

1. Course Overview

The course aims and objectives are to:

- Introduce the fundamentals to understanding and using a triple quadrupole mass spectrometer
- Describe ion production mechanisms in common instrument interfaces
- Describe the coupling of LC to the mass spectrometer
- Discuss solvent, buffers and pH effects on analyte ionisation
- Discuss the creation and importance of vacuum systems
- Describe the process of multiple charging and deconvolution of high molecular weight molecules
- Examine the operational principals and use of the quadrupole mass analyser
- Consider ion detection, tuning and calibration issues
- Describe common triple quadrupole data acquisition modes and their application functionality
- Practically examine all theoretical aspects through the interactive group completion of instrument workshops

By the end of the course you will be able to:

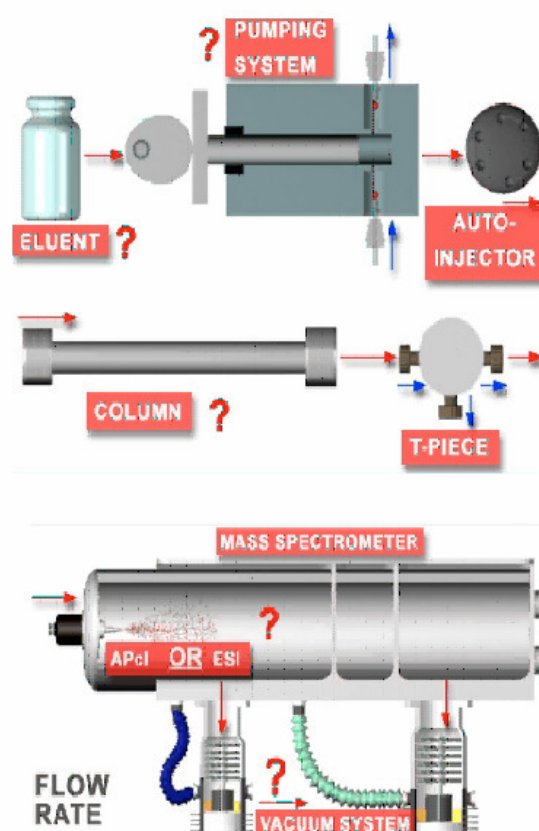
- Characterise and identify compound structures from their unique isotope patterns
- Explain the major ways of producing ions and optimising interface parameters to maximise instrument sensitivity
- Design LC experiments which combine excellent chromatography with maximal mass spectrometer sensitivity
- Identify common adduct ions in the mass spectrum
- Determine the pseudomolecular ion from a multiply charged ion series from first principles and through deconvolution
- Know the difference between mass resolution and mass accuracy and when to maximise both for interpretation purposes
- Understand and optimise the process of in-source and collision cell collisionally induced dissociation
- Decide on, apply and optimise the correct MS/MS data acquisition mode for any given application
- Understand and apply the quantitative mechanism of MRM LC-MS/MS

2. Flow Rates and Flow Splitting

Flow rates used in conventional LC separations using non-mass spectrometric detectors may not be compatible with LC-MS systems depending upon the eluent system and the API interface type used.

Generally, the eluent flow rate used in LC-MS experiments will be governed by several factors including:

- The API interface type used
- The eluent system used
- The vacuum system of the LC-MS instrument used
- The availability of column and HPLC pumping hardware



Perhaps the most limiting factor in the list is the API interface used for a particular application, the two most popular interface types being Electrospray Ionisation (ESI) and Atmospheric Pressure chemical Ionisation (APCI). Each of the interfaces operates via a fundamentally different principle, and so each will operate at widely differing flow rates.

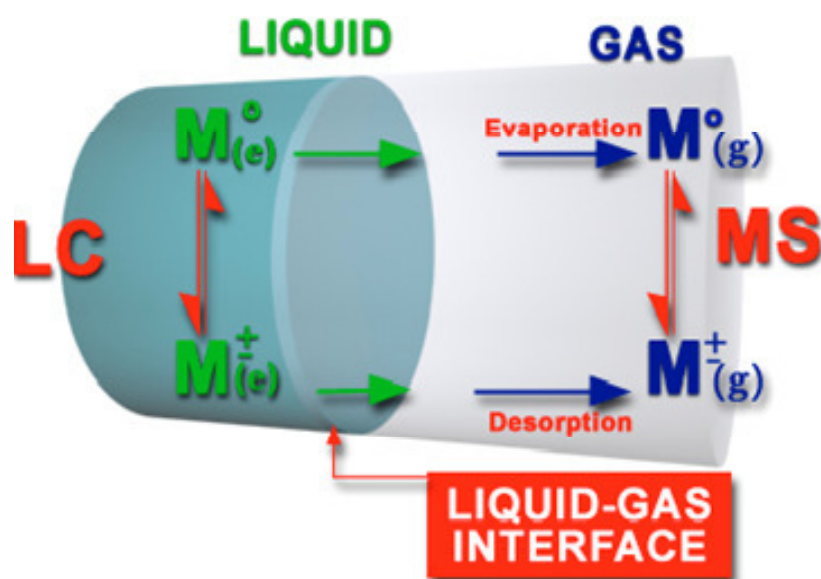
A brief explanation of the similarities and differences between the two API interface types will allow further discussion on the flow rates applicable to modern LC-MS experiments.

