Journal of Current Medical Research and Opinion
Received 28-02-2024 | Revised 29-02-2024 | Accepted 15-03-2024 | Published Online 16-03-2024

DOI: https://doi.org/10.52845/CMRO/2024/7-3-1 ISSN (O) 2589-8779 | (P) 2589-8760
CMRO 07 (03), 2165-2172 (2024)
Original Research

Fourier Transform Infra-Red Spectroscopy and Gas Chromatography/Mass Spectrometry (GC/MS) in Herbal Medicine and Pharmaceutical Drugs Analysis

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Abstract:
Chromatography refers to a method of separation that involves bringing a mixture-carrying mobile phase into contact with a stationary phase that is selectively absorbent. Additionally, it is an essential analytical tool for regulating and standardising phyto therapeutical quality. The separation and analysis of multi-component mixtures, including solvents, essential oils, and hydrocarbons, are accomplished through the use of gas chromatography. You can make the readings mean more by adjusting the temperature; for instance, you can use it to tell distinct compounds that act similarly in the GC process apart. Fundamentally, gas chromatography can quantitatively identify compounds at extremely low concentrations by employing the flame ionisation detector and the electron capture detector, both of which have extremely high sensitivities. Biologically intriguing secondary metabolites abound in plants. Secondary metabolites, in general, are a rich supply of compounds with interesting structures and characteristics. A sample is vaporised and then injected onto the chromatographic column head in gas chromatography, more especially in gas-liquid chromatography. A gaseous mobile phase that is neither acidic nor alkaline carries the sample down the column. An inert material is adsorbed onto the surface of the liquid stationary phase within the column. Adsorption and partitioning are the main principles of gas chromatography. One quick and safe way to analyse things is with FTIR, or Fourier transform infrared spectroscopy. It is a potent tool for the pharmaceutical business, often used in conjunction with chemometrics. Analysing herbal remedies is finding more and more uses for this method. Recent advances and improvements in the quantitative and qualitative study of herbal medicine utilising FTIR are the primary topic of this review. Also, it can be used in quality control labs, manufacturing for process monitoring, or while developing herbal drugs. GC-MS finds extensive application in the pharmaceutical sector for analytical R&D, QC, production, pilot plant departments for API, bulk medicines, and formulations, as well as in quality assurance and control. Method and process development, as well as the detection of API contaminants, make use of it. Medicinal chemistry, pharmaceutical analysis, pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology, and impurity profiling all rely on it.

Keywords: FTIR, Analysis, GC/MS, Pharmaceutical Drugs, Bioactive compounds, Applications

Introduction:

For the traditional medical system, medicinal plants are vital bioresources for medication. They are used to cure a wide range of incurable diseases since they include diverse natural dynamic ingredients [1]. Analysing medicinal plants for their phytoconstituents utilising methods including FTIR, HPTLC, and GC-MS. Fourier transforms Transform Infrared spectroscopy is a vital tool for identifying medications for pharmacopoeia in a number of nations, and it is also one of the most often used methods for classifying chemical components [2]. A wide variety of chemicals can have their molecular characteristics mapped out using this nondestructive analytical technique. The functional groups in the plant extracts were identified using FTIR Spectroscopy, which is a sensitive and reliable approach. The IR area in the 400-4000 cm-1 range was used for this purpose (Figures 1, 2, and 3). It is one of the most important and widely used methods for determining the kinds of chemical groups (functional groups) in substances. One feature of the chemical bond that might be observed in the annotated spectrum is the wavelength of the light that is captivated by it. By employing FTIR, the chemical linkages within the molecules have been anticipated [3]. Without a shadow of a doubt, herbal medicine predates any other medical practices. Eighty percent of the global population still mostly uses botanical medicines, according to the World Health Organisation (WHO). The Food and Drug Administration strictly regulates the use of certain botanicals—like morphine, cocaine, digitalis, etc.—because of their widespread usage in conventional allopathic treatment. These regulations ensure that the botanicals meet strict standards of purity, safety, and effectiveness. Without a doubt, infrared spectroscopy is among the most crucial analytical tools at a scientist's disposal. The versatility of infrared spectroscopy makes it a powerful tool for studying a wide range of samples. New sensitive applications have been created as a result of enhanced technology to evaluate substances that were previously intractable [4]. Lastly, infrared (IR) spectroscopy has many advantages over other spectroscopic methods, the most important of which is that it allows both quantitative and qualitative analysis of almost all chemicals due to their absorption (emission).

