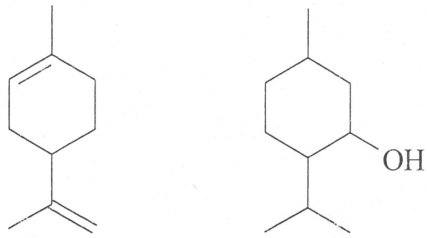
Practice No. 9

ANALYSIS OF DRUGS CONTAINING ESSENTIAL OILS

Essential oils or ethereal oils are volatile compounds found in flowers, leaves, roots, and many other parts of mainly higher plants. Mostly it is a complex mixture of structurally related compounds often of terpene type or aromatic substances formed by the shikimate pathway.

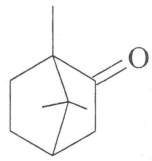
Physical properties: they are liquid at room temperature, but they are volatile. Mostly they are colorless. Their density is generally lower than water, except cinnamon essential oil. Most of them rotate the plane of polarized light. They are soluble in common organic solvents and fats.

Chemical properties: they are a mixture of alcohols, aldehydes, ketones, acids and their esters, ethers, lactones, aliphatic and cyclic monoterpenic, sequiterpenic a diterpenic compounds.



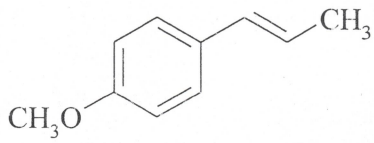
Limonene

Menthol

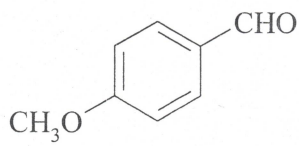


Camphor

Aromatic substances with phenylpropane base are the second group of essential oils according to the chemical structure and they are formed by the shikimate pathway.



Anethole



Anisaldehyde

Monoterpenes and other components of essential oils may be present in plants as glycosides e.g. geraniol, nerol and citronellol are found as glycosides in rose petals, thymol and carvacrol is present as the glucoside and galactoside forms in thyme.

Proof of the presence of essential oils in drugs

Microchemical test is usually carried out in microcrusible which contains a small amount of the drugs. The crucible is covered by a glass plate, containing a drop of the reagent on the bottom side. As a reagent, we use different hydrazine derivatives, such as p-nitrophenylhydrazine, dinitrophenylhydrazine etc., dissolved in 15-30% of acetic acid solution. These reagents react sensitively with aldehydes and ketones and form crystalline precipitates with defined melting points. The crucible is slightly heated. Vapors of essential oils move up (containing mostly aldehydes or ketones) and they react immediately with the drop of reagent to form crystals. Crystals are cleaned and dried. Then their melting point is determined, which is characteristic for each essence.

Carbonyl substances condense with arylhydrazines to form crystalline arylhydrazones with mostly low solubility. This reaction is used as a proof, as well as for the identification of carbonyl compounds.

$$R\text{-}CHO + Ar\text{-}NH\text{-}NH_2 \rightarrow R\text{-}CH = N\text{-}NH\text{-}Ar + H_2O$$

This simple method is not universal for all essential oils. It cannot be used for the detection of essential oils, which are not aldehydes or ketones.

Quantitative determination of essential oils in drugs

Determination of content of essential oils in the drugs is highly dependent on way of implementation and the results of the determination can be compared to each other, only in case when the same amount of the drug was used wuth the same methods and apparatus.

Evaluation is directed by the adjustment of the drug according to the storage of essential oils in the drug. For drugs where essential oils are stored inside the tissue in oil cells, it is recommended to crush the drug into rough powder (*Caryophylli flos, Cinnamoni cortex, Anisi fructus* etc.).

Determination of essential oils in drugs is done by distillation with water or with a mixture of water and 2% ammonium oxalate solution (in the case *Pericarpium aurantia dulce*) or by steam distillation.

In the case of the distillation of the drug with water, the drug is heated in a flask with a certain amount of water, the steam evaporates. This method has the disadvantage that it requires a considerable amount of distillate for the quantitative determination of essential oils.

In the steam distillation, the drug is not in direct contact with water. It is placed under water, so that the escaping steam goes through the drug and carries volatile essential oils. This method, a considerable saponification of essential oils esters does not occur, as in the previous method and it is not necessary to have a large amount of distillate for further evaluation of essential oils.

