Mobile phase:

– *mobile phase A*: *acetonitrile R1*, 5 g/L solution of *phosphoric acid R* (20:80 *V/V*);

– *mobile phase B*: 5 g/L solution of *phosphoric acid R*, *acetonitrile R1* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	55	45
5 - 18	55 → 20	45 → 80
18 - 22	20	80

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µL.

Identification of peaks: use the chromatogram supplied with *valerian dry extract HRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to acetoxyvalerenic acid and hydroxyvalerenic acid.

Relative retention with reference to valerenic acid (retention time = about 19 min): hydroxyvalerenic acid = about 0.2; acetoxyvalerenic acid = about 0.5.

Calculate the percentage content of sesquiterpenic acids, expressed as valerenic acid, using the following expression:

$$\frac{(A_1 + A_2) \times m_2 \times p \times 0.2}{A_3 \times m_1}$$

A_1 = area of the peak due to hydroxyvalerenic acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to acetoxyvalerenic acid in the chromatogram obtained with the test solution;

A_3 = area of the peak due to valerenic acid in the chromatogram obtained with reference solution (b);

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *valerian dry extract HRS* used to prepare reference solution (a), in grams;

p = percentage content of valerenic acid in *valerian dry extract HRS*.

01/2008:1898

VALERIAN DRY HYDROALCOHOLIC EXTRACT

Valerianae extractum hydroalcoholicum siccum

DEFINITION

Extract produced from *Valerian root (0453)*.

Content: minimum 0.25 per cent *m/m* of sesquiterpenic acids, expressed as valerenic acid ($C_{15}H_{22}O_2$; M_r 234.3) (dried extract).

PRODUCTION

The extract is produced from the drug using ethanol (45 to 80 per cent *V/V*) or methanol (40 to 55 per cent *V/V*) by an appropriate procedure.

CHARACTERS

Appearance: brown, hygroscopic powder.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Suspend 1 g of the extract to be examined in 10 mL of *methanol R* and sonicate for 10 min. Filter the supernatant through a membrane filter (nominal pore size 0.45 µm). Use the filtrate as the test solution.

Reference solution. Dissolve 5 mg of *acetoxyvalerenic acid R* and 5 mg of *valerenic acid R* in 20 mL of *methanol R*.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *glacial acetic acid R, ethyl acetate R, cyclohexane R* (2:38:60 *V/V/V*).

Application: 20 µL [or 5 µL] as bands.

Development: over a path of 10 cm [or 6 cm].

Drying: in air.

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min; examine in daylight.

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

Top of the plate	
———	———
Valerenic acid: a violet zone	A violet zone (valerenic acid)
Acetoxyvalerenic acid: a violet zone	A violet zone (acetoxyvalerenic acid)
———	———
	2 faint or very faint violet zones
Reference solution	**Test solution**

TESTS

Loss on drying (*2.8.17*): maximum 6.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. Suspend 1.00 g of the extract to be examined in 50.0 mL of *methanol R1*, sonicate for 10 min and filter.

Reference solution. Dissolve an amount of *valerian standardised dry extract CRS* corresponding to 1.0 mg of valerenic acid in *methanol R1* and dilute to 10.0 mL with the same solvent.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: *acetonitrile R1*, 5 g/L solution of *phosphoric acid R* (20:80 *V/V*);

– *mobile phase B*: 5 g/L solution of *phosphoric acid R*, *acetonitrile R1* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	55	45
5 - 18	55 → 20	45 → 80
18 - 20	20	80

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µL.

Peak identification: use the chromatogram supplied with *valerian standardised dry extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to acetoxyvalerenic acid and valerenic acid.

System suitability: reference solution:

– *relative retention* with reference to valerenic acid (retention time = about 21 min): acetoxyvalerenic acid = about 0.5.

Herbal drugs

Calculate the percentage content of sesquiterpenic acids, expressed as valerenic acid, using the following expression:

$$\frac{(A_1 + A_2) \times m_2 \times p \times 5}{A_3 \times m_1}$$

A_1 = area of the peak due to acetoxyvalerenic acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to valerenic acid in the chromatogram obtained with the test solution;

A_3 = area of the peak due to valerenic acid in the chromatogram obtained with the reference solution;

m_1 = mass of the dried extract to be examined, in grams;

m_2 = mass of *valerian standardised dry extract CRS* used to prepare the reference solution, in grams;

p = percentage content of valerenic acid in *valerian standardised dry extract CRS*.

