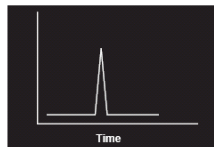


Espectrometria de Massas Sequencial (MS/MS)

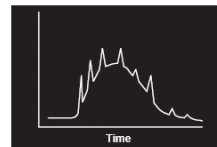
“Tandem Mass Spectrometry”

Álvaro J. Santos Neto
(CROMA/IQSC/USP)

SRM vs. SIM



LC-MS/MS



LC-MS

- Especificidade
- Relação S/N

MS/MS e (MS)ⁿ

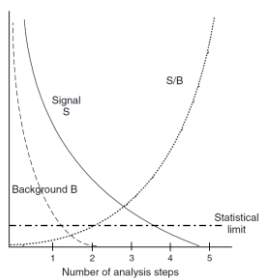
- Instrumentos para *Tandem* MS
 - Setores (BE – MIKES / EBEB / BEBE / BEEB etc)
 - “Tripla” Quadrupolo (QqQ)
 - Ion Trap (IT)
 - Convencional
 - Linear (LIT)
 - Q-ToF
 - IT-ToF
 - Qq-LIT
 - LIT-Orbitrap
 - LIT-FTICR
- Modos de operação
- Alguns aspectos práticos da LC-MS/MS
 - Supressão da ionização

Híbridos

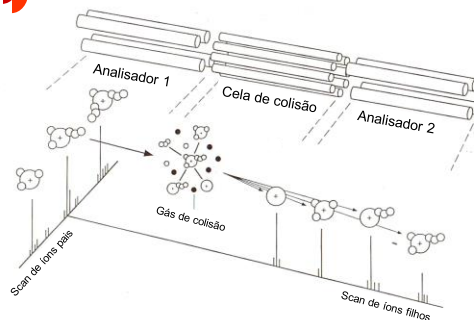
Usos do MS/MS

- | | |
|---------------------------|------------------------------|
| • Quantitativo | • Qualitativo |
| - Redução do ruído | - Informação estrutural |
| - Aumento da seletividade | - Mapa de fragmentação |
| - Melhoria do S/N | - Diferenciação de isômeros |
| | - Identificação de peptídeos |

Qual o verdadeiro ganho?



