Espectrometria de Massas:

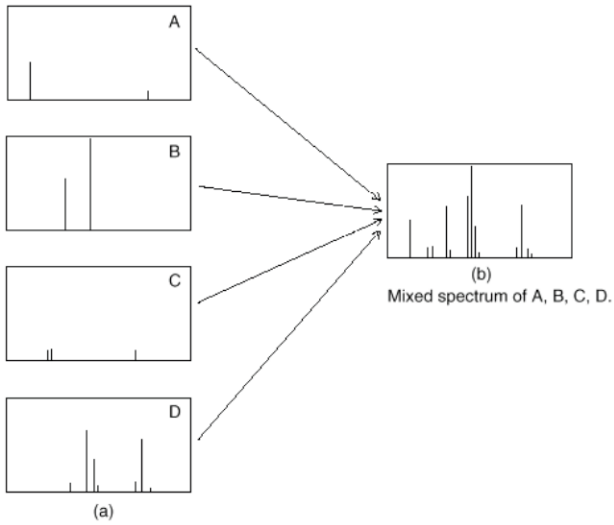
- Introdução
- Sistemas de vácuo
- Fontes de Ionização
- Analisadores de massa
  - Resolução e Exatidão de Massa
- Detectores
- MS/MS

Álvaro José dos Santos Neto

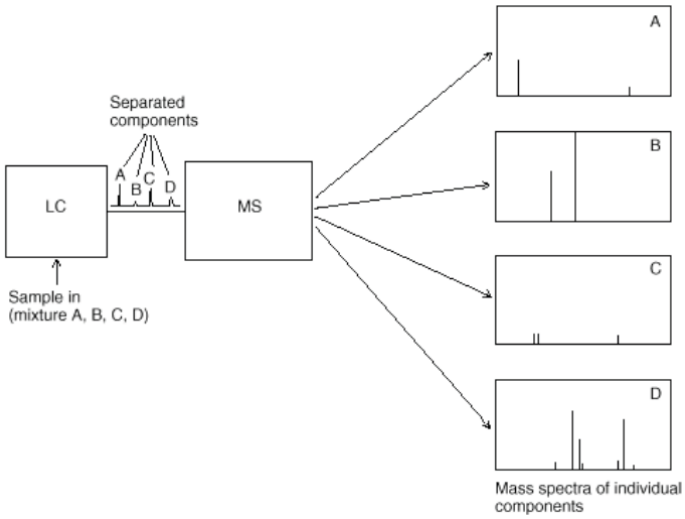
O que é a espectrometria de massas?

Como funciona a espectrometria de massas acoplada à cromatografia?

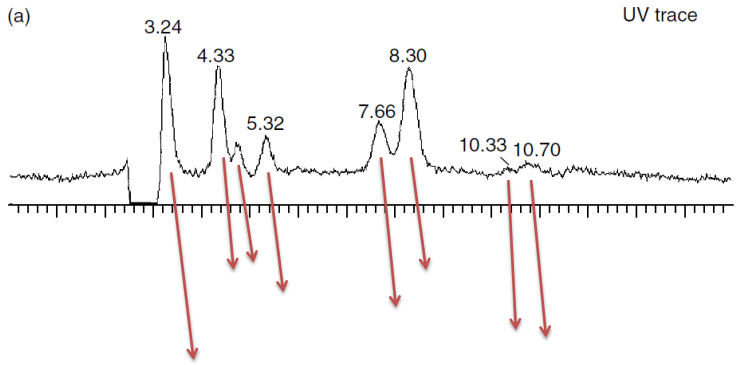
Misturas em MS



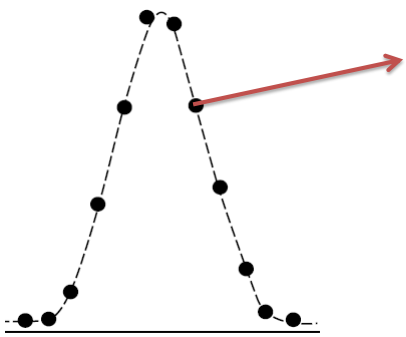
Combinando a cromatografia com a MS



Comparação entre cromatograma de LC-UV e LC-MS (modo TIC)



