.2.1 Extraction

A 50 gm (± 1 %) representative portion of the sample is weighed into a cleavage vessel (suitable, see reference) and 25 mL isooctane added. Then 150 mL of hydrolysis reagent (tin (II)-chloride in hydrochloric acid is added and the vessel is immediately closed with a screw-cap with septum. and agitated in water bath for 2 hr at 80°C . After 30 min the glasses are shaken upside-down in such a way that all sample parts that could have possibly stuck to the cap get contact with the hydrolysis reagent. The reaction mixture is cooled to 30°C in a cooling water bath. One mL of the isooctane-phase is pipetted into a GC vial for analysis.

22.2.1.2.1.1 Nuts, cereals, pulses, oil seeds, cereal products and dried fruits

Weigh out a 50 gm (± 1 %) representative portion of the sample and add 45 mL of water and 25 mL of isooctane and continue with the cleavage reaction a described above

22.2.1.2.2.2 Dried herbs

Weigh out 10 gm (± 1 %) of the sample and add 50 mL of water and 25 mL of isooctane and continue with the cleavage reaction as described above.

22.2.1.2.2 GC Conditions:

GC equipped with auto sample and ECD detector

Column: 60 m x 0.25 mm i.d.1 µm, CP-SIL 8CB

Carrier Gas: Helium, constant flow 1 mL/min

Injection volume: 2 µL

Sample injection: splitless; splitless time 0.20 min

Injector Temperature Program: 60°C, initial time: 0. 2 min

1st ramp: heating rate: 10°C/s

Final temperature: 240°C

Keep 1 min

2nd ramp: heating rate: 10°C/s

Final temperature: 260°C

Keep 5 min

GC Oven Temperature Program: 45°C, initial time: 0. 5 min

1st ramp: heating rate: 5°C/min

Final temperature: 80°C

2nd ramp: heating rate: 20°C/min

Final temperature: 260°C

Keep: 15 min

22.2.1.2.3 Reference:

1. Community Reference Laboratories for Residues of Pesticides Version 2, Document History in page 11EU Reference laboratory for single residue methods

2. Food chemistry (2014), 150, 175-181

NOTE: SOP as per above Reference EU Reference Laboratory for single residue methods is recommended for Fosetyl Aluminium, EthyleneThiourea, Glufosinate, Diquat and paraquat also.

23. ETHYLENE THIOUREA AND ETHYLENE UREA

23.1 Principle:

Ethylene thiourea and ethylene urea are the two major degradation products of dithiocarbamates and are determined by HPLC using a UV detector set at 205 nm.

23.2 Reagents:

(i) Methanol
(ii) Distilled water
(iii) Potassium fluoride
(iv) (suitable, see reference) filter aid
(v) Alumina (neutral)
(vi) Ammonium hydroxide
(vii) n-Hexane (redistilled)

23.3 Apparatus:

(i) Mechanical shaker
(ii) Waring blender
(iii) Rotary vacuum evaporator
(iv) Separatory funnel (250 mL)
(v) Glass column (45 × 1.5 cm i.d.)

23.4 Procedure

23.4.1 Extraction and clean up

(a) Extraction:

A representative ground sample (50 gm) of plant material is blended after adding 15 gm potassium fluoride and 100 mL of methanol: water (3:1 v/v) in a waring blender for two min at high speed. The homogenized material is shaken for 30 min at 25± 2°C on a mechanical shaker and filtered through Buchner funnel with light suction. The filtration was performed by making a 1.5 cm layer of Celite 545 sandwiched between two Whatman No. 1 filter paper. The later is fitted to a filtering flask attached to an aspirator.

Before filtration the Celite layer is washed with solvent mixture methanol: water (3:1 v/v). The filtrate is transferred to a 500 mL conical flask.

(b) Clean up:

The filtrate volume is reduced to approximately 20 mL using a rotary vacuum evaporator at 40°C. The concentrate is transferred to a separatory funnel (250 mL) quantitatively by dilution with 100 mL distilled water. The aqueous mixture is partitioned with n-hexane (3 × 50 mL) by shaking the separatory funnel vigorously for 3min. The aqueous layer is collected into another separatory funnel and the hexane layer was discarded. The aqueous phase is again partitioned with chloroform (2 × 50 mL) by shaking the funnel as above for 3 min. After separation of layers the lower chloroform layer is discarded. The aqueous layer is collected and reconcentrated to 10 mL in a rotary vacuum evaporator at 60°C. The pH of the concentrated extract is adjusted to 8.0 by adding calculated amount of ammonium hydroxide. Chromatographic columns (1.5 cm × 45 cm) are packed with a small plug of cotton and 20 g of activated alumina: (suitable, see reference) filter aid (3:1 w/w). The concentrated alkaline extract is poured slowly into the column and eluted with 100 mL of methanol @ 2-3 mL per min. The elute evaporated to dryness in a rotary vacuum evaporator at 40°C. The residue dissolved in 5 mL, water: methanol (95: 5 v/v) and filtered through membrane filter syringe for ultracleaning. The volume of extract is made upto 10 mL using, water: methanol (95: 5 v/v) and then subjected to HPLC analysis.

23.5 Estimation:

Ethylenethiourea and ethyleneurea residues are determined by using high performance liquid chromatography. Methanol: water (3:97 v/v) is used as mobile phase. ETU is detected at 240 nm and EU is detected at 205 nm.

23.6 Method:

A 20 µL aliquot of the filtered extract is injected into HPLC. The chromatogram thus obtained is compared with that of known standards of ETU and EU for their retention times to identify the peaks in the unknown samples.

23.7 Calculation:

$$\text{ETU/EU } (\mu g/g) = \frac{A_{sam} \cdot V \cdot C}{A_{std} \cdot W} \times F$$

Where,

A sam = Area of the sample

A std. = Area of the standard

V = Volume of the final extract (mL)

W = Weight of the sample (gm)

C = Concentration of standards (ppm)

F = Recovery factor i.e. 100/per cent mean recovery.

23.8 Reference:

1. AOAC. Official method of Analysis. 17th Edition, (2000). pp. 992.31
2. Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol Version 8.1. http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf

24. TRICYCLAZOLE (The LC-MS/MS method for determination of multiresidue analyte may also be used)

24.1 Principle:

The analytical method prescribes the GLC method for estimation of tricyclazole in rice grains and straw.

24.2 Reagents:

- Alumina: Determine moisture content by loss on drying at 110°C, and then add sufficient water to bring total moisture content to 8.0 % and mix thoroughly.

- Acetonitrile, pesticide grade,
- Dichloromethane, analytical reagent, distilled in glass
- Ethyl acetate(AR)
- Methanol(AR)
- Sulphuric acid, 4 N
- Sodium sulfate, anhydrous, analytical reagent, prewashed with methanol
- Tricyclazole, analytical standard. Prepare solutions in acetonitrile at concentration of 1.25, 1.0, 0.5, and 0.2 µg /mL.

24.3 Apparatus:

- Mill Grinder,
- Gas chromatograph, equipped with glass on-column injection and flamephotometric detector.
- Glass vials, 25 mL with aluminum insert screw caps,
- PH Meter
- Rotary vacuum evaporator,
- Folded Filter paper,
- Chromatography columns, 250 mm × 14 mm i.d. equipped with 250 mL solvent reservoir and removable Teflon stopcock.

24.4 Gas chromatographic conditions:

Instrument	:	GC (or equivalent) equipped with flame photometric detector operating in the sulfur mode.
Column	:	Capillary column DB1, 30 mts × 0.3 mm id or

equivalent provided standardization is done.

Column Temperature	:	220°C
Injector temperature	:	250°C
Decto-Detector temperature:		210°C
Nitrogen carrier gas	:	55 mL/min
Hydrogen FPD gas	:	55 mL/min
Air FPD gas	:	60 mL/min
Oxygen FPD gas	:	10 mL/min
Electrometer	:	2.56×10^{-9} A full scale

Retention time of tricyclazole is approximately 5.7 min.

24.5 Experimental Procedures:

i ***Sample Preparation***

(a) Whole rice grain. Grind the whole grain in Mill at the fine setting. Place a representative 25 gm sample in a 250 mL boiling flask. Add 100mL of 4 N sulphuric acid and reflux samples for 1hr. Proceed to (ii) below.

(b) Rice straw. Freeze the rice straw in liquid nitrogen and immediately grind. Place a representative 10 gm sample of straw in a 250 mL boiling flask. Add 125mL of 4 N sulphuric acid and reflux for 1 h. Proceed to (ii) below.

ii. ***Partitioning Cleanup***

Filter the extracts from the rice tissues through folded filter paper. Cool the extracts, neutralize with 50% sodium hydroxide, and adjust the pH to 7.0 with 2 N sodium hydroxide. Transfer the neutralized solution to a 500 mL separatory funnel and extract with two 100 mL portions of ethylacetate. If emulsion persists, transfer the mixture to a centrifuge jar and centrifuge for approximately 10 min. Pass the ethyl acetate extracts through anhydrous sodium sulfate into a 250 mL boiling flask. Evaporate to dryness using a rotary vacuum evaporator at a 50°C.

iii. ***Alumina column procedure***

Prepare a column consisting of 10 gm of 8 % deactivated alumina and 2 cm of anhydrous sodium sulfate (top) in a 14 mm i.d. × 250 mm glass chromatographic column containing 25-30 mL of dichloromethane. Drain the liquid to the top of the column packing. Dissolve the residue from (i.a) or (ii) above in 25 mL of dichloromethane and place on the column. Drain the liquid to the top of the column packing.

Rinse the flask with two 20 mL portions of dichloromethane and pour each rinse over the column. Wash the column with an additional 25 mL of dichloromethane and discard all eluates to this point. Elute the tricyclazole from the column with 70 mL of dichloromethane: methanol (99:1 v/v). Collect the elute in a 125 mL boiling flask andevaporate to dryness using a rotary vaccum evaporator and 50°C water bath. Dissolve the residue in 1.0 mL of acetonitrile and determine tricyclazole by GC-FPD.

iv. ***Gas Chromatography***

The response of the flame- photometric detector is not a linear function of concentration when operated in the sulfur mode. It is therefore imperative to prepare standard response curves for tricyclazole. Establish the instrumental condition desribed in I, c. Inject 3.0 or 5.0 D of the tricyclazole standard solutions (0.2 - 1.25 g/mL). Prepare standard curve for the identical volumes of sample solutions. Dilute samples, if necessary, to obtain responses within the range of the standard curve. Determine the concentrations of sample and recovery from the standard curves and calculate results as described below. Use any acceptable technique for measuring peak response.

24.6 Calculation:

$$\text{Tricyclozole } \mu g/g \text{ (ppm)} = \frac{A_1 \times V_2 \times V_3 \times C}{A_x \times V_1 \times M}$$

Where,

A_1 = Peak area of the sample

V_2 = Volume in µL of standard injected

V_3 = Total volume in mL of the sample solution

C = Concentration in ppm of the standard solution

A_2 = Peak area of the standard

V_1 = Volume in µL of the sample solution injected and

M = Mass in gm of the sample taken for analysis.

24.7 Reference:

1. Zwig, G. Analytical methods for pesticides and plant growth regulator methods. Academic Press, Volume XI (1980), pp. 265-273
2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456
3. J. AOAC International Vol. 90, (2), 485-520

25. TRIADIMIFON AND TRIADIMINOL

25.1 Introduction:

Bayleton fungicide contains triadimefon as its active ingredient. The active component of Bayfidan fungicide is triadimenol.

25.2 Description of method:

The compounds are extracted from cereal samples (green matter, grains, straw) and from hop cones with acetone/water (2:1). Other plant material is extracted with acetone only. Soil samples are extracted by refluxing with methanol/ water (7:3). After filtration, extracts of plant samples are saturated with sodium chloride, and the compounds are then extracted with dichloromethane. Following filtration of soil extracts, the methanol is evaporated, and the aqueous residue is shaken out with dichloromethane. Water samples are directly extracted with dichloromethane.

The dichloromethane extracts are dried on sodium sulphate, and then rotary-evaporated to dryness. The resultant residue of plant samples is cleaned up firstly by column chromatography on silica gel and then by gel permeation chromatography on (suitable, see reference) polystyrene gel. The residue from soil and water samples need not be subjected to column- chromatographic clean up on silica gel. After the compound-containing eluates have been rotary-evaporated, the compounds are measured by gas chromatography using a thermionic nitrogen/ phosphorus detector (TID).

25.3 Apparatus:

- High-speed homogenizer
- Glass jars, 1000 mL, wide neck, with ground joint
- Vacuum filtration flasks, 1000 mL
- Porcelain Buchner funnel, 110 mm i.d. with fast flow-rate filter paper, 110 mm dia.
- Glass funnels, 100 mm dia.
- Round-bottomed flasks, 100 mL, 250 mL, 500 mL, 1000 mL, with ground joint
- Reflux condenser
- Heating mantles for 1000 mL round-bottomed flasks
- Graduated cylinders, 100 mL, 250 mL, 500 mL
- Volumetric flasks, 25 mL, 50 mL, 100 mL with ground joint
- Bulb pipettes, 1 mL, 3 mL, 5 mL and 10 mL
- Separatory funnels, 250 mL, 500 mL, 1000 mL with ground joint
- Test tubes with ground joint, 10 mL
- Rotary vacuum evaporator with water bath, bath temperature of 40 °C
- Chromatographic column, 17.5 mm i.d., 300 mm long, extended outlet with Teflon stopcock.
- Gel permeation chromatograph Autoprep 1002
- Chromatographic column, 25 mm i.d., 600 mm long, packed with 25 x 320 mm Bio
- Beads S-X 3, 200-400 mesh, preswollen in eluting mixture 3; rate of elution : 5.0 mL/min.
- Syringe with Luer-lock fitting, 10 mL

- Syringe, 10 µL
- Gas chromatograph equipped with thermionic N/P detector (TID)
- 1mV Recorder or integrator.

25.4 Reagents:

- Acetone
- Cyclohexane
- Dichloromethane
- Ethyl acetate
- Methanol
- Toluene
- Acetone: water mixture 2 : 1 (v/v)
- Eluting mixture 1. Cyclohexane: ethyl acetate 85:15 (v/v)
- Eluting mixture 2. Cyclohexane: ethyl acetate 20 : 80 (v/v)
- Eluting mixture 3. Cyclohexane: ethyl acetate 1 : 1 (v/v)
- Methanol: water mixture 7 : 3 (v/v)
- Parent compound standard solutions in ethyl acetate : 0.1-100 gtriadimefon/ mL and 0.2-200 g triadimenol/ mL
- Sodium chloride, analytical reagent grade
- Sodium sulphate
- Filter aid, e.g. Celite 545
- Silica gel 60 %, 0.063-0.200 mm, 70-230 mesh (suitable, see reference)
- Bio Beads S-X 3 polystyrene gel, 0.037-0.074 mm, 200-400 mesh
- Glass wool
- Cotton wool, chemically pure
- Air, re-purified
- Nitrogen, re-purified
- Hydrogen, re-purified.

25.5 Analytical procedure

25.5.1 Extraction

25.5.1.1 Cereals and hop cones

Introduce the sample material [50 gm of cereal green matter, 50 gm of cereal grains, 25gm of cereal straw or 10 gm of hop cones] into a 1-1 glass jar, add 450 mL acetone: water mixture (2:1), and macerate with the homogenizer for approx. 3 min. Add approx. 15 gm filter aid, swirl the glass jar several times, and filter hemaceate with gentle vacuum througha Buchner porcelain funnel. Rinse the glassjar and the Filter cake two times with 100 mL portion acetone: water mixture (2:1). Dry the filter cake by vacuum suction, and discard. Transfer the filtrate to a 1-L separatory funnel, saturate with approx. 40 gm sodium chloride, and then shake out with 100 mL dichloromethane. Allow the phases to separate, and discard the lower aqueous phase. Drain the organic phase into a 1-L round-bottomed flask, and rotary-evaporate to a volume of approx. 40 mL.

Add 25 mL dichloromethane, and dry thes olutionon approx. 30 gm sodium sulphate. Next filter through cotton wool plug covered with an approx. 3 cm layer of sodium sulphate in a funnel, and collect in a 500 mL round-bottomed flask. Rinse the1L round bottomed flask and the sodium sulphate three times with 50 mL portions of dichloromethane. Rotary evaporate the filtrate to dryness. Continue to process the residue as described below.

25.5.1.2 Plant material with a high water content, e.g. fruit and vegetables

To 100 gm plant material (apples, bananas, pears, cucumbers, melons, peppers, peaches, tomatoes, grapes, and sugar beet) add 200 mL acetone in a 1-L glass jar, and macerate for ~ 3 min using a homogenizer. Continue to process the sample as described above.

25.5.1.3 Water

Shake out 400 mL water three times with 200 mL portions of dichloromethane. If the water samples available for analysis are of a smaller size (e.g. 100 mL), use appropriately reduced volumes of di chloromethane to extract them. Filter each organic phase separately through a ~ 3 cm layer of sodium sulphate retained in a glass funnel by a loose plug of cottonwool. Collect the filtrate in a 1L round-bottomed flask. Rinse the sodium sulphate three times with 25 mL portions of dichloromethane. Rotary-evaporate the filtrate to dryness, and process the residues as described below.

25.5.2 Column chromatography on silica gel

Pack the chromatographic tube in the following order: 10 mL toluene, cottonwool plug, 15 gm silica gel slurried in toluene (to a level of about 130 mm), ~ 10 mm layer of sodium sulphate, loose plug of glass wool. Drain the level of the toluene down to the top of the sodium sulphate layer.

Dissolve the residue derived from 5.1.1 or 5.1.2 in 10 mL toluene, and pipette the solution onto the column. Allow the solution to trickle down to the top of the sodium sulphate layer, and then rinse the flask two times with 10 mL portions of eluting mixture 1. Rinse the column with the washings (10, 10 mL) and with an additional 80 mL of eluting mixture 2. Collect the eluate in a 250 mL round-bottomed flask, and rotary-evaporate to dryness. Continue to process the residue as described below.

25.5.3 Gel permeation chromatography

Elution volumes ranging from 100 to 130 mL were determined for triadimefon and triadimenol on (suitable, see reference) polystyrene gel using eluting solvent mixture 3 pumped at a flow rate of 5.0 mL/min. The elution volume range should be checked after approx. 500 runs, and re-determined whenever a new gel permeation chromatographic column is used. Collect the compound eluates (elution volume ranging from 100 to 130 mL) in a 100mL round-bottomed flask and rotary-evaporate. Dissolve the residue in a given volume (V_{End}, e.g. 5 mL) of ethyl acetate. Transfer the solutions to a test tube with ground joint (sample solution).

25.5.4 Gas chromatographic measurement

Inject 5 µL ($V_{i.}$) of the sample solution (V_{End}) derived from above step into the gas chromatograph. Then inject 5 µL of the appropriate standard solution (W_{st}). If the sample solution has an excessive content of compound, dilute aliquots of the solution with ethyl acetate to a final volume appropriate to the sensitivity of the detector, and that ensures that the peak areas of the sample solution (F_{gt}) and the standard solution (F_A) are of gt comparable size. Repeat each injection for control.

25.5.4.1 Gas chromatographic conditions

Gas chromatograph: Equipped with a thermionic nitrogen/phosphorus detector (TID).

Columns: 1. Glass tube, 3 mm i.d., 180 cm long, packed with 1.5 % SP 2250+95 % SP 2401 on Supelcoport, 100-120 mesh.

2. Glass tube, 3 mm i.d., 180 cm long, packed with 3.8 % SE 30 on chromosorb W-HP, 80-100 mesh

	Column 1	Column 2
Injection port temperature	250-280°C	280°C
Column temperature	210-225°C	190-205°C
Detector temperature	250-350°C	350°C
Nitrogen carrier gas flow rate	35-45 mL/min	4.5 mL/min
Hydrogen flow rate	4.5 mL/min	4.5 mL/min
Synthetic air flow rate	175 mL/min	175 mL/min
Attenuation and range	1×10^{-11}	1×10^{-11}
Recorder chart speed	5 mm/min	5 mm/min
Injection volume	5µL	5µL
Retention times	2.9-3.6 min	2.7-4.4 min
Triadimefon	3.7-4.5 min	3.5-5.3 min
Triadimenon	250-280°C	280°C

25.6 Evalution:

Quantitation is performed by measuring and comparing the peak areas of the sample solution with those of standard solutions, using an integrator [external standardization method]. Equal volumes (5µL) of the sample solutions and the standard solutions are injected. Detector linear response ranged from 0.5 to 50ng for triadimefon and from 1.0 to 100 ng for triadimenol.

25.7 Reference:

1. Pt'Pflonzenschutz-Nachrichiten Bayer 17, (1984) pp. 86-93

2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456.
3. J. AOAC International Vol. 90, (2), 485-520.

Note: LC-MS-MS method may also be used

26. METALAXYL

26.1 Principle:

The method prescribes a gas chromatographic method for the determination of residues of metalaxyl in food commodities. However, the LC-MS/MS method for determination of multiresidue analyte may also be used.

26.2 Apparatus

26.2.1 Gas Chromatograph

A GC fitted with an alkali-flame inoization detector (AFID) and printer- plotter-cum-integrator. The following operating parameters are suggested, which can be changed provided standardization is done.

Alkali- Flame Inoization Detector (AFID)

Column: Glass, 100 cm length × 2mm I.D.; packed with 3% carbowax 20 M on gas Chrom Q DMCS treated (size: 0.15-0.18 mm)

Temperature

Column Oven	-	185°C
Injection Port	-	240°C
Detector	-	240°C
Flow Rate	-	Nitrogen; 35 mL/ min
Flow Rate	-	Hydrogen; 35 mL/min
Air and Flow Rate	-	230 mL/min

Microlitre Syringe - 10µLcapacityBlender or equivalent Centrifuge

Rotary Evaporative Concentrator Sample Grinder Mechanical Shaker Ultrasonic Bath

Chromatographic Column- glass, 200 cm × 18 mm I.D. with ground glass top

26.3 Reagents:

- Methanol-glass redistilled
- Saturated Sodium Chloride Solution
- AR grade n-hexane- glass redistilled
- Diethyl ether- glass re-distilled
- Dichloromethane- glass redistilled
- Reference Standard Metalaxyl - of known purity
- Sodium Hydrogen Carbonate- AR grade
- Alumina Acidic- W 200, activity grade V (19 % water added)

26.4 Extraction

26.4.1 Vegetable and fruits:

Shred the entire sample with food cutter. Weigh 100 gm representative sample into a 500 mL wide mouth jar and add 200 mL methanol. Macerate with the high speed blender for 2-3 min. Shake the bottle for 2 hr on a mechanical shaker. Filter through Whatman 40 or equivalent filter paper on a Buchner funnel, using suction. Rinse the jar and wash the filter-cake twice with 40 mL of methanol each time. Adjust the volume to 400 mL and take an aliquot of 200 mL corresponding to 50 gm sample.

