

INTERNATIONAL STANDARD

ISO 4389

Third edition
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Tobacco and tobacco products — Determination of organochlorine pesticide residues — Gas chromatographic method

*Tabac et produits du tabac — Dosage des résidus de pesticides
organochlorés — Méthode par chromatographie en phase gazeuse*



Reference number
ISO 4389:2000(E)

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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.ch
Web www.iso.ch

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Foreword

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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 4389 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

This third edition cancels and replaces the second edition (ISO 4389:1997). As a result of extensive examination, the scope of this edition has been extended to tobacco products. Technical Corrigendum 1:1998 has been included.

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

Tobacco and tobacco products — Determination of organochlorine pesticide residues — Gas chromatographic method

1 Scope

This International Standard specifies a method for the gas chromatographic determination of pesticide residues in leaf tobacco, manufactured tobacco and tobacco products.

The method is applicable to the determination of the organochlorine pesticides listed in Table 1. The method is particularly recommended for determination of the substances within the detection limits listed in Table 1.

NOTE 1 The method has been demonstrated to be free from errors that may arise from interfering peaks on the chromatogram that originate from non-organochlorine pesticide substances, when applied to various styles of leaf tobacco. However, the preceding statement cannot be assumed to apply to all forms and variants of tobacco and tobacco products and care should be taken in the interpretation of any unexpected, apparently positive results. Some analysts advocate the use of mass spectrometric confirmation of the chemical structure of compounds detected by chromatography in such circumstances.

NOTE 2 ISO 1750 contains the systematic chemical names and structures corresponding to the common names given in Table 1.

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 document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 648, *Laboratory glassware — One-mark pipettes.*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks.*

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

ISO 4874, *Tobacco — Sampling of batches of raw material — General principles.*

ISO 8243, *Cigarettes — Sampling.*

3 Principle

The pesticide residues from a dried and milled sample, mixed with Florisil®¹⁾, are extracted by *n*-hexane in a special Soxhlet extractor. They are determined without any further clean-up with a gas chromatographic apparatus equipped with an electron-capture detector.

4 Reagents

All reagents shall be suitable for pesticide residue analysis. All solvents shall be checked for purity before use by carrying out a blank determination using exactly the same procedure (extraction and gas chromatography) as used for the test 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.

4.1 Water, degassed, in accordance with at least grade 2 of ISO 3696.

4.2 *n*-Hexane.

4.3 Toluene.

4.4 Reference substances: certified reference materials of minimum purity 95 % (mass fraction) of the substances listed in Table 1.

NOTE *trans*-Chlordane is used as an indicator for chlordane (technical mixture). If α -endosulfan is detected by this method, it is advisable to determine residues of the sum of α -endosulfan, β -endosulfan and endosulfan sulfate by a method suitable for such determinations.

4.5 Internal standard stock solution

Use Mirex,²⁾ an obsolete pesticide which has been superseded (see reference [2]).

NOTE Mirex is a generic name for dodecachloropentacyclo[5.2.1.0^{2,6}.0^{3,9}.0^{5,8}]decane.

Weigh, to the nearest 0,000 1 g, 0,02 g of Mirex into a 100 ml volumetric flask. Dilute to the mark with *n*-hexane (4.2).

4.6 Internal standard solution

Pipette 5 ml of the internal standard stock solution (4.5) into a 200 ml volumetric flask and dilute to the mark with *n*-hexane (4.2) to give a solution containing approximately 5 μ g/ml of Mirex. Store the internal standard solution at between 0 °C and +4 °C and exclude light. Under these conditions the internal standard solution is stable for at least 6 months.

