

CHIMICA ANALITICA II CON LABORATORIO

(AA 2016-17)

8 C.F.U. - Laurea triennale in Chimica

La **spettrometria di massa** è una tecnica analitica che si basa sulla

- generazione di ioni gassosi dalle molecole (o atomi) di analita
- separazione di ioni secondo il rapporto massa/carica (m/z)
- rivelazione di ioni

Le differenze tra i tipi di spettrometro di massa consistono nei diversi modi per svolgere queste tre funzioni

- Lo *spettro di massa* è un diagramma della relativa abbondanza degli ioni in funzione del loro rapporto massa carica

Jürgen H. Gross

Mass Spectrometry

A Textbook

With 357 Illustrations and Tables

http://www.dmf.unicatt.it/~gavioli/corsi/MSFM/ref/Vuoto+Camere/Books/Mass_Spectrometry.pdf

Strumentazione

Tecniche di ionizzazione

EI, CI

FAB

MALDI

ESI

Etc.

Analisi della massa

Strumenti a settore magnetico

Trappole ioniche quadrupolari

Analizzatori a tempo di volo

FT-ICR, orbitrap

Type	Resolving Power (FWHM)
FT-ICR-MS	1,000,000
FT-Orbitrap	100,000
High-Res-TOF	60,000
TOF	10,000
Quadrupole / IonTrap in UltraZoom mode	10,000
Quadrupole / Iontrap	1,000

Sistemi di introduzione del campione

attraverso un sistema cromatografico

GC-MS

LC-MS

su sonda direttamente nella sorgente di ionizzazione, etc

Capitolo 4.3

Magnetic Sector Instruments

da pag. 130

In *Mass Spectrometry: a Textbook*

The Lorentz Force Law can be used to describe the effects exerted onto a charged particle entering a constant magnetic field. The Lorentz Force F_L depends on the velocity v , the magnetic field B , and the charge q of an ion. In the simplest form the force is given by the scalar equation

$$F_L = qvB$$

This is valid if v and B (both are vectors) are perpendicular to each other.

*An ion of mass m and charge q travelling at a velocity v in a direction perpendicular to a homogeneous magnetic field will follow a circular path of radius r_m that fulfills the condition of equilibrium of F_L and **centripetal force** F_c*

$$F_L = qvB = \frac{m_i v^2}{r_m} = F_c$$

Upon rearrangement we obtain the radius r_m of this circular motion

$$r_m = \frac{m_i v}{qB}$$

This shows the working principle of a magnetic sector the radius r_m depends on the momentum mv of an ion, and therefore the momentum depends on m/z .

Thereby, the former potential energy of a charged particle in an electric field is converted into kinetic energy E_{kin} , i.e., into translational motion

$$E_{el} = ezU = \frac{1}{2} m_i v^2 = E_{kin} \quad (4.2)$$

Assuming the ion was at rest before, which is correct in a first approximation, the velocity attained is obtained by rearranging Eq. 4.2 into

$$v = \sqrt{\frac{2ezU}{m_i}} \quad (4.3)$$

Finally, dispersion in momentum causes a dependence of r_m on the square root of mass becoming obvious by substitution of v

~~$$r_m = \frac{m_i}{ezB} \sqrt{\frac{2eU}{m_i}} = \frac{1}{zB} \sqrt{\frac{2m_i U}{e}}$$~~

$$r_m = \frac{m_i}{ezB} \sqrt{\frac{2ezU}{m_i}} = \frac{1}{B} \sqrt{\frac{2m_i U}{ze}}$$

Alternatively, the ratio m_i/q can be expressed as

$$\frac{m_i}{q} = \frac{r_m B}{v}$$

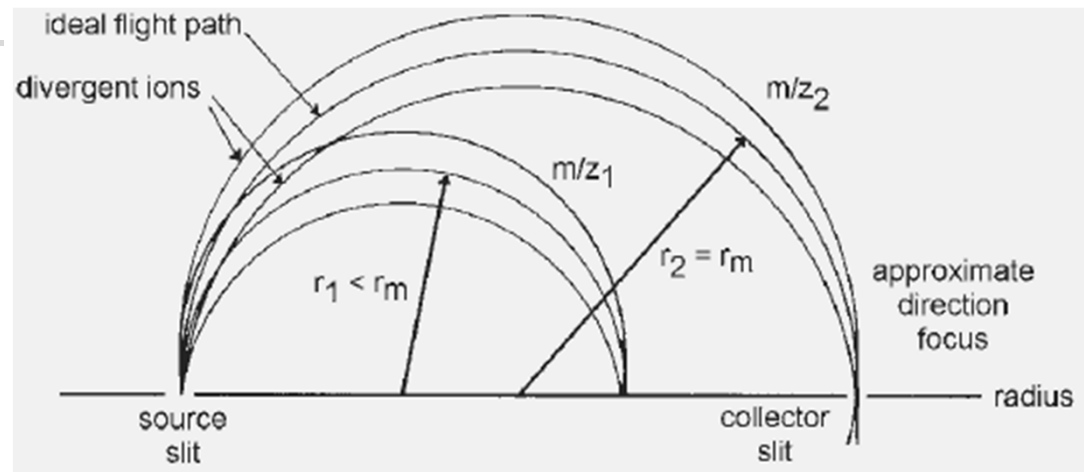
which upon substitution of v as performed above becomes

$$\frac{m_i}{q} = \frac{r_m B}{\sqrt{\frac{2qU}{m_i}}} \Rightarrow \frac{m_i}{q} = \frac{r_m^2 B^2}{2U}$$

This has been formerly known as the *basic equation of mass spectrometry*.

There is *no more justification for a single basic equation* of mass spectrometry because of the various mass analyzers employed.

The focusing action of a homogeneous magnetic field on a beam of ions having the same m/z and the same kinetic energy can best be illustrated by a 180° sector (Fig. 4.18). If the beam is divergent by a half-angle α , the collector slit must be $\alpha^2 r_m$ wide to pass all ions after suffering 180° deflection. This is because the ions come to a first order, i.e., imperfect, focus as they all traverse the magnetic field at the same radius but not all of them entered the field at right angles.



Ions of different m/z fly at a different radius, e.g., the lighter ions of m/z_1 hit the wall while ions of m/z_2 reach the collector slit. To allow for detection of various masses, such an analyzer could either be equipped with a **photographic plate** in the focal plane to become a so-called *mass spectrograph*, **or** it could be designed with **variable magnetic field** to detect different masses at the same point by bringing them subsequently at the collector slit.

Energia cinetica

Lavoro di estrazione

$$e\Delta V = \frac{1}{2}mv^2$$

Forza centrifuga

Forza di Lorentz

$$ma = \frac{mv^2}{R} = qvB$$

$$\frac{m}{e} = \frac{B^2 R^2}{2\Delta V}$$

Per un certo valore della coppia B e V , esisterà un solo valore di massa m per cui il raggio di deflessione r coincide con il raggio di curvatura del tubo R . Di conseguenza gli ioni che hanno questo valore di massa escono dal tubo, gli altri no.

Operando a potenziale V costante e facendo una scansione di campo B è possibile fare uscire dal tubo gli ioni a diversa massa in tempi diversi.

the 180° design demands for large and heavy magnetic sectors. An optimized magnetic sector alone can provide resolutions of $R = 2000\text{--}7000$ depending on its radius. The ***limitation arises from the fact that ions emerging from the ion source are not monoenergetic.*** Ions of different m/z can obtain the same momentum and thus cause overlap of adjacent ion beams at the detector.

- **Example:** Equation 4.13a describes the radius r_m in the magnetic field. Obviously, the value r_m remains constant as long as $m_i U = \text{const}$. If the instrument is set to pass an ion of say m/z 500 and 3000 eV kinetic energy, it will simultaneously allow the passage for ions of m/z 501 having 2994 eV or of m/z 499 having 3006 eV of kinetic energy.

This is why ***obtaining higher resolution requires small kinetic energy distributions.***

The *electrostatic sector* or *electrostatic analyzer* (ESA) produces a **radial electric field** between two oppositely charged plates extending over the ESA angle Φ . An ion passes the ESA midway on a circular path if

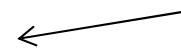
$$F_e = qE = \frac{m_i v^2}{r_e} = F_c$$

F_e = electric force, E = electric field strength, r_e the radius of the ESA.

