CRUDE DRUGS CONTAINING SAPONINS

1. MACROMORPHOLOGICAL EVALUATION

Saponariae albae radix
Primulae radix
Liquiritiae radix
Hippocastani folium
Hippocastani semen
Ginseng radix
Sarsaparillae radix
Hederae folium

2. MICROSCOPIC EVALUATION

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	Primulae radix
	Liquiritiae radix
	Ginseng radix
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(Saponariae albae radix, Primulae radix, Liquiritiae radix, Hippocastani folium, Hippocastani semen)
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(Saponariae albae radix, Primulae radix, Liquiritiae radix, Hederae folium)
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1. MACROMORPHOLOGICAL EVALUATION

Saponariae albae radix
Gipsophila paniculata L.

Soap root
Caryophyllaceae

Ph.Hg.VII.

Consisting of the root and the short rhizome, the entire drug may be as thick as 4 to 8 cm and as long as 40 to 80 cm. White-coloured outside, sometimes twisted. Inside light yellow, radially cracked. The concentrically enclosed xylem is separated by brown cambium from the white bark. The pieces covered with cork are yellowish brown outside, with many, transversally elongated protuberances united almost to a transversal ring.
Store the drug in the pharmacy cut into oblique disces of 2 to 5 mm width or into small pieces.

Primulae radix
Primula vulgaris Huds.
Primula veris L. ssp. *inflata* (Lehm.) Domin

Primrose root
Primulaceae

Ph.Hg.VII.

The short rhizome in 8 to 10 cm long, 4 to 5 mm thick, straight or curved, light brown or brownish red, with cicatrices on the surface which are traces of the leaves and the scape. Filiform cylindrical roots grow out in large number thickly all around from the rhizome. Roots light yellow or brownish, 8 to 10 cm long, l mm thick, finely striated lengthwise, standing out mostly in the same direction from the rhizome, very fragile. White inside.

Liquiritiae rhizoma
Glycyrrhiza glabra L.
Glycyrrhiza glabra ssp. glandulifera
Glycyrrhiza uralensis

Ph.HgVIII., Ph.Eur.

Liquorice root
Fabaceae

Liquorice consists of the cried unpeeled roots and stolons of *Glycyrrhiza glabra* L.
The plants yielding most of the commercial drug are: *Glycyrrhiza glabra* L.varia *typica* Reg. et Herd., a plant about 1.5 m high bearing typical papilionacaeous flowers of a purplish-blue colour. Its fruit is hairless. The underground portion consists of long roots and thin rhizomes or stolons. This plant is grown in Spain, Italy, England, France, Germany, Hungary and the USA. They are sold as "Spanish" liquorice.
G.glabra L. var. *glandulifera* Wald et Kit. is abundant in the wild state in Galicia and central and southern Russia. ("Russian" liqu. Its fruit is glandous. The underground portion consists of a large rootstock, which bears numerous long roots but no stolons.
Description and macroscopic characters Typical "Spanish" liquorice occurs in straight pieces from 5 to 20 mm in diameter. If unpeeled, they have a dark, reddish-brown cork, and the runners, which are more numerous than the roots, bear buds. The peeled drug has a yellow, fibrous exterior. Fracture-fibrous; odour-faint, but characteristic; taste-sweet and almost free from bitterness.

Hippocastani folium
Aesculus hippocastanum L.

Horse-chestnut leaf
Hippocastanaceae

A large deciduous tree with a finely scaly, grey-brown bark. The leaves are opposite, palmate with 5-7 leaflets; these are obovate-lanceolate, up to 22 cm long, tapering to a wedge-shapedbase, strongly ribbbed; the petioles are long; the leaf-buds rather sticky.
The drug has no odor, taste is bitterish acrid.

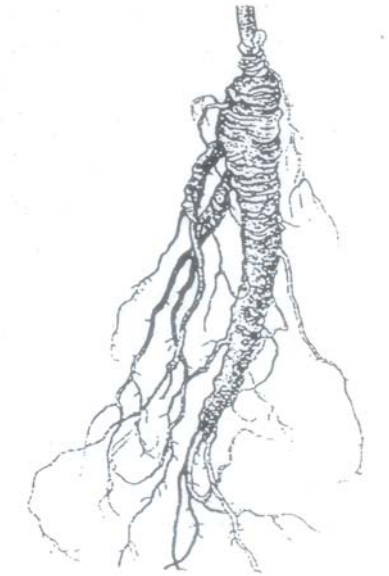
Hippocastani semen
Aesculus hippocastanum L.

Horse-chestnut seed
Hippocastanaceae

The fruit is a leathery prickly capsule. The seeds number 1 to 2. large, nut-like, shining brown, with large whitish scar. They are odorless, their taste is sharp.

Ginseng radix
Panax ginseng C.A. Meyer
Ph.Hg. VIII., Ph. Eur.