1) Florisil® is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

2) Mirex is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Table 1 — List of suitable pesticides with detection limits

Substance	Common name (ISO 1750)	Detection limit µg/g
Aldrin	aldrin	0,02
<i>trans</i> -Chlordane	chlordane	0,02
<i>p,p'</i> -DDE	—	0,02
<i>o,p'</i> -DDT	—	0,04
<i>p,p'</i> -DDT	DDT	0,06
Dieldrin	dieldrin	0,02
α -Endosulfan	endosulfan	0,03
HCB	hexachlorobenzene	0,02
α -HCH or α -BHC	HCH or BHC	0,02
β -HCH or β -BHC	HCH or BHC	0,02
γ -HCH or γ -BHC	gamma-HCH (lindane) or gamma-BHC	0,01
δ -HCH or δ -BHC	HCH or BHC	0,02
Heptachlor	heptachlor	0,02
Heptachlor epoxide	—	0,02
<i>o,p'</i> -TDE or <i>o,p'</i> -DDD	—	0,03
<i>p,p'</i> -TDE or <i>p,p'</i> -DDD	TDE	0,02
<i>o,p'</i> -DDE	—	0,03

4.7 Standard pesticide solutions

Store all pesticide solutions at between 0 °C and +4 °C and exclude light. Under these conditions the solutions are stable for at least 6 months.

4.7.1 Individual standard stock solutions

Weigh, to the nearest 0,000 1 g, 0,02 g of each pesticide reference substance (4.4) into individual 100 ml volumetric flasks. Dilute to the mark with *n*-hexane (4.2) to give individual standard stock solutions containing approximately 200 µg/ml of the individual pesticide.

In the case of β -HCH, the standard stock solution should be made by dissolving the pesticide in toluene (4.3) because of reduced solubility in *n*-hexane.

4.7.2 Mixed stock solution A

Pipette 5 ml of each individual standard stock solution (4.7.1) into a single 200 ml volumetric flask and dilute to the mark with *n*-hexane (4.2) [or toluene (4.3) for β -HCH] to give a solution containing approximately 5 $\mu\text{g/ml}$ of each pesticide.

4.7.3 Mixed stock solution B

Pipette 1 ml of mixed stock solution A (4.7.2) into a 10 ml volumetric flask and dilute to the mark with *n*-hexane (4.2) to give a solution containing approximately 0,5 $\mu\text{g/ml}$ of each pesticide.

4.7.4 Standard calibration solution

Pipette 1 ml of both the mixed stock solution A (4.7.2) and the internal standard solution (4.6) into a 100 ml volumetric flask and dilute to the mark with *n*-hexane (4.2) to give a solution containing approximately 0,05 $\mu\text{g/ml}$ of each pesticide and internal standard.

4.8 Florisil®, 150 μm to 250 μm .

NOTE Florisil® is a special, selected variety of magnesium silicate. The nominal aperture size of 150 μm to 250 μm corresponds to a mesh size range designated as 60 mesh to 100 mesh.

4.8.1 The quality of the Florisil® is one of the most critical features of the test method. The activity of the Florisil® must 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 pre-treated as described in 4.8.2. Only Florisil® that passes the subsequent verification test described in 4.8.3 shall be used.

4.8.2 Heat sufficient Florisil® for the verification test for at least 5 h in a quartz crucible in a muffle furnace at 550 °C. Allow the Florisil® to cool in a desiccator that contains no desiccant and transfer to a round-bottom flask. Add 5 ml of water (4.1) for every 100 g of Florisil®. Mix thoroughly in a rotating flask for approximately 1 h. Allow the Florisil® to equilibrate by storing in a tightly closed glass container for at least 48 h before proceeding as described in 4.8.3.

4.8.3 The activity level of the Florisil® is checked by the extraction of dieldrin from *n*-hexane solution. The solution shall have a concentration equivalent to that of an extract from tobacco containing 1,0 $\mu\text{g/g}$ of this pesticide. The activity level of the pretreated Florisil® is correct if the recovery of dieldrin is better than 95 %.

The activity of the Florisil® shall be checked each time a new portion is prepared.

5 Apparatus

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. All volumetric flasks and pipettes shall comply with class A of ISO 1042 and class A of ISO 648 respectively.

Usual laboratory apparatus and the following items.