ESA acts as an energy dispersive device

$$r_e = \frac{m_i v^2}{qE} = \frac{m_i v^2}{ezE}$$

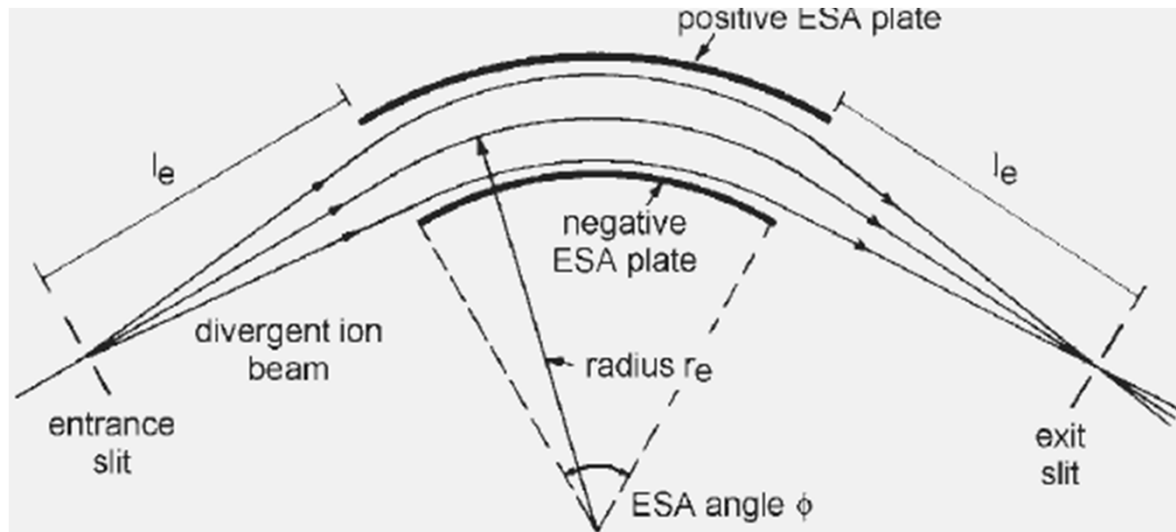
$$v = \sqrt{\frac{2ezU}{m_i}}$$



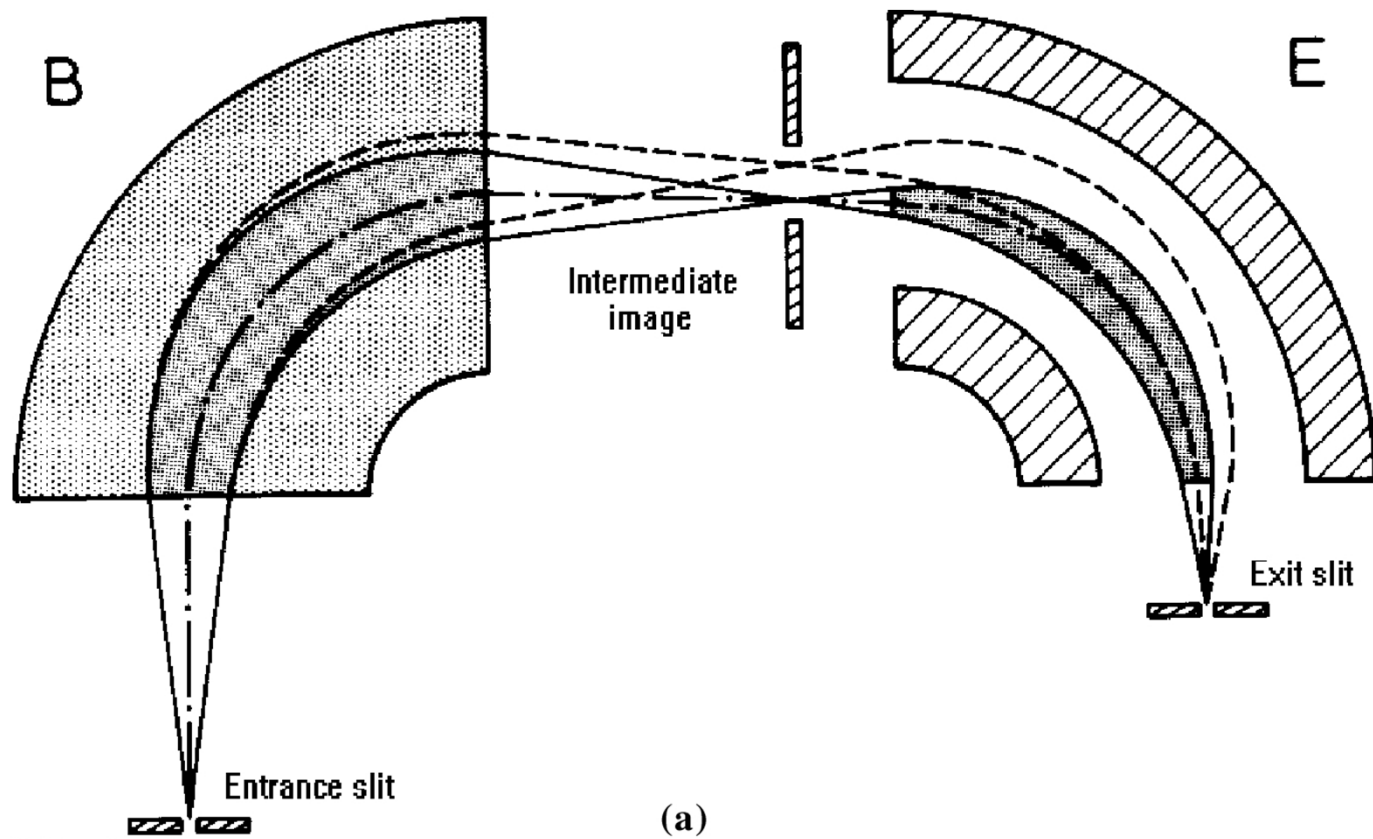
Thus, the **kinetic energy distribution of an ion beam can be reduced.**

The following simple relationship describes the radius of the ESA.

$$r_e = \frac{2U}{E}$$

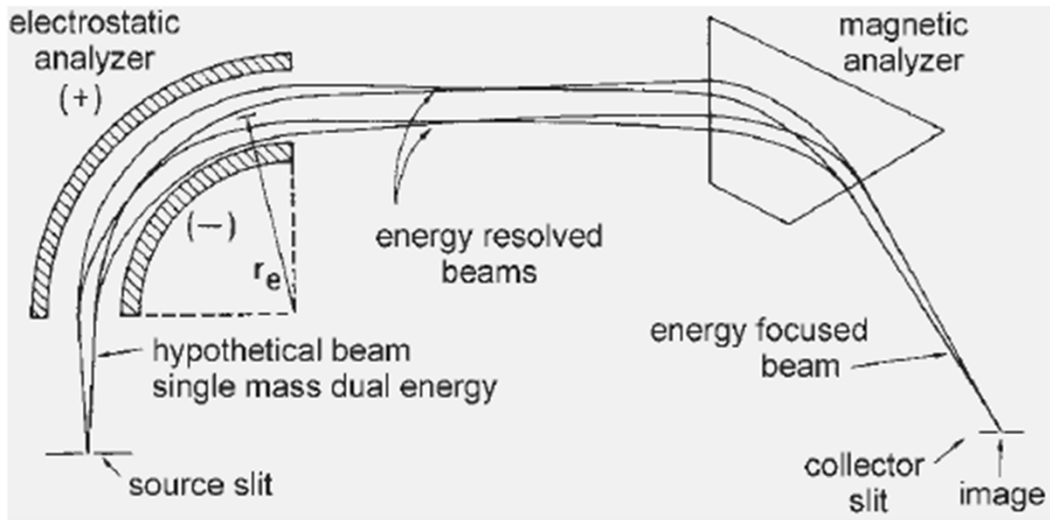


Imagine an *ion drifting towards the outer plate having the same charge sign as the ion*. As it approaches the plate *it is decelerated by the opposed electric field and finally reflected towards the center of the beam*. With its radial component of v inverted it crosses the ideal path at le . In an analogous fashion an *ion approaching the inner plate becomes accelerated by the attractive force*. The resulting higher velocity causes an *increase in centripetal force, and thereby effects **a correction of the flight path in the appropriate sense.***



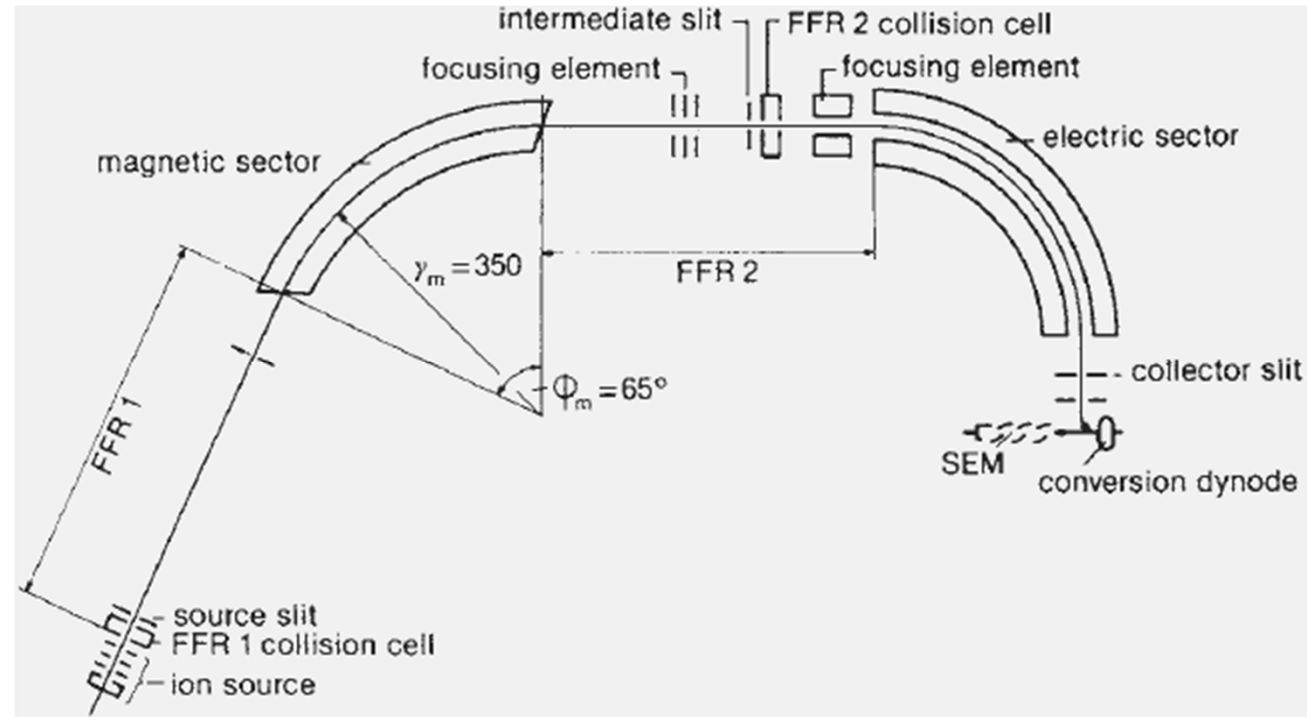
© 2006 Wiley-VCH, Weinheim
 Kellner / Analytical Chemistry
 ISBN: 3-527-30590-4 Fig-25-04-07a

