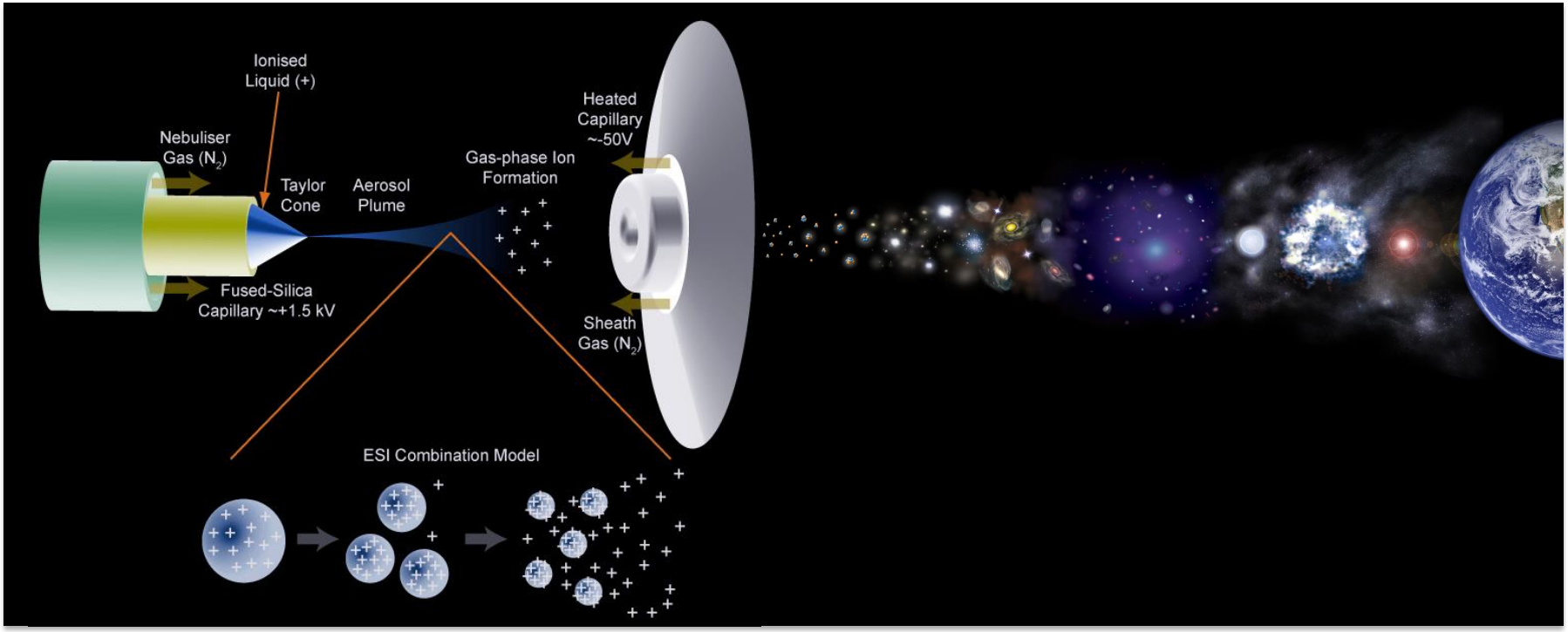
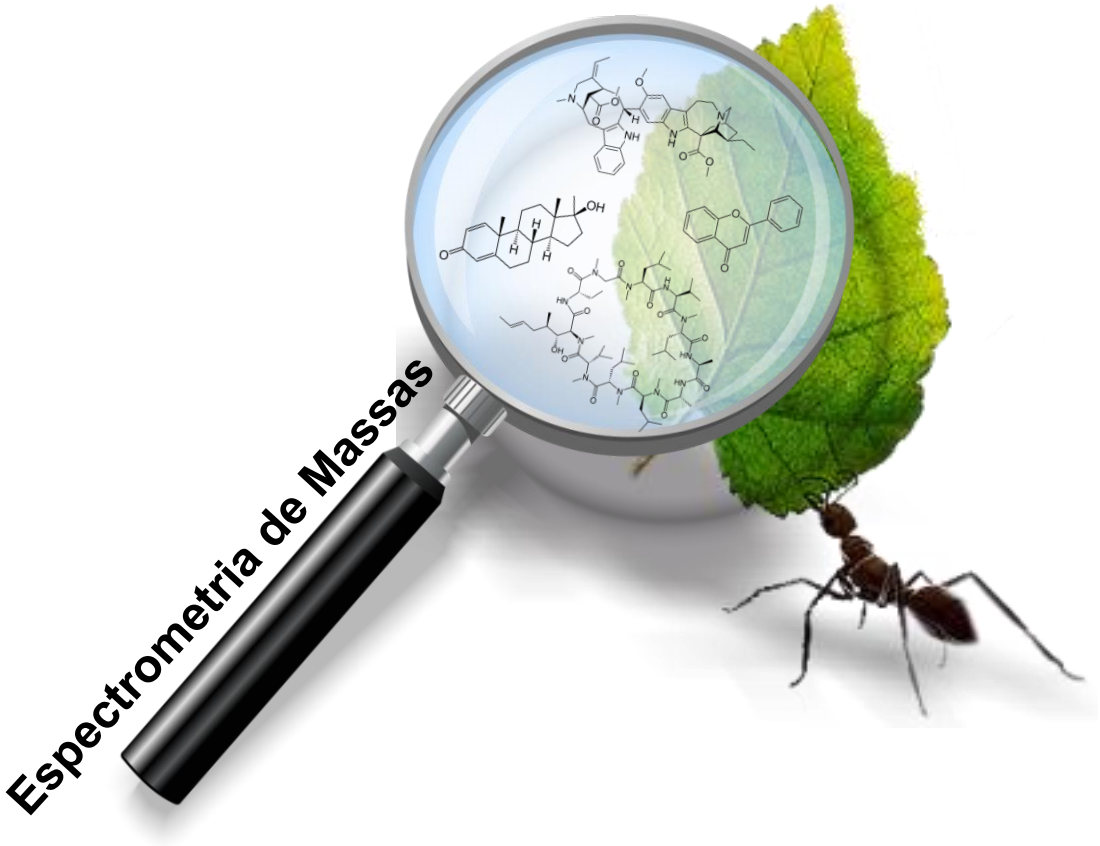
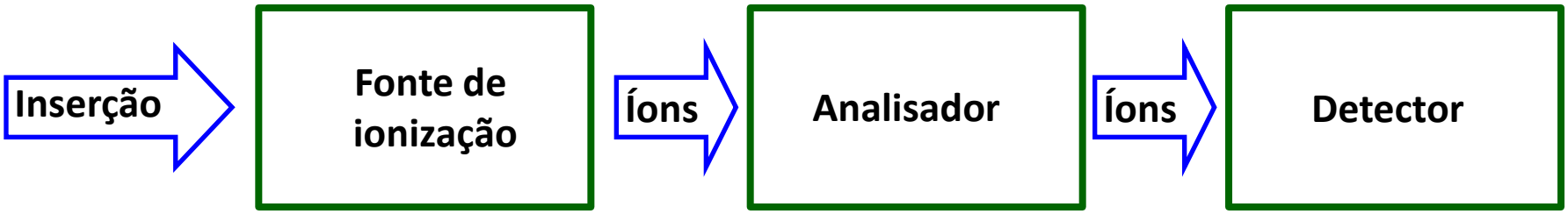
Espectrometria de Massas: um universo de aplicações



Prof. Humberto M. S. Milagre


Espectrometria de Massas

Espectrômetro de Massas



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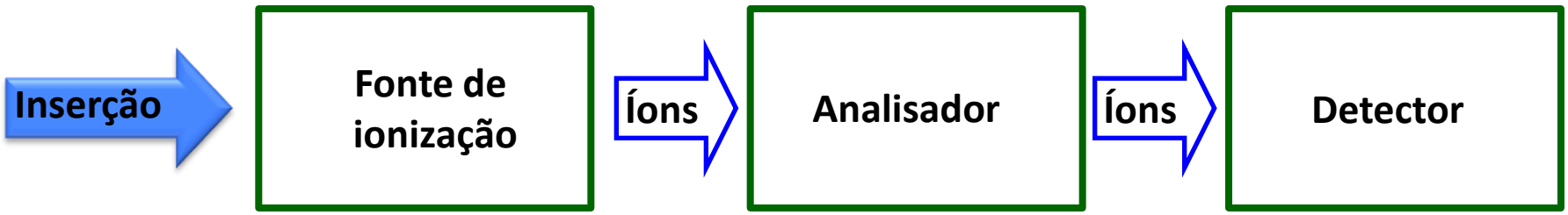
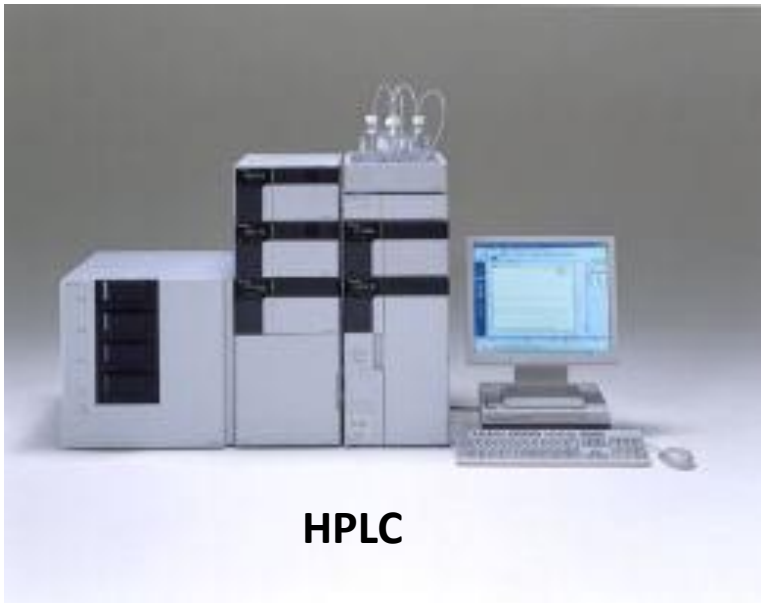


CG

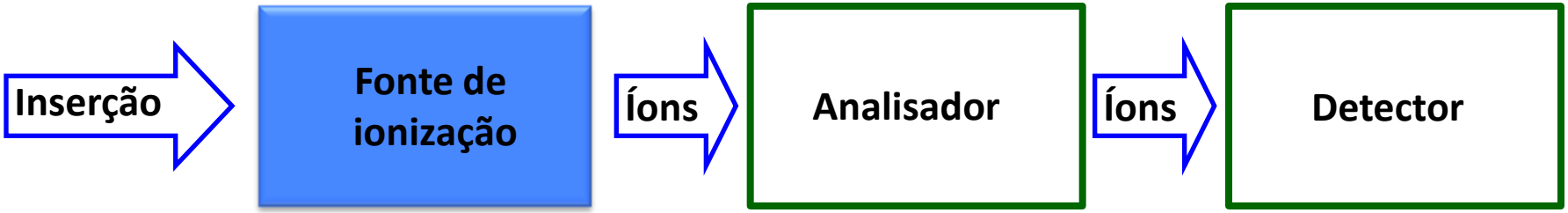


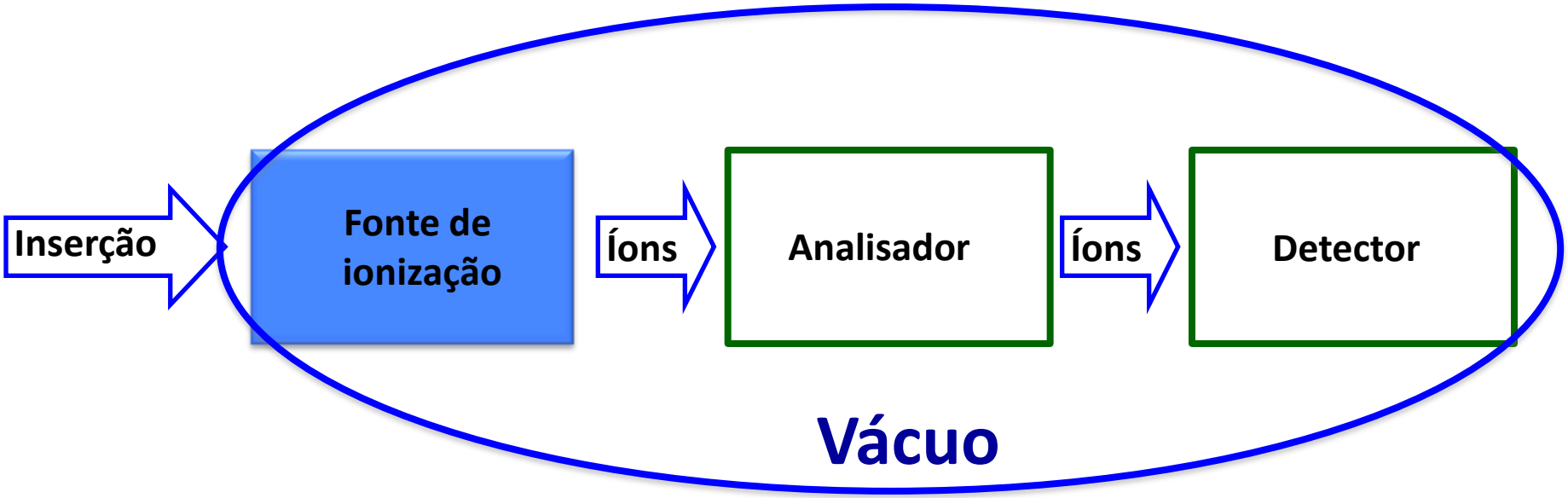
HPLC

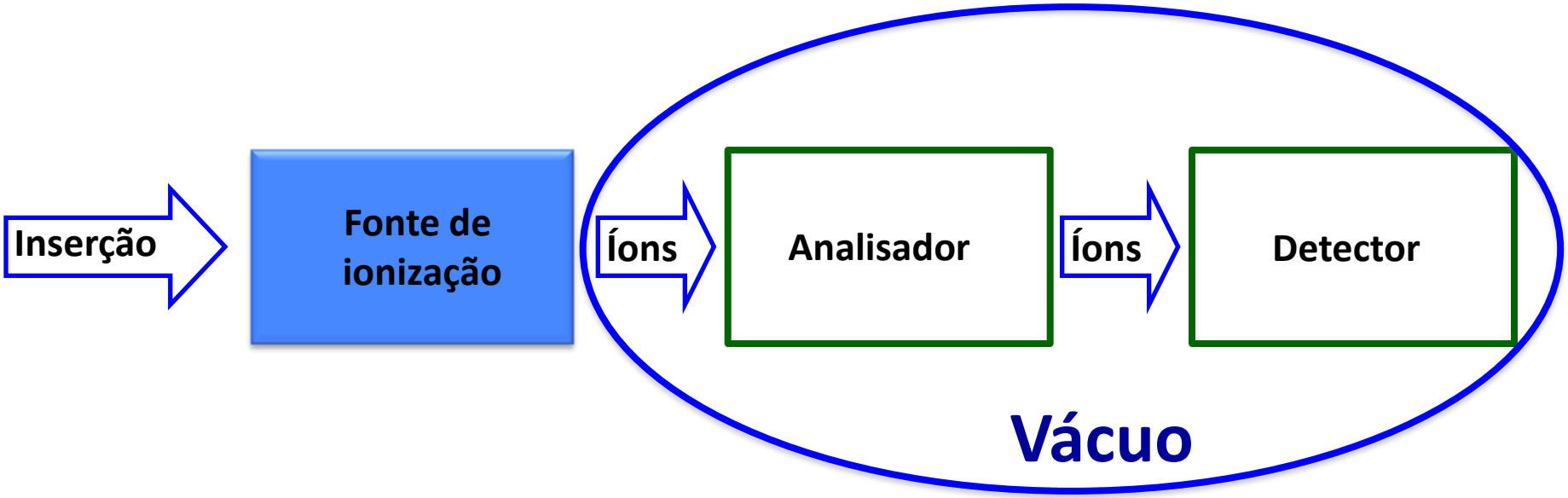
Seringa

Placa de MALDI

Espectrômetro de Massas




Inserção
Fonte de ionização
Íons
Analisador
Íons
Detector
Vácuo


Inserção
Fonte de ionização
Íons
Analisador
Íons
Detector
Vácuo

Fontes de Ionização

a- EI (Electron Ionization")

b- CI ("Chemical Ionization")

c- APCI ("Atmospheric Pressure Chemical Ionization")

d- APPI ("Atmospheric Pressure Photo-Ionization")

e- ESI ("Electrospray Ionization")

f- MALDI ("Matrix-Assisted Laser Desorption Ionization")

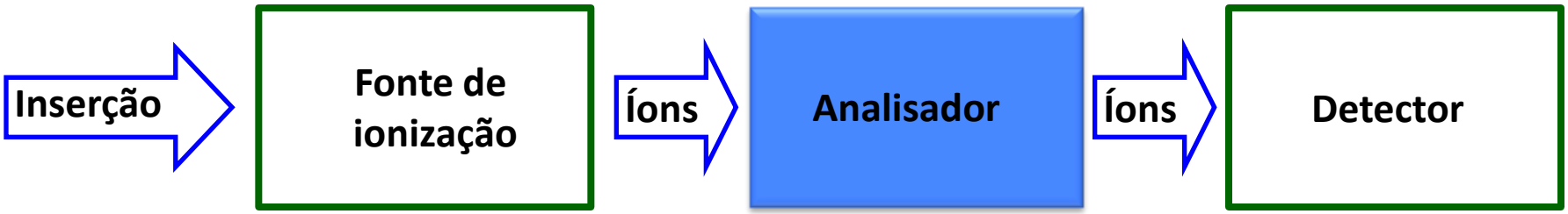
g- DESI ("Desorption Electrospray")

h – DART (Direct Analysis in Real Time")

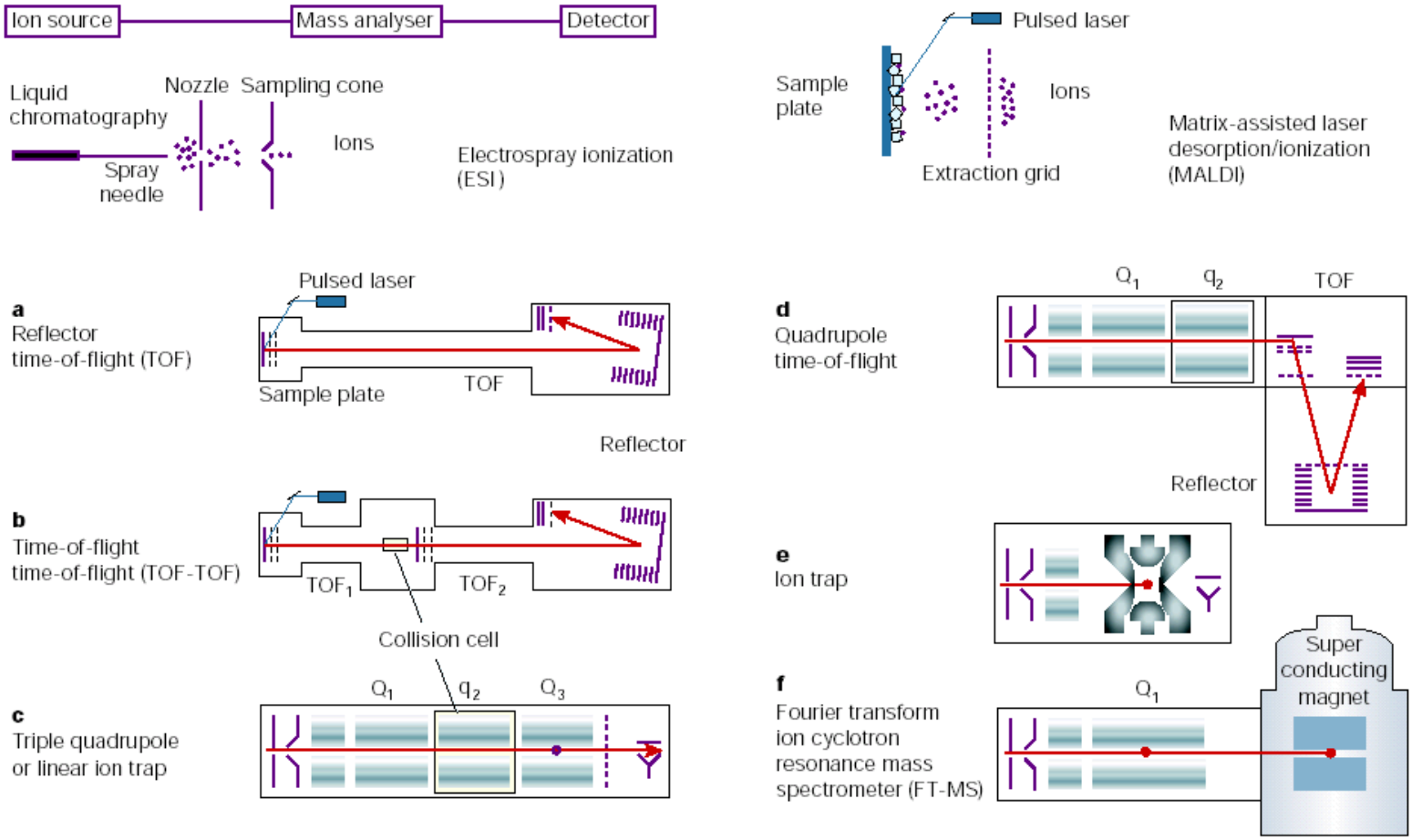
i- ASAP (Atmospheric-pressure Solid Analysis Probe)

j- EASI (Easy Ambient Sonic-Spray Ionization) 

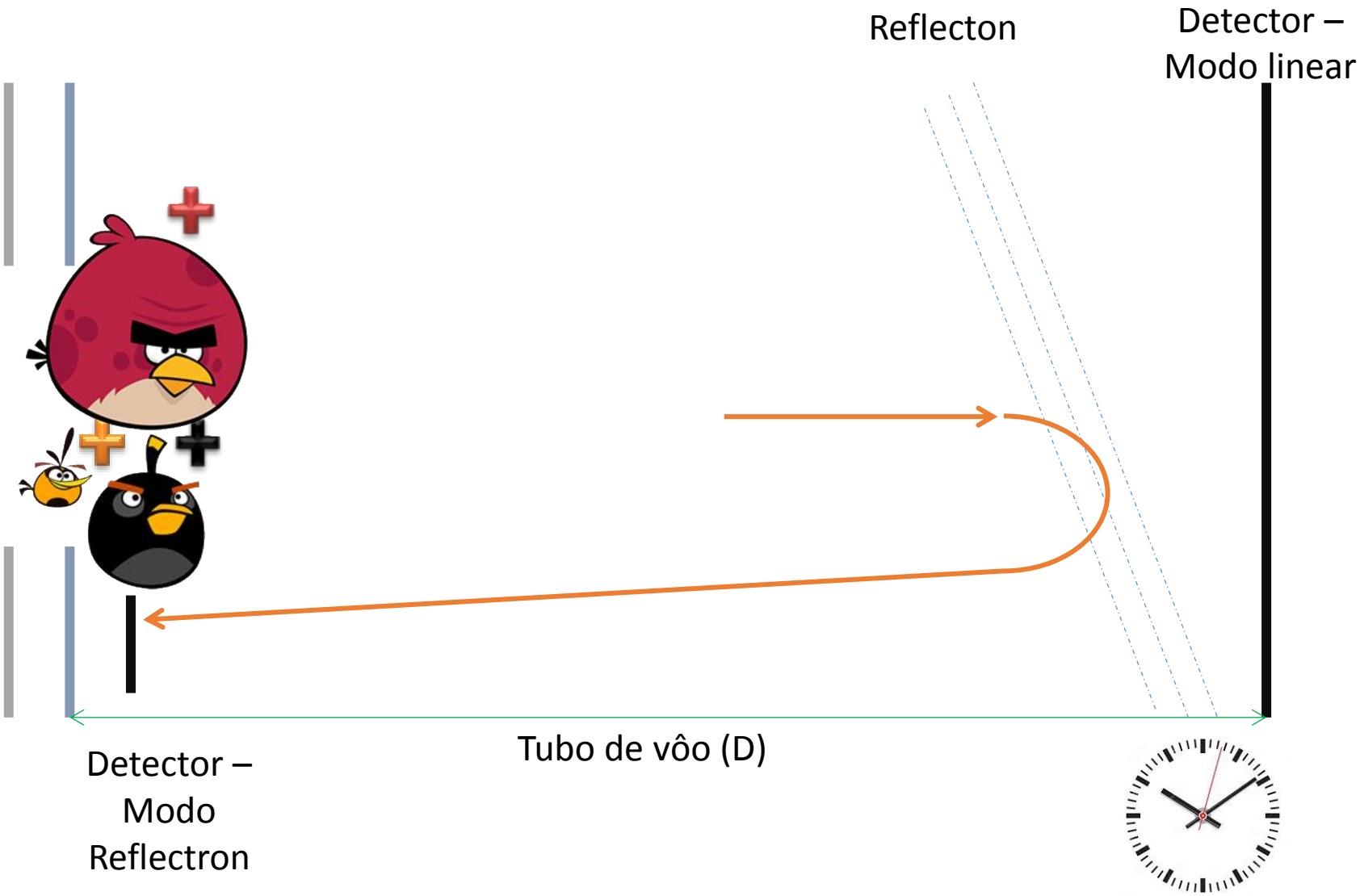
Espectrômetro de Massas



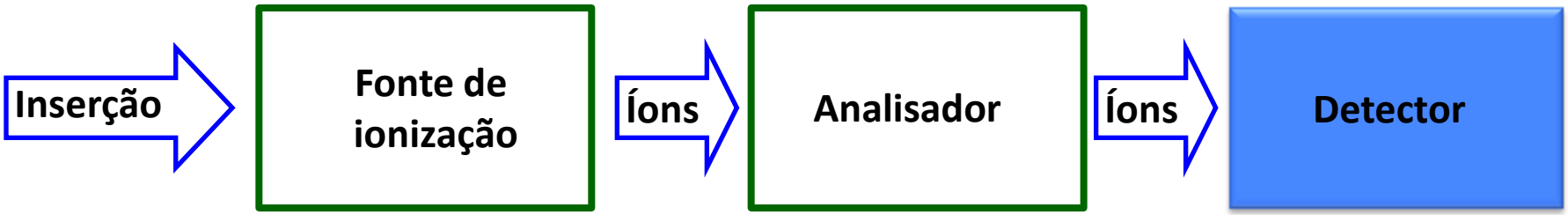
Analisadores



TOF – Time of Flight

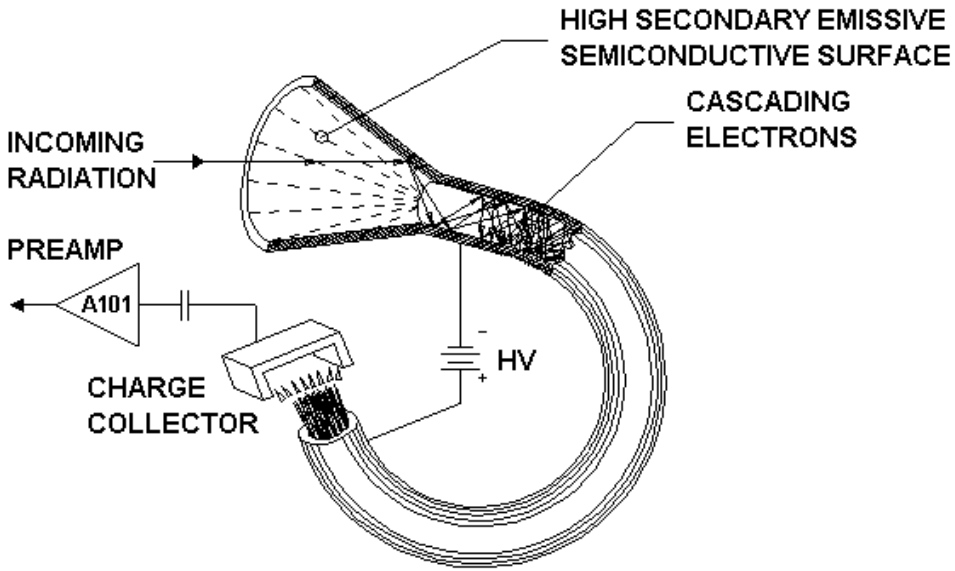


Espectrômetro de Massas



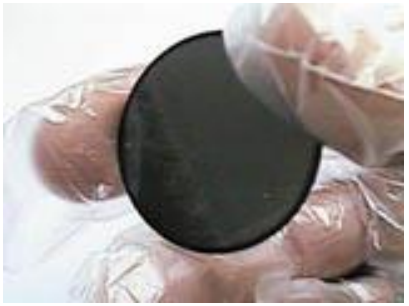
Detectores

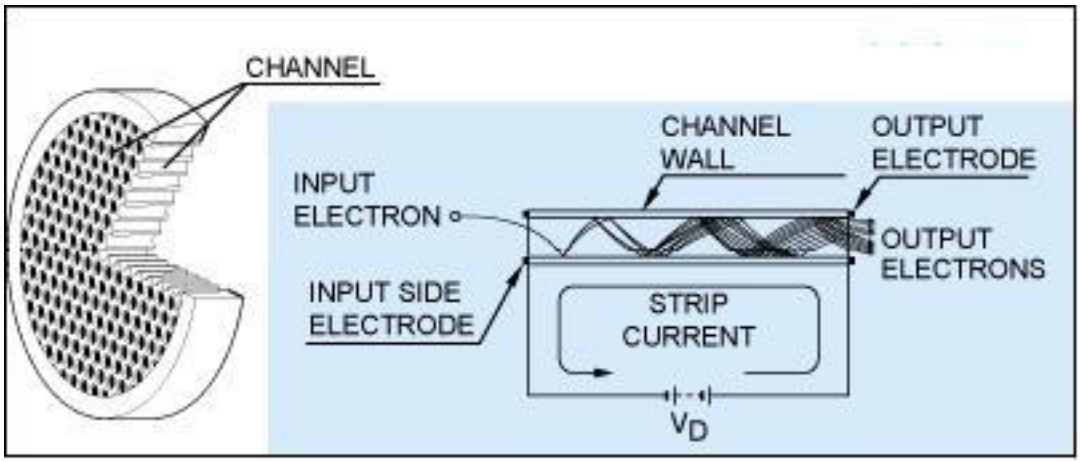
“Continuous Dynode Multipliers”



