WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 2, 444-455. Review Article ISSN 2277–7105

REVIEW ON GAS CHROMATOGRAPHY & ITS HYPHENATION TECHNIQUES

Dr. Nutan Rao*[1] and Kajal D. Gawde[2]

[1]Assistant Professor, Department of Pharmaceutical Chemistry and Quality Assurance and Oriental College of Pharmacy, Sanpada, Navi Mumbai-400705.

[2]Kajal D. Gawde, Oriental College of Pharmacy, Sanpada, Navi Mumbai-400705.

Article Received on
01 Dec. 2017,

Revised on 21 Dec. 2017,
Accepted on 12 Jan. 2018,

DOI: 10.20959/wjpr20182-10757

***Corresponding Author**
Dr. Nutan Rao
Assistant Professor, Department of Pharmaceutical Chemistry and Quality Assurance and Oriental College of Pharmacy, Sanpada, Navi Mumbai-400705.

ABSTRACT

Gas chromatography is a powerful separation technique for gas and vapour mixtures. Combining, separation and on-line detection permits accurate quantitative analysis of complex mixtures, including traces of compounds as low as parts per trillion in some particular cases. The importance of gas chromatography in quality control and process control in the chemical and drug industry, in environmental pollution investigations and in clinical analysis is critical. The review describes gas chromatography and its hyphenation techniques.

KEYWORDS: Gas Chromatography, Hyphenated Techniques, Detectors, column, carrier gas.

INTRODUCTION

A gas chromatograph (GC) is an analytical instrument that is utilized to gauge the substance of various segments in a sample.[1,2] The investigation performed by a gas chromatograph is gas chromatography. Gas chromatography (GC) is a common kind of chromatography used as a piece of analytical science for segregating and investigating exacerbates that can be vaporized without disintegration. Regular employments of GC are trying the immaculateness of a particular substance, or separating of the distinctive segments of a blend.[3-6] In a couple of circumstances, GC may help in identification of a compound. In preparative chromatography, GC can be used to obtain a pure compound from a blend.

Gas chromatography principle: The principle of separation in GC is "partition". The mixture of components to be separated is converted to vapour & mixed with gaseous mobile

phase. The component which is more soluble in stationary phase travel slower & 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. General schematic diagram of GC is given in Figure 1.

