

Appendix F

Theory and Principles of Operation

F.1 Ionization Techniques

Two atmospheric pressure ionization techniques are available:

- Electrospray ionization (see Section F.1.1).
- Atmospheric pressure chemical ionization (see Section F.1.2).

F.1.1 Electrospray Ionization (ESI)

Electrospray ionization takes place as a result of imparting a strong electrical field to the eluent flow as it emerges from the nebulizer, producing an aerosol of charged droplets. These undergo a reduction in size by solvent evaporation until they have reached a charge density sufficient to allow sample ions to be ejected from the droplet's surface ("ion evaporation").

A characteristic of ESI spectra is that ions may be singly- or multiply-charged. Since the mass spectrometer filters ions according to their mass-to-charge ratio, compounds of high molecular weight can be determined if multiply-charged ions are formed.

Eluent flows up to 1 mL/min can be accommodated, although it is often preferable to split the flow such that 100 to 200 $\mu\text{L}/\text{min}$ of eluent enters the mass spectrometer.

F.1.2 Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric pressure chemical ionization generally produces protonated or deprotonated molecular ions from the sample via a proton transfer (positive ions) or proton abstraction (negative ions) mechanism. The sample is vaporized in a heated nebulizer before emerging into a cloud of solvent ions formed within the atmospheric source by a corona discharge. Proton transfer, or abstraction, then takes place between the solvent ions and the sample.

F.2 Ion Optics

Figure F-1 shows the Quattro Premier XE ion optics.

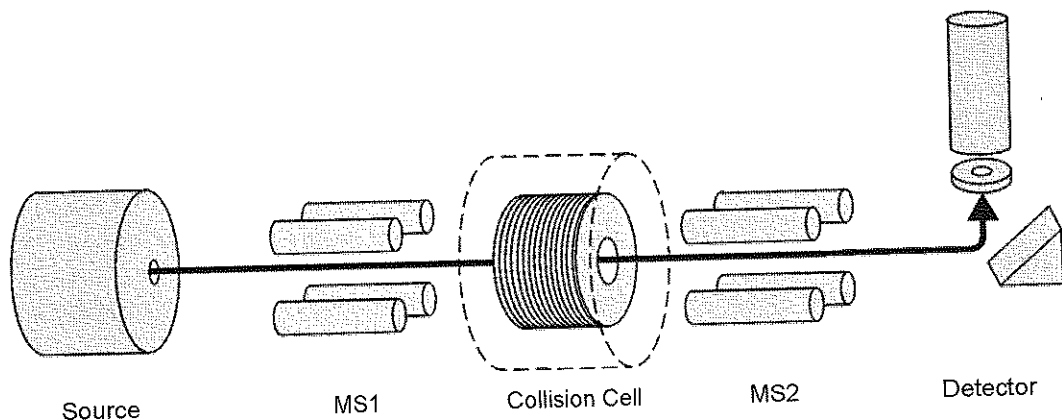


Figure F-1 Ion Optics

F.3 MS Operating Modes

Table F-1 shows the MS operating modes.

Table F-1 MS Operating Modes

Operating Mode	MS1	Collision Cell	MS2
MS1	Resolving (scanning)	Pass all masses	
MS2	Pass all masses		Resolving (scanning)
SIR	Resolving (static)	Pass all masses	

The MS1 mode, in which MS1 is used as the mass filter, is the most common and most sensitive method of performing MS analysis. This is directly analogous to using a single quadrupole mass spectrometer.

The MS2 mode of operation is used, with collision gas present, when switching rapidly between MS and MS/MS operation (for example, survey scan mode). It also provides a useful tool for instrument tuning and calibration before MS/MS analysis, and for fault diagnosis.

The SIR (Selected Ion Recording) mode of operation is used as a quantitation mode when no suitable fragment ion can be found to perform a more specific MRM analysis (see Section F.4.3).

F.4 MS/MS Operating Modes

The four common MS/MS operating modes are summarized in Table F-2.