26.5 Partitioning:

Transfer the corresponding extract-aliquots to a 100 mL separatory funnel and add 20mL saturate sodium chloride solution. Extract the aqueous solution three times with 75 mL of dichloromethane by vigorously shaking the separatory funnel during each

extraction. Collect the dichloromethane phase and filter through a plug of cotton and evaporate to dryness using a rotating evaporator at 40°C.

26.6 Clean-up:

Fill the chromatographic column with n-hexane. Add alumina acidic to a height of 7 cm (30 mL of volume). Drain the solvent to the top of the alumina. Dissolve the residue from partitioning in 5 mL of n-hexane by immersing the flask an ultrasonic bath for 3 min. Transfer this solution to the column. Rinse the flask two times with 5 mL of n-hexane and transfer each portion to the column. Rinse the flask and then the column with 100mL of n-hexane. Elute the active ingredient with 80 mL of the mixture of n-hexane: diethyl either (1:1). Collect the eluate and evaporate to dryness in a rotatary evaporator at 40°C.

Transfer the residue with 3 increments each of 5 mL diethyl either to 25 mL vials and evaporate to dryness in a stream of clean air. Dissolve the residue for gas chromatographic determination in 1 mL n-hexane: ethanol (1:1) mixture.

26.7 Estimation

26.7.1 Preparation of Standard Curve:

Prepare solutions containing 2.5 to 10 (unit) preference standard metalaxyl in one mL of n-hexane-ethanol (1:1) mixture. Inject a minimum of 4 different amounts of metalaxyl ranging from 2.5 to 10 mg for AFID detector. Measure the peak areas and plot them against the active ingredient on a log-log scale.

Inject into the gas chromatograph, with the help of a microlitre syringe, a suitable aliquot of the sample. Identify the peaks by the retention times and measure the peak areas. Estimate the metalaxyl content by comparison with the standard curve.

26.8 Calculation:

$$\text{Calculation Metalaxyl Residue, ppm} = \frac{A1 \times V2 \times V3 \times C}{A2 \times V1 \times M} \times f$$

Where,

A1 = peak area of the samples;

V2 = volume, in µL of standard metalaxyl solution injected;

V3 = total volume, in mL, of sample solution;

C = concentration in ppm, of the standard metalaxyl solution;

$$F = \text{recovery factor} = \frac{100}{\text{Percent mean recovery}}$$

A2 = peak area of standard metalaxyl;

V1 = volume in µL of sample solution injected;

M = and mass, in gm, of sample taken for analysis.

26.9 Reference:

1. Indian Standard 14161: (1994)

2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456

3. J. AOAC International Vol. 90, (2), 485-520

27. TRIAZOLE FUNGICIDES (The LC-MS/MS method for determination of multiresidue analyte may also be used)

27.1 Principle:

Triazole pesticide/ fungicide residues extracted from plant material and soil with acetone are filterd, evaporated to small quantities, diluted with water and saturated sodium chloride solution, and partitioned into dichloromethane. Water samples are diluted with saturated sodium chloride solution, and extracted with dichloromethane. The dichloromethane extracts are rotary evaporated to dryness and the residues are cleaned up by column chromatography on aluminium oxide.Triazoles are determined by gas chramatography using Nitrogen Phosphorus Detector (NPD).

27.2 Experimental

27.2.1 Apparatus:

- Analytical balance with sensitivity of 0.1 mg
- Top loading balance with sensitivity of 1 mg
- Gas chromatograph with Gas Chromatographic column: DB-5, megabore, 30 m long, 0.53 mm i.d. 0.5 µm film thickness (or) similar
- Nitrogen Phosphorus Detector (NPD)
- Mixer grinder
- Laboratory mechanical shaker
- Conical flasks
- Volumetric flasks
- Buchner funnel
- Vacuum flask/filtration flask
- Separatory funnels
- Filter paper, Whatman No.l
- Round bottomed flasks
- Rotary vacuum evaporator
- 40 °C bath temperature
- Chromatographic tubes, 18 mm x 450 mm
- Micro syringe, 10 µL

27.2.2 Reagents:

- Reference standards of known purities of Hexaconazole, Propiconazole, Penconazole, Myclobutanil, Triademifon and Triadiminol

- Acetone.
- Dichloromethane
- Toluene
- n-Hexane
- Sodium chloride
- Anhydrous sodium sulphate
- Aluminium oxide activity grade V: Take 100 gm .Aluminium oxide neutral active in a 300 mL Erlenmeyer flask (with ground joint) and add 15 mL of water drop wise with continuous swirling. Immediately stopper the flask with a ground glass stopper, shake vigorously until all lumps disappeare and store in a tightly stoppered container for at least 2 hr before use.

27.3 Standard solutions:

Prepare stock solution by dissolving 0.01 gm reference pesticide standards in 100 mL n-hexane (add acetone if required for solubilization). For GC determinations prepare a mixed pesticide standard solution by taking suitable aliquots from the above solutions in to 100 mL volumetric flask and diluting them with ethyl acetate.

27.4 Sample preparation:

Finely chop the samples of fruits, vegetables, forages, straw, etc. and mix well in a mixer grinder.

27.5 Extraction:

Plant materials: Weigh 50 gm of ground plant, fruit, vegetable, into 500 mL conical flask and 200 mL acetone. Shake the container for 2 hr on a mechanical shaker at slow to moderate speed. Filter under suction through a Whatman no. 1 filter paper placed on a Buchner funnel attached to a vacuum flask and wash the filter cake with three more 25mL portions of acetone. Pool the filtrate and washings into a 500 mL round bottom

flask and rotary evaporate to about 50 mL volume. Transfer the filtrate quantitatively into 1000 mL separatory funnel, add about 400 mL double distilled water and 50 mL saturated sodium chloride solution. Extract three times with 100 mL portions of redistilled dichloromethane. Filter the dichloromethane phases through anhydrous sodium sulphate into a 1000 mL round bottom flasks and rotary evaporate to near dryness.

Water: Take 500 mL water sample in a separatory funnel, add 50 mL saturated sodium chloride solution and extract successively with three 100 mL portions of dichloromethane. Filter the dichloromethane extract through anhydrous sodium sulphate into 1000 mL round bottom flask and rotary evaporate to near dryness.

27.6 Clean-up:

Place a cotton plug at the bottom of a chromatographic tube and pack it with 5 gm anhydrous sodium sulphate, 30 gm aluminium oxide of activity grade V and 5 gm anhydrous sodium sulphate in tandem using n-hexane. Drain the excess solvent from the column until the level falls to the top of the packing. Transfer the extract from the above step quantitatively using three 5 mL portions of dichloromethane: hexane (60: 40, v/v) solvent mixture and elute the column with 135 mL of the same solvent mixture at a flow rate of approximately 2 drops per second. Collect the eluate in a 250 mL round bottom flask and rotary evaporate to dryness.

27.7 Gas Chromatographic determination:

Dissolve the residue derived from the above step in acetone: hexane (2: 8. v/v) mixture and dilute to suitable volume. Inject 1.0 µL of this solution into GC equipped with Nitrogen Phosphorus Detector (NPD) operated under the following suggested parameters.

Column	:	DB-5, Megabore, 30 m long, 0.53 mm i.d., 0.5 µm film thickness
Injector temperature	:	280°C
Detector temperature	:	290°C

Column oven

Initial Temperature (1)	:	200°C
Initial Hold Time	:	10 min
Ramp rate	:	15°C/min
Temperature (2)	:	225°C
Hold time	:	7 min
Ramp rate	:	15°C/min
Temperature (3)	:	280°C
Final hold time	:	5 min
Carrier gas flow rate	:	10 mL/min
Makeup gas for detector	:	30 mL/min
Hydrogen flow rate	:	3.5 mL/min
Air flow rate	:	110 mL/min

27.8 Calculations:

$$\text{Residue of each triazole (mg/kg)} = \frac{As \times C \times D \times VI}{A_{std} \times W \times V2} \times f$$

Where,

As = peak area of each triazole in sample

Astd = peak area of each triazole when standard injected

C = Concentration of ppm of each triazole standard solution

D = Sample dilution (mL)

W = Weight of sample (g)

VI & V2= Volumes of sample and standard injected

$$f = \text{Recovery factor} = \frac{100}{\text{Percent mean recovery}}$$

27.9 Reference:

1. Indian Standard 34 (1272).
2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456
3. J. AOAC International Vol. 90, (2), 485-520

27.10 ANALYTICAL METHOD

System	:	A GC equipped with mass spectrometer, Auto injector and Interfaced with GCMS solution software.
Column	:	HP-1 MS, (30m length × 0.25mm I.D. × 0.1 µm film thickness)
Injection port		
Temperature	:	200 °C
Injection mode	:	Split
Split ratio	:	1:5
Column oven		
Temeprature	:	Initial temperature 160 °C held for 13 min, ramp
		@10 °C /min to 200 °C held for 5 min,

@50 °C /min to 290 °C held for 6 min.

Column flow (Helium) : 1.5 mL/min

Interface temperature : 300°C

Acquisition mode : EI

Retention time in min (Approximately)

1. Tetraconazole-10.36
2. Penconazole-11.94
3. Tricyclazole -13.83
4. Paclobutrazole-14.35
5. Hexaconazole-15.36
6. Diniconazole-17.87
7. Propiconazole -20.30
8. Tebuconazole-20.75
9. Epoxyconazole-21.75
10. Etoxazole-23.41
11. Fluquiniconazole-24.60
12. Difenconazole-26.30

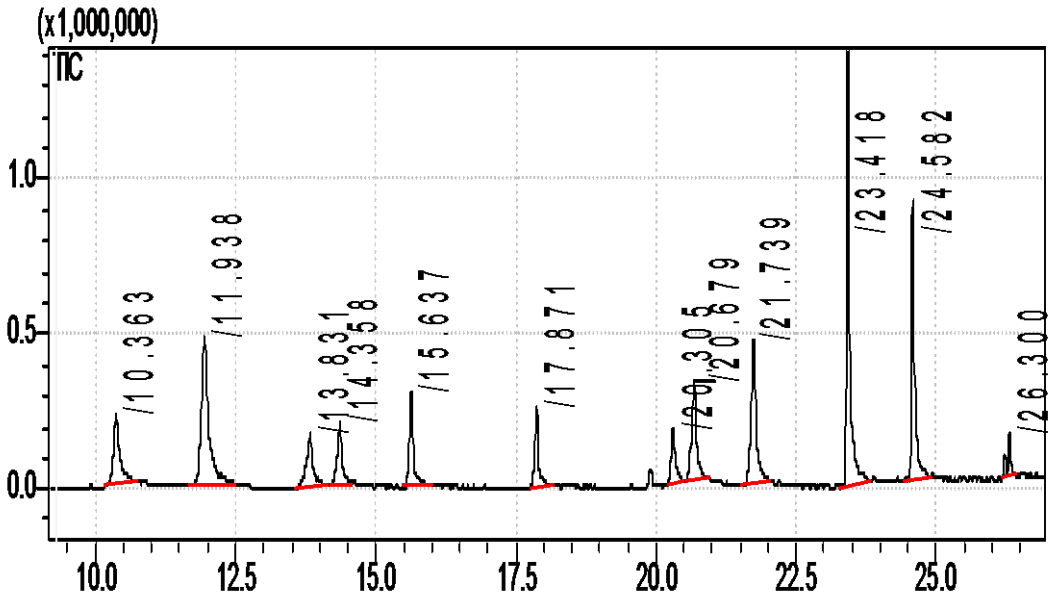

Fig. Representative GC chromatographic profile of standards.

28. CHLORSULFURON AND METSULFURON

28.1 Principle :

This analytical method describes the HPLC method for estimation of residues of chlorsulfuron and metasulfuron in food commodities.

28.2 Reagents:

- Methanol, HPLC quality
- 2-Propanol (isopropanol), HPLC quality
- Toluene, p.a.
- Water, double distilled
- Extraction solution. acetone : buffer solution B (4 : 1 v/v)
- *Mobile phase*: Prepare a mixture consisting of 690 mL cyclohexane + 195 mL isopropanol + 115 mL methanol + 2 mL glacial acetic acid + 1 mL of glacial acetic acid-water mixture (9:1 v/v). Mix well, and de-gas before use in a vacuum filtration unit using water jet pump suction.
- *Conditioning solution*: Prepare a mixture consisting of 200 mL isopropanol + 200mL methanol + 200 mL glacial acetic acid + 20 mL water. Mix well, and de-gas before use in a vacuum filtration unit using water jet pump suction.
- *Stock solutions*: 10 mg/100 mL each of chlorosulfuron and metsulfuron methyl in ethyl acetate. Pipet 1 mL of each stock solution into a 100 mL volumetric flask. Remove the solvent with a gentle stream of nitrogen, dissolve the residue and make up to the mark with mobile phase. Chlorsulfuron and metsulfuron-methyl standard solutions: 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 g/mL of each in mobile phase, de-gassed in an ultrasonic bath. The solutions are stable for approx. two weeks when stored in a refrigerator.
- Hydrochloric acid conc. 10 g/100 g and 1 mol/1 hydrochloric acid p.a.
- Sodium sulphate p.a. anhydrous

- Buffer solution A: 10.6 g/L sodium carbonate anhydrous p.a. and 8.4 g/L sodium hydrogen carbonate anhyhdrous p.a.
- Buffer solution B: 0.82 g/l sodium acetate anhydrous p.a.
- Silica gel disposable cartridge: (suitable, see reference)
- Nitrogen, re-purified

28.3 Procedure

28.3.1 Extraction:

28.3.1.1 Cereals

Homogenize (25 gm of grains) with 150 mL extraction solution in the mixer for 2 min. Suction-filter the supernatant liquid through a fast-flow rate filter paper in a Buchner porcelain funnel. Homogenize the residue in the glass jar and suction- filter two times more as above, each time using 120 mL extraction solution (for cereal grains, use 100 mL each time), finally, rinse the glass jar and the filter with a further 80 mL of extraction solution. Transfer the filtrate to a volumetric flask and make up to a definite volume, e.g. 500 mL (V_{Ex}). Next transfers a tenth of this solution (VR) to a 500 mL separatory funnel, add 100 mL buffer solution A, and proceed to clean up step.

28.3.1.2 Water

Transfer 1 L water into a separatory funnel, adjust the pH to 3-4 with hydrochloric acid (1 M) and extract the water three times with 100 mL portions of dichloromethane. Filter the combined dichloromethane phases through a layer of sodium sulphate, contained in a funnel, into a 500mL round-bottomed flask. Rinse the funnel with 50 mL dichloromethane. Add 1 mL glacial acetic acid and rotary evaporate to ~. 1 mL. Transfer the residue into a 50 mL round bottomed flask, using four 5 mL portions of mobile phase to complete the transfer and rotary-evaporate to 1-2 mL. Evaporate the solution to dryness, using a gentle stream of nitrogen, and proceed in clean up by silica gel cartridge.

28.4 Clean up:

Liquid-liquid partition (only cereals)

Shake the extracts derived from above with 50 mL portions of dichloromethane for 3min. discard the organic phase. Break emulsions, if required, by centrifugation. Transfer the aqueous phase to a beaker and acidify, with vigorous stirring, to pH 5-6 (pH meter) with concentrated hydrochloric acid. Further, adjust the pH to 3.5 using hydrochloric acid (10 % w/w). Transfer the solution back to the separatory funnel, rinse the beaker with 5 mL water and 50 mL toluene, also add the rinsings to the separatory funnel, and shake for 3 min. Separate the aqueous layer, drain the organic phase into a 250 mL centrifuge tube, rinse the separatory funnel with 5 mL water, and add the rinsings to the centrifuge tube. Repeat the extraction two times, each time using 50 mL toluene and adding the organic phases to the centrifuge tube. Add a further 10 mL of water to the centrifuge tube if the phase boundary is difficult to see after the third extraction.

Centrifuge for 15 min at 3800 rpm, and then transfer the upper toluene phase into a 250mL round-bottomed flask, using a 50 mL volumetric pipette. Add 30 mL toluene to the aqueous phase remaining in the centrifuge tube, mix centrifuge for 5 min, and likewise pipette off the top layer into the 250 mL round bottomed flask. Add 1 mL glacial acetic acid to the combined toluene phases, and rotary-evaporate to ~ 1 mL. Transfer the residue into a 50 mL round-bottomed flask, using four 5 mL portions of mobile phase to complete the transfer and rotary evaporate to 1-2 mL. Evaporate the solution to dryness, using a gentle stream of nitrogen as in silica gel cartridge step.

28.5 Silica gel cartridge:

Draw 10 mL of the mobile phase into the glass syringe, attach a silica gel cartridge to the syringe, and force the mobile phase through to condition the cartridge packing. Repeat the conditioning with a further 10 mL portion of mobile phase. Next detach the cartridge, pull the plunger out of the syringe, and re-attach the cartridge, dissolve the residue derived above in 1 mL mobile phase and transfer the solution quantitatively into the syringe with the aid of a Pasteur pipette. Rinse the 50 mL flask with 1 mL mobile phase and also add the rinsings to the syringe. Re-insert the plunger into the syringe and force the liquid through the cartridge, collecting the eluate in a 10 mL test tube. Detach the

cartridge, remove the plunger from the syringe, and re-attach the cartridge. Force a further 5 mL mobile phase through the cartridge, proceeding in a similar manner as above, and collect the eluate in the same test tube.

Evaporate the solution to dryness using a gentle stream of nitrogen.

28.6 High performance liquid chromatographic determination:

Dissolve the residue in mobile phase to an appropriate volume. Inject an aliquot of this solution into HPLC.

Chromatograph : A HPLC system

Injector : Rhoedyne

Column : Zorbax Sil, 4.6 mm i.d. × 25 cm

Column temperature: 25°C.

Mobile phase : Cyclohexane-isopropanol-methanol-acetic acid-water.

Flow rate : 0.5 mL/min.

Conditioning solution: Isopropanol-methanol-acetic acid-water

Detector : Photoconductivity detector (suitable, see reference), operated with a mercury lamp at 254 nm, ATT=5

Recorder : 5 mV; chart speed 5 mm/min.

Injection volume : 20 µL

Retention times for chlorsulfuron: 13 min.

Metasulfuron-methyl: 15 min.

The Zorbax Sil column must be conditioned before use. For this end, pump conditioning solution through the column for 4 hr at a flow rate of 0.7 mL/min. Next equilibrate the column for 3 hr with mobile phase at the same rate.

Moreover, pump conditioning solution at a flow rate of 0.15 mL/min through the system over night, changing to mobile phase at a flow rate of 0.5 mL/min for 1 h before beginning a new series of measurements the next morning.

Quantitation is performed by the calibration technique. Prepare calibration curve as follows. Inject equal volumes of each chlorosulfuron and metasulfuron methyl standard into HPLC. Plot area of the heights of the peaks obtained vs. concentration.

28.7 Simultaneous Determination of Chlorsulfuron and Metsulfuron-methyl by LC-ESI-MS/MS

Instrument : HPLC with Bruker HCT Plus LCMS/MS

Ion source : ESI +Ve

Column : Zorbax SB-C18 3.5µ, 75 x 4.6mm I.D

Mobile phase:

A: 0.1 % Formic acid in Water

B: 100 % Acetonitrile,

Flow Programme:

Ramp B, 5 to 95 % upto15min

Flow : 0.5 mL/min,

Injection Volume: 10 µL

Approximate retention time

Metsulfuron-methyl

R.T = 4 .1 min

MRM: 382->167

Chlorsulfuron

R.T = 4.5 min

MRM: 358->167

References:

1. R.V. Slates: Determination of chlorsulfuron residues in grain, straw and green plants of cereals by high-performance liquid chromatography. J. Agric. Food Chem., (1983), 31(1), pp 113-117.
2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456
3. J. AOAC International Vol. 90, (2), 485-520

29. ANILOPHOS (The LC-MS/MS method for determination of multiresidue analyte may also be used)

29.1 Principle:

The analytical method prescribes a GLC method for estimation of anilophos in rice grains.

29.2 Reagents and apparatus:

- n-Hexane, ethyl acetate, toulene, acetone, acetonitrile, methanol: reagent grade
- Hydrochloric acid, Sodium chloride, Sodium sulfate anhydrous : reagent grade
- Silicagel cartridge column: (suitable, see reference)
- Activated carbon cartridge column: (suitable, see reference)
- Diatomite column: (suitable, see reference)
- Disposable plastic syringe
- GC : with NPD
- Capillry column DB-17; (Daimensions)

29.3 GC Conditions:

- Column : DB-17 (i.e. 0.53 x 15 m film thickness 1 m)
- Temperature : Detector: 280°C; Injector: 250 °C
- Column oven temp : Initial : 100 °C (in min); 30°C/min;
  Final: 270 °C (1 min)
- Carrier gas : He : 20 mL/min
- Combustion gas : H : 3 mL/min. Air : 60 mL/min
- Injector : Spirit - less mode

- Injector volume : 2 µL

29.4 Analytical procedure

29.4.1 Extraction

Twenty five grams of pulverised rice grain are weighed into a round bottom flask accurately and 20 mL of water is added, and allowed to stand for2 hr. shake with 100 mL of methanol by strong shaking for 30 min. The extrcts are combined and concentrated to ~ 20 mL using a rotary evaporator at 40°C. The concentrate is transferred to a separately funnel with 100 mL of ethyl acetate. After strong shake, water layer is transferred to another funnel and extracted by newly 100 mL of ethyl acetate. Both extracts are combined and dried by passing hrough the sodium sulfate anhydrous layer. The extract is concentrated to about 1 mL by using a rotary evaporator at 40°C. The concentrate is dried up by nitrogen flow.

