

Poster # 1945

A Comparison of Information Contained in the Spectra of LC/MS Electron Impact (EI), Chemical Ionization (CI), and Electrospray Ionization (ES) for Selected Chemicals and Natural Products

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Abstract

A series of selected compounds of chemical or natural product origin were introduced into a particle beam interface LCMS system and an atmospheric pressure ionization LCMS system. The compounds were ionized by electron impact, positive and negative chemical ionization and positive and negative electrospray ionization techniques. Compounds were introduced into the mass spectrometer by direct loop injections as well as on column injections. For on column injections, separation prior to mass spectrometry was accomplished by reversed phase HPLC. The CI experiments included both methane and ammonia as reagent gas. The spectra generated under these conditions were examined and compared for their molecular weight information, sensitivity and their ability to match a standardized library spectrum. Results of some experiments made on some of the compounds will be shown in this paper.

Instrumentation – Particle Beam Interface

For particle beam LCMS, the system included the following components. The liquid chromatograph used was an Agilent Model 1100 modular system with quaternary pump, vacuum degasser, 100 vial autosampler and variable wavelength detector. The HPLC column used was a Zorbax SB-C18 (Agilent pn 830990-902), narrow bore 2.1 x 150 mm 3.5 micron. The Genesis II particle beam interface (CSS Analytical Co. Inc.) was attached to an Agilent 5973 MSD so that samples could be analyzed by LC/MS with electron impact and chemical ionization. The Genesis II is an improved particle beam interface, which delivers a higher amount of analyte to the ion source, when compared to previous commercial interfaces. The mass spectrometer used was an unmodified Agilent 5973 Mass Selective Detector (Agilent Technologies, Inc., Palo Alto California) with turbo molecular pump. The Agilent 5973 is a benchtop quadrupole mass spectrometer with mass range of 1.6 to 800 mass units, 10,000 volt HED, and is available with EI or EI/CI capabilities.

Instrumentation – Electrospray Interface

For atmospheric pressure ionization with electrospray (API-ES), the system included the following components. HP 1090 LC with PV-5 solvent delivery system, and diode array detector. The HPLC column used was a Zorbax SB-C18 (Agilent pn 830990-902), narrow bore 2.1 x 150 mm 3.5 micron. HP 5989 MS Engine with 59987A Electrospray interface. High Mass option (2000 amu), HED, Ion Guide, the original equipment “off-axis” sprayer and an orthogonal spray attachment (CSS Analytical Co. Inc.) were utilized to obtain electrospray ionization data.

Instrumentation – Data System

The computer data system used for control of both instrument systems was a Windows 2000 personal computer configured with 128 MB of Ram and the following software: Agilent G1701BA mass spec software and Agilent G1642 multi technique software for the LC. The data system for the Particle Beam instrument was also configured with the CSS Eighty-X Upgrade software for concurrent control of the LC, the PB, and the MS instrument. The data system for the API instrument was also configured with the CSS Eighty-X Upgrade software for concurrent control of the LC, the PB, and the MS instrument. This additional software application allows for complete control of both the mass spec and the HPLC and allows for seamless sequencing of large numbers of samples.

Discussion

Mass spectra acquired by three techniques, Electron Impact (EI), Chemical Ionization (CI), and Atmospheric Pressure Electrospray Ionization (API-ES) were examined and compared for 1) molecular weight information, 2) sensitivity, and 3) matching with mass spectral libraries. All three techniques provided valuable information about the analytes. The spectra from each technique are shown and evaluated. Compound identity obtained from matching with a standardized mass spectra library is shown.

Data gathered by these experiments suggests that when compounds are analyzed by API-ES, the mass spectra will contain strong molecular weight information as shown in Figure 1 for sucrose octaacetate and Figure 2 for pseudoephedrine. Evaluation of the same compounds by CI using NH₃ as reagent gas yields mass spectra which again contain strong molecular weight information. Analysis of the same compounds by EI show little or no molecular weight information.