Ginseng root
Araliaceae



The drug consists of the root ot fhe perennial plant. The plant evolves very slowly, it brings flower only in the 4-5 th year.
The plant is indigenous in East-Asien and Tibet, it is cultivated in Russian and Bulgaria.
The plant is 60 cm in hight and has characteristic complex leaves. Two varieties are known: the yellow and red roots. From the yellow root the cork is removed before the drying (on sun), the red root is put into hot water before drying. The yellow root is the more valuable.
The root is in shape human formig therefore it is named as the "root of life" - it means the immortality.
The grown root is 8-20 cm long and 2 cm thick. Surface is longitudinally wrinkled. At the upper part of root cicatries are visible, the remains of petioles and leaves of shoot from the previous year.
Odour - mild aromatic; in closed pot – very strong, characteristic. Taste - flavouring, first bitter then sweet. fracture - white, mealy.

Sarsaparillae radix
Smilax species

Sarsaparilla root
Liliaceae

The crude drug consists of roots of the tropic plants Smilax species. The roots are washed and dried by sun or fire and put in budles.
The roots are 1-2 m in length and 3-5 mm in diameter, greyish brown coloured, longitudinally wrinkled and inside yellowish white.
Odourless, taste - mucilaginous.

Hederae folium
Hedera helix L.
Ph.Hg. VIII., Ph. Eur.

Hedera leaf Yery
Araliaceae

A woody climbing plant, often creeping and covering large areas on the ground, or climbing up to over 25 m. The leaves are simple, dark green and glossy of 2 kinds: those on flowering shoots entire, ovate to diamond-shaped; the others palmately 3-5 lobed, up to 10 cm long, the lobes triangular.
Odourless taste is bitterish, sharp.

2. MICROSCOPIC EVALUATION

Cross section of Saponariae albae radix

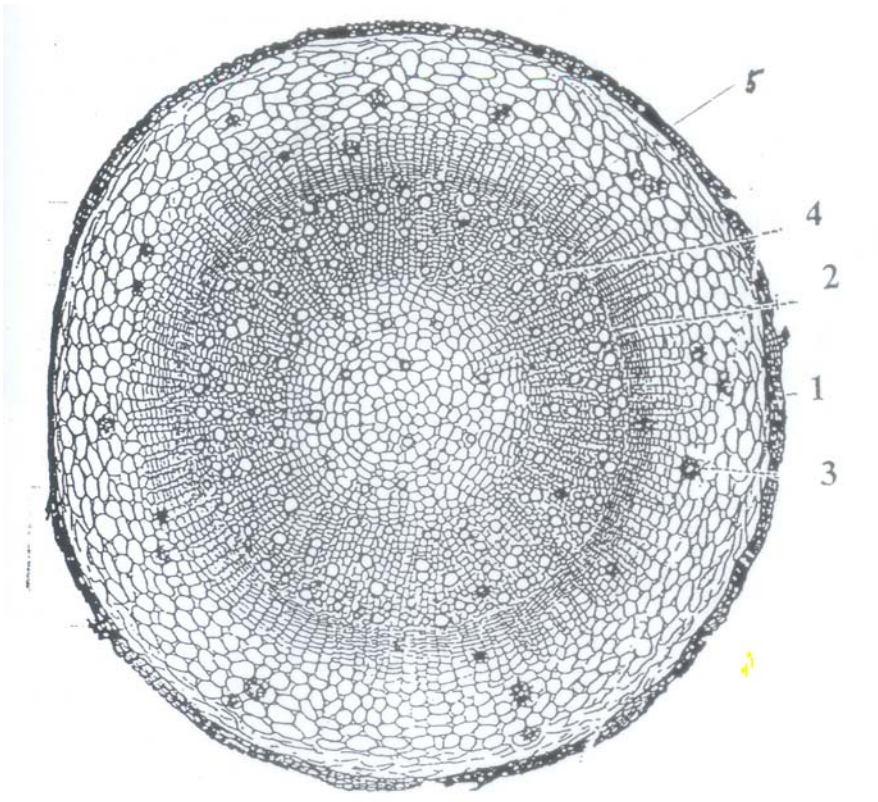
The Soap root is a secondary thickened one below of the periderm the cortex is foundable, which is rich in $Ca(C00)_2$ rosettes.
The medullary rays of phloem and xylem contains $Ca(C00)_2$ rosettes. In the phloem part we find only "soft" phloem constituents. The cambium consists from several cell-lines. The xylem contains trachea, tracheide and parenchymatoes tissue. Xylem fibres are only in the elder roots.

