Liquid Chromatographic Mass Spectrometry

Four different techniques for coupling liquid chromatography with mass spectrometry are available within ASD: particle beam (PB), thermospray (TS), electrospray (ES), and atmospheric pressure chemical ionization (APCI) interfaces. PB and TS ionization are available on a single quadrupole mass spectrometer (see GC-MS). The ES and APCI interfaces are available on a triple quadrupole mass spectrometer (refer to GC-MS-MS).

Liquid chromatographic mass spectrometry (LC-MS) is used for qualitative and quantitative analysis of components in mixtures of organic compounds. LC-MS is useful for the determination of analytes which are nonvolatile, highly polar, or thermally labile, and, therefore, not amenable to GC-MS. Electrospray LC-MS and ES-LC-MS-MS are particularly suited to the analysis of large biomolecules with molecular weights of approximately 100,000 daltons.

Principles of Techniques

Particle Beam Interface

Samples are introduced by flow injection or through an LC column. As the flow injection/LC eluant and analyte elute from the LC column, they are sprayed by a nebulizer into a desolvation chamber heated to 30–80°C, which is the first stage of the PB interface. The LC mobile phase begins to evaporate and the analyte molecules condense to form a beam of particles that passes through two momentum separators and into an electron ionization or chemical ionization MS source. The mobile-phase molecules, which lack sufficient momentum to pass through the two stages of momentum separation, are removed by the vacuum system of the LC-MS instrument and are not introduced into the MS source. The analyte molecules, which have been introduced into the source region of the MS, are ionized by electron ionization (or chemical ionization), mass analyzed, and detected by an electron multiplier.

Thermospray

Samples are introduced into the TS interface by flow injection or through an LC column. The LC effluent, which contains the analytes of interest, forms a superheated, high-velocity aerosol after exiting a capillary heated to approximately 100°C. The aerosol droplets enter a heated source and continue to desolvate. Ions are then formed by a chemical ionization process, by ion evaporation (a volatile buffer in the LC mobile phase charges the droplets), or by use of a filament or discharge. The analyte ions are extracted through the ion exit cone and mass analyzed, while neutral species (e.g., the LC mobile phase) are pumped out of the MS system. Because TS-LC-MS does not require the analytes to be ionized in the gas phase, TS-LC-MS easily ionizes high molecular weight analytes (>800 daltons).

Electrospray

Purified samples are typically introduced via capillary tubing into a grounded needle positioned in an

Examples of Applications

- Analysis of benzidines and nitrogen-containing pesticides in drinking water at parts-per-billion concentrations by PB-LC-MS (U.S. EPA Method 553).
- Identification of nonvolatile, highly polar, or thermally labile contaminants in river water and municipal sewage treatment plant effluents (PB-LC-MS).
- Analysis of biological compounds such as ß-lactams, cephapirin, tetracyclines, spectinomycin, cytidine, and thiamine (PB-LC-MS).
- Detection of azo dyes, fluorescent brighteners, and organophosphorus pesticides at low to subnanogram amounts by TS-LC-MS (U.S. EPA Draft Method 8321).
- Confirmation of pesticides in river and simulated sea waters at 2 to 20 ppb (TS-LC-MS).
- Detection of picomolar amounts of nucleosides (ES-LC-MS).
- Detection and molecular weight determinations of other biomolecules including gramicidin S, cytochrome C, myoglobin, hemoglobin, and bovine serum albumin (ES-LC-MS).
- Detection of environmental contaminants including aldicarb sulfone, terbutryn, propoxur, carbofuran, and azo dyes (ES-LC-MS).
- Analysis of triazine, phenylurea, carbamate, and organophosphorus pesticides at detection limits of 1 to 10 ng (APCI-LC-MS).

electric field of 2 to 4 kV. As the liquid emerges into the electric field, a spray of charged drops that contains the mobile phase and analyte is formed. The spray travels through an inert drying gas, which removes the mobile phase and causes multiple charges to remain on the analyte. The analyte ions then travel through skimmer cones and into the mass analyzer. Multiply charged ions and adduct ions, such as $[M+H]^{+}$, $[M+Na]^{+}$ and $[M-H]^{-}$, are detected by this technique. Because ES-LC-MS does not require the analytes to be ionized in the gas phase, ES-LC-MS easily detects high molecular weight analytes (>800 daltons).