Time of distillation is very individual for both methods and it depends on the character of the drug. The time required for complete distillation of essential oils from the drug is between 3-4 hours. The ratio of the drug and liquid depends largely on the character of the drug and it is individual for every species.

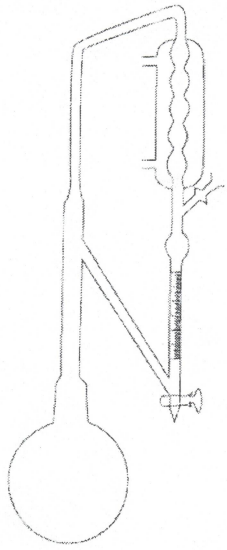
Self-determination of essential oil is established as volume or as weight of distilled essential oils.

Determination of essential oils in the drugs by volume determination is performed using the devices, which are mostly based on continuous distillation of essential oils with water or water vapor. This method has the advantage that it is easier and the time of distillation can be arbitrarily changed.

Determination of content of essential oils ČL 2002

Determination of essential oils in drugs is done by simple distillation of the drug with water and by the measurement of volume of the obtained essential oils. The content of essential oil is expressed in milliliters per 1 kg of the drug.

The described amount of the drug is crushed into the degree shown in the table, and placed with the prescribed liquid and a few pieces of boiling stones into the 1 L boiling flask with ground glass, and it is connected to the apparatus. If it is necessary, xylene is added. The side tube is closed by piece of cotton wool.



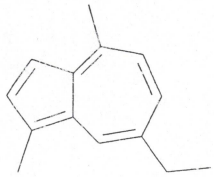
The flask is heated on a boiling nest with occasional shaking. Cooling is stopped after the prescribed distillation period is over; the oils in condenser run and connect to the main volume in the tube. The heating is interrupted and after 5 minutes from the stopping of cooling. Not earlier then 5 minutes, the essential oils are let out into finely divided tube by slight opening of tap and then the volume is measured.

When xylene is used, the volume of oil extracted from weighed amounts of the drug is found by subtracting the volume of xylene from a mixture of xylene and oil, found after distillation.

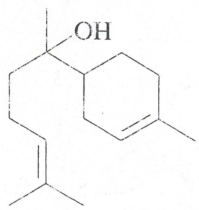
Chamomillae romanae flos

Chamaemelum nobile (L.), Asteraceae

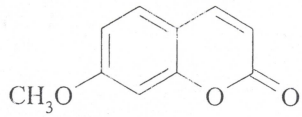
Content compounds: 0.6 – 2.4 % of blue-colored essential oils because of azulene, main compounds of essential oils are chamazulene, α-bisabolol, α-bisaboloxide A and B, farnesene, coumarins (herniarin), flavonoids (apigenin, luteolin, quercitrin), mucilage



Chamazulene



α-bisabolol



Herniarin

Identification

1- Several pieces of the flowers are crushed, then placed into the glass tube. 5 ml of ether are added and it is shaken for few times for 5 minutes. Ether extract is filtered into small ceramic dish and it is dried in a water bath. After evaporation, 3 ml of p-dimethylaminobenzaldehyde solution in acetic and phosphoric acid are added and the mixture is heated for 5 minutes in a water bath. The color of the solution is changed into blue-green (proazulene). (ČsL 3)

Boldo folium

Peumus boldus MOLINA, Monimiaceae

Content compounds: essential oils (containing cineol, p-cymene, linalool, ascaridol), alkaloids of aporphine type (boldine, sparteine), flavonoids

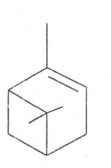
Identification

1- 1.0 g of the powdered drug (IV) is shaken with 80% ethanol for 5 minutes and the extract is filtered. 2 drops of HCl are added to 1 ml of the filtrate and the mixture is dried in a water bath. The residue is dissolved in 5 ml of water and it is filtered into glass tube. To filtrate, 2 drops of HCl and 5 drops of Mayer reagent are added. Precipitate is formed (alkaloids).