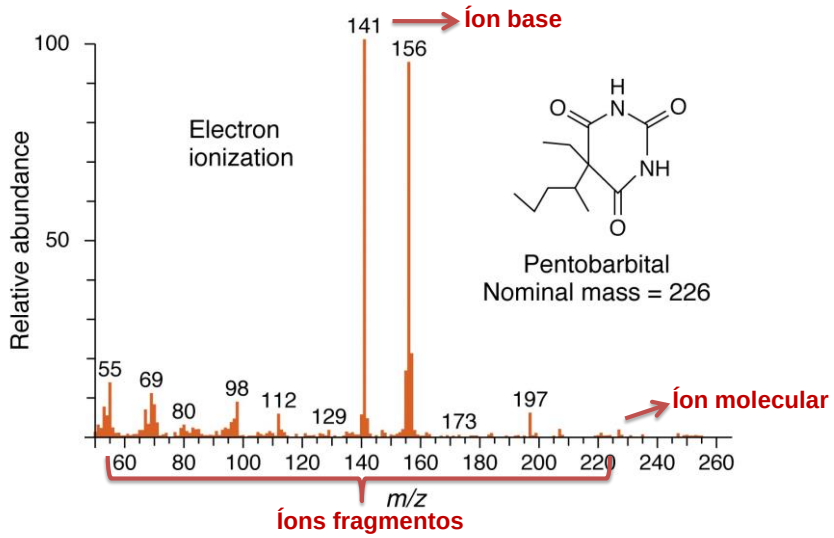
O pico cromatográfico em MS



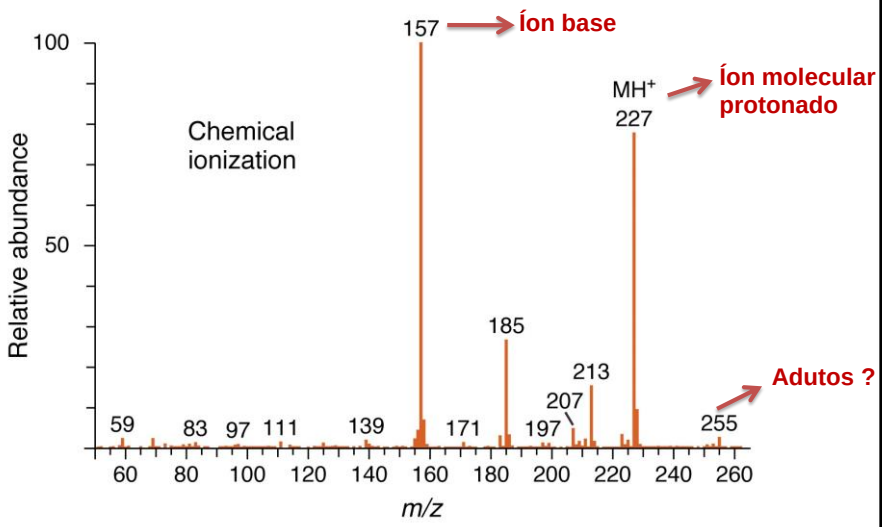
Contém toda a informação de MS:
- Espectro completo (TIC)
- Íons selecionados (SIM)

Ou toda a informação de MS/MS:
- Reações específicas (SRM)
- Perda neutra (*neutral loss*)
- Íons precursores
- Íons produtos

O Espectro de Massas



O Espectro de Massas



Etapas na análise por MS

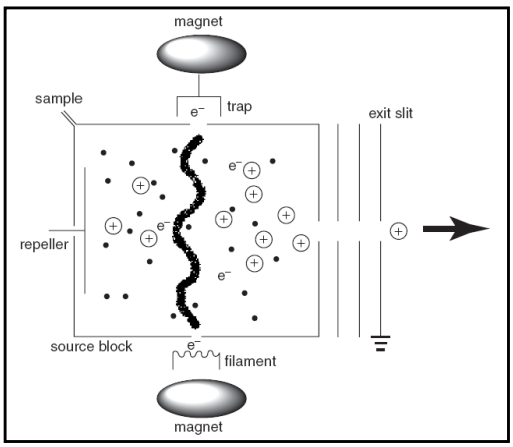
1. Ionização dos analitos
2. Seleção/Separação das massas*
3. Detecção

OBS: etapas 2 e 3 necessitam de vácuo (etapa 1 apenas em alguns casos, e.g. GC-MS)

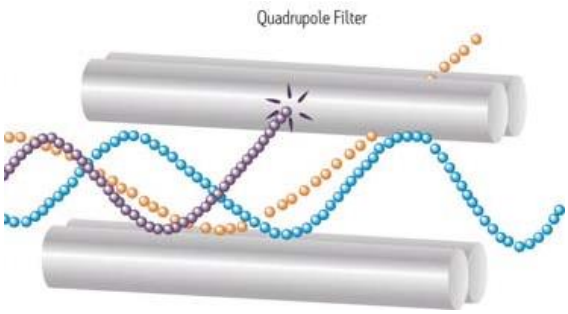
* Na realidade ocorre a seleção/separação por meio da massa/carga (*m/z*).

1. Ionização

$$M + e^- \longrightarrow M^{+\bullet} + 2e^-$$

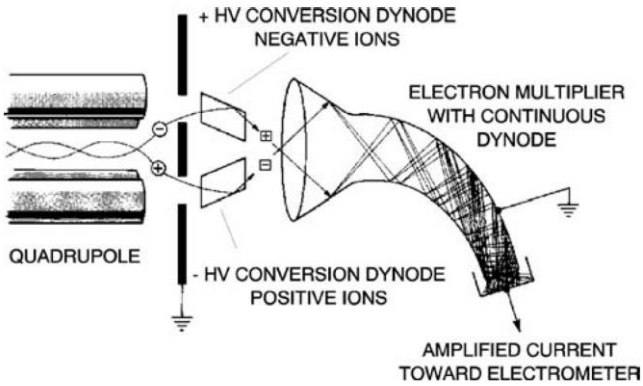


2. Análise/separação



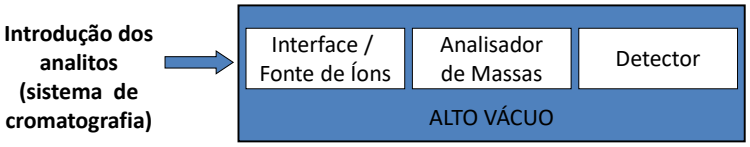
Fonte: http://www.waters.com/waters/pt_PT/What-Types-of-Instruments-Are-Used%3F/nav.htm?cid=10090937

3. Detecção



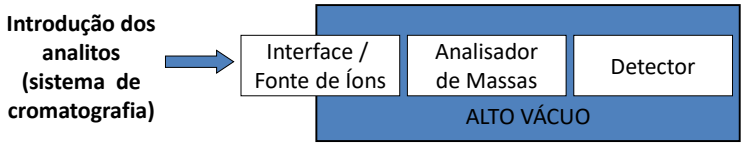
Diagramas de blocos de um MS

Modo de Ionização no Vácuo



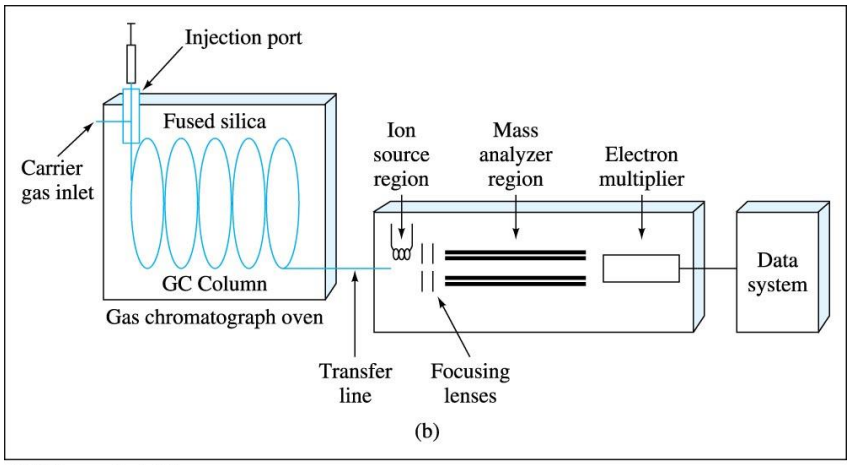
Exemplos: EI, CI, PI, MALDI, TSI, FAB, SIMS, FI/FD

