Original Research Paper

Volume-9 | Issue-3 | March-2019 | PRINT ISSN - 2249-555X

Pharmacy

HEADSPACE GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS) AND IT'S APPLICATIONS-A REVIEW

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ABSTRACT Headspace gas chromatography-mass spectrometry (GC-MS) is a technique used for the concentration and analysis of volatile organic compounds. This technique is relatively simple, fastest and cleanest method that can provide sensitivity similar to dynamic purge and trap analysis. A headspace sample is normally prepared in a vial containing the sample, the dilution solvent, a matrix modifier. Water or a high-boiling organic solvent may be added to dissolve the sample and facilitate release of volatile compounds. The vials are typically heated to drive partitioning of volatiles from the solid or liquid into gas phase which are to be agitated during heating. An aliquot of the vapour in the headspace is delivered to a GC system for separation of all the volatile components. The popularity of this technique has grown and has gained worldwide acceptance for analysis of alcohols in blood and residual solvents in pharmaceutical products.

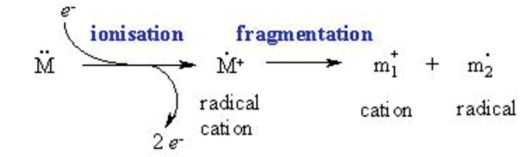
KEYWORDS : volatile organic compounds, dynamic purge and trap analysis, polymers.

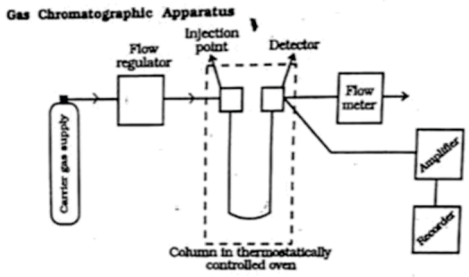
1. INTRODUCTION[1,2]:

Headspace technology is a technique developed in 1980s to elucidate the odour compounds present in the air surrounding various objects. Headspace gas chromatography uses headspace gas injected directly onto a gas chromatographic column. Headspace sampling is excellent for the qualitative or quantitative analysis of volatile species in samples that can be efficiently partitioned into the headspace gas volume from either a liquid or solid matrix. It is also a good technique for the analysis of samples where the entire sample should not be injected into the GC instrument.

Headspace is the gas space in a chromatography vial above the sample. Volatile sample components diffuse into the gas phase, forming the headspace gas.

Gas chromatography is a very popular chromatography technique used to separate volatile compounds or substances that can be vaporized without decomposition. In GC, the mobile phase is a gas(helium or nitrogen) which carries the vapours of the compound through a column with the stationary phase(a thin layer of liquid or polymer on a solid support). Mass spectrometry is an powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample.





2.PRINCIPLE OF HEADSPACE GC-MS

Headspace analysis can be loosely defined as the analysis of characteristic volatile compounds associated with liquids or solids without direct sampling of the matrix. Solid or liquid samples are sealed in headspace vials. Water or a high-boiling organic solvent may be added to the vial to dissolve the sample and facilitate release of volatile compounds to the gas phase. The headspace vials are typically heated to drive partitioning of volatiles from the solid or liquid phase into the gas phase. Sample vials can be agitated during heating. Sampling of the headspace for GC/MS injection is usually performed via a heated gas-tight syringe.

2.1 PRINCIPLE OF GAS CHROMATOGRAPHY[1,2,4]:

The principle of separation in GLC is partition. Gas is used as mobile phase. Liquid which is coated on to a solid support is used as stationary phase. The mixture of components to be separarted is converted to vapour and mixed with gaseous mobile phase. The components are separated according to their partition co-efficient. The components which are more soluble in the stationary phase travels slower and eluted later and vice versa.

2.2 PRINCIPLE OF MASS SPECROMETRY[3]:

A mass spectrometer generates multiple ions from the sample under the investigation. The ions of the compounds are produced by ionization of sample where the vapourized sample is bombarded with a beam of electrons. The produced are accelerated in electric field. They enter into a magnetic field, where the charged particles get attracted and align themselves in a circular path and get separated according to mass-to –charge ratio.

2.INSTRUMENTATION OF HEADSPACE GC-MS:

It is an hyphenated technique in which the headspace Gas Chromatography is coupled with Mass spectrometry.

