THORNAPPLE
FOR HOMOEOPATHIC PREPARATIONS

STRAMONIUM
FOR HOMOEOPATHIC PREPARATIONS

Datura stramonium ad praeparationes homoeopathicas

DEFINITION

Fresh aerial parts of *Datura stramonium* L. (*D. tatula* L.), collected during the flowering period.

CHARACTERS

Fruit may be present. The fruit is a green, spiny capsule.

Macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

A. The stem of thornapple is erect, simple or ramified, glabrous, green, sometimes turning purple and may reach over one meter high. The stalked leaves are oval with coarsely-toothed margin and prominent ribs, green or purple, less marked on the petiole. They measure up to 20 cm long and 15 cm large. The singly flowers with a peduncle are found at the end of lateral stems or at the fork of 2 twigs. The 5-lobed, tubular, gamosepal calyx measures about 4 cm long. The white or sometimes purple corolla is funnel-shaped with a broad, folded, 5-point margin. It may reach up to 7 cm long. The flower bears 5 stamens and an ovary with 2 carpels with axile placentation.

B. Examine a fragment of abaxial epidermis under a microscope using *chloral hydrate solution R*. The abaxial epidermis is stomatiferous and bears covering trichomes and glandular trichomes. The anisocytic or anomocytic stomata (*2.8.3*) are surrounded by 5-8 subsidiary cells. The conical covering trichomes are uniseriate, composed of 3 to 5 cells with warty walls; the short and clavate glandular trichomes have unicellular foot and globular, multicellular head composed of 2 to 7 cells. The epidermis is often accompanied by spongy parenchyma where numerous calcium oxalate clusters of a short diameter (about 10 µm) can be seen.

TESTS

Foreign matter (*2.8.2*): maximum 5 per cent.

Loss on drying (*2.2.32*): minimum 75.0 per cent, determined on 5.0 g of finely-cut drug by drying in an oven at 105 °C for 2 h.

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STOCK

DEFINITION

Thornapple mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Supplement). The mother tincture is prepared with ethanol (45 per cent *V/V*), using the fresh aerial parts of *Datura stramonium* L. , collected during the flowering period.

Adjusted content: minimum 0.01 per cent *m/m* and maximum 0.03 per cent *m/m* of total alkaloids, expressed as hyoscyamine ($C_{17}H_{23}NO_3$; M_r 289.4). The alkaloids are mainly composed of hyoscyamine associated to a small quantity of scopolamine (hyoscine).

CHARACTERS

Appearance: more or less dark orange-brown liquid.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Examine the chromatograms obtained in the test “Mother tincture of Atropa belladonna” and “Mother tincture of Hyoscyamus niger”.

Results: see below the sequence of fluorescent zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other faint, fluorescent zones may be present, in the chromatogram obtained with the test solution.

Top of the plate	
Scopoletin: an intense blue zone ------ Rutin: an orange zone ------	 ----- Several orange zones ----- Several orange zones
Reference solution	**Test solution**

B. Thin-layer chromatography (*2.2.27*).

Proceed as indicated in the test “Atropine”.

Detection A: spray with *potassium iodobismuthate solution R2* until orange or brown zones appear on a yellow background. Examine in daylight.

Results A: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other faint zones may be present particularly in the middle of the chromatogram obtained with the test solution.

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Top of the plate	
Scopolamine bromhydrate: an orange to brown zone ------ ------ Hyoscyamine sulfate: an orange to brown zone	An orange to brown zone (scopolamine) ----- ----- An orange to brown zone (hyoscyamine)
Reference solution	**Test solution**

TESTS

Ethanol (*2.9.10*): 40 per cent V/V to 50 per cent *V/V.*

Dry residue (*2.8.16*): minimum 1.2 per cent *m/m*.

Atropine

Thin-layer chromatography (*2.2.27*).

Test solution. Evaporate 10 mL of mother tincture on a water-bath. Dissolve the residue in 5 mL of *0.05 M sulfuric acid* then filter. Alkalinise with *concentrated ammonia R* then extract with 15 mL of *peroxides free ether R.* Dry the ether layer on *anhydrous sodium sulfate R* and filter. Evaporate to dryness on a water-bath and dissolve the residue in 1 mL of *methanol R*.