Modo de Ionização à Pressão Atmosférica (API)



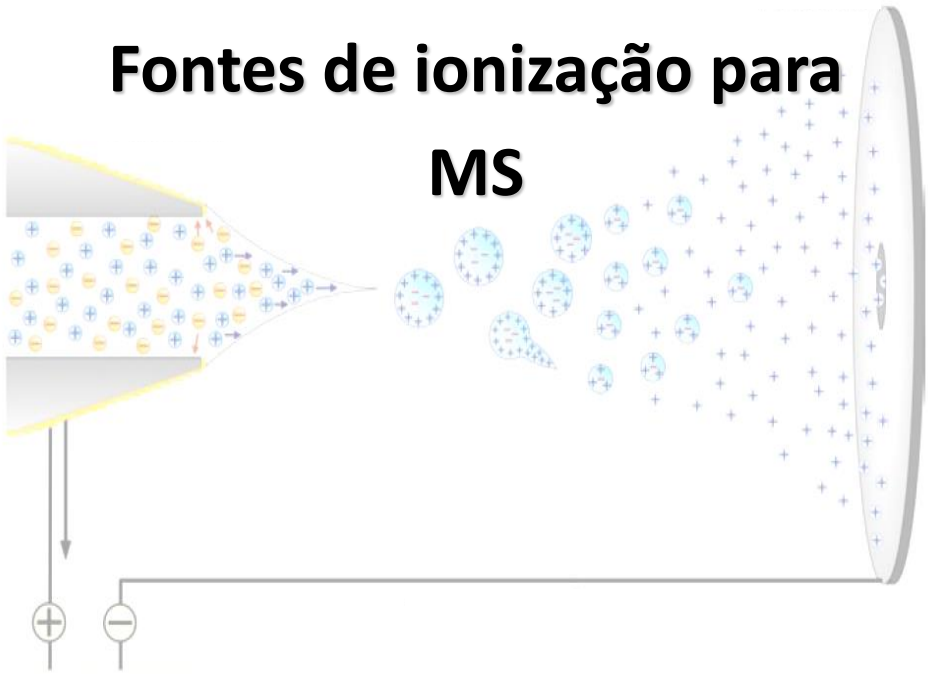
Exemplos: ESI, APCI, APPI, AP-MALDI, DESI, DART, EASI

Esquema de um equipamento – GC-MS



© 2004 Thomson - Brooks/Cole

Fontes de ionização para MS



Fontes de ionização para GC-MS

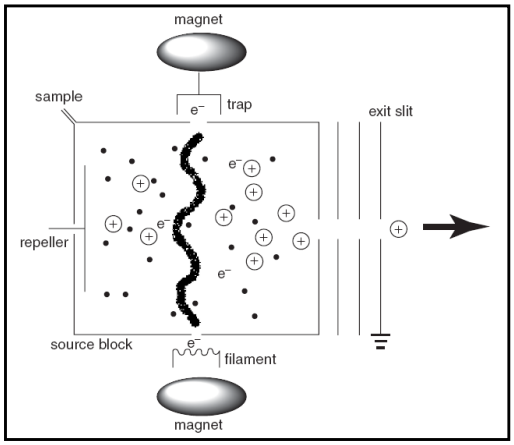
- Ionização por elétrons – EI

  (“Impacto de elétrons” – termo obsoleto*)

- Ionização Química – CI
  - Modo positivo
  - Modo negativo

* http://goldbook.iupac.org/E01998.html

Fonte de EI



- Filamento aquecido
- Energia: 70 eV
- Ionização intensa
  - *"hard ionization"*

$$M + e^- \longrightarrow M^{+\bullet} + 2e^-$$

Reações em EI

- Ionização / Excitação $\quad M + e^- \Longrightarrow M^{\bullet +*} + 2e^-$
- Formação de íon molecular $\quad M^{\bullet +*} \Longrightarrow M^{\bullet +}$
- Fragmentação em íon + radical $\quad M^{\bullet +*} \Longrightarrow A^+ + B^\bullet$
- Fragmentação em íon radicalar + perda neutra $\quad M^{\bullet +*} \Longrightarrow C^{\bullet +} + D$

Alguns mecanismos na formação dos íons

Analito = AB

1. $AB + e^{-*} \rightarrow A^{+} + B^{-} + e^{-}$

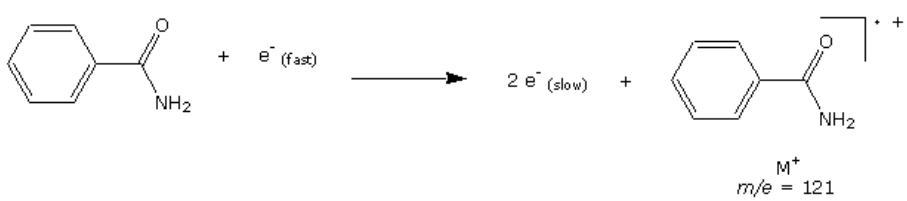
2. $AB + e^{-*} \rightarrow A^{+} + B^{\circ} + 2e^{-}$

3. $AB + e^{-*} \rightarrow [AB^{+\circ *}] + 2e^{-}$, *seguido por:* $[AB^{+\circ *}] \rightarrow AB^{+\circ}$
   - íon molecular

