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Review article *Food Analysis*

Instrumental methods involved in the analysis of preservatives in food and cosmetics products

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ABSTRACT

Analyzing the presence and concentration of preservatives in food and cosmetics is vital for quality control and safety. Various instrumental methods are employed for this purpose, each with its unique set of advantages. High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are widely used for their high accuracy and specificity, often coupled with Mass Spectrometry (MS) for enhanced sensitivity and identification. Fourier Transform Infrared Spectroscopy (FTIR) offers quick, non-destructive analysis, while UV/Visible Spectroscopy provides a straightforward approach for compounds that absorb UV or visible light. For analyzing metal-based preservatives, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is exceptionally sensitive and precise. Other methods like Nuclear Magnetic Resonance (NMR) and Thin Layer Chromatography (TLC) also contribute to the analytical toolbox. The choice of method depends on the type of preservative, the sample matrix, and the required detection limits. Often, a combination of these techniques is employed for a comprehensive analysis.

Keywords: High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Mass Spectrometry (MS), preservatives, quality control

INTRODUCTION

Analysis of food products for the majority of the parameters can be undertakenusing differentinstrumental techniques as described below.

- Chromatographic Techniques
- Hyphenated Techniques
- Spectroscopic Techniques

UV VISIBLE –SPECTROSCOPY1

PRINCIPLE

UV-Visible spectroscopy is based on the principle of the absorption of ultraviolet (UV) and visible light by molecules. When a molecule absorbs UV or visible light, its electrons get excited to higher energy levels. The basic principle involvesthe interaction between electromagnetic radiation and matter.
Basic principle of spectroscopy is the **Beer-Lambert's law.**
In UV-VIS Spectroscopy, a continuum range of wavelengths from 400nm to 800nm is used.

INSTRUMENTATION

Components of UV-vis spectroscopy

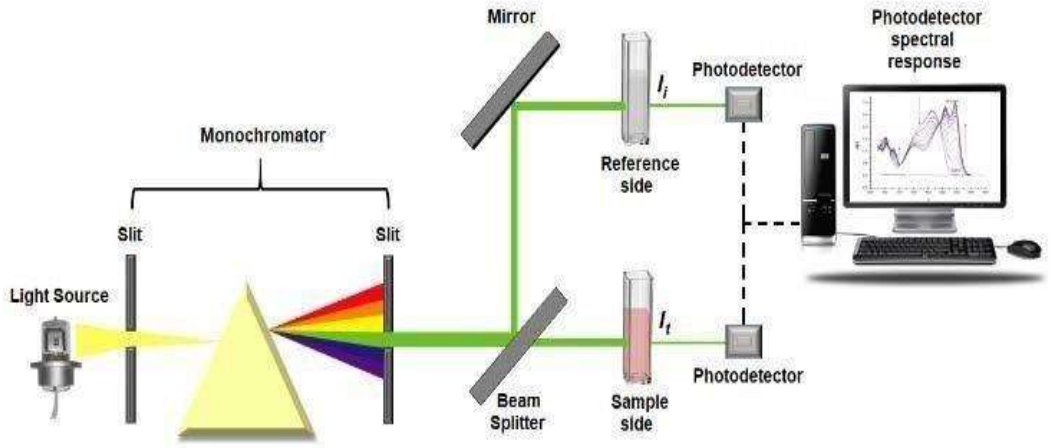

Fig 1: Schematic Diagram of UV-visible Spectroscopy

SOURCE OF LIGHT

- Tungsten halogen lamp
- Hydrogen discharge lamp
- Deuterium lamp
- Xenon discharge lamp
- Mercuryarc

COLLIMATING SYSTEM

The radiation emitted by the source is collimated (maybe parallel) bylenses, mirrorsand slits.

2. Monochromators

It is a device used to isolate the radiation of the desired wavelength from wavelength ofthe continuous spectra.

Converting a polychromatic light or heterochromatic light into monochromatic light.

FILTERS OR MONOCHROMATORS

All monochromators contain the following components parts;

- An entrance slit
- A collimating lens
- A dispersing device (a prism or a grating)
- A focusing lens
- An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the monochromators through the entrance slit. The beam is split into its component wavelength by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromators through the exit slit.

SAMPLE HOLDER

Quartz or fused silica is required for the UV region (wavelengths less than 350 nm)and may be used in the visible region.

DETECTOR

Device which converts light energy into electrical signals, that are displayed on read out devices. The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample. The following types of detectors are employed in instrumentation of absorption spectrophotometer.

