

Electrospray Ion Trap Mass Spectrometry

Introduction

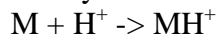
Electrospray Ion Source

The key to using MS for solutions is the ability to transfer your analytes into the vacuum of the mass spectrometer as ionic species. This process is handled by an electrospray ion source in our instrument.

Positive ion electrospray: Electrospray ion sources are soft ionization sources, that is they produce mostly protonated molecular ions, MH^+ . For small molecules, electrospray produces only one peak, the MH^+ peak at mass $M+1$.

You may be familiar with electron impact ionization, which is the normal ionization mode for GC/MS. Electron ionization, EI, is caused by a beam of 70 eV electrons in the source. EI provides enough energy to fragment the molecular ion, so that many fragment ions occur in the spectrum. EI spectra are often quite complicated. Electrospray spectra are much easier to interpret than EI spectra and the molecular weight of your compound is easily determined.

The ions in an electrospray source can be formed in your original solution. For example, quaternary amines, R_4N^+ , are by their nature already are ions. Ions can be formed from basic compounds by protonation by added acids. The proton transfer can occur in your solution or in the droplets produced by the electrospray source.



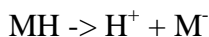
If your compound has several basic sites, like a small peptide with several amine side chains, multiply charged ions may also be formed:



Under these circumstances, several ions will appear in the spectrum, the MH^+ , MH_2^{2+} , and MH_3^{3+} . The MS determines the m/z value, that is the mass divided by the charge. If we take an example of a compound with mass 300 Da, the spectrum will contain ions at $MH^+ = 300+1=301$ Da, $MH_2^{2+} = (300+2)/2=151$ Da, and $MH_3^{3+} = (300+3)/3=101$ Da. Proteins can often produce very high charge states with $z \sim 40$ or more. Small molecules usually show only one predominant charge state. From this discussion, it is easily seen that pH control for the sample solution can have a strong effect on the ionization efficiency and the distribution of the charge states for your analyte ions. As a consequence, sample solutions for electrospray MS usually are buffered or have added acids to enhance and control the formation of ions.

Amines are obvious examples of types of compounds that are easily protonated. However, even alcohols can be protonated in the strongly acidic environment that occurs as the droplets of solution evaporate in the ion source. The spray tip of the ion source must carry the electrospray current, so it acts as the anode of an electrochemical cell. Therefore, if your molecule can't be easily protonated, ions may still be formed by electrochemical processes in the spray tip. Through direct protonation or electrochemical oxidation, most types of compounds can be analyzed. Nonreactive hydrocarbons are not detectable by electrospray ionization.

Negative ion electrospray: Acidic analytes can also be detected using negative ion electrospray.



The detected ion is the M^- ion, at mass $\text{M}-1$. Just like positive ion electrospray, negative ion electrospray is a soft ionization technique that can produce multiply charged ions. For example, DNA readily forms polyanions with a range of z values. Negative ion electrospray is commonly, but not always, run from basic or neutral solutions to increase the formation of negative ions. So just like positive ion electrospray, buffered sample solutions are common.

Buffer Additives: Because of the need to control ion formation, buffers are very common in direct infusion and LC/MS. However, standard buffers like Tris, HEPES, and phosphate buffers are non-volatile and can clog the MS inlet capillary. Therefore, non-volatile additives are necessary. The most common buffer components are formic acid, trifluoroacetic acid, acetic acid, ammonium formate, ammonium acetate, and heptafluorobutyric acid (in that order). For more basic buffers, 10-20 mM tetraethylammonium formate or bicarbonate is common. Often compromises in buffering capacity are made by choosing one of these volatile buffer components. Trifluoroacetic acid can suppress ion formation in electrospray, so its concentration is usually kept <0.1%.

~~MS~~ MS and MS/MS

Electrospray is a soft ionization technique. Electrospray spectra are very simple and molecular weights are easy to determine from the MH^+ parent peaks. Electron ionization, EI, produces fragment rich spectra. The fragment ions are useful to help determine the structure of the compound. On the other hand, in EI some classes of compounds don't produce intense parent peaks, so the molecular weight is difficult to determine. While the ease of molecular weight determination is a strength for electrospray, the lack of structural information from fragment ions can be a draw back. MS/MS techniques can solve this problem.