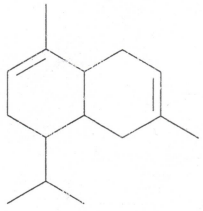
Juniperi fructus

Juniperus communis, Cupressaceae

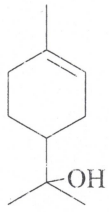
Content compounds: essential oils (α-pinene, camphene, cadinen, alcohols α-terpineol, geraniol, borneol, esters), invert sugar, catechin tannins, flavonoids, leucoanthocyanins



α-pinene



α- cadinen



α-terpineol

Identification:

1- *Proof of the presence of leucoanthocyanins*: 0.5 of the powdered drug is mixed with 10 ml of methanol and it is heated to boiling. The mixture is filtered and 1 ml of filtrate is mixed 1 ml of concentrated sulfuric acid carefully. The red-purple color is formed (ČL97).

2- *Proof of the presence of flavonoids*: The filtrate from previous test is dried in a water bath and the residue is dissolved with 5 ml of water and it is heated again in a water bath for 2 minutes. After cooling down it is filtered and 2 ml of filtrate is mixed with 1 ml of 40% NaOH solution. The yellow-orange color is formed.

Millefolii herba

Achillea millefolium, Asteracae

Content compounds: essential oils (sesquiterpenes, proazulenes, pinenes), bitter compounds, flavonoids (rutin), tannins

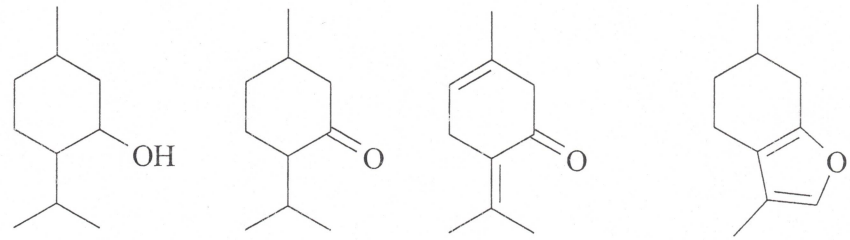
Identification:

1- Approximately 0.50 g of the powdered drug (IV) is shaken with 15 ml of chloroform in round-grinder flask for 5 minutes and extract is filtered over cotton wool. The filtrate is evaporated to cca 1 ml on a water bath and 5 ml of p-dimethylaminobenzaldehyde in acetic and phosphoric acid are added. The mixture is heated for 5 minutes on a water bath. After cooling down, it is shaken in a small separatory funnel with 5 ml of petroleum ether. The color of lower layer is changed into blue or blue-green (azulenogenic compounds).

Menthae piperitae herba

Mentha piperita, Lamiaceae

Content compounds: essential oils (containing menthone, menthol, isomenthone, limonene, cineol, menthofuran, menthylacetate, pulegone, carvone), tannins, flavonoids, organic acids



Menthol Menthone Pulegone Menthofuran

Identification:

Thin layer chromatography on silica gel

Tested solution: 0.2 g of the drug is powdered before use and it is shaken with 2 ml of dichloromethane for several minutes and then it is filtered. The filtrate is evaporated at 40 °C maximally and the residue is dissolved in 0.1 ml of toluene.

Reference solution: 50 mg of menthol, 20 µl of cineol and 10 mg of thymol is dissolved in 10 ml of toluene.

Elution mixture: ethylacetate : toluene (5 : 95)

Detection: anisaldehyde solution (mix 0.5 ml of anisaldehyde, 10 ml of acetic acid, 85 ml of methanol and 5 ml sulfuric acid consensually)

Apply 10 µl of the reference solution and 20 µl of investigated solution separately of TLC layer and it is eluted for a distance of 15 cm. The layer is dried at room temperature. It is observed under UV light at 254 nm. On the chromatogram of tested solution, A visible light colored spot of (carvone, pulegone) is observed, which is under the thymol spot in comparison with reference solution. The layer is then sprayed by detection reagent and it is heated at 100 - 105 °C for 5 - 10 minutes and it is investigated at normal light.

On the reference solution chromatogram, there are visible spots according to the increasing values of R_F: the lower third dark blue to purple spot (menthol), purple-blue to brown spot

(cineol), pink spot (thymol), blue-purple spot (menthylacetate). On tested solution chromatogram, there are visible spots of menthol (very intense) and cineol (poor). There can be other spots between spots of thymol and cineol – light pink spot (carvone), blue-grey spot (pulegone) and grey-green spot (isomenthone). In middle part there is blue-purple spot corresponding to methylacetate by place and color, immediately under that there is a green-blue spot (menthone), closely to the front of the chromatogram there is an intense red-purple spot (hydrocarbons) and there can be other, less intense, spots.