- Barrier layer cell/ Photovoltaic cell
- Photo tube/ Photo emissive tube
- Photomultiplier tube

APPLICATION

- Detection of functional group.
- Detection of extent of conjugation.
- Identifications of an unknown compound.
- Determination of conjugation of geometrical isomers.
- Determination of the purity of a substance

HIGH PREFORMANCE LIQUID CHROMATOGRAPHY2,3

The mixture is dissolved in a solvent [mobile phase] and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture is resolved into its components.

PRINCIPLE

The principle of Separation is Adsorption. Based on mode of separation- 2 types.

- Normal-phase HPLC (NP-HPLC) Stationary phase - Polar in nature Mobile phase –Non-polar in nature
- Reverse-phase HPLC (RP-HPLC) Stationary phase - Non-polar in nature Mobile phase – Polar in nature

INSTRUMENTATION

- Pump
- Sample injector
- Guard column

- Analytical column
- Detector
- Recorders
- Integrator

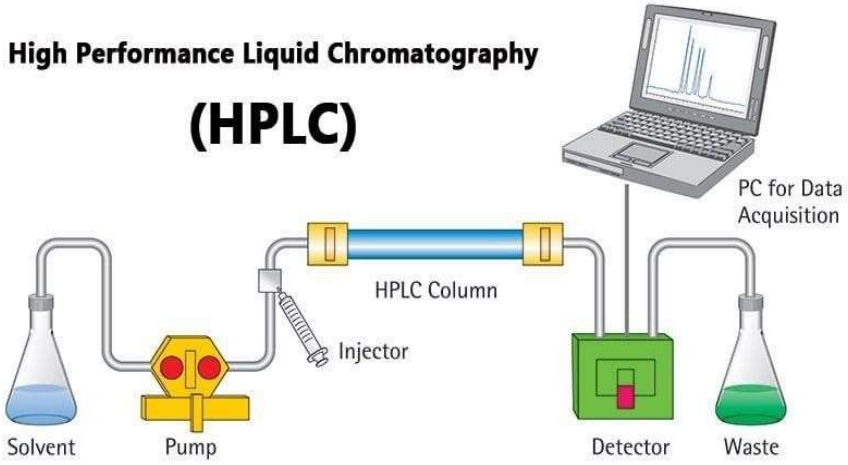

Fig 2: High Performance Liquid Chromatography

APPLICATION

- Qualitative and Quantitative analysis.
- To control the drug stability.

- Checking the purity of a compound
- Multicomponent analysis or Determination of mixture of drug
- Additives, preservatives, antioxidants andstabilizers in processed food products

GAS CHROMATOGRAPHY4

It is a process of separating component(s) from the given crude drug by using agaseous mobile phase.

PRINCIPLE

- The principle of separation in GC is "partition."
- The mixture of component to be separated is converted to vapour and mixed with gaseous mobile phase.
- The component which is more soluble in stationary phase travel slower and eluted later. The component which is less soluble in stationary phase travels faster and eluted out first.
- No two components has same partition coefficient conditions. So the components are separated according to their partition coefficient.

INSTRUMENTATION

- Carrier gas
  - He (common), N2, H2, Argon
- Sample injection port
  - micro syringe
- Columns
- Detectors

- Thermal conductivity (TCD)
- Electron capture detector(ECD)
- Flame Ionization detector (FID)
- Flame photometric (FPD)

