PRO PHARMACOPOEIA TECHNICAL NOTE No 1268 (11threv.)

NOTE ON THE MONOGRAPH

The drug identifications are revised.
Amendment to the identification for the mother tincture, with the addition of TLC allowing for better identification.
Dosage of sterols expressed as epicoprostanol.

AMBRA GRISEA
FOR HOMEPATHIC PREPARATIONS

DEFINITION

Dried intestinal concretions (grey amber) of *Physeter macrocephalus* L.

IDENTIFICATION

Macroscopic characteristics: Ambra grisea presents in the form of balls or lumps that are rounded, irregular, and more or less dark grey in colour, with yellow or black stains. Their weight varies from 50 g to 500 g, but can sometimes be several kilograms.

In its fresh state, grey amber is soft and yellowish or blackish; as it ages, it hardens and becomes grey. Its consistency is waxy, crumbly and a little greasy. Its fracture is irregular, lumpy or lamellar; it reveals several concentric layers.

The general requirements and monographs of the European Pharmacopoeia and the preamble to the French Pharmacopoeia apply.

STRAIN

The mother tincture of Ambra grisea is prepared with an ethanol content of 90% *V/V* from the dried intestinal concretions (grey amber) of *Physeter macrocephalus* L.

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Content: 1.00% *m/m* to 3.00% *m/m* of sterols, expressed as epicoprostanol ($C_{27}H_{48}O$; *Mr* 388.7)

PRODUCTION

Method 1.1.11 (*2371*)

FEATURES

Appearance: amber-coloured liquid.

Characteristic odour reminiscent of cold tobacco.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*)

Solution to be examined. Mother tincture to be examined.

Control solution (a). Dissolve 10 mg of epicoprostanol[1] in *ethanol at 96% R* and make up to 20 ml with the same solvent.

Control solution (b). Dissolve 5 mg of (-)-ambroxide[2] in *ethanol at 96% R* and make up to 20 ml with the same solvent.

Plate: silica gel plate for TLC R (2-10 µm).

Mobile phase: *isopropyl ether R, toluene R* (10:40 *V/V)*

Deposit: 5 µl of each solution in 10 mm strips.

Development: in a pre-saturated double-walled container without saturating paper, over a path of 7 cm.

Drying: in air.

Detection: Spray *anisic aldehyde solution R* and heat at 100-105 °C for 10 min. Examine in daylight.

[1] **Epicoprostanol (5β-Cholestan-3α-ol)**. $C_{27}H_{48}O$; *Mr* 388.7. CAS No [516-92-7] – Sigma Aldrich reference C2882 is suitable.

2 **(-)-ambroxide**. $C_{16}H_{28}O$; *Mr* 236.4. CAS No [6790-58-5] – Sigma Aldrich reference W147108 is suitable.

The general requirements and monographs of the European Pharmacopoeia and the preamble to the French Pharmacopoeia apply.

Results: see below the sequence of paths present in the chromatograms obtained with the control solutions and the solution to be examined. In addition, other low-intensity paths may be present in the chromatogram obtained with the solution to be examined.

Top of the plate	
-------	One bright purple path -------
Ambroxide: one purple path -------	-------
Epicoprostanol: one purple path	One purple band (epicoprostanol) 3 to 4 purple-greyish paths
Control solution	**Solution to be examined**

TEST

Ethanol (*2.9.10*): 85% *V/V* to 95% *V/V*.

Dry residue *(2.8.16):* at least 2.0% *m/m.*.

The general requirements and monographs of the European Pharmacopoeia and the preamble to the French Pharmacopoeia apply.

DOSAGE

Gas chromatography (*2.2.28*).

Silylation reagent[3]*. N,O-Bis(trimethylsilyl)trifluoroacetamide R* containing 0.1% to 1% *trimethylchlorosilane R.*

Blank solution. Take 2.0 ml of *methylene chloride R* and make up to 25.0 ml with *dimethylformamide R*. In a 1.5 ml injection crimp vial, introduce 1.0 ml of the aforementioned solution and add 50 µl of silylation reagent. Vortex for 10 seconds. Heat in a dry bath at 70 °C for 15 min.

Internal standard solution. Dissolve 0.250 g of *(5α)-cholestane R*[4] in *methylene chloride R* and make up to 100.0 ml with the same solvent.

