SIAN
Simpósio de Alimentos e Nutrição

4ª EDIÇÃO
Sustentabilidade e Inovação na Ciência dos Alimentos - Impacto na Bioeconomia
17 E 18 DE JUNHO DE 2019

Espectrometria de Massas: aplicações e tendências dentro das ciências "ômicas"

Laboratório Multiusuários de Química de Produtos Naturais (LMQPN)

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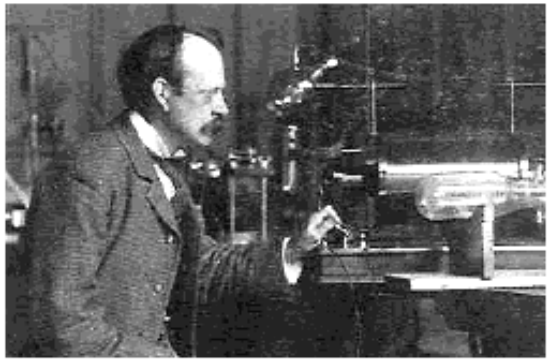
Embrapa

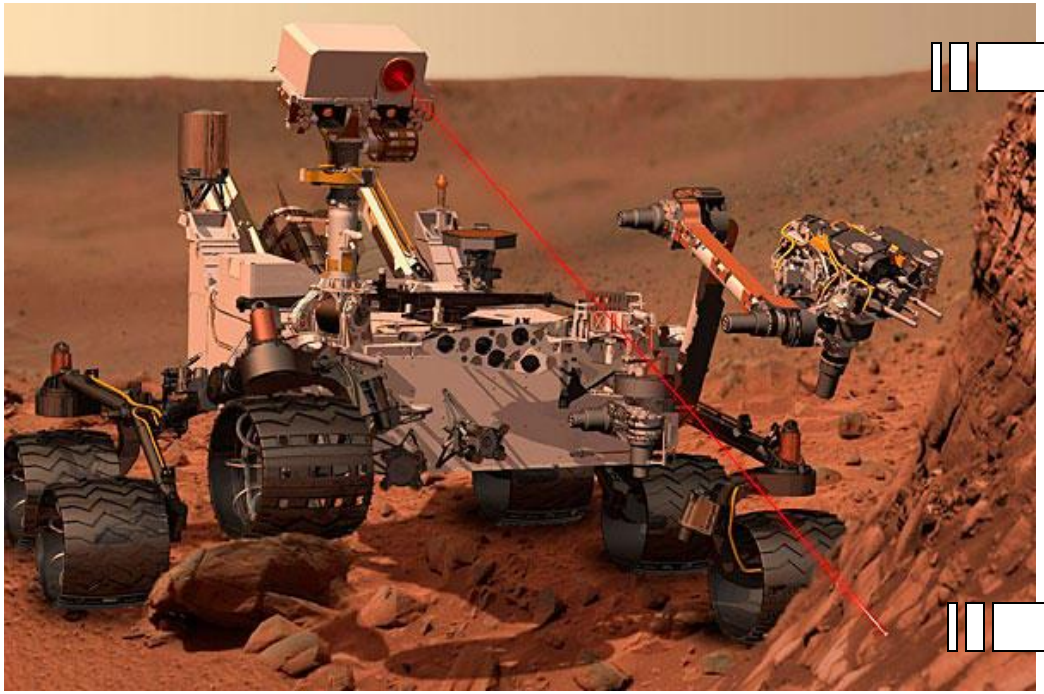
Espectrometria de Massas

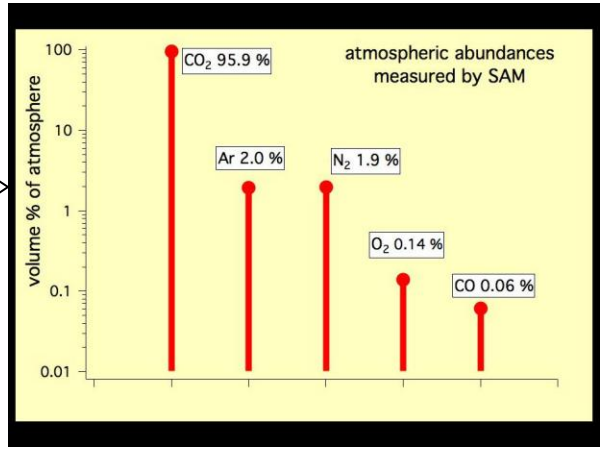
• 1897, Joseph John Thompson descobriu que todos os gases produzem a mesma partícula carregada negativamente e determinou a razão massa carga (*m/z*), a partir da magnitude das deflexões de raios negativamente carregados através de campos magnéticos e elétricos.

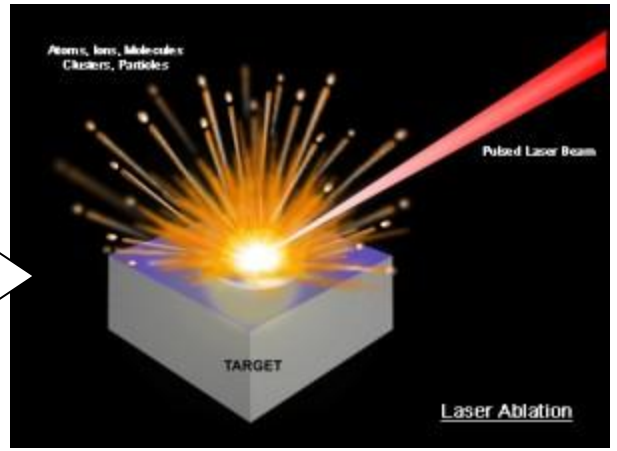
•1911 – J. J. Thompson
Primeiro Espectrômetro de Massas

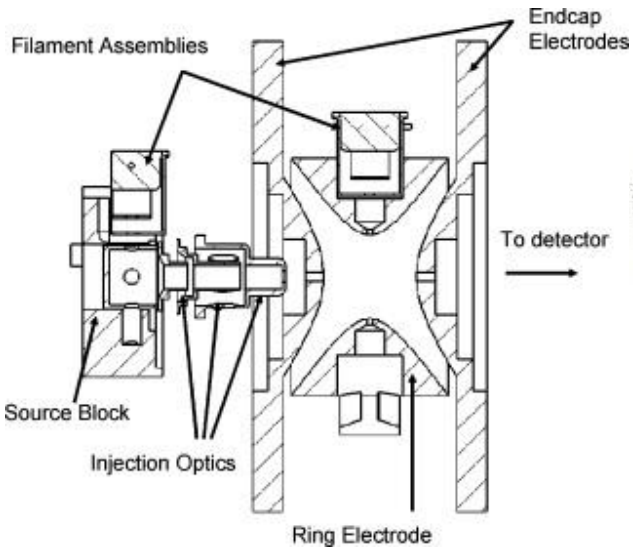


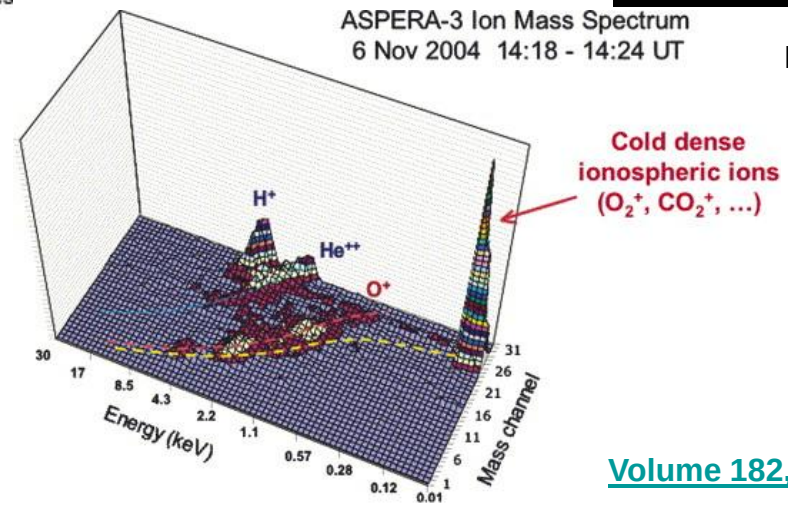
Exploração de Marte







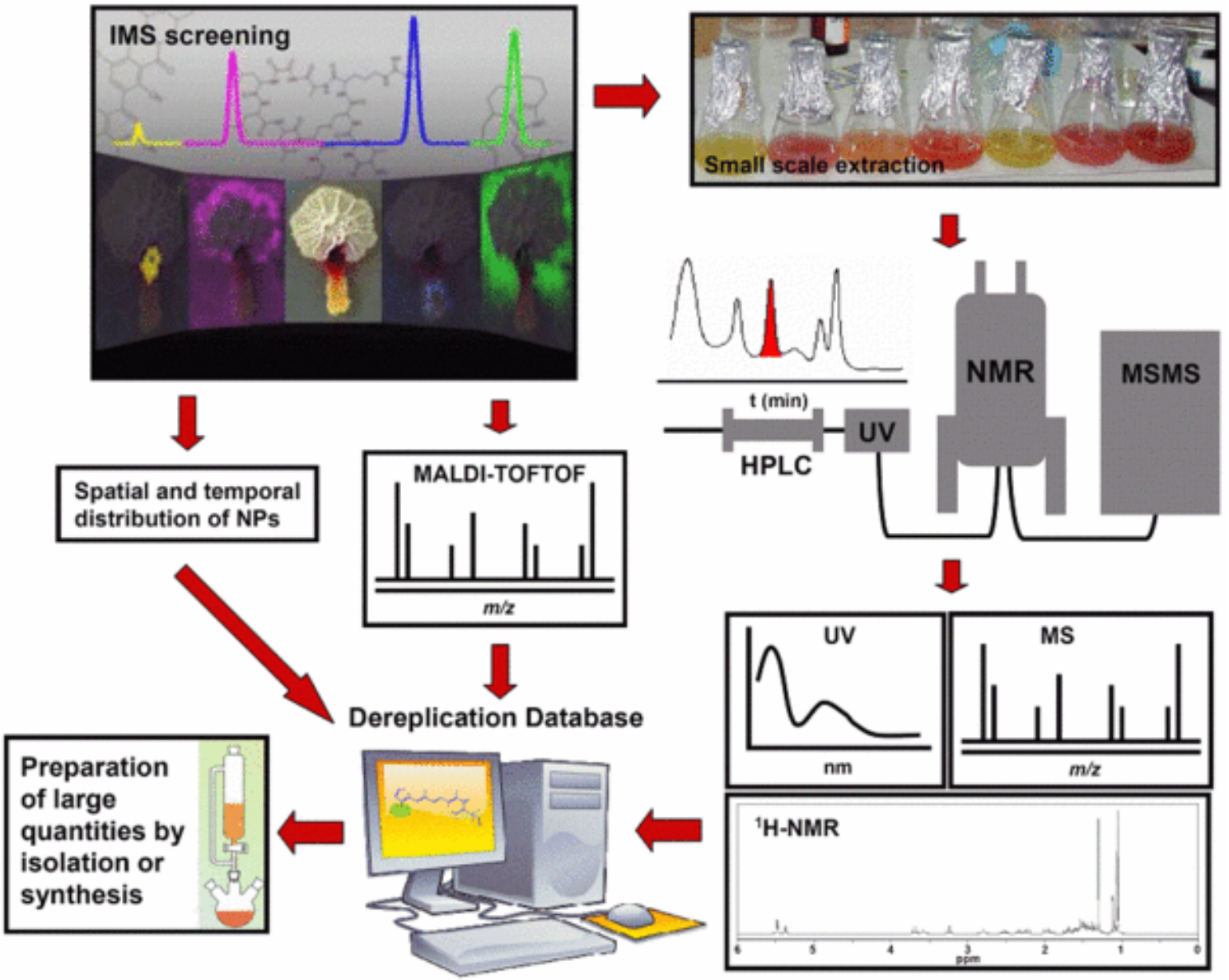




Fonte: NASA/JPL-Caltech

Icarus
Volume 182, Issue 2, June 2006, Pages 308–319

Desenvolvimento e aplicação de imagens em espectrometria de massas



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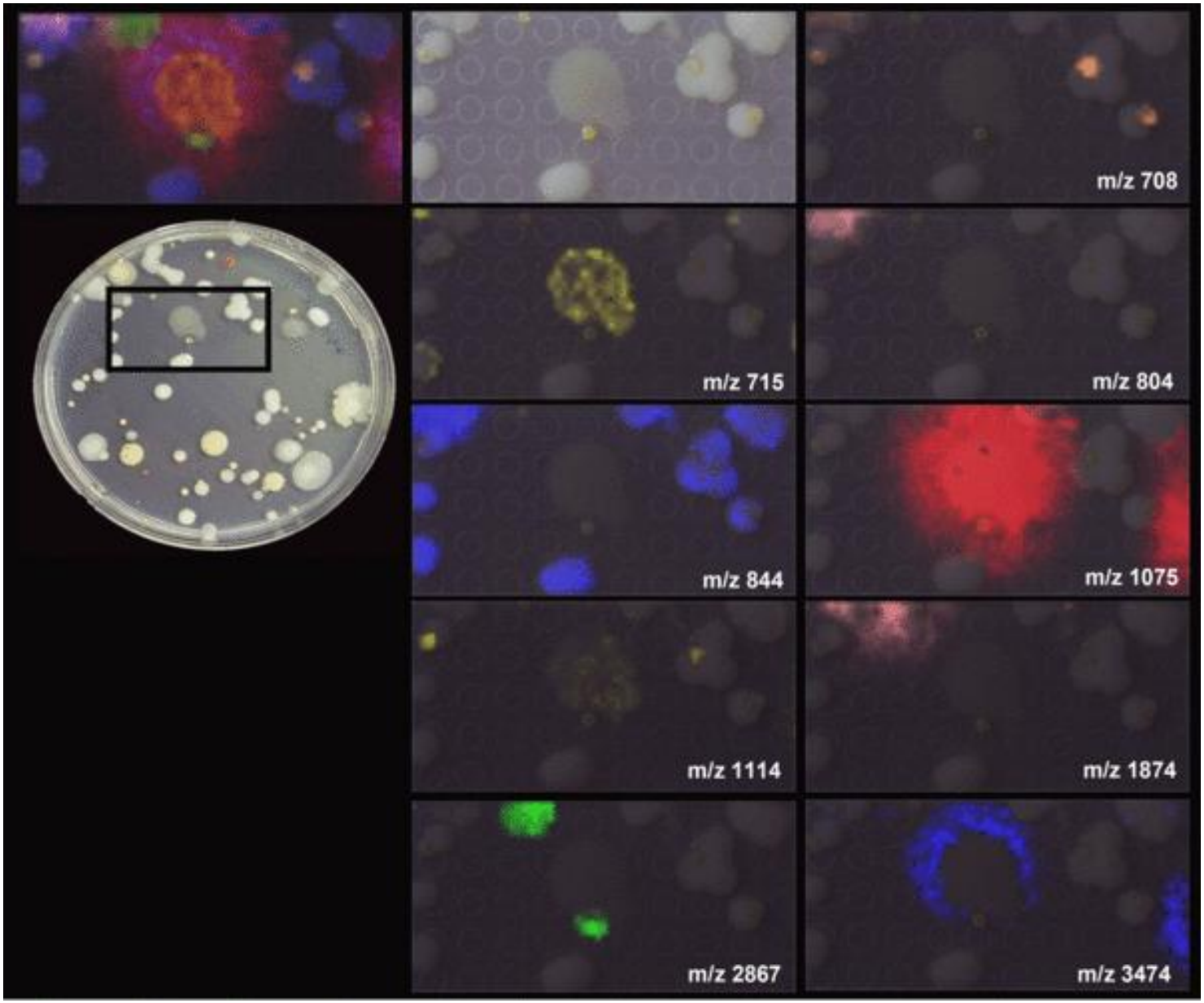
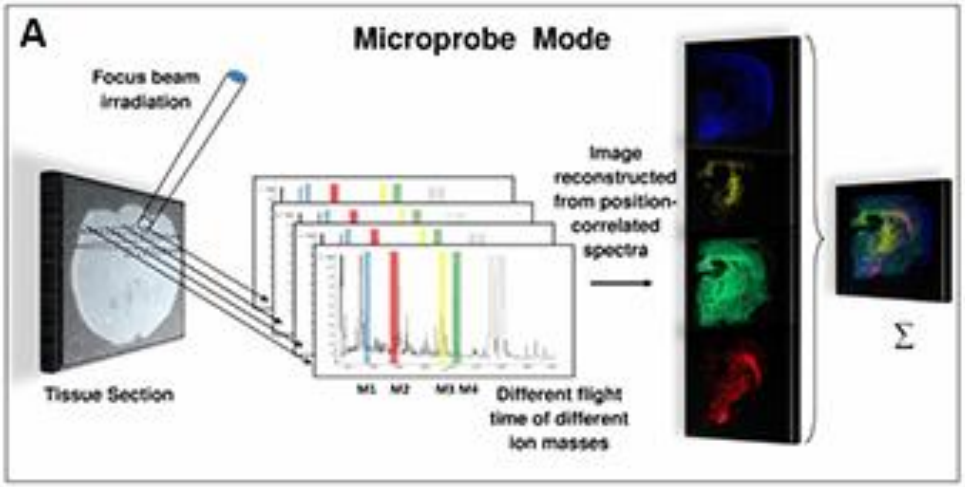
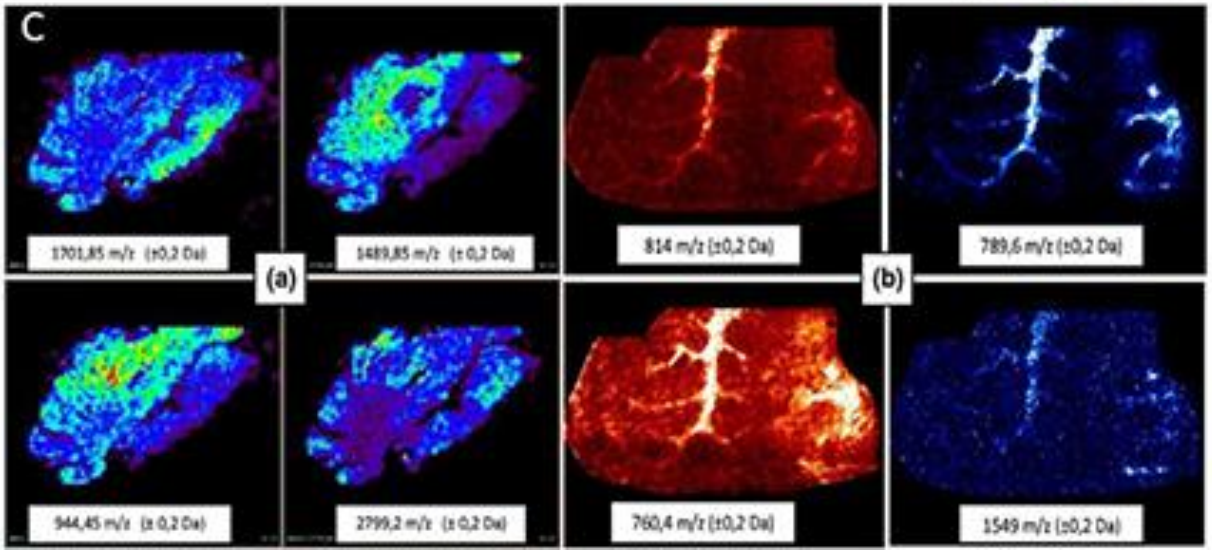


Metodologia desenvolvida para a análise espacial de tecido por espectrometria de massa MALDI





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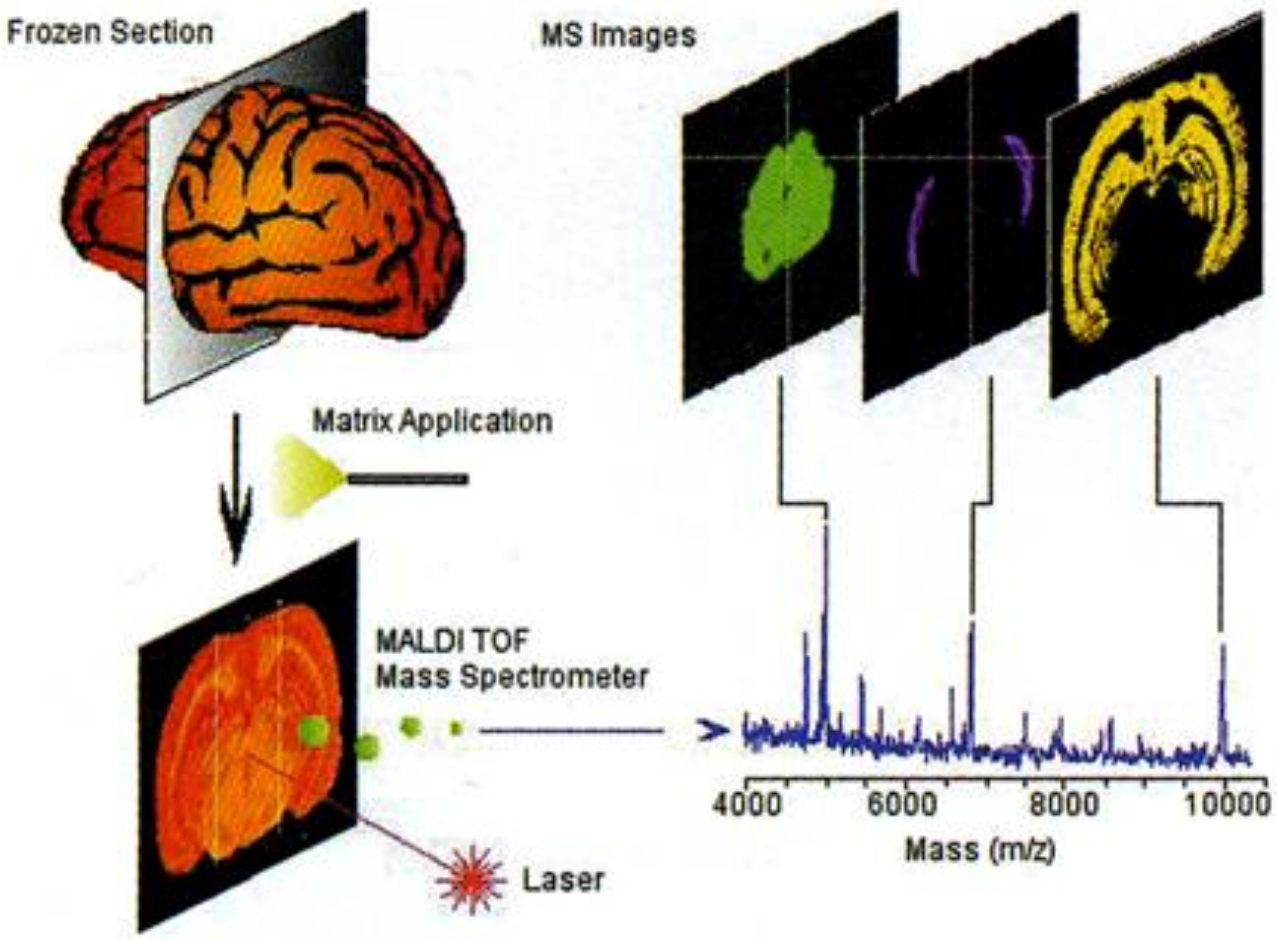
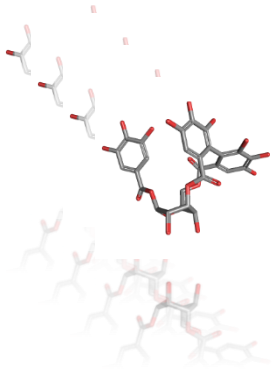
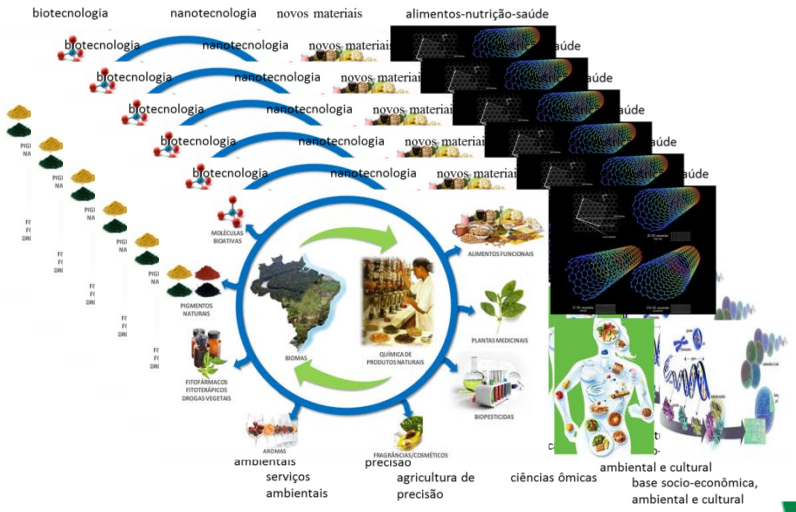


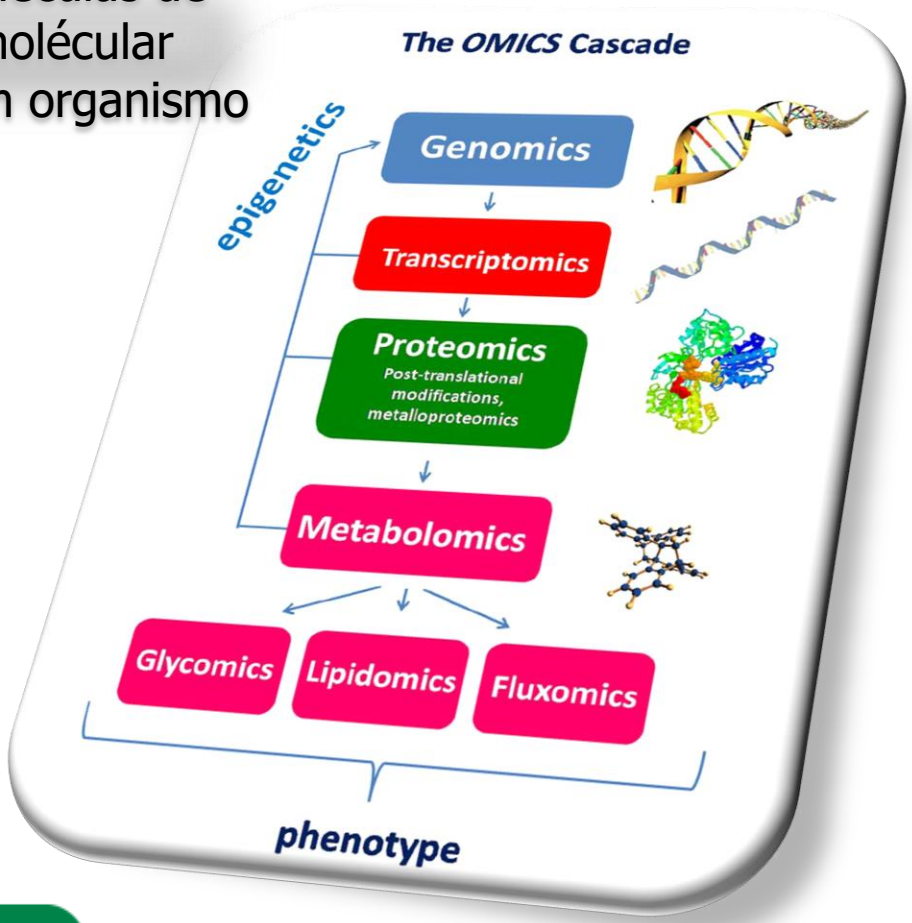
Embrapa

Metabolômica

Conjunto de moléculas de baixo massa molécular produzidas por um organismo







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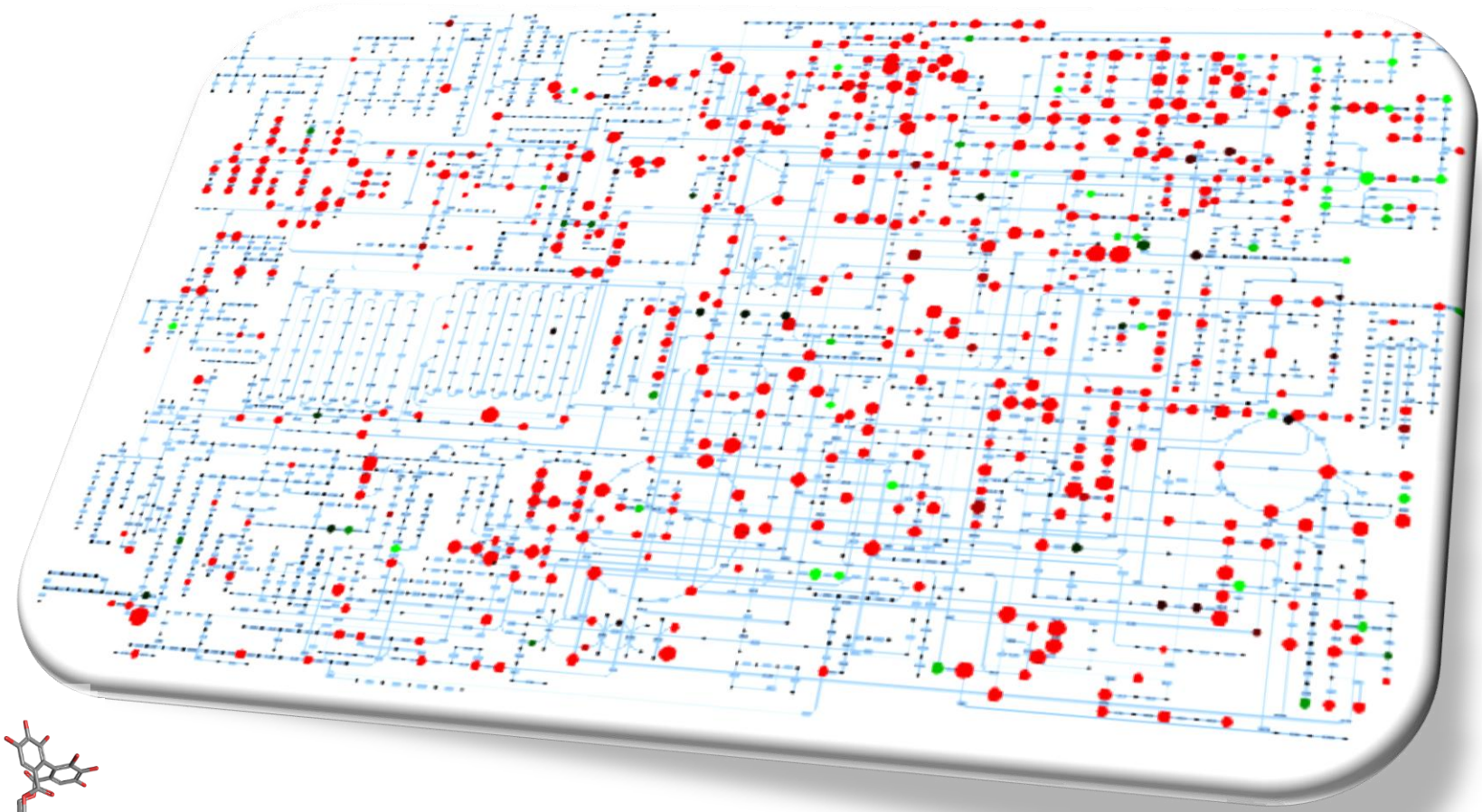
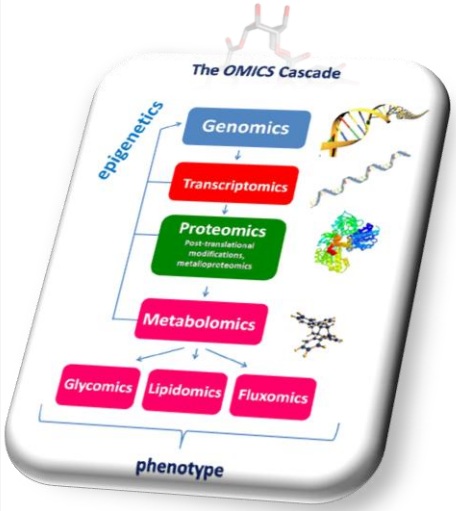