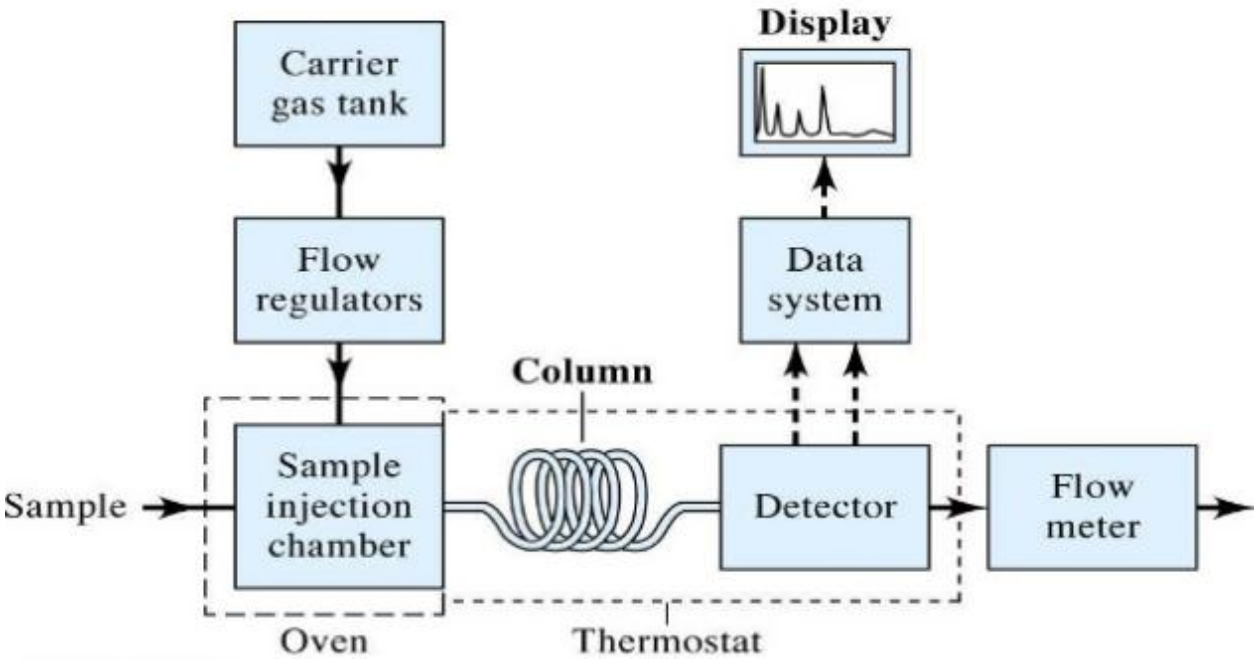

Figure. 1: Schematic diagram of Gas Chromatography.

Physical Components of Gas Chromatography

- Autosamplers
- Inlets
- Detectors

Autosamplers: The autosampler gives the way to bring a sample automatically into the channels. Manual insertion of the sample is possible but no more common. Programmed insertion gives good reproducibility and time-improvement.

Inlets: The column inlet (or injector) gives the way to bring a sample into a continuous stream of carrier gas. The inlet is a piece of equipment appended to the column head The common inlet sorts are: S/SL (split/splitless) injector, on- column inlet, PTV injector, and Gas source inlet or gas switching valve, P/T (Purge-and-Trap) system.[7] The decision of carrier gas (portable stage) is very important. The carrier gas must be chemically inert.

Generally utilized gasses include nitrogen, helium, argon, and carbon dioxide. The decision of carrier gas is regularly depend upon the sort of indicator which is utilized. The carrier gas framework likewise contains an molecular sieve to expel water and different other impurities. So, helium might be more efficient and give the best separation if flow rates are optimized. Helium is non-combustible and works with a more prominent number of detectors. Thus, helium is the most well-known carrier gas utilized. In any case, the cost of helium has gone up significantly over recent years, causing an expanding number of chromatographers to change to hydrogen gas.

Detectors: There are numerous detectors which can be utilized as a part of gas chromatography. Distinctive detectors will give different sorts of selectivity. A non- selective detector reacts to all mixes aside from the carrier gas, a particular indicator reacts to a range of compounds with a typical physical or chemical property and a particular detector reacts to one chemical compound. Detectors can likewise be gathered into concentration dependant detectors and mass flow dependant detectors. The signal from a concentration dependant detector is identified with the grouping of solute in the detector, and does not generally crush the sample dilution with make-up gas will bring down the detectors reaction. Mass flow dependant detectors ordinarily decimate the sample, and is identified with the rate at which solute particles enter the detector. The reaction of a mass flow dependant detector is unaffected by make-up gas. Various types of detectors used in GC are: Mass Spectrometer (GC/MS), Flame Ionization Detector, Thermal Conductivity Detector, Electron Capture Detector, Nitrogen-phosphorus detector, Flame photometric detector (FPD) and Photo-ionization Detector (PID).

Hyphenated Techniques

A Hyphenated technique is combination or coupling of two different analytical techniques with the help of proper interface. The term hyphenated techniques ranges from the combination of separation-separation, separation-identification & identification-identification techniques. The aim of this coupling is obviously to obtain an information-rich detection for both identification and quantification compared to that with a single analytical technique.

Advantages of hyphenated techniques

1. Fast and accurate analysis.
2. Higher degree of automation
3. Higher sample throughput.

4. Better reproducibility.

5. Reduction of contamination due to its closed system separation and quantification achieved at same time.

Following Gas chromatography hyphenated techniques are well known.