In MS/MS analysis, the MH^+ ions formed from the electrospray source are fragmented by adding extra collisional energy. Our MS is based on an ion trap analyzer. In ion traps, ions can be held for long time periods, giving an easy opportunity to fragment the parent MH^+ ions. The trap is always filled with about 1 Torr of helium gas. A small radio-frequency field can be applied to the trap to cause the ions to move faster. The parent ions collide with the helium background gas causing fragmentation. After adding this collisional energy, the resultant ions are scanned in the normal way to determine their m/z . This process is called collision-induced dissociation, CID. CID MS/MS spectra are very similar to EI spectra and can be interpreted in the same way.

MS/MS spectra can be acquired manually by selecting the mass of the parent ion to be fragmented and the amount of collisional energy. MS/MS can also be done automatically. In Auto MS/MS mode, the computer determines the mass of the most intense parent ions and subjects those ions to MS/MS. The user can choose the maximum number of parent ions to be fragmented.

The important parameter for MS/MS based analysis in LC/MS, is that MS/MS takes extra time. If the eluting peaks are too narrow, then there won't be time for MS/MS analysis. As a consequence, some compromise in resolution and retention time may be necessary to do auto MS/MS analysis. Strangely, this means that the best efficiency isn't

always best for MS/MS detection, which is a strange circumstance for most chromatographers.

MS/MS analysis is particularly useful for biopolymers. Proteins, peptides, and oligonucleotides can be sequenced using MS/MS. The auto MS/MS analysis of proteolytic digests of proteins is one of the two MS techniques that have spawned the new field of proteomics. (The other MS technique is MALDI.)

Sample Introduction

Electrospray mass spectrometers can be used in two modes. In direct infusion mode, a dilute solution of the analyte is pumped into the source. No separation takes place and all the components of the sample give peaks in the mass spectrum simultaneously. For simple mixtures this is fine, but for complex samples direct infusion produces a forest of peaks that are difficult to distinguish from the noise. For complex samples, an HPLC is commonly attached to the source. The source then ionizes the analytes separately as they elute from an HPLC column. The sample for LC/MS is usually more concentrated than for direct infusion to compensate for the dilution factor of the HPLC separation. Typical volumes for direct infusion are 20-200 μ L, while HPLC requires 50 nL-5 μ L of more concentrated sample.

?? LC/MS

The most common detector for HPLC is a UV detector. However, a mass spectrometer provides a means of identifying the components in different peaks. MS is a very powerful tool, but your HPLC method and sample preparation must be carefully designed to achieve good detection limits. With careful experimental design, electrospray MS can be as sensitive or even more sensitive than a UV detector.

pH control for the HPLC eluant can have a strong effect on the ionization efficiency and the distribution of the charge states for your analyte ions. As a consequence, HPLC eluants for electrospray MS usually are buffered or have added acids to enhance and control the formation of ions. Volatile additives must be used to avoid clogging the transfer capillary. Most common HPLC packings shouldn't be used at pH<2, so the concentration of added acids, like trifluoroacetic acid (TFA), is limited to about 0.1%.

In negative ion electrospray, neutral or basic mobile phases are often used. However, the most common HPLC packings shouldn't be used at pH>7, so care must be exercised when designing your analysis for negative ion mode.

Flow rates: The electrospray interface can use two different spray needles. The standard interface uses a stainless steel needle. This needle has a relatively large bore, and therefore a relatively large hold-up volume. This large volume causes extra band broadening when the interface is used with capillary HPLC. The flow rate range for the standard source is roughly 4-1000 μ L/min. 10-100 μ L/min is a good starting range.

In HPLC, the volume of the tubing, connections, and detector cells is called the extra-column volume. In other words, the extra-column volume is any volume that does not include the column. Extra-column volume is a source of band broadening. To decrease the extra-column volume, an alternate spray needle is available. This needle is made from

50 μ m ID fused silica tubing. The tip of the needle is specially ground into a conical shape to help increase the efficiency of the source for low flow rates. These “taper-tips” are made by the New Objective Company in Cambridge, MA. One disadvantage of these spray needles is that the tip is very fragile. The small ID also increases the backpressure of the interface. For this reason, the usable flow rate maximum is less than the stainless steel needle. The back pressure from the source should be kept low to avoid rupturing the diode array detector cell. If the source is fitted with the taper-tip fused silica tip, the maximum flow rate is decreased to 50 μ L/min. Using greater than 50 μ L/min will cause a costly repair and long down time. The normal flow rate for 1 mm columns is 50 μ L/min.