Thymi herba - ČL 2002

Thymus vulgaris, Lamiaceae

Content compounds: essential oils (containing thymol, carvacrol, p-cymol, limonene), tannins, flavonoids

Identification:

Thin layer chromatography on silica gel

Tested solution: 0.5 g of the powdered drug is shaken with 3 ml of dichloromethane for 3 minutes and then it is filtered over anhydrous sodium sulfate. The filtrate is used as tested solution.

Reference solution: 5 mg of thymol and 10 µl of carvacrol are dissolved in 10 ml dichloromethane.

Elution mixture: dichloromethane

Detection reagent: anisaldehyde solution (viz Herba menthae)

20 µl of both solutions are applied onto layer and eluted for distance 12 cm. Then the layer is dried at room temperature and it is investigated under UV light and spot are marked. There are visible intense spot of thymol in middle part. The layer is sprayed by detection reagent and it is heated at 100 – 105°C for 10 minutes. Brown-pink spot of thymol is noticeable and light purple spot of carvacrol closely below it. There are other spots between two main spots and the start - order of decreasing R_F: pink (cineol), purple (linalool), grey-brown (borneol) and purple-blue. Near the front is an intense purple-red to grey-purple spot, other spots are near the start.

Salviae herba

Salvia officinalis, Lamiaceae

Content compounds: essential oils (containing α- and β-thujone), tannins, flavonoids (luteolin), bitter compounds, thiamine, nicotinic acid

Melissae herba

Melissa officinalis, Lamiaceae

Content compounds: essential oils (containing citronellal, citral, citronellol, geraniol, linalool, α-caryophyllene, rosemarinic acid), glycosidically bounded chlorogenic and caffeic acid, tannins, flavonoids, triterpens

Aurantii pericarpium dulce

Citrus aurantium L.subsp. aurantium, Rutaceae.

Content compounds: essential oils (limonene, terpineol, pinene, linalool), flavonoids (rutin, hesperidin, eriocitrin, naringin, neohesperidin), coumarins, bitter compounds, carotenoids (violaxanthin)

Anisi vulgaris fructus - ČL 2002

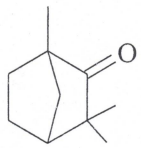
Pimpinella anisum, Apiaceae

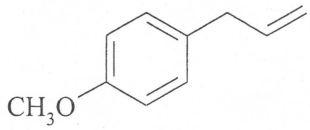
Content compounds: essential oils (mainly anethole, chavicol methylether, p-methoxyphenylacetone, safrole)

Foeniculi fructus

Foeniculum vulgare, Apiaceae

Content compounds: essential oils (trans-anethole, fenchone, anisaldehyde, estragole, α-pinene, limonene), flavonoids, coumarins, proteins, organic acids





fenchone estragole = methylchavicol

Evaluation of essential oils by TLC

Investigated solution I: Essential oils, which were prepared by the distillation of the drug, are poured into a glass tube with cca 2 g of anhydrous sodium sulfate. The condenser and measuring part of apparatus is flushed with small volumes of toluene (totally about 5 ml). The tube is left to stay for 30 minutes with intermittent shaking. Then, it is filtered and the tube and filter are washed by 1 ml of toluene. The filtrate used for chromatography has to be transparent (toluene solution of essential oils).

Investigated solution II: commercial essential oils

Reference solutions: according to what is available

Elution mixture: toluene - ethylacetate (93 : 7)

Detection reagent I: mixture of ethanol and sulfuric acid 95:5

Detection reagent II: vanillin solution in ethanol

Apply 10 µl of the investigated solution and 5 µl of the reference solutions separately of TLC layer and elute for a distance of 12 cm. The layer is dried at room temperature. It is observed under UV light at 254 nm. Layer is sprayed by detection reagent I and after drying by reagent II. The layer is heated at 100 - 105 °C and it is investigated at normal light.