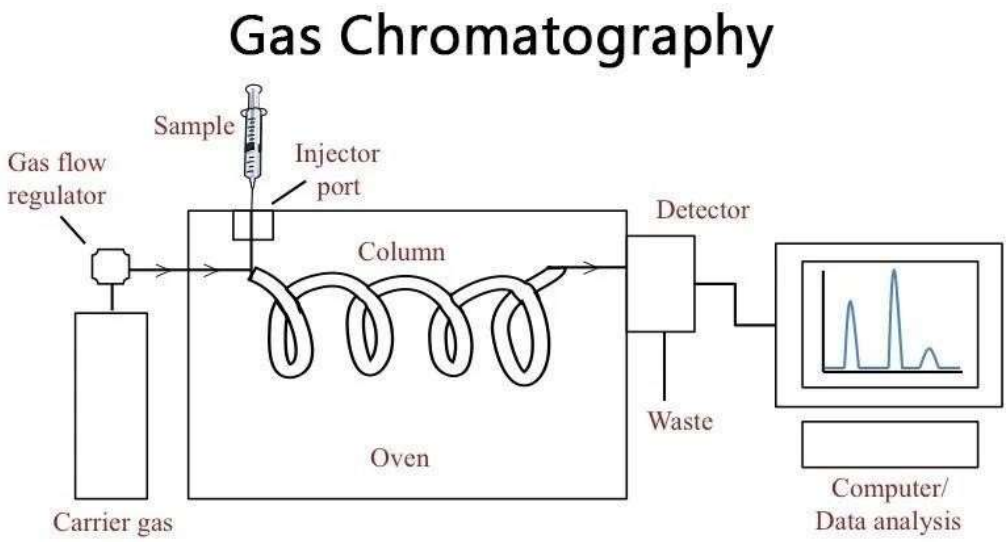

Fig 3: Gas Chromatography

APPLICATIONS

- Cholesterol, Fatty acid profiling and Trans fat analysis.
- Antioxidants and Preservatives like TBHQ, Benzoic acid, Sorbic acid Acetic acid, etc.
- Analysis of residual pesticides and environmental contaminants.
- Characterization of flavours and fragrances.
- The Gas Chromatographic profiling of the essential volatile oils gives a reasonable 'fingerprint' which can be used to characterize the identity ofthe particular oil.

THIN LAYER CHROMATOGRAPHY5,6 PRINCIPLE

- The principle of separation is adsorption. The components move according to theiraffinities towards the adsorbent.
- The component with more affinity towards the stationary phase travels slower. thecomponent with lesser affinity towards the stationary phase travels faster.

PRACTICAL REQUREMENTS

- Stationary phases
- Glass plates
- Preparation and activation of tlc plates
- Application sample
- Development tank
- Mobile phase
- Development technique
- Detecting (or) visualizing agents

Thin Layer Chromatography (TLC) is an important chromatographic technique[7], frequently used for qualitative identification and initial screening of components. A sample is spotted onto the plate or a strip with a micropipette and the plate or the strip is placed in a suitable solvent to develop the chromatogram. The solvent is drawn up the plate by capillary action,which moves the sample components up the plate at different rates, depending upon their solubility and their degree of retention by the stationary phase. Following development, the individual spots are noted or made visible by treatment with a reagent that forms a coloured derivative. For example, amino acids and amines are detected by spraying the plate with a solution of ninhydrin, resulting in a blue or purple coloured spot. If the solute compound is fluorescent, they can be detected by exposing to UV light. The spots generally move at a certain fraction of the rate at which the solvent moves and they are characterized by the Rf value.

Rf VALUE=DISTANCE TRAVELLED BY SOLUTE / DISTANCE TRAVELLED BY SOLVENT FRONT

Rf value is characteristic for a given stationary phase and solvent combination. Since the separation and identification spots on TLC is based upon visual observation, at certain times, if the product analyzed contains large number of components, the method may suffer from poor resolution due to the closely lying or the overlapping spots and poor specificity. Therefore, the results may sometimes be uncertain, misleading or inaccurate.[7]

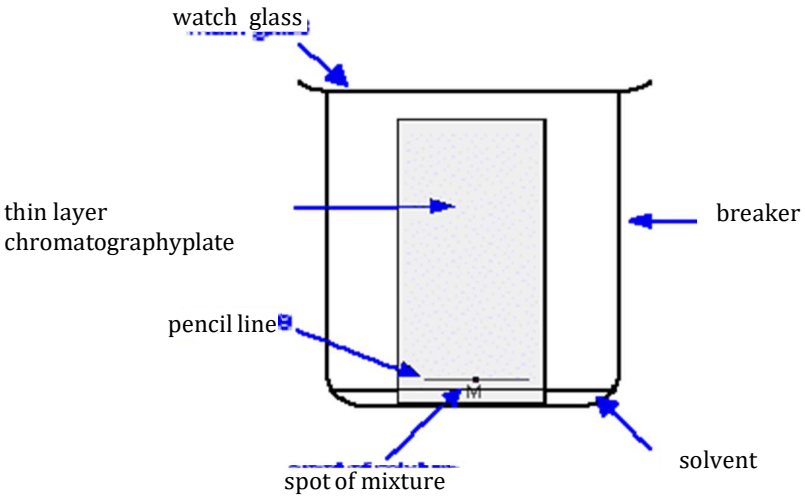

Fig 4: Thin Layer Chromatography

APPLICATION

- Separation of mixture of drugs of chemical or biological origin, plant extracts,etc…
- Separation of carbohydrates, vitamins, antibiotics, proteins, alkaloids, glycosides, etc…
- To detect the presence of foreign substances in drugs.
- To detect decomposition products in drugs.

