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ESSENTIAL OIL EXTRACTION FROM NATURAL PRODUCTS BY CONVENTIONAL METHODS

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I. INTRODUCTION

Essential oils are homogenous mixtures of volatile odorous components of aromatic materials which can be freed by distillation, expression or extraction depending on the nature of the material. They are also called volatile oils, ethereal oils or essences. They are called oils due to their liquid nature at room temperature. They should not be confused with fixed oils which do not volatilise fully when dropped on a piece of filter paper and exposed to heat. Essential oils, on the other hand, evaporate fully without leaving a stain. Because, essential oils differ from fixed oils in both chemical and physical properties.

Essential oils consist of hydrocarbons and their oxygenated derivatives which comprise alcohols, acids, esters, aldehydes, ketones, amines and sulphur compounds. Mono-, sesqui- and even diterpenes constitute the composition of a majority of essential oils. Furthermore, phenylpropanoids, fatty acids and their esters, coumarins, phthalides and paraffins are also encountered in a number of essential oils.

Essential oils are derived mainly from aromatic plants. A few of them are obtained from animal sources *e.g.*, musk, civet, castoreum, or produced by microorganisms. Mosses, liverworts, seaweeds, sponges and fungi have also been shown to contain essential oils.

In plants, essential oils occur in oil cells, secretory ducts or cavities, and glandular hairs. Sometimes, essential oil components are bound with sugars to form glycosides. In such cases, they must be liberated by hydrolysis of the glycosidic form in order to obtain essential oil. Essential oils are frequently associated with gums or resins which are natural exudates of plants.

Fig. 1. Glandular hairs of *Mentha viridis* c. *lavanduliodora* [*from Sacco et al. (1)*].

Essential oils in plant materials can be categorised as superficial or subcutaneous oils. Superficial oils can be released by gently rubbing the leaf surface of Labiatae, Verbenaceae and Geraniaceae plants since oil in these plants is contained in glandular hairs which protrude on the surface and are broken by a slight pressure freeing the oil. The glandular hairs commonly release the oil between the wall and the cuticle which extends considerably. Eventually the cuticle bursts. It may become regenerated and the accumulation repeated, or the hair may degenerate after a single act of excretion.

Subcutaneous oils on the other hand are contained in internal structures such as oil cells, ducts or secretory cavities. The fragrance of flowers is commonly produced by essential oils distributed throughout the epidermis of perianth parts. In some plants, however, the fragrance originates in special glands named osmophors. Osmophors are found in Asclepiadaceae, Aristolochiaceae, Araceae, Orchidaceae and Burmaniaceae. The osmophors have a secretory epidermis tissue usually several layers in depth. The tissue may be compact and vacularised or it may be permeated by intercellular spaces. Oil is contained in modified parenchyma cells in Piperaceae, resin canals in Coniferae and gum canals in Cistaceae and Burseraceae.

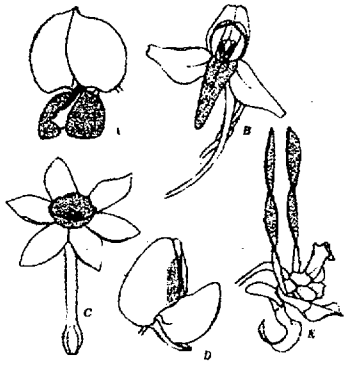

Fig. 2. Osmophors (stippled) in *Spartium junceum* (A), *Platanthera bifolia* (B), *Narcissus jonquilla* (C), *Lupinus cruckshanksii* (D). *Dendrodium minax* (E) [*from Esau (2)*].

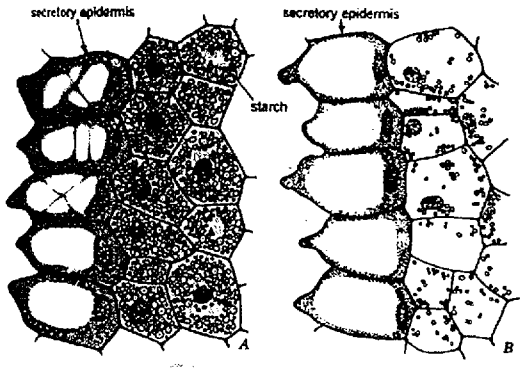

Fig. 3. Sections of secretory tissue of osmophores of *Ceropegia stapeliaeformis* flowers. Before (A) and after (B) emission of fragrance [*from Esau (2)*].

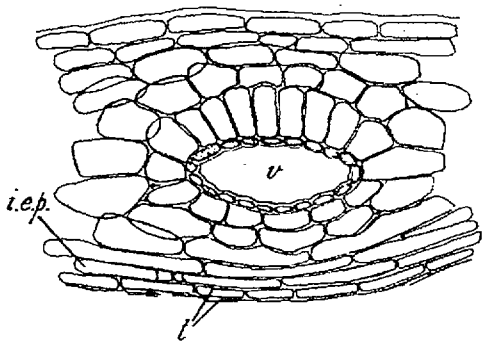

Fig. 4. Schizogenous cavity (vitta) in Fennel fruit [*from Wallis (3)*].

Secretory ducts, cavities or intercellular spaces are called schizogenous, lysigenous or schizolysigenous. Schizogenous cavities are initiated by the dissolution of inner cell wals to form a cavity. The intercellular space remains lined by intercellular material. Essential oil secreted by the surrounding cells accummulates in this cavity. Examples are the resin ducts in the Coniferae and the secretory ducts in the Compositae and the Umbelliferae, Myrtaceae and Graminae.

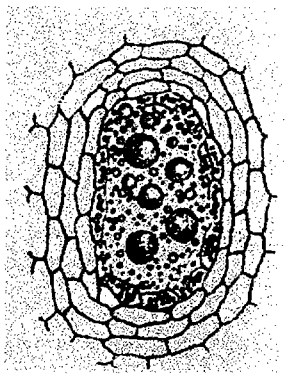

Fig. 5. Lysigenous oil gland of Orange rind [*from Dutta (4)*].

Lysigenous duct or gland arises when central secretory cells disintegrate leaving the secretion, *e.g.* essential oil, surrounded by a layer of secreting cells. Example: Citrus fruits (Rutaceae).

In some plants, the glands may arise schizogenously and subsequently the epithelium breaks down producing a schizolysigenous cavity like in Clove buds.

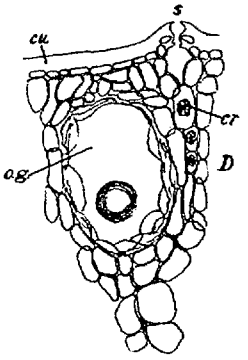

Fig. 6. Schizolysigenous cavity in Clove buds [*from Wallis (3)*].

In order to release a subcutaneous oil the tissue should be broken to expose it. Materials such as pine needles, eucalyptus leaf, aniseeds, cloves, vetiver roots, etc. must be crushed to smell the oil.

Essential oils are obtained by distillation *e.g.* dry distillation, water distillation, water and steam distillation, steam distillation, hydrodiffusion, expression and solvent extraction. Distilled products are called essential oils. However, the products obtained by other methods may be named differently.

Concrete	is obtained by extracting the fresh aromatic plant material with a hydrocarbon solvent. Removal of the solvent under vacuum leaves a solid extract which also contains essential oils.
Absolute	is obtained by extracting the concrete with ethanol. Cooling the ethanolic extract to freezing temperatures (*e.g.* –15°C) to solidify waxes, etc. and evaporating the alcohol *in vacuo* gives a fragrant liquid or semi-liquid rich in essential oil.
Pommade	is the product of enfleurage.
Extrait	is the absolute of a pommade.
Resinoid	also called oleoresin in trade, is a concrete obtained using dried plant material.
Extract	is obtained by extracting the plant material with a polar solvent like ethanol and removing the solvent *in vacuo*.
Tincture	is an alcoholic liquid extract of a plant material.

Balsam is a pathologically induced fragrant exudate of a shrub or tree. It is characterized by high content of benzoic and cinnamic acids and their esters.

Oleoresin is also a pathologically induced natural exudate of a tree or a shrub which comprises resin and high content of essential oil.

Oleogumresin is like oleoresin but it also contains gum.

Essential oils can be extracted from different parts of plants, such as leaves (eucalyptus, cedar, laurel), leafy branches (pine), herbaceous parts (oregano, mint, sage), flowers (rose, jasmin), dried buds (cloves), bark (cinnamon, cassia), wood (sandalwood, cedarwood, rosewood), roots (angelica, vetiver), rhizomes (ginger, orris), bulb (onion, garlic), fruit (anis, fennel, coriander, cumin, juniper), fruit peel (orange, lemon), seeds (cardamom, nutmeg), lichen (oakmoss, treemoss), balsam (storax, peru balsam, myrrh), oleogumresin (mastix), oleoresin (turpentine, opopanax), or animal products (musk, castoreum, civet).

The choice of extraction technique depends upon the nature of the material, the yield and properties of the essential oil and marketability of the product.

Distillation is the most widely used and universally accepted technique for extracting essential oils. Basically, distillation is a separation technique used to vaporise volatile substances from an aromatic matrix at elevated temperatures, and condensing them into a distillate.

II. TECHNIQUES

Essential oil extraction techniques can be classified as

1. Distillation

1.2 Dry Distillation (or Empyreumatic distillation)

1.3 Hydrodistillation

1.3.1 Water distillation

1.3.2. Water and steam distillation

1.3.2.1 Cohobation

1.3.3 Steam distillation

1.3.3.1 Mobile Distillation

1. DISTILLATION

1.2 Dry Distillation is a thermal degradation process used only to produce certain tar oils, such as birch tar oil, cade oil.

In this process, chopped wood (juniper, birch, pine or cedar) is placed on a concave slab. From its centre a pipe leads down to a collector. No air is allowed into the slab. An iron pot just above the stacked wood is filled with coal and burnt. The intense heat so produced brings about thermal degradation which releases the oil from the material and a viscous, dark coloured tarry liquid with a strong smoky odour collects in the collector. This matter usually separates into three layers after 15-20 days. Tar remains at the bottom and an oily layer floats on a watery layer in between. The oil contains methanol, acetic acid and other pyroligneous decomposition products.

1.3 Hydrodistillation

The Principles of Hydrodistillation

Essential oil can be freed from the tissue in which it occurs by heating in the presence of water and steam. Other distillate is then condensed to yield the oil which separates from the water due to difference in density.

During distillation the following physicochemical processes occur in the plant material.

i. Hydrodiffusion, ii. Hydrolysis and iii. Decomposition

i. Hydrodiffusion

In a living plant material, essential oils cannot readily pass through the cell membranes. During distillation, the plant material is soaked with water and the temperature of boiling water dissolves the oil within the glands. The oil-water solution permeates through soaked membranes by osmosis. This is called *hydrodiffusion.* As this oil-water mixture reaches the outer surface of the material it is instantly vaporised by the passing steam. Another important aspect of this process is that the speed of oil vaporisation is not influenced by the volatility of oil components but their degree of solubility in water. Therefore, the higher boiling but more water soluble oxygenated components of an oil distil before the more volatile but less water soluble monoterpene hydrocarbons.

This is true for subcutaneous oils. If the plant material is comminuted then the secreted oil exposed and the distillation proceeds as in the case of superficial oils. In the case of Umbelliferae fruits, composition of the oil changes and the yield of oil increases.

ii. Hydrolysis

Esters are constituents of many essential oils. They constitute the most important odour components in some oils like lavender, lavandin, clary sage. Prolonged boiling with hot water may break ester bonds to form the corresponding alcohol and acid. This chemical reaction is known as hydrolysis. Water distillation, therefore, is not suitable for obtaining oils rich in esters. Such oils should better be obtained by steam distillation.

In many plants, essential oil compounds are bound with sugars in the form of glycosides. Glycosides are non-volatile compounds. They have to be hydrolysed to release their volatile aglycones. Hydrolysis can be achieved by enzymatic or chemical means, or by simply boiling with water. During rose oil distillation rose flowers are subjected to a controlled fermentation to free

volatile alcohols such as citronellol, geraniol and nerol. Bitter almonds and mustard volatile compounds are also released by fermentation.

iii. Decomposition

Decomposition occurs by exposing the plant material to high temperatures and many essential oil components are decomposition products of fatty acids, esters, etc. Blue coloured chamazulen is a thermal decomposition product of colourless matricin in chamomile oil.

All the above processes may occur at the same time depending on the nature of the plant material.

1.3.1 Water distillation

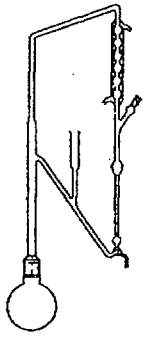

Fig. 7. Clevenger water distillation apparatus

Water distillation is widely used in laboratories to obtain small amounts of oil and in essential oil assays using a Clevenger type distillation apparatus. It is also an industrial process. Rose oil is obtained by water distillation. In cottage industry, direct fired stills are charged with plant materials and water. The amount of the plant material should be loose enough to move freely in the still and the amount of water should be sufficient to last throughout distillation. Otherwise, charring may occur.

In cottage type rose oil distillation, due to low yield of oil, the distillates of several charges are collected and redistilled to obtain rose oil.

In industrial production, water distillation stills are steam-jacketed and live steam can be injected to facilitate and expedite boiling.

1.3.2 Water and steam distillation

This is an improved water distillation technique mainly used by the cottage industry. In this technique, plant material is placed over a perforated grill and the distillation proceeds with the steam generated by boiling water at the bottom part of the still. Solid or liquid fuels are used to generate heat underneat the still.

The oil produced by this technique is more akin to that produced by steam distillation due to the fact that plant material is not in direct contact with extreme heat generated at the bottom of the still as in the case of direct-fired water distillation.

Higher oil yields, less hydrolysis or polymerisation, faster distillation rates and reproducible results can be achieved by water and steam distillation compared to water distillation.

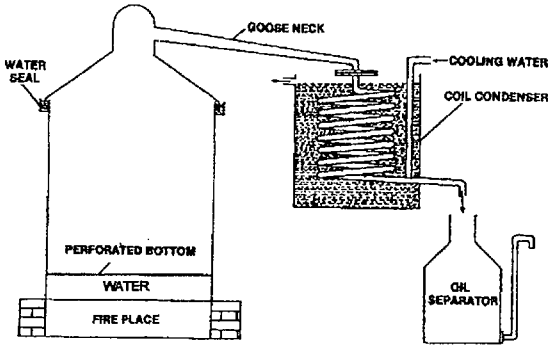

Fig. 8. Water and Steam Distillation Plant [*from Kahol (5)*].

1.3.2.1 Cohobation

Cohobation is often applied in water and water and steam distillation techniques. Cohobation means redistilling the distillate from which oil has been separated by feeding it continuously in to the still. This is done to minimise losses of oxygenated components especially phenols which tend to dissolve in water.

In rose oil distillation, oil-removed distillate waters are redistilled in a separate still to recover more oil. This oil which is rich in phenylethyl alcohol is then mixed with the oil obtained during water distillation to constitute the rose oil.

Except for a few industrial applications, water distillation is used mainly in cottage industry. Attar production in India is also by water distillation. In this technique, distilled oil is trapped in sandalwood oil. Due to the occurrence of thermal decomposition products such oils are often regarded as inferior.

1.3.3 Steam distillation

This is the most widely used essential oil distillation process. The only difference from water and steam distillation is the fact that steam is generated by an external boiler. This allows the control of the amount of steam to be fed into the still and temperature in the still cannot exceed 100°C. Therefore, thermal degradations do not occur and readily marketable oils can be obtained with stills of proper size and design. The only disadvantage of this technique is the high capital expenditure to built a steam distillation facility. The oil-rich vapour condenses in a condenser and oil is separated in a separator called Florentine flask.

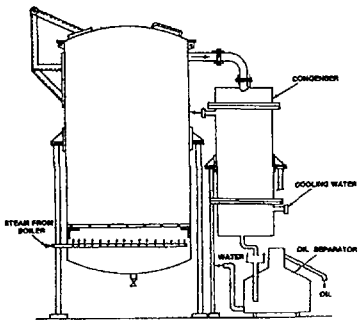

Fig. 9. Steam Distillation Plant [*from Kahol (5)*].

1.3.3.1 Mobile distillation

In the case of large scale cultivation of an aromatic plant, *e.g.* mint, lavender, etc. it is more economical to carry out mobile distillation. In mobile distillation, plant materials which have been mechanically harvested and wilted in the field are transferred into a trailer or tub (as it is commonly called). These mobile units are driven to a central distillation facility which is designed to handle several tubs at a time. Steam is fed into each tub through a steam inlet from the bottom and steam outlet on the top is fitted with a pipe to a condenser. Oil is separated in a Florentine flask. Spent plant materials are discharged and the trailer is driven back to the field for loading.

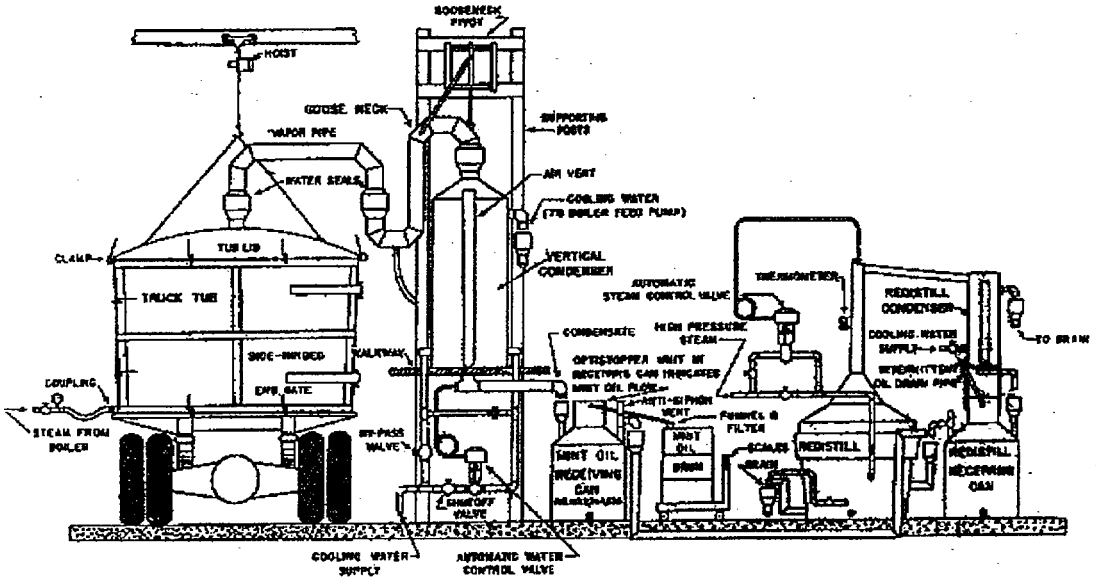

Fig. 10. Mobile Distillation Unit [*from Lawrence (6)*].

1.3.3.2 Continuous distillation

This technique has been in existence at least for the last 25 years in Russia. In the beginning of 90s, pneumatic and endless-screw type continuous distillation plants have been described in the west.

In these systems, countercurrent to the continuous flow of comminuted hot aromatic plant material hot steam is passed through. The steam enriched with oil vapour is sent to the receiver *via* a condenser.

Continuous distillation has been commercially used for distilling cedarwood, fennel, cistus, pine and juniper and for recovering ethanol from fermented grapes.

1.3.4 Hydrodiffusion

This is a steam distillation process but unlike the conventional steam distillation, steam enters the still from the top of the plant charge and diffuse its way through the charge by gravity. Osmotic pressure forces oil to diffuse from oil glands to the surface of the material. The oil is then washed down by the condensed steam. Plant material can be comminuted to increase yield and the technique can be universally used both for superficial and subcutaneous oils.

Usually higher yields of oil are attained with this process however, this is largely due to the extraction of less volatile matters like fatty oils, coumarins, psoralens, chlorophylls, etc. For these reasons, hydrodiffusion has not yet been commercialized.

2. EXPRESSION

Expression or Cold Pressing is confined to citrus oils. It involves inducing physical damage to the essential oil glands on the surface of citrus fruits to release the oil. It is simultaneously washed by the passing water and recovered using an oil-water separator.

Today, there are four major processes that are used to extract citrus oils at commercial scale. These are Pellatrice, Sfumatrice, Brown Peel Shaver and FMC in-line extractor. The latter has become a standard industrial process in recent years due to its high throughput capacity and high efficiency.

2.1 Pellatrice Process

In the Pellatrice process, citrus fruits are fed from a hopper into the abrasive shell of the machine. The fruits are first rotated against the abrasive shell by a slow moving endless screw whose surface rasps the fruit surfaces and bursts essential oil cavities on the surface of the peel. Then, the fruits enter a hopper in which rollers covered with abrasive spikes burst the remaining oil glands. The oil so freed is washed away by a fine spray of water. This emulsion is filtered and passed through centrifugal separators to obtain the oil.

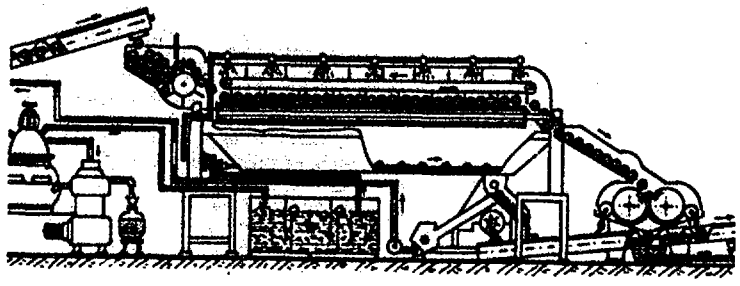

Fig. 11. Pellatrice Polycitrus Extractor (7).

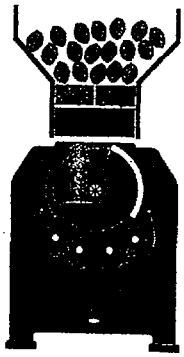

Fig. 12. Pellatrice Speciale Extractor (6,8).

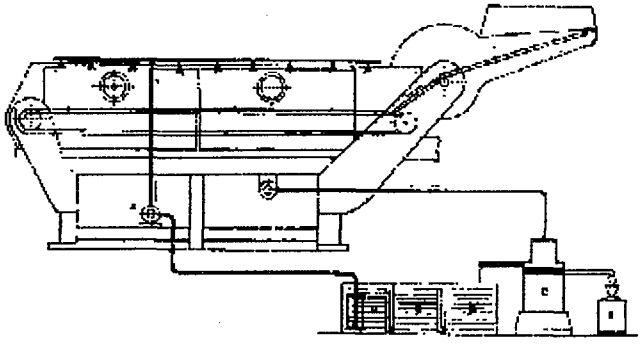

Fig. 13. Sfumatrice Extractor (8).

2.2 Sfumatrice Process

In this process, halved fruits are subjected to a rotating separator to remove the pulp and juice from the peel. The peels are treated with lime solution for 24 hours and then conveyed through horizontal ribbed rollers. They are pressed and bent to release their oil. As in Pellatrice , the oil is washed away and purified as explained above (Fig. 13).

2.3 Brown Peel Shaver

This process is mainly used in North and South America. It involves moving the fruits on a bed of rollers with needle-like projections mounted on a shallow tank. Rolling on the needles on all directions the entire surface of the fruit is punctured and the released oil –water emulsion is washed away by the countercurrent flow of water. Solids are separated by passing through a solids separator and the oil is obtained using a centrifugal separator.

2.4 FMC In-line Extractor

FMC extractor allows for the simultaneous isolation of the oil and the juice. It is an ingenious design. In this process fruit is placed on the lower half of a fixed cup in which metallic fingers interlocked as the top cup forces down on the bottom cup crushing the fruit. As the upper cup moves down on the lower cup holding the fruit, bottom of the fruit is cut and a perforated orifice tube enters into the pulp and sucks the juice. Simultaneously, the metallic fingers peel the fruit and crush it to express its oil. The oil released is washed away from the crushing fruit by strong water jets. The oil is recovered as explained above. The whole process ends in less than a second. FMC extractor holds a line of several cups and fruits rolling down a slope are immediately placed in the cups and processed. A machine with five cups can handle 200-375 fruits in a minute.

Depending on the type and size of the fruit in-line extractor can process 2-7 tons of citrus fruits per hour. Majority of the citrus oil and juice producers in the world use FMC extractor.

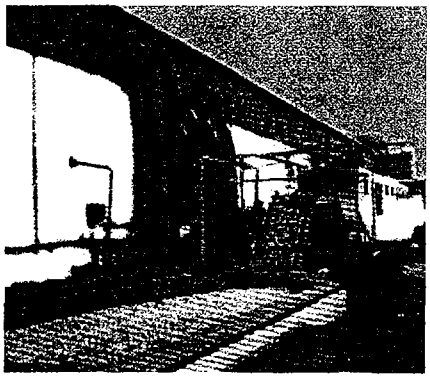

Fig. 14. Washing of Orange fruits before processing (7).

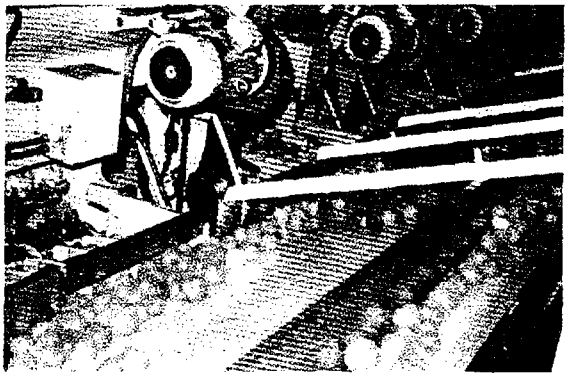

Fig. 15. FMC Extractor (7).

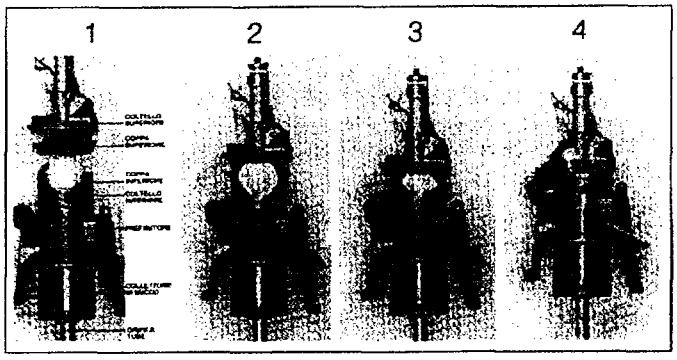

Fig. 16. FMC Extraction process *[from Kunkar (8)]*.

3. EXTRACTION

3.1 Extraction with Hydrocarbon Solvents

The flowers are placed on perforated plates or grills in cylindrical stainless steel extractors of 2000 L capacity. Once the apparatus is loaded with 280-350 kg of raw material the lid is closed and enough solvent (petroleum ether or n-hexane) (*ca.* 1800 L) is introduced to totally immerse the raw material. The solvent is left in contact with the raw material for approximately half an hour, decanted and evaporated. During this time a second washing with the same solvent (1800 L) takes place, lasting slightly shorter (20-25 minutes). The solvent is again decanted and evaporated

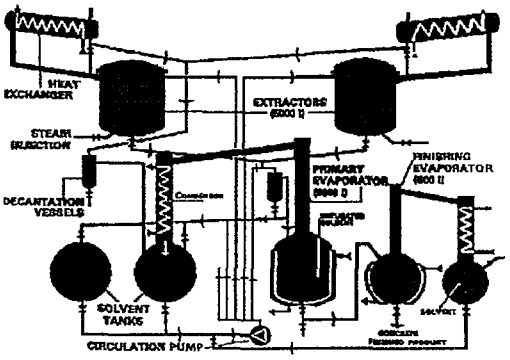

Fig. 17. Concrete Extraction Process [*from Lawrence (6)*].

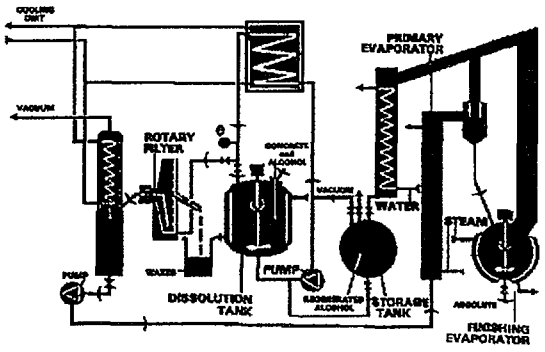

Fig. 18. Process for the preparation of absolutes from concretes [*from Lawrence (6)*].

in the same evaporator. The third washing which is equally important and slightly shorter is also completed in the same manner. The residual solvent remaining on the spent material is stripped by direct injection of steam. The recovered solvent is directed to the solvent tank.

In this manner the evaporator concentrates the equivalent of 6 or 7 loads up to a volume of approximately 200 liters. This "concentrated solution" is then directed , after filtering, into a vacuum evaporator. Vacuum distillation to remove the last traces of solvent is necessary to produce an acceptable product. The slightly coloured and very odorous liquid dough which is removed from the bottom of the vacuum evaporator while warm solidifies to a varying extent according to the raw material. This product is called "Concrete" if the material used is fresh floral. If it has been extracted from dry or viscous products it is more commonly called "Resinoid".

All these products obtained by extraction with hydrocarbon solvents present a problem for perfumery in that they are not entirely soluble in 95% ethanol which is the basis of all fine perfumes. This necessitates extraction of the concrete with alcohol, under certain conditions of mixing and temperature, to make them alcohol-soluble. These products are called "Absolutes".

3.2 Extraction with non-volatile solvents

Two non-volatile solvents are used. These are

- beef tallow or fat : the hard agent
- lard or pork fat : the soft agent

These two animal fats should be very pure. Even then, they undergo a special preparation with a natural anti-oxidizing agent such as Gum of Benzoin or Orange Flower in order to prevent them from turning rancid, which would be catastrophic at the production stage.

These two fats, when mixed together in fixed quantities and having been treated with the anti-oxidizing agent, are called the "prepared fat". This Prepared Fat is the fatty non-olatile solvent used in the manufacture of both pommades prepared with and without the application of heat.

3.2.1 Pommades prepared with applied heat

This is used to extract the odorous components from flowers like Orange flower and the May Rose.

The flowers are saturated in the prepared fat, maintained in the water bath at 50-70°C, and the whole lot is beaten for 15-60 minutes. After each beating, the fat is coarsely filtered to remove the flowers. On average, the ratio of flowers to fat is 5:1. Since the container holds approximately 60 kg of fat, 25 x 12 kg of flowers are added to obtain this ratio.

The frequency of the additions is one in every ninety minutes. The flowers are then either treated with a hydrocarbon solvent to collect the remaining fat or crushed in a press cloth of coarse material, called a "Scourtin".

3.2.2 Manufacture of Pommades in the Absence of Heat (*Enfleurage*)

This technique used to be very popular. It is very rarely used nowadays. It used to be the method of choice for extracting flowers like Jasmin and Tuberose.

A thin layer of the prepared fat is spread on the top and the underneath of a glass plate in a wooden frame called "chassis". The Jasmin flowers are scattered on this at random; the larger Tuberose flowers are evenly spaced. When these frames are piled up in stacks , each stack forming an "assembly", odorous components of the flowers are extracted by the fat with which they are in contact. After 24 hours for Jasmin and 72 hours for Tuberose the exhausted flowers are removed and replaced by fresh ones. The frames are turned over frequently. This process is continued until 1.8 kg of flowers come into contact with 1 kg of fat. The flowers which have been subjected to enfleurage are extracted with hydrocarbon solvents to produce a different type of product.

Both types of pommades are treated in the same manner as concretes and resinoids with 95% ethanol to obtain absolutes.

3.3 Preparation of Absolutes

Pommades, concretes and resinoids are extracted with ethyl alcohol to obtain absolutes.

Absolutes are manufactured in four stages (Fig. 18):

1. *Beating*: Concrete, resinoid or pommade is beaten with ethyl alcohol in a stainless steel or copper beaters containing large blades and levers on a horizontal plane to allow rapid decantation.
2. *Freezing*: Waxes, fats and other materials are removed from the alcoholic extract by freezing to approximately -15°C.
3. *Vacuum filtration*: The matter which has been precipitated at freezing temperatures are removed by suction filtration.
4. *Removal of ethanol*: The clear alcohol solution from which the soluble waxes have been removed is evaporated in a vacuum evaporator to remove ethanol.

The final product is an "absolute essence" or "absolute" in short if it has been extracted from a concrete or pommade. When the raw material is a crude resinoid, it is called "washed resinoid".

A list of most common essential oils and related products are shown in the Annex.

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Annex

SOURCES OF ESSENTIAL OILS

Name of Product	***Source***	***Plant Part Used***	***Process***
Ambrette seed oil	*Hibiscus abelmoschus*	Seeds	SD
Amyris oil	*Amyris balsamifera*	Wood	SD
Angelica root oil	*Angelica archangelica*	Roots	SD
Aniseed oil	*Pimpinella anisum*	Fruits	SD
Basil oil	*Ocimum basilicum*	Flowering tops	SD
Bay oil	*Pimenta racemosa*	Leaves	SD
Bergamot oil	*Citrus bergamia*	Pericarp	Exp.
Bergamot petitgrain oil	*Citrus bergamia*	Leaves, twigs	SD
Bitter orange oil	*Citrus aurantium* subsp. *aurantium*	Pericarp	Exp.
Bitter orange petitgrain oil	*Citrus aurantium* subsp. *aurantium*	Leaves, twigs	SD
Black pepper oil	*Piper nigrum*	Fruits	SD
Boldo oil	*Peumus boldus*	Leaves	SD
Buchu oil	*Barosma betulina*	Leaves	SD
Cade oil	*Juniperus oxycedrus*	Wood	DD
Cajeput oil	*Melaleuca cajeputi*	Leaves	SD
Cananga oil	*Cananga odorata*	Flowers	SD
Caraway oil	*Carum carvi*	Fruits	SD
Cardamom oil	*Elettaria cardamomum* var. *minuscula*	Fruits	SD
Carrot seed oil	*Daucus carota*	Fruits	SD

Cassia oil	*Cinnamomum cassia*	Bark	SD
Catmint oil	*Nepeta cataria*	Aerial parts	SD
Cedarwood oil, Chinese	*Cupressus funebris*	Twigs, leaves	SD
Cedarwood oil, Virginia	*Juniperus virginiana*	Wood	SD
Cedarwood, Texas	*Juniperus mexicana*	Wood	SD
Celery oil	*Apium graveolens*	Fruits	SD
Celery seed oil	*Apium graveolens*	Fruits	SD
Ceylon citronella oil	*Cymbopogon nardus*	Whole aerial plant	SD
Chamomile oil, German	*Matricaria chamomilla*	Flowers	SD
Chamomile oil, Roman	*Anthemis nobilis*	Flowers	SD
Cinnamon leaf oil	*Cinnamomum zeylanicum*	Leaf	SD
Cistus oil	*Cistus ladaniferus*	Aerial parts	SD
Clarysage oil	*Salvia sclarea*	Flowering tops	SD
Clove bud oil	*Syzygium aromaticum*	Buds	WD
Clove leaf oil	*Syzygium aromaticum*	Leaves	WD
Clove stem oil	*Syzygium aromaticum*	Stems	SD
Coriander oil	*Coriandrum sativum*	Fruits	SD
Costmary oil	*Tanacetum balsamita*	Aerial parts	SD
Cubeb oil	*Piper cubeba*	Fruits	SD
Cumin oil	*Cuminum cyminum*	Fruits	SD
Cypress oil	*Cupressus sempervirens*	Needles, twigs	SD
Dalmatian sage oil	*Salvia officinalis*	Flowering tops	SD
Dill seed oil	*Anethum graveolens*	Fruits	SD
Dill weed oil	*Anethum graveolens*	Aerial parts	SD

Elecampane oil	*Inula helenium*	Roots	SD
Eucalyptus citriodora oil	*Eucalyptus citriodora*	Leaves, terminal branches	SD
Eucalyptus oil	*Eucalyptus globulus*	Leaves, terminal branches	SD
Fennel oil	*Foeniculum vulgare*	Fruits	SD
French lavender oil	*Lavandula angustifolia*	Flowering tops	SD
Galbanum oil	*Ferula galbaniflua*	Exudate	WD
Galbanum resinoid	*Ferula galbaniflua*	Exudate	Ext.
Geranium oil	*Pelargonium graveolens*	Herbaceous parts	SD
Guaiacwood oil	*Bulnesia sarmienti*	Wood	SD
Helichrysum italicum oil	*Helichrysum italicum*	Flowring tops	SD
Hyssop oil	*Hyssopus officinalis*	Flowering tops, leaves	SD
Japanese mint oil	*Mentha arvensis*	Whole aerial plant	SD
Jasmin concrete & absolute	*Jasminum officinalis*	Flowers	Ext.
Java citronella oil	*Cymbopogon winterianus*	Whole aerial plant	SD
Juniper oil	*Juniperus communis*	Fruits	SD
Laurel leaf oil	*Laurus nobilis*	Leaves	SD
Lavandin abrialis oil	*Lavandula angustifolia x L.latifolia*	Flowering tops	SD
Lemon oil	*Citrus limon*	Pericarp	Exp.
Lemon petitgrain oil	*Citrus limon*	Leaves, twigs	SD
Lemongrass oil	*Cymbopogon citratus*	Whole aerial parts	SD
Lemongrass oil	*Cymbopogon flexuosus*	Whole aerial plant	SD
Lime oil	*Citrus aurantiifolia*	Pericarp	Exp.
Litsea cubeba oil	*Litsea cubeba*	Fruits	SD

Lovage oil	*Levisticum officinale*	Aerial parts	SD
Mace oil	*Myristica fragrans*	Arils	SD
Mandarin oil	*Citrus reticulata*	Pericarp	Exp.
Mandarin petitgrain oil	*Citrus reticulata*	Leaves, twigs	SD
Manila elemi oil	*Canarium luzonicum*	Exudate	WD
Marjoram oil	*Origanum majorana*	Leave, flowering tops	SD
May rose absolute	*Rosa centifolia*	Flowers	Ext.
Melissa oil	*Melissa officinalis*	Leaves	SD
Myrrh oil	*Commiphora sp.*	Exudate	WD
Myrtle oil	*Myrtus communis*	Leaves	SD
Neroli oil	*Citrus aurantium* subsp. *aurantium*	Flowers	WD
Niaouli oil	*Melaleuca quinquenervia*	Leaves	SD
Nutmeg oil	*Myristica fragrans*	Seeds	SD
Oakmoss concrete & absolute	*Evernia prunastri*	Lichen	Ext.
Olibanum oil	*Boswellia* sp.	Exudate	WD
Oregano oil	*Origanum onites, O.majorana, O.vulgare* subsp. *hirtum, O.minutiflorum, Thymbra spicata*	Aerial parts	SD
Orris oil	*Iris pallida*	Femented rhizome	SD
Osmanthus concrete & absolute	*Osmanthus fragrans*	Flowers	Ext.
Palmarosa oil	*Cymbopogon martini* var.	Whole aerial plant	SD

	motia		
Parsley fruit oil	*Petroselinum sativum*	Fruits	SD
Patchouli oil	*Pogostemon cablin*	Leaves	SD
Pennyroyal oil	*Mentha pulegium*	Whole aerial plant	SD
Peppermint oil	*Mentha x piperita*	Whole aerial plant	SD
Pimento berry oil	*Pimenta dioica*	Fruits	SD
Pimento leaf oil	*Pimenta dioica*	Leaves	SD
Pumilio pine oil	*Pinus mugo*	Needles, twigs	SD
Rose concrete & absolute	*Rosa damascena*	Flowers	Ext.
Rose oil	*Rosa damascena*	Flowers	WD
Rosemary oil	*Rosmarinus officinalis*	Leaves, flowering tops	SD
Rosewood oil	*Aniba rosaeodora* var. *amazonica, A.parviflora*	Wood	SD
Rue oil	*Ruta graveolens*	Whole plant	SD
Sandalwood oil	*Santalum album*	Wood	SD
Sassafras oil	*Ocotea pretiosa*	All parts	SD
Spanish oregano oil	*Corydothymus capitatus*	Flowering branches	SD
Spanish wild thyme oil	*Thymus mastichiana*	Flowering tops	SD
Spearmint oil	*Mentha spicata*	Whole aerial plant	SD
Spike lavender oil	*Lavandula latifolia*	Flowering tops	SD
Star anis oil	*Illicium verum*	Seeds	SD
Storax oil, American	*Liquidambar styraciflua*	Balsam	WD
Storax oil, Levant	*Liquidambar orientalis*	Balsam	WD
Sweet orange oil	*Citrus sinensis*	Pericarp	Exp.

