



Edition: BP 2025 (Ph. Eur. 11.6 update)

Wool Fat



[General Notices](#)

Anhydrous Lanolin

(Ph. Eur. monograph 0134)

Ph Eur

DEFINITION

Purified, anhydrous, waxy substance obtained from the wool of sheep (*Ovis aries*). A suitable antioxidant may be added.

CHARACTERS

Appearance

Yellow, unctuous substance. When melted, it is a clear or almost clear, yellow liquid. A solution in light petroleum is opalescent.

Solubility

Practically insoluble in water, slightly soluble in boiling anhydrous ethanol.

Characteristic odour.

IDENTIFICATION

- A. In a test-tube, dissolve 0.5 g in 5 mL of [methylene chloride R](#) and add 1 mL of [acetic anhydride R](#) and 0.1 mL of [sulfuric acid R](#). A green colour develops.
- B. Dissolve 50 mg in 5 mL of [methylene chloride R](#), add 5 mL of [sulfuric acid R](#) and shake. A red colour develops and an intense green fluorescence appears in the lower layer when examined in daylight, with illumination from behind the observer.

TESTS

Water-soluble acid or alkaline substances

Melt 5.0 g on a water-bath and shake vigorously for 2 min with 75 mL of [water R](#) previously heated to 90-95 °C. Allow to cool and filter through filter paper previously rinsed with [water R](#). To 60 mL of the filtrate (which may not be clear) add 0.25 mL of [bromothymol blue solution R1](#). Not more than 0.2 mL of [0.02 M hydrochloric acid](#) or 0.15 mL of [0.02 M sodium hydroxide](#) is required to change the colour of the indicator.

Water-absorption capacity

Place 10 g of molten wool fat in a mortar and allow to cool to room temperature. Weigh the mortar. Add [water R](#) in portions of 0.2-0.5 mL from a burette, stirring vigorously after each addition to incorporate the [water R](#). Instead of a pestle, use a high-density polypropylene cylindrical rod (120 mm long and 10 mm in diameter, for example). The end-point is reached when visible droplets remain which cannot be incorporated. Weigh the mortar again and determine the amount of water absorbed by weight difference. Not less than 20 mL of [water R](#) is absorbed.

[Acid value \(2.5.1\)](#)

Maximum 1.0, determined on 5.0 g dissolved in 25 mL of the prescribed mixture of solvents.

[Peroxide value \(2.5.5, Method A\)](#)

Maximum 20.

Before adding 0.5 mL of [saturated potassium iodide solution R](#), cool the solution obtained to room temperature.

[Saponification value \(2.5.6\)](#)

90 to 105, determined on 2.00 g while heating under reflux for 4 h.

Water-soluble oxidisable substances

To 10 mL of the filtrate obtained in the test for water-soluble acid or alkaline substances add 1 mL of [dilute sulfuric acid R](#) and 0.1 mL of [0.02 M potassium permanganate](#). After 10 min, the solution is not completely decolourised.

Paraffins

Maximum 1.0 per cent.

The tap and cotton plugs used must be free from grease Prepare a column of anhydrous aluminium oxide 0.23 m long and 20 mm in diameter by adding a slurry of [anhydrous aluminium oxide R](#) and [light petroleum R1](#) to a glass tube fitted with a tap and containing [light petroleum R1](#) (before use, dehydrate the anhydrous aluminium oxide by heating it in an oven at 600 °C for 3 h). Allow to settle and reduce the depth of the layer of solvent above the column to about 40 mm. Dissolve 3.0 g of the substance to be examined in 50 mL of warm [light petroleum R1](#), cool, pass the solution through the column at a flow rate of 3 mL/min and wash with 250 mL of [light petroleum R1](#). Concentrate the combined eluate and washings to low bulk by distillation, evaporate to dryness on a water-bath and heat the residue at 105 °C for periods of 10 min until 2 successive weighings do not differ by more than 1 mg. The residue weighs a maximum of 30 mg.

[Pesticide residues](#)

Maximum 0.05 ppm for each organochlorine pesticide, 0.5 ppm for each other pesticide and 1 ppm for the sum of all the pesticides.

All glassware used is thoroughly washed using phosphate-free detergent as follows. The glassware is immersed in a bath of detergent solution (5 per cent in deionised water) and allowed to soak for 24 h. The detergent is washed off with copious amounts of acetone and hexane for pesticide analysis. It is important to keep glassware specifically for pesticide analyses, it must not be mixed up with glassware used for other applications. The glassware used must be free of chlorinated solvents, plastics and rubber materials, in particular phthalate plasticisers, oxygenated compounds and nitrogenated solvents such as acetonitrile. Use hexane, toluene and acetone for pesticide analysis. Use HPLC grade reagents for ethyl acetate, cyclohexane and water.