HIGH PREFORMANCE THIN LAYER CHROMATOGRAPHY8

- It is also known as High Pressure Thin Layer Chromatography/Planar chromatography or Flat-bed chromatography.
- HPTLC is a well known and versatile separation method, which shows a lot of advantage in comparision to other separation techniques.
- The basic difference between conventional TLC and HPTLC is only particle size and pore size of the sorbents.
- It is a powerful analytical method equally suitable for quantitative analytical tasks Separation may result due to adsorption or partition or by both, phenomenon depending upon the nature of adsorbents used on plates and solvents system used for development.

PRINCIPLE

HPTLC having similar approach and employ the same physical principles of TLC (adsorption chromatography) i.e. the principle of separation is adsorption. The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The components with the lesser affinity towards the stationary phase travels faster. thus the components are separated on a chromatographic plate.

INSTRUMENTAION

1. Selection of chromatographic plate
2. Layer pre-washing
3. Activation of pre- coated plate
4. Sample preparation and application
5. Selection of mobile phase
6. Pre-conditioning
7. Chromatographic development and drying
8. Detection and visualisation
9. Documentation

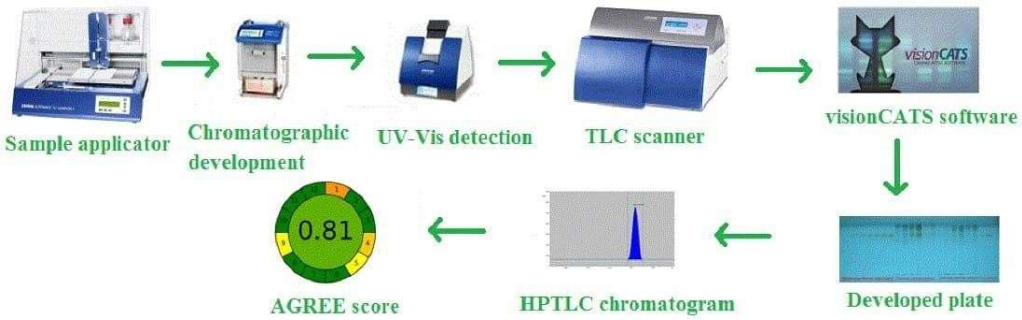

Fig 5: High Performance Thin-Layer Chromatography

APPLICATION

Food Analysis.
Pharmaceutical Industry.
Clinical Application.
Industrial Application.
Forensic Application.
Cosmetics Analysis.

GAS CHROMATOGRAPHY –MASS SPECTROSCOPY (GC-MS)9

The capabilities of integrated gas chromatography-mass spectrometry arealmost unique in meeting the requirements for analytical methods which are not only highly sensitive but also specific and reliable in providing informationon specific compounds as a function of their concentrations.

The GC portion of this system provides separation of volatile organic solutesin a mixture in the gas phase. As each solute exits the GC column, it is divertedinto a mass spectrometer which is capable of monitoring both the amount and identifying the chemical nature of the solute. In this way, both quantitative and qualitative information about the mixture can be obtained.

The MS portion of the system takes each gaseous solute eluting out of gas chromatograph and ionizes it in an electron beam. The ions formed by aspecific solute will depend on the nature of the bonds in the molecule, and it is possible to get both ionized molecules and its fragments. The ions thus formed are then directed down a separator which isolates and counts the ions according to mass. The sequence and relative intensity of the mass peaks give information about the chemical identity of the solute. The absolute intensity of thepeaks provide information about the amount of substance present.

GC-MS PRINCIPLES AND INSTRUMENTATION10

A GC-MS experiment begins with sample preparation, injection and separation on a GC column (as discussed in the previous article). Since the operation of a mass spectrometer requires a highvacuum system, an interface is necessary to direct the molecules from the GC to the mass spectrometer. In the most common type of instrument, the molecules leaving the column enter an ionization chamber where they are bombarded with a stream of energetic electrons, which ionizes and fragments some of the molecules. The ions formed can include the molecular ion (unfragmented) and ions due to fragmentation or rearrangement reactions. The ions are accelerated and rapidly sorted according to the mass to charge ratio (m/z, where m is the mass and z is the charge) in a mass analyzer by use of a magnetic or electric field. The mass analyzer can sort thousands of different ion masses (m/z) per second. Ion abundance is then counted by a detector by measuring the current of electrons generated when the ions strike the detector for each m/z. MS serves as the detector for GC which generates a chromatogram indicating the quantity of each compound as a function of retention time. The underlying dimension of data specific to MS is called a mass spectrum, which is a histogram of the abundance of each ion as a function of m/z and serves as the fingerprint to identify the compound represented by a peak on the chromatogram.

