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Research Article

A REVIEW ON ANALYTICAL METHODS OF EXTRACTION OF ESSENTIAL OILS

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Abstract
A multidisciplinary approach is required for analyzing essential oils. Along with analytical chemistry other branches like phytochemistry, organic synthetic chemistry and biochemistry is strongly required.
In the last thirty years a huge process was in pipeline for calibrating instrumental sensitivity and selectivity improvement. The real big problem for phytochemistry is that the extracts contain a loarge number of compounds along with the essential oil in investigation. Extremely specific methods are required for qualitative and quantitative analysis.
Keywords: extaction, essential oils

INTRODUCTION

Sample preparation steps are very sensitive to the matrix and also responds to 75% of the final error. This is mostly valid when more than one steps are required for the procedure and the final compounded mixture is uncertain. This step is crucial as it has a direct impact on method performances specifically trueness, precision, LOQ (limit of quantitation), linearity, reproducibility and is regarded the rate limiting step for several assay based analytical procedures

After sample processing the complexity starts with dissolution into appropriate solvent to a complicated extraction procedure. For most of the times direct analysis is not possible and derivatization technique is used.

There are several extraction procedures developed for essential oils

Ultra Violet – Visible Spectroscopy

Essential oils are screened by UV visible spectroscopy. The method is deployed both for qualitative and quantitative needs. A beam of light of fixed wavelength is passed through the sample and readings are taken. The reading are analyzed and interpreted. The molecules present in the sample absorb light of specific frequency and is shown as absorbed wavelength.

The frequency of light which is absorbed is called as lambda max. Absorbtion is due to excitation of electrons which corresponds to their energy levels. The electrons are arranged in their energy levels in the molecules and for excitation they require energy. The energy should correspond to the difference in energy of energy levels within electon orbitals. Therefore the frequency of light which is equal to the specified energy level difference is only absorbed while other are transmitted. This shows a sharp increase in the absorbance of that particular wavelength.

This is shown in the fingerprint region where each particular wavelength is used to determine each particular bond. The different types of bonds are also explained via this method. The concentration of sample should be kept very low in order to get a better spectra. The absorbance spectra of one molecule do not correspond or match with any other molecule. This is only valid when the compound is pure in nature. Impurities create disturbances or noise in the spectra.

Sources of radiation

There are two lamps fitted in uv visible spectrophotometer one is tungsten lamp and the other is deuterium lamp. The tungsten lamp is used a source of visible radiation whereas deuterium lamp is used for ultra violet radiation. The instrument must be kept in "on" condition for atleast 10 minutes for stabilization of the radiation.

Wavelength selector

The wavelength selector is most important part of uv spectrophotometer. It consists of entrance slit, collimating lens, dispersive device, a focusing lens and an exit slit.

The polychromatic radiation enters the monocromator and is split by the reflecting grating which finally reaches the exit slit.

Cuvettes

Glass and quartz cuvettes are used. The glass cuvettes cannot be used in UV region due to non consistent transparency. Therefore they are used only in visible region. The advantage of glass cuvettes over the quartz lies in its price. Quartz cuvettes are about fifty times more expensive than the glass quvettes.

Detectors

The most popular type of detector used in UV-Vis spectroscopy is photomultiplier tube. It is extremely popular due to its sensititivity and repeatability of results. In a photomultiplier tube a photon of light falls on the first dianode. For each photon of light absorbed 100 times electrons are emitted and reach the second dynode. Similarly each single electron is multipled hundred times

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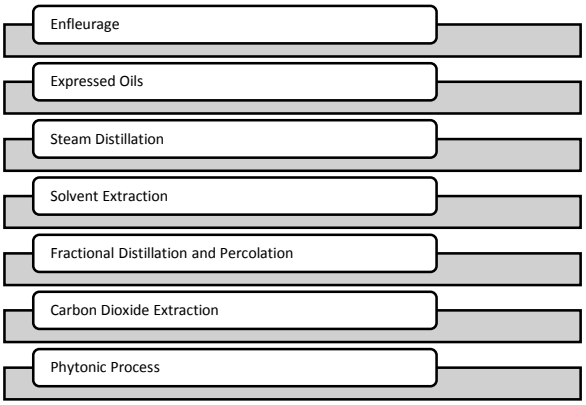