Metabolômica

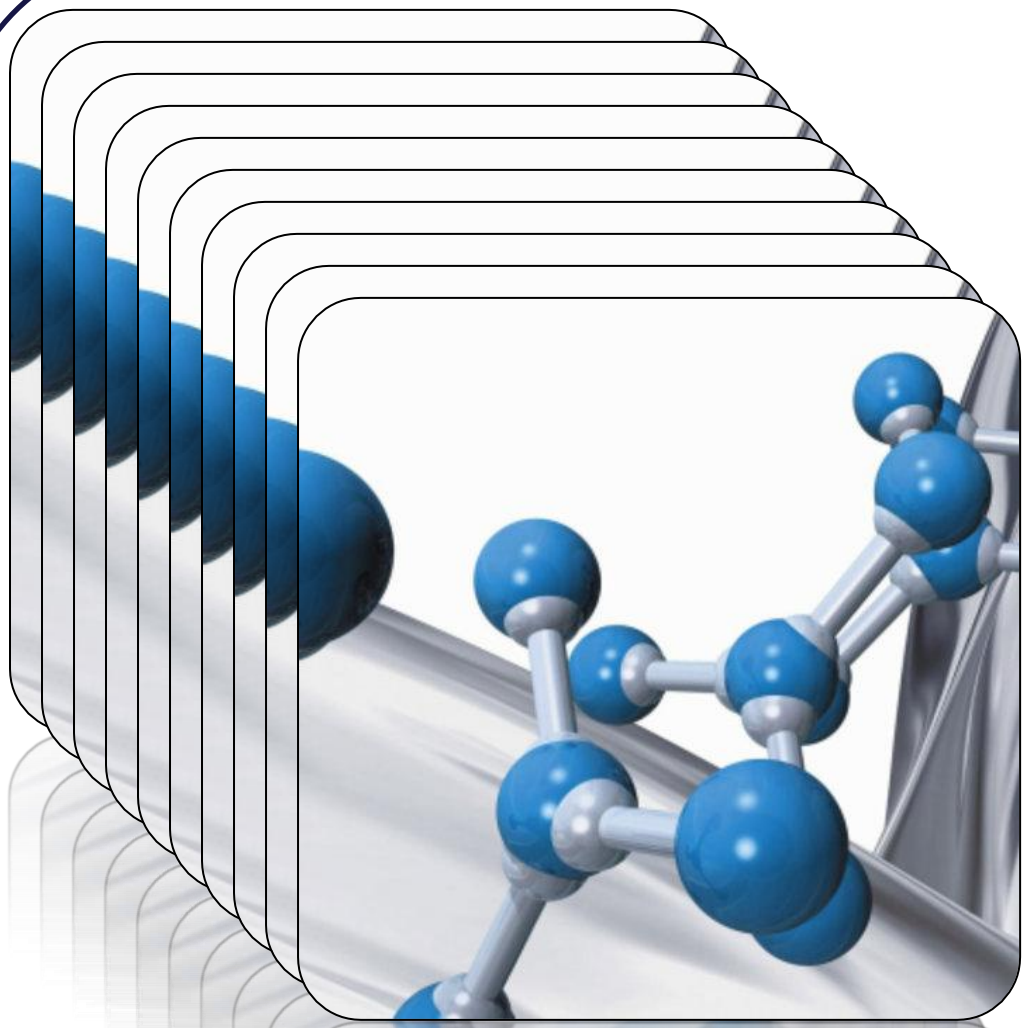
Area of application
Plant breeding and crop quality assessment
Food assessment and safety
Toxicity assessment
Nutrition assessment
Medical diagnosis and assessment of disease status
Pharmaceutical/drug developments
Yield improvement in crops and fermentations
Biomarker discovery
Technological advances in analytical chemistry
Genotyping
Environmental adaptations
Gene function elucidation
Integrated in systems biology

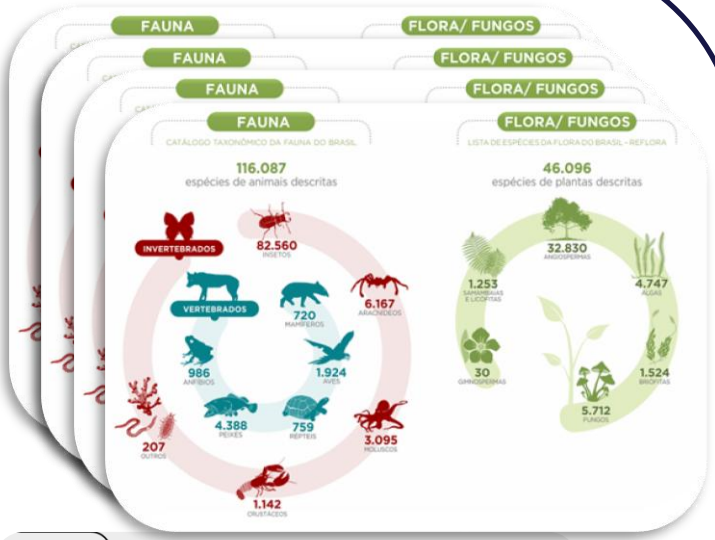


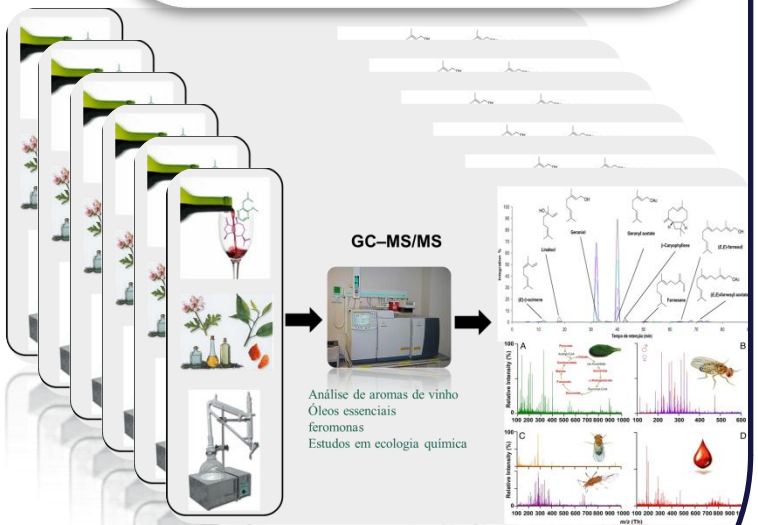
Embrapa

Metabolômica

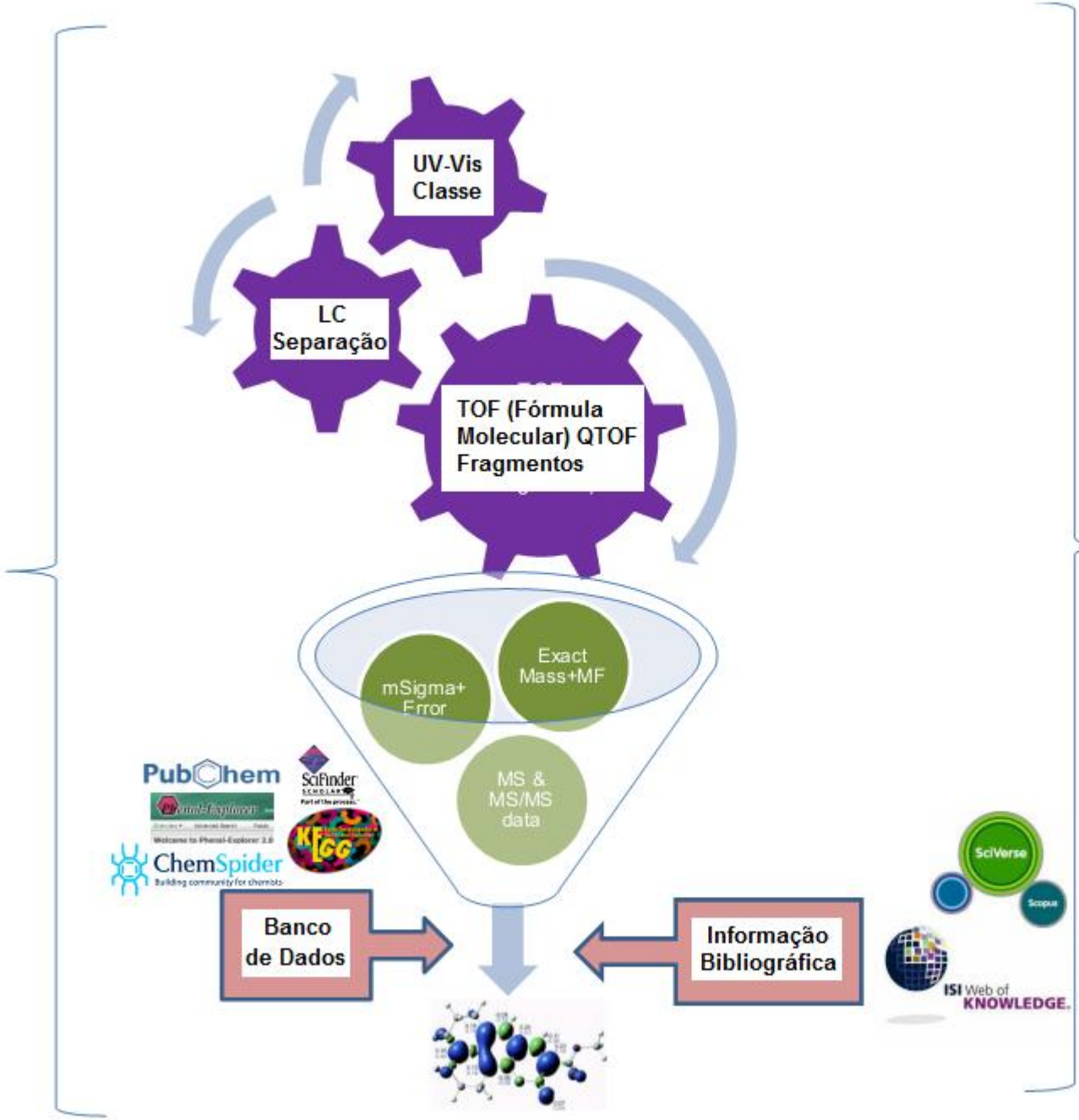
Complexidade







Metodologia de Desreplicação

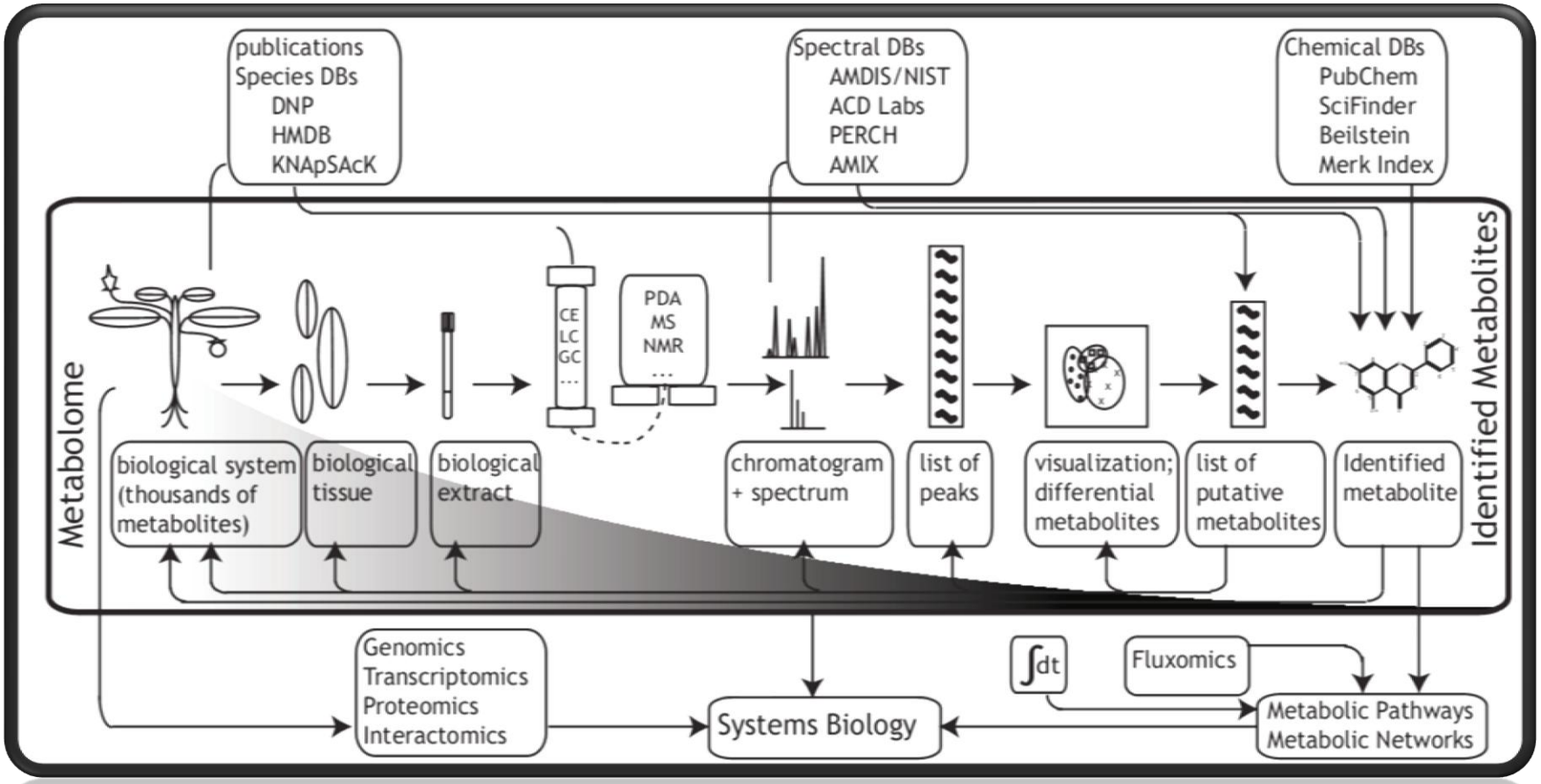


Caracterização

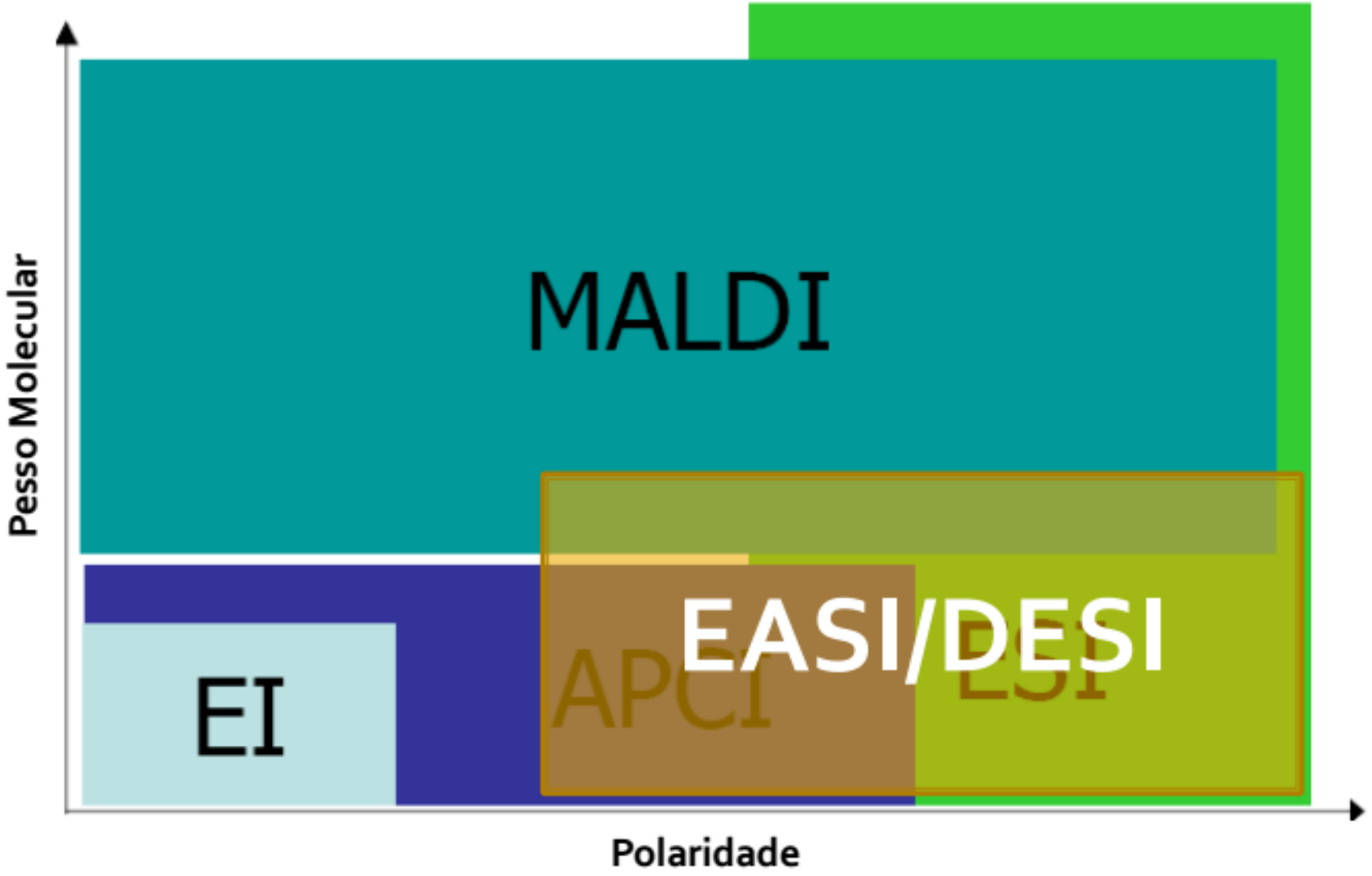
RMN

Metabolômica

Complexidade

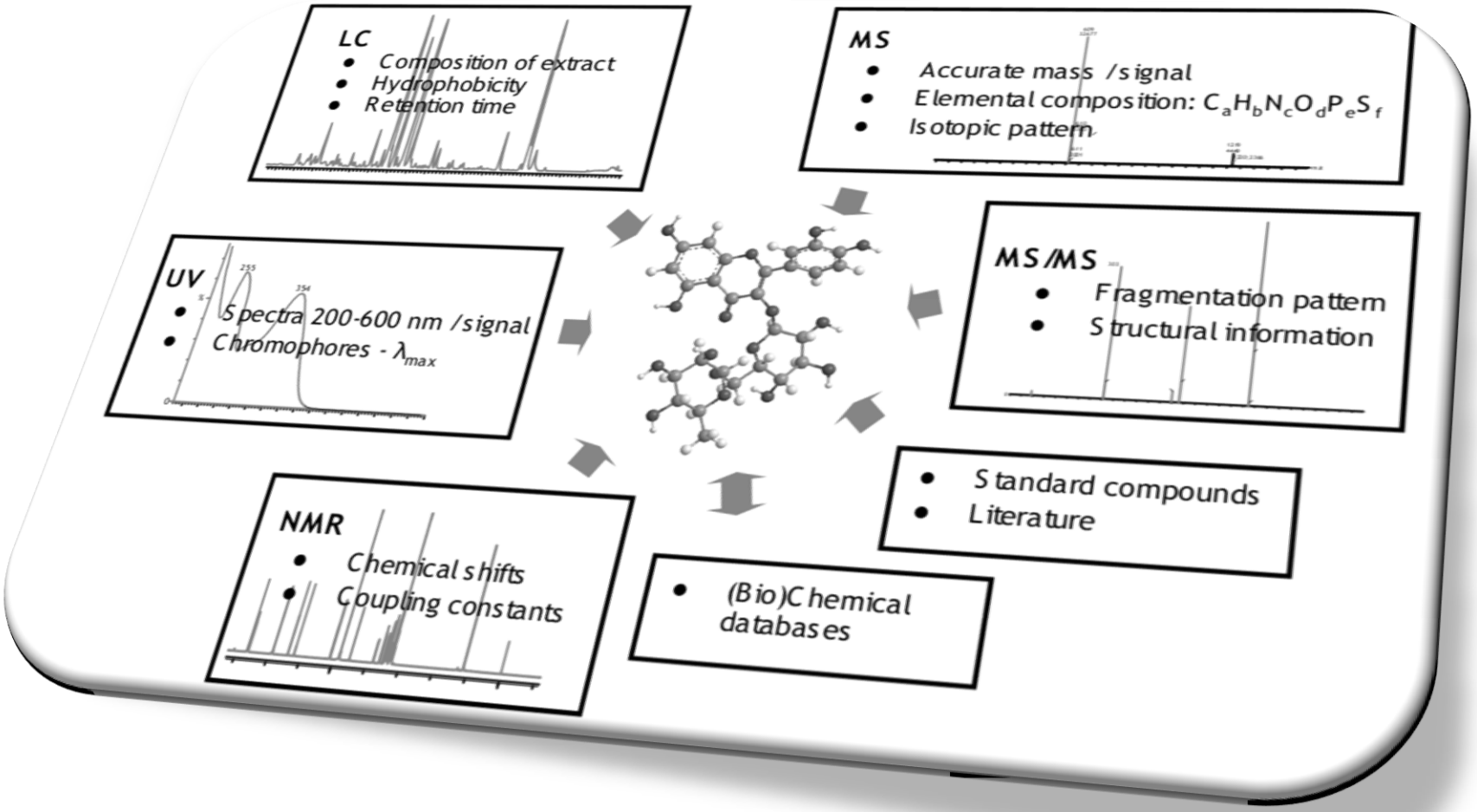


Abrangência da espectrometria de massas



Desafios

- **Grande volume de dados**



Tendências

Espectrometria de Massa em Alta Resolução

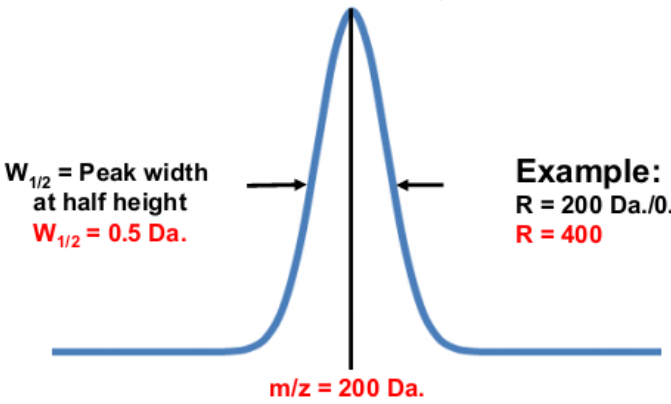
Mass resolution

What is it?

Definition:

Mass resolution = (ion mass)/(mass peak width)

$R = (m/z) / W_{1/2}$



Example:

R = 200 Da./0.5 Da.

R = 400

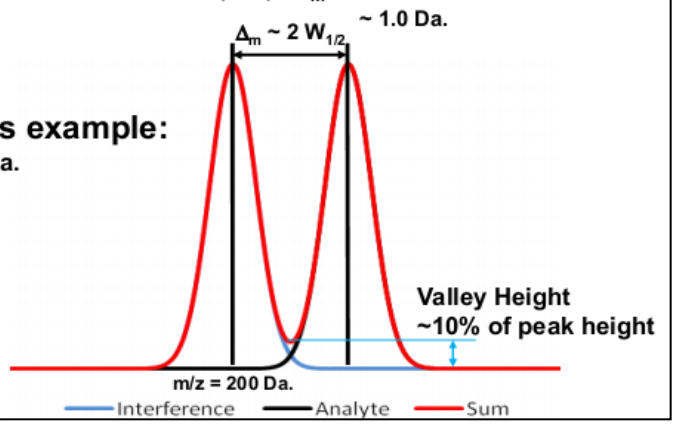
Mass resolution

Older definition – peaks of equal heights

Definition:

Mass resolution = (ion mass)/(mass peak separation)

$R = (m/z) / \Delta_m$



From previous example:

R = 200 Da. / 1.0 Da.

$R_{10\%} = 200$

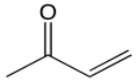
$R_{W1/2} = 400$

Differentiating Structures Using HRMS

Many structures may have the same integer value molecular weight, but different molecular formulas

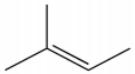
These structures can be differentiated with HRMS

For example:



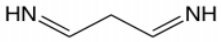
$C_4H_6O_1$

70.0418 amu



C_5H_{10}

70.0783 amu



$C_3H_6N_2$

70.0531 amu

Mass resolution

Older definition – peaks of equal heights

Definition:

Mass resolution = (ion mass)/(mass peak separation)

$R = (m/z) / \Delta_m$

From previous example:

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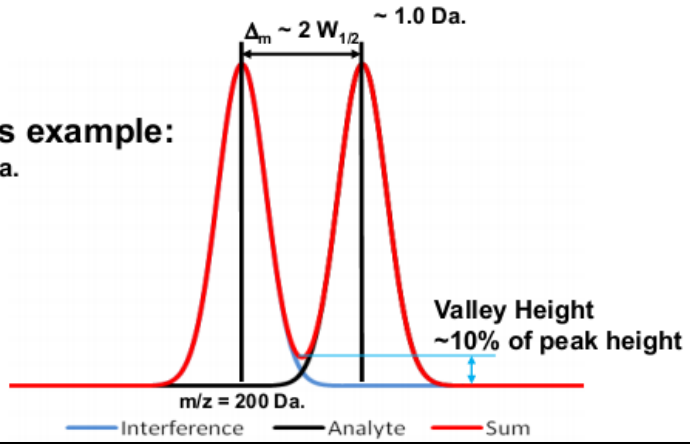
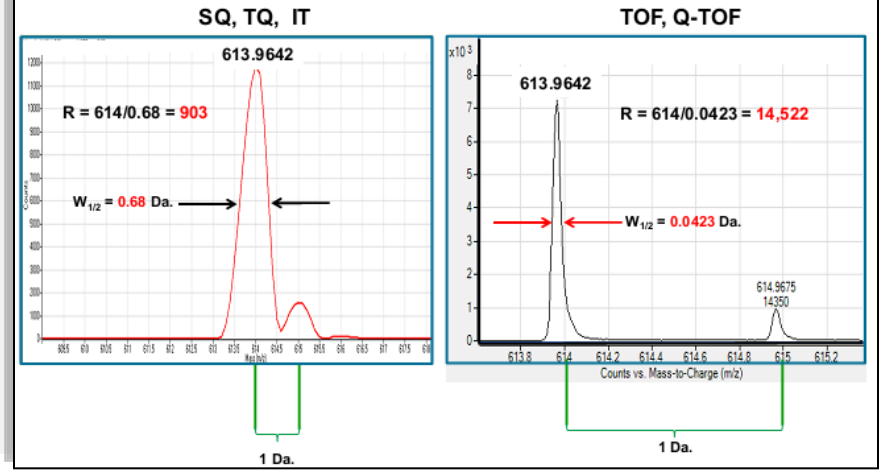


Resolving power & mass accuracy

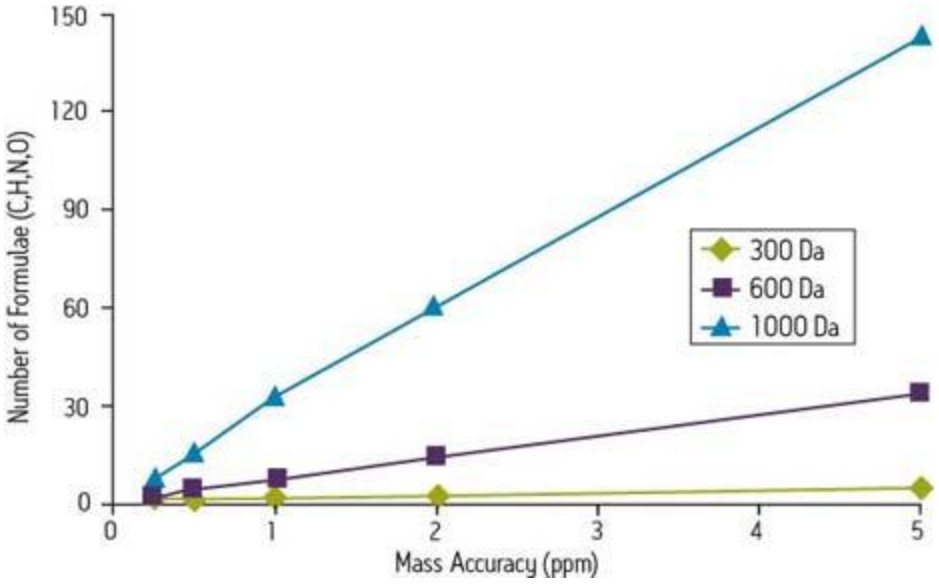
m/z = 613.964203



Mass error

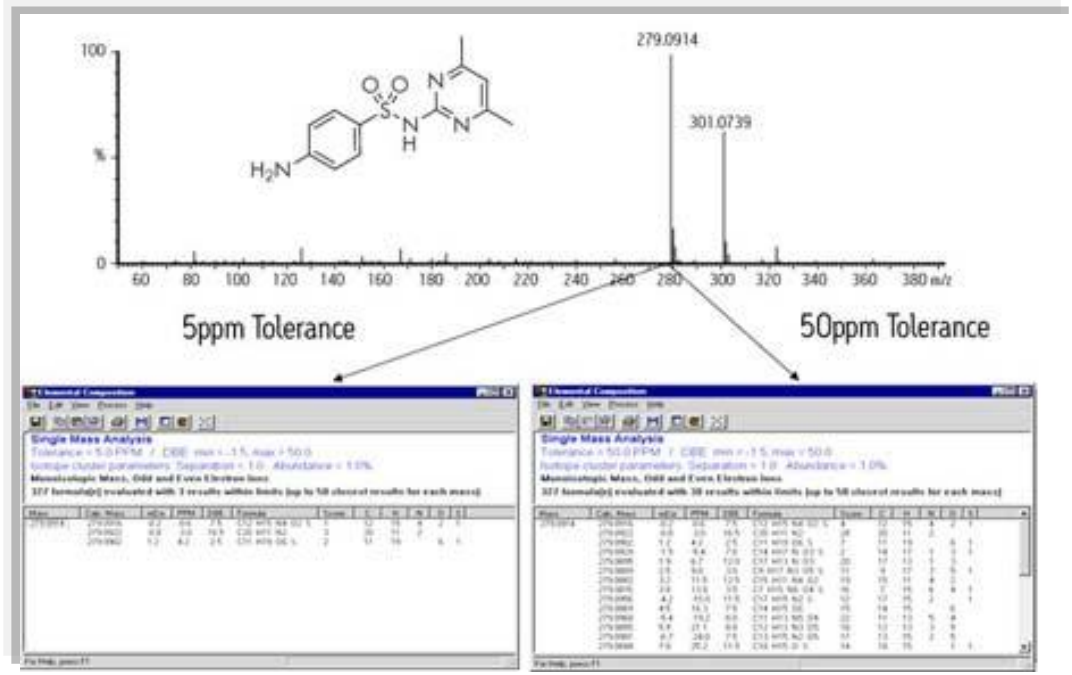
- Mass error = (exact mass) – (accurate mass)
- Mass error in parts per million (ppm) =

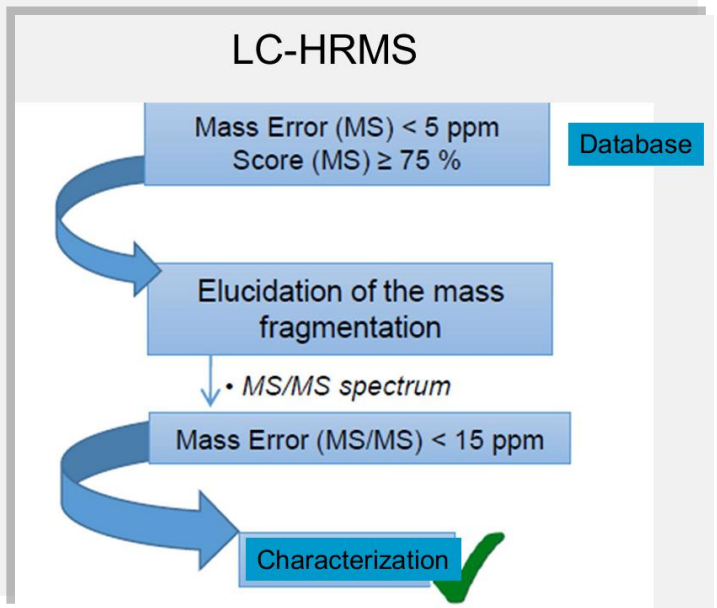
$$\frac{\text{(mass error)}}{\text{(exact mass)}} \times 10^6$$



What will we discuss?

