MORPHOLOGICAL, ANATOMICAL AND CHEMICAL ANALYSIS OF VOLATILE OIL CONTAINING MEDICINAL PLANTS AND HERBAL DRUGS

Volatile oils are the odorous principles found in various plant parts. Because they evaporate when exposed to the air at ordinary temperatures, they are called **volatile oils, ethereal oils, or essential oils.** The last term is applied because volatile oils represent the "essences" or odoriferous constituents of the plants. Volatile oils are colorless as a rule, particularly when they are fresh, but on long standing they may oxidize and resinify, thus darkening in color. To prevent this darkening, they should be stored in a cool, dry place in tightly stoppered, preferably full (not half-emptied), amber glass containers.

Depending on the plant family, volatile oils may occur in specialized secretory structures such as glandular hairs (Labiatae), modified parenchymal cells (Piperaceae), oil-tubes called vittae (Umbelliferae), or in lysigenous or schizogenous passages (Pinaceae Rutaceae). They may be formed directly by the protoplasm, by decomposition of the resmogenous layer of the cell wall, or by the hydrolysis of certain glycosides In the conifers, volatile oils may occur in all tissues; in the rose, they appear in appreciable quantities only in the petals; in cinnamon, only in the bark and the leaves; in the umbelliferous fruits, only in the pericarp; in the mints, only in the glandular hairs of the stems and leaves, and in the orange, one kind of oil occurs only in the flower petals and another kind only in the rind. Volatile oils may act as repellents to insects, thus preventing the destruction of the flowers and leaves; or they may serve as insect attractants, thus aiding in cross-fertilization of the flowers.

Although volatile oils differ greatly in their chemical constitution, they have a number of physical properties in common. They possess characteristic odors, they are characterized by high refractive indices, most of them are optically active, and their specific rotation is often a valuable diagnostic property. As a rule, volatile oils are immiscible with water, but they are sufficiently soluble to impart their odor to water. The aromatic waters are dependent on this slight solubility. Volatile oils, however, are soluble in ether, alcohol, and most organic solvents.

Several points of differentiation exist between volatile oils and fixed oils. Volatile oils can be distilled from their natural sources; they do not consist of glyceryl esters of fatty acids. Hence, they do not leave a permanent grease spot on paper and cannot be saponified with alkalies. Volatile oils do not become rancid as do the fixed oils, but instead, on exposure to light and air, they oxidize and resinify.

Questions on topic"Volatile oils"

1. Give the definition of "Volatile oils"
2. Give the definition of "Terpens"
3. What physical and chemical properties do essential oils have?
4. What do underlie the classifications of the essential oils?
5. Which methods of obtaining of essential oils do you know? How do you choose any of methods of obtaining of essential oils?
6. Which methods of quantitative analysis of essential oils are used?
7. Which physical chemical characterizations are used for standardization of essential oils?
8. What purposes are essential oils employed for?
9. How should herbal drugs contained essential oils be picked up?
10. How should herbal drugs contained essential oils be stored?
11. List the Latin name of plant sources of camphor
12. List the Latin name of plant sources of cineol
13. List the Latin name of plant sources of menthol
14. List the Latin name of plant sources of linalool

METHODS OF OBTAINING OF VOLATILE OILS

Volatile oils are usually obtained by distillation of the plant parts containing the oil. The method of distillation depends on the condition of the plant material. Three types of distillation are used by industrial firms: (1) water, (2) water and steam, and (3) direct steam.

Water distillation is applied to plant material that is dried and not subject to injury by boiling. Turpentine oil is obtained in this manner. The crude turpentine oleo-resin, composed of plant exudates, rainwater, wood chips, pine needles, and other components, is introduced into the distilling chamber and subjected to heat until all volatile matter, both oil and water, is condensed in the condensing chamber. Turpentile oil, consisting almost entirely of terpenes, is not affected by this amount of heat.

Water and steam distillation is employed for either dried or fresh substances that may be injured by boiling. In the case of dried material (cinnamon, clove), the drug is ground and then covered with a layer of water. Steam is passed through the macerated mixture. Because the oil could be impaired by direct boiling, the steam is generated elsewhere and is piped into the container holding the drug. The oily layer of the condensed distillate is separated from the aqueous layer, and the oil may be marketed with or without further processing.

