PRO PHARMACOPOEA TECHNICAL NOTE NO 1293 (11th rev.)

NOTE ON THE MONOGRAPH

The identifications and tests of the plant drug are amended in accordance with the requirements the black cohosh monograph (2069) of the European Pharmacopoeia.
Amendment to the identification for the mother tincture, with the addition of TLC allowing for better identification.
Dosage of triterpenic heterosides expressed as monoammonium glycyrrhizate in the herbal substance and the mother tincture.

ACTAEA RACEMOSA
FOR HOMOEOPATHIC PREPARATIONS

Other homoeopathic name: **Cimicifuga**

DEFINITION

Dried subterranean organs of *Cimicifuga racemosa* (L.) Nutt.

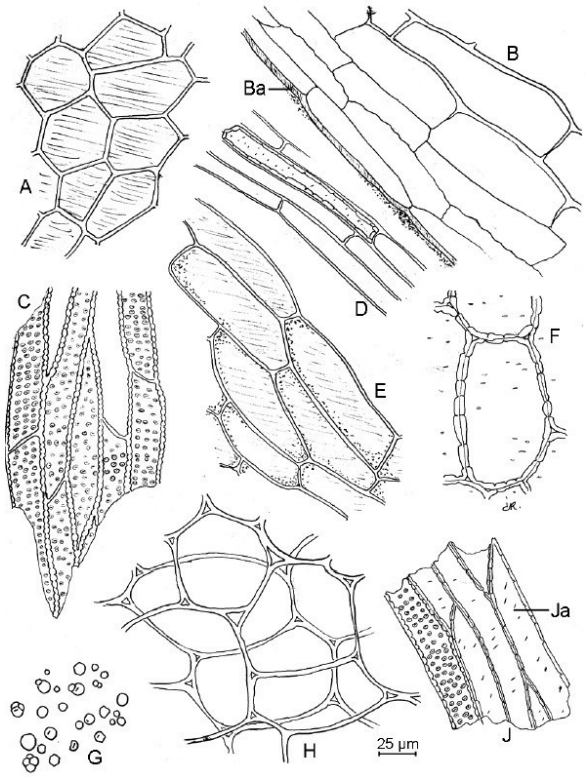
Content: at least 1.0% triterpenic heterosides expressed as monoammonium glycyrrhizate ($C_{42}H_{65}NO_{16}$; M_r 840) (desiccated drug).

IDENTIFICATION

A. *Whole drug*. The rhizome is dark brown, hard, subcylindrical in shape and slightly knotty; it measures 1.5-2.5 cm in diameter and 2-15 cm in length, and it has numerous very close offshoots, both straight and curved, each carrying at their extremities the remains of a concave circular spot or scar. The fracture is horny and the transverse section has a thin cortical area surrounding a ring consisting of numerous clear tapered elements of vascular tissue, alternating with darker medullary rays and a large central stem. The rhizome has roots on its underside, many of which are broken, leaving circular scars. The roots are dark brown with a diameter of 1-3 mm, brittle, noticeably cylindrical or quadrangularly obtuse in shape, and have longitudinal wrinkles; the fracture is short; the cross-section shows a thick cortical area forming a dark brown cylinder whose central part consists of three to six tapered bundles of clearer vascular tissue, joined in the centre and separated by large non-lignified medullary rays.

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

Fragmented drug. Irregular fragments, more or less angular, of rhizome and cylindrical root fragments. The rhizome fragments are hard, horny, and generally present a dark brown surface corresponding to the external surface, and several light brown surfaces that are often striated, correspond to the cut. The root fragments are dark brown, more or less cylindrical, and wrinkled longitudinally. The cross-section of a lighter colour shows a distinct cambial area separating a thick cortical area from the central area, which consisting of three to six tapered bundles of vascular tissue, joined in the centre and separated by wide medullary rays.



Drawing for identification B of pulverised black cohosh

B. Microscopic examination (*2.8.23*). The powder is light brown. Examine under a microscope using *chloral hydrate solution R*. The powder has the following characteristic elements (figure 2069.-1): fragments of the epidermis of the rhizome, with brown polygonal cells [A]; numerous fragments of parenchyma consisting of rounded cells with lightly and regularly thickened walls, with small triangular meatuses between them [H]; clusters of short vessels with tight, bordered pits [C, J], sometimes accompanied by fibres with finely punctuated walls [Ja]; fragments of the parenchyma of the stem of the rhizome having ovoid cells with thickened and canaliculated walls [F]; some fragments of the phloem containing long, isolated sclerotic cells [D]; fragments of root-covering tissue (front view [E], longitudinal section [B]), consisting of brown cells covered with a dark brown cuticle [Ba]. Examine under a microscope using *glycerol R* solution at 50% *V/V*. The powder has numerous simple spherical or polygonal starch granules. measuring 5-10 µm in diameter, or consisting of 2-3 (or, rarely, up to 6) elements; some granules show a slit hilum [G].