29.4.2 Purification

The residue is dissolved in 30 mL of n-hexane (saturated with acetonitrile) and this solution is extracted with two portions of 30 mL acetonitrile (saturated with n- hexane). Acetonitrile layers are combined and concentrated to about 1 mL using a rotary evaporator at 40°C. The concentrate is dried up using a flow of nitrogen.

29.4.3 Activated carbon CC

The residue is dissolved with 10 mL of acetone and transferred to a plastic syringe with an activated carbon cartridge column which is conditioned by loading with 20 mL of acetone, 1 M hydrochloric acid, water and again acetone orderly just before using. The round bottom flask is washed with two portions of 10 mL of acetone and the washing solvent is also transferred to the same plastic syringe, the solutionis loaded and the eluate is concentrated to about 1 mL by using a rotary evaporator at 40°C. Then it is dried up by nitrogen flow.

29.4.4 Silicagel CC

The residue is dissolved with 5 mL of n-hexane: ethyl acetate (95: 5 v/v) and transferred to a syringe with a silicagel cartridge column which is conditioned with 10 mL of n-hexane just before using. The round bottom flask is washed with two portions of 10 mL of n-hexane: ethyl acetate (95: 5 v/v) and the washing combined. The solution is loaded and elute discarded. The column is washed with 10 mL of same solvent and anilophos is eluted with 20 mL of n-hexane: ethyl acetate (8:2 v/v). The column is again washed with 10 mL of n-hexane acetone (2:3 v/v). Each eluate is concentrated to about 1 mL by using a rotary evaporator at 40 °C and dried using a stream of nitrogen. The residue of anilophos is dissolved with small amount of toluene. Volume is made of 5 mL with toluene.

29.4.5 Quantification

Inject 2 µL of each test solution into the GC under conditions described above.

The residue value of anilophos is calculated by the following formula:

$$\text{Residue (mg/kg)} = \frac{[\text{Detected amount (ng)} \times \text{Volume of test solution (mL)}]}{[\text{Sample amount (gm)} \times \text{Injection volume (µL)}]}$$

Reference

1. Hoechst, A.G., Product Development Division, Frankfurt.
2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456.
3. J. AOAC International Vol. 90, (2), 485-520.

30. DICLOFOP-METHYL

30.1 Principle:

The method describes for analysis of diclofop methyl in barley grains, soybeans, wheat grains.

30.2 Outline of method:

The sample material is refluxed for 6 hr with sodium hydroxide solution. Diclofop-methyl is saponified in the process to the sodium salt of 2,[4- (2, 4 dichlorophenoxy) phenoxy]-propionic acid. Next, the reaction mixture is extracted with ethanol, with simultaneous homogenization, and then filtered to separate it from undissolved constituents. The filtrate is acidified with hydrochloric acid, and extracted with a mixture of n-hexane and diethyl ether. The organic phase is washed with water, and extracted with sodium hydrogen carbonate solution. The pH of this solution is then adjusted to 4.5 with hydrochloric acid, and the solution is re-extracted with the hexane-diethyl ether mixture. The organic phase is concentrated, and methylated with diazomethane. Next, diclofop-methyl is extracted with n-hexane from the methanolic solution. The residue remaining after evaporation is taken up in n-hexane from the methanolic solution. The residue remaining after evaporation is taken up in n-hexane, and diclofop-methyl is determined by electron capture gas chromatography.

- Hydrochloric acid, p.a., 5 mol/L
- Potassium hrydroxide solution, 0.5 mol/L potassium hrydroxide p.a.
- Sodium hydroxide solution, 1 mol/L Sodium hydroxide p.a.
- Sodium hydrogen carbonate solution, 5 g/100 mL Sodium bicarbonate p.a.
- Sodium sulphate, p.a., anhydrous
- [Pre-coated (suitable, see reference)
- Argon : methane mixture (95 : 5 v/v)
- Nitrogen, re-purified

30.3 Procedure

30.3.1 Saponification and extraction

Weigh 25 gm of the analytical sample (G) into a 1-1 round-bottomed flask, and reflux with 250 mL sodium hydroxide solution for 6 h. Allow to cool, and add 250 mL ethanol through the condenser. Transfer the mixture to a beaker, homogenize thoroughly with an ultra-Turrax, and filter through a glass filter funnel. Dissolve the filter cake in 150 mL ethanol-water mixture, homogenize with the Ultra-Turrax, and filter again. Combine the filtrates, adjust pH to 2.0 with Hydrochloric acid, and extract three times with 200 mL of hexane-diethyl ether mixture.

30.3.2 Cleanup

Wash the combined hexane-diethyl ether extracts two times with 100 mL ethanol-water mixture. Discard the water phase. Extract the free acid (formed from diclofop-methyl) from the organic phase by extracting three times with 100 mL portions of sodium hydrogen carbonate solution. Combine the aqueous phases, wash with 100 mL n-hexane, and adjust pH to 4.5 with hydrochloric acid. Extract the water phase three times with 100 mL portions of hexane-diethyl ether mixture. Combine the organic phases; wash with 50 mL water, and dry over 20 gm anhydrous sodium sulphate. Then filter and re-wash. Concentrate the combined solutions to approx. 5 mL in a rotary evaporator.

30.3.3 Reaction with diazomcthanc

The apparatus for generating diazomethane consists of a small washbottle about half-filled with diethyl ether, a reaction vessel with dropping funnel, and a methylating vessel filled with the solution derived from above step. The methylating vessel is followed by two more wash bottles in which excess diazomethane is indicated and destroyed, respectively. These two washbottles are filled with 2 mL acetone and acetic acid, respectively. Add 1 mL diethyl ether, 2 mL alkaline diethylene glycol solution and one small stirring rod into the reaction vessel. Place the reaction vessel in a beaker containing hot water, switch on the magnetic stirrer, and slowly pass nitrogen through the apparatus. Then slowly add the methylating reagent dropwise, and continue to

sweep the diazomethane with a nitrogen current into the methylating vessel until the solution is coloured pale yellow.

30.3.4 Isolation of reaction products

Transfer the solution from above to a 100 mL round-bottomed flask, and rotary-evaporate almost to dryness. Transfer the residue remaining after evaporation to a separatory funnel with successive washings of 75 mL methanol-water mixture and 75 mL n-hexane. Shake to partition diclofop-methyl into the hexane phase. Separate the lower phase, and re-extract with 75 mL n-hexane. Combine the hexane phases, wash with 50 mL water, transfer to a 500 mL round-bottomed flask, and concentrate to ~ 5 mL in the rotary evaporator.

Quantitatively rinse the residual solution with n-hexane into an evaporating dish, and evaporate cautiously under infrared light and ventilation. Then rinse the residue with n-hexane into a graduated tube (with ground joint), and make up to 2.5 mL (V_{End}).

30.3.5 Gas chromatographic determination

Operating conditions:

Column: Chromosorb W-HP, 100-120 mesh

Column temperature: 220 °C – Hold 4 min Ramp 5 °C – 260 Hold 10 min

Injection port temperature: 270 °C

Detector : Electron capture detector (^{63}Ni)

Temperature 280°C

Carrier gas flow rate : Helium mixture, 45 mL/min

Attenuation : 10-16

Recorder : 1mV; chart speed 75 cm/h

Injection volume1 : 2 µL

Retention time for diclofop-methyl: 2 min

Capillary Chromatographic Conditions:

Instrument	-	A Gas Chromatograph equipped with Electron Capture Detector, Auto injector coupled with computer software.
Column	-	DB-1, Megabore Column or equivalent (30m × 0.53mm × 1.5µm film thickness).
Gas flow rate		
Nitrogen (N_2)	-	100 Kpa
Temperature conditions		
Oven Initial	-	220 °C
Holding time at 220 °C	-	4 min
Heating rate upto 260 °C	-	20 °C/min
Oven final	-	260 °C
Holding time at 260 °C	-	4 min
Injector	-	270 °C
Detector	-	280 °C
Volume injected	-	1 µL
Column flow rate	-	10mL/min
Injection Mode	-	Split
Split ratio	-	5
Solvent	-	Acetone

Retention time (approximate)

Diclofop Methyl (*Standard*) - 7.40 min

30.4 Evaluation

30.4.1 Method

Quantitation is performed by measuring the peak areas or peak heights of the sample solutions and comparing them with peak areas or heights of standard solutions of known concentration. Equal volumes of the sample solutions and the standard solutions should be injected; additionally, the peaks of the solutions should exhibit comparable areas or heights.

30.5 Recoveries and lowest determined concentration

Recoveries from untreated control samples fortified with diclofop-methyl at levels of 0.01-1.0 mg/kg ranged from 80 to 100 % and averaged 90 %. The routine limit of determination was about 0.01 mg/kg.

30.6 Calculation of residues

The residue R, expressed in mg/kg diclofop-methyl, is calculated from the following equations:

$$R = F_A . V_{End} . W_{st} / F_{st} . V . G$$

(Without cleanup)

$$R = F_A . V_{EndR} - W_{st} . V_{End} / F_{st} . Vl . V_M . G$$

(With cleanup)

Where,

G = sample weight (in gm)

V_{End} = terminal volume of sample solution (in mL)

V_M = portion of volume V_{End} used for cleanup (in mL)

V_{EndR} = terminal volume of cleaned-up sample solution (in mL)

V. = portion of volume V_{End} (without cleanup) or of volume V_{EndR} (with cleanup) injected into gas chromatograph (in L)

W_{st} = amount of diclofop-methyl injected with standard solution (in ng)

F_A = peak area or height obtained from V. (in mm^2 or in mm)

F_{st} = peak area or height obtained from W_{st} (in mm^2 or in mm).

References

1. Hoechst, A.G., Analytical Laboratory, Frankfurt-Hochst, S. Gorbach andK. Kunzler. (Complete reference)

2. Deutsche Forschungs - Gemernschaft HCH Verlogsgell-Schft Weinhein-FRG, Manual of Pesticide Residue Analysis. (1987). pp. 128-133.

3. J. Agric. Food Chem., 2015, 63 (18), 4449–4456.

4. J. AOAC International Vol. 90, (2), 485-520.

31. GLUFOSINATE

31.1 Principle

31.1 Outline of method

Residues of glufosinate-ammonium, glufosinate and the metabolite are extracted from plant and water. Depending on the type of sample material, the extracts are cleaned up by de-fatting with dichloromethane, by precipitation of carbohydrates and proteins with acetone, or by ion exchange chromatography. From water samples, the residues are concentrated on an anion exchange chromatographic column and eluted with formic acid solution. After evaporation of the cleaned-up extracts or of the eluate, the residues are treated with trimethyl orthoacetate, resulting in the formation of the derivatives: methyl 4-[methoxy (methyl) phosphinoyl]-2-acetamidobutyrate from glufosinate, and methyl 3-[methoxy (methyl) phosphinoyl] propionate from the metabolite. The

derivatives are cleaned up on a mini silica gel column and are determined by gas chromatography using a phosphorus-specific flame photometric detector.

31.2 Apparatus

- Homogenizer
- Erlenmeyer flasks, 500 mL and 300 mL
- Watch glass, 10 cm dia
- Hot plate with magnetic stirrer, incl. stirring rod
- Graduated cylinders, 1-1, 100 mL, 50 mL and 2 mL
- Centrifuge
- Volumetric pipets, 20 mL and 10 mL
- Round-bottomed flasks, 100 mL and 50 mL, with ground glass joints
- Rotary vacuum evaporator, 60 °C bath temperature
- Separatory funnels, 1 L and 100 mL
- Filter cartridges for organic solvents, pore size 0.5 fn (suitable, see reference)
- Beakers
- Chromatographic tube 1:15 mm i.d., 30 cm long, with stopcock
- Chromatographic tube 2 : 40 mm i.d., 60 cm long, with stopcock
- Ultrasonic water bath
- Solvent dispensers, 15 mL, 8 mL and 2 mL
- Reflux condenser, jacketed coil type, 30 cm long, with ground joint
- Heating mantles, regulated, for 100 mL and 50 mL round-bottomed flasks
- Disposable syringes, 10 mL, with stainless steel needles (flat tip); needles approx. 15 cm long, straight form and angular bent
- Pasteur pipets
- Pear-shaped flask, 10 mL, with ground joint
- Volumetric flasks, 100 mL, 50 mL and 5 mL
- Gas chromatograph equipped with phosphorus-specific flame photometric detector
- Microsyringes, 100 µL and 10 µL

31.3 Reagents

- Acetone, p.a.
- Dichloromethane, p.a.
- Ethanol, p.a.
- Ethyl acetate, p.a.
- Methanol, p.a.
- Methyl acetate, p.a.
- Toluene, p.a.
- Eluting mixture. methyl acetate : methanol (1:1 v/v)
- Solvent mixture 1. methyl acetate : ethanol (1:1 v/v)
- Solvent mixture 2. methyl acetate : toluene (1:1 v/v)
- Solvent mixture 3. methyl acetate : toluene (7:3 v/v)
- Glufosinate and metabolite standard solutions for fortification experiments: 5 g/mL of each glufosinate-ammonium or 3- methylphosphinico) propionic acid (Riedel-de Haen) in water or, for fortification of fats and oils, in solvent mixture 1.
- Glufosinate derivate standard solution: Methyl 4-[methoxy (methyl) phosphinoyl]-2-acetamidobutyrate in eluting mixture, equivalent to 5 g/ml glufosinate-ammonium. Prepare by weighing glufosinate- ammonium and derivatizing.
- Metabolite derivative standard solution: Methyl 3-[methoxy (methyl) phosphinoyl) propionate in eluting mixture, equivalent to 5 µg/mL metabolite. Prepare by weighing 3-(methylphosphinico) propionic acid and derivatizing.
- Polyethylene glycol solution: 10 g/100 mL polyethylene glycol 400 p.a. in acetone — Glacial acetic acid, p.a.
- Hydrochloric acid, 10 g/100 g HC1 p.a.
- Formic acid, 10 mL/100 mL HCOOH p.a.
- Ammonia solution, 10 g/100 mL and 1 g/100 mL NH3
- Sodium hydroxide solution, 1 mol/L Sodium hydroxide
- Trimethyl ortho acetate
- Cation exchanger, Strong active ion exchanger I

Preparation : Add 2 mL hydrochloric acid to 1000 gm cation exchange in a beaker, allow to stand for 30 min, decant, wash the resin with water until neutral (pH indicator paper), and further treat, in the following sequence with :

Ammonia solution (9 g/100 mL), allow standing for 5 min, washing to neutral with water;

Ethanol-water mixture (1:1 v/v), allow to stand for 30 min; Hydrochloric acid (conversion to H form), wash with water until neutral.

Cation exchange column Pour 6 gm of the damp cation exchanger as prepared above into a chromatographic tube.

- Anion exchanger, Strong basic: (suitable, see reference)

  Preparation: Allow the resin (in the chloride form as supplied) to swell in water for 1 h. Pour approx. 100 gm of the swollen resin into a chromatographic tube (type 2), and wash with 750 mL sodium hydroxide solution to convert it into the OH~ form. Wash with water until the eluate is neutral to pH indicator paper.

  Anion exchange column: Pour 6 gm of the damp anion exchanger as prepared above into a chromatographic tube pH indicator paper

- RP-18 disposable cartiridge: (suitable, see reference)Silica gel, deactivated with 4 % water: Heat silica gel 60, 0.063-0.200 mm, for 6 hr at 130°C, allow to cool in a desiccator, and store in a tightly stoppered container in the desiccator. To 100 gm dried silica gel in a 300 mL Erlenmeyer flask (with ground joint), add 4 mL water dropwise from a pipette, with continuous swirling. Immediately stopper flask with ground stopper, shake vigorously for 5 min until all lumps have disappeared, next shake for 2 hr on a mechanial shaker, and then store in a tightly stoppered container.

- Quartzwool
- Air
- Helium

- Hydrogen

31.4 Procedure

31.4.1 Extraction

31.4.1.1 Plant material with water content (apples, asparagus, bananas, beans, Chinese cabbage, kiwi fruit, lemons, mirabellas, oranges, plums, potatoes, sour cherries, sugar beet) and soil

Homogenize 25 gm of the analytical sample (G) with 200 mL water in a 500 mL Erlenmeyer flask. Cover the flask with a watch glass, and magnetically stir the homogenate for 30 min at 25±2°C, prepare the volume of the homogenate (V), and centrifuge a 60 mL portion at 3000 r.p.m. for 10 min. Pipet 20 mL of the supernatant (V_X) into a 50 mL round bottomed flask and rotary-evaporate to dryness, using a 60 °C bath temperature. Add 5-10 mL ethyl acetate to the residue and rotary-evaporate to dryness again to remove residual traces of water. Repeat if required until the residue is absolutely dry.

31.4.1.2 Plant material with high content of water soluble carbohydrates or proteins (almonds, caraway, maize, peas, soybeans, wheat)

Homogenize 25 gm of the analytical sample (G) with 200 mL water in a 500 mL Erlenmeyer flask. Cover the flask with a watch glass, and magnetically stir the homogenate for 30 min at room temperature. Measure the volume of the homogenate (V_{Ex}), and centrifuge a 60 mL portion at 300 r.p.m. for 10 min. Take 40 mL of the supernatant (V) and add 40 mL acetone to precipitate carbohydrates and proteins (total volume, V_2). Centrifuge the mixture again. Pipet 40 mL of the supernatant (V_3) into a 100mL round bottomed flask and rotary-evaporate to dryness, using firstly room temperature, then a 60°C bath temperature. Add 5-10 mL ethyl acetate to the residue and rotary-evaporate to dryness again to remove residual traces of water. Repeat if required until the residue is absolutely dry.

31.4.1.3 Fat containing plant material (rape seed, sunflower seeds)

Homogenize 25 gm of the analytical sample with 200 mL water in a 500 mL Erlenmeyer flask (total volume of the homogenate, V_{Ex}). Cover the flask with a watch glass, and magnetically stir the homogenate for 30 min at 25°C. Allow solid particles to settle, then decant 40 mL of the supernatant (V_{R1}) into a centrifuge tube, and add 40 mL acetone (total volume of the mixture, V^). Mix, and centrifuge the mixture at 3000 rpm for 5 min. Next, transfer 40 mL of the supernatant as (V^) into a 100 mL separatory funnel and shake with 20 mL dichloromethane. Separate the lower organic phase and re- extract it two times with 10 mL portions of water. Combine the aqueous phases in a 100 mL round bottomed flask and rotary evaporate to dryness, initially at 25°C, then at 60°C bath temperature. Add 5-10 mL ethyl acetate to the residue and rotary evaporate to dryness again to remove residual traces of water. Repeat if required until the residue is absolutely dry.

31.4.1.4 Fats and oils (primrose oil, sunflower oil)

Dissolve 20 gm of the analytical sample (G) in 100 mL dichloromethane (use the homogenizer for dissolution of solid fat if required). Add 100 mL water (V_{Ex}) and homogenize for 5 min. Decant ~ 60 mL of the aqueous supernatant into a centrifuge tube and centrifuge at 3000 rpm for 5 min. Force 25 mL of the supernatant (V_{R1}) through a RP-18 disposable cartridge by forcing through 3 mL water. Collect both the aqueous eluate and the wash in a 50 mL round-bottomed flask and rotary evaporate to dryness, using a 60 °C bath temperature. Add 5-10 mL ethyl acetate to the residue and rotary-evaporate to dryness again to remove residual traces of water. Repeat if required until the residue is absolutely dry.

31.4.1.5 Water

Before concentrating glufosinate and its metabolite on an anion exchange column, the cations present in the water must be exchanged for H^+. For this purpose, the ion exchange columns are connected in series, so that the eluate from the cation exchanger flows directly onto the anion exchanger.

Using a 1L separatory funnel, transfer 1L of the water sample onto the cation exchange column at a rate of 5-10 mL/min. Wash both columns with 100 mL of distilled or deionized water, and discard the eluates. Remove the cation exchange column. Next elute glufosinote and its metabolite with 70 mL dilute formic acid into a 100 mL round-bottomed flask. Add 100 ^polyethylene glycol solution to the eluate and rotary-evaporate to dryness, using a 60 °C bath temperature. Add 5-10 mL ethyl acetate to the residue and rotary-evaporate to dryness again to remove residual traces of water. Repeat if rquired until the residue is absolutely dry.

31.4.2 Derivatization

Suspend the residue derived from extraction step in 2 mL glacial acetic acid by dipping the flask for 10 min in an ultrasonic bath. Add 8 mL trimethyl orthoacetate, dip the flask for a further 5 min in the ultrasonic bath, and then reflux the mixture for 4 h with occasional swirling to prevent suspended solids from shaking onto the walls of the flask.

Allow to cool, add 15 mL toluene, and rotary-evaporate to a residual volume of approx. 1mL (do not evaporate to dryness!) with 40 °C bath temperature. Repeat the evaporation with toluene two more times to completely remove residual acetic acid and derivatization reagent.

Make up the 0.5-1 mL residue to 3 mL with toluene, dip the flask for 1 min in the ultrasonic bath, and draw up the flask contents, including the suspended solid material, into a 10 mL disposable syringe. Rinse the flask with 5 mL ethyl acetate, dip the flask for approx. 2 min in the ultrasonic bath, and also draw up the rinsing into the syringe. Mix the contents well by shaking the syringe.

31.4.3 Insert a quartz wool plug into the bottom of a Pasteur pipet and add 0.6 gm silica gel. Condition the column with 5 mL solvent mixture 2 delivered from a disposable syringe with a 15 cm long stainless steel needle. Using the needle, stir the silica gel solvent mixture until it is free of air bubbles. Filter the solution derived from derivative. Then step in the first syringe through a filter cartridge mounted on the syringe and, with the aid of an angular bent needle, transfer the filtrate onto the mini silica gel column. Rinse the flask from with 10mL methyl acetate. Drawn up the rinsing into the syringe, filter and transfer it onto the column in a similar manner. When the column has run dry,

apply gentle suction to dry the packing. Next, elute the derivatives of glufosinate and its metabolite from the column with eluting mixture, collecting exactly 5 mL eluate (V_{End}) in a 5 mL volumetric flask.