Methods for Fourier Transform Infrared Spectroscopy:

Although FTIR was initially developed as a spectroscopic method for determining the functional groups of chemical components, it has lately found extensive application in the identification, quality control, and manufacturing process supervision of herbal medicine. Herbal remedies' efficacy is proportional to the concentration of their active ingredients, which might vary greatly. Consequently, there must be strict measures for the quality assurance of herbal remedies. This article aims to provide an update on the latest advancements in using Fourier transform infrared spectroscopy for the analysis of herbal medicines. Infrared Technique Using the Fourier Transform The measurement of the amount of infrared light absorbed (or emitted) by a sample as a function of wavelength is the fundamental premise of infrared spectroscopy [4]. There are two ways to measure infrared light: transmission and reflectance. Transmission is the more common method. Molecular structures can be better understood with the use of infrared spectroscopy. The infrared (IR) spectrum of a polyatomic molecule is derived from vibrations of its molecules, which in turn are affected by bond strengths, atomic masses, and intra- or intermolecular interactions. Consequently, an organic compound's comprehensive infrared spectrum serves as a distinct fingerprint that stands out from the infrared absorption patterns of other compounds, including isomers. To rephrase, the vast majority of substances may be positively identified using their infrared spectra alone when reference spectra are accessible [5]. There is a wealth of structural information in the infrared spectrum. The structure of organic compounds has traditionally been studied using infrared spectroscopy. Low noise, fast speed, high repeatability, easy operation, low expense, etc. have contributed to FTIR spectroscopy's rapid development in recent years. The application of Fourier transform infrared spectroscopy (FTIR) to the evaluation of herbal properties has grown in recent years, thanks to its associations with related scientific fields, such as mathematics and computers, as well as with other methods like two-dimensional correlation analysis (2D-IR). In the mid-infrared region, between 4000 and 400 cm−1, most molecules display infrared bands. The chemical bonding atoms, their conformation, and their surrounding environment are all characterised by the location and strength of vibrational bands, which in turn reveal the underlying molecular motion. As a result, bands in a specific spectral

region are produced by a particular submolecular group [6]. These distinct bands are used as a foundation to interpret vibrational spectra empirically. The utilisation of distinctive absorption bands also allows for the identification of individual compounds. The superior performance of Fourier transform infrared spectrometers has led to their near-total replacement of dispersive instruments. The utilisation of this method has considerably enhanced the process of IR spectrum acquisition. The use of fingerprint spectra (FPS) for the identification of herbal medications dates back to early 1987 [7]. To isolate the herb's constituents, we use a series of solvents with varying polarities—petroleum ether (or cyclohexane), chloroform, ethanol, and water—to extract them. Then, we analyse their UV/FTIR fingerprint spectra. For the purpose of ensuring the efficacy of herbal remedies, three different approaches are used to gather IR FPS. The first approach uses solvents with varying polarity to remove components. Following the solvent evaporation, the components are combined with KBr powder and compressed into a pellet. Subsequently, the IR FPS of the samples are obtained. Thanks to the samples, we can tell many herbal remedies apart. The second approach involves collecting FTIR spectra after mixing herbal medicine powders with KBr and pressing the mixture into a pellet. The final technique involves taking reflectance spectra straight from plant sources. The first approach provides more resolution in herbal medication identification than the other two, despite the fact that the latter two are more convenient. Fast thermal infrared spectroscopy (FTIR spectroscopy) is a commonly used method for the rapid evaluation of food ingredients. One study used methanol and an acetone/water mixture to extract procyanidins from red and white grape seeds [8]. The degree of polymerization (DPn) was estimated using FTIR spectroscopy and a partial least squares (PLS1) regression model with eight latent variables (LVs). The RMSECV was 11.7%. If you want your deciduous perennial fruit crops to break buds and bloom properly, you need to chill them in the winter. A change in the sugar and amino acid profiles is linked to the emergence of dormant buds, according to recent studies. It seems that the levels of carbohydrates, pectin, and cellulose in the meristems are correlated with these wavenumbers [9]. This FTIR signature has the potential to shed light on the optimal timing and concentrations of applications of bud break regulators, like hydrogen cyanamide, as well as the impact of various physiological and environmental factors on bud dormancy breaking and shoot outgrowth.

