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Animal feeding stuffs — Determination of residues of organophosphorus pesticides — Gas chromatographic method

Aliments des animaux — Détermination des résidus de pesticides organophosphorés — Méthode par chromatographie en phase gazeuse

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 734 10 79
E-mail copyright@iso.ch
Web www.iso.ch

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14182 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 10, *Animal feeding stuffs*.

Annexes A and B of this International Standard are for information only.

Animal feeding stuffs — Determination of residues of organophosphorus pesticides — Gas chromatographic method

1 Scope

This International Standard specifies a gas chromatographic method for the determination of the residues of organophosphorus pesticides in animal feeding stuffs.

The method is applicable to animal feeding stuffs containing residues of one or more of the following organophosphorus pesticides: azinphos-ethyl, azinphos-methyl, bromophos, carbophenothion, chlorpyrifos, chlorpyrifos-methyl, diazinon, dimethoate, ethion, fonofos, malathion, methidathion, parathion, parathion-methyl, pirimiphos-ethyl and pirimiphos-methyl.

The lower limit of determination for these organophosphorus pesticides is 0,01 μg/g.

NOTE The method is probably equally applicable to other organophosphorus pesticides such as methaccrifos and fenitrothion, but it has not been validated for these pesticides.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative documents referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696, *Water for analytical laboratory use — Specification and test methods.*

ISO 6498, *Animal feeding stuffs — Preparation of test samples*.

3 Principle

A test portion is extracted with acetone. The filtered extract is diluted with water and a saturated sodium chloride solution. The pesticides are partitioned in dichloromethane. The concentrated extract is purified on a chromatographic column of 10 % water-deactivated silica gel. Gas chromatographic determination is carried out with a phosphorus-selective detector or a mass-selective detector.

4 Reagents and materials

Use only reagents of recognized analytical grade and with a purity suitable for pesticide residue analysis.

Check the purity of the reagents by performing a blank test under the same conditions as used in the method. The chromatogram should not show any interfering impurity.

WARNING — Some of the organic solvents are suspected carcinogens. Use with care.

4.1 Water, complying with at least grade 3 in accordance with ISO 3696.

4.2 Hexane

4.3 Acetone

4.4 Dichloromethane

4.5 Ethyl acetate

4.6 Silica gel, with a mass fraction of water of 10 %.

Activate silica gel 60**,** particle size 63 µm to 200 µm, at 130 °C overnight and cool in a desiccator. After cooling to room temperature, pour the silica gel into an air-tight glass container and add sufficient distilled water to bring the final mass fraction of water to 10 %. Shake the container mechanically or by hand vigorously for 30 s and allow to stand for 30 min with occasional shaking. After 30 min the silica gel is ready for use. It may not be stored for more than 6 h.

4.7 Eluting solvent, dichloromethane in hexane (50 % volume fraction).

Mix equal volumes of dichloromethane (4.4) and hexane(4.2).

4.8 Inert gas, e.g. nitrogen.

4.9 Sodium sulfate, anhydrous.

4.10 Sodium chloride saturated solution

4.11 Pesticide reference standards, as follows:

— azinphos-ethyl [*S*-(3,4-dihydro-4-oxobenzo[*d*][1,2,3]triazin-3-ylmethyl) *O*,*O*-diethyl phosphorodithioate];

— azinphos-methyl [*S*-(3,4-dihydro-4-oxobenzo[*d*][1,2,3]triazin-3-ylmethyl) *O*,*O*-dimethyl phosphorodithioate];

— bromophos [*O*-4-bromo-2,5-dichlorophenyl *O*,*O*-dimethyl phosphorothioate];

— carbophenothion [*S*-4-chlorophenylthiomethyl *O*,*O*-diethyl phosphorothioate];

— chlorpyrifos [*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate];

— chlorpyrifos-methyl [*O*,*O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate];

— diazinon [*O*,*O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate];

— dimethoate [*O*,*O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate];

— ethion [*O*,*O*,*O'*,*O'*-tetraethyl *S*,*S'*-methylene di(phosphorodithioate)];

— fonofos [*O*-ethyl *S*-phenyl ethylphosphonodithioate];

— malathion [diethyl (dimethoxythiophosphorylthio)succinate];