The test consists of the isolation of pesticide residues by size-exclusion chromatography ([2.2.30](#)) followed by solid phase extraction and identification by gas chromatography coupled with an electron capture detector or a thermionic detector.

ISOLATION OF THE PESTICIDE RESIDUES

As detector, use a UV-visible spectrophotometer set at a wavelength of 254 nm to calibrate the chromatographic column for gel permeation.

Calibration is extremely important in gel permeation chromatography (GPC) to check that the pressure, solvent flow rate, solvent ratio, temperature and column conditions remain constant. The gel permeation column is to be calibrated at regular intervals using a standard mixture prepared as follows: into a 1000 mL volumetric flask, introduce 50.00 g of [maize oil R](#), 0.20 g of [methoxychlor R](#) and 50.0 mg of [perylene R](#). Dilute to 1000.0 mL with a mixture of equal volumes of [cyclohexane R](#) and [ethyl acetate R](#).

To calibrate the column, set the mobile phase at a flow rate of 5 mL/min with a mixture of equal volumes of [cyclohexane R](#) and [ethyl acetate R](#). Inject 5 mL of the standard mixture and record the resulting chromatogram. The retention times for the analytes must not vary by more than ± 5 per cent between calibrations. If the retention time shift is greater than ± 5 per cent, take corrective action. Excessive retention time shifts may be caused by:

- poor laboratory temperature control;
- the pump containing air; this can be verified by measuring the flow rate: collect 25 mL of column eluate in a volumetric flask and record the time (300 ± 5 s);
- a leak in the system.

Changes in pressure, in mobile phase flow rate or in column temperature conditions, as well as column contamination, can affect pesticide retention times and are to be monitored. If the flow rate or column pressure are outside desired bands the precolumn or column is to be replaced.

Test solution In a volumetric flask, dissolve 1 g of the substance to be examined, accurately weighed, in a mixture of 1 volume of [ethyl acetate R](#) and 7 volumes of [cyclohexane R](#). Add 1 mL of an internal standard (2 ppm, either [isodrin R](#) or [ditalimphos R](#)) and dilute to 20 mL. *The internal standard solutions are used to establish that recoveries of the pesticides from the GPC purification stage, evaporation and solid phase extraction stage are at acceptable levels. Recovery levels of the internal standard solutions from the wool fat are determined by comparing the peak areas of the wool fat extracts with peak areas of solutions of the internal standards.*

Precolumn:

- size: $l = 0.075$ m, $\varnothing = 21.2$ mm;
- stationary phase: [styrene-divinylbenzene copolymer R](#) (5 μ m).

Gel permeation column:

- size: $l = 0.3$ m, $\varnothing = 21.2$ mm;
- stationary phase: [styrene-divinylbenzene copolymer R](#) (5 μ m).

Mobile phase [ethyl acetate R](#), [cyclohexane R](#) (10:70 V/V).

Flow rate 5 mL/min.

Detection Spectrophotometer at 254 nm.

Inject 5 mL of the test solution. Discard the first 95 mL (19 min) of eluate containing the substance to be examined. Collect the next 155 mL of eluate (31 min) containing any pesticide residues in an evaporating vessel.

Place the 155 mL of the eluate collected from the gel permeation chromatography column into an evaporating vessel. Place this vessel in an autoevaporator setting the water-bath temperature at 45 °C and the nitrogen pressure at 55 kPa. Evaporate the eluate down to 0.5 mL.

To prepare the solid phase extraction cartridges take some [magnesium silicate for pesticide residue analysis R](#) and heat it in a muffle furnace at 700 °C for 4 h to remove moisture and polychlorinated biphenyls. Subsequently allow the magnesium silicate to cool for 2 h and transfer it directly to an oven at 100-105 °C, and allow to stand for 30 min. Transfer the magnesium silicate to a stoppered glass jar and allow to equilibrate for 48 h. This material may be used for up to 2 weeks. After that period the magnesium silicate is to be reactivated, by heating at 600 °C for 2 h in a muffle furnace. Remove the magnesium silicate from the furnace, cool and store in a stoppered glass jar. The magnesium silicate is deactivated by adding 1 per cent of [water R](#). After the water has been added, shake the magnesium silicate intermittently over 15 min just prior to use. The deactivated magnesium silicate is suitable for use for up to 1 week. It is essential that only deactivated magnesium silicate is used.