For samples of drinking water, evaporate the 5 mL of eluate to approx. 100 fusing a 40°C water bath and a gentle stream of nitrogen, and make up to 1.5 mL (V_{End}) with eluting mixture.

For most other sample materials, a second cleanup on a mini silica gel column is recommended. Transfer the eluate to a pear-shaped flask using 3 mL toluene and rotary-evaporate (40°C bath temperature) to ~ 1 mL. The methanol must be removed completely. Make up to 3 mL with toluene, and mix with 5 mL methyl acetate. Rechromatograph the mixture on a mini silica gel column as described above, with the exception of the filtration step.

31.4.4 Gas-chromatographic determination

Inject an aliquot of the solution derived from 4.3, e.g. 5 µL (V.), into the gas chromatograph.

Operating conditions:

Column	:	Fused silica capillary, 0.53 mm i.d., 10 m long; coated with Carbowax 20 M, film thickness 0.25 a (Restek) or equivalent
Column temperature	:	Isothermal at 150 °C for 2 min, programmed to rise at 39°C/ min from 150 to 240°C, then isothermal at 240 °C for 4 min
Injection Port temperature:		225 °C
Detector	:	Flame photometric detector, equipped with 526-nm phosphorus filter, Temperature 225 °C
Gas flow rates	:	Helium carrier, 28 mL/min (2314 cm/s), Hydrogen, 75 mL/min Air, 125 mL/min

Injection volume : 5 µL

Retention times for :

Metabolite derivative - 42 s

Glufosinate derivative - 4 min 18 s

Alternative Conditions:

Column : Fused silica capillary, 0.53 mm i.d., 8 m long; coated with RTX- 2330, film thickness 0.25 ffl (suitable, see reference) or equivalent, provided standardization is done.

Column temperature : Isothermal at 140 °C for 2 min, programmed to rise at 39°C/min from 140 to 240°C, then isothermal at 240 °C for 4 min

Gas flow rates : Helium carrier, 25 mL/min (180 cm/s) Helium purge gas, 10 mL/min Hydrogen, 70 mL/min Air, 120

Retention times for :

Metabolite derivative - 1 min 24 s

Glufosinate derivative - 5 min

31.5 Evaluation

31.5.1 Method

Quantitation is performed by measuring the peak areas or peak heights of the sample solutions and comparing them with the peak areas or peak heights obtained from the

derivative standard solutions. Equal volumes of the sample solutions and the derivative standard solutions should be injected; additionally, the peaks of the solutions should exhibit comparable areas or heights.

31.5.2 Recoveries and lowest determined concentration

The recoveries from untreated control samples, fortified with glufosinate-ammonium or metabolite at levels of 0.05 to 10 mg/kg, ranged from 64 to 116 %. The routine limit of determination was 0.05 mg/kg for plant material, fats, oils and soil, 0.05 to 0.1 mg/kg for animal matrices.

$$R = \frac{F_A . V_{Ex} . V_{R2} . V_{End}}{W_{St} / F_{St} . V_{R1} . V_{R3} . V_j . G}$$

Where,

G = Sample weight (in gm)

V_{Ex} = total volume of homogenate or (for fats and oils) volume of water added to the organic phoase for extraction (in mL)

V_{R1} = portion of volume V_{Ex} used for further processing (in mL)

V_{R2} = total volume of the aqueous solution with acetone added (in mL)

V_{R3} = portion of volume V_{R2} used for further processing (in mL)

V_{End} = terminal volume of sample solution from 6.3 (in mL)

V_j = portion of volume V_{End} injected into gas chromatograph (in µL).

W_{St} = amount of glufosinate- ammonium equivalents or metabolite equivalents, respectively, injected with derivative standard solution or “fortified calibration solution” (in ng)

F_A = peak area or height obtained from V_j (in mm^2 or integerator counts)

F_{St} = peak area or height obtained from W_{St} (in mm^2 or integratorcounts)

For water,

$$R = F_A . V_{End} . W_{St} / F_{St} . V_j . G$$

Where,

G = sample volume (in 1)

V_{End} = terminal volume of sample solution (in mL)

V_j = portio of volume V_{End} injected into gas chromatograph (in µL)

W_{St} = amount of glufosinate-ammonium equivalents or metabolite equivalents, respectively, injected with derivative standard solution or "fortified calibration solution" (in mg)

F_A = peak area or height obtained from V_j (in mm^2 or integrator counts)

F_{St} = peak area or height obtained from W_{St} (in mm^2 or integrator counts)

To calculate glufosinate as free acid, multiply the results for glufosinate-ammonium by a factor of 0.91, and the result for the metabolite by a factor of 1.19.

Reference:

Hoechst, A.G., Product Development, Division C, Frankfurt/Main, H. Sochor.

NRL established method for glyphosate, AMPA, glufosinate

32. LINURON (The LC-MS/MS method for determination of multiresidue analyte may also be used)

Principal

32.1 Outline of method

Linuron is alkali hydrolyzed in the presence of the analytical material under reflux conditions. The resultant aniline is simultaneously steam-distilled and extracted from the distillate with isooctane. Following cleanup by column chromatography, the aniline is acetylated with acetic anhydride. The final gas-chromatographic determination can be performed with a GC equipped with either an alkali flame ionization detector or an electrolytic conductivity detector.

32.2 Apparatus

- Pyrex round-bottomed flask, 2-1, with NS 29 ground joint
- Round-bottomed flasks, 250 mL and 100 mL, with NS 29 ground joint
- Blender apparatus, for hydrolysis, distillation and extraction
- Heating mantles, for 2 L and 250 mL round-bottomed flasks
- Chromatographic tube, 1 cm i.d., 20 cm long
- Rotary vacuum evaporator, 45 °C bath temperature
- Gas chromatograph equipped with alkali flame ionization detector or electrolytic
- Conductivity detector
- Microsyringe, 10 µL

32.3 Reagents

- Ethanol, distilled in glass
- Dichloromethane, distilled in glass
- n-hexane, distilled in glass
- Isooctane, pure
- n-hexane : ethanol mixture (1:1 v/v)
- Eluting mixture 1. n-hexane : dichloromethane (7:1 v/v)
- Eluting mixture 2. n-hexane : dichloromethane (1:4 v/v)
- Standard solution : 1µg/mL
- Acetic anhydride, p.a.

- Sodium hydroxide solution, 40 g/ 100 mL Sodium hydroxide p.a.
- Aluminium oxide, basic, W 200, activity grade III: to 100 gm aluminium oxide add 7gm water.
- Antifoam liquid, e.g(suitable, see reference)
- Compressed air, re-purified
- Helium
- Hydrogen, re-purified
- Nitrogen, re-purified.

32.4 Procedure

32.4.1 Hydrolysis, distillation and extraction

Weigh 50 gm of the comminuted analytical sample into a 2 L Pyrex round-bottomed flask. Then add 300 mL distilled water, 200 mL sodium hydroxide solution, 10 mL (suitable, see reference)and a few boiling chips. Place the flask in a heating mantle, half-fill the U-tube of the Bleidner apparatus with water and isooctane, and connect the flask to the lower arm of the Bleidner apparatus. Attach a 250 mL round-bottomed flask filled with 100 mL isooctane to the upper arm of the Bleidner apparatus. Heat both flasks for 14 h at a temperature that will ensure condensation of equal amounts of water and isooctane (this can be checked easily from the volumes of the immiscible solvent phases in the capillary of the Bleidner apparatus).

On completion of the distillation-extraction step, let the isooctane cool to room temperature.

32.4.2 Cleanup on an aluminium oxide column

Fill the chromatographic tube with isooctane, and slowly add aluminium oxide, activity grade III, to a level of approx. 12-14 cm (10 mL). Drain the supernatant isooctane to the level of the adsorbent. Next add the whole of the isooctane phase from the Bleidner extraction to the column. Wash the column with 40 mL of eluting mixture 1. Then elute the anilines with 40 mL of eluting mixture 2 into a 100 mL round bottomed flask.

32.4.3 Acetylation

To the elute collected from the aluminium oxide column add 10 mL acetic anhydride. Attach the flask to the rotary evaporator, and rotate without vacuum for 10 min in a water bath heated to 45°C. Next evaporate the eluate to dryness under vacuum. Rinse the flask walls with 5 mL of deionized water, and rotary-evaporate to dryness at a water bath temperature of 45°C.

32.4.4 Gas-chromatographic determination

Dissolve the residue in 1 mL of hexane-ethanol mixture. Inject an aliquot of the solution into the gas chromatograph.

Operating conditions

Gas chromatograph

Column	:	DB Wax 15 × 0.25 mm id or equivalent,
Column temperature	:	Programmed to rise from 150 °C to 260 °C at a rate of 8°C/min, Hold 15 min
Injection port temperature:		240 °C
Detectors	:	a) Electrolytic conductivity detector
Carrier gas flow rate	:	Helium, 5.0 mL/min
Oven temperature	:	740°C
Catalyst	:	Granulated nickel
Absorber for acidic gases	:	Strontium hydroxide
Purge gas flow rate	:	Helium, 2.0 mL/min
Reactant gas flow rate	:	Hydrogen, 50 mL/min
Transfer line temperature:		235 °C
Voltage	:	30V

Attenuation	:	4
Sensitivity	:	4 V full-scale deflection
		b) Alkali flame ionization detector (AFID)
Gas flow rates	:	Nitrogen carrier, 30 mL/min
		Hydrogen, 35 mL/min
Temperature	:	Air, approx. 230 mL/min
Sensitivity	:	240 °C
Recorder	:	16.10^{-12} A full-scale deflection
		1mV; chart speed 1 cm/min
Injection volume	:	105 µL for electrolytic conductivity detector
		0.1-1 µL for AFID

$$\text{Linuron µg/g (ppm)} = \frac{A1 \times V2 \times V3 \times C}{A2 \times V1 \times M} \times f \times 1.22$$

where,

A1 = peak area of the sample

V2 = volume (in µL) of standard linuron injected solution.

V3 = Total volume in mL of the sample

C = Concentration in µg/mL of standard linuron injected solution.

A2 = Peak area of the standard linuron solution

V1 = Volume in µL of the sample solution.

M = Mass of the sample.

F = Recovery factor

Reference

1. Zwig, G. (Edit).Analytical method for pesticides, plant growth regulators. Academic Press, New York-Sen Francisco, London. Volume 5 (1967), pp. 434-437.

2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456.

3. J. AOAC International Vol. 90, (2), 485-520.

33. DIQUAT AND PARAQUAT (The LC-MS/MS method for determination of multiresidue analyte may also be used)

33.1 Principle

The sample is extracted by refluxing with 18 N Sulphuric acid, filtered, neutralized, and cleaned up by means of an ion exchange column. Diquat or paraquat is eluted from the column with saturated ammonium chloride and measured after alkaline reduction with sodium dithionite to a free radical having a strong absorption peak at 380 nm for diquat or 394nm for paraquat.

33.2 Reagents

- *Ion exchange resin.* Dowex 50-W-X8, 200-400 mesh, hydrogen form. Place the resin in a large chromatography column and backwash the resin with water to remove the fine particles.

- *Sodium dithionite* (sodium hydrosulfite), purified powder.

- *Caprylic alcohol.*

- *EDTA* (Ethylenedinitrilotetraacetic acid disodium salt).

- Diquat

- *dibromide and Paraquat dichloride* analytical standards.

33.3 Equipment

- Boiling flasks, 1000 mL, standard taper, with heating mantles and reflux condensers.
- *Chromatography columns,* 10 x 200 mm, equipped with Teflon stopcocks and a reservoir at the top.
- *Spectrophotometer*(suitable, see reference).
- *pH meter.*

33.4 Experimental procedure

33.4.1 Preparation of sample

Macerate a representative sample in a food chopper or blender. Transfer a 50 gm sample to a 1000 mL boiling flask and add boiling stones and 40 mL deionized water. Carefully add 40 mL concentrated Sulphuric acid. Attach a water condenser to the flask and heat at reflux temperature for 30-60 min. Using an asbestos glove, occasionally swirl the flask during reflux. If the sample foams excessively, add 1-5 mL caprylic alcohol through the top of the condenser. After refluxing, add 600 mL of water and filter with suction through Whatman No. 1 filter paper in a Buchner funnel. Transfer the filtrate to a 100 mL beaker and add approximately 70 mL of 50 % sodium hydroxide and 3 gm EDTA. Stir and adjust the solution to pH 9.

33.4.2 Cleanup

Place a glass wool plug in the bottom of a chromatography column (10 × 200 mm) and add 6 mL settled ion exchange resin in water. Insert a glass wool plug above the resin column. Rinse the column with 25 mL of 6 M sodium chloride followed by 50 mL of water. Transfer the sample extract to a two-neck flask fitted with a stopper and glass tube extending to the bottom of the flask. Position the flask above the column and connect the glass tube in the two-neck flask to the top of the column with Tygon tubing

and a rubber stopper fitted with glass tubing. Force the extract through the column at a rate of 5-10 mL/minute using air pressure (3 psi) at the side neck of the two-neck flask. Rinse the column with 25 mL of water, followed by 25 mL of 0.5 M ammonium chloride. Elute the diquat or paraquat with 5 M ammonium chloride at a flow rate of 1 mL per 90 s. Collect exactly 25 mL of eluate.

33.4.3 Measurement

After mixing, transfer a 15 mL portion of eluate to a test tube. Add 3 mL of freshly prepared sodium dithionite solution (0.2 % in 0.5 N sodium hydroxide) Stopper and mix very gently. Immediately measure the absorbance at the maximum (approximately 380nm for diquat or 394 nm for paraquat) and at 4 nm on each side of the maximum. Use 4 or 5 cm cells and a reference solution containing a mixture of 15 mL of 5 M ammonium chloride and 3 mL of sodium dithionite solution. With each set of determinations, measure the absorbances of a standard solution containing 0.5 µg/mL diquat or paraquat in 5 M ammonium chloride. Correct the absorbance of the sample for background as follows:

$$A_{corr.} = \frac{A_{ms}}{2A_{ms} - (A_{hs} + A_{ls})} [2A_m - (A_h + A_i)]$$

where,

A_m = observed absorbance of sample at maximum (A_m);

A_l = observed absorbance of sample at 4 nm lower than maximum (A_1);

A_h = observed absorbance of sample at 4 nm higher than maximum (A_h);

A_{ms} = absorbance of standard diquat or paraquat at A_m;

A_{hs} = absorbance of standard diquat or paraquat at A_h.

The factor A_{ms} / {2 A_{ms} - (A_{hs} + A_{ls}) is a constant that is calculated from the average absorbances of the standard solution measured before and after each set of samples.

33.4.4 Limit of detection

The limit of detection of the method is 0.01 ppm when a 50 gm sample is used.

Reference

Zwig, G. Analytical methods of pesticides - Plant growth regulators. Academic Press, New York, San Francisco-London. Vol. X (1980). pp. 321-325.

Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol Version 8.1

http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf

34. ATRAZINE AND SIMAZINE

34.1 Scope

This analytical method describes gas chromatographic (GLC) method for determination of atrazine and semazine residues in food, and water sample. The limit of determination (is this detection) of the compound is 0.05 µg/g by the GLC method.

34.2 Apparatus

- Waring Blender
- Laboratory shaker (Rotary action)
- Chromatographic column, 25 cm long × 2 cm
- Rotary vacuum evaporator
- Air blower
- Water bath

34.3 Gas Chromatograph

Equipped with a nitrogen specific detector and operating under the following suggested parameters. These parameters may be varied according to the available facilities, provided standardization is done:

- Column: A fused Silica capillary DB 5 Column 30m × 0.25 id mm id DB5

- Temperatures: Column oven: 140°C-1 min Ramp @ 2 ° - 240 °C Hold 10 min ramp @10 °C to 260 hold 10 min or any equivalent column, provided standardization is done.
- Injector : 300 °C
- Detector : 240 °C
- Carrier gas (nitrogen) flow : 0.7mL/min rate
- Retention time:Atrazine : 6.0 min approximately
- Semazine : 7.0 mixture approximately
- Microliter syringe : 1.0 µL capacity.

34.4 Reagents

- Chloroform - AR grade Petroleum Ether- boiling range 40-60 °C
- Ethyl Ether - 5 percent (v/v) in carbon tetrachloride.
- Carbon tetrachloride
- Acetonitrile - AR grade
- Methanol - AR grade
- Sodium acetate buffer solution - Mix equal volumes of 2 N acetic acid and 1 N sodium hydroxide solution
- Reactivated basic alumina - Mix 90 gm basic alumina with 10 mL water thoroughly and allow standing overnight.
- Acetic Acid - 2N.
- Sodium hydroxide solution -1 N.
- Sodium sulphate Anhydrous
- Ethyl acetate -AR grade
- Reference standard Atrazine and simazine is of known purity.

34.5 Extraction:

34.5.1 Grain, Straw, Hay (Low Moisture)

Transfer a suitable quantity (50-100 gm) finely ground sample into a waring blender, add 100 mL chloroform and homogenize for 5 min. Transfer the contents quantitatively to a stoppered 1000 mL conical flask, add 100 mL chloroform and shake the slurry vigorously on a rotary-action laboratory shake for one hour. Filter the extract by decanting, through a layer of anhydrous sodium sulphate mounted on a Waterman No. 1 or equivalent filter paper and a funnel. Note the exact volume of the filtrate obtained, Evaporate an aliquot of the extract, equivalent to 50 gm of the crop sample, carefully to dryness on a water bath in a 250mL beaker, with the help of a gentle stream of air.

34.5.2 Fruits and vegetables

Transfer a suitable quantity (100 gm) of the finely chopped fruit or vegetable sample into a Waring blender along with about 100 g anhydrous sodium sulphate and 100 mL of chloroform. Blend the mixture for 5 min. Follow the procedure as in grains.

34.5.3 Crops containing Fat (Oilseeds and Nuts)

Follow the extraction procedure described above. Evaporate chloroform completely on the water bath with the help of steam of air and dissolve the residue in 50 mL petroleum ether. Transfer the solution to a 250 mL separatory funnel and wash the beaker two times with 20 mL portions of petroleum ether transferring the washings into the separatory funnel. Extract the petroleum ether solution in the separatory funnel three times with 25 mL portions of acetonitrile. Pool the acetonitrile extract, and transfer to a second 250 mL separatory funnel and wash with 50 mL petroleum ether. Discard the petroleum ether layer. Transfer the acetonitrile solution quantitatively, directly to the round bottom flask of the rotary vacuum evaporator and completely evaporate the acetonitrile under slightly reduced pressure and with a water bath at 50°C.

34.5.4 Water

Transfer 500 mL of the water sample into a 1 L separatory funnel and extract the aqueous layer three times with 60 mL portions of chloroform. Pool the chloroform extracts in a 250 mL beaker and evaporates the contents to dryness on a water bath with the help of a stream of air.

34.5.6 Clean up

Add 25 gm reactivated basic alumina to the chromatographic column, tap gently to eliminate channeling and to achieve uniform packing. Dissolve the extracted sample residue in 10 mL carbon tetrachloride, transfer on to the column and allow to penetrate into the alumina. Wash the beaker with 10 mL of carbon tetra-chloride and transfer to the column allowing to penetrate as before. This operation shall be further repeated with another 5 mL of carbon tetrachloride. When the solvent has just penetrated into the column, add 80 mL of carbon tetrachloride. After the solvent has drained down, place a clean 250 mL beaker as receiver, and add 100 mL of 5 percent ethyl ether in carbon tetrachloride collecting the complete eluate in the beaker. Evaporate the contents of the beaker to dryness on a water bath with a stream of air.

34.6 Gas chromatographic method

34.6.1 Principle

The residue of atrazine or simazine extracted from the sample after clean up is dissolved in ethyl acetate and estimated by GC equipped with a nitrogen specific detector. The content of atrazine and simazine in the sample is determined by comparing the response with the response of a known standard of similar concentration.

34.7 Calculations:

$$\text{Residue of atrazine/simazine} = \frac{A1 \times V2 \times V3 \times C}{A2 \times V \times M} \times f$$

Where

Aj = peak area of the samples;

V2 = volume, in µL of standard solution injected;

V3 = total volume, in mL, of sample solution;

C = concentration in ppm, of the standard solution;

F = recovery factor = 100 / percent mean recovery

A2 = peak area of standard simazine or atrazine

V, = volume in µL of sample solution injected; and

M = mass, in gm, of sample taken for analysis.

References

1. Indian Standard 13829 (1993).

2. Zwig, G. Analytical methods for pesticides - Plant growth regulators. Academic Press, Volume X, pp. 513-524.

3. Sao Paulo, Determination of herbicide residues in soil by small scale extraction Eclet.Quim.Vol 27 (2002).

4. J. Agric. Food Chem., 2015, 63 (18), 4449–4456.

5. J. AOAC International Vol. 90, (2), 485-520.

35. METRIBUZIN

35.1 Principle

The method describes a typical method for metribuzin (4-amino-6-tert-butyl-(3-methylthio)-l,2,4-triozin-5 (4H)-one) in plant material, and water.

Plant samples are extracted with acetonitrile and water samples are extracted with a mixture of ethyl acetate and dichloromethane. The acetonitrile extract from cereal grains is cleaned up by shaking out with petroleum ether. Following evaporation of the extract, the residue is dissolved in methanol and determined by gas chromatography using a nitrogen specific alkali flame ionization detector.

