International Standard 4389

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Tobacco and tobacco products — Determination of organochlorine pesticide residues (Reference method)

Tabac et produits du tabac — Détermination des résidus de pesticides organochlorés (Méthode de référence)

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 4389 was developed by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, and was circulated to the member bodies in October 1979.

It has been approved by the member bodies of the following countries :

Australia	France	South Africa, Rep. of
Austria	Germany, F.R.	Spain
Belgium	Greece	Sri Lanka
Brazil	India	Switzerland
Bulgaria	Italy	Thailand
Czechoslovakia	Netherlands	United Kingdom
Egypt, Arab Rep. of	Poland	Yugoslavia
Ethiopia	Romania	

No member body expressed disapproval of the document.

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Tobacco and tobacco products — Determination of organochlorine pesticide residues (Reference method)

WARNING. Acetonitrile and benzene are toxic. Strict precautions should be taken for the protection of personnel.

1 Scope and field of application

This International Standard specifies a reference method for the determination of organochlorine pesticide residues in tobacco and tobacco products.

The method is applicable to the determination in tobacco and tobacco products of a large number of organochlorine pesticides and some of their isomers and breakdown products.

The method is particularly recommended for determination of the substances within the detection limits listed in the table.

NOTE — ISO 1750 contains the systematic chemical names and structures corresponding to the ISO common names in the table.

The application of this method is limited in the presence of polychlorobiphenyls or camphechlor.

2 References

ISO 1750, *Pesticides and other agrochemicals — Common names.*

ISO 4874, *Tobacco and tobacco products — General conditions of sampling.*

3 Principle

Extraction of the pesticide residues from the sample with a mixture of acetonitrile and water. Re-extraction from the resultant solution with hexane. Clean-up of the concentrated hexane extract by absorption onto and elution from a column of Florisil using first hexane and then benzene. Determination of the pesticide residues in the hexane and benzene fractions by gas chromatography.

Table — List of substances with detection limits

Substance	ISO 1750 Common name	Detection limit µg/g
aldrin	aldrin	0,10
α-chlordane or β-chlordane	—	0,05
pp'-DDE	—	0,10
op'-DDT	—	0,10
pp'-DDT	—	0,10
dieldrin	dieldrin	0,10
endosulfan	endosulfan	0,10
endosulfan sulphate		0,20
endrin	endrin	0,10
HCB	hexachlorobenzene	0,05
α-HCH or α-BHC	—	0,05
β-HCH or β-BHC	—	0,05
γ-HCH or γ-BHC	gamma-HCH or gamma-BHC	0,05
δ-HCH or δ-BHC	—	0,05
heptachlor	heptachlor	0,10
heptachlor epoxide	—	0,10
op'-TDE or *op'*-DDD	—	0,10
pp'-TDE or *pp'*-DDD	TDE	0,10

4 Reagents

4.1 General

All the reagents shall be of analytical reagent quality. All solvents shall be checked for purity before use by carrying out a blank determination using exactly the same procedure (extraction, concentration, clean-up and gas chromatography) as is used during the determination on a sample. The chromatogram obtained from the solvents shall have a baseline without noticeable peaks that could interfere with those from the pesticide residues being determined.

NOTE — Grades of reagents and solvents specially purified for pesticide residues analysis are commercially available.

Water used shall be distilled water or water of at least equivalent purity.

4.2 ***n*-Hexane.**

4.3 Benzene.

4.4 Sodium sulphate, anhydrous.

4.5 Acetonitrile/water mixture.

Mix 5 volumes of acetonitrile and 2 volumes of water.

4.6 Sodium chloride, 20 g/l solution.

4.7 Florisil, 60 to 100 mesh.

NOTE — Florisil is the trade name of a special selected variety of magnesium silicate. The mesh size range designated as 60 to 100 mesh corresponds to a mesh aperture size range of 250 to 150 µm.

4.7.1 Requirement

The quality of the Florisil is one of the most critical features of the method of test. The activity of the Florisil needs to be sufficient to retain impurities present in the extract from the sample while allowing the pesticide residues to be eluted. The Florisil shall first be pretreated as described in 4.7.2. Only Florisil that passes the subsequent verification test described in 4.7.3 shall be used.

4.7.2 Pretreatment

Heat the Florisil for 5 h in a muffle furnace at 500 to 550 °C. Allow the Florisil to cool in a tightly sealed container that contains no desiccant. Add rapidly, drop by drop and with constant mixing, 5 ml of water for every 100 g of Florisil. Mix thoroughly in a rotating flask for 30 min. Allow the Florisil to equilibrate by storing in a completely sealed container for at least 48 h before proceeding as described in 4.7.3.

4.7.3 Verification of activity level

Prepare a standard pesticide solution in hexane containing all the pesticides under investigation, each at a concentration equivalent to 10 times the lower limit of detection for the pesticide concerned (see the table).

NOTE — The concentration value of 10 times the lower detection limit is based on a 95 % recovery and also ensures that at least twice the lower limit is present for the subsequent check by gas chromatography.

