# WILD YAM
# FOR HOMOEOPATHIC PREPARATIONS

# DIOSCOREA VILLOSA
# FOR HOMOEOPATHIC PREPARATIONS

**Dioscorea villosa ad praeparationes homoeopathicas**

## DEFINITION

Dried, underground organ of *Dioscorea villosa* L.

*Content*: minimum 1.8 per cent of total saponosides, expressed as diosgenin ($C_{27}H_{42}O_3$; $M_r$ 414.6) (dried drug).

## CHARACTERS

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

## IDENTIFICATION

A. Greyish-white rhizome, 7 cm to 8 cm long and 5 mm to 10 mm in diameter, bearing numerous rootlets. Surface showing scars corresponding to the insertion points of stems. Neat fracture, horny appearance.

B. Reduce wild yam to a powder (355). The powder is blackish-brown. Examine under a microscope using *chloral hydrate solution R.* Numerous fragments of cellulose parenchyma with ovoid cells with intercellular air-spaces or with polyhedral cells without air-spaces; fragments of isolated wood vessels with pitted or reticulate decorations; scarce fragments of coating tissue composed of polyhedral cells with dark brown cell-walls. Examine under a microscope using a solution of *glycerol* (50 per cent *V/V*) *R*. Very numerous ovoid or rounded starch granules, either free or within parenchyma cells.

C. Thin layer chromatography (*2.2.27*).

*Test solution.* Add 30 mL of *ethanol* (65 per cent *V/V*) *R* to 3 g of powdered drug (355). Heat under a reflux condenser on a water-bath at 60 °C for 15 min. Allow to cool. Filter. Shake 5 mL of the filtrate with 10 mL of *methylene chloride R.* Filter the organic layer with *anhydrous sodium sulfate R* then evaporate under reduced pressure. Dissolve the residue in 0.5 mL of *methanol R*.

*Reference solution.* Dissolve 10 mg of *diosgenin R* and 5 mg of *hederagenin R* in 10 mL of *methanol R*.

*Plate*: *TLC silica gel plate R*.

*Mobile phase*: *methanol R, methylene chloride R* (5:95 *V/V*).

*Application*: 20 µL, as bands.

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

*Development*: over a path of 10 cm.

*Drying*: in air.

*Detection A*: spray with a solution of *sulfuric acid* (10 per cent *m/V*) *R* in *ethanol* (*96 per cent*) *R*. Heat at 100-105 °C for 10 min. Examine in ultraviolet light at 365 nm.

*Results A*: 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 blue zone |
| ----- | ----- |
| Diosgenin: a purplish-blue zone | A purplish-blue zone (diosgenin)<br>A purple zone |
| ----- | ----- |
| Hederagenin: a yellowish-green zone | A greenish-yellow zone |
| **Reference solution** | **Test solution** |

*Detection B*: examine in daylight.

*Results B*: 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 in the chromatogram obtained with the test solution.

| **Top of the plate** | |
|---|---|
| | A pink zone |
| ----- | ----- |
| Diosgenin: a pinkish-brown zone | A pinkish-brown zone (diosgenin)<br>A pink zone |
| ----- | ----- |
| Hederagenin: a purplish-pink zone | A pink zone |
| **Reference solution** | **Test solution** |

TESTS

**Foreign matter** (*2.8.2*): complies with the test.

**Loss on drying** (*2.2.32*): maximum 12.0 per cent, determined on 1.0 g of powdered drug (355), by drying in an oven at 105 °C for 2 h.

**Total ash** (*2.4.16*): maximum 10.0 per cent, determined on 1.000 g of powdered drug (355).

ASSAY

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

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*Test solution*. Add 10 mL of *ethanol* (70 per cent *V/V*) *R* to 1.000 g of powdered drug (355). Heat under a reflux condenser on a water-bath for 30 min. Allow to cool and filter. Rinse the filter. Combine the filtrate and the rinsing solution in a 10.0 mL volumetric flask, and dilute to 10.0 mL with *ethanol* (70 per cent *V/V*) *R*. In a 100.0 mL volumetric flask, place 5.0 mL of this solution and dilute with *methanol R.* Mix. Take 1.0 mL of this solution and evaporate to dryness. Dissolve the residue in 10.0 mL of *sulfuric acid R.* Leave in contact for 1 h. Repeat the process twice in order to obtain three solutions.