Princípio de operação



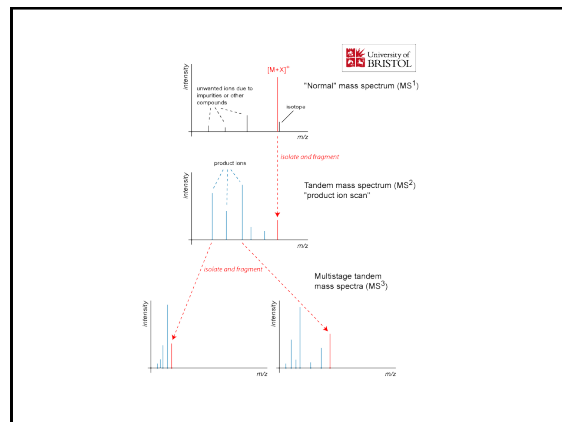
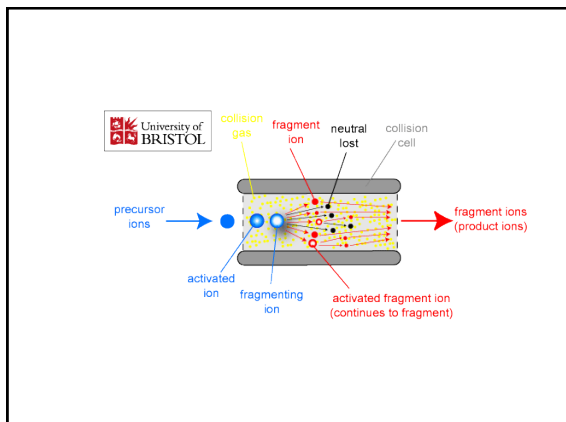
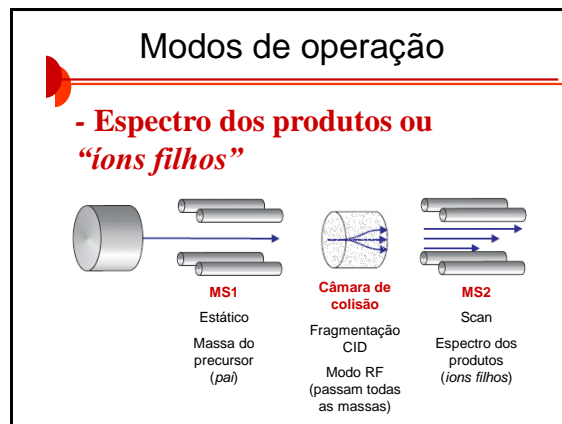
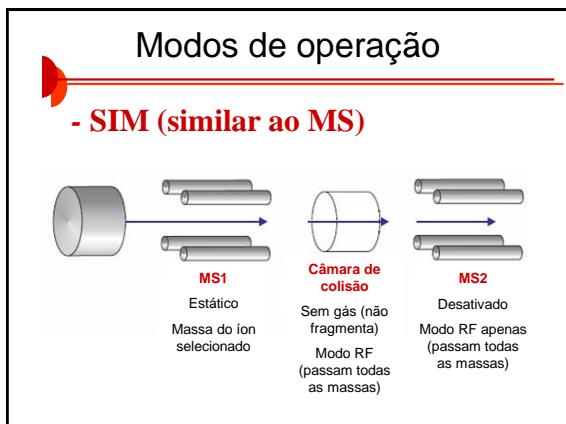
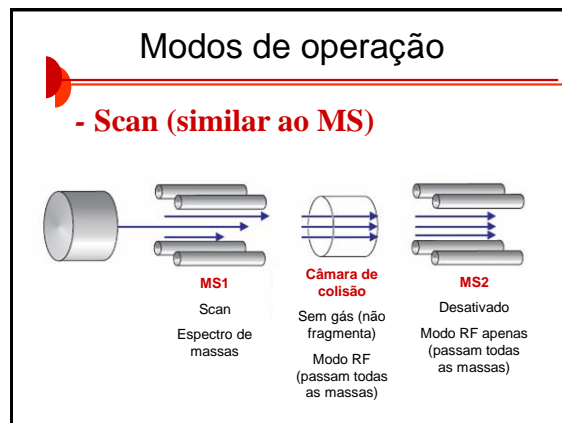
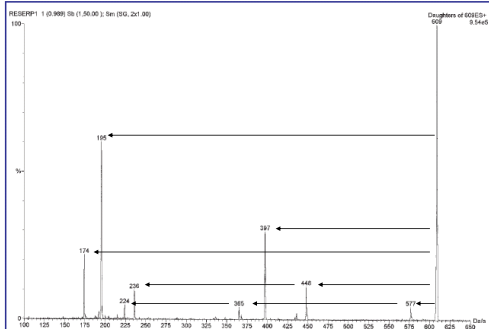


TABLE 3.1. The Standard Tandem MS Modes

Mode	Purpose	Experiment
Product ion	To get structural information on ions produced in the ion source	MS1 selects one precursor ion, MS2 acquires a full mass spectrum of the fragment ions produced
Precursor ion	To find compounds that produce a common fragment	MS1 is scanning all precursor ions, MS2 selects one fragment ion
Neutral loss	To find compounds that lose a common neutral species	MS1 and MS2 are both scanning at a fixed m/z difference
Selected reaction monitoring	To monitor a selected reaction	MS1 selects one precursor ion, MS2 selects one fragment ion

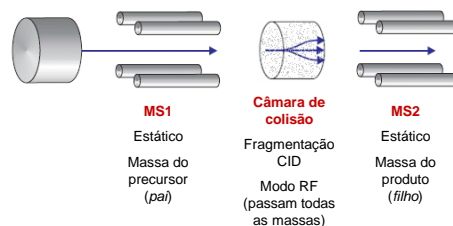


Ex: Determinação dos fragmentos obtidos a partir do íon de m/z 609 da reserpina ("ion pai").