Tagetes oil	*Tagetes minuta*	Whole plant	SD
Tansy oil	*Tanacetum vulgare*	Whole plant	SD
Tarragon oil	*Artemisia dracunculus*	Flowering tops	SD
Tea tree oil	*Melaleuca alternifolia*	Leaves, terminal twigs	SD
Thyme oil	*Thymus vulgaris, T.zygis*	Herbal parts	SD
Treemoss concrete & absolute	*Evernia furfuracea*	Lichen on pine branches	Ext.
Trilobed sage oil	*Salvia fruticosa*	Leafy branches	SD
Tuberose concrete & absolute	*Polyanthes tuberosa*	Flower buds	Ext.
Vanilla absolute	*Vanilla planifolia*	Cured beans	Ext.
Verbena oil	*Lippia citriodora*	Leaves	SD
Vetiver oil	*Vetiveria zizanioides*	Roots	SD
Violet leaf absolute	*Viola odorata*	Leaf	Ext.
Ylang-Ylang oil	*Cananga odorata*	Flowers	SD

SD= Steam Distillation, WD= Water Distillation, DD= Dry Distillation,
Exp.= Expression, Ext.= Extraction

ESSENTIAL OIL EXTRACTION FROM NATURAL PRODUCTS: MODERN METHODS

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I. INTRODUCTION

Essential oils are mixtures of volatile compounds biosynthesized by living organisms or produced by processing the material such as fermentation. They can be liberated from an aromatic matrix by distillation, expression or extraction.

Conventional essential oil extraction techniques such as water distillation, steam distillation, water and steam distillation, expression, solvent extraction have been dealt with in the previous lecture. Now, I shall focus on the most recent techniques applied in the extraction of essential oils from natural products. The techniques I shall try to cover in my lecture may not necessarily be used for the commercial production of essential oils but are considered as valuable new applications for micro-analysis of essential oils in plant or other biological materials. These techniques can be listed as follows:

1. Headspace Trapping Techniques
    1.1. Static Headspace Techniques
    1.2 Vacuum Headspace Techniques
    1.3 Dynamic Headspace Techniques
        1.3.1 Closed-loop Stripping Method
        1.3.2 Direct Sampling Method
2. Solid Phase Micro Extraction (SPME)
    2.1 Headspace SPME
    2.2 Immersion SPME
3. Extraction with Liquified Gasses
    3.1 Supercritical Fluid Extraction (SFE)
    3.2 Phytosol® (Phytol) Extraction

4. Poroplast Technique
5. Likens-Nickerson Simultaneous Distillation-Extraction (SDE)
6. Microwave Distillation
7. Controlled Instantaneous Decompression (CID)
8. Thermomicrodistillation
9. Microdistillation
10. Molecular Distillation (Short-Path Distillation)
11. Microscale Spinning Band Distillation
12. Membrane Extraction Techniques

    12.1Pervaporation

    12.2Liquid Membrane Extraction

II. DRAWBACKS OF DISTILLATION

1. Common distillation techniques are capable of isolating volatile chemicals having boiling points within a relatively narrow range. For this reason, losses of both very light and heavy fractions are often unavoidable during the distillation of natural aromatic materials.
2. During distillation, chemicals contained in natural materials may undergo thermal reactions and artefacts are often formed. Therefore, distilled oil contains both natural compounds as well as their thermal decomposition products. As water and steam distillation processes are universally accepted such oils are considered as natural pure oils. The oil distilled may not therefore represent the true odour of the aromatic material from an olfactory point of view.
3. Distillation does not separate closely boiling substances regardless of differences in their chemistry.
4. Distillation is inappropriate if the recovery of trace amounts from a bulk mixture is desired even when the boiling points are markedly different.
5. Distillation is quite inefficient in recovering aroma chemicals from dilute aqueous media, such as hydrosols and fruit juices.

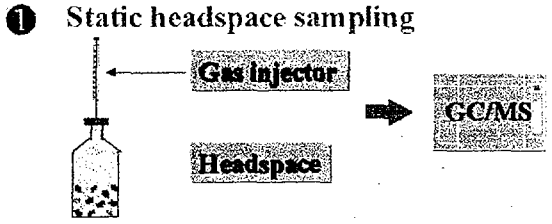
1. HEADSPACE TRAPPING TECHNIQUES

Headspace trapping techniques are used to capture the odour emitted by aromatic materials. Odour can be sampled either directly or trapped on an adsorbent material. The trapped odorous components can be freed by solvent extraction or thermal desorption prior to analysis by modern instrumental techniques.

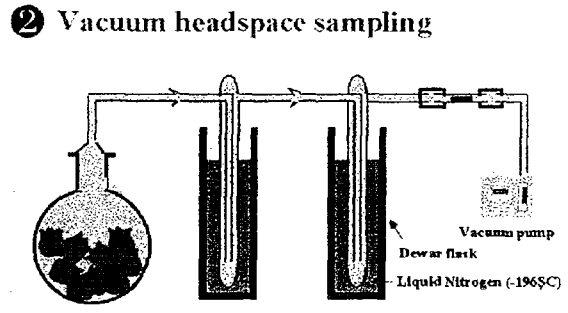
Headspace trapping techniques can be classified as follows:

1. Static headspace sampling
2. Vacuum headspace sampling
3. Dynamic headspace sampling

1.1 In the **static headspace sampling technique**, analyte is kept in a closed vial and the air (headspace air) above the solid or a liquid sample is sampled by a gas syringe or directed on to the gas chromatography column or more usually first concentrated on an adsorbent trap. Heat may be applied to enhance the release of volatiles from the analyte. Although a very rapid method, it does not give a comprehensive profile of the volatiles as some important compounds may be missed.



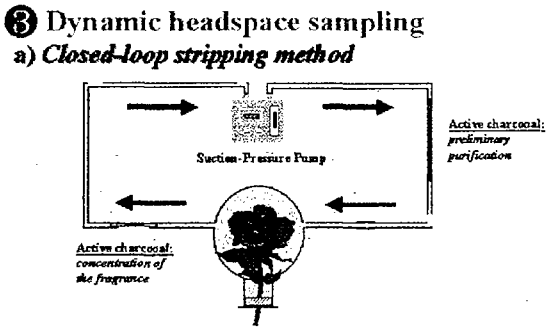
1.2 **Vacuum headspace sampling technique** involves suction of the headspace air *via* a vacuum pump through condensers cooled with liquid nitrogen to condense odorous principles. This technique is also used by some perfumery companies for commercial scale production of fragrances.



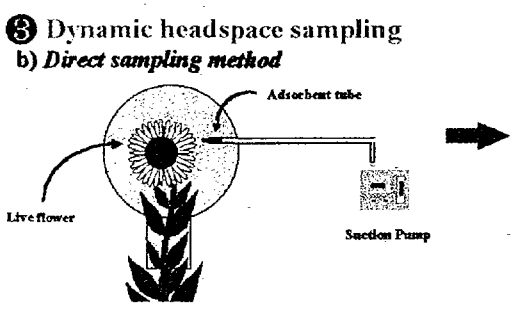
1.3 **Dynamic headspace sampling** involves sweeping the analyte with a stream of air or gas and adsorption of the volatiles from the gas stream on an adsorbent trap. Hydrophobic traps are preferred Tenax, Porapak Q, Chromosorb 101-105 or activated charcoal being the most popular. Volatiles from the trap can either be removed by thermal desorption or by solvent extraction. In fragrance analysis solvent extraction is the preferred method.

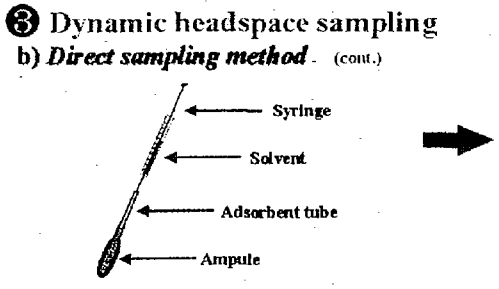
Dynamic headspace sampling techniques can be applied in one of the following ways:

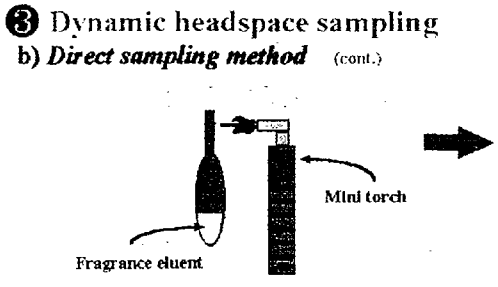
1.3.1. ***Closed-loop Stripping Method:*** The analyte is placed in the middle of a closed circuit system in which clean air is continuously pumped through the analyte and odorous components in the headspace air are trapped on an adsorbent material.

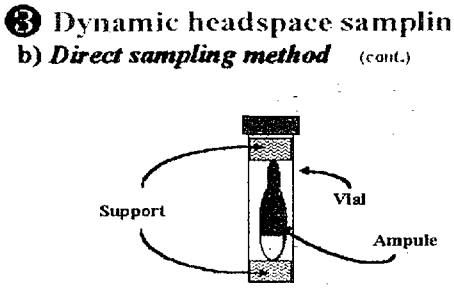


1.3.2. ***Direct Sampling Method:*** The analyte, which may be a living flower, is placed in a glass container and the headspace air is sucked *via* a suction pump through an adsorbent tube in which the odorous components are trapped.





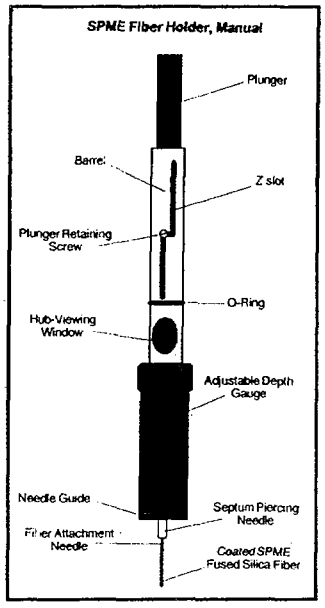




2. SOLID PHASE MICRO EXTRACTION

Solid Phase Micro Extraction (SPME) is a micro sampling technique which has found wide application in environmental as well as flavour and fragrance research. It is a solvent-free method which is used to trap flavours and fragrances either from aqueous samples (immersion SPME) or from the vapour space above a liquid or a solid sample (headspace SPME). Recently its applications to trap odours of living flowers and fragrance applied on skin have been reported.

SPME functions on the principle that analytes adsorbed on a high-boiling polymer film coated on a fused silica fiber. Since it is an equilibrium technique it does not extract analytes exhaustively, hence does not disturb the concentration of a liquid. Agitation of the liquid facilitates rapid extraction. For the headspace samples, faster mass transport rates are attained and volatiles are extracted faster than semi-volatiles.



Analytes adsorbed on the fiber are thermally desorbed directly in the injection port of a gas chromatograph into a capillary column for analysis. SPME assembly consists of a specially designed injector (also called SPME holder) which enables the coated fiber to move in and out of the needle. During injection into a vial for sampling or injection port for analysis, the fiber is concealed in the needle and exposed during sampling and thermal desorption.

Several polar coatings are commercially available such as polydimethylsiloxane (PDMS), polyacrylate (PA), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carbowax (CW), carbowax/divinylbenzene (CW/DVB), carboxene/polydimethylsiloxane (C/PDMS).

SPME can be used with the following combinations: gas chromatography/flame ionization or nitrogen phosphorous or flame photometric or atomic emission detectors, or with mass spectrometry or Fourier-transform infrared spectrophotometry. Multidimensional gas chromatography/mass spectrometry system is used for enantioselective analysis of chiral flavour and fragrance components, olfactory gas chromatography is another area of application. SPME can also be used in combination with HPLC and capillary electrophoresis. In short, it can be adapted to use with any instrumental analytical technique available.

A fragrance emits its aromatic molecules into the air based on their diffusivity. Diffusivity is independent of molecular weight, boiling point, odour treshold or odour value. Molecules with high diffusivity are continuously emitted to form an aura around the source. Conventional headspace sampling and SPME can both be used efficiently to trap the odour components. Since trapping of volatile molecules on adsorbents require elution with a solvent in conventional headspace techniques, success of the experiment depends on the adsorbing power of the adsorbent

and eluting power of the solvent used. Impurities may come from the solvent or the adsorbent material, confusing an inexperienced analyst with the detection of unusual compounds.

SPME can be efficiently used for headspace sampling since contaminations or impurities mentioned above do not occur and highly comparable results are obtained. Therefore, SPME has proved itself as a highly efficient and simple sample preparation technique which may be expected to replace conventional headspace techniques.

3. EXTRACTION WITH FLUIDIZED GASSES

3.1 SUPERCRITICAL FLUID EXTRACTION (SFE)

The critical point of a pure substance is defined as the highest temperature and pressure at which it can exist in vapour-liquid equilibrium. At pressures and temperatures above this point, a single homogeneous fluid which forms is said to be supercritical. A substance in the supercritical phase is neither a true liquid nor a true gas and has some properties of each. These properties can be varied within wide limits by a suitable choice of pressure and temperature. In other words, supercritical fluids can dissolve a wide variety of organic compounds and their solvent power can be raised near their critical points by small pressure and temperature changes. At high temperatures and low pressure the density is low and the supercritical fluid behaves more like a gas, but at low temperature and high pressure the density is increased and it assumes the properties of a liquid. Pressure-temperature relationship of carbondioxide is illustrated in Fig.1. Supercritical carbondioxide (sCO_2) is considered to exist at pressures above 73.8 bar (or atm or 7.4 Mpa) and temperatures above 31.2°C.

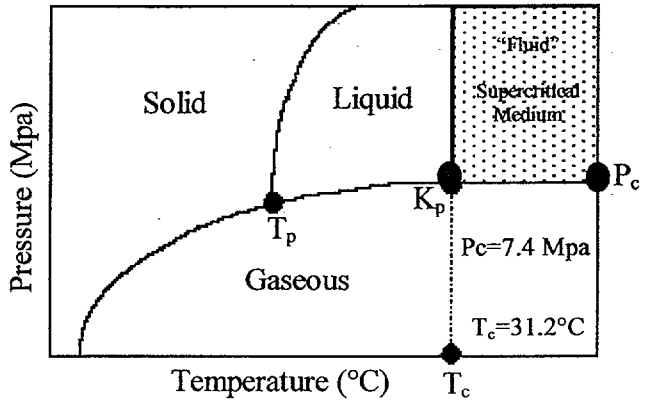


Figure 1. Pressure-Temperature Relationship of CO_2

Supercritical fluids possess superior mass transfer properties by virtue of their low viscosities and high solute diffusivities along with the ability to penetrate microporous materials. Among the variety of gasses that can be rendered supercritical, carbondioxide has become the most popular and most widely used since it is harmless, non-flammable, cheap, abundantly

available, non-corrosive and has a low boiling point. It possesses lipophylic solvent properties and its dissolution capacity can be controlled by modifying temperature and pressure. The polarity of supercritical carbondioxide can be modified by the addition before or during the process polar solvents such as water, methanol, ethanol, acetone. They are normally used at low concentrations, *i.e.*, 1-5 mole%. All these enable fluid carbondioxide to acquire the selectivity of any known or unknown solvent.

The principle and procedure of supercritical fluid extraction is illustrated in Fig. 2. The system consists of a CO_2 source, a pump, a preheater, an extraction vessel, a reduction valve (or restrictor) and a collecting vessel or separator. The quality of CO_2 is critical. High-purity carbondioxide is required for successful operations. It must be free from water, hydrocarbons and halocarbons. To pressurize CO_2 to supercritical state a high pressure pump is necessary. To do this, reciprocating (like those used in HPLC) or syringe pumps can be used. Syringe pumps are prefered since they can provide pulseless flow and can be easily filled with CO_2.

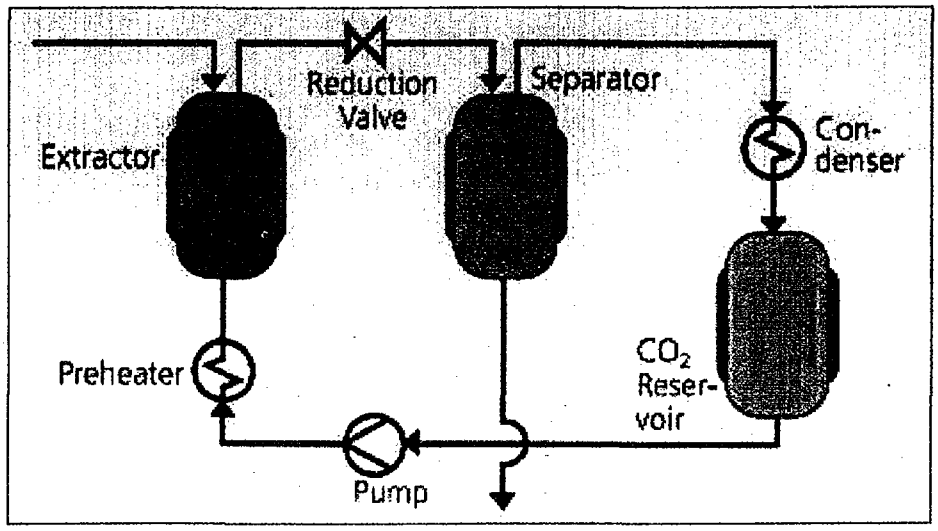


Figure 2. Supercritical Fluid Extraction

Sample to be extracted must be reduced in size to increase the surface area to enhance extraction efficiency. It may, in some instances, be necessary to adjust its pH. If the sample is semi-solid, gel or liquid, it has to be immobilized on a solid support such as diatomaceous earth and anhydrous sodium sulfate. Liquid products can also be processed as such by feeding into a counter-current extraction column from the top. They slowly fall to the bottom in counter-current against the rising supercritical gas. At the base of the column, the residue is discharged and the separated components are separated out in the separator. In this case, the evaporator must be replaced by a counter-current extraction column. This process can be run continuously.

SFE is performed in a high pressure system in which the solute is kept in contact with supercritical carbondioxide fluid for 20-30 minutes. As the supercritical carbondioxide passes through the restrictor. The change in pressure in the restrictor causes the pressure of the supercritical fluid to decrease. Depressurizing at a separate compartment brings the fluid into gaseous state which can either be condensed back to fluid for further use or released to the atmosphere. In commercial operations the former choice is preferred. At reduced temperature and pressure conditions the extract precipitates out in the separator. The extract-free CO_2 stream, leaving the separator is then recycled back to the extractor. The analyte may be collected in a solvent or an analyte trap in the case of small scale operations.

SFE produces extracts which represent the almost true composition of the odour of the sample (*e.g.*, spice or aromatic material) due to the fact that no oxidation takes place and high temperatures are not attained during the process and decomposition products are not formed. An undesirable feature may be the hydrolysis of esters due to acidic nature of fluid carbondioxide.

Supercritical carbondioxide is useful for non-polar to semi-polar compounds. As mentioned above, modifiers can be added prior to extraction by adding them directly on the sample or during the process through a separate syringe pump in order to potentiate the polarity of the solvent. This enables supercritical carbondioxide to extract polar compounds as well.

Liquid carbondioxide is a good solvent for compounds whose molecular weight is below 250. This includes compounds such as hydrocarbons and oxygenated monoterpenes found in essential oils. The solubility for compounds whose molecular weight is between 250 and 400 in liquid carbondioxide is at best extremely poor, while compounds with molecular weights over 400 are almost insoluble in liquid carbondioxide. This means that liquid carbondioxide cannot extract waxes, polyphenols, carbohydrates, carotenoids, chlorophylles, etc.

Supercritical CO_2 extraction technique can be used to deterpenate citrus oils. To do this, citrus oil is adsorbed on a silica gel column and sCO_2 is passed to elute the hydrocarbons while oxygenated constituents are retained on silica gel at low temperature and low pressure. Elution of the oxygenated constituents is achieved by increasing the temperature and pressure of the CO_2.

For the extraction of essential oils, supercritical carbondioxide alone can be used. Optimum operating conditions can be decided after experiments. For rosemary oil, best oil yields were attained at 40°C and 100 bar.

If SFE is compared with conventional techniques such as solvent extraction and steam distillation, many advantages over the conventional techniques are apparent.

Solvent extraction technique has the following drawbacks. It is time consuming, labour-intensive, requires large volume of solvent, leaves solvent residue and is environmentally unfriendly.

Disadvantages of steam distillation technique are as follows. It is time consuming, energy intensive, needs large amount of sample and is not suitable for thermally unstable compounds.

On the other hand, SFE can offer the following advantages:

1. Higher diffusion rates than liquid solvents
2. Lower viscosities than liquid solvents
3. Higher vapour pressure than liquid solvents
4. Solubility and (to some extent) selectivity can be controlled by modifying the parameters
5. Low polarity of CO_2 can be changed with modifiers (or co-solvents)
6. Suitable for heat-labile compounds
7. Does not leave any solvent residue.

Disadvantages of SFE are as follows:

1. CO_2 has low polarity and hence cannot extract polar compounds. However, in the case of essential oils, this is not a disadvantage since most essential oil components can be extracted with non-polar solvents.
2. Presence of water may cause problems. However, a small amount of water is added as modifier to increase polarity.
3. Unpredictability of matrix effect.
4. Expensive. Requires highly specialized and expensive equipment

Extraction with supercritical carbondioxide has been used commercially for many years. Typical examples include decaffeination of coffee and tea, production of nicotine/tar-free tobacco, production of extracts from tobacco, hops, spices, fats and oils both from plant and animal sources, and cholesterol-free food products. In recent years, the technique is utilized for sample preparation of residues such as pesticides, herbicides, fungicides, aflatoxins in food and plant materials as well as for the extraction of phytochemicals, essential oils and flavour and fragrance components.

3.2 *PHYTOSOL EXTRACTION TECHNIQUE*

The use of refrigerant gasses for extraction of plant materials has been known for some time. Harmful effects to the ozone layer of chloro fluoro hydrocarbons negatively affected their use. Recently, a patented method has been developed which involved the use of a refrigerant gas harmless to the environment. The method called "advanced Phytonics" use 1,1,1,2-tetrafluoroethane with or without modifiers. The solvents are named "Phytosols®". Phytosol A consists only of the gas, Phytosol B is a mixture of the gas with butane/isobutane, Phytosol C consists of the gas with dimethylether as modifier. Boiling point of 1,1,1,2-tetrafluoroethane or HFC 134 as known in trade is –26.2°C. It is a non-toxic, odourless gas whose pressure in liquid state at room temperature is 5 bar which can be compared to the pressure of a bottle of Champagne (6 bar). Plant material is kept in contact with this fluid at room temperature. Due to their low viscosity phytosols have good penetrating properties into the material to be extracted. Sucking the extracting fluid into the collection vessel due to pressure drop, gas can either be released to the atmosphere or compressed for recycling or stored for future use (Fig. 3). The extract so obtained is said to be more representative of the flavour properties of the original material, in higher yields and at lower cost of production compared to conventional extraction and distillation techniques and SFE. Phytosol extracts resemble more to essential oils than concretes since Phytosols essentially extract non-polar materials. Even with modifiers Phytosols cannot extract polar substances. Phytosol extracts have been approved for use in foods in England and EU. This technique is also called Phytol Extraction Technique.

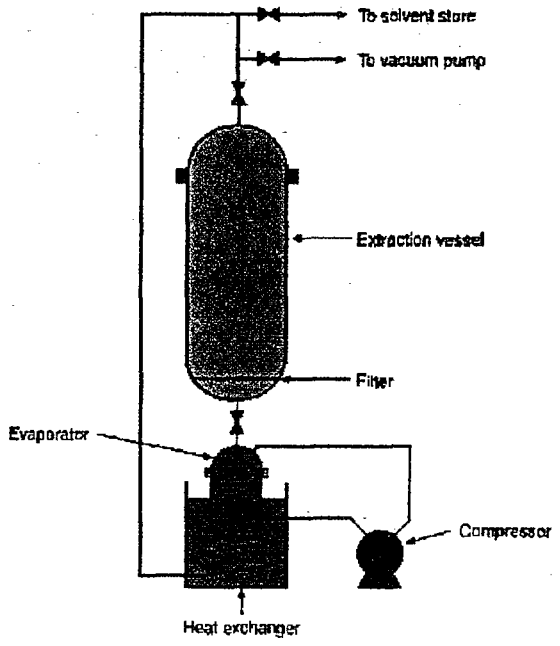


Figure 3. Phytosol ® Extraction

4. POROPLAST EXTRACTION TECHNIQUE

Liquid-liquid extraction can be defined as a partition technique where the constituents of a solution are separated due to their different partition coefficients between the two immiscible liquids. Its application to essential oils is possible in the case of aromatic waters or distillation waters which are usually decanted and discarded during the distillation process.

If such waters which contain water soluble aromatic constituents of an essential oil are extracted with an immiscible solvent such as *n*-hexane, chloroform, methylene chloride, etc., after removal of the solvent under vacuum a certain amount of the distilled oil can be recovered. This is possible to achieve at small scale operations using a separating flask at laboratory.

However, industrial applications are more complex. In all liquid-liquid contactors used in industry, one phase is dispersed in the other in the form of spherical droplets. Spherical shape has a limited surface area for a given volume. By agitating the solution, size reduction of the droplets can be achieved but then emulsion formation obstruct phase separations. Sophisticated and expensive equipment are required to solve this problem. However, since small volumes are used in flavour and fragrance industries, such technologies may not prove to be cost-effective.

Poroplast extraction technique was invented in Russia in 1972. It is a liquid-liquid extraction technique. However, in this case, one of the liquid phases is immobilized. Transfer of solute takes place when a moving aqueous phase passes through a bed of stationary phase with low polarity non-specifically held on the hydrophobic surface of a porous support. Depending on the purpose and experimental design, water soluble components can be extracted into an organic phase or vice versa. This technique has found industrial application both for recovery of essential oils from aqueous media as well as for the preparation of terpeneless oils. The technique can be likened to partition chromatography.

Porous support which holds the stationary phase is teflon (PTFE=Polytetrafluoroethylene) powder made into a sponge through a special heat treatment. For the extraction of essential oil from aqueous dispersions, teflon support is packed in a column and wetted by an organic solvent. Aqueous dispersion is pumped into the column. Aromatic constituents are transferred from the aqueous phase to the organic phase. After a certain amount of aqueous phase is passed, the organic phase saturated with oil is replaced by passing fresh solvent through the column. The process continues until the planned amount of aqueous dispersions is treated. When the process is over, organic and aqueous layers form in the separator. The exhausted aqueous phase is discarded and the organic phase saturated with aroma constituents is continuously sent to the product recovery unit where the recovered essential oil is collected and the stripped solvent is returned to the solvent reservoir (Figure 4).

Due to efficiency of the extraction process, aromatic chemicals can be recovered even from very dilute aqueous dispersions (100-500 ppm). The amount of solvent used is only about 0.1-1% of the aqueous phase in the process.

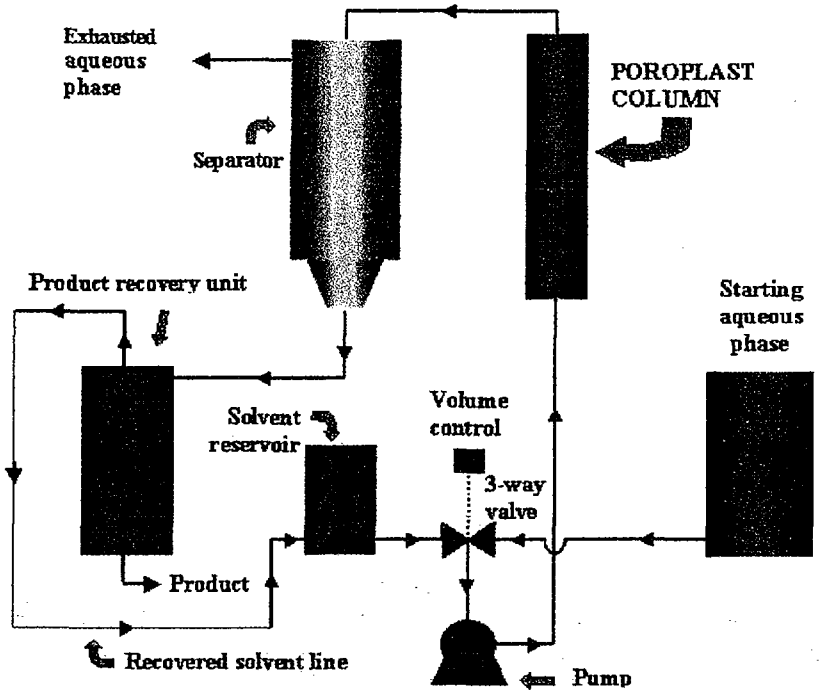


Figure 4. Poroplast Extraction Technique

Another industrial application of the poroplast extraction technique is deterpenation of citrus oils. The process involves initial coating of the porous teflon support with citrus oil. The oil is, then, eluted with aqueous alcohol which pushes oxygenated constituents of the oil leaving behind hydrophobic non-polar hydrocarbons which are retained on teflon. After reduction of alcohol in the aqueous alcohol phase to ca. 30%, the recovered alcohol is returned to the process and the aqueous dispersion of oil is passed through a second poroplast column which had been charged with a low-boiling organic solvent such as methylene chloride. The oxygenated constituents are retained in the organic stationary phase as described in the first technique. The stripped organic phase is then vacuum distilled to remove the solvent leaving behind the deterpenified oil.

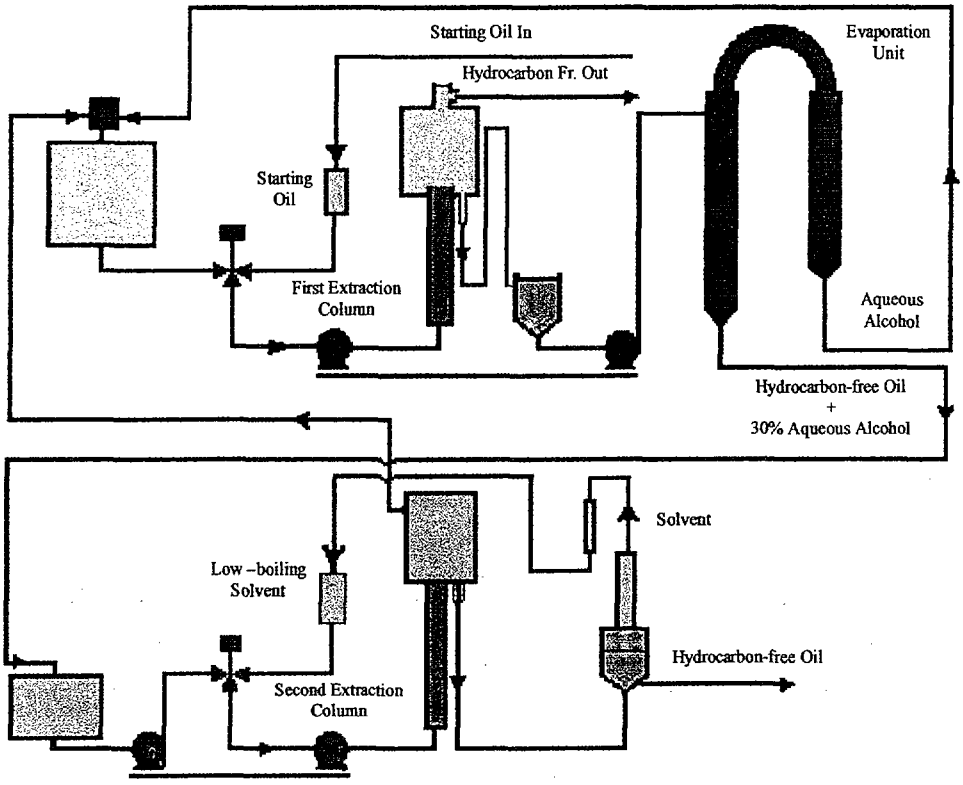


Figure 5. Poroplast Deterpenation of Citrus Oil

5. LIKENS-NICKERSON SIMULTANEOUS DISTILLATION-EXTRACTION (SDE) TECHNIQUE

This technique utilizes a specially designed all glass distillation system which allows the simultaneous countercurrent extraction of the distillate with an immiscible solvent. In this ingenious design, aromatic plant material is distilled with water in the distillation flask and the immiscible solvent is boiled in the receiver flask. Both vapours condense on the same condenser in the middle of the assembly and mix in a central port. Organic volatiles in the distillate are continuously extracted with the solvent which flows back to the receiver flask. Water also overflows back to the distillation flask. Distillation and simultaneous liquid-liquid extraction continues until the volatiles in the distillate are exhausted. The exhaustively extracted and continuously concentrated volatiles in the organic solvent can be analyzed directly by a GC or GC/MS system. The major advantage of the system is that it requires only a small amount of material.

Two different designs of this apparatus are available for high-density (*e.g.*, chloroform, methylene chloride) and low-density (*e.g.*, hexane, heptane) solvents (Fig. 6). SDE is widely used in flavour and fragrance, and food industries as a micro sample preparation technique.

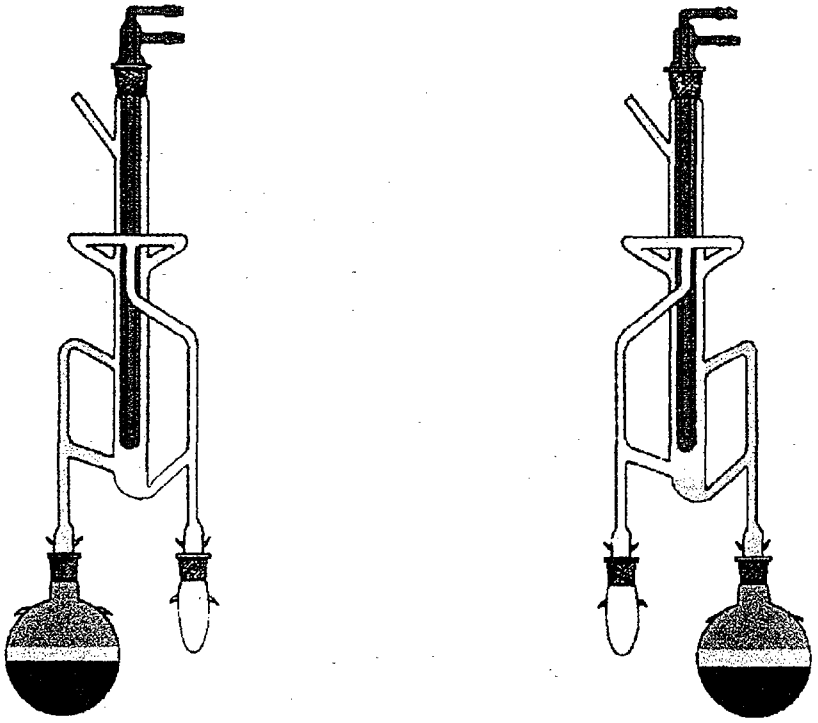


Figure 6. High and Low Density SDE Apparatus

6. MICROWAVE DISTILLATION

Microwave extraction technique has been successfully used to extract aromatic constituents from aromatic materials using microwave transparent solvents like hexane, ethanol or their mixtures, etc. Microwave energy causes a sudden increase in temperature and oil glands in aromatic plant material are ruptured to release oil to the surrounding solvent. Extraction is completed in a short time with good yields. However, the complete removal of solvent poses a serious problem.

Microwave distillation is applied on fresh plant materials. When they are exposed to microwave energy in a closed system water in the material causes generation of steam which is enough to distil the oil released from ruptured oil glands through a cooler to a receiver or into a cooled trap of methylene chloride to recover the oil.

In the pioneering paper by Craveiro et al, microwave distillation is compared with steam distillation using fresh *Lippia sidoides* leaves. 30-40 g of material is microwave-distilled for 5 minutes to complete the recovery of oil compared to 60-90 minutes of steam distillation.

These methods have not yet been commercialized.

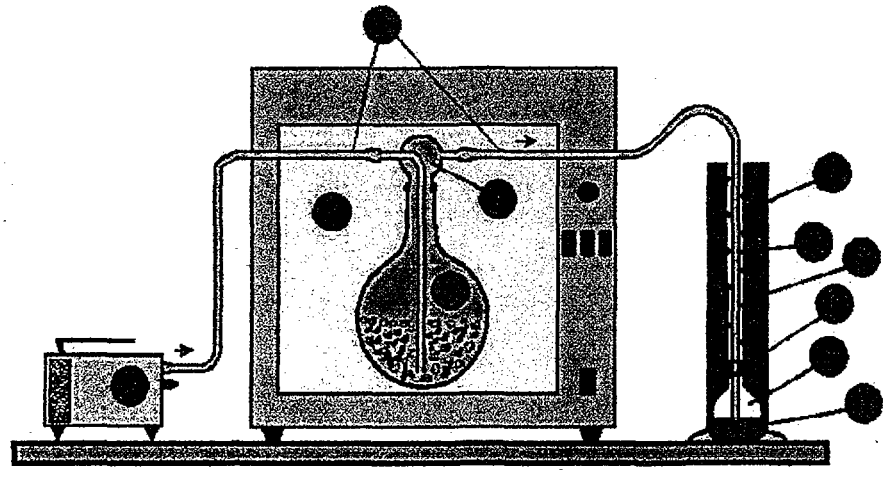


Figure 7. Microwave Distillation

7. CONTROLLED INSTANTANEOUS DECOMPRESSION (CID)

This new technique was described only last year for the extraction of essential oil from rosemary leaves.