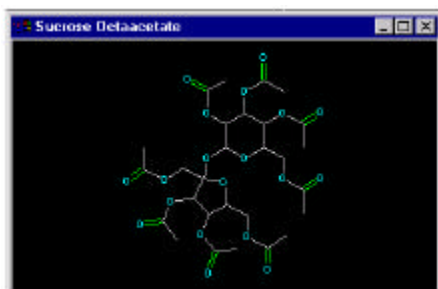


Figure 1a.

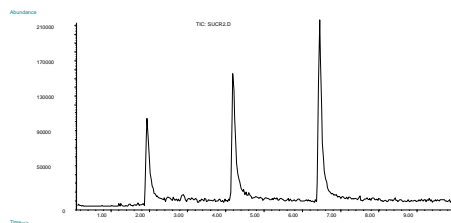


Figure 1b.

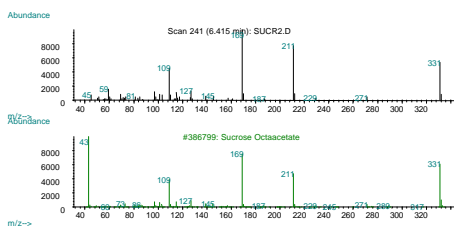


Figure 1c.

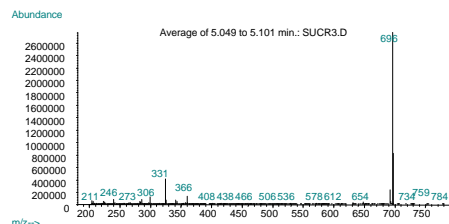


Figure 1d.

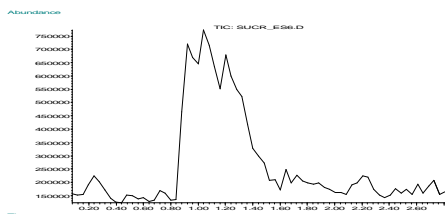


Figure 1e.

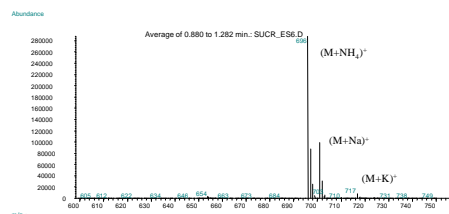


Figure 1f.

Figure 1. Sucrose Octaacetate. 1a. Structure. 1b. Total ion chromatogram by loop injection. 1c. Spectrum analyzed by electron impact and its library match selected from the Wiley 7th / NIST98 Library. 1d. Spectrum analyzed by NH₃ positive chemical ionization. 1e. Total ion chromatogram by ESI. 1f. Spectrum analyzed by atmospheric pressure positive electrospray ionization.

The analysis of sucrose octaacetate (Figure 1.) by mass spectrometry with electron impact ionization yields a spectrum rich in structural information. Ions are present that indicate that it is a sugar and also that the sucrose has been acetylated. This is confirmed by the Library match selected from the Wiley 7th / NIST98 with good matching quality. Note that the molecular ion for sucrose octaacetate is not observed. This is important because in higher molecular weight acetylated sugars, with higher degree of polymerization (d.p.), the same m/z 331 ion present in the spectrum of sucrose octaacetate is present. The NH₃ chemical ionization spectrum better defines the d.p. of the sugar due to the intense molecular ion with NH₄⁺. The spectrum of sucrose octaacetate obtained by atmospheric pressure positive electrospray ionization also contains a strong molecular ion with NH₄⁺ due to the addition of ammonium acetate to the mobile phase.