http://alfa-img.com/show/electron-multiplier.html

MCP - Micro-Channel Plates



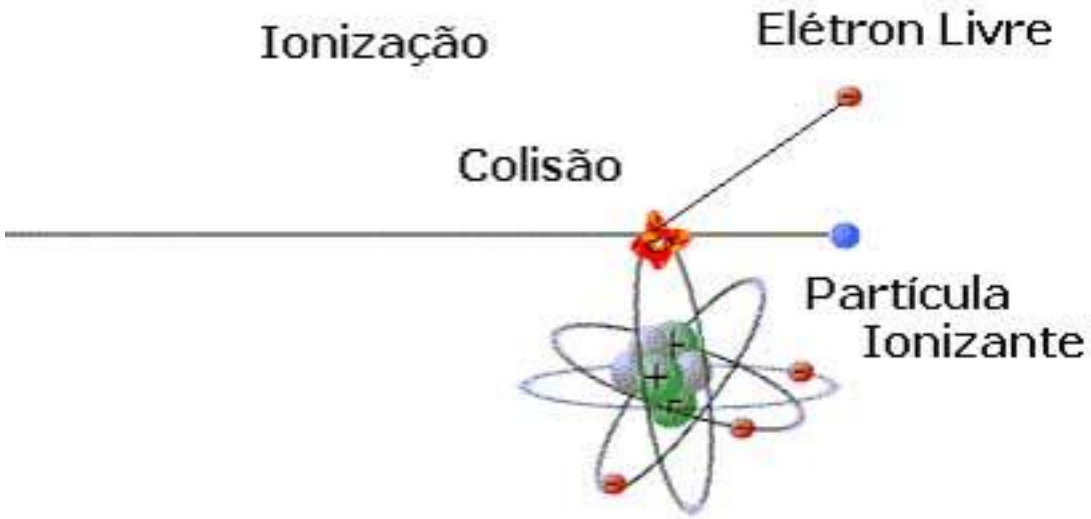


Por que ionizar?

http://luke1428.com/a-15-yr-old-life-should-never-end-this-tragically/

EI : Electron Ionization 70 eV

(Dempster & Nier)



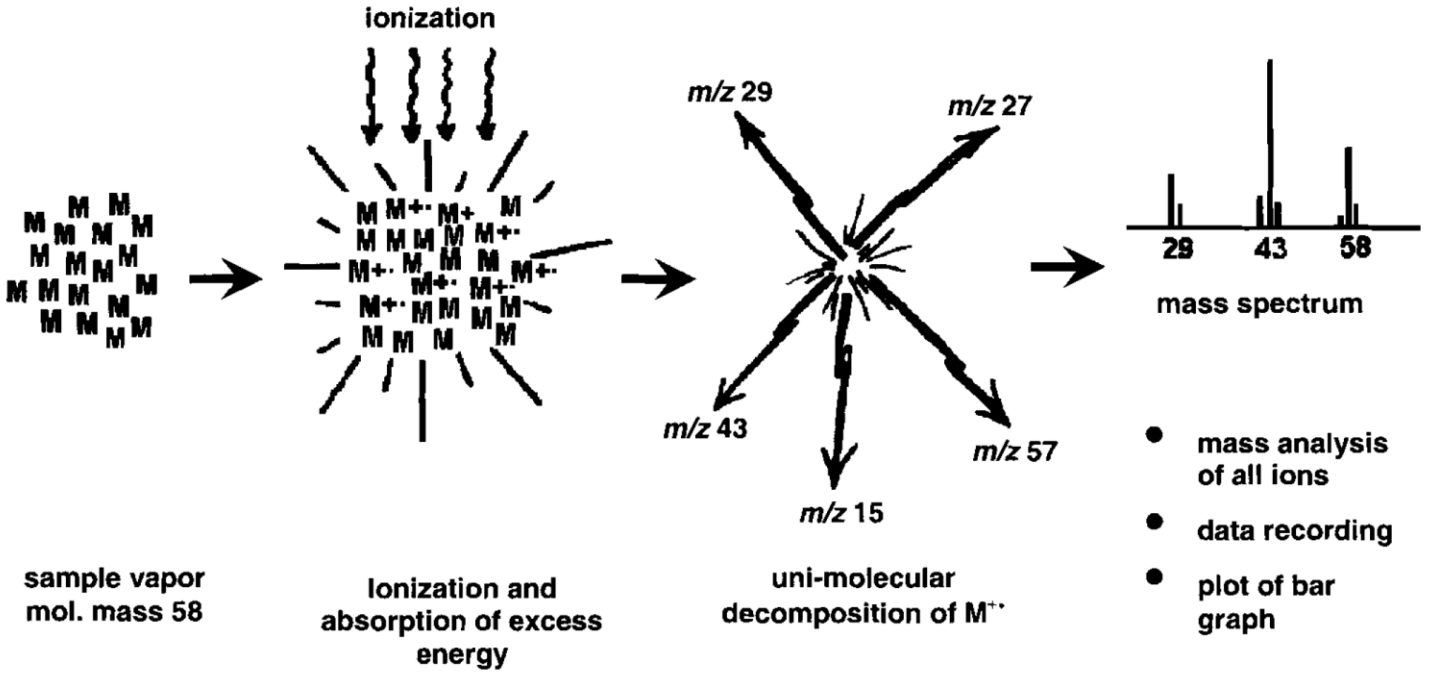
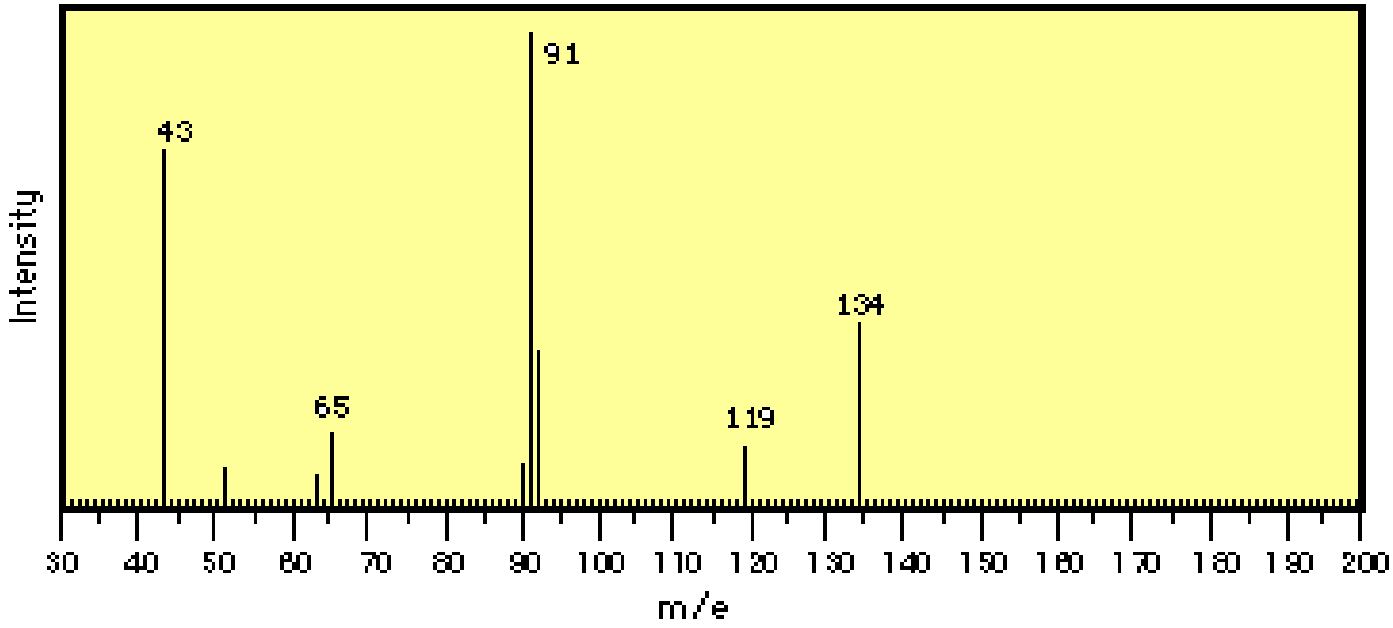
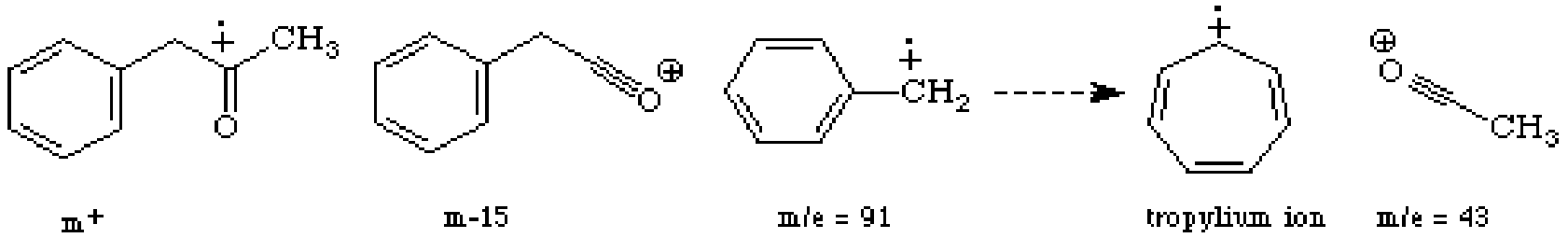


Figure 1-3. Conceptual illustration of gas-phase ionization of analytes followed by ion separation according to the m/z value.

Espectro de Massas: Ionização por Elétrons (EI)





https://www.colourbox.com/image/red-question-mark-inside-cracked-eggshell-image-3506306

Revolução em Espectrometria de Massas

The Nobel Prize in Chemistry 2002

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

ESI

John B. Fenn

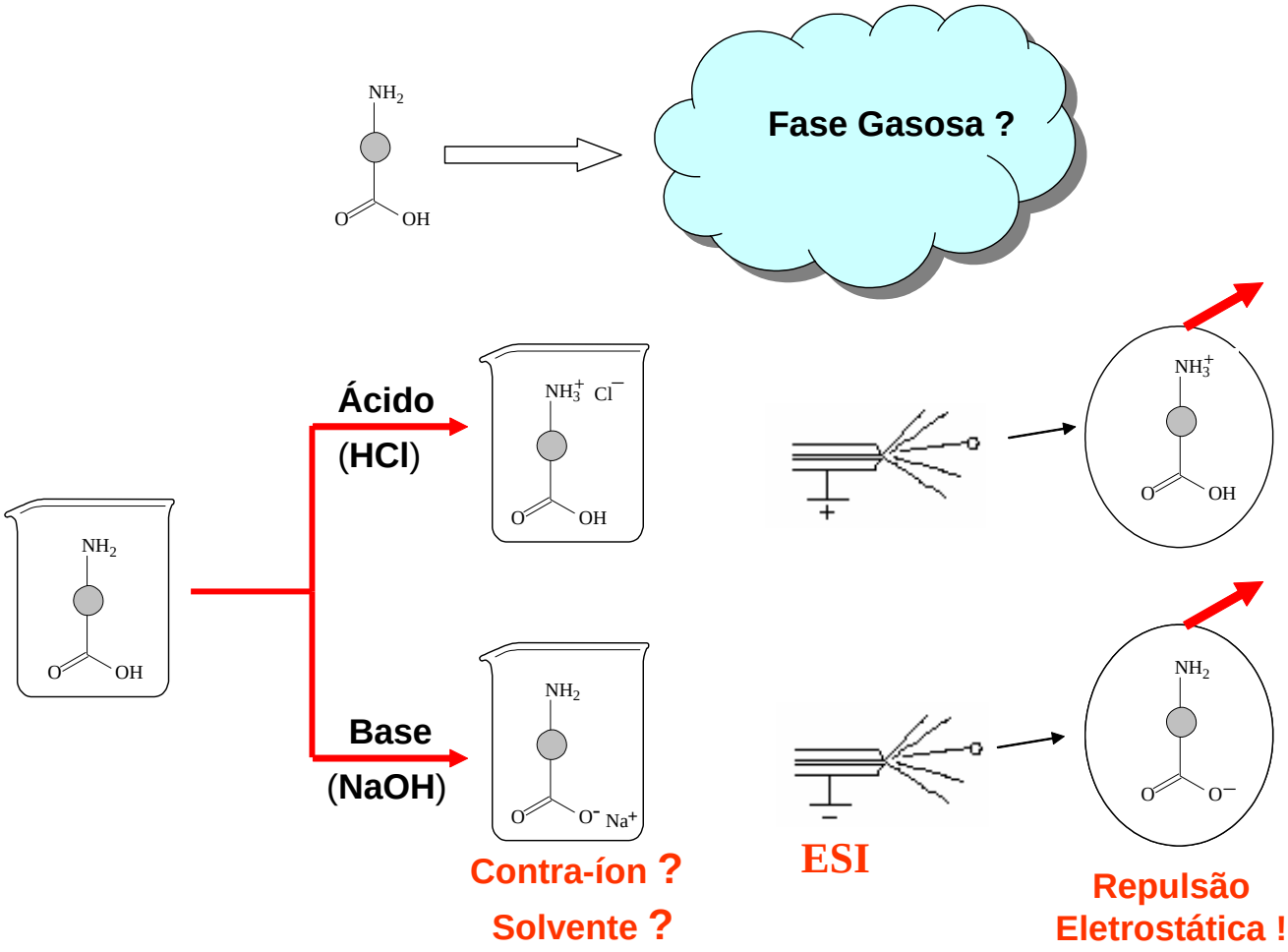
Virginia Commonwealth University
Richmond, VA, USA

MALDI (Proteínas)

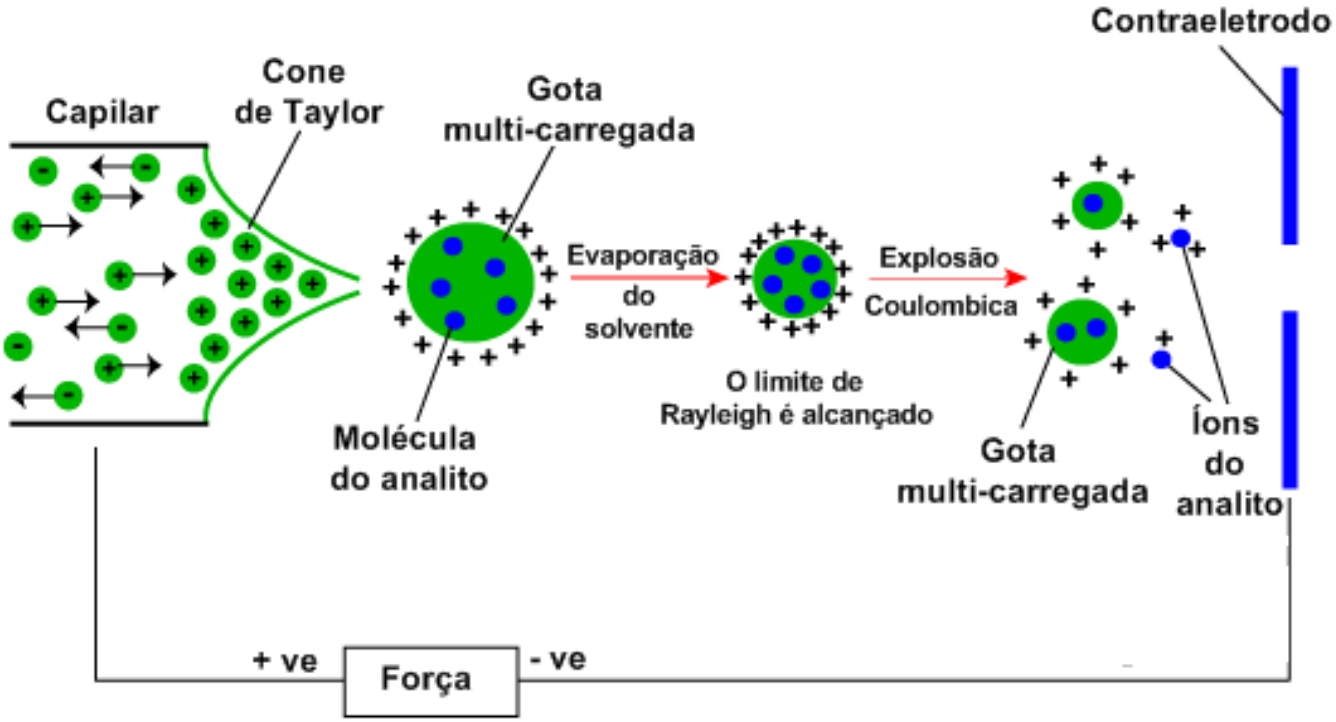
Koichi Tanaka

Shimadzu Corp.
Kyoto, Japan

ESI – Electrospray Ionization

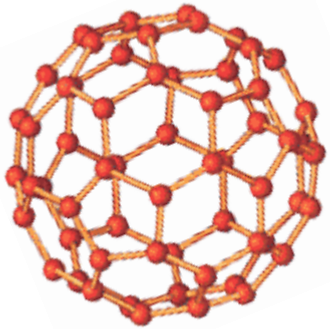


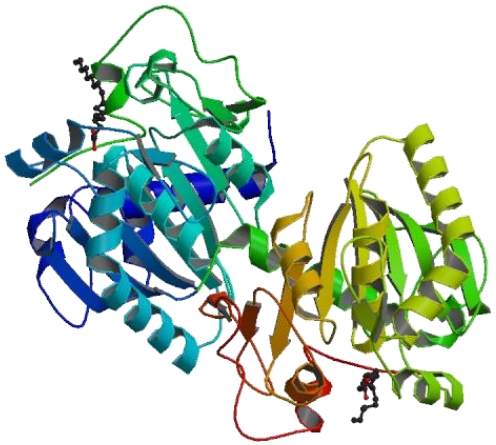
ESI – Electrospray Ionization

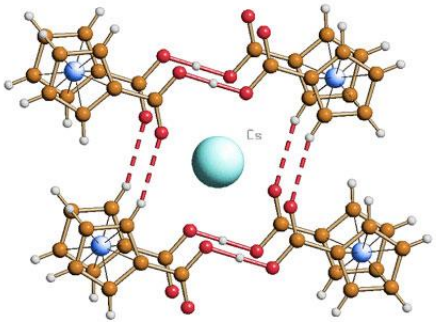


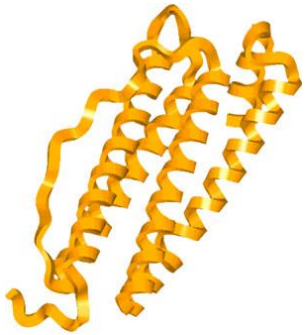
ESI – Electrospray Ionization

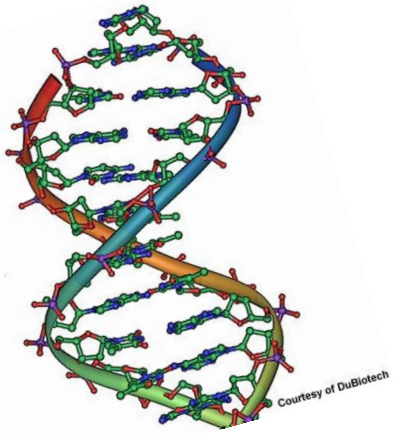
Fenn's Nobel lecture: "*Electrospray Wings for Molecular Elephants*"



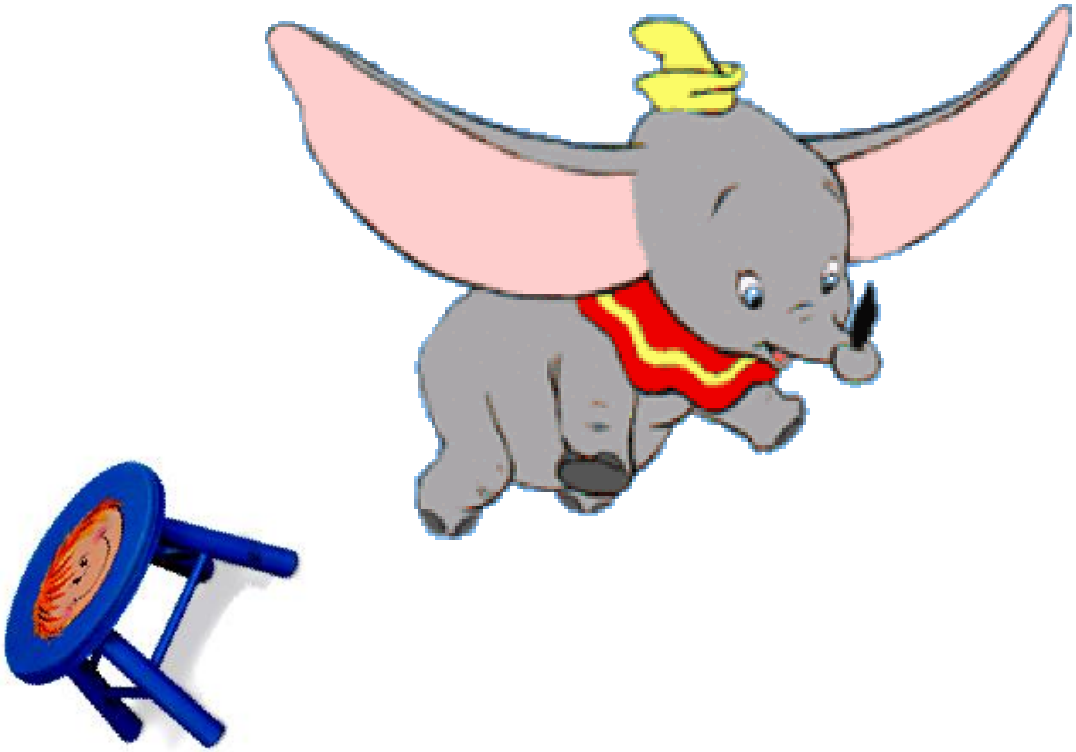


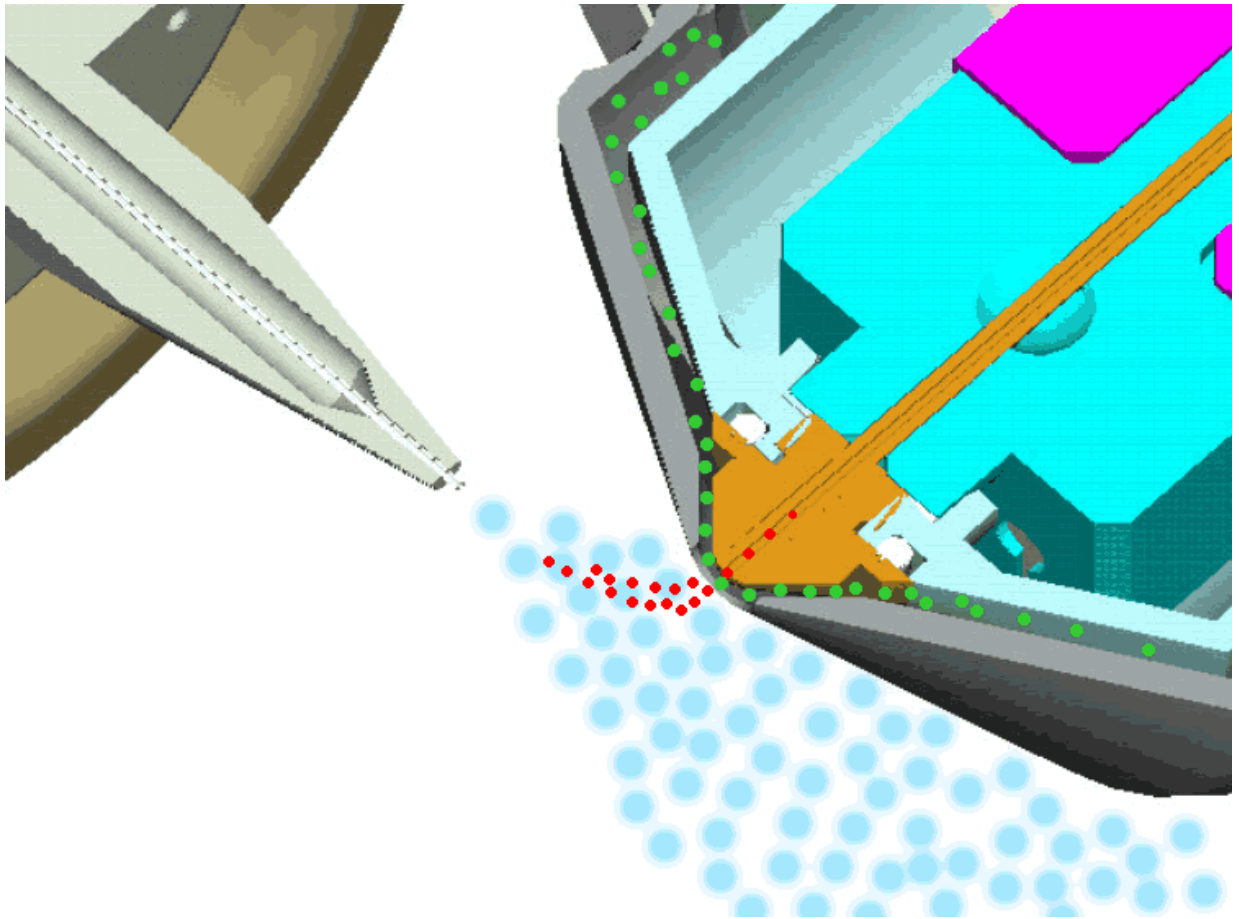


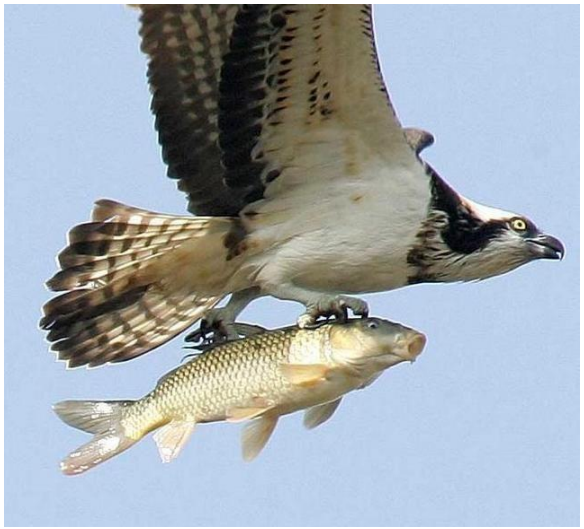




http://karlshuker.blogspot.com.br/2013/12/youll-believe-elephant-can-flypossibly.html