(a)



Configurazione diretta EB
 $R > 10000$

Configurazione
 Inversa BE
 $R > 70000$



- La separazione di ioni con diverso rapporto m/z si può conseguire con :
la variazione del raggio di curvatura che porterà gli ioni ad esser separati nello spazio, mentre
la variazione di B o V porterà gli **ioni con diverso m/z ad esser separati nel tempo** (i.e. possono esser rilevati uno dopo l'altro da un detector a posizione fissa dietro una fenditura. La risoluzione dipende dalle fenditure in ingresso a B ed in uscita al detector (compromesso selettività/sensibilità).
- Prestazioni in termini di risoluzione di massa sono migliorabili con **analizzatore elettrostatico (ESA)**, che focalizza gli ioni con ugual m/z ma diversa energia cinetica (si migliora la risoluzione senza perder segnale) → **strumenti a doppia focalizzazione (HR, masse accurate)**.
- Elevato range di masse, alta risoluzione, buona capacità di quantificazione... ma costi alti.

Es.

[Mass Spectrometry Instruments & Equipment](#) › [Mass Spectrometry Systems & Components](#) › DFS™ Magnetic Sector GC-HRMS System

DFS™ Magnetic Sector GC-HRMS System

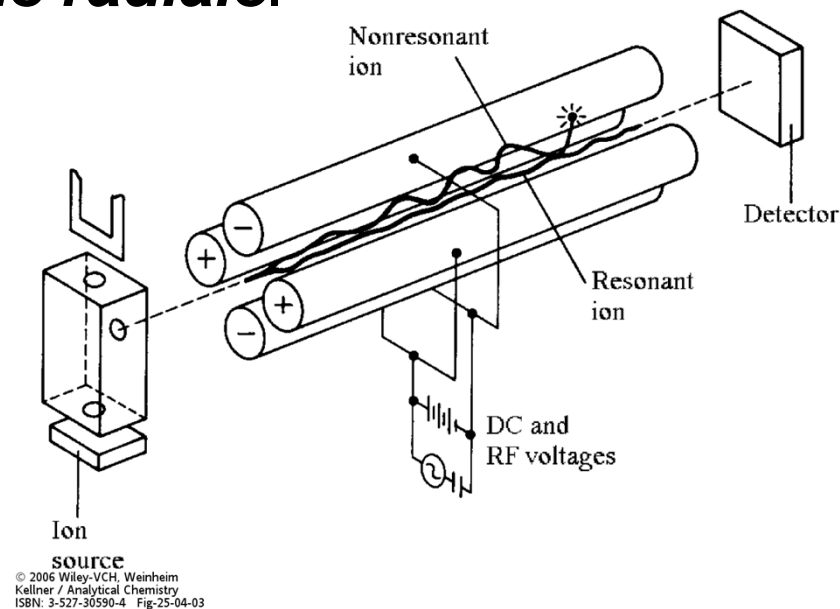


Thermo Scientific™ Related applications: [Industrial Mass Spectrometry](#)

Achieve outstanding sensitivity for analysis of dioxins and persistent organic pollutants (POPs) with the Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS system. The DFS double-focusing system is the highest performance mass spectrometer ever built for target compound analysis. Injecting as little as 20fg of tetrachlorodibenzo-p-dioxin (TCDD) onto the column generates an exceptional signal/noise ratio (greater than 200:1). A product resulting from 50 years experience developing magnetic sector MS, the DFS system has all the performance you need, with a footprint that fits in a small laboratory.

Analisi di massa quadrupolo

Ioni generati dalla sorgente ionica sono estratti elettrostaticamente e introdotti in un analizzatore di massa (filtro) a quadrupolo. È un dispositivo che consiste di **quattro barre di acciaio con sezione circolare o iperboliche, posizionate parallelamente con disposizione radiale**.



QuickTime™ and a decompressor are needed to see this picture.

**Capitolo 4.4 Linear Quadrupole
Instruments da Pag 145**
In Mass Spectrometry: a Textbook

“Since the Nobel Prize-awarded discovery of the **mass-analyzing and ion-trapping properties of two- and three-dimensional electric quadrupole fields** [103,104] and the concomitant construction of a *quadrupole (Q) mass spectrometer*, this type of instrument has steadily gained importance.

Starting from GC-MS applications, where rapid scanning devices were required, quadrupole analyzers made their way into the MS laboratories, although the early systems offered poor resolving power and low mass range, e.g., m/z 1–200.

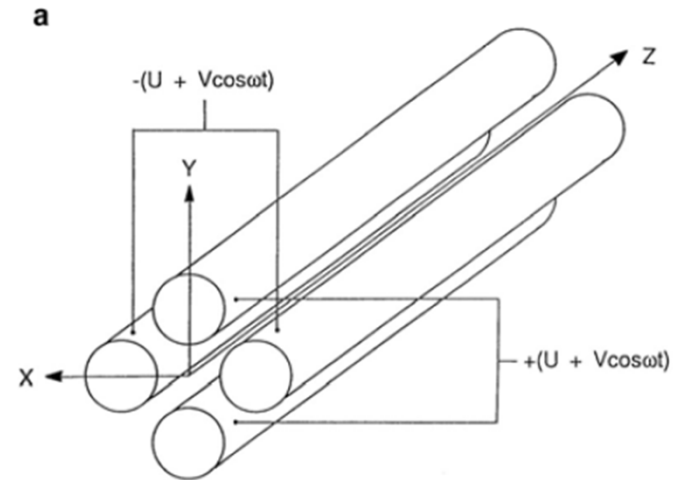
Modern quadrupole instruments cover the m/z 2000–4000 range with good resolving power and represent some kind of standard device in LC-MS.

The **advantages** of quadrupoles are that they :

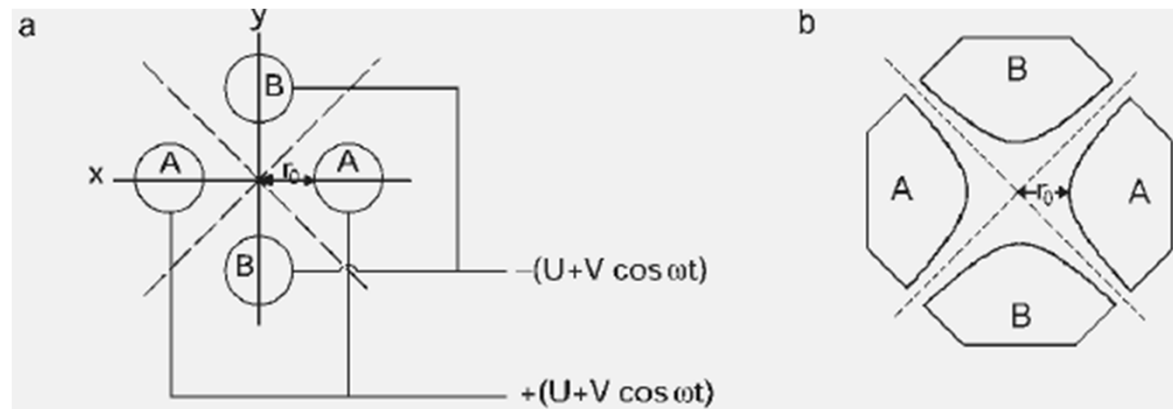
- i) have high transmission,
- ii) are light-weighted, very compact and comparatively low-priced,
- iii) have low ion acceleration voltages, and
- iv) allow high scan speeds, since scanning is realized by solely sweeping electric potentials.»

A linear quadrupole mass analyzer consists of four hyperbolically or cylindrically shaped rod electrodes extending in the z-direction and mounted in a square configuration (xy-plane).

The **pairs of opposite rods are each held at the same potential** which is composed of a DC and an AC component.



Cross section of a quadrupole **(a)** for the cylindrical approximation and **(b)** for the hyperbolic profile of the rods. The **electric field is zero along the dotted lines, i.e., along the asymptotes in (b)**.



r_0

As an **ion enters the quadrupole assembly in z-direction**, an attractive force is exerted on it by one of the rods with its charge actually opposite to the ionic charge. **If the voltage applied to the rods is periodic, attraction and repulsion in both the x- and y-directions are alternating in time, because the sign of the electric force also changes periodically in time.**

If the applied voltage is composed of a DC voltage U and a radiofrequency (RF) voltage V with the frequency ω **the total potential Φ_0** is given by

$$\Phi_0 = U + V \cos \omega t$$

Thus, the equations of motion are

$$\frac{d^2 x}{dt^2} + \frac{e}{m_i r_0^2} (U + V \cos \omega t) x = 0$$

$$\frac{d^2 y}{dt^2} - \frac{e}{m_i r_0^2} (U + V \cos \omega t) y = 0$$

The electric field is zero along the asymptotes in case of the hyperbolic electrodes. It is therefore *possible that **an ion may traverse the quadrupole without hitting the rods, provided its motion around the z axis is stable with limited amplitudes in the xy-plane.*** Such conditions can be derived from the theory of the **Mathieu equations**, as this type of differential equations is called.