07/2010:0453

VALERIAN ROOT

Valerianae radix

DEFINITION

Dried, whole or fragmented underground parts of *Valeriana officinalis* L. *s.l.*, including the rhizome surrounded by the roots and stolons.

Content:

– *essential oil*: minimum 4 mL/kg (dried drug);

– *sesquiterpenic acids*: minimum 0.17 per cent *m/m*, expressed as valerenic acid ($C_{15}H_{22}O_2$; M_r 234.3) (dried drug);

IDENTIFICATION

A. The rhizome is yellowish-grey or pale brownish-grey, obconical or cylindrical, up to about 50 mm long and 30 mm in diameter; the base is elongated or compressed, usually entirely covered by numerous roots. The apex usually exhibits a cup-shaped scar from the aerial parts; stem bases are rarely present. When cut longitudinally, the pith exhibits a central cavity transversed by septa. The roots are numerous, almost cylindrical, of the same colour as the rhizome, 1-3 mm in diameter and sometimes more than 100 mm long. A few filiform fragile secondary roots are present. The fracture is short. The stolons show prominent nodes separated by longitudinally striated internodes, each 20-50 mm long, with a fibrous fracture.

B. Reduce to a powder (355) (*2.9.12*). The powder is pale yellowish-grey or pale greyish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: cells containing a pale brown resin or droplets of essential oil; groups of small, rectangular sclereids with thick walls and a narrow, channelled branched lumen; occasional groups of larger, thinner-walled sclereids from the stem bases; lignified, reticulately-thickened vessels, singly or in small groups; thin-walled, elongated cells of the piliferous layer, some with root hairs; occasional fragments of cork. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows abundant starch granules, mainly compound with up to 4-6 components but frequently separated to form single granules, rounded or irregular and up to about 15 µm in diameter; most of the granules show a rather indistinct cleft or radiate hilum.

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

Test solution. Suspend 1 g of the powdered drug (355) (*2.9.12*) in 10 mL of *methanol R* and sonicate for 10 min. Filter the supernatant through a membrane filter (nominal pore size 0.45 µm). Use the filtrate as the test solution.

Reference solution. Dissolve 5 mg of *acetoxyvalerenic acid R* and 5 mg of *valerenic acid R* in 20 mL of *methanol R*.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *glacial acetic acid R, ethyl acetate R, cyclohexane R* (2:38:60 *V/V/V*).

Application: 20 µL [or 5 µL] as bands of 10 mm [or 8 mm].

Development: over a path of 10 cm [or 6 cm].

Drying: in air.

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min; examine in daylight.

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

Top of the plate	
———	———
Valerenic acid: a violet zone	A violet zone (valerenic acid)
Acetoxyvalerenic acid: a violet zone	A violet zone (acetoxyvalerenic acid)
———	———
	2 faint or very faint violet zones
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 5 per cent of stem bases and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of well homogenised powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 12.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 5.0 per cent.

ASSAY

Essential oil (*2.8.12*). Use 40.0 g of freshly powdered drug (500) (*2.9.12*), a 2000 mL flask, 500 mL of *water R* as the distillation liquid and 0.50 mL of *xylene R* in the graduated tube. Distil at a rate of 3-4 mL/min for 4 h.

Sesquiterpenic acids. Liquid chromatography (*2.2.29*).

Test solution. Place 1.50 g of the powdered drug (710) (*2.9.12*) in a 100 mL round-bottomed flask with a ground-glass neck. Add 20 mL of *methanol R1*. Mix and heat on a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Place the filter with the residue in the 100 mL round-bottomed flask. Add 20 mL of *methanol R1* and heat on a water-bath under the reflux condenser for 15 min. Allow to cool and filter. Combine the filtrates and dilute to 50.0 mL with *methanol R1*, rinsing the round-bottomed flask and the filter.

Reference solution. Dissolve an amount of *valerian dry extract HRS* corresponding to 1.0 mg of valerenic acid in *methanol R1* and dilute to 10.0 mL with the same solvent. Sonicate for 10 min and filter through a membrane filter (nominal pore size 0.45 µm).

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

Herbal drugs