5.1 Rotary evaporator.

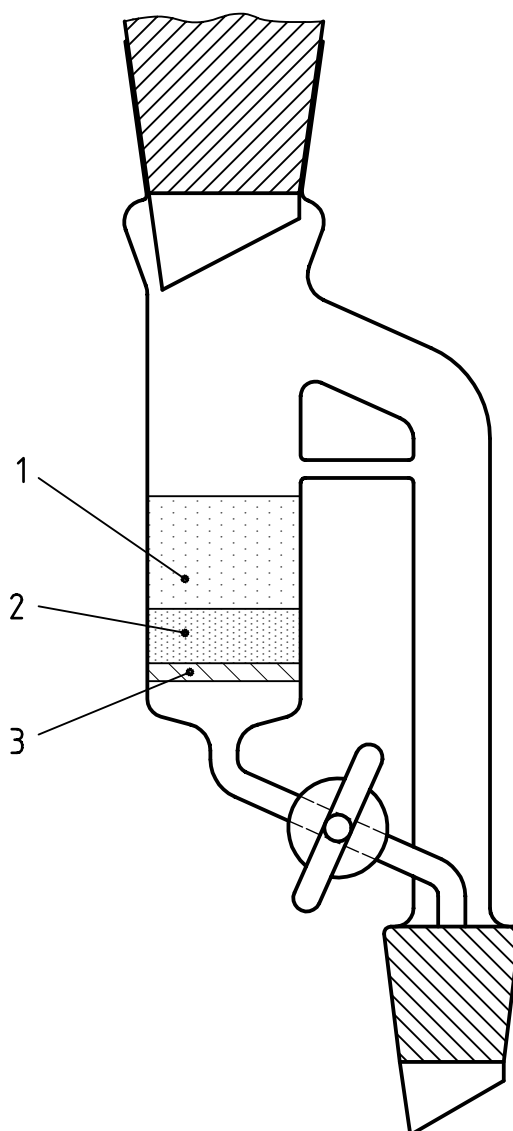
5.2 Tobacco mill, with 2 mm mesh.

5.3 Oven, with ventilation.

5.4 Muffle furnace.

5.5 Heating mantles.

5.6 Soxhlet extractor, for continuous extraction (see Figure 1).



Key

- 1 Tobacco + Florisil®
- 2 Florisil®
- 3 Filter disk porosity 1

NOTE Distillation rate 200 ml/h.

Figure 1 — Apparatus used for tobacco extraction

5.7 Reflux condenser.

5.8 Desiccator.

5.9 Quartz crucible.

5.10 Gas chromatograph

Operate the gas chromatograph in accordance with the manufacturer's instructions. The injection port, oven and detector shall each be equipped with a separate heating unit.

The following conditions have been found to be satisfactory on a particular make of instrument and are given for guidance. If other conditions are used they should be validated prior to use.

a) Temperatures

The injection port temperature shall be between 180 °C and 210 °C. The detector temperature shall be between 290 °C and 340 °C. If any other conditions are used they shall be sufficient to achieve satisfactory separation of all components and similar to that given in the specimen chromatogram (see Figure A.1 for an example). A suitable temperature programme is

— initial temperature	40 °C
— initial time	2 min
— temperature profile 1	20 °C/min from 40 °C to 150 °C
— temperature profile 2	3 °C/min from 150 °C to 270 °C
— final time	15 min at 270 °C
— total GC run time	62,5 min

b) Gas flow rates

Gas flow rates should be set according to the instrument manufacturer's guidance and the analyst's experience. Suitable gas flow conditions are

— carrier gas	helium, 4 ml/min
— make-up gas	nitrogen, 30 ml/min
— septum purge	5 ml/min
— split vent	30 ml/min

c) Injection mode

Use 2 µl splitless with split valve closed for 1 min after injection.

5.10.1 Injection device

Use an automated injector or any suitable alternative means of injection.

For manual injection, the use of a microsyringe capable of injecting 1 µl to 5 µl portions is recommended. Before solutions are injected with the syringe, rinse it at least ten times with *n*-hexane then five times with the solution. After injection, rinse the syringe five times with *n*-hexane.