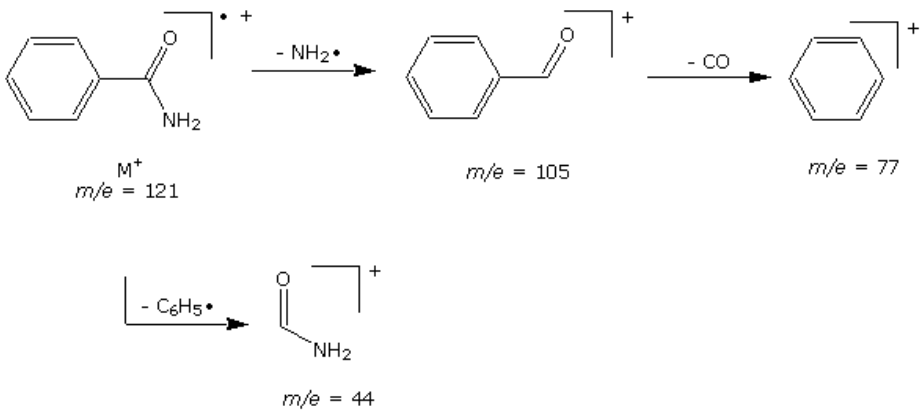
4. $AB + e^{-*} \rightarrow [AB^{2+*}]'' + 3e^{-}$, *seguido por:* $[AB^{2+*}]'' \rightarrow A^{+} + B^{+}$
   - pequena abundância

5. $AH + e^{-*} \rightarrow AH^{*} + e^{-}$, *seguido por:* $AH^{*} + AH \rightarrow [AH+H]^{+} + A^{-}$
   - 'autoionização química'

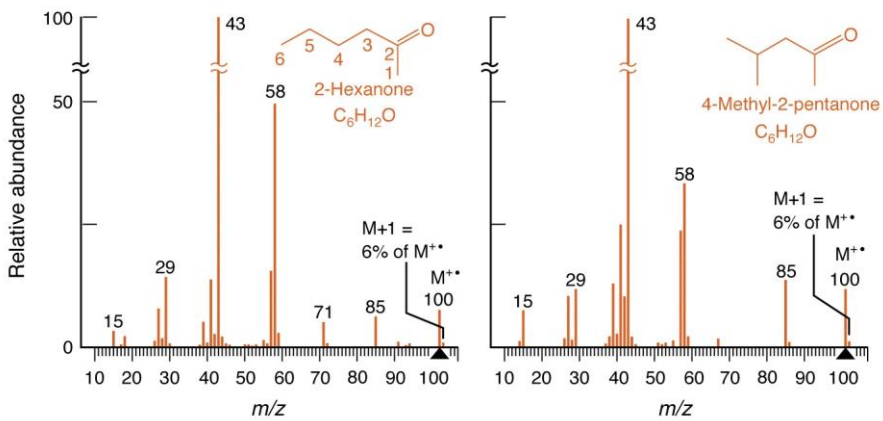
O processo de ionização em GC-EI-MS



O processo de fragmentação em GC-EI-MS



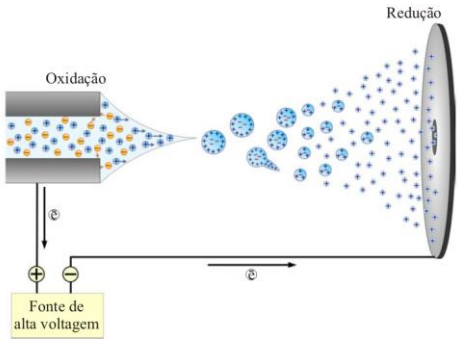
Espectro de massas por EI (70 eV) de duas cetonas isoméricas com composição $C_6H_{12}O$.

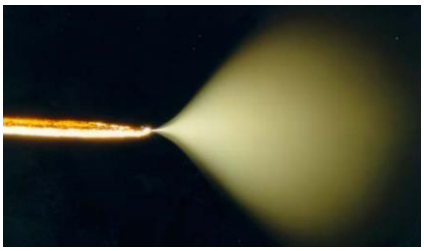


Fontes de ionização para o LC-MS

- Ionização por *electrospray* – ESI
  (eletronebulização)
  - Modo ESI "puro"
  - Modo ESI "com assistência pneumática" – atualmente o mais usado
- Ionização Química a Pressão Atmosférica – APCI
- Fotoionização a Pressão Atmosférica – APPI

Acoplamento LC-ESI-MS

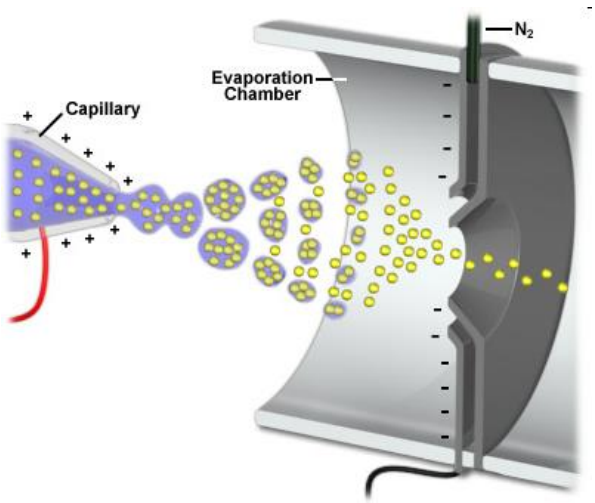




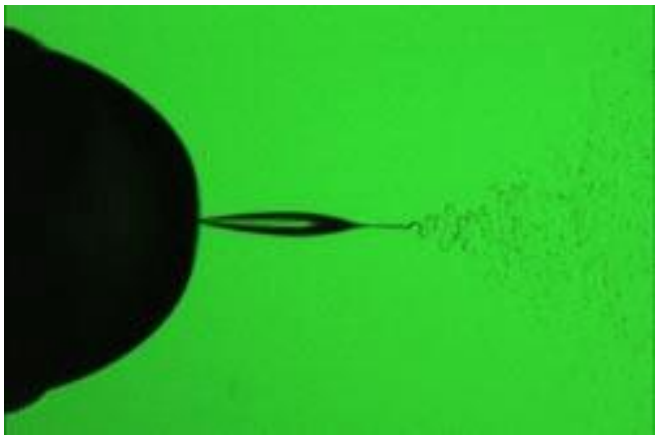
- Melhor opção para análise ácidos e bases fracas
- Mais compatível com baixas vazões