— methidathion [*S*-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl *O*,*O*-dimethyl phosphorodithioate];

— parathion [*O*,*O*-diethyl *O*-4-nitrophenyl phosphorothioate];

— parathion-methyl [*O*,*O*-dimethyl *O*-4-nitrophenyl phosphorothioate];

— pirimiphos-ethyl [*O*-2-diethylamino-6-methylpyrimidin-4-yl *O*,*O*-diethyl phosphorothioate];

— pirimiphos-methyl [*O*-2-diethylamino-6-methylpyrimidin-4-yl *O*,*O*-dimethyl phosphorothioate];

NOTE The common names and the chemical names (between square brackets) according to IUPAC nomenclature, are in accordance with ISO 1750 [1].

4.12 Internal standard: tributylphosphate.

4.13 Pesticide standard solutions

4.13.1 Stock solutions, of concentration 1 000 µg/ml.

Prepare a stock solution of each pesticide reference standard (4.11) and of the internal standard (4.12) as follows.

Weigh, to the nearest 0,1 mg, a mass of a pesticide reference standard (4.11) or the internal standard (4.12) which will result in a solution with a content of reference standard or internal standard of 1 000 µg/ml. While weighing, observe the cleanness of the standard material. Transfer the weighed mass into a volumetric flasks, dissolve in ethyl acetate (4.5) and dilute to volume with ethyl acetate.

These solutions are stable for 6 months when stored at 4 °C in the dark.

4.13.2 Intermediate solutions, of concentration 10 µg/ml.

Pipette 1 ml of each stock solution (4.13.1) into individual 100 ml volumetric flasks. Dilute to volume with ethyl acetate (4.5). The solutions are stable for 1 month when stored at 4 °C in the dark.

NOTE The stability of properly stored pesticide standards is very widely known. Investigations have shown that all neat pesticide standards tested are stable for 15 years when stored at –18 °C and that stock solutions of pesticide standards in toluene of 1 mg/kg are stable for at least 3 years when stored at –18 °C.

A recommended practice for longer storage is as follows. Transfer portions of the prepared standard solutions to amber vials with PTFE-lined screwcaps. Weigh the vials and store at –20 °C. When needed, remove a vial from the freezer, bring to room temperature and weigh. If accumulated loss in mass (due to evaporation) is 10 % or more of the prefrozen net mass, discard the vial. Weigh and refreeze stock standards and intermediate solutions that are in use for more than 1 month (usually in 25 ml vials). Otherwise, the prepared standard solutions (usually in 2 ml vials) may be stored at 4 °C and shall be discarded after 1 month.

4.13.3 Working solutions, of concentration 0,5 µg/ml.

Pipette 5 ml of each intermediate solution (4.13.2) into 100 ml volumetric flasks and dilute to volume with ethyl acetate (4.5). The solutions are stable for 1 month when stored at 4 °C in the dark (see 4.13.2).

4.14 Blank sample solutions, of the same type as the samples being analysed, but free of positives, resulting from previous determinations.

5 Apparatus

Before use, wash all glassware thoroughly with detergent free of interfering substances, rinse with water, then with acetone and dry.

Avoid the use of plastics containers and do not lubricate the stopcocks with grease, otherwise impurities could be introduced into the solvents.

Usual laboratory equipment and in particular the following.

5.1 Separating funnels, of capacities 500 ml and 1000 ml, with polytetrafluoroethylene (PTFE) stopcocks and stoppers.

5.2 Filtering flasks, of capacity 500 ml.

5.3 Büchner funnel, made of porcelain, with internal diameter 90 mm.

5.4 Graduated tubes, of capacity 10 ml, with polytetrafluoroethylene (PTFE) stoppers.

5.5 Glass chromatographic tube, about 300 mm in length, 8 mm to 10 mm internal diameter, with coarse fritted plate of porosity grade P 100 (pore size index 40 μm to 100 μm [2]) or glass wool plug.

5.6 Rotary vacuum evaporator, provided with round-bottom flasks of capacities 100 ml and 500 ml, and a water bath set at 40 °C.

