Electrospray Ionization – ESI

ESI is an atmospheric pressure, soft ionization technique that utilizes high voltages to generate quasi-molecular ions, $[M + H]^+$ or $[M – H]^-$, from non-volatile, polar liquid-phase analytes. The ions generated are then desolvated over a defined region and introduced in the mass spectrometer inlet as distinct gas phase ions. Electrospray ionization can be used on small molecules ranging in molecular weights from 50-2000 Da. Multiple charging of peptides and proteins can be achieved to enable analysis of very large molecular weight species at lower molecular weights, and data can be transformed to enable parent ion molecular weight determination.

Advantages

- Because it is a soft ionization technique samples with large masses can be analyzed

- Able to analyze large biological samples such as proteins, peptides, DNA, etc.
- Can be equipped to a quadruple, ion trap, and liquid chromatography
- No matrix interference or limitation
- Multiple charging allows for analysis of high mass ions with a relatively low m/z range instrument.
- ESI can also provide multiple ionization modes positive and negative.

Disadvantages

- Characterization using smaller fragments can be very difficult
- Difficulty with analyzing mixtures
- Multiple charges can result in very confusing mass spectra.
- Low-high mass ranges typically less than 200,000 Da.
- Does not work well for non volatile salts (buffers)
- Not useful for non-polar compounds

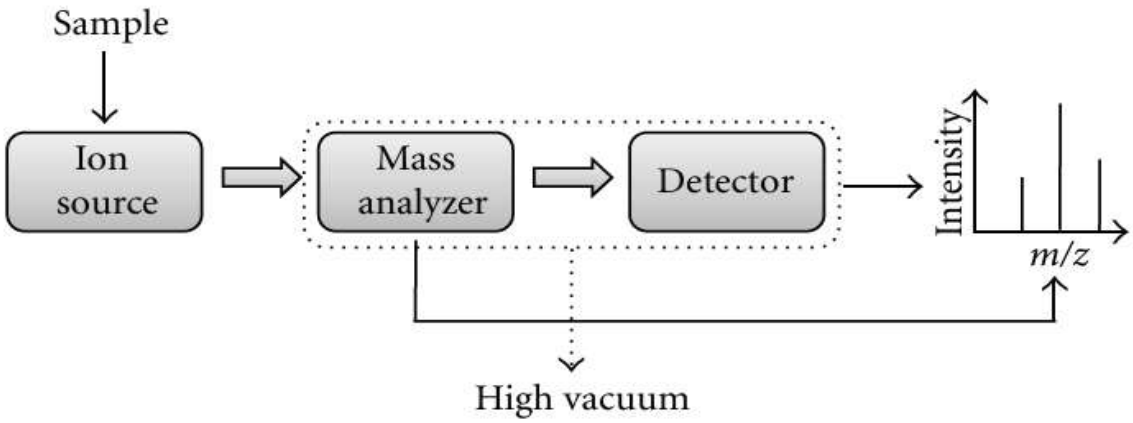
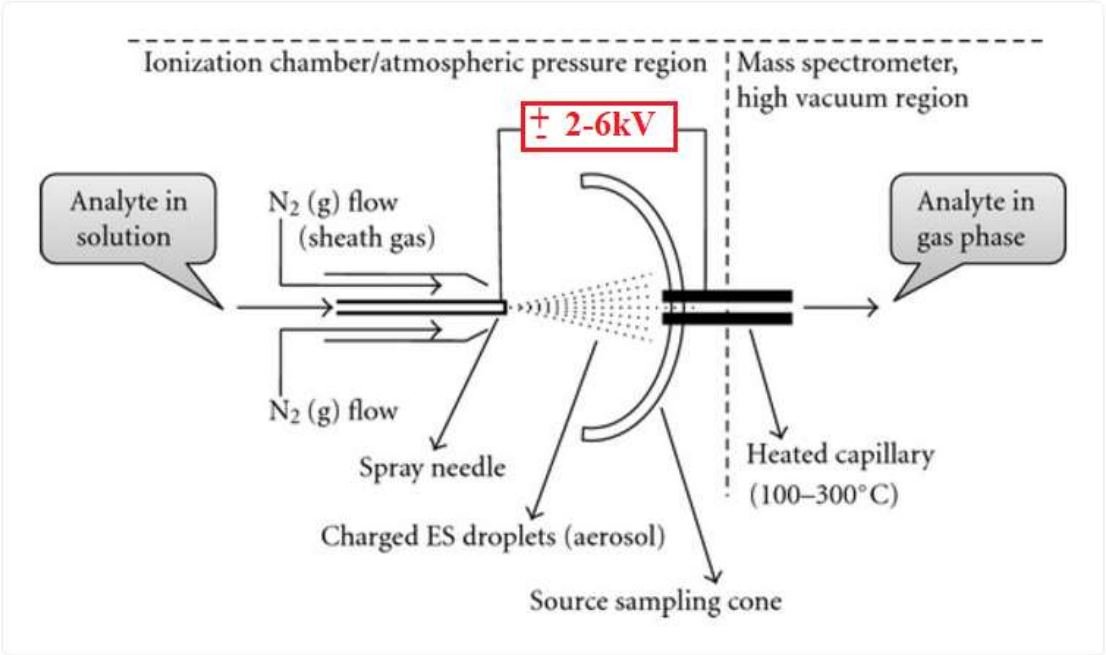