The process involves subjecting the partially humidified plant material for a short period of time to a steam pressure varying from 0.5 to 3 bar followed by a rapid decompression to a vacuum (about 15 mbar) for 200 msec each time. The vapour in the plant material created by autovaporization produces a mechanical strength which ruptures the oil cells. Due to pressure differences in the extractor and vacuum chamber, in each opening of the pressure valve oil-rich vapour is instantaneously sucked into the vacuum chamber where it instantly condenses (Fig. 8). This continues until pressure difference in both chambers becomes equal. The oil so obtained is devoid of hydrocarbons. In other words, it is rich in oxygenated compounds which is a desired feature as monoterpene and sesquiterpene hydrocarbons are believed to spoil odour quality of a given essential oil and negatively affect its alcohol-soluble properties.

A pressure of the order of 3 bar and a process time of 10 minutes was found to be sufficient to recover 97% of essential oil from a plant material.

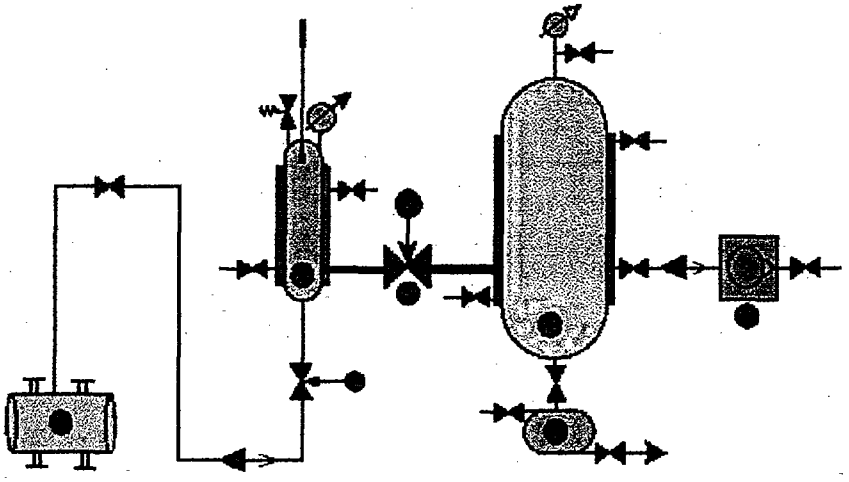


Figure 8. Schematic Diagram of Controlled Instantaneous Decompression Unit

8. THERMOMICRODISTILLATION (TAS Method)

The so-called TAS (=Thermomikro-Abtrenn-, Transfer- und Auftrage- Verfahren für Substanzen) oven is applied to materials that become volatile at high temperatures without decomposition was developed by Stahl (4,5). In this very simple technique developed as an accessory to Thin Layer Chromatography which had also been developed by Stahl, narrow part of a pasteur pipette is plugged with glass wool, small amount of aromatic plant powder is placed in the middle and a few miligrams of humid indicator silica is positioned just before the stopper (aluminium foil). The system is placed in a heating device (TAS oven) specially designed to embrace the pipette fully and heated. Heat generates steam by evaporating water from silica, which strips the volatiles in the plant material and the steam rich in volatiles condense on the surface of a TLC plate where it leaves a spot (Fig. 9). When developed in a suitable solvent system essential oil components are separated.

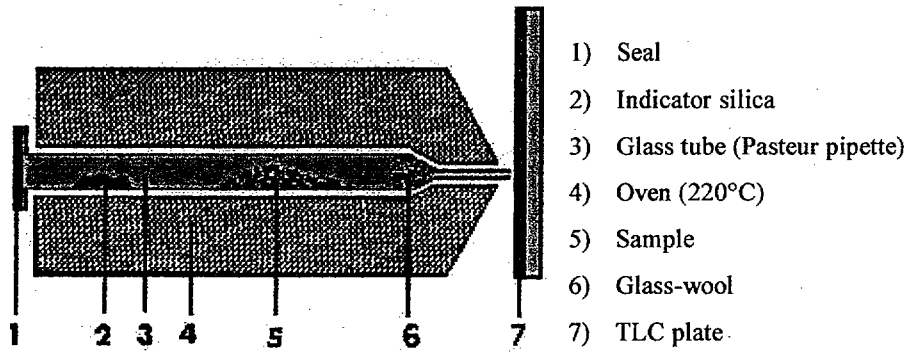


Figure 9. Schematic View of TAS Unit

9. MICRODISTILLATION

The company Eppendorf offers a new micro-distillation system (MicroDistiller) allowing rapid, programmable distillation of up to six samples at the time. A micro-capillary is used to connect the sample vial with the phase separation vial. The system has 6 heating and 6 cooling units for parallel operations. The material to be distilled (150-500 mg) is placed in a 15 ml capacity vial with 10 ml of water and sealed with a cap having a small slit on top. The vial is placed in a heating compartment. The vial is connected to the receiving vials placed in cooling holes by a short fused silica capillary column. The receiving vial contains 1 ml of distilled water, approx. 1-2 g of NaCl to break the formation of emulsion and approx. 300 µl of organic solvent to extract organic volatiles. The organic layer is decanted and directly analysed.

Fully automated system enables overall control of the distillation process. A thermal gradient can be programmed for distillation. This is, normally, not possible to achieve in the usual steam or water distillation processes. Distillation period varies according to the temperature programme and satisfactory distillations can be performed between 1-2 hours. Electronic operation permits different distillation modes and programmes (99 programmes). Peltier technique eliminates the use of water for cooling. The apparatus occupies a small space and can be operated at bench-top connectable to a PC (Fig. 10).

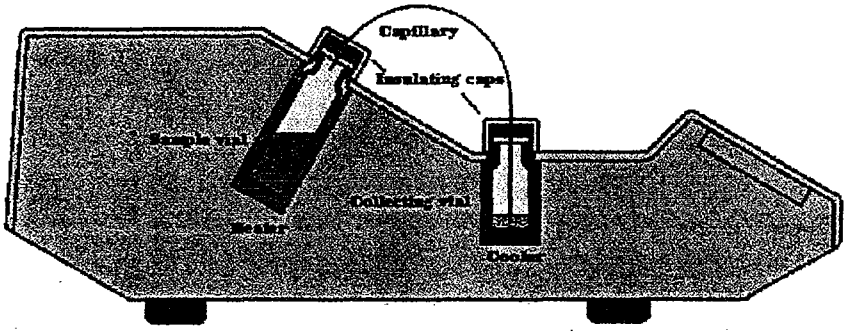


Figure 10. Schematic View of MicroDistiller Unit

Overall Advantages

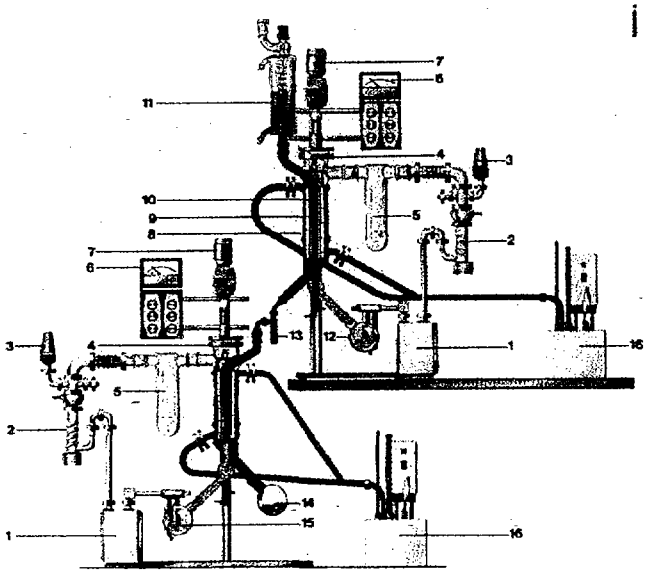
- requires less than 1 gr or 1 ml of material, which makes it excellent for working with herbarium samples.
- fast preparation and set-up.
- fast isolation and readiness for analysis.
- cheaper and more efficient in long term use
- easy operation

10. SHORT PATH DISTILLATION (Molecular Distillation)

Short path distillation is a continuous fractionating process without steam injection and the most preservative distillation method available. It is resorted to when traditional methods prove not to be sufficient. This separation method can either be used on micro-scale or industrial scale. In this method, low evaporating temperature and short residence time allow distillation of heat sensitive materials. Its main application is in the high vacuum range. The material is heated only for a short time and the temperature is very low which prevents decomposition. Central part of the equipment is a vertical, double jacketed cylindrical evaporator, which is heated by circulating heat

transfer oil. In the centre of the cylindrical evaporator, there is a condenser which circulates chilled water.

The material to be distilled is charged through the double-jacketed feed vessel positioned above the evaporator. The material is charged into the evaporator under vacuum using a valve which can be precisely adjusted. When the material flows down by gravity into the evaporator which is under high vacuum (*ca.* 10^{-3} mbar) [*ca.* 0.0007 mmHg] created by both rotary and diffusion pumps, rotating roller-wipers distribute the material on the whole heated surface of the evaporator and a very thin film forms throughout the operation. While the evaporated molecules reach the condenser through a short path, the vapour is condensed and collected separately. The non-evaporated liquid is collected in another flask. Depending on the vacuum applied, evaporator temperature, rotating speed of the roller-wipers and physical properties, average residence time of the material on the surface of the evaporator cylinder is very short (*ca.* within 1 minute). The most volatile parts of the vapour, which are not condensed on the internal condenser surface, are removed *via* vacuum nozzle and trapped in a cold trap to avoid contamination of the vacuum pump. The operating condition is that the free path of the molecule is longer than the distance between the heated and cooled surfaces. Therefore the probability of a vapour molecule colliding with another molecule is smaller than the probability to travel without collision to the cold surface resulting in efficient separation also called as molecular distillation by the inventors. Therefore, it is only possible to separate the material into two cuts except that the trapped into the cold trap, in single stage Short Path Distillation (SPD) unit. However, it is also possible to set-up multiple-stage unit for further fractions (Fig. 11).



1) Rotary-vane pump
2) Oil-diffusion pump
3) Gauging tube
4) Evaporation head
5) Cold trap
6) Vacuum-measuring unit
7) Variable-speed motor
8) Evaporator surface
9) Roller-wiper system
10) Internal condenser
11) Feed vessel
12) Product-receiving flask (Distillate I)
13) Pressure lock
14) Product-receiving flask (Residue)
15) Product-receiving flask (Distillate II, Final product)
16) Oil-circulation thermostats

Figure 11. Two-Stage Short-Path Distillation System

There are many different applications of SPD for a wide range of different products following different operating modes.

The main application areas are in flavour and fragrance, pharmaceutical and other chemical industries.

11. MICROSCALE SPINNING BAND DISTILLATION

Spinning-band column is an efficient fractional distillation unit. Microscale spinning band distillation system can purify and isolate very small amounts of material for analysis. This system provides a minimal pressure drop obtained with the spinning band configuration. In spinning band distillation unit, there is a inert close fitting helical band which is located inside the distillation column. This helical band is connected to a high-speed motor rotating at >3000 rpm. The spinning of the helical band mechanically forces the two phases to contact. This action causes excellent fractionation especially under vacuum. The spinning-band distillation column provides an high number of theoretical plates. The higher the spinning band rotates, the greater the number of theoretical plates resulting in better separation. One of the most important advantages is the separation of liquids with boiling points as close as 0.5°C. The other main advantage is its very small hold-up volume, typically 100 µl or less.

Micro-scale adiabatic spinning-band distillation column is 20 cm long with 7 mm bore size. 10 to 25 ml boiling flask sizes accommodate a wide range of sample volume. Micro-scale spinning-band distillation system can fractionate complex samples with its 30 theoretical plate efficiency maximum. The system uses 1 ml collecting vials in a rotating PTFE basket for fraction collection (Fig.12).

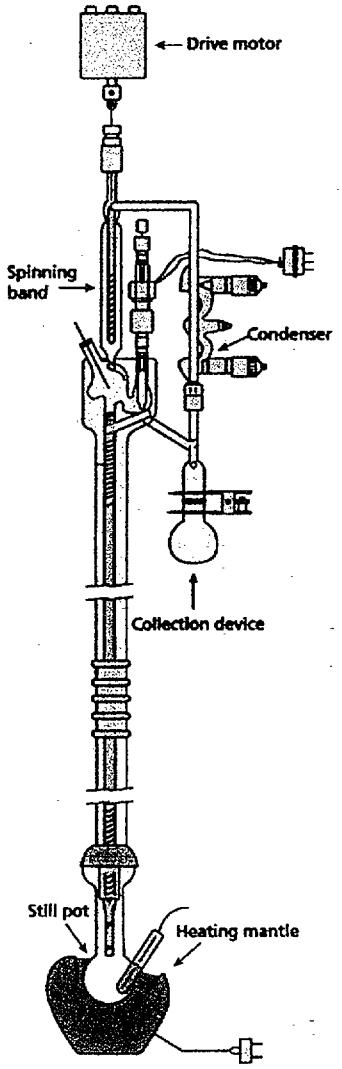


Figure 12. Spinning-Band Distillation Unit

12. MEMBRANE EXTRACTION TECHNIQUES

In all living systems the transfer of solutes is achieved through membrane processes. Cell membranes are very efficient and highly selective in performing extremely delicate micro-transports either through concentration differences or pressure differences.

Membranes used in separation processes have been developed in order to perform similar processes in industry. There are several membrane extraction and distillation techniques. Here, I shall mention only two which are relevant to our topic. These are pervaporation and liquid membrane extraction.

12.1 **Pervaporation** is a membrane distillation technique applied to aqueous or non-aqueous solutions containing small amounts of volatile impurities. It is used at commercial scale for the removal of water from 95% ethanol in order to bring its strength to over 99% by the use of hydrophilic membranes. For the removal of organic volatiles from aqueous solutions hydrophobic membranes are utilized.

The basic principle of pervaporation for the removal of volatile organic compounds (VOC) from aqueous media is as follows: Hot aqueous feed flows alongside a non-porous hydrophobic membrane which consists of a thin film of silicone rubber on teflon. Hydrophobic membrane prevents water from entering the matrix while organic matter diffuses through it. Vaporization occurs as the permeating volatile matter pass through the membrane due to vacuum applied on the other side of the membrane. Before the vacuum pump there is a condenser to recover the permeate as liquid (Fig. 13).

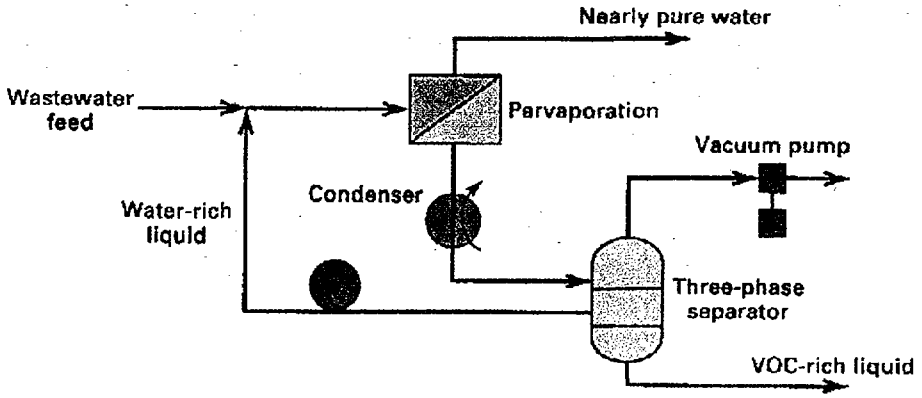


Figure 13. Schematic Diagram of Pervaporation Process

Pervaporation process is chiefly used for removal of small amounts of water from organic liquids and volatile organics (VOC) from water particularly in environmental cleaning of streams. Separation of flavouring volatiles from apple juice concentrate has been a recent application.

12.2 In membrane technology, the membrane used is not always a solid. A liquid film which permits selective solute transport can also be considered a membrane and the process is named "**Liquid Membrane Extraction (LME)**". It consists of three liquid systems (Fig. 14).

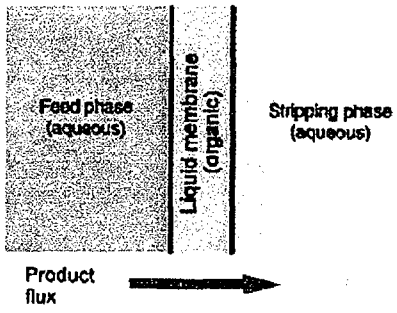


Figure 14. Liquid Membrane Extraction System

Liquid membranes selectively partition low molecular weight compounds into an organic phase. Separation is achieved by selective transport of the product into a second aqueous phase where it is retained.

Emulsion liquid membranes are successfully used for this purpose. They are formed by dispersing the aqueous stripping phase under high sheer within an organic solvent which forms a film around the stripping phase droplets. The emulsion thus formed is stabilised by the addition of a surfactant and is dispersed in the feed phase containing the solute (Fig. 15).

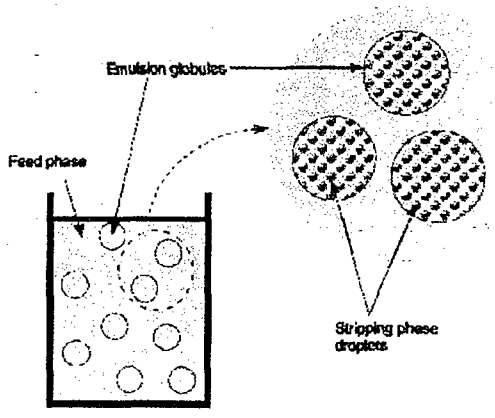


Figure 15. Emulsion Liquid Membranes

The two aqueous phase are not in physical contact with each other and the solute is transported into the stripping phase droplets by diffusion through the stabilised solvent film.

After the extraction process, a mixer-settler is used to recover the emulsion which is then broken to release the stripping phase containing the solute by applying an alternating electric field to the emulsion.

Solvents frequently used in liquid membrane extractions are paraffin, heptane, toluene or their mixtures which are mixed with a selective liquid carrier to enhance solute solubility.

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DISTILATION OF ESSENTIAL OILS AND SOME GENERAL CONSIDERATIONS

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GENERAL NATURE AND OCCURRENCE

Essential oils are the volatile constituents of plant materials which collectively are responsible for imparting the characteristic odour associated with the plant material itself.

Thus the pleasant odour of jasmines, the fresh smell of lemon grass, the lasting fragrance of sandalwood, the pungent sweetness so characteristic of mint or cinnamon are all attributable to the essential oils within the plant material.

The essential oils are, generally speaking, completely volatile in steam. These odour-giving constituents of plants occur in many different plant sites.

Chemical Composition: Essential oils being volatile in steam are distinguished thus from the chemically different "fixed" or "fatty" oils such as coconut oil or palm oil. These are composed predominately of triacylglycerols which have different or identical fatty acids esterified to the three hydroxyl positions on the glycerol molecule. The essential oils are mixtures of chemical entities of a variety of chemical types. Each essential oil has its own characteristic array of chemical compounds ranging in number from a few, to in some instances, as much as a hundred.

The most common chemical classes of compounds found in essential oils are the **Terpenoids**. Widely distributed throughout the plant kingdom these are a group of compounds of extraordinarily diverse chemical structure bound by a common origin. All of them are formed by the union of two or more chemical entities called "isoprene" or some variation of the isoprene unit itself.

The complexity arises out of the fact that the terpenoids that exist in an essential oil include the terpene hydrocarbons as well as their oxygenated derivatives such as alcohols, aldehydes, ketones, acids and esters.

Another class of compounds called by the general term **Phenylpropanoid**s also exist in many essential oils alongside the terpenoids. Phenylpropanoids are

compounds which have a phenol nucleus to which is attached a three carbon chain.

Besides these two classes of chemical compounds simple aromatic as well as aliphatic compounds also form the volatile fraction of the plants and hence are distilled over. Hence these, too, are included within what is recognised as, the chemical constituents of natural essential oils.

ECONOMIC IMPORTANCE OF THE ESSENTIAL OIL INDUSTRY

The essential oil industry may not be regarded as a major contributor to the national economy of many a producer country but it is evident that the number of consumer goods that are processed which require a small quantum of a product derived from essential oils, is considerable. However, given adequate incentive and good management, this industry, with essential oils produced to quality specifications, would enable even a small developing country to enhance its economic gains to a substantial degree. The technology involved is relatively simple and the variations possible enable innovations to be made to accommodate a diverse range of conditions.

Essential-oil-producing plant species grow abundantly in the tropical areas of the world. Most of the tropics belong to the developing countries where the essential oils industry thrives sometimes as a mere cottage-scale industry. If one were to scan this particular region of the globe across several continents, *i.e.* Asia, Africa and Latin America, one would see the production of essential oils linked with a great variety of distillation hardware. Sometimes quite basic still designs are employed, and yet the oil finds its way sometimes to even the sophisticated markets of Europe and America, albeit with very marginal benefits to the producers themselves.

Agronomic expertise is available or can be procured. The industry itself is not always capital intensive and local design and fabrication of distillation equipment can cut capital costs down to the absolute minimum. The labour intensive nature of the industry is an advantage where labour availability is a

factor. Price fluctuations so common to the industry can also be circumvented if economic cultivation and harvesting techniques are adopted, field distillations are carried out wherever feasible and quality ensured for the products. The development of downstream processing of products such as isolates and aroma chemicals from essential oils is an exercise that could also be profitable in the correct circumstances, for even a developing country producer.

DISTILLATION TECHNOLOGY AND THE PRODUCTION OF ESSENTIAL OILS

The methods and equipment employed for the recovery of essentials oils in the developing countries are often obsolete, resulting in poor and inconsistent quality of oils and consequent poor returns.

The raw materials and crude oils thus produced are invariably exported to the developed countries where they are subjected to refining fractionation and blending to the exact standards and specific needs, consumed internally or exported, thereby earning enormous profits.

Because of large-scale urbanisation of most of the developed countries, a large variety of medicinal and aromatic plants and cheap labour are abundantly available in the developing countries and hence merits the establishment of small-scale industries in these countries, based on simple technologies for the extraction and refinement of intermediate products, export these to the developed countries in the first phase. At a later stage, when necessary confidence, expertise and infrastructure are available, the intermediates can be further processed to semi-finished products for internal consumption and or export.

GENERAL METHODS OF PRODUCING ESSENTIAL OILS

Depending on the nature of plant material, its end-use and the state of development of the country of origin the following techniques are in practice in the recovery of essential oils:

1- Extraction with supercritical carbon dioxide
2- Distillation in water
3- Cold Pressing
4- Extraction with non volatile solvent (enfleurage).
5- Distillation by passage of steam

1- Extraction with supercritical carbon dioxide: This method is the latest development and is confined to a few developed countries due to high capital cost and sophistication. This technique therefore may not find favour in most of the developing countries, in the near future.

2- Distillation in water: In this method the plant material is completely immersed in an appropriate volume of water. The water is then brought to boiling by the application of heat directly to the vessel containing the plant material and the water, or by the use of a steam jacket or a steam coil. The main feature is that the plant material is always in contact with the boiling water.
This method has several disadvantages

- release of the volatile oil is incomplete
- sensitive compounds could undergo hydrolysis more easily
- compounds such as aldehydes present in the oil may tend to polymerise
- distillation requires a larger number of stills as charges have to be less dense
- the method is energy expensive
- the method skilled labour intensive
- is applicable only when the charges are relatively small

In many cases where this method was in use in commercial scale distilleries it is now giving way to other methods.

3- Cold pressing: This operation is used in very limited cases, such as production of essential oils from Citrus fruit rinds.

4- Extraction with non volatile solvents (enfleurage): The method of enfluerage is now obsolete. It was one used particularly in the south of France for the processing of the essence of flowers such as Jasmine where the fragrance is considered sensitive to heat. The method is now replaced by solvent extraction with light hexane.

5- Distillation by passage of steam: This method is by far the most widely accepted method for the production of essential oils on a commercial scale. The plant material is supported within a still body and packed uniformly to provide for the smooth passage of steam through it.

The suspension of the plant material could be on a grid above the base of the still, or set into the still body in a "basket" or "cartridge".

The steam is generated externally with a boiler or steam generator, and this way there is a good measure of control over the rate of entry of the steam. The steam, which must be partially "wet", interacts with the plant material causing the essential oils within the cell membranes to diffuse out. The oil constituents then form mixtures with the water vapour. These mixtures are volatile at temperatures below 100°C, and hence distil over with the steam to be condensed. Thus the presence of a sufficient amount of moisture in the steam is an obvious requirement, to ensure complete extraction of the essential oils.

The process of direct steam distillation has several advantages over the other variants heretofore described;

1- The boiling point of the steam-essential oil mixture is almost 100°C at atmospheric pressure and guarantees adequate volatility of the components to be distilled without the risk of overheating, as the saturation temperature is not exceeded during the distillation process.

2- The condensation of both oil and water in the condenser is at the same time an effective method of separation by virtue of the poor solubility of the oil in water. The losses caused by the low solubility of the oil in water can be minimized by reusing the distilled water to produce steam.
3- Steam protects the essential oil from oxidation.
4- The plant parts are swollen up by the steam, thus facilitating the release of the oils through the dilated pores.
5- The process is cheap, technically simple and optimal as regards operational safety, as the use of water provides protection against explosion and fire.
6- It is more energy efficient.
7- It provides for better control of the distillation rate.
8- There is the possibility for varying the steam pressure which in turn enables the employment of low pressures for oils with substantially high volatile constituents and higher pressures for those with low volatile constituents such as the sesquiterpenes.
9- The method is better suited to commercial scale operations, as results are more consistent and reproduceable.

The only disadvantages are the risk of decomposition by heat and hydrolysis, though the desirable hydrolytic cleavage of glycosidically bound essential oil components can also be advantageous here. The conversion of matricin into chamazulene is mentioned as an example of a conversion of a substance into a steam-volatile component under the conditions of steam-distillation.

Although steam distillation of aromatic plants is well known and simple but data on optimum conditions, time of distillation, steam rate, etc., needs to be worked out, on individual raw materials, since every plant material is unique by itself, and cannot be generalised.

Higher steam rates and larger periods of distillation beyond optimum levels render the production of essential oils uneconomical.

All plant materials are to be distilled immediately after harvesting, to prevent loss of volatile oils due to evaporation, oxidation and resinification. However, if it must be stored before distillation, it should be dried under shade and as far as possible, free from air circulation.

STORAGE OF ESSENTIAL OILS

Essential oils when stored improperly deteriorate in quality, due to oxidation, resinification, hydrolysis, polimerization etc.

Moisture, air, heat and light activates these processes. Essential oils should therefore be free from moisture an air. Essential oils freed from moisture should be stored in well-fitted, tightly closed, conical top containers of aluminium at low temperatures and protected from light.

DESIGN OF STEAM DISTILLATION UNITS

Following are the essential equipment required for a steam distillation unit

a- Steam Boiler
b- Tank Still
c- Condenser
d- Oil Separator

a- Steam Boiler: Availability of the kind of fuel will generally determine the choice of the type of boiler. Oil fired boilers are very convenient to operate and start up. They are most amenable to automate instrumentation and require minimum supervision. Coal and fuel wood fired boilers are simpler in construction, take longer to start up and require manual operation and supervision.

Many of these boilers can be converted for firing with locally available waste materials and spent essential oil plant material.

b- Tank Still: Height to diameter ratio of tank still is an important design variable to be considered. Most of the commercial stills are designed with a ratio of 1 to 1.5. Ratios lower than 1 result in uneven distribution of steam over the whole crossection of the tank whereas a ratio exceeding 1.5 may cause steam oil vapour to reflux back into the tank due to excessive tank height. Taller tank stills should be, preferably, insulated to overcome this problem. In order to design a still of a particular required capacity, the bulk density of the plant material to be distilled should be known in advance. Alteration in the bulk density due to shade drying should be noted.

Particular attention should be paid to proper location and design of steam sparger in the tank bottom to ensure even distribution of steam. Tank walls should be fabricated with adequate metal thickness so that they do not buckle while transporting and erecting the still. Considerable saving in sheet metal thickness can be made by welding stiffening rings on the outside of the tank body which provide the structural support.

Top lid of the still can be closed with a metal flange (Figure 1). When shell and tube type condensers are used with the still, water seals of 8 inches depth are adequate. An electric hoist or chain pulley system is generally used to lift the top lid. Grid bottom may be lifted out with hoist along with the spent plant material to discharge the still.

c- Condenser: Two designs of condensers are most popular in the essential oil industry. These are older submerged coil type and more modern shell and tube type (Figure 2). Optimum design of condenser is critical because an undrsized condenser will allow steam – oil vapours to escape uncondensed and over sizing the condenser means unnecessary extra capital cost.

Heat removal capacity of a condenser is expressed by the following equation;

$$Q = U \times A \times \Delta T_{log}$$

Where Q= Heat removal rate

U= Heat transfer co-efficient of condenser

A= Area available for heat transfer

ΔT_{log}= Log mean temperature difference between cooling water and condensate

The main difference between a submerged coil type condenser and a shell and tube type is the higher value of heat transfer co-efficient (U) available in the case of shell and tube design. This is attributed to high degree of turbulence in the cooling water of a shell and tube design whereas cooling water is almost stagnant in the tank of a submerged coil condenser. According to theory of convective heat transfer, increased turbulence is conducive to higher heat transfer rates.

Thus it is clear that for a fixed duty or condensation rate and same cooling water temperature, total cooling area (A) required in the case of a shell and tube condenser will be lower than the coil type. This difference may be as much as 30% in some cases. But inspite of this the coil type condenser has its own unique advantages.

- Fabrication of shell and tube type condenser is more complicated compared to coil type
- A running water supply is essential to operate the shell and tube condenser which is not necessary for the coil type

d- Oil Separator: The condensate that merges from a steam distillation, (that is the distillate), is a mixture of essential oil and water. The essential oil may eventually settle either as an upper layer (lighter than water) or as a lower layer (heavier than water). Sometimes it settles as both an upper layer and a lower layer

due to the fact that the lighter fractions which come over first form a single upper layer while the heavier fractions agglomerate at the bottom as a lower layer.

The functions of the oil separator or Florentine Vessel is to separate the oil from the water phase and should be designed to accommodate all of the situations above. Accordingly three types of separators are in common use. They are;

Type 1: for separating oils less dense than water
Type 2: for separating oils denser than water
Type 3: for separating oils which have both a less dense as well as a denser component in the distillate

The essential oils are in general only sparingly soluble in water and this is the property that makes it possible to effect the separation from the aqueous phase. As the oil phase of the distillate condenses it separates into distinct layers due to the differences in the specific gravity and the removal of these layers, or layer as the case may be, is facilitated by the use of the Florentine vessel or flask. The design of the Florentine Vessel must also take into account the fact that the volume of the distillate aqueous phase is considerably larger than that of the oil phase.

Hence the Florentine Vessel is designed to enable the continuous removal of the water phase during distillation. Oftimes several Florentine Vessels are connected in series in a "cascade arrangement" to facilitate separation as well as the continuous removal of the water phase. This water may be discarded provided the residual quantity of oil dissolved in it is negligible. If however the water-soluble oil content is economically significant, -as in the case of phenolic oils such as cinnamon bark oil and sandalwood oil which are also costly, then provision must be made for economic recovery of the residual oil that is contained in the "discard" waters. In some cases this is carried out by running the water into large underground storage sumps of cement and brick, and allowed to remain there for long periods. After many distillations substantial quantities of oil is

recovered from the storage tanks. In other instances such as in the case of sandalwood and vetiver oils, the discard water is returned to the distillation still.

The design of the three main types of oil separators or Florentine Vessels are shown in Figure 3.

One factor which requires special mention is the importance of the temperature of the condensate in obtaining an effective separation of the oil-water phase. When the specific gravity of the oil is only marginally less than that of the water it is preferable if the condensate flows into the separator at around 40-45°C. This is because the specific gravity of oil decreases relatively more than water with increased temperature. The greater the differential between the respective specific gravities the better the separation of the two phases into layers. Where the specific gravity of the oil is marginally greater than water at ambient temperatures, the distillate should run as cold as possible to effect the most rapid separation.

PARAMETERS OF STEAM DISTILLATION

The yield and quality of essential oil from steam distillation is affected by the various process parameters. It advisable to keep them in mind while designing such systems. Some of the important parameters are being listed below;

a- Materials of construction
b- Steam pressure
c- Time of distillation
d- Condition of raw material
e- Solubility of oil in water

Essential oils which are corrosive in nature should be preferably distilled in stills made of resistant materials like aluminium, copper or stainless steel. The tank still can be made from a cheaper metal like mild still or Galvanised Iron and condenser and separator made from a resistant but expensive material like

stainless steel. The logic behind this contention is that since only vapour is present in tank still, the rust and other products or corrosion may not be carried over into the oil. This can result in considerable saving in the capital cost of the equipment. Only expensive food grade spice oils are distilled in all stainless steel systems. Aluminium has also found application as a material of construction of distillation stills since ancient times, its availability is getting reduced and with the arrival of superior alloys like stainless steel it is slowly disappearing from the scene.

Since the heat content and temperature of steam depend upon its pressure, a change in steam pressure can alter the distillation characteristics. High boiling constituents of essential oils will normally require high pressure steam to distil over. Tough plant materials like roots and seeds yield their essential oil much more easily if high pressure steam is employed to distil them. This is because high pressure steam is much more effective in breaking down the plant tissues to liberate the essential oil.

Different constituents of the essential oil get distilled in the order of their boiling points. Thus the highest boiling fractions will be last to come over when, generally, very little oil is distilling. If the distillation is terminated too soon, the high boiling constituents will be lost. In many essential oil plants like vetiver, these high boiling fractions are valuable due to the quality of their aroma. Thus the time of distillation must be chosen with due care.

Condition of raw material is important because some materials like roots and seeds will not yield essential oil easily if distilled in their natural state. These materials have to be crushed, powdered or soaked in water to expose their oil cells. Chopping of plants will also change the packing density of the material when placed in distillation still. Air drying and wilting the herb prior to distillation also has considerable effect on distillation. Shade drying for 24 hours of Citronella grass prior to distillation has the effect of better oil separation and reduced emulsion formation.

