BUSH CLOVER, DRY FOR HOMOEOPATHIC PREPARATIONS

LESPEDEZA CAPITATA SICCUM FOR HOMOEOPATHIC PREPARATIONS

Lespedeza capitata siccum ad praeparationes homoeopathicas

DEFINITION

Dried, flowering, aerial part, of *Lespedeza* capitata Michx.

Content: minimum 0.50 per cent of total flavonoids, expressed as isoorientin ($C_{21}H_{20}O_{11}$; M_r 448.4) (dried drug).

IDENTIFICATION

A. Ramified stem all over the upper part of the plant; alternate, compound leaves with three entire leaflets, elliptical-ovate, more or less acuminate, 4.5 cm long and about 1.8 cm large; both sides covered with silky hairs, more numerous on the underside. Petiole shorter than the petiolule of the middle leaflet. Numerous inflorescences arranged in sub-globular spikes; compact and borne by a short peduncle; very numerous and tight flowers, measuring about 1 cm long; calyx presenting 5 teeth almost equal; creamy white corolla, sometimes tinged with purple. Ten stamens; 9 united into a tube opening backwards and 1 free, posterior stamen.

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

Test solution. Add 10 mL of *methanol R* to 1.0 g of powdered drug (355), Heat to boiling, under a reflux condenser for 10 min. Allow to cool and filter.

Reference solution. Dissolve 2.5 mg of *orientin R*, 2.5 mg of *isoorientin R* and 10.0 mg of *rutin R* in 10.0 mL of *methanol R.*

Plate: *TLC silica gel plate R*.

Mobile phase: *glacial acetic acid R, anhydrous formic acid R, water R, ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application: 20 µL as bands of 20 mm.

Development: over a path of 12 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 *macrogol 400 R* in *methanol R.* Allow the plate to dry in the air for about 30 min. Examine in ultraviolet light at 365 nm.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

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	
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Orientin: a yellow zone	A yellow zone (orientin)
Isoorientin: a yellow zone	A yellow zone (isoorientin)
Rutin: an orange zone	An orange zone (rutin)
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	A yellow zone
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of finely-cut drug, by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 6.0 per cent.

ASSAY

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Stock solution. Place 0.400 g of powdered drug (355) into a 100 mL round-bottomed flask and add 40 mL of *ethanol* (60 per cent *V/V*) *R.* Heat on a water-bath at 60 °C, for 30 min; stirring regularly. Allow to cool. Filter through a plug of absorbent cotton into a 100.0 mL volumetric flask. Transfer the cotton plug into the round-bottomed flask containing the residue. Dilute the whole content with 40 mL of *ethanol* (60 per cent *V/V*) *R.* Heat again on a water-bath at 60° C for 10 min. Allow to cool. Filter on a paper filter. Rinse the round-bottomed flask and the paper filter with 6 mL of *ethanol* (60 per cent *V/V*) *R*. Combine the filtrates and the washings into the 100.0 mL volumetric flask and dilute to 100.0 mL with *ethanol* (60 per cent *V/V*) *R*.

Test solution. Place 5.0 mL of stock solution into a round-bottomed flask. Evaporate to dryness, under reduced pressure and dilute the residue with 10 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. Then add 10 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R* and dilute to 25.0 mL with *glacial acetic acid R*.

Compensation liquid of the test solution. Place 5.0 mL of stock solution into a round-bottomed flask. Evaporate to dryness, under reduced pressure and dilute the residue with 10 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R.* Add 10 mL of *anhydrous formic acid R* and dilute to 25.0 mL with *glacial acetic acid R.*

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Reference stock solution. Place 5.0 mg of *isoorientin R* into a 100.0 mL volumetric flask, and dilute to 100.0 mL with a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R.* In a 25.0 mL volumetric flask, place 10.0 mL of this solution and dilute to 25.0 mL with the same solvent.

Reference solution. Place 10.0 mL of reference stock solution into a 25.0 mL volumetric flask and add 10.0 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R.* Dilute to 25.0 mL with *glacial acetic acid R.*

Compensation liquid of the reference solution. Place 10.0mL of reference stock solution into a 25.0 mL volumetric flask, add 10.0 mL of *anhydrous formic acid R* and dilute to 25.0 mL with *glacial acetic acid R.*

Thirty min after the addition of the last reagent, measure the absorbance of the test solution at 410 nm, in comparison with the compensation liquid of the test solution, and the absorbance of the reference solution in comparison with the compensation liquid of the reference solution.