Powder of Saponariae albae radix

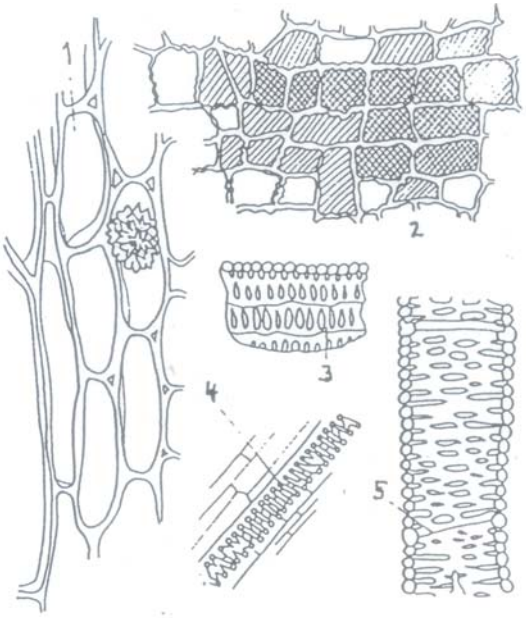
The powder can be characterized by the $Ca(C00)_2$ rosettes and tracheas of pitted, spiral walls as well as oblique perforated transversal walls.

In the case of unpealed drug we can also see the part of cork.

Cross section of Saponariae albae radix



1 = periderm
2 = phloem body
3 = $Ca(C00)_2$ rosettes
4 = wood body
5 = cambium



Powder of Saponariae albae radix

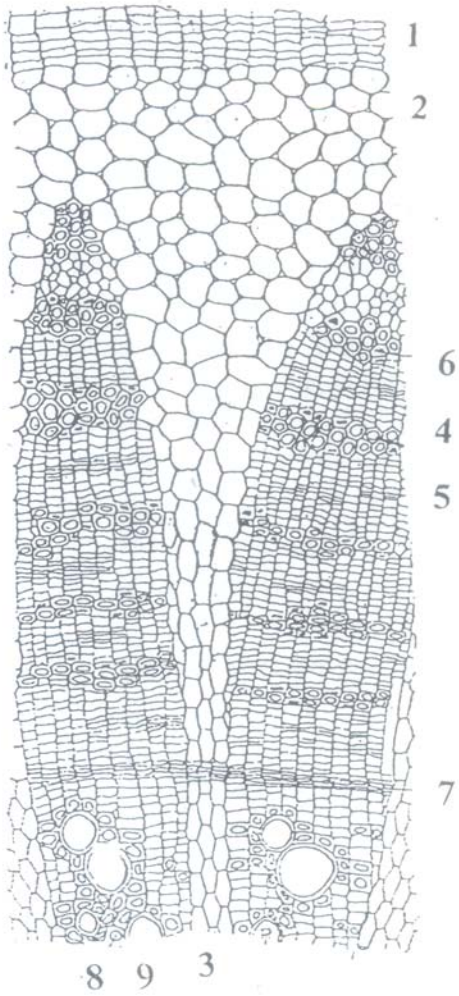
1 = Cortex-parenchym with cells containing Ca oxalate rosettes
2 = cork in surface view
3 = trachea of pitted thickened wall
4 = trachea of spiral thickened wall
5= trachea of oblique perforated transversal walls

Cross section of Liquiritiae radix

Below the periderm is the cortex-parenchym (dilatation zone). Then we find the phloem wood which is devided by phloem and pit medullary rays.
The parenchym-cells of the phloem wood contain starch. The phloem wood consists from hard and soft parts. The sieve tubulars of the soft-phloem are function-able, close to the cambium but far from cambium we find non-functional sieve tissue (keratenchym).
The hard phloem consists from sclerenchymatous bast fibre bundles and locular fibres containing single Ca oxalate crystals.
The cambium is building from several cell-lines. The wood part is devided by pit and xylem medullary rays. In the xylem medillary rays tracheides, tracheas, xylem fibres and locular fibres are findable.

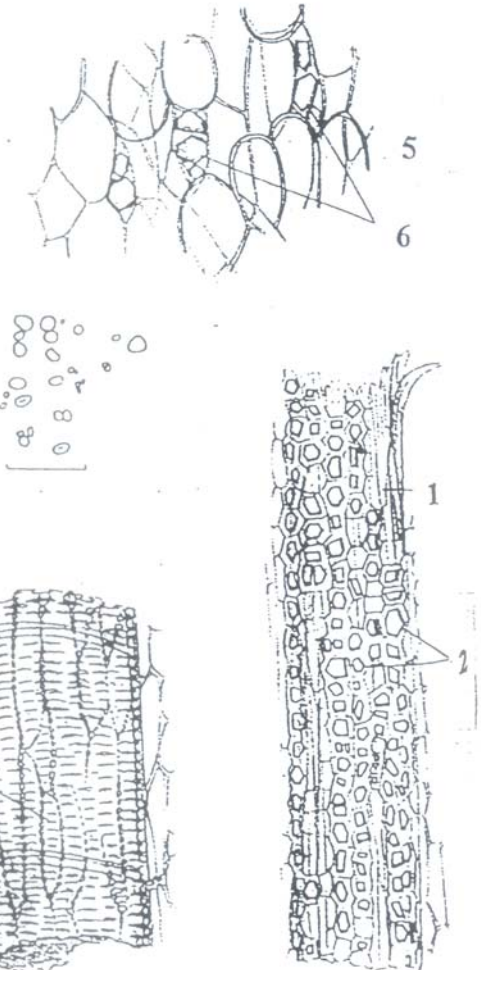
Powder of Liquiritiae radix

The powder is characterized by sclerenchymatous phloem fibre bundles and locular fibres containing simple Ca oxalate crystals.
The parenchym fragiles contain starches. At the unpeeled drug we find also cork-part.