1. **GC-MS**
2. **GC-IR**
3. **GC-NMR**
4. **GC-AES**
5. **GC-FTIR**
6. **GC-MS/MS**
7. **GC*GC/MS**
8. **GC-ICP-MS**
9. **GC-TOF-MS**

1. GC-MS: GC is able to separate the volatile and semi volatile compounds but it is unable to identify them whereas MS can identify the compound by giving its structural information at molecular level but it is unable to separate them. Therefore, the combination of these two techniques took place shortly after the development of GC.[9] GC-MS was the first technique to be hyphenated and this technique can confirm the organic volatile, semi-volatile compounds and residual solvents with great resolution. For the analysis of the compound by GC-MS the compound should possess the property such as volatility and thermal stability.[10] These two techniques are highly compatible with each other, the sample is in the vapour phase in both the techniques. But there is incompatibility between two techniques i.e. GC is operated at high pressure (760 torr) and in this the carrier gas is present whereas in case of mass spectroscopy it operates at a vaccum 10^{-6} to $l0^{-5}$ torr.[11]

Instrumentation and Working: When vaporized analyte is carried through the GC column with the help of heated carrier gas, the separation occurs in column only. Carrier can also be called as the mobile phase e.g. helium. Distinguishable interactions of analyte between mobile phase and stationary phase lead to separation of the compounds. The separation of the analyte also depends on the dimensions of column (length, diameter, film thickness), type of carrier gas, column temperature (gradient) and the properties of the stationary phase. The sample travels through the length of the column. The difference in the boiling point and other chemical properties lead to separation of the components of the mixture. The components

will be having differences in elution time and retention time due to their different adsorption or difference in the partition between mobile phase and the stationary phase respectively. Then the separated components of the mixture will enter into the MS through an interphase. This is followed by ionization, mass analysis and detection of mass-to charge (m/z) ratios of ions generated from each analyte by the mass spectrometer. An interface like effusion separator, jet/orifice separator & membrane separator can be used to connect GC with MS. The process of ionisation not only ionise the molecule but also break the molecule into the fragments. Two widely used Ionization techniques in GCMS are the electron impact ionization (EI) and the alternative chemical ionization (CI) in either positive or negative modes[16] The molecular ion of analyte form a finger print spectrum which is different from other analytes. GC-MS is important tool in analytical chemistry because these techniques accurately separate, identify and provide information about structure and composition using very less sample. The advantage of this technique is sometimes two different analytes will have the same mass spectrum but the retention time of both the analytes is different so such type of analytes can be separated or analysed with the help of GC-MS.

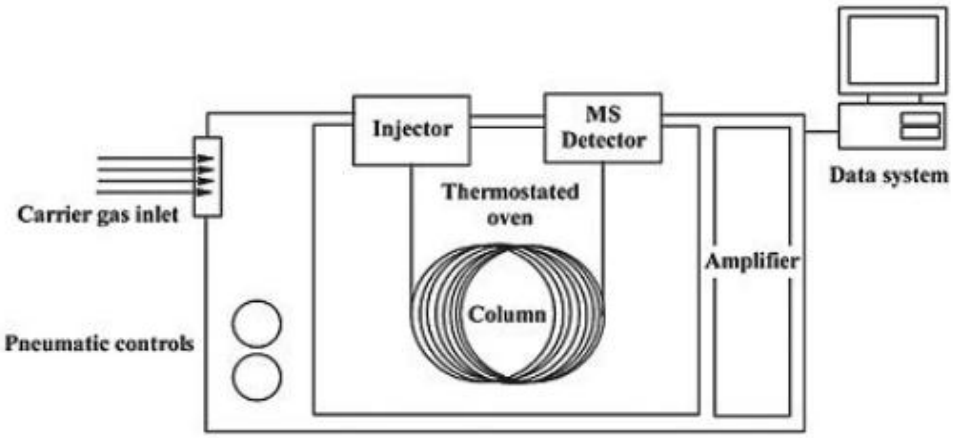

Figure. 2. Schematic diagram of GC-MS.

Applications

1. Quantification of pollutants in drinking and waste water using official U.S. Environmental Protection Agency (EPA) methods.

2. Quantification of drug in metabolites and urine is done for the pharmacological and forensic use.

3. Identification of unknown organic compounds in hazardous waste dumps and reaction products by synthetic organic chemistry.

