LC-MS: Why use it, and what is it?

Introduction

An LC-MS is an HPLC system with a mass spec detector. The HPLC separates chemicals by conventional chromatography on a column. Usually the method will be reverse phase chromatography, where the metabolite binds to the column by hydrophobic interactions in the presence of a hydrophilic solvent (for instance water) and is eluted off by a more hydrophobic solvent (methanol or acetonitrile). As the metabolites appear from the end of the column they enter the mass detector, where the solvent is removed and the metabolites are ionised. The metabolites must be ionised because the detector can only work with ions, not neutral molecules. And ions only fly through a very good vacuum, so removal of the solvent is a vital first step. The mass detector then scans the molecules it sees by mass and produces a full high-resolution spectrum, separating all ions that have different masses. Why chromatography?

Since mass spectrometers can differentiate a lot of chemicals all mixed together on the basis of mass, it is tempting not to bother separating chemicals first with hplc. There are however two good reasons why chromatography is a very good thing.

- Most biologically interesting chemicals exist as isomers. Isomers have exactly the same mass and cannot normally be differentiated by a mass detector, no matter how expensive it is. Therefore it helps if you can additionally separate the isomers before hand by chromatography.
- When a mixture of chemicals enters the process of ionisation, the chemicals can interact and affect one another's chances of getting properly ionised. This is called ion suppression. It is usually a problem where you are trying to detect one minor, or poorly ionised chemical in the presence of a large amount of something else, maybe a buffer from the sample. Some pre-purification of the ionisation mixture can get the suppressed away from the suppressors. There are ways to recognise ion suppression.

And why is the chromatography usually reverse-phase?

The first thing that happens to the flow from the HPLC when it enters the mass detector is that the solvent is squirted through a fine needle and evaporated away, to leave metabolites floating free. If the solvent contains non-volatile salts, they will appear, solid and horrible and clog up the system. Therefore ion-exchange chromatography is not really compatible with LC-MS. If you must have salts present, low concentrations of volatile things, such as ammonium acetate, can be tolerated.

Why bother with mass spectroscopy?

HPLC separates things, but provides little extra information about what a chemical might be. In fact, it is hard in hplc to be certain that a particular peak is pure, and contains only a single chemical. Adding a mass spec to this tells you the masses of all the chemicals present in the peak, which can be a very good starting point for identifying them, and an excellent method to check for purity. You can also use even a simple mass spec as a mass-specific detector, specific for your chemical of interest.
More sophisticated mass detectors such as triple quadrupole and ion-trap instruments can be set up to carry out more detailed structure-dependant analyses on what is eluting from the HPLC system.

After HPLC separation the sample goes straight into a mass detector. Mass specs detect ions in a vacuum, so the first tasks in the LC-MS are to

- remove the solvent and ionize the metabolites.
- get the ions into a vacuum.

**Historical note:**

*GC-MS came before LC-MS because it is comparatively easy to pump off a small amount of GC carrier gas, but quite difficult to pump off all the vapour that can be created from even a small amount of liquid. One ml water will produce 1.3 litres of vapour at room temperature and pressure...*

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