Hyphenated Techniques for the Analysis of Mixtures

Hyphenated techniques combine at least two different analysis techniques into one instrument. They are configured to take advantage of the unique abilities of each of the two techniques and provide a capability not possible with each individual instrument. This section focuses on the combination of separation and mass spectrometric techniques into single instruments, specifically gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) (Figure 1). In these instruments, the mass spectrometer is used as a detector for identifying and quantitating components of mixtures that have been separated on the chromatographic or electrophoretic instrument. The value of mass spectrometric detection is that it has the capability of providing structural information and hence the identity of a chemical substance. Mass spectrometers can also be used to obtain information about the quantity or concentration of chemical substances in samples.

![Figure 1. Schematic of a hyphenated chromatographic-mass spectrometric instrument.](image)

In chromatographic systems that use regular detectors (e.g. flame ionization detector, UV-Vis detector, electron-capture detector), components of mixtures are identified by their retention time (i.e. time of elution from the chromatographic column). This approach works well when conditions can be established for completely separating all components of the mixture (Figure 2), although even then one can never be completely sure whether a peak represents a pure compound or whether something else is co-eluting at the same time.
Many real-world samples are challenging to analyze because the analyte(s) of interest is present in a matrix that contains many other chemical substances. Examples of such samples include but are not limited to biological tissues and fluids, environmental, pharmaceutical and food samples. In these cases, it is sometimes difficult to find conditions that completely separate the analyte(s) completely from other constituents of the sample. As such, GC-MS, LC-MS and CE-MS methods come in handy. If a high resolution mass spectrometer is employed, unequivocal characterization of the analytes in the mixture can be accomplished because the instrument provides the exact mass of the species.

GC-MS, LC-MS, and CE-MS require that the analyte be present in the gas phase. In GC-MS, the compounds are already present in the gas phase as they elute from the chromatographic column. Because of this, an electron ionization (EI) ion source serves to create gas-phase ions for analysis. Alternately, chemical ionization (CI) can be employed if a soft ionization technique is necessary. Liquid chromatography and capillary electrophoresis perform separations in the liquid phase. As compounds elute from an LC or EC system, the solvent must be stripped away as the compounds are ionized. Electrospray ionization (ESI) can accomplish this task when flow-rates are low (≤ 0.2 ml/min). For higher flow rates (0.2 to 2 mL/min), atmospheric pressure chemical ionization (APCI) can be used. For information on these ionization techniques, refer to the text on ion sources.

Data Output from a Hyphenated System

Another advantage of hyphenated techniques is that there are various ways to examine the data that is obtained. In GC-MS, the mass spectrometer usually records an individual mass spectrum in a second or less. Therefore, numerous mass spectra are recorded over the entire chromatogram. A common way to present the data is as a total ion chromatogram (TIC). In this case, the ion count in each individual mass spectrum is totaled up and that number is plotted as a function of retention time. The TIC looks similar

![Figure 2. A chromatogram showing well resolved peaks, where retention time can be used to help identify a compound.](image-url)
to a chromatogram that is obtained using something like a flame ionization (in GC) or UV (in LC) detector (Figure 3).

The data from a total ion chromatogram can be used for quantitative study, where the area under a peak can be related through a calibration curve to the amount of a particular compound. It is also possible to examine the individual mass spectrum at any point in the chromatogram to obtain qualitative information about each compound (Figure 4). The mass spectrum contains information that can be used to identify a compound. The process of identifying a compound is often done by comparing the spectrum of a peak in the chromatogram to spectra in a library. For example, the National Institute of Standards and Technology publishes a library that contains mass spectra for over 180,000 known compounds. The section on data interpretation provides more information on how to use a mass spectrum for qualitative purposes.

Another way of presenting the information is to select specific m/z ions to monitor in each mass spectrum and produce a separate chromatographic plot for each ion. These plots are called extracted
ion chromatograms (XIC). In XIC it is often possible to resolve two compounds that elute together from the separation technique. In the chromatogram shown in Figure 3, peaks 2, 3 and 4 overlap. If the compound that causes each peak in the chromatogram has a unique m/z molecular or fragment ion, it is possible to produce four distinct XICs as shown in Figure 5. An XIC can be used for quantitative study as the area under each peak will be proportional to the amount of the compound present in the sample.

Figure 5. Four extracted ion chromatograms for the total ion chromatogram shown in Figure 3.