5.10.2 Column

A fused silica capillary column of length 30 m and of internal diameter 0,32 mm is recommended; stationary phase DB-5³⁾ (5 % methyl phenyl silicone); thickness of the stationary phase 0,25 µm. The performance of the column should be sufficient to achieve satisfactory separation of all components and similar to that given in the specimen chromatogram (see Figure A.1 for an example).

5.10.3 Detector

An electron-capture detector shall be used with a sensitivity sufficient to detect (twice baseline noise) a 2 µl injection of a 0,001 5 µg/ml solution of *p,p'*-DDT.

6 Sampling and preparation of test sample

6.1 Sampling

Sample the tobacco in accordance with ISO 4874 or all the cigarettes in accordance with ISO 8243. Pay particular attention to ensuring that the test sample is representative of the product as received.

6.2 Preparation of test sample

Dry the tobacco in the oven (5.3) set at 50 °C for 2 h to obtain a mass fraction of moisture of approximately 5 % after drying.

Grind the tobacco through a 2 mm mesh (5.2), taking care to avoid heating above 50 °C. Alternatively the tobacco may be received in a milled form in which case ensure that the mass fraction of moisture is less than 10 %.

Store the tobacco in sealed containers and exclude light. If samples are kept for longer than one month prior to analysis, they shall be stored in a freezer at a temperature below – 8 °C.

7 Procedure

7.1 Test portion

Weigh, to the nearest 0,01 g, 5 g tobacco test portions into 50 ml beakers. Add 5 g of pretreated Florisil® (4.8) and mix thoroughly. Carry out the procedures described in 7.2 and 7.3.

7.2 Extraction

Add 5 g of pretreated Florisil® (4.8) to a Soxhlet extractor. Transfer the test portion as prepared in 7.1 without mixing so that two separate layers are formed.

For recovery determinations, appropriate pesticide standard solutions should be added at this stage, by means of a pipette, to the top of the test portion layer.

Transfer 60 ml of the *n*-hexane (4.2) and 1 ml of the internal standard solution (4.6) to a suitable round-bottom flask of capacity 150 ml to 250 ml.

Assemble the extraction apparatus, ensuring good seals at all joints, and turn on the heating mantles (5.5).

³⁾ DB-5 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Regulate the heating element and the tap on the Soxhlet extractor to give a distillation rate of at least 200 ml per hour. The level of *n*-hexane above the tobacco shall be kept constant by adjusting the tap on the Soxhlet extractor. Do not allow the round-bottom flask to become dry. Total extraction time is 4 h 30 min.

After extraction, allow to cool for at least 30 min and take an aliquot portion of the extract for analysis by gas chromatography. No volumetric adjustment is made.

7.3 Linearity

Pipette aliquot portions of 10 ml, 5 ml and 1 ml of mixed stock solution A (4.7.2) into three individual 100 ml volumetric flasks. Add 1 ml of the internal standard solution (4.6) to each volumetric flask and dilute to the mark with *n*-hexane (4.2). Pipette an aliquot portion of 1 ml of mixed stock solution B (4.7.3) into two individual volumetric flasks of capacities 100 ml and 200 ml. Add 1 ml of the internal standard solution (4.6) to the 100 ml volumetric flask and 2 ml of the internal standard solution to the 200 ml volumetric flask. Dilute both up to the mark with *n*-hexane. This procedure provides pesticide concentrations of approximately 0,5 µg/ml, 0,25 µg/ml, 0,05 µg/ml, 0,005 µg/ml and 0,002 5 µg/ml.

These solutions shall be used to check the linearity of the electron-capture detector response. This need only be checked when using a detector for the first time, or after any servicing of the detector or associated electronic circuitry.

7.4 Calibration

If the detector response was found to be linear, a single-level calibration may be used. For single-level calibration, the standard calibration solution (4.7.4) should be used.

7.5 Gas chromatography

Set up the gas chromatograph (5.10) and equilibrate the system. Check that reproducible results are obtained from triplicate injections of the standard calibration solution (4.7.4). The single value shall not differ from the mean value by more than ± 5 %. Carry out duplicate injections of each sample bracketed by single injections of the standard calibration solution and calculate the mean values.