Modos de operação

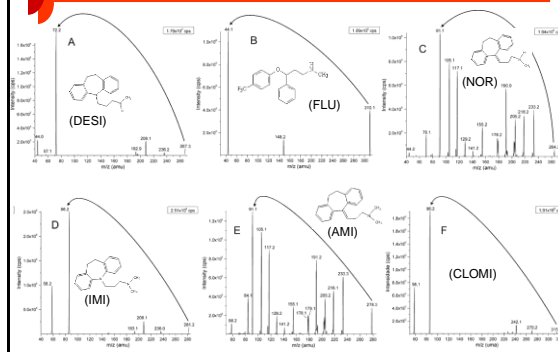
- Monitoramento de Reação Seleccionada (SRM)



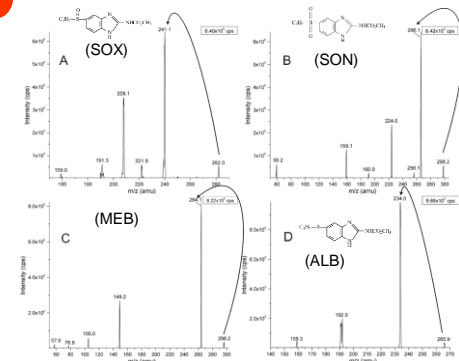
Ex: Monitoramento da transição: m/z 609 \rightarrow m/z 195 para a reserpina.



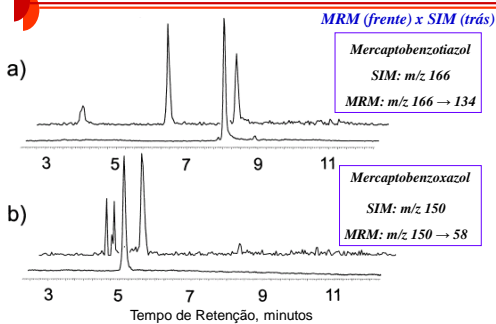
Fragmentação dos antidepressivos



Fragmentação dos anti-helmínticos

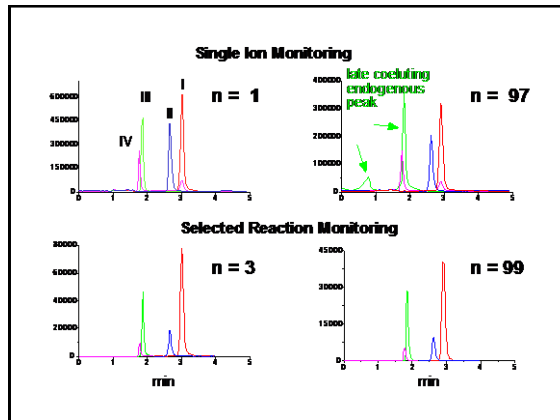
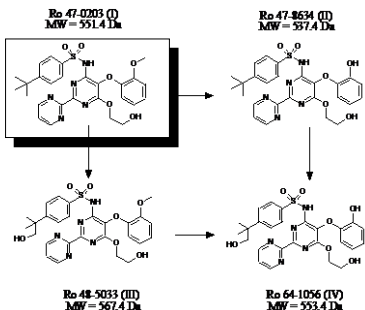


QqQ – Aplicação



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

Há diferença na seletividade entre SIM/LC-MS e SRM/LC-MS ?



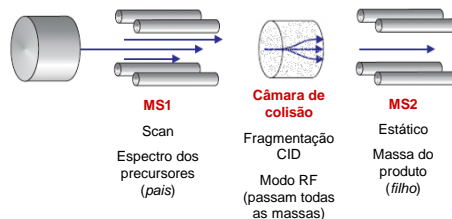
Condições de SRM

Analites	MRM transition		Orifício potencial (V)	Collision energy (eV)
	Precursor ion (m/z)	Product ion (m/z)		
desipramine	267.4	72.2	48.5	20.1
fluoxetine	310.3	44.1	48.5	20.1
nortriptyline	264.4	91.1	48.5	20.1
imipramine	281.4	86.1	48.5	20.1
amitriptyline	278.5	91.1	48.5	20.1
clomipramine	315.4	86.1	48.5	20.1
albendazole sulfóxido	282.2	241.1	55	14.8
albendazole sulfone	298.2	266.1	55	14.8
mebendazole	296.2	264.1	55	14.8
albendazole	266.2	234.1	55	14.8

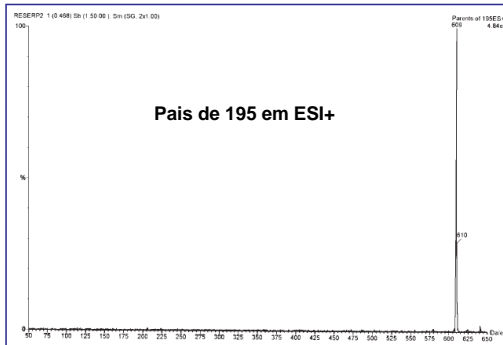
MS instrument: Sciex API III+
 Ion Spray Voltage: 3500 V
 Nebulizing gas pressure: 40 psi
 Collision Gas Thickness (Ar): 210x10¹³ atoms cm⁻²
 Dwell time: 100 ms