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

C. Examine the chromatograms obtained in the substitution test with *Cimicifuga americana* Michx., *C. foetida* L., *C. dahurica* (Turcz.) Maxim. or *C. heracleifolia* Kom.

Results B: use the chromatograms provided with *Actaea racemosa ERV* and the chromatogram obtained with control solution (a) to identify the paths corresponding to *A. racemosa*.

See below the sequence of paths present in the chromatograms obtained with control solutions (a) and (b) and the solution to be examined. In addition, other low-intensity paths may be present in the chromatograms obtained with control solution (a) and the solution to be examined.

Top of the plate		
-------	-------	-------
Actein: one brown path	Actein: one brown path	One brown path (actein)
23-epi-26-deoxyactein: one brown path		One brown path (23-epi-26-deoxyactein)
-------	-------	-------
One purple path		One purple path
One purple path		One purple path
One brown path		One brown path
Control solution (a)	**Control solution (b).**	**Solution to be examined**

TEST

Foreign elements (*2.8.2*): maximum 5%.

Loss on drying (*2.2.32*): maximum 12%, determined in the oven at 105 °C for 2 hours with 1.000 g of pulverised drug (355) (*2.9.12*).

Total ash (*2.4.16*): maximum 10%.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 5%.

Substitution for *Cimicifuga americana* Michx., *C. foetida* L., *C. dahurica* (Turcz.) Maxim. or *C. heracleifolia* Kom. Thin-layer chromatography (*2.2.27*).

Solution to be examined. Add 10 ml of *ethanol at 50% V/V R* to 0.50 g of pulverised black cohosh. (*2.9.12*). Shake well, then treat with ultrasound for 10 min and centrifuge. Use the supernatant.

Control solution (a). Add 10 ml of *ethanol at 50% V/V R* to 0.50 g of *Actaea racemosa ERV*. Shake well, then treat with ultrasound for 10 min and centrifuge. Use the supernatant.

Control solution (b). Dissolve 2 mg of*actein R* in *methanol R* and top up to 10 ml with the

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

same solvent.

Plate: *silica gel plate F_{254} for TLC R* (2-10 µm).

Mobile phase: *anhydrous formic acid R, ethyl formate R, toluene R* (20:30:50 *V/V/V*).

Deposit: 2 µl in paths of 8 mm (see table *Deposit schedule 1*).

Strip	1	2	3	4	5	6	7
Volume of deposit (µl)	2	2	2	-	2	2	2
Solution	Control solution (a)	Control solution (b).	Solution to be examined	Blank	Control solution (a)	Control solution (b).	Solution to be examined
After development, the plate is cut along strip 4 (blank). Strips 1 to 3 are used to detect substitutions for *C. americana*, *C. foetida*, *C. dahurica* or *C. heracleifolia* (detection A); strips 5 to 7 for identification D (detection B).							

Table – *Deposit schedule 1*

Development: over a path of 6 cm.

Drying: in air.

System compliance: control solution (b):
- the R_F of the path due to actein is located between 0.35 and 0.40 (detection B).

Detection A: examine under ultraviolet light at 254 nm.

Results A: the chromatogram obtained with the solution to be examined does not show, between R_F 0.2 and R_F 0.35, attenuation paths with a fluorescence more intense than those in the chromatogram obtained with control solution (a).

Detection B: treat with *sulphuric acid R* solution at 10% V/V in *methanol R*; heat to 100 °C for 5 min. Allow to cool to room temperature and examine in daylight.

Falsification by Cimicifuga americana Michx., C. foetida L., C. dahurica (Turcz.) Maxim. and/or C. heracleifolia Kom. Thin-layer chromatography (*2.2.27*) according to the indications of test involving substitution with *Cimicifuga americana* Michx., *C. foetida* L., *C. dahurica* (Turcz.) Maxim. or *C. heracleifolia* Kom., with the following modifications.

Control solution (c). Dissolve 2 mg of *cimifugine R* in *methanol R* and top up to 10 ml with the same solvent.

Deposit: 2 µl of control solutions (b) and (c), 20 µl of the solution to be examined and of control solution (a), in paths of 8 mm (see table *Deposit schedule 2*).