Table F-2 MS/MS Operating Modes

Operating Mode	MS1	Collision Cell	MS2
Daughter (Product) Ion Spectrum	Static (at parent mass)	Pass all masses	Scanning
Parent (Precursor) Ion Spectrum	Scanning		Static (at daughter mass)
Multiple Reaction Monitoring (MRM)	Static (at parent mass)		Static (at daughter mass)
Constant Neutral Loss Spectrum	Scanning (synchronized with MS2)		Scanning (synchronized with MS1)

F.4.1 Daughter (Product) Ion Mode

The daughter (product) ion mode is shown in Figure F-2. It is the most commonly used MS/MS operating mode.

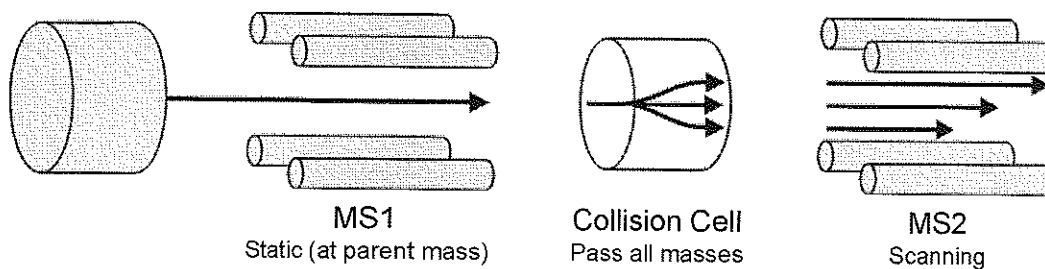


Figure F-2 Daughter (Product) Ion Mode

Typical Applications

- Structural elucidation (for example, peptide sequencing)
- Method development for MRM screening studies:
 - Identification of daughter ions for use in MRM transitions.

- Optimization of CID tuning conditions to maximize the yield of a specific daughter ion to be used in MRM analysis.

Figure F-3 shows an example of daughters of the specific parent at m/z 609 from reserpine in Electrospray positive ion mode.

