CRUDE DRUGS CONTAINING ORGANIC AND INORGANIC PLANT ACIDS AND THEIR D ERIVATIVES

1. MACROMORPHOLOGICAL EVALUATION

Capsici fructus - paprika, red Hungarian pepper
Rosae pseudo-fructus - dog rose /rose hip
Hibisci sabdariffae flos - roselle
Equiseti herba - can-washing grass, horse tail
Pulmonariae folium - lung-wort
Polygoni avicularis herba - knotgrass

2. MICROSCOPIC EVALUATION

Cross section: Pericarp of *Rosae pseudo-fructus*
Pericarp of *Capsici fructus*

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1. MACROMORPHOLOGICAL EVALUATION

Capsici fructus
Capsicum annuum L. var. *minimum* Paprika fruit
Capsicum frutescens L. – chili - Solanaceae

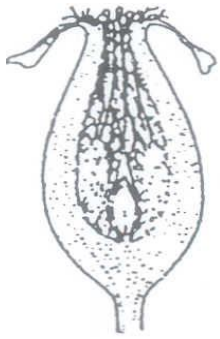
Ph.Hg.VIII., Ph.Eur.

The crude drug is formed by the red riped fruit of *Capsicum annuum* L. var. *minimum* or *C. frutescens.* The fruit is a more or less inflated berry. It is formed from 2 or 3 carpels. Inside it is hollow (cavernous), the placentae and the dividing walls are white and have spongy consistance. The seeds sit on the dividing walls and placentae. Themselves the seeds are not pungent. The wall of the fruit (the pericarp) has a bright red colour, inside it is full of blisters (anatomical: giant cells) which secrate the capsaicin - the pungent substance

The fruit is 6-12 cm in length, 4-6 cm in diameter. It has a bend stalk (pedicle) and sit in a calix-ring of 5 teeth. Before grinding the stem and stalk are removed, but the seeds are grinded into the powder to get red colour. (Carotenoids are lipophil and soluble in the fatty oil of seeds.)
The drug has a characteristically spicy taste and causes a charp burning sensation on the tongoue.

Rosae pseudo-fructus Dog rose, Rose hip
Rosa canina L. Rosaceae

Ph.Hg.VIII., Ph.Eur.



Hip is a false fructus. It means that the fruit is formed not only from pistil (=carpel), but also the receptacle.
The hip is bright red and its wall is wrinkled. On its top sepal (=calyx) - leaves are visible, and on its other end the rest of pedicle can be found.
Crossing it in half, the "seeds", the real fruits, the achenes are found, their wall is hard. Further we see the rigid bristle hairs covering the false fruit from inside.

Dog rose *(Cynosbati pseudofructus sine seminibus)* consists of the rose hips made up by the succulent (fleshy) receptacle and the remains of the dried sepals, with the achenes removed („sine seminibus”).

It is 1-2 cm in length and l cm in wide. It consists of fragments of the fleshy, hollow, urceolate receptacle, bearing the remains of the reduced sepals, light pink to orange-pink, the convex outer surface shiny and strongly wrinkled; bearing on its lighter inner surface abundant bristle-like hairs

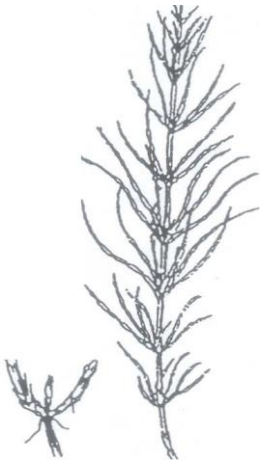
Hibisci sabdariffae flos Roselle
Hibiscus sabdariffa L. *Rosaceae*

Ph.Hg.VIII., Ph.Eur.

The calyx is joined in the lower half to form an urceolate structure, the upper half dividing to form 5 long, acuminate recurved tips. The tips have a prominent; slightly protruding midrib and a large, thick nectary gland about 1 mm in diameter. The epicalyx consist of 8 to 12 small, obovate leaflets which are adnate to the base of the calyx. The calyx and epicalyx are fleshy, dry easily fragmented and coloured bright-red to deep-purple, somewhat lighter at the base of the inner side.