In the method of **direct steam distillation,** applicable to fresh plant drugs (peppermint, spearmint), the crop is cut and placed directly into a metal distilling tank on a truck bed. The truck is driven to a distilling shed, where steam lines are attached to the bottom of the distilling tank The plant material is still green and contains considerable natural moisture; therefore, maceration is unnecessary. Steam is forced through the fresh herb and carries the oil droplets through a vapor pipe attached at the top of the tank to the condensing chamber.

During steam distillation certain components of a volatile oil tend to hydrolyze, whereas other constituents are decomposed by the high temperatures Ideal distillation methods utilizing steam should provide for the highest possible diffusion rate of steam and water through plant membranes and should thus keep the hydrolysis and decomposition at a minimum.

Some volatile oils cannot be distilled without decomposition and are usually obtained by **expression** (lemon oil, orange oil) or possibly by other mechanical means. In the United States, the general method for obtaining citrus oils involves puncturing the oil glands by rolling the fruit over a trough lined with sharp projections that are long enough to penetrate the epidermis and pierce the oil glands located in the outer portion of the peel **(ecuelle** method). A pressing action on the fruit removes the oil from the glands, and a fine spray of water washes the oil from the mashed peel while the juice is extracted through a center tube that cores the fruit. The resulting oil-water emulsion is separated by centrifugation. A variation in this process is to remove the peel from the fruit before the oil is extracted.

Often the volatile oil content of fresh plant parts (flower petals) is so small that oil removal is not commercially feasible by the above-mentioned methods. In such instances, an odorless, bland, fixed oil or fat is spread in a thin layer on glass plates. The flower petals are placed on the fat for a few hours; then, repeatedly, the old petals are removed, and a new layer of petals is introduced. After the fat has absorbed as much fragrance as possible, the oil may be removed by extraction with alcohol. This process is known as **enfleurage** and was formerly used extensively in the production of perfumes and pomades.

Investigation of volatile oils and determination of their authenticity.

The authenticity of the volatile oils is established by their physico-chemical properties. Colour, odour, taste, density, optical rotation, refractive index, solubility in alcohol, acidic and essential numbers are determined.

Colour is determined by placing 10 ml of oil into a transparent glass cylinder with diameter 2-3 cm and watching it in passing light.

Odour: 2 drops are placed on the stripe of filtered paper with length of 12 cm and width of 5 cm and the odor of tested oil is compared with the odor of control specimen every 15 minutes. During 1 hours the odor must similar to the odor of reference specimen.

Taste. Taste is determined by putting the stripe of filtered paper with the drop of oil on it to the tongue.

Density. Density of volatile oils is identified by picnometer. Their density is varies in limits of

0,69 to 1,188. (see table 11.1)

Optical rotation. The optical rotation of a liquid is the angle though, which the plane of polarization of light is rotated, when the polarized light is passed through a sample of the liquid. This rotation may be either clockwise or anti clockwise. In addition the fundamental effects of the molecules of liquid under investigation, the observed rotation is dependent on the thickness of the layer examined, its temperature and the nature of the light employed. The pharmacopoeias use sodium light, a layer 1 cm thick and a temperature of 20^{o}C (degrees sentigrade). The optical rotation is determined in the polarimeter. The optical rotation refracts the composition of optically active substances, contained in volatile oils. It is the sum of rotation of the ingredients of volatile oils.

The direction of the rotation is a useful criterion of purity. Examples, to illustrate the range of values found are caraway oil +74° to 80°, peppermint oil -16° to 30°, cinnamon oil 0° to -2°. (see table 11.1)

Determination of Refractive Index (Ph. Eur.)

The *refractive index* (*n*) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance.It varies with the wavelength of the light used in its measurement. Refractive indices, n_{20}^{D}, are stated in terms of the wave-length of the sodium D-line (589.3 nm) at a temperature of 19.5° to 20.5° unless otherwise specified.

Measurements of refractive index of volatile oils can be found in the pharmacopoeias. Oil of cassia has refractive index of about 1,61; oil of lemon - 1,474-1,476; lavender oil - 1,460 - 1.474 etc. (see table 11.1).

Acid Value *(Ph. Eur.)*

The ***acid value*** is the number of mg of potassium hydroxide required to neutralise the free acid in 1 g of the substance when determined by the following method, unless otherwise specified in the monograph.