- What is mass resolution and mass accuracy
- Why and when they are important
- How to use resolution and mass accuracy for practical analysis





A Liquid Chromatography Mass Spectrometry based Metabolome Database for Tomato

Sofia Moco, Raoul J. Bino, Oscar Vorst, Harrie A. Verhoeven, Joost de Groot, Teris A. van Beek, Jacques Vervoort and Ric C.H. De Vos *Plant Physiology* 141: 1205-1218 (2006)

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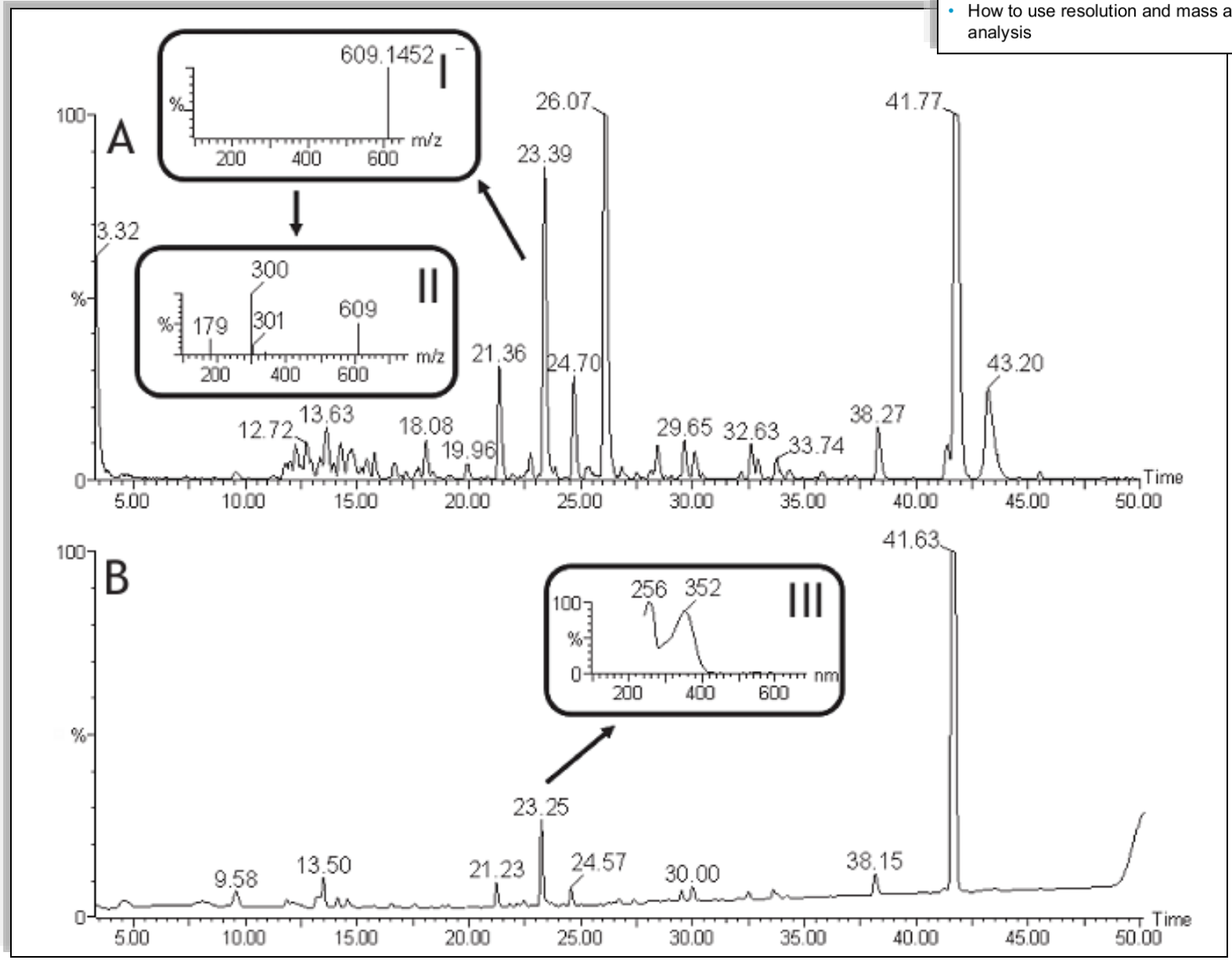

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Ret (min)		Av m/z	UV/Vis	MS/MS fragments	Mol Form	Theo. Mass	Mean Δ (ppm)	Putative ID
Av	StDev							
9.45	0.09	341.0883	-	179, 135	$C_{15}H_{18}O_9$	341.0878	1.52	Caffeic acid-hexose I
9.75	0.08	325.0930	294sh, 313	163	$C_{15}H_{18}O_8$	325.0929	0.25	Coumaric acid-hexose I
10.32	0.08	341.0883	310	179, 161, 135	$C_{15}H_{18}O_9$	341.0878	1.58	Caffeic acid-hexose II
11.35	0.08	341.0883	302sh, 318	281, 251, 233, 221, 179, 161, 135	$C_{15}H_{18}O_9$	341.0878	1.53	Caffeic acid-hexose III
12.08	0.06	355.1036	290sh, 313	193, 177, 145	$C_{16}H_{20}O_9$	355.1035	0.31	Ferulic acid-hexose I
12.58	0.07	341.0883	-	181, 179, 137, 135	$C_{15}H_{18}O_9$	341.0878	1.49	Caffeic acid-hexose IV
13.32	0.05	341.0883	-	281, 221, 181, 179, 161, 137, 135	$C_{15}H_{18}O_9$	341.0878	1.39	Caffeic acid-hexose V
13.43	0.07	353.0878	300sh, 327	191, 173, 127	$C_{16}H_{18}O_9$	353.0878	0.01	3-Caffeoylquinic acid
13.71	0.07	325.0929	285	163, 119	$C_{15}H_{18}O_8$	325.0929	0.05	Coumaric acid-hexose II
14.41	0.10	353.0878	295sh, 327	179, 173	$C_{16}H_{18}O_9$	353.0878	-0.08	5-Caffeoylquinic acid (S)
15.90	0.05	355.1036	-	193, 175, 160	$C_{16}H_{20}O_9$	355.1035	0.42	Ferulic acid-hexose II
15.98	0.06	341.0886	-	179	$C_{15}H_{18}O_9$	341.0878	2.26	Caffeic acid-hexose VI
16.76	0.07	353.0880	323	191, 173, 161, 127	$C_{16}H_{18}O_9$	353.0878	0.49	4-Caffeoylquinic acid
19.53	0.25	1272.5901	-	1227, 1095, 1065, 933, 866, 770	$C_{57}H_{95}NO_{30}$	1272.5866	2.75	(Esculeoside B)FA
21.42	0.04	741.1870	256, 299sh, 351	301, 271, 255	$C_{32}H_{38}O_{20}$	741.1884	-1.82	Quercetin-hexose-deoxyhexose-pentose
22.83	0.06	1314.6001	-	1269, 1137, 1107, 974, 770, 752	$C_{59}H_{97}NO_{31}$	1314.5972	2.21	(Lycoperoside G)FA or (Lycoperoside F)FA or (Esculeoside A)FA I
23.43	0.04	609.1451	256, 299sh, 355	301, 271, 255	$C_{27}H_{30}O_{16}$	609.1461	-1.59	Quercetin-glucose-rhamnose (S)
25.48	0.16	1314.6005	-	1269, 1137, 1107, 975, 908, 866, 812, 770, 752, 275, 179, 161, 149, 143, 125, 113	$C_{59}H_{97}NO_{31}$	1314.5972	2.54	(Lycoperoside G)FA or (Lycoperoside F)FA or (Esculeoside A)FA II

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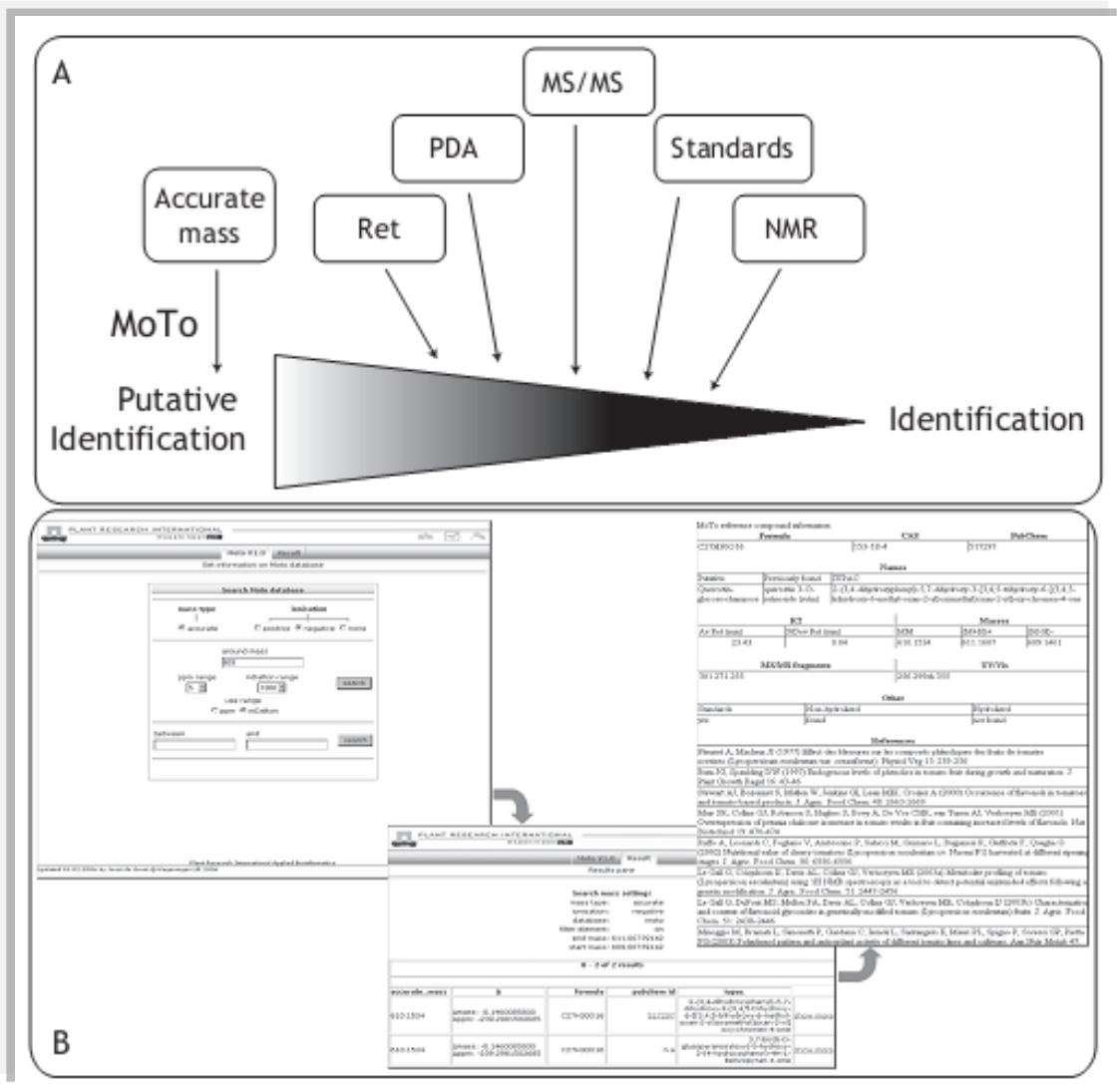
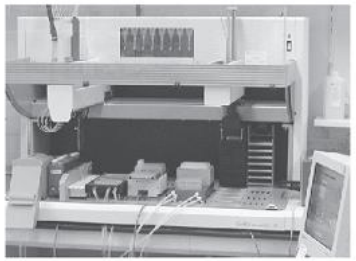


Growth and harvest of plant material

Freezing and grinding

STEPS 1-2

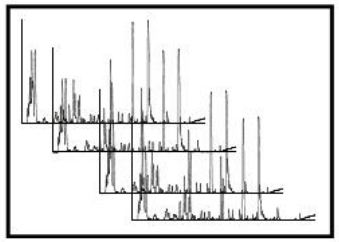


Extraction, centrifugation and filtration STEPS 3-7

Transfer vials to autosampler

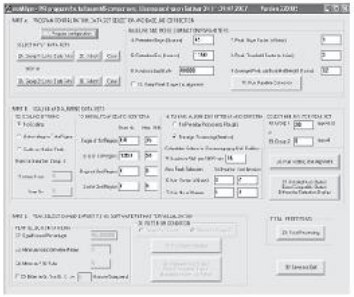
LC-PDA-QTOF MS

STEPS 8-9



LC-MS profiles

Data processing



MetAlign for mass peak extraction and alignment over samples

STEPS 10-14

Output:

CSV file

STEP 15

Data analyses

- t-tests
- multivariate analyses tools
- correlation analyses
- .....

Identification of relevant mass peaks

STEPS 16-19

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J. Am. Soc. Mass Spectrom. (2016) 27:1897–1905
DOI: 10.1007/s13361-016-1469-y

CRITICAL INSIGHT

Untargeted Metabolomics Strategies—Challenges and Emerging Directions

Alexandra C. Schrimpe-Rutledge,[1,2,3,4] Simona G. Codreanu,[1,2,3,4] Stacy D. Sherrod,[1,2,3,4] John A. McLean[1,2,3,4]

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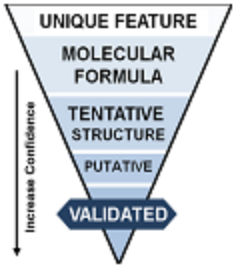
[3]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37235, USA
[4]Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, TN 37235, USA



Abstract. Metabolites are building blocks of cellular function. These species are involved in enzyme-catalyzed chemical reactions and are essential for cellular function. Upstream biological disruptions result in a series of metabolomic changes and, as such, the metabolome holds a wealth of information that is thought to be most predictive of phenotype. Uncovering this knowledge is a work in progress. The field of metabolomics is still maturing; the community has leveraged proteomics experience when applicable and developed a range of sample preparation and instrument methodology along with myriad data processing and analysis approaches. Research focuses have now shifted toward a fundamental understanding of the biology responsible for metabolomic changes. There are several types of metabolomics experiments including both targeted and untargeted analyses. While untargeted, hypothesis generating workflows exhibit many valuable attributes, challenges inherent to the approach remain. This Critical Insight comments on these challenges, focusing on the identification process of LC-MS-based untargeted metabolomics studies—specifically in mammalian systems. Biological interpretation of metabolomics data hinges on the ability to accurately identify metabolites. The range of confidence associated with identifications that is often overlooked is reviewed, and opportunities for advancing the metabolomics field are described.

Keywords: Metabolomics, Untargeted, Targeted, Discovery, Global, Validation, Identification, Bioinformatics

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J. Am. Soc. Mass Spectrom. (2016) 27:1897–1905
DOI: 10.1007/s13361-016-1469-y

CRITICAL INSIGHT

Untargeted Metabolomics Strategies—Challenges and Emerging Directions

Alexandra C. Schrimpe-Rutledge,[1,2,3,4] Simona G. Codreanu,[1,2,3,4] Stacy D. Sherrod,[1,2,3,4] John A. McLean[1,2,3,4]

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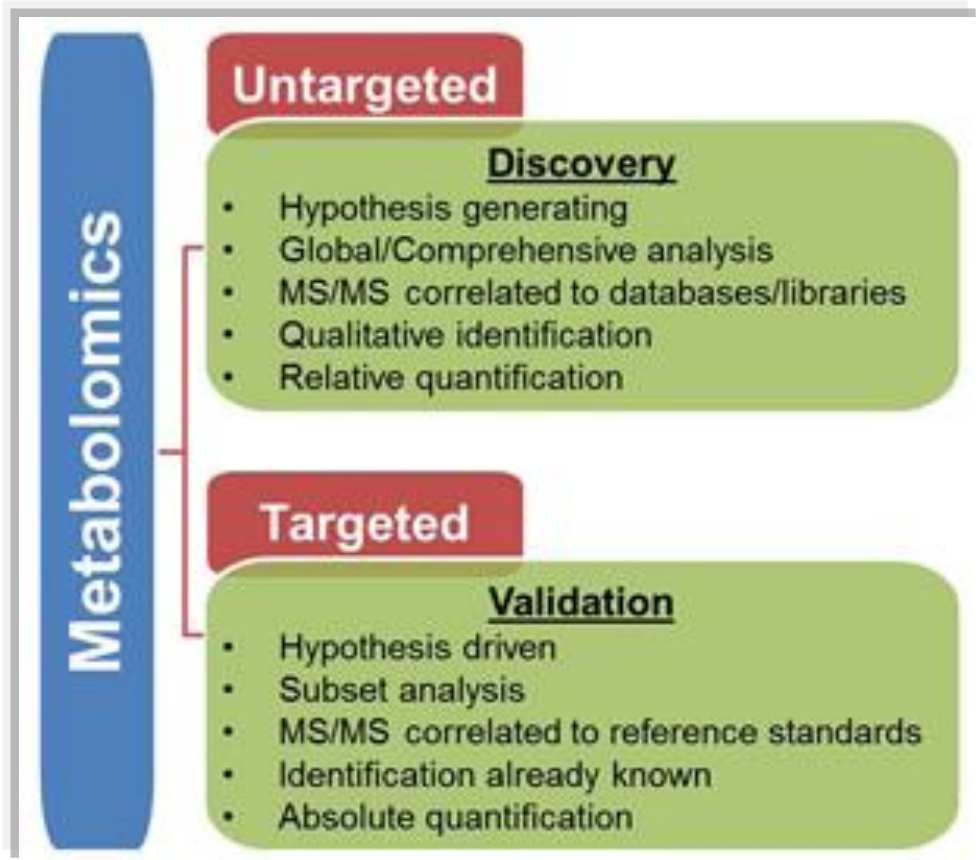
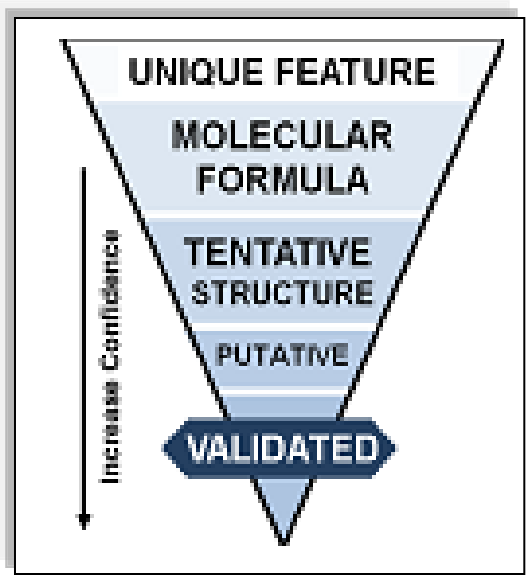
[2]Center for Innovative Technology, Vanderbilt University, Nashville, TN 37235, USA
[3]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37235, USA
[4]Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, TN 37235, USA





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CRITICAL INSIGHT

Untargeted Metabolomics Strategies—Challenges and Emerging Directions

Metabolite identification confidence

Increasing confidence

Level 5

61M+ compounds (PubChem)

Unique Feature
Mass measurement accuracy, ± ppm

- unit resolution..... 354 ± 1 Da..... 200k molecules
- 100 ppm..... 354.16 ± 0.03 D.....44k molecules
- 10 ppm..... 354.158 ± 0.003 Da.....11k molecules
- 1 ppm..... 354.1579 ± 0.0003 Da.....10k molecules
- Heuristic Filtering

Level 4

Molecular Formula
Isotope abundance distribution, charge state and adduct ion determination

$C_{20}H_{22}N_2O_4$ .....10k isomeric structures

Level 3

Tentative Structure
MS1 m/z database match

Orthogonal information

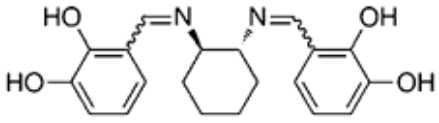
- fragmentation data (MS/MS)
- retention time
- collision cross section
- spatial/temporal location
- optical spectroscopy
- NMR

Level 2

Putative Identification
MS/MS spectrum match

Level 1

Validated Identification
Reference standard confirms structure



.....1 confirmed identification

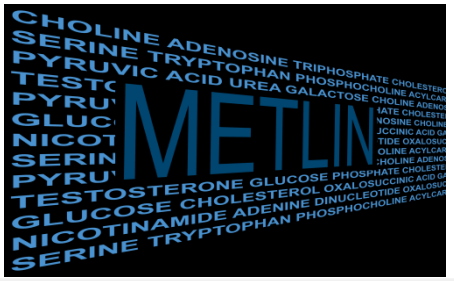


Table 2. Confidence Annotation, Statistical Evaluation, and Selected Bioinformatics Tools

Confidence	Utility	Statistical treatment/ Approach	Bioinformatic tools
Levels 5 and 4 (Unique m/z feature or Molecular formula)	Ranking significant differences	t-test Principle Component Analysis (PCA) Partial Least Squares (PLS) Modeling	Most statistical software packages
	Data visualization and prioritization	Cloud Plot/Volcano Plot Self-organizing Map (SOM)	XCMS Online [38] Metabolite Expression Dynamics Inspection (MEDI) [39]
	Pathway/network analysis without formal annotation	Pathway and network prediction	*mummichog* [45]
Level 3 (Tentative structure)	Matching parent ion exact mass and fragmentation patterns	MS1 database and MS2 spectral library	ChemSpider, METLIN, HMDB, MassBank, mzCloud, LipidBlast, GNPS, NIST [16–19]
Levels 2 and 1 (Putative identification or Validation)	Integration with known biology	Pathway and network analyses	MetaboAnalyst [40] Kyoto Encyclopedia of Genes and Genomes (KEGG) [41] Mbrole (Metabolite Biological Role) [42] MetaCyc/BioCyc [43]

Bases de Dados de Produtos Naturais que podem ser utilizadas para busca de substâncias em meios virtuais

Database	Number of entries	Additional information	Refs
Super Natural II	355,000	2D structures; vendor information for over 215,000 compounds	*
Universal Natural Product Database	197,201	3D structures assembled from other available Chinese databases	289
Chinese Natural Product Database	53,000	Has been used in a virtual screen for PPAR-γ agonists	290
Drug Discovery Portal	40,000	Not all natural products, but all based on available samples	49
iSMART	20,000	Based on components from traditional Chinese medicines	291, 292
Database from historical medicinal plants, DIOS	6,702	Successfully used in several virtual screening campaigns	293
AfroDb	1,000	Compounds from African medicinal plants	294
NuBBE	640	Compounds from Brazilian sources	295‡

2D, two-dimensional; 3D, three-dimensional; iSMART, integrated systems biology-associated research with traditional Chinese medicine; PPAR-γ, peroxisome proliferator-activated receptor-γ. *See the Super Natural II database. ‡See the NuBBE database.

Embrapa

A.L. Harvey, R. Edrada-Ebel, R.J. Quinn, The re-emergence of natural products for drug discovery in the genomics era, Nat. Rev. Drug Discov. 14 (2015) 111–129. doi:10.1038/nrd4510.

Substances | Get References | Get Reactions | Tools | Send to SciPlanner

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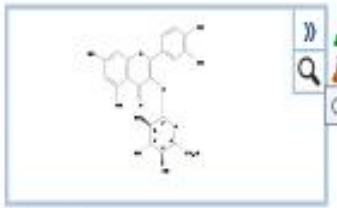
~816

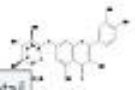


Click to view detail

C21 H18 O13
β-D-Glucopyranosiduronic acid, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-1-benzopyran-3-yl

Spectra
Experimental Properties

2. **Substance Detail**
38934-20-2
~46



C21 H18 O13
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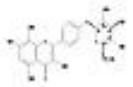
3. **Substance Detail**
54869-23-7
~46



C21 H18 O13
β-D-Glucopyranosiduronic acid, 4-(3,5,7,8-tetrahydroxy-4-oxo-4*H*-1-benzopyran-2-yl)phenyl

4. **Substance Detail**
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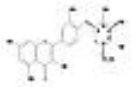
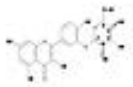
~29



C21 H18 O13
β-D-Glucopyranosiduronic acid, 2-hydroxy-4-(3,5,7-trihydroxy-4-oxo-4*H*-1-benzopyran-2-yl)phenyl

5. **Substance Detail**
328006-77-5
~29



C21 H18 O13
β-D-Glucopyranosiduronic acid, 2-hydroxy-5-(3,5,7-trihydroxy-4-oxo-4*H*-1-benzopyran-2-yl)phenyl

Experimental Properties

6. **Substance Detail**
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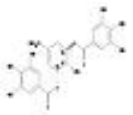
~21

7. **Substance Detail**
95753-52-9
~14



C21 H18 O13
Benzoic acid, 3,4,5-trihydroxy-, 1,1'-[(1*R*,2*S*,3*R*)-5-carboxy-2-hydroxy-4-cyclohexene-1,3-diyl] ester

Spectra

8. **Substance Detail**
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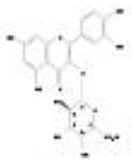

C21 H18 O13
β-D-Galactopyranosiduronic acid, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-1-benzopyran-3-yl

Analysis | **Refine**

Analyze by: Substance Role

Click bar to view only those substances within the current answer set

Biological Study	36
Preparation	30
Occurrence	27
Properties	23
Uses	22
Analytical Study	12
Formation, Nonpreparative	8
Reactant or Reagent	7
Process	4

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Sort by: Number of References | Answers per Page [50] | View:

0 of 47 Substances Selected

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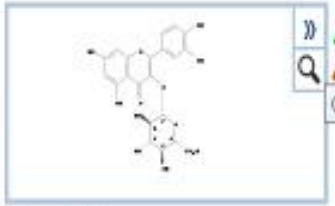
   ~816
   
   C21 H18 O13
   β-D-Glucopyranosiduronic acid, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-1-benzopyran-3-yl
   Spectra
   Experimental Properties

2. **Substance Detail**
   38934-20-2
   ~46
   
   Click to view detail
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   β-D-Glucopyranosiduronic acid, 2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4-oxo-4*H*-1-benzopyran-7-yl

3. **Substance Detail**
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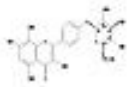
   ~46
   
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   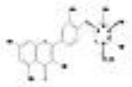
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   β-D-Glucopyranosiduronic acid, 2-hydroxy-4-(3,5,7-trihydroxy-4-oxo-4*H*-1-benzopyran-2-yl)phenyl

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   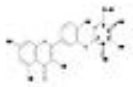
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   β-D-Glucopyranosiduronic acid, 2-hydroxy-5-(3,5,7-trihydroxy-4-oxo-4*H*-1-benzopyran-2-yl)phenyl
   Experimental Properties

6. **Substance Detail**
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   C6 H10 O7
   

7. **Substance Detail**
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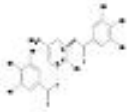
   ~14
   
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   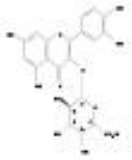
   C21 H18 O13
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Analysis | Refine

Analyze by: Substance Role

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Occurrence	27
Properties	23
Uses	22
Analytical Study	12
Formation, Nonpreparative	8
Reactant or Reagent	7
Process	4

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Table 1
Freely and commercially available web-resources for MS-based metabolomics.