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

Strip	1	2	3	4	5	6	7	8	9

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

Volume of deposit (µl)	20	2	2	20	-	20	2	2	20
Solution	Control solution (a)	Control solution (b).	Control solution (c)	Solution to be examined	Blank	Control solution (a)	Control solution (b).	Control solution (c)	Solution to be examined
After development and examination for detection of *C. americana* (detection A), the plate is cut along strip 5 (blank). Strips 1 to 4 are used to detect falsifications by *C. foetida* (detection B), strips 6 to 9 to detect falsifications by *C. dahurica* and/or *C. heracleifolia* (detection C).									

Table – *Deposit schedule 2*

System compliance:: control solution (b):
- le R_F of the path due to actein is located between 0.35 and 0.40 (detections B and C).

Detection A: examine under ultraviolet light at 254 nm.

Results A: absence of more than 10% of *C. americana*.

Compare the chromatogram of *C. americana* provided with *Actaea racemosa ERV* with the chromatograms obtained with the solution to examined and with control solution (a). The chromatogram obtained with the solution to be examined has no attenuation paths with fluorescence at R_F 0.3 (in capitals below in the chromatogram of *C. americana*). The presence of this path in the chromatogram obtained with the solution to be examined indicates falsification of more than 10% by *C. americana*.

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

Top of the plate	
-------	-------
One weak path	One weak path
2 weak paths	2 weak paths
One weak path	One weak path
-------	-------One dark path
One weak path	One weak path
One dark path	One dark path
One dark path	One dark path
Control solution (a)	***C. americana* (10%)**

Detection B: dissolve 4.5 g of *boric acid R* in 150 ml of *anhydrous ethanol R* (solution A); dissolve 5 g of*oxalic acid R* in 50 ml of*anhydrous ethanol R* (solution B); combine solutions A and B and mix well; treat the plate with this recently prepared solution and heat to 120 °C for 5 min; examine in ultraviolet light at 365 nm.

Results B: absence of more than 5% of *C. foetida*.

Compare the chromatogram of *C. foetida* provided with *Actaea racemosa ERV* with the chromatograms obtained with the solution to be examined and with control solutions (a), (b) and (c). The chromatogram obtained with the solution to be examined has no attenuation paths with an intense fluorescence between R_F 0.03 and R_F 0.06, nor at the position of the path in the chromatogram obtained with control solution (c) (in capitals below in the chromatogram of *C. foetida*). The presence one or both of these paths in the chromatogram obtained with the solution to be examined indicates falsification at more than 5% by *C. foetida*.

Top of the plate			
-------	-------	-------	-------
Actein: one weak whitish path	Actein: one weak whitish path		One weak whitish path (actein)
-------	-------	-------	-------
One bluish path			One bluish path
		Cimifugine: one intensely fluorescent path	One intensely fluorescent path(cimifugine)
One brownish path One bluish path			One brownish path One bluish path
			One fluorescent path
Control solution (a)	**Control solution (b).**	**Control solution (c)**	***C. foetida* (5%)**

Detection C: dissolve 8 g of *antimony trichloride R* in 200 ml of *methylene chloride R*; treat with this solution and heat at 120 °C for 10 min; examine in ultraviolet light at

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

365 nm.

Results C: absence of more than 5% of *C. heracleifolia* and/or *C. dahurica*.

Compare the chromatograms of *C. heracleifolia* and *C. dahurica* provided with *Actaea racemosa ERV* with the chromatograms obtained with the solution to be examined and with control solutions a) and b). The chromatogram obtained with the solution to be examined has not paths with intense fluorescence just above the path due to actein (below in the chromatogram of *C. heracleifolia* or *C. dahurica*). The presence of this path in the chromatogram obtained with the solution to be examined indicates falsification at more than 5% by *C. heracleifolia* and/or *C. dahurica*.

Top of the plate		
		One intensely fluorescent path
Actein: one weak brownish path	Actein: one weak brownish path	One weak brownish path (actein)
One brownish path		One brownish path
One bluish path		One bluish path
Control solution (a)	**Control solution (b).**	**Solution to be examined**

DOSAGE

Liquid chromatography (*2.2.29*).

Solution to be examined. In a 125 ml screw-cap vial, introduce 4.000 g of pulverised black cohosh (355) (*2.9.12*), then add 25.0 ml of a mixture of equal volumes of *methanol R* and *water R*. Treat with ultrasound for 45 min and shake for 15 min. Filter through a membrane filter (nominal pore diameter 0.45 µm).

Control solution (a). Using ultrasound, dissolve 20.0 mg of *Actaea racemosa for dosage SCR* (containing monoammonium glycyrrhizate) in *methanol R* and top up to 10.0 ml with the same solvent.