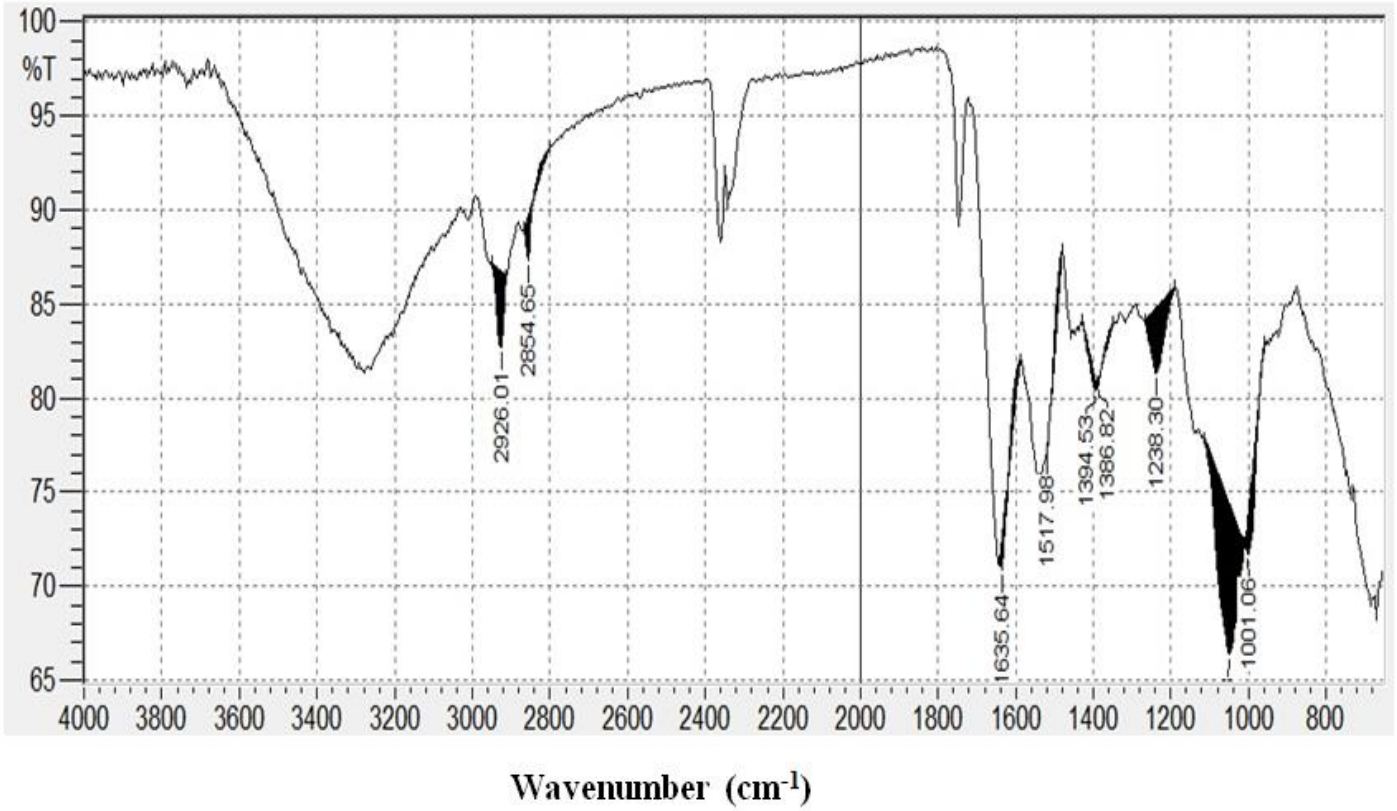

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of Glycine max