Percolate the prepared standard pesticide solution through a column of the pretreated Florisil, as described in 7.3, and determine the amount of each pesticide in the eluates, using the gas chromatographic procedure described in 7.4 and 7.5.

The activity level of the pretreated Florisil is correct and it is satisfactory for use if the recovery of each pesticide is better than 90 %.

The activity of the Florisil should be checked each time it is used.

4.8 Standard pesticide solutions.

It is recommended that solid pesticides of guaranteed purity should be used. Weigh at least 10 mg of each solid pesticide so as to ensure a precision of at least 1 % (for example 10 ± 0,1 mg). Prepare stock solutions, in benzene or hexane as appropriate, of each pesticide at a concentration which will allow for subsequent dilution for calibration (7.5) and for checking the Florisil activity in accordance with 4.7.3. Store the stock solutions in a refrigerator. These solutions are stable for at least 6 months.

From the stock solutions prepare suitable dilute solutions of each pesticide, in the appropriate solvent, as required for calibration.

4.9 Internal standard (optional).

Mirex can be used as an internal standard, if necessary.

NOTE — Mirex is a generic name for dodecachloropentacyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decane.

5 Apparatus

NOTE — It is essential to clean all glassware very thoroughly before use and to avoid the use of plastics containers and stopcock grease, otherwise impurities may be introduced into the solvents.

Ordinary laboratory apparatus not otherwise specified, and

5.1 Conical flasks, of capacity 500 ml, with ground glass stoppers.

5.2 Separating funnels, of capacity 250 ml, with PTFE stopcocks.

5.3 One-mark volumetric flasks, of capacity 25 ml, complying with class A of ISO 1042.

5.4 One-mark pipettes, of capacity 5 ml, 25 ml and 50 ml, complying with class A of ISO 648.

5.5 Pasteur pipettes.

5.6 Rotary evaporator, equipped with flasks of various capacities, or

5.7 Kuderna-Danish evaporator (see figure in annex).

5.8 Chromatographic column, 10 mm internal diameter, equipped with a PTFE stopcock and sintered glass disc, porosity grade P 100 (see ISO 4793).

5.9 Gas chromatograph.

5.9.1 Basic requirements

The gas chromatograph shall be set up and the parameters optimized to suit the particular makes of instrument and detector in use. The injection port, oven and detector shall each be equipped with a separate heating unit. The dead volume of the detector should be as small as possible and shall be less than 5 % of the total volume flowing each minute to the detector. On-column injection is recommended.

The gas chromatograph used shall be capable of being set up in accordance with the detailed instructions and recommendations given in 5.9.2 to 5.9.4 inclusive.

5.9.2 Temperatures

The temperature of the injection port should be 30 °C higher than the column temperature, for example, for a column at 210 °C, the injection port should be at 240 °C.

Detector temperatures shall be 180 to 350 °C, depending on the type of detector.

5.9.3 Injection device

Use an automatic injection system or any suitable alternative means of injection.

For manual injection the use of a spring-loaded microsyringe, capable of injecting 1 to 5 µl portions, is recommended. Before solutions are injected with the syringe, rinse it 10 times with pure solvent, then 5 times with the solution. After injection rinse the syringe 5 times with pure solvent.

5.9.4 Column

5.9.4.1 Dimensions

The chromatograph shall be equipped with a glass chromatographic column of sufficient length (2 to 4 m) to effect adequate separation of the pesticide residues in the sample under examination.

5.9.4.2 Packing

The support and stationary phase shall be one of the following alternatives :

a) 1,5 g of SP-2250 and 1,95 g of SP-2401 on each 100 g of Supelcon 100/120;

b) 1,5 g of OV-17 and 1,95 g of QF-1 on each 100 g of Varaport 30 (or equivalent support).

NOTES

1 SP-2250 is a poly(methylphenylsiloxane) (50 % phenyl).
SP-2401 is a fluoropropylsilicone.
Supelcon 100/120 is a silanized diatomaceous earth (100/120 indicates a mesh size in the range 150 to 125 µm).
OV-17 is a poly(methylphenylsiloxane).
QF-1 is a trifluoropropylmethylsilicone.
Varaport 30 is a silanized diatomaceous earth.

2 If a more positive identification and determination is required, a second column of different polarity should be used.

3 The stationary phases and support media are specified by the designations under which they are commercially available. This form of specification is necessary because the substances cannot be sufficiently specified for the purposes of this International Standard on the basis of generic descriptions as given in note 1.

5.9.4.3 Detector

An electron-capture detector shall be used.

5.9.4.4 Carrier gas

Pure nitrogen, pure helium, or an argon and methane mixture, 90 + 10 or 95 + 5 (by volume) shall be used. If the flow of gas through the column is less than 25 ml/min, additional carrier gas shall be added at the exit of the column to ensure a sufficiently high gas flow rate through the electron-capture detector (purge gas).

The carrier gas supply shall be purified by a molecular sieve included in the gas supply line.

6 Sampling and preparation of sample

6.1 Sampling

Sample the tobacco or tobacco product in accordance with ISO 4874. Give particular attention to ensuring that the test sample is representative of the product as received.