Scrivendo le equazioni in forma adimensionale, si ottiene

$$\frac{d^2 x}{d\tau^2} + (a_x + 2q_x \cos 2\tau)x = 0$$

$$\frac{d^2 y}{d\tau^2} + (a_y + 2q_y \cos 2\tau)y = 0$$

In cui i parametri a e q sono

$$a_x = -a_y = \frac{4eU}{m_i r_0^2 \omega^2}, \quad q_x = -q_y = \frac{2eV}{m_i r_0^2 \omega^2}, \quad \tau = \frac{\omega t}{2}$$

For a given set of U , V and ω the overall ion motion can result in a stable trajectory causing ions of a certain m/z value or m/z range to pass the quadrupole.

Ions oscillating within the distance $2r_0$ between the electrodes will have stable trajectories. These are transmitted through the quadrupole and detected thereafter.

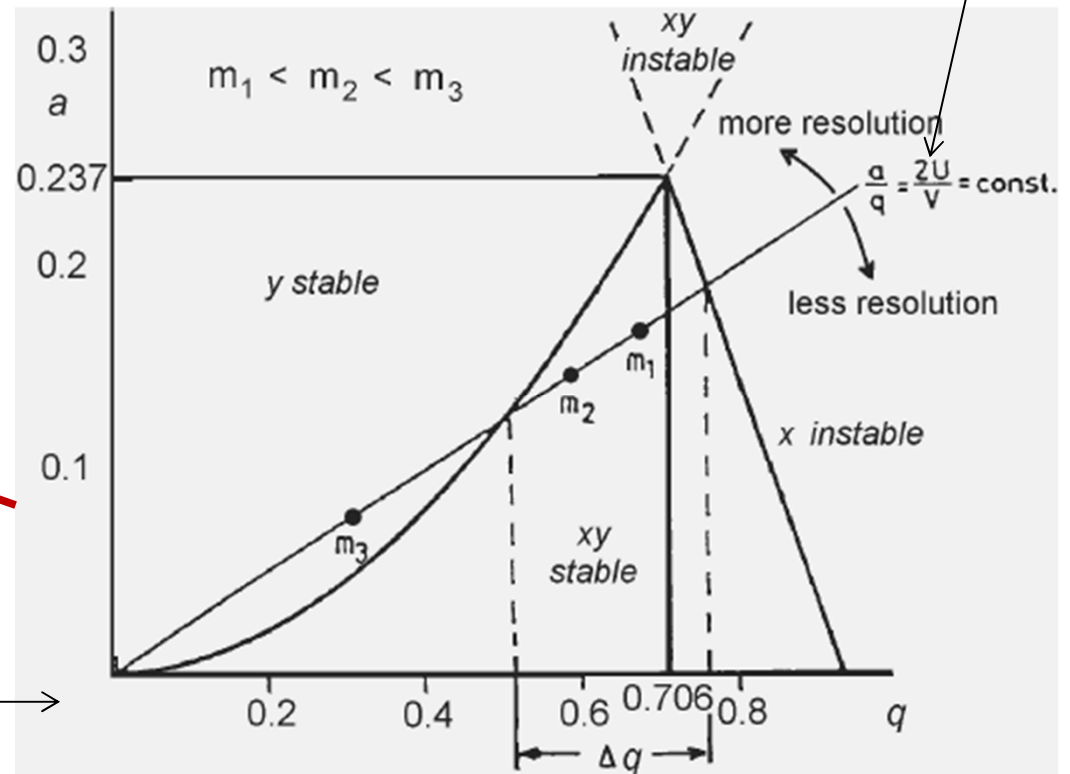
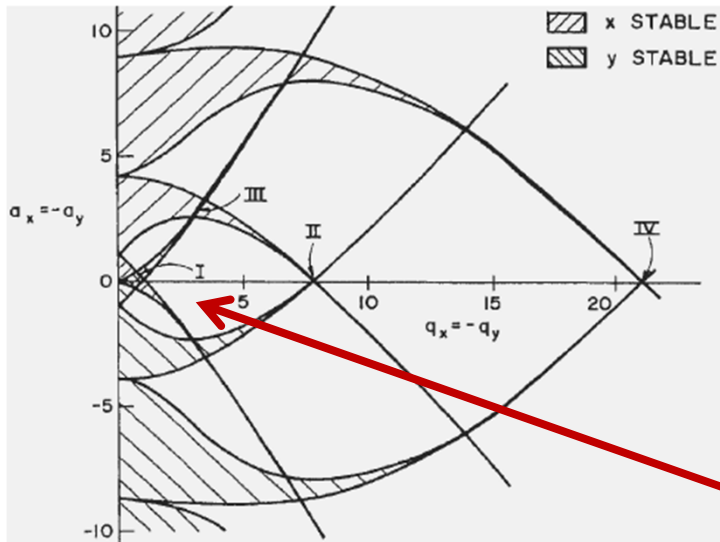
The path stability of a particular ion is defined by the magnitude of the RF voltage V and by the ratio U/V .

By plotting the parameter a (ordinate, time invariant field) versus q (abscissa, time variant field) one obtains the **stability diagram of the two-dimensional quadrupole field**. This reveals the existence of regions where

- i) both x - and y trajectories are stable, either
- ii) x - or (iii) y - trajectories are stable, and
- iv) no stable ion motion occurs

- region I is of special interest for the normal mass-separating operation of the linear quadrupole

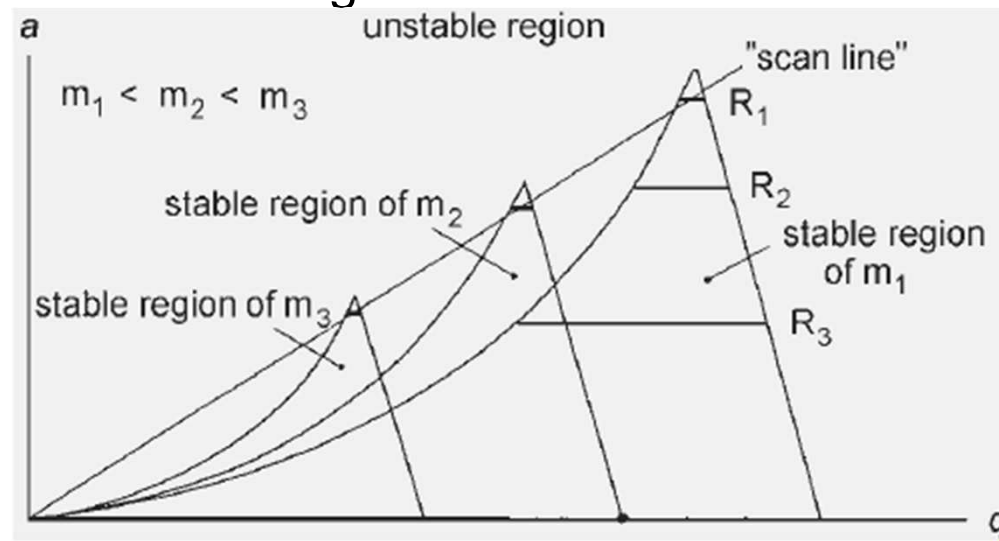
Per questo valore di a/q
Il filtro fa passare sia m_1 che m_2



Detail of the upper half of region I of the stability diagram for a linear quadrupole analyzer.

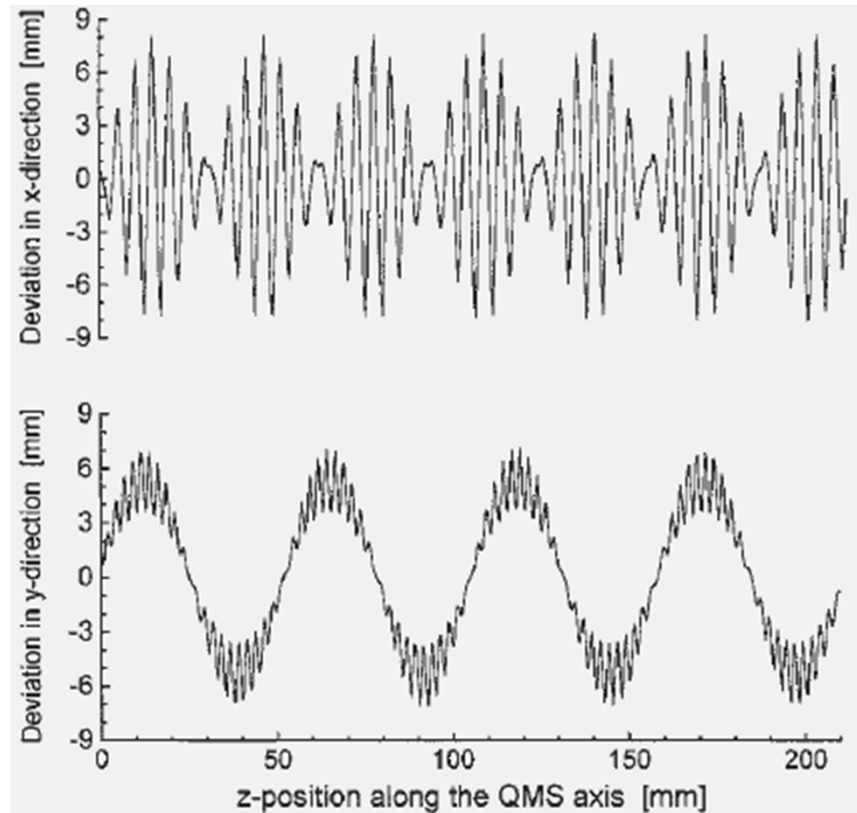
If the **ratio a/q** is chosen **so that $2U/V = 0.237/0.706 = 0.336$** , **the xy -stability region shrinks to one point**, the apex, of the diagram (cf. Eq. 4.26, Fig. 4.34). By reducing a at constant q , i.e., reducing U relative to V , an increasingly wider m/z range can be transmitted simultaneously. **Sufficient resolution is achieved as long as only a small m/z range remains stable**, e.g., one specific $m/z \pm 0.5$ for *unit resolution*.