Unless otherwise specified in the monograph weigh 10 g of the substance being examined and add 50 ml of a mixture of equal volumes of *ethanol (96%)* and *ether* that has been neutralised with 0.1M *potassium hydroxide VS* using 0.5 ml of *phenolphthalein solution R1* as indicator. When the substance has completely dissolved, titrate with 0.1M *potassium hydroxide VS*, shaking constantly until a pink colour that persists for at least 15 seconds is produced. Calculate the acid value from the expression 5.610*v*/*w*, where *v* is the volume, in ml, of potassium hydroxide solution required and *w* is the weight, in g, of substance taken (see table 11.1).

Ester Value *(Ph. Eur.)*

The ***ester value*** is the number of mg of potassium hydroxide required to saponify the esters present in 1 g of the substance.

Calculate the ester value by subtracting the acid value from the saponification value.

Hydroxyl Value *(Ph. Eur.)*(Former ester after acetylation)

The ***hydroxyl value*** of a substance is the number of mg of potassium hydroxide required to neutralize the acetic acid liberated by the hydrolysis of 1 g of the acetylated substance.
Determine the *saponification value.*.

Acetylate by the following method. To 10 g in a 200-ml Kjeldahl flask add 20 ml of *acetic anhydride*. Support the flask on a sheet of heat resistant material in which a hole about 4

cm in diameter has been cut and heat with a small, naked flame, not more than 25 mm in height and which does not impinge on the bottom of the flask. Boil gently under a reflux air condenser for 2 hours, allow to cool, pour into 600 ml of *water* contained in a large beaker, add 0.2 g of *pumice powder* and boil for 30 minutes. Cool, transfer to a separating funnel and discard the lower layer. Wash the acetylated product with three or more 50-ml quantities of a warm, saturated solution of *sodium chloride* until the washings are no longer acidic to *litmus paper*. Finally shake with 20 ml of warm *water* and remove the aqueous layer as completely as possible. Pour the acetylated substance into a small dish, add 1 g of powdered *anhydrous sodium sulphate*, stir thoroughly and filter through a dry, pleated filter paper. Determine the *saponification value* of the acetylated substance. Calculate the acetyl value from the expression:

$a_V = \frac{1335 \cdot (b-a)}{(1335-a)}$, where

a is the saponification value of the substance and ***b*** is the saponification value of the acetylated substance.

Table 11.1. ***Volatile oils composition, and numerical data*** (Pharmacopoeia of the USSR)

Source	Content, not less*	Specific gravity	Specific rotation, α	Acid value	Ester value	Saponification value	Refractive index
Aetheroleum Juniperi	1,0 %	0.865-0.885	–l,8 to -13°	3	1-12	212	1,472-1,479
Aetheroleum Menthae piperitae	1,2%	0.897-0.912	+20 to -32°	0,5-5	16,7-21,5	-	1,463-1,470
Aetheroleum Thymi	1,2%	0.9140	–5 to -84°	1.9	28	-	1,490-1,500
Aetheroleum Eucalypti	2,0%	0.9072-0.918	+4,7 to +11°	1,2-2,9	5,42-20,0	-	1,458-1,470
Aetheroleum Carvi	3,0%	0.905-0.915	+75 - +85°	-	-	-	
Aetheroleum Coriandri	0,6%	0.864-0.877	+9 to +12°	2	11,2	186-190	1,463-1,470
Aetheroleum Foeniculi	2,0 %	0.960-0.980	+11 to +21°	-	-	-	1,530-1,540
Aetheroleum Anisi	1,5 %	0.979-0.991	–2 to 0°	-	-	-	1,552-1,556
Aetheroleum Therebinthinae		0.915-0.922	+10,8 - +10,9°	>7	44,6	19,5	1,467-1,478