Cross section of Liquiritiae radix

1 = periderm
2 = outer cortex
3 = pit-medullary ray
4 = phloem fibre
5 = phloem parenchym
6 = locular fibre
7 = cambium
8 = xylem fibre
9 = trachea

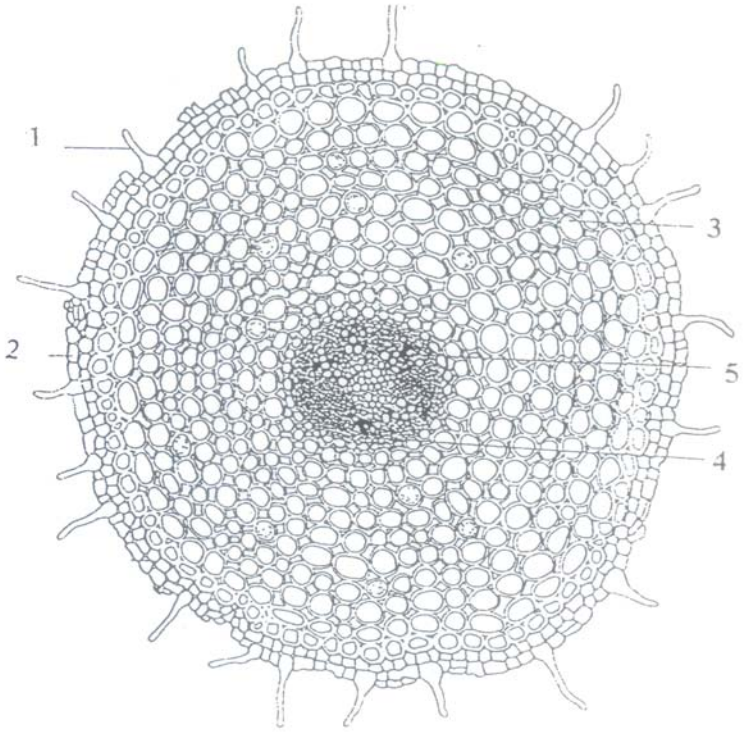


Powder of Liquiritiae radix

1 = phloem fibre
2 = locular fibres
3 = starch
4 = trachea of pittily thikened wall
5 = cortex parenchym
6 = simple Ca $(C00)_2$ crystals

Cross section of Primulae radix

A part of the cells of rhyzoderm transformed to root-hairs.
Inside of rhizoderm we find the wide primery cortex-parenchim. It consists from 15-20 cell-layers, with pitted-thickened wall and full of starch.
The central cylinder is devidid from cortex-parenchym by endodermis. The endodermis consists of thin walled cells with radial walls thickened by Caspary-dotes.
The central clinder contains the xylem and phloem boundles which give xylem, phloem rings if the the root is elder.