Thus, the width (Δq) of the stable region determines the resolution.



By **varying the magnitude of U and V at constant U/V ratio, an $U/V = \text{constant}$ linked scan is obtained** allowing ions of increasingly higher m/z values to travel through the quadrupole.

Overall, the quadrupole analyzer rather acts as a **mass filter** than as a momentum (B sector) or energy (ESA) spectrometer; hence the widespread use of the term **quadrupole mass filter**.



Ion trajectory simulations allow for the visualization of the ion motions while travelling through a quadrupole mass analyzer.

Projection of a 3D trajectory simulation of a stable ion onto the x- and y coordinate.

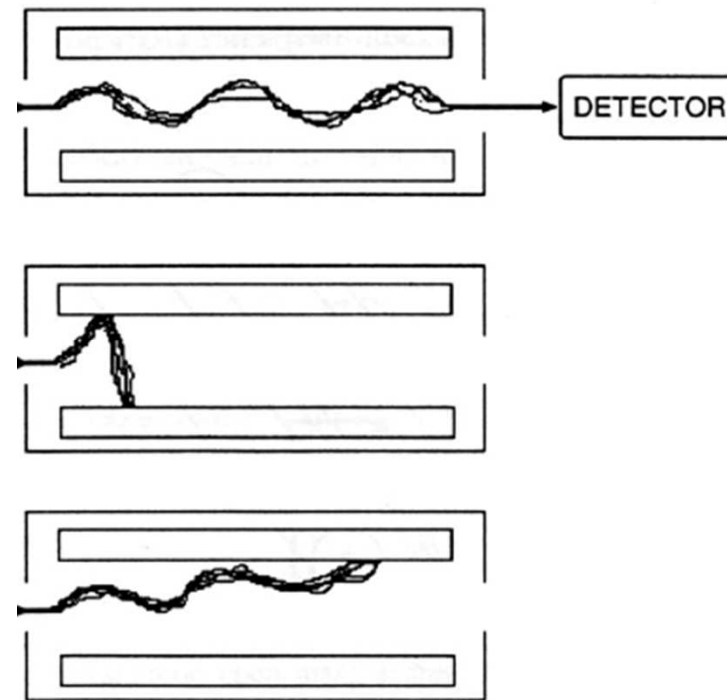
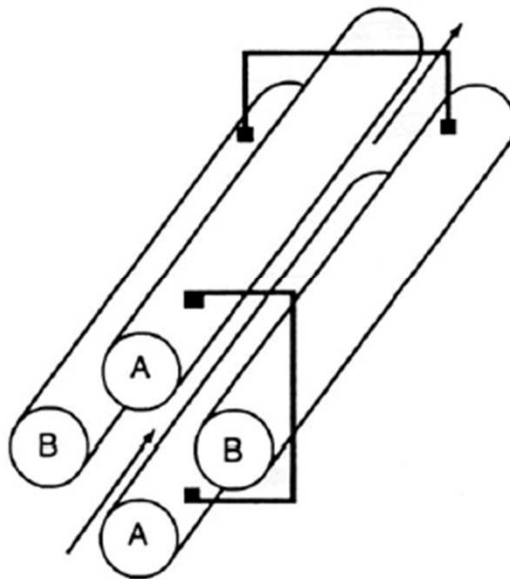


The optimum number of oscillations to achieve a certain level of performance can be determined (best performance is obtained when ions of about 10 eV kinetic energy undergo a hundred oscillations).

Note: Standard quadrupole analyzers have rods of 10–20 mm in diameter and 15–25 cm in length. The radiofrequency is in the order of 1–4 MHz, and the DC and RF voltages are in the range of some 10^2 – 10^3 V. Ions of about 10 eV kinetic energy undergo approximately 100 oscillation during their passage

- Le traiettorie degli ioni di un particolare m/z sono stabili e riescono ad attraversare il quadrupolo fino al detector, mentre per le altre le oscillazioni si amplificano e collidono sulle barre.

- Quadruple analyzer



Conceptual explanation of the way a
Quadrupole Mass Filter works.

<https://www.youtube.com/watch?v=aYOCb6GnXio>

0

<https://www.youtube.com/watch?v=qxPb9vFWdqo>

Trappole ioniche quadrupolari

Filtri a quadrupolo

Risoluzione sull'unità di massa fino a circa 2000 Da,

Facilità operativa,

Scansioni veloci,

Costi contenuti

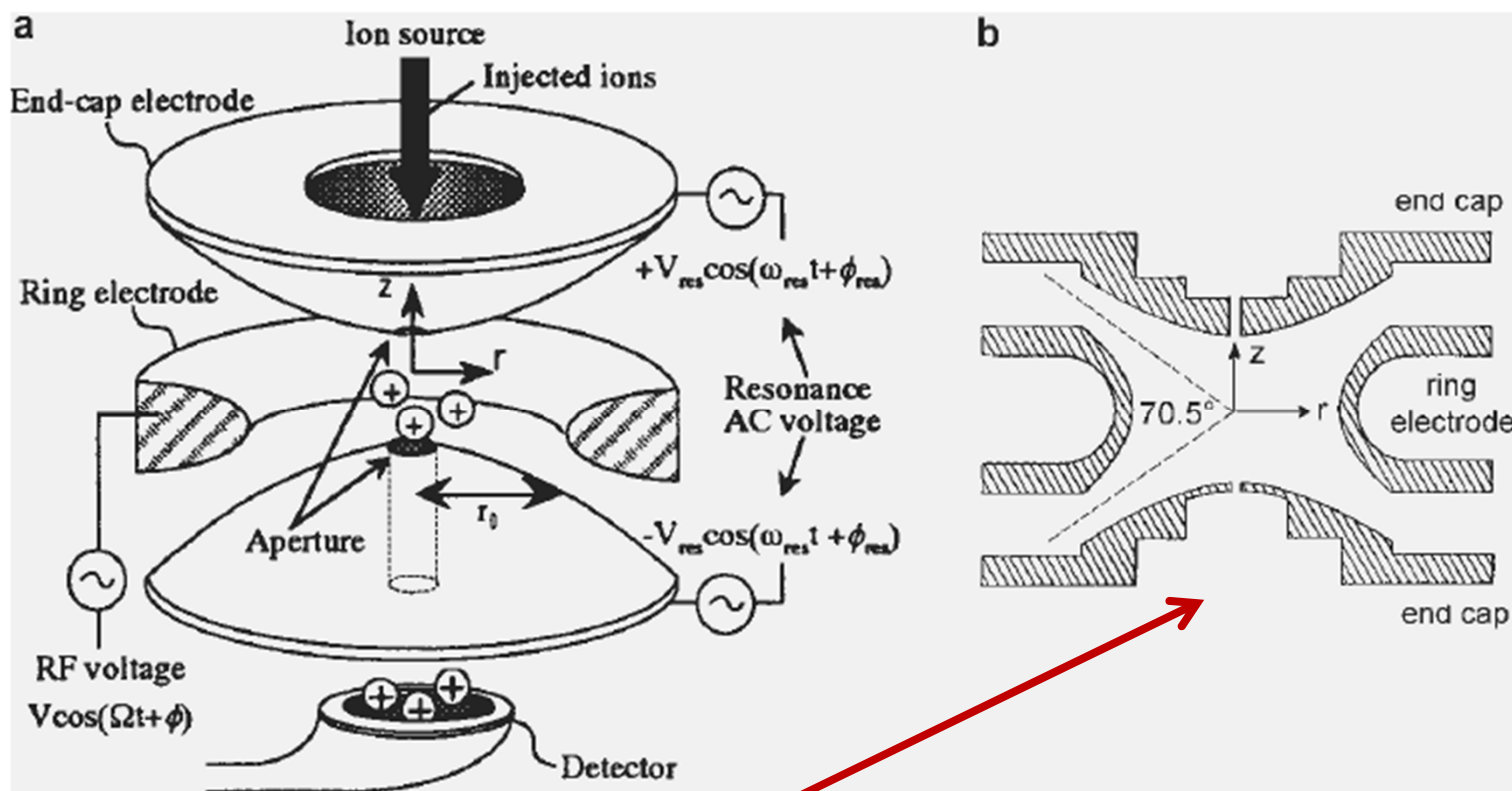
Sviluppo: trappola ionica quadrupolare tridimensionale

Trappola ionica: un elettrodo ad anello cilindrico su cui è applicato un campo quadrupolare, con due elettrodi *end cap*.