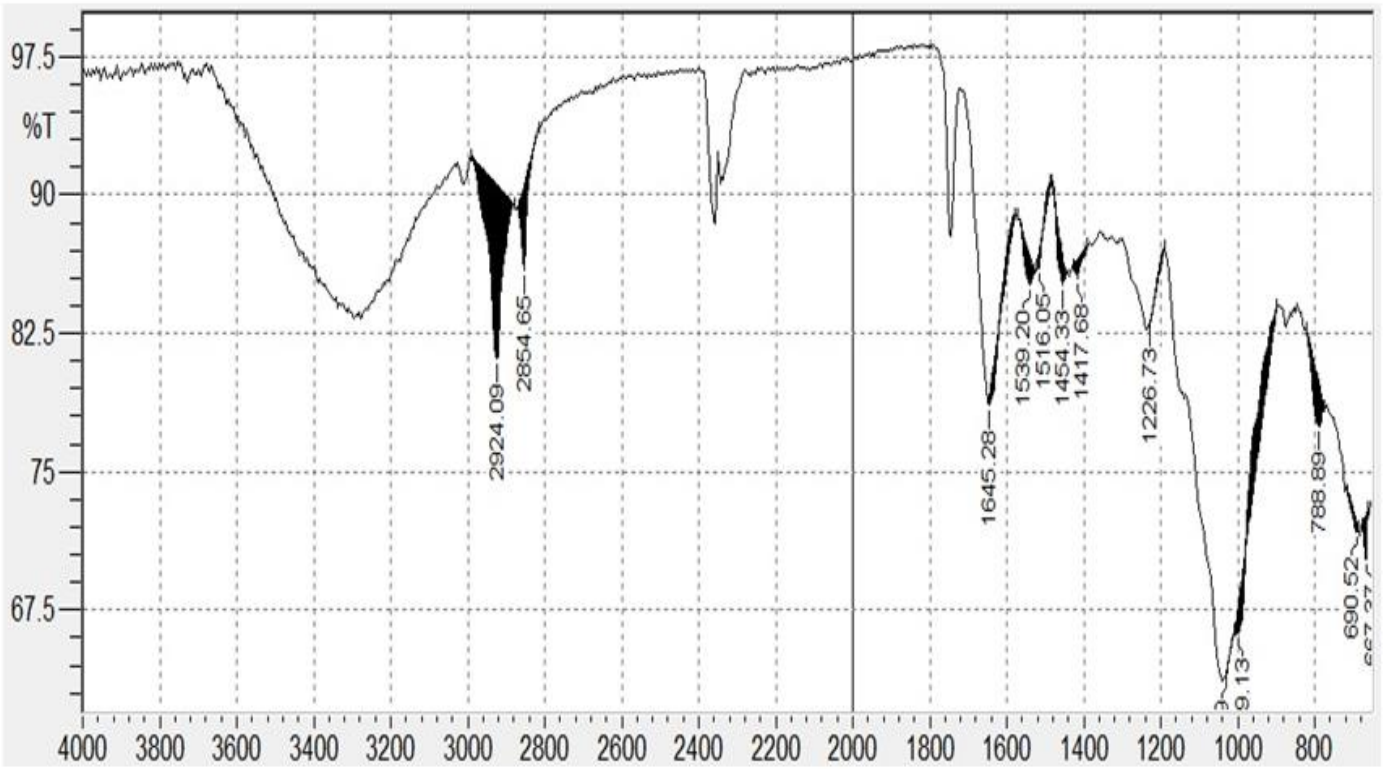

Figure 2. Fourier-transform infrared spectroscopic profile solid analysis of *Diplotaxis cespitosa*