35.2. Apparatus

- Blender, E.G.
- Glass suction filter
- Round-bottomed flasks, 1 L, 500 mL and 50 mL
- Polyethylene bottle, 500 mL
- Shaking machine,
- Rotary vacuum evaporator, bath temperature of 50°C
- Separatory funnels, 1 L and 500 mL
- Gas chromatograph equipped with thermionic nitrogen detector

35.3 Reagents

- Acetonitrile, redistilled
- Dichloromethane, redistilled
- Ethyl acetate, redistilled
- Helium
- Air
- Methanol, redistilled
- Sodium sulphate, analytical reagent grade, anhydrous
- Petroleum ether, boiling range 40-60°C
- Hydrogen
- Metribuzin standard solutions

35.4 Analytical procedure

35.4.1 Extraction

For plant material like barley (green and straw), potato tubers, potato tops, alfalfa, asparagus, tomatoes, tomato tops macerate 100 gm of the prepared plant sample (G) with 200 mL of acetonitrile for about 2 min in the blender. Filter the macerate through a suction filter.

Reblend the filter cake with 200 mL of acetonitrile, and filter the macerate through a suction filter. Rinse the blender jar and the suction filter with a total of 150 mL of acetonitrile, and combine these washings with the filtrate in a 1 litre round-bottomed flask. Carefully rotary evaporate the combined solutions until they are free of acetonitrile; immediately chill the aqueous residues in an ice bath.

35.4.2 Grains

Macerate a 50 gm portion of ground grains with 100 mL of acetonitrile for about 5 min in the blender. Macerate through a suction filter. Reblend the filter cake with 100 mL of acetonitrile, and filter the macerate through a suction filter. Rinse the blendor jar and the suction filter with a total of 150 mL of acetonitrile. Combine these washings with the filtrate in a 1L seperatory funnel.

35.4.3 Water samples

Extract a water sample (500 mL) three times with 100 mL portions of a 9:1 dichloromethane: ethylacetate mixture by shaking in a separatory funnel. Filter the combined lower dichloromethane ethyl acetate phased over approx. 10 gm of sodium sulphate through a fluted filter and rotary-evaporate just to dryness.

35.4.4 Cleanup of extracts

35.4.4.1 Plant material

After chilling it in an ice bath, filter the aqueous residue derived through a fluted filter into a 500 mL separatory funnel. Rinse the round bottomed flask and fluted filter with approx. 100 mL of water and add the washing to the separatory funnel. Shake out the combined filtrates successively with 200, 200 and 100 mL protions of dichloromethane. Filter the combined dichloromethane phases over approx. 10 gm of sodium sulphate through a fluted filter into a 1 litre round-bottomed flask, and rotary- evaporate just to dryness.

35.4.1.2 Grains

Shake out the combined acetonitrile solutions derived above, successively with 100 and 100 mL portions of petroleum ether. Discard the petroleum ether phases. Rotary-evaporate the acetonitrile phase just to dryness.

35.4.5 Gas-chromatographic determination

Transfer the residue derived from plant material, grains, soil and water into a 50 mL round-bottomed flask using methanol to complete the transfer, rotary evaporate the solution just to dryness, and dissolve the residue in 2.0 mL of methanol. If the sample solution has an excessive content of metribuzin, dilute it to another appropriate final volume. Inject an aliquot of this solution (V_j) into the GC in operated with the following conditions:

Column glass tube 180 cm long, 2.3 mm id packed with 8 % DC - 550 + 2Y DC on gas chrom Q 80-100 mesh.

Column temperature 230 °C.

Detector 390 °C.

Injection port temperature 360 °C.

35.5 Evaluation

35.5.1 Procedure

The gas chromatogram was evaluated by measuring the peak area of the sample and comparing it with that obtained for the standard solutions of metribuzin. Equal volumes of the extract solutions and the standard solutions should be injected.

35.6 Calculation of residues

The residue R in mg/ kg metribuzin is calculated by applying the following equation:

$$R = \frac{F_A . V_{End} . W_{st}}{F_{St} . V_j . G} . F$$

Where

F = recovery factor determined by analyst

F_A = peak area of sample obtained from V_j (mm^2)

FSt = peak area of standard obtained from W_{St} (mm^2)

G = sample weight (gm)

V_{End} = terminal volume of methanol solution (mL)

V_j = portion of terminal volume

V_{End} injected into gas chromatograph (µL)

W_{St} = amount of parent compound injected with the standard solution (ng).

35.7 Comments

All the steps of the analytical procedure and the final determination should be conducted and completed on one and the same day to avoid conversion of metribuzin to its metabolites in the extracts.

Reference

1. Pflenzenschutz Sodium hydroxiderichten Bayer 31 (1978), pp. 84-97.
2. J. Agric. Food Chem., 2015, 63 (18), 4449–4456.
3. J. AOAC International Vol. 90, (2), 485-520.

36. 2, 4-D

36.1 Principle/Scope

The analytical method prescribes gas liquid chromatographic method for determination of 2, 4-D residues in food commodities.

36.2 Apparatus

- GLC fitted with an electron capture detector (Ni63) and printer-plotter cum integrator. The following operating parameters are suggested, which can be changed provided standardization is done:
- Column:Glass column of DB 1 30m × 0.25 mm id GC
- Temperature180 °C

- Column oven Injector 250°C
- Detector 280 °C
- Carrier gas Nitrogen
- Flow rate 20
- Miro syringe 2 µL

Note- Approximate retention time for 2, 4-D Me is 4 min and the minimum detectable level is 0.01 ppm under the suggested operating parameters at the single to noise ratio 1:3

- High speed blender
- Analytical balance capable of weighing 0.1 mg
- Mechanical shaker
- Vacuum rotary evaporator
- Stoppered bottles 500 mL
- Round bottom flasks 500 mL
- Buchner funnel and flask
- Volumetric pipettes 1 mL and 10 mL
- Separatory funnels 500 mL
- Measuring cylinder 10 mL, 250 mL
- Diazald kit (suitable, see reference) or suitable apparatus for diazomethane preparation (Fig. 1).

36.3 Reagents

- Acetone
- Chloroform
- Diethyl ether

- Ethyl alcohol 99.9 percent purity
- Hydrocholoric acid 2.0 N
- Sodium bicarbonate AR grade
- Sodium hydroxide AR grade
- N-methyl N-nitrosotoluene-4-sulphonamide 20 per cent w/v solution in diethylether
- Anhydrous sodium sulphate AR grade
- Filter paper Whatman No.41
- Neutral alumina (60-100 mesh) colum chromatographic grade
- Florisil (60-100 mesh) colum chromatographic grade
- 2,4-D acid analytical standard of high purity

36.4 Diazomethane

36.4.1 Diazomethane preparation

Diazomethane is very toxic; its preparation should be carried out only in a fume cupboard (hood) provided with a powerful exhaust system. The use of a screen of safety glass is recommended.

Add 50 mL of 96 % ethanol to a solution of 10 gm of potassium hrydroxide in 15 mL of water. Place this solution in a 500 mL distillation flask equipped with a dropping funnel and an efficient double surface condenser. Connect the other end of condenser to a conical flask of 200 mL capacity to act as receiver charge. Fill the conical flask with 40 mL of ether and arrange that the inlet tube of the smaller receiver dips below the surface of the ether. Cool the receiver in an ice-salt mixture. Heat the distillation flask in a water bath at 60-65°C, place a solution of 43 gm of N-methyl N-nitrosotoluene-4-sulphonamide in about 250 mL of ether in dropping funnel and introduce it into the distillation flask over a period of 45 min. Adjust the rate of addition so that it is about equal to the rate of distillation. When the dropping funnel is empty, add more ether (capacity 30 mL)

gradually for completing ether distilling in colourless form, the combined ethereal solutions in the receivers contain 5.9-6.1 gm of diazomethane.

For smaller quantities of diazomethane dissolve 2.14 gm of N-methyl N- nitrosotoluene-4 sulphonamide in 30 mL of ether. Cool in ice. And add a solution of 0.4 gm of potassium hrydroxide in 10 mL of 96 % ethanol. If a precipitate forms, add more ethanol until it just dissolves. After 5 minute, distill the etheral diazomethane solution from a water bath. The etheral solution contains 0.32-0.35 gm of diazomethane.

36.4.2 Precautions

Notes: The nitroso compound from which diazomethane can be derived are all toxic and carcinogenic and should be handled with caution as they cause skin irritation.

36.5 Esterfication of 2, 4-D Acid

Prepare a stock solution of 10 ppm of 2, 4-D acid in ethanol. To 1 mL of the above solution, add 3 mL of diazomethane solution and keep the mixture at 25°C in the fuming cupboard for 10 min. Esterified solution is evaporated near to dryness at 30°C and taken in suitable volume of acetone.

36.6 Extraction

36.6.1 Grains straw, oilseeds, fruits, vegetables, and nuts

Take 25 gm of homogenized sample in 500 mL stoppered bottle, add 100 mL of acidified ether and shake in mechanical shake for 15 min. Filter the organic layer and re-extract the sample with 100 mL of acidified ether, filter and combined the filtrates, concentrate the combined filtrate to 50 mL on a rotary vacuum evaporator at 25°C.

The concentrated extract is partitioned two times with 100 mL and 50 mL of saturated sodium bicarbonate solution, the collected aqueous layer is further acidified (pH 2-3) using 2 N hydrochloric acid and partitioned two times with 150 mL of chloroform. Collect the chloroform layer each time and dry over anhydrous sodium sulphate. The chlorform extract is evaporated to near dryness and the residues taken in a small amount of methanol for column clean up.

36.7 Clean-up

Column clean up for grains, straws, fruits, vegetables and nuts. A chromatographic column (1.8 cm id) is packed with 5 gm of neutral alumina (activated at 110°C) in between two layers of 2 gm of anyhydrous sodium suphate using hexane is washed with 50 mL of diethyl ether. Transfer the sample soution completely into the column using 3mL of ether. Elute the residues using 100 mL of ether and methanol (90: 10) mixute at a flow rate of 1 mL/min. Collect elute, concentrate to dryness and take in 2 mL of ethanol for esterification.

36.8 Derivatization

The samples are esterified by the addition of 3 mL of freshly prepared diazomethane. Evaporate the sample solution near to dryness and take in suitable volume of acetone.

36.9 Estimation

Inject 0.2 µL of reference standard solution of 2, 4-D methyl ester followed by the samples. Identify the peaks by their retention times and measure the peak heights/areas. Calculate the amount of residues of 2, 4-D as its ester from the preconstructed calibration curve.

36.10 Calculation

$$\text{2, 4-D methyl ester residues} = \frac{A1 \times V2 \times V3 \times C}{A2 \times V \times M}$$

Where,

Aj = peak area of the samples;

V2 = volume, in µL of standard solution injected;

V3 = total volume, in mL, of sample solution;

C = concentration in ppm, of the standard solution;

A2 = peak area of standard simazine or atrazine

V, = volume in µL of sample solution injected; and

M = mass, in g, of sample taken for analysis.

Calculations:

Residues of 2, 4-D µg/g = Amount of 2, 4-D ME × 221/235

Reference:

Indian Standard. IS 14857: 2000.

Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol Version 8.1 http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf

37. PENDIMETHALIN

37.1 Principle

Residues of Prowl {Pendimethalin} N-(l-Ethylpropyl)-3,4-dimethyli 2,6-dinitrobenzenamine) is extracted from the crop material with aqueous acidified methanol, with chloroform-methanol and from oil with n-hexane. After removal of many co-extractives by various solvent partitionings, final clean up is achieved on florosil.

The residues of Pendimethalin are measured by GLC using an electron capture detector.

37.2 Reagents

- Pendimethalin standard of known purity
- Solvents : n-hexane, benzene, acetonitrite, methanol, chloroform, pyridine, acetic anhydride
- Chromatographic adsorbent: Florosil 60-100 mesh
- Acid extraction solvent: aqueous acid - methanol 20 mL of concentrated hydrochloric acid and 200 mL methanol dissolved in water and made to 1 L with water.
- Acid salt solution: 8.3 mL of concentrated hydrochloric acid and 120 g of sodium chloride dissolved in water and made to 1 L with water.

37.3 Apparatus

- Gas chromatograph: suitable for use with glass columns and equipped with an on-column injection system and Ni electron capture detector.
- Gas chromatograph column: 180 cm borosilicate glass tube (2 mm id 16 nm o.d.) designed to fit the chromatograph and packed with 5 % EGSS-X on gas chrom. Q 100-120 mesh.
- Chromatographic tubes : 10 mm i.d. x 250 mm, and 20 mm i.d.x 250 mm with reservoir with Teflon stopcock.
- Rotary evaporatior
- Standard solution: Prepare standard solution of Prowl 1µg/mL separately.

37.4 Extraction

Extraction of Pendimethalin from sample of vegetables, corn, cereal, grains, peanuts, soybeans, cotton.

Weigh 25 gm of sample into 32 ounce narrow mouth bottle. Add 500 mL of aqueous acid-methanol and shake the contents for 6 hr using a slow reciprocating shaker. After shaking filter with sunction until about 150 mL of filtrate is obtained. Transfer 50 mL portion to a seperatory funnel, add 50 mL of 0.1 N HCl and extract the solution two times with 100 mL portion of hexane. Transfer the combined hexane layers to an evaporation flask and remove the solvent using a rotatry evaporator. Dissolve the residue in 100 mL of hexane, transfer the solution to a seperatory funnel and extract three times 25 mL of acetonitrile. Transfer the acetonitrile layers to an evaporation flask and remove the solvent using a rotary evaporator.

Deactivation of florosil: Weigh 500 gm of florosil into a shallow tray and dry in an oven at 130°C for 3 hr. After cooling at 25°C, transfer 500 gm of the dried material to a 1 gallon bottle. Add 30 mL of water tightly stopper and mix throroughly. Let it stand for 24 hr.

37.5 Clean up

Dissolve the contents of the flask in 10 mL hexane and transfer to a fresh florosil column. Open the stopcock and adjust the flow rate to 1 -2 drops for second until the solvent reach the top of the packing. Repeat the rinse and wash with two additional portions of hexane. Elute the compound from the column with 150 mL of hexane-benzene (80:20

v/v) using a flow rate of 3-4 drops per second. Evaporate the contents of the flask to dryness in a rotary evaporator. Dissolve the residue in 5 mL benzene. Analyse by GLC as per procedure.

Gas chromatograph with ^{63}N electron capture detector, A DB 5 GLC column 30m × 0.53 × 1.5 micron

Column temperature : 240 °C

Injection port temperature : 260 °C

Detector temperature : 270°C

Gas flow rate : 25 mL/min

Retention time : 6 min

37.6 Calculation

$$\text{Pendimethalin residue (mg/kg)} = \frac{A1 \times V2 \times V3 \times C}{A2 \times Vj \times M} \times f$$

Where,

A1 = Peak area of the sample

V2 = Volume in µL standard solution injected

V3 = Total volume in mL of sample solution

C = Concentration in ppm of the standard solution

F = Recovery factor = 100/per cent recovery

Vj = Volume in µL the sample solution injected

A2 = Peak area of the standard

M = Mass in gm of the sample taken for analysis.

Reference

1. Gunter Zwig, Analytical methods for pesticides and plant growth regulators. Academic Press, Volume X (1978), pp. 461-482.
2. J. Chromatogr. A, (2012),1270, 283-295
3. J. Agric. Food Chem., 2015, 63 (18), 4449–4456.
4. J. AOAC International Vol. 90, (2), 485-520.

38. ORGANO CHLORINE PESTICIDES IN WATER BY GAS CHROMATGRAPHIC METHOD

38.1 Principle

The analytical method describes determination of aldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, chlordane, 4,4-dichlorodiphenyldichloroethane, 4,4'- dichlorodiphenyldichloroethylene, 4,4'-dichlorodiphenyltrichloroethane, dieldrin, endosulfan-I, endosulfan-II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, and heptochlor expoxide in water.

Principle measured volume of test portion (1 L) is extracted with dichloromethane by shaking in separatory funnel or mechanical tumbling in bottle. Dichloro methane extract is separated, dried with anhydrous Sodium sulfate, solvent exchanged with methyl tert-butyl ether (MTBE), and concentrated to 5 mL. Pesiticides are separated and measured by capillary gas chromatography with electron-capture detection.

38.2 Apparatus

- Separatory funnel – 2 L, with TFE - fluorocarbon stopcock and ground-glass or TFE fluorocarbon stopper.
- Tumber bottle - 1.7 L, with TFE-fluorocarbon lined screw cap. Cut liners to fit screw cap from sheets and extract overnight with methane before use.
- Kuderna Danish (K-D) apparatus (1) Concentrator tube -10 or 25 mL, graduated (Kontes 570050-1025 or 570050-2525, or equivalent). Check calibration of concentrator tube at volumes used in method. Use ground-glass stoppers to percent evaporation of extracts (2) Evaporation flask - 500 mL (suitable, see reference). Attach to concentrator tube with springs.

- Snyder columns - Three-ball macro ((suitable, see reference); 2-ball micro (suitable, see reference)).
- Vials - Glass, 5-10 mL capacity, with TFE-fluorocarbon lined screw caps. Separatory funnel shaker - capable of holding 2 L separatory funnels and shaking them with rocking motion to thoroughly mix funnel contents ((suitable, see reference)
- Tumbler- Capable of holding and tumbling bottles, (b), end-over-end at 30 turns/ min.
- Boiling stones - Carborundum, No. 12 granules (suitable, see reference)
- Heat 30 min. at 400 °C before use. Cool and store in desiccator.
- Water bath - Heated, capable of control ± 2°C. Use bath in hood.
- Balance - Analytical, capable of accurately weighing to nearest 0.0001 gm.
- Gas chromatography -Temperature programmable system suitable for use with capillary columns, including syringes, analytical columns, gases, detector, and strip chart recorder.
- Data system is recommended for measuring peak areas.
- Primary column; 30m x 0.25 mm id DB-5 fused-silica capillary column, 0.25 µm film thickness (J&W Scientific, Inc.).
- Conformation column; 30 m x 0.25 mm id DB-1701 fused silica capillary column, 0.25 µm film thickness (J&W Scientific Inc.)
- Operating conditions;
- injection volume 2 µL
- He carrier gas at 30 cm/s linear velocity
- injector 250°C
- detector 320 °C
- Oven programmed from 60-300 °C at 4°C/min

- Electron capture detector.

38.3 Reagents

- Standard solutions - Use standards of test compounds with purity > 96 % to prepare stock solutions at 1 mg/mL in methyl tert-butyl ether (MTBE). Commercially prepared stock standards may be used at any concentration if they are certified by manufacturer or independent source. Store solutions at room temperature and protect from light. Replace stock standard solutions after 2 months or sooner if comparison with laboratory control standards indicates degradation.
- Internal standard solution - Prepare pentachloronitrobenzene (Purity > 98 %) stock solution at 0.1 mg/mL in MTBE.
- Add 5 µL stock solutions to 5 mL test portion extract to give final internal standard concentration of 0.1 µL pentachloronitrobenzene/ mL of extract.
- Surrogate solution - Prepare 4, 4'-dichlorobiphenyl (purity >96 %) stock solujtion at 0.5 mg/mL in MTBE. Add 50 µL stock solution to 1 L test sample prior to extraction to produce surrogate concentration of 25 g] 4,4'-dichlorobiphenyl/L in test sample and, assuming quantitative recovery, 5.0 g/mL in extract.
- Instrument performance solution - Prepare individual stock standard solutions containing chlorothalonil, chlorpyrifos, DCPA, and δ-BHC at 0.10 µL/mL in MTBE.
- For assessing instrument performance, combine 50 µL hlorothalonil stock solution, 2 µL chlorpyrifos stock solution, 50 µL DCPA stock solutions, and 40 µL δ-BHC stock solutions in 100 mL volumetric flask and dilute to volume with MTBE.
- Solvents - Acetone, methylene chloride, and MTBE Distilled-in-glass quality, or equivalent.
- Phosphate buffer- pH 7. Mix 29.6 mL 0.1 M hydrochloric acid and 50 mL 0.1 M dipotassium hydrogen phosphate.

- Sodium sulfate - Granular, anhydrous, ACS grade. Heat in shallow tray for > 4 at at 450°C to remove interfering organic substances.
- Sodium chloride - Crystals ACS grade. Heat in shallow tray for > 24 h at 450°C to remove interfering organic substances.
- Reagent water - Water reasonably free of contamination that would prevent determination of any analyte of interest.
- Preservative - Mercuric chloride solution. 10 mg Mercuric chloride (ACS grade) /mL reagent water.
- Sodium thiosulfate – Sodium thiosulfate, Granular, anhydrous, ACS grade.

38.4 Preparation of laboratory sample bottles

Add 1 mL preservative, to glass laboratory sample bottle. If residual chlorine is expected to be present in laboratory samples, add 80 mg Sodium thiosulfate to laboratory sample bottle before collection.

38.5 Laboratory Sample Collection

Collect 1 L grab laboratory samples in glass bottles by conventional sampling practices. Since bottles contain preservative and Sodium thiosulfate, do not prerinse bottles with laboratory sample before collection. Add laboratory sample to bottle containing preservative, seal laboratory sample bottle, and shake vigorously 1 min. Refrigerate laboratory samples at 4°C from time of collection until extracted. Protect from light. Extract laboratory samples within 7 days of sample collection.

38.6 Laboratory sample preparation

Automated extraction method - Add preservative, (CQ) to any laboratory samples not previouslyh preserved. Mark water meniscus on side of laboratory sample bottle for later determination of volume. Add 50 µL surrogate stock solution, C(c) to laboratory sample. If mechanical separatory funel shaker is used, pour entire laboratory sample

into 2 L separatory funnel. If mechanical tumbler is used, pour entire laboratory sample into tumbler bottle. Adjust sample to pH 7 by adding 50 mL phosphate buffer, C. check pH and add Sulphuric acid or Sodium hydroxide if necessary.

Add 100 gm sodium chloride, seal, and shake to dissolve salt. Add 300 mL dichloromethane to tumbler bottle or sepaatory funnel, seal, and shake 30 s to rinse inner walls. Transfer rinse to test portion contained in separatory funnel or tumbler bottle, seal, and shake 10 s, venting periodically. Repeat shaking and venting until pressure release is not observed during venting. Reseal and place test portion container in appropriate mechanical mixing device (separatory funnel shaker or tumbler). Shake or tumble test portion for 1 hr.

