Application of gas chromatography in pharmaceutical analysis

Chromatography is a physical method of separation in which components to be separated are distributed between two phases, one of which is stationary phase while the other mobile phase move in a definite direction. The stationary phase may be a solid or a liquid supported on a solid or a gel. The mobile phase may be gaseous or liquid. The basis for gas chromatography separation is the distribution of a sample between two phases. one of these phases is a stationary bed of large surface area, and the other phase is a gas which percolates through the stationary bed. The physical state of the mobile phase distinguishes the fundamental type of a chromatographic separation. Liquid chromatography (LC), gas chromatography (GC) and super critical fluid chromatography (SFC) all named for the state of their respective mobile phases.

The first person to chromatography was Tswett (1872-1919) the Russian chemist. He used chromatography from the Greek for colour – chroma and write- graphein to describe his work on the separation of coloured plant pigments. Until 1930’s chromatography in the form of thin-layer and ion-exchange chromatography became a regularly used technique. In 1940 development of partition chromatography and paper chromatography followed by the first disclosure of effective gas chromatography (GC) by Martin and his co-worker James in 1953. GC is a technique for separating volatile substances by percolating a gas stream over a stationary phase. It is a technique that revolutionized analytical chemistry. GC has been applied successfully to numerous compounds in variety of fields. Headspace GC has been used since the 1980s, but only recently has it become part of mainstream of pharmaceutical analysis. In this essay GC technical aspect

and its application for pharmaceutical quantitative analysis has been explained. Moreover, the comparative advantage over other techniques and the disadvantage of using GC has been also discussed and reached on some conclusion.

2. Gas Chromatography

2. 1 Technical Aspect

In GC the components to be separated are carried through the column by an inert gas. Here the mobile phase is a gas, often nitrogen, but sometimes helium, hydrogen or occasionally another gas. It is called the “ carrier gas”. GC is equipped with standard oven for temperature programming, split/split less injection ports and flame ionization detector. The sample mixture is partitioned between the carrier gas and a non volatile solvent (stationary phase) supported on an inert size-graded solid. The solvent selectively retards the sample components, according to their distribution coefficient, until they form separate bands in the carrier gas. These component bands leave the column in the gas stream and are recorded as a function of time by a detector. This elution technique has the following advantages :

The column is continuously regenerated by the inert gas phase.

Usually the sample components are completely separated and mixed only with an inert gas making collection and quantitative determinations easy.

The analysis time is very short.

In general GC is a powerful and widely used technique for the separation, identification and quantitation of components in a mixture.

In this technique a sample is converted to the vapor state and a flowing stream of carrier gas sweeps the sample into a thermally -controlled column. In GC the column is usually packed with solid particles that coated with a non-volatile solvent. Retention time is defined from injection of the sample to time a specific sample component is detected. After exiting the column the separated components are detected and a detector response is recorded. Polarity and boiling points of the components are also vital properties in GC separation. While polarity is the major factor governing separation; the boiling points of components of the sample also play a significant role in determining the retention time. Components with higher volatility have lower boiling point.

2. 2 Advantages of GC

2. 2. 1. Speed

The entire analysis is completed less than half an hours. The use of gas as the moving phase has the advantage of rapid equilibrium between the moving and stationary phases and allows high carrier gas velocities to be employed. Separations requiring only seconds have been reported, however, analysis time of minutes duration is more common in GC. Preparative scale separations, or resolution of wide-boiling complex samples may require hours.

2. 2. 2. Resolution

The separation of some compounds such as methyl esters of stearic, oleic and linoleic acids by other techniques is extremely difficult or impossible. The boiling point differences are negligible in that the compounds vary only

in degree of unsaturation. By using selective solvents, however, GC can provide resolution impossible by distillation or other techniques.

2. 2. 3. Qualitative Analysis

The retention time in GC is that time from injection to peak maxima. This property is characteristic of the sample and the liquid phase at a given temperature. With proper flow and temperature control, it can be reproduced to within 1% and used to identify each peak. Several compounds has only one retention time. This retention time is not influenced by the presence of other components.

2. 2. 4. Quantitative Analysis

The area peak produced for each on chromatogram is proportional to concentration of the peak in GC analysis. This can be used to determine the exact concentration of each component. Accuracy attainable with GC depends upon, detector, integration method and sample concentration.