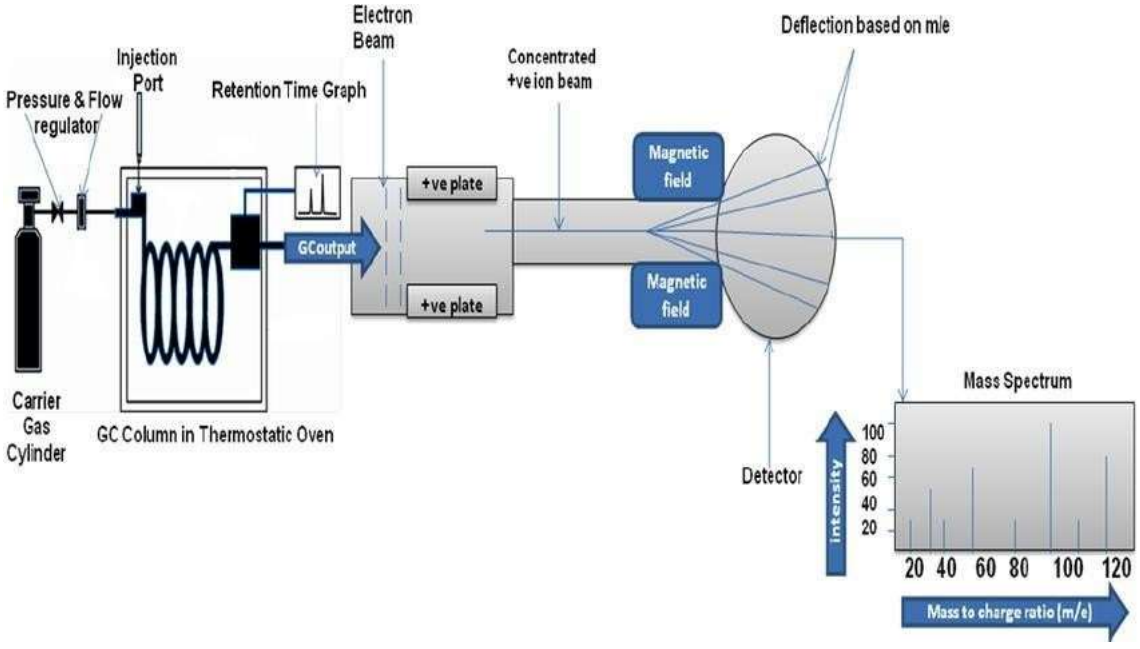

Fig 6: GC-MS

APPLICATIONS

- Metabolic profiling.
- Analysis of biologically important aromatic amines.
- Analysis of pesticides in foodstuffs.
- Identification of volatile components.
- Environmental and forensic application.
- Miscellaneous.

LIQUID CHROMATROGRAPHY–MASS SPECTROSCOPY (LC-MS)11

Liquid Chromatography-Mass Spectrometry (LC-MS) is an analytical technique that combines the physical separation capabilities of HPLC coupled with the confirmation by mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally, its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals in a chemical mixture.

PRINCIPLE

The LC-MS technology involves use of an HPLC, wherein the individual componentsin a mixture are first separated followed by ionization and separation of the ions on the basis of their mass/charge ratio. The separated ions are then directed to a photo or electron multiplier tube detector, which identifies and quantifies each ion. The ion source is an important component in any MS analysis, as this basically aids in efficient generation of ions for analysis. To ionize intact molecules, the ion source could be APCI (Atmospheric Pressure Chemical Ionization), ESI (Electronspray Ionization), etc. to name a few popular ones. The choice of ion source also depends on the chemical nature of the analyte of interest i.e. polar or non-polar.

The major advantages of this technology include sensitivity, specificity and precision as analysis is done at molecular level. Also, structural details of the analyte can be deciphered.