Figure 1: Methods of extraction of essential oils

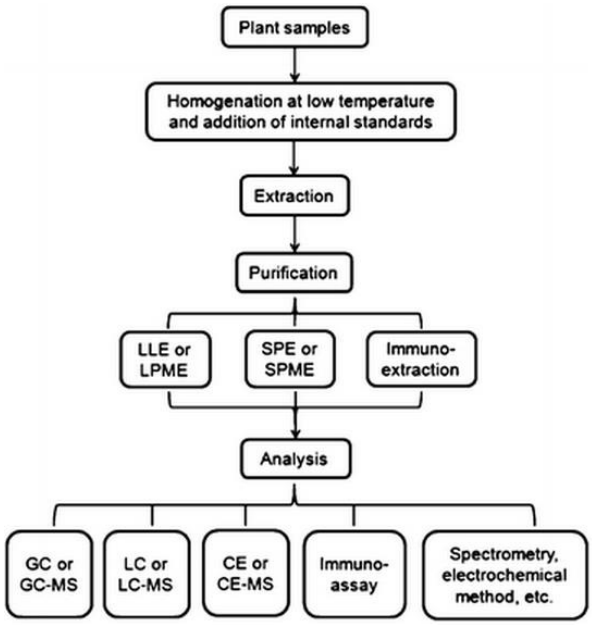

Figure 2: Strategies of Determination

to reach third dynode. Finally each photon of light gives 106107 electron signlas, which are easy to measure.

There are other types of detectors available for the instrument but the most popular is photomultiplier tube.

Infra-red spectroscopy

Infra Red region is between 0.78 to 1000micro meter. In the infra red spectroscopy, wavelength is measured in wavenumbers.

Wavenumber= 1 / wavelength in centimeters

The energy of infra red radiation is very less as compared to ultra violet radiation therefore they are cannot excite any electrons, but they are able to produce vibrations in the molecules. All of the atoms of molecule are in constant vibrational or rotational state. They are constantly in motion. The radiation when falls on the molecule produces vibration

Number of vibrational modes

Change in dipole moment is a requirement for observing vibrational mode. If the molecule do not have any dipole moment then it cannot have any significant absorbtion. A molecule can vibrate in a lot number of way depending upon the field around it.

For molecules with N number of atoms the single side or linear hwill have 3N minus 5 degress of vibrational modes. Example water molecule is non linear and has 3 degress of vibrational freedom.

Each frequency of light has a certain energy associated with it and is proportional to the frequency, this means the larger frequency means larger frequency.

Bond stretching

The bonds in a covalent are not rigid but are held together with the attraction between the corresponding electrons and protons. The two nuclei are constantly in vibration both in forward and backward directions. This forward and backward direction

The energy required to make the vibration is dependent upon many factors like length of the bond, mass of the atoms, number of bonds, polarity etc. All these requires different amount of energies. The amount of energy required from bond to bond varies depending upon all of these factors.

Bond bending

The bond along with stretching also bends a little bit allowing a different type of vibration.

Instrumentation of IR spectrophotometer

Instrumental Components

The IR instrument contains souce. The Nernst glower is the a cylinder having platinum wires sealed at the ends. The Nernst glower can reach temperature more than 2200K.

Globar source is made up of silicon carbide rod which can be heated less than Nernst globar and can reach upto 1500K. Due to this high temperature arching is a frequent problem and therefore water cooling of electrical contacts are needs. The output obtained from spectral is camparable with Nernst glower but have short wavelengths but the output becomes large.

The incandescent wire source is also used in IR spectrophotometer which is a tightly bound nichrome wire. This nichome wire heated upto 1100K where it starts radiating desired frequency of IR radiations. The intensity of this is lower than the Nernst globar but has longer working time as compared to earlier.