4. Used for drug analysis, pesticide and herbicide detection

2. GC-IR[12]

GC-IR technique is hyphenation of gas chromatography and Infrared spectroscopy. This technique is very sensitive, very expensive; sample recovery is also possible because IR is a non-destructive technique. GC carries out the separation part whereas IR performs the function of identification. Gas chromatography separates components of the analyte. These components will travel through the column. These two techniques are linked through glass column or vacuum tubes. Interface used in this technique is internally gold coated small glass pipe connected to column by narrow tubing. Light pipe is heated in order to get rid of condensation and maximize path length for enhanced sensitivity. Effluent from GC is directly forwarded into the heated pipe of IR at atmospheric pressure. Infrared red spectroscopy identifies the compound by detecting the functional groups.

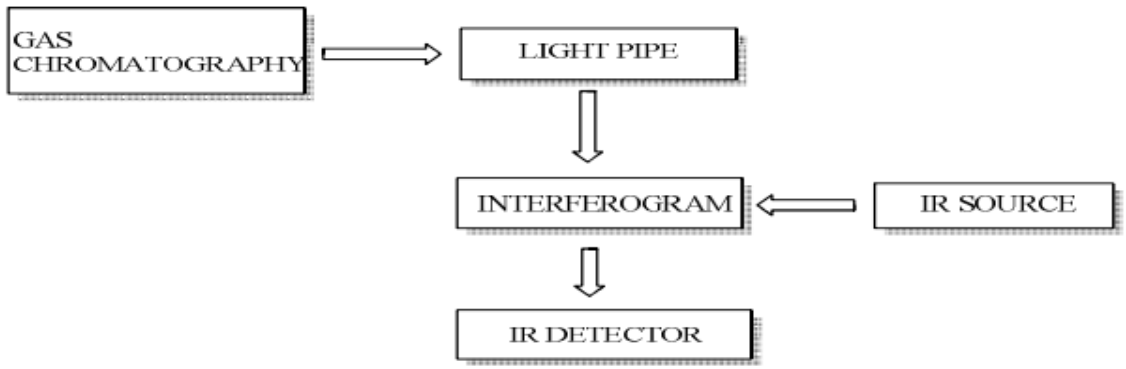

Figure. 3. Schematic diagram of GC-IR.

Application: In pharmaceutical industry & DNA analysis of blood samples and other fluids.

3. GC-NMR: In this technique the GC is combined with NMR. NMR performs the identification of the components and GC is used for the separation of the components. The hyphenation of this technique provides the structural information of the separated components.[13,14]

Instrumentation: The problem involved in combination of these two techniques is the physical state of sample. The samples used for analysis are liquid or in solid state in case of NMR, whereas in GC it is in the gaseous state. If the carrier gas is used for analysis in NMR, it will show the low Signal to-noise ratio of the signal obtained at atmospheric pressure. To overcome this sensitivity problem microcells and computers are used to improve the signal-to-noise ratio.[15] Some other modifications are also performed which include use of stronger magnets and advanced microprobes.[16] The analytes having boiling point above 65°C are

condensed in the capillary connection i.e transfer capillary and probe head. This problem can be solved by use of transfer capillary which will be heated by bifilar coil. This coil is constructed from zero susceptibility wire which is combined with strong magnetic field.

Applications

1. Constitutional and Configurational isomers can be separated. Enantiomers show the same spectra at different retention time.
2. Identification of stereoisomers in a complex mixture.

4. GC-AES: This technique is combination of gas chromatography with atomic emission spectroscopy. Atomic emission spectroscopy is one of the elemental analysis techniques. GC performs the separation of the components and with the help of AES, the elemental identification of the components is performed. Elemental composition of every peak separated by GC is determined.