FIGURE 2: The basic components of the ESI-mass spectrometer.



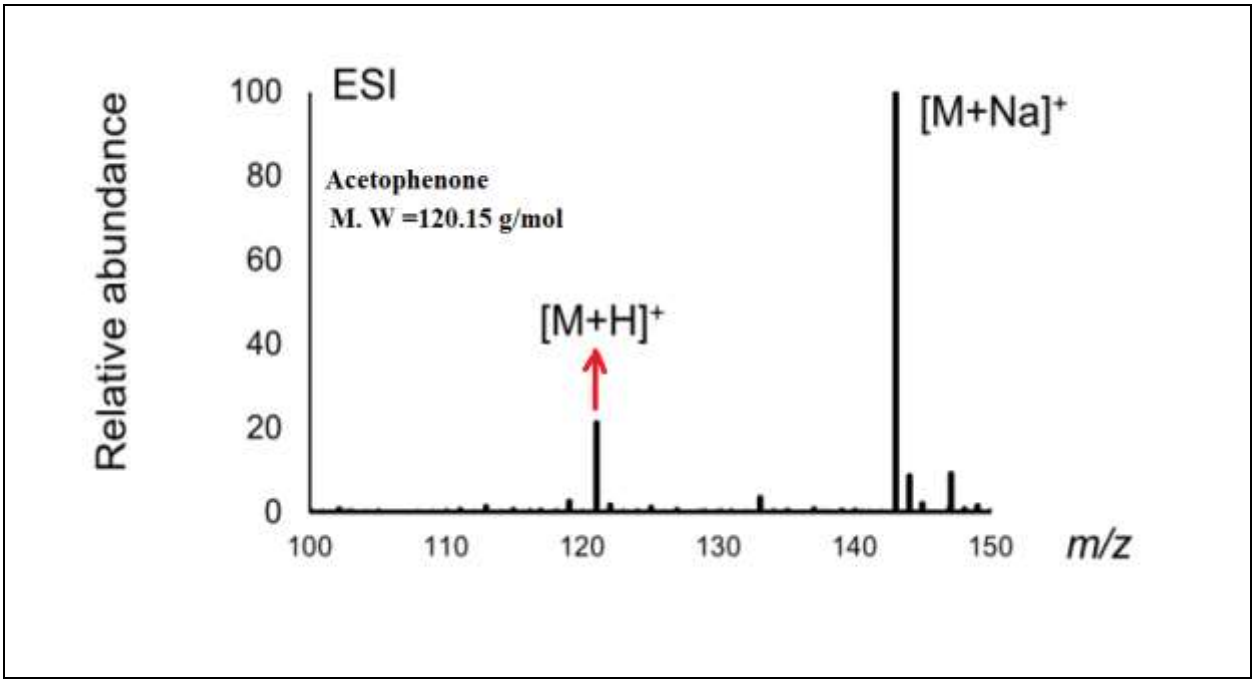
A schematic representation of the ESI-ion source.