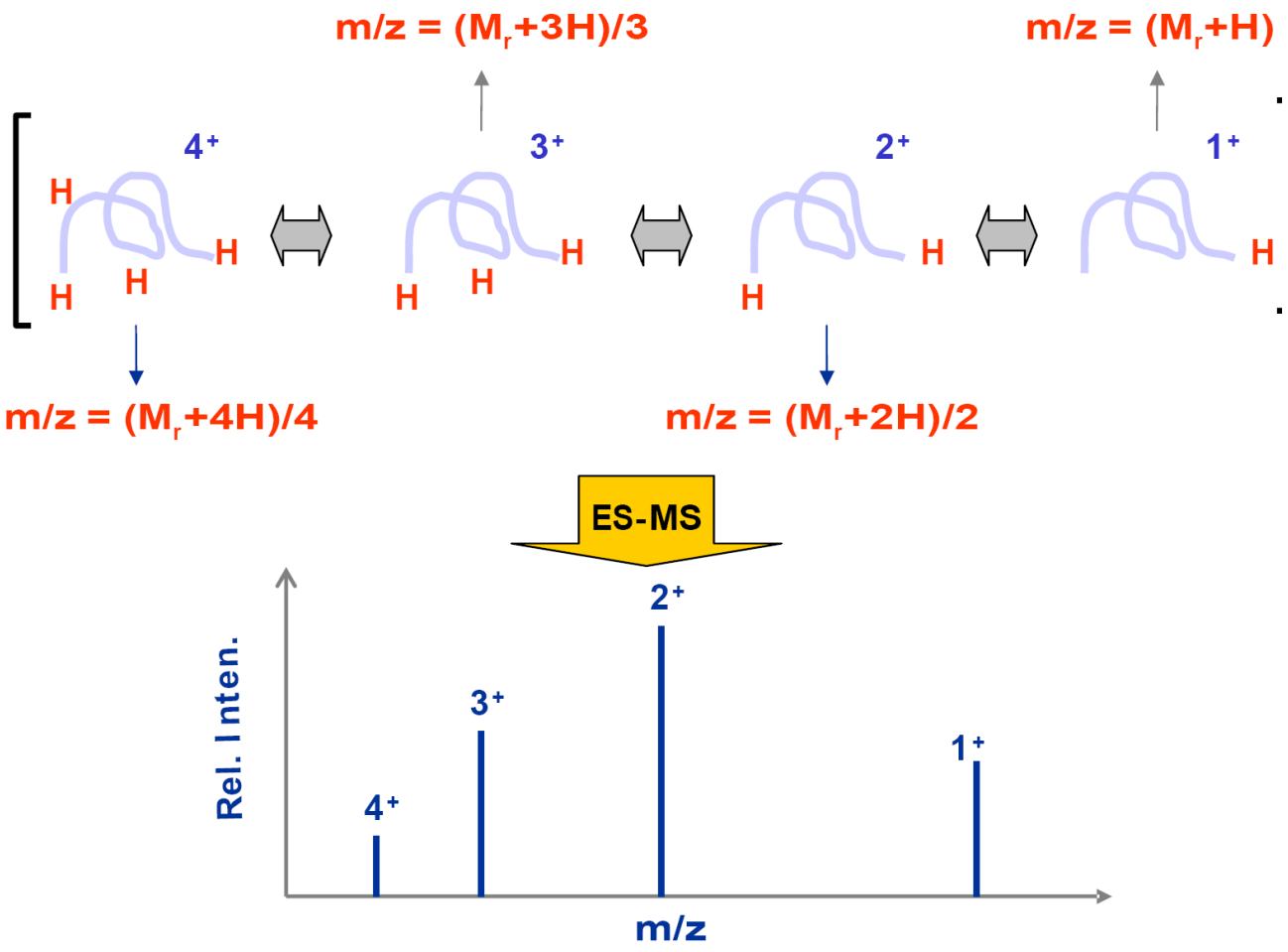






https://www.reddit.com/r/funny/comments/14yj9l/i_can_show_you_the_world/

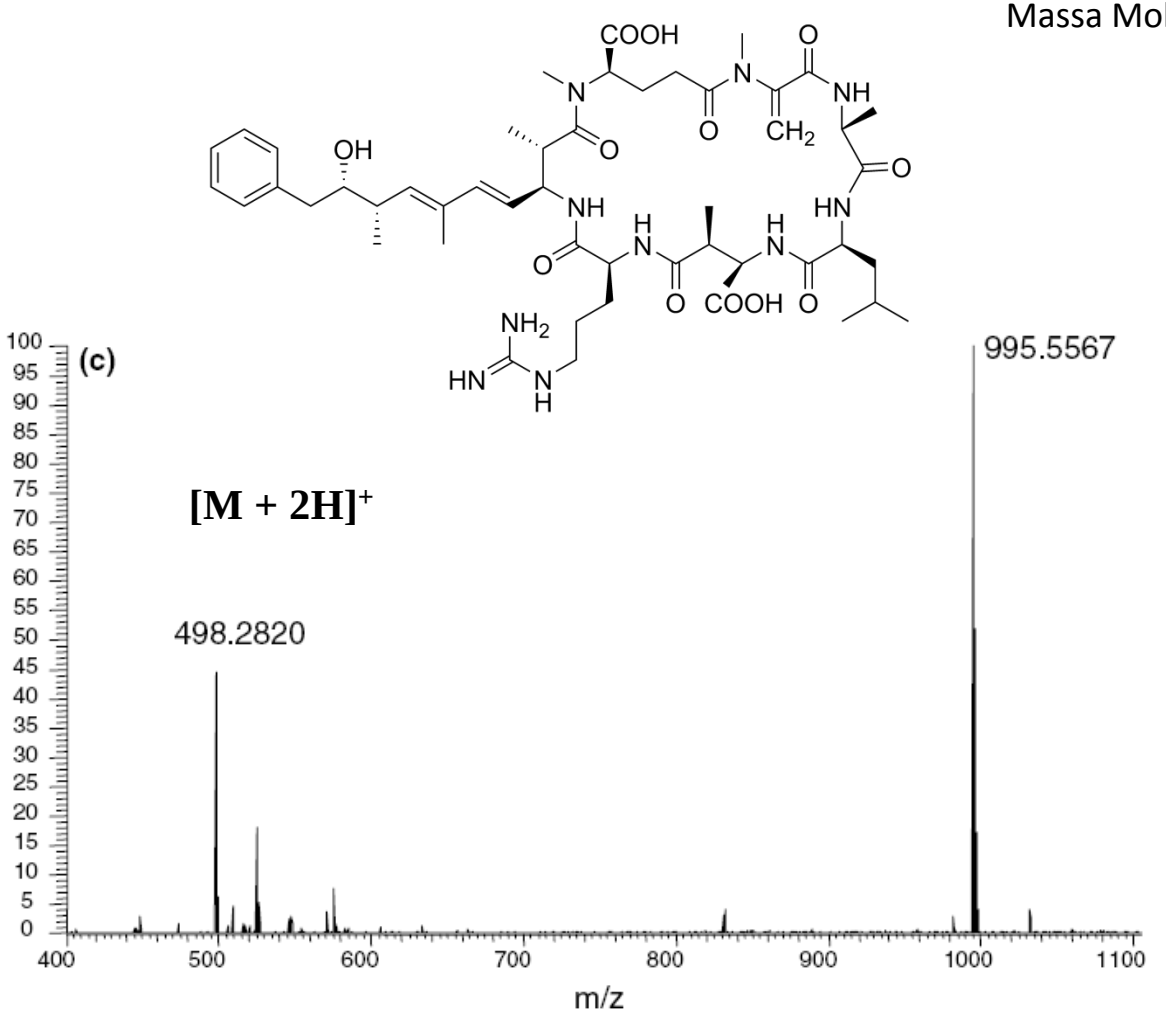
ESI gera íons multicarregados



Caracterização de peptídeos

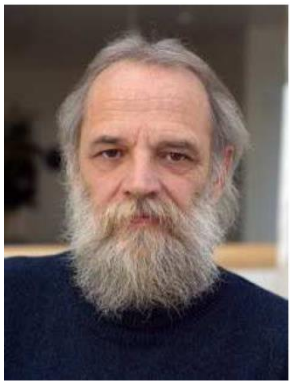
Massa Molecular: 994.00 Da

Espectro de ESI-MS:



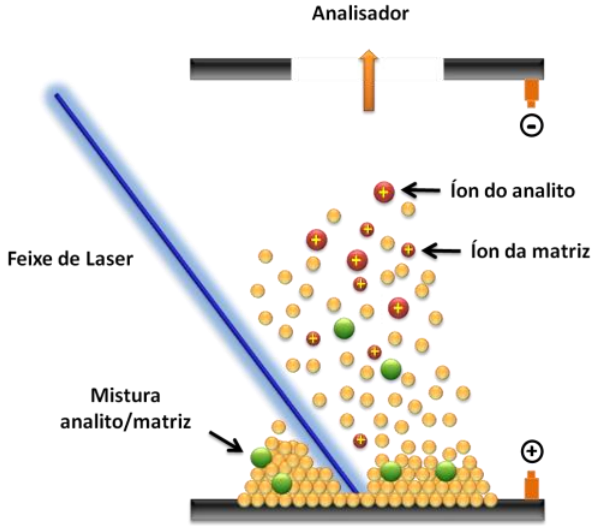
Cong, L. et al. Analytica Chimica Acta **2006**, *569*, *157*.

MALDI – Matrix-Assisted Laser Desorption/Ionization



Karas & Hillenkamp

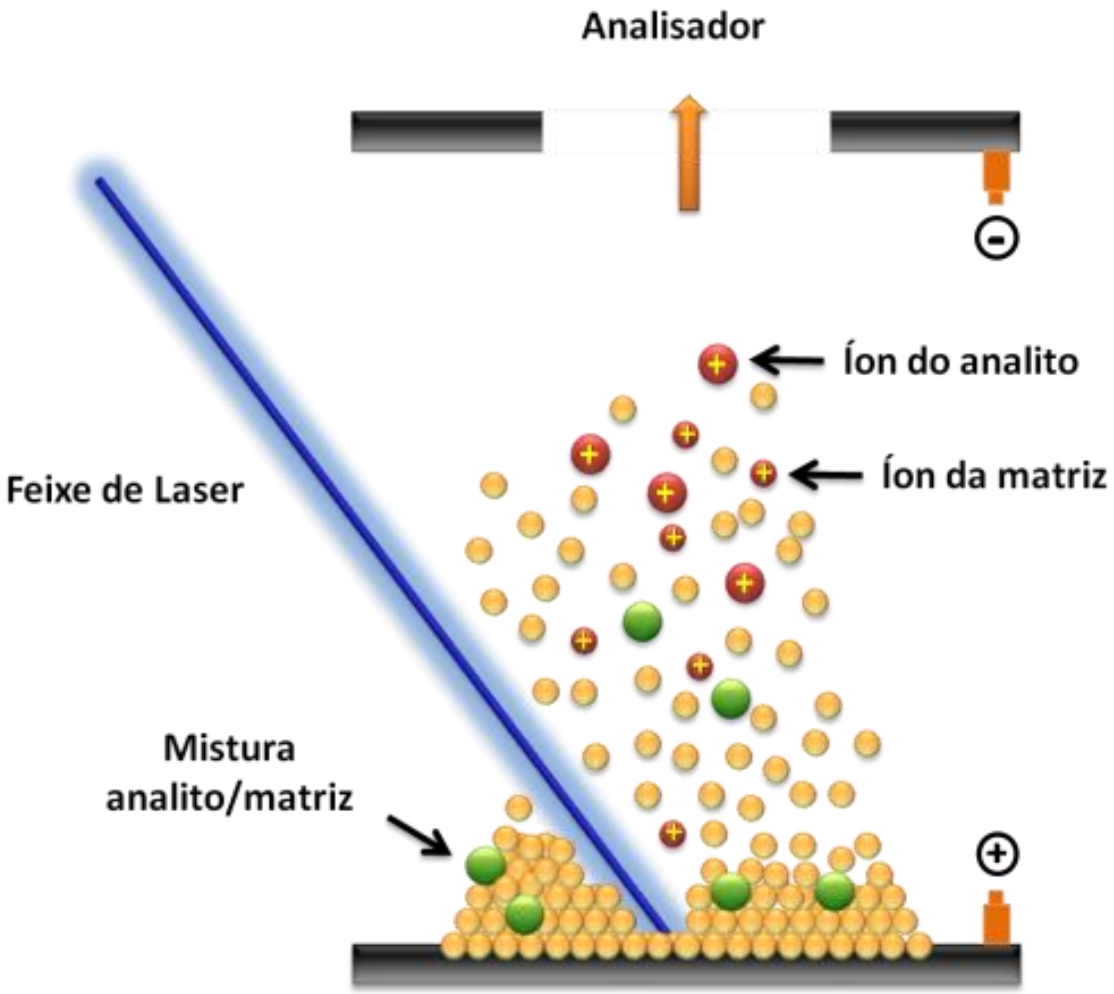
Koichi Tanaka



MALDI – Matrix-Assisted Laser Desorption/Ionization

2011

MALDI – Matrix-Assisted Laser Desorption/Ionization



MALDI – Matrix-Assisted Laser Desorption/Ionization

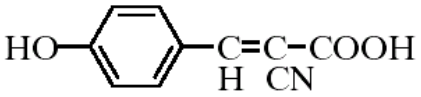
Table 1.1 Some common lasers used for MALDI.

Laser	Wavelength	Energy (eV)	Pulse width
Nitrogen	337 nm	3.68	<1 ns to a few ns
Nd:YAG μ3	355 nm	3.49	5 ns
Nd:YAG μ4	266 nm	4.66	5 ns
Er:YAG	2.94 μm	0.42	85 ns
CO_2	10.6 μm	0.12	100 ns + 1 μs tail

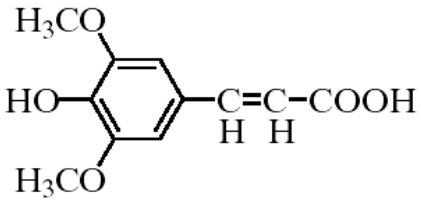
Nd:YAG (neodymium-doped yttrium aluminium garnet; Nd:$Y_3Al_5O_{12}$)

MALDI – Matrix-Assisted Laser Desorption/Ionization

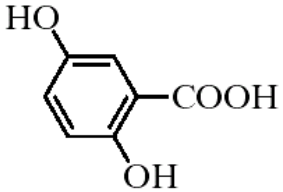
Matrizes - MALDI



(1)



(2)



(3)

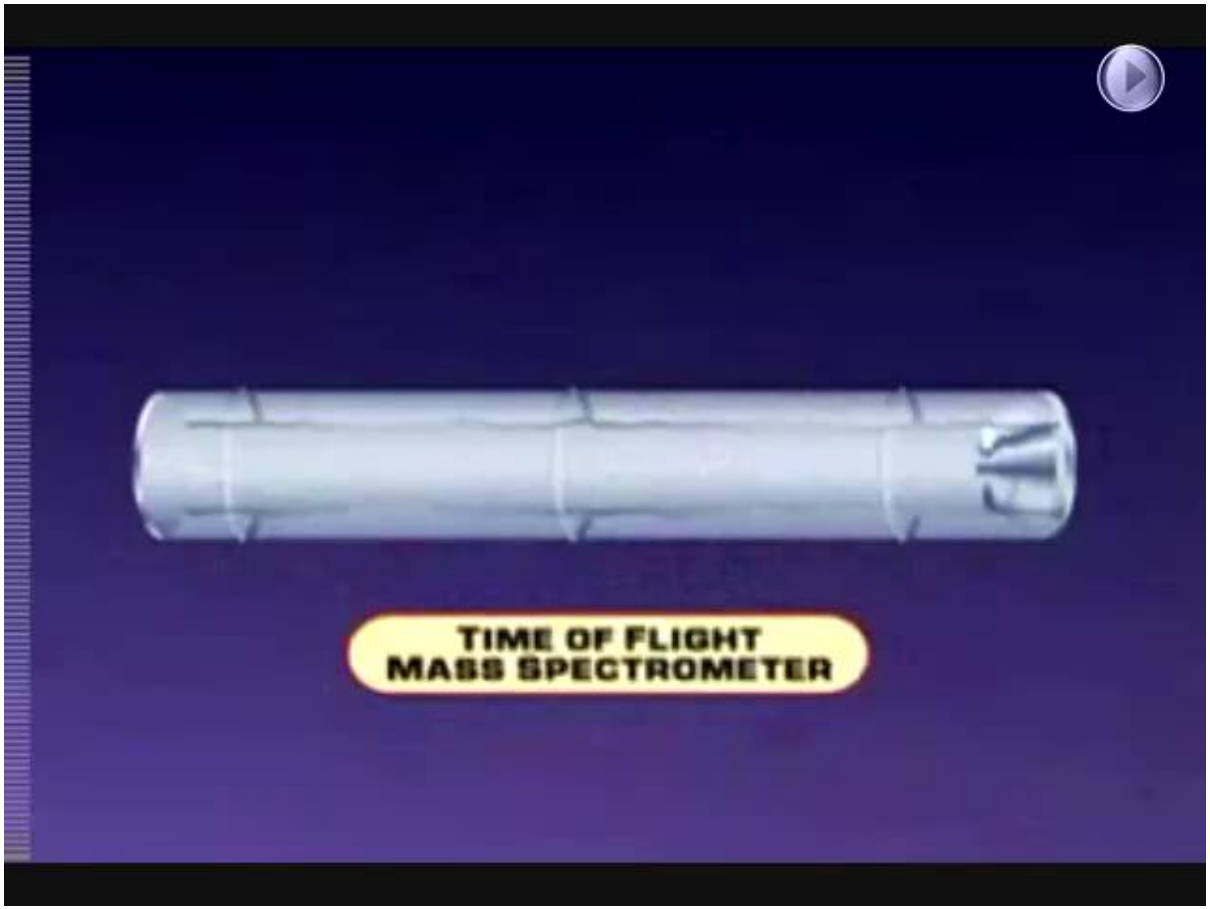
(1) α-cyano-4-hydroxycinnamic acid

(2) 3,5-dimethoxy-4-hydroxycinnamic acid

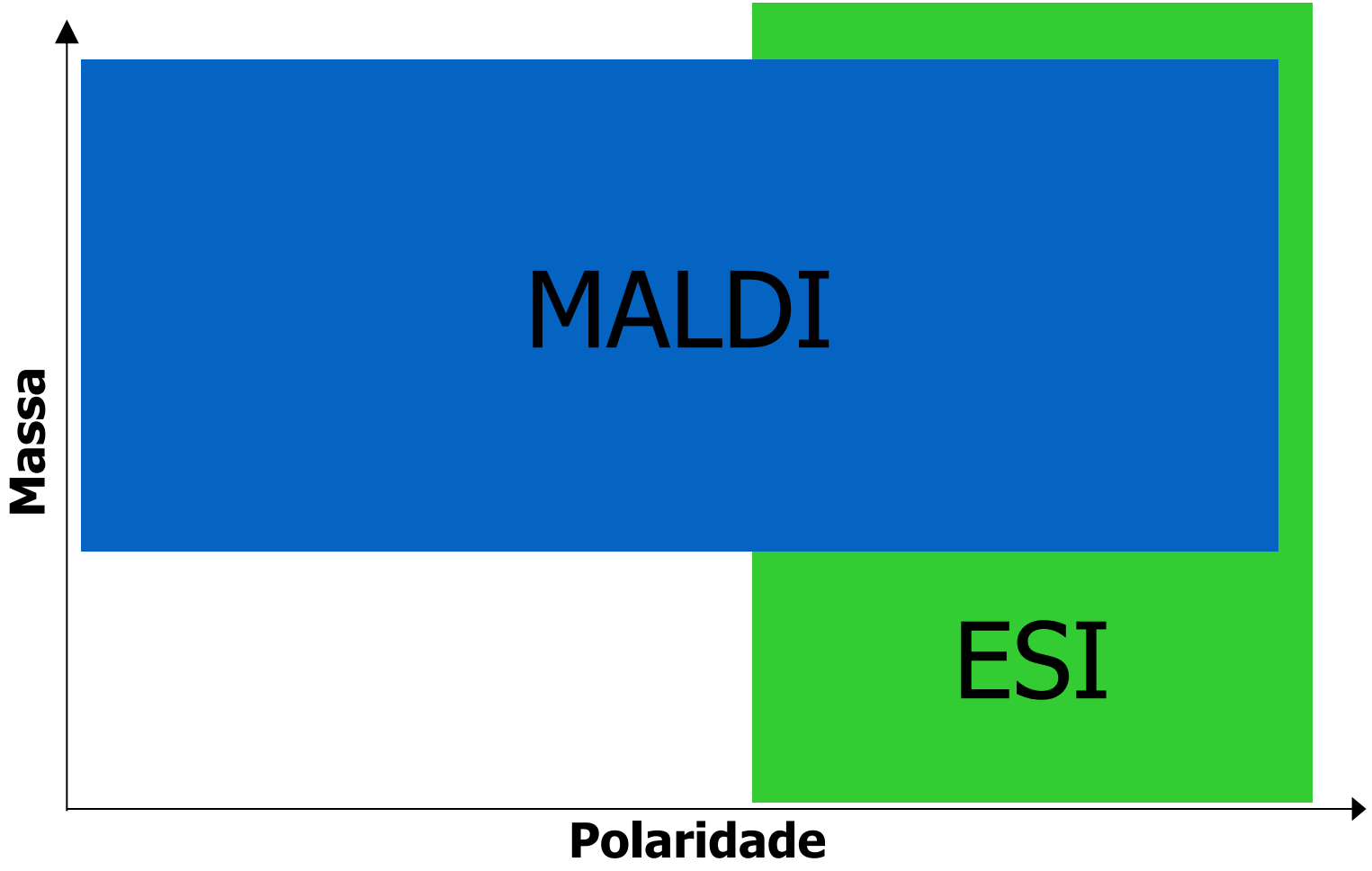
(3) 2,5-dihydroxybenzoic acid.

Razão matriz-amostra: 100:1 a 5000:1

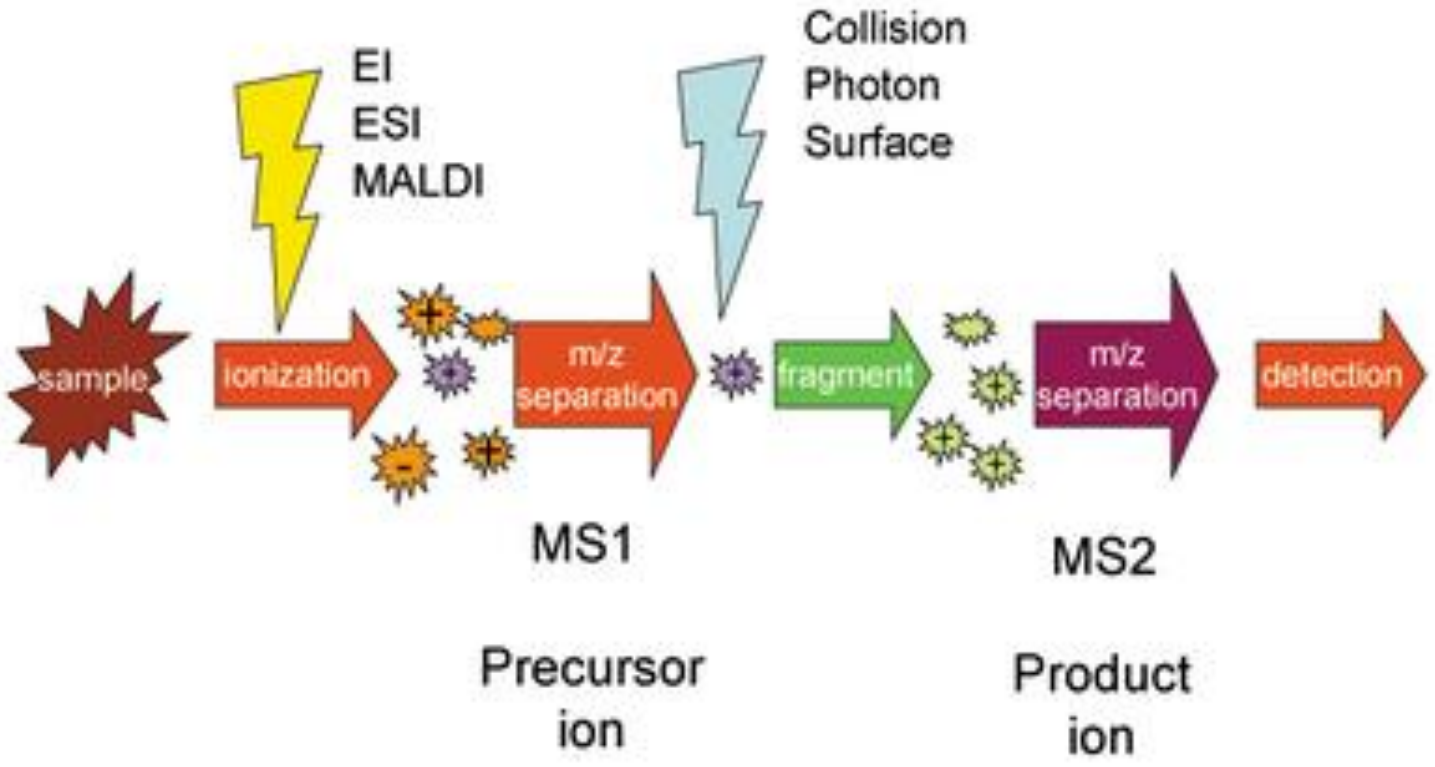
MALDI – Matrix-Assisted Laser Desorption/Ionization

