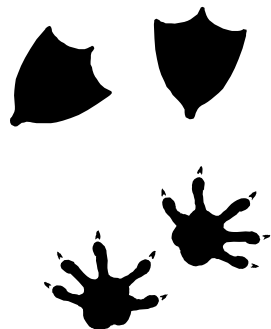




SHERLOCK'S ANALYTICAL PAWPRINT



**A Quick Reference Handbook of Analytical Methods
for the Pharmaceutical
and Medical Device Industries**





Welcome to Katz Analytical Services

*We are a **GLP** and **cGMP** compliant analytical laboratory
located in the Minneapolis / St. Paul metropolitan area,
dedicated to providing
a broad range of state of the art
analytical characterization, bioanalytical and consulting solutions
to the Medical Device, Pharmaceutical, Biotechnology, and related industries.*

*This guide is designed
to provide you with an overview of the analytical techniques
of greatest relevance to these industries.*



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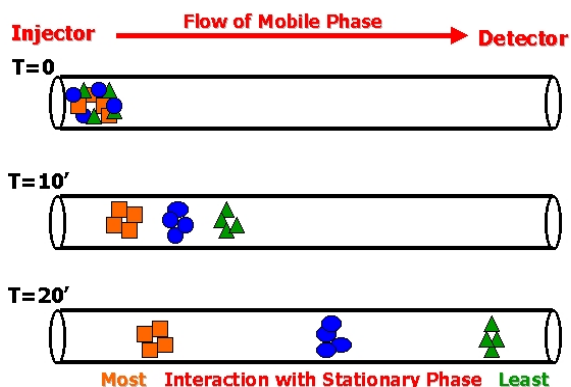
GAS CHROMATOGRAPHY (GC)

Principle:

Gas Chromatography is an instrumental method for the separation and identification of volatile compounds. In principle, a liquid sample is introduced into a heated injector, vaporized, carried through a separation column by an inert gas, and detected as a series of peaks along a time line on a recorder as components leave the column.

Chromatographic separation involves the use of a stationary phase and a mobile phase. The mobile phase consists of an inert gas such as helium, hydrogen or nitrogen. The stationary phase is a solid or a liquid coated on a solid (often a viscous material such as siloxane, or wax), and contained in a coiled column. The column is housed in a heated oven, preceded by the injection port. Components of a mixture carried in the mobile phase are attracted differently to the stationary phase and, therefore, move through the column at different rates.

Components that interact more strongly with the stationary phase spend proportionally *less* time in the mobile phase and therefore move through the column more *slowly*. Normally the column is chosen such that its polarity matches that of the sample. When this is the case, the interaction and elution times can be defined according to Raoult's law and the relationship between vapor pressure and enthalpy of vaporization. This translates to the rule of thumb that retention times generally correlate with boiling points. Other variables that affect retention are column temperature and carrier gas flow rate



There are several different types of detectors common to gas chromatography instruments. The choice of detector is determined by the general class of compounds being analyzed and the sensitivity required. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas, FIDs are sensitive primarily to hydrocarbons.

Identification and Quantitation:

Each component of the mixture reaches the detector at a different time, called the retention time, and produces a peak on a timeline. The area under the peak is related to the amount of the component present in the mixture. The identification of components is based on the analysis and comparison to a set of controlled standards.

Sensitivity:

ppb to ppm and higher.

Typical Applications:

- Nutraceutical purity analysis
- Volatiles analysis
- Trace component analysis
- Residual solvent analysis



GAS CHROMATOGRAPHY – MASS SPECTROMETRY (GC – MS)

Principle:

Gas Chromatography when coupled to Mass Spectrometry provides a highly versatile tool that can be used to separate, quantify and also identify the molecular composition of unknown volatile organic compounds and gases. By combining the sensitivity of mass spectrometry and the high resolving power of gas chromatography, complex mixtures can be analyzed.

A gas chromatography system is first used to separate the volatile compounds in a mixture. The separated compounds can then be quantified using a mass spectrometer detector. While many different detectors can be used for identification of the separated compounds, the mass spectrometer combines high sensitivity with the unique property of being able to determine the molecular composition.

