Considerations for Adapting an HPLC Method for MS Detection

by HPLC Innovations Team

Summary
Most HPLC methods can be coupled with mass spectral detection, provided appropriate attention is given to pump capabilities, column diameter, and system plumbing, and to sample matrix and mobile phase composition.

Greater sensitivity, highly reliable compound identification, and/or other anticipated advantages prompt many inquiries about adapting traditional HPLC methods for LC/MS detection and, increasingly, LC/MS is used in parallel with UV/VIS, ELSD or fluorescence detection. Most HPLC methods, except those requiring nonvolatile additives (e.g., ion exchange chromatography), can be coupled with MS detection. The following considerations apply when LC/MS is interfaced through either of two popular sample ionization methods: electrospray (ESI) or atmospheric pressure chemical ionization (APCI).

Pump and Column Considerations

Generally, an ESI or APCI interface nebulizes the column effluent at atmospheric pressure, using a strong electric field (ESI) or pneumatically (APCI), and electrostatically directs ions generated from evaporating droplets into the mass spectrometer. During ESI, charged droplets evaporate, increasing the charge density at the droplet surface. As desolvation continues, repulsion between the like charged ions becomes greater than the droplet surface tension, and ions leave the droplet. In APCI, a heated vaporizer quickly desolvates the droplets. Ionized solvent molecules collide with analyte molecules, forming analyte ions. The flow rate into the ionization interface is limited by the fixed diameter of the MS transfer capillary line, and by the interface's ability to effectively evaporate the effluent. A typical interface can accept flows of up to 500µL/min., but performs best at approximately 20µL/min. Micro-flow and nano-flow LC pumps capable of pumping at low flow rates are well suited for use with microbore and capillary columns (≤ 1mm ID), and with MS detection. A standard pump may be used, provided the flow is properly split to accommodate both the column ID and the MS interface.

Flow to the MS is reduced by splitting the flow, with an intent to optimize both chromatography and ionization at the MS interface. The point at which the flow is split is determined by the inner diameter of the column and the destination of the diverted flow (Figure 1). Splitting is least complicated when flow is diverted directly to waste. If an additional detector is to be used, that detector will require specific flow rates based on instrument configuration. When using a pump that has significant void volume in the solvent mixing chamber, and is not capable of pumping at low flow rates, the position of the split tee should be determined by the diameter of the column and the volume of sample. When using a microbore or capillary LC column, place the tee between the pump and the injection point (Figure 1a), for proper pump function at higher flow rates (1-2 mL/min.) and to allow reasonable time for the mobile phase to reach the injection point when using a gradient. The entire sample will enter the reduced flow, directly before the column. Often, to force flow through the microbore or capillary column, backpressure will be required in the flow path directed away from the mass spectrometer. This can be accomplished by using small diameter tubing or an in-line backpressure regulator, or including an old column in this path, to make it more restrictive to flow. If the goal is to avoid sample overload on the column, place the tee between the injection point and the column (Figure 1B). When using a column of inner diameter larger than 2.1mm, split the flow at a point between the column and the MS interface (Figure 1C).

In the cases illustrated in Figure 1B and 1C, the sample will be split, and this must be considered when preparing the sample. Splitting the flow makes quantification difficult due to band broadening (caused primarily by system void volume), and great precision is required in determining the split ratio and plumbing the MS as a second detector. Decrease peak broadening by using short lengths of narrow diameter tubing between components, and by using low void volume tees and unions.

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REFERENCES

1 *Basic HPLC and CE of Biomolecules* Robert L. Cunico, Karen M. Gooding and Tim Wehr, Bay Bioanalytical Laboratory, Inc., 1998. pages 88-94.