Specimen chromatograms of the standard calibration solution and a tobacco extract are given in Figures A.1 and A.2.

8 Expression of results

The amounts of pesticides are determined by the internal standard method.

The response factor, E_p , for the respective pesticide is given by the equation

$$E_p = \frac{c_{\text{pst}} \cdot A_{\text{ist}}}{A_{\text{pst}} \cdot c_{\text{ist}}}$$

where

A_{pst} is the peak area or the peak height of the respective pesticide in the standard calibration solution (4.7.4);

A_{ist} is the peak area or the peak height of the internal standard (Mirex) in the standard calibration solution;

c_{pst} is the concentration, in micrograms per millilitre, of the respective pesticide in the standard calibration solution;

c_{ist} is the concentration, in micrograms per millilitre, of the internal standard solution in the standard calibration solution.

The content, R_p , of the respective pesticide residue, expressed in micrograms per gram of dried tobacco, is given by the equation

$$R_p = \frac{A_p \cdot E_p \cdot Q_{\text{ist}} \cdot 100}{A_i \cdot m \cdot (100 - w)}$$

where

A_p is the peak area or peak height for the respective pesticide in the sample extract (7.2);

A_i is the peak area or peak height for the internal standard in the sample extract;

Q_{ist} is the quantity of internal standard added to the extraction solution, in micrograms (approximately 5 µg);

m is the mass of the tobacco test portion (7.1), in grams;

w is the mass fraction of moisture of dried tobacco (6.2), as a percentage.

9 Repeatability, reproducibility and recovery

Details of a collaborative study on the precision of the method are given in annex B.