Name	Link	Note
"Freely available" software		
Pathway		
AraCyc	http://www.arabidopsis.org/biocyc/index.jsp	*Arabidopsis*
BioCyc	http://biocyc.org/	Organisms
EcoCyc	http://ecocyc.org/	*Escherichia coli*
KaPPA-View3	http://kpv.kazusa.or.jp/kpv3/guestIndex.jsp	*Arabidopsis*, rice, tomato, lotus
KEGG	http://www.genome.jp/kegg/ligand.html	Organisms
MetaCyc	http://metacyc.org/index.shtml	Organisms
MetaCrop	http://metacrop.ipk-gatersleben.de	Crops
PlantCyc	http://www.plantcyc.org/	Plants
Analytical data (samples)		
ARMeC	http://www.armec.org/MetaboliteLibrary/index.html	*Arabidopsis*, potato
KOMICS	http://webs2.kazusa.or.jp/komics/	Tomato
McGill-MD	http://metabolomics.mcgill.ca/	Crops
MotoDB	http://appliedbioinformatics.wur.nl/moto/	Tomato
Analytical data (standard		
Publication and literature		
BioMeta	http://biometa.cmbi.ru.nl/	
ChEBI	http://www.ebi.ac.uk/chebi/	
HMDB	http://www.hmdb.ca/	
KNApSAcK	http://kanaya.naist.jp/KNApSAcK/KNApSAcK.php	
MassTRIX	http://mips.gsf.de/proj/metabolomics/	
MMCD	http://mmcd.nmrfam.wisc.edu/	
MMsINC	http://mms.dsfarm.unipd.it/MMsINC.html	
PubChem	http://pubchem.ncbi.nlm.nih.gov/	
UCI ChemDB	http://cdb.ics.uci.edu/CHEM/Web/	
Compound family		
Flavonoid viewer	http://www.metabolome.jp/software/FlavonoidViewer/data/mass	Flavonoids
LIPID MAPS	http://www.lipidmaps.org/index.html	Lipids
LIPIDBANK	http://lipidbank.jp/	Lipids
"For a commercial fee" database		
Adams library	Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, 4th Edition (2007)	
Dictionary of natural medicines	http://dnp.chemnetbase.com/dictionary-search.do?method=view&id=2714206&si=	
DiscoveryGate	http://www.symyx.com/products/databases/discoverygate/index.jsp	
MASS Frontier	http://www.thermo.com/com/cda/product/detail/1,1055,11442,00.html	
NIST	http://www.nist.gov/srd/onlinelist.htm	
Scifinder	http://www.cas.org/SCIFINDER/SCHOLAR/index.html	

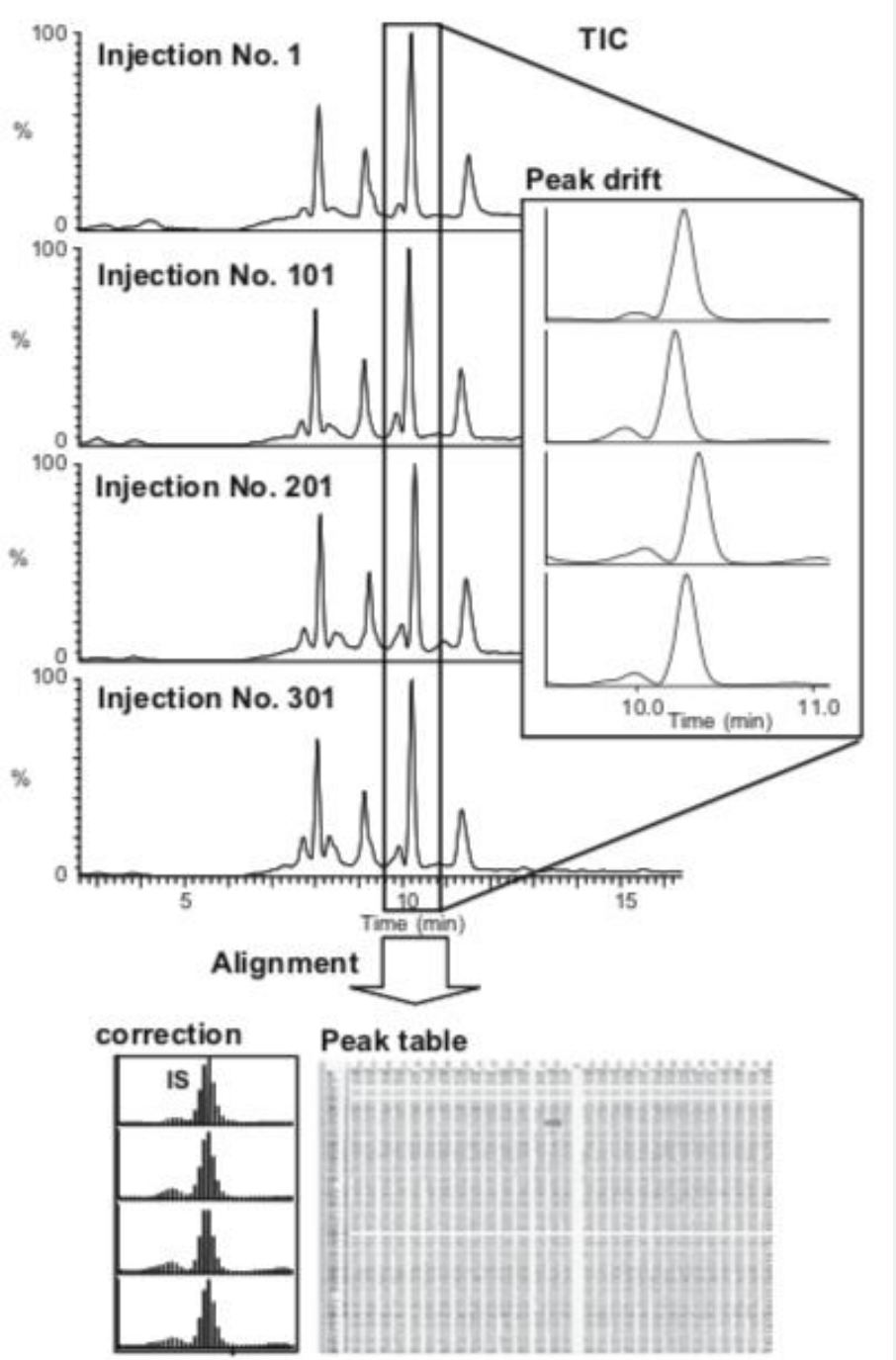

Fig. 1. Schematic overview of peak drift and correction in LC–MS.

Phytochemistry 70 (2009) 450–456

BMC Bioinformatics

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Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry

Tobias Kind and Oliver Fiehn*

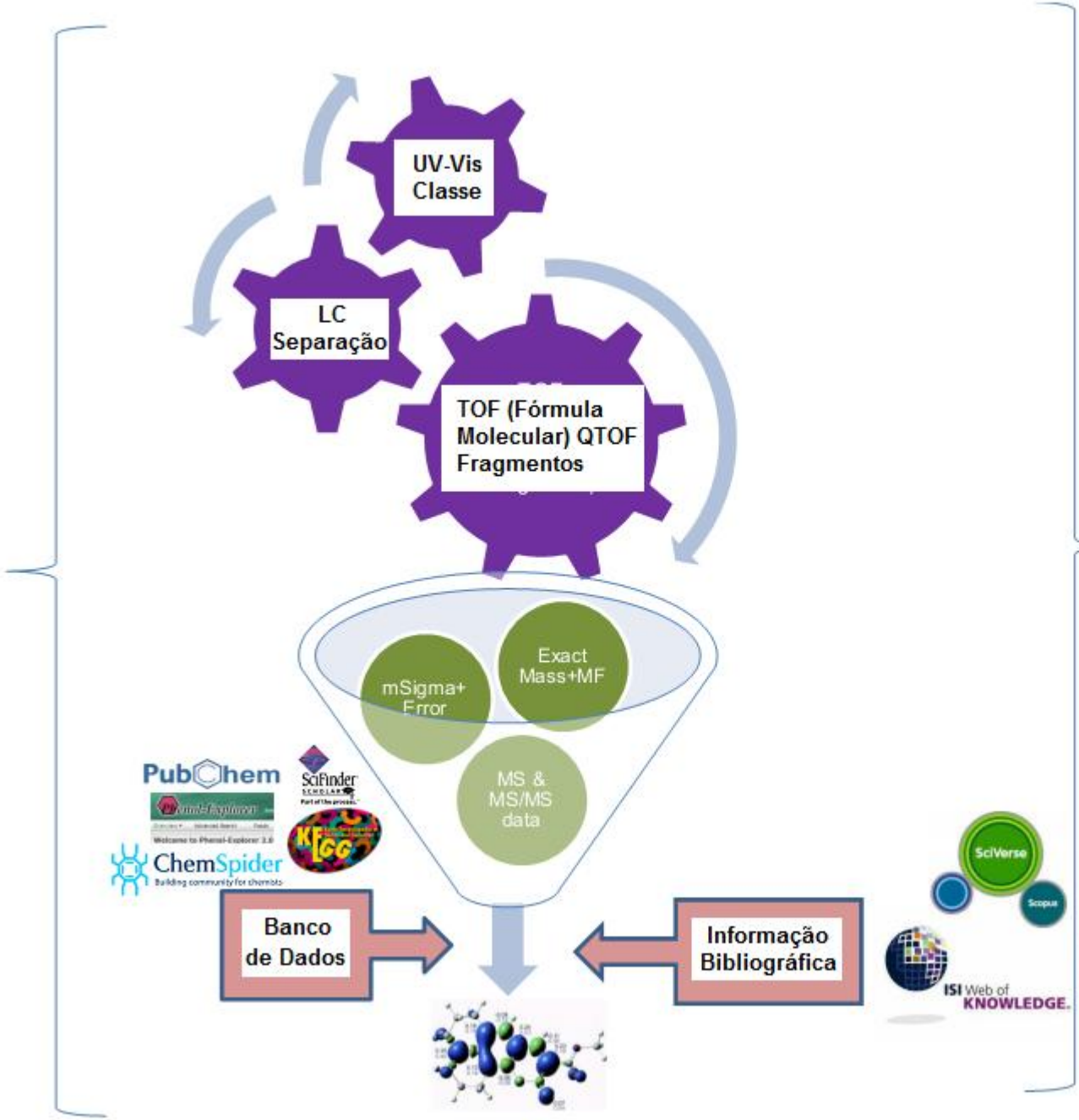
Address: University of California Davis, Genome Center, 451 E. Health Sci. Dr., Davis, CA 95616, USA

Email: Tobias Kind - tkind@ucdavis.edu; Oliver Fiehn* - ofiehn@ucdavis.edu

* Corresponding author

Embrapa

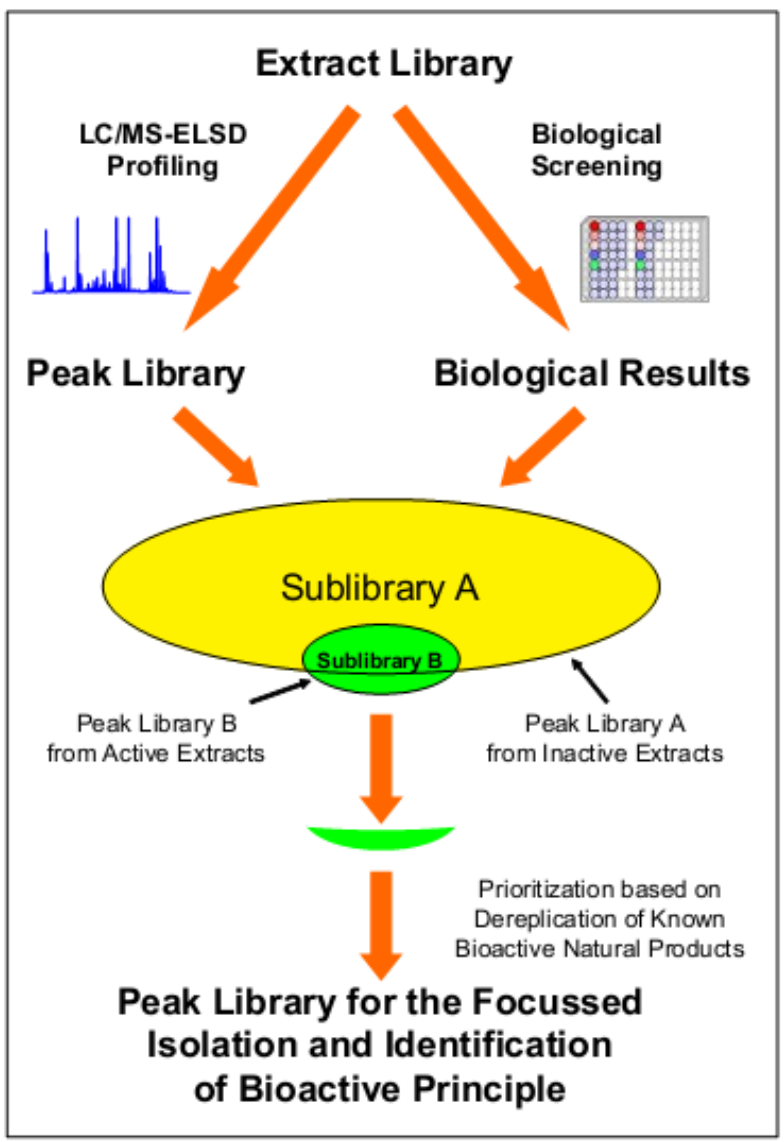
Metodologia de Desreplicação

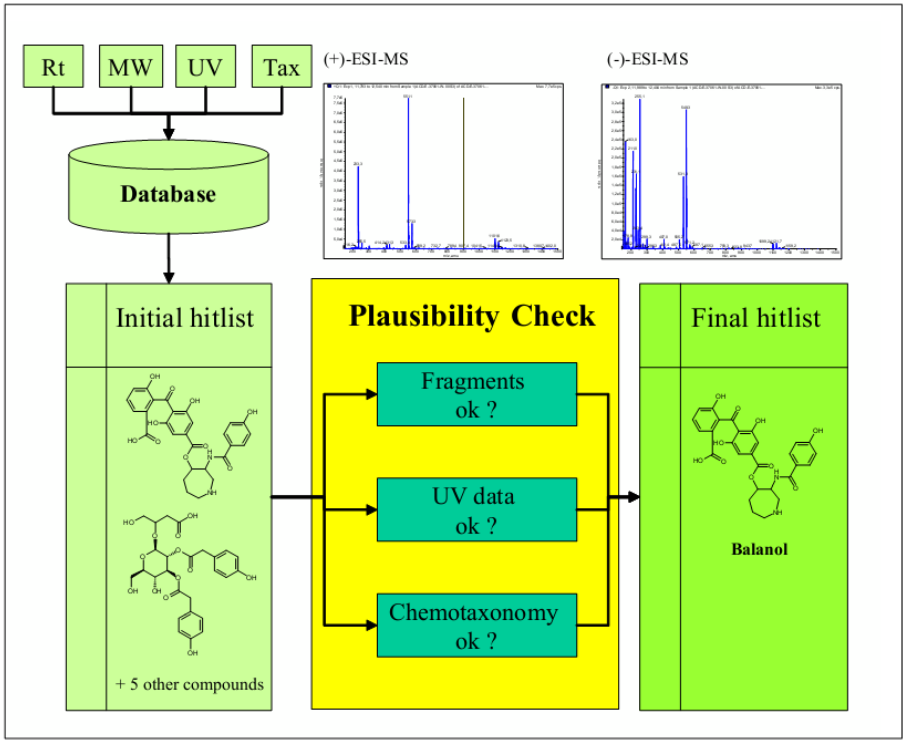


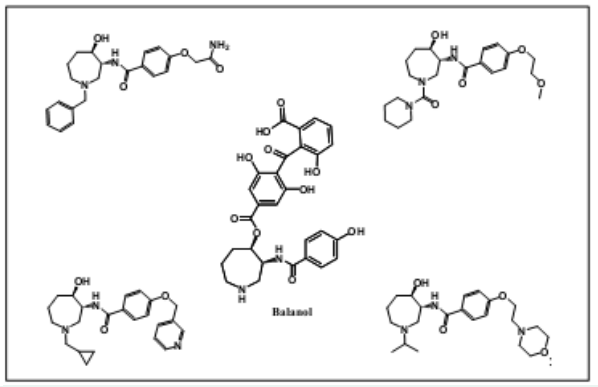
Caracterização

RMN

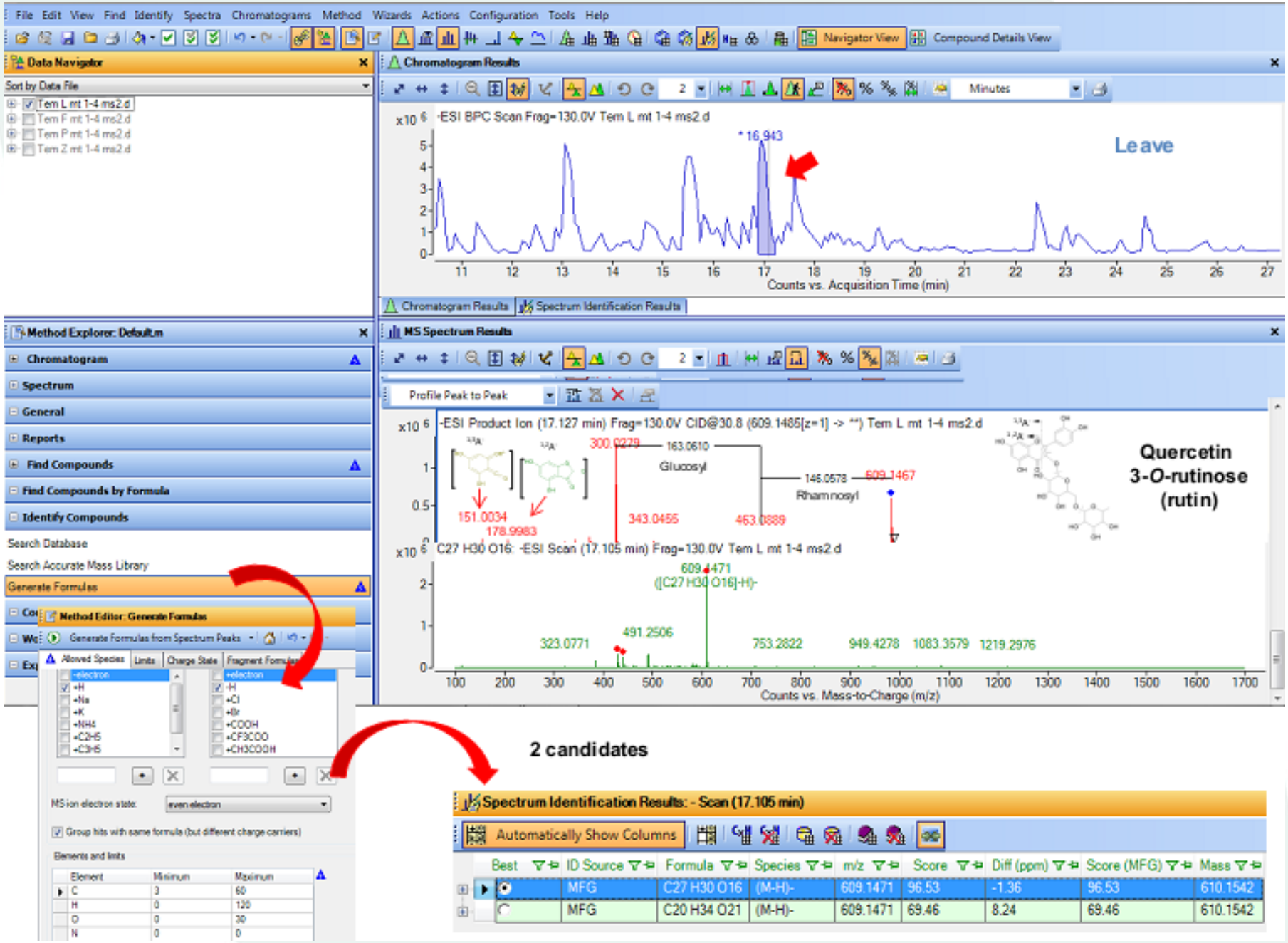
Bases de Dados de Produtos Naturais que podem ser utilizadas para busca de substâncias em meios virtuais







D. Wolf, K. Siems, Burning the Hay to Find the Needle – Data Mining Strategies in Natural Product Dereplication, Chim. Int. J. Chem. 61 (2007) 339–345. doi:10.2533/chimia.2007.339.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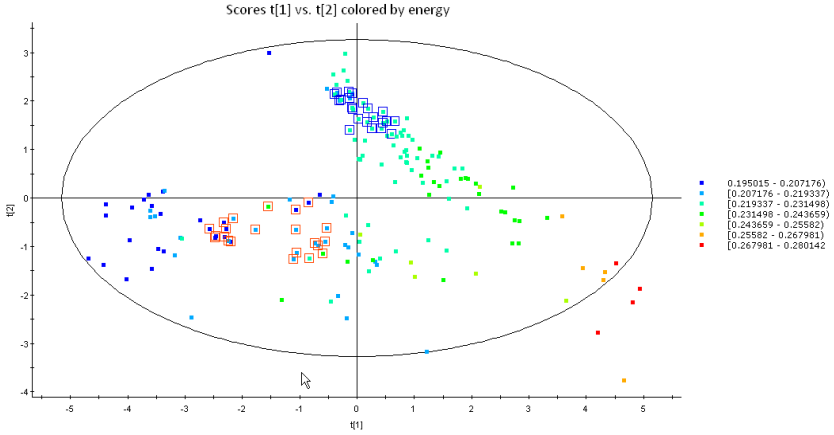
Navigator View
Compound Details View
Data Navigator
Sort by Data File
Tem L mt 1-4 ms2.d
Tem F mt 1-4 ms2.d
Tem P mt 1-4 ms2.d
Tem Z mt 1-4 ms2.d
Chromatogram Results
-ESI BPC Scan Frag=130.0V Tem L mt 1-4 ms2.d
16.943
Leave
Minutes
Counts vs. Acquisition Time (min)
Chromatogram Results
Spectrum Identification Results
Method Explorer: Default.m
Chromatogram
Spectrum
General
Reports
Find Compounds
Find Compounds by Formula
Identify Compounds
Search Database
Search Accurate Mass Library
Generate Formulas
MS Spectrum Results
Profile Peak to Peak
-ESI Product Ion (17.127 min) Frag=130.0V CID@30.8 (609.1485[z=1] -> **) Tem L mt 1-4 ms2.d
300.0229
163.0610
Glucosyl
146.0578
Rhamnosyl
609.1467
151.0034
178.9983
343.0455
463.0889
Quercetin
3-O-rutinose
(rutin)
C27 H30 O16 -ESI Scan (17.105 min) Frag=130.0V Tem L mt 1-4 ms2.d
609.1471
([C27 H30 O16]-H)-
323.0771
491.2506
753.2822
949.4278
1083.3579
1219.2976
Counts vs. Mass-to-Charge (m/z)
Method Editor: Generate Formulas
Generate Formulas from Spectrum Peaks
Allowed Species
Limits
Charge State
Fragment Formulas
MS ion electron state:
even electron
Group hits with same formula (but different charge carriers)
Elements and limits
Element
Minimum
Maximum
C 3 60
H 0 120
O 0 30
N 0 0
2 candidates
Spectrum Identification Results: - Scan (17.105 min)
Automatically Show Columns
Best
ID Source
Formula
Species
m/z
Score
Diff (ppm)
Score (MFG)
Mass
MFG C27 H30 O16 (M-H)- 609.1471 96.53 -1.36 96.53 610.1542
MFG C20 H34 O21 (M-H)- 609.1471 69.46 8.24 69.46 610.1542

Quimiometria

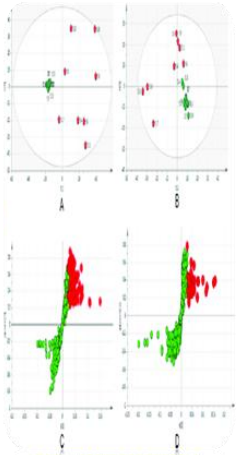
Obs num	Group	v1	v2	V3	V4	V5	V6	V7	V8	V9	V1
1	N	348	323	62	365	20	59	3	11	5	
2	T					12	69	4	8	3	
3	T	143	245	61	26	1	100	1	4	1	
4	N	291	66	45	339	9	56	1	9	1	
5	N	312	435	1	313	84	172	4	33	31	
6	T	208	352	1	117	10	113	1	7	1	
7	T	217	442	51	102	1	28	1	8	1	
8	T	64	207	7	1	8	229	4	1	1	
9	T	368	974	320	321	53	122	1	39	10	
10	T	300	408		370	51	23	2	9	11	
11	T	196	450	308	276	16	45	1	8	2	
12	T	259	331	71	163	2	37	1	6	3	
13	N	219	66	46	282	9	23	3	9	1	
14	N					9	160	2	33	74	
15	N	211	217	42	512	13	18	1	2	1	
16	T	239	521	1	313	20	74	1	33	22	



Coleta e preparo de amostra

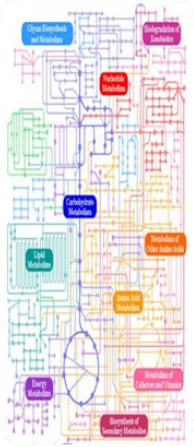
Análise instrumental

Processamento de dados



Análise estatística

Identificação dos metabólitos



Interpretação biológica

Aplicações

Introdução

- **Baraúna: Aspectos Gerais**

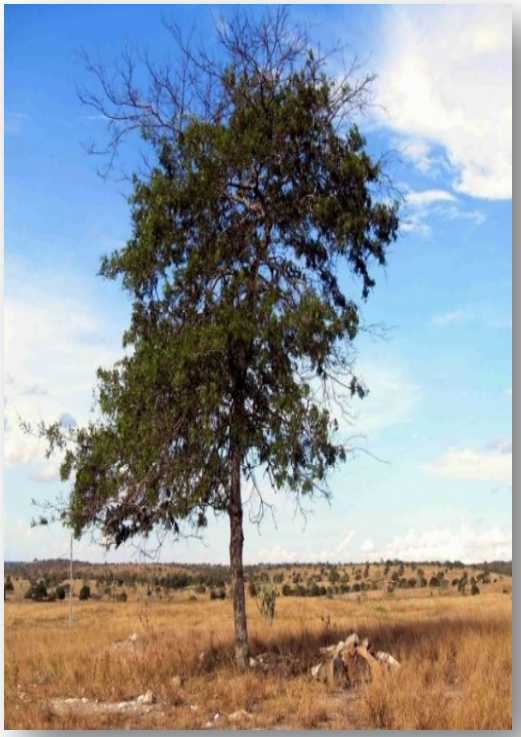
Família Anacardiaceae

braúna, braúna-parda e braúna-do-sertão

A madeira é dura, pesada, de alta resitência e durabilidade

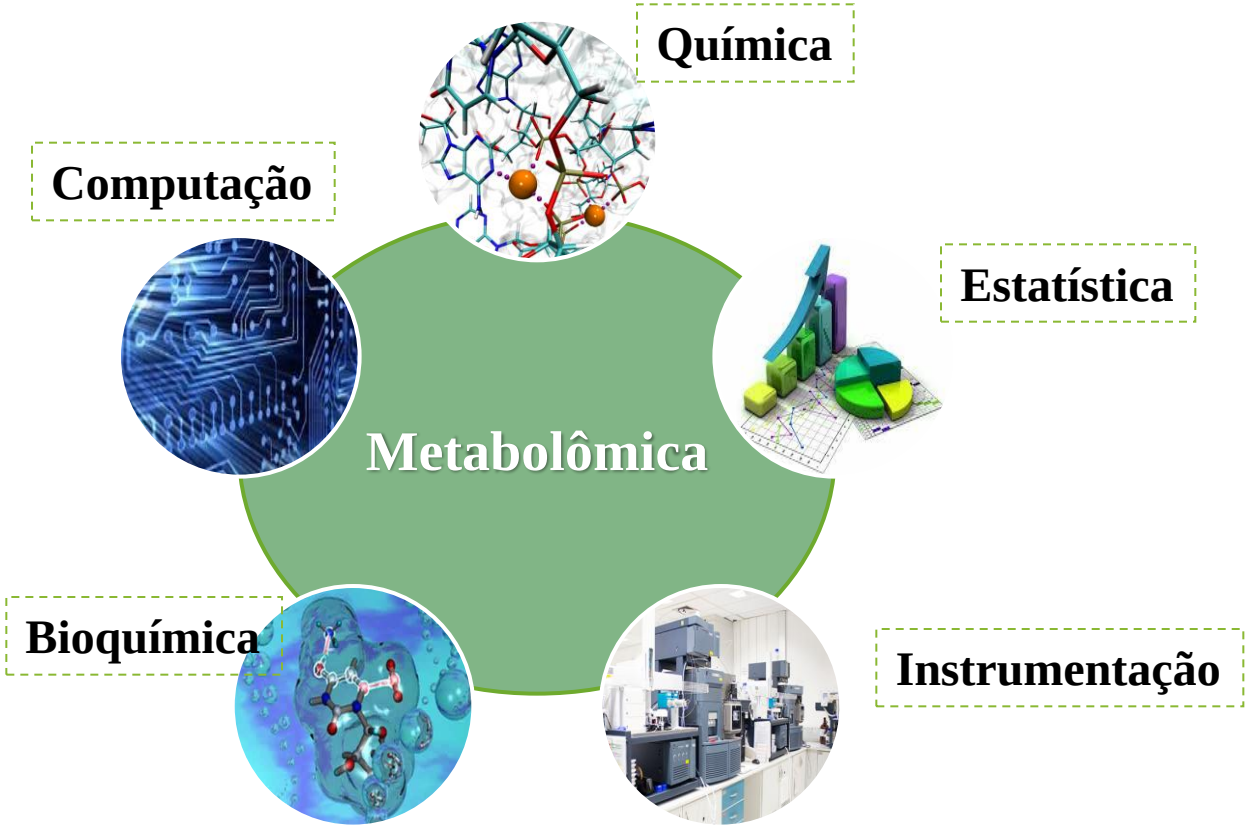
Árvore espinhenta de crescimento lento

Galhos espessos e bem distribuído numa copa de poucas e pequenas folhas



✓ Santos, Raí Vinícius, and Maria Betânia Moreira Amador. "Baraúna: Diálogo entre natureza e sociedade." Periódico Eletrônico Fórum Ambiental da Alta Paulista 9.7 (2013).