In this technique, a sample first flows through the GC column as in a normal gas chromatography separation process. As the various sample components emerge from the column opening, they flow into a capillary column interface. This device is the connection between the GC column and the Mass Spectrometer. The interface acts as a separator and concentrates the sample via removal of the helium carrier.

The sample then enters the ionization chamber. Two methods exist for ion production. The most frequently used method is electron impact (EI). The alternative is chemical ionization (CI). For *electron impact* ionization a collimated beam of electrons impact the sample molecules causing the loss of an electron from the molecule. A molecule with one electron missing is represented by M^+ and is called the molecular ion (or parent ion). When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Alternatively, *chemical ionization* begins with the ionization of methane (or other gas), creating a radical which in turn will impact the sample molecule to produce MH^+ molecular ions. Some of the molecular ions fragment into smaller daughter ions and neutral fragments. Both positive and negative ions are formed but only positively charged species are detected.

Less fragmentation occurs with CI than with EI, hence CI yields less information about the detailed structure of a molecule, but does yield the molecular ion. Sometimes the molecular ion cannot be detected by EI detection, therefore the two methods complement one another. Once ionized, a small positive potential is used to repel the positive ions out of the ionization chamber.

Identification and Quantitation:

The next component of the mass spectrometer is a mass analyzer (filter), which separates the positively charged particles according to their mass. Several types of separating techniques exist: quadrupole filters, ion traps, magnetic deflection, time-of-flight, radio frequency, cyclotron resonance and isoelectric focusing, to name a few. The most common mass analyzers are quadrupoles and ion traps.

After the ions are separated according to their masses, they enter a detector and then on to an amplifier to boost the signal.

The detector sends information to a computer which records all the data produced, converts the electrical impulses into visual displays and a hard copy is displayed. Identification of a compound based on its mass spectrum relies on the fact that every compound has a unique fragmentation pattern. A library of known mass spectra (several thousand compounds in size) is stored on the computer and can be searched using computer algorithms to assist the analyst in identifying the unknown. It is important to incorporate all other available structural information (chemical, spectral, sample history) into the interpretation wherever appropriate.

Sensitivity:

ppt to ppm and higher.

Typical Applications:

- Reverse engineering
- Trace analysis
- Volatile component verification

HEADSPACE ANALYSIS

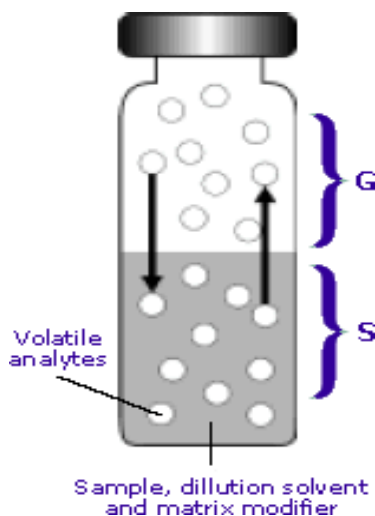
Principle:

Headspace Analysis is a technique where the vapors in the gas above and in equilibrium with a solid or liquid is sampled, as opposed to sampling the solid or the liquid directly. The advantage of this approach is that gas chromatography can be used as the analytical technique, as opposed to liquid chromatography, providing four to five orders of magnitude greater sensitivity.

A headspace sample is normally prepared in a vial containing the sample, the dilution solvent, a matrix modifier and the headspace. Volatile components from complex sample mixtures can be extracted from non-volatile sample components and isolated in the headspace or gas portion of a sample vial.

The procedure involves the extraction of a volume of the equilibrium gas over the sample (usually about 10 ml) by a syringe through a vial containing a bed of an appropriate absorbent. The vial is then placed in line with a gas chromatography column, the vial is heated. The vaporized sample is swept onto the column and the components are separated via conventional GC methods.

Illustration of a headspace sample:



Phases of the Headspace Vial

G = the gas phase (headspace)

The gas phase is commonly referred to as the headspace and lies above the condensed sample phase.

S = the sample phase

The sample phase contains the compound(s) of interest. It is usually in the form of a liquid or solid in combination with a dilution solvent or a matrix modifier.