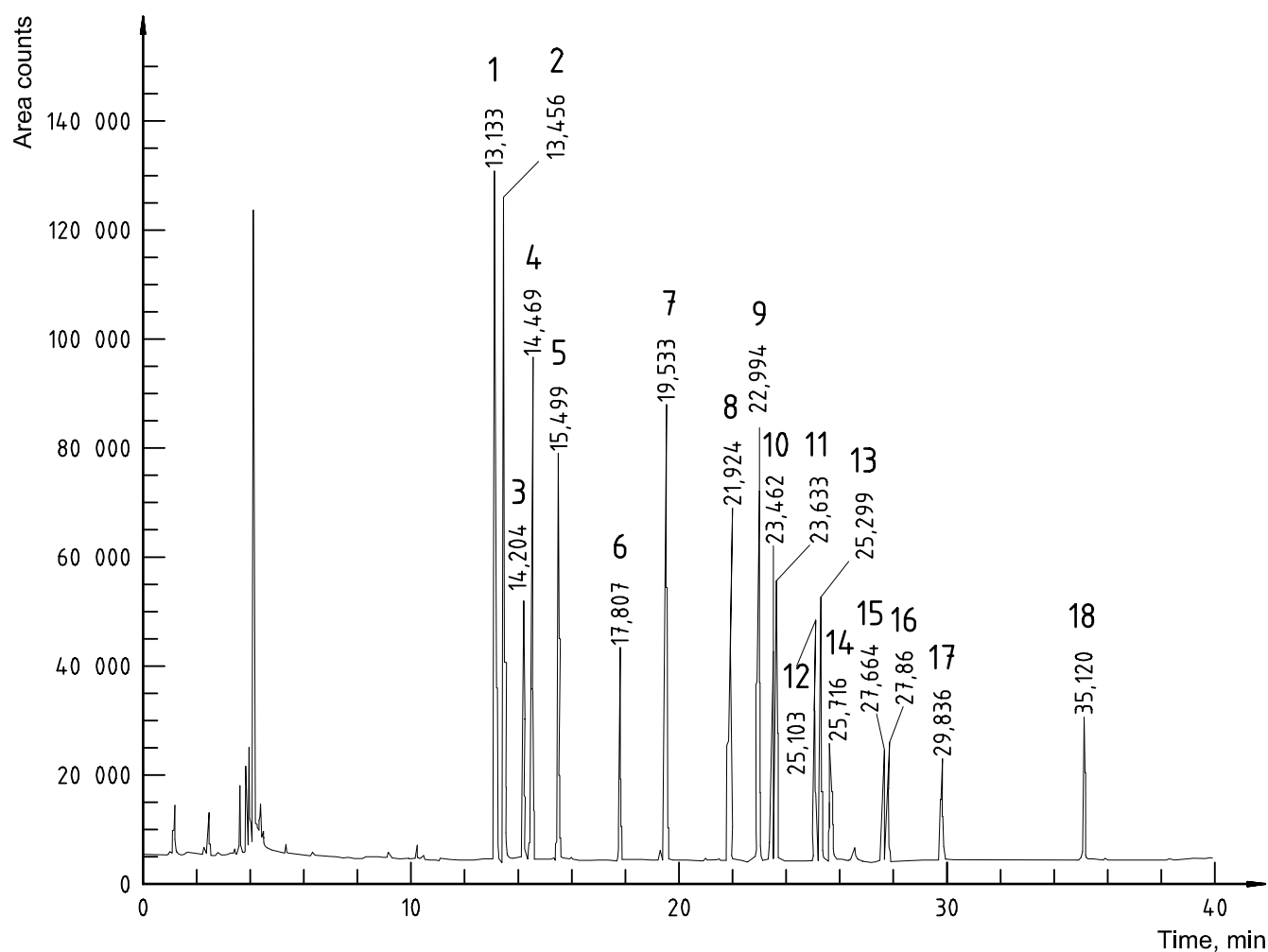
10 Test report

The test report shall show the method used and the results obtained, in micrograms per gram of dried tobacco. It shall indicate the amounts of each of the individual pesticide residues identified. It shall also mention any operating conditions not specified in this International Standard or regarded as optional, as well as any circumstances that may have influenced the results.

The report shall include all details required for complete identification of the sample.

Annex A (informative)

Examples of chromatograms



Key

1	α -HCH	10	<i>o,p'</i> -DDE
2	HCB	11	α -Endosulfan
3	β -HCH	12	Dieldrin
4	γ -HCH	13	<i>p,p'</i> -DDE
5	δ -HCH	14	<i>o,p'</i> -DDD
6	Heptachlor	15	<i>p,p'</i> -DDD
7	Aldrin	16	<i>o,p'</i> -DDT
8	Heptachlor epoxide	17	<i>p,p'</i> -DDT
9	<i>trans</i> -Chlordane	18	Mirex

Figure A.1 — Chromatogram of standard calibration solution containing 0,05 $\mu\text{g/ml}$ of each pesticide