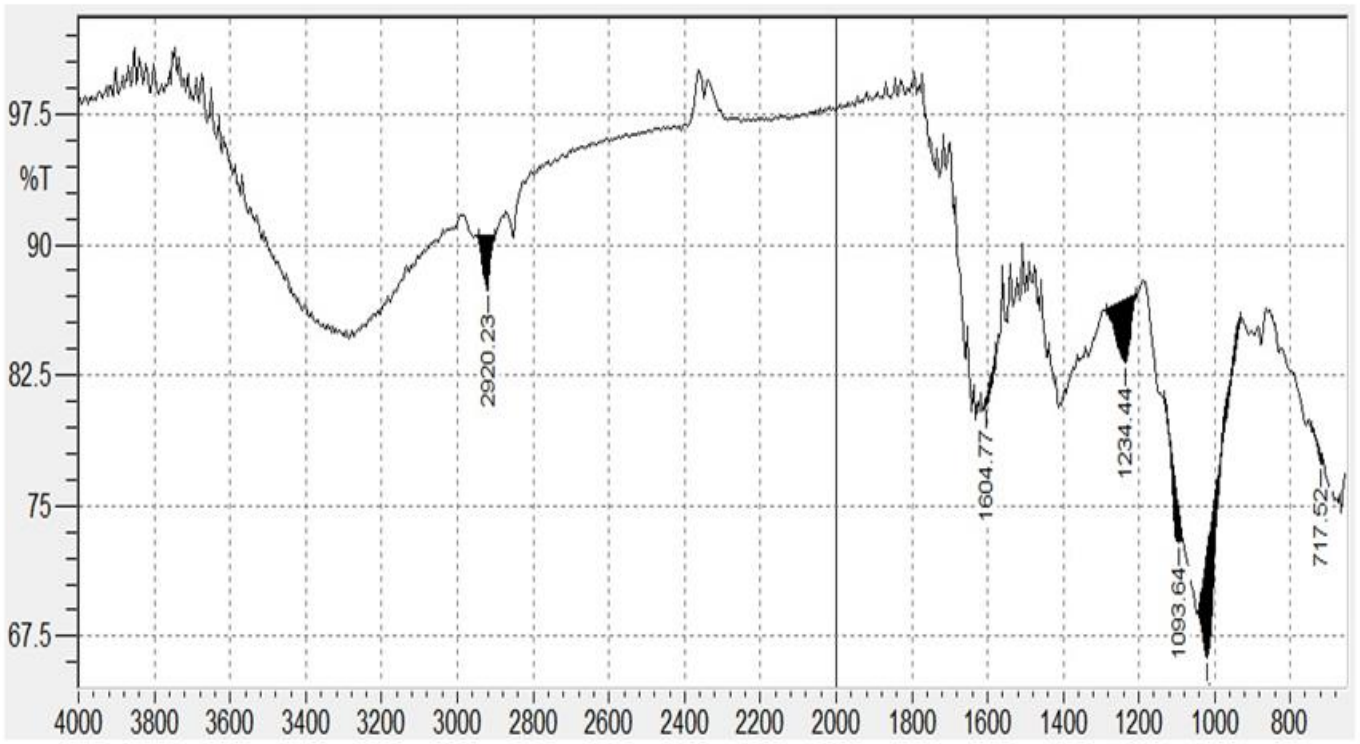

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Malva parviflora*

Gas Chromatography-Mass Spectrometry (GC-MS):

When testing clinical samples for steroid hormones, gas chromatography-mass spectrometry (GC-MS) has traditionally been the technique of choice. Low concentration analytes, like estradiol and testosterone, can be detected with this method in specific patient populations [10]. The steroid hormones work wonderfully with GC-MS because they are either lipophilic or nonpolar. If you want to assess the relative abundance of three of the most noticeable or repeatable ions following mass fragmentation, and you need to make sure they fall within certain ratios, then you should utilise a GC-MS, which contains one quadrupole by definition, for selected ion monitoring or SIM analysis [11]. Chromatography run durations might be lengthy for steroid hormones because they all share fragment ions, which means they need to be separated before entering the mass spectrometer. Furthermore, clinical samples often contain steroid hormones in polar sulphate or glucuronide conjugates that cannot be detected with GC-MS. Before the parent compound can be studied, the conjugates must be eliminated via hydrolysis, which might be acidic or enzymatic. Even at very low concentrations, gas chromatography is a very effective tool for the separation and analysis of complicated mixtures of chemicals [12]. Compounds having boiling points lower than 200ºC benefit greatly from it. Forensics, identifying unknown samples, geochemical research, petrochemical analysis, clinical toxicology, pharmaceutical drug detection, fire investigation, environmental analysis, explosives detection, flavour and fragrance analysis in food and beverages, and many more applications are just a few of the many uses for gas chromatography–mass spectrometry. Airport security uses GC-MS to detect chemicals in luggage and on humans, demonstrating its adaptability. The GC-MS method completely destroys samples during analysis, however it only requires very little amounts of samples for identification.