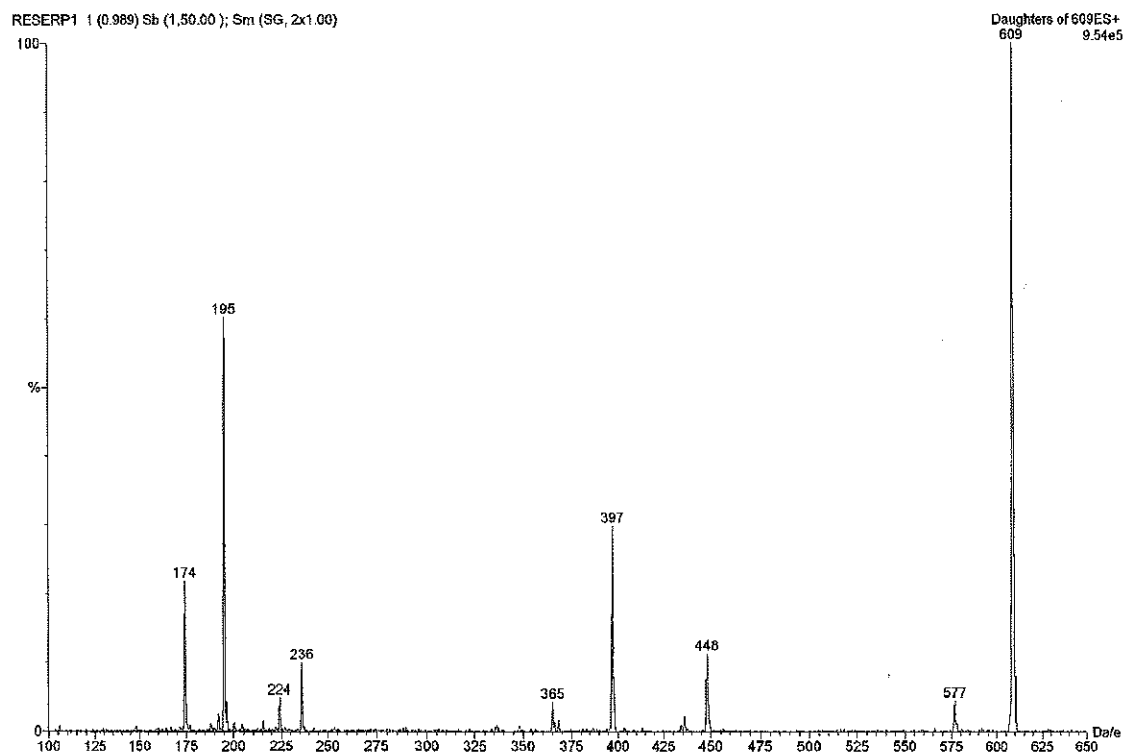


Figure F-3 Daughters of the Specific Parent at m/z 609 from Reserpine in Electrospray Positive Ion Mode

F.4.2 Parent (Precursor) Ion Mode

The parent (precursor) ion mode is shown in Figure F-4.

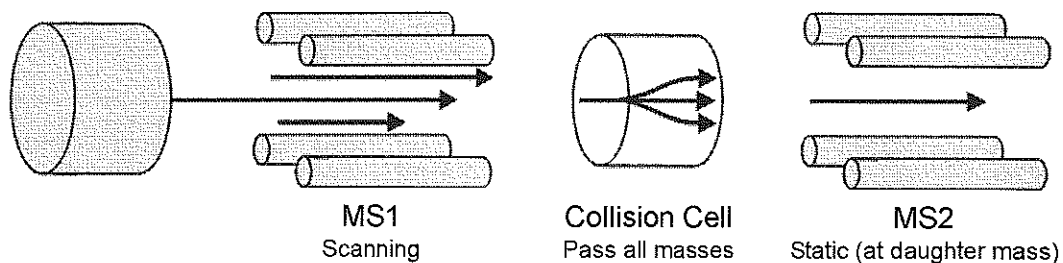


Figure F-4 Parent (Precursor) Ion Mode

A typical application is for structural elucidation, that is, complementary or confirmatory information (for daughter scan data).

Figure F-5 shows an example of parents of the specific daughter ion at m/z 195 from reserpine in electrospray positive ion mode.

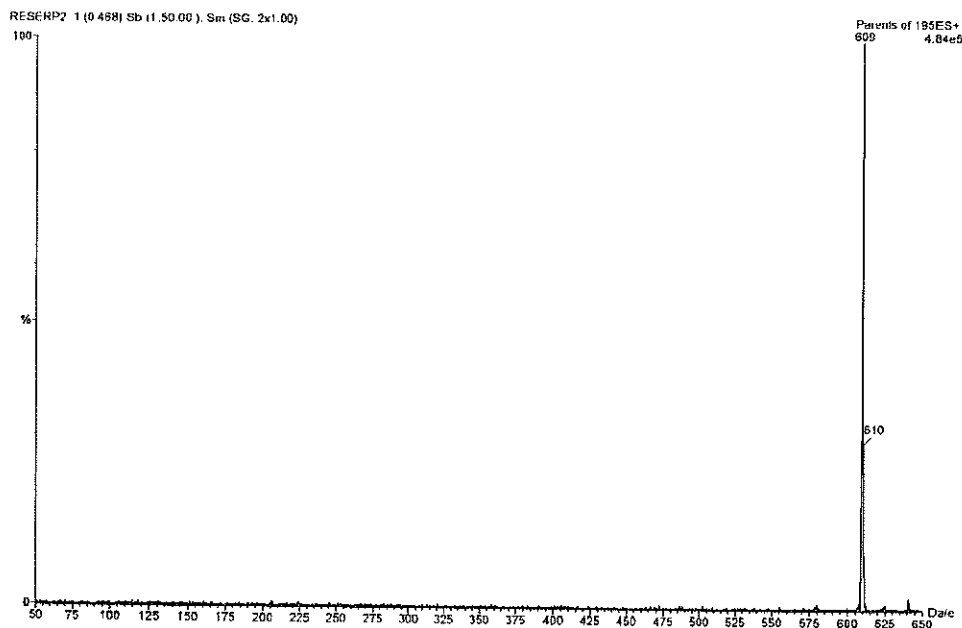


Figure F-5 Parents of the Specific Daughter Ion at m/z 195 from Reserpine in Electrospray Positive Ion Mode