INSTUMENTATION

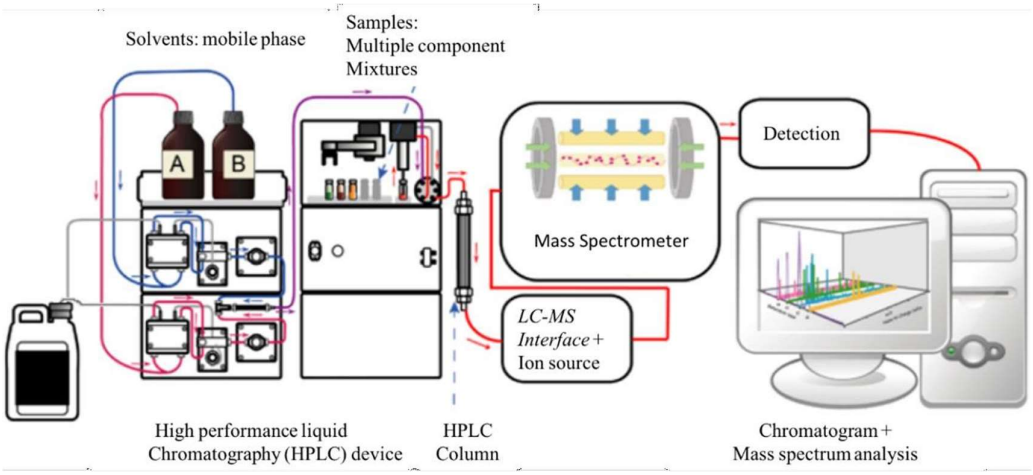

Fig 7: LC-MS

APPLICATION

Mycotoxins: LC-MS is routinels used for detection of: Toxins produced by different fungi, e.g. *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., etc. Some of the mycotoxins regularly analyzed in food samples include Aflatoxins B1, B2, G1 and G2, ochratoxin A, etc.

Residual drugs and antibiotics in different food products.

Banned dyes and colourants e.g. Sudan dyes in different food products.

Residual pesticides in raw and processed food products.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)12

A method of obtaining an infrared spectrum by measuring the interferogram of a sample using an interferometer, then performing a fourier transform upon the interferogram to obtain the spectrum.

PRINCIPLE

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum.

This absorption corresponds specifically to the bonds present in the molecules. The Frequency range are measured as wave numbers typically over the range 4000-600 cm^{-1}.

FTIR is particularly useful for identification of organic molecular group and compounds due to the range of functional group,side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range.

INSTRUMENTATION

Source of infrared energy
Interferometer
Sample
Detector
Computer

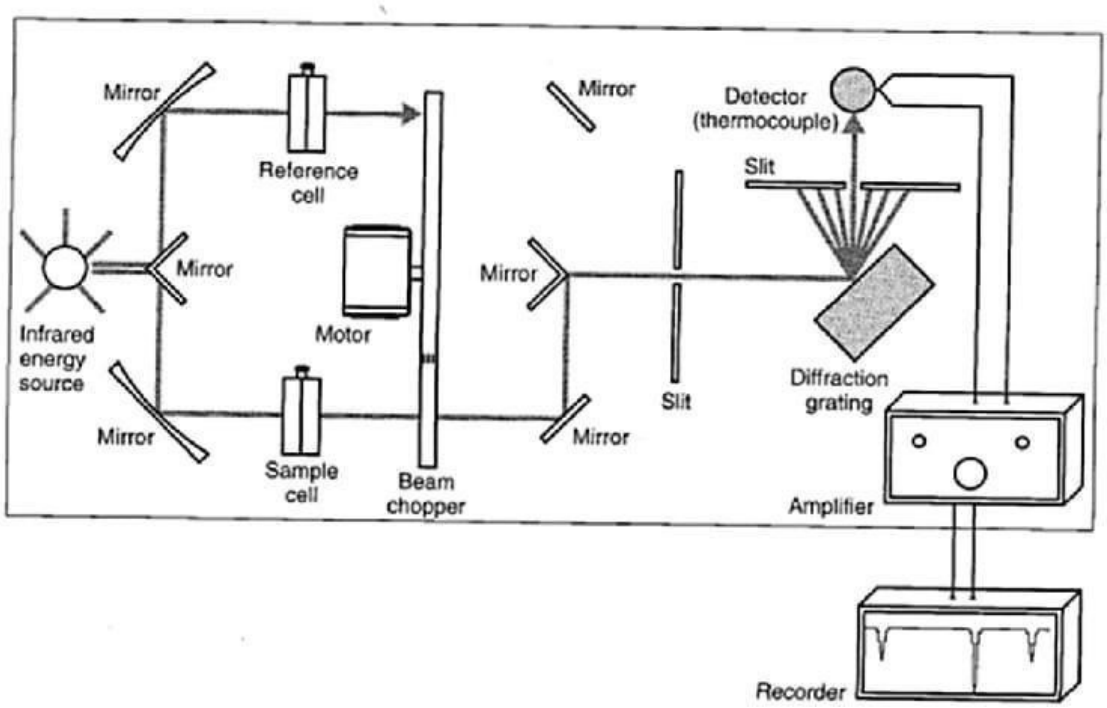

Fig 8: FTIR Spectroscopy

APPLICATION

- Identification of an organic compound.
- Structure determination.
- Study of chemical reaction .
- Study of complex molecules
- Detection of impurities in a compound.
- Conformational analysis.