*Test solution.* In a 20.0 mL volumetric flask, dissolve 2.50 mg of *diosgenin R* in *methanol R* and dilute with the same solvent. Mix. Take 1.0 mL of this solution and evaporate to dryness. Dissolve the residue in 10.0 mL of *sulfuric acid R*. Leave in contact for 1 h. Repeat the process twice in order to obtain three solutions.

*Compensation liquid: Sulfuric acid R.*

Measure the absorbance values of the three test solutions and of the three reference solutions at 410 nm, in comparison with the compensation liquid.

Calculate the percentage content of total saponosides, expressed as diosgenin from the expression:

$$\frac{A_1 \times m_2}{A_2 \times m_1} \times 1000$$

$A_1$ = average of the absorbance values of the test solution at 410 nm,
$A_2$ = average of the absorbance values of the reference solution at 410 nm,
$m_1$ = mass of the dried drug sample in grams,
$m_2$ = mass of diosgenin sample in the reference solution in grams.

## STOCK

DEFINITION

Wild yam mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Authority Supplement). The mother tincture is prepared with ethanol (65 per cent *V/V)*, using the dried, underground organ of *Dioscorea villosa* L.

*Content*: minimum 0.20 per cent *m/m* of total saponosides, expressed as diosgenin ($C_{27}H_{42}O_3$; $M_r$ 414.6).

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

CHARACTERS

*Appearance:* bright yellow liquid.

IDENTIFICATION

Thin layer chromatography (*2.2.27*).

*Test solution.* Shake 5 mL of mother tincture with 10 mL of *methylene chloride R*. Filter the organic layer on *anhydrous sodium sulfate R* then evaporate under reduced pressure. Dilute the residue in 0.5 mL of *methanol R.*

*Reference solution*. Dissolve 10 mg of *diosgenin R* and 5 mg of *hederagenin R* in 10 mL of *methanol R.*

*Plate*: *TLC silica gel plate R.*

*Mobile phase*: *methanol R*, *methylene chloride R* (5:95 *V/V*).

*Application*: 20 µL, as bands.

*Development*: over a path of 10 cm.

*Drying*: in air.

*Detection A*: spray with a solution of *sulfuric acid* (10 per cent *V/V*) *R* in *ethanol* (*96 per cent*) *R.* Heat at 100-105 °C for about 10 min. Examine in ultraviolet light at 365 nm.

*Results A*: 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 blue zone |
| ----- | ----- |
| Diosgenin: a purplish-blue zone | A purplish-blue zone (diosgenin)<br>A purple zone |
| ----- | ----- |
| Hederagenin: a yellowish-green zone | A greenish-yellow zone |
| **Reference solution** | **Test solution** |

*Detection B*: examine in daylight.

*Results B*: 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 in the chromatogram obtained with the test solution.

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| Top of the plate | |
|---|---|
| | A pink zone |
| ----- | ----- |
| Diosgenin: a pinkish-brown zone | A pinkish-brown zone (diosgenin) |
| | A pink zone |
| ----- | ----- |
| Hederagenin: a purplish-pink zone | A pink zone |
| **Reference solution** | **Test solution** |

TESTS

**Ethanol** (*2.9.10*): 60 per cent *V/V* to 70 per cent *V/V.*

**Dry residue** (*2.8.16*): minimum 0.6 per cent *m/m*.

ASSAY

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

*Test solution.* In a 100.0 mL volumetric flask, place 5.00 g of mother tincture and dilute to 100.0 mL with *methanol R*. Mix. Take 1.0 mL of this solution and evaporate to dryness. Dissolve the residue in 10.0 mL of *sulfuric acid R*. Leave in contact for 1 h. Repeat the process twice in order to obtain three solutions.

*Reference solution.* Weigh 2.50 mg *diosgenin R*. Dissolve in *methanol R* and dilute to 20.0 mL with the same solvent. Mix. Take 1.0 mL of this solution and evaporate to dryness. Dissolve the residue in 10.0 mL of *sulfuric acid R.* Leave in contact for 1 h. Repeat the process twice in order to obtain three solutions.

*Compensation liquid*: *Sulfuric acid R.*

Measure the absorbance of the three test solutions and the three reference solutions at 410 nm, in comparison with the compensation liquid.

Calculate the percentage content *m/m* of total saponosides, expressed as diosgenin, from the expression:

$$\frac{A_1 \times m_2}{A_2 \times m_1} \times 500$$

$A_1$ = average of the absorbance values of the test solution at 410 nm,
$A_2$ = average of the absorbance values of the reference solution at 410 nm,
$m_1$ = mass of the mother tincture sample in grams,
$m_2$ = mass of diosgenin sample in the reference solution in grams.

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