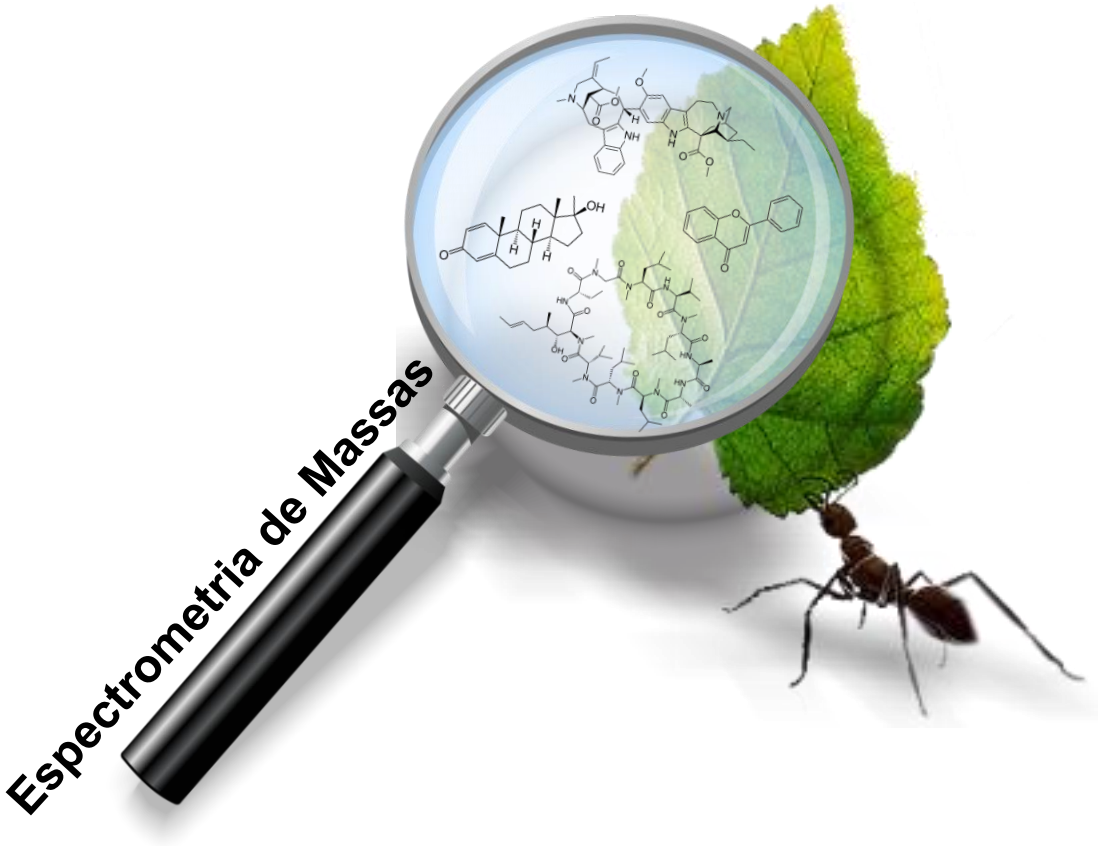


MALDI X ESI

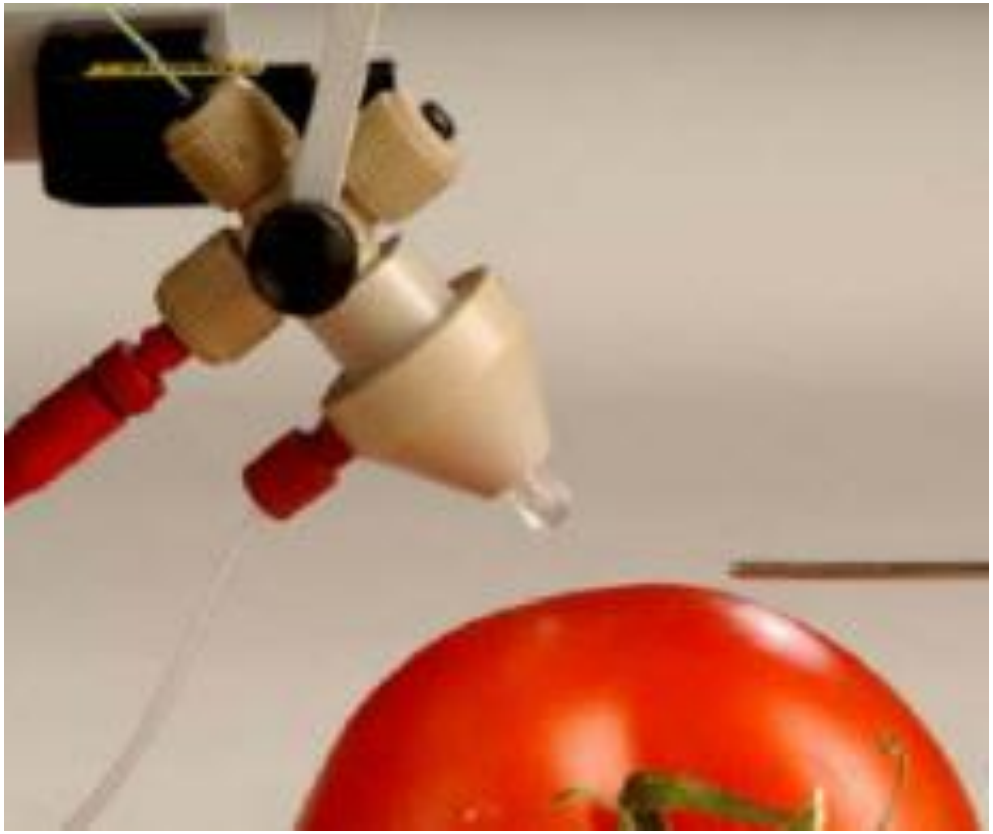



MS/MS ???
MS2 ????
MSn ????


EI
ESI
MALDI
Collision
Photon
Surface
sample
ionization
m/z
separation
fragment
m/z
separation
detection
MS1
MS2
Precursor
ion
Product
ion

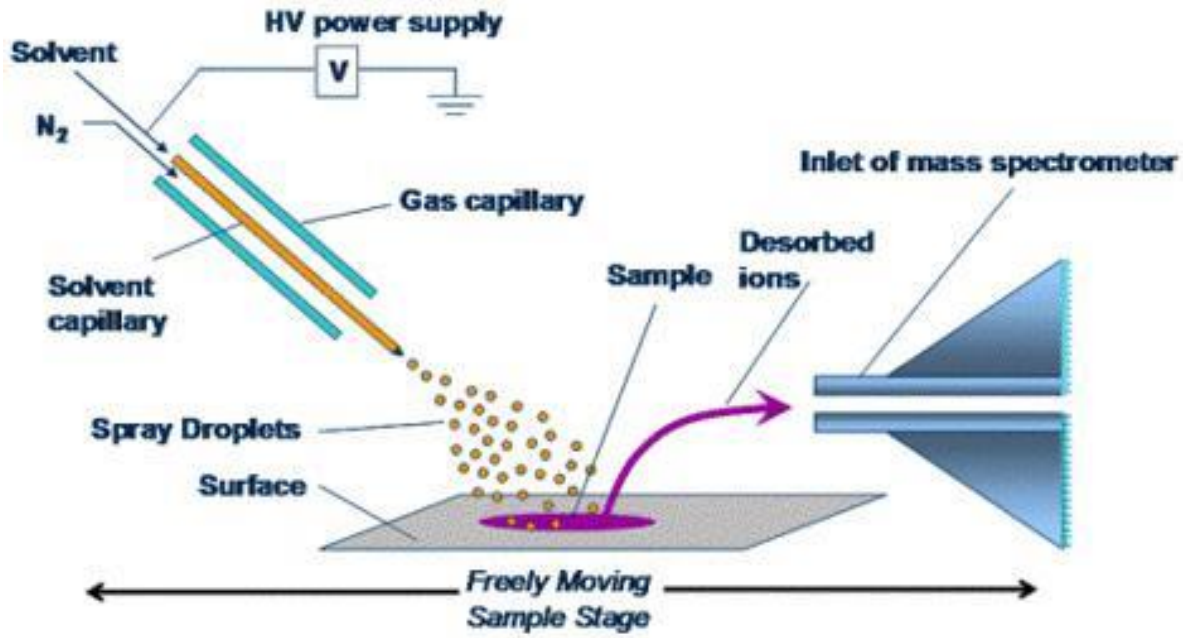

Espectrometria de Massas

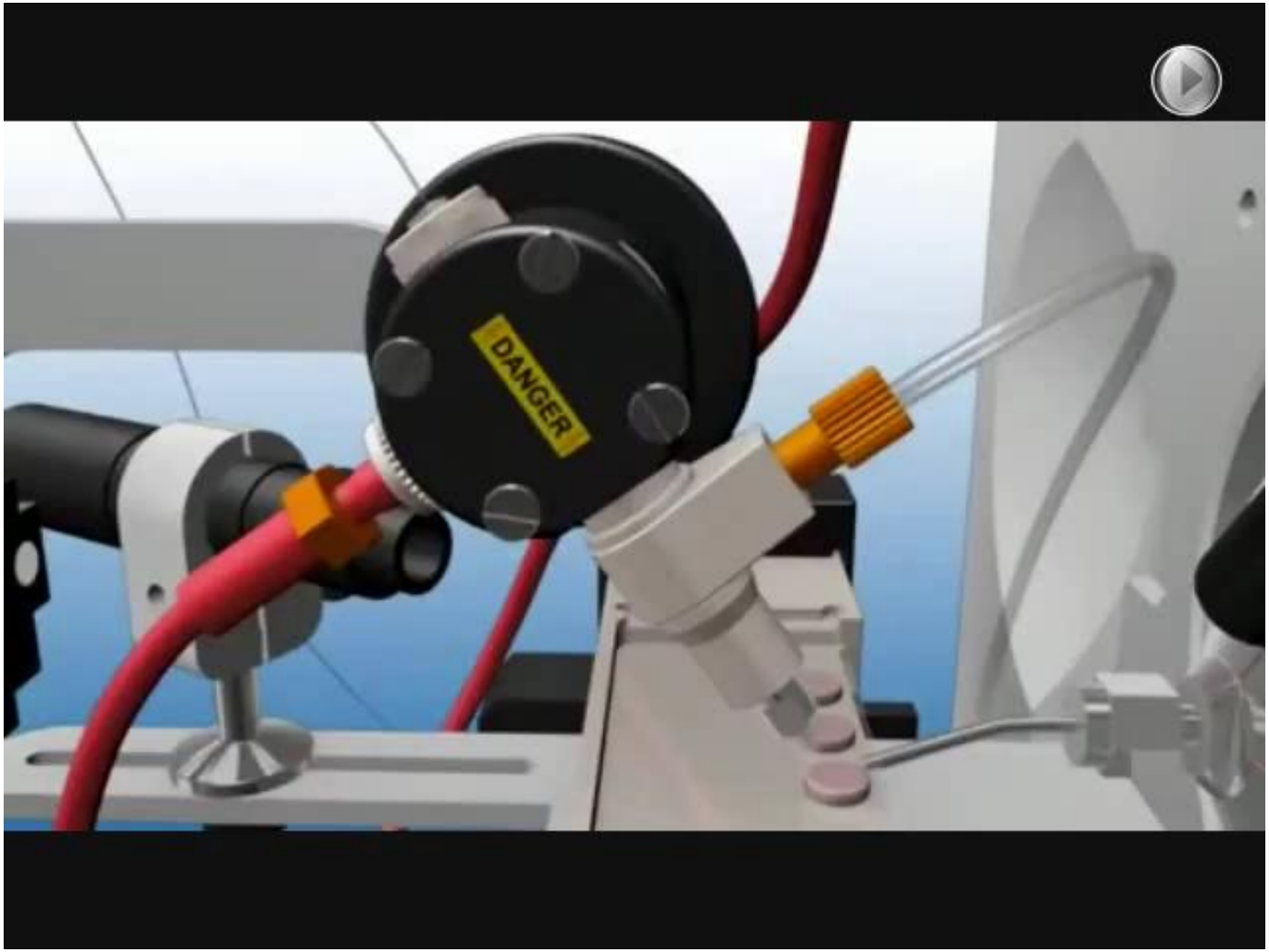
Fontes modernas de Ionização



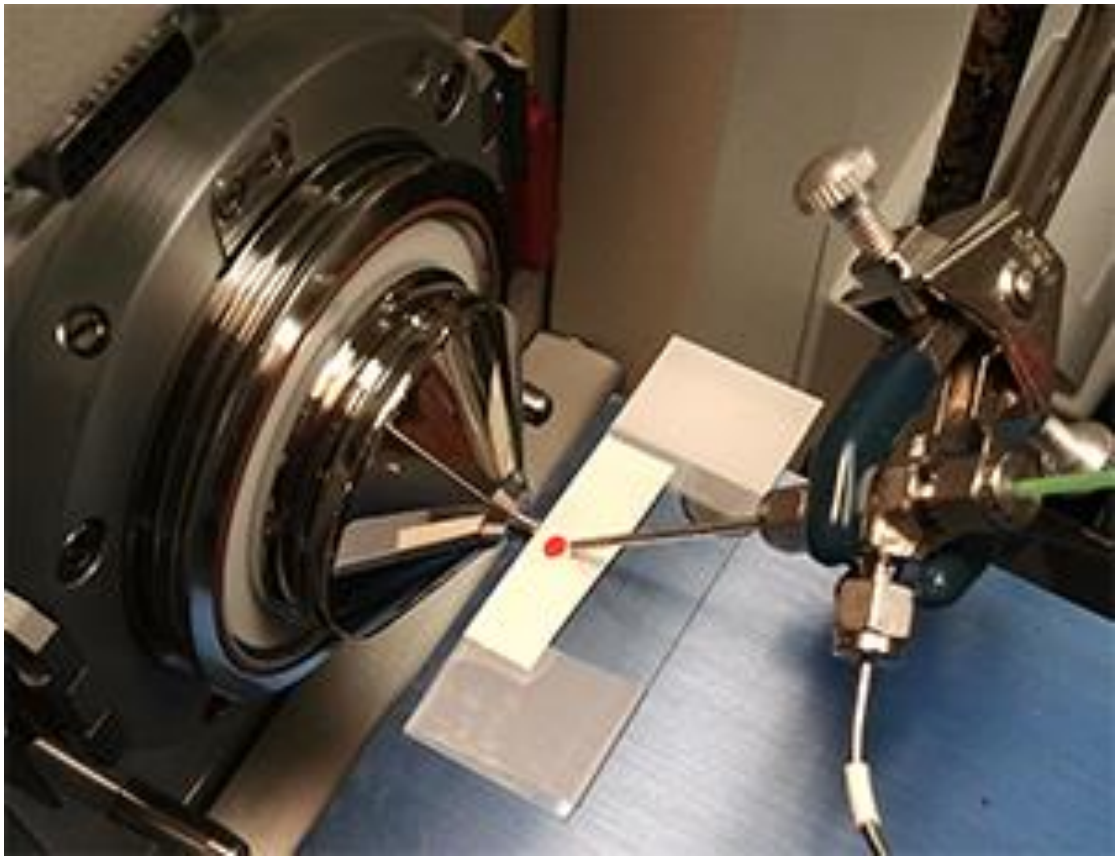
Desorption Electronspray Ionization - DESI

Prof. Graham Cooks



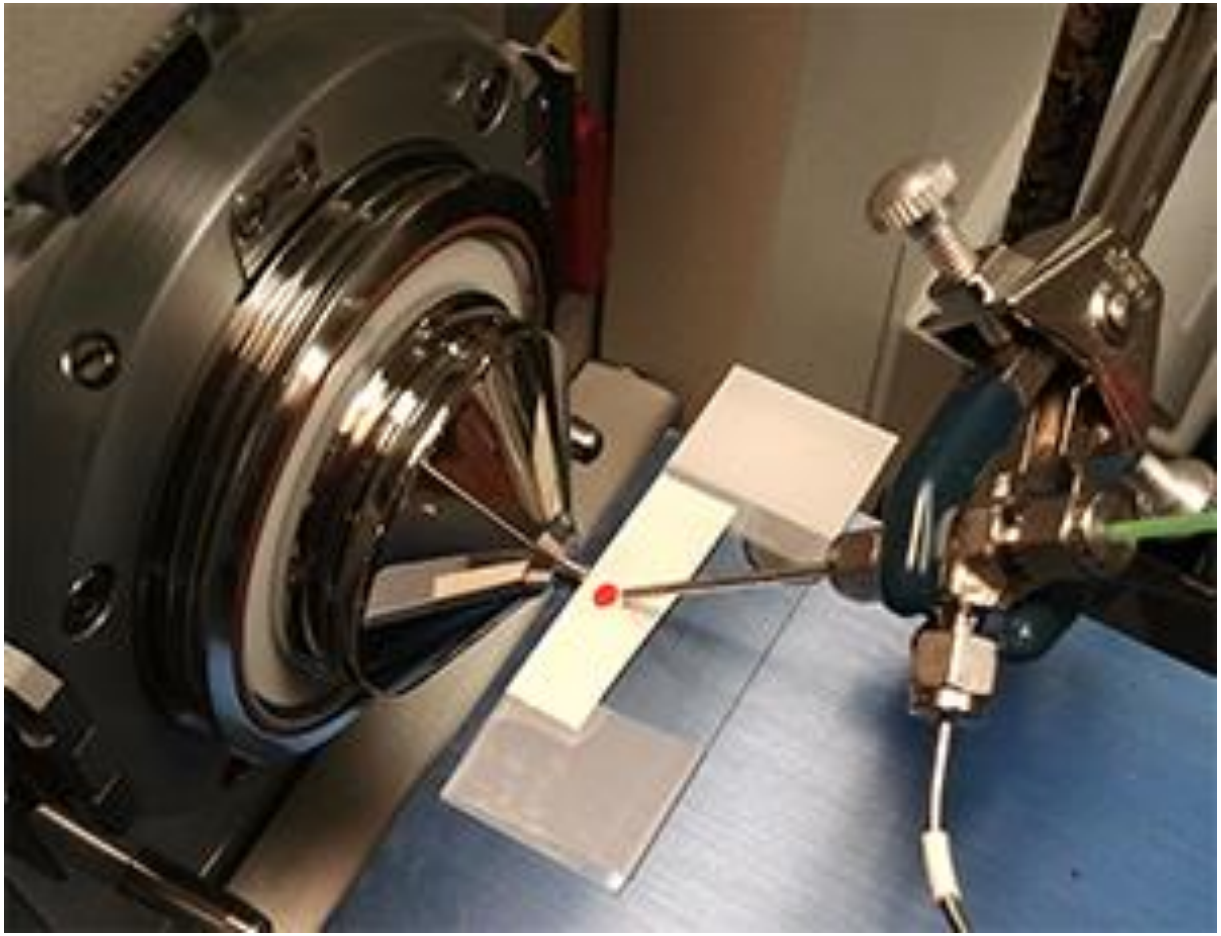

DANGER

Desorption Electronspray Ionization - DESI



Desorption Electronspray Ionization - DESI

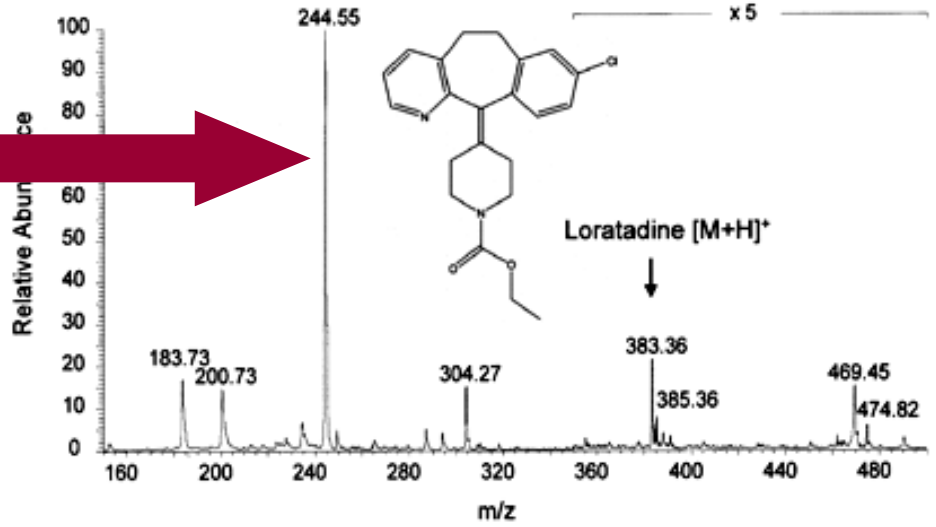
Desorption Electronspray Ionization - DESI

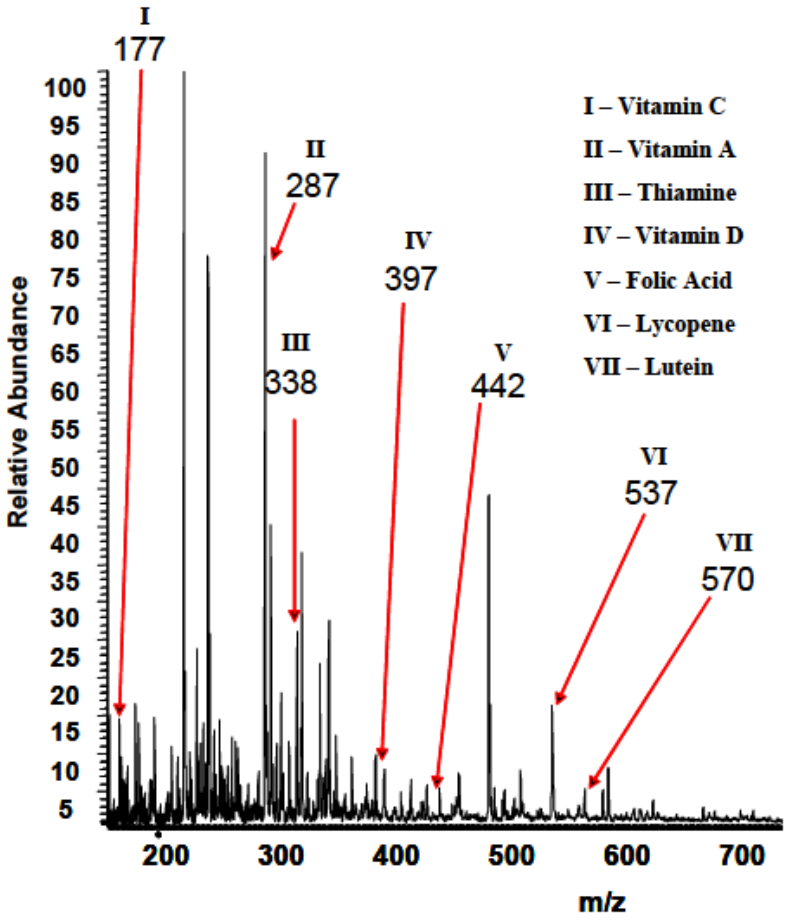


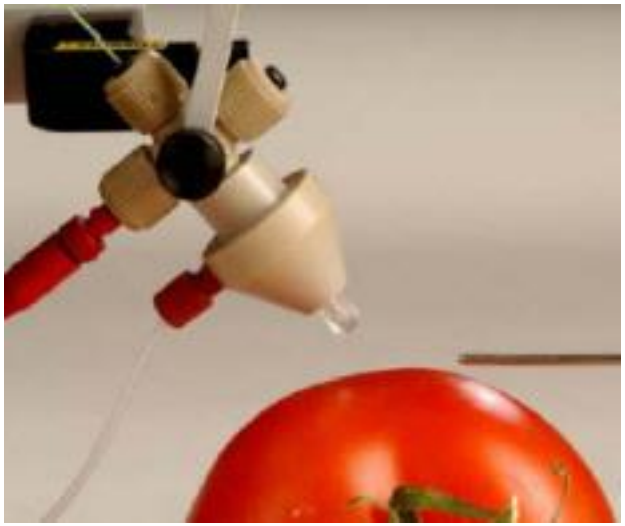
Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization

Zoltán Takáts, Justin M. Wiseman,
Bogdan Gologan, R. Graham Cooks*

SCIENCE VOL 306 15 OCTOBER 2004




I
177
II
287
III
338
IV
397
V
442
VI
537
VII
570
I – Vitamin C
II – Vitamin A
III – Thiamine
IV – Vitamin D
V – Folic Acid
VI – Lycopene
VII – Lutein
Relative Abundance
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
200
300
400
500
600
700
m/z



Pesticides

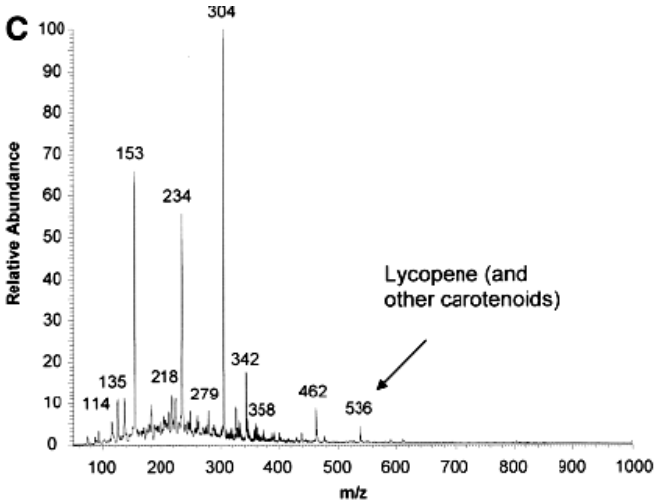


Fig. 3. (**A**) Positive-ion DESI spectrum of *Conium maculatum* seed section with the sample held under ambient conditions. The signal at *m/z* 126 corresponds to protonated γ-coniceine (molecular weight 125), an alkaloid present in the plant. The inset shows the MS/MS spectrum of *m/z* 126. (**B**) The intensity distribution of *m/z* 126 across a stem cross section. (**C**) DESI mass spectrum of the tomato shown in photograph S2. (*1*) The peak at *m/z* 536 is due to lycopene and/or other carotenoids. Methanol-water was sprayed onto the tomato surface and desorbed ions were transferred to the ion trap mass spectrometer.