Trappola (o gabbia) ionica quadrupolare
Ion Trap

Mass spectrometry: an introduction p.155

The *quadrupole ion trap* (QIT) creates a **three-dimensional RF quadrupole field to store ions within defined boundaries**. Its invention goes back to 1953, however, it took until the mid-1980s to access the full analytical potential. Modern QITs cover ranges up to about m/z 3000 with fast scanning at unit resolution, and in addition, offer “zoom scans” over smaller m/z ranges at higher resolution.

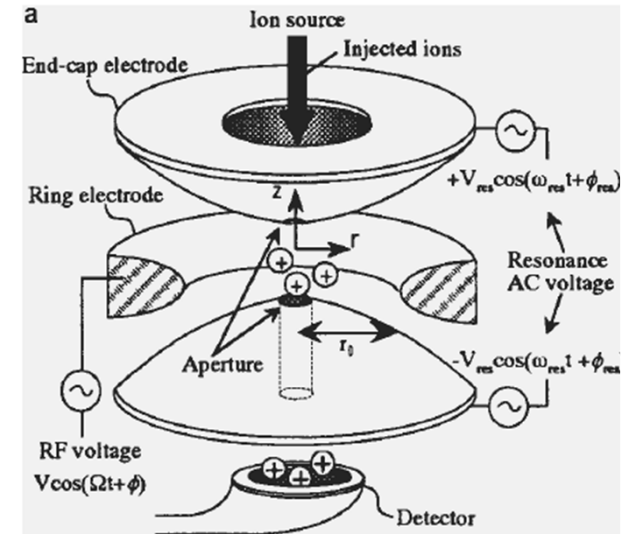
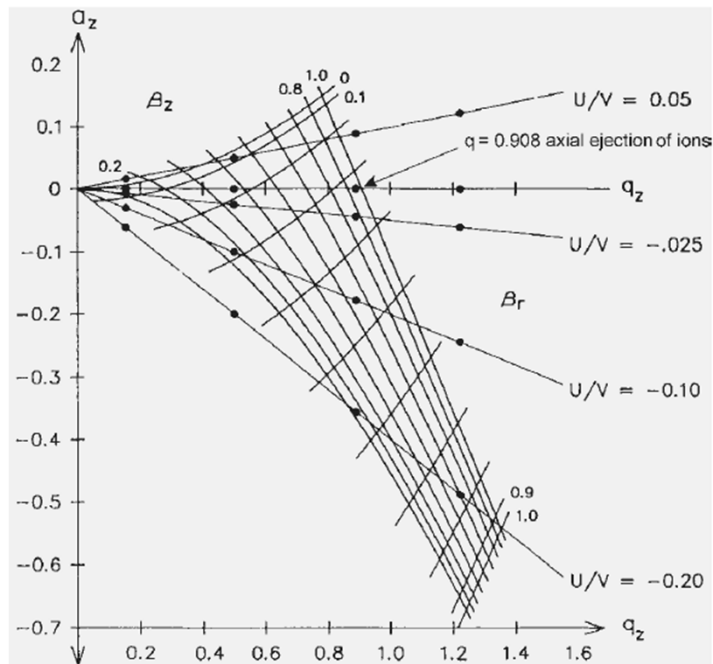


a section through the rz -plane of the QIT closely resembles that of the entrance of linear quadrupole with hyperbolic rods

The end caps are electrically connected and the DC and RF potentials are applied between them and the ring electrode. The **working principle** of the QIT is based on *creating stable trajectories for ions of a certain m/z or m/z range while removing unwanted ions by colliding them with the walls or by axial ejection from the trap due to their unstable trajectories.*

For the QIT, the electric field has to be considered in three dimensions.

To remain stored in the QIT, an **ion** has to be *simultaneously stable in the r and z directions.*



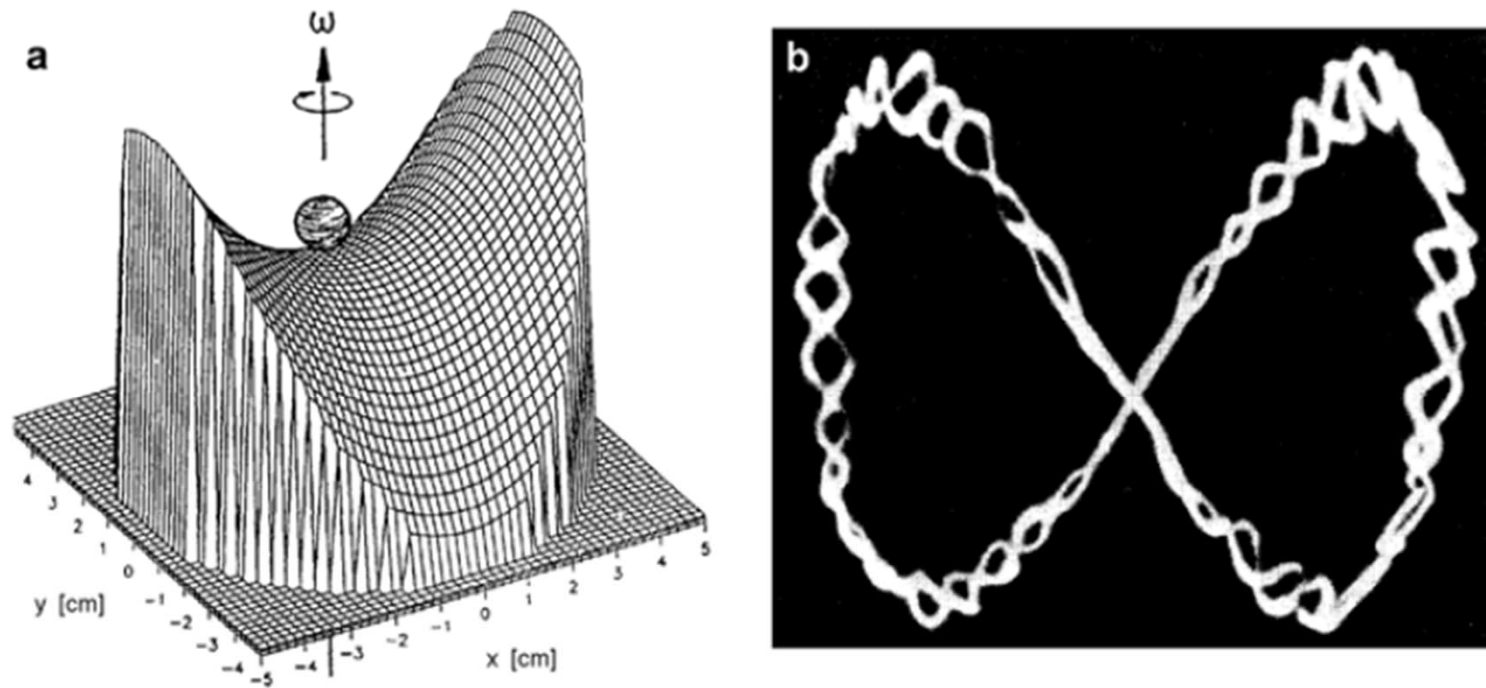
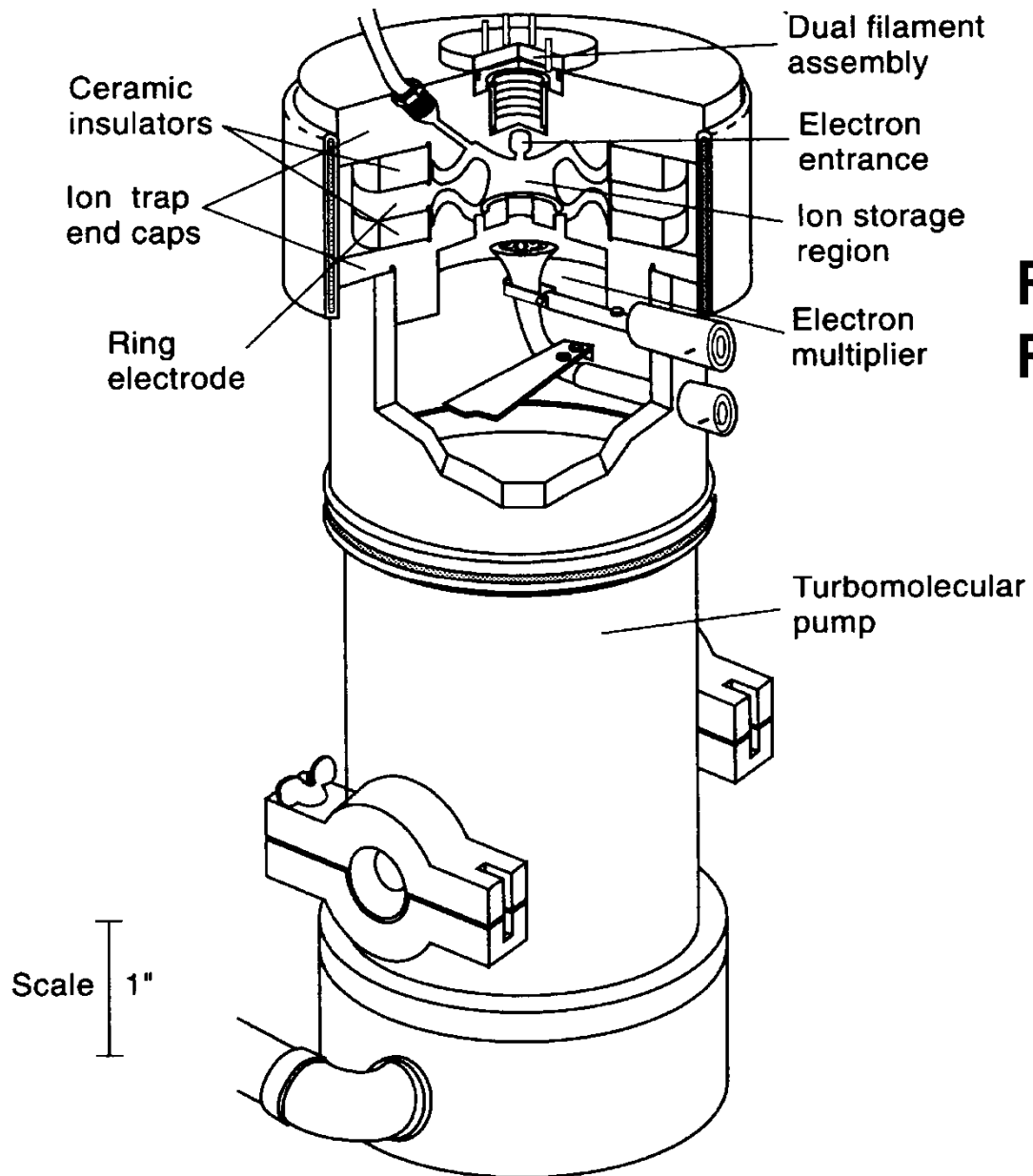