3.1 GAS CHROMATOGRAPHY[1,2,4]:

3.1.1 Carrier Gas: The choice of carrier gas determines the efficiency of chromatographic separation. Commonly used carrier gases are hydrogen, nitrogen, helium and argon. Hydrogen has better thermal conductivity, low density. Helium has excellent thermal conductivity but it is expensive. Nitrogen is inexpensive but has reduced senstivity.

3.1.2 Flow regulators and flow meters: Flow regulators are used to deliver the gas with uniform pressure. Flow meters are used to measure the flow rate of carrier gas. They are Rotameter and Soap bubble meter.

3.1.3 Injection devices: Samples for introducing into the column can be of any type i.e, either gas, liquid or solid in nature. Gases can be introduced into the column by valve devices. Liquids can be injected through loop or septum devices. Solid samples are dissolved in a suitable solvent and then they are injected through a septum.

3.1.4 Columns: Column is one of the important parts of GC which decides the separation efficiency. Columns are made up of glass or stainless steel. Stainless steel columns have the advantage of long lifr and can be easily handled without fragility. Glass columns have the advantage that they are inert and do not react with any kind of sample.

Analytical column have a length of 1-1.5 meters and a outer diameter of 3-6mm. whereas preparative columns are larger when compared to analytical columns and have a length of 3-6 meters and a outside diameter of 6-9mm.

3.1.5 Detectors: A detector uses some property by which it can detect the difference between a pure carrier gas and a eluted component. Commonly used detectors are i) Thermal conductivity detector- it is based upon thermal conductivity difference between carrier gas

3.2 MASS SPECTROMETER[3]: A typical mass spectrometer consists of

3.2.1 Inlet system: For a mass spectrometry, a sample size of about 1µ mole is required. The sample is converted to its gaseous state in the inlet system. To achieve this, the system is generally heated so that less volatile samples get vapourised. The rate at which the sample is introduced into the ionisation chamber must be constant. It involves different types of handling systems for transfer of different samples. Liquids are handled by a hypodermic needle injection, whereas for gases it involves gas from gas bulb to a meeting reservoir and then to expansion reservoir. From the sample reservoir, the gaseous samples are leaked into the ionisation chamber or ion source through a pinhole reservoir of about 0.013 to 0.050 mm diameter.

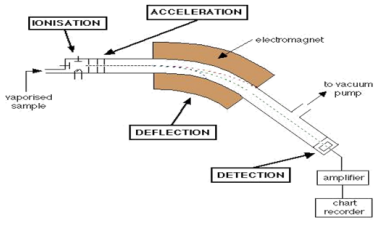
3.2.2 The ion source: From the inlet system, the sample is made to introduce into ionisation chamber where a beam of electrons is put across the molecules of samples. The molecules become ionised. Gas phase ion source includes electron impact ionization, chemical ionization, field ionisation. Desorption techniques include field ionisation, electron spray ionisation, matrix assisted laser desorption or ionisation, plasma desorption, fast atom bombardment. and that of component. ii) Flame ionisation detector-based upon the electrical conductivity of carrier gases. Iii) Argon ionisation detector- depends on the excitation of argon atoms to a metastable state, by using radioactive energy.

3.2.3 The Electrostatic Accelerating system: The positive ions formed in the ionisation chamber are withdrawn by the electric field which exist a between accelerator plate and the repeller plate. A strong electrostatic field between them of 400-4000V accelerates the ions to their final velocities. Whenever the mass spectrometer is started to record the spectrum, the second accelerator is charged to an initial potential of 4000 volts. Then, this charge is permitted to leak off to ground at a controlled rate over a period of 25 minutes.

3.2.4 Magnetic field: As the accelerated particles from the electrical field ener the magnetic field, the force of the magnetic field require them to move in a curved path. The radius of this curvature,r, is dependent upon mass,m, the accelerating voltage, V, the electron charge,e, and the strength of the magnetic field,H.

3.2.5 Ion separator (Analyzer): It is that part of mass spectrometer which separates ions according to their masses.

3.2.5.1 Single focussing magnetic deflection: It consists of an evacuated horse shoe shaped metal or glass tube consisting of a permanent magnet or the electromagnet which deflects the ion beam in a circular path.



3.2.5.2 Double focussing: It is generally employed whenever highest resolution is required. This type of instrument is used in the determination of precise molecular weights. Double beam instruments where two ion beams from independent sources pass side by side through a common mass analyser and are detected by separate collectors. Such instruments may be used to compare samples directly to investigate a single sample under different ionising conditions or to compare a sample with a standard. The resolving power of this double focussing mass spectrometer is of the order 30000. Such resolving capability enables high molecular weight fragments, which differ by only one mass unit.