CTV

Analyst

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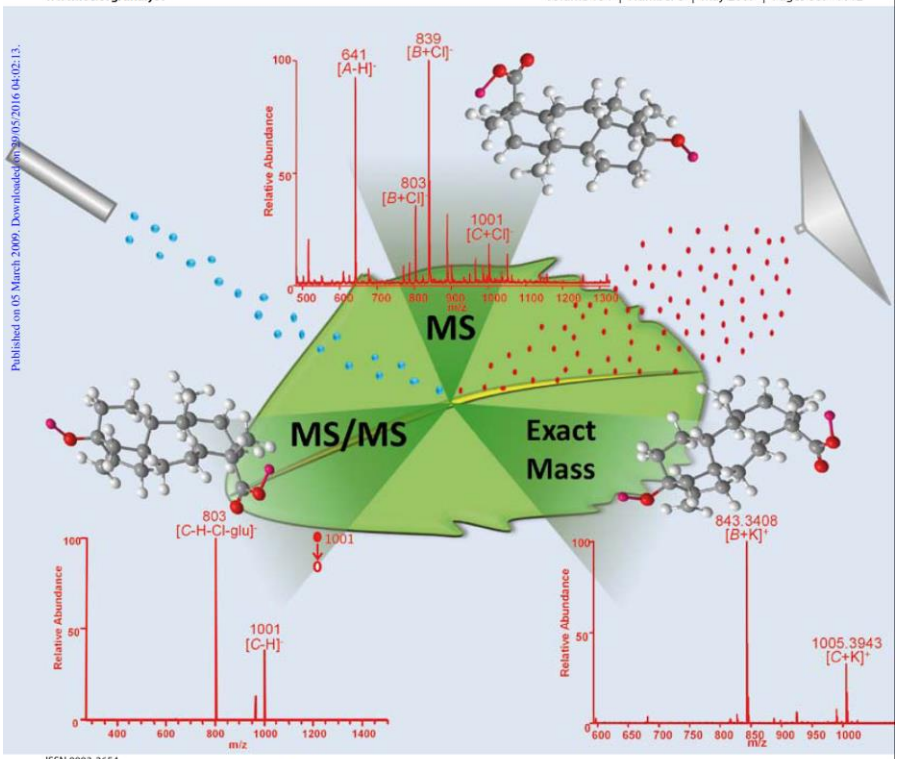

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PAPER
R. Graham Cooks *et al.*
Direct analysis of *Stevia* leaves for diterpene glycosides by desorption electrospray ionization mass spectrometry

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Microwave-enhanced electroanalytical processes: generator–collector voltammetry at paired gold electrode junctions

0003-2654(2009)134:5;1-F

PAPER www.rsc.org/analyst | Analyst

Direct analysis of *Stevia* leaves for diterpene glycosides by desorption electrospray ionization mass spectrometry†

Ayanna U. Jackson,[a] Alessandra Tata,[b] Chunping Wu,[a] Richard H. Perry,[a] George Haas,[c] Leslie West[c] and R. Graham Cooks*[a]

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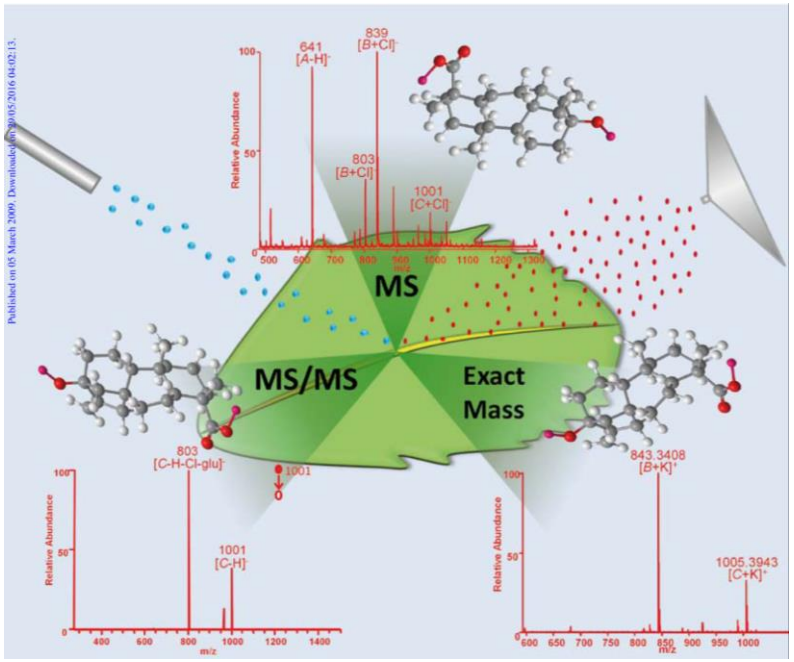


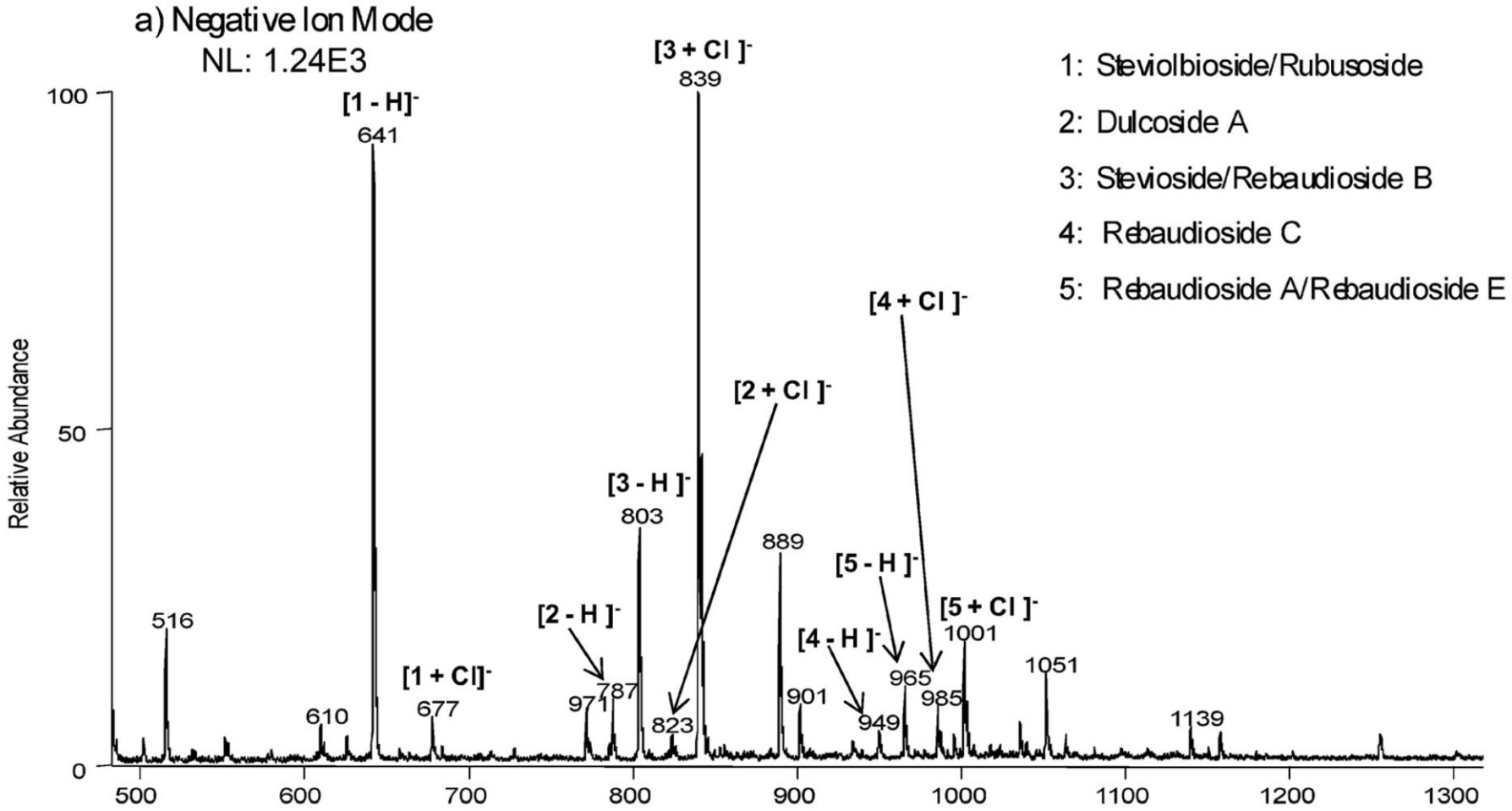
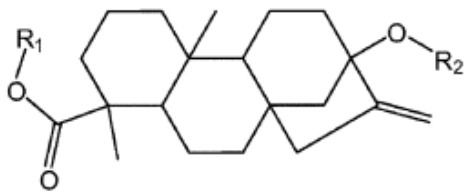

a) Negative Ion Mode
NL: 1.24E3
1: Steviolbioside/Rubusoside
2: Dulcoside A
3: Stevioside/Rebaudioside B
4: Rebaudioside C
5: Rebaudioside A/Rebaudioside E
Relative Abundance
[1 - H]-
641
[3 + Cl]-
839
[4 + Cl]-
[2 + Cl]-
[3 - H]-
803
889
[5 - H]-
[5 + Cl]-
1001
[2 - H]-
[4 - H]-
516
[1 + Cl]-
677
610
787
971
823
901
965
985
949
1051
1139
100
50
0
500
600
700
800
900
1000
1100
1200
1300

Table 1 Summary of sweet glycosides and observations



Sweet glycoside	MW (Da)	R_1	R_2[a]	Sweetening potency[3]	Concentration in leaf (w/w)[4]	Glycoside ratio in leaf[4]	Confirmed by MS/MS using DESI[b]	Approx. glycoside ratio in leaf by DESI
Steviolbioside	642	H	glc-glc	100–125	<0.4%	0.3–3%	Y	~41%[c]
Rubusoside	642	glc	glc	100–120	<0.4%	N/A	Y	~41%[c]
Stevioside	804	glc	glc-glc	150–300	4–14%	43.1–79.6%	Y	~31%[c]
Rebaudioside A	966	glc	$glc(glc)_2$	250–450	2–4%	7.6–9.9[d]	Y	~9%[c]
Rebaudioside B	804	H	$glc(glc)_2$	300–350	<0.4%	0–0.02%	Y	~31%[c]
Rebaudioside C	950	glc	glc(rham)(glc)	120–500	1–2%	0.5–6.0%	Y	~6%
Rebaudioside D	1128	glc-glc	$glc(glc)_2$	250–450	<0.4%	0–0.4%	Y	~4%
Rebaudioside E	966	glc-glc	glc-glc	150–300	<0.4%	5.6–43.2%[d]	Y	~9%[c]
Rebaudioside F	936	glc	glc(xyl)(glc)	N/A	<0.4%	0.04–0.1%	Y	~4%
Dulcoside A	788	glc	glc-rham	50–120	0.4–0.7%	0.2–0.4%	Y	~5%

[a] glc = glucose, rham = rhamnose, xyl = xylose. [b] Compound detected in negative ion mode by conventional DESI analysis. [c] Overlap due to isomers. [d] Glycoside ratios calculated together.

Table 2 Exact mass measurements of *Stevia* glycosides

Sweet glycosides	Molecular formula	Theoretical *m/z* (Da)	Experimental *m/z* (Da)	*m/z* Error (Δ ppm)
Steviolbioside/rubusoside	$C_{32}H_{50}O_{13}$	641.31787 $(M - H)^-$	641.315 $(M - H)^-$	−4.467
		677.29454 $(M + Cl)^-$	677.295 $(M + Cl)^-$	0.676
		665.31436 $(M + Na)^+$	Not observed	N/A
		681.28830 $(M + K)^+$	681.288 $(M + K)^+$	−0.440
Stevioside/rebaudioside B	$C_{38}H_{60}O_{18}$	803.37069 $(M - H)^-$	803.372 $(M - H)^-$	1.633
		839.34737 $(M + Cl)^-$	839.349 $(M + Cl)^-$	1.947
		827.36692 $(M + Na)^+$	827.370 $(M + Na)^+$	2.942
		843.34086 $(M + K)^+$	843.341 $(M + K)^+$	−0.146
Rebaudioside A/rebaudioside E	$C_{44}H_{70}O_{23}$	965.42351 $(M - H)^-$	965.424 $(M - H)^-$	0.506
		1001.39992 $(M + Cl)^-$	1001.403 $(M + Cl)^+$	2.807
		989.41974 $(M + Na)^+$	Not observed	N/A
		1005.39368 $(M + K)^+$	1005.394 $(M + K)^+$	0.342
Rebaudioside C	$C_{44}H_{70}O_{22}$	949.42860 $(M - H)^-$	949.427 $(M - H)^-$	3.109
		985.40528 $(M + Cl)^-$	985.409 $(M + Cl)^-$	3.718
		973.42564 $(M + Na)^+$	Not observed	N/A
		989.39958 $(M + K)^+$	989.397 $(M + K)^+$	−2.478
Rebaudioside F	$C_{43}H_{68}O_{22}$	935.41295 $(M - H)^-$	Not observed	N/A
		971.38963 $(M + Cl)^-$	971.389 $(M + Cl)^-$	−0.643
		959.40999 $(M + Na)^+$	Not observed	N/A
		975.38393 $(M + K)^+$	975.384 $(M + K)^+$	0.634
Dulcoside A	$C_{38}H_{60}O_{17}$	787.37577 $(M - H)^-$	Not observed	N/A
		823.35190 $(M + Cl)^-$	823.354 $(M + Cl)^-$	1.881
		811.37282 $(M + Na)^+$	Not observed	N/A
		827.34678 $(M + K)^+$	827.345 $(M + K)^+$	−1.305

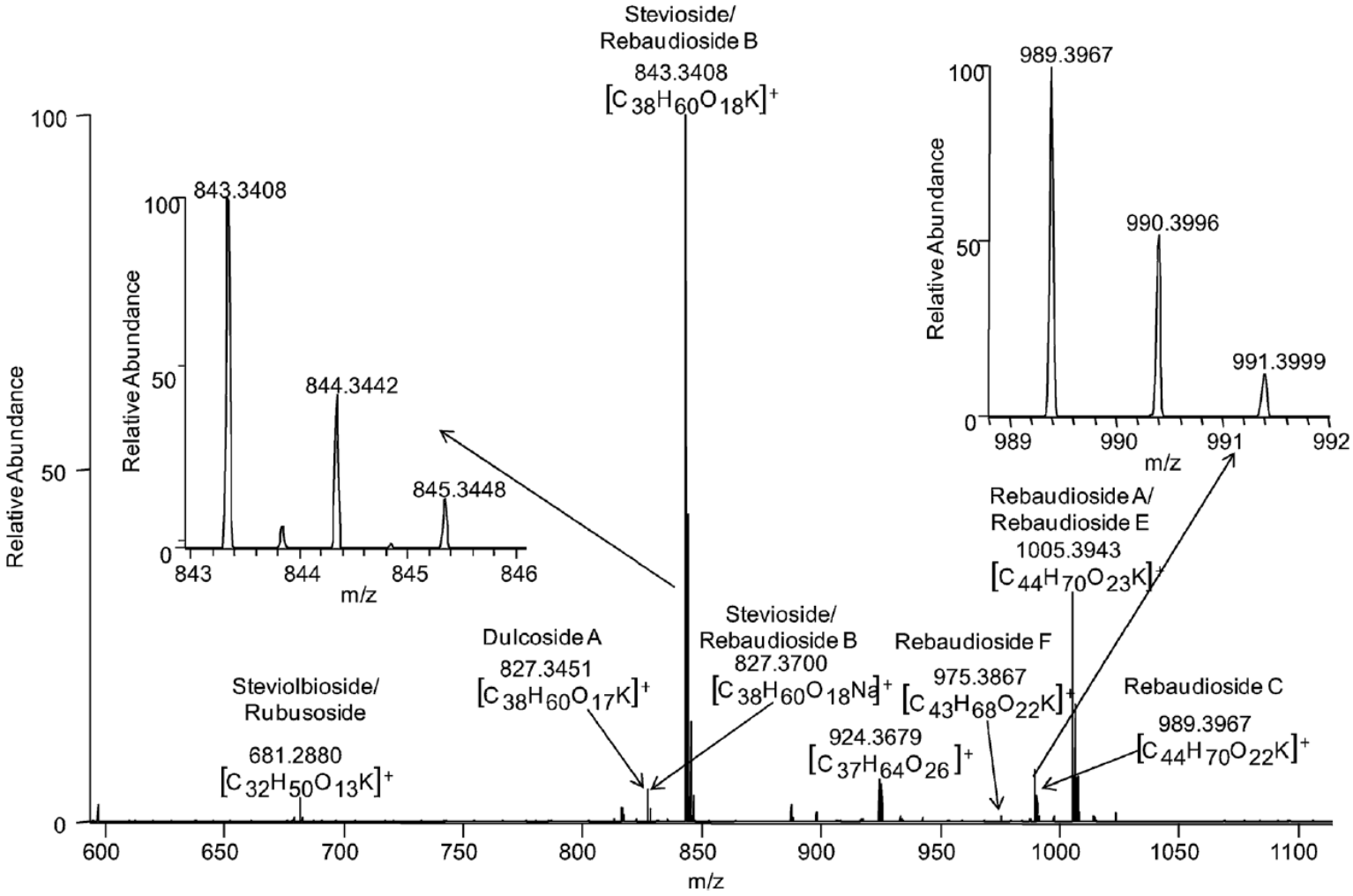
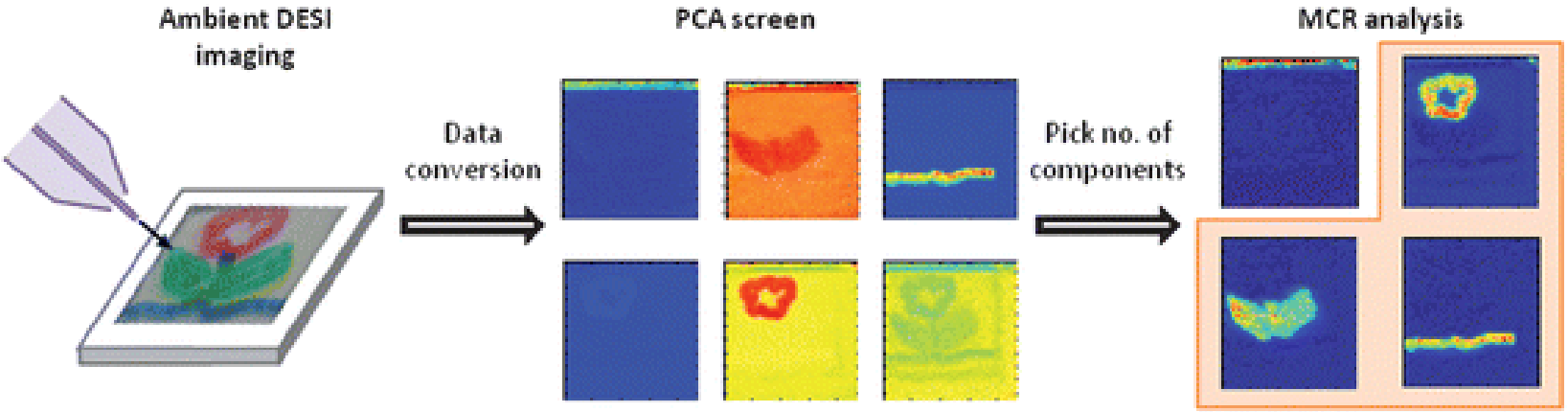


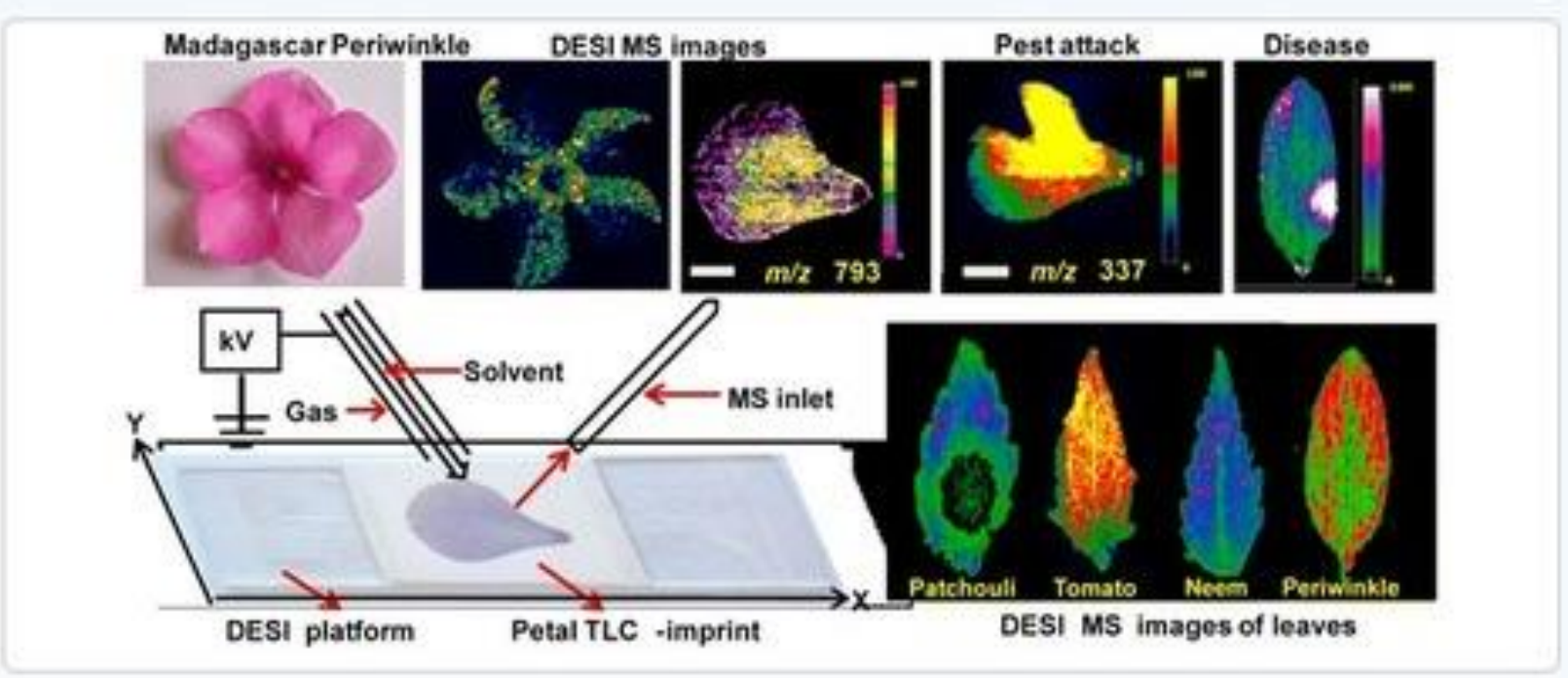
Fig. 2 DESI LTQ-Orbitrap mass spectrum of a *Stevia* leaf fragment in positive ion mode using a spray solvent of MeOH : H_2O (20 : 80) 106.7 pmol/μL MRFA (for lock mass analysis). The various glycosides observed are labeled. Insets illustrate the potassium isotope profile of select species.

DESI-Imaging-MS



http://pubs.rsc.org/is/content/articlelanding/2012/an/c2an35430f#!divAbstract

DESI-Imaging-MS



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Article

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Understanding the Molecular Signatures in Leaves and Flowers by Desorption Electrospray Ionization Mass Spectrometry (DESI MS) Imaging

R. G. Hemalatha and T. Pradeep*

DST Unit on Nanoscience and Thematic Unit of Excellence, Department of Chemistry, Indian Institute of Technology Madras, Chennai, India