Interface Similarities

In general all API interfaces have to be able to accommodate the following processes:

- Evaporate liquids into gases
- Ionise neutrals into charged species or transfer charged species in solution to gas phase ions
- Evacuate large amounts of eluent and remove the resulting vapour from the system in order to maintain the required level of vacuum within the mass spectrometer

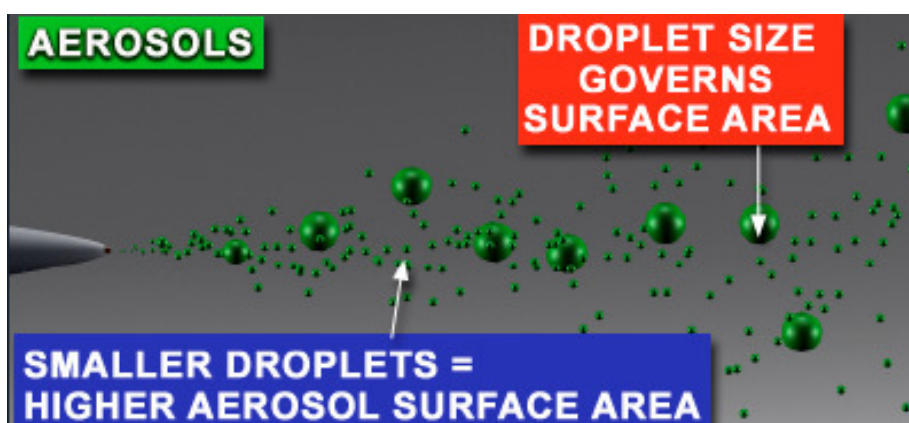
The figure shown below highlights the change of state that applies to all LC-MS interfaces. Whether the analyte is neutral in the liquid phase [$M^0_{(l)}$] or charged [$M^{\pm}_{(l)}$] it must make its way to a surface to evaporate into the gas phase. Once in the gas phase a neutral analyte [$M^0_{(l)}$] must be ionised so that mass analysis can occur.



It is the pathway between the two sides of the diagram that will determine the differences between the types of interface used.

The energy requirements for the thermodynamic change of state are independent of the path taken from one side of the diagram to the other and will be similar for all interface types. It is the way that the energy is applied to the system that will differentiate the interface types.

Aerosols are of great importance in LC-MS as they provide the surface area from which the analyte species will evaporate into the gas phase. The higher the surface area, the greater the amount of evaporation, and hence the greater the number of analyte species available for sampling into the mass analyser.

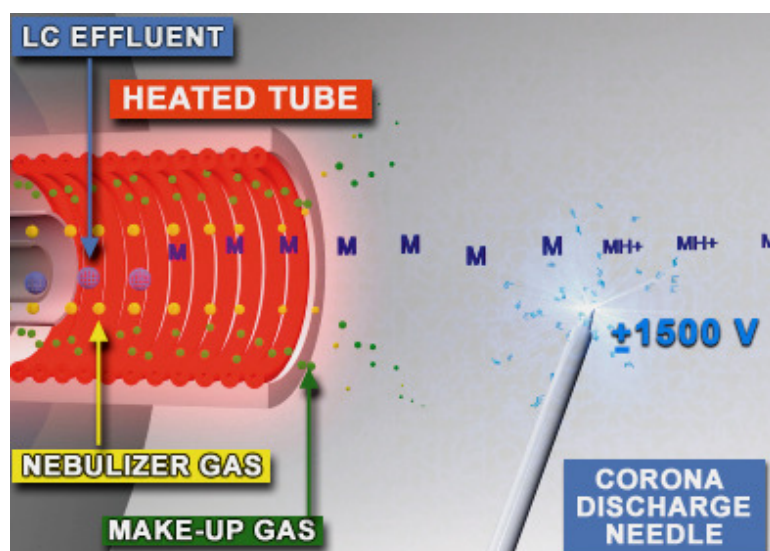


2.1 Atmospheric Pressure chemical Ionisation (APCI)

In APCI the eluent is introduced into the interface using a capillary of similar design to the ESI source. However, no potential is applied to the capillary, but instead the liquid emerges from the capillary surrounded by a flow of inert, heated nebulising gas into a heated region.

The combination of nebulising gas and heat forms an aerosol that begins to rapidly evaporate. A pin is placed within the heated region that has a high potential applied to it and produces an electrical discharge that ionises solvent molecules.

A combination of collisions and charge transfer processes cause an ionised gas plasma to be formed. Sample molecules that elute in the gas phase into this plasma may be ionised via transfer of protons to give either a positive or negative ion depending upon the proton affinity of the analyte species relative to the solvent gas plasma molecules.



Liquid flow rate is important here for two main reasons:

- The flow of eluent into the heated region will determine the rate of evaporation needed to ensure that all species are in the gas phase as they encounter the discharge pin.
- Stable droplet formation is required in terms of droplet size and formation rate to ensure that a stable plasma of ionised solvent molecules exists around the discharge pin, allowing the same discharge conditions to affect all analyte molecules within the sample.

Essentially APci is a high flow rate technique optimising at flow rates of over 1.0mL/min. Below 0.4mL/min. an unstable analyte signal is usually observed due to the instability of the gas plasma caused by non-reproducible discharge processes. At high flow rates, in the region of 2-4mL/min., the heating and drying gas should be adjusted to ensure that all analyte species are in the gas phase as they encounter the discharge region of the interface, allowing for maximum ion generation and therefore optimised signal intensity.