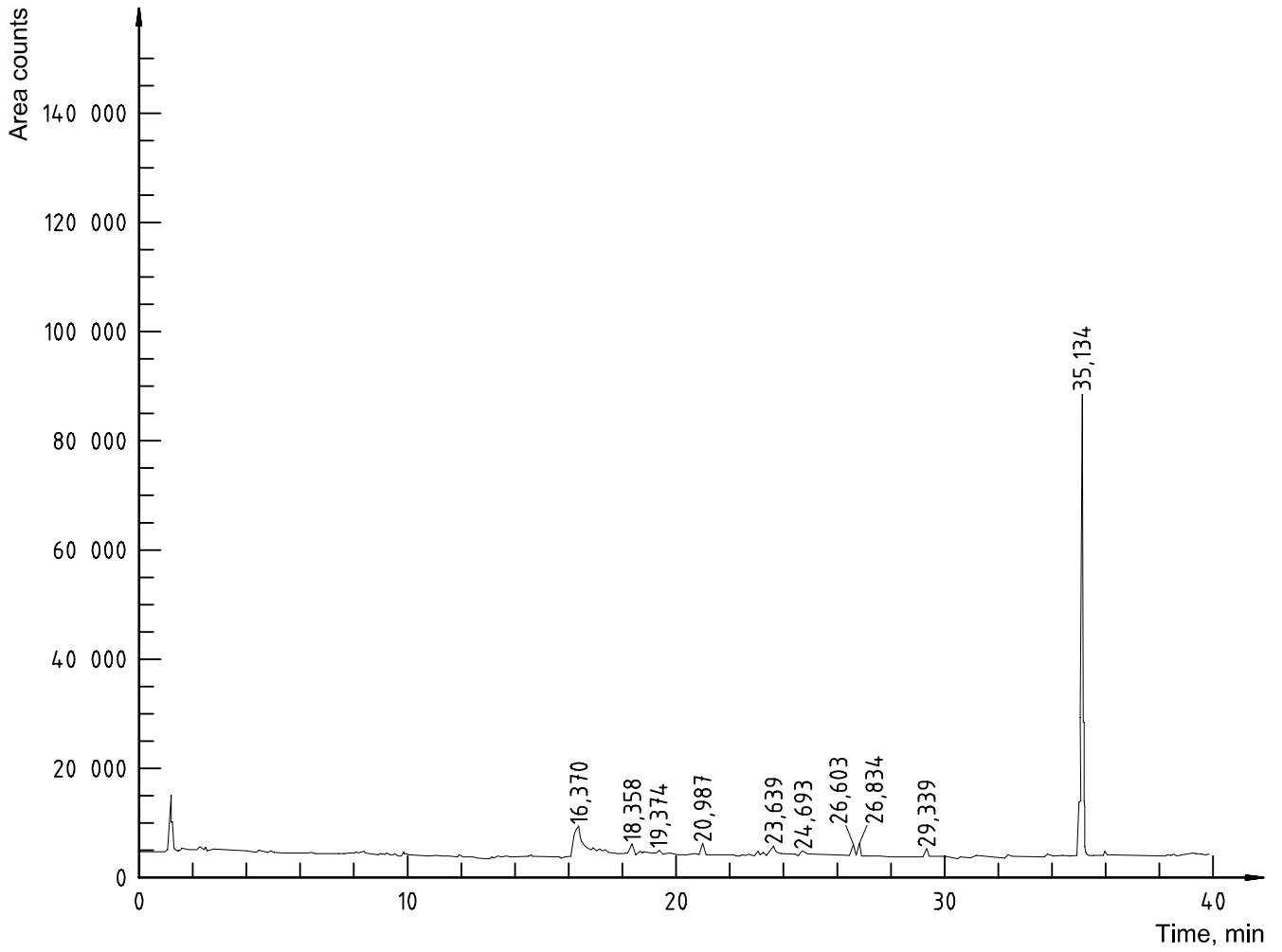


Figure A.2 — Chromatogram of a blank tobacco sample

Annex B (informative)

Results of interlaboratory tests

In a collaborative study involving 12 laboratories and samples with four spiking levels (F_1 , F_2 , F_3 , F_4), the values given in Table B.1 for mean recovery (Rec), standard deviation of repeatability (s_r) and standard deviation of reproducibility (s_R) were obtained.

Each laboratory investigated two samples at each spiking level. As each sample was determined in duplicate, this represents a total of 48 determinations per pesticide at each level. For further details about the statistical evaluation of results, see reference [3].

Apart from the 17 pesticides mentioned in Table B.2, the possible analysis of endrin and β -endosulfan was also investigated by the joint experiment. Results given in reference [3] show that these two pesticides cannot be analysed by the method.