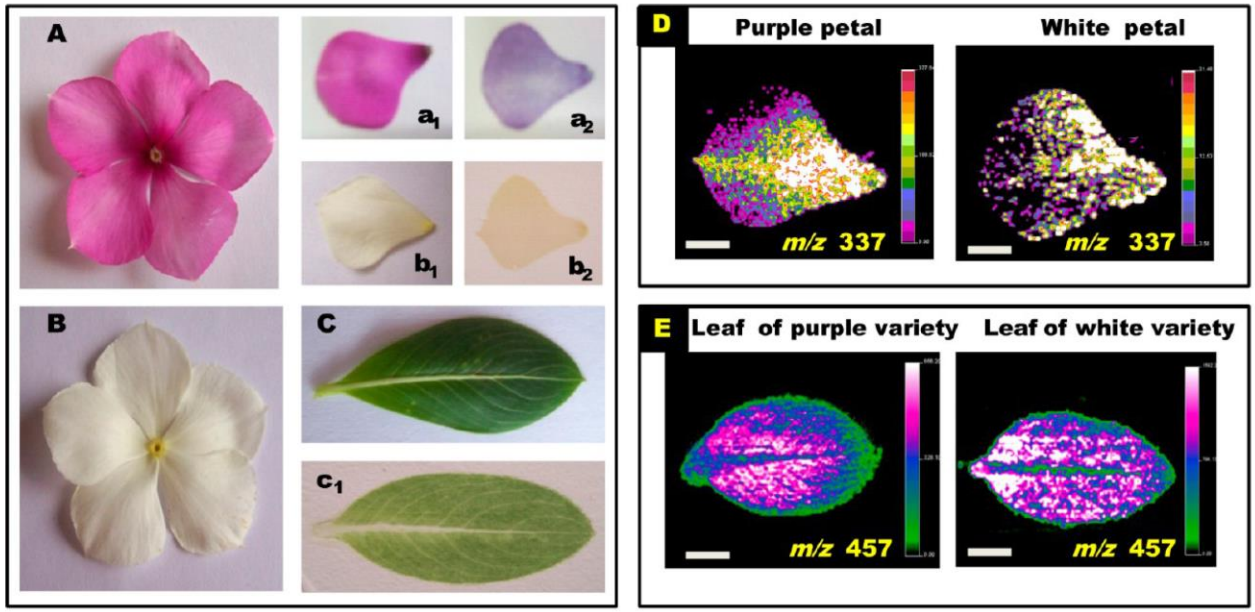
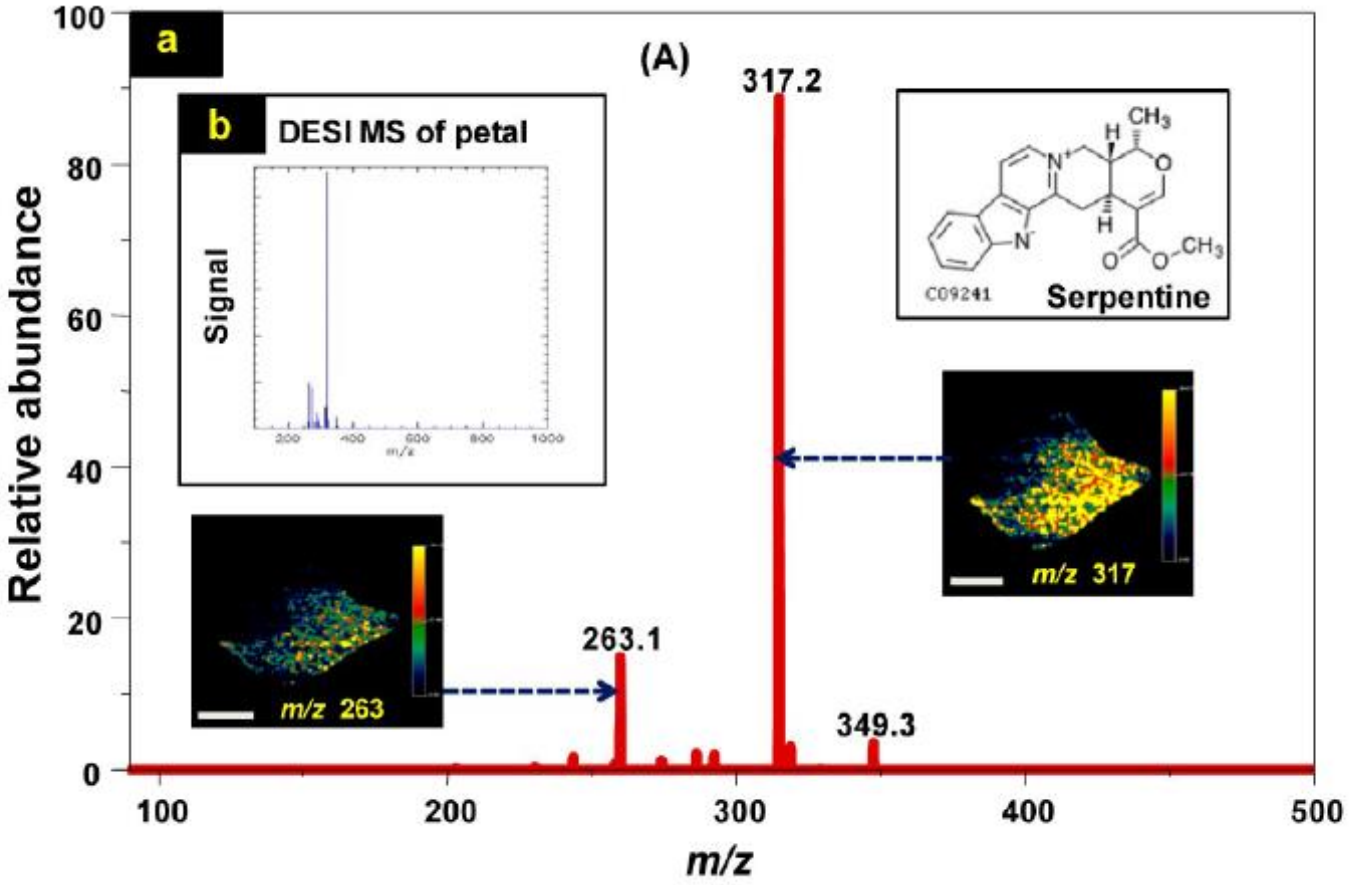
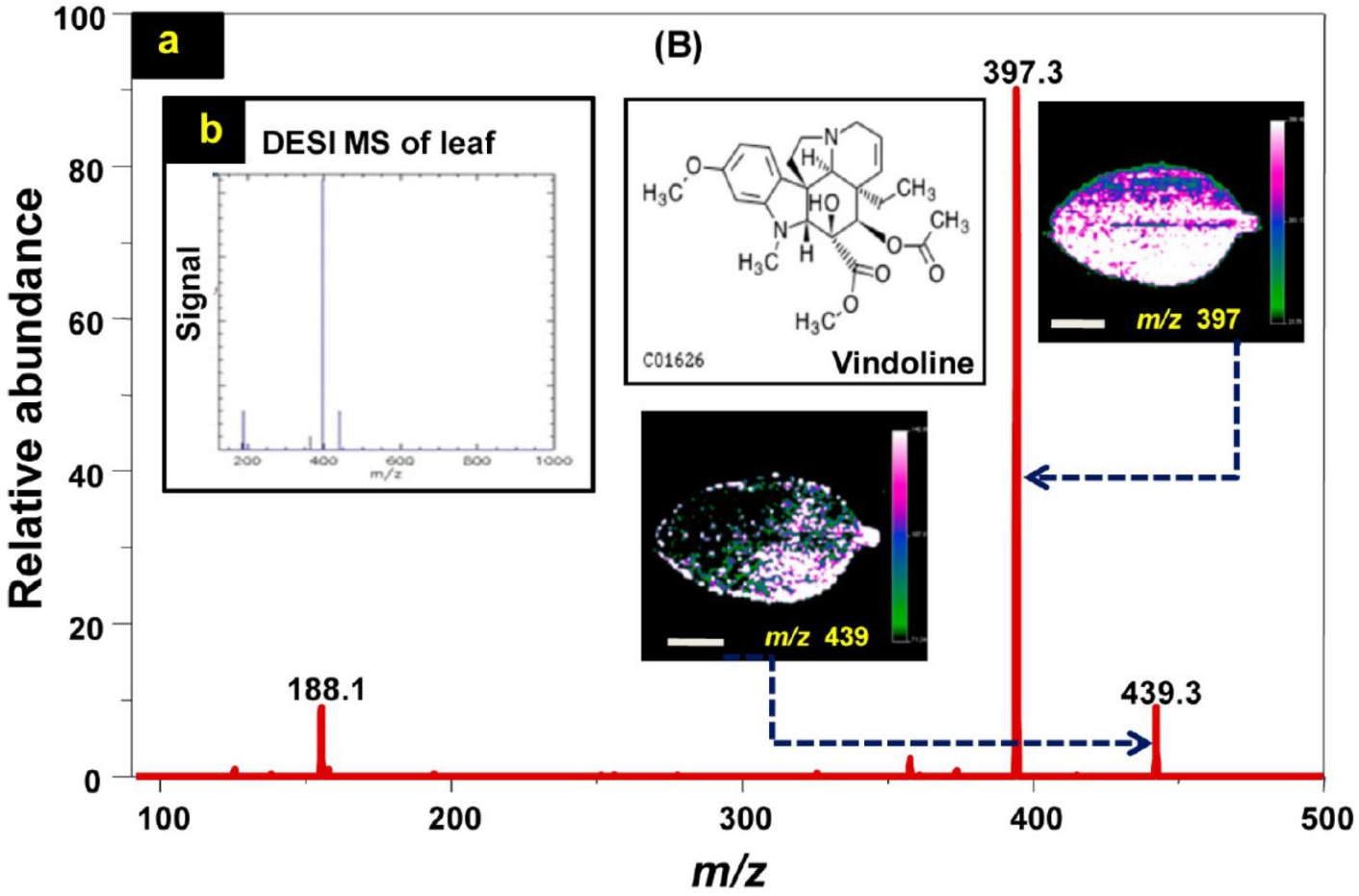
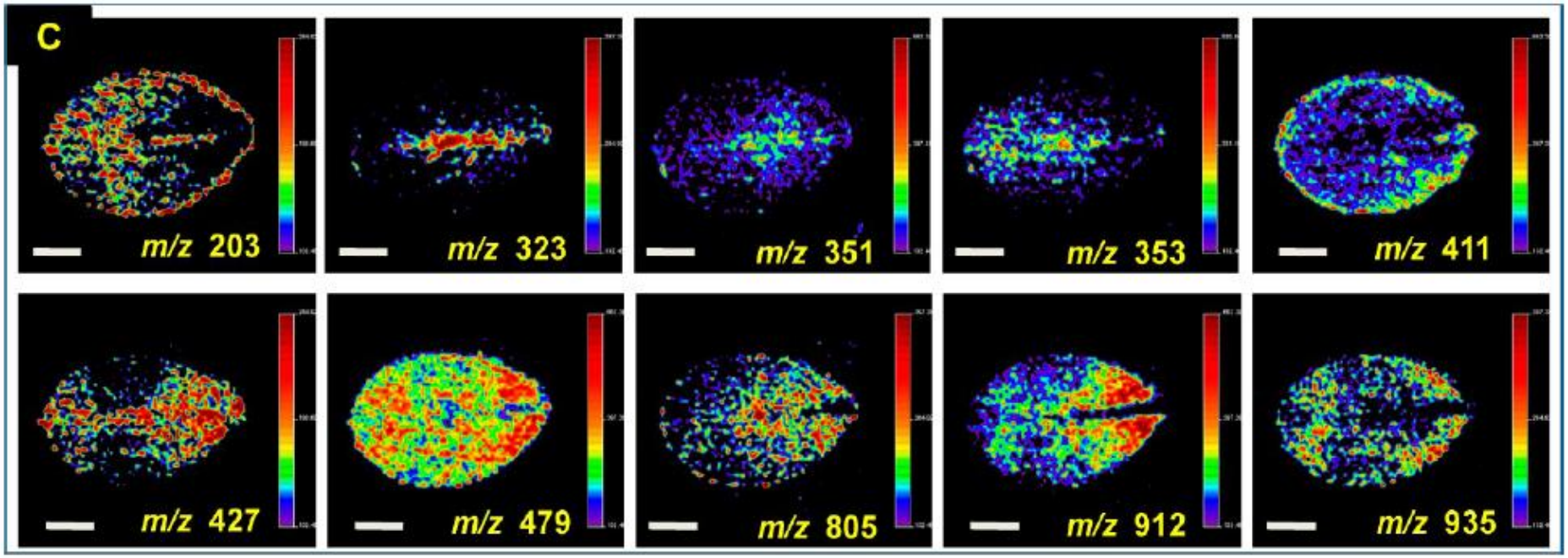
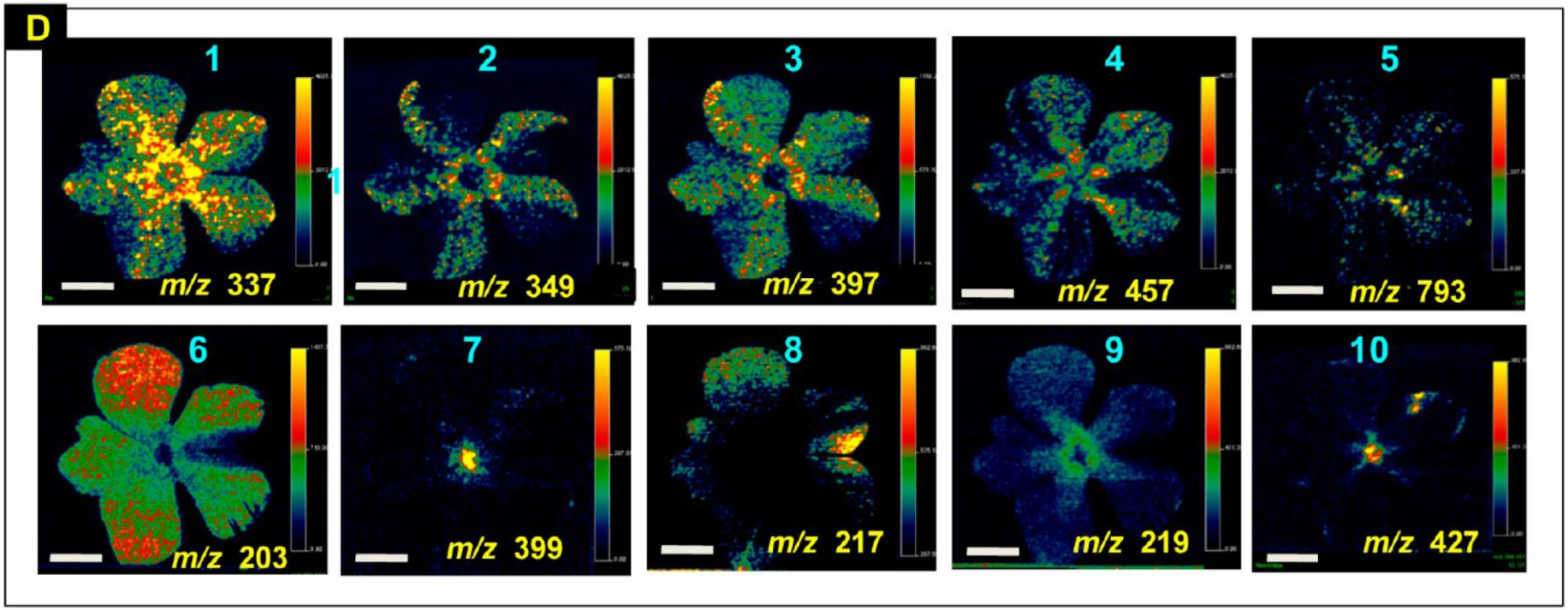


Figure 1. Photographs of flowers, petals, and a leaf of Madagascar periwinkle *C. roseus* and their TLC imprints: Images of (A) pink flower, (a_1) single petal of a pink flower, and (a_2) TLC-imprint of a pink petal. Images B, b_1, and b_2 correspond to the same data for a white flower. Images C and c_1 correspond to a leaf and its imprint. Imprints do not correspond to the same petals or leaf whose photographs are shown. Images D and E correspond to one of the DESI MS images collected from petal and leaf showing the difference in spatial distribution between purple and white varieties of periwinkle, using the ion at *m/z* 337 and 457, respectively. Scale bars of both the images in D and E are the same (5 mm).


a
(A)
b
DESI MS of petal
Signal
317.2
263.1
349.3
m/z 263
m/z 317
C09241
Serpentine
Relative abundance
m/z
100
80
60
40
20
0
100
200
300
400
500


a
b
DESI MS of leaf
Signal
m/z
(B)
397.3
C01626
Vindoline
m/z 397
m/z 439
188.1
439.3
Relative abundance
m/z
100
80
60
40
20
0
100
200
300
400
500


C
m/z 203
m/z 323
m/z 351
m/z 353
m/z 411
m/z 427
m/z 479
m/z 805
m/z 912
m/z 935


D
1
m/z 337
2
m/z 349
3
m/z 397
4
m/z 457
5
m/z 793
6
m/z 203
7
m/z 399
8
m/z 217
9
m/z 219
10
m/z 427

Espectrometria de Massas: Aplicação em Cirurgias

CANCER DIAGNOSTICS

Intraoperative Tissue Identification Using Rapid Evaporative Ionization Mass Spectrometry

Júlia Balog,[1]* László Sasi-Szabó,[2]* James Kinross,[3,4] Matthew R. Lewis,[3] Laura J. Muirhead,[3,4] Kirill Veselkov,[3] Reza Mirnezami,[4] Balázs Dezső,[5] László Damjanovich,[2] Ara Darzi,[4] Jeremy K. Nicholson,[3†] Zoltán Takáts[3†]

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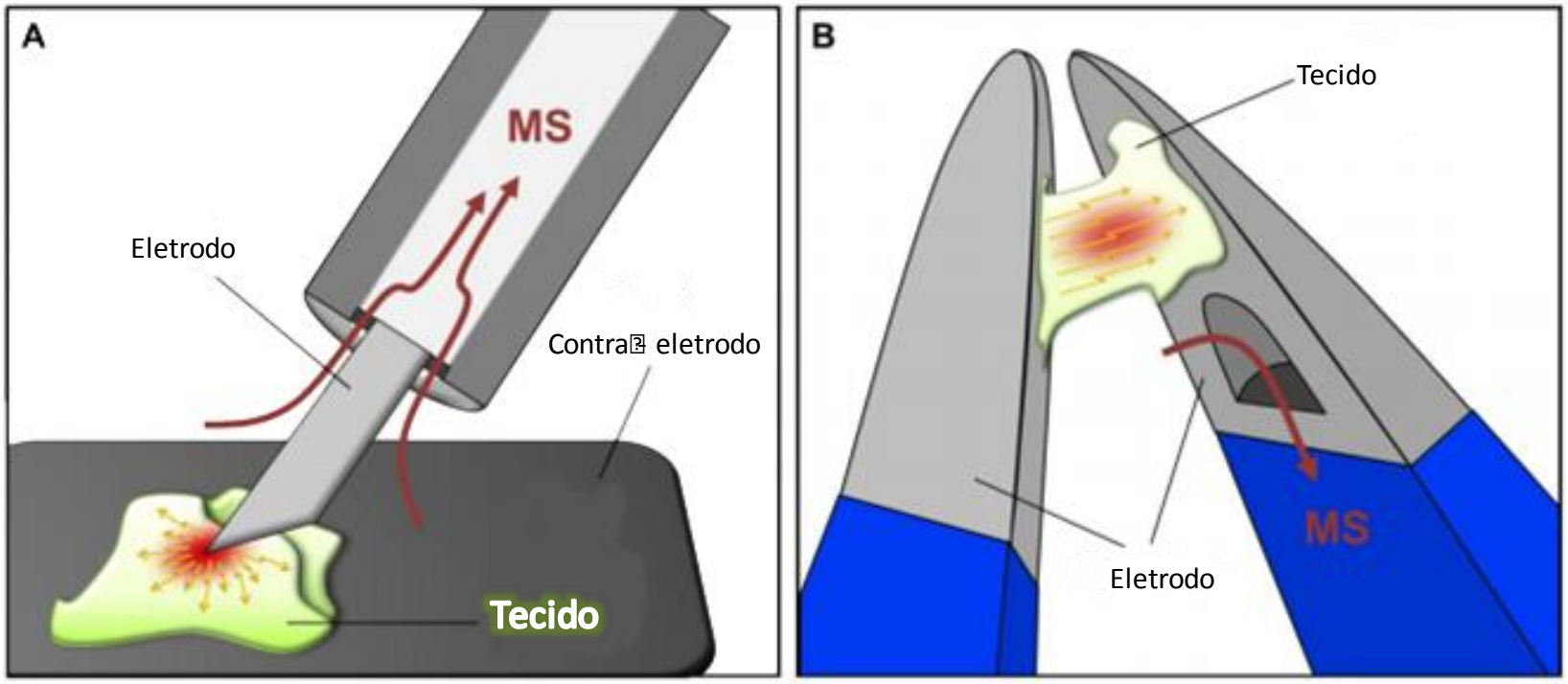
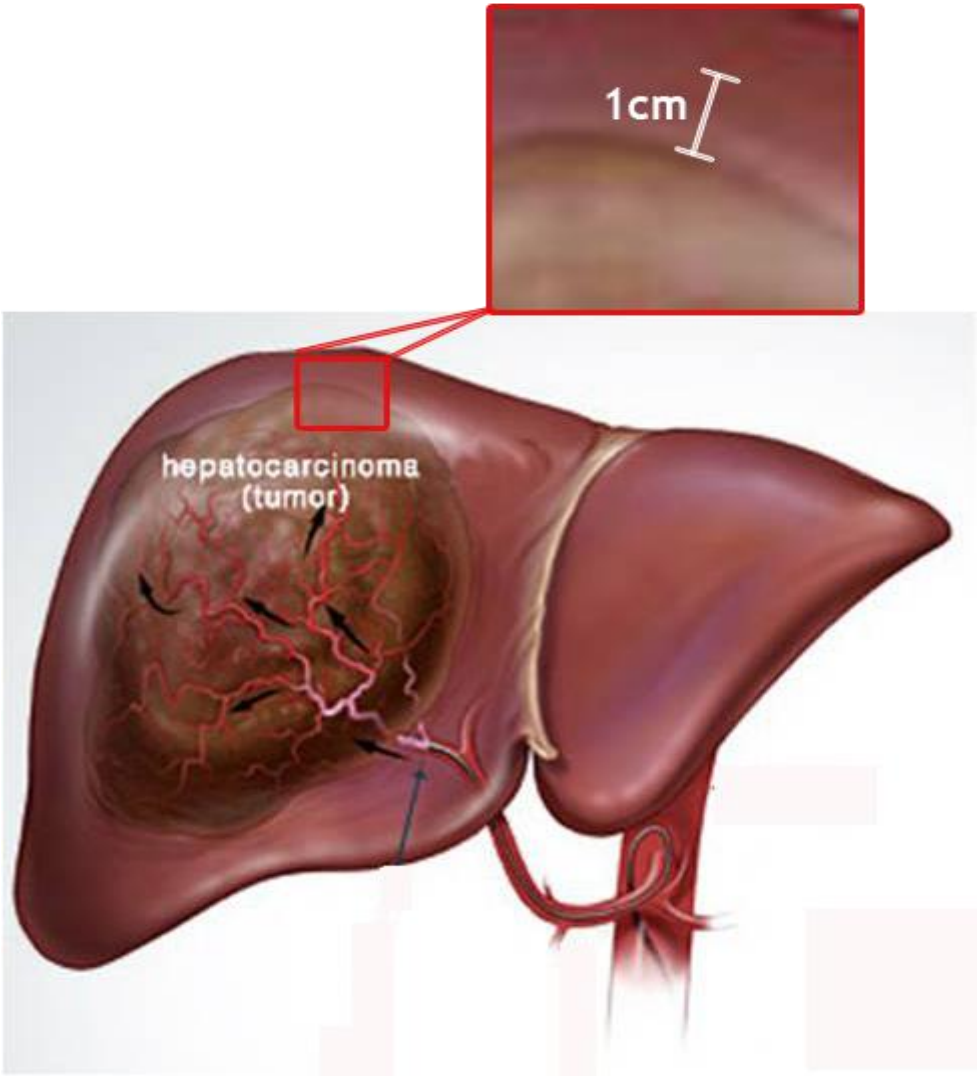
iKnife



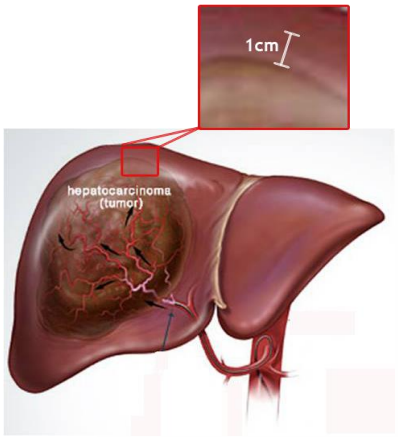
Monopolar (Bisturi)

Bipolar (Pinça)

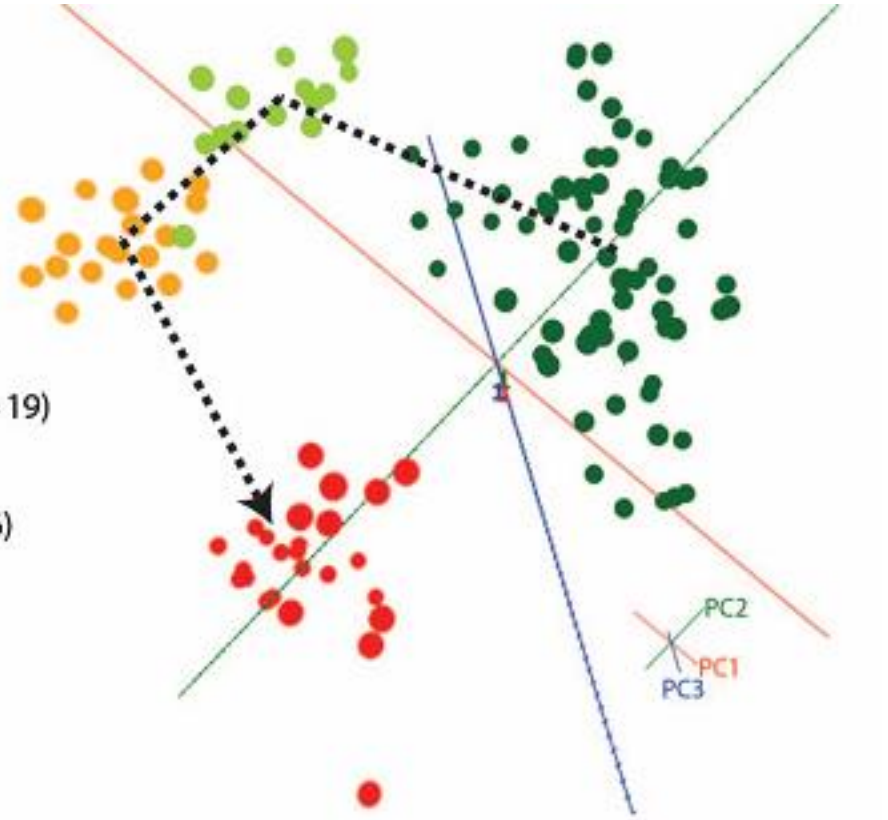

B


1cm
hepatocarcinoma
(tumor)

Diferentes regiões do fígado

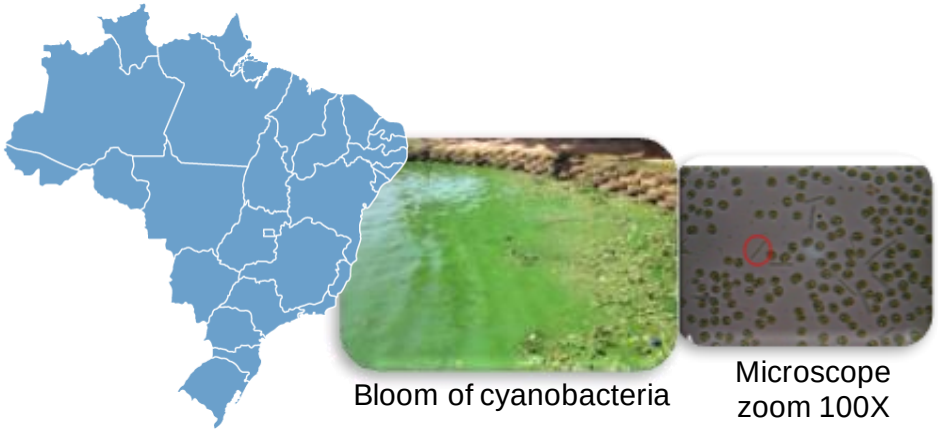


Cholangiocellular carcinoma ($n = 27$)
<1 cm border line, cancerous part ($n = 19$)
Healthy liver parenchyma ($n = 111$)
<1 cm border line, healthy part ($n = 16$)



Espectrômetro de Massas

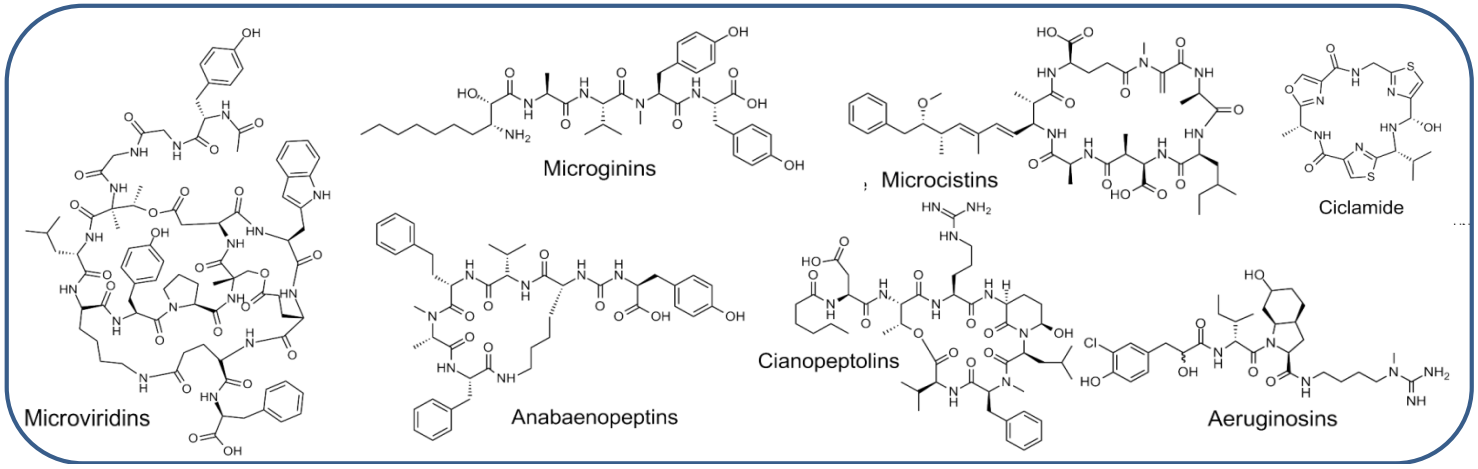
Identificação e caracterização de cianopeptídeos em florações de cianobactérias: análise direta por MALDI-TOF-MS.