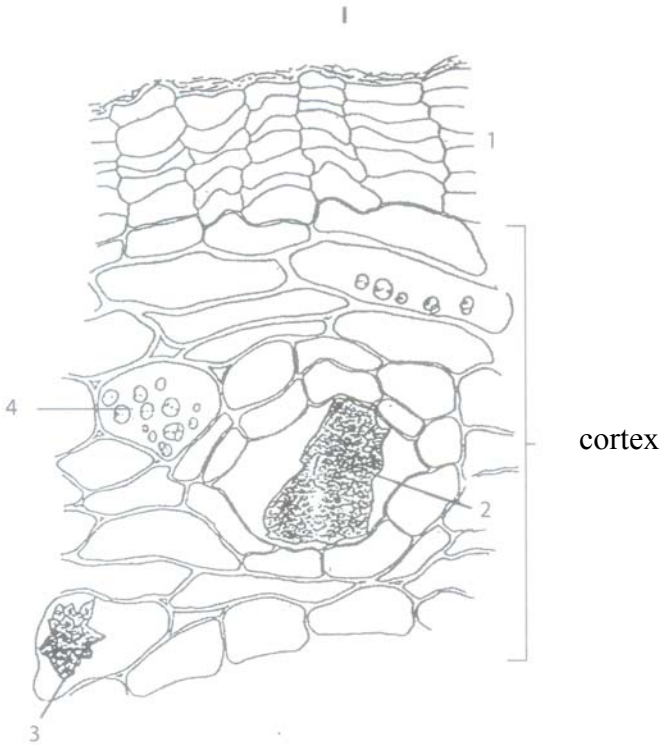


Cross section of Primulae radix

1 = root-hair
2 = rhizoderm
3 = cortex parenchym
4 = endoderm
5 = xylem vessels

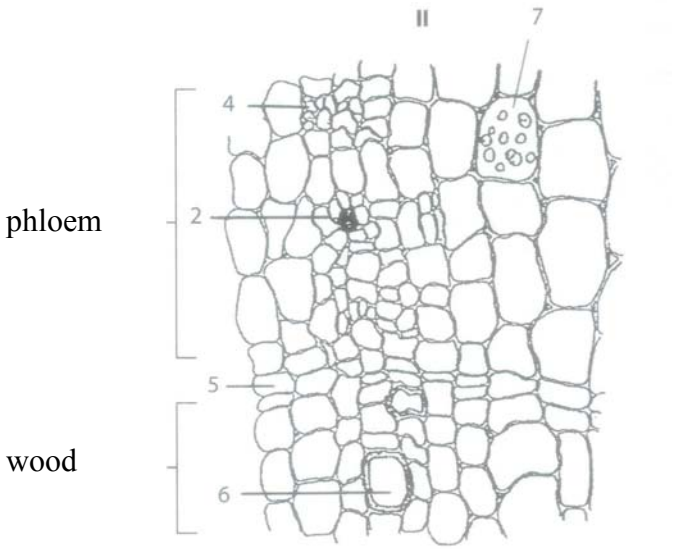
Cross section of *Ginseng radix*

Below of the periderm the cortex is foundable, which consists from the external dilatation zone (cortex-parenchym) and the phloem-wood, which is separated from the xylem-wood by the cambium. The xylem-wood is devided by xylem and pitty medullary rays.



Cross section of *Ginseng radix*

I. Periderm and cortex-part
1 = periderm
2 = schizogene oil cell system and
3 = calcium oxalate clube-shaped crystals (rosettes)
4 = starches in parenchima



II. Phloem- and wood - parts
2 = oil holder
4 = sive-tube group bark in internal bark
5 = cambium
6 = trachea in xylem body
7. = starches in parenchyma

3. PHISICOCHEMICAL AND CHEMICAL QUALITATIVE INVESTIGATIONS

3.1. Detection of saponins (triterpenes, sterins) by LiebErmann-Bourchard reation.

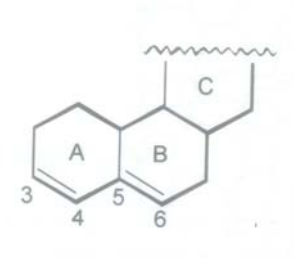
Samples

- Saponariae albae radix
- Primulae radix
- Liquiritiae radix
- Hederae folium
- Hippocastani folium
- Hippocastani semen

Agitate 1 g of powdered drug (IV) with 10 ml of chloroform for 2 minutes, filter through a filter paper and evaporate the filtrate in a porcelain dish to dryness. Dissolve the residue in 1 ml of glacial acetic acid and transfer cautiously over
5 ml of cc. sulphuric acid in a test tube.
The interface of both liquids becomes brown or reddish brown; the colour of the glacial acetic acid layer in various influenced by the drug samples.

Essence of reaction

A conjugated double boundle system is formed by the loose of water molecule from the secundary alcohol group sitting in the third position of the triterpene ring-system.



3.2. Foaming test

Samples

Saponariae albae radix

Shake the powdered drug with water (1 + 10); the liquid foams strougly and the foam is lasting.

3.3 TLC detection of saponins

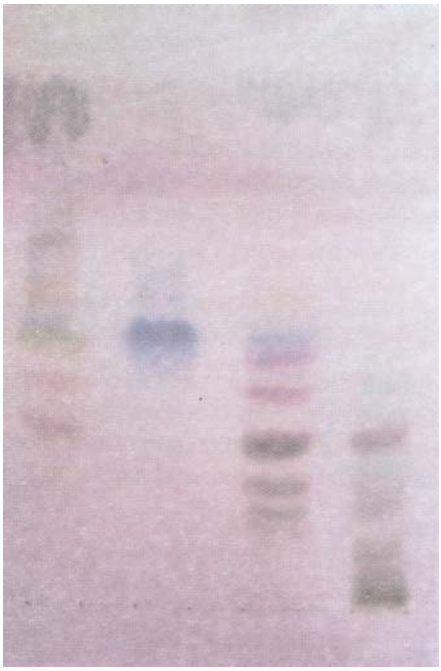
Prepare of samples

Boil 1 g of crude drug powder with 20 ml of 70% ethanol on water bath for 20 minutes.
Filter and evaporate the extract to 4,0 ml; 5µl of the solution is investigated by TLC, beside saponin reference solution (0,1% 10µl).