Detectors

There are three major classes of detectors

Thermocouples have a pair of junction of different metals. For example bismuth and antimony can be used in pair. A potential difference is maintained between the ends and noted down. The difference in temperature is noted to draw interpretations.

Pyroelectric detectors are also used in IR spectroscopy and forms the second class. A crystalline pyroelectric material is used. The pyroelectric material which is used should be like that it creates a potential difference when external electric field is applied. The degree of polarization is dependent upon temperature. So, by putting the pyroelectric material between two electrode a temperature dependent capacitor formation is made. The effect of heating of incident IR radiation causes the change of capacitance of the material. Pyroelectric detectors are used

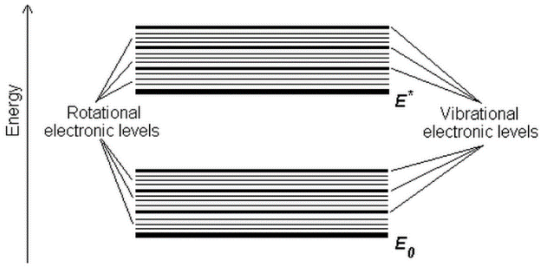

Figure3: Electronic Transitions

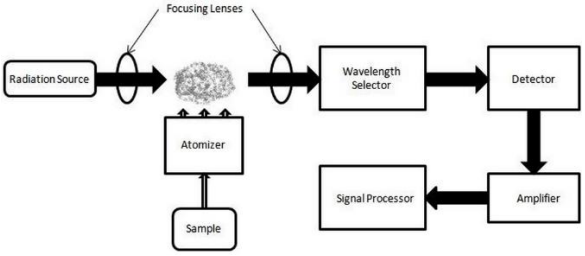

Figure 4: Instrumentation of UV VIsible Spectrophotometer

to obtain fast response time. They are mostly used in Fourier Transform Infra Red instruments.

Photoelectric detectors such as mercury cadmium detector comprixe a solid coat of semiconducting material deposited on a glass surface which is sealed in an evacuated envelope. The absorption of IR promotes non conducting valence from the ground level to the higher level. Absorption of IR promotes the conducting state. The electric resistance decreases. The characteristic of these detectors have a large sensitity on the results obtained.

Dispersive IR Instrument

These instruments are often double beam instruments which can employ diffraction gratings on the dispersion of radiation.

The radiation from the source is flicked between the reference and sample paths. The dispersive instrument is often called as grating or scanning spectrophotometer. A grating used is similar to large number of prisms kept in line. It separates the wavelength of individual light into its constitutent. Each wavelength is measured one at a time. A short pulse is not used. The X-axis of dispersive of an infrared spectrum is typically nanometers which can be converted to a unit wavelength by dividing it by 10 and a calibration is applied.

The sources of IR instrument are heated to get the desired radiation. The sources should be inert as they are susceptible to react at high temperatures. The use of monochromator lies in separating the radiation into its constituent radiations. For most of the times, double beam arrangement is used in such instruments the reason lies in radiation differences at different times. As the readings are highly sensitive therefore a slight change in voltage of the supplier unit might effect the radiation of the souce. One of the beam passes throught the reference while the other one passes through the sample. Both the beams are then analyzed.

After the incident radiation travels through the sample species, the emitted radiation is dispersed by monochromator. This combination of prisms and dispersive gratings give the instrument its name as discpersive IR spectrophotometer. There is requirement of a device to convert this signal into readable formats which is done by detectors. They convert the analog signal into the digital signal for faster processing. Several mathematical algorithms are already mounted within the instrument to read the signal.