3.2.5.3 Cycloidal focussing: In cycloidal focussing, ions while passing through crossed magnetic and electric fields will produce a cycloidal path.

3.2.5.4 Quadrapole mass spectrometer: It consists of 4 voltage cylindrical carrying rods parallel to the direction of ion beam.

A DC voltage and a RF is applied to rods, generating an oscillating electrostatic field in the region between rods. Ions having correct m/z ratio undergo stable oscillation and travel down quadrapole axis with a crockscrew type trajectory and reach the detector.

3.2.5 Detector and Readout devices: Ions after passing from the analyser, reaches the detector which produces a signal. The signal is further amplified to give the m/z value. Readout system usually employed is a direct writing recording oscilloscope consisting of 3-5 galvanometers.

3.3 COUPLING OF GC-MS[11]: The main interfaces types for GC-MS are:

3.3.1 Jet separator: In these separators, the GC flow is introduced into an evacuated chamber through a restricted capillary. At the capillary tip a supersonic

expanding jet of analyte and carrier molecules are formed and its core area sampled into mass spectrometer. The jet interface is very versatile, inert and efficient.

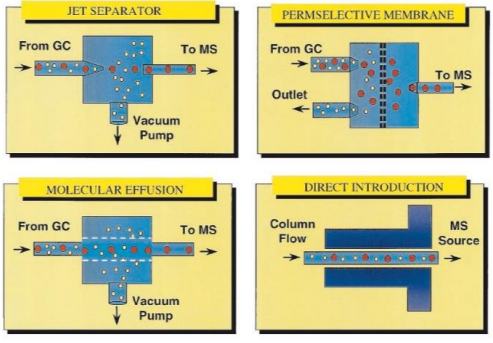


Plate 1. Interfaces for GC-MS coupling. The analyte and carrier gas molecules are represented by the red and yellow spheres, respectively.

3.3.2 Permselective membrane Interface: It is made of a silicone-rubber membrane that transmits organic non polar molecules and acts as a barrier for non-organic carrier gases.

3.3.3 Molecular effusion interface: It is based on the molecular filtering of thee gas effluent by means of a porous glass frit. The column effluent passes through a fritted tube situated in a vacuum chamber. Small molecules transverse through the microscopic pores in the tube wall and are evacuated whereas high molecular mass molecules are transferred to the ion source.

4 TYPES OF HEADSPACE SAMPLING[6]:

4.1 Static headspace sampling: It is the simplest method, particularly when carried out manually with a gas-tight syringe. For this purpose the sample must be thermostatted and allowed to reach an equilibrium. The headspace sample should be small in comparision with the total jet interface is very versatile, inert and efficient.

headspace volume to avoid the action of sampling from changing the sample characteristics and the sampled volume must be compatible with the capillary column and its operating conditions. Gas samples of 10-2000µl are possible in this way provided a split is used and the column is preferably operated under programmed conditions. It has been a primary tool for analysis of volatile organic compounds in environmental and flavour and fragrance analysis for decades.

4.2 Dynamic headspace sampling[10]: Dynamic headspace sampling involves the passing of carrier gas through a liquid sample, followed by

trapping of the volatile analytes on a sorbent and the desorption onto a GC. This is a well-known, validated technique, which is also routinely performed by non-specialists, and it is the method of choice for analysis of extremely low (ppb and ppt) concentrations of volatile organic compounds in aqueous marices.

4.3 Purge and trap sampling: A measured amount of sample is placed in a sealed vessel. The sample is purged with inert gas, causing volatile compounds to be swept out of the sample. The volatile compounds are retained in an adsorbent trap, which allows the purge gas to pass

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through to vent. The volatiles are desorbed by heating the trap: they are injected into the GC by backflushing the trap using the GC carrier gas. Then, separation and detection is performed by normal GC operation.

5.1 Determination of Organic Volatiles in Water by Headspace GCMS[11]: The static headspace technique was applied to analyse organic volatiles in wastewater. Traditionally the dynamic headspace technique (purge and trap) is used for this application. The static headspace is a good alternative as a sample introduction technique for the analysis of volatiles in water. Especially for the more 'heavy' compounds (from toluene onwards) detection limits of 10 - 100 ppt can easily be achieved. The minimal detection limit is 10 ppt. For aromatic compounds like toluene up to naphthalene further optimisation efforts (e.g. sample amount, splitless injection, injection with high pressure pulsation) will have a good chance to be successful.