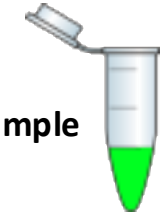


Bloom of cyanobacteria

Microscope zoom 100X

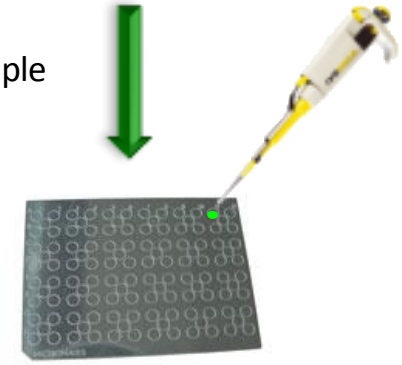
Cyanobacteria with different morphology





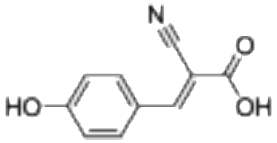
Cyanobacteria sample

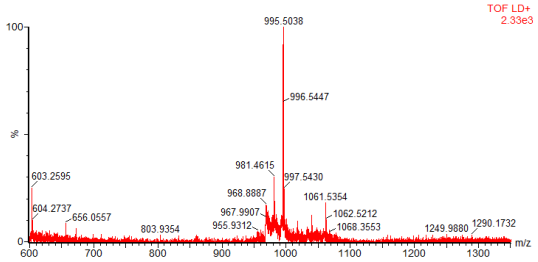
1,0 µL of sample



MALDI plate

1,0 µL of saturated matrix solution in H_2O/CH_3CN 50%



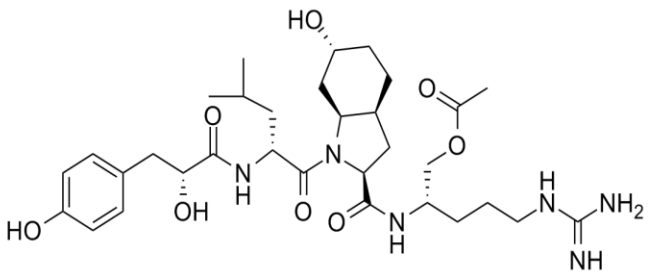


MALDI-TOF

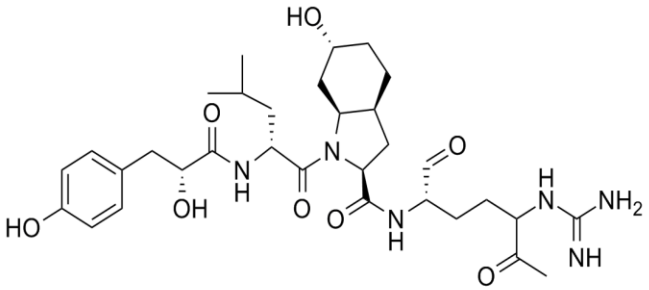
Tabela 2- m/z referentes aos peptídeos identificados na floração por MALDI-TOF-MS. *Valores descritos na literatura

Oligopeptideo	$[M + H]^+$ Form. Mol.	Teórica *m/z* $[M + H]^+$	MALDI-MS *m/z* $[M + H]^+$	MALDI-MS Erro(ppm)
Aeruginosina 602	$C_{30}H_{47}N_6O_7$	603.35007	603.3510	1.54
Aeruginosina 298A	$C_{30}H_{49}N_6O_7$	605.36572	605.3663	0.95
Aeruginosina 644	$C_{32}H_{49}N_6O_8$	645.36064	645.3586	-3.16
Aeruginosina 646	$C_{32}H_{51}N_6O_8$	647.37620	647.3776	2.03
Cianopeptolina 972	$C_{46}H_{73}N_{10}O_{13}$	973.53530	973.5425	7.39
Cianopeptolina 986 A	$C_{47}H_{75}N_{10}O_{13}$	987.55096	987.5488	-2.18
MC-LR	$C_{49}H_{75}N_{10}O_{12}$	995.55604	995.5626	6.59
MC-HilR	$C_{49}H_{73}N_{10}O_{13}$	1009.53000	1009.5429	7.52
MC-RR	$C_{49}H_{76}N_{13}O_{12}$	1038.57309	1038.5757	2.51
MC-YR	$C_{52}H_{73}N_{10}O_{13}$	1045.53531	1045.5437	8.03
Cianopeptolin 1071	--	1072*	1072.6240	--
Microviridin 1707	--	1707.75*	1707.6777	--

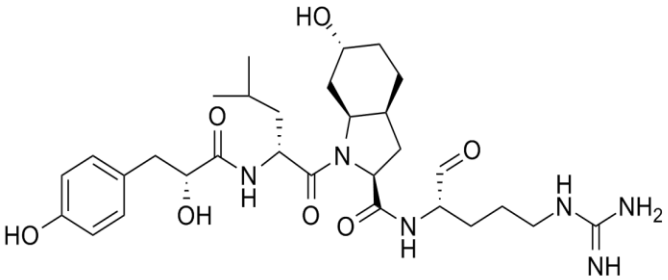
Estrutura das aeruginosinas identificadas na floração de Americana



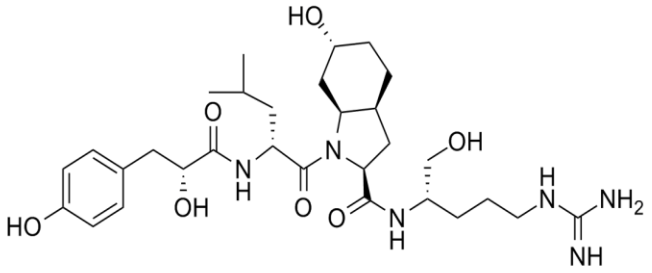
aeruginosin 646



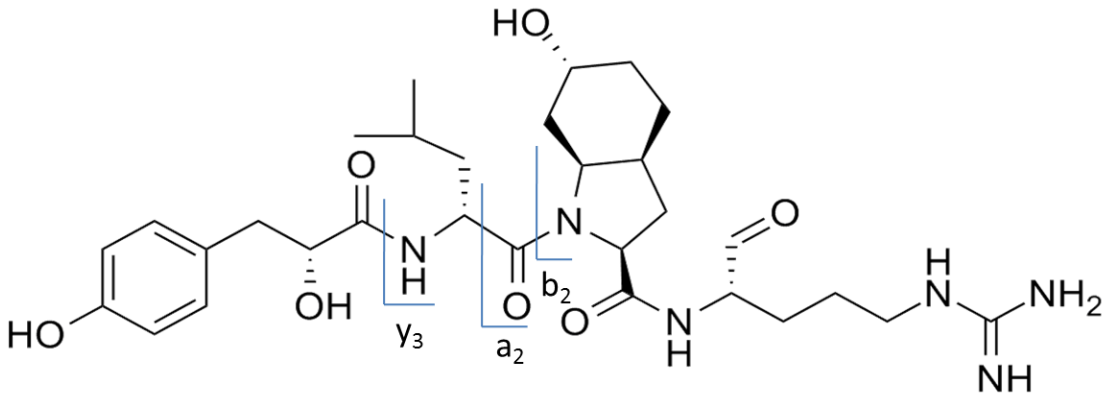
aeruginosin 644

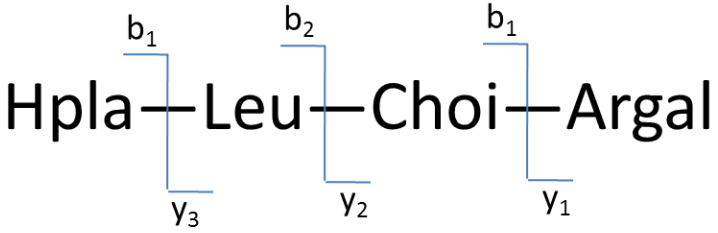


aeruginosin 602



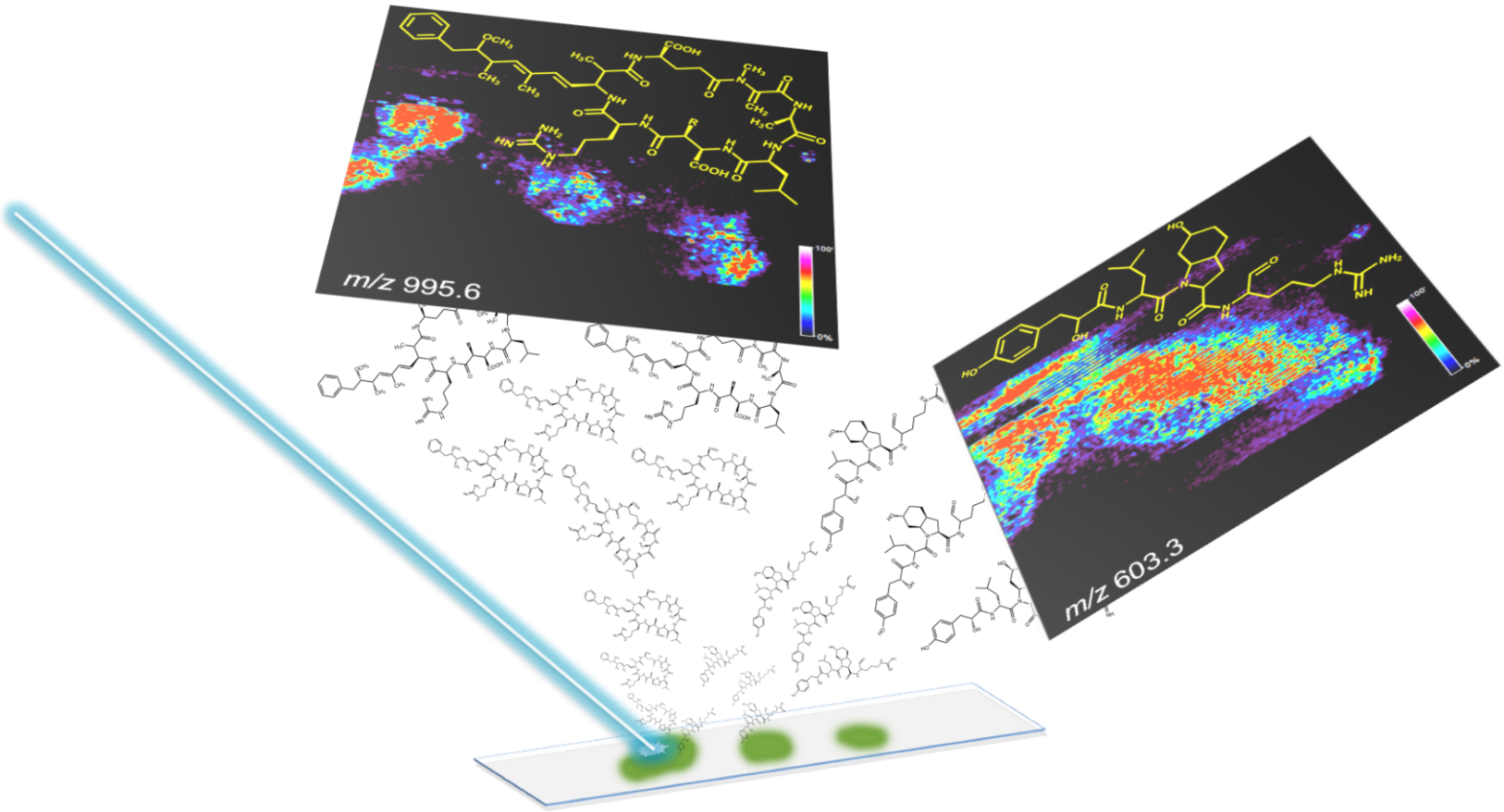
aeruginosin 298A





Aeruginosina 602 - Hpla-Leu-Choi-Argal		
Fragmento	Tipo de íon	*m/z*
M+H		603
M-H_2O+H		585
M-CH_3N_2+H		543
M-Argal	b3	445
Choi-Argal-NH_2+H		309
Choi-Argal-NH_2-H_2O+H		291
Choi-Argal-CH_3NH2-H_2O+H		266
Hpla-Leu+H	b2	278
Hpla-Leu-CO+H	a2	250
Leu-Choi-fragmento		221
Choi imonio		140
Argal-fragmento		100
Leu-imonio		86

MALDI Imaging Mass Spectrometry of Freshwater Cyanobacteria: Spatial Distribution of Toxins and other Metabolites.



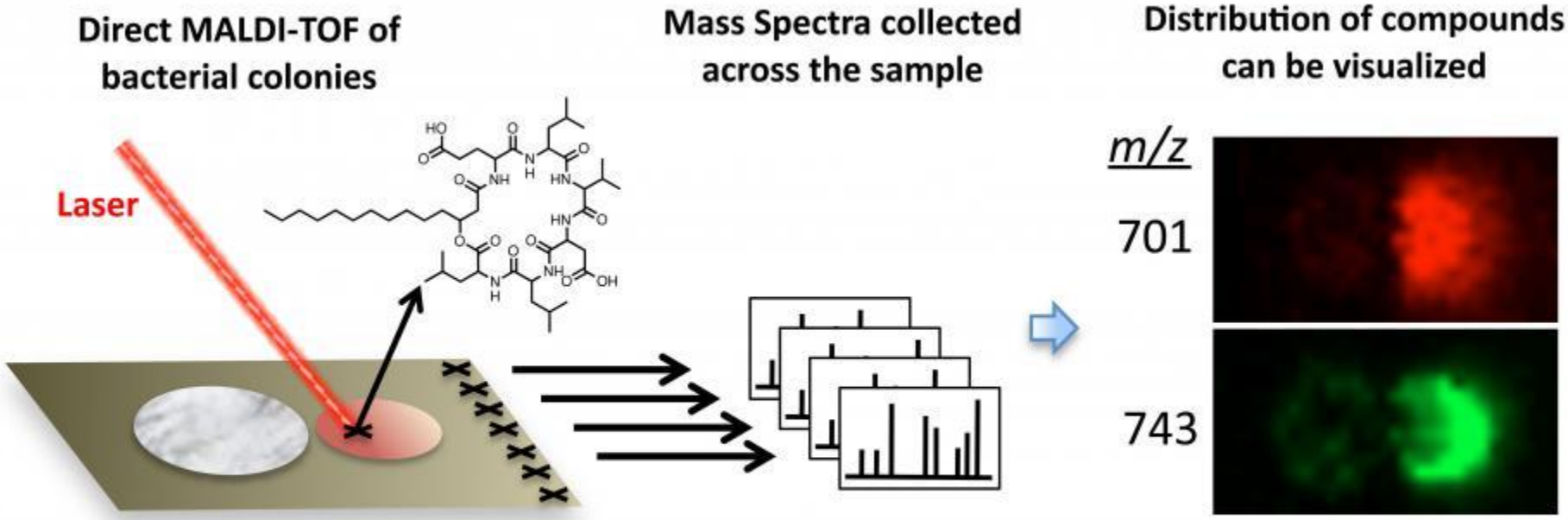
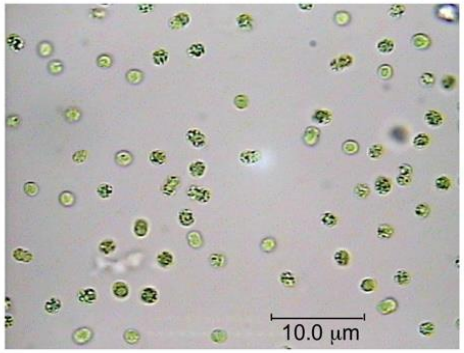
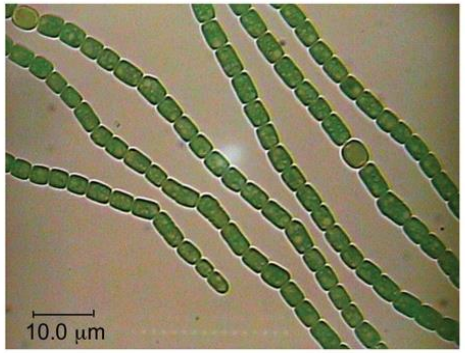


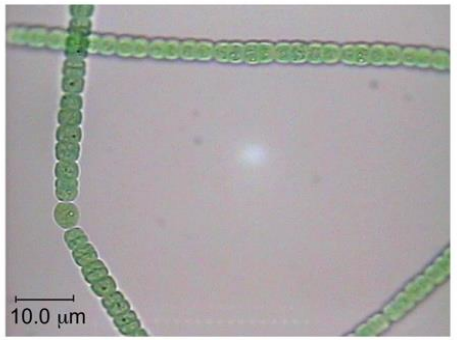
Fig. 4 Overview of the MALDI-TOF imaging mass spectrometry workflow. Distributions of chemicals in the colonies are directly sampled and visualized.