Take a 6 mL empty solid phase extraction cartridge and weigh into the cartridge 1 g of the deactivated magnesium silicate.

At this stage the GPC fraction still contains about 10 per cent of the substance to be examined, so further clean-up is necessary. A separate isolation procedure is carried out a) for organochlorine and synthetic pyrethroid pesticides and b) for organophosphorus pesticides. Place a preconditioned solid phase extraction cartridge containing 1 g of deactivated [magnesium silicate for pesticide residue analysis R](#) onto a vacuum manifold.

Condition the cartridge by adding 10 mL of [toluene R](#) and allowing the solvent to elute through the cartridge. Place the 0.5 mL of the solvent fraction from the evaporating vessel on the preconditioned cartridge. Elute the pesticide fractions from the cartridges using 20 mL of either of the 2 different solvent systems shown below:

- a) for determination of the organochlorine and synthetic pyrethroid pesticides: [toluene R](#); a very small amount of the substance to be examined is co-eluted;
- b) for determination of the organophosphorus pesticides: a mixture of 2 volumes of [acetone R](#) and 98 volumes of [toluene R](#); this solvent system is used to elute all the pesticides including the more polar organophosphorus pesticides; unfortunately, some of the substance to be examined is co-eluted with this solvent system, which can interfere with the electron capture detector.

Collect the eluate from the extraction cartridges in 25 mL glass vials. Quantitatively transfer the eluate to an evaporating vessel, washing the vial with 3 quantities, each of 10 mL, of [hexane R](#).

Place the evaporating vessel on the autoevaporator and evaporate the solid phase extraction fractions down to 0.5 mL. The water-bath temperature is set at 45 °C and the nitrogen pressure is 55 kPa.

Examine the residues by gas chromatography ([2.2.28](#)) using electron capture and thermionic detectors as described below.

Recovery Calculate the recovery correction factor (R_{cf}) of the internal standards ([ditalimphos R](#) or [isodrin R](#)) added to the test solution using the following expression:

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A_1 = peak area of an internal standard 1 ppm in solution;

A_2 = peak area of internal standard extracted from the test solution.

5 mL of the 20 mL test solution containing 1 mL of 2 ppm internal standard concentrated to 0.5 mL is equivalent to 1 ppm of the internal standard in the solution.

If the recovery of the internal standards falls outside the range of 70 per cent to 110 per cent the test is not valid.

Reference solutions Prepare reference solutions of pesticides using the pesticides standards at a concentration of 0.5 ppm (see composition of reference solutions A to D in Table 0134.-1). Commercially available pesticides may be purchased. The individual standards have a concentration of 10 ppm.

Table 0134.-1. – Composition of the reference solutions

Reference solution A (0.5 ppm or 0.5 mg/L) (organochlorine and synthetic pyrethroid pesticides) Cyhalothrin R Cypermethrin R o,p'-DDE R p,p'-DDE R p,p'-DDT R Deltamethrin R Endrin R Heptachlor R Heptachlor epoxide R Hexachlorobenzene R Lindane R Tecnazene R	Reference solution B (0.5 ppm or 0.5 mg/L) (organochlorine and synthetic pyrethroid pesticides) Aldrin R o,p'-DDT R o,p'-DDD R p,p'-DDD R Dieldrin R α-Endosulfan R β-Endosulfan R Fenvalerate R α-Hexachlorocyclohexane R β-Hexachlorocyclohexane R δ-Hexachlorocyclohexane R Methoxychlor R Permethrin R
Reference solution C (0.5 ppm or 0.5 mg/L) (organophosphorus pesticides) Bromophos-ethyl R	Reference solution D (0.5 ppm or 0.5 mg/L) (organophosphorus pesticides) Bromophos R

Reference solution A
(0.5 ppm or 0.5 mg/L)
(organochlorine and
synthetic pyrethroid
pesticides)

[Carbophenothion R](#)
[Chlorfenvinphos R](#)
[Diazinon R](#)
[Dichlofenthion R](#)
[Ethion R](#)
[Fenchlorphos R](#)
[Malathion R](#)
[Propetamphos R](#)

Reference solution B
(0.5 ppm or 0.5 mg/L)
(organochlorine and
synthetic pyrethroid
pesticides)