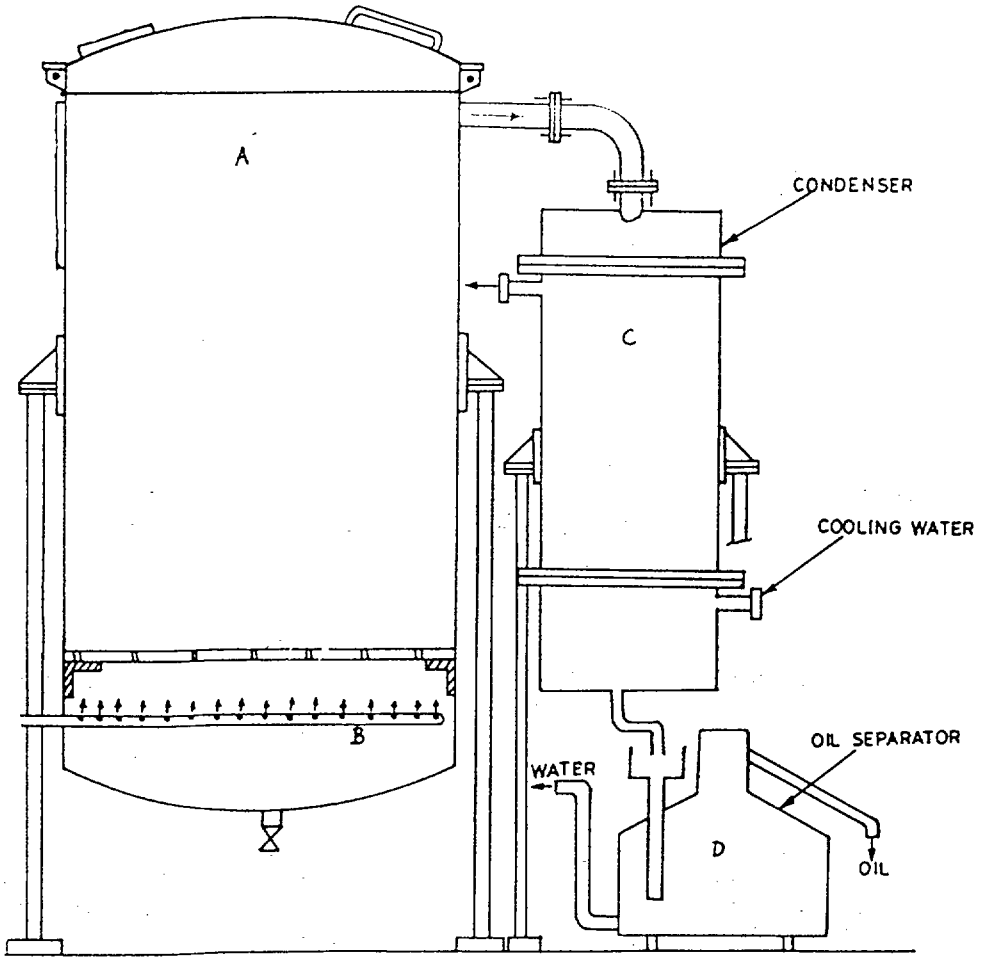


Figure 1. Distillation still

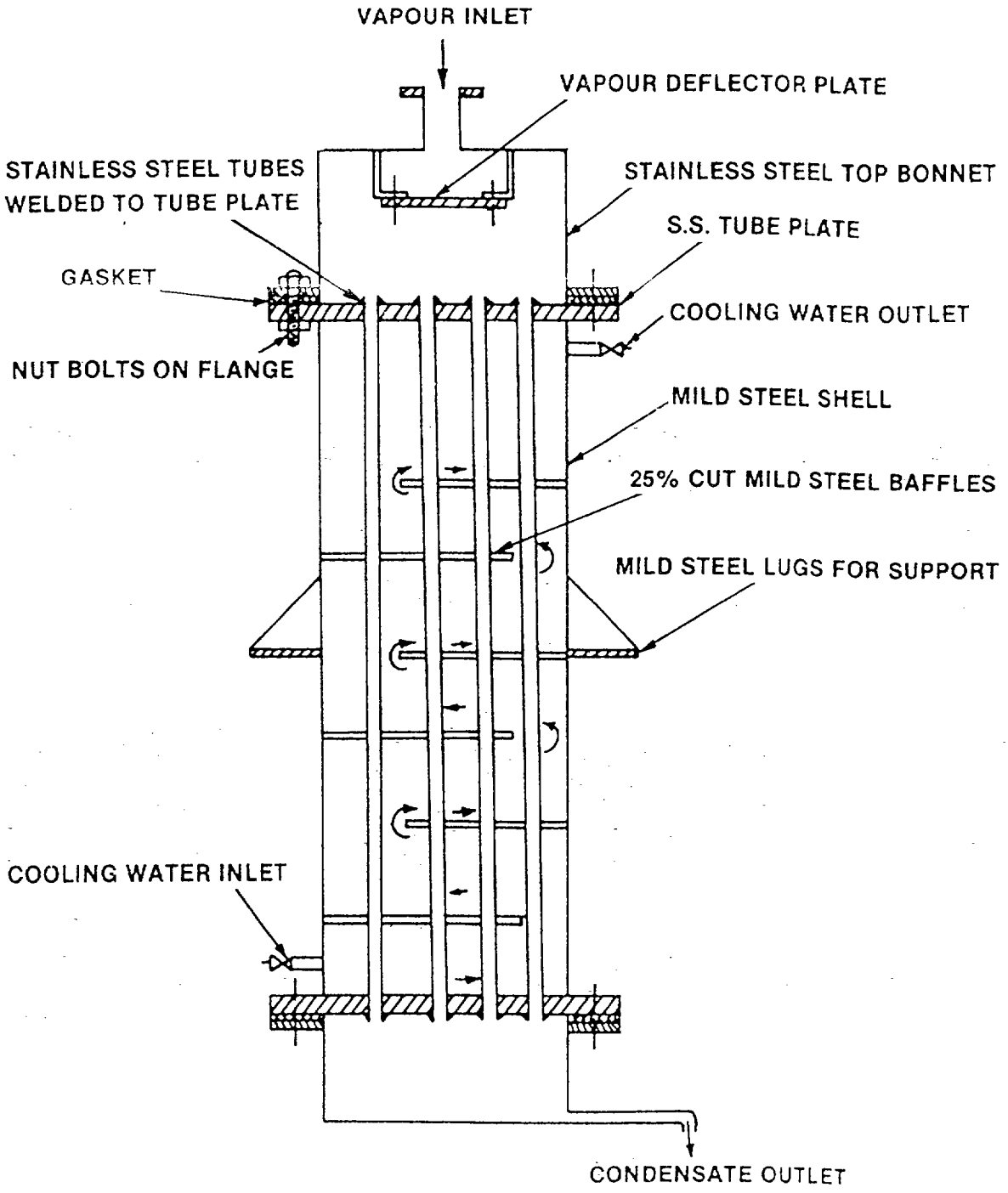
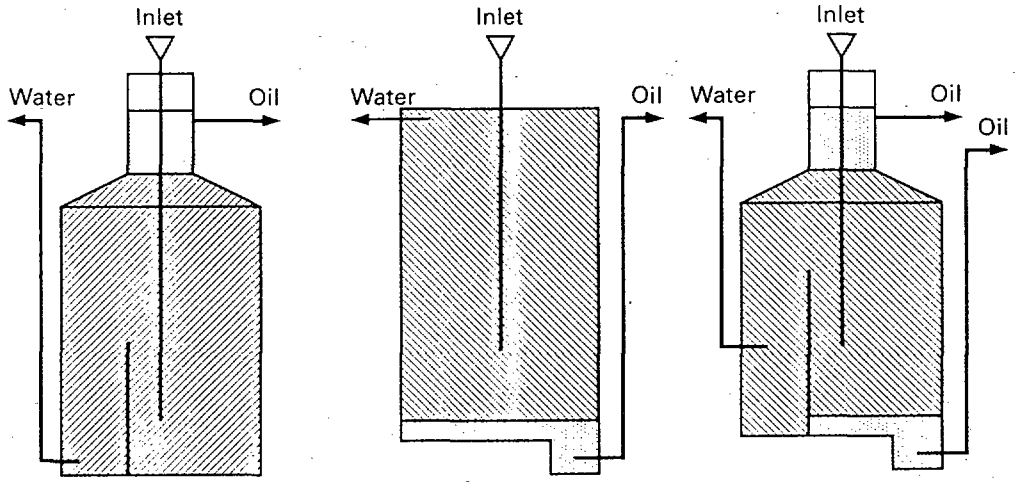
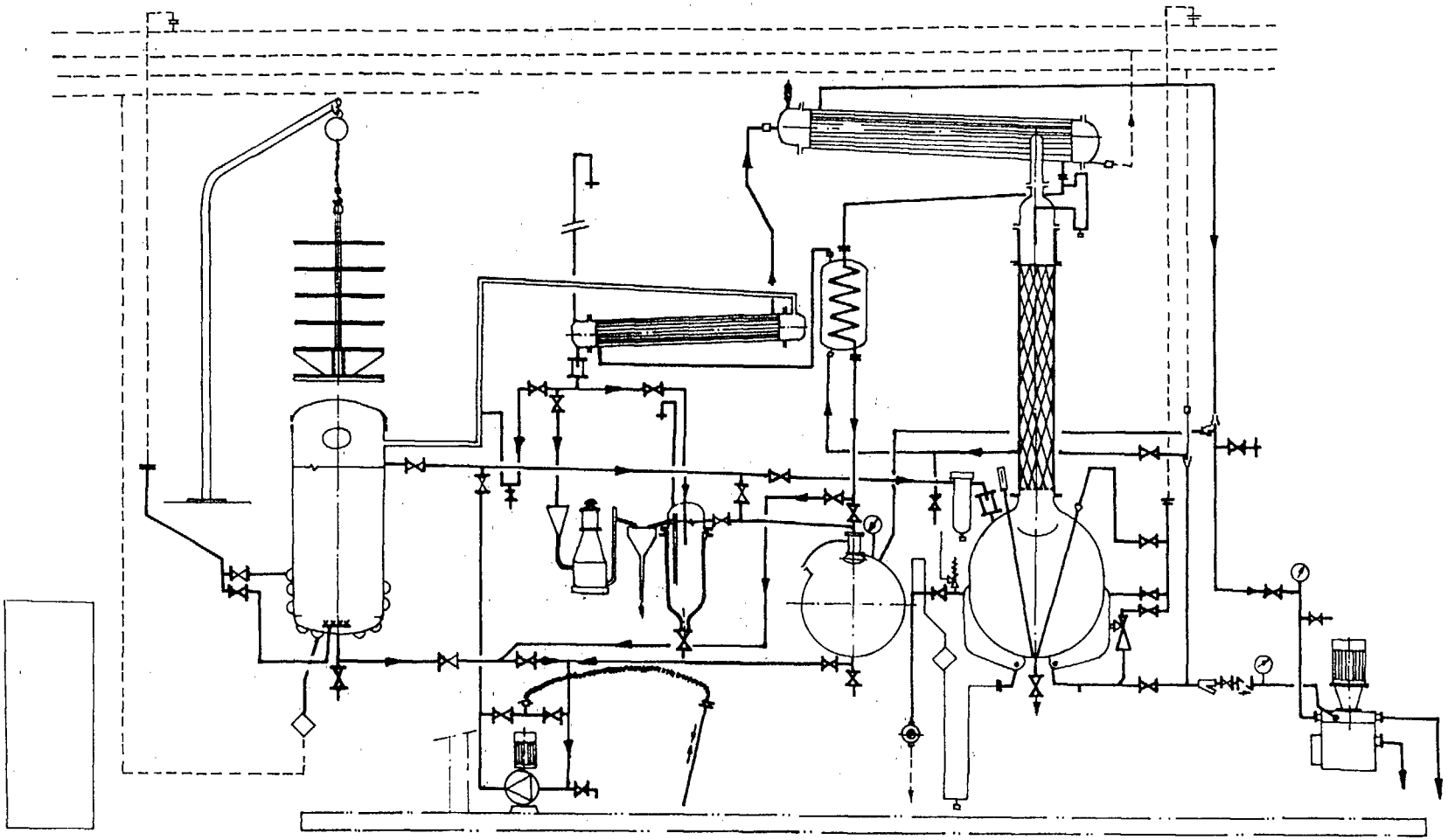


Figure 2. Construction detail of condenser

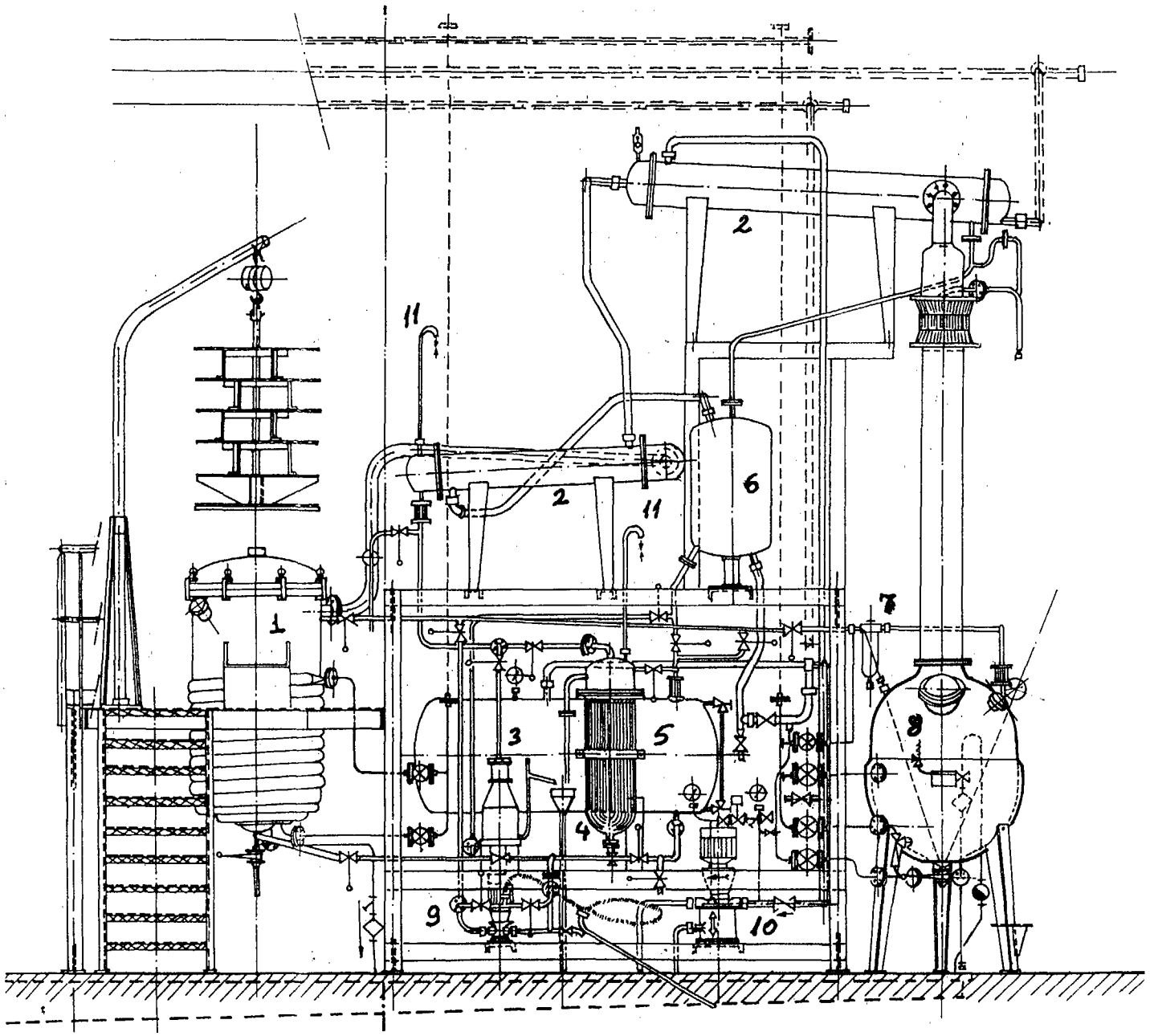


Type 1 Type 2 Type 3

Figure 3. Separator types



Flow Diagram of Multipurpose Pilot Plant



1- Extractor
2- Condenser
3- Florentine flask
4- Phase separator
5- Solvent tank
6- Cooler
7- Miscella filter
8- Evaporator
9- Circulation pump
10- Vacuum pump
11- Air vent

Multipurpose Pilot Plant

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FRACTIONAL DISTILLATION IN THE ESSENTIAL OIL INDUSTRY

Prof.Dr. Mustafa Kara

INTRODUCTION

Distillation is a method of separating the components of a solution which depends on the distribution of the substances between a vapour and a liquid phase, applied to cases where all components are present in both phases. The theory and practice of distillation (vapour-liquid phase equilibria, mass and heat transfer and hydrodynamics) are covered in detail in chemical engineering. The mode of operation (continuous, batch) the capacity and the internal structures of distillation columns (plate, packing) vary widely. In the chemical and petrochemical industries the capacity is usually very large and the same mixture is always distilled, therefore, the continuous operation with plate column is common. However, in the medicinal and aromatic plants research and production units the quantity of material to be processed is usually small and versatile. This requires a small capacity, batch distillation unit with a packed column.

In the plant extraction, often water, organic solvents (hexane, alcohol) or a mixture of solvents hexane-heptane, water-alcohol) are used. Distillation in the only process in most cases, if not all, to recover and purify the used solvents.

In the essential oil industry many essential oils, when distilled from the plant material, are contaminated with volatile products arising from the decomposition of complex plants substances under the influence of hot water or steam. Also, oil itself may contain as normal constituents substances somewhat objectionable odor (certain aldehydes or sulphur compounds). In order to improve the odor of such oils, they must be freed from undesirable compounds by redistillation.

In the construction of distillation units either glass or stainless steel is commonly used. The liquid charge is usually heated by high pressure steam (at least 5 atm) or for higher temperature requirements by hot oil bath. The vapour in the condenser is condensed by cooling water, however for some special cases (less than 5-10°C) chilled water is necessary.

The boiling temperatures of liquids are functions of pressure as shown in Figure 1 for linalool, the main constituents of linalool oil. This implies, in the essential oil industry distillation is carried out under reduced pressure, not to expose the oil to higher temperatures. All the joints in the unit must be absolutely airtight to keep the desired vacuum unchanged.

A schematic diagram of a batch distillation system is shown in Figure 2. The system consists of a spherical or cylindrical, steam or hot oil heated steel, a packed column, a shell and tube condenser and two product receivers. Vapours from the still on their way to the condenser can flow countercurrently to a portion of the condensate returned to the column as reflux. The condensed and cooled overhead products are collected in the receiver. These receivers are so connected to the condenser that while one receiver remains under vacuum and collects the fraction distilling over at a given temperature the other receiver may be opened to draw off the previous fraction.

It is out of scope of this short note to discuss all the aspects of distillation process. Since the mode of operation is batch and the packed columns are frequently used in the plant based production industry, these two points will be discussed very briefly.

Batch Distillation:

Batch distillation is often used for separating small quantities and large variety of liquids, therefore, the unit must be versatile. In a typical batch distillation a batch of liquid to be processed is charged to the still and the system is first brought to steady-state under total reflux (at total reflux no overhead product is withdrawn and all the condensate from the condenser is returned to the column), after which part of the condensate is continuously withdrawn as an overhead product and the remainder is refluxed. The predetermined reflux ratio is adjusted by a reflux timer controller. Nothing is withdrawn from the still until the distillation is completed. The entire column operates as an enriching section. Obviously as time proceeds the composition of the material being distilled becomes less rich in the more volatile components, and the distillation must be stopped after a certain time to attain a desired average composition and yield of the more volatile components in the overhead product.

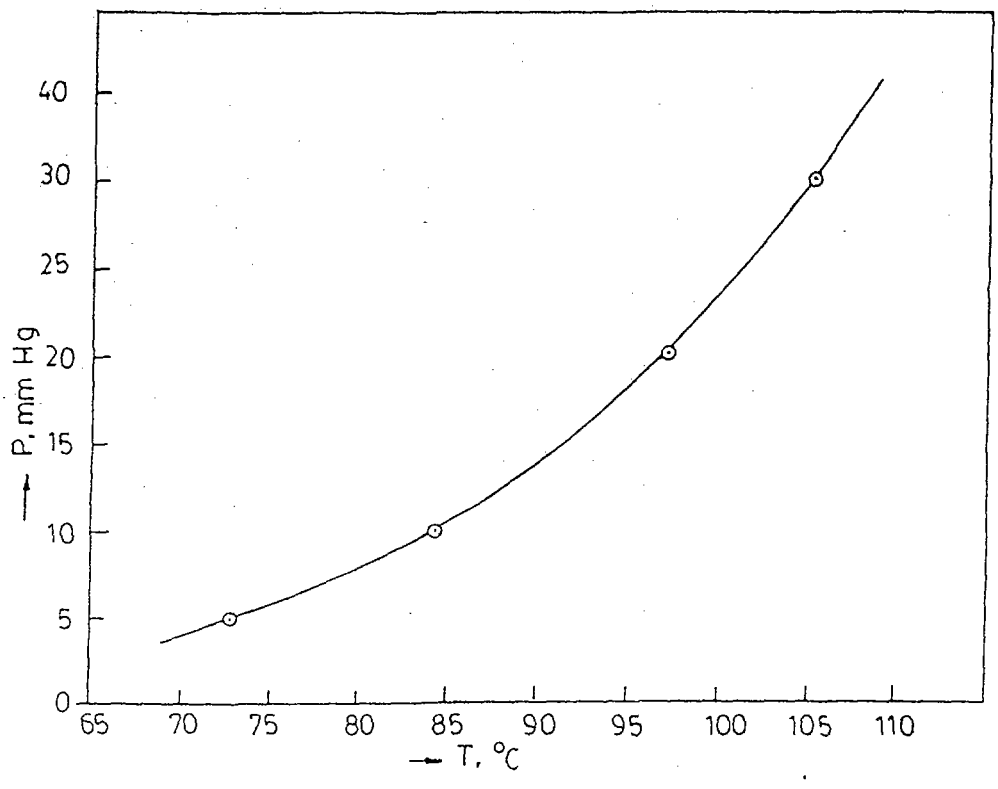

Figure 1. Vapour pressure of linalool

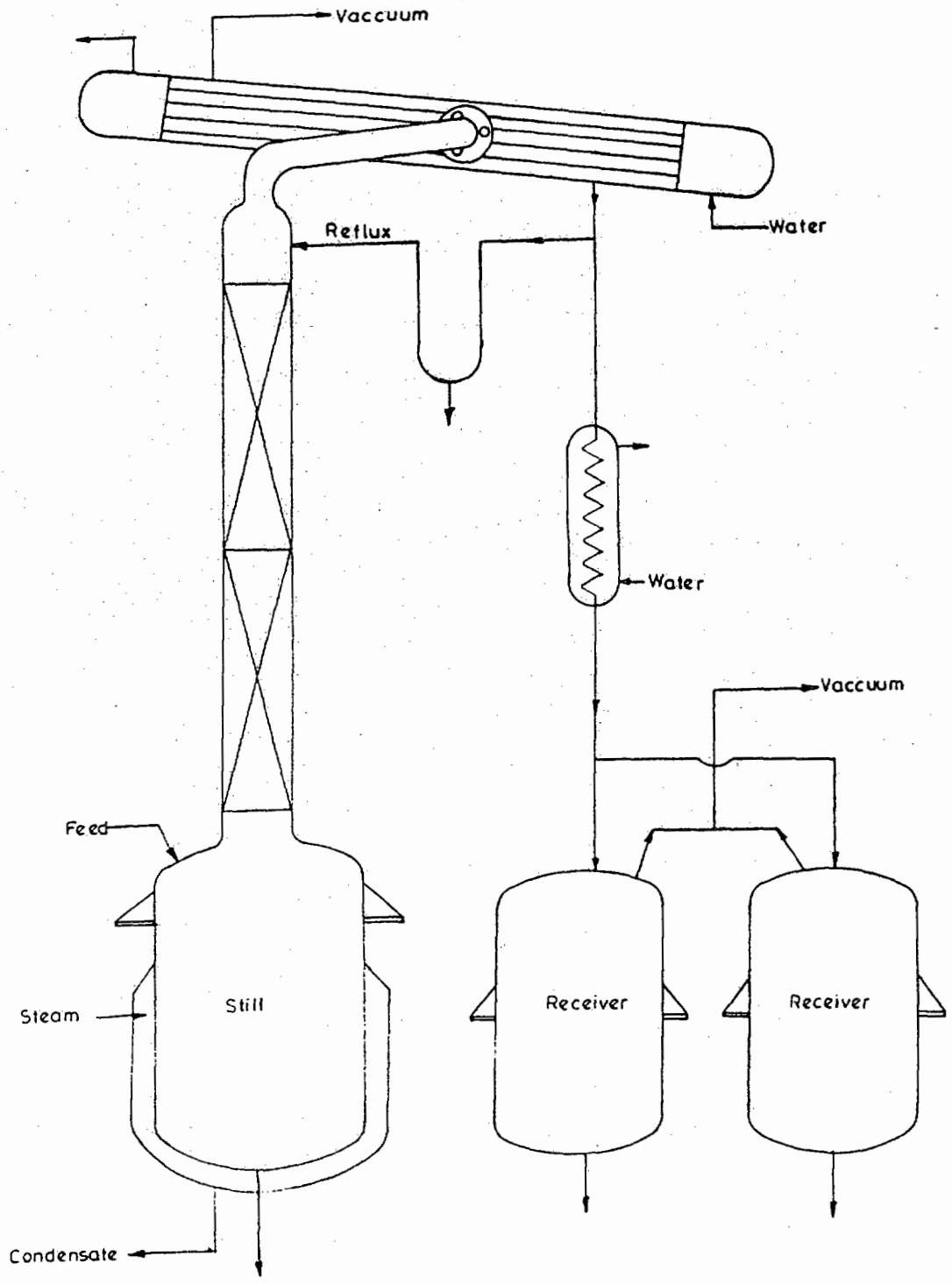

Figure 2. Schematic diagram of a batch distillation system

Since a batch distillation is usually carried out in an existing column equivalent to a known number of theoretical plates, it is necessary to determine the reflux ratio required to give the desired distillate purity.

Batch distillations may be carried out in two ways:

1. Constant Overhead Product Composition, Variable Reflux Ratio:

If it is desired to maintain a constant overhead product composition, the amount of reflux returned to the column must be constantly increased throughout the run. As the time proceeds, the reboiler is gradually depleted of the lighter component, and the increased difference in composition between the bottom and top of the column makes the required separation constantly more difficult to attain. Finally the points is reached at or near total reflux where the desired product composition can no longer be made, the overhead product, now the intermediate cut, is collected in a separate receiver. The distillation is terminated when the composition similar to that of the original charge, is usually added to the next batch for further processing.

2. Variable Overhead Product Composition, Constant Reflux Ratio:

This simpler type of operation is perhaps more common, as no changes in operation are required during the course of the run. In this case product composition vary as shown in Figure 3, the shape of the curves being functions of relative volatility, reflux ratio, and number of theoretical plates. The distillation proceeds until the average composition of the distillate represents the desired composition and yield, the overhead product is then diverted to another receiver and an intermediate cut is withdrawn until the remaining still liquid meets the required specification.

An alternate procedure sometimes practiced with laboratory distillations is to establish total reflux, then to withdraw as product for a short length of time a proportion of the reflux liquid, after which total reflux is again established. After a new steady-state is attained, another increment of product is withdrawn, and so forth.

Column Packing:

The purpose of a distillation column is to achieve an extensive liquid-vapour interface so that equilibrium between ascending vapour and descending liquid can be rapidly attained. The column packing should offer the following characteristics:

1. Provide for large interfacial surface area between liquid and vapour per unit volume of packed space.

2. Possess desirable fluid-flow characteristics. The packing must permit passage of large volumes of liquid through small column cross section without flooding, with low pressure drop for the vapour and low liquid holdup.
3. Be chemically inert to fluids being process.
4. Have structural strength to permit easy handling and installation.
5. Represent low cost.

Column packings can be fabricated from stainless steel, ceramic as well as from glass for glass columns, in various shapes and sizes, some of which are shown in Figure 4.

In the distillation of essential under vacuum conditions, pressure drop through the column packing should be as low as possible. Thus, the liquid in the still will not be exposed to high temperatures and consequently the decomposition of liquid will be minimized. The pressure should not be higher than 5 to 10 mm Hg as measured in the still above the boiling liquid. Pressure drop through the packings is about 1 to 2 mm Hg for random packings and 0.01 to 0.8 mm Hg for structured ones per theoretical plate.

The requirement of good contact between liquid and vapour is hard to meet, especially in large columns. Ideally the liquid, once distributed over the top of the packing, flows in thin films overall the packing surface all the way down the column. Actually the films tend to grow thicker in some places and thinner in others, so that the liquid collects into small rivulets and flows along localized paths through the packing. Especially at low liquid rates much of the packing surface may be dry or, at best, covered by a stagnant film of liquid. This effect is known as chaneling, and it is the chief reason for the poor performance of large packed columns. Channeling may be minimized by having the diameter of the column at least 8 times the packing diameter. If the ratio of column diameter to packing diameter is less than 8:1, the liquid tends to flow out of the packing and down the walls of the column. Unfortunately, many randomly packed columns fail to achieve the product specifications when highly purified overhead product is desired. most, if not all, of the troubled columns employ a bed height greater than 6 meters and suffer from inadequate redistribution of liquid in the bed. Therefore, addition of a liquid redistributor at the center of the column reduces the channaling and improves the column performance.

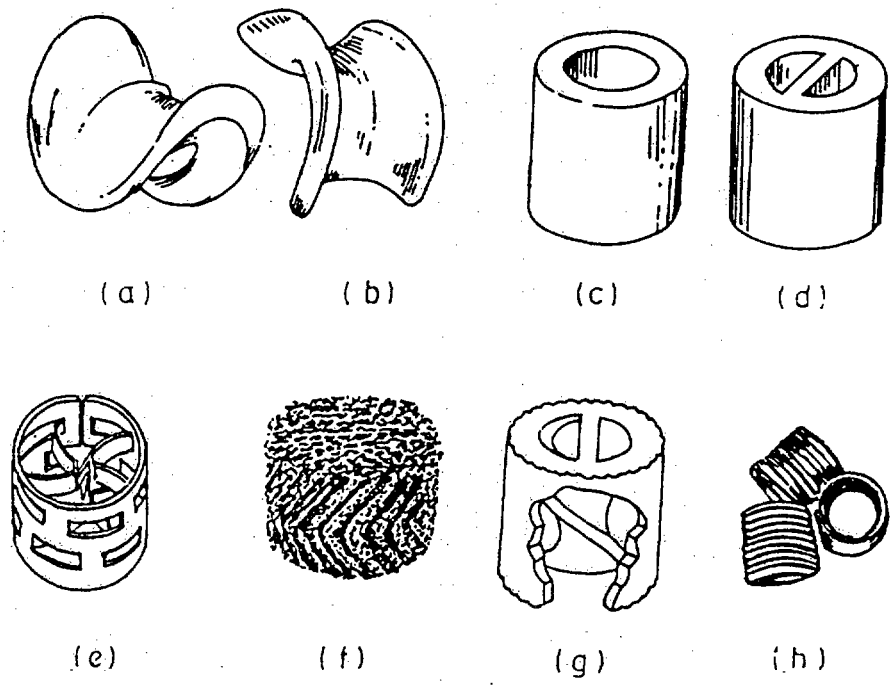

Figure 3. Typical distillation column packings: a) Berl saddle; b) Intalox saddle; c) Rasching ring; d) Lessing ring; e) Pall ring; f) Knit mesh-Hyperfil; g) Double-spiral ring; h) Helices

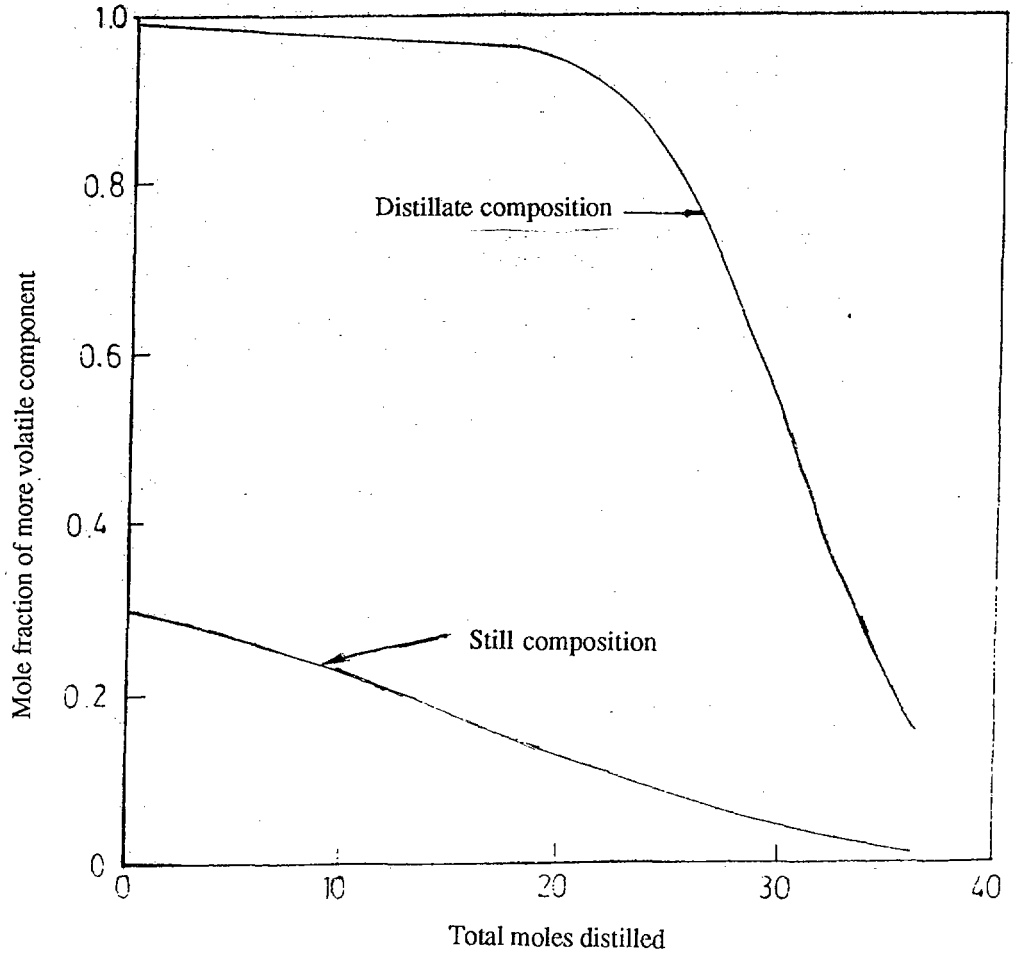

Figure 4 Composition variation in batch distillation at constant reflux ratio

Columns are packed either randomly or regularly. Random packings are carefully dumped into the column during installation and allowed to fall at random. Generally the random packings offer larger specific surface and larger pressure drop but they cost less per unite volume. Regular packings are of great variety and offer the advantages of higher efficiency, lower pressure drop for the vapour and greater possible fluid flow rates, usually at the expense of more costly installation than random packing. Knitted or otherwise woven wire screen, rolled as a fabric into cylinders or other metal gauzelike arrangements provide a large interfacial surface of contected liquid and vapour and a very low vapour pressure drop, especially useful for vacuum distillation as in the essential oil industry. Moreover, HETP (Height equivalent to a theoretical plate) for random packings is between 0.40 and 1.50 meters, for regular packings is much lower, 0.10 to 0.75 meters.

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QUALITY IMPROVEMENT of ESSENTIAL OILS

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PHARMACOPOEIAL TECHNICS FOR TESTING ESSENTIAL OILS

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INTRODUCTION

Many crude drugs are used for pharmaceutical purposes because of their essential oils .Essential oils separated from the drugs are also used as drugs in themselves.Various essential oil containing crude drugs are used as spices and condiments. The most common use for the essential oil drugs as well as for the separated oils is for flavoring purposes .In addition to their pharmaceutical uses , the essential oils are used widely as flavor for foods and confections and in the spice , perfume and cosmetic trades.

The quality of an essential oil containing crude drug or essential oil is established by reference to the descriptions of Pharmacopoeia monographs.

The European Pharmacopoeia (EP , Ph.Eur.) is widely used internationally.There are a lot of monograph on essential oils and essential oil containing drugs EP .Evaluated methods are included to test the essential oils and essential oil containing drugs.These methods are not expensive but given valuable results.

Testing techniques will be examine in 2 categories as crude drugs and essential oils.

The outline of the analysis applied for crude drugs are showed in Table - 1

Macroscopic and microscopic analysis are very useful and important. The details concerning these methods not included the text.

For identifications mostly TLC methods are used . The colour reactions are used very rare (Matricaria flower) .TLC,GC or sometimes very simple tests are used for adulterations (Extractable matter , ...) .

The volatile oil content of crude drug is determined by a distillation method. Sometimes , the essential oil separated by distillation is used for GC analysis to determine the active substances of the essential oil .

The outline of the analysis applied for essential oils are showed in Table - 2 .

TLC and GC methods (Table -3 ,4) are used to identify the main components of the essential oils .

Measurements of refractive index , optical rotation , freezing point ,acid value .. . are particularly valuable for purity of essential oils.

Simple (Sol. in alcohol , test for foreign esters ,fatty oils and resinified essential oils , ...) or developed (measuring absorbance ,...) tests are used for adulterations .

GC and some other methods (Assay of 1,8 – cineol in essential oils , determination of carvon ,...) are used for quantitative analysis of the essential oils .

CHARACTERS

Odour , Taste

Macroscopic , Microscopic

IDENTIFICATION

Macroscopy , Microscopy

TLC , Colour reactions

FOREIGN MATTER

WATER or LOSS ON DRYING

ASH

Total , Insoluble in hydrochloric acid

OTHER TESTS

Adulteration (TLC , GC)

Extractable matter (water , alcohol)

ASSAY

Essential oil

Active substance (UV , GC)

Table - 1

Outline of the Testing Methods for Essential oil Containing Crude Drugs

CHARACTERS

Colour , odour ,taste,solubility

IDENTIFICATION

TLC , Chromatografic profile

TESTS

Relative density

Refractive index , Optical rotation

Freezing point

Acid value , Ester v. Hydroxyl v.

Water , Foreign esters

Fatty oils and resinified essential oils

Specific react.(phellandren, aldehydes,..)

Water soluble portion

Residue on evaporation

Absorbance

Solubility in alcohol ,carbon disulfide , potassium hydroxide

Chromatografic profile

ASSAY

GC

Others (1,8 cineol , aldehydes..)

Table - 2

Outline of the Testing Methods for Essential oils

Adsorbent	silicagel silicagel GF_{254}
Extracting solvent	toluene
Solvent system	ethyl acetate 5 - 15 v toluene 85 - 95 v
Developing	15 cm
Visualization	UV light 254 or 365nm vanillin reagent * anisaldehyde reagent ** 100 - 105 C 5 - 10 minutes

***) Carefully add , dropwise,2 ml of sulfuric acid R to 100 ml of a 10 g/l solution of vanillin R in alcohol R.Use within 48 h of preparation.**
****) Mix the following order , 0.5 ml anisaldehyde R , 10 ml of glacial acetic acid R ,85 ml of methanol R , 5 ml of sulfuric acid R .**

Table - 3

Outline of the TLC method used for Essential Oils in EP

Column	fused-silica capillary 25 - 60 m 0.25 mm int. diameter coated macrogol 20000
Carrier gas	helium
Flow rate	1.5 ml/minute
Detector	FID
split ratio	1/100
Inj. port t.	200°-270° C
Detector t.	200°-270° C

Table -4

Outline of the GC Method Used for Essential Oils in EP

Essential oil containing

CRUDE DRUGS

Most of the texts in this part
have been taken from
European Pharmacopoeia (EP , Ph. Eur.)
The text numbers are identical
to the second chapter
(Methods of analysis) of Ph.Eur.

2.2.27. THIN-LAYER CHROMATOGRAPHY

Principle. Thin-layer chromatography is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support (plate) of glass, metal or plastic. Solutions of analytes are deposited on the plate prior to development. The separation is based on adsorption, partition, ion-exchange or on combinations of these mechanisms and is carried out by migration (development) through the thin-layer (stationary phase) of solutes (solutions of analytes) in a solvent or a suitable mixture of solvents (mobile phase).

APPARATUS

Plates. The chromatography is carried out using coated plates as described under *4.1.1. Reagents.*

Pre-conditioning of the plates. It may be necessary to wash the plates prior to separation. This can be done by migration of an appropriate solvent. The plates may also be impregnated by procedures such as development, immersion or spraying. At the time of use, the plates may be activated, if necessary, by heating in an oven at 100 °C to 105 °C for 1 h.

A chromatographic tank with a flat bottom or twin trough, of inert, transparent material and of a size suitable for the plates used and provided with a tightly fitting lid. For horizontal development the tank is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to the stationary phase.

Micropipettes, microsyringes, calibrated disposable capillaries or other application devices suitable for the proper application of the solutions.

Fluorescence detection device to measure direct fluorescence or the inhibition of fluorescence.

Visualisation reagents to detect the separated spots by spraying, exposure to vapour or immersion.

METHOD

Vertical development. Line the walls of the chromatographic tank with filter paper. Pour into the chromatographic tank a sufficient quantity of the mobile phase for the size of the tank to give after impregnation of the filter paper a layer of appropriate depth related to the dimension of the plate to be used. For saturation of the chromatographic tank, replace the lid and allow to stand at 20 °C to 25 °C for 1 h. Unless otherwise indicated, the chromatographic separation is performed in a saturated tank.

Apply the prescribed volume of the solutions in sufficiently small portions to obtain bands or circular spots at an appropriate distance from the lower edge and from the sides of the plate. Apply the solutions on a line parallel to the lower edge of the plate with an interval of at least 10 mm between the spots.

When the solvent has evaporated from the applied solutions, place the plate in the chromatographic tank ensuring that the plate is as vertical as possible and that the spots or bands are above the surface of the mobile phase. Close the chromatographic tank, maintain it at 20 °C to 25 °C and protect from sunlight. Remove the plate when the mobile phase has moved over the prescribed distance. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

Horizontal development. Apply the prescribed volume of the solutions in sufficiently small portions to obtain circular spots 1 mm to 2 mm in diameter, or bands 5 mm to 10 mm by 1 mm to 2 mm, at an appropriate distance from the lower edge and from the sides of the plate. Apply the solutions on a line parallel to the lower edge of the plate with an interval of at least 5 mm between the spots. When the solvent has evaporated from the applied solutions, introduce a sufficient quantity of the mobile phase into the trough of the chamber using a syringe or pipette, place the plate horizontally in the separating chamber and connect the mobile phase direction device according to the manufacturer's instructions. If prescribed, develop the plate starting simultaneously at both ends. Close the chamber and maintain it at 20 °C to 25 °C. Remove the plate when the mobile phase has moved over the distance prescribed in the monograph. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development perpendicular to that of the first development.

VISUAL ESTIMATION

Identification. The principal spot in the chromatogram obtained with the test solution is compared visually to the corresponding spot in the chromatogram obtained with the reference solution by comparing the colour, the size and the retention factor (R_f) of both spots.