Equiseti herba Common horsetail
Equisetum arvense L. Equisetaceae

Ph.Hg.VIII., Ph.Eur.



The crude drug is formed by the sterile airial shoots of the plant. It is branched from verticils. It is 10-50 cm in lenght and 0,5 cm in thickness. The stem-members are tubular, the knots are compact. The surface of the shoots is ribbed. They are green or greenishgray.
The stemknots are rounded by leaves-cod and on the top of the cod brown teeth are seen. The number of the ribs and teeth are the same.. The crude drug is odorless, having an initating soapy taste.
It can not be adulterated by *E. palustre* respectively to its toxicaI alkaloid content.

Pulmonariae herba Lung-wort

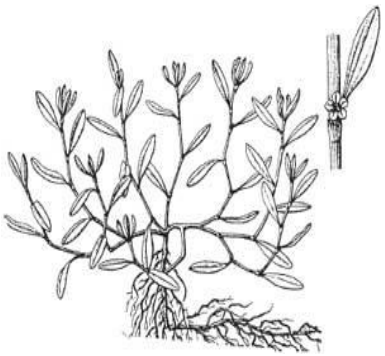
Pulmonaria officinalis L. Boraginaceae

Pulmonaria officinalis (Boraginaceae) has perrenial rhizome. The radical leaves are heart-shaped, white spotted, with long stalk. They are covered by bristle hairs. Its purple funnuliformed flowers stand in a cyme or in curls.
In can be mistaken with *P. molissima*, it is covered by glandular hairs, and adulterated by *Symphytum officinale*.

Polygoni avicularis herba Knotgrass

Polygonum aviculare L. Polygonaceae

Ph.Hg.VIII., Ph.Eur.

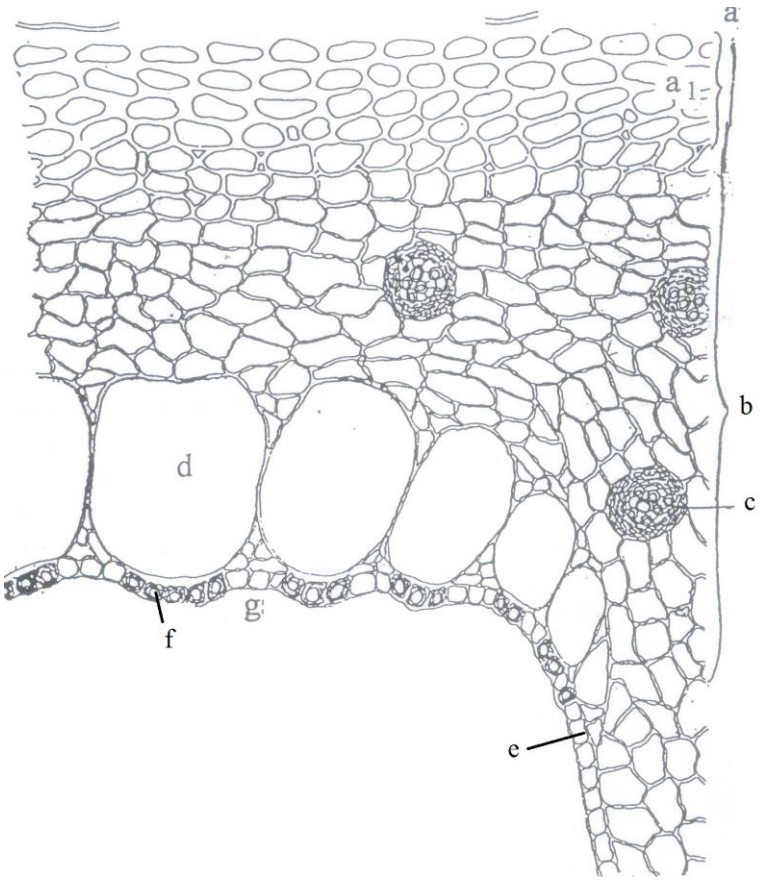


The stem is 0.5 mm to 2 mm thick, branched, with nodes, cylindrical or slightly angular and longitudinally striated. It bears sessile or shortly petiolate, glabrous entire leaves, which differ widely in shape and size. The sheath-like stipules (ochrea) arc lacerate and silvery. The small axillary flowers have 5 greenish-white perianth segments, the tips of which are of ten coloured red. The fruits are 2 mm to 4 mm, brown to black triangular nuts, usually punctate or striate.