5.2 For the analysis of aroma compounds to authenticity testing of honey[12]: The volatile profiles of 43 authentic honey samples of different botanical and geographical origins were
obtained by means of gas chromatography–mass spectrometry. A qualitative analysis of the volatile compounds identified was performed in order to assess the marker compounds (if/when existing) for both botanical and geographical origin. The results seem to indicate the existence of certain marker compounds for the floral origins assessed (e.g. acacia, chestnut, eucalyptus, heather, lavender, lime, rape, rosemary and sunflower).

5.3 For the determination of volatile compounds in cow's milk[13]: The composition of the volatile fraction of milk from cows was investigated in a survey of milk samples using a headspace sampling technique and gas chromatography coupled to mass spectrometry analysis (GC-MS). Forty-one compounds in milk were isolated and identified from GC-MS headspace analysis. Quantitatively, the most representative chemical class was ketones (eight compounds, 170 μg/kg), followed by aldehydes (nine compounds, 63 μg/kg), alcohols (eight compounds, 36 μg/kg), and lower amounts of hydrocarbons (six compounds), sulphur compounds (three compounds), esters (four compounds) and terpenes (three compounds).

5.4 Characterization of Espresso Coffee Aroma[14]: The aromas of three espresso coffee (EC) samples from different botanical varieties and types of roast (Arabica coffee, Robusta natural blend, and Robusta Torrefacto blend (special roast by adding sugar)) were studied by static headspace GC−MS. Seventy-seven compounds were identified in all of the EC samples. Among them, 13 key odorants have been quantified and correlated with their flavor notes by applying multivariate statistical methods.

5.5 For the analysis of polycyclic aromatic hydrocarbons in groundwater samples[15]: Groundwater samples from a former gas plant site were investigated by headspace GC/MS and general parameters with regard to organic pollution. The contamination plume was distinguished from background with GC/MS headspace and dissolved organic carbon analyses. Headspace GC/MS analyses of these samples revealed the presence of several aromatic and heterocyclic compounds typical to coal tar leachates. Selected ion monitoring GC/MS was used to establish the relative contamination level of seven selected polycyclic aromatic hydrocarbons (PAH) from 9 sampling wells. Three wells showed a high contamination level and therefore, they could be attributed to the vicinity of contamination sources. Well samples downgradient from the pollution source showed decreasing contamination levels for all compounds except acenaphthene.

5.6 Analysis of Biofluids for Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL)[16]: This analytical procedure allows for the rapid detection and differentiation of GHB and GBL in biofluids. The use of headspace autosamplers to introduce the analytes to the GCs has the advantage of eliminating the need for time-consuming injector and column maintenance that occurs from direct injections. Tests were performed to determine the extent of the conversion of GHB to GBL by the use of concentrated sulfuric acid alone. GHB-spiked aqueous samples were subjected to the concentrated sulfuric acid treatment at varying lengths of time (1-30 rain).The GC-MS analyses used an HP 7694 headspace autosampier and 6890 GC. The MS was an HP 5973 operated in full-scan EI mode (m/z 35-200).

5.7 Simultaneous determination of formic acid and formaldehyde in Pharmaceutical excipients using headspace GC/MS[17]: Formic acid and its esters, as well as formaldehyde, are trace impurities that are often present in pharmaceutical excipients. These trace impurities can potentially react with amino and/or hydroxyl groups in drugs to form significant levels of degradants. Samples were dissolved or dispersed in acidified ethanol to convert formic acid and formaldehyde to ethyl formate and Diethoxymethane, respectively. Identification was conducted using a GC/MS system. The limits of quantitation of the method were 0.5 ppm for formic acid and 0.2 ppm for formaldehyde. The precision of the method was demonstrated by the acceptable R.S.D. (≤10%) over a linear range of 0.5–10,000 ppm. The accuracy of the method was within 80– 120% over the linearity range.

6.CONCLUSION:

Headspace GC-MS assay provides a practical, sensitive and reliable method for the accurate measurement of volatile compounds which was usually very difficult by using other methodologies. It provides an attractive approach for trace anesthetics analysis and alcohol analysis in clinical and forensic laboratories.

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