5.7 Mechanical shaker or **high-speed blender**

5.8 Gas chromatographic system

5.8.1 Components

The system shall comprise:

— splitless or on-column injection system;

— column;

— phosphorus-selective detector or mass-selective detector;

— electrometer;

— mV recorder or integrator;

— data-handling software and computer system.

Each injection port, column oven and detector shall be provided with an independent heating device controlled to the nearest 0,1 °C.

The chromatographic system shall be adjusted and the parameters optimized according to the characteristics of the instrument used.

5.8.2 Conditions

The injection port and the detector temperatures shall be 220 °C to 240 °C and 180 °C to 380 °C, respectively, according to the manufacturer's instructions.

For organophosphorus separations with a capillary column, a temperature programme for the column oven is recommended.

5.8.3 Injection device

An autosampler or any other adequate injection device may be used.

For manual injections, use a microsyringe of capacity 1 µl to 5 µl, with a needle length suitable for the mode of injection (splitless or on-column).

Before injecting the solution into the gas chromatograph, rinse the syringe ten times with pure solvent, then five times with solution. After injection, rinse the syringe five times with pure solvent.

5.8.4 Column

The use of capillary columns coated with nonpolar to mid-range polarity stationary phases, e.g. SE-30, SE-54, OV-17, or equivalent, is recommended.

Standard glass columns, of length 2 m to 4 m and of internal diameter 2 mm to 4 mm, packed with 10 % DC-200 on Chromosorb WHP 0,15 mm to 0,18 mm particle size, or a mixture of 2 % QF1 and 1,5 % DC-200 on Chromosorb WHP 0,125 mm to 1,15 mm particle size, or any other stationary phases and inert supports recommended for organophosphorus residue analyses could be used as an alternative.

The temperature programme for the column shall be chosen to separate the mixture of pesticides specified in clause 1 into individual components (see annex A).

After installing a new column, it shall be conditioned for at least 48 h at a temperature slightly above the proposed highest operating temperature, with carrier gas flowing through it.

5.8.5 Detector

Use a phosphorus-selective detector [flame photometric detector (FPD) or nitrogen-phosphorus detector (NPD) in P mode] or mass-selective detector (MSD), with a minimum detection limit of 50 pg of P compounds.

5.8.6 Carrier gas

Use pure nitrogen, pure helium or pure hydrogen.

Dry the carrier gas by passing it through 0,5 nm molecular sieve traps, previously activated at 350 °C for 4 h to 8 h, installed in the carrier gas line.

Reactivate the molecular sieves each time a new gas cylinder is assembled and as often as needed.

5.8.7 Auxiliary gases

Use hydrogen and air.

5.8.8 Verification of the linearity of the system

Check the linearity of the system from 0,1 ng to 2 ng of parathion.

Prepare working solutions with parathion contents ranging from 0,05 µg/ml to 1,0 µg/ml. Inject 2 µl.

Plot the peak size (area or height) against the mass, in nanograms, of parathion injected. The graph shall be a straight line that passes through the origin. If not, establish the range of concentrations within which the detector response is linear.

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [3].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

Grind a portion of the well-mixed laboratory sample (dry or low-moisture products, e.g. cereals and cereal products, oilseeds and oilseed meals, mixed feeds, hay, etc.) so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

Chop high-moisture products (e.g. grasses, silages, etc.) into small pieces and mix thoroughly to obtain homogeneous samples.

8 Procedure

8.1 General

Carry out the following steps on both the prepared test sample (clause 7) and a blank sample (4.14) having a matrix of the same type as the sample being analysed, for use in preparing the reference calibration solution.

8.2 Test portion

Weigh, to the nearest 0,1 g, 50 g of the prepared test sample (clause 7) for dry or low-moisture products, or 100 g for high-moisture products, into a 1000 ml conical flask.

8.3 Extraction

Add enough water (4.1) to the test portion so that a total amount of water of about 100 g is obtained. Let the sample soak for about 5 min. Add 200 ml of acetone (4.3). Close the flask tightly and shake for 2 h on a mechanical shaker or homogenize for 2 min in a high-speed blender.

Filter the suspension with suction through a Büchner funnel (5.3), fitted with filter paper of medium porosity, into a 500 ml filtering flask (5.2). Wash the conical flask or the blender cup and the residue on the filter paper with two 25 ml portions of acetone, collecting the washings into the same filtering flask (5.2).