2. MICROSCOPICAL EVALUATION

Capsici fructus

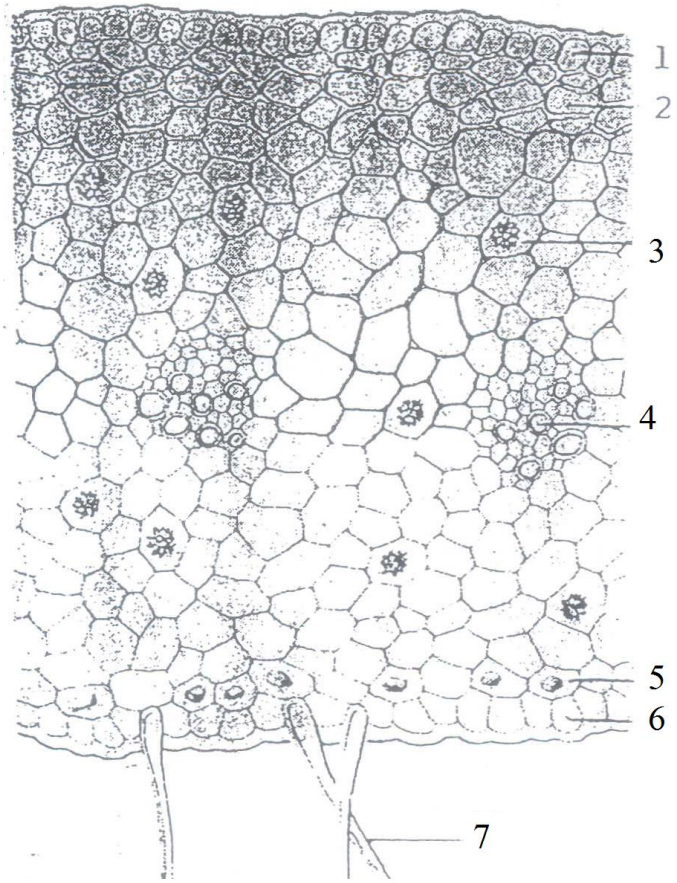
The pericarp consists of 3 parts: **exocarp**, **mesocarp** and **endocarp**. The unicelled exocarp is covered by **cuticule**. The. first part of mesocarp is the hypoderm which is followed by parenchymatous tissue and ended in giant cells. The size of them is 1-2 mm. .
The mesocarp contains little collateral closed **vascular bundles** and fixed **oildrops** and **chromoplasts** in its paremchymatous cells. The endocarp consists of **sclereid** and parenchymatous cells. The cells of sclereid have **pitted thickened walls** and direct touch with the giant cells, the cells of thin wall form a parenchymatous wedge among the giant cells.



a: exocarp
a1: hypoderm
b: mesocarp
c: vascular bundle
d: giant cell
e: endocarp
f: sclereid

Rosae pseudo-fructus

The external cell line of the false fruit is the epiderm covered by cuticule. Below of the epiderm we find the hypoderm which is followed by the parenchymatous tissue. Its cell contain **chromoplasts** and **$Ca(COO)_2$ cluster crystals**. Among the cells little collateral closed **vascular bundles** can be find. The parenchym cells laying in neighbourhood of inner (internal) epiderm contain single Ca oxalic crystal. The inner epiderm consists from cells having thickened wall. Some of them transformed to **bristle hairs**. The basis of the bristle hairs sinks down into the epiderm so it is similar to stonecells; the neckpart of hairs becomes thin.



1. cuticule and outer epiderm
2. hypoderm
3. Ca $(COO)_2$ crystal rosett
4. collateral vascular bundle
5. single Ca $(COO)_2$ crystal
6. inner epiderm
7. bristle hair

3. PHYSICAL-CHEMICAL AND CHEMICAL QUALITATİVE INVESTIGATIONS

3.1 Detection of carotenoids with colour reaction in paprika

Shake 0,1 g of the crude drug powder with 5 ml petroleum ether, for a few minutes.