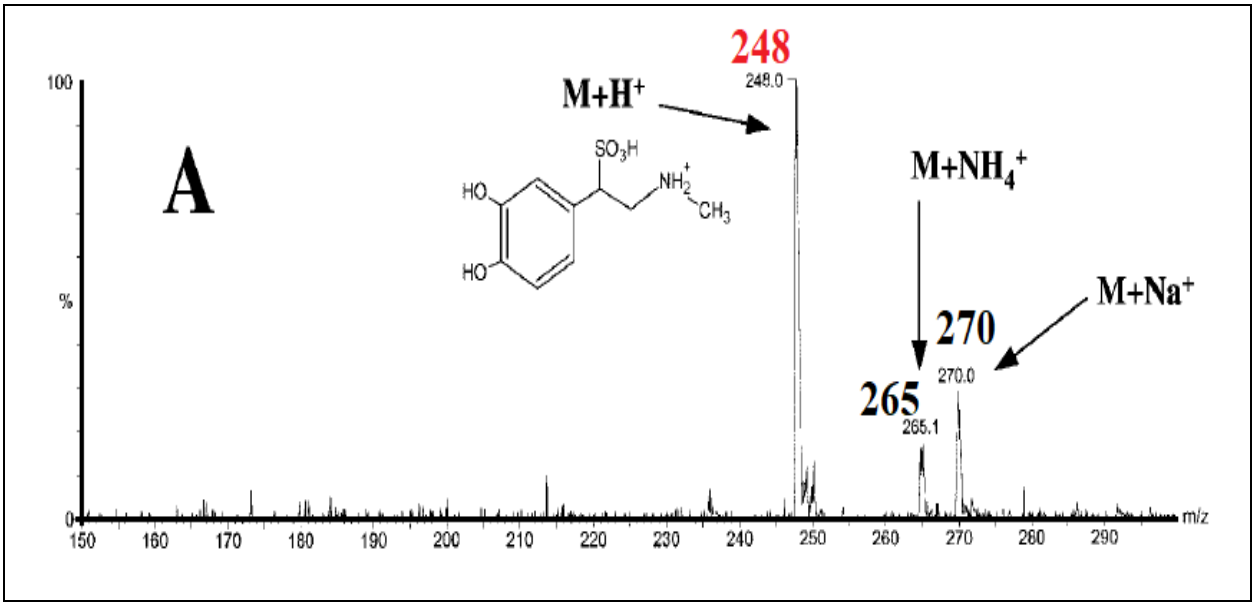
POLARITY SELECTION

Positive mode:

Positive ion electrospray analysis is commonly used to generate positively charged molecular ions through protonation to yield [M+H]+, where M signifies the molecular compound, and H, the additional proton. For positive-ion mode, 0.1% formic acid or acetic acid is usually added into the analyte solution to enhance protonation and increase sensitivity. The m/z value at which one detects a singly charged ion is therefore 1 Dalton, Da, higher in mass than the molecular weight. Basic chemical functionalities that are most frequently ionized in the positive mode include basic nitrogen, pyridinic, furans and inorganic cations (Functional groups that readily accept H+ (such as amide and amino groups found in