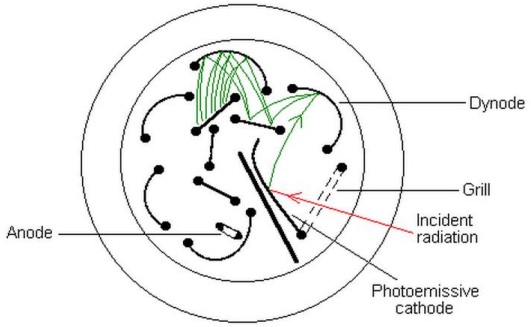

Figure 5: Cross section of Photomultiplier Tube

FTIR spectrometers

A common FTIR spectrophotometer consists of source, interferometer, detector and amplifier. The components of FTIR spectrophotometer consists of source, interferometer, sample compartment, detector, amplifier etc. The radiation is produced by source which is then passed through amplifier, finally the amplified signal is converted from analog to digital signal. The usage of fourier transformation is used by the UNIX powered machine.

The Fourier transformation is a mathematical method to reduce the noise. The spectra obtained from fourier transformation is easy to interpret with greater reliability. The major difference between the normal dispersive type spectrophotometer and the FTIR spectrophotometer is the presence of Michelson interferometer

A typical interferometer contains two mirrors placed perpendicularly to each other and a beam splitter. One of the mirror is a stationary mirror and the other one is rotating mirror. The beamsplitter is designed for transmission of half of the light and reflection of the other part of the light. The striking of the transmitted and the reflected light is made on the stationary mirror and the movable mirror. After reflection from the mirrors, the two beams of light combine with each other at the beam splitter.

If both of the beams travel by the same distance between two mirrors nd the beamsplitter, the condition is defined as zero path difference. The mirror displacement comes in existence when the rotating mirror moves away from the zero path difference and then the beam strikes.

The extra distance can be understood as the optical path difference and is represented by delta

$$\delta = 2\Delta$$

Equation 1

It is established that when the optical path difference is multiple of wavelengths, additive type of interference occurs and the crests of the wavelength overlaps with the

crests of other wavelength and same with the troughs. This results in the maximum intensity at the detector. The condition is therefore describe as below

$$\delta = n\lambda$$

Equation 2

Where n is an integer. On the other hand if the optical path difference is half the wavelength then the complete opposite type of situation comes into existence and here both the rays nullify each other. The situation can be descried by the following equation.

$$\delta = (n + \frac{1}{2})\lambda$$

Equation 3

These above two situations are two extreme situations but if the optical path difference is neither n-folds wavelengths nor (n+1/2)-fold wavelengths, the interference should e between the constructive and destructive. Therefore the intermediate intensity of the signal is obtained. Since, the mirror moves back and forth, the variable amount of intensity is obtained which gives rise to a cosine type of wave.

Fourier transform to spectrum

The interferogram is a function of time and the output against the time domain. The time is transformed to get the frequency domain, which is then deconvolved to the product a spectrum.

Fourier transform is a mathematical method to transform one function into another function. The equation below is a common form of Fourier transform with unitary normalization constants

$$F(\omega) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} f(t) e^{-i\omega t} dt$$

Equation 4

The following equation describes the fourier transformation when applied to real even functions

$$F(\nu) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} f(t) \cos(2\pi\nu t) dt$$

Equation 5

Chromatography

Chromatography is used to separate the mixture of components into individual components. It is high precision, high accuracy technique which is widely used in determination of content of essential oils. There are two phases one is stationary phase and the other is mobile phase. The difference in solubility of the solute in the running solvent creates the difference and isolates components.

The movement of components of solute in the mobile phase is controlled by the level of their interactions with the mobile and the stationary phase. More the adhesive property of the component with the stationary as compared with mobile phase results in low velocity, whereas if the component of solute if having higher solubility will result in high velocity.

The distribution of the solute in the mobile and stationary phase is called as partition coefficient which is defined as below

$$\kappa = \frac{C_s}{C_m}$$

Equation 6

Where C_s is the concentration of solute in the stationary phase and C_m is the concentration in mobile phase. The mobile phase carries the component molecules across the column. The retention time is defined by this particular time which is required from sample injection to the highest peak. The area of each peak is proportional to the amount of the solute.