[Chlorpyrifos R](#)
[Chlorpyrifos-methyl R](#)
[Coumaphos R](#)
[Phosalone R](#)
[Pirimiphos-ethyl R](#)
[Tetrachlorvinphos R](#)

Reference solution E
(electron capture detector
calibration mixture)

[Aldrin R](#) (0.01 mg/L)
[Cypermethrin R](#) (0.1 mg/L)
[o,p'-DDD R](#) (0.01 mg/L)
[Deltamethrin R](#) (0.1 mg/L)
[Endrin R](#) (0.01 mg/L)
[β-Hexachlorocyclohexane R](#)
(0.01 mg/L)

Reference solution F
(thermionic detector
calibration mixture)

[Chlorfenvinphos R](#)
(0.05 mg/L)
[Diazinon R](#) (0.05 mg/L)
[Ethion R](#) (0.05 mg/L)
[Fenchlorphos R](#) (0.05 mg/L)
[Propetamphos R](#) (0.05 mg/L)

Reference solution G
(internal standard organo-
phosphorus pesticide)

[Ditalimphos R](#)
(2 ppm or 2.0 mg/L)
[Ditalimphos R](#)
(1 ppm or 1.0 mg/L)

Reference solution H
(internal standard organo-
chlorine pesticide)

[Isodrin R](#)
(2 ppm or 2.0 mg/L)
[Isodrin R](#)
(1 ppm or 1.0 mg/L)

At the same time prepare solutions of pesticides equivalent to the limit of detection of the method (see recommended compositions in Table 0134.-1). These reference solutions are used to optimise the electron capture detector and thermionic detector to achieve the detection limits of the method (reference solutions E and F).

To prepare the reference solutions at the different concentrations use a calibrated pipette and volumetric flasks. To prepare the internal standard solutions G and H use a four-place analytical balance, pipette and volumetric flasks.

IDENTIFICATION AND QUANTIFICATION OF THE PESTICIDE RESIDUES

To identify any pesticide residues compare the chromatograms obtained with chromatograms obtained with reference solutions A to D.

The identity of the pesticides can be confirmed by spiking samples or overlaying chromatograms using an integration package on a computer. The interpretation of pesticides in trace residue analyses is extremely complex. The detectors, particularly the electron capture detector, are prone to interference, both from the substance to be examined itself, and from solvents, reagents and apparatus used in the extraction. These peaks can easily be misinterpreted or quoted as a false positive. Confirmation of pesticides can be achieved by running samples and standards on different capillary columns (see chromatographic systems A or B described below). The peaks can be identified by using the information in Table 0134.-2.

A knowledge of the different responses the pesticides have with the 2 detectors is useful in identification of unknown peaks.

Table 0134.-2. – Elution order of the pesticides on chromatographic systems A and B

Chromatographic system A	Chromatographic system B
Tecnazene	Tecnazene
α-Hexachlorocyclohexane	Hexachlorobenzene
Hexachlorobenzene	α-Hexachlorocyclohexane
β-Hexachlorocyclohexane	Diazinon
Lindane	Lindane
Propetamphos	Propetamphos

Chromatographic system A	Chromatographic system B
δ-Hexachlorocyclohexane	Heptachlor
Diazinon	Dichlofenthion
Dichlofenthion	Aldrin
Chlorpyrifos-methyl	Chlorpyrifos-methyl
Heptachlor	Fenchlorphos
Fenchlorphos	β-Hexachlorocyclohexane
Aldrin	δ-Hexachlorocyclohexane
Malathion	Pirimiphos-ethyl
Chlorpyrifos	Chlorpyrifos
Bromophos	Bromophos
Pirimiphos-ethyl	Malathion
Heptachlor epoxide	Heptachlor epoxide
Chlorfenvinphos (E)	<i>o,p'</i> -DDE
Chlorfenvinphos (Z)	Chlorfenvinphos (E)
Bromophos-ethyl	α-Endosulfan
<i>o,p'</i> -DDE	Chlorfenvinphos (Z)
α-Endosulfan	Bromophos-ethyl
Tetrachlorvinphos	<i>p,p'</i> -DDE
Dieldrin	Dieldrin
<i>p,p'</i> -DDE	Tetrachlorvinphos
<i>o,p'</i> -DDT	<i>o,p'</i> -DDT
Endrin	Endrin
β-Endosulfan	<i>o,p'</i> -DDD
<i>o,p'</i> -DDD	<i>p,p'</i> -DDD
<i>p,p'</i> -DDD	β-Endosulfan
Ethion	Ethion
Carbophenothion	<i>p,p'</i> -DDT
<i>p,p'</i> -DDT	Carbophenothion
Methoxychlor	Methoxychlor
Phosalone	Cyhalothrin
Cyhalothrin (2 isomers)	<i>cis</i> -Permethrin
<i>cis</i> -Permethrin	Phosalone
<i>trans</i> -Permethrin	<i>trans</i> -Permethrin
Coumaphos	Cypermethrin (4 isomers)
Cypermethrin (4 isomers)	Coumaphos
Fenvalerate (2 isomers)	Fenvalerate (2 isomers)
Deltamethrin	Deltamethrin