SOLID PHASE EXTRACTION TECHNIQUE13

- "A solid phase extraction" consists of bringing a liquid or gaseous test sample in contact with a solid phase, whereby the analyte is selectively adsorbed on the surface of the solid phase.
- Other solvents added to remove possible adsorbed matrix components.
- Eluting solvent added to desorb analyte selectively

PRINCIPLE

- Partition of compounds between two phases of solid and liquid.
- Solid phase extraction normally involves bringing an aqueous sample into contact with a solid phase or sorbent, whereby the compound is selectively adsorbed onto the surface of the solid phase. The solid phase sorbent is usually packed into small tubes or cartridges. By careful selection of the sorbent, the organic compounds should be retained by the sorbent in preference to other extraneous materials present in the sample. This extraneous material can be washed from the sorbent by the passing of an appropriate solvent. Subsequently, the compounds of interest can then be eluted from the sorbent using a suitable solvent. This solvent is then collected for analysis.

THE PROCESS OF SOLID PHASE EXTRACTION COMPLETES IN FOUR STEPS:

1. Conditioning of sorbent
2. Loading step
3. Washing of sorbent
4. Elution

CONDITIONING OF SORBENT

In this step, a solvent is passed through the sorbent to wet the packing materials and solvate the functional groups of the sorbent. During this process, the air present in the column is removed and void spaces are filled with solvent.
A commonly used conditioning solvent is methanol which is then followed with water or an aqueous buffer. If the sorbent dries for more than several minutes under a vacuum, the sorbent must be reconditioned.

LOADING OF SAMPLE

In this step, the sample and analyte are applied to the column. The mechanism of retention holds the analytes on the column while the sample is added. The mechanisms of retention include Van der Waals interaction, hydrogen bonding, dipole-dipole forces, size exclusion, and cation and anion exchange. During this step, analytes are concentrated on the

sorbent. Similarly, some of the matrix compounds may also be retained.

WASHING OF SORBENT

In this step, a suitable solvent is used to remove the sample matrix and other impurities from the interstitial spaces of the column while retaining the analytes. If the sample matrix is aqueous, an aqueous buffer or a water-organic solvent may be used.

ELUTION OF ANALYTES

In this step, an appropriate solvent is used to disrupt the analyte-sorbent interactionso that analyte is eluted from the sorbent column. The eluting solvent should remove as little as possible of the other substance sorbed on the column

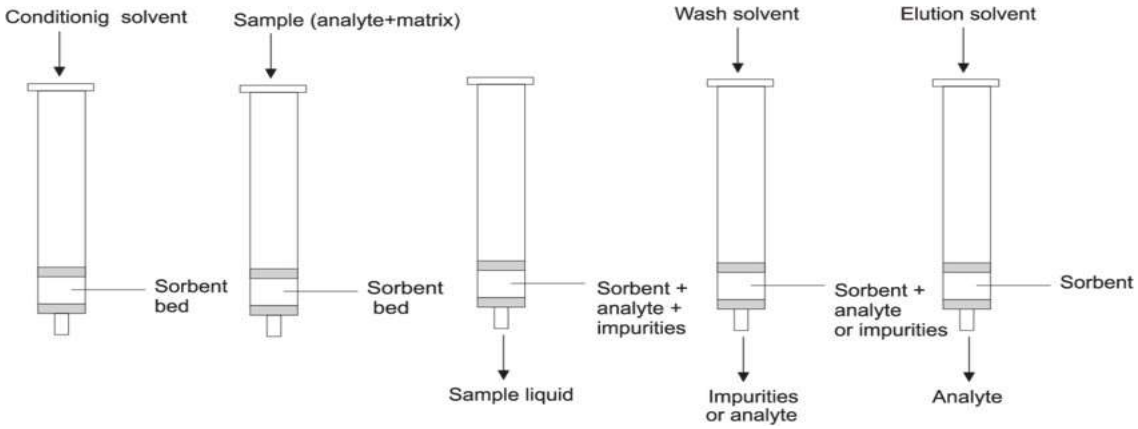

Fig 9: Solid Phase Extraction

APPLICATION

- The concentration of trace organic pollutants from water.
- Purification of drugs of abuse from blood and urine.
- Extraction of organic compounds from food and beverages.
- Impurity profiling of pharmaceutical.
- Applications to biological fluids.