After extraction, pour contents of tumbler bottle into a 2 L separatory funnel if tumbler bottle are used. Let organic layer separate from water phase for > 10 min. If emulsion interface between layers is more than one-third volume of solvent layer, complete phase separation mechanically. Collect dichloromethane extract in 500 mL Erlenmeyer flask containing ca 5 gm anhydrous Sodium sulfate. Swirl flask to dry extract; let flask sit 15min. Determine original laboratory sample volume by refilling laboratory sample bottle to mark and transferring water to 1000 mL graduated cylinder. Record sample volume to nearest 5 mL.

Manual extraction method - Add preservative (CQ) to labroatory samples not previously preserved. Mark water meniscus on side of tumbler bottle for later determination of laboratory sample volume. Add 50 µL surrogate stock solution, C(c) to laboratory sample, pour entire contents into 2 L separatory funnel. Adjust to pH 7 by adding 50 mL phosphate buffer, C (f). Check pH and add H_2 SO_4 or Sodium hydroxide if necessary. Add 100 gm Sodium chloride to contents, seal, and shake to dissolve salt. Add 60 mL dichloromethane to tumbler bottle, seal and shake bottle 30s to rinse inner walls.

Transfer rinse to separately funnel and extract laboratory sample by vigorously shaking funnel for 2 min with periodic venting to release excess pressure. Let organic layer separate from water phase for > 10 min. If emulsion interface between layers is more than 1/3 volume of solvent layer, complete phase separation mechanically. Collect dichloromethane extract in 500 mL Erlenmeyer flask containing ca 5 gm anhydrous sodium sulfate. Add second 60 mL portion of dichloromethane to separately funnel and repeat extraction procedure a second time, combining extracts in Erlenmeyer flask.

Perform third extraction in same manner. Swirl flask to dry extract; let flask sit for 15 min. Determine original laboratory sample volume by refilling laboratory sample bottle to mark and transferrng water to 1 L graduated cylinder. Record laboratory sample volume to nearest 5 mL.

38.7 Extract concentration

Assemble K-D concentrator by attaching 25 mL concentrator tube to 500 mL evaporation flask. Decant dichloromethane extract into concentrator. Rinse remaining Sodium sulfate with two 25 mL portions of dichloromethane and decant rinses into concentrator.

Add 1 or 2 clean boiling stones to evaporation flask and attach macro-Snyder column. Prewet column by adding ca 1 mL dichloromethane to top. Place K-D apparatus on 65-75°C water bath so that concentrator tube is partially immersed in hot water and entire lower, rounded surface of flask is bathed with hot vapour. Adjust vertical position of apparatus and water temperature as required to complete concentration in 15- 20 min. At proper rate of distillation, balls of column will actively chatter, but chambers will not flood. When apparent volume of liquid reches 2 mL, remove K- D apparatus and let it drain and cool > 10 min.

Remove Snyder column and rinse flask and its lower joint with 1-2 mL MTBE, collecting rinse in concentrator tube. Add 5-10 mL MTBE and fresh boiling stone.

Attach micro-Snyder column to concentrator tube and prewet column by adding ca 0.5mL MTBE to top. Place micro K-D apparatus on water bath so that concentrator tube is partially immersed in hot water. Adjust vertical position of apparatus and water temperature as required to complete concentration in 5-10 min. When apparent volume of liquid reaches 2 mL, remove apparatus from bath and let it drain and cool.

Add 5-10 mL MTBE and boiling stone and reconcentrate to 2 mL. Remove micro K-D apparatus from bath and let it drain and cool. Remove micro-Snyder column, and rinse walls of concentrator tube while adjusting volume to 5.0 mL with MTBE.

Add 5 µL internal standard stock solutions, C to laboratory sample extract, seal, and shake to distribute internal standard. Transfer extract to appropriate-size TFE fluorocarbon-sealed, screw cap vial and store at 4°C until analysis. A 14-day maximum extract storage time is recommended.

38.8 Caliberation of Gas Chromatograph with Electron-Capture Detector

Table 1 summarizes retention times and detection limits observed using this method of analysis of chlorinated pesticides.

38.9. Calculations

The concentration (C) in the sample can be calculated from the following equation:

$$C\ (\mu g/L) = \frac{(A)\ (V)}{(Vt)\ (Vs)}$$

where,

A = Amount of the material injected (ng)

Vi = Volume of extract injected (µL)

Vt = Volume of the total extract (µL)

Vs = Volume of the water extract (mL)

Table 6. Relative retention times and estimated method detection limits for chlorinated pesticide

Analyte	Relative retention time		Estimated MDL
	Primary	Confirmation	
Aldrin	1.18	1.12	0.075
a -BHC	0.93	0.97	0.020
β -BHC	0.98	1.187	0.070
γ -BHC	1.03	1.22	0.0100
d -BHC	0.99	1.04	0.0150
a -chlordane	1.31	1.31	0.0075
γ -chlordane	1.28	1.29	0.0015
4,4'- ODD	1.42	1.38	0.0025
4,4'- DDE	1.35	1.32	0.0100
4,4-DICHLORODIPHENYLTRICHLOROETHANE	1.48	1.48	0.0600

Dieldrin	1.35	1.35	0.020
Endosulfan I	1.30	1.28	0.0150
Endosulfan II	1.40	1.45	0.0150
Endosulfan sulphate	1.47	-	0.0150
Heptachlor	1.11	1.04	-
Heptachlor expoxide	1.24	1.24	0.075

Reference

1. AOAC Official method. 17th Edition. 990.06 (2000).

2. J. Chromatogr. A, 1218, 38(23), 2011, 6780-6791.

39. N-METHYL CARBAMOYLOXIMES AND N-METHYL CARBAMATES IN FINISHED DRINKING WATER

39.1 Scope

The analytical method prescribes HPLC method for determination of aldicarb, aldicarb sulfone, aldicarb sulfoxide, baygon, carbaryl, carbofuron, 3-hydroxy carbofuron, methiocarb, methomyl, and oxamyl in drinking water.

39.2 Principle

Water sample is filtered and measured volume is directly injected onto reverse- phase LC column. Analytes are separated by gradient elution chromatography. After elution from LC column, analytes are hydrolyzed with Sodium hydroxide at 95°C. Methylamine formed during hydrolysis is reacted with o-phthalaldehyde and 2-mercaptoethanol to form highly fluorescent derivative, which is detected by fluorescence detector. Estimated method detection limits range from 0.5 µg/L for methomyl to 4.0µg/L for methiocarb; estimated method detection limits for 8 other compounds range from 1.0 to 2.0 µg/L.

39.3 Apparatus

(a) Grab sample bottle. 60mL, borosilicate glass, screw-cap vials (suitable, see reference) and caps with PTFE-faced silicone septa (suitable, see reference). Before use, wash vials and septa with soap and water, followed by 3 tap water rinses and 3 deionized water rinses.

(b) Balance- Analytical, capable of accurately weighing to nearest 0.0001 gm.

(c) Macrofilters. - 47 mm 0.45 µm, nongridded, cellulose acetate filters for water phases; 47 mm, 0.5 µ m nongrided, PTFE filters for organic phases.

(d) Microfilters- 13 mm stainless steel filter holder and 13 mm diameter, 0.2 m polyster filters (suitable, see reference).

(e) Hypodermic syringe- 10 µL, glass, with Luer-Lok tip.

(f) Syringe valve- 3 way.

(g) Syringe needle- 7-10cm long, 17gauge, with blunt tip.

(h) Microsyringes - Various sizes.

(i) Solution storage bottles- Amber glass, 10-15 mL capacity with TFE-fluorocarbon-lines screw cap.

(j) LC system- Capable of injecting 200-400 µL aliquots and performing binary linear gradients at constant flow rate. Data system is recommended for measuring peak areas. Primary column: 250 mm × 4.6 mm id stainless steel packed with 5 µm Beckman Ultrasphere ODS. Mobile phase linear gradient from methanol water (15+85) to methanol in 32 min at 1.0 mL/min. Confirmation column: 250 mm × 4.6 mm id stainless steel packed with 5 µm supelco LC-1. Mobile phase linear gradient from methanol: water (15:85) to methanol in 32 min at 1.0 mL/ min.

(k) Postcolumn reactor - Reactor constructed with PTFE tubing and equipped with pumps capable of mixing 0.5 mL/min OPA reaction solution, C (i), and 0.5 mL/min Sodium hydroxide, C (f), into mobile phase. Reactor must contain mixing tees and two 1.0 mL delay coils, one thermostated at 95°C.

(l) Fluorescence detector. Capable of excitation at 230 nm and detection of emission energies > 418 nm.

39.4 Reagents

(a) Standard solutions- Use standards of test compounds with purity > 96 % to prepare stock solutions at 1.00 µg/mL in methanol. Commercially prepared stock standards may be used at any concentration if they are certified by manufacturer or independent source. Transfer stock standard solutions into TFE fluorocarbon-sealed screw-cap vials. Store at room temperature protected from light. Replace stock standard solutions after 2 months, or sooner if comparison with laboratory control standards indicates degradation.

(b) Instrument performance solution- Combine 20 µL 3-hydroxycarbofuran stock solution, (a), and l.0 mL aldicarb sulfoxide stock solution, (a) in 10 mL volumetric flask and dilute to volume with methanol.

(c) Reagent water. Distilled Water reasonably free of contamination that would prevent determination of analytes.

(d) Water- LC grade.

(e) Methanol- LC grade. Filter B(C), and degas with helium before use.

(f) Sodium hydroxide.-0.05M. 2.0gm Sodium hydroxide/l.0L reagent water, Filter B and degas with helium before use.

(g) Mercaptoethanol: acetonitrile- (1:1). Mix 10.0 mL 2-mercaptoethanol and 10.0 mL acetonitrile. Store in borosilicate glass vial or bottle with PTFE-lined cap. (Caution: Strong odor. Store in hood.)

(h) Sodium borate.-0.05N. 19.1gm Borax or sodium borate.$10H_2O$/1.0 L reagent water, Sodium borate dissolves completely at room temperature if prepared day before use.

(i) OPA reaction solution- 100 ± 10 mg o-phthaladehyde (mp 55-58°C)/10µL methanol (e) add to l.0L 0.05M borax or sodium borate solution, (h). mix, filter, B

(c), and degas with helium. Add 100 µL 2-mercaptoethanol, (g), and mix. Prepare solution fresh daily.

(j) Helium- For degassing solutions and solvents

(k) Monochloroacetic acid buffer- pH 3. Mix 156mL 2.5M monochloroacetic acid and l00mL 2.5M potassium acetate.

(l) Sodium thiosulfate- Sodium thiosulfate . granular, anhydrous. ACS grade.

(m) Buffered reagent water- Mix l0 mL monochloroacetic acid buffer, (k), and 1 L reagent water, (c).

(n) Internal standard solution- Prepare 4-bromo-3.5 dimethylphenyl N-methylcarbamate (BDMC) (Purity 98 %, Aldrich Chemical Co.) stock solution at 0.1 mg/mL in methanol.

39.5 Preparation sample Bottles

Add 1.8 mL monochloroacetic acid buffer, C (k), to sample bottle, B (a). If residual chlorine is expected, add 5 mg Sodium thiosulfate to bottle before sample collection.

39.6 Sample collection

Collect 60 mL grab samples in glass bottles by conventional sampling practices. Because bottles contain buffer and Sodium thiosulfate, do not prerinse with sample before collection. Add sample to bottle, seal, and shake vigorously 1 min.

Refrigerate samples at 4°C from time of collection until storage. Store at -10°C until analyzed. Analyze samples within 28 days of collection.

39.7 Sample Preparation

Adjust pH of sample or standard to pH 3 ± 0.2 by adding 1.5 mL 2.5 M monochloroacetic acid buffer, C (k), to 50 mL sample. This step should not be necessary if sample pH was adjusted during sample collection. Fill 50 mL volumetric flask to mark with sample. Add 5 µL internal standard stock solution, C (n), to the 50 mL of sample (final concentration l0 µg/L). Affix 3 -way valve to l0 µL syringe. Place clean filter in filter holder, B (d), and

affix filter holder and 7-10 cm syringe needle to syringe valve. Rinse needle and syringe with reagent water, C (C). Prewet filter by passing 5 mL reagent water through filter. Empty syringe and check for leakes. Draw l0 mL sample into syringe and expel through filter. Draw another l0mL sample into syringe, expel through filter, and collect last 5 mL for analysis. Rinse syringe with reagent water. Discard filter. Inject 400 µL of collected sample into LC system under conditions in B (j).

39.8. Calibration of LC system

Table 7. Presents retention times and estimated method detection of 10 carbamate pesticides.

Table 7

Analyte	Retention time	EDL
Aldicarb	21.4	1.6
Aldicarb sulfone	12.2	2.0
Aldicarb sulfoxide	17.5	2.0
Baygon	23.4	1.0
Carbaryl	25.4	2.0
Carboftiron	24.4	1.5
3 -hydroxycarboruron	19.0	2.0
Methiocarb	28.6	4.0
Methomyl	14.8	0.50
Oxamyl	14.6	2.0

39.9 Calculation :

$$C\ (\mu g/L) = \frac{(A)\ (V)}{(V_t)\ (V_s)}$$

Where,

A= Amount of the material injected (ng)

V= Volume of extract injected (µL)

V_t= Volume of the total extract (µL)

V_s= Volume of the water extract (mL)

Reference

1. Official methods of Analysis.10.5.02.

2. J. Chromatogr. A, 1218, 38(23), 2011, 6780-6791.

40. DETERMINATION OF ALACHLOR, ATRAZINE AND BUTACHLOR IN WATER

40.1 Scope

This is a gas chromatographic (GC) method applicable to the determination of certain nitrogen and phosphorus contining pesticides in ground water and finished drinking water. The following compounds can be determined using this method:

40.2 Equipment and supplies (all specifications are suggested)

- Sample Bottie-Borosilicate. 1 L volume with graduations, fitted with screw caps lined with TFE-fluorocarbon. Protect samples from light. Amber bottles may be used. The container must be washed and dired before use to minimize contamination. Cap liners are cut to fit from sheets (suitable, see reference) and extracted with methanol overnight prior to use.

Glassware

- Separatory funnel-2000 mL, (suitable, see reference) with TFE- fluorocarbon lined screw cap. Cap liners are cut to fit from sheets (suitable, see reference)and extracted with methanol overnight prior to use.

- Flask Erlenmeyer-500 mL. Concentrator Tube, Kuderna-Danish (K-D)-l0 mL, graduated (suitable, see reference). Calibration must be checked at the volumes

employed in the test. Ground glass stoppers are used to prevent evaporation of extracts.

- Evaporative flask, K-D-500 mL (suitable, see reference). Attach to concentrator tube with springs.

- Snyder column, K-D-Three-ball macro (suitable, see reference).

- Snyder column, K-D-Two-ball micro (suitable, see reference).

- Vials- glass, 5-10 mL capacity with TFE fluorocarbon lined screw cap.

- Separatory Funnel Shaker (Optional)- Capable of holding 2 L spearatory funnels and shaking them with rocking motion to achieve thorough mixing of Separatory funnel contents ((suitable, see reference)
- Tumbler-Capable of holding tumbler bottles and tumbling them end-over- end at 30 turns/min. (suitable, see reference)
- Boiling stones- Carborundum, #12 granules (suitable, see reference)Heat at 400 °C for 30 min prior to use. Cool and store in desiccator.

- Water Bath- Heated, capable of temperature control (±2°C). The bath should be used in a hood.

- Balance- Analytical, capable of accurately weighing to the nearest 0.0001gm.

- Gas Chromatograph- Analytical system complete With temperature programmable GC suitable for use with capillary columns and all required accessories including syringes, analytical columns, gases, detector and stripchart recorder. A data system is recommended for measuring peak areas. Table no. 8 lists retention times observed for method analytes using the columns and analytical conditions described below.

- Column 1 (primary column) - 30 m long × 0.25 mm I.D DB-5 bonded fused silica capillary column, 0.25 µm film thickness or equivalent Helium carrier gas flow is

established at 30 cm/s, linear velocity and oven temperature is programmed from 60-300 °C at 4°C/min. Data presented in this method were obtained using this column. The injection volume was 2 µL in splitless mode with a 45 s delay the injector temperature was 250 °C and the detector temperature was 300°C.

- Column 2 (confirmation column) -30 m long × 0.25 mm I.D DB 1701 bonded fused silica column, 0.25µ m film thickness (suitable, see reference) or equivalent. Helium carrier gas flow is established at 30 cm/sec, linear velocity and oven temperature is programmed from 60-300 C at 4°C/min.

- Detector-Nitrogen-phosphorus(NPD)

40.3 Reagents and standards

Warning: When a solvent is purified, stabilizers added by the manufacturer are removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed thus potentially reducing the shelflife.

- Acetone, Methylene Chloride, Methyl tert-Butyl Ether (MTBE) - Distilled-in-glass quality or equivalent.

- Phosphate buffer, pH7- Prepare by mixing 29.6mL 0.1 M hydrochloric acid and 50mL 0.1 M dipotassium phosphate.

- Sodium chlorides Crystal, ACS Grade - Heat treat in a shallow tray at 400 °C for a minimum of four hr to remove interfering organic substances. Store in a glass bottle (not plastic) to avoid hydrogen phthalate (K_2HPO_4) contamination.

- Sodium sulfate-Granular, Anhydrous, ACS Grade - Heat in a shallow tray at 400 °C for a minimum of four hr to remove interfering organic substances. Store in a glass bottle (not plastic) to avoid phthalate contamination.

- Sodium thiosulfate, Granular, Anhydrous, ACS Grade.

- Triphenylphosphate (TPP), 98 % purity - For use as internal standard (available from Aldrich Chemical Co.).

- 1,3-Dimethyl-2-nitrobenzene, 98 % purity - For use as surrogate standard (available from Aldrich Chemical Co.).

- Mercuric Chloride, ACS Grade (Aldrich Chemical Co.) - For use as a bactericide optional.

- Reagent Water - Reagent water is defined as a water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Reagent water used to generate the validation data in this method was distilled water obtained from the (suitable, see reference).

- Stock Standard Solutions (1.00 µg/mL) — Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

  Prepare stock standard solutions by accurately weighing approximately 0.01 gm of pure material. Dissolve the material in MTBE, and dilute to volume in a 10 mL volumetric flask. The stock solution for simazine should be prepared in methanol. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96 % or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source. Transfer the stock standard solutions into TEE-fluorocarbon-sealed screw cap amber vials. Store at 25±2 °C and protect from light.

  Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks or QC samples indicate a problem.

- Internal Standard Solution - Prepare the internal standard solution by accurately weighing approximately 0.05 g of pure TPP. Dissolve the TPP in MTBE and dilute to volume in a 100 mL volumetric flask. Transfer the internal standard solution to a TEE-fluorocarbon-sealed screw cap bottle and store at room temperature. Addition of 50 µg/mL of the internal standard to 5mL of sample extract result in a final TPP concentration of 5.0µg/mL. This solution should be replaced when ongoing QC indicates a problem.

Note*:* TPP has been shown to be an effective internal standard for the method analytes.

- Surrogate Standard solution - Prepare the surrogate standard solution by accurately weighing approximately 0.025 gm of pure 1,3-dimethyl-2-nitrobenzene. Dissolve the 1, 4-dimethyl-2-nitrobenzene in MTBE and dilute to volume in a 100 mL volumetric flask. Transfer the surrogate standard solution to a TEEfluorocarbon- sealed screw cap bottle and store at room temperature. Addition of 50 µL of the surrogate standard solution to a 1 L sample prior to extraction results in a 1,3-dimethyl-2-nitrobenzene concentration in the sample of 12.5 µg/L. Solution should be replaced when ongoing QC indicates a problem.

  Note: 1,3-dimethyl-2-nitrobenzene has been shown to be an effective surrogate standard for the method analytes.

- Laboratory Performance Check Solution - Prepare the laboratory performance check solution by adding 5 µL of the vernolate stock solution. 0.5 mL of the bromacil stock solution, 30 µL of the prometon stock solution. 15 µL of the atrazine stock solution, 1.0 mL of the surrogate solution, and 500 µL of the internal standard solution to a 100 mL volumetric flask. Dilute to volume with MTBE and thoroughly mix the solution. Transfer to a TEE-fluorocarbon-sealed screw cap bottle and store at room temperature.

  Verify calibration standards periodically (at least quarterly), by analyzing a QCS.

40.4 Extraction (Manual method)

- Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Add preservative to LRBs and LFBs. Fortify the sample with 50 µL of the surrogate standard solution. Pour the entire sample into a 2 L separately funnel.

- Adjust the sample to pH 7 by adding 50 mL of phosphate buffer. Check pH. Add acid or base if necessary to obtain pH 7.

- Add 100 gm sodium chloride to the sample, seal, and shake to dissolve salt.

- Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by vigorously shaking the funnel for two min with periodic venting to release excess

pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, entrifugation, or other physical methods. Collect the methylene chloride extract in a 500 mL Erlenmeyer flask.

- Add a second 60 mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner. Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

40.5 Extraction (Autotmated method)

- Mark the water meniscus on the side of the sample bottle for later determination of sample bottle for later determination of sample volume. Add preservative to LRBs and LFBs. Fortify the sample with 50 µL of the surrogate standard solution. If the mechanical separately funnel shaker is used, pour the entire sample into a 2 L separatory runnel. If the mechanical tumbler is used, pour the entire sample into a tumbler bottle.

- Adjust the sample to pH 7 by adding 50 mL of phosphate buffer. Check pH.

- Add acid or base if necessary to obtain pH 7. Add 100 g sodium chloride to the sample, seal, and shake to dissolve salt. Add 300 mL methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer the solvent to the sample contained in the separatory funnel or tumbler bottle, seal, and shake for 10 s, venting periodically. Repeat shaking and venting until pressure released is not observed. Reseal and place sample container in appropriate mechanical mixing device (separatory funnel shaker or tumbler). Shake or tumble the sample for one hour. Complete mixing of the organic and aqueous phases should be observed within about two min after starting the mixing device.

- Remove the sample container from the mixing device. If the tumbler is used, pour contents of tumbler bottle into a 2 L separatory funnel. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 500 mL Erlenmeyer flask Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

40.6 Extract Concentration

- Assemble a K-D concentrator by attaching a 25 mL concentrator tube to a 500mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements are met.