Atmospheric Pressure Chemical Ionization

In APCI, liquid effluent containing the analytes of interest passes through a heated capillary and forms a spray. The analytes are then ionized at atmospheric pressure as they interact with a corona discharge from an electrode that is external to the heated capillary. The analyte ions are directed into the MS source by voltages applied to a tube lens. Because APCI is a soft, chemical ionization process, it is well-suited to the analysis of small, polar compounds with molecular weights <1000 daltons.

Samples

Form. Liquids or solids (dissolved in appropriate solvents).

Size. The amount of sample required is determined by the LC-MS interface used. For PB-LC-MS, 10 to 1000 ng of analyte are required for full scan, MS analysis. TS, ES, and APCI LC-MS analyses require 0.1 to 100 ng of analyte. Smaller quantities of analyte may be detected if the MS is operated in the selected ion monitoring mode.

Preparation. Most samples must be extracted and concentrated. Some fractionation may be required for complex samples. Some aqueous solutions may be run as received by ES or APCI LC-MS.

Limitations

General Limitations of LC-MS

Solids must be freely soluble in the carrier solvent/LC mobile phase and liquids must be miscible with the LC mobile phase. Typical eluants are methanol and acetonitrile for reverse phase LC-MS. Hexane, dichloromethane, and chloroform may be suitable solvents for regular phase PB-LC-MS. ES and APCI LC-MS are better suited to aqueous solvents and mobile phases than PB-LC-MS.

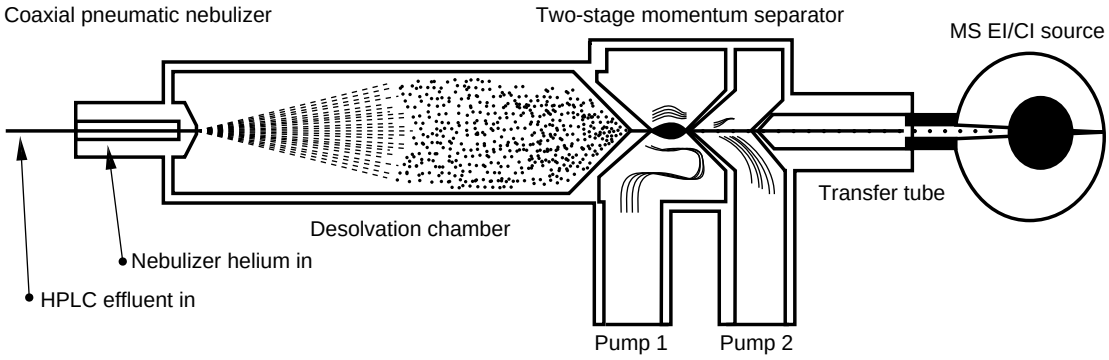
LC-MS response may be compound dependent; not all analytes have comparable detection limits.

Particle Beam Limitations

Analyte response is affected by organic content of the LC mobile phase and the presence of co-eluting interferents and matrix components. Accurate quantitation by PB-LC-MS is difficult and requires ^{13}C-labeled internal standards; both linear and nonlinear calibration curves have been observed. Transport across the PB interface is inefficient; thus, instrument detection limits are restricted to nanograms of analyte injected. PB interfaces can accommodate LC flow rates of 0.2 to 1 mL/min. Very high molecular weight compounds (>800 daltons) cannot be analyzed by PB-LC-MS because analytes must be sufficiently volatile to be ionized in the source of the MS.