Uses of GC-MS: Research on environmental hazards:

In spite of a dramatic improvement in reliability, the price of GCMS equipment has dropped. This method is highly useful for screening for contaminants such as chlorophenols in water and soil, PAHs, unleaded petrol, dioxins, dibenzofurans, organo-chlorine pesticides, herbicides, phenols, halogenated pesticides, and sulphur in the air. In bio-mass studies, it can screen for lignin breakdown products; in spinach, it can screen for pesticides [13]. Derivatization is unnecessary for the study of carbamazepine and its metabolites in treated sewage water and steroids, as well as decacyclene, ovalene, and even C60 degradation.

Academic Investigations:

There is a once in a lifetime chance to characterise and identify newly synthesised or derivatized chemicals using GC-MS, a unique and powerful method. Chemistry, polymers, nanotechnology, and biotechnology are just a few of the many scientific fields that make use of it. GC-MS is useful for research and has been published in scholarly journals around the world [14–16]. For complicated investigations, GC-MS provides a high-tech analytical platform that is vital in metabolomics [19, 20]. A wide variety of metabolites, both main and secondary, in plants, animals, and microbes may be studied in depth with this technique, which allows researchers to probe the metabolome to greater depths. Specifically, the problem of untargeted metabolite identification is well-solved by High-Resolution Accurate Mass (HRAM) GC-MS.

Practical Uses in Industry:

Analysing aromatic solvents, inorganic gases, amino alcohols in water, contaminants in compounds like styrene, glycol, diols, and xylene, and allergens in cosmetics are just a few of the many uses for GC-MS in the industrial sector. For industrial uses, GC-MS is also essential for characterising formic acid within acetic acid. Acetic acid is an important intermediate, especially in the field of coal chemical synthesis. The wide range of products made from it, including synthetic fibres, textiles, polyethylene, cellulose acetate, and polyvinyl, shows how it is used in many different industries [17].

Analysis of Biological Samples:

All the way from single cells to whole organisms, biofluids, organs, tissues, tissue extracts, and cellular organelles are all part of the vast spectrum of biological samples [18]. Analysing a wide range of biotic components—including amino acids, fatty acids, and metabolites in biological fluids—and xenobiotics—including pharmaceuticals, drug metabolites, toxicants, and specific metabolic products generated by toxicants—is best accomplished using gas chromatography and gas

chromatography-MS. Measurement of fatty acids in biological samples is important because of the linkages between these compounds and many disorders. Fatty acids play important functions in biological systems. Analytical procedures include selecting columns and internal standards, as well as extraction and derivatization methods. The separation of different fatty acids is made possible by using GC-MS instruments in conjunction with specialised capillary columns, but liquid-liquid extraction and solid-phase microextraction are the most used extraction methods [19]. Fatty acids of different isomers and chain lengths seen in biological samples are commonly analysed using high-polarity columns such as the HP-88, DB-FFAP, and SLB-IL series.

Findings from Biological and Pesticide Analyses:

Medicines such as anaesthetics, anticonvulsants, antihistamines, anti-epileptic medicines, tranquil hypnotics, narcotics, alcohols, residual solvents, and bio-analysis of blood and urine are the sole applications of GC-MS. Adulteration detection, fatty acid profiling in microorganisms, free steroid presence, blood pollutants, serum metabolites, organo-chlorinated pesticides in river water, drinking water, soft drinks by head space, pesticides in sunflower oil, etc., could all be addressed with this technique.

Analysing Food:

The complex organic components of food, including lipids, proteins, carbohydrates, vitamins, and minerals, are essential to human survival. Pesticides, chemical pollutants, natural poisons, veterinary medications, and packaging materials are among the contaminants that food product analysis seeks to identify and quantify [20]. One of analytical chemistry's greatest contributions to human advancement is its ability to detect and measure food components. On top of that, food analysis can help with things like process control, quality assurance, and detecting adulteration. Xenobiotic chemicals can be found in foods not only in their inherent components, but also in them due to environmental factors, packaging, and farming practices. These xenobiotics are harmful even at low concentrations, and several of them are quite dangerous [21, 22]. Direct one-dimensional GC procedures, using a column such as 30 m × 0.25 mm × 0.25 µm, are effective for food samples that are not very complicated to moderately complex. Vapour separation in food is a common use of this method, which produces peak capacities between 400 and 600 atomic mass units. Methods such as GCGC work well for both targeted and unknown studies in the field of food analysis when dealing with extremely complicated samples [23].