Instrumentation: In this process, analytes are first atomized using either ICP or microwave irradiation (high temperatures), where the atoms are transferred to electronically excited state. Later, these electrons return to the lower energy levels emitting photons at certain wavelengths that are characteristic of the particular element. In both the techniques, sample is in gas phase so the techniques are complementary to each other. The GC effluent is directly introduced into the Quartz atomization furnace via heated nickel transfer line. The interface is simple but in practice the conditions has to be optimised eg. Quartz furnace, heating with flame or a thermostat, or using the graphite furnace as the atomization device for obtaining good sensitivity and selectivity.[17]

Applications

1. Identification and Quantification of the compounds.
2. GC-AES coupled with microwave-induced plasma (MIP) can be used to study organic polymers and to perform speciation analysis of organotin compounds in human urine. This provides a method suitable for rapid sensitive screening of human urine samples without dilution of the sample.

5. GC-FTIR[18]

For novel structures or new chemical entities, it is possible that no matched reference spectra can be found in MS databases. Manual interpretation of mass spectra requires sound

knowledge on organic mass spectrometry and dedicated experience and often not possible to suggest any candidate structures. With the help of the molecular spectroscopy, FT-IR, information on functional groups or structural moieties with specific infrared absorptions is complementary to MS and can be very valuable for structure elucidation, as already being used as stand-alone.

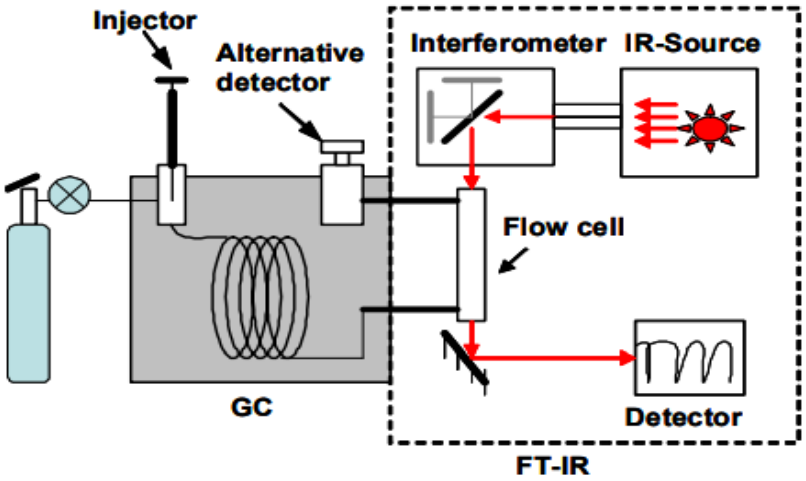

Figure. 4: Schematic diagram of GC-FTIR.

Instrumentation & working: On line GC-FTIR coupling, the effluent from the GC flows through a heated transfer-line into the light pipe. A schematic drawing of a typical GC-FTIR is shown in figure. The interferograms are scanned continuously to record either 'on-the-fly' gas-phase vapour IR spectra or trapped component spectra. This makes it possible to reconstruct a chromatogram in real time by a vector technique called the Gram-Schmidt method. After the acquisition is finished, the spectra of each GC peak can be normalized and searched by comparison with an IR spectra library. The combination of a gas chromatograph with both FT-IR and MS detectors on one instrument allows the simultaneous measurement of one peak by two supplementary detections. In fact, at each retention time, two different chromatograms were obtained. The sample passes the IR detector without destruction and is registered by the subsequent MS detector.

Application: Used for analysis of polychlorinated dibenzo-p-dioxins, dibenzofurans, aromatic polymers, petroleum.

6. GC-MS/MS

In this technique the gas chromatography is coupled with tandem mass spectrometry. This technique is sensitive as well as specific and can be used for ultra-trace analysis. For

qualitative identification with MS/MS, production scan, precursor ion scan and neutral loss with a triple quadrupole or product scan with an ion trap can be used. In recent years, the sensitivity of the quadruplole & the scanning speed has increased.[16]

Applications

1. Identification of trace unknown impurities.
2. Used for determination of contaminations in environment and foods such pesticides and Poly Chlorinated Biphenyls (PCB's) in foods and biological samples.

7. GC*GC-MS

In this technique, two dimensional GC is coupled with mass spectroscopy. This coupling will lead to the better resolution of the peaks in GC. Sometimes in one dimensional GC, the analyte is unevenly distributed along the whole retention time.