*** -** Content of volatile oil in herbal drugs (Pharmacopoeia's requirements)

Determination of Cineole *(Ph. Eur.)*

Weigh 3.00 g of the oil, recently dried with *anhydrous sodium sulphate*, into a dry test tube and add 2.10 g of melted o-*cresol*. Place the tube in the apparatus for the *determination of freezing point*, and allow to cool, stirring continuously. When crystallisation takes place there is a small rise in temperature; note the highest temperature reached (*t*1).Remelt the mixture on a water bath ensuring that the temperature does not exceed *t*1 by more than 5° and place the tube in the apparatus maintained at a temperature 5° below *t*1. When recrystallisation takes place, or when the temperature of the mixture has fallen 3° below *t*1, stir continuously; note the highest temperature at which the mixture freezes (*t*2). Repeat the operation until the two highest values obtained for *t*2 do not differ by more than 0.2°. If supercooling occurs, induce crystallisation by the addition of a small crystal of a complex consisting of 3.00 g of *cineole* and 2.10 g of melted

o-*cresol*. If *t*2 is below 27.4°, repeat the determination after the addition of 5.10 g of the complex. Determine the percentage w/w of cineole corresponding to the freezing point (*t*2) from the Table, obtaining intermediate values by interpolation. If 5.10 g of the cineole–*o*-cresol complex was added, calculate the percentage w/w of cineole from the expression 2 (*A*– 50), where *A* is the value corresponding to a freezing point of *t*2 taken from the Table11.2

Table 11.2.

t_2°	cineole, % w/w	t_2°	cineole, % w/w	t_2°	cineole, % w/w	t_2°	cineole, % w/w
24	45,5	32	56,0	40	67,0	48	82,0
25	47,0	33	57,0	41	68,5	49	84,0
26	48,5	34	58,5	42	70,0	50	86,0
27	49,5	35	60,0	43	72,5	51	88,5
28	50,5	36	61,0	44	74,0	52	91,0
29	52,0	37	62,5	45	76,0	53	93,5
30	53,5	38	63,5	46	78,0	54	96,0
31	54,5	39	65,0	47	80,0	55	99,0

Assay of Volatile Oils

Fixed oils and resinified volatile oils *(Ph. Eur.)*

Allow 0.05 ml of the substance being examined to fall onto a filter paper. The substance evaporates completely within 24 hours without leaving a translucent or greasy mark.

Foreign esters *(Ph. Eur.)*

Heat 1 ml with 3.0 ml of a freshly prepared 10% w/v solution of *potassium hydroxide* in *ethanol (96%)* on a water bath for 2 minutes. No crystals form within 30 minutes, even after cooling.

Odour and taste *(Ph. Eur.)*

Mix 0.15 ml with 5 ml of *ethanol (90%)* and stir in 10 g of *sucrose*, in powder. The odour and taste are similar to those of the plant or the parts of the plant from which the volatile oil was obtained.

Residue on evaporation *(Ph. Eur.)*

The residue on evaporation is the percentage by weight of the oil that remains after evaporation when determined by the following method. Unless otherwise specified in the monograph place 5 g of the oil in a heat-resistant glass evaporating dish, which has been weighed after heating on a water bath for 1 hour and cooling in a desiccator; place the evaporating dish over a water bath on a cover with holes 70 mm in diameter and maintain the water level in the water bath so that it is about 50 mm below the cover throughout the test. Vigorously boil the water in the water bath in a draught-free atmosphere for the time specified in the monograph. Allow the evaporating dish to cool in a desiccator and weigh.

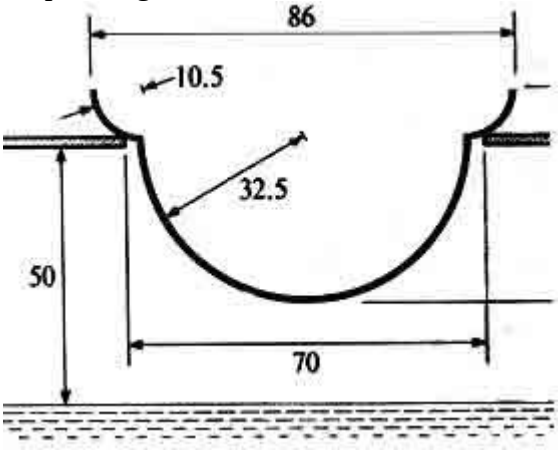

Fig. 11.1. Apparatus for determination of residue on evaporation of volatile oils. *Dimensions in mm*

Solubility in ethanol *(Ph. Eur.)*

Weigh 1 ml of the oil with an accuracy of 5 mg into a 25- or 30-ml glass-stoppered cylinder and place in a constant-temperature device maintained at 19.8° to 20.2°. Using a burette with a capacity of not less than 20 ml, add ethanol of the strength specified in the monograph by 0.1-ml increments until solution is complete and then by 0.5-ml increments to 20 ml, shaking frequently and vigorously. Record the volume of the ethanol required to produce a clear solution. Continue to add more of the ethanol in the same manner. If the solution becomes cloudy or opalescent before 20 ml of the ethanol has been added, record the volume at the point at which the cloudiness or opalescence appears and, if appropriate, the volume at which it disappears. If a clear solution is not obtained when 20 ml of the ethanol has been added, repeat the test using the next highest concentration of ethanol.