2. 2. 5. Sensitivity

A major reason for the extensive analytical application of GC is the sensitivity available. The simplest forms of thermal conductivity cells can determine down to 0. 1 %. The flame detector easily sees parts per million, and the specific electron capture and phosphorus detectors can measure parts per billion. An advantage of this extreme sensitivity is the small size sample or micro liters of sample are sufficient for complete analysis. This is indeed trace analysis is also easily achieved. It is simple to operate and

understand. Interpretation of the data obtained is also rapid and straight forward. The cost of GC is very low compared to the data obtained.

3. Application of GC in Pharmaceutical Analysis

The major success of the application of GC in pharmaceutical quantitative analysis is firstly due to the very high efficiencies of separation power, secondly to the extreme sensitivity of the detection of even very small amounts of separated species and finally to the precision and accuracy of the data from quantitative analyses of very complex mixtures. GC analyses are also easy to automate from sample introduction to separation. Because of the above main advantages and its short analysis time and reliable results GC is used as quality control purposes in the pharmaceutical industry. In fact pharmaceutical analysis generally involves two steps; separation of the compound of interest and quantitation of the compounds. The better the separation the easier the quantitation. GC detectors have different responses to each compound. In order to determine quantitative amounts of various compounds in a separation the detector must be calibrated using standards. Standard solutions of sample are injected and the detector response recorded. Comparison of the standard and sample retention times allows qualitative analysis of the sample. Comparison of the peak area of the standards with that of the sample allows quantitation of analyte. Due to this fact, GC is widely used as a routine analytical technique in pharmaceutical quantitative analysis mostly used in for the determination of organic volatile impurities and nicotine level during drugs formulation.

3. 1. Determination of Organic Volatile Impurities by GC

Organic Volatile impurities are residual solvents that are used in and are produced during the synthesis of drug substances, or in excipients used in the production of drug formulations. Many of these residual solvents generally cannot be completely removed by standard manufacturing processes or techniques and are left behind, preferably at low levels. Organic solvents such as acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene frequently used in pharmaceutical industry for the manufacturing of Active Pharmaceutical ingredients therefore , in manufacturing drug substances and from one or more steps of the synthetic process, volatile solvents can be retained in the end products. Most of the time ethanol and acetone are used in the preparation of the polymeric coating of tablets. On other hand isopropyl alcohol is used in the crystallization of the active ingredient while ethyl acetate is a process solvent for the gel forming polymer. Low levels of these organic solvents are inevitably present in the drug product even after drying process. These organic volatile residuals affect physiochemical properties of a drug, such as particle size, dissolution rate and stability, but also can present a serious potential health hazard. Very often these solvents, referred to as residual solvents, are carried to the pharmaceutical preparation concerned and making their determination very important. Therefore, GC is superior to other techniques for analysis of these residual solvents. It provide good retention and separation at low oven temperatures. Due to the above fact the content of residual organic solvents in pharmaceutical industry is routinely measured by GC technique.

3. 2. Determination of Nicotine by GC

Because of its rapid and accurate analytical result; GC is used to determine the nicotine level in pharmaceutical drugs formulation. GC applications in combination with other techniques are also vital in pharmaceutical industries for isolation and characterization of volatile compounds. Currently the use of GC in pharmaceutical quantitative analysis is very usual and include the analysis of samples of active pharmaceutical ingredients and their intermediates as well as in- process testing for residual solvents to optimize the drying process.

4. Discussion and Conclusion

4. 1. Discussion

The disadvantage of GC are that the components of the sample must be volatile at temperature at which they will not decompose. As there are more involatile materials than there are volatile, and volatility immediately places a serious limitation on the field of application. In addition to these GC is also strongly retained components travel very slowly, or in some cases do not move at all. However, this difficulty can be overcome by using temperature programming of the column to decrease elution time. Temperature programming is the increase of temperature during an analysis to provide a faster and more adaptable analysis.

4. 2. Conclusion

Even though, GC has a few limitation in field of application due to its high detector sensitivity and high resolving power it is generally used extensively in pharmaceutical industry both in research and quality control purposes.