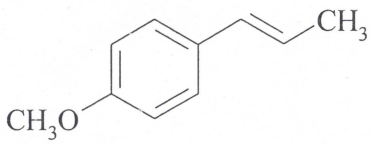
Oleum anisi

Pimpinella anisum, Apiaceae

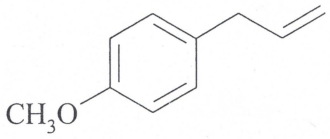
Main components of essential oils: anethole, methylchavicol (red-brown color)

Other compounds: R_F = 0.95

Comment: determined R_F = 0.64



Anethole



Estragole = methylchavicol

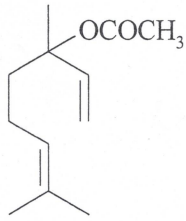
Oleum bergamotae

Citrus aurantium, ssp. bergamia , Rutaceae

Main components of essential oils: linalyl acetate (R_F =0.75, grey-blue color)

Other compounds: geraniol, terpineol (mixture R_F = 0.25; grey-blue color), linalool (R_F = 0.2; blue color)

Comment: determined R_F = 0.5



linalyl acetate

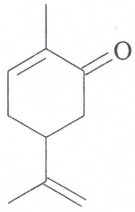
Oleum carvi

Carum carvi, Apiaceae

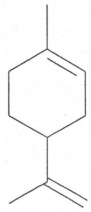
Main components of essential oils: carvone (R_F = 0.46, red-purple color)

Other compounds: carveol, dihydrocarveol (R_F = 0.27; green-blue color), limonene (turquoise color)

Comment: determined R_F = 0.35



Carvone



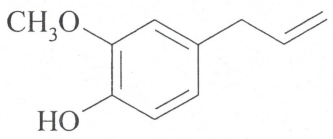
Limonene

Oleum caryophylli

Syzigium aromaticum, Myrtaceae

Main components of essential oils: eugenol (R_F = 0.47; yellow-brown color)

Other compounds: β-caryophyllene (R_F = 0.84; purple)



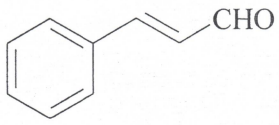
eugenol

Comment: determined R_F = 0.5

Oleum cinnamomi

Cinnamomum, Lauraceae

Main components of essential oils: cinnamon aldehyde (R_F = 0.31; blue-green color)



Cinnamon aldehyde

Other compounds: β-caryophyllene (R_F = 0.84; purple)

Comment: determined R_F = 0.35

Oleum citri

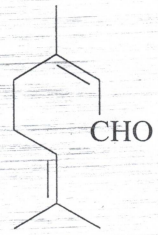
Citrus, Rutaceae

Main components of essential oils: citral (R_F = 0.42; blue-purple color)

Other compounds: β-caryophyllene (R_F = 0.84; purple)

limonene (R_F = 0.41; turquoise color)

Comment: determined R_F = 0.46



citral

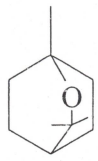
Oleum eucalypti

Eucalyptus globulus, Myrtaceae

Main components of essential oils: eucalyptol = cineol (R_F = 0.4; blue color)

Other compounds: citral (R_F = 0.42); piperitone

Comment: determined R_F = 0.35



cineol

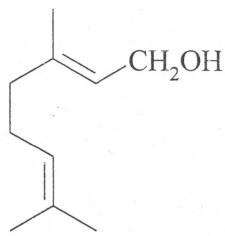
Oleum geranii

Pelargonium, Geraniaceae

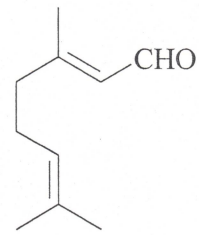
Main components of essential oils: geraniol (R_F = 0.25; grey-blue color)

Other compouns: citral (R_F = 0.42); piperitone

Comment: determined R_F = 0.24; green-blue-brown color



geraniol



citral

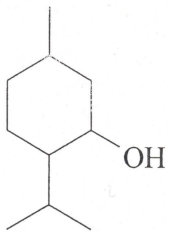
Oleum menthae

Mentha piperita, Lamiaceae

Main components of essential oils: menthol (R_F = 0.3; blue color)

Other compounds: piperitone (R_F = 0.35; orange); cineol (R_F = 0.4; blue)

Comment: determined R_F = 0.33



menthol

Oleum chamomillae

Matricaria recutita, Asteraceae

Main components of essential oils: chamazulene (R_F = 0.95; red-purple color)

Other compounds:			
	bisabolol-oxide	yellow-green	R_F = 0.2
	terpenic alcohols	purple	R_F = 0.25
	bisabolol	purple	R_F = 0.35
	farnesene	blue-purple	R_F = 0.99

Oleum boldo

Peumus boldus, Monimiacea

Main components of essential oils: *cineol* (R_F = 0.4; blue color)