- Dry the extract by pouring it through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate. Collect the extract in the K-D concentrator, and rinse the column with 20-30 mL methylene chloride.

- Alternatively, add about 5 g anhydrous sodium sulfate to the extract in the Erlenmeyer flask; swirl flask to dry extract and allow to sit for 15 min. Decant the methylene chloride extract into the K-D concentrator. Rinse the remaining sodium sulfate with two 25 mL portions of methylene chloride and decant the rinses into the K-D concentrator. Add one to two clean boiling stones to the evaporative flask and attach a macro Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 65-70°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapour. Adjust the vertical position of the appratus and the water temperature as required to complete the concentration in 15-20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 2 mL, remove the K-D apparatus and allow it to drain and cool for at least10 min.

- Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of MTBE. Add 5-10 mL of MTBE and a fresh boiling stone. Attach a micro-Snyder column to the concentrator tube and prewet the column by adding about 0.5 mL of MTBE to the top. Place the micro K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in five to 10 min.
- When the apparent volume of liquid reaches 2 mL, remove the micro K-D from the bath and allow it to drain and cool. Add 5-10 mL MTBE to the micro K-D and reconcentrate to 2 mL. Remove the micro K-D from the bath and allow it to drain and cool. Remove the micro Snyder column, and rinse the walls of the concentrator tube while adjusting the volume to 5.0 mL with MTBE.
- **Note:** If methylene chloride is not completely removed from the final extract, it may cause detector problems. If the internal standard calibration procedure is used, add 50 µL of the internal standard solution to the sample extract, seal, and shake to distribute the internal standard. Transfer extract to an appropriate sized TFE-fluorocarbon-sealed screw-cap vial and store, refrigerated at 4°C, until analysis by GC-NPD.

40.7 Gas Chromatography

- Table 8 shows the retention times observed using this method.
- Inject 2 µL of the sample extract. Record the resulting peak size in area units.
- If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze. If using IS calibration, add an appropriate amount of IS so that the extract concentration will match the calibration standards.

40.8 Identification of Analytes

Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds,

within limits, to the retention time of a standard compound, then identification is considered positive.

The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard derviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms. Identification requires expert judgement when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e. broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternative techniques to help confirm peak identification, need be employed. For example, more positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used e.g., mass spectrometry, or the use of a second chromatography column.

40.9 Calculations

The concentration (C) in the sample can abe calculated from the following equation:

$$C\ (\mu g/L) = (A)(Vi) / (Vt)(Vs)$$

Where,

A = Amount of material injected (ng)

Vi = Volume of extract injected (µL)

Vt = Volume of total extract (µL)

Vs = Volume of water extracted (mL)

References

1. ASTM Annual Book of Standards, Part 11, Volume 11.02, D3694-82. "Standard Practice for Preparation of Sample Containers and for Preservation". American Society for Testing and Materials, Philadelphia, PA, (1986).

2. National Institute for Occupational Safety and Health ,"Carcinogens - Working with Carcinogens", Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Publication No. 77-206, august (1977).

3. Occupational Safety and Health Administration, OSHA 2206, "OSHA Safety and Health Standards, General Industry", (29 CFR1910), (Revised, January 1976).

4. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, (1979).

5. American Society for Testing and Materials, ASTM Annual Book of Standards, Part 11, Volume 11.01, D3370-82, "Standard Practice for Sampling Water", Philadelphia, PA, (1986).

6. Munch, J.W "Method 525.2 - Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Chromatography/ Mass Spectrometry".

7. USEPA, National Exposure Research Laboratory, Methods for the Determination of Organic Compounds in Drinking Water, Supplement 3. Cincinnati, Ohio, 45268, (1995).

Table 8: Retention times for method analytes

Analyte	**Retention Time[8]**	
	Column 1	**Column 2**
l,3-Dimethyl-2-Nitrobenzene (surrogate) Atrazine	14.48 31.58	30.00
Alachlor	35.96	34.10
Butachlor	41.45	39.00

Reference:

1. Official Method of Analysis AOAC. 17th Edition, 991.07 (2000).

2. J. Chromatogr. A, 1218, 38(23), 2011, 6780-6791.

NOTE: It is suggested to follow IS: 14543-2004, Amendment No.7

41. DETERMINATION OF 2,4-D IN DRINKING WATER BY LIQUID-LIQUID MICROEXTRACTION, DERIVATIZATION, AND FAST GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

Scope

41.1 Summary of Method

A 40-mL volume of sample is adjusted to pH > 12 with 4 N sodium hydroxide and allowed to sit for one hour at room temperature to hydrolyze derivatives. Following hydrolysis, a wash step using a hexane: MTBE mixture is performed as a sample cleanup and to remove Dacthal. The aqueous sample is then acidified with Sulphuric acid to a pH of less than 1 and extracted with 4-mL of methyl tert-butyl ether (MTBE). The chlorinated acids that have been partitioned into the MTBE are then converted to methyl esters by derivatization with diazomethane. The target esters are separated and identified by fast capillary column gas chromatography using an electron capture detector (GC/ ECD). Analytes are quantified using a procedural standard calibration technique with an internal standard.

Note: since many of the analytes contained in this method are applied as a variety of esters and salts, it is imperative to hydrolyze them to the parent acid prior to extraction.

41.2 Apparatus and Equipment

- Sample Containers - Amber glass bottles, approximately 40 mL, fitted with PTFE (polytetrafluoroethylene) lined screw caps.
- Extraction Vials - 60mL clear glass vials with PTFE lined screw caps.
- Autosampler Vials - 2.0mL vials with screw or crimp cap and a PTFE faced seal.

- Standard Solution Storage Containers - 10 to 20mL amber glass vials with PTFE lined screw caps.
- Pasteur Pipettes - Glass, disposable. Pipettes - Class A, 2.0mL and 4.0mL glass, or adjustable volume dispensers.
- Volumetric Flasks - Class A, suggested sizes include 5 mL, 10 mL, and 100 mL.
- Micro Syringes - Various sizes.
- Balance - Analytical, capable of weighing to the nearest 0.0001 gm.
- Diazomethane Generator - See Figure I for a diagram of an all glass system custom made for these validation studies.
- Micro molar generators are also available from commercial sources (Aldrich Cat. #: Z10, 889-8 or equivalent).(Do you want to mention commerciall name)
- Gas Chromatograph - Capillar GC, if the fast GC option is used the modifications should include a high pressure (> 50 psi) split/splitless injector, fast temperature ramps oven (50 °C /min) and a low volume (150 mL) micro ECD detector.
- Additionally, a data system capable of fast sampling (20 points/peak) is required.
- Primary GC Column - RTX-1701, 180 µm i.d., fused silica capillary with chemically bonded (14 % cyanopropylphenyl-methylpolysiloxane), or equivalent bonded, fused silica column. Confirmation GC Column - DB-5, 180 µm i.d., fused silica capillary with chemically bonded (5 % phenyl-methylpolysiloxane), or equivalent bonded, fused silica column.

41.3 Reagents and Standards

- Reagent Water - Purified water which does not contain any measurable quantities of any target analytes or interfering compounds greater than 1/3 the MRL for each compound of interest. (MTBE)-High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).
- Acetone - High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).

- Carbitol (Diethylene Glycol Monoethyl Ether) - High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).
- Hexane: MTBE (90:10, v/v) Wash Solvent - High purity, unpreserved, demonstrated to be free from analytes and interferences (HPLC grade or better).
- Hexane - High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).
- Sodium sulfate, Sodium sulfate - Pesticide grade, granular, anhydrous. Interferences have been observed when lower quality grades have been used. If interferences are observed, it may be necessary to heat the sodium sulfate in a shallow tray at 400 °C for up to 4 hr to remove phthalates and other interfering organic substances. Alternatively, it can be extracted with methylene chloride in a Soxhlet apparatus for 48 hr. Store in capped glass bottle rather than a plastic container.
- Acidified Sodium sulfate - Acidify by slurrying 100 gm of muffled sodium sulfate with enough ethyl ether to just cover the solid. Add 0.5mL concentrated Sulphuric acid dropwise while mixing thoroughly. Remove the ether under vacuum. Mix 1gm of the resulting solid with 5 mL of reagent water and measure the pH of the mixture. The pH must be below pH 4. Store in a desiccator or at 100°C to keep the reagent dry.
- Copper II Sulfate Pentahydrate, $Cu_2SO_4.5H_2O$ - ACS reagent grade or better.
- 4 N Sodium Hydroxide Solution - Dissolve 16 gm sodium hydroxide (Sodium hydroxide) pellets (ACS grade or equivalent) in reagent water and dilute to 100mL.
- potassium hrydroxide Solution (37 %, w/v) - Dissolve 37 gm of potassium hrydroxide pellets (ACS grade or equivalent) in reagent water and dilute to 100 mL. Sodium Sulfite, Na SO - ACS reagent grade, used as a dechlorinating agent in this method.
- Diazald Solution - Prepare a solution containing 5 g diazald (ACS reagent grade) in 50 mL of a 50:50 (v/v) mixture of MTBE: carbitol. This solution is stable for

one month or longer when stored at 4 ^{o}C in an amber bottle with a PTFE lined screw cap.

- Sulphuric acid - Concentrated, ACS reagent grade.
- Silica Gel - ACS reagent grade, 35-60 mesh.
- Hydrogen - 99.999 % pure or better, GC carrier gas.
- Nitrogen - 99.999 % pure or better.

41.4 Procedure Sample Extraction and Hydrolysis:

- Remove the samples from storage and allow them to equilibrate to room temperature. Place 40 mL of the water sample into a precleaned 60-mL, glass vial with a PTFE lined screw cap using a graduated cylinder.
- Add 10 µL of surrogate standard (100 ug/mL, 2,4-dichlorophenylacetic acid in acetone) to the aqueous sample.
- Add 1 mL, of the 4 N Sodium hydroxide solution prepared to each glass vial. Check the pH of the sample with pH paper or a pH meter. If the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 4 N Sodium hydroxide solution. Let the sample sit at room temperature for 1 hour, shaking the contents periodically.
- Note : The method is for 2,4,D in H_2O Since many of the herbicides contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction. This step must be included in the analysis of all extracted field samples, LRBs, LFMs and calibration standards. Failure to perform this step may result in data that are biased low for some targets in field samples.
- Following hydrolysis, add 5 mL of (90:10, v: v) hexane: MTBE and shake vigorously for three min. Allow the phases to separate for approximately 5 min then remove and discard the top hexane/MTBE layer. This wash aids in sample cleanup and removes any Dacthal from the sample which would interfere with the quantitation of the Dacthal metabolites.

- Adjust the pH to approximately 1 by adding concentrated Sulphuric acid. Cap, shake and then check the pH with a pH meter or narrow range pH paper. Add additional Sulphuric acid as needed to properly adjust the pH. Quickly add approximately 2 g of copper II sulfate pentahydrate and shake until dissolved. This colors the aqueous phase blue and allows the analyst to better distinguish between the aqueous phase and the organic phase in this micro extraction.

- Quickly add approximately 16 g of muffled sodium sulfate and shake until almost all is dissolved. Sodium sulfate is added to increase the ionic strength of the aqueous phase and thus further drive the chlorophenoxy acids into the organic phase. The addition of salt also decreases the solubility of MTBE in the aqueous phase and allows greater volumetric recovery. The addition of this salt and the copper II sulfate pentahydrate should be done quickly so that the heat generated from the addition of the acid will help dissolve the salts.

- Add exactly 4.0-mL MTBE and shake vigorously for three min. Allow the phases to separate for approximately 5 min.

41.5 Sample Methylation with Diazomcthanc. Generation of Diazomethane

- Assemble the diazomethane generator in a hood. The collection vessel is a 10- or 15mL, glass vial equipped with a PTFE lined screw cap that is maintained at 0-5 C.

- Add a sufficient amount of MTBE (approximately 7 mL) to tube 1 to cover the first impinger. Add 10 mL of MTBE to the collection vial. Set the nitrogen flow at 5-10 mL/min. Add 4-mL Diazald solution and 3 mL of 37 % potassium hrydroxide solution to the second impinger. Connect the tubing and allow the nitrogen flow to purge the diazomethane from the reaction vessel into the collection vial for 30min. Cap the vial when collection is complete and maintain at 0-5°C. When stored at 0-5°C, this diazomethane solution may be used over a period of 72 hr. Several commercial sources of glassware are available for diazomethane generation. (suitable, see reference). Using a Pasteur pipette, transfer the sample extract (upper MtBE layer) to a 7- mL, screw cap vial. Add 0.6 g acidified sodium sulfate and shake. This step is included to dry the MtBE extract.

- Using a Pasteur pipette, transfer the extract to a second, 7mL glass vial. Add 250 µL of the diazomethane solution prepared to each vial. The contents of the vial should remain slightly yellow in color indicating an excess of diazomethane. Additional diazomethane may be added if necessary. Let the esterification reaction proceed for 30 min.
- Remove any unreacted diazomethane by adding 0.1 g of silica gel. Effervescence (evolution of nitrogen) is an indication that excess diazomethane was present. Allow the extracts to sit for 0.5 hour.
- Transfer the extract to an autosampler vial. A duplicate vial may be filled using the excess extract.
- Analyze the sample extracts as soon as possible. The sample extract may be stored up to 21 days if kept at 0 °C or less. Keep the extracts away from light in amber glass vials with PTFE lined caps.

41.6 Gas Chromatography

If the fast GC option is used, several important changes from "conventional GC" should be made to aid in the rapid analysis of the analytes. The instrument should have a fast temperature ramp (50°C/minute) oven and a high pressure (> 50 psi) split/splitless injector. Additionally, the column diameter and film thickness should be decreased and the carrier gas should be changed to hydrogen.

Use of Hydrogen Safely - Although hydrogen can be used safely as a carrier gas, the potential for fire or explosion does exist if the gas system is mishandled. If you are unsure of the safety guidelines for using hydrogen as a carrier gas, seek advice from your instrument manufacturer regarding its use.

41.7 Analysis of Extracts

Establish operating conditions as described. Confirm that retention times, compound separation and resolution are similar to those summarized in Table 9.

Table 9:

Chromatographic conditions and average retention time data for the primary column (RTX-1701, 40m x 180 µm i.d.)

Peak Number	Compound	Average Tr (min)[a]	%RSD
1.	2,4-D	12.37	0.002

41.8 Calculations

$$C\ (\mu g/L) = \frac{(A)\ (V_i)}{(V_t)\ (V_s)}$$

Where,

A=Amount of material injected (ng)

V_i=Volume of the extract injected (µL)

V_t=Volume of the total extract (µL)

V_s=Volume of water extracted (mL)

Reference

Official Method of Analysis AOAC. 17th Edition. 10.7.03(2000).

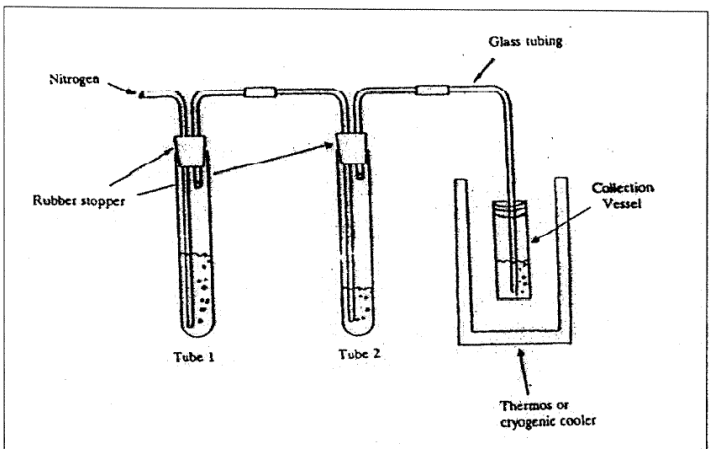

Fig. 2 Diazomethane solution generator

42. DETERMINATION OF ISOPROTURON IN WATER BY SOLID-PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY

42.1 Scope

Residue method for analysis of isoproturon in drinking water, using a styrene- divinyl benzene co-polymer solid phase extraction followed by liquid chromatography with diode array detection is described.

42.2 Apparatus:

A. Off-line solid phase concentrator-manual or automated.
B. Polypropylene cartridges: filled with 200 mg stryene - divinyl benzene e.g(suitable, see reference)
C. HPLC/UV system - suited for solvent - gradient elution with automatic sample injection and equipped with a UV - diode array detector, allowing the reconding of spectra between 200 and 400 (with λ_{max} = 230nm) and a data processing system.
D. Capillary vials - 5 mL, amber glass.

42.3 Reagents:

A. Certified reference materials of the Isoproturon: Standard solution of isoproturon is prepared in acetonitrile.
B. Solvents pesticide grade.

42.4 Sample extraction:

Pass a 500 mL volume of drinking water over the conditioned sorbent at a flow rate of 5-10 mL/min. Dry the solvent under nitrogen or air for 5 min. Elute the sorbent with 10mL methnol: acetonitrile (60:40). Soak the sorbent with 2 mL eluent for 30 min. Pass the rest of the eluent through sorbent through the sorbent at 3 mL/min. and collect the elute in the same vessel. Dry the combined elute under a gentle stream of nitrogen to approximately 200 µL, which is taken up in a 500 µL syringe. Adjust the volume to 1000 µL with water. Use an aliquot of this extract for HPLC/UV analysis.

42.5 HPLC/UV determination:

Use the following conditions of HPLC/UV for analysis. Column Supelcosil ABZ +(25 cm × 2.1 or 4.6 mm i.d 5 µm particles) combined with ABZ + guard column (2 cm, 4.6 mm id, 3 or 5 fn particles), both from supelceo gradient from acetonitrile: water (5:95) to acetonitrile: water (63:37) in 60 min to 100 % acetonitrile in 5 min., held for 5 min. the identity of each compound was confirmed, by the retention time, which was required to the within 1 % or < 10/5 from the retention time of the standards.

42.6 Calculation

The concentration (C) in the sample can be calculated from the following equation:

$$C\ (\mu g/L) = \frac{(A)\ (Vi)}{(Vt)\ (Vs)}$$

Where,

A= Amount of material injected (ng)

Vi= Volume of the extract injected (µL)

Vt = Volume of the total extract (µL)

Vs=Volume of water extracted (mL)

Reference:

AOAC International, 85 (2): 375-383 (2002).

43. NITROGEN-AND PHOSPHORUS-CONTAINING PESTICIDES IN FINISHED DRINKING WATER

The analytical method prescribes residue analysis of Dichlorvos (DDVP), Phorate, Chlorpyrifos. Methyl, Chlorpyrifos, Monocrotophos, Dimethoate, Malathion, Parathion, methyl, Parathion, ethyl, Ethion, Phosphamidon Atrazine, Simazine in water.

43.1 Principle

Measured volume of sample (1 L) is extracted with dichloromethane by shaking in separatory funnel or by mechanical tumbling in bottle. Dichloro methane extract is separated, dried with anhydrous Sodium sulfate solvent exchanged with (MTBE), and concentrated to 5 mL. Pesticides are separated and measured by capillarycolumn gas chromatographyusing a nitrogen-phosphorus detector.

43.2 Apparatus

(a) Grab sample bottles 1 L borosilicate glass with TFE fluorocarbon- lined screw caps (suitable, see reference)). Extract liners overnight with methanol before use.

(b)Separatory funnel 2 L, borosilicate glass with TFE fluorocarbon stopcock and ground-glass or TFE-fluorocarbon stopper.

(c)Tumbler bottle.-1.7 L, low extractable borosilicate glass with TFE flurorcarbon- lined screw caps (suitable, see reference). Cut liners to fit screw cap from TFE-fluorocarbon sheets (suitable, see reference); extract liners overnight with methanol before use.

(d) Kuderna-Danish (L-D) apparatus:
(l) Concentrator tube.-10 or 25 mL. Borosilicate glass, graduated, standard taper 19/22. Check calibration of concentrator tube at volumes used in method. Use ground-glass stoppers to prevent evaporation of extracts.
(2) Evaporation flask- 500 mL, borosilicate glass, standard taper 24/40 top, standard taper 19/22 bottom, capable of attachment to concentrator tube with springs.
(3) Snyder columns- 3-ball macro, 218 mm, standard taper 24/40 or 2-ball micro, 170 mm, standard taper 19/22.

NOTE: Instead of KD (Khuderman Danish) concentrator flash evaporator may also be used.

(e)Vials- Glass, 5 to 10 mL capacity, with TFE-fluorocarbon lined screw caps.

(f)Separatory funnel shaker.-(Optional) Capable of holding 2 L separatory funnels and

shaking them with rocking motion to thoroughly mix funnel contents (Eberbach Co. ann Arbor, ML or other suppliers).

(g)Tumbler. - Capable of holding tumbler bottles and over and at 30 rpm (Associated Design and Manufacturing Co. Alexandria. VA. Meets these specifications).

(h)Boiling stones- Carborundum, No. 12 granules. Heat 30 min at 400 °C before use. Cool and store in desiccator.
(i)Water bath. Heated, capable of control ±20 °C Use bath in hood.

(j)Balance- Analytical, capable of accurately weighing to nearest 0.0001 g.

(k)Gas chromatograph.-Temperature-programmable system for use with capillary columns, including syringes, analytical columns. Gases, detector, and strip chart recorder. Data system is recommended for measuring peak areas. Primary column: 30m × 0.25 mm id DB-5 fused-silica capillary column, 0.25 μm film thickness (suitable, see reference). Confirmation column: 30m × 0.25 mm id DB210 fused-silica capillary column, 0.25 μm film thickness (suitable, see reference).

Operating conditions:
Injection volume 2 μL splitless with 45 s delay;
He carrier gas at 30 cm/s linear velocity;
Injector 250°C,
detector300°C,
Oven programmed from 60 to 300 °C at 4°C/min;
Nitrogen-phosphorus detector.

43.3 Reagents:

(a)Standard solutions -Use standards of test compounds with purity 96 % to prepare stock solutions at 1 mg/mL in MTBE. Commercially prepared stock standards may be used at any concentration if they are certified by manufacturer or independent source. These stock standards may be available from U.S. Environmental Protection Agency Toxic and Hazardous Materials Repository. Research Triangle Park. NC Store solutions at

room temperature and protect from light. Replace stock solutions after 2 months, Or sooner if comparision with degradation.