Electrospray – ESI

Vazões: 100 nL/min a 5 μL/min

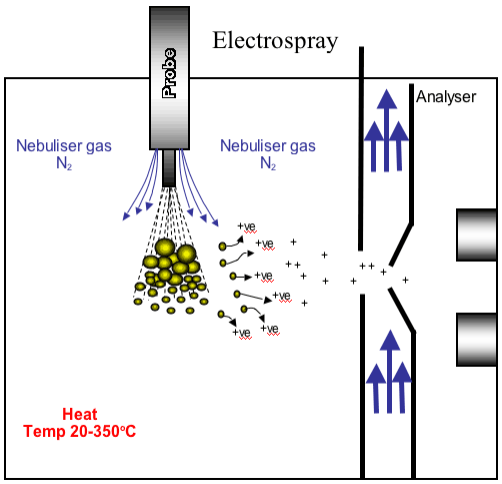


Electrospray - ESI



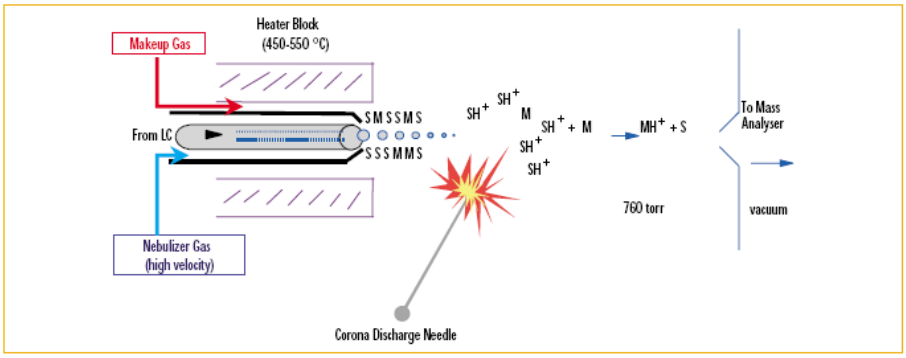
ESI com assistência pneumática ("~~IonSpray~~")

Vazões: 5 µL/min a 0,2-1,0 mL/min

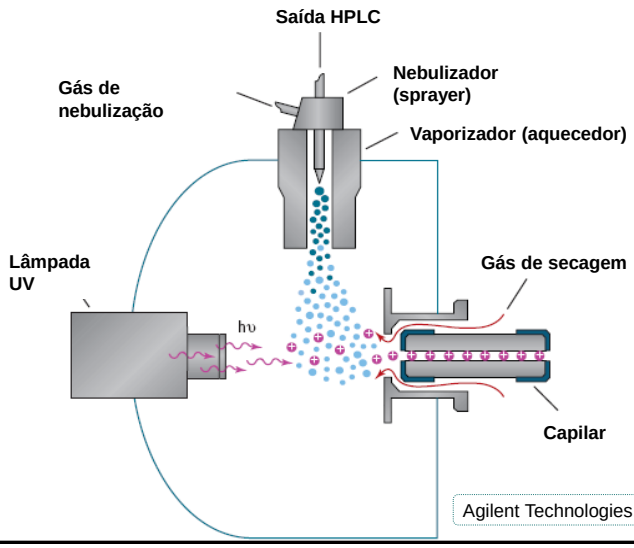


Ionização Química a Pressão Atmosférica (APCI)

Vazões: ~1,0 mL/min



Fotoionização a Pressão Atmosférica (APPI)

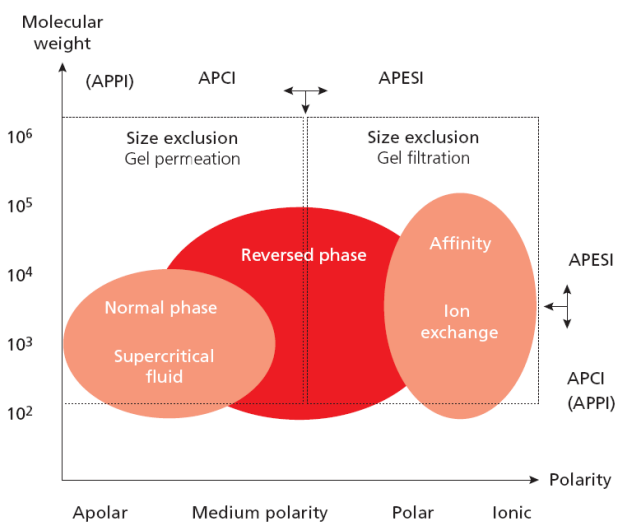


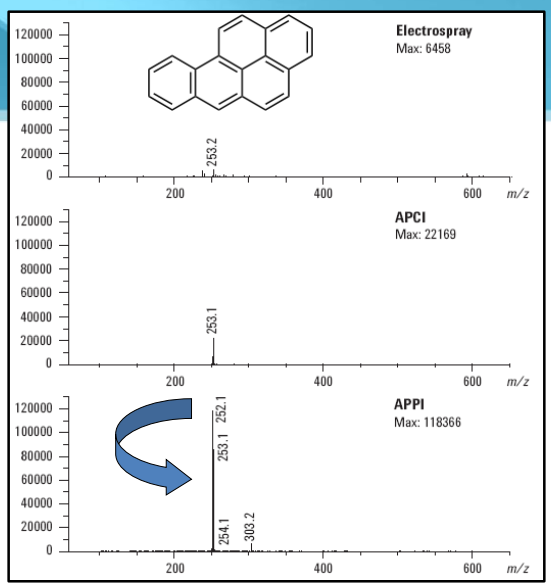
- Pode ser usado um dopante

Ex. Tolueno

Agilent Technologies, www.agilent.com/chem, 2001.

Escolha da técnica de ionização está intimamente relacionada ao modo de separação em LC.





Baixa abundância do íon $[M+H]^+$ em *m/z* 253.

Íon $[M+H]^+$ mais abundante
Presença de M^+ em *m/z* 252
Isótopo C_{13} em m/z 254

Abundância 5 x maior que APCI e 20 x maior que ESI.

Análise de benzo[a]pireno;100 picomoles; modo positivo; injeção em fluxo.

Agilent Technologies, www.agilent.com/chem, 2001.