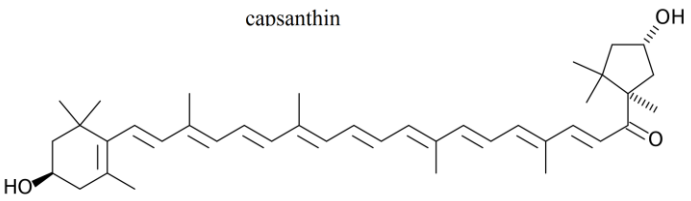
Filter the extract and evaporate the solvent on waterbath (petroleum ether is inflammable!)

The colour of the residue (carotenoids) turns blue or greyesh-blue from several drops of 80 % sulphuric acid.

Reaction way:

Extraction: the carotenoids (terpen derivatives) are apolar compounds so they are soluble in petroleum ether.

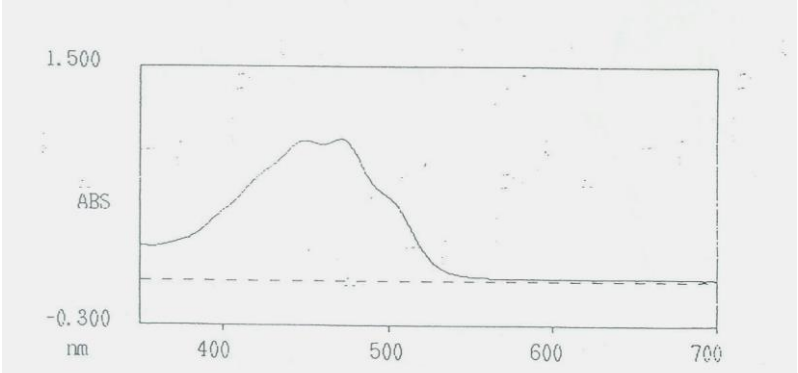
Colour reaction: The carotenoids of paprika are the capsantin and capsorubin contain keto- and hydroxyl groups; so in their structure the number of double bounds increase regarding to the water-affinity of 80 % H_2SO_4.



3.2 Spectroscopic investigation of carotenoids in paprika

Dilute 0,05 ml of the red petroleum etheric solution obtained during the determination of capsaicin content (see 4.3.) 100 times with petroleum ether (to 5 ml) and run its spectrum between 350-700 nm; against the solvent.

Mark the characteristic λ_{max} values of carotinoids in the spectrum.



Characteristic spectrum of paprika-carotenoids

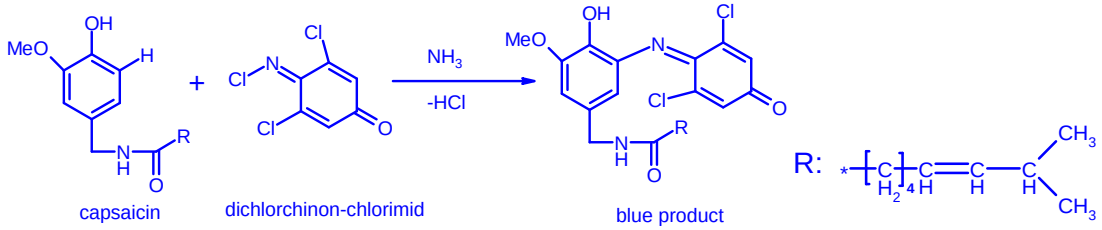
3.3 TLC investigation of capsaicin in paprika (Ph. Hg.VII)

Prepare of the sample
Shake 0,5 g of paprika-powder and 20 ml of aceton in ultrasonic bath for a few minutes. Filter it and chromatografy 40 µl of the solution beside l0 µl of capsaicin standard solution (2,5 mg/10 ml $CHCl_3$).

Chromatographic condition
Adsorbent: Kieselgel 60 F_{254} (Merck 0,2 mm)
Developing system: chloroform-methanol (95:5)
Reagent: l % of dichlorchinon-chlorimid in methanol (fresh!) then NH_3 steam,
investigate inVIS

Evaluation of chromatogram
Among more red plots the blue plot of capsaicin is visible (Rf 0,75), but the blue colour soon disappears.