Once the sample phase is introduced into the vial and the vial is sealed, volatile components diffuse into the gas phase until the headspace has reached a state of equilibrium as depicted by the arrows. The sample is then taken from the headspace.



Headspace analysis is commonly used with either gas chromatography (GC) or gas chromatography—mass spectrometry (GC-MS) for the analysis of volatile materials.

HEADSPACE GC-MS

Headspace GC-MS (HS/GC-MS) is used for the analysis of volatile organic compounds in solid and liquid samples, such as residual solvents and hydrocarbons.

A typical experiment consists of heating a sample containing a solvent in a headspace vial and analyzing the compounds in the headspace by GC-MS.

Sensitivity:

ppm range

Typical Applications:

- Unknown identification
- Residual solvent analysis
- Analysis of odors, aerosols, perfumes



INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY (ICP-MS)

Principle:

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is a highly sensitive analytical technique with a high linear dynamic range (from ultra-trace level detection to large main component detection). It is capable of analyzing all elements from Li to U. In ICP-MS, sampled material is transferred by an argon gas flow to an inductively coupled plasma, in which an effective temperature of 8000 – 10,000 K results in the atomization and ionization of the analyte material. From there, the analyte ions are extracted into a mass spectrometer, with which the elemental composition of the material is determined.

The high-energy argon plasma consists of both electrons and positively charged argon ions. In the plasma, the analyte material is split into individual atoms. These atoms will then lose electrons and become singly charged positive ions. Most elements ionize very efficiently (> 90%) in the hot plasma.

Identification and Quantitation:

The resulting positive ion beam is cooled by passing it through a series of apertures, and then enters the quadrupole mass analyzer. In the quadrupole, the ions are separated on the basis of their mass-to-charge ratio. Each element has its own characteristic isotopes and masses and will therefore produce its own mass spectrum. After passing through the quadrupole, the ions hit a special detector that contains two stages, allowing for simultaneous measurements of both high and very low signals. This allows simultaneous detection of main components and ultra-trace elements in a single run, which makes the ICP-MS an ideal tool for the analysis of completely unknown samples.



Sensitivity:

ppt for elements ranging from Lithium to Uranium.

Typical Applications:

- Bioaccumulation of metals in tissue and biological fluids
- Low level metals analysis
- Extractables and leachables assessment



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Principle:

In HPLC an analyte is passed through a column of a stationary phase (usually a tube packed with small round particles with a certain surface chemistry) by pumping a liquid (mobile phase) at high pressure through the column. The sample to be analyzed is introduced in a small volume to the stream of mobile phase and its progress through the column is impacted (slowed down) by specific chemical or physical interactions with the stationary phase as it travels the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time and provides a unique identifying characteristic for a given analyte.

The use of pressure increases the analyte's speed, giving the components less time to diffuse within the column, leading to improved resolution (the definition of the peak in the resulting chromatogram). Common solvents used include any miscible combinations of water or various organic liquids (the most commonly used being methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the analyte components.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry, with the ability to separate, identify and quantify the compounds that are present in any sample that can be dissolved in a liquid. Trace concentrations of compounds, as low as “parts per trillion” (ppt), are easily obtained. HPLC can be applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, and environmental samples.

Identification and Quantitation:

Quantification of compounds by HPLC is the process of determining the unknown concentration of a compound in a known solution. It involves injecting a series of known concentrations of a standard compound solution onto the HPLC for detection. The chromatograph of these known concentrations will result in a series of peaks that correlate to the concentration of the compound injected. These known concentrations can be plotted as a graph of area vs. concentration.



To then quantify the amount of the analyte, the height and area of the peak are proportional to the concentration of the corresponding component. A calibration curve is created using standards of known concentrations. The concentration of the analyte of interest can then be determined from the peak area of the detected analyte as it relates to the calibration curve of known standards.

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)

Liquid chromatography-mass spectrometry (LC-MS, or alternatively HPLC-MS) combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications and has very high sensitivity and selectivity. Generally its application is oriented towards the specific detection and potential identification of chemicals / compounds in the presence of other chemicals (in a complex mixture).

Sensitivity:

ppt and higher

Typical Applications:

- Extractables and leachables from packaging materials
- Process and product purity analysis
- Identification and characterization of unknowns