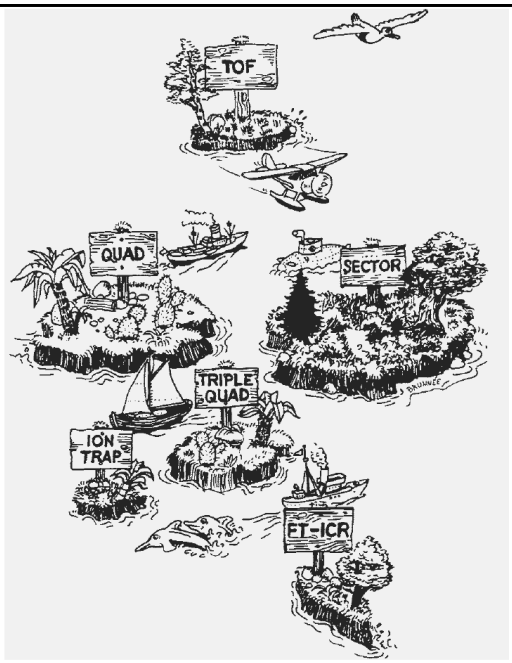


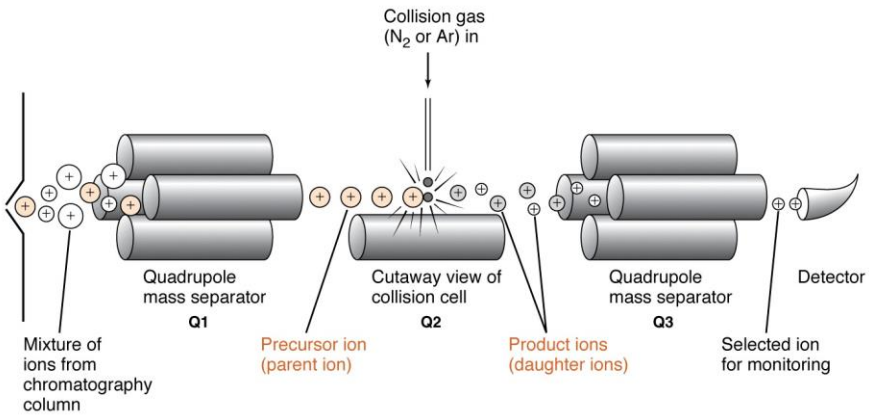
Fig. 4.1. Mass spectrometer islands. A cartoon by C. Brunnée. Reproduced from Ref. [2] with permission. © Elsevier Science, 1987.

Analisadores para MS

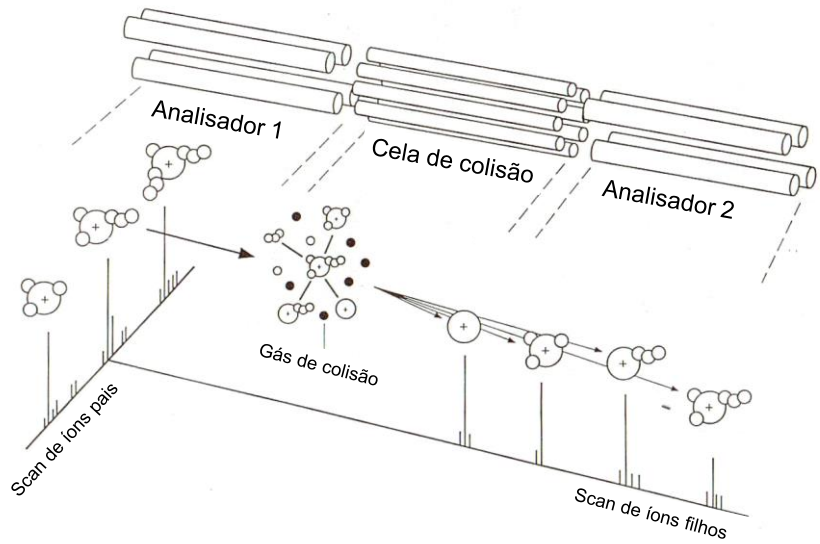
- Setor Magnético e Setor Eletrostático
- Quadrupolo
- *Ion Trap*
- Tempo de Voo
- FT-ICR
- (FT) Orbitrap

MS/MS (sequencial)

MS^n onde *n* indica o número de estágios de MS

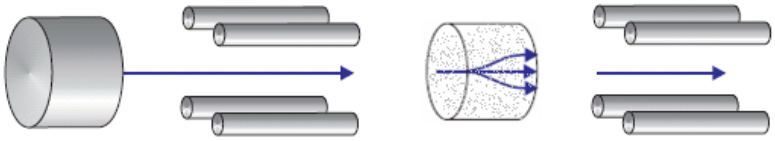


Princípio de operação



Modos de operação

- Monitoramento de Reações Selecionadas (SRM)



MS1

Estático

Massa do precursor (*pai*)

Câmara de colisão

Fragmentação CID

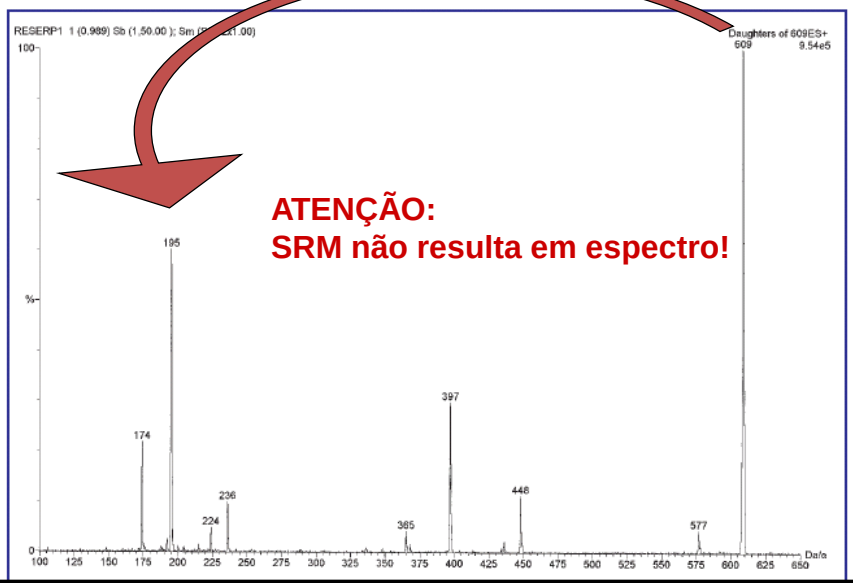
Modo RF (passam todas as massas)

MS2

Estático

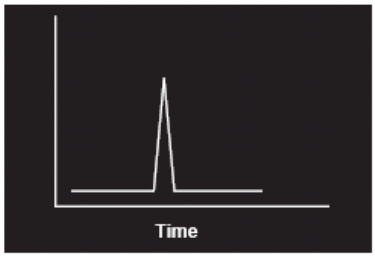
Massa do produto (*filho*)

Ex: Monitoramento da transição: *m/z* 609 → *m/z* 195 para a reserpina.