Reaction mechanism



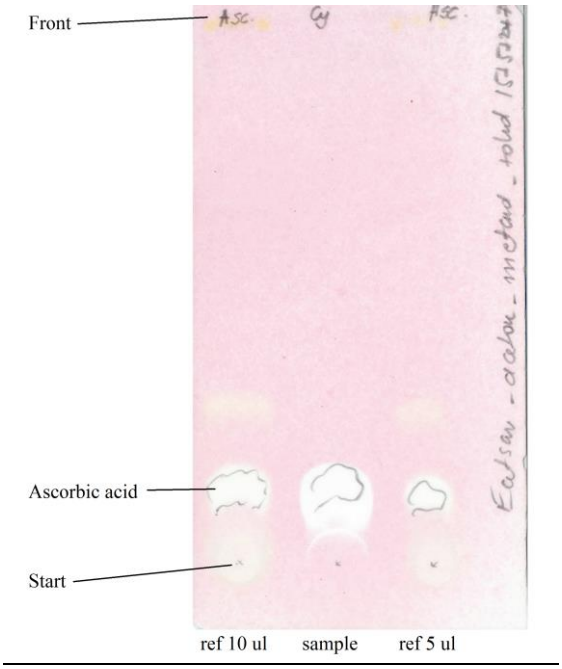
3.4 Detection of ascorbic acid in Rosae pseudo-fructus by TLC

Test solution: To 5 g of the powdered drug add 25 ml of alcohol R. Shake for 25 min and filter. 20 µl of this solution is used on layer.
Reference solution: 2 µl of standard is used (10mg of ascorbic acid in 5.0 ml of alcohol 60 %)

TLC parameters:
Absorbent: Kieselgel 60 F_{254} (0.2 mm, Merck)
Development system: acetic acid-aceton-methanol-toluene (5 : 5 : 20 : 70)
Evaluation: UV 254
Reagent: dichlorophenolindophenol-Na in alcohol (0.2 g/l)

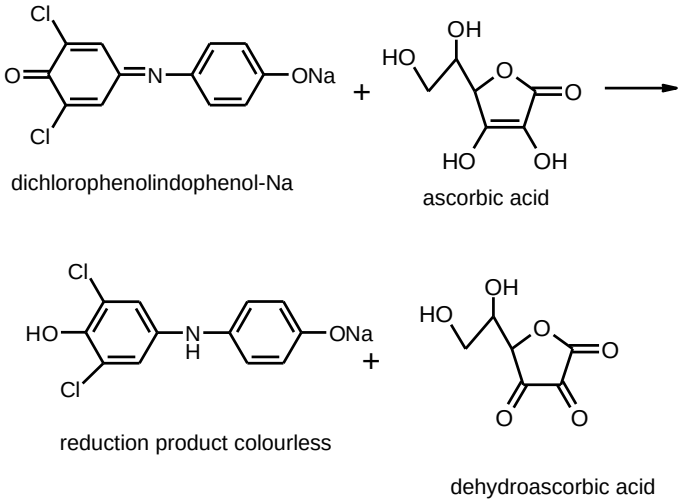
The chromatogram obtained with the solution shows a quenching zone similar in position to the principal zone in the chromatogram obtained with the reference solution. Spray with a 0.2 g/l solution of dichorophenolindophenol, sodium salt in alcohol. Examine in daylight. The

chromatogram obtained with the test solution shows a white zone on a pink background similar in position and colour to the principal zone in the chromatogram obtained with the reference solution. The chromatogram also shows an intense orange-yellow zone near the solvent front and a yellow zone in the upper third (carotenoids).



Reaction mechanism

The ascorbic acid reduces the coloured reagent, so the pink background turnes white.



4. QUANTITATIVE EVALUATIONS

4.1 Determination of ascorbic acid (vitamin-C) content (Ph.Hg.VII.)

Rosae pseudo-fructus

Principle of determination

The spectrophotometric method is carried out from the wateric extract of hip, in presence of Fe^{3+} and α-α'-dipridyl in a buffer medium. The vitamin-C reduce the Fe^{3+} to Fe^{2+}, and a Fe^{2+} dipridyl complex forms, which has red colour, and its intensity is proportional with the concentration of the complex.