Control solution (b). Take 5.0 ml of control solution (a) and top up to 10.0 ml with *methanol R.*

Control solution (c). Take 2.0 ml of control solution (a) and top up to 10.0 ml with *methanol R.*

Control solution (d). Take 1.0 ml of control solution (a) and top up to 20.0 ml with *methanol R.*

Control solution (e). Dissolve 500 mg of *dry Actaea racemosa extract for system compliance ERV* in *methanol R* and top up to 10.0 ml with the same solvent; treat with ultrasound and filter through a membrane filter (nominal pore diameter 0.45 µm).

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

Column:
- *dimensions*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm)[1];
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: solution of *anhydrous formic acid R* at 0.1% *V/V* in *water R*;
- *mobile phase B*: *acetonitrile R*.

Time (min)	Mobile phase A (*V/V* %)	Mobile phase B (*V/V* %)
0 - 8	80	20
8 - 15	80 → 68	20 → 32
15 - 55	68 → 36	32 → 64
55 - 65	36 → 5	64 → 95
65 - 70	5	95
70 - 75	5 → 80	95 → 20

Flow rate: 1.6 ml/min.

Detection: evaporative light-scattering detector; the following settings have worked to satisfaction; if the adjustment parameters of the detector are different, make the adjustments necessary to meet the system compliance criterion (signal-to-noise ratio):
- carrier gas: *nitrogen R*;
flow rate: 1.5 l/min;
- temperature of the evaporative detector: 40 °C;
- temperature of the canister: 40 °C;

Injection: 20 µl.

Identification of peaks: use the chromatogram obtained with control solution (e) to identify peaks 1 to 12 to be quantified.

Relative retention in relation tomonoammonium glycyrrhizate (retention time = approx. 23.4 min): peak 1 = approx. 0.73; peak 2 = approx. 0.96; peak 3 = approx. 1.12; peak 4 = approx. 1.16; peak 5 = approx. 1.22; peak 6 = approx. 1.24; peak 7 = approx. 1.28; peak 8 = approx. 1.36; peak 9 = approx. 1.43, peak 10 = approx. 1.55; peak 11 = approx 2.05; peak 12 = approx. 2.10.

System compliance:
- *signal-to-noise ratio*: at least 4.0 for the peak due to monoammonium glycyrrhizate in the chromatogram obtained with control solution (d).

Plot a calibration curve, putting on the horizontal axis the decimal logarithm of the concentration (in milligrams per millilitre) of control solutions (a), (b), (c), and (d) (adjusted for the assigned percentage of monoammonium glycyrrhizate content of *Actaea racemosa for dosage SCR*) and on the vertical axis the decimal logarithm of the corresponding peak area.

1 Zorbax Eclipse XDB-C18 Agilent reference 990967-902 is suitable.

calculate the content (%) of each peak using the following expression:

$$\frac{10^{A} \times 2,5}{m} \qquad 10^{A} \text{ x } 2.5/m$$

A = decimal logarithm of the concentration of each peak in the chromatogram obtained with the solution to be examined, determined from the calibration curve;

m = mass of the plant drug to be examined used to prepare the solution to be examined, in grams.

Calculate the content (%) of triterpenic heterosides by calculating the sum of the contents (%) corresponding to peaks 1 to 12.

STRAIN

DEFINITION

The mother tincture of Actaea racemosa is prepared with an ethanol content of 65*V/V* from the dried subterranean organs of *Cimicifuga racemosa* (L.) Nutt.,

---

~~*(1038)* and the supplementary clarification of the French Pharmacopoeia Authority).~~

Content: at least 0.080% triterpenic heterosides expressed as monoammonium glycyrrhizate ($C_{42}H_{65}NO_{16}$; M_r 840).

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

PRODUCTION

Method 1.1.10 (*2371*)

FEATURES

Appearance: yellow amber-coloured liquid.

Odour: faintly reminiscent of liquorice.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*) according to the indications of identification test C of the drug with the following modification.

Solution to be examined. Mother tincture to be examined.

Results: see identification C of the drug.

TEST

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

Dry residue (*2.8.16*): minimum 0.80% *m/m.*

DOSAGE

Liquid chromatography (*2.2.29*) according to the dosage indications for the drug with the following modification.

Solution to be examined. Put 8.000 g of the mother tincture of Actaea racemosa into a 100 ml flask and evaporate to dryness. Take the residue in 10.0 ml increments, with a mixture of equal volumes of *water R* and *methanol R*. Filter through a membrane filter (nominal pore diameter 0.45 µm).

calculate the content (%) of each peak using the following expression:

$$\frac{10^{A}}{m}$$

A = decimal logarithm of the concentration of each peak in the chromatogram obtained with the solution to be examined, determined from the calibration curve;

m = mass of the mother tincture to be examined used to prepare the solution to be examined, in grams.

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

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