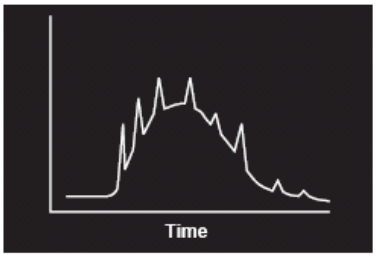


ATENÇÃO:
SRM não resulta em espectro!

SIM vs. SRM



LC-MS/MS

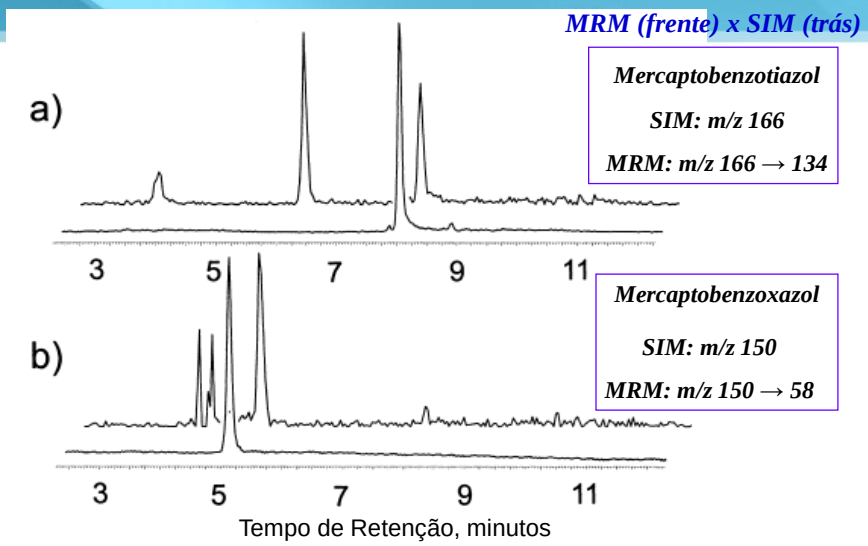


LC-MS

- Especificidade

- Relação S/N

QqQ – Aplicação



Trends Anal. Chem., *2001, 20, 533-542.*

Analisadores para MS

- Setor Magnético e Setor Eletrostático
- Quadrupolo
- *Ion Trap*
- Tempo de Voo
- FT-ICR
- (FT) Orbitrap

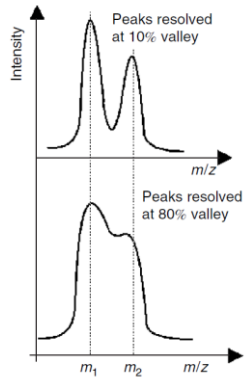


Figure 2.1
Diagram showing the concepts of peak resolution and valley.