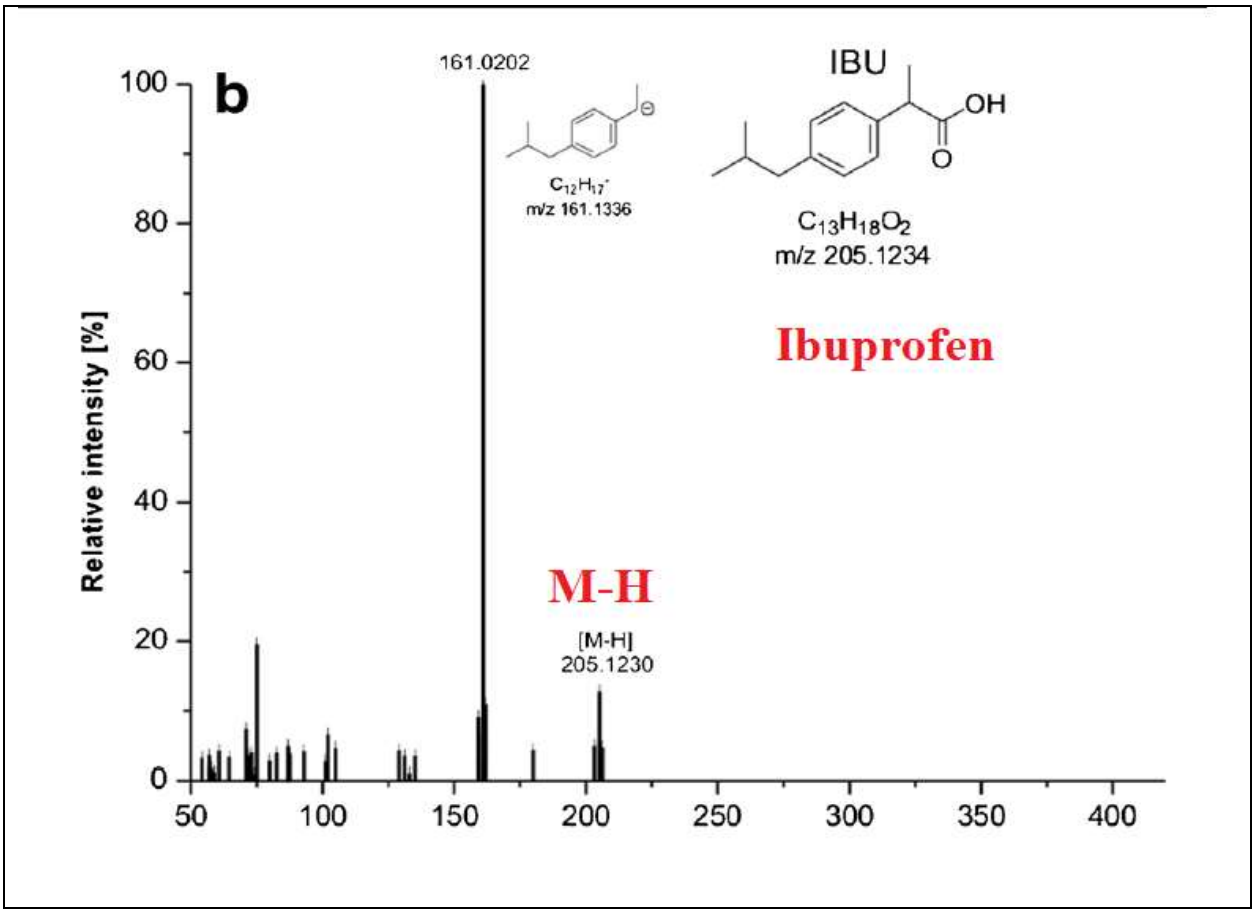
peptides and proteins) can be ionized using positive mode ESI).





Negative mode:

Negative ion electrospray analysis commonly used to generate negatively charged molecular ions through deprotonation to yield [M-H]-, where M signifies the molecular compound, and H, the lost proton. For negative-ion mode, 0.3% NH_4OH is usually added into the analyte solution to help deprotonation and increase sensitivity. The m/z value where one would detect a singly charged deprotonated molecular ion is 1 Da lower in mass than the molecular weight. Acidic chemical functionalities that are most frequently ionized include neutral nitrogen, pyrrole, carboxylic acids and inorganic anions (Functional groups that readily lose a proton (such as carboxylic acids and hydroxyls as found in nucleic acids and sugars) should be ionized using negative mode ESI).



Adduction:

In special instances, sample adduction may be required to produce efficient ionization. Adduct formation is carried out in the solution phase prior to sample introduction. In negative ion ESI, the

ionization of polar, neutral molecules or very weakly acidic species that do not generate stable negative ions through deprotonation often times form adducts with chloride ions. The resultant ion of increased molecular weight, +35 in the case of chloride, but possessing a single negative charge. In positive mode ESI, the addition of a salt, in very small quantities, less than 1mM, can facilitate ionization through the formation of a positively charged adduct with sodium, lithium, or ammonium. The observed positive ions result in a molecular weight increase equivalent to that of the salt ion, +23 for sodium, , and typically carry a single positive charge.