Chromatographic condition

Adsorbent: Kieselgel 60 F_{254}
Developing system: $CHCl_3$
Reagent for spray: sulphur acidic anise aldehyde reagent.
Warm the layer for 10 minutes at 100°C

TLC investigation of saponins



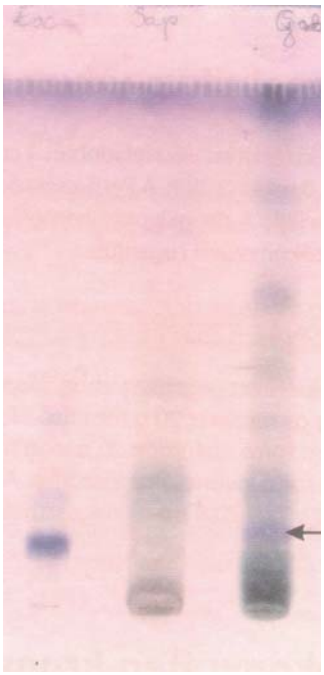
1. 2. 3. 4.

1. Hederae folium	10 µl
2. Aescin standard 2%	10 µl
3. Hippocastani semen	5 µl
4. Saponariae albae radix	10 µl

Chromatographic parameters:

Adsorbent:	Kieselgel G60 F_{254}
Developing system:	n-butanol-acetic acid-H_2O (3:1:1)
Dying reagent:	sulphuric acidic anis-aldehyde
	spry and warm it at 100°C for 5 minutes
Evaluation:	visually

TLC investigation of saponins in Ginseng radix
(According to prescribtion of Ph.Hg. VIII. and Ph. Eur.)



← **Ginsenosid Rb1**

1. **2.** **3.**

1. Aescin standard 2%	5 µl
2. Saponin 0,1% (Fluka)	10 µl
3. Ginseng radix extract	10 µl

Chromatographic parameters:

Adsorbent:	Kieselgel G60 F_{254}
Developing system:	ethyl acetate- H_2O -1-butanol (25:50:100 V/V)
	use the upper phase!
Dying reagent:	sulphuric acidic anis-aldehyde
	spray and warm it at 100°C for 5 minutes
Evaluation:	visually

4. QUANTITATIVE EVALUATIONS

4.1. Determination of foam number

Samples

Saponariae albae radix
Liquiritiae radix
Hederae folium

Definiation

The biggest dilution of extract (relative to 1 g of the crude drug) which gives 1 cm high foam if its 10 ml is shaked for 15 sec in test tube of 16 mm.

Description of determination

Warm 0.25 g of crude drug + 100 ml of water (30 min on water bath, filter, completeto 100 ml with water) Basis solution.

1. test tube	**2. tube**	**3. tube**	**4. tube**	**5. tube**	
0.4 ml	1.0 ml	2.0 ml	4.0 ml	8.0 ml	of stock solution
9.6 ml	9.0 ml	8.0 ml	6.0 ml	2.0 ml	of water

Shake the tubes for 15 sec, leave to stand 15 min!
Search the dilution, where the thickness of the foam is 1.0 cm!

Calculation

$$F = \frac{10}{p}$$

F = foam number of crude drug
p = is the amount (g) of crude drug in the test tube.
in wich the foam 1 cm thick

4.2. Determination of haemolytic index (Ph.Hg.VII.)

Reagents

Buffer solution (pH = 7.4). In a volumetric flask, dissolve 1.743 g of potassium hydrogen-phosphate (KH_2PO_4), 9.596 g of disodium hydrogen-phosphate ($Na_2HPO4 . 2H_2O$) and 9.00 g of sodium chloride to 1000 ml in water.
2 per cent blood suspension. Agitate in a widemouthed glass-stoppered flask (containing a few glass-pearls) fresh bovine blood until the separation of fibrin is completed. Filter the blood then through a double layer of gauze in a vessel placed into a 200 water bath, then dilute a 10.00 ml portion in a volumetric flask with 200 buffer solution to 500 ml. Prepare blood suspension freshly on the day of the test.
R-saponin solution, 0.02 per cent. Dissolve 0.0200 g of saponin in a volumetric flask with the buffer solution to 100 ml. Prepare the solution freshly on the day of the test.

Preparation of Extract

Wash the powdered (IV) and air-dry drug in a quantity specified by the different monographs, and weigh accurately into a 250 ml flask with 100 g of the buffer solution. Heat the content of the flask covered by a small glass funnel on hot water bath for 30 minutes, while the content of the flask is agitated four times by concentric swinging. Filter the hot extract into a 100 ml volumetric flask through a 0.15 g piece of cotton plug, placed between two layers of gauze (about 7x7 cm).