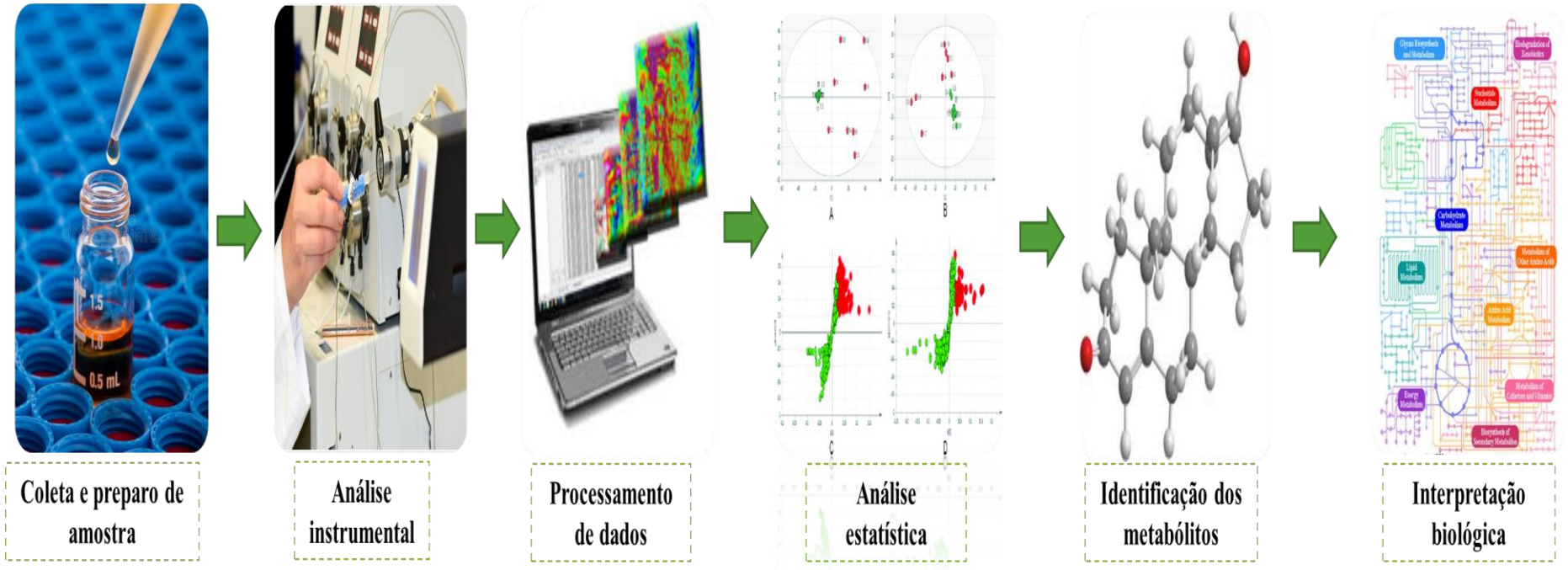
❑ Abordagem metabolômica



✓ FORCISI, S. et al. Liquid chromatography-mass spectrometry in metabolomics research: Mass analyzers in ultra high pressure liquid chromatography coupling. **Journal of Chromatography A**, v. 1292, p. 51–65, 2013.

1. Introdução

- **Abordagem metabolômica**



- ✓ Canuto GAB, Costa JL da, Cruza PLR da, Ana Rosa L. de Souza ATF, Klassen A, Rodrigues KT, Tavares MFM. METABOLÔMICA: DEFINIÇÕES, ESTADO-DA-ARTE E APLICAÇÕES

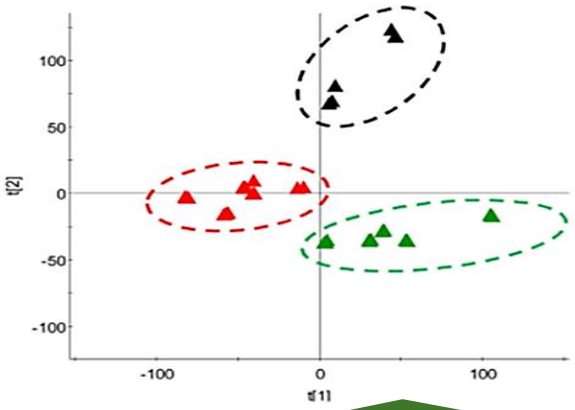
3. Metodologia

- **Análise estatística da atividade citotóxica**

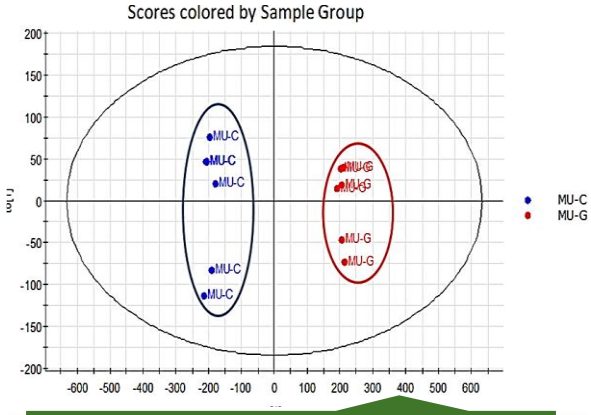
- Foram analisadas a partir da média ± desvio padrão (DP) da porcentagem de inibição do crescimento celular;
- *GraphPad* Prism 5.

3. Metodologia

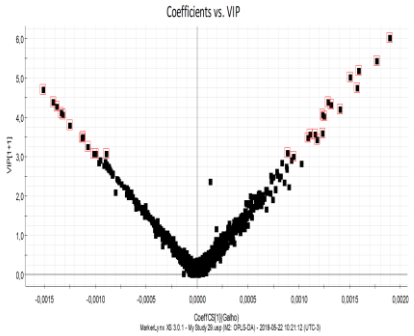
❑ Análise Multivariada dos dados



Análise de Componentes Principal (PCA)

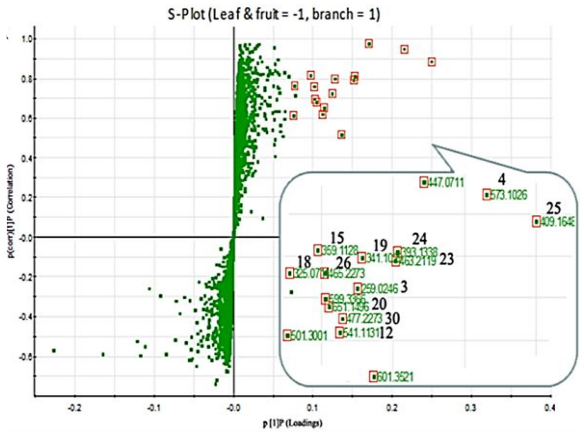


Análise Discriminante Ortogonal dos Mínimos Quadrados Parciais(OPLS-Da)



Variável de Importância na Projeção (VIP)

- Interpretaçãe grande volumes de dados
- (UPLC-QTOF-MS^{E})
- Determinação dos possíveis biomarcadores



S-plot

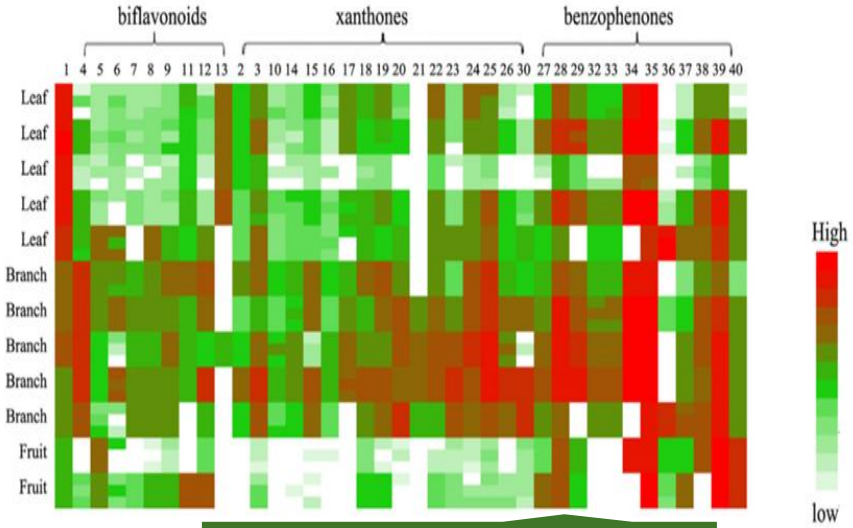
✓ Long, C. lin. (2016). Comparative UPLC-QTOF-MS-based metabolomics and bioactivities analyses of Garcinia oblongifolia. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1011, 179–195.

3. Metodologia

- **Análise Multivariada dos dados**

- Método Hierárquico de Análise de Cluster;

- Contribui para um melhor entendimento da variação dos metabólitos nas diferentes partes da planta;



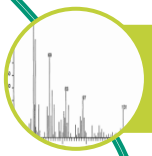
Mapa de Calor (Heat map)

- Software utilizado GENE-E.

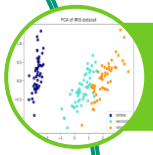
✓ Long, C. lin. (2016). Comparative UPLC-QTOF-MS-based metabolomics and bioactivities analyses of Garcinia oblongifolia. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1011, 179–195.

3. Metodologia

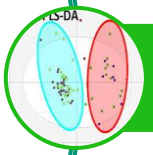
- **Parâmetros de análise quimiométrica**



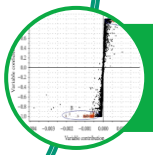
Intervalo de tempo de retenção de 0,70-7,0 min e intervalo de massa de 110-1200 Da no modo ES$^-$



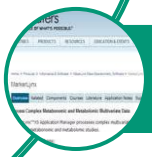
O modelo utilizado na análise de componentes principais (PCA) foi o pareto;



A análise discriminante dos mínimos quadrados ortográficos parciais (OPLS-DA) foi utilizada para validar o modelo de PCA e identificar os metabólitos diferenciais;



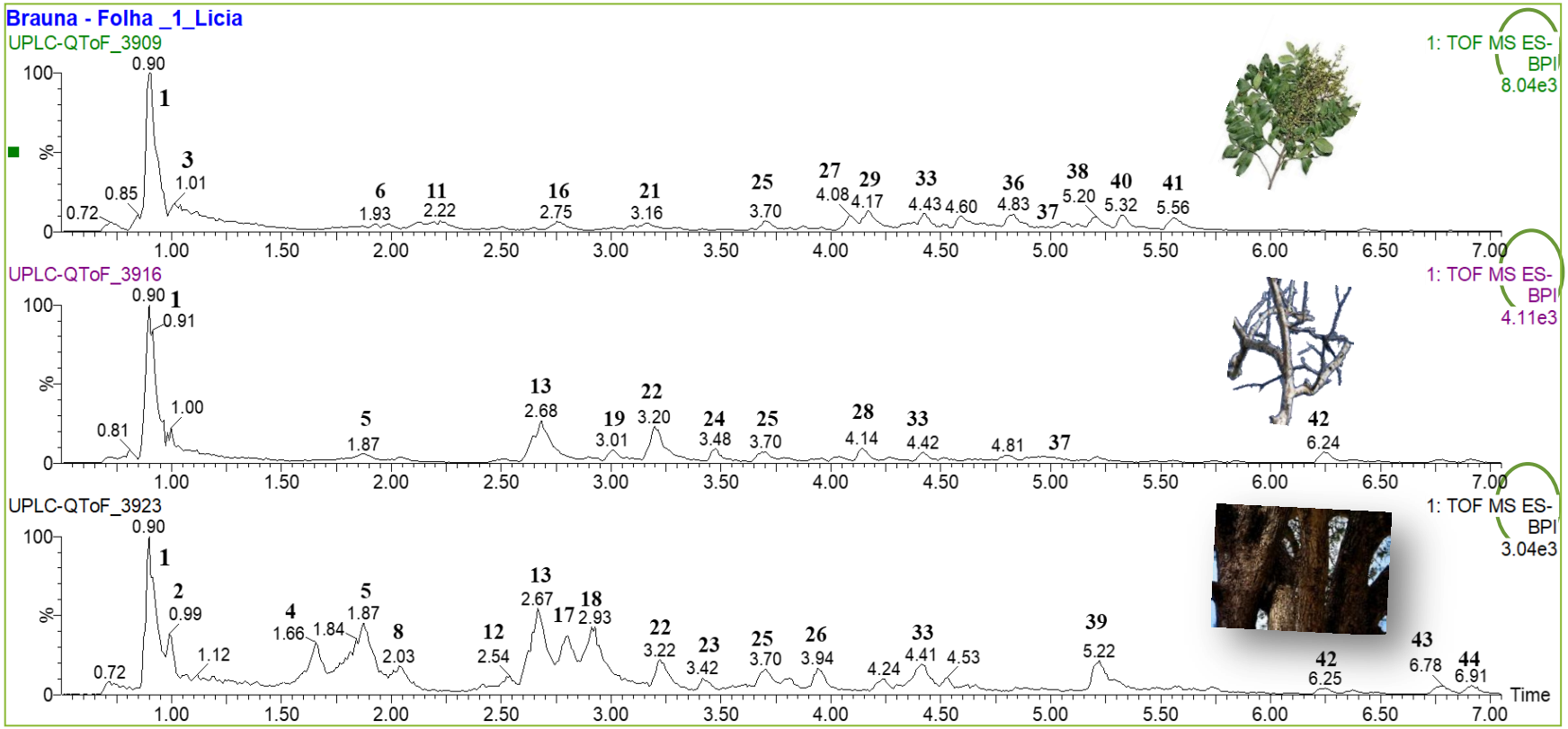
Os biomarcadores foram selecionados pela variável de importância na projeção (VIP) juntamente com o S-Plot.



O Heat map foi utilizado para corroborar com os resultados obtidos de PCA, OPLS-DA, VIP e S-PLOT.

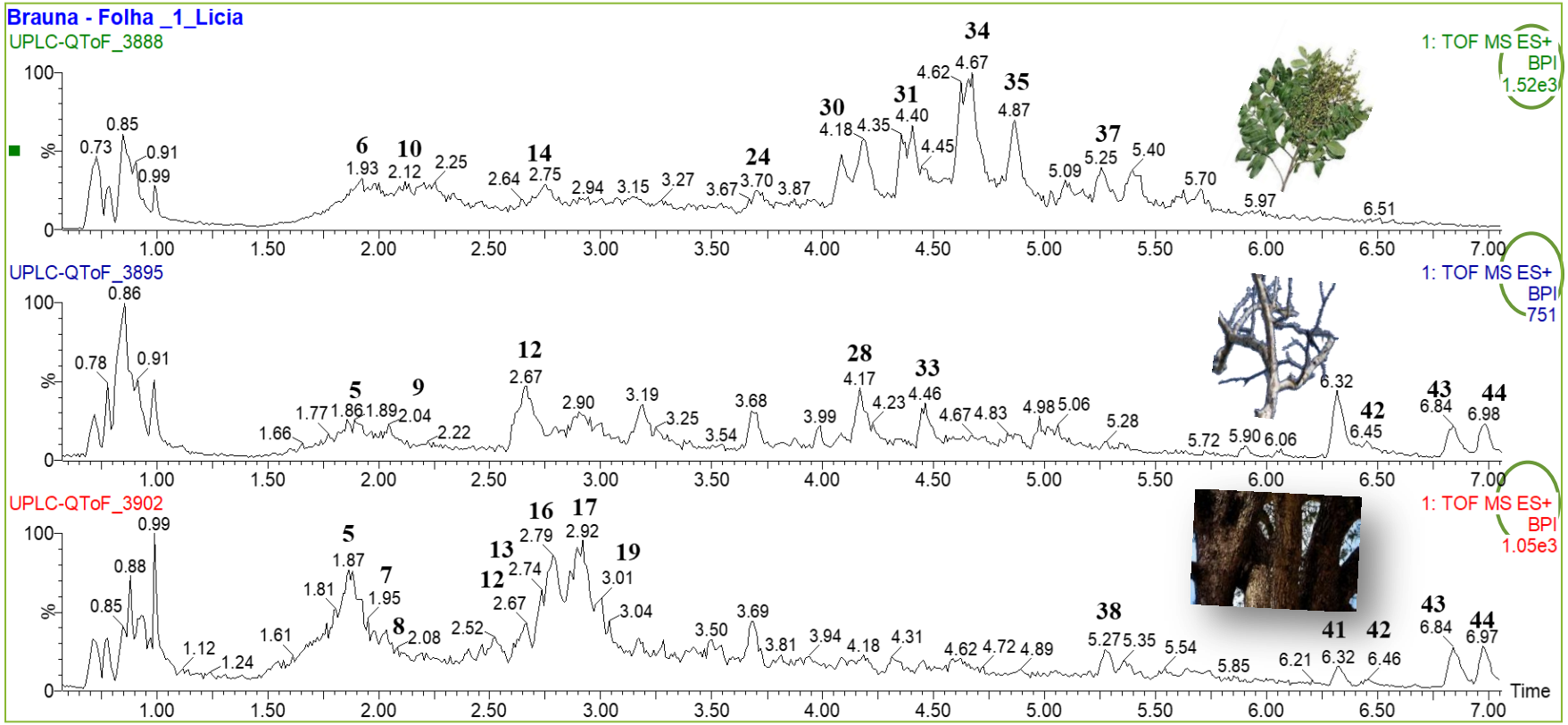
4. Resultados e Discussão

- **Cromatogramas dos extratos de folha, galho e casca (modo ESI$^-$)**



4. Resultados e Discussão

- **Cromatogramas dos extratos de folha, galho e casca (modo ESI$^+$)**



4. Resultados e Discussão

- **Tentativa de identificação dos metabólitos secundários presentes nos extratos de folha, galho e casca**

Picos	t_r (min)	Modo de ionização negativo			Modo de ionização positivo			Fórmula molecular	Tentativa de identificação	*S. brasiliensis* Folha	*S. brasiliensis* Galho	*S. brasiliensis* Casca	Referências
		MS $[M-H]^-$	MS/MS	ppm	MS $[M+H]^+$	MS/MS	ppm						
1	0,90	191,0549	173,0440 127,0401 85,0333	-3,7	-	-	-	$C_7H_{12}O_6$	Ácido quínico[a]	+	+	+	(ABU-REIDAH et al., 2015a; SRIVASTAVA, 2016)
2	0,99	191,0189	133,0167 111,0075	-1,6	-	-	-	$C_6H_8O_7$	Ácido cítrico[a]			+	(OJEWOLE, 2003)
3	1,00	191,0496	133,0151 85,0302	-3,7	-	-	-	$C_{14}H_8O$	n.i	+	+		-
4	1,66	343,0655	191,0497 169,0125 125,0265	-5,2	-	-	-	$C_{14}H_{16}O_{10}$	Ácido quínico galoil isômero I[a]			+	(ABU-REIDAH et al., 2015a; ERSAN et al., 2016)
5	1,87	343,0634	191,0522 169,0082 125,0193	-2,9	345,0817	153,0187 125,0203	-1,4	$C_{14}H_{16}O_{10}$	Ácido quínico galoil isômero II[a]		+	+	(ABU-REIDAH et al., 2015a; ERSAN et al., 2016)
6	1,93	465,1231	271,0438 169,0129 125,0238	-2,8	467,1385	315,0734 153,0202	-3,4	$C_{18}H_{26}O_{14}$	n.i	+			-
7	1,95	-	-	-	345,0824	153,0210	0,6	$C_{14}H_{16}O_{10}$	Ácido quínico galoil isômero III			+	(ABU-REIDAH et al., 2015a; ERSAN et al., 2016)
8	2,03	343,0835	191,0540 169,0121 125,0247	2,3	345,0824	153,0209	0,6	$C_{14}H_{16}O_{10}$	Ácido quínico galoil isômero IV			+	(ABU-REIDAH et al., 2015a; ERSAN et al., 2016)

4. Resultados e Discussão

- **Tentativa de identificação dos metabólitos secundários presente nos extratos de folha, galho e casca**

Picos	t_r (min)	Modo de ionização negativo			Modo de ionização positivo			Fórmula molecular	Tentativa de identificação	*S. brasiliensis* Folha	*S. brasiliensis* Galho	*S. brasiliensis* Casca	Referências
		MS $[M-H]^-$	MS/MS	ppm	MS $[M+H]^+$	MS/MS	ppm						
9	2,04	-	-	-	315,0710	153,0193 125,0159	-1,9	$C_{13}H_{14}O_9$	Levoglucosan galato isômero I[a]	+			(ABU-REIDAH et al., 2015a)
10	2,12	-	-	-	315,0726	153,0215 125,0114	3,2	$C_{13}H_{14}O_9$	Levoglucosan galato isômero II[a]	+			(ABU-REIDAH et al., 2015a)
11	2,22	449,1292	271,0503 169,0157 125,0159	-0,7	-	-	-	$C_{18}H_{26}O_{13}$	n.i	+			-
12	2,54	495,0789	343,0688 191,0524 169,0148	2,8	-	-	-	$C_{21}H_{20}O_{14}$	Ácido quínico digaloil isômero I			+	(FRAIGE et al., 2017; SANTOS et al., 2017)
13	2,65	353,0856	191,0531 179,0337 135,0439	-4,8	355,1042	163,0403 89,0397	3,7	$C_{16}H_{18}O_9$	Ácido clorogênico[a]		+	+	(HASSAN et al., 2011; WILLEMS et al., 2016)
14	2,73	-	-	-	497,0946	479,0812 309,0629 153,0195	3,0	$C_{21}H_{20}O_{14}$	Ácido quínico digaloil isômero II			+	(FRAIGE et al., 2017; SANTOS et al., 2017)
15	2,74	-	-	-	315,0729	153,0209 125,0215	4,1	$C_{13}H_{14}O_9$	Levoglucosan galato isômero III	+			(ABU-REIDAH et al., 2015a)
16	2,75	389,1086	271,0392 169,0164 125,0276	0,5	-	-	-	$C_{16}H_{22}O_{11}$	n.i	+			-

4. Resultados e Discussão

- **Tentativa de identificação dos metabólitos secundários presente nos extratos de folha, galho e casca**

Picos	t_r (min)	Modo de ionização negativo			Modo de ionização positivo			Fórmula molecular	Tentativa de identificação	*S. brasiliensis* Folha	*S. brasiliensis* Galho	*S. brasiliensis* Casca	Referências
		MS $[M-H]^-$	MS/MS	ppm	MS $[M+H]^+$	MS/MS	ppm						
17	2,78	495,0750	343,0645 191,0562 169,0143	-5,0	497,0954	479,085630 9,0629 153,0203	4,6	$C_{21}H_{20}O_{14}$	Ácido quínico digaloil isômero III			+	(FRAIGE et al., 2017; SANTOS et al., 2017)
18	2,93	495,0759	343,0643 191,0616 169,0119	-3,2	497,0956	479,0872 309,0630 153,0201	5,0	$C_{21}H_{20}O_{14}$	Ácido quínico digaloil isômero IV			+	(FRAIGE et al., 2017; SANTOS et al., 2017)
19	3,01	359,0991	169,0140	3,6	-	-	-	$C_{15}H_{20}O_{10}$	etil-*O*-β-D-(6'-*O*-galoil)-glucopiranosídeo		+		(SANTOS et al., 2017)
20	3,01	-	-	-	497,0926	479,0812 309,0629 153,0195	-1,0	$C_{21}H_{20}O_{14}$	Ácido quínico digaloil isômero IV			+	(FRAIGE et al., 2017; SANTOS et al., 2017)
21	3,16	321,0239	169,0121 125,0238	-2,5	-	-	-	$C_{14}H_{10}O_9$	Ácido digálico[a]	+			(ABU-REIDAH et al., 2015a)
22	3,21	453,1016	313,0551 179,0317 169,0128	-3,8	-	-	-	$C_{20}H_{22}O_{12}$	2-hidroxi-4-metoxifenol 1-*O*-β-D-(6'-*O*-galoil) glucopiranosídeo		+	+	(SANTOS et al., 2017)
23	3,48	633,0762	463,0793 300,9986 169,0133	5,4	-	-	-	$C_{27}H_{22}O_{18}$	Corilagina[a]		+		(SRIVASTAVA, 2016) Padrão analítico

4. Resultados e Discussão

- **Tentativa de identificação dos metabólitos secundários presente nos extratos de folha, galho e casca**

Picos	t_r (min)	Modo de ionização negativo			Modo de ionização positivo			Fórmula molecular	Tentativa de identificação	*S. brasiliensis* Folha	*S. brasiliensis* Galho	*S. brasiliensis* Casca	Referências
		MS $[M-H]^-$	MS/MS	ppm	MS $[M+H]^+$	MS/MS	ppm						
24	3,69	635,0938	457,0819 169,0127 125,0114	-0,8	-	-	-	$C_{20}H_{28}O_{23}$	n.i			+	-
25	3,70	631,0961	479,0884 169,0130 125,0231	4,1	633,1121	319,0495 153,0235	4,6	$C_{28}H_{24}O_{17}$	n.i	+	+		-
26	3,94	923,1716	481,1007 169,0107 125,0255	4,9	-	-	-	$C_{46}H_{36}O_{21}$	n.i			+	-
27	4,08	787,1006	635,0993 617,1226 465,0739 169,0145	1,5	-	-	-	$C_{34}H_{28}O_{22}$	tetra-*O*-galoil-glucose isômero I[a]	+			(BERARDINI; CARLE; SCHIEBER, 2004)
28	4,14	787,1068	615,0982 169,0111 125,0196	-2,5	-	-	-	$C_{45}H_{24}O_{14}$	n.i		+		-
29	4,16	787,1042	635,0993 169,0068 617,1226	6,0	-	-	-	$C_{34}H_{28}O_{22}$	tetra-*O*-galoyl-glucose isômero II[a]	+			(BERARDINI; CARLE; SCHIEBER, 2004)
30	4,17	-	-	-	467,0858	153,0191	6,9	$C_{20}H_{18}O_{13}$	*O*-galoilnorbergenina[a]		+		(ABU-REIDAH et al., 2015a)
31	4,18	615,0983	-	-0,5	617,1168	303,0540 153,0210	4,1	$C_{28}H_{24}O_{22}$	Miricitrina *O*-galato isômero I[a]	+			(ABU-REIDAH et al., 2015a)

4. Resultados e Discussão

- **Tentativa de identificação dos metabólitos secundários presente nos extratos de folha, galho e casca**

Picos	t_r (min)	Modo de ionização negativo			Modo de ionização positivo			Fórmula molecular	Tentativa de identificação	*S. brasiliensis* Folha	*S. brasiliensis* Galho	*S. brasiliensis* Casca	Referências
		MS $[M-H]^-$	MS/MS	ppm	MS $[M+H]^+$	MS/MS	ppm						
32	4,40	-	-	-	617,1122	303,0524	-3,4	$C_{28}H_{24}O_{22}$	Miricitrina *O*-galato isômero II[a]	+			(ABU-REIDAH et al., 2015a)
33	4,41	939,1135	787,1022 769,0886 617,0902 467,0977	3,3	-	-	-	$C_{41}H_{32}O_{26}$	Penta-*O*-galoil-β-D[a]	+	+	+	(ERSAN et al., 2016)
34	4,46	-	-	-	771,1117	153,0194	9,3	$C_{34}H_{26}O_{21}$	di-*O*-galoil-2,3-(S)-hexahidroxidifenol-silo-quercitol[a]		+		(ABU-REIDAH et al., 2015a)
35	4,67	463,0877	301,0325		465,1042	303,0537	1,9	$C_{21}H_{20}O_{12}$	Quercetina 3-O-hexosídeo[a]	+			(BERARDINI; CARLE; SCHIEBER, 2004)
36	4,83	615,0992	463,0869 301,0328 169,0112	1,0	617,1132	303,0486	-1,8	$C_{28}H_{24}O_{16}$	Quercetina-3-O-(6''-O-galoil)-β-hexosídeo	+			(SALDANHA; VILEGAS; DOKKEDAL, 2013)
37	5,05	1091,1239	939,1112 769,1029 393,2011 433,0771	2,4	-	-	-	$C_{48}H_{36}O_{30}$	Hexagaloil-hexosídeo[a]	+	+		(ABU-REIDAH et al., 2015a)
38	5,20	585,0894	469,2008 301,0325 169,0166	2,4	587,1027	457,0462 303,0493 153,0224	-1,7	$C_{27}H_{22}O_{15}$	Quercetina galoil pentosídeo[a]	+			(ERSAN et al., 2016)