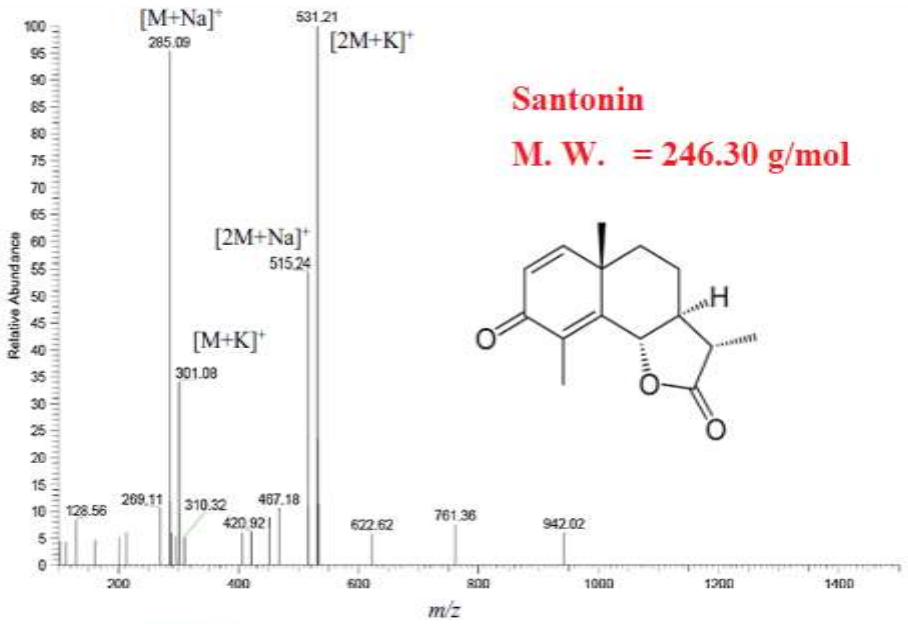


Figure 4. Potassium adduct of santonin observed after triboionization. $[M + Na]^+$ (*m/z* 285.09), $[M + K]^+$ (*m/z* 301.08), $[2M + Na]^+$ (*m/z* 515.24), and $[2M + K]^+$ (*m/z* 531.21) were observed