The two dimensional GC lead to the better resolution. With the help of two dimensional GC, components of the analytes are properly separated so that, even the trace amount of the components will be identified in the MS.[19]

Applications

1. Large number of samples can be analyzed at the same time.
2. Analysis of petroleum, PCBs, complex extracts and food samples is performed by this technique.

Advantages

1. Unprecedented selectivity.
2. High sensitivity.
3. Enhanced separation power and increased speed.

8. GC-ICP-MS[8]

Individual ICP-MS [Inductively coupled plasma-Mass spectrometry] does not provide information on the chemical structure of the analyte at molecular level since all forms of the analytes are converted to positively charged atomic ions in the plasma. However, as an excellent elemental analyser (ICP) with resolution on masses (MS), ICP-MS can also be used as gas chromatographic detector. Resulting from this hyphenation, target analytes are separated into their constituent chemical forms or oxidation states before elemental analysis. In GC-ICP-MS, where the sample is gaseous, the transfer line should be inactivated and

heated to eliminate sample degradation and condensation and will guide the sample directly into the ICP torch. In this way, the sample is maintained at constant high temperature from the end of the chromatographic column in the GC oven to the tip of the ICP injector. It is almost a universal detector (only H, He, Ag, F, Ne cannot be directly measured), fits perfectly with a wide range of GC carrier gases and flows, and is capable of quantification with isotope dilution. A picogram (pg) level of sensitivity can be achieved. GC-ICP-MS is very useful technique for speciation analysis such as sulphur speciation & organometallic speciation.

9. GC-TOF-MS[8]

Recent advancements in instrumental optics design, the use of fast recording electronics and improvements in signal processing have led to a booming of the TOF-MS for investigation of organic compounds in complex matrices. GC-TOF-MS with high resolution of about 7000 is capable of achieving a mass accuracy as good as 5 ppm for small molecules. This allows not only isobaric ions to be easily mass-resolved but also the measurements of accurate masses for elemental composition assignment or mass confirmation, which adds one more powerful means for identification in GC-MS besides mass spectrum database searching and tandem mass spectrometry. High resolution detection in GC-TOF-MS offers not only the high mass accuracy of molecular and fragment ions but also the accurate isotopic distribution with regards to isotope intensities and isotope-resolved information for element assignments. It is extremely helpful for unknown compounds for which no Library spectrum is available for database searching. With the help of software tools, a carbon number prediction filter can be applied to reduce the number of possible elemental compositions based on the relative abundance of the isotopic peak corresponding to ^{13}C (relative to the ^{12}C peak, each ^{13}C isotope contributes 1.1% to the ^{13}C peak). The nitrogen rule can also be used to determine whether the ion is an ‘‘even-electron ion’’ (for instance, protonated or deprotonated molecule) or an ‘‘odd-electron ion’’ (for instance, radical cation or anion). With these considerations, possible elemental compositions can be obtained when it is searched in available databases (e.g., Index Merck, Sigma Aldrich, Chem-Spider, Pubchem, Reaxys) and a chemical structure can be proposed. Both accurate masses and isotopes of fragment ions should be in agreement with the chemical structure assigned. However, in order to secure this identification, a reference standard will be required in a final step to check the GC retention time and to confirm the presence of fragment ions experimentally by GC-TOF-MS analysis.

Application: Target screening of organic pollutants in water, pesticide residues in food, anabolic steroids in human urine and xenoestrogens in human-breast tissues.

Disadvantage: Despite its excellent mass accuracy and sensitivity for qualitative studies, GC-TOF-MS is not as robust as other MS detectors such as triple quadrupoles for quantification due to its limited dynamic range.

CONCLUSION

Many individual instrumental analytical techniques are already well developed and able to provide specific information about analytes in samples, although each of them has its advantages and disadvantages. The complexity of real-world sample matrices often exceeds the analytical capabilities of any conventional chromatographic separations. The development and employment of more comprehensive coupling techniques to enable a deeper insight into the composition of natural and synthetic matrices has become a necessity.