Calculate the percentage content of total flavonoids, expressed as isoorientin (dried drug), from the expression:

$$\frac{A_1 \times m_2 \times 80}{A_2 \times m_1}$$

A_1 = absorbance of the test solution,
A_2 = absorbance of the reference solution,
m_1 = mass of the drug sample in the test solution, in grams,
m_2 = mass of *isoorientin R* sample in the reference solution, in grams.

STOCK

DEFINITION

Bush clover (dry) mother tincture is prepared with ethanol (55 per cent *V/V*), using the dried, flowering, aerial part of *Lespedeza capitata* Michx.

Content: minimum 0.040 per cent *m/m* of total flavonoids, expressed as isoorientin ($C_{21}H_{20}O_{11}$; M_r 448.4).

PRODUCTION

Method 1.1.10 (2371). Drug fragmented into segments, about 1 cm long. Maceration time: 3-5 weeks.

CHARACTERS

Appearance: orange-brown liquid.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 2.5 mg of *orientin R,* 2.5 mg of *isoorientin R* and 10.0 mg of *rutin R* in 10 mL of *ethanol R.*

Plate: *TLC silica gel plate R*.

Mobile phase: *glacial acetic acid R, anhydrous formic acid R, water R, ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application: 20 µL as bands of 20 mm.

Development: over a path of 12 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 *macrogol 400 R* in *methanol R.* Allow the plate to dry in the air for about 30 min. Examine in ultraviolet light at 365 nm.

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	
	A greenish-yellow (solvent front)
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	A dark green zone (vitexin)
	An orange zone (isoquercitroside)
Orientin: a yellow zone	A yellow zone (orientin)
	A dark green zone (isovitexin)
Isoorientin: a yellow zone	A yellow zone (isoorientin)
Rutin: an orange zone	An orange zone (rutin)
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	Three dark green to yellowish-green zones (faint)
	A yellow zone
Reference solution	**Test solution**

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

TESTS

Ethanol (*2.9.10*): 50 per cent *V/V* to 60 per cent *V/V.*

Dry residue (*2.8.16*): minimum 1.0 per cent *m/m*.

ASSAY

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Stock solution. In a 20.0 mL volumetric flask, place a sample *m* accurately weighed of about 6.500 g of mother tincture and dilute to 20.0 mL with *glacial acetic acid R*.

Test solution. Place 1.0 mL of stock solution into a 25.0 mL volumetric flask. Add 10 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. Add 10 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R*, and dilute to 25.0 mL with *glacial acetic acid R*.

Compensation liquid of the test solution. Place 1.0 mL of stock solution into a 25.0 mL volumetric flask. Add 10 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R.* Add 10 mL of *anhydrous formic acid R* and dilute to 25.0 mL with *glacial acetic acid R.*

Reference stock solution. In a 100.0 mL volumetric flask, place 5.0 mg of *isoorientin R* and dilute to 100.0 mL with a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. In a 25.0 mL volumetric flask, place 10.0 mL of this solution and dilute to 25.0 mL with the same solvent.

Reference solution. Place 10.0.mL of reference stock solution into a 25.0 mL volumetric flask, add 10.0 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R.* Dilute to 25.0 mL with *glacial acetic acid R.*

Compensation liquid of the reference solution. Place 10.0mL of reference stock solution into a 25.0 mL volumetric flask, add 10.0 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *glacial acetic acid R.*

Thirty min after the addition of the last reagent, measure the absorbance of the test solution at 410 nm, in comparison with the compensation liquid of the test solution, and the absorbance of the reference solution in comparison with the compensation liquid of the reference solution.

Calculate the percentage content *m/m* of total flavonoids, expressed as isoorientin, from the expression:

$$\frac{A_1 \times m_2 \times 80}{A_2 \times m_1}$$

A_1 = absorbance of the test solution,
A_2 = absorbance of the reference solution,
m_1 = mass of the mother tincture sample in the test solution, in grams,
m_2 = mass of *isoorientin R* sample in the reference solution, in grams.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.