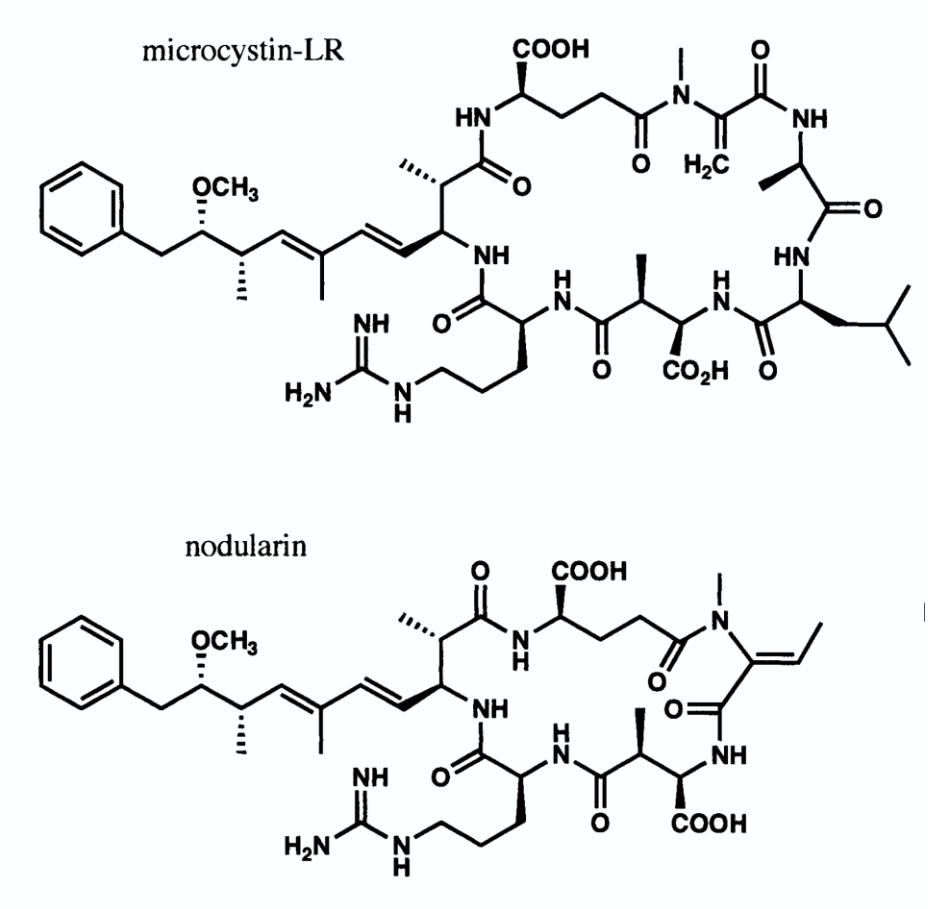
Microcystis aeruginosa PCC 7820



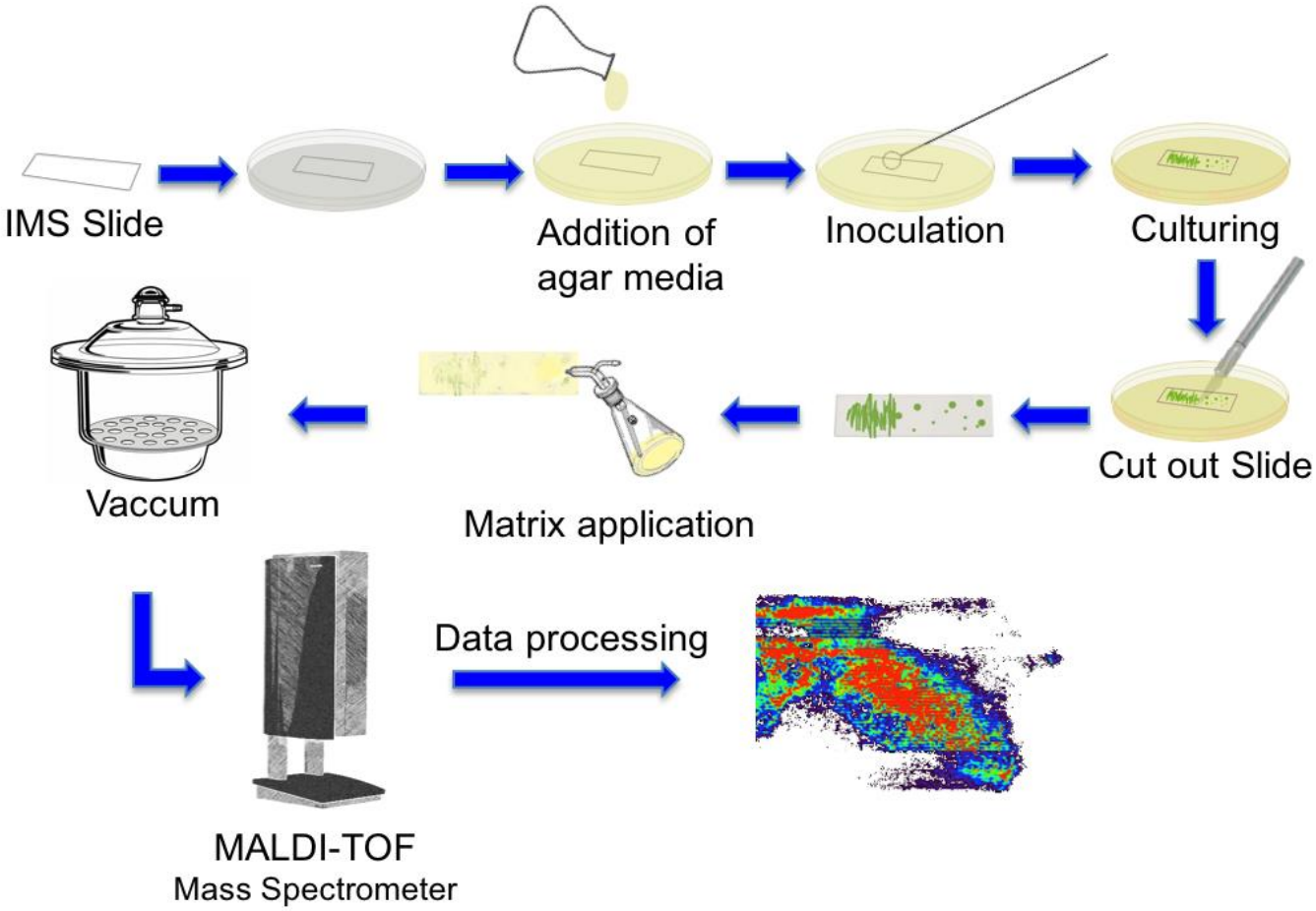
Anabaena cylindrica PCC 7122

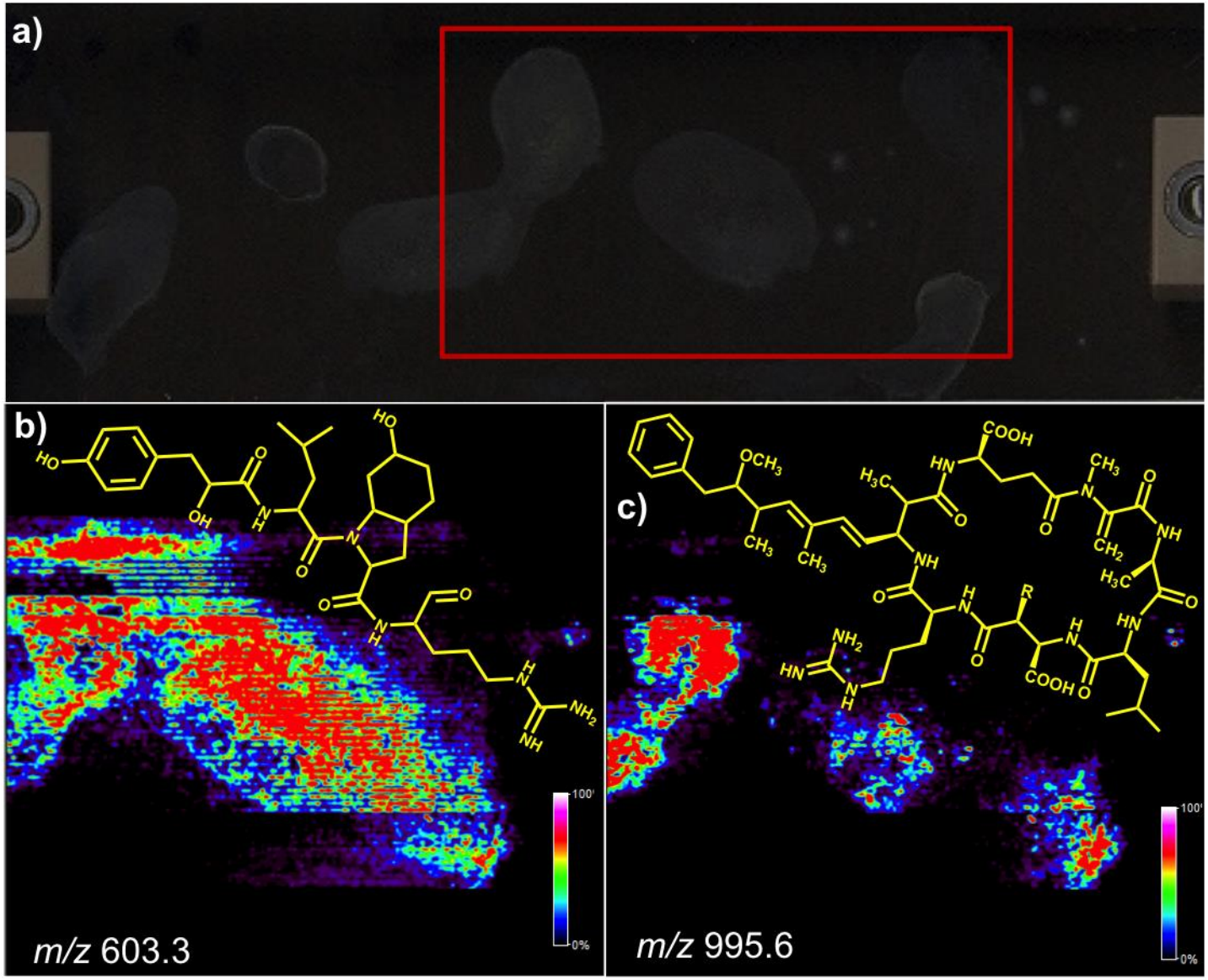


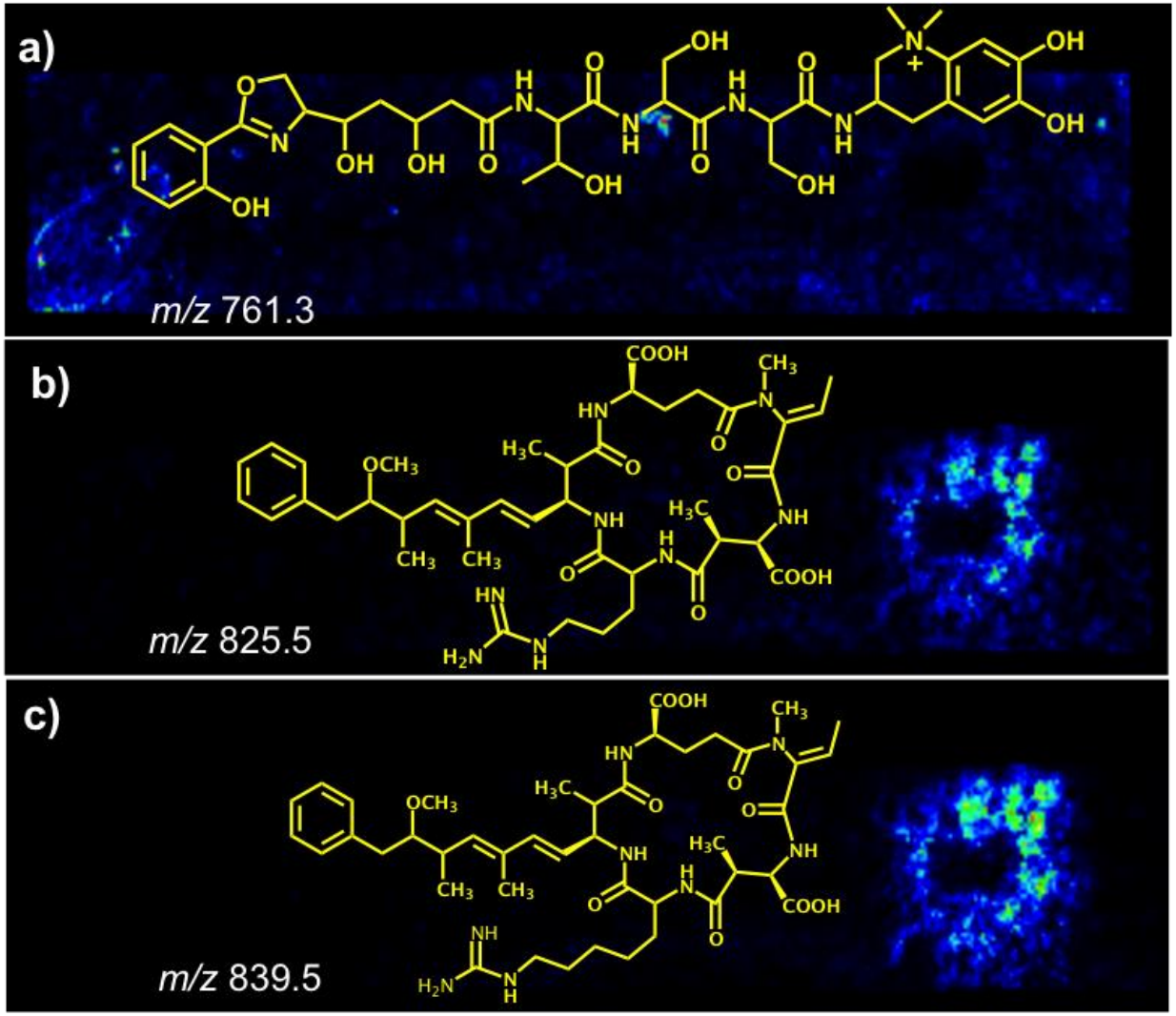
Nodularia harveyana PCC 7804

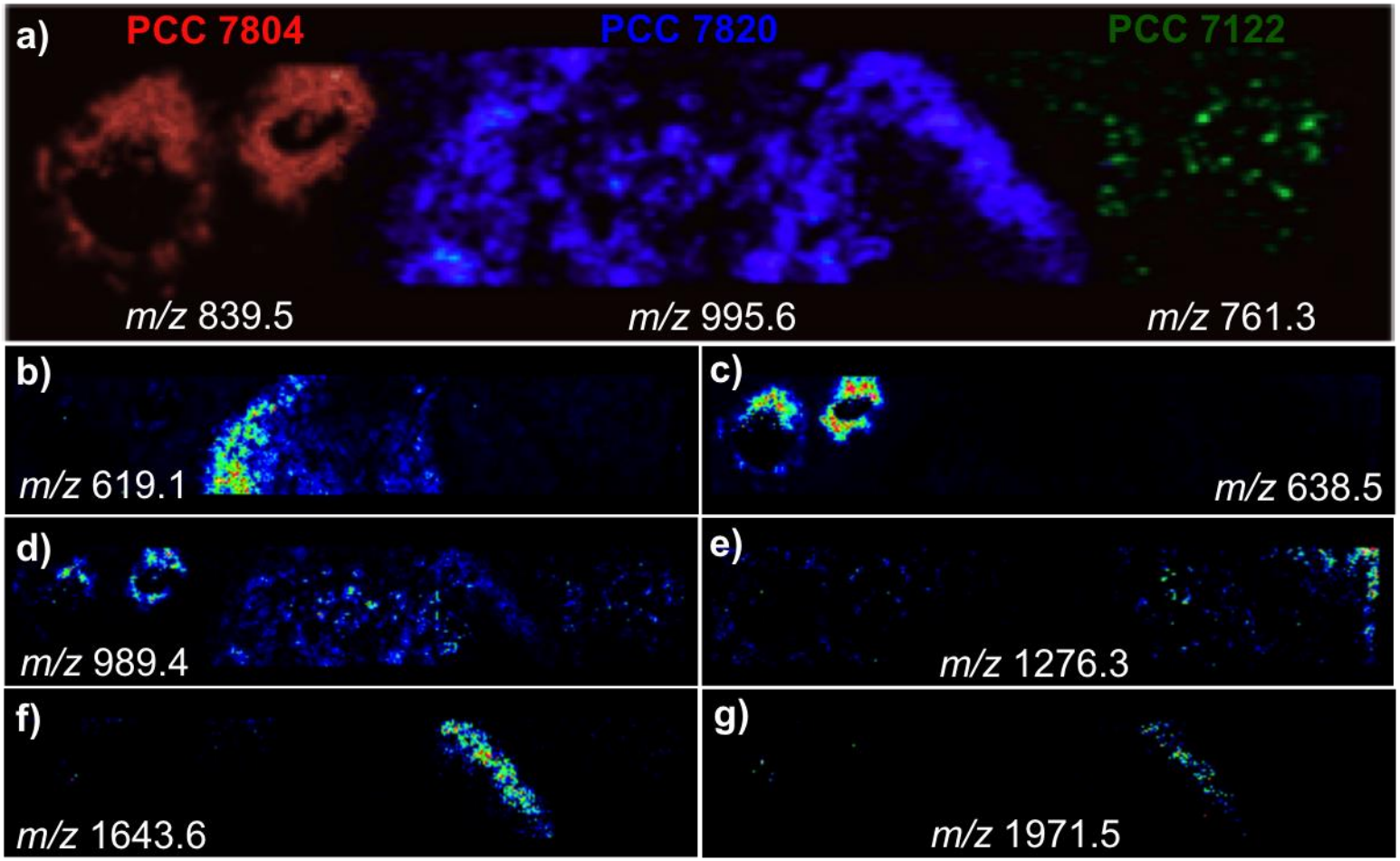

microcystin-LR
COOH
HN
O
N
O
NH
H2C
OCH3
NH
HN
NH
H2N
N
H
CO2H

nodularin
O
COOH
N
OCH3
NH
NH
H2N
N
H
COOH

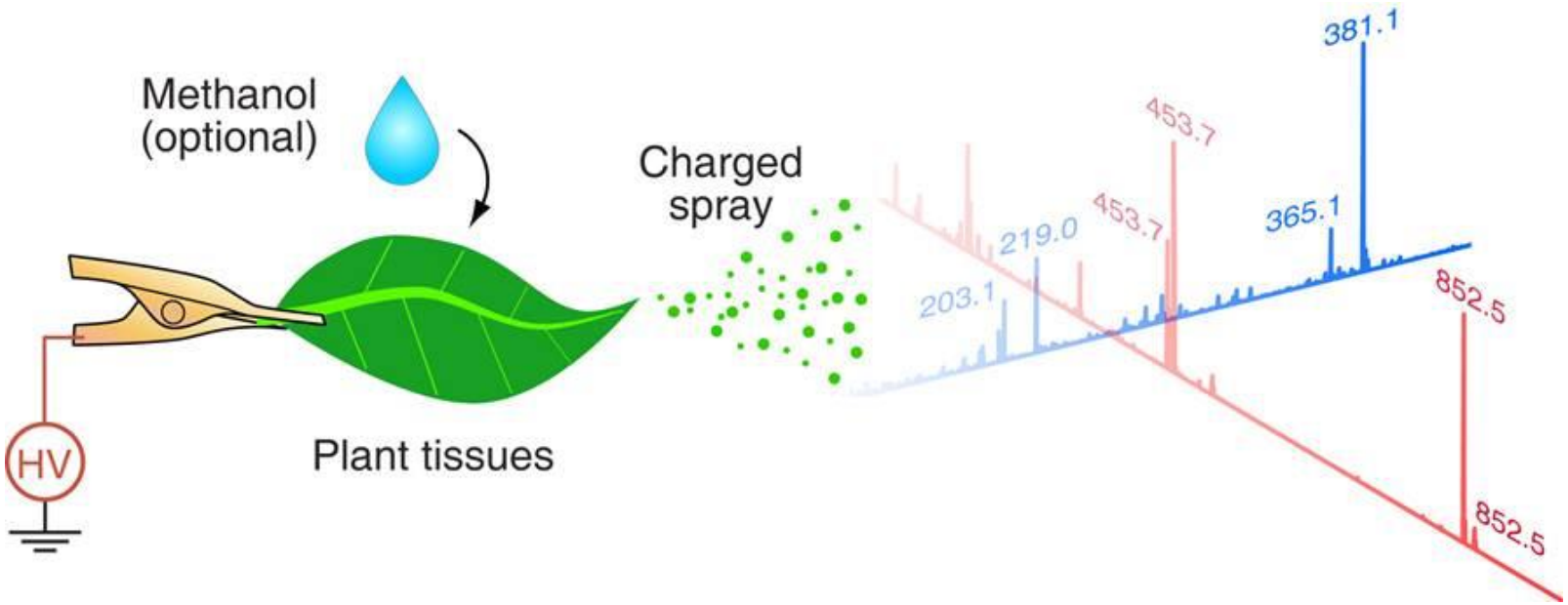

IMS Slide
Addition of agar media
Inoculation
Culturing
Cut out Slide
Matrix application
Vaccum
Data processing
MALDI-TOF
Mass Spectrometer


a)
b)
c)
m/z 603.3
m/z 995.6
100%
0%

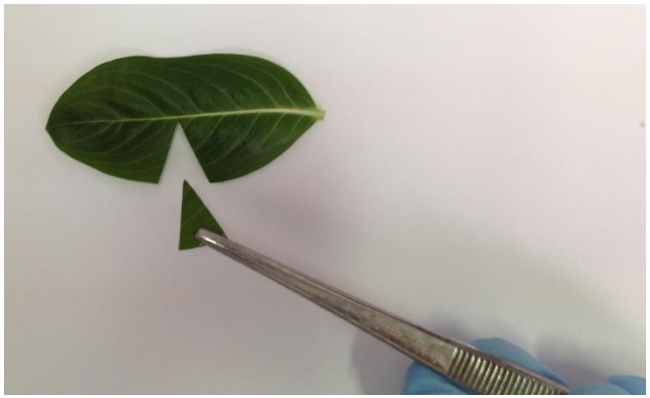

a)
m/z 761.3
b)
m/z 825.5
c)
m/z 839.5

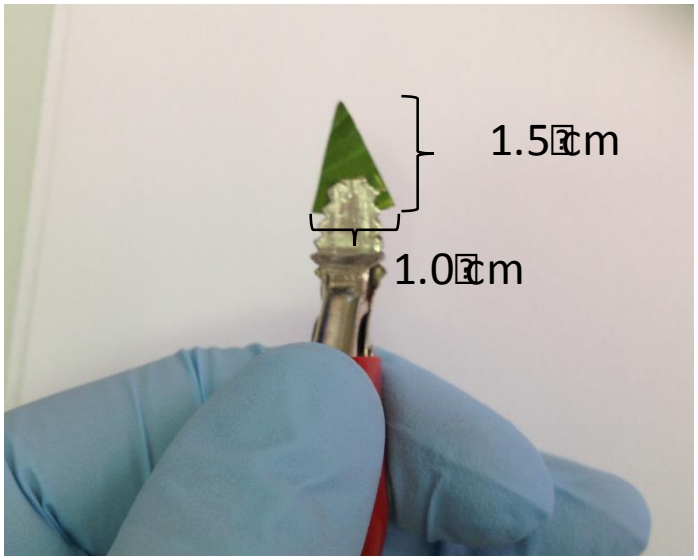

a)
PCC 7804
PCC 7820
PCC 7122
m/z 839.5
m/z 995.6
m/z 761.3
b)
m/z 619.1
c)
m/z 638.5
d)
m/z 989.4
e)
m/z 1276.3
f)
m/z 1643.6
g)
m/z 1971.5

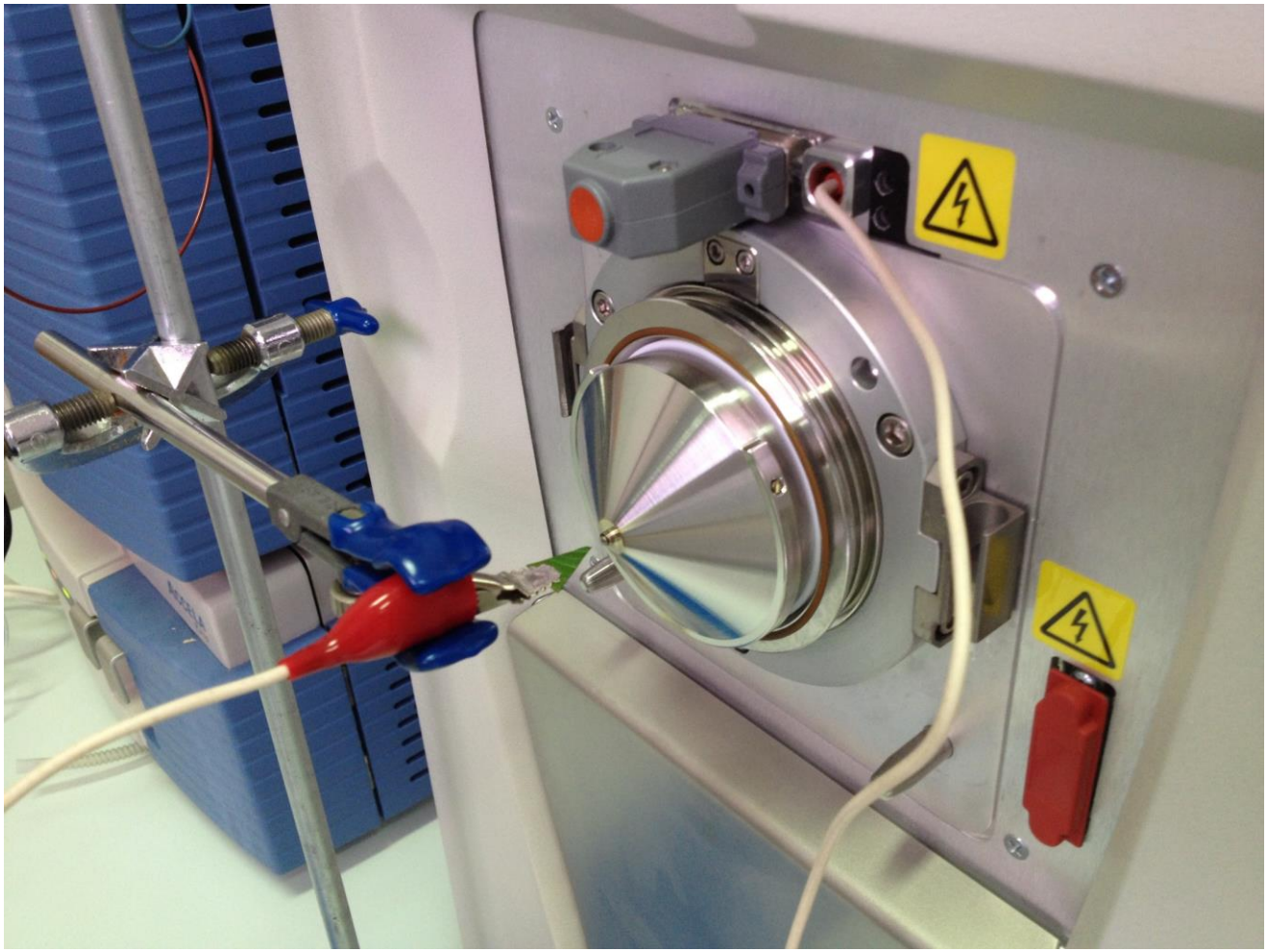
Leaf Spray

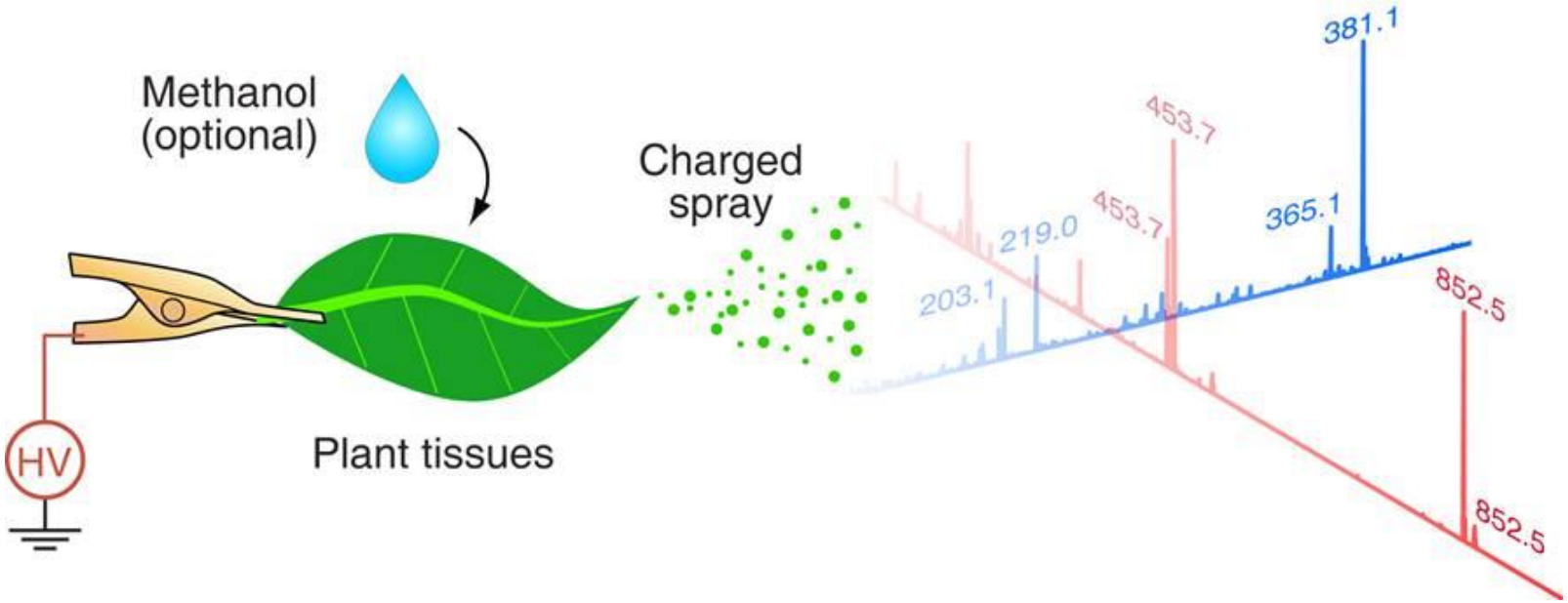


Anal. Chem. 2011, 83, 7608–7613

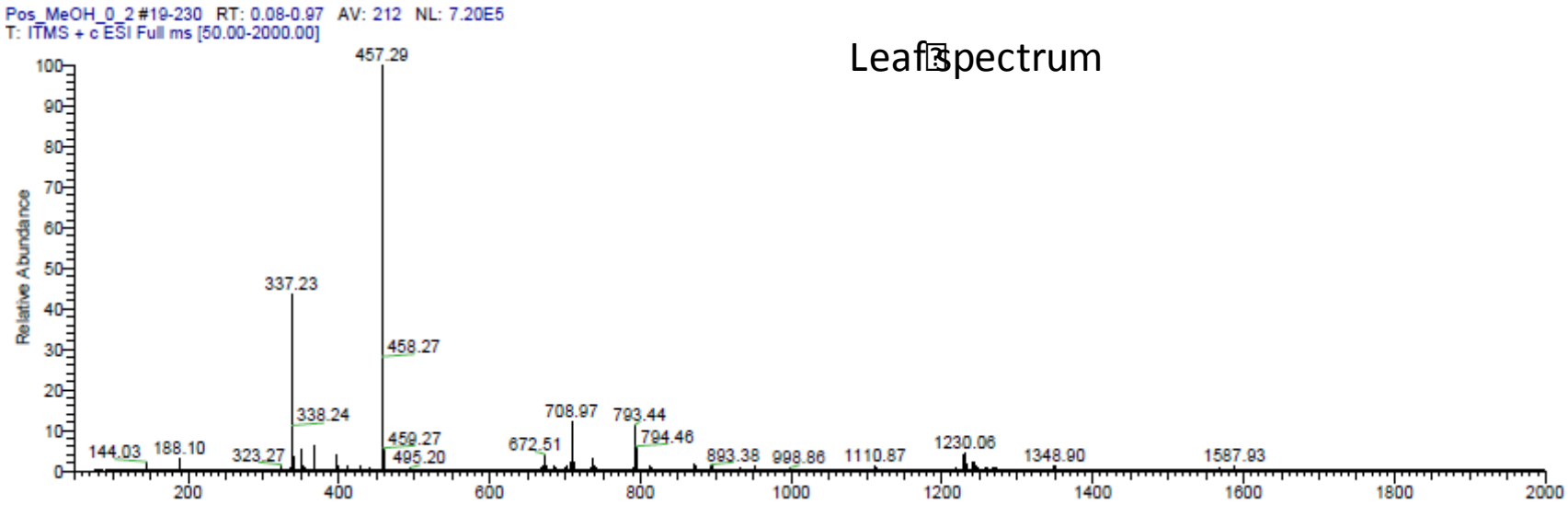



1.5 cm
1.0 cm

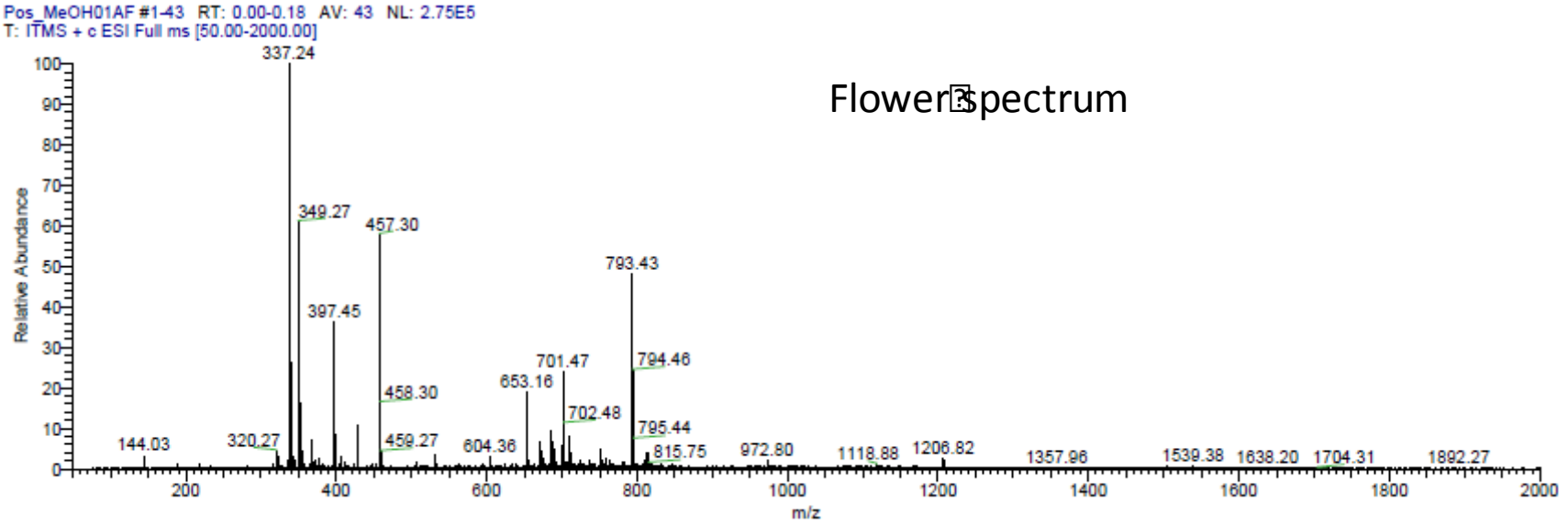



Methanol
(optional)
Charged
spray
HV
Plant tissues
381.1
453.7
219.0
453.7
365.1
203.1
852.5
852.5

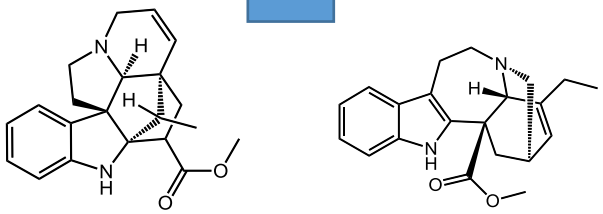
Leaf Spectrum



Flower Spectrum



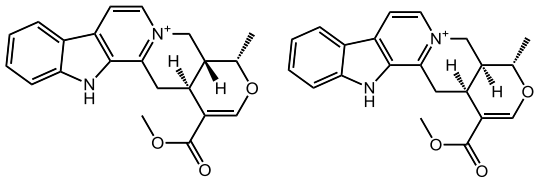
A ser avaliado por MS tandem



Vindolinina/Catharantina

$C_{21}H_{24}N_2O_2$

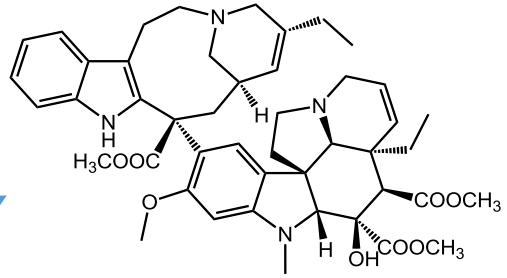
m/z $[M+H]^+$ = 337.1910



Serpentina/Alstonina

$C_{21}H_{21}N_2O_3^+$

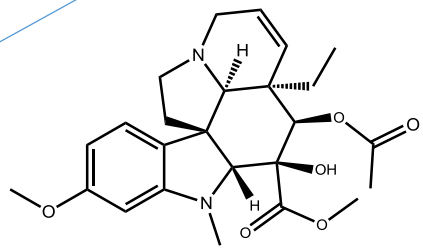
m/z $[M]^+$ = 349.1547



3,4-Anidrovimblastina

$C_{46}H_{56}N_4O_8$

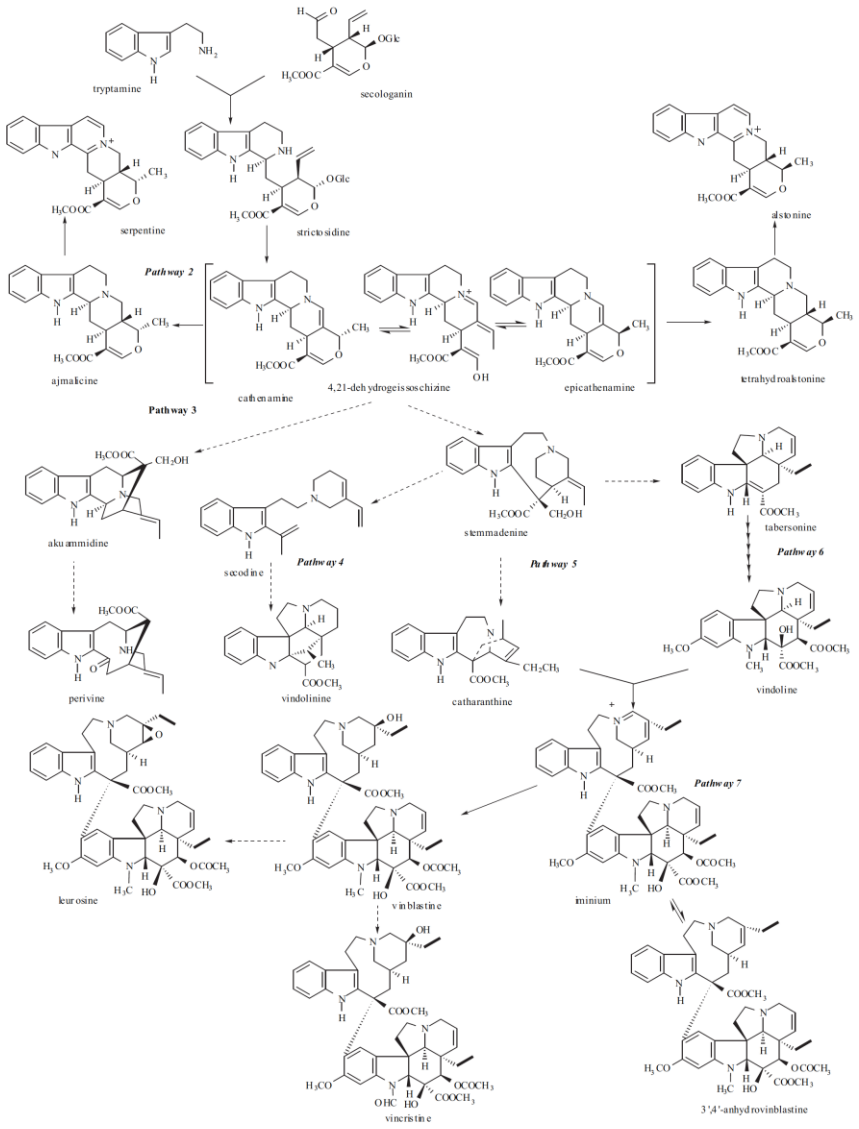
m/z $[M+H]^+$ = 793,4171



Vindolina

$C_{25}H_{32}N_2O_6$

m/z $[M+H]^+$ = 457.2339


tryptamine
secologanin
serpentine
strictosidine
ajmalicine
Pathway 2
cathenamine
4,21-dehydrogeissoschizine
epicathenamine
tetrahydroalstonine
alstonine
Pathway 3
akuammidine
stemmadenine
tabersonine
Pathway 6
Pathway 4
Pathway 5
secodine
perivine
vindolinine
catharanthine
vindoline
Pathway 7
leurosine
vinblastine
iminium
vincristine
3',4'-anhydrovinblastine

2005

2009

2007

2013

2011

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Aerial view of Institute of Chemistry - UNESP

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Biocatalysis and Mass Spectrometry

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