Modos de operação

- Espectro dos precursores ou "íons pais"

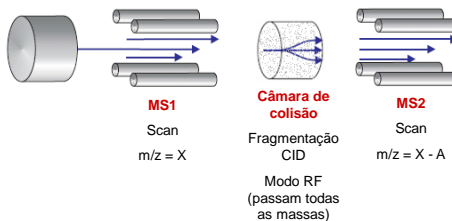


Ex: Determinação dos íons ("íons pai") que originaram um determinado íon fragmento ("íon filho").

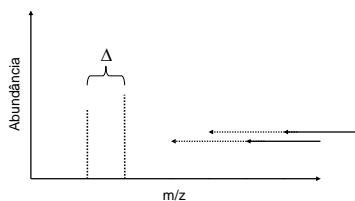


Modos de operação

-Perda Neutra (constante)



Perda Neutra



Exemplo:

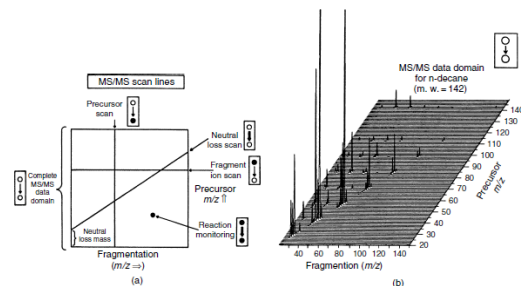
Caracterização de metabólitos de fármacos

Perda de glucoronídeo = 176 u.m.a.

Perda de sulfato = 80 u.m.a.

Perda de glicose = 162 u.m.a.

Opções de MS/MS em QqQ



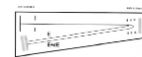
Resumo de modos de operação

	MS1	Cela de colisão	MS2
Espectro dos íons filhos	→ Precursor		↗
Espectros dos íons pais	↗	→	→ Produto
SRM	→ Precursos	→	→ Produto
Perda Neutra	↗ Sincronizado MS2		↗ Sincronizado MS1

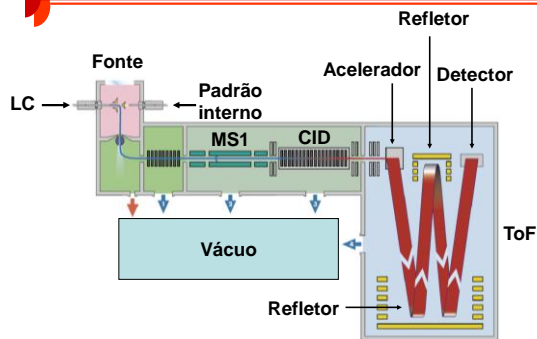
Análise por Q-ToF

• Características de um ToF:

- Aquisição veloz do espectro
- Virtualmente sem limite de massas
- Boa resolução : 10000 a 50000
 - para melhor MRP (ortogonal e com refletor)
 - geralmente < 20000
- Exatidão: 20 a 100 ppm
- Exige calibração interna para melhorar exatidão



Q-ToF: esquema

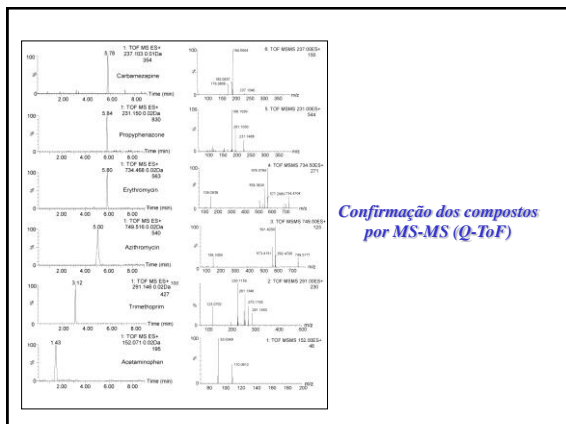


Tandem no espaço – Q-ToFMS

- QqToF: ToF no lugar do último quadrupolo

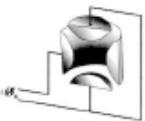
Vantagens:

