Waters Alliance™ Systems for LC/MS ESI/APCI Applications

Salicylamide Characterization Using Electrospray Mass Spectrometry

Highlights: Salicylamide, an analgesic compound, was studied by Electrospray (ESI) mass spectrometry using a Waters Alliance LC/MS System Featuring a Platform LC Detector. In addition to the molecular weight information that was obtained by ESI, fragmentation was induced by "In-Source" Collision Induced Dissociation (CID) which yielded some additional structural information.

ESI is a widely used method of analysis combining both the universality and selectivity of MS with liquid sample introduction that is used to solve many complex analytical problems. ESI is a solvent-assisted ionization technique that produces ions at atmospheric pressure by nebulizing and vaporizing the mobile phase solvent and liberating ions that are already in solution. Pseudo-molecular ions, [M+H]+ or [M-H]-, are normally produced with little or no fragmentation.

The ESI process involves flowing the analyte dissolved in mobile phase through a metal capillary needle at a high electric potential relative to the walls of the atmospheric pressure region. The analyte must be initially ionized in solution. The electric potential causes the mobile phase to explode into a fine spray of charged droplets. These droplets undergo evaporation assisted by a drying gas. Eventually upon evaporation, analyte ions are expulsed from the shrinking droplets and are transported to the analyzer region of the mass spectrometer through a low pressure transport region. ESI is a very "soft" ionization technique generating pseudo-molecular ions even for very thermally labile and involatile molecules. ESI-MS is also considered to be a very sensitive technique.

Experimental Conditions: Salicylamide was dissolved in warm water at a concentration of 10 mg/ml and infused at 3 ul/min using a Harvard Syringe pump into a Platform LC Detector with the ESI probe installed. Acquisition by positive ion ESI was at a mass range up to 200 Daltons. Mass axis calibration was performed to 1000 Da using PEG1000. The cone voltage was switched from high to low (20V to 80V) to produce the molecular ion and fragmentation respectively.