Table B.1 — Results of the collaborative study

Pesticide	F_1				F_2				F_3				F_4				Detection limit µg/g
	Spiking level µg/g	Rec. %	s_r %	s_R %	Spiking level µg/g	Rec. %	s_r %	s_R %	Spiking level µg/g	Rec. %	s_r %	s_R %	Spiking level µg/g	Rec. %	s_r %	s_R %	
α -HCH	0,099 5	79	12	27	0,497 5	102	14	18	0,995 0	105	8	13	4,975 0	95	11	18	0,02
β -HCH	0,100 0	89	17	25	0,500 0	99	10	15	1,000 0	100	6	11	5,000 0	86	5	14	0,02
γ -HCH (Lindane)	0,100 5	100	26	34	0,502 5	101	15	16	1,005 0	109	9	19	5,025 0	100	7	29	0,01
δ -HCH	0,102 0	62	13	27	0,510 0	88	7	18	1,020 0	96	9	15	5,100 0	90	12	22	0,02
HCB	0,100 0	109	9	19	0,500 0	105	16	18	1,000 0	105	8	13	5,000 0	88	8	16	0,02
Heptachlor	0,102 0	100	13	19	0,510 0	105	13	15	1,020 0	107	12	16	5,100 0	101	13	25	0,02
Aldrin	0,100 0	83	13	24	0,500 0	100	14	14	1,000 0	107	8	14	5,000 0	103	7	25	0,02
Heptachlor epoxide	0,102 5	84	15	29	0,512 5	89	16	19	1,025 0	97	16	18	5,125	91	11	24	0,02
<i>trans</i> -Chlordane	0,099 5	86	8	23	0,497 5	96	6	14	0,995 0	102	4	10	4,975 0	99	6	18	0,02
<i>o,p'</i> -DDE	0,101 0	105	12	25	0,505 0	106	9	11	1,010 0	110	4	7	5,050 0	101	8	19	0,03
α -Endosulfan	0,101 0	73	14	36	0,505 0	81	9	15	1,010 0	83	10	18	5,050 0	77	10	23	0,03
Dieldrin	0,101 5	84	15	34	0,507 5	87	23	27	1,015 0	93	13	22	5,075 0	95	14	31	0,02
<i>p,p'</i> -DDE	0,100 0	104	10	21	0,500 0	115	6	17	1,000 0	120	4	15	5,000 0	112	6	21	0,02
Endrin	0,099 5	53	12	35	0,497 5	59	30	31	0,995 0	58	19	41	4,975 0	78	26	43	0,03
β -Endosulfan	0,100 0	12	17	24	0,500 0	12	19	21	1,000 0	14	15	21	5,000 0	12	9	22	0,02
<i>o,p'</i> -DDD	0,100 5	111	9	20	0,502 5	109	6	10	1,005 0	114	5	10	5,025 0	100	9	12	0,03
<i>p,p'</i> -DDD	0,101 0	117	14	29	0,505 0	113	8	20	1,010 0	116	6	19	5,050 0	119	13	25	0,02
<i>o,p'</i> -DDT	0,101 5	106	11	27	0,507 5	109	10	18	1,015 0	109	7	14	5,075 0	102	8	10	0,04
<i>p,p'</i> -DDT	0,100 5	104	13	24	0,502 5	111	8	21	1,005 0	117	5	22	5,025 0	116	11	21	0,06

Table B.2 — List of equivalent IUPAC names

Pesticide	Chemical name according to IUPAC
α -HCH	1,3,5/2,4,6-hexachlorocyclohexane
β -HCH	1,2,4,5/3,6-hexachlorocyclohexane
γ -HCH (Lindane)	1,2,3,4,5,6-hexachlorocyclohexane
δ -HCH	1,2,3/4,5,6-hexachlorocyclohexane
HCB	hexachlorobenzene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Aldrin	(1 <i>R</i> ,4 <i>S</i> ,4a <i>S</i> ,5 <i>S</i> ,8 <i>R</i> ,8a <i>R</i>)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene
<i>trans</i> -Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
<i>o,p'</i> -DDE	1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethylene
α -Endosulfan	(1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylene-bismethylene) sulfite-I
Dieldrin	(1 <i>R</i> ,4 <i>S</i> ,4a <i>S</i> ,5 <i>R</i> ,6 <i>R</i> ,7 <i>S</i> ,8 <i>S</i> ,8a <i>R</i>)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
<i>p,p'</i> -DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene
<i>o,p'</i> -DDD	1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
<i>p,p'</i> -DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
<i>o,p'</i> -DDT	1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
<i>p,p'</i> -DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane

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ICS 65.160

Price based on 15 pages

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