Reference solution. Dissolve 50 mg of *hyoscyamine sulfate R* in 9 mL of *methanol R.* Dissolve 15 mg of *scopolamine bromhydrate R* in 10 mL of *methanol R.* Add 1.8 mL of scopolamine bromhydrate solution to 8 mL of hyoscyamine sulfate solution and dilute to 10 mL with *methanol R*.

Plate: *TLC silica gel plate R.*

Mobile phase: *concentrated ammonia R, water R, acetone R* (3:7:90 *V/V/V*).

Application: 20 µL as bands.

Development: over a path of 10 cm.

Drying: at 100-105 °C for 10 min then allow to cool.

Detection B: after “*Detection A*”, spray with *sodium nitrite solution R* until the yellow background disappears. Examine in daylight after 15 min.

Results B: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore possible secondary zones present in the chromatogram disappear.

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Top of the plate	
Scopolamine bromhydrate: an orange to brown zone ------ ------ Hyoscyamine sulfate: a brown to reddish-brown zone	 ------ ------ A brown to reddish- brown zone (hyoscyamine) but absence of a greyish-blue zone (atropine)
Reference solution	**Test solution**

Mother tincture of Atropa belladonna*l* Mother tincture of Hyoscyamus niger.

Test solution. Mother tincture.

Reference solution. Dissolve 1 mg of *scopoletin R* and 5 mg of *rutin R* in 20 mL of *ethanol (96 per cent) R.*

Plate: TLC silica gel plate R.

Mobile phase: glacial acetic acid R, water R, butanol R (10:10:40 *V/V/V*).

Application: 20 µL as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: first spray with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R* then with a 50 g/L solution of *400 R macrogol* in *methanol R.* Allow the plate to dry for 30 min. Examine in ultraviolet light at 365 nm.

Results: the presence of an intense blue, fluorescent zone in the front of the chromatogram shows adulteration by the mother tincture of *Atropa belladona* L.; the absence of orange zones in the half bottom part of the chromatogram shows adulteration by *Hyoscyamus niger* L.

ASSAY

Evaporate 100.0 g of mother tincture at low temperature until about a 10 g residue is obtained. Transfer the whole quantity of residue into a separating funnel, using a few millilitres of *ethanol* (70 per cent *V/V*) *R*. Add 5 mL of *concentrated ammonia R* and 25 mL of *water R.* Extract successively with fractions of a mixture of 1 volume of *methylene chloride R* and 3 volumes of *peroxide free ether R*, until complete extraction of alkaloids. Evaporate a few millilitres of the last organic fraction to dryness. Dissolve the residue in *0.25 M sulfuric acid R* and check the absence of alkaloids with *potassium tetraiodomercurate solution R*. Combine the organic layers and extract the alkaloids several times with a solution of *0.25 M sulfuric acid*. Separate both layers by centrifugation if need be, then transfer the acid fractions into a second separating funnel. Alkalinise them with *ammonia R* and shake successively at least with 3 quantities each of 30 mL of *methylene chloride R.* Combine the organic layers; add 4 g of *anhydrous sodium sulfate R* and allow to stand for 30 min, shaking

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from time to time. Allow the organic layer to separate. Filter. Wash the anhydrous sodium sulfate with 3 quantities each of 10 mL of *methylene chloride R*. Combine the organic layers, evaporate to dryness on a water-bath and dry in an oven at 100-105 °C for 15 min. Allow to cool. Dissolve the residue in a few millilitres of *methylene chloride R*, add 20.0 mL of *0.01 M sulfuric acid R.* Remove the methylene chloride by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide R* in presence *of methyl red mixed indicator R.*

Calculate the percentage content *m/m* of total alkaloids, expressed as hyoscyamine from the expression:

$$\frac{0.5788\ (20 - n)}{m}$$

n = volume of 0.02 M sodium hydroxide used, in millilitres,
m = mass of the sample in grams.

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