6.2 Preparation of test sample

The test sample should preferably be in the form of cut tobacco. If the test sample cannot be prepared in this form, powder the laboratory sample, taking care to avoid heating it.

7 Procedure

7.1 Test portions

7.1.1 Weigh, to the nearest 0,1 g, duplicate 20 g test portions of the prepared test sample. Carry out the procedure described in 7.2 to 7.6 inclusive on each of the duplicate test portions.

7.1.2 Concurrently with taking the test portions as described in 7.1.1, take another test portion and use it for the determination of the water content of the test sample.

7.2 Extractions

7.2.1 Acetonitrile extraction

Transfer the test portion to a 500 ml conical flask (5.1). Add 280 ml of acetonitrile/water mixture (4.5), stopper the flask and shake it for 1 h. Alternatively, place the test portion and the acetonitrile/water mixture in a laboratory macerator and macerate for 2 min.

Filter the contents through a fast filter paper, previously washed with acetonitrile/water mixture, and collect all the filtrate.

7.2.2 Re-extraction into hexane

Take in a pipette 50 ml of the filtrate and transfer it to a 250 ml separating funnel (5.2). Add 25 ml of the hexane (4.2) and 150 ml of the sodium chloride solution (4.6) and shake gently for 1 min. Allow the phases to separate and then transfer the aqueous layer into a second separator.

Add 25 ml of the hexane to the second separator, shake it for 1 min and allow the phases to separate. Run off the aqueous layer to waste and combine the hexane layer with that from the first separator.

Dry the combined hexane layers by passing them through a column, diameter 2 cm, height 2 cm, of the anhydrous sodium sulphate (4.4). Wash the column with a further 20 ml of hexane.

Transfer the total dried hexane solution to the rotary evaporator (5.6) and heat at a temperature of 40 °C and an absolute pressure of 53 mbar. Alternatively, transfer the solution to the Kuderna-Danish evaporator (5.7) and heat on a steam bath. Reduce the volume of the solution to about 1 ml.

7.3 Clean-up and preparation of test eluate

Fill a chromatographic column (5.8) to a height of 5 cm to 6 cm with the hexane (4.2). Add 2 g of the Florisil (4.7), followed by anhydrous sodium sulphate (4.4) to a height of about 2 cm. Drain the hexane from the column until the hexane level coincides with the top of the column packing.

Transfer the concentrated solution (7.2.2) from the evaporator quantitatively to the column using a pasteur pipette (5.5), washing the evaporator, flask and pipette as necessary with hexane. Drain the column until the solution level in the column coincides with the top of the column packing.

Elute the column with hexane by adding just enough hexane to allow 25 ml of eluate to be collected in a 25 ml volumetric flask (5.3). This is fraction A.

Add benzene (4.3) to the column and collect 25 ml of eluate in a second 25 ml volumetric flask. This is fraction B.

NOTE — Normally, dieldrin, endrin, heptachlor epoxide, and the endosulphans are found in fraction B.

7.4 Gas chromatography

Inject in turn triplicate 1 to 5 µl portions of fraction A and fraction B on to the gas chromatograph (5.9) and obtain in triplicate the chromatograms for each fraction. For each of the triplicate injections use the detector response corresponding to each pesticide residue, and the calibration curve (see 7.5), to calculate the concentration of the pesticide residue.

For mean values of 1,0 part per million or greater, the range of the triplicate values should not exceed 10 % of their mean. For mean values of less than 1,0 part per million, the range of the triplicate values should not exceed 20 % of their mean. If for any set of triplicate values this closeness of agreement is not obtained, the corresponding result for the test portion is invalid.

Provided that this requirement for closeness of agreement is complied with, use the mean of each set of triplicate values for calculation of the result (see clause 8).

7.5 Calibration

Prepare from the standard pesticide solutions (4.8) a number of dilute pesticide solutions covering a range of concentrations of each pesticide under examination. Inject triplicate 1 to 5 µl portions of the first standard pesticide solution into the gas chromatograph and determine the mean detector response from the chromatograms (peak height or peak area). Proceed similarly for each of the standard pesticide solutions. Plot a curve for each pesticide residue relating the mean detector response to the concentration of each standard solution.

NOTE — The response of the electron-capture detector is not always linear.

In the process of testing the samples it is vital to alternate injections of standard and sample solutions to check the calibration. To maintain the detector response within its linear range dilute the extract if necessary. Use Mirex (see 4.9) as an internal standard if required, to increase the precision of the results.

8 Expression of results

8.1 Method of calculation and formula

Calculate for each test portion the value for each pesticide residue, in micrograms per gram of the sample (parts per million), using the formula

$$\frac{C' \times 280 \times 25}{50 \times m} \times \frac{100}{(100 - W)}$$

where

C' is the concentration (mean of triplicate values, see 7.4), in micrograms per millilitre, of pesticide residue in the final extracts as read from the calibration curve;

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

W is the water content, as a percentage by mass, of the test sample.