Thermospray Limitations

TS-LC-MS produces largely chemical ionization spectra. The use of a buffer in the mobile phase, such as ammonium acetate, is necessary to aid ionization. In TS-LC-MS, molecular ions and adduct ions are observed; little molecular fragmentation occurs. Thus, this technique is most useful for molecular weight



Particle beam LC-MS interface (courtesy of Hewlett-Packard).

determination and confirmation of the presence of a particular analyte. Analytes detected by TS-LC-MS must have basic (H^+ acceptor) sites to show good sensitivity (0.1 to 10 ng detected). TS-LC-MS is compatible with LC mobile-phase flow rates of 1 to 2 mL/min. Because some heating is used in the ionization process, TS-LC-MS may not be amenable for use with analytes which easily decompose at moderate temperatures.

Electrospray Limitations

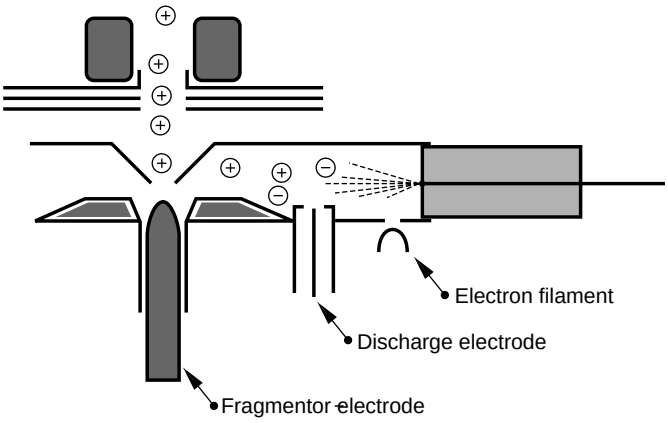
ES-LC-MS can produce multiply charged ions; thus, ES spectra may be complex to interpret. Adduct ions are often observed in ES spectra. Because there is little fragmentation of analytes, ES-LC-MS is not ideal for structural identification. MS-MS or adjustment of the skimmer cone voltages used in ES can induce greater fragmentation of the analyte ions to provide more structural information. ES-LC-MS is compatible with flow rates of 1 to 1000 µL/min.

APCI Limitations

APCI is a soft ionization technique and, therefore, is not well-suited to structural determinations. MS-MS or adjustment of the skimmer cone voltages in APCI-LC-MS can induce fragmentation of the analyte ions to provide more structural information. Picogram detection limits are achievable. APCI-LC-MS can easily accommodate flow rates of 1 to 2 mL/min, making it easy to transfer traditional LC analyses to an MS detector.

Estimated Analysis Time

The time required for an analysis depends on many factors, including the complexity of the sample and the data objectives of the client. Verification of the structure of a well isolated analyte may take less than an hour. However, one or more days may be required to characterize a complex mixture. Analyzing a single, complex sample may significantly contaminate the LC-MS source and interface. It may be necessary to clean the source and interface between each sample, which will add significantly to the analysis time. Although methods can be developed using HPLC, the specific interface required for analysis may dictate changes in buffers or solvent systems, necessitating further method development for LC-MS analysis.



Thermospray LC-MS interface (courtesy of Hewlett-Packard).

Capabilities of Related Techniques

High performance liquid chromatography (HPLC) can be used to screen samples and determine general chromatographic conditions for LC-MS analyses; however, the specific interface required for analysis may dictate changes in the solvent/buffer system prior to LC-MS analysis.

Gas chromatography–mass spectrometry (GC-MS) may be used to identify and quantitate analytes that are thermally stable, nonpolar, and volatile. GC-MS may also be used to analyze polar compounds that have been appropriately derivatized.

Matrix-assisted laser desorption–ionization coupled to time-of-flight mass spectrometry (MALDI-TOF-MS), an ion trap, or a quadrupole MS may also be used to provide molecular weight information for biomolecules. This technique is currently under development by ASD.

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