Solvent

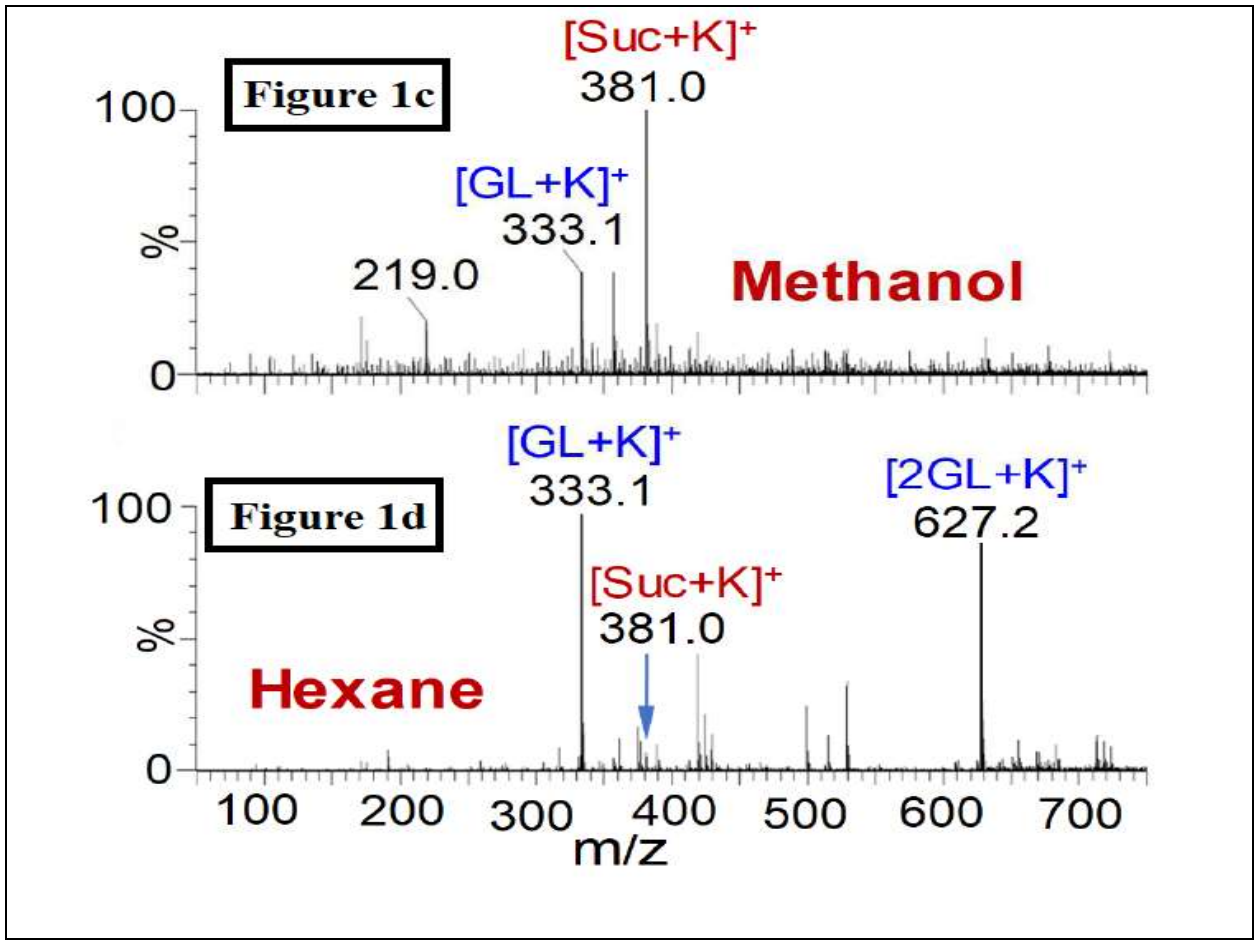
1- Nonpolar solvents like hexane have been considered unsuitable for electrospray ionization mass spectrometry (ESI-MS)["non-ESI-friendly"] due to their limited electrical conductivity.

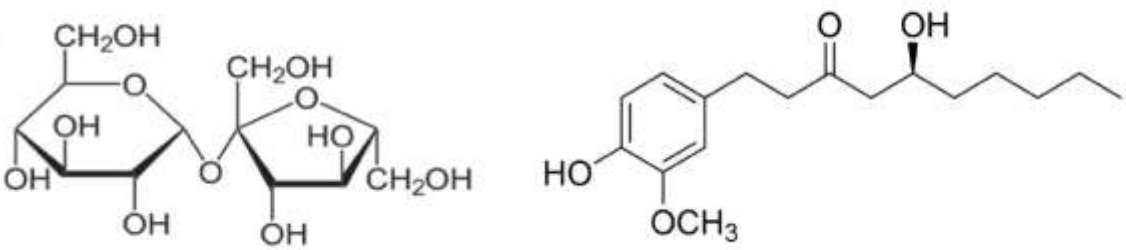
2- Polar solvents such as methanol, water and acetonitrile are commonly used for ESI-MS analysis.

3- Alternatively, analytes in nonpolar solvents have also been recently achieved using ESI-MS with conductive nanomaterials. Without the use of additional conductive solvents and materials, direct ionization of analytes in nonpolar solvents under ESI conditions is still challenging.

Example:

The peak of polar sucrose at m/z 381 was dominated in the mass spectrum of ginger tissue (Figure 1c) by use of methanol, while the peaks of low-polar analytes such as [6]-gingerol and dimer of [6]-gingerol were dominated in the mass spectrum when hexane was loaded as spray solvent (Figure 1d). Based on the molecular structures of sucrose and[6] gingerol, sucrose has various hydrophilic hydroxy and [6]-gingerol has a hydrophobic tail and benzene ring, as shown in Figure 1g.





Sucrose (Suc) Mw: 342

Polar

[6]-gingerol (GL) Mw: 292

Low polar

Figure 1g.