The retention factor (R_f) is defined as the ratio of the distance from the point of application to the leading edge of the spot and the distance travelled by the solvent front from the point of application.

Verification of the separating power for identification. Normally the performance given by the suitability test described in *4.1.1. Reagents* is sufficient. Only in special cases an additional performance criterion is prescribed in the monograph.

Related substances test. Secondary spot(s) in the chromatogram obtained with the test solution is (are) compared visually to either the corresponding spot(s) in the chromatogram obtained with the reference solution containing the impurity(ies) or the spot in the chromatogram obtained with the reference solution prepared from a dilution of the test solution.

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Verification of the detecting power. The detecting power is satisfactory if a spot or band is clearly visible in the chromatogram obtained with the most dilute reference solution.

QUANTITATIVE MEASUREMENT

The requirements for resolution and separation are given in the particular monographs.

Substances responding to UV-Vis irradiation separated by thin-layer chromatography can be determined directly on the plate using appropriate instrumentation. While moving the plate or the measuring device, examine the plate by measuring the reflectance or transmittance of the incident light. Similarly fluorescence may be measured using an appropriate optical system. Substances containing radionuclides can be quantified in three ways: either directly by moving the plate alongside a suitable counter or vice versa (see *Radiopharmaceutical preparations 125)*, by cutting the plates into strips and measuring the radioactivity on each individual strip using a suitable counter or by scraping off the stationary phase, dissolving it in a suitable scintillation cocktail and measuring the radioactivity using a liquid scintillation counter.

Apparatus. The apparatus for direct measurement on the plate consists of:

- a device for exact positioning and reproducible dispensing of the amount of substances on the plate,
- a mechanical device to move the plate or the measuring device along the *x*-axis or the *y*-axis,
- a recorder and a suitable integrator or a computer,
- *for substances responding to UV-Vis irradiation*: a photometer with a source of light, an optical device able to generate monochromatic light and a photo cell of adequate sensitivity are used for the measurement of reflectance or transmittance. In the case where fluorescence is measured, a monochromatic filter is required in addition, to select a particular spectral region of the emitted light,
- *for substances containing radionuclides*: a suitable counter for radioactivity. The linearity range of the counting device is to be verified.

Method. Prepare the solution of the substance to be examined (test solution) as prescribed and, if necessary, prepare the reference solutions of the substance to be determined using the same solvent as in the test solution. Apply the same volume of each solution to the plate and develop.

Substances responding to UV-Vis irradiation: Prepare and apply not fewer than three reference solutions of the substance to be examined, the concentrations of which span the expected value in the test solution (about 80, 100 and 120 per cent). Spray with the prescribed reagent, if necessary, and record the reflectance, the transmittance or fluorescence in the chromatograms obtained with the test and reference solutions. Use the measured results for the calculation of the amount of substance in the test solution.

Substances containing radionuclides: Prepare and apply a test solution containing about 100 per cent of the expected value. Determine the radioactivity as a function of the path length and report the radioactivity in each resulting peak as a percentage of the total amount of radioactivity.

Unless otherwise prescribed, the results of the determination are not valid if the resolution (R_s) between measured peaks in the chromatograms is less than 1.0.

The **resolution** (R_s) is calculated from the formula:

$$R_s = \frac{1.18(z_b - z_a)}{b_{0.5a} + b_{0.5b}}$$

$$z_b > z_a$$

z_b, z_a = distances in millimetres, along the baseline between the point of sample application and perpendiculars dropped from the maxima of two adjacent peaks,

$b_{0.5a}$, $b_{0.5b}$ = peak widths, in millimetres, at half height.

If the limit set for impurities is performed by photometric measurement, then the signal-to-noise ration (S/N) is an important parameter for the determination of the Limit of Detection.

The **signal-to-noise ratio** (S/N) is calculated from the equation:

$$\frac{S}{N} = \frac{2H}{h}$$

H = height of the peak corresponding to the component concerned, in the chromatogram obtained with the prescribed reference substance, measured from the maximum of the peak to the base of the signal observed over a distance equal to twenty times the width at half-height.

h = maximum amplitude of the background noise in a chromatogram obtained after application of a blank, observed over a distance equal to twenty times the width at half-height of the peak in the chromatogram obtained with the prescribed reference substance and situated equally around the place where this peak would be found.

2.8.2. FOREIGN MATTER

Vegetable drugs should be free from moulds, insects and other animal contamination.

Unless otherwise prescribed, the amount of foreign matter is not more than 2 per cent *m/m*.

Foreign matter is material consisting of any or all of the following:

1) *Foreign organs*: matter coming from the source plant but not defined as the drug,
2) *Foreign elements*: matter not coming from the source plant and either of vegetable or mineral origin.

DETERMINATION OF FOREIGN MATTER

Weigh 100 g to 500 g of the substance to be examined, or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. Examine for foreign matter by inspection with the unaided eye or by use of a lens (6 ×). Separate foreign matter and weigh it and calculate the percentage present.

2.2.13. DETERMINATION OF WATER BY DISTILLATION

The apparatus (see Figure 2.2.13.-1) consists of a glass flask (*A*) connected by a tube (*D*) to a cylindrical tube (*B*) fitted with a graduated receiving tube (*E*) and reflux condenser (*C*). The receiving tube (*E*) is graduated in 0.1 ml. The source of heat is preferably an electric heater with rheostat control or an oil bath. The upper portion of the flask and the connecting tube may be insulated.

Method. Clean the receiving tube and the condenser of the apparatus, thoroughly rinse with water, and dry.

Introduce 200 ml of *toluene R* and about 2 ml of *water R* into the dry flask. Distil for 2 h, then allow to cool for about 30 min and read the water volume to the nearest 0.05 ml. Place in the flask a quantity of the substance, weighed with an accuracy of 1 per cent, expected to give about 2 ml to 3 ml of water. If the substance has a pasty consistency, weigh it in a boat of metal foil. Add a few pieces of porous material and heat the flask gently for 15 min. When the toluene begins to boil, distil at the rate of about two drops per second until most of the water has distilled over, then increase the rate of distillation to about four drops per second. When the water has all distilled over, rinse the inside of the condenser tube with *toluene R*. Continue the distillation for 5 min, remove the heat, allow the receiving tube to cool to room temperature and dislodge any droplets of water which adhere to the walls of the receiving tube. When the water and toluene have completely separated, read the volume of water and calculate the percentage present in the substance using the formula:

$$\frac{100\,(n_2 - n_1)}{m}$$

m = the mass in grams of the substance to be examined,

n_1 = the number of millilitres of water obtained in the first distillation,

n_2 = the total number of millilitres of water obtained in the two distillations.

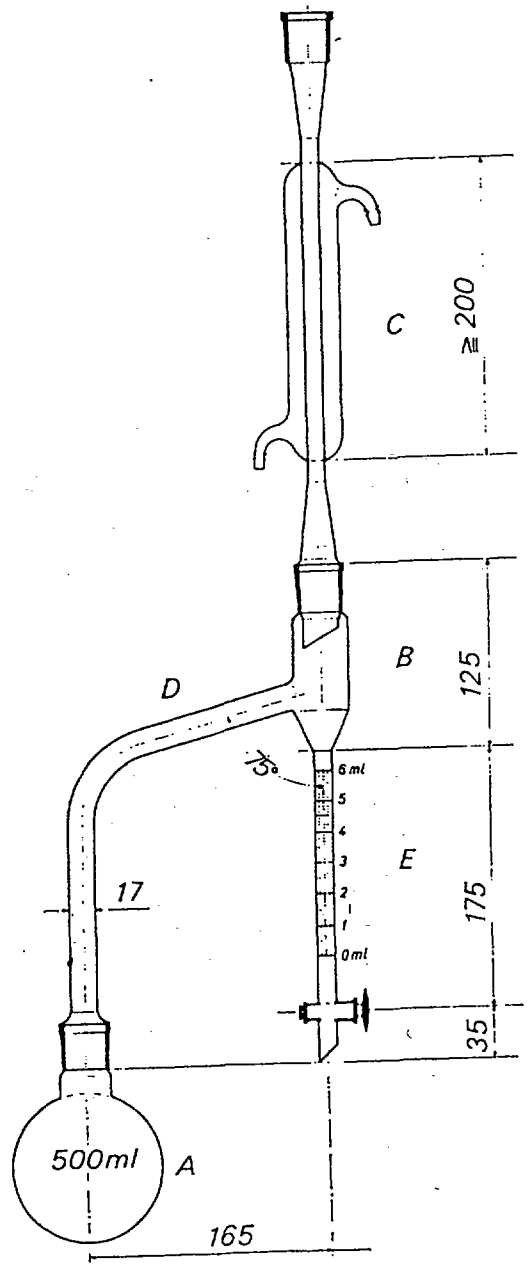


Figure 2.2.13.-1. – *Apparatus for the Determination of Water by Distillation*

Dimensions in millimetres

2.2.32. LOSS ON DRYING

Loss on drying is the loss of mass expressed as per cent *m/m*.

Method. Place the prescribed quantity of the substance to be examined in a weighing bottle previously dried under the conditions prescribed for the substance to be examined. Dry the substance to constant mass or for the prescribed time by one of the following procedures.

a) "in a desiccator": the drying is carried out over *diphosphorus pentoxide R* at atmospheric pressure and at room temperature;

b) *"in vacuo"*: the drying is carried out over *diphosphorus pentoxide R*, at a pressure of 1.5 kPa to 2.5 kPa at room temperature;

c) *"in vacuo"* within a specified temperature range: the drying is carried out over *diphosphorus pentoxide R*, at a pressure of 1.5 kPa to 2.5 kPa within the temperature range prescribed in the monograph;

d) "in an oven" within a specified temperature range: the drying is carried out in an oven within the temperature range prescribed in the monograph;

e) "under high vacuum": the drying is carried out over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa, at the temperature prescribed in the monograph.

If other conditions are prescribed, the procedure to be used is described in full in the individual monograph.

Ash

Use Method I unless otherwise directed in the monograph.

Method I

For crude drugs Incinerate 2 to 3 g of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cool and weigh. If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot *water*, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°. Calculate the percentage of ash with reference to the air-dried drug.

For other substances Carry out the above method using 1 g, unless otherwise stated. Calculate the percentage of ash.

Method II

Heat a silica or platinum crucible to red heat for 30 minutes, allow to cool in a desiccator and weigh. Unless otherwise directed in the monograph, evenly distribute 1 g of the substance being examined in the crucible, dry at 100° to 105° for 1 hour and ignite to constant weight in a muffle furnace at a temperature not exceeding 600°. Allow the crucible to cool in a desiccator after each ignition.

2.8.1. ASH INSOLUBLE IN HYDROCHLORIC ACID

Ash insoluble in hydrochloric acid is the residue obtained after extracting the sulphated or total ash with *hydrochloric acid R*, calculated with reference to 100 g of drug.

To the crucible containing the residue from the determination of sulphated or total ash, add 15 ml of *water R* and 10 ml of *hydrochloric acid R*, cover with a watch-glass, boil the mixture gently for 10 min and allow to cool. Filter through an ashless filter, wash the residue with hot *water R* until the filtrate is neutral, dry, ignite to dull redness, allow to cool in a desiccator and weigh. Reheat until the difference between two consecutive weighings is not more than 1 mg.

2.8.12. DETERMINATION OF ESSENTIAL OILS IN VEGETABLE DRUGS

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, using xylene to take up the essential oil; the aqueous phase is automatically returned to the distillation flask.

Apparatus. The apparatus comprises the following parts:

(a) a suitable round-bottomed flask with a short, ground-glass neck having an internal diameter of about 29 mm at the wide end,

(b) a condenser assembly (see Figure 2.8.12.-1) that closely fits the flask, the different parts being fused into one piece; the glass used has a low coefficient of expansion; the stopper *K'* is vented and the tube *K* has an orifice of diameter about 1 mm that coincides with the vent; the wide end of the tube *K* is of ground-glass and has an internal diameter of 10 mm; a pear-shaped swelling, *J*, of 3 ml capacity; the tube *JL* is graduated in 0.01 ml; the bulb-shaped swelling *L* has a capacity of about 2 ml; *M* is a three-way tap; the junction *B* is at a level 20 mm higher than the uppermost graduation,

(c) a suitable heating device, allowing a fine control,

(d) a vertical support with a horizontal ring covered with insulating material.

Method. Use a thoroughly cleaned apparatus. Carry out the assay according to the nature of the drug to be examined. Place the prescribed volume of distillation liquid in the flask, add a few pieces of porous porcelain and attach the condenser assembly. Introduce *water R* through the filling funnel *N* until it is at the level *B*. Remove the stopper *K'* and introduce the prescribed quantity of *xylene R*, using a pipette with its tip at the bottom of the tube *K*. Replace the stopper *K'* and ensure that the orifice coincides with the vent. Heat the liquid in the flask to boiling and adjust the distillation rate to 2 ml to 3 ml per minute, unless otherwise prescribed.

To determine the rate of distillation, during distillation lower the level of the water by means of the three-way tap until the meniscus is at the level of the lower mark (a) (see Figure 2.8.12.-2). Close the tap and measure the time taken for the liquid to reach the upper mark (b). Open the tap and continue the distillation, modifying the heat to regulate the distillation rate. Distil for 30 min. Stop the heating and after at least 10 min read off the volume of xylene in the graduated tube.

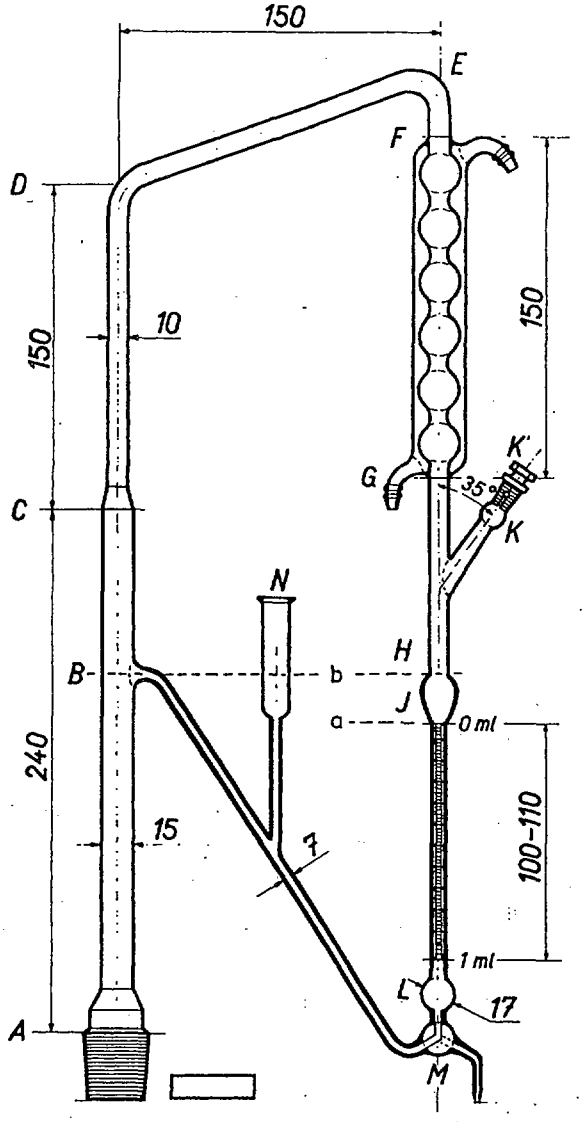


Figure 2.8.12.-1. – *Apparatus for the Determination of Essential Oils in Vegetable Drugs*

Dimensions in millimetres

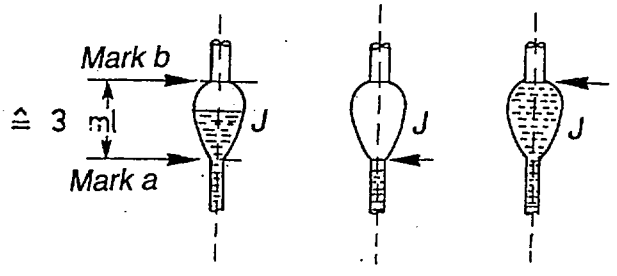


Figure 2.8.12.-2

Introduce into the flask the prescribed quantity of the drug and continue the distillation as described above for the time and at the rate prescribed. Stop the heating and after 10 min read the volume of liquid collected in the graduated tube and subtract the volume of xylene previously noted. The difference represents the quantity of essential oil in the mass of the drug taken. Calculate the result as millilitres per 100 g of drug.

When the essential oil is to be used for other analytical purposes, the water-free mixture of xylene and essential oil may be recovered as follows: remove the stopper *K'* and introduce 0.1 ml of a 1 g/l solution of *sodium fluoresceinate R* and 0.5 ml of *water R*. Lower the mixture of xylene and essential oil into the bulb-shaped swelling *L* by means of the three-way tap, allow to stand for 5 min and lower the mixture slowly until it just reaches the level of the tap *M*. Open the tap anticlockwise so that the water flows out of the connecting tube *BM*. Wash the tube with *acetone R* and with a little *toluene R* introduced through the filling funnel *N*. Turn the tap anticlockwise in order to recover the mixture of xylene and essential oil in an appropriate flask.

MATRICARIA FLOWER

Matricariae flos

D. Place 0.1 ml of the test solution used in identification test C in a test-tube, add 2.5 ml of a solution prepared by dissolving 0.25 g of *dimethylaminobenzaldehyde R* in a mixture of 5 ml of *phosphoric acid R*, 45 ml of *acetic acid R* and 45 ml of *water R*. Heat on a water-bath for 2 min and allow to cool. Add 5 ml of *light petroleum R* and shake. The aqueous layer has a distinct greenish-blue or blue colour.

VALERIAN ROOT

Valerianae radix

Extractable matter. To 2.00 g of the powdered drug (250) add a mixture of 8 g of *water R* and 12 g of *alcohol R* and allow to macerate for 2 h shaking frequently. Filter, evaporate 5 g of the filtrate to dryness on a water-bath and dry at 100 °C to 105 °C. The residue weighs not less than 0.1 g (20.0 per cent).

HOP STROBILE

Lupuli flos

Matter extractable by alcohol (70 per cent *V/V*). To 10.0 g of the powdered drug (355) add 300 ml of *alcohol (70 per cent V/V) R* and heat for 10 min on a water bath under a reflux condenser. Allow to cool, filter and discard the first 10 ml of the filtrate. Evaporate 30.0 ml of the filtrate to dryness on a water-bath and dry in an oven at 100 °C to 105 °C for 2 h. The mass of the residue is not less than 0.250 g (25.0 per cent).

cetylpyridinium chloride CRS. Examine the substances in the solid state.

C. To 5 ml of *dilute sodium hydroxide solution R* add 0.1 ml of *bromophenol blue solution R1* and 5 ml of *chloroform R* and shake. The chloroform layer is colourless. Add 0.1 ml of solution S (see Tests) and shake. The chloroform layer becomes blue.

D. Solution S gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*Method II, 2.2.2*).

Acidity. To 50 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 2.5 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Amines and amine salts. Dissolve 5.0 g with heating in 20 ml of a mixture of 3 volumes of *1 M hydrochloric acid* and 97 volumes of *methanol R* and add 100 ml of *2-propanol R*. Pass a stream of *nitrogen R* slowly through the solution. Gradually add 12.0 ml of *0.1 M tetrabutylammonium hydroxide* and record the potentiometric titration curve (*2.2.20*). If the curve shows two points of inflexion, the volume of titrant added between the two points is not greater than 5.0 ml. If the curve shows no point of inflexion, the substance to be examined does not comply with the test. If the curve shows one point of inflexion, repeat the test but add 3.0 ml of a 25.0 g/l solution of *dimethyltetradecylamine R* in *2-propanol R* before the titration. If the titration curve after the addition of 12.0 ml of the titrant shows only one point of inflexion, the substance to be examined does not comply with the test.

Water (*2.5.12*): 4.5 per cent to 5.5 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*). Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 2.00 g in *water R* and dilute to 100.0 ml with the same solvent. Transfer 25.0 ml of the solution to a separating funnel, add 25 ml of *chloroform R*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 50 g/l solution of *potassium iodide R*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous layer with three quantities, each of 10 ml, of *chloroform R* and discard the chloroform layers. To the aqueous layer add 40 ml of *hydrochloric acid R*, allow to cool and titrate with *0.05 M potassium iodate* until the deep-brown colour is almost discharged. Add 2 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10.0 ml of the freshly prepared 50 g/l solution of *potassium iodide R*, 20 ml of *water R* and 40 ml of *hydrochloric acid R*.

1 ml of *0.05 M potassium iodate* is equivalent to 34.0 mg of $C_{21}H_{38}ClN$.

1997:0380

CHAMOMILE FLOWER, ROMAN

Chamomillae romanae flos

DEFINITION

Roman chamomile flower consists of the dried flower-heads of the cultivated double variety of *Chamaemelum nobile* (L.) All. (*Anthemis nobilis* L.). It contains not less than 7 ml/kg of essential oil.

CHARACTERS

Roman chamomile flower has a strong and characteristic odour.

It consists of flower-heads with a white to yellowish-grey colour, being composed of solitary hemispherical capitula, made up of a solid conical receptacle bearing the florets, each subtended by a transparent small palea.

It has the macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

A. The capitula have a diameter of 8 mm to 20 mm; the receptacle is solid; the base of the receptacle is surrounded by an involucre consisting of two or three rows of compact and imbricated bracts with scarious margins. Most florets are ligulate, but a few pale yellow tubular florets occur in the central region. Ligulate florets are white, dull, lanceolate and reflexed with a dark brown, inferior ovary, a filiform style and a bifid stigma; tubular florets have a five-toothed corolla tube, five syngenesious, epipetalous stamens and a gynoecium similar to that of the ligulate florets.

B. Separate the capitulum into its different parts. Examine under a microscope, using *chloral hydrate solution R*. All parts of the flower-heads are covered with numerous small yellow glistening glandular trichomes. The involucral bracts and paleae have epidermal cells in longitudal rows, sclerified at the base and they are covered with conical trichomes, about 500 µm long, each composed of three or four very short base cells and a long, bent, terminal cell about 20 µm wide. The corolla of the ligulate flowers consists of papillary cells with cuticular striations. The ovaries of both kinds of florets have at their base a sclerous ring consisting of a single row of cells. The receptacle and the ovaries contain small clusters of calcium oxalate. The pollen grains have a diameter of about 35 µm and are rounded and triangular with three germinal pores and a spiny exine.

C. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution. To 0.5 g of the powdered drug (710) add 10 ml of *methanol R* and heat with shaking in a water-bath at 60 °C for 5 min. Allow to cool and filter.

Reference solution. Dissolve 2.5 mg of *apigenin R* and 2.5 mg of *apigenin-7-glucoside R* in 10 ml of *methanol R.*

Apply separately to the plate as bands 10 µl of each solution. Develop over a path of 10 cm using a mixture of 17 volumes of *glacial acetic acid R*, 17 volumes of *water R* and 66 volumes of *butanol R.* Dry the plate at 100 °C to 105 °C for 5 min and spray the warm plate with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, using about 10 ml for a plate 200 mm square. Spray the plate with the same volume of a 50 g/l solution of *macrogol 400 R* in *methanol R.* Allow to stand for about 30 min and examine in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows in the upper third a zone of yellowish-greenish fluorescence (apigenin) and in the middle third a zone of yellowish fluorescence (apigenin-7-glucoside). The chromatogram obtained with the test solution shows a zone of yellowish-green fluorescence corresponding in position and fluorescence to the apigenin and a zone of yellowish fluorescence corresponding in position and fluorescence to the apigenin-7-glucoside obtained with the reference solution; above the apigenin-7-glucoside zone there is a zone of brownish fluorescence (luteolin); immediately below the apigenin-7-glucoside zone there is a zone of light brownish fluorescence (apiin); immediately below the apiin zone there is a zone of bright blue fluorescence and below this zone a zone of bright blue fluorescence; other faint zones may be present.

TESTS

Diameter of the flower-heads. Not more than 3 per cent of flower-heads have a diameter smaller than 8 mm.

Deteriorated flower-heads. Brown or darkened flower-heads are absent.

Water (*2.2.13*). Not more than 10.0 per cent, determined by distillation using 20.0 g of whole drug.

Total ash (*2.4.16*). Not more than 8.0 per cent.

ASSAY

Carry out the determination of essential oils in vegetable drugs (*2.8.12*). Use 20.0 g of whole drug, a 500 ml round-bottomed flask, 250 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 3 ml to 3.5 ml per minute for 3 h.

STORAGE

Store in a well-closed container, protected from light.

1997:0313

CHARCOAL, ACTIVATED

Carbo activatus

DEFINITION

Activated charcoal is obtained from vegetable matter by suitable carbonisation processes intended to confer a high adsorption power.

CHARACTERS

A black, light powder free from grittiness, practically insoluble in all usual solvents.

IDENTIFICATION

A. When heated to redness it burns slowly without a flame.

B. It complies with the test for adsorption power (see Tests).

TESTS

Solution S. To 2.0 g in a conical flask with a ground-glass neck add 50 ml of *dilute hydrochloric acid R.* Boil gently under a reflux condenser for 1 h, filter and wash the filter with *dilute hydrochloric acid R.* Evaporate the combined filtrate and washings to dryness on a water-bath, dissolve the residue in *0.1 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Acidity or alkalinity. To 2.0 g add 40 ml of *water R* and boil for 5 min. Cool, restore to the original mass with *carbon dioxide-free water R* and filter. Reject the first 20 ml of the filtrate. To 10 ml of the filtrate add 0.25 ml of *bromothymol blue solution R1* and 0.25 ml of *0.02 M sodium hydroxide.* The solution is blue. Not more than 0.75 ml of *0.02 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid-soluble substances. To 1.0 g add 25 ml of *dilute nitric acid R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (10) and wash with 10 ml of hot *water R.* Evaporate the combined filtrate and washings to dryness on a water-bath, add to the residue 1 ml of *hydrochloric acid R*, evaporate to dryness again and dry the residue to constant mass at 100 °C to 105 °C. The residue weighs not more than 30 mg (3 per cent).

Alkali-soluble coloured substances. To 0.25 g add 10 ml of *dilute sodium hydroxide solution R* and boil for 1 min. Cool, filter and dilute the filtrate to 10 ml with *water R.* The solution is not more intensely coloured than reference solution GY_4 (*Method II, 2.2.2*).

Alcohol-soluble substances. To 2.0 g add 50 ml of *alcohol R* and boil under a reflux condenser for 10 min. Filter immedi-

1999:1304

CORIANDER

Coriandri fructus

DEFINITION

Coriander consists of the dried cremocarp of *Coriandrum sativum* L. It contains not less than 3 ml/kg of essential oil, calculated with reference to the dried drug.

CHARACTERS

The cremocarp is brown or light brown and is more or less spherical, about 1.5 mm to 5 mm in diameter, or oval form 2 mm to 6 mm long.

It has the macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

A. The mericarps are usually tightly connected. The cremocarp is glabrous and has ten wavy, slightly raised primary ridges and eight straight, more prominent secondary ridges. The stylopod crowns the apex. The mericarps are concave on the internal surface. A small fragment of the pedicel may be present.

B. Reduce to a powder (355). The powder is brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows numerous oil droplets; fragments of endosperm with small thick-walled regular cells containing microcrystals and microrosettes of calcium oxalate and oil droplets; fragments of endocarp with very narrow cells having a parquetry arrangement and usually associated with a layer of thin-walled rectangular sclereids of the mesocarp; fragments from the sclerenchymatous layer of the mesocarp with short, strongly thickened, pitted, fusiform cells occurring in layers with the cells of adjacent layers approximately at right angles to one another; fragments of parenchyma with small, thick-walled cells; occasional fragments of vascular bundles.

C. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution. Shake 0.50 g of the freshly powdered drug (355) with 5.0 ml of *hexane R* for 2 min to 3 min and filter over 2 g of *anhydrous sodium sulphate R*.

Reference solution. Dissolve 15 µl of *linalol R* and 25 µl of *olive oil R* in 5.0 ml of *hexane R* immediately before use.

Apply to the plate as bands 20 µl of the test solution and 10 µl of the reference solution. Develop over a path of 10 cm using a mixture of 5 volumes of *ethyl acetate R* and 95 volumes of *toluene R*. Allow the plate to dry in air and develop again in the same conditions. Allow the plate to dry in air. Spray the plate with *anisaldehyde solution R* and examine in daylight while heating at 100 °C to 105 °C for 5 min to 10 min. The chromatogram obtained with the reference solution shows in the lower half a violet to greyish-violet zone (linalol) and in the upper half a bluish-violet zone (triglycerides). The chromatogram obtained with the test solution shows zones similar in position and colour to the zones in the chromatogram obtained with the reference solution. Several violet-grey to brownish zones, including the zone corresponding to geraniol, are between the starting point and the zone due to linalol in the chromatogram obtained with the reference solution. It may also show several faint violet-grey zones between the zone due to triglycerides and that due to linalol in the chromatogram obtained with the reference solution.

TESTS

Foreign matter (*2.8.2*). It complies with the test. None of the cremocarps show perforations due to animals.

Loss on drying (*2.2.32*). Not more than 10.0 per cent, determined on 1.000 g of the powdered drug (355) by drying in an oven at 100 °C to 105 °C for 2 h.

Total ash (*2.4.16*). Not more than 8.0 per cent.

ASSAY

Carry out the determination of essential oils in vegetable drugs (*2.8.12*). Use a 500 ml round-bottomed flask, 200 ml of *water R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Reduce the drug to a coarse powder and immediately use 30.0 g for the determination. Distil at a rate of 2 ml/min to 3 ml/min for 2 h.

STORAGE

Store protected from light.

1997:0759
corrected 1999

CORTICOTROPIN

Corticotropinum

DEFINITION

Corticotropin is a substance obtained from the anterior lobe of the pituitary gland of pigs and contains the corticotrophic peptide which increases the rate at which corticoid hormones are secreted by the adrenal glands. The purified material is dried by a suitable method. The potency is not less than 70 I.U./mg.

2.8.8. ODOUR AND TASTE OF ESSENTIAL OILS

Mix three drops of the essential oil with 5 ml of *90 per cent V/V alcohol R* and stir in 10 g of powdered *sucrose R*. The odour and taste are similar to that of the plant or parts of the plant from which the essential oil has been obtained.

2.2.5. RELATIVE DENSITY

The relative density d_{20}^{20} of a substance is the ratio of the mass of a certain volume of the substance to the mass of an equal volume of water, both weighed at 20 °C.

Determine the relative density d_{20}^{20} with precision to the number of decimals prescribed in the monograph, using a pycnometer, a density bottle, a hydrostatic balance or a hydrometer. The thrust of air is disregarded during the weighing; this may introduce an error of 1 unit in the third decimal place.

Two other definitions are commonly used.

The relative density d_4^{20} of a substance is the ratio of the mass of a certain volume of the substance at 20 °C to the mass of an equal volume of water at 4 °C.

The density ρ_{20} of a substance is the ratio of its mass to its volume at 20 °C. It is expressed in kilograms per cubic metre ($1\ kg.m^{-3} = 10^{-3}\ g.cm^{-3}$).

The numerical relationships between the relative density and the density expressed in kilograms per cubic metre are:

$$\rho_{20} = 998.202\ d_{20}^{20} \text{ or } d_{20}^{20} = 1.001\,80 \times 10^{-3}\ \rho_{20}$$

$$\rho_{20} = 999.972\ d_4^{20} \text{ or } d_4^{20} = 1.000\,03 \times 10^{-3}\ \rho_{20}$$

$$d_4^{20} = 0.998\,230\ d_{20}^{20}$$

2.2.6. REFRACTIVE INDEX

The refractive index n_λ^t of a medium with reference to air is equal to the ratio of the sine of the angle of incidence of a beam of light in air to the sine of the angle of refraction of the refracted beam in the given medium.

Unless otherwise prescribed, the refractive index is measured at 20 ± 0.5 °C, with reference to the wavelength of the D-line of sodium (λ = 589.3 nm); the symbol is then n_D^{20}.

Refractometers normally determine the critical angle. In such apparatus the essential part is a prism of known refractive index in contact with the liquid to be examined.

To calibrate the apparatus, use the reference liquids listed in Table 2.2.6.-1 below. The value of the refractive index of each reference liquid is stated on the label.

Table 2.2.6.-1

Reference liquid	$\Delta n/\Delta t$ (temperature coefficient)
Trimethylpentane CRS	– 0.00049
Carbon tetrachloride CRS	– 0.00057
Toluene CRS	– 0.00056
Methylnaphthalene CRS	– 0.00048

When white light is used, the refractometer is provided with a compensating system. The apparatus gives readings accurate to at least the third decimal place and is provided with a means of operation at the temperature prescribed. The thermometer is graduated at intervals of 0.5 °C or less.

2.2.7. OPTICAL ROTATION

Optical rotation is the property displayed by certain substances of rotating the plane of polarisation of polarised light.

The specific optical rotation $[\alpha_m]_\lambda^t$ is the rotation, expressed in radians (rad), measured at the temperature t and at the wavelength λ given by a 1 metre thickness of liquid or solution containing 1 kilogram of optically active solute per cubic metre of solution. For practical reasons the specific optical rotation $[\alpha_m]_\lambda^t$ is normally expressed in milliradians metre squared per kilogram ($mrad.m^2.kg^{-1}$)

The Pharmacopoeia adopts the following conventional definitions.

The *angle of optical rotation* of a liquid is the angle of rotation α, expressed in degrees (°), of the plane of polarisation at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C using a layer of 1 decimetre; for a solution, the method of preparation is prescribed in the monograph.

The *specific optical rotation* $[\alpha]_D^{20}$ of a liquid is the angle of rotation α, expressed in degrees (°), of the plane of polarisation at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C in the liquid substance to be examined, calculated with reference to a layer of 1 decimetre and divided by the density expressed in grams per cubic centimetre.

The *specific optical rotation* $[\alpha]_D^{20}$ of a substance in solution is the angle of rotation α, expressed in degrees (°), of the plane of polarisation at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C in a solution of the substance to be examined and calculated with reference to a layer of 1 decimetre containing 1 gram of the substance per millilitre. The specific optical rotation of a solid is always expressed with reference to a given solvent and concentration.

In the conventional system adopted by the Pharmacopoeia the specific optical rotation is expressed in degree millilitres per decimetre gram [(°) .ml.dm^{-1}.g^{-1}].

The conversion factor from the International System to the Pharmacopoeia system is the following :

$$[\alpha_m]_\lambda^t = [\alpha]_\lambda^t \times 0.1745$$

In certain cases specified in the monograph the angle of rotation may be measured at temperatures other than 20 °C and at other wavelengths.

The polarimeter must be capable of giving readings to the nearest 0.01°. The scale is usually checked by means of certified quartz plates. The linearity of the scale may be checked by means of sucrose solutions.

Method. Determine the zero of the polarimeter and the angle of rotation of polarised light at the wavelength of the D-line of sodium (λ = 589.3 nm) at 20 ± 0.5 °C. Measurements may be carried out at other temperatures only where the monograph indicates the temperature correction to be made to the measured optical rotation. Determine the zero of the apparatus with the tube closed; for liquids the zero is determined with the tube empty and for solids filled with the prescribed solvent. Carry out at least five measurements and calculate the average.

Calculate the specific optical rotation using the following formulae, dextrorotation and laevorotation being designated by (+) and (-) respectively.

For liquids: $$[\alpha]_D^{20} = \frac{\alpha}{l \cdot \rho_{20}}$$

For solids: $$[\alpha]_D^{20} = \frac{100\alpha}{l \cdot c}$$

Calculate the content c in g/l or the content c' in per cent *m/m* of a dissolved substance using the following formulae:

$$c = \frac{1000\alpha}{l \cdot [\alpha]_D^{20}} \qquad c' = \frac{100\alpha}{l \cdot [\alpha]_D^{20} \cdot \rho_{20}}$$

α = angle of rotation in degrees read at 20 ± 0.5 °C,

l = length in decimetres of the polarimeter tube,

ρ_{20} = density at 20 °C in grams per cubic centimetre. For the purposes of the Pharmacopoeia, density is replaced by relative density (*2.2.5*),

c = concentration of the substance in g/l,

c' = content of the substance in per cent *m/m*.

2.2.18. FREEZING POINT

The freezing point is the maximum temperature occurring during the solidification of a supercooled liquid.

Apparatus. The apparatus (see Figure 2.2.18.-1) consists of a test-tube about 25 mm in diameter and 150 mm long placed inside a test-tube about 40 mm in diameter and 160 mm long. The inner tube is closed by a stopper which carries a thermometer about 175 mm long and graduated in 0.2 °C fixed so that the bulb is about 15 mm above the bottom of the tube. The stopper has a hole allowing the passage of the stem of a stirrer made from a glass rod or other suitable material formed at one end into a loop of about 18 mm overall diameter at right angles to the rod. The inner tube with its jacket is supported centrally in a 1 litre beaker containing a suitable cooling liquid to within 20 mm of the top. A thermometer is supported in the cooling bath.

Method. Place in the inner tube sufficient quantity of the liquid or previously melted substance to be examined, to cover the thermometer bulb and determine the approximate freezing point by cooling rapidly. Place the inner tube in a bath about 5 °C above the approximate freezing point until all but the last traces of crystals are melted. Fill the beaker with water or a saturated solution of sodium chloride, at a temperature about 5 °C lower than the expected freezing point, insert the inner tube into the outer tube, ensuring that some seed crystals are present, and stir thoroughly until solidification takes place. Note the highest temperature observed during solidification.

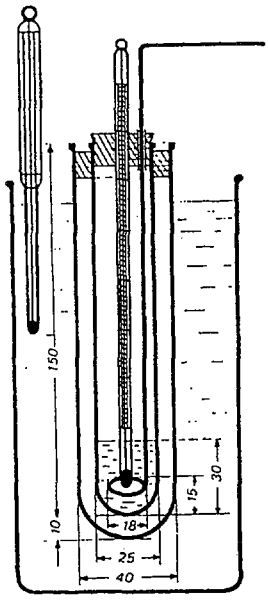


Figure 2.2.18.-1. – *Apparatus for the Determination of Freezing Point*
Dimensions in millimetres

2.5.1. ACID VALUE

The acid value I_A is the number that expresses in milligrams the quantity of potassium hydroxide required to neutralise the free acids present in 1 g of the substance.

Dissolve 10.00 g of the substance to be examined, or the quantity prescribed (*m* g) in 50 ml of a mixture of equal volumes of *alcohol R* and *ether R*, previously neutralised with *0.1 M potassium hydroxide*, unless otherwise specified, using 0.5 ml of *phenolphthalein solution R1* as indicator. When the substance to be examined has dissolved, titrate with *0.1 M potassium hydroxide* until the pink colour persists for at least 15 s (*n* ml of *0.1 M potassium hydroxide*).

$$I_A = \frac{5.610\ n}{m}$$

2.5.2. ESTER VALUE

The ester value I_E is the number that expresses in milligrams the quantity of potassium hydroxide required to saponify the esters present in 1 g of the substance. It is calculated from the saponification value I_S and the acid value I_A.

$$I_E = I_S - I_A$$

2.5.3. HYDROXYL VALUE

The hydroxyl value I_{OH} is the number that expresses in milligrams the quantity of potassium hydroxide required to neutralise the acid combined by acylation in 1 g of the substance.