Fig. 4.43. Visualization of ion motion in the ion trap. (a) Mechanical analogue of the QIT. (b) Photograph of ion trajectories of charged aluminum particles in a quadrupole ion trap.

1. First, ***the full m/z range of interest is trapped within the QIT.*** The trapped ions may either be created inside the QIT or externally.
2. Then, with the end caps grounded an ***RF-voltage scan (V) is applied to the ring electrode causing consecutive ejection of ions in the order of their m/z values.*** This is known as *mass-selective instability (ejection)* mode.

QUADRUPOLE ION TRAP

GC sample inlet

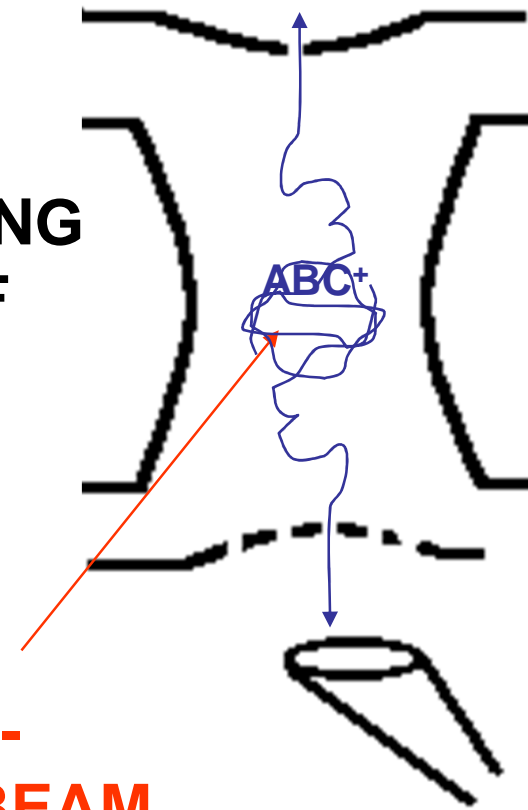


END CAPS

RING
RF

e-
BEAM

DETECTOR



Trappole ioniche quadrupolari

L'end-cap superiore contiene fori per l'introduzione di ioni o elettroni nella trappola, mentre l'end-cap inferiore contiene fori per gli ioni che vengono espulsi all'elettromoltiplicatore. Gli ioni che si formano nella trappola o che sono generati da una sorgente esterna, sono immagazzinati nella trappola. Aumentando il potenziale con radiofrequenza, le traiettorie degli ioni a m/z successivi sono resi instabili e espulsi dalla trappola, e rilevati dall'elettromoltiplicatore (si rilevano gli ioni instabili!!!)

Spettrometria di massa in tandem

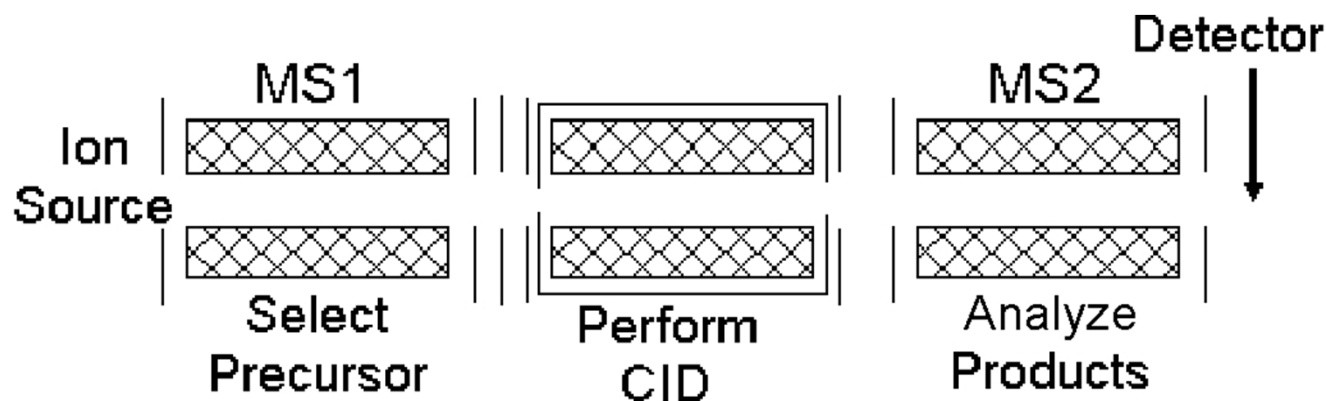
Negli studi sulla struttura delle molecole, spesso si vogliono maggiori informazioni sugli ioni generati nel processo di ionizzazione. Ciò vale in particolare per le ionizzazioni “soft”. Un aumento dell’energia interna di uno ione generato con una ionizzazione soft, porterà alla frammentazione di questi ioni. Si può ottenere ciò in diversi modi; ad esempio con attivazione per collisione con molecole di gas neutro (**collision-induced dissociation, CID**), mentre altri approcci prevedono la fotodissociazione indotta da laser o da superfici. A seguito dell’aumento di energia interna, si ha frammentazione unimolecolare



M_p^+ è lo ione precursore, m_d^+ lo ione prodotto e m_n rappresenta molecole neutre. Avviene fuori dalla sorgente di ioni. Alcuni frammenti si formano nella sorgente, altri nel percorso accelerato verso il detector (processo probabilistico)

Spettrometria di massa in tandem

Nel caso di una collision-induced dissociation, CID, la frammentazione è indotta in una cella di collisione in cui c'è una pressione più alta. Configurazione strumentale:



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Kellner / Analytical Chemistry
ISBN: 3-527-30590-4 Fig-25-04-09

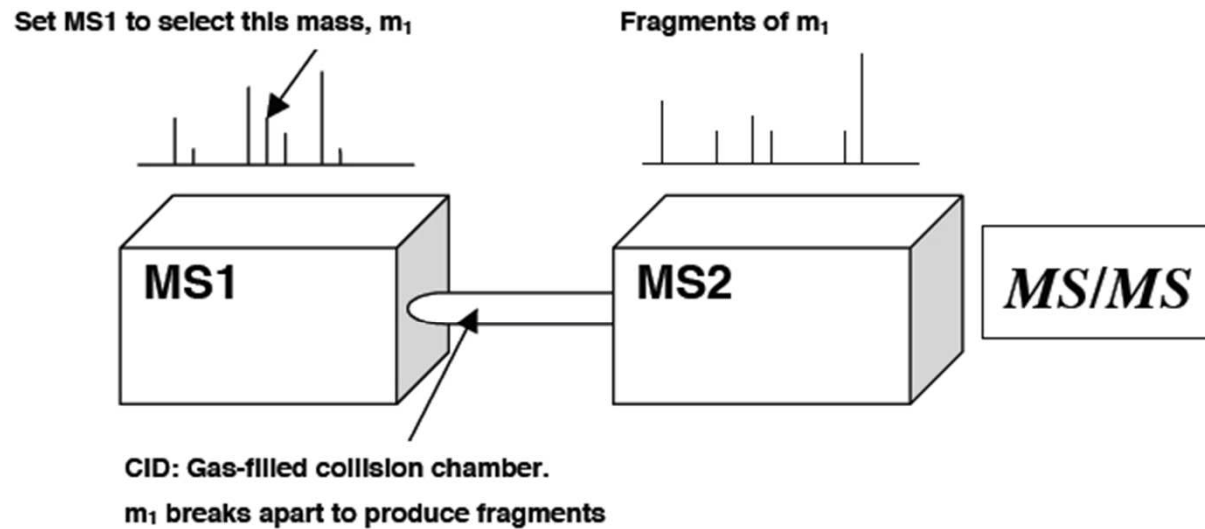
Due analizzatori di massa : uno per selezionare il precursore tra tutti gli ioni, ed uno per analizzare gli ioni prodotti a seguito delle collisioni (è un tandem...)