- Alta resolução (alcançando valores maiores do que 20000);
- Boa exatidão de massas (< 5 ppm);
- Possibilidade de registrar o espectro de massas completo para cada pulso de íons injetados no sistema;
- Data Dependent Analysis (DDA)



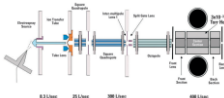
Análise por Ion Trap

- Características de um IT:
 - $m/z < 4000$
 - Resolução 3000 a 5000
 - Geralmente unidade
 - Exatidão: 200 a 300 ppm
 - Baixo custo
 - MS^n ($n < 4$ ou 5)
 - Versátil (*Data Dependent Analysis*)
 - *Charge-space effects*

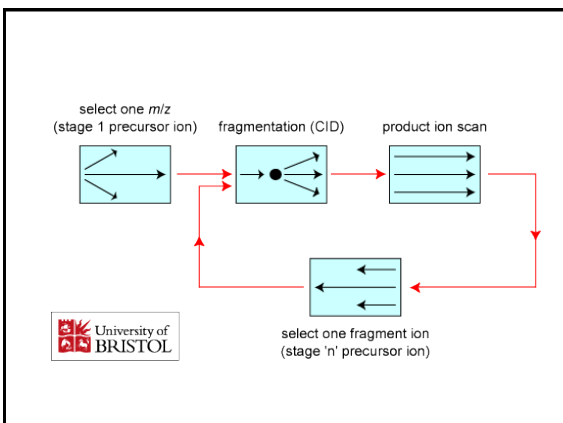


Etapas da análise

- Aprisionamento
- Isolamento
- Excitação (induz fragmentação)
- Ejeção



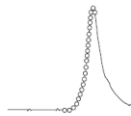

[Video](#)



QqQ vs. Ion Trap

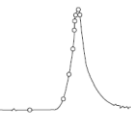

Triplo Quadrupolo

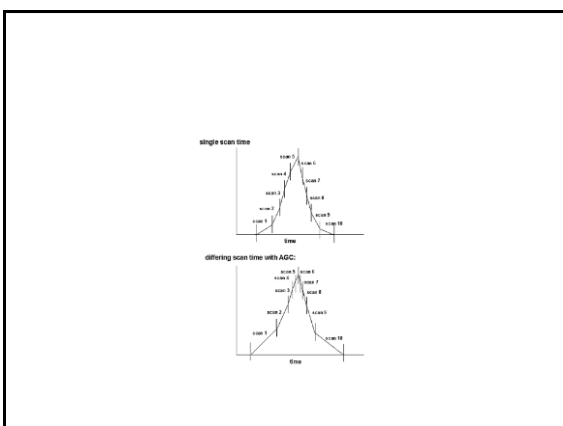
Espaçamento uniforme

Ion Trap

Pontos concentram no topo

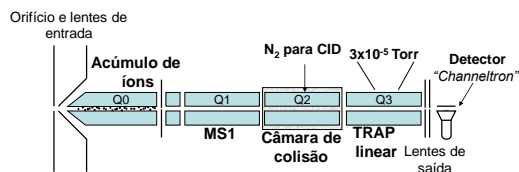


Outros instrumentos em Tandem

- IT_{linear}-FTICR: LTQ FT Ultra®
- IT_{linear}-FTOrbitrap: LTQ Orbitrap®
- QTRAP®



QTRAP – Varredura de Íons-Produto

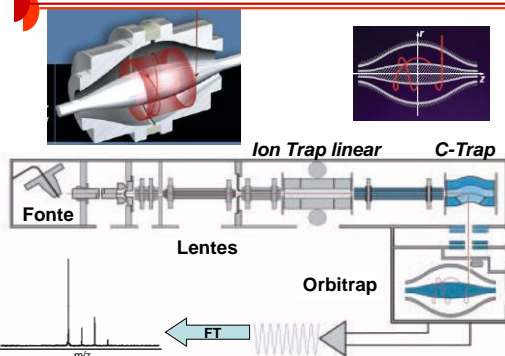


1. Seleção do ion precursor em Q1
2. Fragmentação em Q2
3. Aprisionamento dos produtos em Q3
4. Aprisionamento concomitante em Q0