METHOD A

Introduce the quantity of the substance to be examined shown in Table 2.5.3.-1 (m g) into a 150 ml acetylation flask fitted with an air condenser, unless another quantity is prescribed in the monograph. Add the quantity of *acetic anhydride solution R1* stated in Table 2.5.3.-1 and attach the air condenser.

Table 2.5.3.-1

Presumed value I_{OH}	Quantity of sample in grams	Volume of acetylating reagent in millilitres
10 - 100	2.0	5.0
100 - 150	1.5	5.0
150 - 200	1.0	5.0
200 - 250	0.75	5.0
250 - 300	0.60 or 1.20	5.0 or 10.0
300 - 350	1.0	10.0
350 - 700	0.75	15.0
700 - 950	0.5	15.0

Heat the flask in a water-bath for 1 h keeping the level of the water about 2.5 cm above the level of the liquid in the flask. Withdraw the flask and allow to cool. Add 5 ml of *water R* through the upper end of the condenser. If a cloudiness appears add sufficient *pyridine R* to clear it, noting the volume added. Shake the flask and replace in the water-bath for 10 min. Withdraw the flask and allow to cool. Rinse the condenser and the walls of the flask with 5 ml of *alcohol R*, previously neutralised to *phenolphthalein solution R1*. Titrate with *0.5 M alcoholic potassium hydroxide* using 0.2 ml of *phenolphthalein solution R1* as indicator (n_1 ml of *0.5 M alcoholic potassium hydroxide*). Carry out a blank test under the same conditions (n_2 ml of *0.5 M alcoholic potassium hydroxide*).

$$I_{OH} = \frac{28.05\ (n_2 - n_1)}{m} + I_A$$

METHOD B

Introduce the prescribed quantity of the substance to be examined (m g) into a perfectly dry 5 ml conical flask fitted with a ground-glass or suitable plastic stopper and add 2.0 ml of *propionic anhydride reagent R*. Close the flask and shake gently to dissolve the substance. Allow to stand for 2 h unless otherwise prescribed. Remove the stopper and transfer the flask and its contents into a wide-mouthed 500 ml conical flask containing 25.0 ml of a 9 g/l solution of *aniline R* in *cyclohexane R* and 30 ml of *glacial acetic acid R*. Swirl the contents of the flask, allow to stand for 5 min, add 0.05 ml of *crystal violet solution R* and titrate with *0.1 M perchloric acid* until an emerald-green colour is obtained (n_1 ml of *0.1 M perchloric acid*). Carry out a blank test under the same conditions (n_2 ml of *0.1 M perchloric acid*).

$$I_{OH} = \frac{5.610\ (n_1 - n_2)}{m}$$

To take account of any water present, determine this (y per cent) by the semi-micro determination of water (*2.5.12*).

The hydroxyl value is then given by the equation:

$$I_{OH} = \text{(hydroxyl value as determined)} - 31.1\ y$$

2.8.5. WATER IN ESSENTIAL OILS

Mix 10 drops of the essential oil with 1 ml of *carbon disulphide R*. The solution remains clear on standing.

2.8.6. FOREIGN ESTERS IN ESSENTIAL OILS

Heat 1 ml of the essential oil for 2 min on a water-bath with 3.0 ml of a freshly prepared 100 g/l solution of *potassium hydroxide R* in *alcohol R*. No crystals are formed within 30 min, even after cooling.

2.8.7. FATTY OILS AND RESINIFIED ESSENTIAL OILS IN ESSENTIAL OILS

Allow 1 drop of the essential oil to fall onto filter paper. The drop evaporates completely within 24 h without leaving any translucent or greasy spot.

2.8.9. RESIDUE ON EVAPORATION OF ESSENTIAL OILS

The residue on evaporation of an essential oil is the percentage by mass of the oil which remains after evaporation on a water-bath under the conditions specified below.

Apparatus. The apparatus (see Figure 2.8.9.-1) consists of:

- Water-bath with a cover having holes of 70 mm diameter,
- Evaporating dish of heat-resistant glass which is inert to the contents,
- Desiccator.

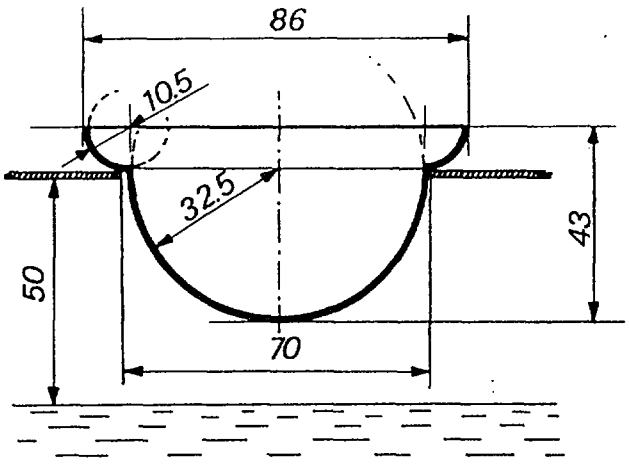


Figure 2.8.9.-1.
Dimensions in millimetres

Method. Weigh the evaporating dish after having heated it on the water-bath for 1 h and cooled it in the desiccator. Weigh into the evaporating dish 5.00 g of the essential oil, unless otherwise prescribed. Heat the oil on the vigorously boiling water-bath in a draught-free atmosphere for the prescribed time. Allow to cool in the desiccator and weigh.

During the test, the level of water in the bath is maintained about 50 mm beneath the level of the cover.

2.8.10. SOLUBILITY IN ALCOHOL OF ESSENTIAL OILS

Place 1.0 ml of the essential oil in a 25 ml or 30 ml glass-stoppered cylinder. Place in a constant temperature device, maintained at a temperature of 20 ± 0.2 °C. Using a burette of at least 20 ml capacity, add the alcohol of the strength prescribed in the monograph by increments of 0.1 ml until solution is complete and then continue adding by increments of 0.5 ml to a total of 20 ml, shaking frequently and vigorously. Record the volume of alcohol added when a clear solution has been obtained and, if the solution becomes cloudy or opalescent before 20 ml of alcohol has been added, record the volume added when the cloudiness or opalescence appears and, where applicable, the volume added when the cloudiness or opalescence disappears.

If a clear solution has not been obtained when 20 ml of alcohol of the prescribed strength has been added, repeat the test using the next highest concentration of alcohol.

An essential oil is said to be *"soluble in n volumes or more of alcohol of given strength t"* when the clear solution in n volumes remains clear when compared with the undiluted oil after further addition of alcohol of the same strength up to a total of 20 volumes of alcohol.

An essential oil is said to be *"soluble in n volumes of alcohol of given strength t, becoming cloudy when diluted"* when the clear solution in n volumes becomes cloudy in n_1 volumes (n_1 less than 20) and stays so after further gradual addition of alcohol of the same strength up to a total of 20 volumes of alcohol.

An essential oil is said to be *"soluble in n volumes of alcohol of given strength t with cloudiness between n_1 and n_2 volumes"* when the clear solution in n volumes becomes cloudy in n_1 volumes (n_1 less than 20) and stays so after further gradual addition of alcohol of the same strength up to a total of n_2 volumes of alcohol and then becomes clear (n_2 less than 20).

An essential oil is said to be *"soluble with opalescence"* when the alcoholic solution shows a bluish tinge, similar to that of a standard of opalescence freshly prepared as follows: mix 0.5 ml of *silver nitrate solution R2* and 0.05 ml of *nitric acid R*; add 50 ml of a 12 mg/l solution of *sodium chloride R*; mix and allow to stand protected from light for 5 min.

2.2.28. GAS CHROMATOGRAPHY

Gas chromatography is a method of separation in which the mobile phase is a gas (the carrier gas) and the stationary phase, contained in a column, is either a solid or a liquid coated on a solid inert support or a liquid film evenly coated on the walls of the column.

Gas chromatography is based on mechanisms of adsorption and/or partition.

Apparatus. The apparatus consists of a gas supply, a sample injection port, a chromatographic column, a detector and a recorder. The column is usually of glass or stainless steel and contains the stationary phase. The carrier gas flows through the column at a controlled rate and then through the detector.

The determination is carried out either at a constant temperature or according to a given temperature programme.

The detector used must enable the amounts of the substances of interest present in the column eluate to be determined. It is usually based on flame ionisation, thermal conductivity, thermionic or electron capture phenomena.

Method. Equilibrate the column, injection port and detector at the prescribed temperatures. Prepare the solution of the substance to be examined and the reference solution or solutions as prescribed. Using the reference solutions, determine suitable instrument settings and the quantities to be injected to produce an adequate response. Carry out replicate injections to verify the repeatability of response and check, if required, the number of theoretical plates.

Inject the solutions and record the resulting chromatograms. Carry out replicate injections to verify the repeatability of response. Determine the peak areas or, alternatively when the symmetry factor calculated as shown below is between 0.80 and 1.20, the peak heights corresponding to the compo-

nents of interest. In applications requiring temperature programmes, peak area determinations are to be used. Where an internal standard is used, check that no peak of the substance to be examined is masked by that of the internal standard.

From the values obtained, calculate the content of the component or components being determined. When prescribed, the percentage of one or more components of the substance to be examined is calculated by determining the area of the peak or peaks as a percentage of the total area of all peaks, excluding those due to solvents or any added reagents (normalisation procedure). The use of a wide-range amplifier and an automatic integrator is then recommended.

The **symmetry factor** of a peak may be calculated from the expression:

$$\frac{b_{0.05}}{2A}$$

$b_{0.05}$ = width of the peak at one-twentieth of the peak height,

A = distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

The **resolution** (R_s) may be calculated from the formula:

$$R_s = \frac{1.18\,(t_{Rb} - t_{Ra})}{b_{0.5a} + b_{0.5b}}$$

$$t_{Rb} > t_{Ra}$$

t_{Rb} and t_{Ra} = distances, in millimetres, along the baseline between the point of injection and the perpendiculars dropped from the maxima of two adjacent peaks,

$b_{0.5a}$ and $b_{0.5b}$ = peak widths, in millimetres, at half height.

The results of the determination are not valid unless the resolution between measured peaks in the chromatogram is greater than 1.0, unless otherwise prescribed.

The **number of theoretical plates** (n) may be calculated from data obtained under isothermal conditions from the formula:

$$n = 5.54\left(\frac{t_R}{b_{0.5}}\right)^2$$

t_R = distance, in millimetres, along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak in question,

$b_{0.5}$ = peak width, in millimetres, at half height.

The **mass distribution ratio** D_m (also known as the capacity factor) is defined as:

$$D_m = \frac{\text{amount of solute in stationary phase}}{\text{amount of solute in mobile phase}} = K \times \frac{V_s}{V_m}$$

K = the equilibrium distribution coefficient,

V_s = the volume of the stationary phase,

V_m = the volume of the mobile phase.

The mass distribution ratio of a component may be determined from the chromatogram using the formula:

$$D_m = \frac{t_R - t_{R'}}{t_{R'}}$$

t_R = distance in millimetres along the baseline between the point of injection and a perpendicular dropped from the maximum of the peak corresponding to the component,

$t_{R'}$ = distance in millimetres along the baseline between the point of injection and a perpendicular dropped from the maximum of the peak corresponding to an unretained component.

The **signal-to-noise ratio** (S/N) is calculated from the equation:

$$S/N = \frac{2H}{h_n}$$

H = height of the peak corresponding to the component concerned in the chromatogram obtained with the prescribed reference solution,

h_n = absolute value of the largest noise fluctuation from the baseline in a chromatogram obtained after injection of a blank and observed over a distance equal to twenty times the width at half-height of the peak in the chromatogram obtained with the prescribed reference solution, and situated equally around the place where this peak would be found.

2.8.11. ASSAY OF 1,8-CINEOLE IN ESSENTIAL OILS

Weigh 3.00 g of the oil, recently dried with anhydrous *sodium sulphate R*, into a dry test-tube and add 2.10 g of melted *cresol R*. Place the tube in the apparatus for the determination of freezing point (*2.2.18*) 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 at a temperature that does not exceed t_1 by more than 5 °C and place the tube in the apparatus, maintained at a temperature 5 °C below t_1. When crystallisation takes place, or when the temperature of the mixture has fallen 3 °C below t_1, stir continuously. Note the highest temperature at which the mixture crystallises (t_2). Repeat the operation until two highest values obtained for t_2 do not differ by more than 0.2 °C. If supercooling occurs, induce crystallisation by adding a small crystal of the complex consisting of 3.00 g of *cineole R* and 2.10 g of melted *cresol R*. If t_2 is below 27.4 °C, repeat the determination after the addition of 5.10 g of the complex.

The content of cineole corresponding to the highest temperature observed (t_2) is given in Table 2.8.11.-1. If 5.10 g of the complex has been added, calculate the cineole content per cent *m/m* from the expression:

$$2\,(A–50)$$

where A is the value found in the Table.

The content of cineole, corresponding to the highest temperature observed (t_2), is obtained, where necessary, by interpolation.

Table 2.8.11.-1

t_2 °C	cineole per cent *m/m*	t_2 °C	cineole per cent *m/m*	t_2 °C	cineole per cent *m/m*	t_2 °C	cineole per cent *m/m*
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

Determination of Aldehydes

To 1 g of the oil in a glass-stoppered tube (approximately 150 mm × 25 mm) add 5 ml of *toluene* and 15 ml of *hydroxylamine solution in ethanol (60%)*, shake vigorously and titrate immediately with 0.5M *potassium hydroxide in ethanol (60%) VS* until the red colour changes to yellow. Continue shaking and neutralising until the full yellow colour of the indicator is permanent in the lower layer after shaking vigorously for 2 minutes and allowing to separate; the reaction is complete in about 15 minutes. This procedure gives an approximate value for the aldehyde content of the oil.

Repeat this procedure, using as the colour standard for the end-point of the titration the titrated liquid of the first

Determination of Carvone

To 1.5 g of the oil in a glass-stoppered tube (approximately 150 mm × 25 mm) add 10 ml of *hydroxylamine solution in ethanol (90%)*. Titrate with 1M *potassium hydroxide in ethanol (90%) VS* until the red colour changes to yellow. Place the tube in a water-bath at 75° to 80° and, at 5-minute intervals, neutralise with 1M *potassium hydroxide in ethanol (90%) VS*; after 40 minutes complete the titration to the full yellow colour of the indicator. This procedure gives an approximate value for the carvone content of the oil.

Repeat this procedure, using as the colour standard for the end-point of the titration the titrated liquid of the first determination with the addition of 0.5 ml of 1M *potassium hydroxide in ethanol (90%) VS*. Calculate the content of carvone from the second determination. Each ml of 1M *potassium hydroxide in ethanol (90%) VS* is equivalent to 0.1514 g of carvone, $C_{10}H_{14}O$.

Determination of Esters

Boil a convenient quantity of *ethanol (96%)* thoroughly to expel carbon dioxide and neutralise it to *phenolphthalein solution*. Unless otherwise directed in the monograph, weigh 2 g or other suitable quantity of the substance being examined so that the volume of 0.5M *ethanolic potassium hydroxide VS* added is at least twice that theoretically required, dissolve it in 5 ml of the neutralised ethanol contained in a hard-glass flask and neutralise the free acid in the solution with 0.1M *ethanolic potassium hydroxide VS* using 0.2 ml of *phenolphthalein solution* as indicator. Add 25 ml of 0.5M *ethanolic potassium hydroxide VS* and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of *water* and titrate the

EUCALYPTUS OIL

Eucalypti aetheroleum

Aldehydes. Place 10 ml in a glass-stoppered tube 25 mm in diameter and 150 mm long and add 5 ml of *toluene R* and 4 ml of *alcoholic hydroxylamine solution R*. Shake vigorously and titrate immediately with *0.5 M potassium hydroxide in alcohol (60 per cent V/V) R* until the red colour changes to yellow. Continue the titration with shaking; the end-point is reached when the pure yellow colour of the indicator is permanent in the lower layer after shaking vigorously for 2 min and allowing separation to take place. The reaction is complete in about 15 min. Repeat the titration using a further 10 ml of the substance to be examined and, as a reference solution for the end-point, the titrated liquid from the first determination to which has been added 0.5 ml of *0.5 M potassium hydroxide in alcohol (60 per cent V/V) R*. Not more than 2.0 ml of *0.5 M potassium hydroxide in alcohol (60 per cent V/V) R* is required in the second titration.

Phellandrene. To 1 ml add 2 ml of *glacial acetic acid R* and 5 ml of *light petroleum R1*. Add 2 ml of a saturated solution of *sodium nitrite R* and shake gently. No crystalline precipitate is formed in the upper layer within 1 h.

LAVENDER OIL

Lavandulae aetheroleum

Water-soluble portion. Introduce 20 ml of *saturated sodium chloride solution R* into a 50 ml measuring cylinder and carefully add slowly 10 ml of the essential oil. Note the zone of contact of the two layers, mix and allow to stand. The volume of the essential oil does not change.

LEMON OIL

Limonis aetheroleum

Absorbance (*2.2.25*). Dissolve 0.250 g in *alcohol R*, mix and dilute to 100.0 ml with the same solvent. Measure the absorbance over the range 260 nm to 400 nm. If a manual instrument is used, measure the absorbance at 5 nm intervals from 260 nm to about 12 nm before the expected absorption maximum, then at 3 nm intervals for three readings and at 1 nm intervals to about 5 nm beyond the maximum and finally at 10 nm intervals to 400 nm. Plot a curve representing the absorption spectrum with the absorbances as ordinates and the wavelengths as abscissae. Draw as a base-line the tangent between *A* and *B* (see Figure 0620-1). The absorption maximum *C* is situated at 315 ± 3 nm. From *C* draw a line perpendicular to the axis of abscissae and intersecting *AB* at *D*. Deduct the absorbance corresponding to point *D* from that corresponding to point *C*. The value obtained is 0.20 to 0.96 and for Italian-type lemon oil it is not less than 0.45.

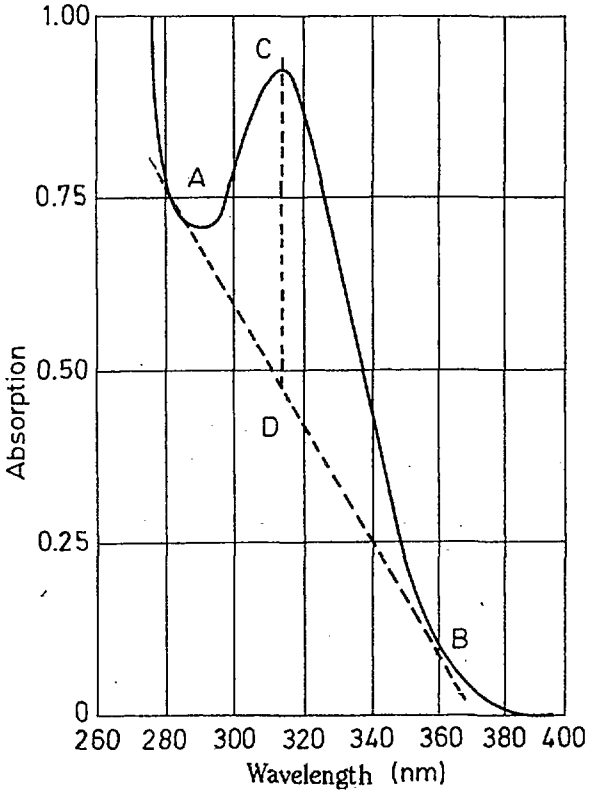


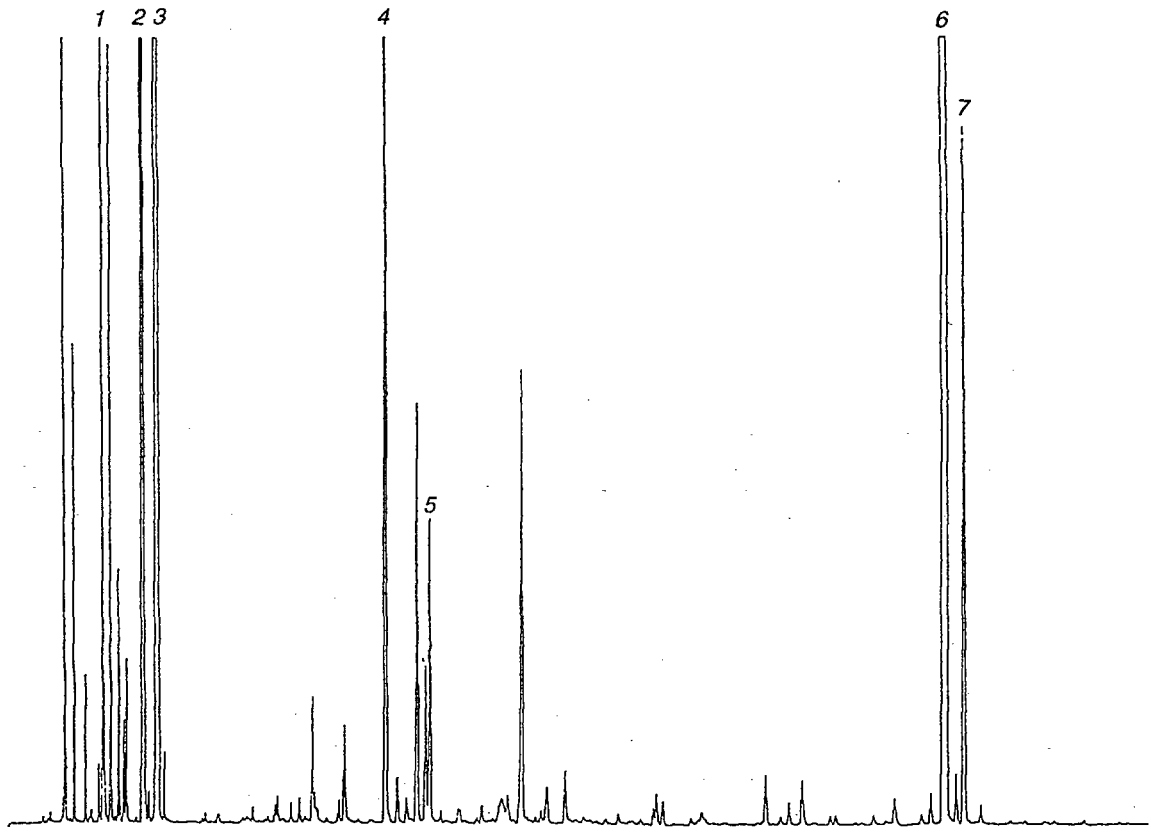
Figure 0620-1.–*Typical spectrum of Lemon Oil*

Adulterants. Examine the chromatograms obtained in the identification test in ultraviolet light at 254 nm. The chromatogram obtained with reference solution (b) shows a quenching zone corresponding to citral. In the chromatogram obtained with the test solution, any quenching zone above the zone due to bergamotin (methyl anthranilate and menthyl salicylate) or any quenching zone at about the same level as citropten (chalcones) is not more intense than the quenching zone in the chromatogram obtained with reference solution (b). Examine in ultraviolet light at 365 nm. The chromatogram obtained with reference solution (b) shows a bright-blue fluorescent zone corresponding to citropten. In the chromatogram obtained with the test solution, any violet or blue fluorescent zone above the zone due to bergamotin is not more intense than the fluorescent zone in the chromatogram obtained with reference solution (b). Expose the plate to hydrochloric acid vapour and examine in daylight. Neither bright red zones (chalcones) nor bright blue or yellow zones (other adulterants) appear in the lower third or in the middle of the chromatogram obtained with the test solution, but may appear in the upper third of the chromatogram.

THYME OIL

Thymi aetheroleum

The following type chromatogram is given for information and guidance only; it does not form a mandatory part of the monograph.



1. β-myrcene
2. γterpinene
3. *p*-cymene
4. linalol
5. terpinen-4-ol
6. thymol
7. carvacrol

Figure 1374.-1. – *Type chromatogram for thyme oil (chromatographic profile test)*

Determine the percentage content of the components of the normalisation procedure.

The percentages range between the following values:

β-Myrcene	1.0 per cent to 3.0 per cent
γ-Terpinene	5.0 per cent to 10.0 per cent
p-Cymene	15.0 per cent to 28.0 per cent
Linalol	4.0 per cent to 6.5 per cent
Terpinen-4-ol	0.2 per cent to 2.5 per cent
Thymol	36.0 per cent to 55.0 per cent
Carvacrol	1.0 per cent to 4.0 per cent

200 ml of *water R* as the distillation liquid and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 3 ml to 4 ml per minute for 2 h.

STORAGE

Store in a well-closed container, protected from light.

1997:0405

PEPPERMINT OIL

Menthae piperitae aetheroleum

DEFINITION

Peppermint oil is obtained by steam distillation from the fresh overground parts of the flowering plant of *Mentha* × *piperita* L.

CHARACTERS

A colourless, pale yellow or pale greenish-yellow liquid with a characteristic odour and taste followed by a sensation of cold, miscible with alcohol, with ether and with methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution. Dissolve 0.1 g of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 10 mg of *thymol R*, 10 µl of *menthyl acetate R*, 20 µl of *cineole R* and 50 mg of *menthol R* in *toluene R* and dilute to 10 ml with the same solvent.

Apply separately to the plate as bands 10 µl of the reference solution and 20 µl of the test solution. Develop over a path of 15 cm using a mixture of 5 volumes of *ethyl acetate R* and 95 volumes of *toluene R*. Allow the plate to dry in air until the odour of the solvent is no longer perceptible and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution may show quenching zones (carvone, pulegone) situated just below the level of the zone (thymol) in the chromatogram obtained with the reference solution. Spray with *anisaldehyde solution R* and examine in daylight for 5 min to 10 min while heating at 100 °C to 105 °C. The chromatogram obtained with the reference solution shows, in order of increasing R_f value: an intense blue to violet zone (menthol) in the lower third; a violet-blue to brown zone (cineole); a pink zone (thymol); and a violet-blue zone (menthyl acetate). In the chromatogram obtained with the test solution: there is a zone due to menthol (the most intense) and a faint zone due to cineole; at R_f values between those of the cineole and thymol zones in the chromatogram obtained with the reference solution, there may be light pink or greyish-blue or greenish-grey zones (carvone, pulegone, isomenthone); in the middle of the chromatogram, there is a violet-blue zone (menthyl acetate) and just below it a greenish-blue zone (menthone); an intense violet-red zone (hydrocarbons) appears near the solvent front and below it a brownish-yellow zone (menthofuran); other less intensely coloured zones also appear.

B. Examine the chromatograms obtained in the test for chromatographic profile. The retention time of the principal peaks in the chromatogram obtained with the test solution is similar to that of the principal peaks in the chromatogram obtained with the reference solution. Carvone and pulegone may be absent from the chromatogram obtained with the test solution.

TESTS

Acid value (*2.5.1*). Not more than 1.4, determined on 5.0 g dissolved in 50 ml of the prescribed mixture of the solvents.

Relative density (*2.2.5*): 0.900 to 0.916.

Refractive index (*2.2.6*): 1.457 to 1.467.

Optical rotation (*2.2.7*). The angle of optical rotation is - 10° to - 30°.

Fatty oils and resinified essential oils (*2.8.7*). It complies with the test for fatty oils and resinified essential oils.

Chromatographic profile. Examine by gas chromatography (*2.2.28*).

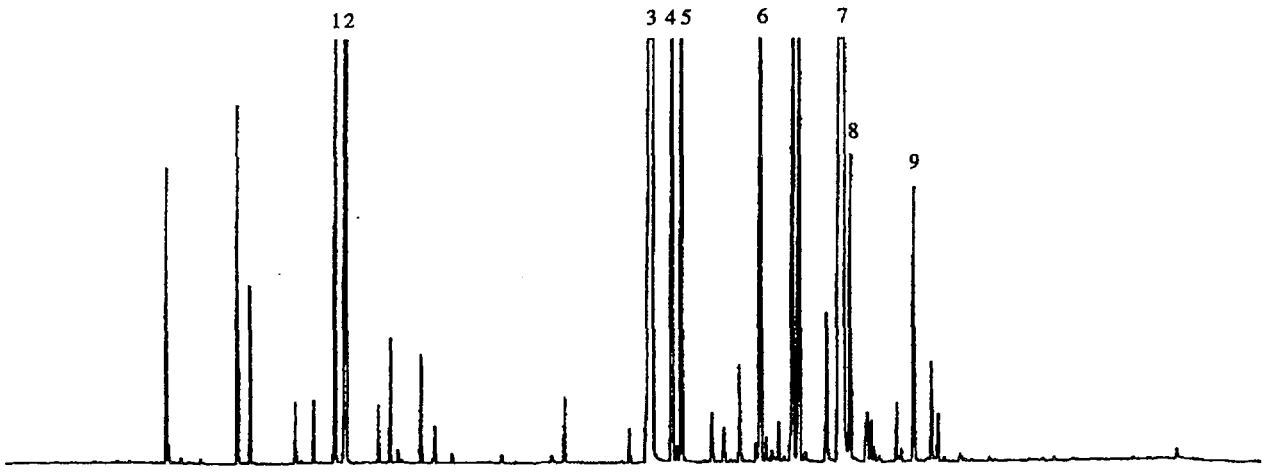
Test solution. The substance to be examined.

Reference solution. Dissolve 0.1 g of *limonene R*, 0.2 g of *cineole R*, 0.4 g of *menthone R*, 0.1 g of *menthofuran R*, 0.1 g of *isomenthone R*, 0.4 g of *menthyl acetate R*, 0.6 g of *menthol R*, 0.2 g of *pulegone R* and 0.1 g of *carvone R* in 1 ml of *hexane R*.

The chromatographic procedure may be carried out using:

- a fused-silica capillary column 60 m long and about 0.25 mm in internal diameter coated with *macrogol 20 000 R* as the bonded phase,
- *helium for chromatography R* as the carrier gas at a flow rate of 1.5 ml per minute,
- a flame-ionisation detector,
- a split ratio of 1/100,

maintaining the temperature of the column at 60 °C for 10 min, then raising the temperature at a rate of 2 °C per



1. limonene
2. cineole
3. menthone
4. menthofuran
5. isomenthone
6. menthyl acetate
7. menthol
8. pulegone
9. carvone

Figure 405-1.–*Type chromatogram for peppermint oil*

The type chromatogram is given for information and guidance in application of the analytical method. It is not part of the requirements of the monograph.

minute to 180 °C and maintaining at 180 °C for 5 min and maintaining the temperature of the injection port and of the detector at 220 °C.

Inject about 0.2 µl of the reference solution. When the chromatograms are recorded in the prescribed conditions, the components elute in the order indicated in the composition of the reference solution. Record the retention times of these substances.

The test is not valid unless: the number of theoretical plates calculated from the limonene peak at 110 °C is at least 30 000; the resolution between the peaks corresponding to limonene and cineole is at least 1.5.

Inject about 0.2 µl of the test solution. Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution (disregard the peak due to hexane).

Determine the percentage content of the components by the normalisation procedure.

The percentages are within the following ranges:

Limonene	1.0 to 5.0 per cent
Cineole	3.5 to 14.0 per cent
Menthone	14.0 to 32.0 per cent
Menthofuran	1.0 to 9.0 per cent
Isomenthone	1.5 to 10.0 per cent
Menthyl acetate	2.8 to 10.0 per cent
Menthol	30.0 to 55.0 per cent
Pulegone	not more than 4.0 per cent
Carvone	not more than 1.0 per cent

The ratio of cineole content to limonene content is greater than two.

STORAGE

Store in a well-filled, airtight container, protected from light and heat.

1997:0682

PEPSIN POWDER

Pepsini pulvis

DEFINITION

Pepsin powder is prepared from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases, active in acid medium (pH 1 to 5). It has an activity not less than 0.5 Ph. Eur. U. per milligram, calculated with reference to the dried substance. Pepsin powder is prepared in conditions designed to minimise the degree of microbial contamination.

CHARACTERS

A white or slightly yellow, crystalline or amorphous powder, hygroscopic, soluble in water, practically insoluble in alcohol and in ether. The solution in water may be slightly opalescent with a weak acidic reaction.

TBAM-ICS/UNIDO Training Course on Quality Improvement of Essential Oils
15-19 November 1999, Eskişehir, Turkey

OLFACTORY EVALUATION OF ESSENTIAL OILS

Magda CHMIELEWSKA

Pollena Aroma
Warsaw, Poland

They haven't got no noses,
The fallen sons of Eve;
Even the smell of roses
Is not what they supposes;
But more than mind discloses
And more than men believe.

The brilliant smell of water,
The brave smell of a stone,
The smell of dew and thunder,
The old bones buried under,
Are things in which they blunder
And err, if left alone.

The wind from winter forests,
The scent of scentless flowers,
The breath of brides' adorning,
The smell of snare and warning,
The smell of Sunday morning,
God gave to us for ours.

And Quoodle here discloses
All things that Quoodle can,
They haven't got no noses,
They haven't got no noses,
And goodness only knowses
The Noselessness of Man.

G. K. Chesterton

OLFACTORY EVALUATION OF ESSENTIAL OILS

Magdalena Chmielewska
Chief Perfumer of „Pollena-Aroma"
Warsaw, Poland

I. INTRODUCTION

The history of fragrance is as old as mankind itself. Fragrance is the essence of luxury and refinement. Even before people began to decorate themselves, their utensils and their surroundings, they were trying to please each other and their gods through the use of fragrances. In the beginning, they simply used flowers, herbs and resins. But soon they discovered that the resins and balms gave off their fragrances more readily and more strongly under the influence of heat. „Perfumum", became the name of one of the most precious of cultural luxuries. Perfumum suggests that fragrances originally were used as gifts to the gods.

The world knew of fragrant substances gradually and their preparation at least for thousand years before the birth of Christ. The techniques of pressing, boiling, drying, powderizing, maceration in fat, and even a simple form of distillation are ancient crafts.

There was a great need for fragrant substances in antiquity, because the humans who populated the world in those days, from China to Egypt, from Persia to Rome, perfumed themselves and their surrounding with a lavishness that would appear to us grotesque. Those who could afford it took baths in perfumed water. Not only was the body scented, but also the hair, the clothing, the bed – including the bedmate, the favorite slave and horse. Also the tiles of the houses and the temples, the sides of the tents, the sedan chairs, and even the sails of the ships were perfumed.

Perhaps the first treatise on odours is that of Theophrastus who wrote his „Concerning odours" in the second or third century B.C. But history of perfumery or rather perfume as we understand today an alcoholic solution of fragrance materials started in 1367 when „Queen of Hungary Water" was created and idntroduced in Europe by the Polish born Queen of Hungary Elisabeth. It was based on rosemary, thyme and some other herbs and was known and used as a parfume and medicine until end of last century. The next milestone in history of contemporary fragrances was „Aqua mirabilis" later developed as „Eau de Cologne" by Jean-Antoine Farina in 1690 existing to our times in hundreds of modifications and remaining most popular men's daily fragrance.

It was in the XVIII century when the revolution in perfumery happend. Three different but strictly connected phenomena appeared on the perfumery field. First was formation of professional perfumery houses, which manufactured and distributed ready made perfumes making these available on the market and attracting more users. At the same time fragrance row materials producers started their activities producing essential oils and creating fragrance compounds used by perfumery houses. And finally organic chemistry emerged with the first synthetic organic products that nature can be imitated by chemistry.

First companies which specialised in the manufacture of fragrance raw materials and compouding were established in Grasse in France, Germany, Great Britain and USA. Among the biggest compouders operating now in the international markets, the oldest is Givaudan – Roure, Bush Boake Allen and Harman – Reimer. Others are Firmanich, Quest, Dragoco, Takasago and IFF.

Based on actual production of essential oils one can say that the production of turpentine and pine oils amounts more than 80% of the production of all essential oil combined. The next oils which tonnage of world production is high are:

Citronella oils, Mentha oils, Lemongrass oil, Sweet orange oil, Lemon oil, Clove leaf oil, Lavandin oils, Boise de Rose oil, Spearmint oil, Lime oil.

The most important centres of production of essential oils are located in Zanzibar and Pemba, Grenda, Nossi-Be, Moluccan islands, USA, Madagascar, Reunionisland, Comoro islands, Marocco, Jamaica, Formosa and Brazil.
If somebody is unaccustomed to smelling perfumery materials, may at first find some difficulty with odour description because no all of the raw materials posses odour which can easily be related to natural smells. Smelling – exercises will, however, become easier with practice, particulary when somebody is familiar with the meanings of the descriptive terms discussed in the notes on odour descritpion and classification. Nomenclature is always thorny subject, inviting controversy, and in cases where we are aware there is considerable disagreement as to exactly how certain words should be defined. Several years ago when the American Society of Perfumers tried to agree upon definitions of fragrance terms, they suggested the following for *Essential oils*:
Voilatile oils obtained by various processes from leavs, petals, twigs, roots, bark, seeds, woods, fruit peels, etc.
But not everybody is satisfied with this definition. Maybe it should be:
Aromatic oils derived from raw materials of botanical origin by such processes as distillation, expression, extraction, maceration, etc. Which isolate and concentrate the basic aroma principle of raw material.

II. NATURAL FRAGRANCE RAW MATERIALS

Natural fragrance raw materials are derived mainly from aromatic plants. A few of them are obtained from animal sources, e.g. musk, civet, etc. Or produced by microorganisms. Mosses, liverworths, seaweeds and fungi have also been shown to contain essential oils. Natural fragrance materials from plants can be divided into the following categories according to the method of production:
Essential oils obtained from natural plant raw materials

- by distillation with water or steam (most of the oils – e.g. lavender, rose, rosemary, citronella, sandalwood, vetiver, mint, ylang – ylang, cinnamon barle, neroli, geranium and many others)
- by mechanical expression (mainly from epicarp of citrus fruits – e.g. lemon, organe, grapefruit, bergamot)
- by dry distillation (very few products – e.g. birch tree, bark oil)

The essential oils when separated from water by physical means can be further processed (by distillation or fractional destillation) to give *Rectified essential oils* (e.g. mint oils) *Terpenless, Sesquiterpenless*, or *X-less* essential oils (e.g. terpenless citruis oils, bergaptene free bergamot oild), *Folded* (concentrated) essential oils (2,3 – fold orange oil), and *Isolates* (e.g. citral ex Litsea Cubeba) and side products of above processes i.e. aromatic waters (e.g. rose water, lavender water), *Terpenes* (e.g. lemon terpenes) and *Residues.*
Extraction products can also be classified according to the solvent and the method of extraction *Tinctures* (obtained by maceration of raw material in ethand e.g. Tonka Tincture), *Concretas* (obtained by treating natural raw materials with a non-aqueous, non –polar solvent and its evaporation e.g. rose, oak moss), *Pomades* (obtained by diffusion of odorieferous constituents of the flowers with cold or hot fats e.g. tuberose), *Resinoids* (obtained by treating dried natural raw material, usually resins, with a solvent e.g. Labdarum, Olibarum, Myrrh) and *Absolutes* (obtained from concrets, pomades or resinoids by extraction with ethand and its evaporation from the extract e.g. rose lavender, violet leaf, jasmine).
Sources of most important essential oils and other perfumery natural raw materials as decribed above are listed in ISO DRAFT STANDARD No 4720.3. „Nomenclature" which gives the name of species, and the parts of the plant used for production of the material . Apart form their unique odour value which in practice cannot be replaced by synthetics, natural fragrance raw materials are more and more important in various uses where no synthetics are allowed. Although not that important in fragrance compounding, it is a crucial factor in flovours where natural origin is a basic criteria for the material to be used in food products. Numerous problems appeard when such critiria were introduced as very often it is difficult to recognise individual chemical comopounds obtained from natural sources from its synthetic equivalent. „Nature identical" description was introduced for synthetic products indentical with those obtained from natural raw materials. Most sophisticated methods are used to verify origin of the material including most modern isotope tests and chiral analysis which for time being can prove the origin of the product correctly.