Transfer the filtrate to a 1 000 ml separating funnel. Wash the filtering flask (5.2) with 100 ml of dichloromethane (4.4) and transfer this to the separating funnel. Add 250 ml of water (4.1) and about 50 ml of saturated sodium chloride solution (4.10) into the separating funnel. Stopper and shake for 2 min.

Allow the phases to separate and draw off the lower phase (dichloromethane) into a 500 ml separating funnel. Repeat twice with 50 ml of dichloromethane (4.4) and combine the extracts in the same 500 ml separating funnel.

Wash the dichloromethane extract with two 100 ml portions of water, discarding the washings.

Filter the dichloromethane extract through a filter paper containing about 20 g of sodium sulfate (4.9) into a 500 ml flask of a vacuum evaporator. Rinse the separating funnel and the sodium sulfate with two 10 ml portions of dichloromethane and add to the flask.

Concentrate to about 2 ml under vacuum at a temperature not exceeding 40 °C. Transfer the solution to a 10 ml graduated tube using 1 ml to 2 ml of hexane (4.2) and concentrate under nitrogen to about 1 ml.

Do not allow the solution to dry out or losses of pesticides may occur because of volatility or poor solubility.

8.4 Column clean-up

8.4.1 Preparation of the column

Transfer 5 g of 10 % water-deactivated silica gel (4.6) into a glass chromatographic tube (5.5). Add 5 g anhydrous sodium sulfate (4.9) on the top of the silica gel. Wash the prepared column with 20 ml of hexane (4.2).

NOTE Prepacked silica or Florisil cartridges (e.g. Millipore-SEP PAK) could be used instead of a silica gel column, after checking for efficiency and absence of interferences.

8.4.2 Purification

Transfer quantitatively the concentrated extract (8.3) to the top of the prepared column (8.4.1) using 1 ml to 2 ml portions of hexane (4.2).

Elute the organophosphorus pesticides with 50 ml of eluting solvent (4.7) and collect the eluate into a 100 ml flask of a vacuum evaporator.

Concentrate the eluate as in 8.3 but using ethyl acetate (4.5) instead of hexane and dilute the final solution to 10 ml with ethyl acetate for chromatography.

When an internal standard method is used, add 0,5 ml of the intermediate solution of tributylphosphate (4.13.2) to the final extract before diluting to 10 ml with ethylacetate.

Keep the blank extract to prepare the reference calibration solution (8.5).

8.5 Gas chromatography

Equilibrate the gas chromatographic system under the recommended operating conditions (5.8). Inject 1 µl to 2 µl of working standard solution (4.13.3), then the same volume of the sample extract (8.4.2). Dilute the sample extract if necessary.

Identify the individual pesticide peaks on the basis of retention times.

Determine the amount of pesticides by comparing the size of the sample peaks with those of the known amount of the corresponding pesticide peak in the working standard solution.

If the results correspond to or exceed the maximum residue limits (MRLs), prepare a reference calibration solution by adding to the blank extract appropriate amounts of intermediate solutions (4.13.2) of those pesticides identified in the sample solution, so that the size of the peaks of this reference solution is within 25 % of the size of the peaks in the sample solution. Dilute to 10 ml with ethyl acetate (4.5). Inject into the gas chromatograph the same volume as for the sample solution.

Determine the amount of pesticides by comparing the size of the sample peaks with those of the known amount of the corresponding pesticide peak in the reference calibration solution.

9 Expression of results

9.1 Calculation

Calculate the content of each individual pesticide residue in the test sample by the equation:

$$w = \frac{A \cdot m_S \cdot V}{A_S \cdot m \cdot V_i}$$

where

w is the individual pesticide residue content, in micrograms per gram, of the test sample;

A is the size of the sample peak;

A_S is the size of the corresponding standard pesticide peak in the working standard solution or the reference calibration solution;

m_S is the mass, in nanograms, of standard pesticide injected into the gas chromatograph;

V is the final volume, in millilitres, of the sample extract taking into account any dilution that is necessary;

V_i is the volume, in microlitres, of the sample extract injected into the gas chromatograph;

m is the mass, in grams, of the test portion.