The analysis of pseudoephedrine (Figure 2.) by mass spectrometry with electron impact ionization yields a spectrum that is dominated by the presence of m/z 58 which is indicative of a C3 amine. This ion is not, however, selective for pseudoephedrine, and in fact is quite common. Despite this ambiguity the library match selected from the Wiley 7th / NIST98 with good matching quality does identify pseudoephedrine. The NH₃ chemical ionization spectrum better defines the molecular weight of pseudoephedrine do to the intense molecular ion with H⁺. The spectrum of pseudoephedrine obtained by atmospheric pressure positive electrospray ionization also contains a strong molecular ion with H⁺ and is almost identical to the NH₃ chemical ionization spectrum.

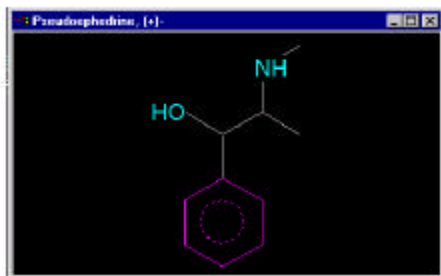


Figure 2a.

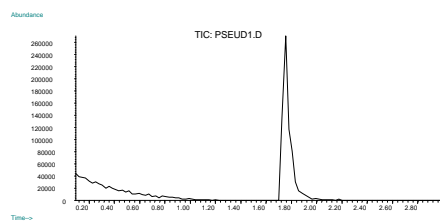


Figure 2b.

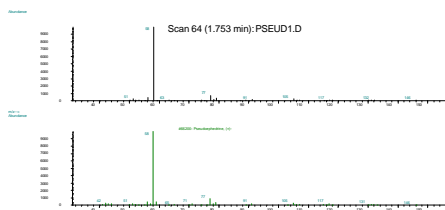


Figure 2c.

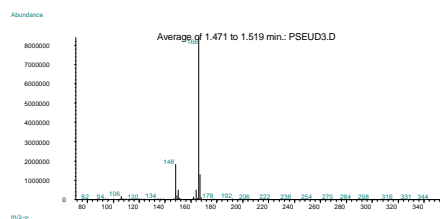


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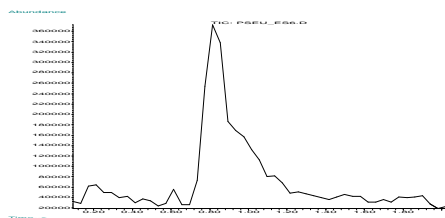


Figure 2e.

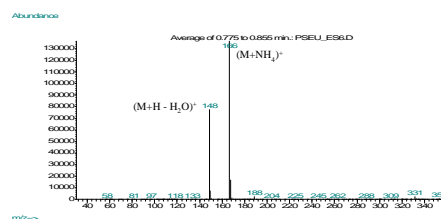


Figure 2f.

Figure 2. Pseudoephedrine. 2a. Structure. 2b. Total ion chromatogram by loop injection. 2c. Spectrum analyzed by electron impact and its library match selected from the Wiley 7th / NIST98 Library. 2d. Spectrum analyzed by NH₃ positive chemical ionization. 2e. Total Ion chromatogram by ESI. 2f. Spectrum analyzed by atmospheric pressure positive electrospray ionization.

Conclusion

Almost all new commercial LC/MS systems today are atmospheric pressure ionization instruments. Despite this, it is important to remember that the analysis of a true unknown is an extremely difficult task and may require the utilization of more than one technique. Nothing helps more than the direction that can be obtained from a standardized database of mass spectra with which to match. Commercial and standardized libraries are not yet prevalent for CI and API-ES. CI and API-ES are typically used to obtain molecular weight information. For this reason CI and API-ES are commonly used as a confirmation tool to confirm an identity obtained from matching an unknown spectrum with a mass spectral database.

To put it all in perspective, an evaluation of our experiments showed the following:

1. Electron impact is good for identifying small molecules that have some volatility.
2. Chemical ionization is good for identification of many fragile and/or ambiguous oligomeric molecules that have some volatility.
3. Atmospheric pressure ionization gives limited information and lacks the databases to specifically identify unknowns, but has advantages in sensitivity especially for large polar molecules and may be the only way to perform LC/MS when the molecule has no volatility.