As said above natural raw materials after decades of their replecement with synthetic chemicals are becaming more important. Their use in perfumery and the demond and production are expanding. List of basic natural raw materials necessary for the starting stage of a creative perfumery laboratory is given in Annex 1.
All natural raw materials can devide into parts according to odour type and suggested use:

1. Fresh citrus

Bergamot
Cadrat
Lemon
Lime

2. Sweet citrus

Orange bitter
Orange sweet
Grapefruit
Mandarin
Tangerin

3. Woody

Sandalwood
Cedarwood
Vetiver
Oakmoss
Patchouli
Cyperus
Guaiac wood

4. Spicy

Cascarilla
Nutmeg
Clove
Lovage herb
Ginger
Carnation
Cinnamon Bark
Pimenta Berry
Pepper black
Bay
Cardamon

5. Orange Flower nots

Petitgrain Paraguay
Petitgrain sweet orange
Orange Flower water absolute
Orange flower absolute
Lemon petitgrain
Petitgrain bigarade
Neroli

6. Anise nots

Fermal sweet
Star anise
Basil
Estragon
Carawy

7. Rose gruop

Rose absolute
Rose oil
Geranium
Palmarosa

8. Citronella type

Citronella Ceylon
Citronella Java
Eucalyptus citriodora
Lemongrass
Litsea cubeba
Melissa
Verbena

9. Camphoraceous – agrestic

Camphor oil, white
Lavandin
Spike lavender
Sage,Spanish
Rosemary
Lavender
Eucaliptus
Hyssop
Laurel leaf
Myrtle

10. Herbaceous

Buchu leaf
Arnica
Valerian
Marigold absolute
Blackcurrant
Chamomile
Tagetes
Zdrawetz
Sage dary
Artemisia
Rue

11. Balsamic – amber

Ambra
Labdanum
Peru balsam oil
Tolu balsam
Styrax
Tonka absolute
Copaiba balsam

12. Animal notes

Castoreum
Civet
Musk
Costus

13. Resinous

Olibarum
Benzoin
Opoponax
Galbaum
Myrrha
Eleni

14. Minty notes

Peppermint oil
Spearmint oil
Mentha arvensis
Eucalyptus globulus

15. Floral

Mimosa
Cananga
Ylang-ylang
Champaca
Osmanthus fragrans
Orris absolute
Violet flower absolute
Gardenia
Tuberose
Jasmin

16. Conifer – fresh

Pine oil
Turpentine
Pinus pumilio
Juniperberry
Fir needle
Thuja

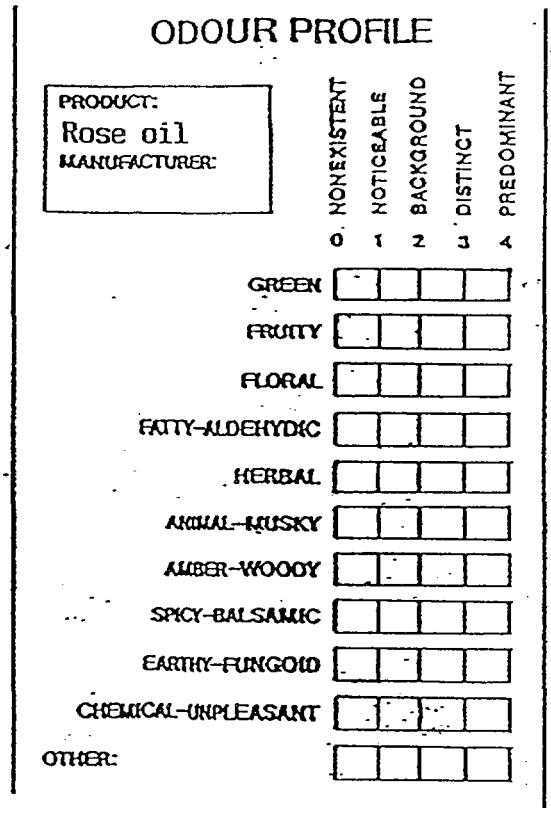
17. Rosewood

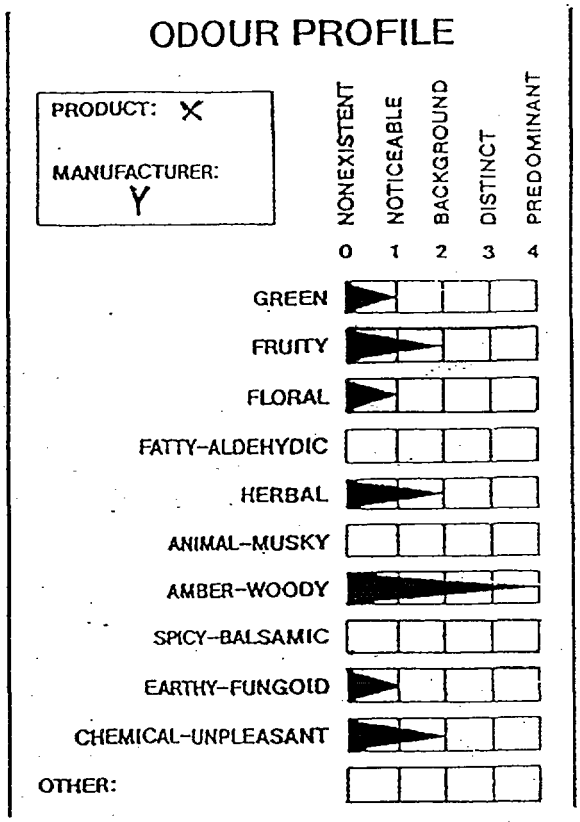
Boise de rose oil
Ho
Linaloe
Coriander
Niaouli
Amyris

18. Various

Calamus
Almond, bitter
Brich
Ambrette sead
Atractylis
Tobaco leaf
Wintergreen
Horseradish
Cognac oil

This division describe above includes natural raw materials which are use or know in Pollena-Aroma. Sense of smell and odour descriptions are based on associations i.e. there are no objective uses the words „like" i.e. „like rose", „like jasmine", or just adjectives like „fatty", „green", „sweet", „warm", „fruity", „floral" etc.
For general descriptions, very often the terminology taken from music is used. „Notes", „accords", „harmony", „tone" are terms used to describe individual odours or their combination.
Brud Odour Profiles as an easy method of odour comparison, based on mean results of odour evaluation by a team of perfumers, was the best way of classification of the products.
The idea of the Odour Profiles is shown below





These systems apply both two raw materials, natural and synthetic, and fragrance compounds and semiproducts (specialities and bases).
Many other methods were elaborated and published including a variety of odour profiles, hedonic systems, computerised programmes based on big panels work, up to multidimensional spherical models of odours. Most of these very complicated methods, created with special computer programmes, were of pure scientific interest, without much practical use. For routine work many fragrance compouding companies use their own systems of odour descriptions and classification for internal use by their perfumers.

Jasmin and Rose in Perfumery.

Jasmin absolute, Blossoms are producing in countires such as France, Egypt, Algeria, Marocco, italy, Turkey, China and India. Jasmine is delicate. It can not tolerate frost, and it intensely dislikes the prolonged drought that can occur in the African and Maditerranean countries. Further, the blossom will not give up its fragrance substance, the jasmin absolute, unless it is handled with extremely meticolous care.
With precious drop of absolute, the perfumer possesses a gem. Jasmine absolute is an olfactory fiant, an essential ingredient of epoch – making fragrance creations such as Arpega/Lanvin, Joy/Paton, Diorella/Dior, Charlie/Revlon, and Miss Dior/Dior.
All these perfumes, tremendous successes throughout the world, would have been impossible without jasmine.

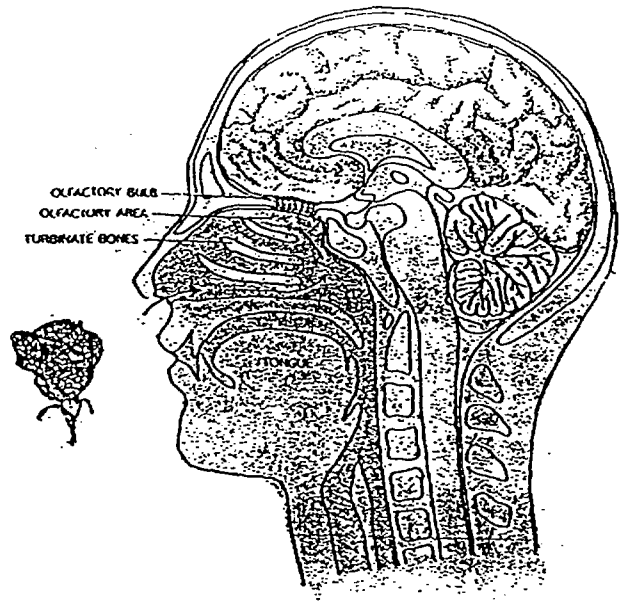
Rose oil, absolute is producing in France, Turkey, Marocco, Bulgaria. Rose absolute is rich, warm, spicy-floral and very deep rose odour with a more or less pronounced honeylike undertone. Its diffusive power is only realized when the absolute is diluted or used at the concentration of a few percent or even less in a perfume base. Rose absolute is use in high-class perfumes. Apart from strictly rosy florals, it forms important parts of the conventional rose – jasmin complex which is found in countless fashion perfums today.
The odour of rose oil is warm, deepfloral, slightly spicy and rich, truly reminescent of red roses, often with mances in the spicy and honeylike notes.
Both rose oil and rose absolute are main contributors to the fragrances of a variety of famous, high quality perfumes.

III. SENSORY EVALUATION

1. The sens of smell

One of the most ancient iventions in the course of the development of animals and humans is their olfactory sense, their sense of smell. From the primitive beginning human being used his nose as a source of most important information on food sources, danger, stranger etc. Our sense of smell can warn us against harmful substances and provoke protective reactions, e.g. flight. Inhalation of irritationg gases, e.g. such strong vapors as mustard oil or vinegar, can even cause us to hold our breath.
Life on the Earth began in the water of the oceans, in an age in which only a portion of the mineral salts had veen leached into the sea from the Earth's crust. Even today, the blood serum of human beings consists of a composition of salts that is similiar to that of diluted sea water. As life came ashore from the oceans, lakes and rivers of the world, it left its aqueous environment, from which it had proviously been able to obtain information. Now, the environment was geseous, and a biological trick was necessary in order to be able to receive the information blown in by the new environmental medium, air. It was necessary fro the air to pass over a thin mucous layer, in which the substances carried in the air could be captured in order to then be tasted in the same manner as before.

As a means of remote perception, the sense of smell undoubtedly was originally even more important that vision, which only later developed its full capabilities. In the case of human beings, there is not much left of the capabilities once offered by the sense of smell, yet nevertheless, olfactory perceptions still pay a significant role in our feelings and our response to the environment, even if we are frequently not fully aware of these influences. Odours can excite us or cheer us up, yet they can alco have a repulsive, clabilitating effect. In the form of sexual odorants, which are called pheromones, odours play an important role in relations between the sexes, just as the messenger substances produced by our bodies, the hormones. But as in ancient times people follow the example of our ancestors and try to change to odour of their bodies and environment for nearly the same reasons as primitive humanoids. We use air fresheners or aromaterapy burners to create „good" or rather commonoly accepted in our society) room odour. We wear perfumes, use soaps and shampoos to change odour of our body or breath aiming to become similar to other people of our social group applying some minor differences to save our individuality.
Our sense of smell is located in two regions of our nose mucous membranes, each about 3 – 4 cm in size, on the roof and the upper part of both walls of the nosal cavity, protruding from the center into the lateral regions of the nose cavity in the form of thin plates of bone, covered by mocous membrane.
There is no difficulty about seeing the nose. But some of those parts of it which are directly concerned in its sensitivity to smells, that is in detecting smells are very small indeed.



Since the value of a fragrance depends upon its effect on the nose, the ultimate test must obviously be by that member. It is not enough for a product to meet all analytical tests, if the nose of an experienced specialist says „no". To lighten the load on the creative perfumer, whose chief role is the creation fo finished fragrances, special panels of odour evaluators function in quality control of raw materials, intermediated, and fragrance products, to make sure that all of them pass required odour standards, usually by comparsion with reference samples. These evaluators need not have the same abilities in the creative sens, but they generally have the most sensitive and perceptive noses in the business. They of course use GC systems in their own laboratories as adjuncts, but their odour judgments are based mostly on the classical „smelling paper" studies in which the odour of the material being evaluated is observed over a period of hours or even days. This reveals much of importance which might not be readily apparent from a cursory sniff.
In spite of the greate influence that laboratory instruments have had upon research and quality control in the fragrance field, it must never be thought that they have replaced the human nose and its olfactory system as the final arbiter of fragrance value. True, instruments have made thins easier, but only as adjuncts.
They are, when used correctly, rapid, sensitive, and precise, but they cannot substitute for human judgment. In addition the nose is much more sensitive than the finest GLC yet divised. However, human perception of odour is often colored by subjectivity, and there are certain physiological defect in even the most highly trained olfactory system. If disregarded, this can lead to serious errors. It is by the careful combination of the two methods of testing that best results are obtained.

2. The Selection of Panellist for Odour Evaluation Board.

The best panel can be selected from non-professional company employesses previously tested for ability in odour differentiation. According to the size of the company and number of samples to be tested, the number of panellists can vary, but most useful is to have about twenty selected and trained people from whom 6 – 9 colled at random for each session. This system minimizes the routine boredom which affects results from people permanently smelling samples in a control department.
A few tests can easily show the ability of the panellists to smell, recognize, discriminate, and memorize odours. Test for Odour Differentiation (will be described with deatels). The odour differentiation test is a simple triangle method with four sets of samples prepared according to Table The candidates are presented with eight sets of three samples for each of four combinations of materials. The candidate is requested to specify which of the three samples in each set is different

from two others or state that there is no difference within the set. A minimum of seven correct out of eight answers is necessary to accept the candidate.

Test for Odour Discrimination

Sandalwood oil and 8 parts sandalwood oil with 1 part cedarwood oil

Pine needle oil and 6 parts pine needle oil with 1 part terpineol

Bergamot oil and 10parts bergamot oil with 1 part terpinyl acetate

α-Amylocynamic aldehyde and 35 parts α-amylcinnamic aldehyde with 1 part benzaldehyde.

Test for Odour Recognition.

We cannot expect the candidates for panellist to name aroma chemicals or oils which they have never met before. But there are odours all round us which can be very easily associated with certain products or situations. A panellist should have the ability to associate odours and remember them. During the test, the candidate receives the samples, left alone in a separate room – should indicate some identity for each one.
It should be emphasized that a variety of answers are acceptable. An organic chemist's answers for vanilin and anethol will be most „vanilin" and „anethol", but they can also be „ice cream" and „confectionery" which are perfectly acceptable. It is usual that within ten samples we give two very similar (e.g. lemon and orange oils, peppermint and menthol, or rose and geranium). Each good answer is given to points. A fair one (e.g. fruit for lemon oil) will get one point. Hence, a maximum 20 points are available and 15 is accepted as minimum.

Odour Memory.

The initial experience in smelling an odour may be hedonic, a feeling state rather than a sensation, whereas in vision the sequence may be the reverse. The stress on feeling is thought to be the reason that odour memory seems to be exceptionally good.
Perhaps for various reasons olfactory memory may be more difficult to study than visual and auditory. In the case of pictorial and verbal material it is possible to make direct comparisons between „memory rerconstruction" and the original, preserved material. Only in exceptional circumstances is this possible with odour. It is less easy with odours to formulate precisely what it is one is trying to remember, and to know explicitly and within definable limits that one has remembered it. In many ways olfactory associative overtones. It is well known that when auxiliary cues are removed people may fail to identify even the simplest and most familiar odours. Even when the name of the substance is known and it is basically familiar, a searching process may occur which, during a reasonable span of time,

still fails to strike upon the required word. At other times there is almost a snap decision and the right word seems to be almost intsantaneously available. These observations reflect common experience and are familiar in other forms of memory involving other series.

3. Tringle Odour Evaluation Test.

For each test, we need 6 – 9 panellists (preferably 7). Each of them is presented with coded paper strips dipped in appropriate products. There are two ways of preparation of the test according to the number of tests to be made and working time available for test preparation. The simple way is presentation of strips marked A,B and C to all panellist in the same order, e.g., each blotter represents the same material. More complicated in preparation and calculation of results, but more objective (expecially if panel is working in the same room) is the presentation of strips dipped for each sample in different sequences so that each set of three strips represents a different combination.
For very simple and quick work, if more samples are to be evaluated, only one set of strips is prepared for each product and panellists smell them in turn. Panelists are requested to specify which of three strips has a different odour from two of the others (or which two strips are the same). With the questionnaire shown below, a quick record of results is collected. Panellists should not know the sources of evaluated samples to avoid any preferences which may affect results. A final conclusion from results obtained from the panel should be made by the panel secretary. According to literature sources on statistical data evaluation, there is a direct relation between the number of panellists (or tested sample sets) and the minimum number (or percentage) of correct answers for reliabel reslut. Where the samples are evalueted by seven panellists correct answers would be five (as minimum).

IV. THE PRACTICAL ASPECT OF QUALITY CONTROL

In the practical sense, quality control involves all the departments in a fragrance and flavor company. Usually we think of the responsibility for quality control falling on two groups of technicians, the subjective and the objective group. In the subjective group are the gragrance quality control laboratories, which compare flavour and fragrance against established standards, and perhaps examine the gross physical characteristics of a material such as colour, fluidity, clarity, etc. In the objective control groups are the analysts who measure the physical and chemical characteristics of the materials, and the instrumental groups that compare the various physico-chemical properties against reference samples, check molecular structure, and obtain quantitative and qualitative information.
First and most important is the selection of reference standard for each raw material. It is fortunate for our industry that years ago at least one of the leading buyers of fragrance raw materials took the time and spent the money to go to all the major areas of the world where aroma raw materials are produced to take authentic samples of actual fild distillations. These were carefully preserved, and analyzed to establish the odour, as well as physical, chemical and instrumental specifications to guide buyers in quality selection. In the typical essential oil company, almost all departments are involved in selecting standards and all have an equal share in assuring that raw materials meet those quality standards.
The Purchasing Department must find a source of supply for all raw materials the company needs, and its tasks is to bring the highest quality materials into the plant at the most reasonable cost. Purchasing obtains samples of materials for approval, then routes the samples to those who will measure quality, subjectively and objectively. Perfumers get involved in raw material selection in strange ways. The quality control laboratory may have rejected a sample of lavandin because it was thin, and contained too much cineol and camphor.
The perfumer has prevailed upon Purchasing to continue buying this grade in spite of a poor compromise between quality and cost because in the particular finished product being made the impact of cineol/camphor is very necessary, and the lavender character is not that important. The control chemist enters the discussion, his opinion being that it would be better to add cineol and camphor to the compound if necessary, but the requirements and recomendations of the perfumer will previal in such a discussion against all the other departments.
There are of course many reasons why natural products vary from year to year, from supplier to supplier, and from one geographical area to another. A difference in rainfall, temperature during the

growing season, varying sunlight, harvesting conditions, a change in processing equipment – all these and other factors may change the nature of the essential oil produced.
For known essential oils of commerce, specifications have been drawn and published by authoritative organizations, the most important for essential oils TC 54 Commitee of the International Standards Organization (ISO). Essential oil and aroma chemical monographs published by the Essential Oil Association of USA have now become almost obsolete but can still be safety used for referencing 9 volume set of Flavor and Fragrance Ingredient Data Sheet Set compiled by the Flavour and Extract Manufacturers Association (FEMA), The Reseach Institute for Fragrance Materials (RIFM) and the Fragrance Materials Association of the United States (FMA) contain safety information on over 1500 raw materials used in the fragrance and flavour industries. International Fragrance Association (IFRA) and International Organization of the Flavour Industry (IOFI) publish safety and use limits data on essential oils and aroma chemicals in their regularly updated „Code of Practice". FEMA produces FEMA GRAS List (GRAS stands for Generally Recognized As Safe). Of the 1783 substances listed in the list ca. 1400 have been identified in nature. The remaining substance have not yet been identified in nature byt their chemical structures suggest that they will probably be identified as natural constituents. Pharmacopoeias and Food Chemicals Codex provide monographs for essential oils used in food and pharmaceuticals. International Federation of Essential Oils and Aroma Trades (IFEAT) has published a guideline for classification and labelling of essential oils for transport and handling. Flash points of essential oils are also indicated in the guideline. An essential oil is expected to conform to such specifications in order to fetch the market value and be appreciated by the buyers.

V. FULL SENSORY TEST (in a practical manner).

Evaluation of raw materials (also essential oils) is closely combined with odour descritpions used and the general classification and evaluation systems in company. However when starting a fragrance laboratory one shall consider two ways of evaluation of fragrance compound. First of them, a comperative one is used for the comparison of raw material with another one – standard or target sample. The second which can be considered objective or hedonic is based on ranking the sample (or samples) with the aim of selecting the best one of the group or degree of approval for a specific purpose.
There are many more or less complicated methods to achive the purpose of odour evaluation. All of them are based on panel work. People for the panel selected according to their odour discrimination, ability and odour memory.
Evaluation of raw materials odour quality, and fragrance compound odour in comparison with target sample (market product, customers sample etc.) can be performed by a so called triangle method and 8-points scale of similarity. The samples shall be evaluated by 5 – 9 members panel selected and trained for the purpose. The triangle method is used to answer the basic question „Is the sample evaluated identical with standard or target sample or different?"
Evaluation with method 8-point scale of similarity is necessary to evaluate the difference between two samples which not be identical but are similar enough to replace each other. For that purpose an 8-point scale differentation test has been elabrated with the following descritpion of marks:
1 – totally different
2 – different with some note of standard
3 – different top or base note
4 – distinct different note
5 – weak stable different note
6 – weak passing different note
7 – just noticeable difference
8 – no difference, identical with standard
With trained panel and proper calculation of mean values, the average result of a minimum of 5,5 may allow one to accept the product. This particular method is especially useful when samples of natural raw materials are tested which due to sources, season, harvesting and method of production always show small differences in odour.

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Annex 1. Basic natural fragrance raw materials in creative perfumery laboratory

Ambrette seed extract
Anis oil
Armoise oil
Badian oil
Basil oil
Benzoin Siam resinoid
Bergamot oil
Bois de Rose oil
Calamus oil
Chamomile oil
Camphor oil
Cananga oil
Caraway oil
Cardamon oil
Carrot seed oil
Castoreum
Cedarwood oil
Celery seed oil
Citronella oil
Clary sage oil
Clove bud oil
Coriander oil
Costus root extract
Eucalyptus oil
Fir balsam
Galbanum resinoid
Geranium oil
Ginger oil
Grapefruit oil
Jasmine absolute
Juniper berry oil
Labdanum resinoid
Lavandin oil
Lavender oil
Lemon oil
Lemon terpenes
Lemongrass oil
Mandarine oil
Myrrh resinoid
Neroli oil
Nutmeg oil
Oakmoss absolute
Olibanum resinoid
Opoponax resinoid
Orange oil
Orange terpenes
Palmarosa oil
Patchouli oil
Pepper oil
Peppermint oil
Petitgrain oil
Piment oil
Pine needle oil
Rose oil
Rosemary oil
Sandalwood oil
Spearmint oil
Styrax resinoid
Tagetes oil
Thyme oil
Tuberose absolute
Vetiver oil
Ylang-ylang oil

ODOUR PROFILE

PRODUCT:

MANUFACTURER:

	NONEXISTENT 0	NOTICEABLE 1	BACKGROUND 2	DISTINCT 3	PREDOMINANT 4
GREEN					
FRUITY					
FLORAL					
FATTY-ALDEHYDIC					
HERBAL					
ANIMAL-MUSKY					
AMBER-WOODY					
SPICY-BALSAMIC					
EARTHY-FUNGOID					
CHEMICAL-UNPLEASANT					
OTHER:					

GAS CHROMATOGRAPHY (GC) AND

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC/MS) SYSTEMS

Gas Chromatography (GC)

Gas chromatography is a chromatographic technique that is used to separate volatile organic compounds. In gas chromatography, the sample is vaporized in injection port and injected onto the head of a chromatographic column.

There are two types of gas chromatography

- Gas-Solid Chromatography (GSC)
- Gas-Liquid Chromatography (GLC)

Gas-Liquid Chromatography is widely used in all fields and is usually shortened as gas chromatography (GC). Gas-liquid chromatography is based upon the partition of the analyte between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid.

A gas chromatograph consists of the following units:

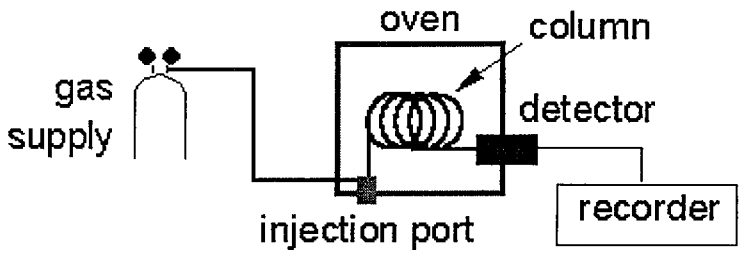
- Injection port
- Carrier gas (Mobile phase)
- Column oven
- Chromatographic column for separation
- Detector

The injection port is maintained at a higher temperature than the boiling point of the least volatile component in the sample mixture. The injection port consists of a rubber septum through which a syringe needle is inserted to inject the sample.

Mobile phases are generally inert gases such as helium, argon, or nitrogen. Since the partitioning behavior is dependant on temperature, the separation column is usually contained in a thermostat-controlled oven.

Separating components with a wide range of boiling points are accomplished by starting at a low oven temperature and increasing the temperature over time to elute the high-boiling point components. Most columns contain a liquid stationary phase on a solid support. Separation of low-molecular weight gases is accomplished with solid adsorbents.

Schematic of a gas chromatograph



Sample Injection System

Sample injection is very important step for GC analysis. The most common method of sample injection involves the use of a microsyringe to inject a liquid or gaseous sample through a silicone rubber diaphragm or septum into a flash vaporized port located at the head of the column.

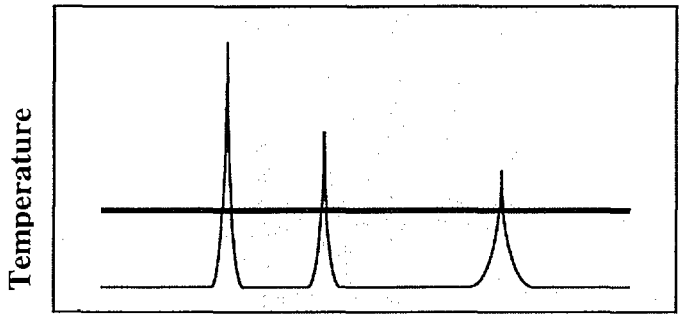
There are two types of injectors. One is for packed column and the other one is for capillary column. The capillary column injection methods are:

- Split injection
  - Split & Splitless injector
  - PTV injector
- Splitless injection
  - Split & Splitless injector
- Direct injection
  - On-column injector
  - Wide-bore capillary column attachment
- Solvent cut injection
  - injector or moving needle injector
  - Solventless sample
- Cool injection
- Septumless injection

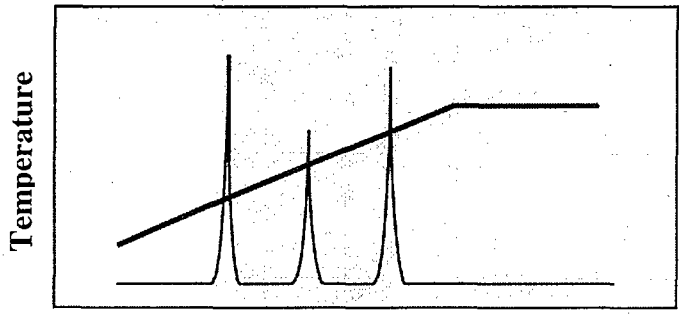
Column Oven

The column oven temperature should be high enough for the analysis to be completed in reasonable time and low enough to obtain the desired separation of sample mixture. There are two types of oven temperature methods.

1. Isothermal Analysis



2. Temperature Programming Analysis



Detectors

Detector is to indicate the presence and measure the amount of component eluted out from the column. The ideal gas chromatography detector should have the following characteristics:

- Adequate sensitivity
- Good stability and reproducibility
- A temperature range from room temperature to at least 400°C
- A short response time that is independent of flow rate
- High reliability
- Easy to use
- Similarity in response toward all analytes
- Nondestruction of sample

Types of Detector

There are several detectors used for different kind of applications. Some of the well known detectors are given below.

❖ ***Flame Ionization Detector (FID)***

The FID detector is one of the most widely used and generally applicable detectors for gas chromatography.

- Detects any compounds that can be oxidized in hydrogen/air flame.

❖ ***Thermal Conductivity Detector (TCD)***

This detector is based upon changes in the thermal conductivity of the gas stream brought about by the presence of analyte molecules.

- Detects any component including N_2 and O_2 except the gas used for the carrier gas.

❖ ***Electron Capture Detector (ECD)***

ECD operates in much the same way as a proportional counter for measurement of X-radiation.

- Sensitive to electronegative functional groups. It is insensitive toward amines, alcohols and hydrocarbons. Preferred in pesticide analysis.

❖ ***Flame Termionic Detector (FTD)***

- Selective to organic compounds containing phosphorus and nitrogen.

❖ ***Flame Photometric Detector (FPD)***

- Sensitive to organic compounds containing phosphorus and sulfur.

❖ ***Atomic Emission Detector (AED)***

The newest commercially available gas chromatographic detector is based upon atomic emission .

Chromatographic Column

There are two types of column used for the separation of volatile mixture in GC applications.

1. Packed Columns

The packed columns are fabricated from glass, stainless steel, copper, aluminum and Teflon tubes having 2 to 3 m length and 2 to 4 mm inside diameter.

2. Capillary Columns

Open tubular or capillary columns are of two basic types, namely, *wall-coated open tubular* (WCOT) and *support coated open tubular* (SCOT). Generally, the efficiency of a SCOT column is less than that of a WCOT column but significantly greater than that of a packed column. The newest WCOT columns are *fused silica open tubular* columns (FSOT).

The Stationary Phase

Desirable properties for the immobilized liquid phase in a gas-liquid chromatographic column include:

- Low volatility
- Thermal stability
- Chemical inertness
- Good solvent characteristics

The retention time for a solute on a column depends upon its partition ratio, which in turn is related to the chemical nature of the stationary phase.

Gas Chromatography / Mass Spectrometry (GC/MS)

Mass spectrometers use the difference in mass-to-charge ratio (*m/z*) of ionized atoms or molecules to separate them from each other. Mass spectrometry is therefore useful for quantitation of atoms or molecules and also for determining chemical and structural information about molecules. Molecules have distinctive fragmentation patterns that provide structural information to identify structural components. In mass spectrometry, a substance is bombarded with an electron beam having sufficient energy to fragment the molecule. The fragments are accelerated in a vacuum through a magnetic field and are sorted on the basis of mass-to-charge ratio.

A very low concentration of sample molecules is allowed to leak into the ionization chamber, which is under a very high vacuum, where they are bombarded by a high-energy electron beam. The molecules fragment and the positive ions produced are accelerated through a charged array into an analyzing tube. Ions having the proper mass-to-charge ratio will follow the path of the analyzer, exit through the slit and collide with the Collector.

The output of the mass spectrometer shows a plot of relative intensity *vs* the mass-to-charge ratio (*m/z*). The most intense peak in the spectrum is termed the base peak and all others are reported relative to it's intensity.

The process of fragmentation follows simple and predictable chemical pathways and the ions which are formed will reflect the most stable cations and radical cations which that molecule can form. The highest molecular weight peak observed in a spectrum will typically represent the parent molecule, minus an electron, and is termed the molecular ion (M^+). Generally, small peaks are also observed above the calculated molecular weight due to the natural isotopic abundance of ^{13}C, ^{2}H, etc. Many molecules with especially labile protons do not display molecular ions; an example of this is alcohols, where the highest molecular weight peak occurs at *m/z* one less than the molecular ion (M-1). Fragments can be identified by their mass-to-charge ratio, but it is often more informative to identify them by the mass which has been lost.

Purposes of mass spectrometry is:

- Molecular weight determination
- Structural characterization
- Gas phase reactivity study

The general operation of a gas chromatography-mass spectrometry can be explained in brief as follows:

1. Injection of a sample mixture in to the injection port of GC.

2. Separation of the sample mixture in the GC column.

3. Creation of gas-phase ionization.

4. Separation of the ions according to their mass-to-charge ratio.

5. Measurement of the quantity of the ions of each mass-to-charge ratio.

6. Storing of the mass spectrums of the compounds.

7. Interpreting of the mass spectrums of the compounds.

In general, a mass spectrometer consists of four basic components. These are an inlet unit, an ion source, a mass-selective analyzer, and an ion detector (Figure 1). Since mass spectrometers operate in a high-vacuum, system works with high vacuum pumps such as turbo molecular or diffusion pumps.

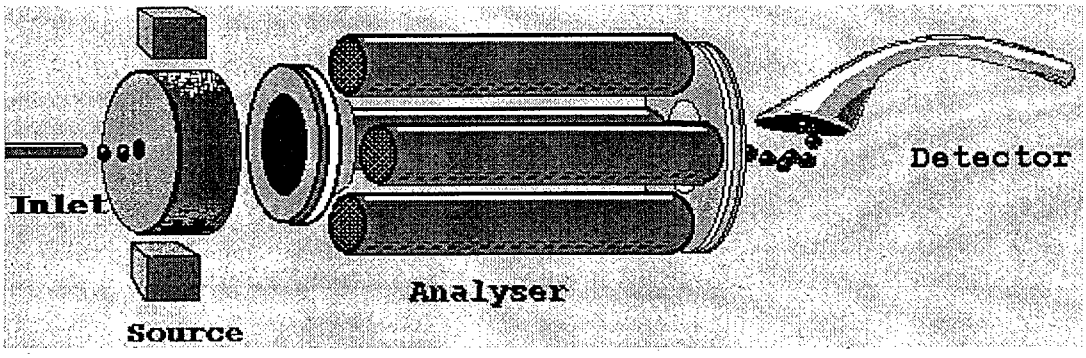


Figure 1. Basic Components of a Mass Spectrometer

Inlet System

Sample can be introduced to the mass spectrometer directly *via* solids probe, or in the case of mixtures, by the intermediary of chromatography device (*e.g.* Gas chromatography, Liquid chromatography, Capillary electrophoresis, etc.).

Ion Source and Ionization Methods

Once the sample molecules are subjected to ionization, ions formed by different methods. These ionization methods are:

1. ***Electron Impact Ionization (EI)***: Electron impact ionization is widely used in mass spectrometry for relatively volatile samples that are insensitive to heat and have relatively low molecular weight. The spectra, usually containing many fragment-ion

peaks, are useful for structural characterization and identification. Small impurities in the sample are easy to detect.

$$M + e(70eV) \text{ -----> } M^{+}. + 2e$$

EI ionization method is suitable for non thermolabile compounds. The volatility of the sample is required. Sample molecules in vapor state are bombarded by fast moving electrons, conventionally 70 eV energy. This results in ion formation.

An EI source uses an electron beam, usually generated fron a tungsten filament, to ionize gas-phase atoms or molecules. Sample can be introduced to the EI source *via* a gas chromatography device, for example in the case of mixtures, or directly *via* a solids probe device. The quantities needed for an experiment is usually less than a microgram of material.

EI mass spectra, in most of cases, contain intense fragment ion peaks and much less intense molecular ion peak. When the molecular ion peak is not observed in the mass spectrum, chemical ionization can be used in order to get molecular ion information.

2. ***Chemical Ionization (CI):*** For organic chemists, Chemical Ionization is especially useful technique when no molecular ion is observed in EI mass spectrum, and also in the case of confirming the mass to charge ratio of the molecular ion. Reagent gas (*e.g.* ammonia) is first subjected to electron impact. Sample ions are formed by the interaction of reagent gas ions and sample molecules. This phenomenon is called ion-molecule reactions.

   Positive ions and negative ions are formed in the CI process. Depending on the setup of the instrument (source voltages, detector, etc.) only positive ions or only negative ions are recorded.

   In CI, ion molecule reactions occur between ionized reagent gas molecules (G) and volatile analyte neutral molecules (M) to produce analyte ions. Pseudo-molecular ion MH^{+} (positive ion mode) or $[M-H]^{-}$ (negative ion mode) are often observed.

   Positive ion mode:

$$GH^{+} + M \text{ ------> } MH^{+} + G$$

   Negative ion mode:

$$[G-H]^{-} + M \text{ ------> } [M-H]^{-} + G$$

   Proton transfer is one of the simple processes observed in positive CI:

$$RH^{+} + M \text{ -----> } MH^{+} + R$$

CI is applied to similar samples; it is used to enhance the abundance of the molecular ion. For both ionization methods, the molecular weight range is 50 to 800 Dalton. In rare cases it is possible to analyze samples of higher molecular weight. Accuracy of the mass measurement at low resolving power is ±0.1 Dalton and in the high resolution mode, ±5 ppm.

The main reagent gases used in CI are Ammonia, Methane, and Isobutane. Choice of reagent gas affect the extend of fragmentation of the quasi-molecular ion.

Two factors determine the choice of the gas to be used:

- Proton affinity PA
- Energy transfer

NH_3 (ammonia) is the most used reagent gas in CI because of the low energy transfer of NH_4^+ compare to CH_5^+ for example. With NH_3 as reagent gas, usually MH^+ and MNH_4^+ are observed.

Negative Ion Chemical Ionization:

Three mechanisms can be underlined:

1- Electron capture reaction due to attainment of slow moving, low energy "thermalized" electrons which may be transfered more efficiently to sample molecules.