Strumentazione per MS-MS

I più comuni:

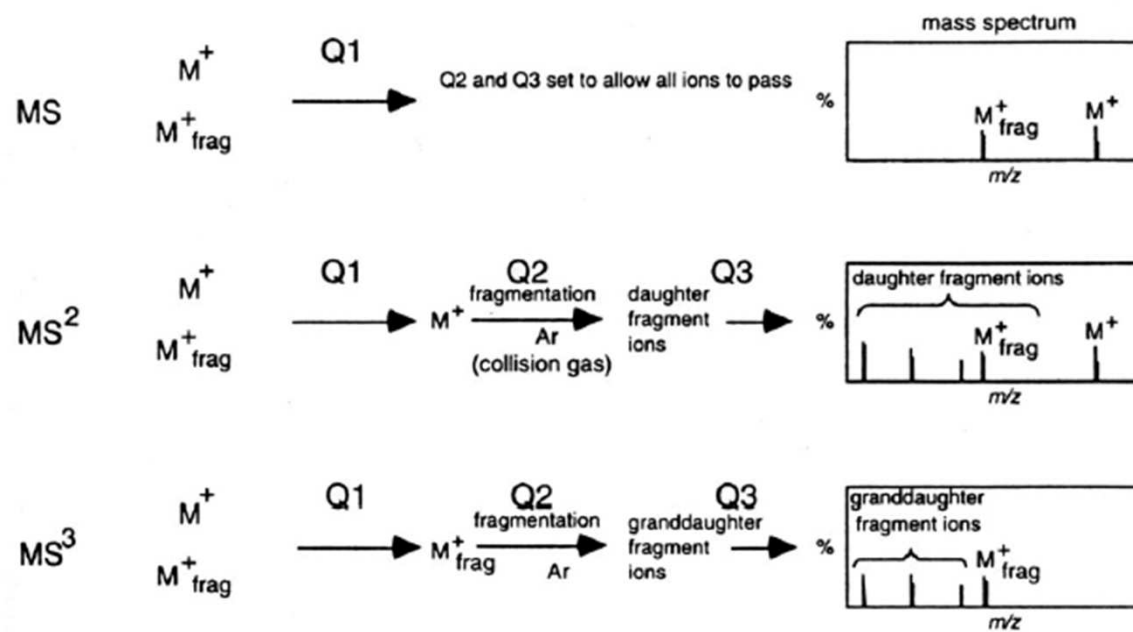
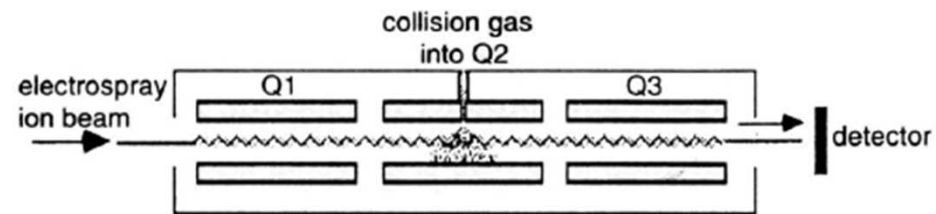
- Q-Q-Q (triplo quadrupolo, Q- q_{coll} -Q)
- Quadrupole ion trap (seleziona nella trappola ione con specifiche m/z , eccito con impulso RF lo ione selezionato e provocho dissociazione (CID); possibilità
- Q-TOF , (Q- q_{coll} -TOF, masse accurate ed eccellente sensibilità)

Spettrometria di massa in tandem



Simple Schematic of a Two Mass Spectrometer Tandem MS/MS

- Tandem mass spectrometry



Product-ion scan (m_1^+ specified)

If we do an MS/MS experiment to find out all of the product ions, m_2^+ that result from the decomposition of a specified parent ion m_1^+ , then this is called a **product-ion scan**. This is the most common and well-known MS/MS experiment. It is used to determine structurally significant fragment ions for a selected precursor ion.

Precursor-ion scan (m_2^+ specified)

If we perform an MS/MS experiment that tells us all of the possible precursor ions m_1^+ that decompose to produce a specified product ion m_2^+ , then this is called a **precursor-ion scan**. This is useful when you know that a particular product (fragment) ion mass is characteristic of a class of compounds, and you would like to identify the mixture components that belong to that compound class.

Mass-Selective Instability Mode

First, the full m/z range of interest is trapped within the QIT. The trapped ions may either be created inside the QIT or externally. Then, with the end caps grounded an RF-voltage scan (V) is applied to the ring electrode *causing consecutive ejection of ions in the order of their m/z values*. This is known as *mass-selective instability (ejection)* mode. Thus, they hit the detector located behind a hole in the center of one of the endcaps.

Altri modi di scansione

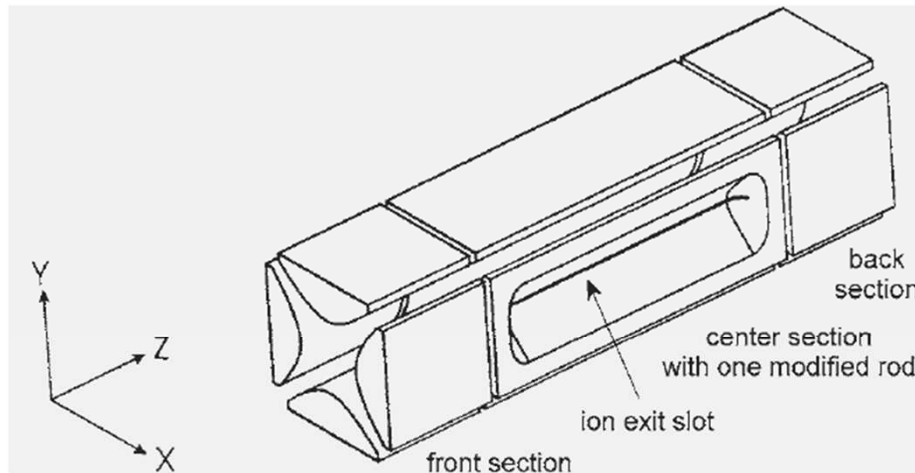
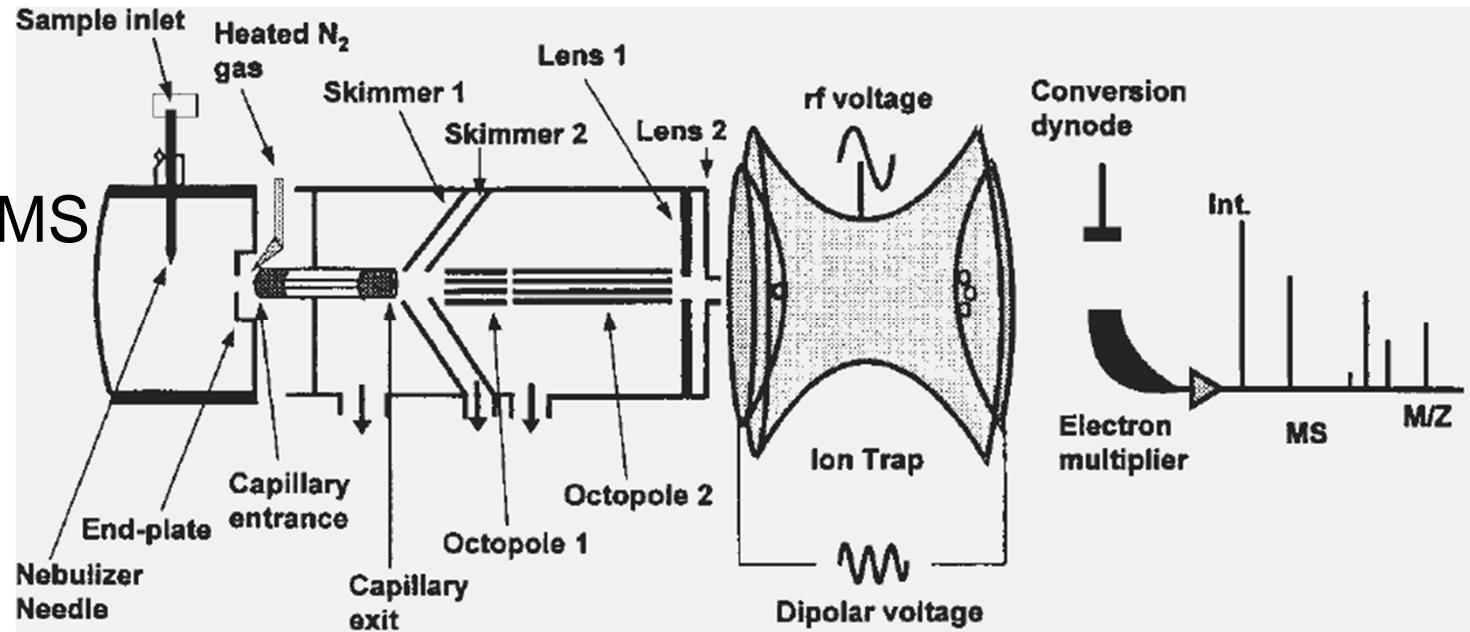
(Mass selective stability mode; lento e poco sensibile)

Resonant ejection

Axial modulation

<https://www.youtube.com/watch?v=3uUwa1DDoHQ>

LC-ESI-QIT-MS



Quadrupolo lineare
 Modificato per fungere
 Da trappola ionica

Fig. 4.39. Schematic of a linear quadrupole ion trap with scanning capability. Applying higher potential to the front and back sections creates a trapping potential for ions in the center section. Ions are exiting through the slot in one of the four rods. Adapted from Ref. [135] by permission. © Elsevier Science, 2002.