PRO PHARMACOPOEIA TECHNICAL NOTE No. 1292 (rev. 11)

NOTE ON THE MONOGRAPH

Ink without the sac is taken into account in the definition of the drug.
Modification of the identification for the mother tincture with the addition of TLC allowing a better identification of amino acids.
Determination of homarine expressed as tyrosine in the mother tincture.

SEPIA OFFICINALIS
FOR HOMEOPATHIC PREPARATIONS

DEFINITION

The drug Sepia officinalis consists of the dried ink sac or dried ink of *Sepia officinalis* L.

The sac is a bag-shaped organ comprising two parts with distinct functions:

- a secretory part which is a gland with a lamellar structure consisting of a very large number of melanocytes arranged in bays within a highly vascularised chorion. These pigment cells will slough off and release the pigment produced.

- an excretory part comprising a reservoir where the secreted liquid accumulates. This reservoir is related to the terminal sphincter excretory channel connected to the rectum.

The secreting and excretory parts are anatomically differentiated organs wrapped in a thick fibrous tunic.

The ink is a black, thick, neutral reaction liquid, with a very faintly salty taste.

CHARACTERISTICS

Miscible with water in the fresh state, the ink is practically insoluble in the dry state.

DETAILS

A. Stir 0.2 g of ink into 1 ml of *R water* and 1 ml of *diluted R sodium hydroxide solution.* Heat carefully. An odour of methylamine will develop. Place a moistened *red litmus paper R* above the tube. The paper turns blue.

B. Insert 1 g of ink, 3 g of *sodium hydroxide R*, 1 g of *sodium thiosulphate R* and 1 ml of *water R* into a porcelain crucible. Mix and heat carefully until dry. Dry for 30 min at 300 °C. After cooling, add 40 ml of *sodium thiosulphate solution R* at 50 g/l and acidify with *glacial acetic acid R*. Filter.

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

Extract the filtrate with 20 ml of *ether* R. Separate the ethereal phase and evaporate it in a water bath. Add to the residue a few drops of *dimethylaminobenzaldehyde sulphuric solution R*. Heat gently and then add a few drops of water. A violet-blue to dark blue colour will appear.

TEST

Colloidal solution. Stir 0.5 g of powdered ink into 5 ml of water. Dissolution is partial and the solution is viscous.

Ashes. Calcify 0.5 g of ink for 5 min. Cool. Add 3 drops of concentrated hydrogen peroxide solution R. Dry and then calcify at 800°C for 15 min. The ash rate is a minimum of 11.0 per cent and a maximum of 13.0 per cent.

STRAIN

DEFINITION

The mother tincture of Sepia officinalis prepared at an ethanol content of 65% V/V from the dried ink sac or the dried ink of *Sepia officinalis* L.

Content: at least 0.050 percent *m/m* of homarine expressed as tyrosine ($C_9H_{11}NO_3$; M_r 181.2).

PRODUCTION

Method 1.1.11 (2371).

CHARACTERISTICS

An almost colourless to pale yellow solution.

Foul-smelling odour.

DETAILS

Thin-layer chromatography *(2.2.27)*.

Solution to be examined. Mother tincture.

Control solution (a). Dissolve 1 mg of *valine R* in 10 ml of *ethanol at 60% V/V R.*

Control solution (b). Dissolve 1 mg of alanine *R* in 10 ml of *ethanol at 60% V/V R.*

Plate: silica gel plate for TLC R.

Mobile phase: glacial acetic acid R, water R, acetone R, butanol R (10:20:35:35 *V/V/V/V*) *(mixture to be prepared extemporaneously)*

Application: 10 µL in bands.

Development: over a course of 7 cm.

Drying: in air.

Detection: Spray a solution of *ninhydrin R* at 1 g/l in *butanol R* and heat at 100-105 °C for 10 min.

Results: see below the sequence of bands present in the chromatograms obtained with the control solution and the Solution to be examined. In addition, other weak stripes may also be present in the chromatogram obtained with the Solution to be examined.

Top of the plate	
Valine: a pink band Alanine: a pink band	A pink band A pink band A pink band
Control solution	**Solution to be examined**

TEST

Ethanol (*2.9.10*): 60% *V/V* and 70% *V/V*.

Dry residue (*2.8.16*): minimum 0.15% *m/m*.

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

DOSAGE

Liquid chromatography *(2.2.29)*.

Solution to be examined. Dissolve 1.000 g of mother tincture in *phosphoric acid R at 0.2% V/V* and top up to 10.0 ml with the same solvent.

Control solution. Dissolve 12.0 mg of *tyrosine R* in *phosphoric acid R at 0.2% V/V* and top up to 100.0 ml with the same solvent (solution 1). Dissolve 12.0 mg tryptophan SCR in *phosphoric acid R at 0.2% V/V*and top up to 25.0 ml with the same solvent (solution 2). Take 10.0 ml of solution 1 and 0.5 ml of solution 2 and then top up to 20.0 ml with *phosphoric acid R at 0.2 per cent V/V*.

Column:
- *dimensions:* l = 25 cm, 0 = 4.6 mm.
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm), Uptisphere ODB.type.
- *temperature:* 30 °C.

Mobile phase:
- *mobile phase A: solution of phosphoric acid R at 0.2% V/V,*
- *Mobile phase: methanol R.*

Time (min)	Mobile phase A (vol.%)	Mobile phase A (vol.%)
0 - 4	100	0
4 - 6	100 → 10	0 → 90
6 - 11	10	90
11 - 13	10 → 100	90 → 0

Flow rate: 1.0 ml/min.
Detection: spectrophotometer at 274 nm.
Injection: 20 µL.
System compliance: control solution:
- *Resolution:* at least 4.0 between peaks due to tyrosine and tryptophan.
- *Repeatability:* relative standard deviation of maximum 0.62 for tyrosine after 3 injections.

Calculate the total *m/m* percent content in homarine, expressed as tyrosine, using the formula:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1 \times 20}$$

where:
A_1 = peak surface due to homarine in the chromatogram obtained with the solution to be examined,
A_2 = peak surface due to tyrosine in the chromatogram obtained with the control solution,
m_1 = mass of mother tincture to prepare the solution to be examined, in grams.
m_2 = mass of *tyrosine R* used to prepare the control solution (a), in grams,
p = tyrosine percentage content in tyrosine *R*.

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