Once the pesticides have been identified, calculate the content of each pesticide using the following expression:

- C_p = concentration of identified pesticide (ppm);
 P_p = peak area of the individual pesticide in the test sample obtained;
 C_e = concentration of the individual pesticide in the external standard (ppm);
 P_e = peak area of the individual pesticide in the external standard;
 D = dilution factor;
 R_{cf} = recovery correction factor.

The dilution factor (D) can be defined as follows:

- V_1 = volume of sample obtained after the 2nd evaporation stage;
 m = sample weight;

V_2 = GPC injection volume;
 V_3 = sample volumetric flask volume.

Chromatographic system A:

Precolumn:

- material: deactivated silica;
- size: $l = 4.5\text{ m}$, $\varnothing = 0.53\text{ mm}$.

Column:

- material: fused silica;
- size: $l = 60\text{ m}$, $\varnothing = 0.25\text{ mm}$;
- stationary phase: [phenyl\(5\)methyl\(95\)polysiloxane R](#) (film thickness $0.25\text{ }\mu\text{m}$).

Carrier gas [helium for chromatography R](#).

Linear velocity 25 cm/s .

Pressure 180 kPa .

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	75
	1 - 5	75 → 175
	5 - 30	175 → 275
	30 - 40	275 → 285
	40 - 55	285
Injection port		300
Detector		350

Detection Electron capture or thermionic specific detector.

Injection $2\text{ }\mu\text{L}$.

Chromatographic system B Which may be used for confirmation analysis:

Precolumn:

- material: deactivated silica;
- size: $l = 4.5\text{ m}$, $\varnothing = 0.53\text{ mm}$.

Column:

- material: fused silica;
- size: $l = 60\text{ m}$, $\varnothing = 0.25\text{ mm}$;
- stationary phase: [cyanopropyl\(7\)phenyl\(7\)methyl\(86\)polysiloxane R](#) (film thickness $0.25\text{ }\mu\text{m}$).

Carrier gas [helium for chromatography R](#).

Linear velocity 25 cm/s .

Pressure 180 kPa .

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	75
	1 - 5	75 → 175
	5 - 30	175 → 275
	30 - 40	275 → 285
	40 - 55	285
Injection port		300
Detector		350

Detection Electron capture or thermionic specific detector.

Injection 2 µL.

Chlorides

Maximum 150 ppm.

Boil 1.0 g with 20 mL of [ethanol \(90 per cent V/V\) R](#) in a round-bottomed flask fitted with a reflux condenser for 5 min. Cool, add 40 mL of [water R](#) and 0.5 mL of [nitric acid R](#) and filter. To the filtrate add 0.15 mL of a 10 g/L solution of [silver nitrate R](#) in [ethanol \(90 per cent V/V\) R](#). Allow to stand for 5 min protected from light. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by adding 0.15 mL of a 10 g/L solution of [silver nitrate R](#) in [ethanol \(90 per cent V/V\) R](#) to a mixture of 0.2 mL of [0.02 M hydrochloric acid](#), 20 mL of [ethanol \(90 per cent V/V\) R](#), 40 mL of [water R](#) and 0.5 mL of [nitric acid R](#).

[Loss on drying \(2.2.32\)](#)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

[Sulfated ash \(2.4.14\)](#)

Maximum 0.15 per cent.

Ignite 5.0 g and use the residue to determine the sulfated ash.

STORAGE

At a temperature not exceeding 25 °C.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter [5.15](#)). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for wool fat used in water-emulsifying ointments and lipophilic creams.

Water-absorption capacity

(see Tests).

Drop point ([2.2.17, Method A](#))

To fill the metal cup, melt the wool fat on a water-bath, cool to about 50 °C, pour into the cup and allow to stand at 15-20 °C for 24 h. The drop point is typically 38 °C to 44 °C.

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