Prescription

Boil 2,00 g of the crude drug powder (V) -measured with 1 mg of accuracy - with a mixture of 2,0 ml 2 M acetic acid and 60 ml water in a flask of 200 ml (1,2). Put in the neck of the flask a condenser or a funnel.

Boil the reaction mixture for five minutes (3) then let it cool and filter through cotton-wool into a volumetric flask of 200 ml. After repeated washing of the crude drug, complete the content of the volumetric flask to the signe. For the determination, use 10,0 ml of the solution (extract).

Measure into volumetric flask of 100 ml: 10,0 ml of extract, 2,00 ml of R iron /III/ ammonium-sulhpuric solution, 10,0 ml of citric acid solution (of 1 % in water), 0,40 ml α-α'–dipyridyl solution (of 1 % in 96 % ethanol) and 10,0 ml of ammonium-acetate solution (of 20 % in water). Shake the reaction mixture and keep on a dark place for 120 minutes, while the red colour is fully developing (4). Complete its volume with water and measure its absorbance in thickness of 1 cm, at 525 nm.

Reference solution is prepared like the investigation-solution with the difference that instead of dipyridyl solution water is used.

Remarks

(1) The evaluation is made from a wateric extraction of the crude drug.

(2) The acetic acid makes free up the bound ascorbic acid (salt), and gives a suitable pH.

(3) In the Pharmacopoeas, during the boiling, CO_2 gase-flow is recommended to prevent the possible (accidental) oxidative effect of the air.

(4) The iron II - forming in equivalent quantity with vitamin-C - gives a complex of red colour with α- α'-dipyridyl.

Calculation: Ascorbic acid content (m/m %) of hip

$$\% = \frac{100 \times c}{m \times 10^{6}} \times 100 \qquad m = \frac{g\ (weighted)}{20}$$

$$\% = \frac{c}{m \times 100} \qquad \% = \frac{c}{10}$$

m = weight (g) of crude drug in 100 ml solution

Simplified equation: $$\% = \frac{2{,}86 \times A}{g\ (weighted)}$$

A = abszorbance at thickness of 1 cm

4.2 Determination of ascorbic acid content by Ph.Eur.

Rosae pseudofructus

Test solution. In a round-bottomed flask, weigh 0.500 g of the freshly powdered drug (710). Add a solution of 1.0 g of oxalic acid R in 50.0 ml of methanol R. Boil under a reflux condenser for 10 min, and cool in iced water until the temperature reaches 15 °C to 20 °C. Filter. Transfer 2.0 ml of the filtrate to a 50 ml conical flask. Add successively, with gentle shaking after each addition, 2.0 ml of dichlorophenolindophenol standard solution R and then, exactly 60 s later, 0.5 ml of a 100 g/l solution of thiourea R in alcohol (50 per cent V/V) R and 0.7 ml of dinitrophenylhydrazine-sulphuric acid solution R. Heat under a reflux condenser at 50 °C for 75 min, and place immediately in iced water for 5 min. Add dropwise 5.0 ml of a mixture of 12 ml of water R and 50 ml of sulphuric acid R, taking care to carry out the addition over a period of not less than 90 s and not more than 120 s while maintaining vigorous stirring in iced water. Allow to stand for 30 min at room temperature and measure the absorbance (2.2.25) at 520 nm using solution A as compensation liquid.

Solution A. Treat 2.0 ml of the filtrate obtained during the preparation of the test solution as described but adding the dinitrophenylhydrazine-sulphuric acid solution R just before the absorbance is measured.

Reference solution. Dissolve 40.0 mg of ascorbic acid R in a freshly prepared 20 g/l solution of oxalic acid R in methanol R and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with the methanolic solution of oxalic acid. Treat 2.0 ml of the solution as described above for the filtrate obtained during the preparation of the test solution. Measure the absorbance (2.2.25) at 520 nm using solution B as compensation liquid.

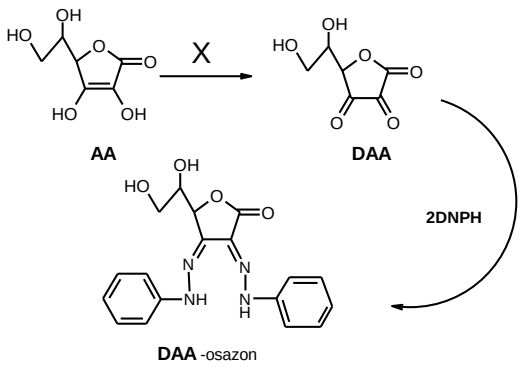
Solution B. Treat 2.0 ml of the reference solution as described above for solution A.