2- Electron transfer from ionized reagent gas (*e.g.* NH_2- may transfer an electron to a molecule having a greater electron affinity than NH_2).

3- Reagent gas ions participate in true CI reactions (e.g. proton abstraction, according to relative acidities).

Molecular ions observed in negative ion chemical ionization mass spectra are usually M^- or $[M-H]^-$

Particle induced desorption techniques

For compounds that are not volatile and thermolabile soft ionization techniques can be used. Among these techniques are particle induced desorption. We will describe in this entry only: Fast Atom Bombardment (FAB), Laser Desorption (LD), Plasma Desorption (PDMS), and Liquid Secondary Ion Mass Spectrometry (L-SIMS).

4- ***Fast Atom Bombardment (FAB):*** Fast atom bombardment ionization (FAB or sometimes called liquid secondary ionization MS, LSIMS) is a softer ionization method than EI. The spectrum often contains peaks from the matrix, which is necessary for ionization, a few fragments and a peak for a protonated or deprotonated sample molecule. FAB is used to obtain the molecular weight of sensitive, nonvolatile compounds. The method is prone to

suppression effects by small impurities. The molecular weight range is 100 to 4000 Da. Exact mass measurement are usually done by peak matching. The accuracy of the mass is the same as obtained in EI, CI.

5- ***Matrix-assisted laser desorption (MALDI-TOF):*** MALDI is used to determine the molecular weight of peptides, proteins, oligonucleotides, and other compounds of biological origin as well as of small synthetic polymers. The amount of sample needed is very low (pmoles or less). The analysis can be performed in the linear mode (high mass, low resolution) up to a molecular weight of *m/z* 300,000 (in rare cases) or reflectron mode (lower mass, higher resolution) up to a molecular weight of 10,000. The analysis is relatively insensitive to contaminants. Mass accuracy (0.1 to 0.01%) is not as high as for other mass spectrometry methods. Recent development in Delayed Extraction TOF allow higher resolving power and mass accuracy. Some structural information for small molecules can be obtained in a "Post-Source Decay" mode, or by collisional activation.

In FAB a high-energy beam of neutral atoms, typically Xe or Ar, strikes a solid sample causing desorption and ionization. It is used for large biological molecules that are difficult to get into the gas phase. FAB causes little fragmentation and usually gives a large molecular ion peak, making it useful for molecular weight determination.

The atomic beam is produced by accelerating ions from an ion source though a charge-exchange cell. The ions pick up an electron in collisions with neutral atoms to form a beam of high energy atoms.

MALDI is a LIMS method of vaporizing and ionizing large biological molecules such as proteins or DNA fragments. The biological molecules are dispersed in a solid matrix such as nicotinic acid.

6- ***Electrospray ionization (ESI):*** Electrospray ionization (ESI) allows production of molecular ions directly from samples in solution. It can be used for small and large molecular-weight biopolymers (peptides, proteins, carbohydrates, and DNA fragments), and lipids. Unlike MALDI, which is pulsed, it is a continuous ionization method that is suitable for using as an interface with HPLC or capillary electrophoresis. Multiply charged ions are usually produced. ESI should be considered a complement to MALDI. The sample must be soluble, stable in solution, polar, and relatively clean (free of nonvolatile buffers, detergents, salts, etc.). Electrospray ionization is installed on the four-sector tandem instrument in Chemistry and available on two Finnigan LCQ instruments (Chemistry and Medicine) and the Finnigan TSQ 7000 (Medicine).

The ESI source consists of a very fine needle and a series of skimmers. A sample solution is sprayed into the source chamber to form droplets. The droplets carry charge when the exit the capillary and, as the solvent evaporates, the droplets disappear leaving highly charged analyte molecules. ESI is particularly useful for large biological molecules that are difficult to vaporize or ionize.

7- ***Electron-Capture ionization:*** Electron-capture (sometimes called negative ion chemical ionization or NICI) is used for molecules containing halogens, NO2, CN, etc, and it usually

requires that the analyte be derivatized to contain highly electron-capturing moieties (e.g., fluorine atoms or nitrobenzyl groups). Such moieties are generally inserted into the target analyte after isolation and before mass spectrometric analysis. The sensitivity of NICI analyses is generally two to three orders of magnitude greater than that of PCI or EI analyses. Little fragmentation occurs during NICI, and this mode of ionization is generally employed for quantitative analyses of trace amounts of compounds of known structure in conjunction with the use of heavy isotope-labeled internal standards.

8- ***Field ionization:*** Molecules can lose an electron when placed in a very high electric field. High fields can be created in an ion source by applying a high voltage between a cathode and an anode called a field emitter. A field emitter consists of a wire covered with microscopic carbon dendrites, which greatly amplify the effective field at the carbon points.

9- ***Laser ionization (LIMS):*** A laser pulse ablates material from the surface of a sample, and creates a microplasma that ionizes some of the sample constituents. The laser pulse accomplishes both vaporization and ionization of the sample.

10- ***Plasma-desorption ionization (PD):*** Decay of ^{252}Cf produces two fission fragments that travel in opposite directions. One fragment strikes the sample knocking out 1-10 analyte ions. The other fragment strikes a detector and triggers the start of data acquisition. This ionization method is especially useful for large biological molecules.

11- ***Resonance ionization (RIMS):*** One or more laser beams are tuned in resonance to transitions of a gas-phase atom or molecule to promote it in a stepwise fashion above its ionization potential to create an ion. Solid samples must be vaporized by heating, sputtering, or laser ablation.

12- ***Secondary ionization (SIMS):*** A primary ion beam; such as $^{3}He^{+}$,$^{16}O^{+}$, or $^{40}Ar^{+}$; is accelerated and focused onto the surface of a sample and sputters material into the gas phase. Approximately 1% of the sputtered material comes off as ions, which can then be analyzed by a mass spectrometer. SIMS has the advantage that material can be continually sputtered from a surface to determine analyte concentrations as a function of distance from the original surface (depth profiling).

13- ***Spark source:*** A spark source ionizes analytes in solid samples by pulsing an electric current across two electrodes. If the sample is a metal it can serve as one of the electrodes, otherwise it can be mixed with graphite and placed in a cup-shaped electrode.

14- ***Thermal ionization (TIMS):*** Thermal ionization is used for elemental or refractory materials. A sample is deposited on a metal ribbon, such as Pt or Re, and an electric current heats the metal to a high temperature. The ribbon is often coated with graphite to provide a reducing effect.

Ion Analyzer

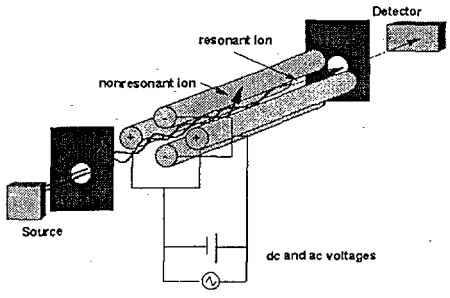
Molecular ions and fragment ions are accelerated by manipulation of the charged particles through the mass spectrometer. Uncharged molecules and fragments are pumped away. EI ionization produces singly charged particles, so the charge (z) is one.

Different mass analyzer designs are used for the separation of ions which are accelerated from the ionization chamber. The mass spectrometers are named according to the analyzer that is mounted to the system.

- **Magnetic-Sector Mass Spectrometer Analyzer :** The ions are accelerating to travel in a magnetic field for separation according to their mass-to-charge ratio. There are two type magnetic field analyzer used in GC/MS.
  - Single focusing
  - Double foucusing

  Double focusing is usually achieved by the use of carefully selected combination of electrostatic and magnetic fields.
- **Quadrupole Mass Filters :** A quadrupole mass filter consists of four parallel metal rods. Two opposite rods have an applied potential. The applied voltages affect the trajectory of ions traveling down the flight path centered between the four rods.

Schematic of a quadrupole filter



- **Fourier-Transform Mass Spectrometry (FT-MS)**
- **Time-of-Flight Mass Spectrometry (TOF-MS)**
- **Ion Cyclotron Resonans (ICR)**

Detector

There are many types of detectors, but most work by producing an electronic signal when struck by an ion. The mass analyzer sorts the ions according to *m/z* and the detector records the abundance of each *m/z*. Regular calibration of the *m/z* scale is necessary to

maintain accuracy in the instrument. Calibration is performed by introducing a well known compound into the instrument.

EI ionization introduces a great deal of energy into molecules. It is known as a "hard" ionization method. This is very good for producing fragments which generate information about the structure of the compound, but quite often the molecular ion does not appear or is a smaller peak in the spectrum.

There are several type ion detectors for GC/MS systems:

- Channeltron
- Daly detector
- Electron multiplier tube (EMT)
- Faraday cup
- Microchannel plate

A few important steps should be considered while the interpreting a mass spectrum.

1. Check the molecular ion peak.
2. Try to calculate the molecular formula.
3. Calculate the total number of rings plus double bonds.
4. Postulate the molecular structure consistent with abundance and *m/z* of fragments.

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GAS CHROMATOGRAPHY-FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER

IR spectroscopy is the measurement of the wavelength and intensity of the absorption of mid-infrared light by a sample. Mid-infrared light (2.5 - 50 μm, 4000 – 200 cm^{-1}) is energetic enough to excite molecular vibrations to higher energy levels. The wavelength of IR absorption bands are characteristic of specific types of chemical bonds, and IR spectroscopy finds its greatest utility for identification of organic and organometallic molecules.

A mathematical method called a Fourier transform is used to do the conversion from the time-domain spectrum to the conventional frequency-domain spectrum. The detector simultaneously measures all of the frequencies that pass through the cell and routes the information to the computer which decodes the information using a Fourier-transform. The decodec spectrum is directed to the readout device.

Fourier-transform infrared spectrophotometer is particularly useful in those circumstances where spectra of low-concentration samples are required and in those cases where a spectrum must be obtained rapidly. FT-IR spectrophotometers can be used as detectors for chromatography. Small amount volatile mixtures can be separated in gas chromatography easily. These compounds are then subjected to infrared spectroscopic measurement by using an FT-IR spectrometer. The detector measures all the frequencies while the compound passing through the cell after the separation in the chromatographic column. Only the Fourier-transform technique makes it possible to get the spectra of the samples which is flowing through the cell in a very short time.

Mechanism of IR Absorption

The transition moment for infrared absorption is:

$$R = < X_i \mid u \mid X_j \, dt >$$

where X_i and X_j are the initial and final states, respectively
u is the electric dipole moment operator:

$$u = u_o + (r\text{-}r_e)(du/dr) + \ldots \text{ higher terms.}$$

u_o is the permanent dipole moment, which is a constant, and since $< X_i \mid X_i > = 0$, R simplifies to:

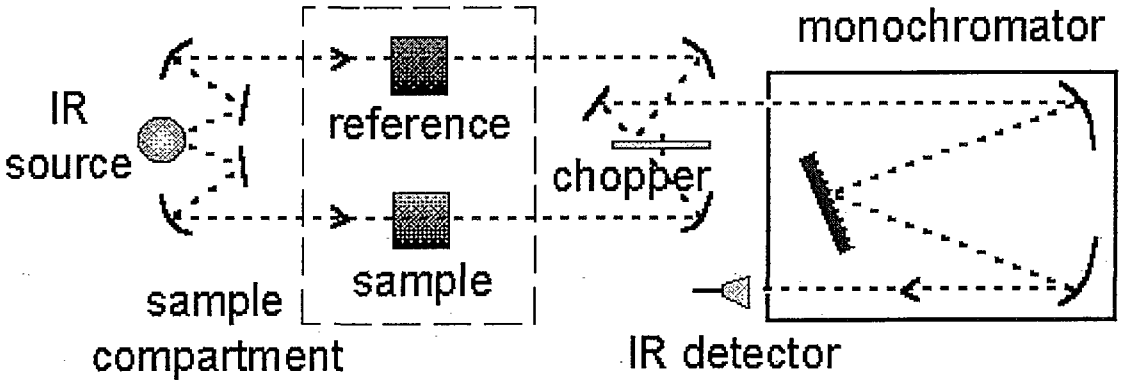
$$R = < X_i \mid (r\text{-}r_e)(du/dr) \mid X_j >$$

The result is that there must be a change in dipole moment during the vibration for a molecule to absorb infrared radiation.

Dispersive Infrared Spectrometers

Common light sources are tungsten lamps, Nernst glowers, or glowbars. Dispersive IR spectrometers use a grating monochromator to select wavelengths and are commonly used when a single wavelength is desired to monitor the kinetics of a reaction or as a GC or LC detector.

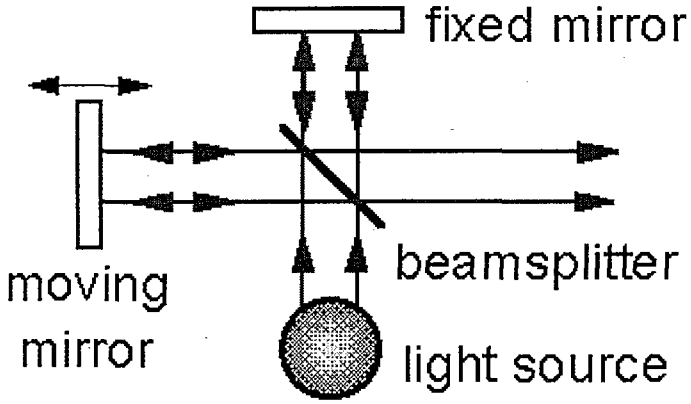
Schematic of a dispersive IR absorption spectrophotometer



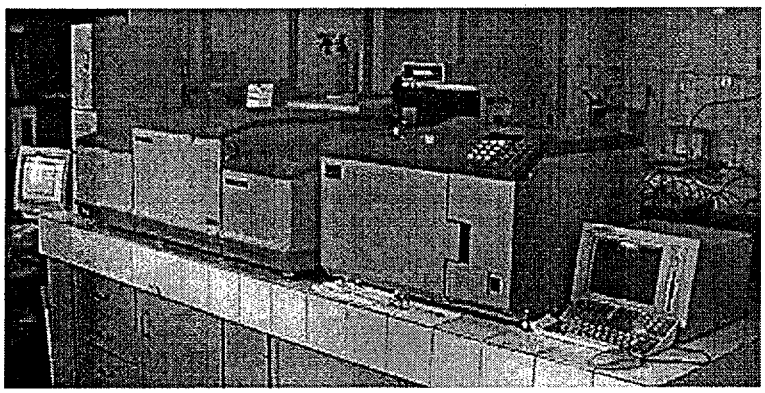
Fourier-Transform Infrared (FTIR)

Modern IR instruments more commonly use Fourier-transform techniques with a Michelson interferometer.

Michelson Interferometer Design



Picture of a GC/FT-IR spectrophotometer



Interferometers

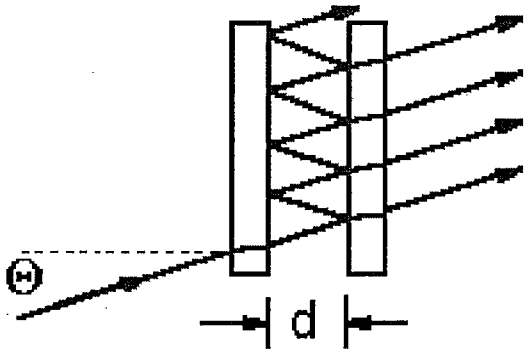
The purpose of an interferometer is similar to that of a filter or monochromator, i.e., to isolate a specific portion of the electromagnetic spectrum. Unlike prism or grating monochromators, interferometers are not dispersive instruments, but use interference to selectively transmit a certain wavelength. The links below lead to descriptions of three interferometer designs.

Types of Interferometers

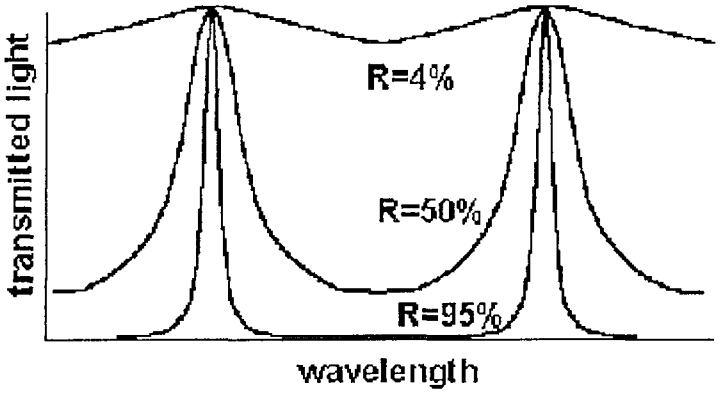
Fabry-Perot interferometer (Etalon)

Used in high-resolution applications, such as atomic spectroscopy or measurement of narrow-band laser linewidths.

Fabry-Perot interferometers that cannot be scanned are called etalons.



Schematic of a Fabry-Perot etalon

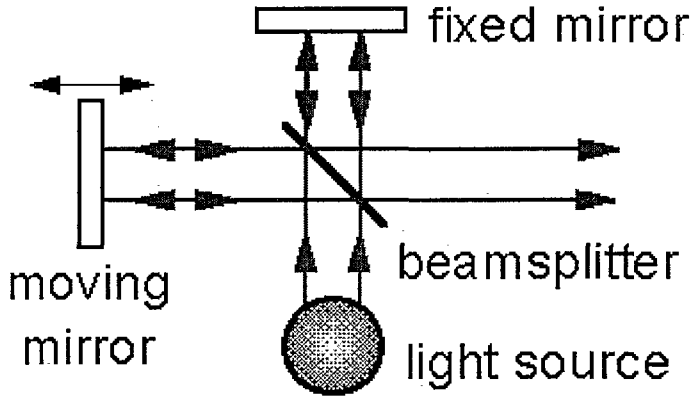


Transmission through a Fabry-Perot interferometer as a function of wavelength

Michelson interferometer

Used in Fourier-transform infrared absorption spectrophotometers (FT-IR).

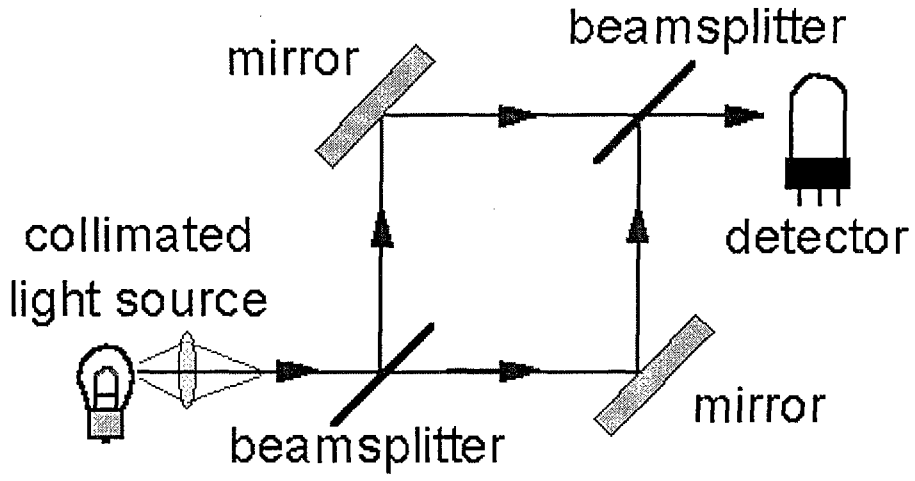
Schematic of a Michelson Interferometer



Mach-Zender Interferometer

Used to measure refractive index changes in gases and in interference microscopes to image transparent samples.

Schematic of a Mach-Zender Interferometer



Advantage of FT-IR

In principle, a well designed interferometer has several basic advantages over a classical dispersive instrument.

- **Multiplex Advantage (*Fellget Advantage*)**

  All frequencies are measured simultaneously in an interferometer, whereas they are measured successively in a dispersive spectrometer. A complete spectrum can be obtained very rapidly, and many scans can be averaged at the time taken for a single scan of a dispersive spectrophotometer.

- **Throughput Advantage (*Jacquinot Advantage*)**

  For the same resolution the energy throughput in an interferometer can be higher than in a dispersive spectrometer where it is restricted by the slits. In combination with the multiplex advantage, this leads to one of the most important features of an FT-IR spectrophotometer; the ability spectrophotometer to achieve the same signal-to-noise ratio as a dispersive spectrometer in a much shorter time.

- **Connes Advantage**

  The frequency scale of an interferometer is derived from a helium neon laser which acts an internal reference for each scan. The frequency of this laser is known very accurately and is very stable. As a result, the frequency calibration of interferometers is much more accurate and has much better long-term stability than the calibration of dispersive instruments.

- **Negligible Stray Light**

  Because of the way in which the interferometer modulates each frequency, there is no direct equivalent of the stray light found in dispersive spectrophotometers.

- **Constant Resolution**

  Resolution is the same at all wavelengths. In a dispersive instruments, the resolution varies due to of the slit program.

- **No Discontinuities**

  Since there are no grating or filter changes, there is no discontinuitiy in the spectrum.

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CHIRAL SEPARATION BY MULTIDIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Chirality has become vitally important in the pharmaceutical, chemical, and agricultural industries. The differences which make compounds chiral can produce critically different pharmacological effects in biological systems. As a result, demand for stereoselective separation techniques and analytical assays to evaluate the enantiomeric purity of chiral compounds, has increased. Chiral Chromatography has become a necessary tool - not only for the analytical determination of enantiomeric purity, but also for the isolation of pure enantiomers.

In an achiral environment, enantiomers exhibit identical physical and chemical properties. The two enantiomers have the same melting and boiling points, solubility, chromatograms, IR and NMR spectra, although, a mixture of the two may have a different melting point and boiling point.

Many of the traditional separation methods, which are fundamentally based on differences in analyte solubility or volatility, are inadequate for the resolution of optical isomers.

Optical Activity and Chirality

Any material that rotates the plane of polarized light is said to be *optically active*. If a pure compound is optically active, the molecule is non-superimposable on its mirror image. If a molecule is superimposable on its mirror image, the compound does not rotate the plane of polarized light; it is *optically inactive*. The property of nonsuperimposability of an object on its mirror image is called *chirality*. If a molecule is not superimposable on its mirror image, it is called *chiral*. If it is superimposable on its mirror image, it is *achiral*. The relationship between optical activity and chirality is absolute.

If a molecule is nonsuperimposable on its mirror image, the mirror image must be different molecule, since superimposability is the same as identity. In each case of optical activity of a pure compound there are two and only two isomers, called *enantiomers* (sometimes enantiomorphs), which differ in structure only in the left- and right-handness of their orientation. Enantiomers have identical physical and chemical properties except in two important respects:

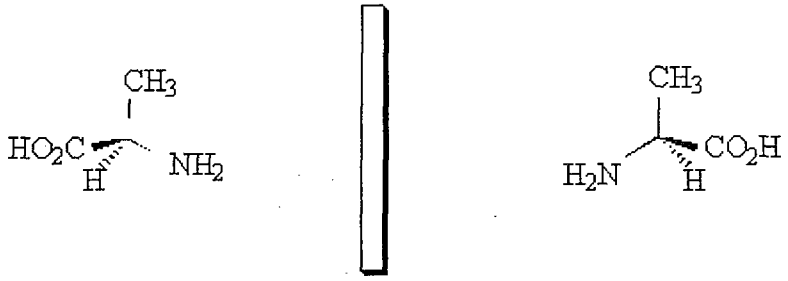
1. They rotate the polarized light in opposite light in opposite directions, though in equal amounts. The isomer that rotates the plane to the right (clockwise) is called the *dextrorotatory-isomer* and is designed (+), which one that rotates the plane to the left (counterclockwise) is called the *levorotatory-isomer* and is designed (-). Because they differ in this property they are often called *optical antipodes* or *enantiomers*. In a mixture of equal amounts of two antipodes, the rotations cancel each other out exactly. Such equimolar antipode mixture are optically inactive (±) and are also referred to as racemic mixture.

2. They react at different rates with other chiral compounds. One enantiomer undergoes the reaction at a convenient rate while the other does not react at all. This is the reason why many compounds are biological active while their enantiomers are not. Enantiomers react at the same rate with achiral compounds.

Although pure compounds are always optical active if they are composed of chiral molecules, mixtures of a equal amounts of enantiomers are optical inactive since the equal opposite rotations cancel. Such mixtures are called *racemic mixtures* or *racemates*. Their properties are not always the same as those of individual enantiomers. The properties in the gaseous or liquid state or solution usually are the same, since such a picture is nearly ideal, but properties involving a solid state, such as melting points, solubilities, and heat of fusion are often different.

Chiral compounds are ones which exist in two forms (enantiomers) which are non-superimposable mirror images of each other, and so are asymmetrical.

An example:



mirror plane

The term "stereoisomer" is a general one for all isomers that differ only in the orientation of the atoms in space. Stereoisomers include not only the mirror image enantiomers, but also geometric (*cis/trans*) isomers and diastereoisomers (isomers of drugs with more than one chiral center that are not mirror images of one another). Diastereoisomers and geometric isomers are both chemically distinct and pharmacologically different (unless they are interconverted *in vivo*) and are generally readily separated without chiral techniques. Geometric isomers and diastereoisomers therefore should, with the rare exception of cases where *in vivo* interconversion occurs, be treated as separate drugs and developed accordingly. In general, geometric isomers have been developed as single isomers. Practice with respect to diastereoisomers has been variable.

Non-superimposable means that, if you turn one enatiomer round and then try putting in on top of the other, the groups will not be in the same place in each enantiomer. The four bonds shown in each diagram are supposed to represent three dimensions: two bonds are in the plane of the paper, one goes back behind the plane, the other comes out. A chiral centre is one which results in a compound having this kind of asymmetry, and these can most commonly be recognised by the fact the central carbon atom (not drawn in the diagram above, but at the point where the four bonds join) is bonded to four different groups.

In a chiral environment such as a biological entity, optical isomers may exhibit dramatically different activity (*e.g.*, bioefficacy or biotoxicity). Such stereoisomers usually require specialized chiral techniques such as multidimensional gas chromatography-mass spectrometry for their correct separation, measurement, characterization and identification. They are often readily distinguished by biological systems, however, there are many cases in which enantiomers have been shown to have different pharmacokinetic behavior (absorption;

distribution, biotransformation. and excretion) and quantitatively or qualitatively different pharmacologic or toxicologic effects.

Today, chromatographic methods for the separation of enantiomers are indispensable and broaden the scope of modern methodology in flavor and fragrance compound analysis. Multidimensional gas chromatography-mass spectrometry offers a fascinating potential for rapid and accurate differentiation of chiral and fragrance and flavor substances. With the MD-GC/MS technique, it is possible to investigate the chiral components of complex mixtures of natural substances without additional sample preparation. Coupling an achiral precolumn to a chiral main column allows direct stereo-differentiation of the chiral components which are present in complex substance mixture, including a wide variety of flavor extracts, blossom extracts or essential oils.

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TBAM
Supercritical Fluid Extraction (SFE) System Report

Material : *Origanum majorana*
Quantity : 1.9906 gr
Date : 17.11.1999
Description : SFE extraction of *Origanum majorana*

SFX 22 System Isco Inc.
Mode : Alternating
Fluid : CO_2
Extraction
Temperature : 50°C
Pressure : 100 bar
Volume : 20 mL
Flow Rate : 1.5 mL/min
Modifier : No
Trap Solvent : Hexane (5 mL), (in CO_2 trap)
Restrictor Temp.
Body : 40 °C
Tip : 40 °C
Static Extraction : 0 min
Dynamic Extraction : 20 mL

GC/MS CONDITIONS

System : Hewlett Packard G1800A GCD System
Column : HP- Innowax Silica Capillar
(60 m x 0.25 mm i.d., 0.25 μm film thickness)
Temperatures
Column : 60°C for 10 min // 4°C/min to 220°C // 220°C for 10 min,
1°C/min to 240°C // 240°C for 20 min.
Injection : 250°C
Carrier Gas : Helium (1 mL/min)
Split Ratio : 50:1
Electron Energy : 70 eV
Mass Range : 35-425 m/z

Library Search : TBAM Library of Volatile Constituents and Wiley GC/MS Library

TBAM

MICRO-DISTILLATION / SAMPLE FORM

Program NO: ..... **Program Name:** ..... **DATE:** ...../...../1999

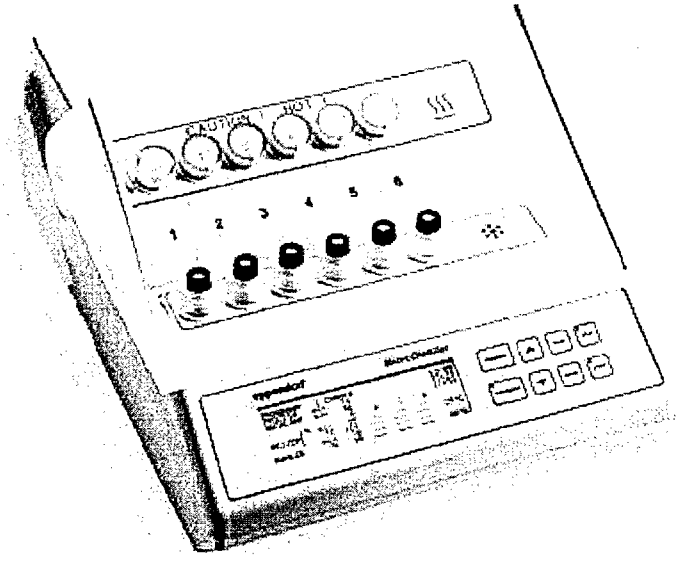
PROGRAM:	
Sample: (code)	
Physical Prop: (type, color, quant)	
Distillate: (color, smel, quant)	
NOTES:	

Cycle		A	B	C	D	Post-run
HEATER	°C/min	5-20	5-20	5-20	5-20	
	°C	25-120	25-120	25-120	25-120	
	min	1-999	1-999	1-999	1-999	1-999
COOLER	°C	-9-15	-9-15	-9-15	-9-15	

°C	A	B	C	D	Post-run
120					
110					
100					
90					
80					
70					
60					
50					
40					
30					
20					
10					
0					
-10					
	.....min	.....min	.....min	.....min	.....min

Eppendorf-Netheler-Hinz MicroDistiller®

Pioneering work on microdistillation has been performed in the early eighties'.[1,2] Very recently Eppendorf-Netheler-Hinz has introduced a new system: The MicroDistiller®.[3-5] This distillation system enables qualitative and quantitative separation and enrichment of organic and inorganic substances from complex matrixes on benchtop. Rapid and programmable simultaneous distillation of six (different) samples are possible. Electronic operation eliminates the need of cooling water. Short satisfactory distillation periods (1-2 h) can be manipulated. Sample amounts can drop up to 100 mg in solid, liquid or paste form and can have many applications. This simple system is very useful in essential oil isolation especially if only small amounts are available.



[1] Stahl E, Schild W. *Isolierung und Charakterisierung von Naturstoffen*, Gustav Fischer Verlag, Stuttgart, 28-32,1986.

[2] Wagner H, Blandt S. *Plant Drug Analysis*, 2nd Edition, Springer-Verlag, Berlin, 149, 1996

[3] Briechle R, Dammertz W, Guth R, Volmer W. *Bestimmung Ätherischer Öle in Drogen.* GIT Fachz. Lab. 41, 749-53, 1997.

[4] Brunn W, Schuster K, Volmer W. *Mikrodestillation– eine Neue Methode für Probenaufbereitung und Screening.* CLB – Chemie in Lab. Biotech. 48, 428-30,1997.

[5] Giesselmann G, Volmer W. *Vereinfachungen in der Cyanid Analytik*, LaborPraxis 21 (7/8), 36-43, 1997.

TBAM GC/MS ANALYSIS REPORT

Analysis Number : .....

GC/MS Conditions

System : Hewlett Packard G1800A GCD
Column : Innowax FSC (60 m x 0.25 mm Ø, with 0.25 µm film thickness)
Carrier gas : Helium (1 mL/min)

GC Temperatures

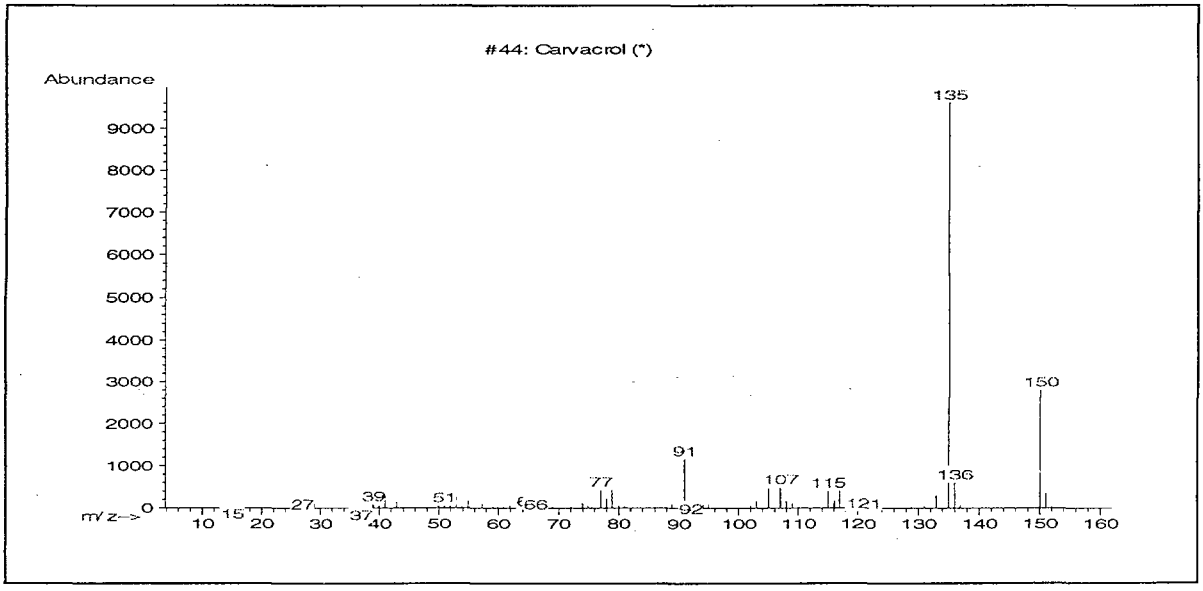
Injection : 250°C
Column : 60°C for 10 min; 4°C/min to 220°C; 220°C for 10 min (Total : 60min)
Mass range : 35-425 m/z
Split ratio : 50:1
Electron energy : 70 eV
Library search : TBAM Library of Essential Oil Constituents
Wiley GC/MS Library

Sample Information

Sample : Essential Oil of *Origanum majorana*
Preparation technique :
Injection quantity :
Sample code :
Analysis date :

Main constituents and relative percentages

Carvacrol . . . . . .
Thymol . . . . . .



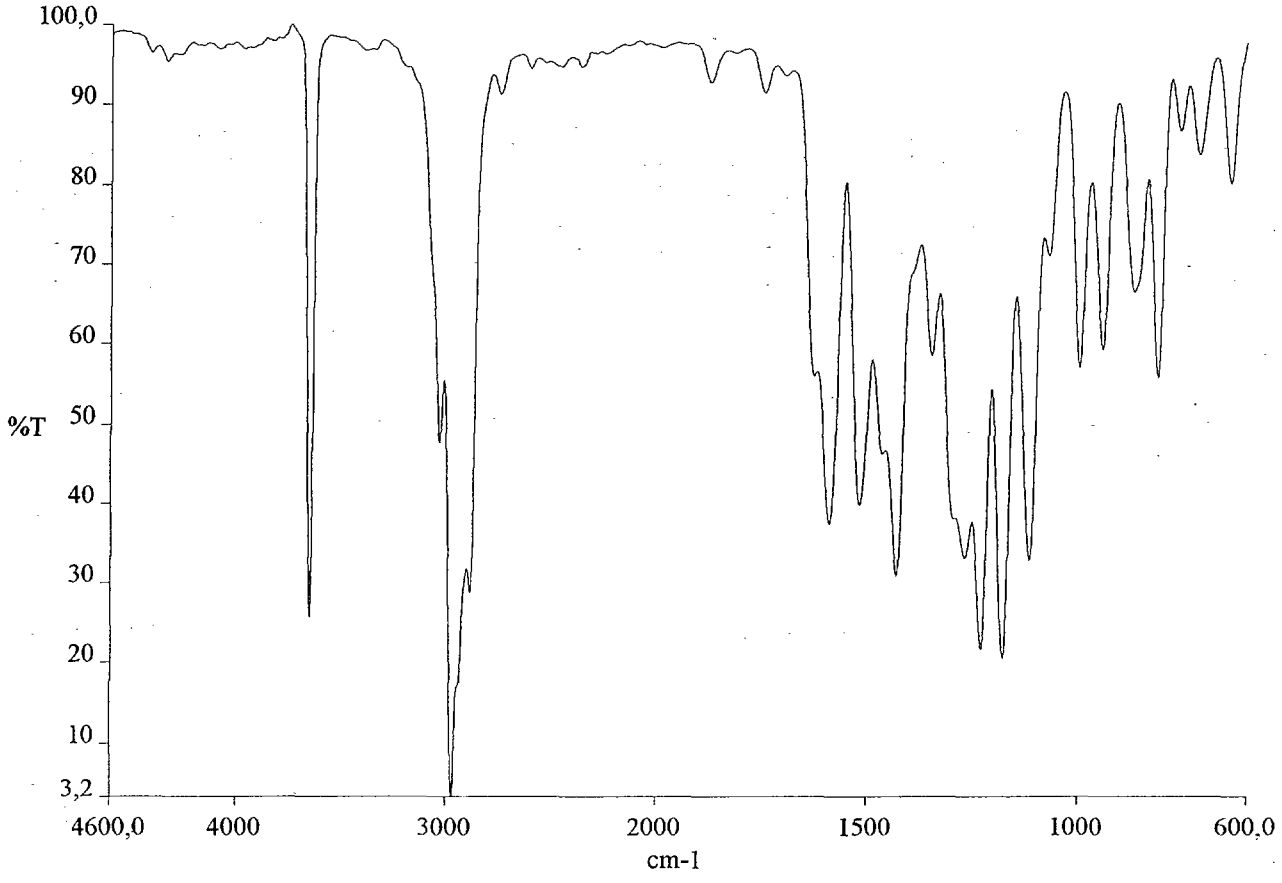
Mass Spectrum of Carvacrol

TBAM GC-FT/IR Analysis Report

Source : *Origanum vulgare* ssp. *hirtum*
Description : Essential Oil

Spectrum Name : Carvacrol

Instrument Model : Spectrum 2000
Detector : mb050MCT:Rightback:GCIR
Spectrum Type : % T
Scan Mode : Interferogram



TBAM GC-FT/IR Analysis Report

Source : *Origanum onites*
Description : Essential Oil

Spectrum Name : Linalool

Instrument Model : Spectrum 2000
Detector : mb050MCT:Rightback:GCIR
Spectrum Type : % T
Scan Mode : Interferogram