The following definitions are applied.

Soluble in n *volumes or more of ethanol (*a*%)*: when the clear solution in *n* volumes remains clear when compared with the undiluted oil after further addition of ethanol of the same strength up to a total of 20 volumes of the ethanol.

Soluble in n *volumes of ethanol (*a*%), becoming cloudy when diluted*: when the clear solution in *n* volumes becomes cloudy in *n*1 volumes (where *n*1 is less than 20) and remains so after further gradual addition of ethanol of the same strength up to a total of 20 volumes of the ethanol.

Soluble in n *volumes of ethanol (*a*%), with cloudiness between* n1 *and* n2 *volumes*: when the clear solution in *n* volumes becomes cloudy in *n*1 volumes (where *n*1 is less than 20) and remains so after further gradual addition of ethanol of the same strength up to a total of *n*2 volumes (where *n*2 is less than 20) and then becomes clear.

Soluble with opalescence: when the ethanolic solution shows a bluish tinge similar to that produced by mixing 0.5 ml of *silver nitrate solution R2* with 0.05 ml of *nitric acid*, adding 50 ml of a 0.0012% w/v solution of *sodium chloride*, mixing and allowing to stand protected from light for 5 minutes.

Water *(Ph. Eur.)*

Mix 0.5 ml with 1 ml of *carbon disulphide*. The solution remains clear on standing

Method of quantitative determination of volatile oil in the herbal drugs

The quantitative determination of volatile oil in the raw material is made by volumetrec method. This methods is based on water and steam distillation of volatile oil from the plant raw material with the following measuring of it's volume. The raw material is placed in distillation flask with water, which is connected to the receiver and to a condenser. Steams of water and volatile oils are condensed in the refrigerator and the liquid flows into the receiver. The oil is settled in a graduated joint of the receiver and water flows back through the smaller joint into the flask. After finishing of distillation and cooling the volume of the oil is measured.

The determination of essential oils in vegetable drugs is carried out by steam distillation in a special apparatus in the conditions described below. The distillate is collected in the graduated tube, the aqueous phase is returned to the distillation flask.

Apparatus. The apparatus comprises the following parts:

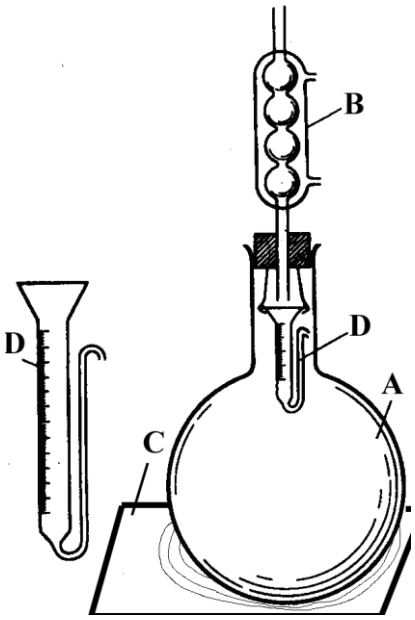

Fig. 11.2. Apparatus of Ginsberg

(A) - a suitable round-bottomed flask with a long, ground-glass neck having an internal diameter of about 29 mm at the wide end;
(B) - a condenser that closely fits the flask,
(C) - a heating device
(D) - a receiver, the tube is graduated in 0.01 ml.

Place 10 g of cut crude drug (weigh out with precision ±0,01 g) into a flask and add 300 water, introduce the receiver in to the flask and attach the reflux condenser, heat to the temperature of boiling and distil for the prescribed time. Stop the heating, allow to cool and read the volume of volatile oil collected in the receiver.

Calculate the result as millilitres per 100 g of drug from the expression:

$$X = \frac{a \cdot 100 \cdot 100}{b \cdot (100 - w)} =$$

a - volume of a volatile oil, ml; ***b*** - mass of vegetable drugs, g; ***w*** – loss on drying, %.