You need to be aware of the tip that is installed in the source, to determine a good operating flow rate. If you are unsure, assume that the taper-tip is being used. Also when cleaning the source you should be careful to avoid touching the end of the spray needle.

?? **Direct Infusion**

Solutions may be directly input into the electrospray source using the syringe pump or a peristaltic pump. This mode, which does not require the HPLC, is called direct infusion. The important parameter here is the flow rate.

Flow rates: The electrospray interface can use two different needles. Please see the discussion on flow for LC/MS, above. The flow rate range for the standard source is roughly 4-1000 μ L/min. 10-100 μ L/min is a good starting range. If the source is fitted with the taper-tip, the usable flow rate maximum is less than the stainless steel needle. The seal that is made to the syringe or the tubing of the pump limits the maximum pressure. If the source is fitted with the taper-tip fused silica tip, the maximum flow rate is decreased to around 25 μ L/min for infusion.

You need to be aware of the tip that is installed in the source, to determine a good operating flow rate. If you are unsure, assume that the taper-tip is being used.

?? **Electrospray Source Settings**

Nebulizer Settings: Flows of nitrogen gas are used to nebulize the analyte solution and dry the droplets. There are several gas flow rates that need to be set for proper operation of the source. Nitrogen gas is used because it is relatively cheap and very pure. The nebulizer pressure determines the nebulization efficiency. 15 psi is a good starting point. Use the table below to determine approximate values for the flow rate you are using. When the nebulizer is working properly, you will see a fine, even mist at the tip of the spray needle. The spray cone should be stable and not show fluctuations in size.

The dry gas has two purposes. This gas is heated to provide for efficient evaporation of the solvent. The dry gas also acts as a barrier that helps keep the transfer capillary clean. If the dry gas flow is not sufficient, droplets and contaminants can clog or contaminate the transfer capillary. The vacuum system must be turned off to clean the capillary, requiring at least two hours before the instrument can be used again. Therefore, we must always insure the dry gas flow is 5-9 L/s before we begin spraying anything from the ESI needle.

The dry gas flow is decreased to 2 after use to conserve on nitrogen gas.

Typically, higher flows and dry gas temperatures are needed as the water content of the analyte solution is increased. Higher flows and temperatures are needed at higher flow rates.

Ion Trap Source Tuning: The ion optics that transfer and focus the ions into the ion trap have a variable efficiency for ions of different masses. Therefore, the source settings must be optimized for different mass ranges. Luckily, the Agilent Ion Trap has a “Smart” mode that chooses the proper settings based on the expected weight of your analyte and its stability toward collision-induced dissociation. This smart mode is usually sufficient and is used exclusively in these instructions. In “Smart” mode, you simply input the approximate mass of your analyte. Then you choose a value for the stability. Values near 100% provide the best sensitivity, but only if your compounds are not susceptible to CID. Most compounds are relatively easy to fragment to some extent, so a good starting setting is around 30%. If you are running something similar to tryptic peptides, a mass of 1000 and a stability of 18% works well.

In-source CID usually decreases the sensitivity of the MS, because you are spreading the intensity of the molecular ion into several peaks. In other words, you are taking one tall peak and splitting it into several shorter peaks that are harder to see above the noise. On the other hand, in-source CID can be helpful. A small amount of in-source CID can decrease solvent adduct formation or break up dimers and other complexes that may form in solution. In very complex mixtures, in-source CID can increase the sensitivity, for example, if your compound is at high mass and is very stable towards CID while the matrix components are not. In any event, we usually tune the source for a small amount of CID.

The amount of in-source CID is determined by the potential difference between the end of the transfer capillary and skimmer 1. Settings that give the total voltage difference above 90 V are likely to produce some in-source CID for complex molecules like peptides. Voltage differences above 120 V may be necessary for aromatics and other stable molecules if you want to promote CID. You can read this voltage difference by switching the tuning mode to “Expert” and noting the “Cap Exit” value.

You also need to choose narrow or wide range. If you are analyzing a known compound and want the optimal sensitivity, choose the narrow range setting. For complex mixtures use the wide setting. The wide setting will prevent discrimination at the high and low mass end of the spectrum.