F.4.3 Multiple Reaction Monitoring (MRM) Mode

The MRM mode (Figure F-6) is a highly selective MS/MS equivalent of SIR. As both MS1 and MS2 are static, greater dwell time on the ions of interest is allowed, and therefore better sensitivity compared to scanning MS/MS.

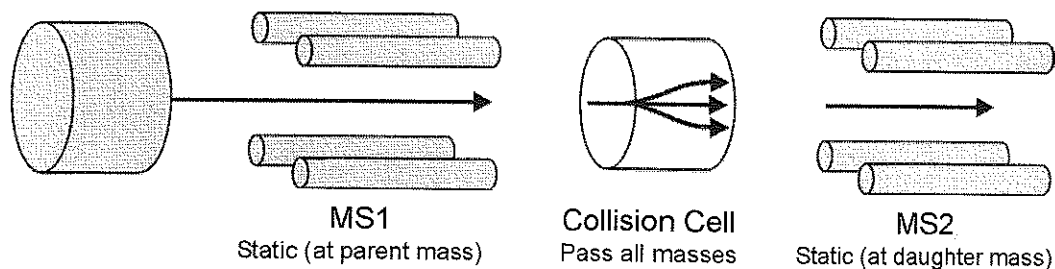


Figure F-6 MRM Mode

A typical application is for quantification of known analytes in complex samples:

- Drug metabolite and pharmacokinetic studies.
- Environmental, for example, pesticide and herbicide analysis.
- Forensic or toxicology, for example, screening for target drugs in sport.
- MRM does not produce a spectrum as only one transition is monitored. As in SIR, a chromatogram is produced.

F.4.4 Constant Neutral Loss Mode

The constant neutral loss mode is shown in Figure F-7. It detects the loss of a specific neutral fragment or functional group from an unspecified parent or parents.

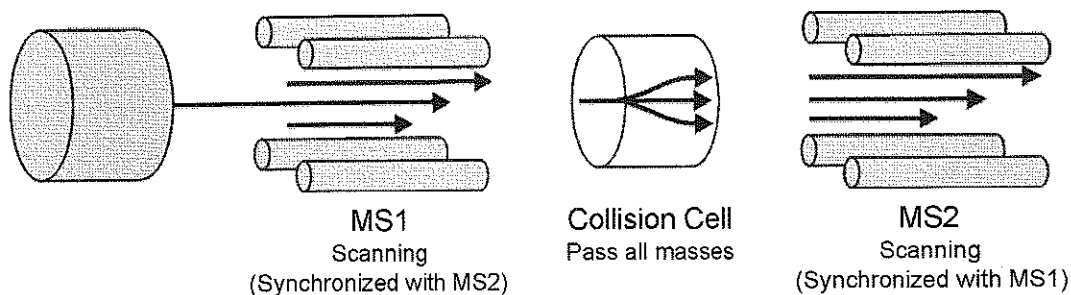


Figure F-7 Constant Neutral Loss Mode

A typical application is for screening mixtures for a specific class of compound that is characterized by a common fragmentation pathway, indicating the presence of compounds containing a common functional group.

The scans of MS1 and MS2 are synchronized. When MS1 transmits a specific parent ion, MS2 "looks" to see if that parent loses a fragment of a certain mass. If it does, it registers at the detector.

The result is that the spectrum shows the masses of all parents that actually lost a fragment of a certain mass.

F.4.5 Source and Collision Cell T-Wave Devices

The T-Wave devices are stacked ring electrode ion guides with opposite phases of radio frequency voltage applied to adjacent plates to confine ions radially (Figure F-8).