Analysis of Beverages:

Beverages include a wide variety of liquids that are meant for human consumption, including water, milk, tea, juice, smoothies, and soft drinks. They can be alcoholic or non-alcoholic. Producers and consumers alike place a premium on beverage safety testing [24] for reasons such as confirming product uniformity, preventing adulteration, and tracking contamination. To obtain optimal output while minimising waste, it is necessary to assess essential parameters such as citric acid, malic acid, acetic acid, ascorbic acid, lactic acid, glucose, fructose, sucrose, iron, or ethanol; then, corrective steps can be taken. One powerful analytical tool for alcoholic beverage analysis is gas chromatography (GC). Due to the samples' liquid condition within an alcohol or alcohol/water matrix, minimal sample preparation is usually necessary. The texture and flavour of alcoholic drinks are greatly affected by their ethanol concentration, which can range from 721% (v/v) for wine to 2050% (v/v) for liqueurs and from 3% to 6% (v/v) for beer [25]. Aldehydes, acids, alcohols, and other trace-level flavour components are volatile chemicals in alcoholic beverages, which are well-suited to GC's capabilities. The mass selective detector (MSD) and flame ionisation detector (FID) are two examples of detectors that can be used. The Stabilwax®-DA capillary column is well-known for its stability and polarity, making it ideal for analysing acids, esters, and other flavour components in alcoholic beverages. By using large volume injection (LVI) methods, it is possible to analyse trace-level flavour components in addition to compounds at greater concentrations, such as esters and alcohols, all at once. Peak areas are used to represent peak concentration in capillary GC analysis, and when these areas deviate from the standard graph, it means that substances beyond the standard are present. When used in combination with comparisons of resolution times, this method helps to quantify the sample amount in the peak [26].

Analysis of Flavour and Aroma:

Research on Pharmaceutical Goods:

In the pharmaceutical industry, gas chromatography (GC) plays an essential role in

analysing drugs, evaluating drugs, and controlling drug product quality. Remainder solvent evaluation, analysis of different functional groups, pharmaceutical compound purity percentage determination, drug of abuse identification, identifying complex mixtures in natural products during pharmaceutical R&D, and metabolomic studies are just a few of the many analyses that rely on it. For routine assessments, GC with flame ionisation detection (FID) or electron capture detection (ECD) provides enough sensitivity for residual solvent monitoring. Recent research using static headspace (SHS) or headspace (HS) methods has shown that targeted chemicals are more selective, more sensitive, and less affected by matrix interference. In the pharmaceutical industry, GC-MS is widely used for the detection and quantification of a wide range of pharmaceuticals, such as antibiotics, TB medications, anticancer treatments, antivirals, central nervous system stimulants, general anaesthetics, sedatives, steroids, and major and minor tranquillizers [26]. Assuring the efficacy, safety, and quality of pharmaceutical goods relies heavily on this analytical approach.

Conclusion:

Because of its efficiency, portability, and automation, GC-MS has had a profound effect on many domains, including research, industry, and academia, and it produces results that are both speedy and reproducible. Its sensitivity, selectivity, and total separation make it useful in many contexts, from quality assurance to planetary research. When it comes to compound synthesis, stability testing, and impurity profiling, GC-MS is indispensable; as a result, it is a key tool in medicinal chemistry, pharmaceutical analysis, pharmacognosy, process control, and pharmaceutical biotechnology. It helps in pharmaceutical research, quality control, and making different parts of drugs. Although computational techniques can help with data analysis, the complex nature of data interpretation means that GC-MS relies on human skill to deduce molecular structures from mass spectra. While the difficulty of analysing complex materials necessitates continuous technological and instrument development, future advancements could be ushered in by further investigation into the potential of GC-MS.

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