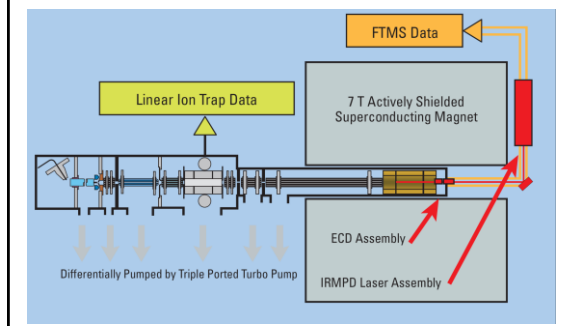
Scan Type	Q1	Q2	Q3
Q1 Scan	Resolving (Scan)	RF-only	RF-only
Q3 Scan	RF-only	RF-only	Resolving (Scan)
Product Ion Scan (PIS)	Resolving (Fixed)	Fragment	Resolving (Scan)
Precursor Ion Scan (PI)	Resolving (Scan)	Fragment	Resolving (Fixed)
Neutral Loss Scan (NL)	Resolving (Scan)	Fragment	Resolving (Scan Offset)
Selected Reaction Monitoring mode (SRM)	Resolving (Fixed)	Fragment	Resolving (Fixed)

Enhanced Product Ion (EPI)	Resolving (Fixed)	Fragment	Trap/scan
MS3	Resolving (Fixed)	Fragment	Isolation/frag trap/scan
Time delayed frag capture Product Ion (TDF)	Resolving (Fixed)	Trap/No frag	Frag/trap/scan
Enhanced Q3 Single MS (EMS)	RF-only	No frag	Trap/scan
Enhanced Resolution Q3 Single MS (ERMS)	RF-only	No frag	Trap/scan
Enhanced Multiply Charge (EMC)	RF-only	No frag	Trap/empty/scan

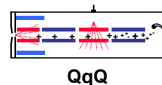
LTQ Orbitrap®



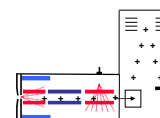
LTQ FT®



Qual analisador é melhor para confirmação de estrutura ?

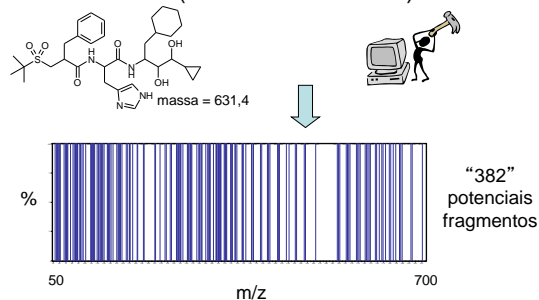


- Sensibilidade
- Velocidade de scan
- Capacidade de MS/MS
- MSⁿ
- Exatidão
- Resolução

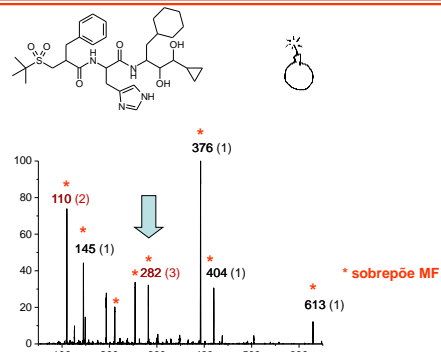


Fragmentação calculada por computador - "Remikeren"

(Mass Frontier® - MF)



Espectro do "Remikeren" em um QqQ

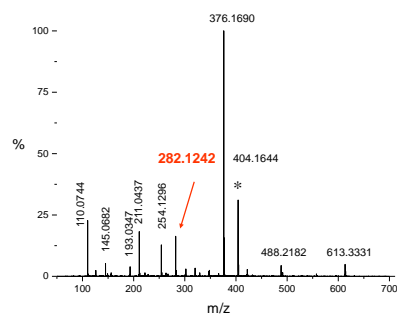


Estruturas propostas pelo MF para m/z 282

(A)	(B)	(C)
$C_{18}H_{28}NO_3$	$C_{18}H_{26}NO_2$	$C_{18}H_{26}NO_2S$
282.2069	282.1242	282.1164
A-C = 90.5 mmu	B-C = 7.8 mmu	

Espectro do "Remikeren" em um Q-ToF

Medidas exatas de massas



Predito versus Medido

m/z 282.1242

(A)	(B)	(C)
$C_{18}H_{28}NO_3$	$C_{18}H_{26}NO_2$	$C_{18}H_{26}NO_2S$
282.2069	282.1242	282.1164
82.7 mmu	0.0	7.8 mmu
293 ppm	0.1 ppm	28 ppm

E o MSⁿ ?!

