Instrumental Techniques

Mass Spectrometry (MS)

Mass spectrometry is today regarded as an established routine detection technique. MS detectors can be coupled to various separation techniques such as liquid chromatography (LC), thin layer chromatography (TLC), or gas chromatography (GC) where the hyphenation with LC is by far the most frequent setup. In contrast to more simple detectors, i.e. UV, RI, FL etc, MS generates data about molecular masses and detailed structural parameters and thereby offers the possibility to discriminate between co-eluting peaks in selected ion monitoring mode. The latter reduces the requirement for chromatographic retention and resolution before detection, yet it is always better to have retained and completely resolved peaks to prevent ion suppression or ion enhancement effects.

Mass analyzers can be quadrupole, magnetic sector, time-of-flight, ion trap or ion cyclotron resonance type. A quadrupole mass analyser consists of four parallel rods that have fixed DC and alternating RF potentials applied to them. The HPLC system handles dissolved analytes under ambient pressure (760 Torr) and delivers the sample to the MS, where the detection of the gaseous, ionized samples is performed under high vacuum conditions (10-5-10-6 Torr). The transfer of the analyte solution from the LC to the MS is accomplished via an interface. The interface stepwise converts the sample to an aerosol, ionizes it and removes the solvent. Ions are then focussed and passed along the middle of the quadrupoles. Their movement will depend on the electric fields so that only ions of a particular mass to charge ratio (m/z) will have a stable path to the detector. The RF is varied to bring ions of different m/z into focus on the detector and thus build up a mass spectrum.

Depending on the physical properties and the molecular mass of the molecules different types of interfaces are used, where they vary among each other in how they ionize the molecules and the pressure applied during this process. At present all the common ionization techniques operate under ambient pressure; i.e. electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), matrix assisted laser desorption/ionization (MALDI) and the less prominent atmospheric pressure photo Ionization (APPI). ESI and APCI are by far the most widely used in LC-MS hyphenation. The more esoteric techniques, electron ionization (EI) and chemical ionization (CI) work under high vacuum conditions with the advantage of being suitable for GC-MS hyphenation. Quadrupole mass spectrometers commonly have two configurations when used with liquid-chromatography, either as a simple single quadrupole system or placed in tandem. The latter principle, the triple quadrupole mass spectrometer, enables ion fragmentation studies (tandem mass spectrometry or MS/MS) to be performed.

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Electrospray Ionization (ESI)
In ESI mode liquid solutions of charged or polar substances, delivered with a HPLC system, are sprayed utilizing a metal capillary ("spray needle") and a nebulizer gas (nitrogen) in the MS. Resulting droplets are dried (desolvatization) and volatilized isolated analyte ions are transferred to the detector. Thermal stress is very low hence the analyte molecules do not decompose. ESI is almost unlimited regarding molecule size and suitable for medium to strong polar molecules, e.g., amines, carboxylic acids, heteroaromatics, and sulfonic acids. ESI is applied when fragmentations are unwanted and molecular masses of biomolecules have to be determined. ESI-MS is well suited for hyphenation with LC, and as long as flow rates do not exceed maximum 1-2 mL/min attainable sensitivity is very high, however more common is flow rates between 1-500 µL/min.

In liquid solution molecules are either already ionized or becomes protonated or deprotonated by additives in the sample solution and the mobile phase. To achieve best sensitivity, mobile phases used should be set at a pH where analytes are ionized, and a rule of thumb is to use neutral to basic pH (7-9) for acids whereas more acidic pH (3-4) is advisable for basic compounds. If the analytes of interest have multiple pKa values and may change their ionisation state, other pH values may be more beneficial both in terms of ionisation of the analyte and behaviour in the column. Thus depending on the choice of solvent and additives either positive and/or negative ESI mode can be used. Typically, positive mode is applied in combination with more basic molecules, while acid compounds are analyzed in negative mode. 0.1% formic acid is commonly added to the mobile phase in positive ESI mode to provide a low pH (□3) to protonate the analytes(s). Acidic analytes will be neutralised under such conditions wherefore negative ESI mode is preferred and higher mobile phase pH is recommended. Volatile buffers like ammonium acetate or ammonium formate are used in the pH range 4.5-7 to deprotonate the analyte(s), and for high pH it possible to use either ammonium carbonate or ammonium hydroxide (aqueous ammonia).

For both negative and positive ESI it is a prerequisite that all solvents and additives are volatile in order to avoid contamination of the mass spectrometer and that the total mobile phase ionic strength is adequate (generally 2-25 mM) to prevent unnecessary down-time for cleaning of detector. Strong acids like hydrochloric acid or nitric acid are unsuitable for two reasons: They form ion pairs with analyte molecules (analyte signal suppression) and display strong oxidizing properties. Trifluoroacetic acid (TFA) is a special case: It is widely used as an ion pairing reagent to improve the liquid chromatographic separation of peptides or proteins. On the other hand, TFA can cause strong ion suppression in mass spectrometry (mainly in negative ESI mode) and also contaminates the LC-MS system.

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Unfortunately both a quantitative estimation of these effects as well as general recommendations is not possible, as their strength strongly depend on the MS system used. Triethylamine as an alternative additive behaves in a similar manner. If the use of TFA is unavoidable, a weak acid (such as propanoic acid) or isopropanol can be added to the eluent to decrease a signal suppression effect.

Buffers do not only adjust the pH of the eluent and lead to ionization of a target molecule; they can also form adducts with the analyte. Adducts [M+buffer], e.g. with ammonium, alkali, halogens, formate or acetate, will lead to the detection of an additional peak in the MS spectrum; even a complete suppression of the analyte signal is possible when the vapour pressure of the resulting adduct (mainly alkali) is decreased significantly. As a reason of this and in order to keep the ESI source clean, volatile buffers are recommended. Non-volatile salts like phosphates, borates, sulphates or citrates will precipitate in the MS source, block it and cause tedious cleaning procedures!

Atmospheric pressure chemical ionization (APCI)
This technique is complementary to ESI and also useful for LC-MS hyphenation. It does not require a mobile phase with conducting properties wherefore acetone or acetic acid esters can be used as solvents and thus allows for a coupling of APCI with normal phase chromatography. In APCI mode, the analyte solution is vaporized prior to the ionization. Subsequently solvent molecules (aqueous-organic, e.g. methanol, propanol, acetonitrile, acetone etc., combined with 2 – 20 mM of a volatile organic buffer such as formic or acetic acid, ammonium acetate, ammonium formate or triethylamine) become ionized with a corona needle where their charge is then transferred to the analyte molecules via proton transfer or abstraction. APCI is suitable for the analysis of less polar, weakly ionizable substances with small or medium molecular weight (analytes without acidic or basic functional groups, e.g. hydrocarbons, alcohols, aldehydes, ketones, esters) and therefore complementary to ESI, as long as the sample is thermally stable and vaporizable. Fragmentations are generally observed with APCI. Highest sensitivity is achieved with acetonitrile, methanol or water as solvents, and where the degree of analyte ionization can be optimized via eluent pH. As for ESI flow rates up to maximum 1-2 mL/min can be tolerated.