Informative Test of Extract

Transfer 0.2, 0.4, 0.6, 0.8, 1.00 and 1.2 ml of drug extract into 6 test tubes, make up the content of the test tubes with buffer solution to 5.0 ml, and add 5 ml of blood suspension to every test tube:

Test tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Drug extract (ml)	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Buffer solution (ml)	4.8	4.6	4.4	4.2	4.0	3.8	3.6
Blood suspension (ml)	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Seal the mouth of the test tube with the dry fingertip, washed in soap and water and dried with alcohol, homogenize its content by turning the mouth of the test tube downwards. Strong agitation should be avoided in order to prevent foaming. Observe the results of the informative test in 2 hours. The limit is indicated by the test tube the content of which is still transparent, but that of the next one in the order of decreasing drug amount is already opalescent.

Determination of Apparent Hemolytic Index (hi)

Since, after the informative test of 2 hours, it may be expected that a hemolysis may be produced also by an amount of drug extract inferior to that determined as the limit, prepare a series of 12 test tubes of the same drug extract. The 12th member of the test tube series will be the limit dilution of the informative test thus transfer a gradually decreasing quantity of the extract into the following tubes each dose being inferior to the previous one by 0.05 ml. (The first test tube will therefore contain 0.55 ml less drug extract than the 12th.) Make up the content of each test tube with buffer solution to 5.0 ml and then mix with 5.0 ml of blood suspension. Continue the test in the way described at the informative test with the difference that the limit dilution (h) should be determined in 6 hours.
Calculate the apparent hemolytic index (hi) of the drug from the value H with the help of the following formula:

$$hi = \frac{10}{p}$$

where p = drug content of the extract filled into the test tube, expressed in ction of free cinnamic acid
*
In order to obtain the hemolytic index (HI) of the drug, the apparent hemolytic index (hi) must be multiplied with the factor (F) of the blood employed

$$HI = hi \times F$$

Determine the blood factor (F) as follows. In the way described for the drug extract prepare 0.02 per cent R-saponin solution with the buffer solution and dispense into 7 test tubes, starting from 1 ml and increasing the doses by 0.5 ml, and complete with buffer solution to 5.0 ml; dispense finally 5.0 mg (related to the drug dried at 1000).
In order to obtain the hemolytic index (HI) of the drug, the apparent hemolytic index (hi) must be multiplied with the factor (F) of the blood employed

$$HI = hi \times F$$

Determine the blood factor (F) as follows. In the way described for the drug extract prepare 0.02 per cent R-saponin solution with the buffer solution and dispense into 7 test tubes, starting from 1 ml and increasing the doses by 0.5 ml, and complete with buffer solution to 5.0 ml; dispense finally 5.0 ml of blood suspension into each test tube. Perform the informative test and the subsequent detailed test in the way described for the drug extract. The apparent hemolytic index (hi_s) of R-saponin is thus established. The blood factor can be obtained by dividing the hemolytical index of standard saponin (25 000) with the apparent hemolytic index (hi_s)

$$F = \frac{25.000}{hi_s}$$

Ginseng radix-Examine by liquid chromatography (Ph. Eur. 6.)

Test solution. Reduce about 50 g to a powder. Place 1.00 g of the powdered drug and 70 ml of a 50 per cent *V/V* solution of *methanol* in a 250 ml round-bottomed flask. After adding a few grains of pumice, boil on a wather-bath under a reflux condenser for 1 h. After cooling, centrifuge and collect the supernatant liquid. Treat the residue as described above. Mix the collected liquids and evaporate to dryness under reduced pressure at a temperature not exceeding 60°C. Tace up the residue with 20.0 ml of acetonitrile (20 per cent *V/V*) and water (80 per cent *V/V*). Dilute 2.0 ml of the solution to 10.0 ml.

Reference solution. Dissolve 3.0 mg of *ginsenoside* Rgl 3.0 mg *ginsenoside R*e, 3.0 mg of *ginsenoside Rf* and 3.0 mg *of ginsenoside Rbl* in 10 ml methanol.