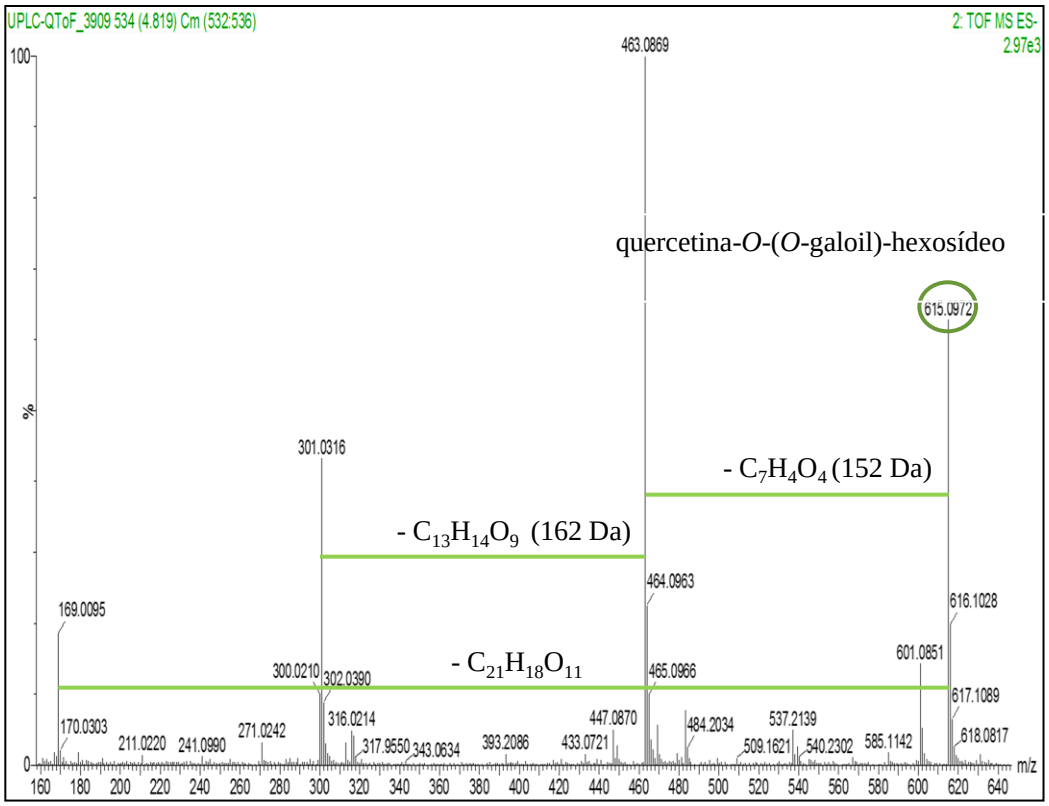
4. Resultados e Discussão

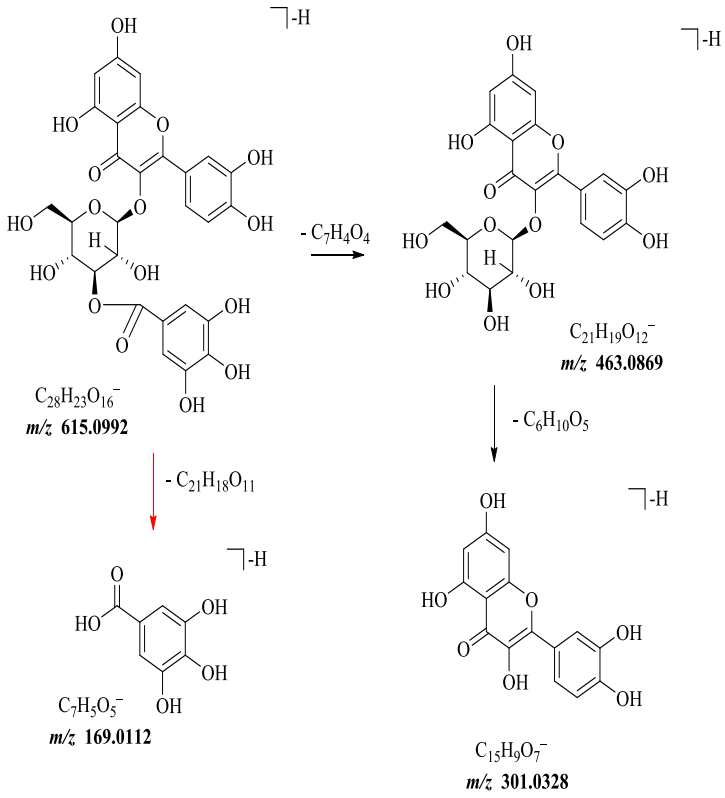
- **Tentativa de identificação dos metabólitos secundários presente nos extratos de folha, galho e casca**

Picos	t_r (min)	Modo de ionização negativo			Modo de ionização positivo			Fórmula molecular	Tentativa de identificação	*S. brasiliensis* Folha	*S. brasiliensis* Galho	*S. brasiliensis* Casca	Referências
		MS $[M-H]^-$	MS/MS	ppm	MS $[M+H]^+$	MS/MS	ppm						
39	5,22	619,1095	449,0901 169,0164 125,0459	1,1	621,1203	451,1079 301,0754 153,0187	-6,6	$C_{31}H_{24}O_{14}$	n.i			+	-
40	5,32	523,2187	469,2070 169,0137 125,0575	1,5	-	-	-	$C_{26}H_{36}O_{11}$	n.i	+			-
41	5,55	349,0580	198,0759 197,0420 169,0096 124,0149	5,7	-	-	-	$C_{16}H_{14}O_9$	Etil 2,4-dihidroxi-3-(3,4,5-trihidroxibenzoil) oxibenzoato	+			(DORTA et al., 2014)
42	6,24	525,1210	389,1068 371,1021 169,0185 135,0057	4,6	527,1376	417,1063 137,0260	6,4	$C_{30}H_{22}O_9$	Urundeuvina A isômero I[a]		+	+	(VIANA; BANDEIRA; MATOS, 2003) Padrão analítico
43	6,78	525,1205	389,1053 371,0912 169,0130 135,0101	3,6	527,1379	417,1101 137,0269	9,3	$C_{30}H_{22}O_9$	Urundeuvina A isômero II[a]		+	+	Padrão analítico
44	6,92	523,1061	387,0897 135,0090	6,1	525,1228	415,0859 389,1020 137,0258	8,0	$C_{30}H_{20}O_9$	n.i			+	-

4. Resultados e Discussão

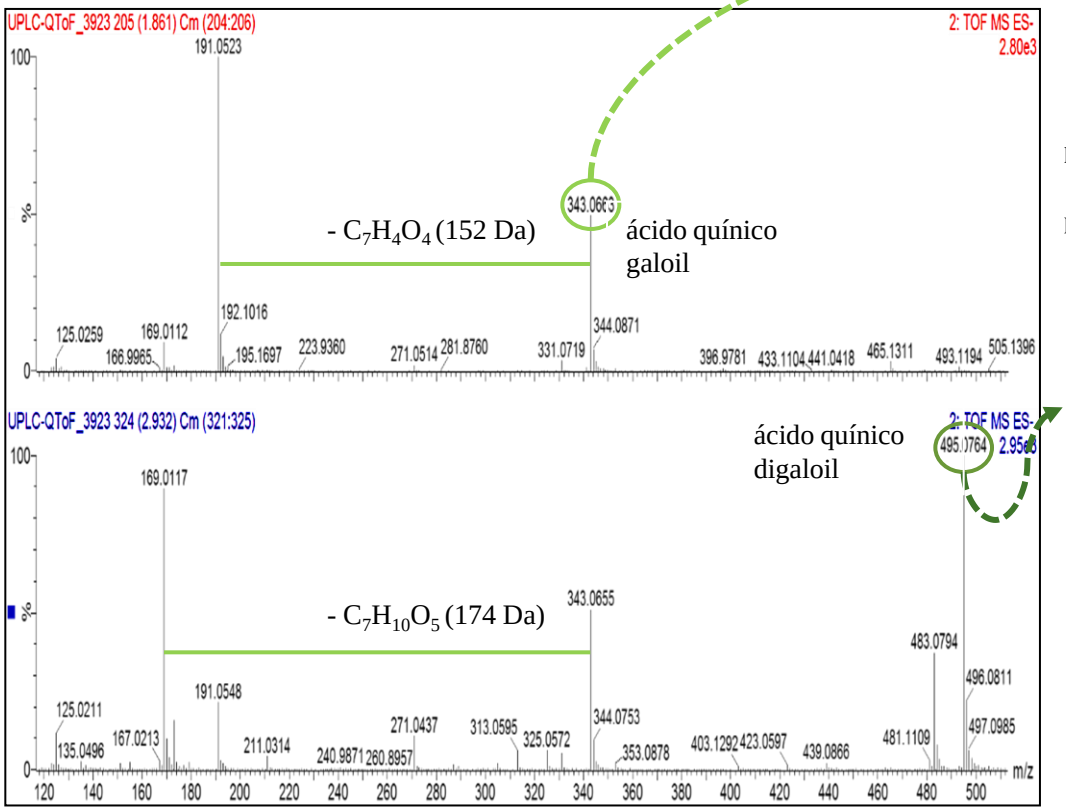
- **Espectros de massas**

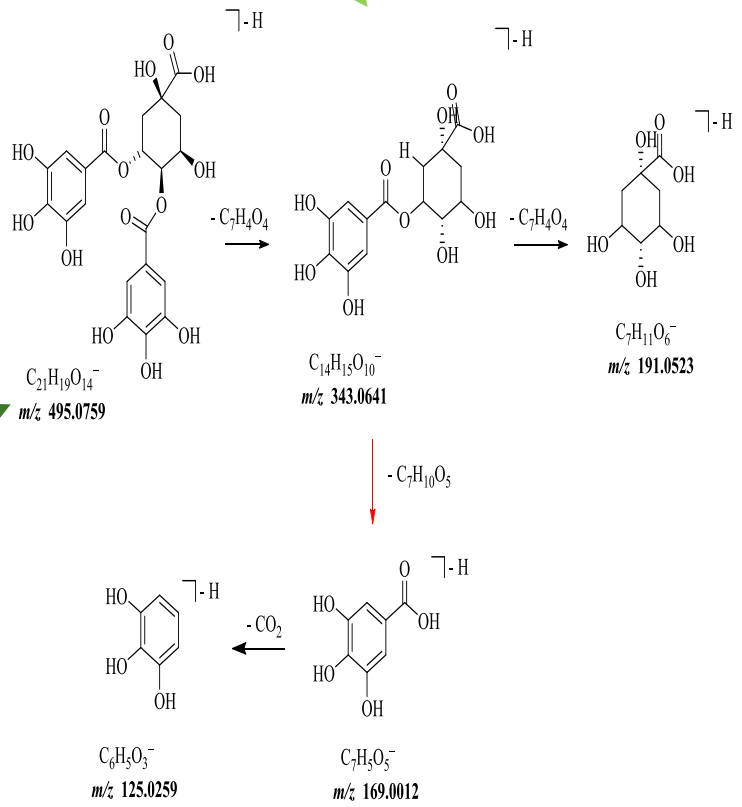




4. Resultados e Discussão

- **Espectros de massas**





4. Resultados e Discussão

Atividade Citotóxica

- Percentual de inibição da proliferação celular (%) de extratos de *S.brasiliensis* em concentração de 100 µg/mL.

Inibição da proliferação celular (%)										
Extrato	**HL60**	**DP%**	**HCT-116**	**DP%**	**PC3**	**DP%**	**SF-295**	**DP%**	**RAJI**	**DP%**
Galho	**72,17**	8,44	0	-	16,37	2,36	34,04	7,63	4,97	1,87
Folha	**88,68**	1,35	**77,55**	2,46	39,22	3,49	**74,25**	4,46	53,64	14,47
Casca	**83,16**	0,24	57,80	5,21	33,49	1,33	**73,94**	3,17	17,34	7,47

4. Resultados e Discussão

❑ Atividade Citotóxica

- Atividade citotóxica *in vitro* dos extratos de folha e casca em células tumorais e não tumoral.

	CI_{50} µg/mL (Intervalo)*						
Amostra	**SF-295** (Glioblastoma)	**PC3** (Próstata)	**HL60** (Leucemia)	**RAJI** (Colorretal)	**HCT-116** (Colorretal)	**SW-620** (Colorretal)	**L929** (Fibroblasto murino)
Casca	**>100**	**>100**	**58,75** (52,98 - 65,13)	**>100**	**93,64** (85,06 - 103,1)	**25,68** (16,25 - 40,57)	**82,0** (51,43 - 131)
Folha	**78,57** (52,92 – 116,6)	**71,54** (33,95 – 150,7)	**52,58** (47,19 - 58,59)	**55,90** (34,93 - 89,45)	**61,73** (53,60 - 71,10)	**65,46** (55,11 - 77,75)	**49,53** (33,62 - 72,97)
Doxorrubicina[a]	**0,25** (0,22-0,28)	**0,44** (0,34-0,54)	**0,01** (0,005-0,01)	**0,46** 0,45-0,47	**0,11** (0,08-0,14)	**0,03** (0,02-0,05)	**0,99** (0,92-1,08)

* Valores de CI_{50} com um intervalo de confiança de 95% obtido por regressão não-linear a partir de três experimentos independentes, realizados em duplicata em seis linhagens tumorais e uma linhagem não tumoral.

[a]Doxorrubicina foi usada como controle positivo

4. Resultados e Discussão

Atividade Citotóxica

- Valores Índice de seletividade IC_{50} (células não tumorais)/IC_{50}(célula tumoral) nas linhagens tumorais.

Índice de seletividade IC_{50} (células não tumorais)/IC_{50}(célula tumoral)

	SF-295 (Glioblastoma)	PC3 (Próstata)	HL60 (Leucemia)	RAJI (Colorretal)	HCT-116 (Colorretal)	SW-620 (Colorretal)
Casca	ND	ND	1,4	ND	0,9	**3,2**
Folha	0,6	0,7	0,9	0,9	0,8	0,8

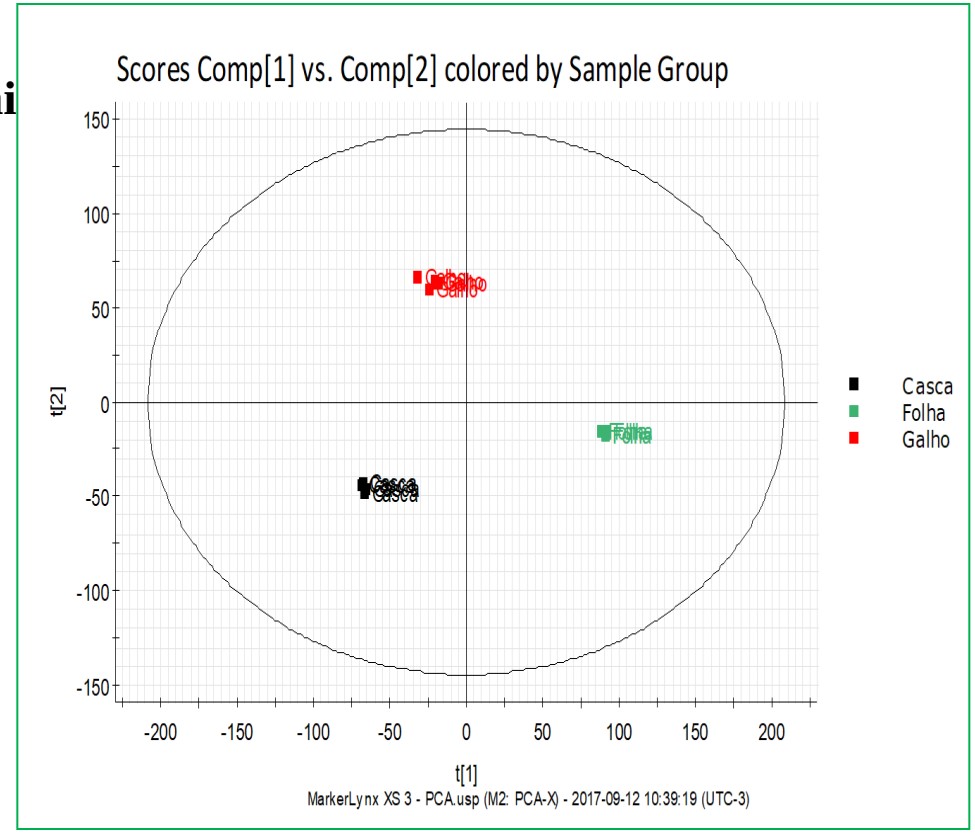
4. Resultados e Discussão

❑ Análise Quimiométrica

- **Análise de Componentes Principai**

- Amostras foram claramente separadas e agrupadas em três grupos;

- O gráfico de PCA-X representou 77,73% da variância total (poder de discriminação das amostras) (R2X[1] = 0.5256 e R2X[2] = 0.2517).

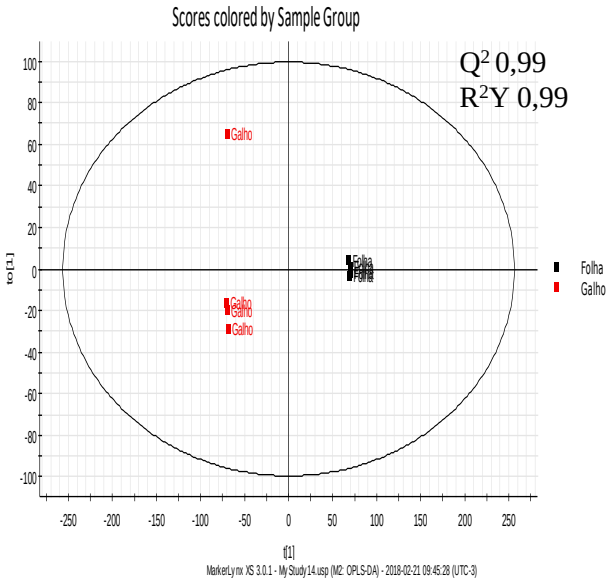


4. Resultados e Discussão

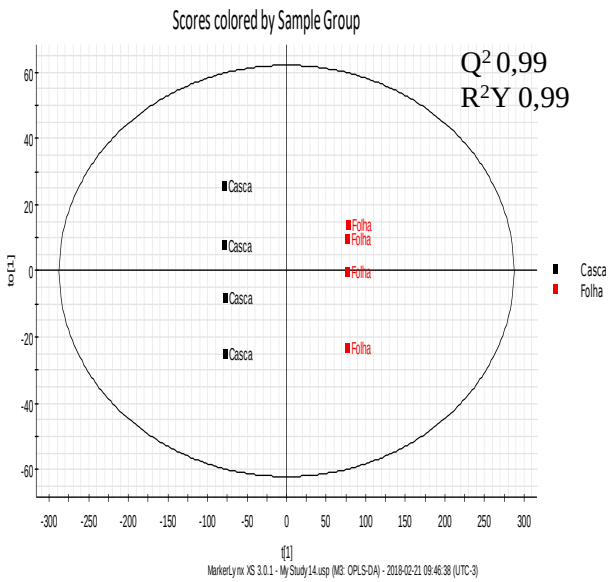
Análise Quimiométrica

- **Análise Discriminante Ortogonal dos Mínimos Quadrados Parciais (OPLS-Da)**

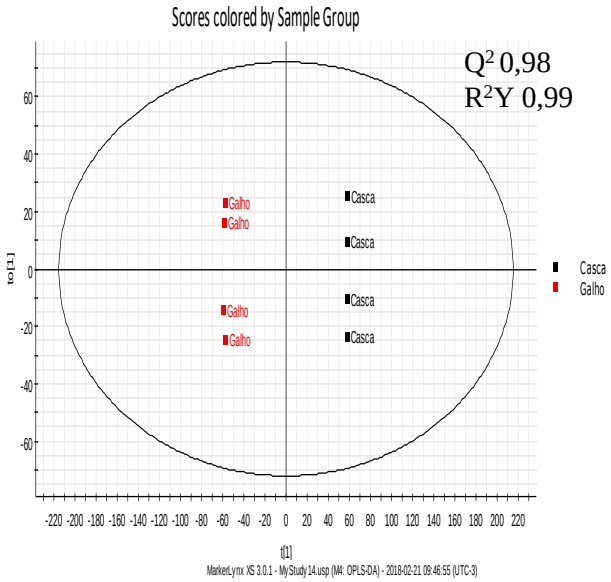
Galho vs Folha



Casca vs Folha



Galho vs Casca

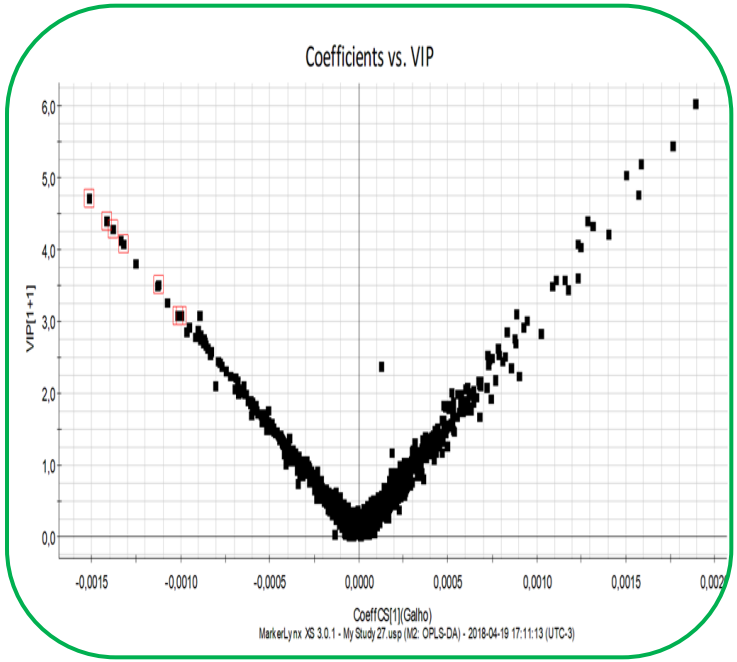


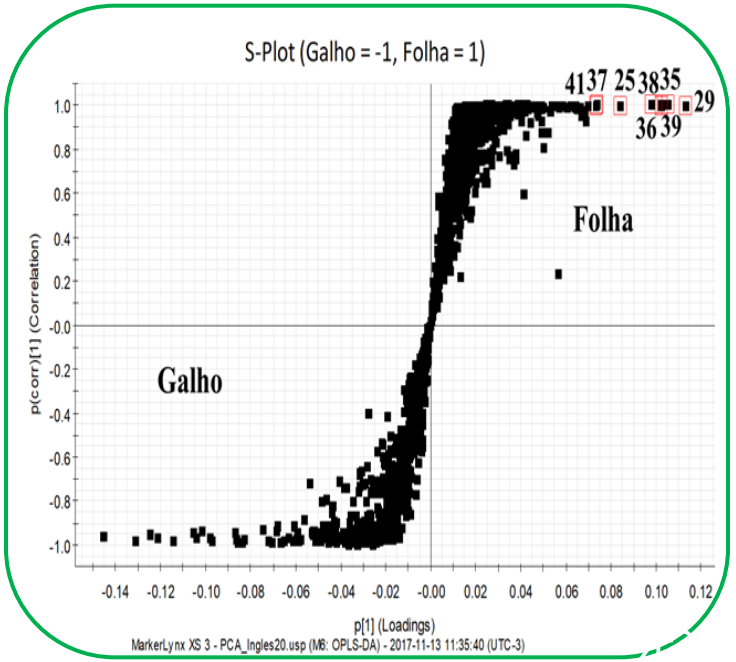
4. Resultados e Discussão

❑ Análise Quimiométrica

- **Potenciais Biomarcadores**

Galho vs **Folha**

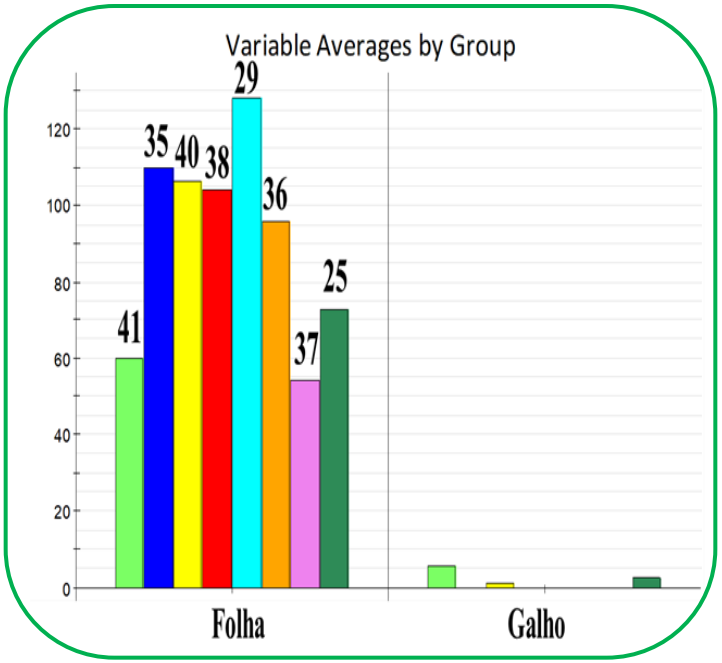




4. Resultados e Discussão

Análise Quimiométrica

- **Potenciais Biomarcadores**



25. n.i

29. tetra-O-galoil-glucose isômero I

35. quercetina-O-hexosídeo

36. quercetina-O-(O-galoil)-hexosídeo

37. hexagaloil hexosídeo

38. quercetina galoil pentosídeo

40. n.i

41. etil 2,4-diidroxi-3-(3,4,5-triidroxibenzoil)oxibenzoato

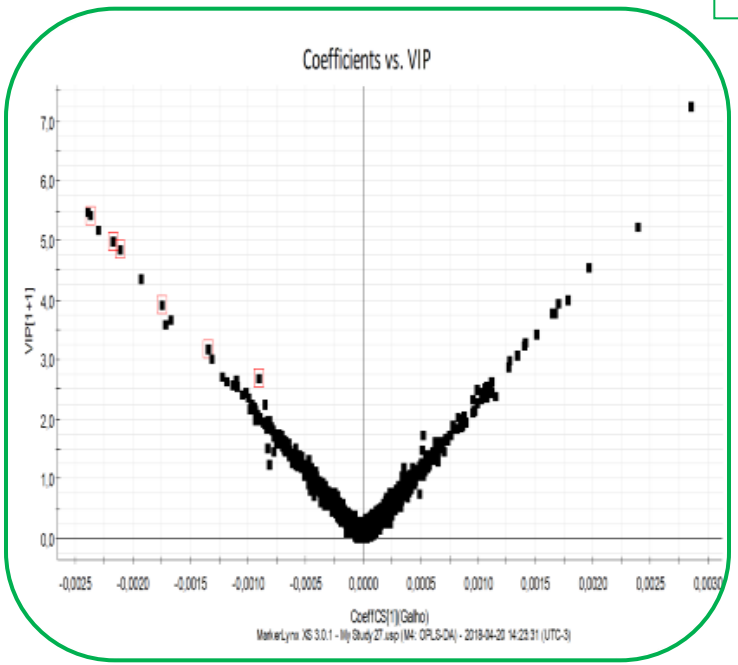
- ✓ A quercetina e seus derivados possuem propriedades antioxidante, anti-histamínico e antiinflamatória (KAHRAMAN et al., 2003; LESJAK et al., 2018; MAMANI-MATSUDA et al., 2006).

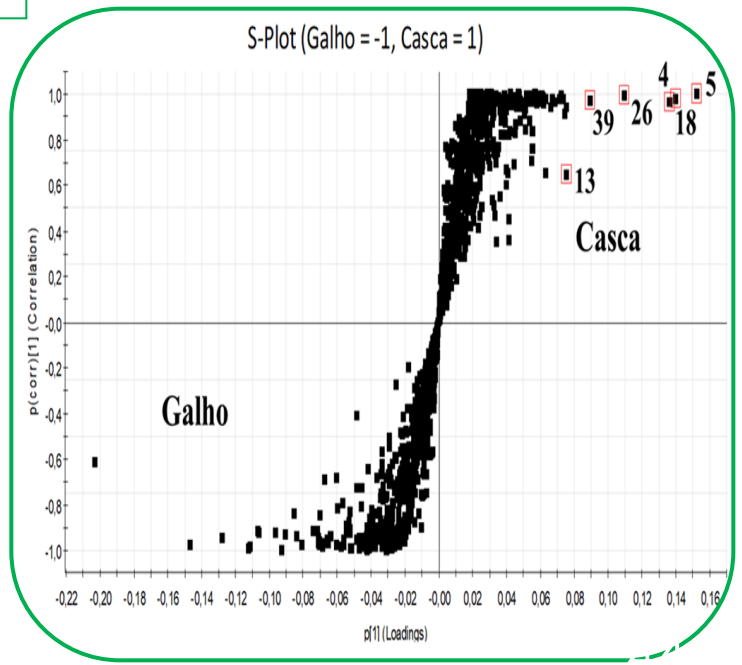
4. Resultados e Discussão

❑ Análise Quimiométrica

- **Potenciais Biomarcadores**

Galho vs **Casca**

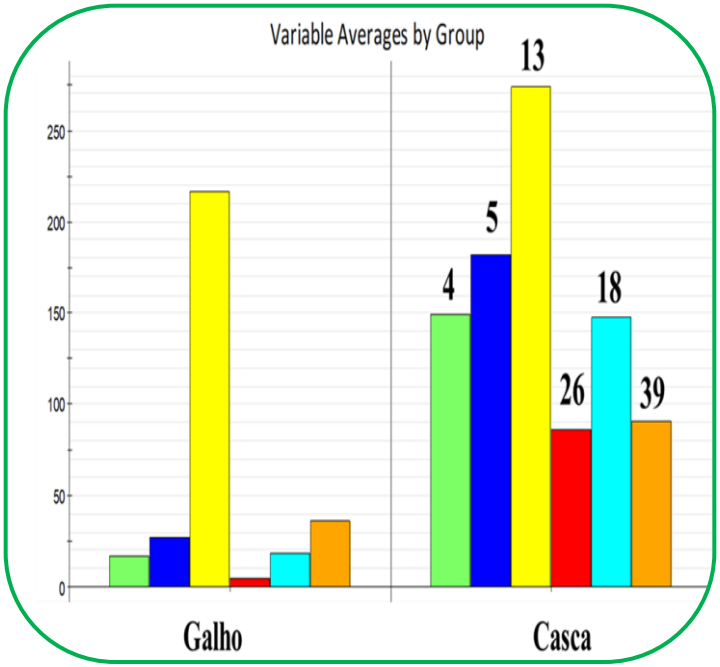




4. Resultados e Discussão

❑ Análise Quimiométrica

- **Potenciais Biomarcadores**



4. ácido quínico galoil isômero I

5. ácido quínico galoil isômero II

13. ácido clorogênico

18. ácido quínico digaloil isômero

26. n.i

39. n.i

- ✓ Derivados do ácido galoil quínico possuem considerável atividade antioxidante. Quanto maior o número de unidades de galoil, maior a atividade observada (BARATTO et al., 2003).