Exemplo

N_2^+

m/z = 28,006148

CO^+

m/z = 27,994915

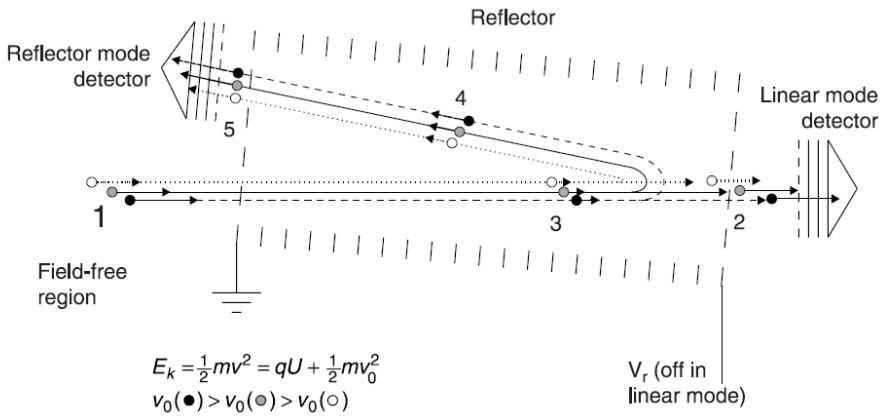
Δm = 0,011233

Requer grau de exatidão de pelo menos 200 ppm

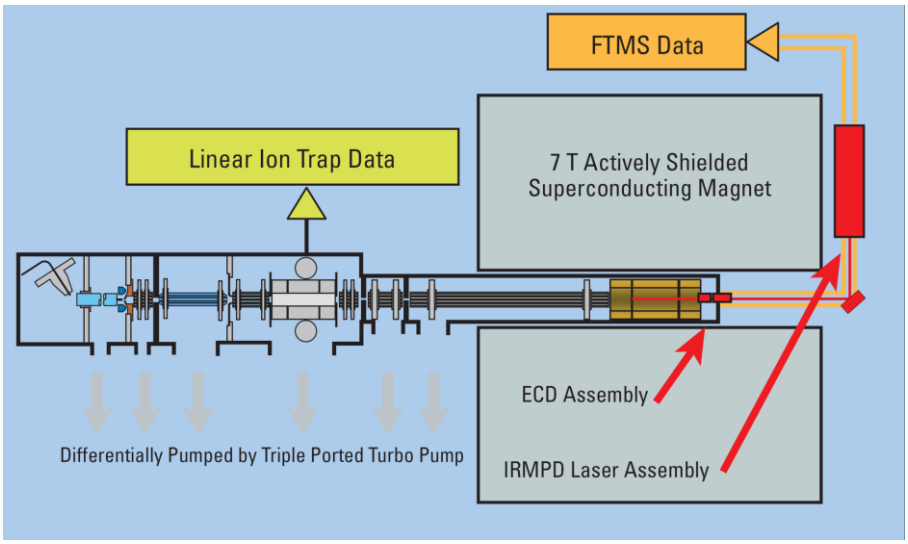
RP (10% vale) ≈ 2.500

RP (FWHM) ≈ 4.250

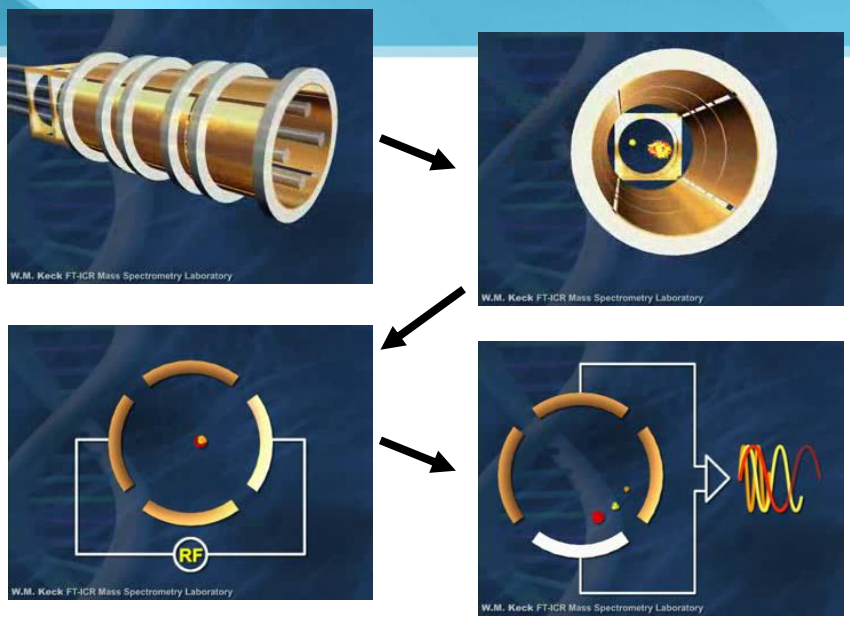
TOF de alta resolução – *Reflectron*



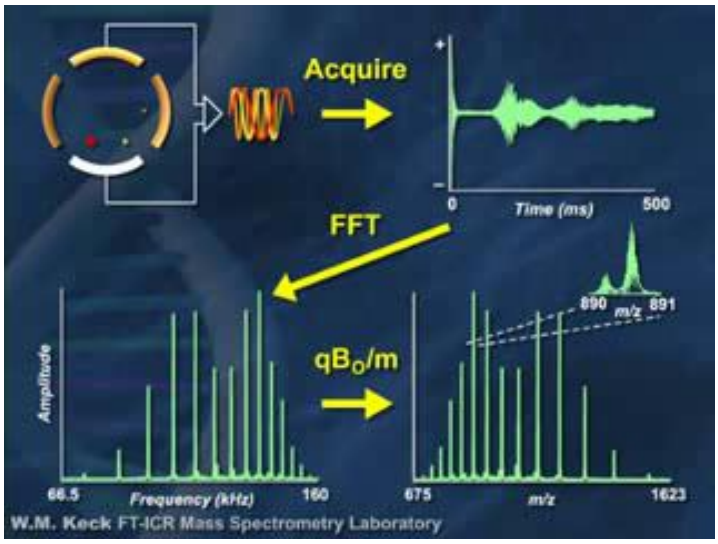
Ressonância Ciclotrônica de Íons com Transformada de Fourier (FT-ICR)



FT-ICR

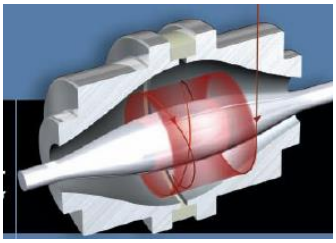

W.M. Keck FT-ICR Mass Spectrometry Laboratory
W.M. Keck FT-ICR Mass Spectrometry Laboratory
RF
W.M. Keck FT-ICR Mass Spectrometry Laboratory
W.M. Keck FT-ICR Mass Spectrometry Laboratory

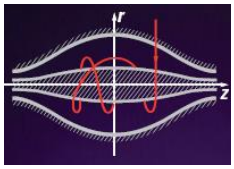
FT-ICR

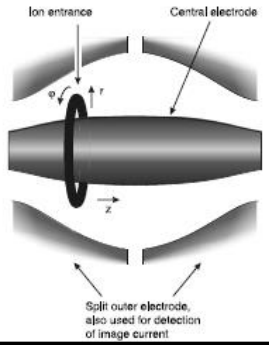

Acquire
+
-
0
Time (ms)
500
FFT
890
m/z
891
qB$_O$/m
Amplitude
66.5
Frequency (kHz)
160
675
m/z
1623
W.M. Keck FT-ICR Mass Spectrometry Laboratory

Orbitrap

- Aprisionador eletrostático orbital axialmente harmônico
- Também utiliza FFT
- Mais nova tecnologia de análise de massas (2002)



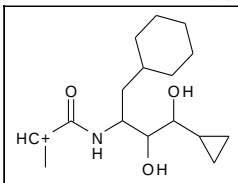
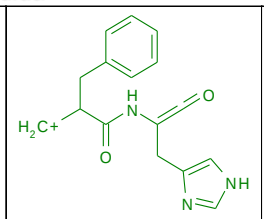
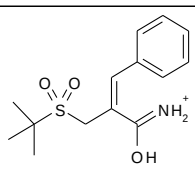




http://planetorbitrap.com/q-exactive#.UoNbeBpwp28

Qual a importância da Exatidão de Massa?

$m/z_{\text{medida}} = 282.1242$

		
(A)	(B)	(C)
$C_{16}H_{28}NO_3$	$C_{16}H_{16}N_3O_2$	$C_{14}H_{20}NO_3S$
282.2069	282.1242	282.1164
82.7 mmu	0.0	7.8 mmu
293 ppm	0.1 ppm	28 ppm