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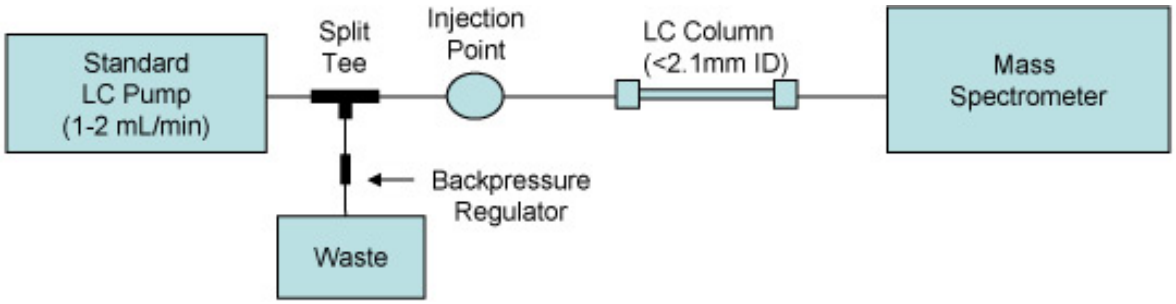
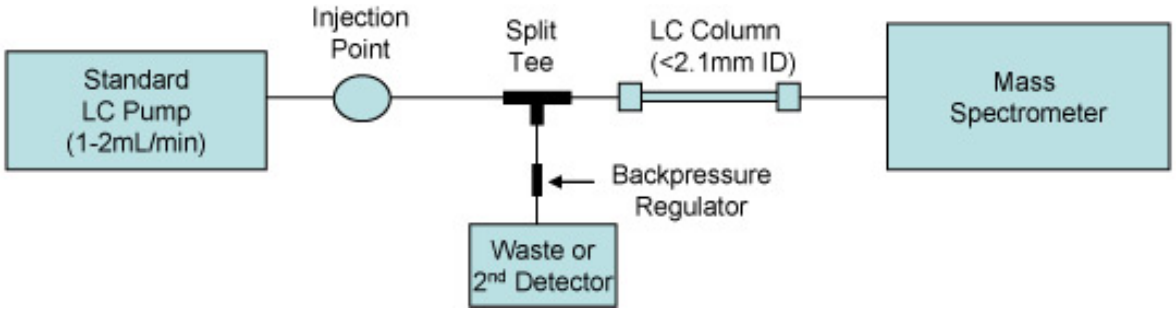
Figure 1 Pathways for diverting excess flow from a high flow pump away from an LC/MS ionization interface.

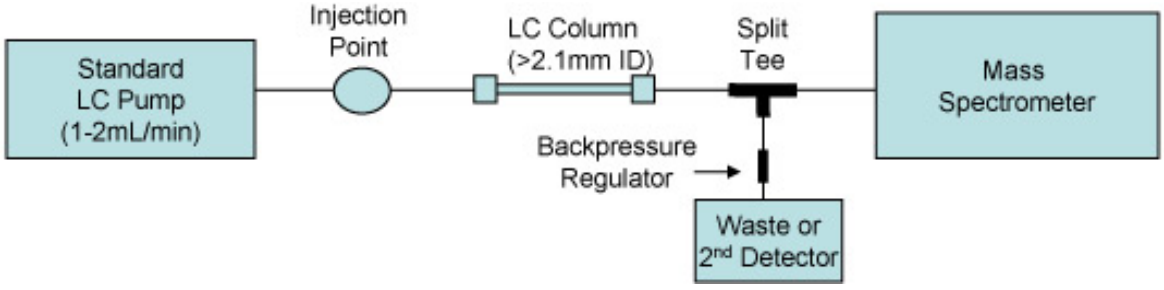
A. Narrow ID column: excess flow directed to waste.



B. Narrow ID column: excess flow directed to waste or to a second detector.



C. Column ID greater than 2.1mm: excess flow directed to waste or to a second detector.



Sample Matrix and Mobile Phase Considerations

Ionization is a competitive process: analytes, matrix components, and mobile phase components compete for charge produced in the ion source. Often, compounds other than analytes preferentially form ions. Consequently, extensive sample clean-up is essential for many samples, especially those in complex matrices, such as biological fluids. Clean-up can be accomplished by liquid-liquid extraction, solid phase extraction, size exclusion chromatography, or other techniques. To avoid clogging the ionization interface and to maximize analyte ionization, the mobile phase should contain only volatile components. Water is difficult to evaporate in the interface, so higher concentrations of organic solvents are beneficial. Adding 1-2% organic solvent to a totally aqueous mobile phase can improve desolvation, if chromatographic separation is not compromised.

Additives often are incorporated into a mobile phase, for pH control or for ion pairing. When the analysis is to be adapted for MS, it is necessary to replace inorganic salts with volatile additives. Trifluoroacetic acid (TFA) is notorious for causing ion suppression in ESI, for example, by ion pairing with basic analytes. Use formic acid to reduce pH (approximately 0.15%, v/v). Volatile additives can be included with aqueous or organic solvents to maintain ionic strength throughout a gradient elution. Formic acid often can be substituted for TFA, or add a mixture of 75% propionic acid / 25% 2-propanol to a TFA-containing mobile phase to reduce ion suppression.[1] Use ammonium acetate, or other ammonium solutions, to adjust a mobile phase to higher pH; a 10mM concentration typically is sufficient to maintain a constant pH. Ion pairing reagents, such as triethylamine (TEA), often can be avoided by using a base-deactivated HPLC column.

Note that despite thorough efforts to reduce ionization interferences, mass spectra often include m/z values of common adducts, from the matrix. For example, the m/z value of a molecular ion $[M+H]^+$ might not be observed, but the sodium adduct $[M+Na]^+$ will be seen. Adduct m/z values can be interpreted in the same manner as protonated ion m/z values. In positive ion mode, sodium, potassium, or ammonium adduct ions are common

and, in negative ion mode chloride adduct ions are common.

Useful Literature

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