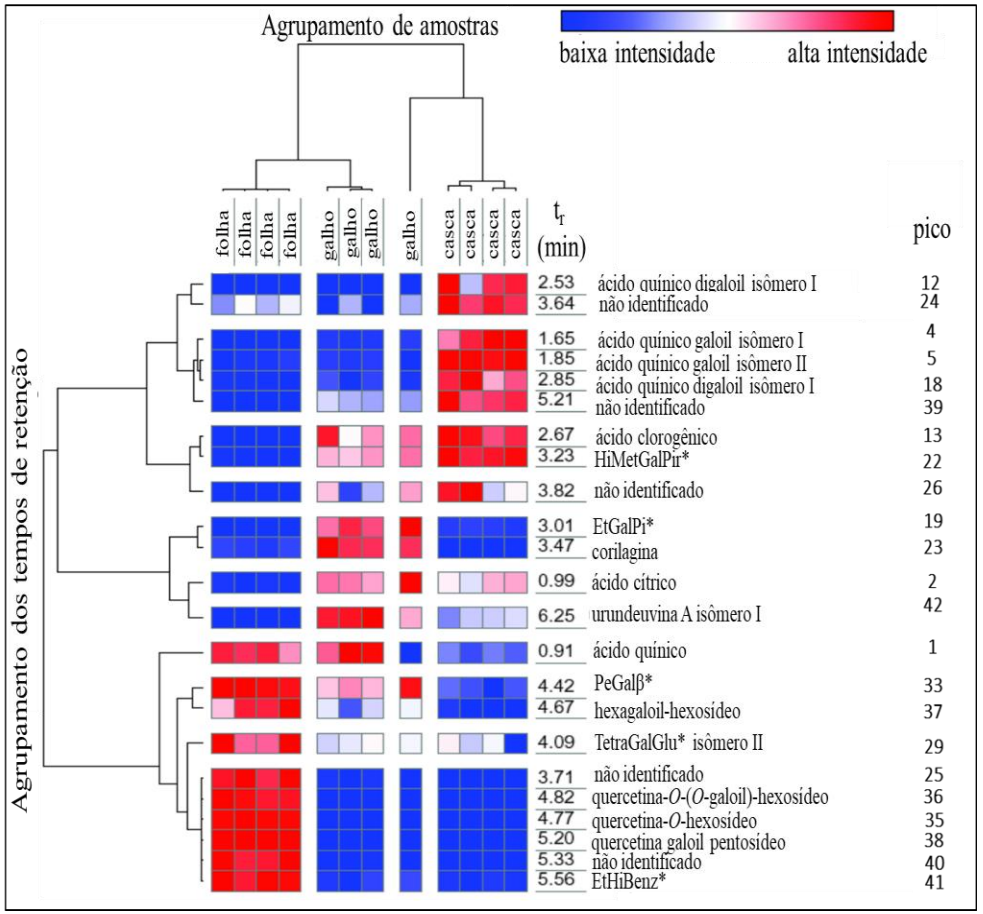
- ✓ Regulação da expressão de genes relacionados à apoptose (LI et al., 2017; YAMAGATA et al., 2017);
- ✓ Diminuição da proliferação em células cancerígenas no pulmão (YAMAGATA et al., 2017);
- ✓ Diminuição da taxa de crescimento de células HT29 em 46% (NAM et al., 2017).

4. Resultados e Discussão

❑ Análise Quimiométrica

- **Mapa de Calor (Heat map)**

  - As amostras das folhas foram agrupadas devido à maior quantidade relativa de ácido quinico.
  - As amostras de cascas foram agrupadas pela maior quantidade de isômeros de ácido quínico galoil e digaloil, corroborando com os resultados de PCA.



Introdução – O cajueiro

Nome da família: *Anacardiaceae*

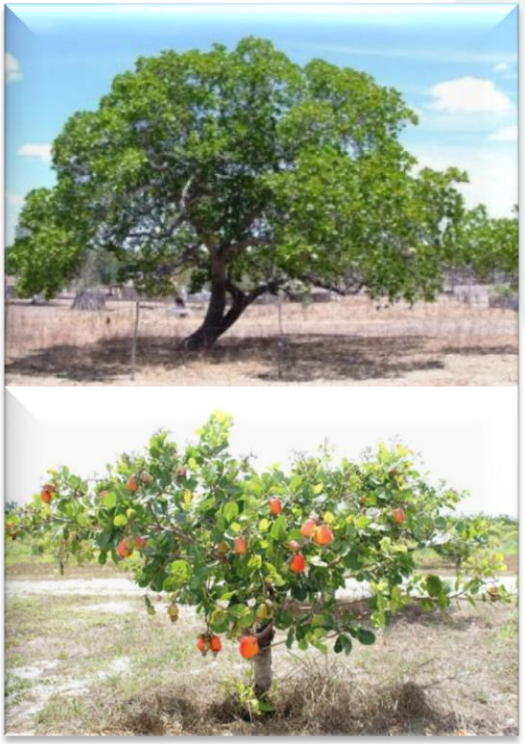
Nome científico: *Anacardium Occidentale Linn*

Nome popular: Cajueiro

Centro de origem: Brasil

- Adaptada ao clima quente e seco;
- Classificada pelo porte;
- Dois tipos de cajueiro: comum e anão-precoce.

Figura 1 – Tipos de cajueiro.



Fonte: Elaborada pelo autor.

SANTOS-SEREJO, J. A. DOS et al. **Fruticultura Tropical: espécies regionais e exóticas**. Brasília, DF. Embrapa Informação Tecnológica. 2009.

Materiais e Métodos

- **Coleta das amostras:**

Tabela 2 – Descrição das amostras analisadas

Denominação	Amostra	Característica
Clone 1	CCP 76	Resistente
Clone 2	BRS 226	Resistente
Clone 3	BRS 189	Resistente
Clone 4	BRS 265	Suscetível

Fonte: Elaborada pelo autor

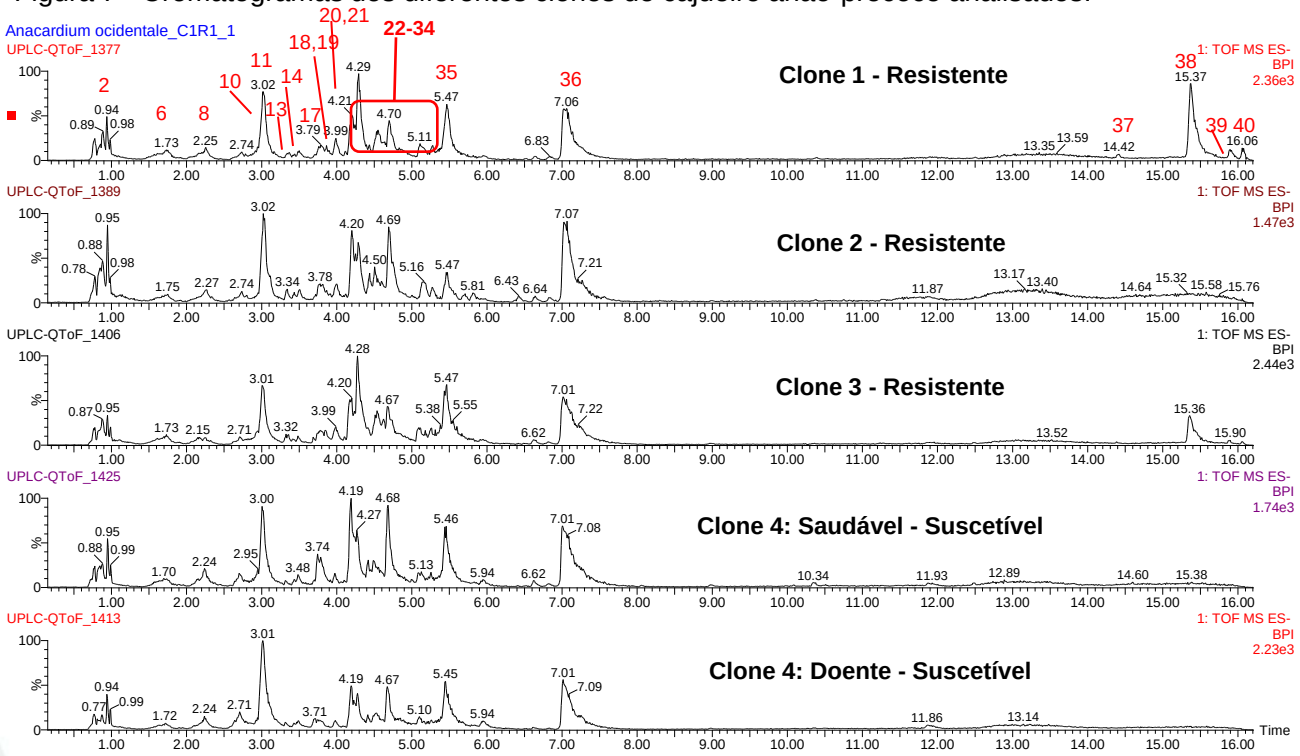
- Planta doente
- Planta saudável

Local de cultivo das plantas

Resultados e Discussão

- **Perfil químico via UPLC-ESI-QTOF-MS(/MS) dos extratos de folhas da *Anacardium Occidentale L.***

Figura 7 - Cromatogramas dos diferentes clones de cajueiro anão-precoce analisados.



Fonte: Elaborada pelo autor

Resultados e Discussão

- **Perfil químico via UPLC-ESI-QTOF-MS(/MS) dos extratos de folhas da *Anacardium Occidentale L.***

Tabela 6 continuação – Constituintes identificados ou tentativamente identificados em folhas de *Anacardium Occidentale L.* das plantas saudáveis (C1, C2, C3, C4_SA) e da planta doente (C4_CA).

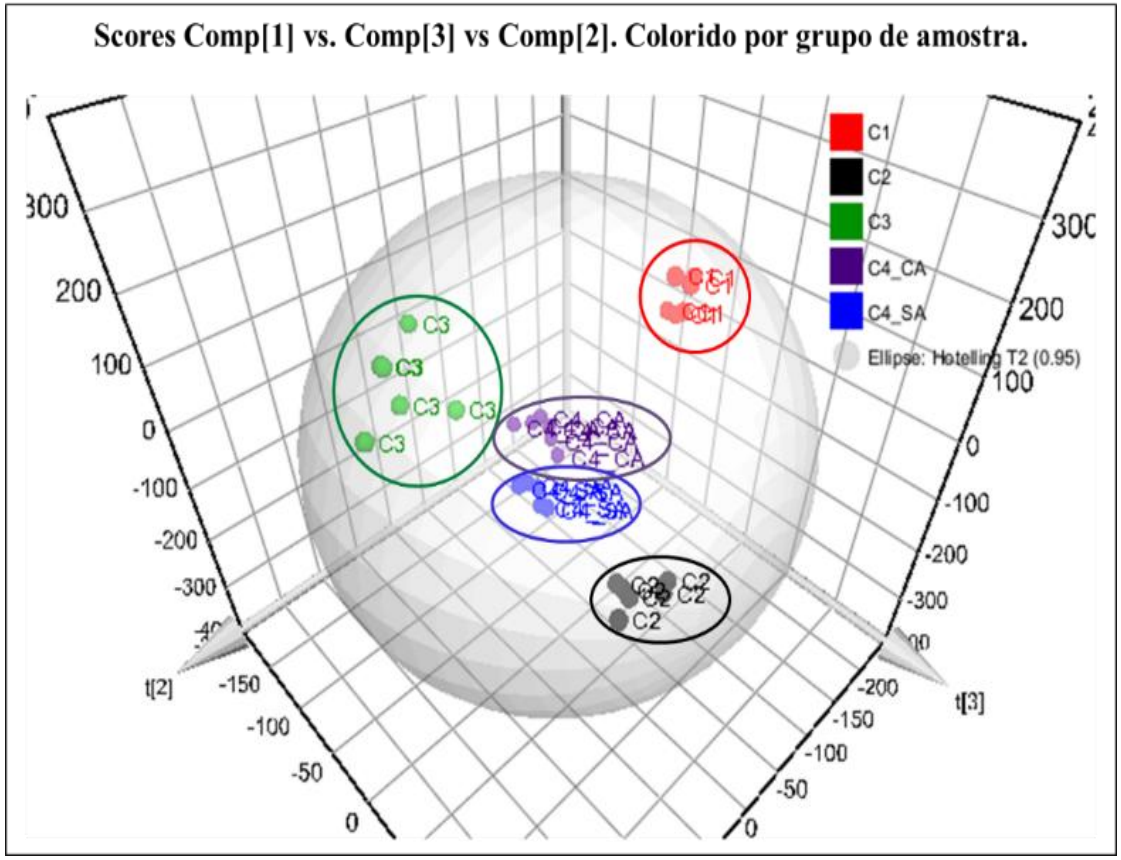
Pico no.	t_R (min)	$[M-H]^-$ Calculado	$[M-H]^-$ Observado	Fragmentos de íons (MS/MS)	Fórmula Molecular	Erro ppm	Tentativa Identificação	Referências	Clone 1	Clone 2	Clone 3	Clone 4 Saudável	Clone 4 Doente
30	4,57	1091,1213	1091,1285	939,1167;769,0932; 617,0867; 169.0123	$C_{48}H_{36}O_{30}$	6,6	Hexagaloil hexosídeo	(GRACE et al., 2016; ERŞAN et al., 2016)	+	+	+	+	+
31	4,59	599,1037	599,1074	285,0398;169,0123; 125,0252	$C_{28}H_{24}O_{15}$	6,2	Cianidina 3-*O*-(2'' galoil)-galactosídeo	(ABU-REIDAH et al., 2015)	-	+	-	-	-
32	4,69	447,0927	447,0909	301,0343; 300,0271	$C_{21}H_{20}O_{11}$	-4,0	Quercetina 3-*O*-ramnosídeo	(ABU-REIDAH et al., 2015)	+	+	+	+	+
33	4,75	585,0880	585,0923	301,0341; 169,0125	$C_{27}H_{22}O_{15}$	7,3	Quercetina galoil pentosídeo	(ERŞAN et al., 2016)	+	+	+	+	+
34	5,27	585,0880	585,0912	301,0350; 169,0152	$C_{27}H_{22}O_{15}$	5,5	Quercetina galoil pentosídeo dímero	(ABU-REIDAH et al., 2015)	-	+	-	-	-
35	5,44	349,0560	349,0562	198,0495;197,0426; 169,0128; 124,0166	$C_{16}H_{14}O_9$	0,6	2,4-di-hidroxi-3-(3,4,5-tri-hidroxibenzoil) oxibenzoato de etilo	(DORTA et al., 2014)	+	-	+	+	+
36	7,02	537,0822	537,0809	417,0616; 375,0501	$C_{30}H_{18}O_{10}$	-2,4	Amentoflavona ou agatisflavona	(ABU-REIDAH et al., 2015)	+	+	+	+	+
37	14,41	341,2117	341,3125	297,2204;119,0514; 106,0428	$C_{22}H_{30}O_3$	5,3	Ácido anacárdico (15:3)	(ERŞAN et al., 2016)	+	-	-	-	-
38	15,38	369,2430	369,2406	325,2503	$C_{24}H_{34}O_3$	-6,5	Ácido anacárdico (17:3)	(ERŞAN et al., 2016)	+	-	+	-	-
39	15,90	345,2430	345,2428	301,2550	$C_{22}H_{34}O_3$	-0,6	Ácido anacárdico (15:1)	(ERŞAN et al., 2016)	+	-	-	-	-
40	16,06	371,2586	371,2582	327,2686	$C_{24}H_{36}O_3$	-1,1	Ácido anacárdico (17:2)	(ERŞAN et al., 2016)	+	-	-	-	-

Fonte: Elaborada pelo autor.

Resultados e Discussão

- **Análises quimiométricas:**

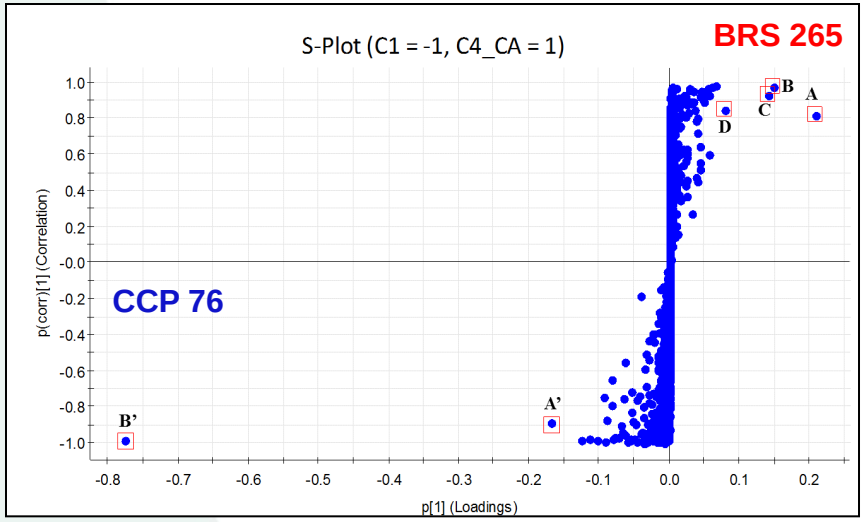
Figura 9 – Gráfico em 3D da Análise de Componentes Principais para as amostras de *Anacardium Occidentale L*. analisados por UPLC-ESI-QTOF-MS(/MS).



Fonte: Elaborada pelo autor

Resultados e Discussão

Figura 11 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 1 relacionados aos dos clone 4 - doente.



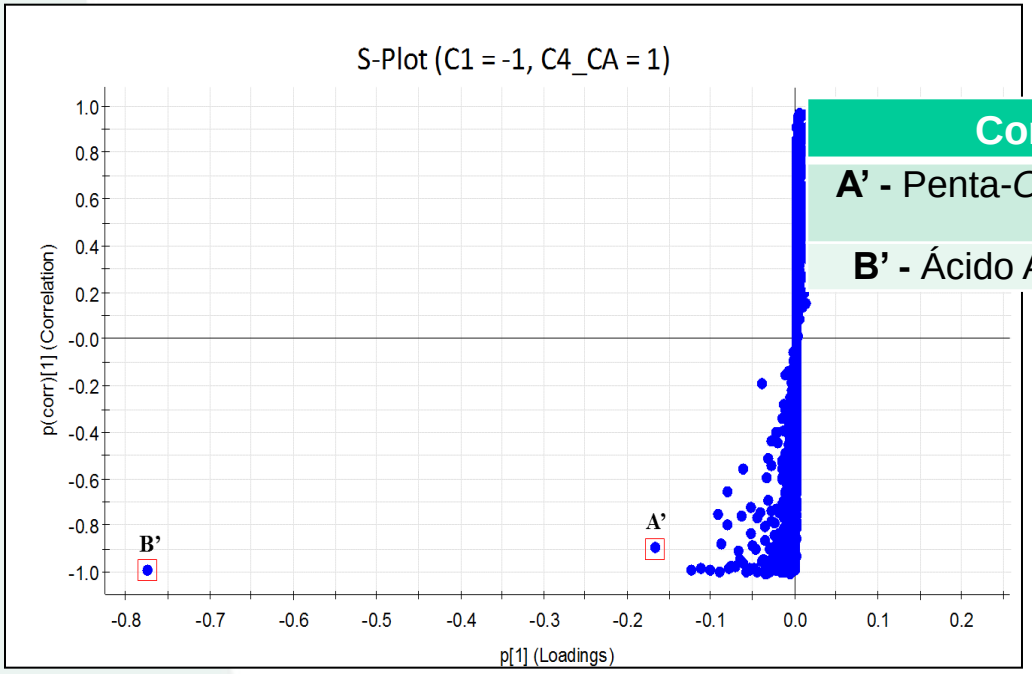
Compostos	t_r	*m/z*	p[1]	p(corr)[1]
D - Monogalato de dímero de procianidina tipo B	3,71	729,1516	0,0769052	0,851377
B – Dímero de Procianidina tipo B	2,74	577,1382	0,145079	0,978857
C - 2,4-di-hidroxi-3-(3,4,5-tri-hidroxibenzoil) oxibenzoato de etilo	5,44	349,0543	0,136453	0,934055
A - Catequina	3,01	289,0669	0,202476	0,817749
A' - Penta-*O*-galoil-glucosídeo	4,28	939,1139	-0,167484	-0,886497
B' - Ácido Anacárdico (17:3)	15,39	369,2391	-0,775554	-0,98636

Fonte: Elaborada pelo autor

Resultados e Discussão

Biomarcadores de **Resistência**

Figura 12 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 1 relacionados aos dos clone 4 - doente.



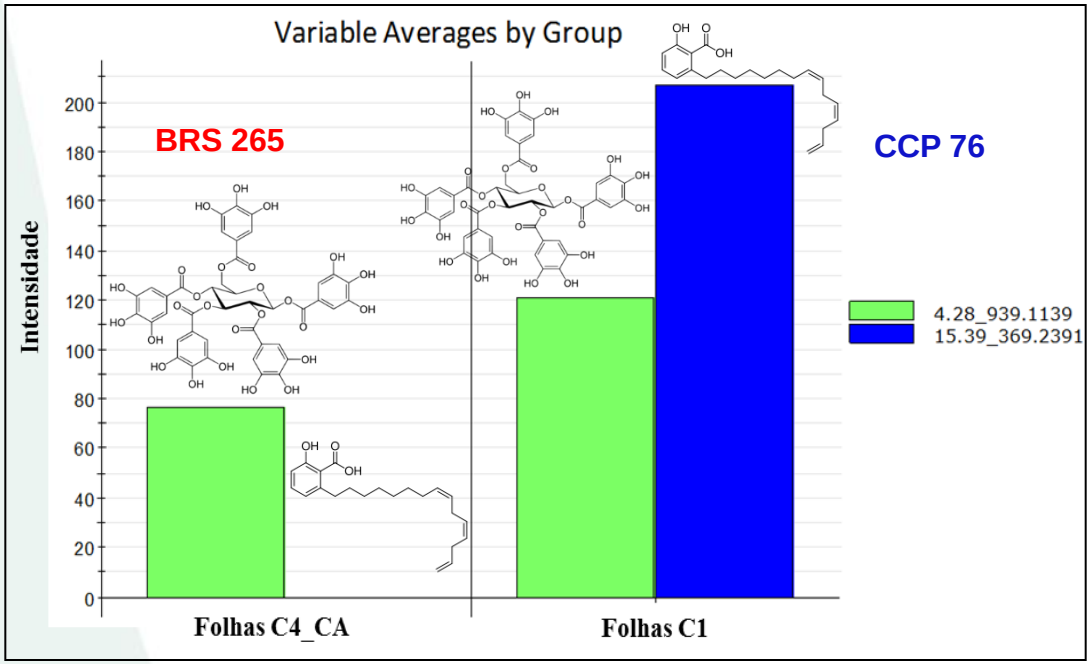
Compostos	t_r	*m/z*
A' - Penta-*O*-galoil-glucosídeo	4,28	939,1139
B' - Ácido Anacárdico (17:3)	15,39	369,2391

Fonte: Elaborada pelo autor

Resultados e Discussão

Biomarcadores de **Resistência**

Figura 13 - Variação média dos discriminantes do extrato etanólico do clone 1 em relação ao clone 4 doente.



Fonte: Elaborada pelo autor

ÁCIDOS ANACÁRDICOS:

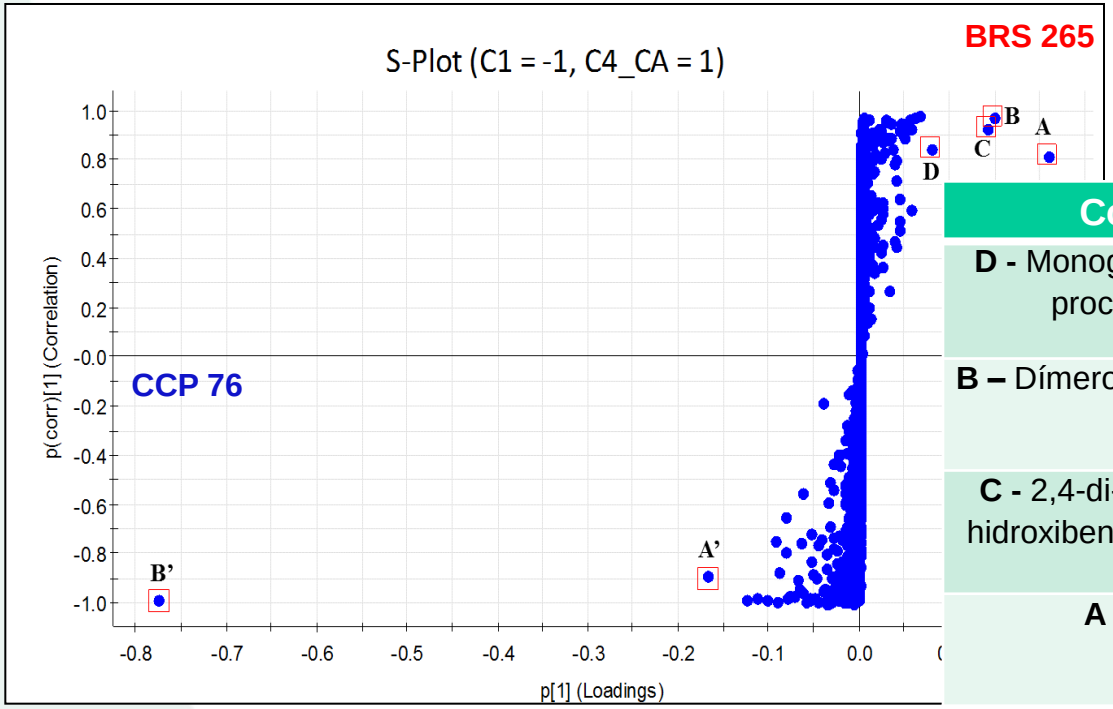
Inibição de germinação de conídios e crescimento micelial de fungos
(MUZAFFAR et al., 2016)

Compostos	t_r	*m/z*
A' - Penta-*O*-galoil-glucosídeo	4,28	939,1139
B' - Ácido Anacárdico (17:3)	15,39	369,2391

Resultados e Discussão

Biomarcadores de **Suscetibilidade**

Figura 14 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 1 relacionados aos dos clone 4 - doente.



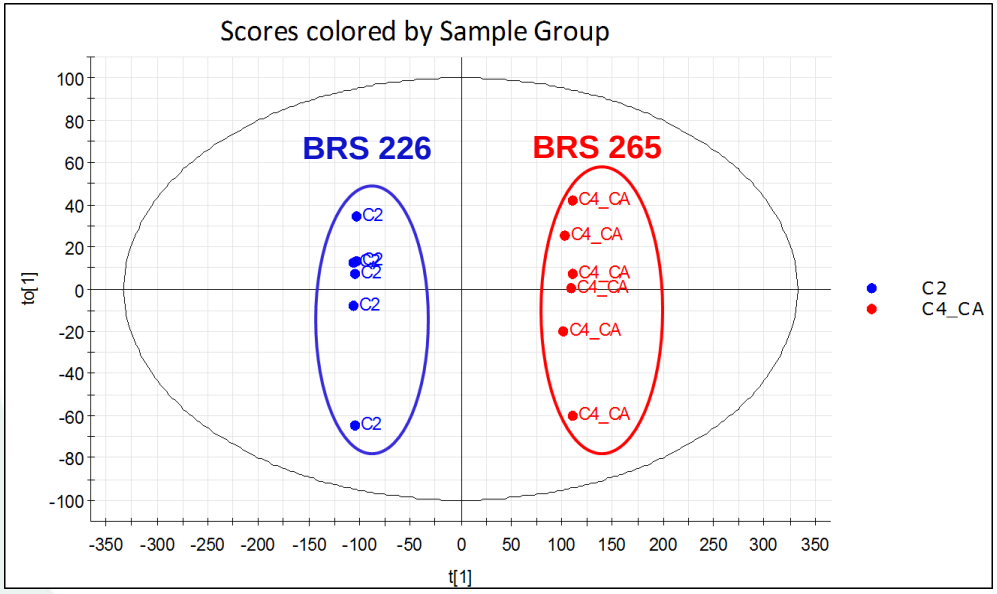
Compostos	t_r	*m/z*
D - Monogalato de dímero de procianidina tipo B	3,71	729,1516
B – Dímero de Procianidina tipo B	2,74	577,1382
C - 2,4-di-hidroxi-3-(3,4,5-tri-hidroxibenzoil) oxibenzoato de etilo	5,44	349,0543
A - Catequina	3,01	289,0669

Fonte: Elaborada pelo autor

Resultados e Discussão

- **Análise Discriminante por Projeções Ortogonais a Estruturas Latentes (OPLS-DA):**

Figura 15 – Gráfico de scores da OPLS-DA para os extratos de folhas do clone 2 relacionados aos dos clone 4 - doente.

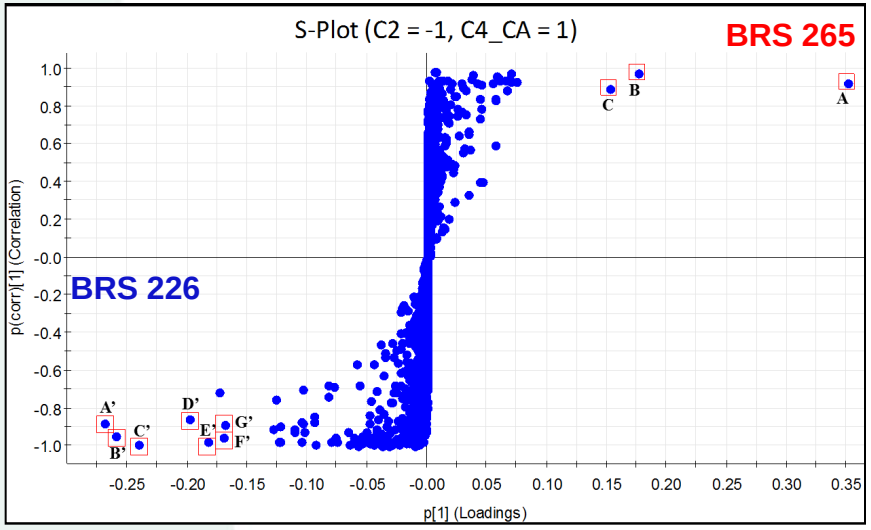


Fonte: Elaborada pelo autor

$R^2Y = 0,99$
$Q^2 = 0,99$

Resultados e Discussão

Figura 16 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 2 relacionados aos dos clone 4 - doente.