Solution to be examined. In a 50 ml round-bottomed flask, evaporate to dryness, under reduced pressure, 1.750 g of the mother tincture to be examined. Redissolve the residue in 5 ml of *dimethylformamide R*. Transfer quantitatively into a 50.0 ml graduated flask. Rinse the round-bottomed flask twice with 5 ml of *dimethylformamide R* and add the rinses to the 50.0 ml flask. Add 4.0 ml of the internal standard solution and make up to 50.0 ml with *dimethylformamide R*. In an 1.5 ml injection crimp vial, introduce 1.0 ml of the aforementioned solution and add 50 µl of silylation reagent. Vortex for 10 seconds. Heat in a dry bath at 70 °C for 15 min.

Control solution. Dissolve 50.0 mg of epicoprostanol[5] in *dimethylformamide R* and make up to 20.0 ml with the same solvent (solution 1). Take 5.0 ml of solution 1, add 4.0 ml of the internal standard solution and make up to 50.0 ml with *dimethylformamide R*. In a 1.5 ml injection crimp vial, introduce 1.0 ml of the aforementioned solution and add 50 µl of silylation reagent. Vortex for 10 seconds. Heat in a dry bath at 70 °C for 15 min.

Resolution solution. Dissolve 50.0 mg of *cholesterol R*in *dimethylformamide R* and make up to 20.0 ml with the same solvent (solution 2). Take 5.0 ml each of solutions 1 and 2 and make up to 50.0 ml with *dimethylformamide R*. In a 1.5 ml injection crimp vial, introduce 1.0 ml of the aforementioned solution and add 50 µl of silylation reagent. Vortex for 10 seconds. Heat in a dry bath at 70 °C for 15 min.

Column:
- *material*: fused silica;
- *dimensions*: *l* = 60 m, ⊘ = 0.25 mm;
- *stationary phase:* (5%-phenyl)-methylpolysiloxane (film thickness 0.25 µm)[6].

Carrier gas: helium for chromatography R.

[3] **Silylation reagent.** BSTFA + 1% TMCS Thermo Fisher reference TS38831 is suitable.
[4] **5α-cholestane**. $C_{27}H_{48}$; *Mr* 372.7; CAS No [481-21-0]; Sigma Aldrich reference C8003 is suitable.
[5] **Epicoprostanol** (5β-Cholestan-3α-ol). $C_{27}H_{48}O$; *Mr* 388.7; CAS No [516-92-7]; Sigma Aldrich reference C2882 is suitable.
[6] **DB-5MS UI.** Agilent reference 122-5562UI is suitable.

The general requirements and monographs of the European Pharmacopoeia and the preamble to the French Pharmacopoeia apply.

Pressure: 38 psi.

Split ratio: 1:10

Temperature:

	Interval (min)	Temperature (°C)
Column	0 – 2,0	280
	2,0 – 12,0	280 → 320
	12,0 – 20,0	320
	20,0 – 22,2	320 → 280
	22,2 – 24,0	280
Injection chamber		300
Detector		300

Detection: flame ionisation.

Injection: 1.0 µl.

Recording: 20 min.

Relative retention compared to epicoprostanol (retention time = approximately 16.9 min): (5α)-cholestane = approximately 0.83; ambrein = approximately 1.05; cholesterol = approximately 1.08.

System compliance:
- *resolution*: at least 1.5 between the peaks due to epicoprostanol and cholesterol (resolution solution).
- *repeatability*: relative standard deviation no more than 0.62% after 3 injections of the control solution.

Calculate the sterol *m/m* content percentage, expressed as epicoprostanol, using the expression:

$$\frac{(A_1 + A_2) \times A_3 \times m_2 \times p}{A_4 \times A_5 \times m_1 \times 4}$$

A_1 = peak area due to epicoprostanol in the chromatogram obtained with the solution to be examined;
A_2 = peak area due to ambrein in the chromatogram obtained with the solution to be examined;
A_3 = peak area due to (5α)-cholestane in the chromatogram obtained with the control solution;
A_4 = peak area due to epicoprostanol in the chromatogram obtained with the control solution;
A_5 = peak area due to (5α)-cholestane in the chromatogram obtained with the solution to be examined;
m_1 = mass of mother tincture used to prepare the solution to be examined, in grams;
m_2 = mass of epicoprostanol used to prepare the control solution, in grams;
P = epicoprostanol content (%) in the control epicoprostanol.

The general requirements and monographs of the European Pharmacopoeia and the preamble to the French Pharmacopoeia apply.