Figure 1. Wiley Library Reference Spectrum of Salicylamide

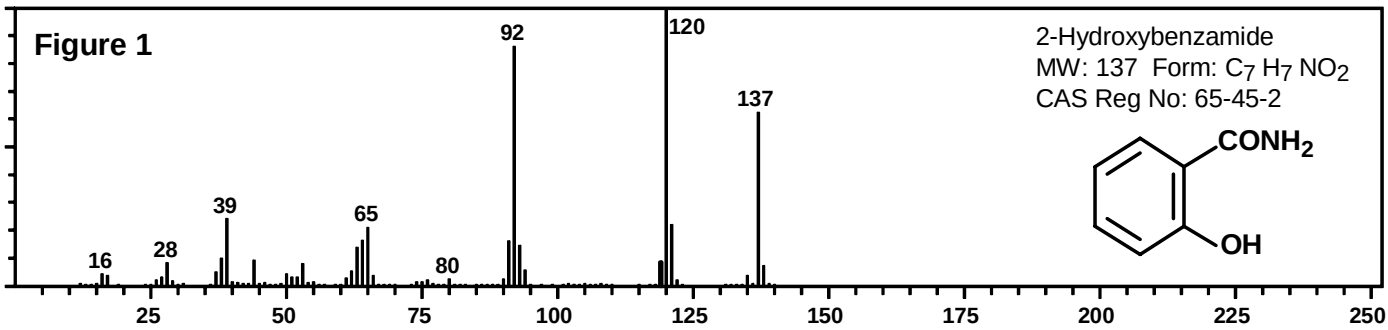


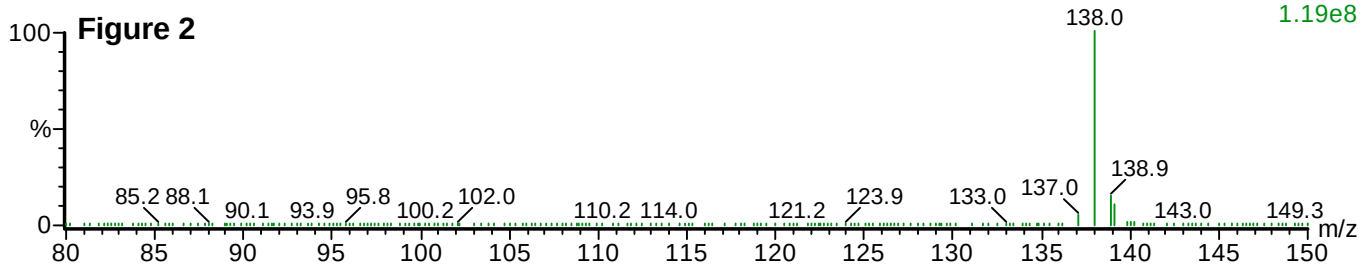
Figure 1 shows the Wiley Library spectrum of salicylamide generated by Electron Ionization (EI). EI is a much "harder" ionization technique which imparts more energy into the molecular ion. Reproducible fragmentation of this species results. Structural information gleaned from the resulting mass spectrometric "fingerprint" can then be utilized for identification purposes. This EI spectrum of salicylamide is shown here to contrast the nature of the information obtained from ESI (see reverse side).

Figure 2 illustrates the ESI spectrum of salicylamide at a low cone voltage (20 V). The ion at m/z 138 is the protonated molecular ion (protonated molecule). By this method, molecular weight information can easily be obtained for target analytes.

Sample Description

SALICYLAMIDE 1 (0.067) Cn (Cen,2, 80.00, Ar)

Scan ES+
1.19e8



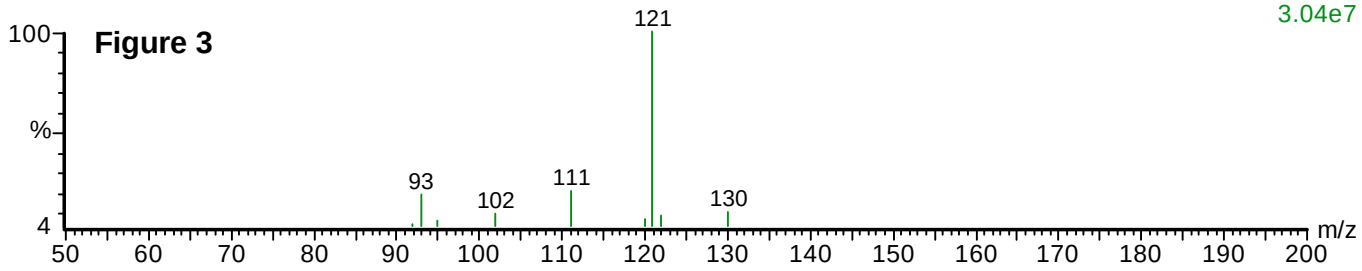
The "In-Source" CID process (also referred to as 'Cone Voltage Fragmentation') imparts excess energy to pseudo-molecular ions resulting in limited fragmentation. An increased voltage is placed on the skimmer (sample cone) which is located in a region of moderate vacuum. This voltage accelerates the ions through this region so that energy-transferring ion-molecule reactions occur between these ions and the neutral surrounding molecules. When energy from these collisions is transferred to the pseudo-molecular ions, the ions fragment. These fragmented ions can then be utilized for known compound confirmation.

Figure 3 shows a salicylamide spectrum acquired using a cone voltage of 80 V. The generation of fragment ions is obvious, yet , there are differences between the EI spectrum (more information rich) and this In-source CID spectrum. Some fragment ions in this spectrum, however, are likely to have come from a small contaminant in the salicylamide solution.

Sample Description

SALICYLAMIDE 80V 31 (0.617) Cn (Cen,2, 80.00, Ar); Cm (11:38)

Scan ES+
3.04e7



The Waters Alliance LC/MS system Featuring the Platform LC Detector also utilizes Atmospheric Pressure Chemical Ionization (APCI) as well as ESI to generate pseudo-molecular ions from which the molecular weight of target compounds can be deduced. In-Source CID fragmentation can be produced in either case. This LC/MS system also includes a Waters 996 Photodiode Array detector, the 2690 Solvent and Sample Management system and Micromass' Windows NT based MassLynx software.