ATOMIC –ABSORPTION SPECTROSCOPY14

Atomic absorption spectrometry is a very popular method for assessing the concentration of metals and minerals that may be present in the food products. This technique allows measuring all the elements of periodic table. It encompasses a wide variety of techniques and provides rapid, sensitive and selective determination of elemental composition.

Atomic absorption spectrometer has five basic components, which are:

1. A light source (cathode lamp)
2. A sample cell (absorption cell)
3. Monochromator
4. Detector
5. Output unit

In this technique, the elements in the sample are brought into their ionized form in solution by using the wet digestion, dry ashing or suitable microwave assisted digestion system and then aspirated through a nebulizer into the high temperature flame where the sample gets converted into gaseous atoms. The source of light is usually a hollow cathode lamp, which is composed of the element being measured. Each element requires a different lamp. The hollow cathode lamp produces emission lines specific for the element used to construct the cathode. The lamp is filled with an inert gas like argon or neon. When a potential is applied, the gas is ionized and is driven towards thecathode and cause the metal atoms to sputter all the surface of the cathode and produce specific atomic emission lines.

The characteristic emission lines produced by the source i.e. hollow cathode lamp are absorbed by the atoms which get excited and are raised to higher energy level. As the sample passes through the flame, the beam of light passes through the monochromator. The monochromator isolates the specific spectrum line emitted by the light source through spectral dispersion and focuses it upon a photo multiplier detector where light signal is converted into an electrical signal. The process of electrical signal is fulfilled by a signal amplifier. The signal could be displayed for readout or further fed into a data station. The greater amount of sample present, the higher the absorbance energy. Different flames can be achieved by using different mixtures of gases, depending, on the desired temperature and burning velocity.

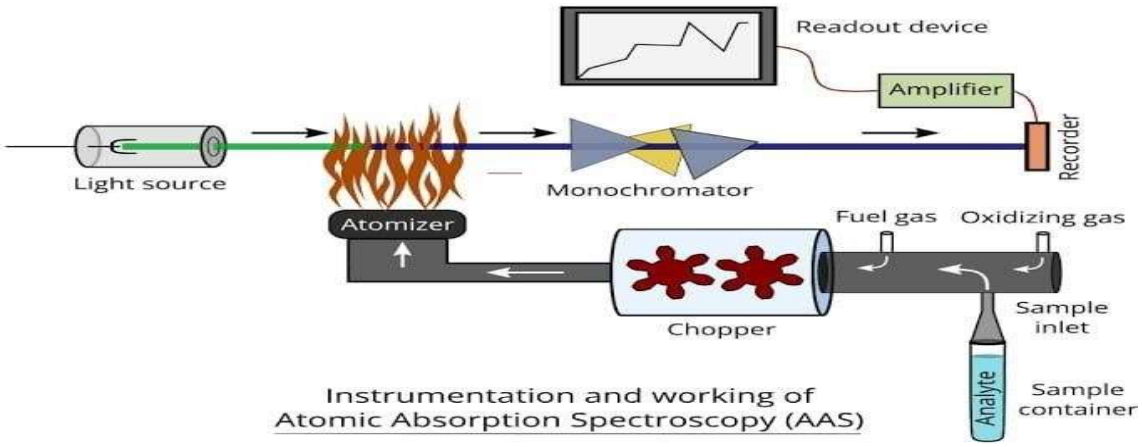

Fig 10: Schematic of an Atomic-Absorption Experiment

Some elements can only be converted to atoms at high temperatures. Even at high temperatures, if excess oxygen is present, some metals form oxides that do not re-dissociate into atoms. To inhibit their formation, conditions of the flame may be modified to achieve a reducing, non-oxidizing flame. The most widely used flames are air-acetylene and nitrous oxide-acetylene flame. The nitrous oxide-acetylene flame has a higher temperature as compared to air-acetylene flame.

APPLICATION

- Atomic spectroscopy is used for quantitative analysis of metal elements in water, soil, plant material, and ceramics.
- In health care, it is used to analyze ionic metal elements in blood, saliva, urine samples. The elements analyzed routinely include sodium, potassium, magnesium,calcium, and zinc.
- To determine heavy metals like iron, manganese, copper, zinc, mercury, lead, nickel, and in urine and blood.
- To determine metal elements like copper, nickel, and zinc in the food industry.
- To estimate Lead in petroleum products.
- To determine metal concentrations in groundwater and bore well samplings before using for drinking and irrigation.

CONCLUTION

To ascertain the impacts of preservatives' action on human health, various analytical techniques including UV-Visible Calorimetry, HPLC, GC, LC-MS, and electrophoresis were used to identify preservatives on food and cosmetic products.

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