(b) Internal standard solution. Prepare 2 nitrotoluene (purity 98 % stock solution at 0.25 mg/mL in MTBE add 50 µL stock solution to 5 mL sample extract to give final internal standard concentration of 2.5 µg/mL.

(c) Surrogate solution-Prepare 1.3-dimethyl-2-nitrobenzene (DMNB) (purity 98 %) stock solution at 0.25 mg/mL in MTBE. Add 50µL stock solution to 1 L sample prior to extraction to produce surrogate concentration of 12.5 µg/mL in sample and assuming quantitative recovery, 2.5 µg/mL in extract.

(d)Instrument performance slution.Using standard solutions (a) combine 5 µL vernolate stock solution. 0.5 mL bromacil stock solution. 30 µL prometon stock solution and 15 µL atrazine stock solution in 100mL volumetric flask, and dilute to volume with MTBE.

(e)Solvents.-Acetone, methylene chloride, methyl tert-butyl ether (MTBE). Distilled-in-glass quality or equivalent.

(f)Phosphate buffer.-pH7 Mix 29.6 mL 0.1 M HCI and 50mL 0.1 M dipotassium hydrogen phosphate (K_2HPO_4).

(g)Sodium sulfate.-Granular, anhydrous. ACS grade. Heat in shallow tray for > 4hr at 450 °C to remove interfering organic substances.

(h)Sodium chloride.-Crystals. ACS grade. Heat in shallow tray for >4h at 450 °C to remove interfering organic substances.

(i) Regent water.-Water reasonably free of contamination that would prevent determination of any analyte of interest.

(j)Preservative-Mercuric chloride solution. 10 mg Mercuric chloride (ACS grade) mL reagent water,

(k)Sodium thiosulfate.-Sodium thiosulfate. Granular, anhydrous. ACS grade.

43.4 Preparation of sample Bottles

Add 1 mL preservative, CQ, to glass sample bottle. If residual chlorine is expected to be present in samples, add 80mg Sodium thiosulfate, to sample bottle before collection.

43.5 Sample Collection:

Collect 1 L grab laboratory samples in glass bottles by conventional sampling practices. Because bottles contain preservative and Sodium thiosulfate do not prerinse bottles with sample before collection. Add sample to bottle containing preservative, seal sample bottle, and shake vigorously 1 min. Regrigerate samples at 4 °C from time of collection until extracted. Protect from light. Samples are stable for 14 days when stored under these conditions. Extracts, stored at 4 °C away from light, are stable for 14 days.

43.6 Sample Preparation

(a) Automated extraction procedure-add preservative, CQ, to any samples not previously preserved. Mark water meniscus on side of sample bottle for later determination of sample volume. Add 50 µL surrogate stock solution to sample. If mechanical separatory funnel shaker is used, pour entire sample into separatory funnel. If mechanical tumbler is used, pour entire sample into tumbler bottle. Adjust sample to pH 7 by adding 50 mL phosphate buffer, check pH and add sulphuric acid or Sodium hydroxide if necessary. Add 100 g Sodium chloride to sample, seal, and shake to dissolve salt. Add 300 mL dichloromethane to sample bottle, seal, and shake 30s to rinse inner walls.

Transfer solvent to sample contained in separatory funnel or tumbler bottle, seal, and shakes 10 s, venting periodically. Repeat shaking and venting until pressure release is not observed during venting. Reseal and place sample container in appropriate mechanical mixing device (separatory funnel shaker or tumbler). Shake or tumble sample for 1 h. After extraction, pour contents of tumbler bottle into 2 L separatory

funnel. Let organic layer separate from water phase for 10 min. if emulsion interface between layers is more than on-thrid volume of solvent layer, complete phase separation mechanically. Collect dichloromethane extract in 500mL Erlenmeyer flask containing ca 5 g anhydrous sodium sulfate. Swiril flask to dry extract; let flask sit 15 min. determine original sample volume by refilling sample bottle to mark and transferring water to 1000 mL graduated cylinder. Record sample volume to nearest 5mL.

(b) Manual extraction method.- Add preservative (CQ) to samples not previously preserved. Mark water meniscus on side of sample bottle for later determination of sample volume. Add 50 µL surrogate stock solution to sample. Pour entire sample into 2 L separately funnel. Adjust sample to pH 7 by adding 50mL phosphate buffer, check pH, and add sulphuric acid or Sodium hydroxide. If necessary add 100 g sodium chloride to sample, Seal and shake to dissolve salt. Add 60mL dichloromethane to sample bottle, seal and shake bottle 30s to rinse inner walls.

Transfer solvent to separately funnel and extract sample by vigorously shaking funnel for 2 min with periodic venting to release excess pressure. Let organic layer separate from water phase for 10 min. if emulsion interface between layers is more than on-third volume of solvent layer, complete phase separation mechanically. Collect dichloromethane extract in 500 mL Erlenmeyer flask containing ca 5 g anhydrous sodium sulfate. Add second 60 mL portion of dichloromethane to sample bottle and repeat exraction procedure a second time, combining extracts in Erlenmeyer flask. Perform third extraction in same manner. Swirl flask to dry extract. Let flask sit for 15 min. determine original sample volume by refilling sample bottle to the mark and transferring water to 1 L graduated cylinder. Record sample volume to nearest 5 mL.

43.7 Extract Concentration

Asemble K-D concentrator by attaching 25 mL concentrator tube to 500mL evaporation flask. Decant dichloromethane extract into concentrator. Rinse remaining Sodium sulfate with two 25 mL portions of dichloromethane and decant rinses into concentrator. Add 1 or 2 clean boiling stones to evaporation flask and attach macro-snyder column. Pre wet column by adding ca 1 mL dichloro methane to top. Place K-D apparatus on 65-70°C

water bath so that concentrator tube is partially immersed in hot water and the entire lower, rounded surface of flask is bathed with hot vapor. Adjust vertical position of apparatus and water temperature as required to complete concentration in 15-20 min. at proper rate of distillation. Balls of column will actively chatter, but chambers will not flood. When apparent volume of liquid reaches 2 mL, remove K-D apparatus, let drain and cool 10min.

Remove snyder column; rinse flask and its lower joint with 1-2 mL MTBE collecting rinse in concentrator tube. Add 5-10 mL MTBE, collecting rinse in concentrator tube Add 5-10 mL MTBE and fresh boiling stone. Attach micro-snyder column to concentrator tube and prewet column by adding ca 0.5 mL MTBE to top. Place micro K-D apparatus on water bath so that concentrator tube is partially immersed in hot water. Adjust vertical position of apparatus and water temperature as required to complete concentration in 5-10 min. when apparent volume of liquid reaches 2 mL remove apparatus from bath and let it drain and cool.

Add 10mL MTBE and boiling stone and reconcentrate to 2 mL. When apparent volume of liquid reaches 2 mL. Remove apparatus from bath and let it drain and cool. Add second 10 mL MTBE and boiling stone and reconcentrate to 2 mL when apparent volume of liquid reaches 2 mL, remove apparatus from bath and let it drain and cool.

Add third 10 mL MTBEand boiling stone and reconcentrate to µL Remove micro K-D apparatus from bath and let it drain and cool. Remove micro-snyder column; rinse walls of concentrator tube while adjusting volume to 5.0 mL with MTBE. Add 50 µL internal standard stock solution C (b) to sample extract, seal, and shake to distribute internal standard. Transfer extract to appropriate-size TFE- fluorocarbon-sealed screw-cap vial. Store at 40 °C until analysis. A 14-day maximum storage time is recommended.

43.8 Calibration of gas chromatograph with Nitrogen Phosphorus Detector.

Table 10 summarizes retention times observed using this method of analysis of organic nitrogen and phosphorus pesticides.

Table 10: Retention times for method 8141A analytes employing 30-m columns[3]

Compound	RT (min)	
	DB-5	DB-210
Dichlorvos (DDVP)	7.45	6 .99
Phorate	12.27	16 .57
Chlorpyrifos. Methyl	15.94	20.45
Chlorpyrifos	17.06	22 .22
Monocrotophos	19.08	15 .98
Dimethoate	18.11	17 .21
Malathion	19.83	21 .75
Parathion, methyl	20.15	20.45
Parathion, ethyl	21.38	22 .22
Ethion	22.55	27 .12
Phosphamidon	22.77	20 .09
Atrazine	13.98	17 .63
Simazine	13.85	17.41

43.9 Calculations:

The concentration (C) in the sample can be calculated from the following equation:

$$C\ (\mu g\ /L) = \frac{(A)\ (V1)}{(Vt)\ /\ (Vs)}$$

Where,

A = Amount of the Standard injected (ng)

V1 =Volume of the extract injected (µL)

Vt =Volume of total extract (µL)

Vs =Volume of the water extract (mL).

Reference:

EPA method 8141 A. United States Environmental Protection Agency 8141.

Organophosphorus compounds by gas chromatography capillary column technique.

Or

BIS method (IS 13428) may also be followed.

J. Chromatogr. A, 1218, 38(23), 2011, 6780-6791.

44. LABORATORY SAFETY

44.1 General Safety Rules

The rules of safety as applied to laboratory operations are the professional, moral, and legal responsibility of the analyst. The practice of safety requires the desire on the part of individual analysts to protect themselves and their associates by following a set of rules.

1. Proper eye protection is required for anyone entering the laboratory. Specific eye protection should be used according to the work to be performed.
2. All laboratory personnel shall familiarize themselves with the location and use of all emergency equipment.
3. No one may work alone in the laboratory. Exceptions are on a case by-case basis or as indicated in the laboratory's safety program. Supervisors will judge the risk of the work to be performed and provide specific permission to work alone in the laboratory.
4. Before starting a sample analysis or research project, the procedure must be reviewed for possible hazards, and the necessary precautions taken to eliminate or counteract the hazard.
5. Unauthorized analyses or experiments are not to be conducted.
6. All accidents and hazardous conditions must be brought to the supervisor's attention.

44.2 Personal Protection

1. Safety glasses must be worn at all times in laboratory areas. Any exceptions to this rule must be included in the laboratory safety program. Contact lenses

should not be worn in the laboratory. Gases and vapors can be concentrated under contact lenses and cause permanent eye damage. In the event of a chemical splash into the eye, it is often nearly impossible to remove the contact lens to irrigate the eye because of involuntary spasm of the eyelid. If it is absolutely essential to wear contacts, the supervisor must be made aware of this and adequate safety precautions must be taken. Where splashing chemicals or flying particle hazards exist, appropriate glasses with side shields, goggles, or face shields should be used. Specialized eye wear must also be used to protect against laser, ultraviolet, or other intense light sources.

2. Appropriate protective clothing, specifically rubber gloves, should be worn when handling:

   acids or bases or caustic chemicals

   silicone-based lubricants

   blood

   items suspected of being contaminated in some manner

3. Ear plugs/hearing protectors should be worn when:

   working in a high noise level area

   working with systems that could produce a sudden sonic shock -using some types of ultrasonic cleaners

   using other equipment emitting high frequency noise

4. Safety shoes should be worn when moving or lifting heavy items.

5. Loose clothing, neckties, scarves, jewelry, and long hair should be confined or bound so that they will not become entangled in equipment.

6. Hands should be washed after handling chemicals and specimens, before eating, and before leaving work at the end of the day.

7. Smoking, eating, and drinking should not be permitted in the laboratory.

8. Laboratory refrigerators are not to be used for personal food storage, e.g.,

lunches.

9. Cosmetics should never be applied in the laboratory.

44.3 Housekeeping

Good housekeeping in the laboratory helps prevent accidents. "A PLACE FOR EVERYTHING AND EVERYTHING IN ITS PLACE", makes for safety and efficiency.

1. Aisles should be kept free of obstructions (cartons, carts, chairs, etc.).

2. Hoods should be kept clear and clean and should not be used as a common storage area.

3. Sinks must be kept clean.

4. Chipped or broken glassware should be discarded into marked containers for disposal.

5. Work surfaces should be cleaned after each analysis.

6. All spills must be cleaned up immediately.

7. Broken glass should be removed with a brush and dust pan or with cardboard. Absorbent cotton, held with tongs, may be used to pick up fine pieces of broken glass. A towel should never be used to clean up broken glass.

8. Laboratory services (air, gas, water, etc.) are to be turned off at the service cock when not in use.

9. Equipment should be returned to its proper place after use.

10. Materials should always be stacked neatly.

11. Toxic and corrosive materials should be rinsed from containers before the containers are given to the person washing glassware.

12. Good lighting is especially important around work areas, storage areas, and stairways.

44.4 Special Precautions

1. Unlabeled chemicals must be removed and disposed of properly.

2. When performing a taste test, the material being tested should not be swallowed.

3. A pipet bulb should be used instead of mouth suction to pipet chemical or to start a siphon.

4. All liquid reagents, particularly corrosive ones, should be poured from their containers with the use of a pouring rod.

5. Mercury spills inust be cleaned up immediately. If the spills run into inaccessible areas, the mercury should be covered with sulfur to reduce vaporization.

6. When lifting heavy objects, the feet should be set apart to obtain a firm footing. One foot should be put alongside the object and one foot behind it.The back should be kept straight and the chin tucked in so the head and neck continue the straight back line. The object should be gripped firmly with the palms of the hands and held close to the body with arms and elbows tucked into the sides of the body to keep the body weight centered, and then the object is lifted straight with a thrust of the rear foot.

44.5 Laboratory Hazards

Laboratories contain a greater variety of hazards than do most workplaces.
Therefore, an analyst must work defensively at all times, considering each operation for its intrinsic dangers, and building into each experimental setup methods of control, security, and escape. Serious accidents, affecting health, eyesight, and life, rarely happen, but they are always due to carelessness and they are preventable. A convenient common sense question to ask before performing an analysis is "What would happen if ... ?" Answers to this question require some knowledge of hazards associated with the chemicals and equipment involved.

44.6 Hazards Associated With Use of Chemicals

The hazards associated with chemical use are physical or chemical. Fires, explosions, and exothermic reactions are the most serious immediate dangers in a laboratory. Dangers that usually become apparent over somewhat longer periods are due to contamination by toxic materials, poisoning, and asphyxiation. The analyst must be alert to possibilities for all these hazards and avoid them.

44.6.1 Physical Hazards

Physical hazards include flammability; explosions; and means of high pressure containment, such as compressed gas cylinders.

44.6.1.1 Flammability

Flammable substances are among the most common causes of serious laboratory accidents. In discussing flammability, a few definitions are needed.

44.6.1.2 Flash Point: The lowest temperature at which a liquid gives off vapor in sufficient concentration to form an ignitable mixture with air near the surface .of the liquid within the test vessel.

44.6.1.3 Ignition Temperature: The minimum temperature required to initiate or cause self- sustained combustion independent of the heat source.

44.6.1.4 Limits of Flammability: A flammable liquid may be above its flash point and

yet not ignite in the presence of an adequate energy source. The reason for this is that each flammable gas and liquid (as a vapor) has two fairly definite limits defining the range of concentrations in mixtures with air that will propagate flame and explode.

44.6.1.5 The lower flammable limit: (lower explosive limit [LEL]) is the minimum concentration (percent by volume) of the vapor in air below which a flame is not propagated when an ignition source is present. Below this concentration, the mixture is too lean to burn. The upper flammable limit upper explosive limit [DEL]) is the maximum concentration (percent by volume) of the vapor in air above which a flame is not propagated. Above this concentration, the mixture is too rich to burn.

44.6.1.6 Spontaneous Ignition or Combustion: This phenomenon takes place when a substance reaches its ignition temperature without the application of external heat. Materials susceptible to spontaneous combustion include oily rags, dust accumulations, organic materials mixed with strong oxidizing agents(such as nitric acid, chlorates, permanganates, peroxides, and persulfates), alkali metals such as sodium and potassium, finely divided pyrophoric metals, and phosphorics. The basic precautions for safe handling of flammable materials include the following:

Flammable substances should be handled only in areas free of ignition sources.

Flammable substances should never be heated by using an open flame, steam baths, water baths, oil baths, heating mantles, and hot air baths are the preferred heat sources.

When transferring flammable liquids in metal equipment, static-generated sparks should be avoided by bonding and the use of ground straps.

Ventilation should be provided. This is one of the most effective ways to prevent the formation of flammable mixtures.

44.6.1.7 Explosions

Explosions usually result from extremely rapid exothermic reactions of mixtures of compounds. As heat is evolved, reaction rates increase and often large volumes of expanding gases are produced in fractions of a second. Many reactions can take place explosively, but oxidations are among the most common sources of accidents.

Explosions can also result from reactions initiated by the cleavage of weak bonds in sensitive compounds. Such explosions can be induced by heat or mechanical shock.

An especially treacherous source of explosions is peroxides formed by autoxidation of common solvents. An example is ether in an opened bottle that has been left in the laboratory for a few months. Ethers should never be left for long periods in partially filled containers or in the light.

Potentially explosive substances should not be subjected to friction. They should not be stored in screw cap or ground glass-stoppered containers, and the mixture should be kept away from stopcocks and stirrer packing glands. Many compounds containing high proportions of oxidizable or oxidizing groups are best stored wet.

The use of explosive materials demands special safety measures and handling techniques that are thoroughly understood and followed. Guidelines for use in any laboratory operation that might involve explosive materials are as follows:

The analyst must wear safety glasses that have a cup-type shield affixed to the frame; a face shield with a "snap-on" throat protector in place when in a hazardous, exposed position; gloves when it is necessary to reach behind a shielded area.

Shields, barricades, or guards must be placed around the hazardous area. Warning signs should be posted in the hazardous area.

Miscellaneous protective devices should be available as required to prevent exposure of any part of the body to injury.

44.6.1.8 Means of Containment

Compressed gases contained in cylinders present a unique hazard because they have the potential for simultaneous exposure to both mechanical and chemical hazards. Therefore, they must be handled with care. Compressed gas cylinders must not be dropped or thrown and must be firmly secured at all times and properly identified. They should be protected from heat and direct sunlight and the head should be covered with a valve-protecting cap during storage or when being transported. Pressure-relief devices to protect equipment attached to cylinders of flammable, toxic, or otherwise hazardous gases should be vented to a safe place.

Cylinders should be placed so that the cylinder valve is accessible at all times. The main cylinder valve should never be left open when the equipment is unattended or not operating.

44.6.2 Chemical Hazards

Many chemicals found in the laboratory are known to be toxic or corrosive or both. Their toxic effects are classified as acute or chronic hazards. Acutely hazardous chemicals are capable of producing immediate or slightly delayed effects, such as burns, inflammations, allergic responses, and damage to the eyes, lungs, or 'nervous system. The effects of chronic hazards are delayed or develop only after exposure over long periods of time. Carcinogenic effects are usually chronic. The major classes of corrosive chemicals are strong acids and bases, dehydrating agents, and oxidizing agents.

44.6.2.1 Exposure to chemicals may occur by:

44.6.2.1.1 Inhalation:

Poisoning by absorption through the mucous membrane of the mouth, throat, and lungs is produced by inhalation of toxic vapors, mists, gases, or dusts. Inhaled gases or vapors may pass rapidly into the capillaries of the lungs and be carried into the circulatory system. The degree of injury resulting from exposure to toxic substances depends on the toxicity of the material and its solubility in tissue fluids, as well as on its concentration and the duration of exposure. Several chemicals, e.g., mercury and its derivatives, and some of the common solvents, e.g., benzene, are cumulative or chronic poisons that can produce body damage through exposure to small concentrations over a long period of

time.

Work that involves toxic gases, vapors, and dusts must be carried out in a fume hood to prevent the escape of such material into the working place. Chemicals of unknown toxicity should never be smelled.

44.7 Safety Measures of Handling EC Detector General

The BCD contains Nickel (a maximum of 15mCi) radioactive material. Although beta particles at this energy level have little penetrating power - the surface layer of the skin or a few sheets of paper will stop most of them - they may be hazardous if the isotope is ingested or inhaled. Radioactive leak tests must be performed at the required intervals. The inlet and outlet fitting must be capped when the detector is not in use.
Corrosive chemicals must not be introduced into the detector, and the effluent from the detector must be vented outside the laboratory environment.

44.8 Ecd Warnings

1. Owners may not open the ECD cell.
2. Owners shall not modify the cell in any manner.
3. Owners shall not use any solvent, including water, to internally clean the cell.
4. Owners shall not interface with or attempt to defeat the overheat circuitry that may be supplied with the ECD.
5. Owners shall not transfer the ECD to another person or another location except as described in the applicable Regulations.
6. Owners must perform a radioactive leak test at least every 6 months.
7. Owners must maintain record as required by your local Agency (the NCR or in certain states, a state agency).
8. Owners must notify the in case of incidents or failures that might lead to a hazardous condition.

44.9 Safety

1. For radioactive lead testing, wipe-test of ECD cell is required at intervals of no longer that 6 months.
2. If a leak detects more 0.005 mCi (microcurie) of removable radioactive cell or if the cell is damaged in such a way as to indicate that it may no longer adequate shield the radioactive material inside, you must immediately suspend operation of your chromatograph until the cell has been repaired. "Never dismental the ECD cell. You can remove the ECD cell for repair.
3. Never eat, drink, or smoke when handing ECDs.
4. Always wear safety glasses when working with or near open ECDs.
5. Wear protective cloths such as laboratory jackets, safety glasses, and gloves, and follow good laboratory practices. Wash hands thoroughly with a mild non-abrasive cleaner after handling of ECDs.
6. Cap the inlet and outlet fitting when the ECD is not in use.
7. Connect the ECD exhaust vent to fume hood or vent it to the outside.
8. It is mandatory to get the prior permission for import / procure and use of ECD from Atomic Energy Regulatory Board, Govt. of India, Mumbai. Very few Make and Model of ECD are approved by AERB for use in our country.

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Note:

~Flash evaporator may be used in place of The KD (Khuderman Danish) concentrator.

~Wherever alternate methods are suggested in addition to the existing methods, they need to be re-validated in the respective food laboratories, so as to make them adoptable.

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