Retention time is given by the following equation in seconds

$$t_R = t_S + t_M$$

Equation 7

Where t_s is the total time taken by analyst in the stationary phase and t_m is the dead time.

Band broadening and Column Efficiency

The band broadening adversely effects the column efficiency. The quantitative terms are described in the rate theory. The sources of band broadening is from multiple path of analyte rising through the column packing, the diffusion process among molecules and the effect of rate of mass transfer between the phases. The degree of band broadening often increases with the age of column but there are measures that can be taken to slow the aging process and to maintain the column and good instrument conditions for better efficiency and minimum band broadening.

The band broadening was understood firstly by Van Deemter, where he also gave the equations for better understanding of the correction. He described the individual terms and also a composite curve was derived which related the plate height with the linear velocity. To optimize the separation it is needed to get an understanding of the factors affecting it. For example the following figure shows the decrease in performance when moving from volume 20 to 80 microliters.

Eddy Diffusion

The primary factor which relates the band broadening is Eddy diffusion. The eddy diffusion describes the variations in both of the phases between the chromatographic column.

Eddy diffusion takes the fact that analyte molecule can take more than one path within the column. These multiple paths arise due to inhomogeneties in column packing and the small sized pores in the particle size of the material by which column packing has taken place.

Longitudinal Diffusion

A band of solutes under investigation contained in the injection solvent will tend to disperse in each of the direction due to the presence of concentration gradient at the outer side of the band.

This factor is known as longitudinal diffusion as inside tubes, the greatest scope of broadening is along the axis of flow. The band broadening in all tybings of the system but the worst effect will be encountered within the column itself.

The diffusion where longitudinal factors are present wherever HPLC system containing internal volumes with the larges than required at some instances are

Longevity of the tube

Widening of the tube (internal radius)

Unions which are joined by tubings

Incorrectly connected Zero Dead Volumen fittings

The usage of wrong column nuts and ferrules

A flow of detector that has a large volume inside.

Mass transfer

As the analyte molecule passes through the stationary phase to reach the surface of the packing material, there is also a little diffusion in the process. Analyte molecule entering the diffused hole but not able to penetrate more deeply will be held at that point to different extents. This is the cause of broadening of the peak. The porosity of the material is responsible for the error. This is therefore a very important factor of Van Deemter equation The surface area of the packing material is adversely effected by the same.

The non-linear analyte residence time in the stationary phase is also differential, which again cause the difference in the elution time and broadening of band. These effects may be minimized by the reduction of size of the material of packing and to make the pores as shallow as possible. The effects of transfer of mass are also less at lower speed of the mobile phase.

Optimization of flow rate

Although the particle size change is not having any marked effect on the efficiency and resolution of this type of differential separation, yet the study of results should be made carefully.

The reduction of particle size even when used by traditional column internal diameters has a pronounced effect on the height of plate. It can be usefully used for improvement of the resolution of the separations, especially where the options of selection have failed to reduce the peaks of separated baselines.

The height of plates obtained with very small particle are extremely low. By combination of the short column with traditional internal diameter and the presence of silica packaging material. Starting with the mobile phase investigation it can be seen that the flow rate of the mobile phase has an effect on broadening of band. It is important to mention that the comparisons of other variables affecting resolution and the flow rate would be used as a separation only.

Nuclear Magnetic Resonance

Nuclear magnetic resonance is a powerful technique which provides exact information about the number of hydrogen or carbon atoms and their position depending upon the instrument used. The most popular is H-NMR. The signals of NMR are interpreted to get the number, position as well as the surrounding of the protons. NMR is the name of phenomenon which takes place when the static magnetic fields interact with the nuclei of the atom.

Most of the matter examined with the technique are composed of molecules. The molecules are further composed of atoms. Each of the molecule has an electron cloud around its atoms. The proton also has a proterty of spinning which also generates a small magnetic field around it. All of the nuclei do not posses spin, only the nuclei with odd number nucleons possess spin.

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