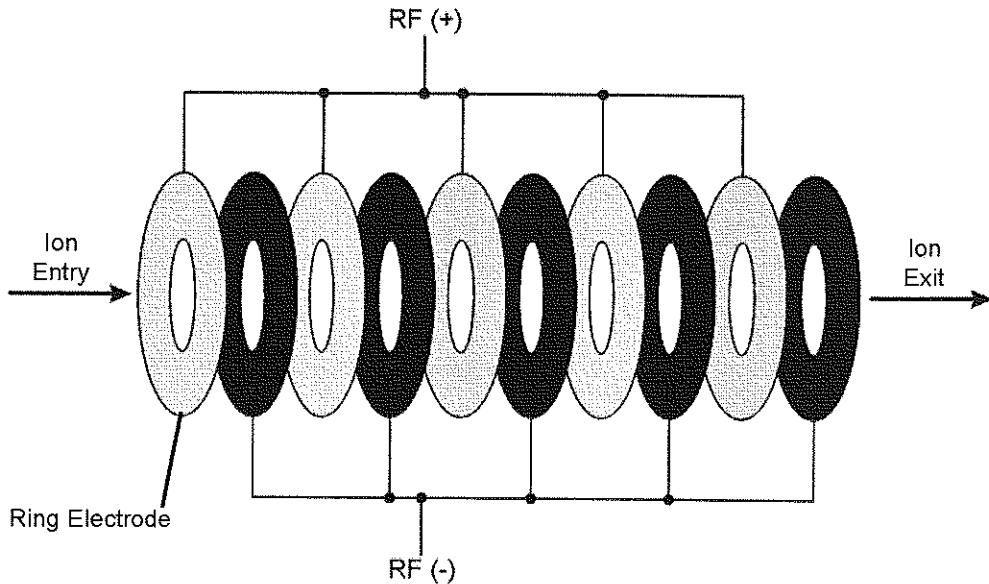


Figure F-8 T-Wave Schematic Diagram

The presence of gas in these devices causes an axial slowing of ions, which can have adverse effects for fast acquisitions such as reduced sensitivity resolution and increased cross-talk. To reduce the residence time of ions in these optics, a travelling voltage wave (T-Wave) moves along the device by application of a transient d.c. voltage to successive ring electrodes. Through appropriate choice of wave amplitude and velocity, ions "surf" on the front of this wave, reducing their transit time (Figure F-9).

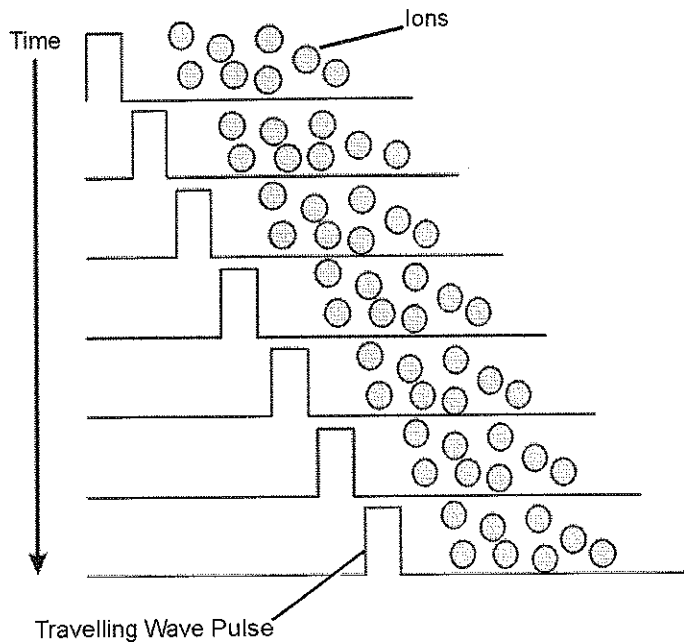


Figure F-9 Travelling Wave Pulse

Under normal operating conditions, the wave parameters are fixed by MassLynx to give optimal performance. However, under certain operating conditions, manual control may be necessary (see Section C.19).