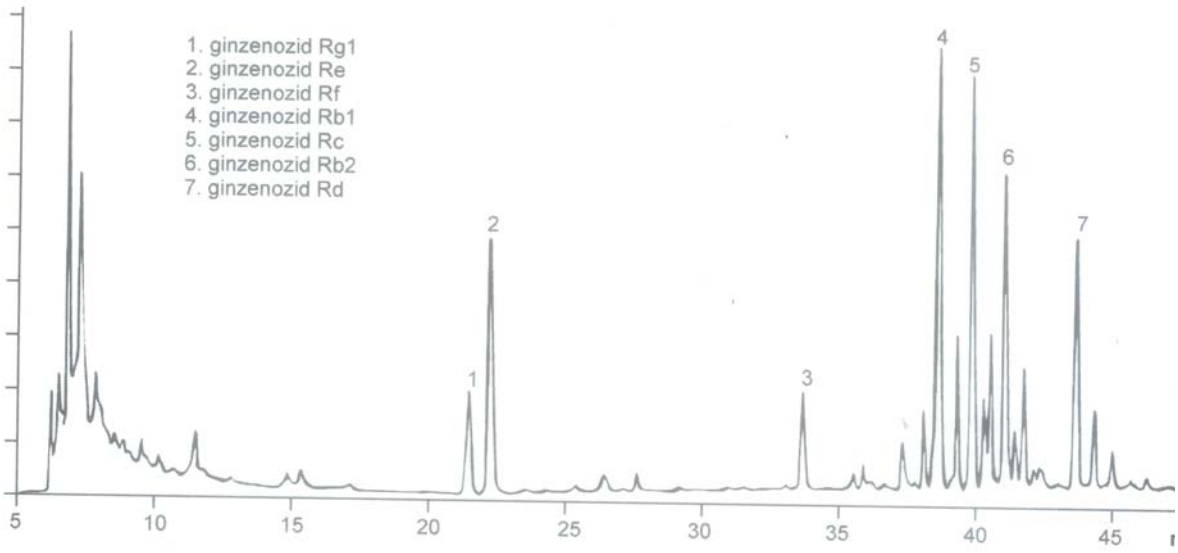
Column:
size: *l* = 0,125 m, Ǿ = 4,6 mm,
stationary phase: *octadecylsilyl silica gel for chromatography* (5 µm),
temperature: 25°C

Mobile phase:
Mobile phase A: *Water*, pH set to 2 with H_3PO_4
Mobile phase B: *Acetonitrile*

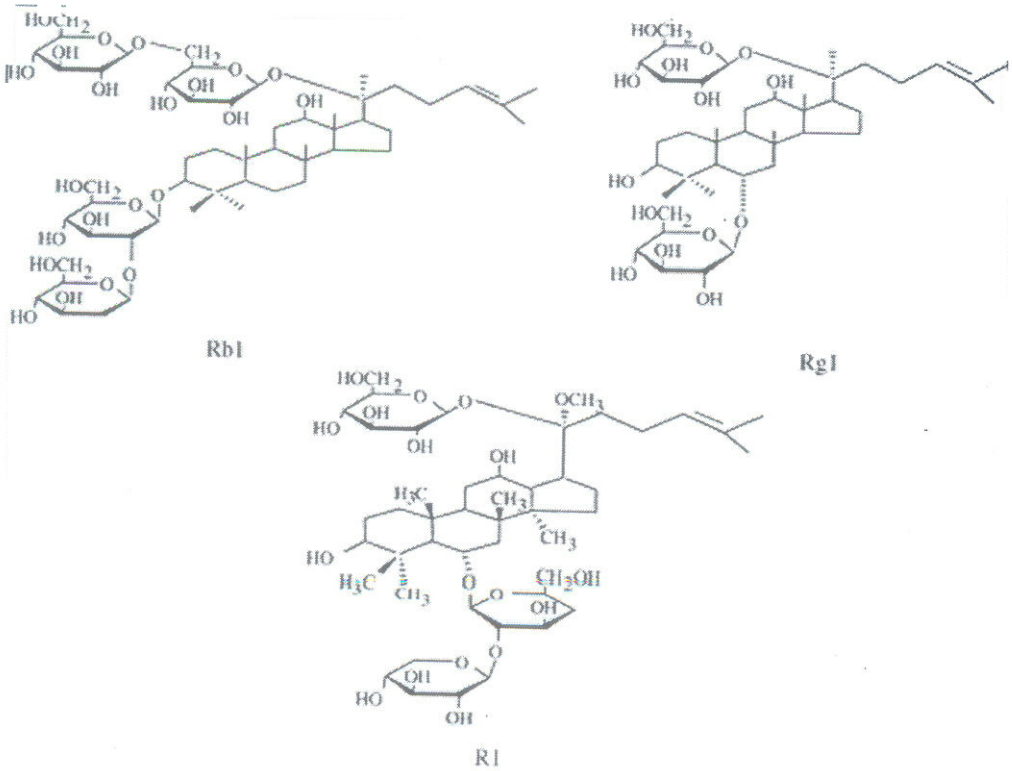
Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0-8	80	20
8-40	80 → 60	20 → 40
40-45	60 → 40	40 → 60
45-47	40 → 0	60 → 100
47-52	0	100
52-55	0 → 80	100 → 20

Flow rate: 1.0ml/min.
Detector: Spectrophotometer at 203 nm.
Equilibration time: 20 min.
Injection: 20µl.
Elution order: As given above in *Reference solution*. Record the retention times of compounds.
Resolution: Minimum 1.0 between the peaks due to *ginsenoside Rgl* and to *ginsenoside Re.*

Identify the peaks due to *ginsenoside Rbl* (4) and *ginsenoside Rgl* (1) on the chromatogram of the test solution.



HPLC Chromatogram of a *Ginseng radix* test solution



Results in report

- Result of test tube reactions, summerized in table
- Haemolytic index
- TLC of saponins
- Foam number