REFERENCES

1. Mahendra Kumar T, et al. Evaluation of the isotopic abundance ratio in biofield energy treated resorcinol using gas chromatography-mass spectrometry technique. Pharm Anal Acta., 2016; 7: 481.
2. Arnoldi S, et al. Validation study of analysis of 1-phenyl-2-propanone in illicit methamphetamine samples by dynamic headspace gas chromatography mass spectrometry. J Chromatogr Sep Tech., 2016; 7: 322.
3. Nimmanwudipong T, et al. Determination of intramolecular 13c isotope distribution of pyruvate by Headspace solid phase microextraction-gas chromatography-pyrolysis-gas chromatography-combustion-isotope ratio mass spectrometry (HS-SPMEGC-Py-GC-C-IRMS) Method. J Anal Bioanal Tech., 2015; 7: 293.
4. hen Z, et al. Utilization of a matrix effect to enhance the sensitivity of residual solvents in static headspace gas chromatography. J Chromatogr Sep Tech., 2015; 6: 289.
5. okhart M, et al. Determination of organochlorine pesticides in wildlife liver and serum using gas chromatography tandem quadrupole mass spectrometry. J Chromatogr Sep Tech, 2015; 6: 286.
6. Albert K, et al. (2015) Investigating insect adhesion secretions by gas chromatography mass spectrometry. J Chromatograph Separat Techniq, 2015; S6: 001.
7. Goswami J. Different separation or experimental techniques for clinical chromatography: small review. J. Chromatogr Sep Tech., 2015; 6: 297.

8. Guo X, Lankmayr E, Hyphenated Techniques in Gas Chromatography, Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Austria, 14-19.
9. Ronald A. Hites, Gas Chromatography Mass Spectrometry, Handbook of Instrumental Techniques for Analytical Chemistry, 609-610.
10. Singh S, Handa T, Narayanam M, Sahu A, Junwal M, Shah RP. A critical review on the use of modern sophisticated hyphenated tools in the characterization of impurities and degradation products, Journal of Pharmaceutical and Biomedical Analysis, 2010.
11. Settle FA, Handbook of instrumentation technique for analytical chemistry. Delhi, pearson education, 2004.
12. Ghulinyan *et al*. Gas Chromatography Infrared Spectrometry (GC/IR). Appl Phys Lett 2006; 88: 241103.
13. Buddrus J, Herzog H. Coupling of Chromatography and NMR Study of Flowing Gas Chromatographic Fractions by Proton Magnetic Resonance. Organic Magnetic Resonance, February, 1981; 15(2): 211-213.
14. Herzog H, Buddrus J. Coupling of Chromatography and NMR. Part 5: Analysis of High-Boiling Gas-Chromatographic Fractions by On-line Nuclear Magnetic Resonance. Chromatographia, 18(1): 31-33.
15. Milazzo B, Petrakis L, Brown P. Microsampling Techniques for Combined Gas Chromatography and High-Resolution Nuclear Magnetic Resonance Spectroscopy. Applied Spectroscopy, Sep., 1968; 22(5): 574-575.
16. Grynbaum M, Kreidler D, Rehbein J, Purea A, Schuler P, Schaal W, Czesla H, Webb A, Schurig V. Albert K. Online Coupling of Gas Chromatography to Nuclear Magnetic Resonance Spectroscopy: Method for the Analysis of Volatile Stereoisomers. Analytical Chemistry, Apr 2007; 79(7): 2708-2713.
17. Dirkx W, Lobinski R, Adams F. Speciation Analysis of Organotin by GC-AAS and GC-AES after Extraction and Derivatization, In: Quality Assurance for Environmental Analysis, Ph. Quevauviller, Maier EA and Griepink B, (Ed Elsevier), Amsterdam, The Netherlands 357-409.
18. Bruker Optics. (2009). GC-FT-IR in the ng Range. Application Note # AN-76E.
19. Frysinger G, Gaines R. Comprehensive Two-Dimensional Gas Chromatography with Mass Spectrometric Detection (GC×GC/MS) Applied to the Analysis of Petroleum. Journal of High Resolution Chromatography, May1999; 22(5): 251–255.