Calculate the percentage content of ascorbic acid from the expression:

$$\frac{2.5 \times A_1 \times m_2}{A_2 \times m_1}$$

A_1 = absorbance of the test solution,
A_2 = absorbance of the reference solution,
m_1 = mass of the substance to be examined, in grams,
m_2 = mass of ascorbic acid used, in grams.

Reaction mechanism of determination of ascorbic acid. content by Ph Eur.



Dichlorophenolindophenol reagent oxidised the ascorbic acid to dehydroascorbic acid, then the excess of reagent is reduced by tiocarbamid. DNPH forms with dehydroascorbic acid a coloured product (osazon). Its intensity is proportional with concentration of vitamin C.

4.3. Determination of capsaicin content

Principle of determination
The capsaicin content is determined by photometric method based on a condensation colour reaction. The intensity of the blue reaction product (indophenolblue) depends on the capsaicin concentration.

Extraction and purification
Measure 1 g of paprika powder - with mg accuracy - in an Erlenmeyer flask and shake in 40 ml aceton for 30 minutes (1). Filter the extract on filterpaper. After pressing and washing the extracted crude drug with 10 ml of aceton, concentrate the united acetonic solution to 1-2 ml by vacuum distillation (Rota). Wash the residue with 2 x 10 ml of aceton into shaking funnel. Add to the acetonic solution 10 ml of 0,5% NaCl solution and 10 ml of petroleum ether. Shake it for 1 minute, then separate the two phases (2). Flow the lower phase into a volumetric flask of 50 ml. Shake the upper (red) petroleum etheric phase with 10 ml ethanol (of 57 % in water) containing 0,5% of NaCl (3). Flow the lower phase again into the volumetric flask and complete the volume to the signe with aceton - *stock solution.*
(The upper petroleum etheric phase is for the spectroscopic detection of the carotenoids).

Test solution:
Measure 10,0 ml of the stock solution into volumetric flask of 25 ml and add *5,0* ml of 8,2 % (G/V) sodium acetate solution and 3,0 ml of 0,12% (m/V) methanolic 2,6-dichlorchinonchlorimid solution. Complete its volume to the signe with *water,* shake it and leave it stand for 30 minutes in dark place /4/. Determine its absorbance (A) at 620 nm in 1 cm thickness against reference solution.

Reference solution is prepared like the investigating solution but instead of 10 ml of stock solution, 10 ml of water is used.

Remarks
(1) The extraction is carried out on room temperature because the capsaicin is thermolabil.
(2) The colouring substances (carotenoids - apolaric compounds) are removed by petroleum ether.
(3) From the petroleum etheric phase traces of capsaicin are obtained back.
(4) See the reaction mechanism in 3.3. point.

Calculation
The specific coefficient of the blue reactionproduct: $A^{1\%}/ 1\ cm = 471$

$$X = \frac{A}{471} \times \frac{1000}{4} \qquad X = 0{,}53 \times A$$

(X = quantity (mg) of capsaicin in l0,0 ml of stock solution - 200 mg of crude drug.
(Stock solution: 1,0 g crude drug/50 ml)

Precentage (%) capsaicin content of paprika: % = x/2m m = mass (g) of starting crude drug

4.4 Determination of colouring power of roselle by spectrophotometric method

To 1.0 g of the powered drug add 25 ml of boiling water in a 100 ml flask and heat for 15 min on a water-bath with frequent shaking. Filter the hot mixture into a 50 ml graduated flask. After cooling dilute to 50 ml with water. Dilute 5 ml of this solution to 50 ml with water. Measure the absorbance at 520 nm using water as the compensation liquid. The absorbance is not less than 0.350 for the whole drug and not less than 0.250 for the cut drug.

Report

1. UV - spectrum of carotenoids
2. TLC of capsaicin detection and ascorbic acid
3. Ascorbic acid content
4. Capsaicin content
5. Colouring power