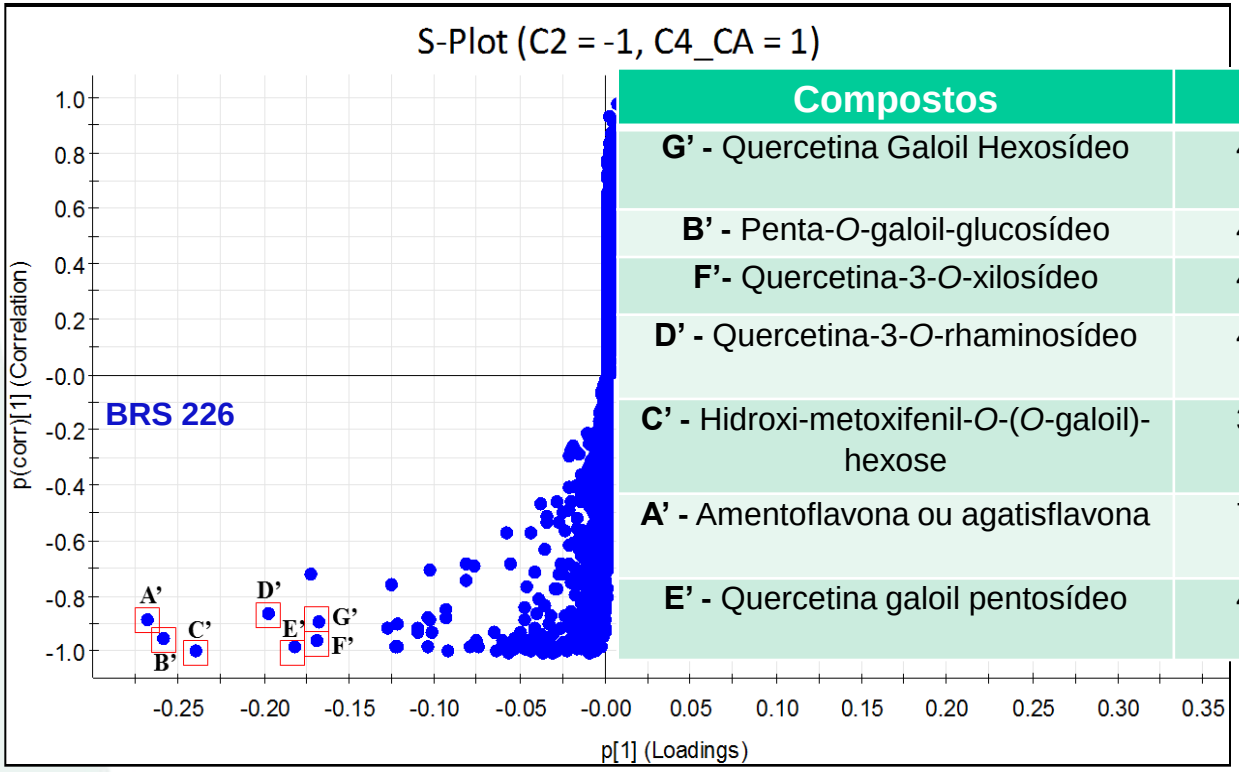
Fonte: Elaborada pelo autor

Compostos	t_r	*m/z*	p[1]	p(corr)[1]
A - Catequina	3,01	289,0669	0,350639	0,922335
B - Dímero de procianidina tipo B	2,74	577,1382	0,175672	0,973503
C - 2,4-di-hidroxi-3-(3,4,5-tri-hidroxibenzoil) oxibenzoato de etilo	5,44	349,0543	0,15251	0,890879
G' - Quercetina Galoil Hexosídeo	4,24	615,0998	-0,168821	-0,886397
B' - Penta-*O*-galoil-glucosídeo	4,28	939,1139	-0,259856	-0,946644
F'- Quercetina-3-*O*-xilosídeo	4,44	433,0781	-0,170444	-0,948913
D' - Quercetina-3-*O*-rhaminosídeo	4,68	447,0900	-0,197768	-0,854247
C' - Hidroxi-metoxifenil-*O*-(*O*-galoil)-hexose	3,10	453,1048	-0,241299	-0,989756
A' - Amentoflavona ou agatisflavona	7,02	537,0800	-0,268911	-0,876031
E' - Quercetina galoil pentosídeo	4,74	585,0909	-0,183283	-0,974708

Resultados e Discussão

Biomarcadores de **Resistência**

Figura 17 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 2 relacionados aos dos clone 4 - doente.

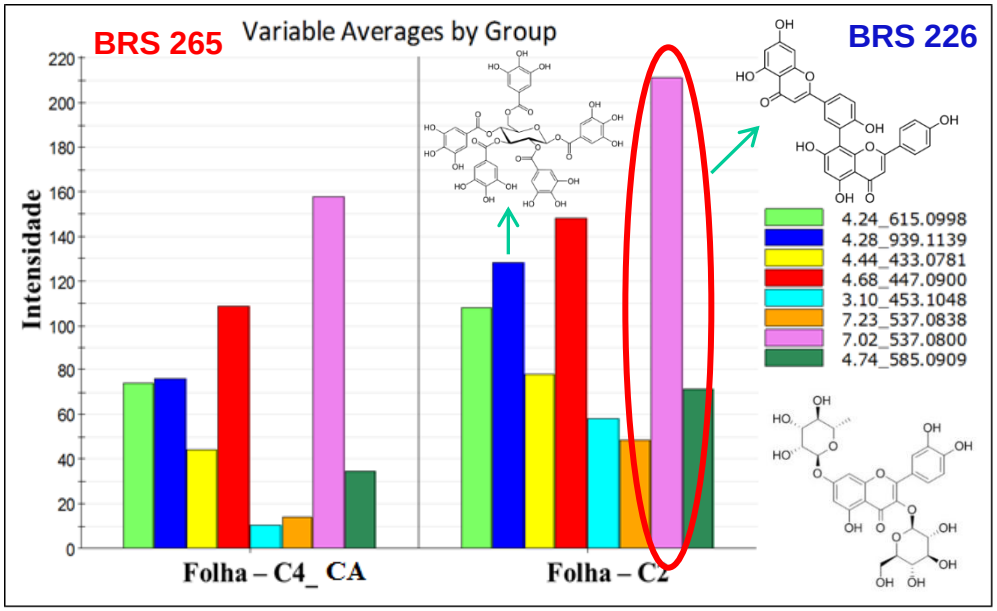


Compostos	t_r	*m/z*
G' - Quercetina Galoil Hexosídeo	4,24	615,0998
B' - Penta-*O*-galoil-glucosídeo	4,28	939,1139
F'- Quercetina-3-*O*-xilosídeo	4,44	433,0781
D' - Quercetina-3-*O*-rhaminosídeo	4,68	447,0900
C' - Hidroxi-metoxifenil-*O*-(*O*-galoil)-hexose	3,10	453,1048
A' - Amentoflavona ou agatisflavona	7,02	537,0800
E' - Quercetina galoil pentosídeo	4,74	585,0909

Fonte: Elaborada pelo autor

Resultados e Discussão
Biomarcadores de **Resistência**

Figura 18 - Variação média dos discriminantes do extrato etanólico do clone 2 em relação ao clone 4 doente.



Fonte: Elaborada pelo autor

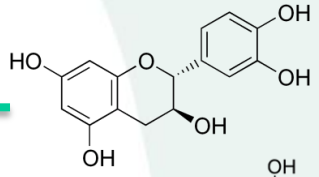
AMENTOFLAVONA:

Atividade antifúngica (JUNG et al., 2006)

Compostos	t_r	m/z
G' - Quercetina Galoil Hexosídeo	4,24	615,0998
B' - Penta-O-galoil-glucosídeo	4,28	939,1139
F'- Quercetina-3-O-xilosídeo	4,44	433,0781
D' - Quercetina-3-O-rhaminosídeo	4,68	447,0900
C' - Hidroxi-metoxifenil-O-(O-galoil)-hexose	3,10	453,1048
A' - Amentoflavona ou agatisflavona	7,02	537,0800
E' - Quercetina galoil pentosídeo	4,74	585,0909

Resultados e Discussão

Biomarcadores de **Suscetibilidade**



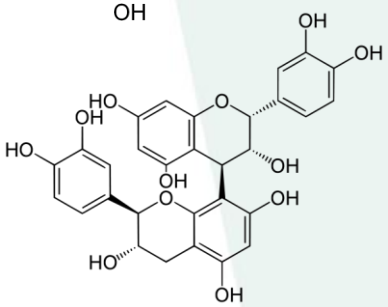
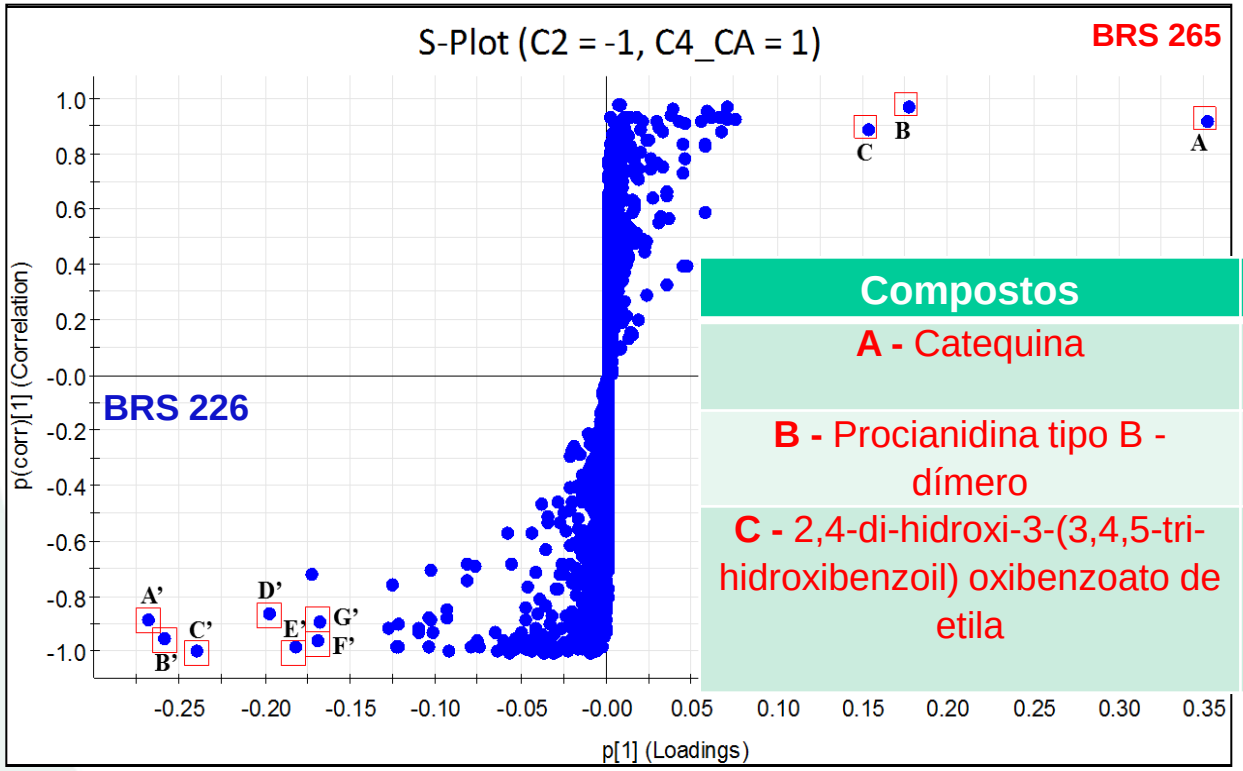


Figura 19 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 2 relacionados aos dos clone 4 - doente.



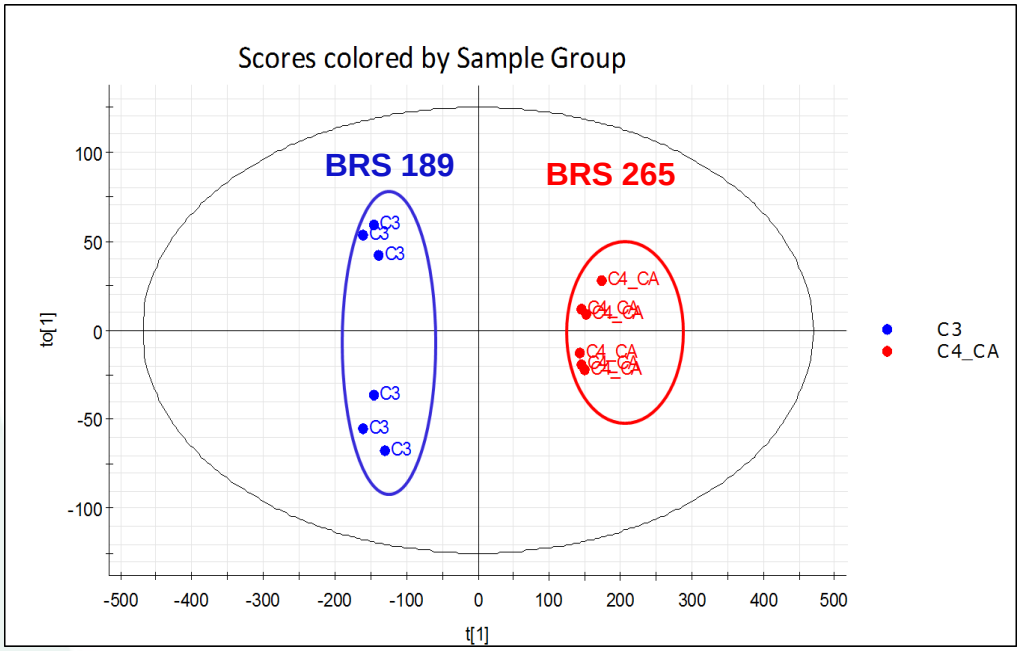
Compostos	t_r	*m/z*
A - Catequina	3,01	289,0669
B - Procianidina tipo B - dímero	2,74	577,1382
C - 2,4-di-hidroxi-3-(3,4,5-tri-hidroxibenzoil) oxibenzoato de etila	5,44	349,0543

Fonte: Elaborada pelo autor

Resultados e Discussão

- **Análise Discriminante por Projeções Ortogonais a Estruturas Latentes (OPLS-DA):**

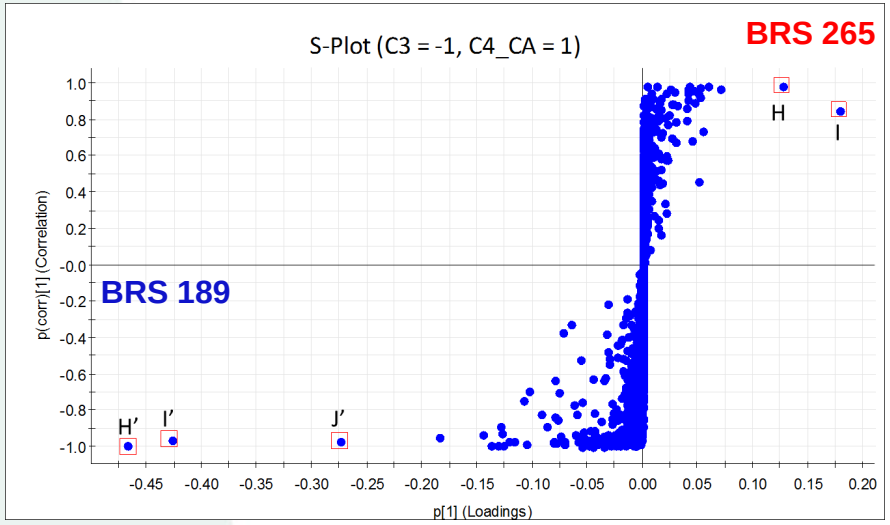
Figura 20 – Gráfico de scores da OPLS-DA para os extratos de folhas do clone 3 relacionados aos dos clone 4 - doente.



$R^2Y = 0,99$
$Q^2 = 0,98$

Resultados e Discussão

Figura 21 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 3 relacionados aos dos clone 4 - doente.



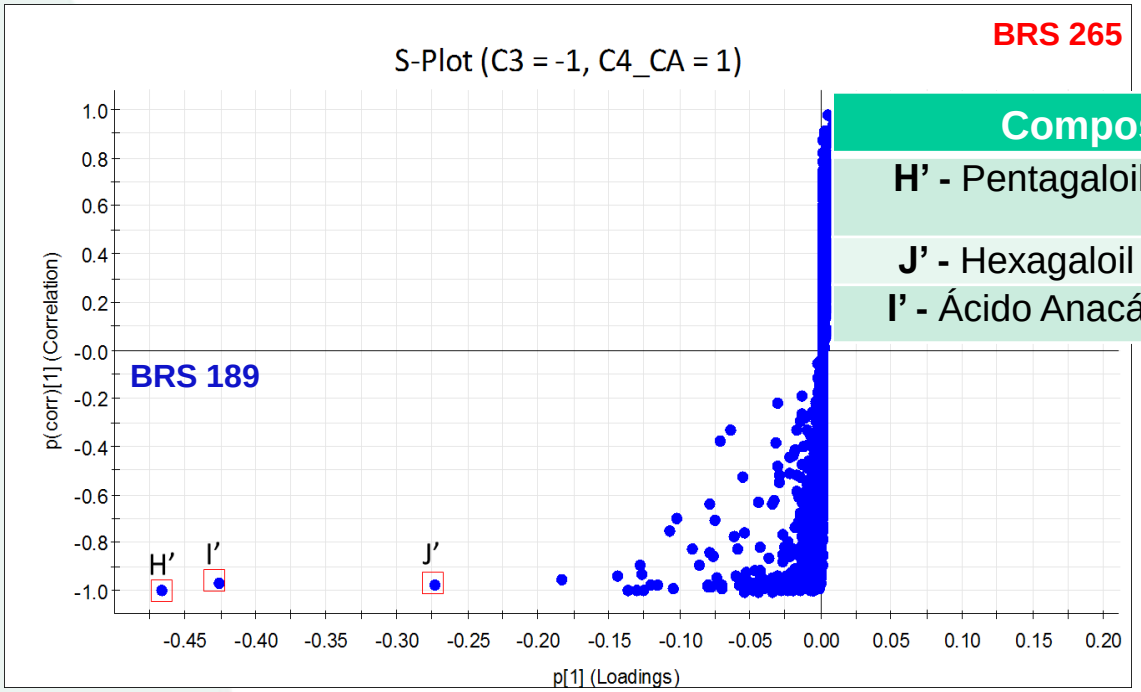
Fonte: Elaborada pelo autor

Compostos	t_r	*m/z*	p[1]	p(corr)[1]
I – Catequina	3,01	289,0669	0,178502	0,849393
H - Procianidina tipo B – dímero	2,74	577,1382	0,127435	0,982202
H' - Pentagaloil hexosídeo	4,28	939,1139	-0,467289	-0,989771
J' - Hexagaloil Hexosídeo	4,57	1091,1316	-0,274869	-0,97266
I' - Ácido Anacárdico (17:3)	15,39	369,2391	-0,42684	-0,962733

Resultados e Discussão

Biomarcadores de **Resistência**

Figura 22 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 3 relacionados aos dos clone 4 - doente.

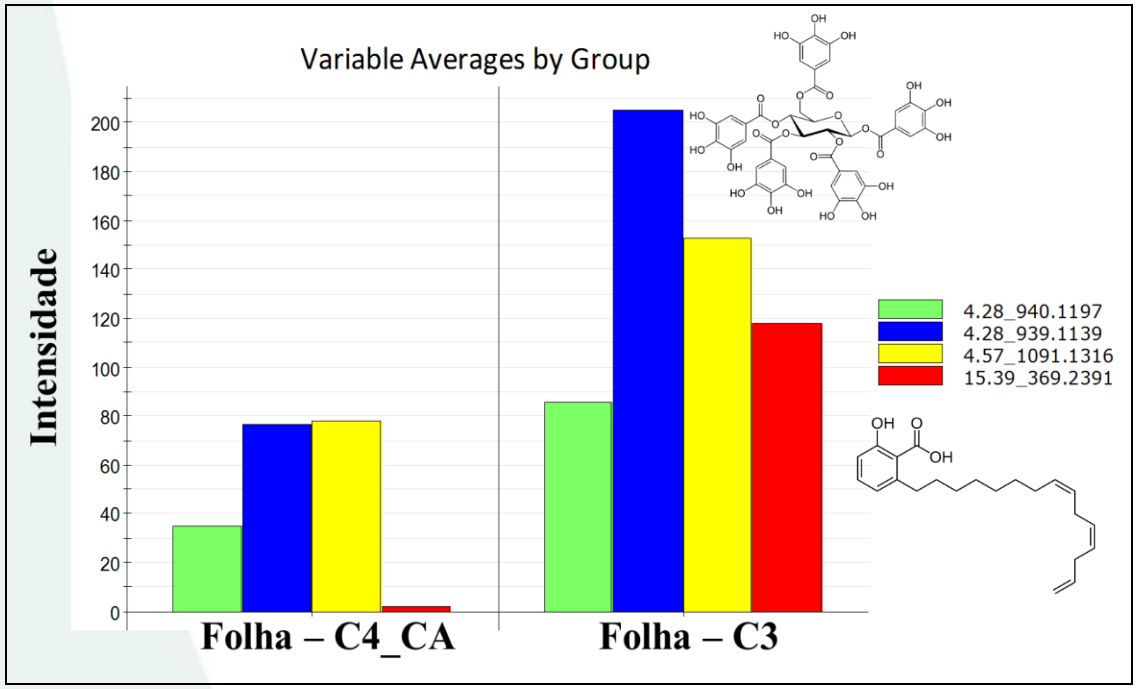


Compostos	t_r	*m/z*
H' - Pentagaloil hexosídeo	4,28	939,1139
J' - Hexagaloil Hexosídeo	4,57	1091,1316
I' - Ácido Anacárdico (17:3)	15,39	369,2391

Fonte: Elaborada pelo autor

Resultados e Discussão
Biomarcadores de **Resistência**

Variação média dos discriminantes do extrato etanólico do clone 3 em relação ao clone 4 doente.



Penta-*O*-galoil-glucosídeo

Hexagaloil glucosídeo

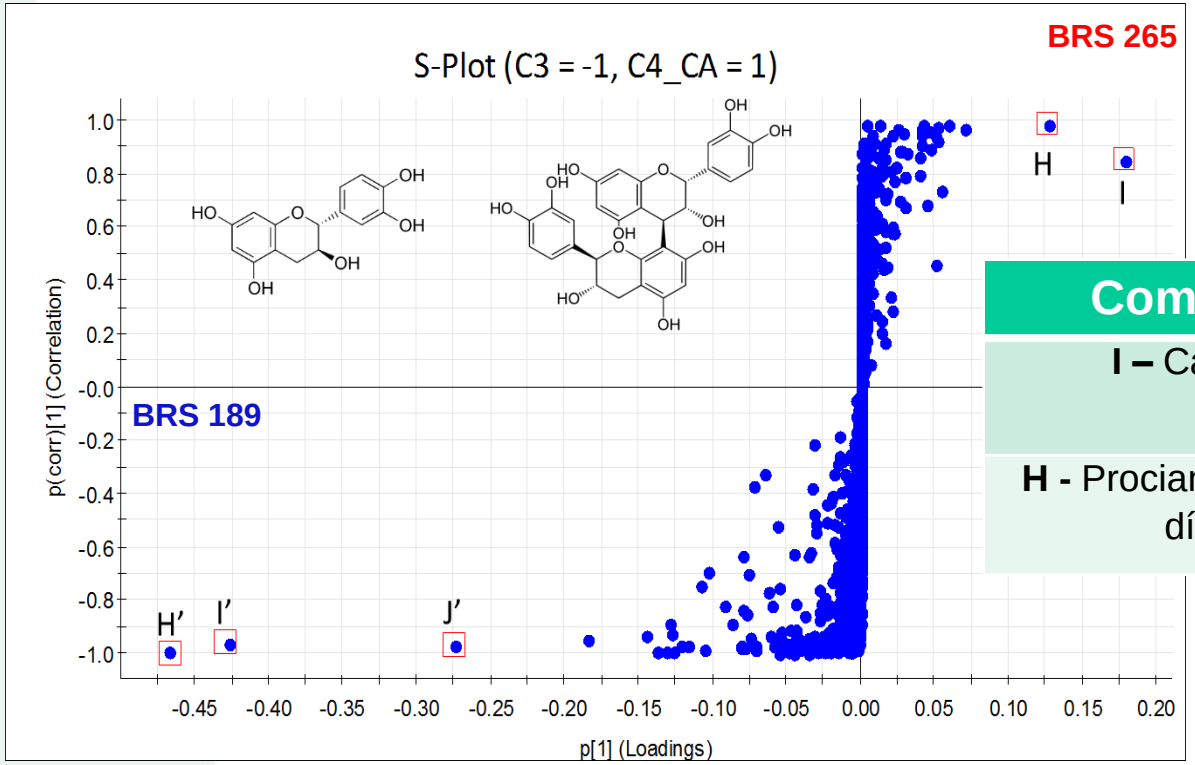
Ácido anacárdico (17:3)

Fonte: Elaborada pelo autor

Resultados e Discussão

Biomarcadores de **Suscetibilidade**

Figura 24 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 3 relacionados aos dos clone 4 - doente.



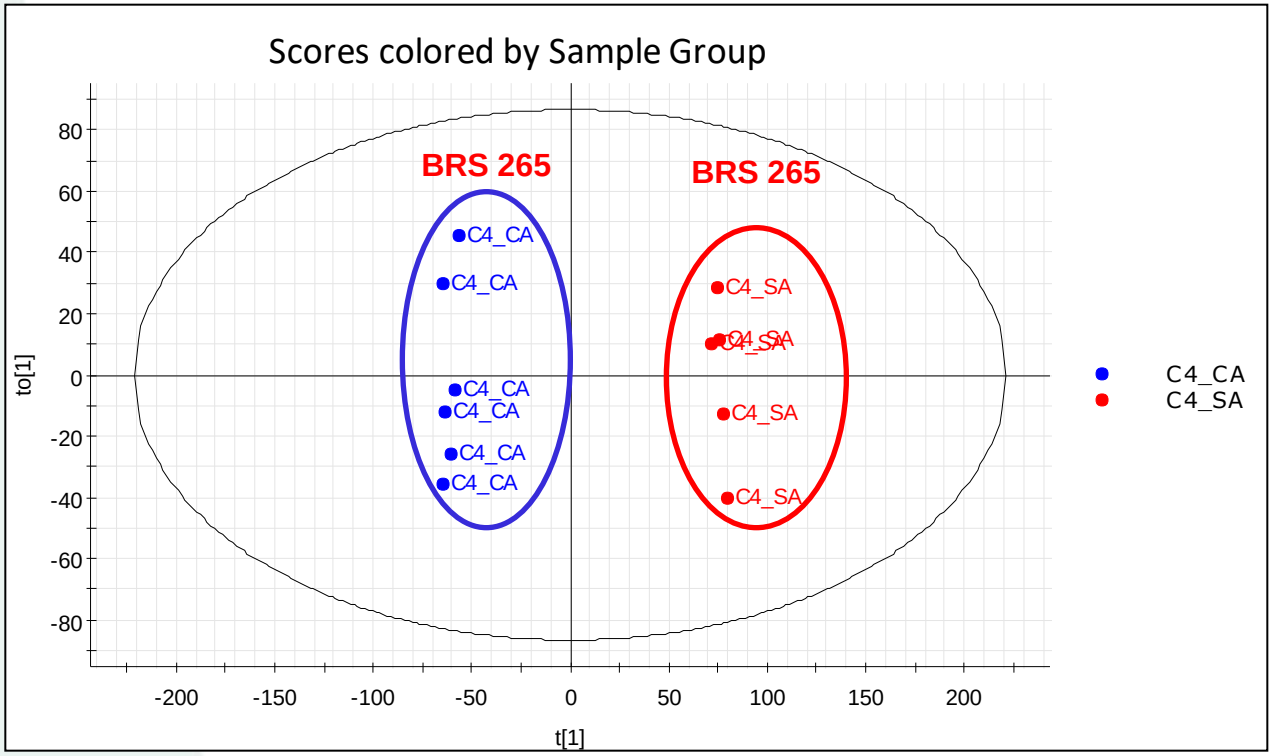
Compostos	t_r	*m/z*
I – Catequina	3,01	289,0669
H - Procianidina tipo B – dímero	2,74	577,1382

Fonte: Elaborada pelo autor

Resultados e Discussão

Biomarcadores de **Suscetibilidade**

Figura 25 – Gráfico de scores da OPLS-DA para os extratos de folhas do clone 4 (saudável) relacionados aos dos clone 4 (doente).



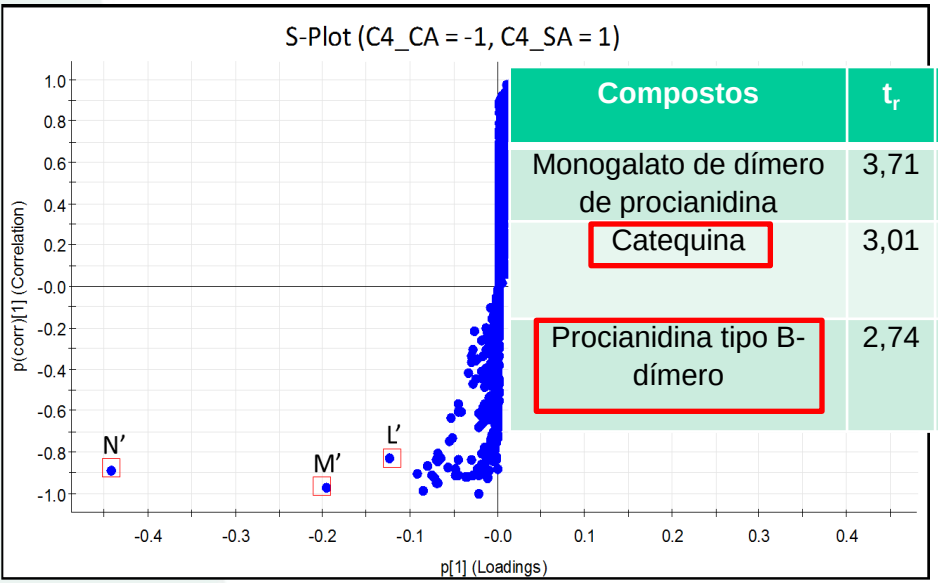
Fonte: Elaborada pelo autor

$R^2Y = 0,99$
$Q^2 = 0,97$

Resultados e Discussão

Biomarcadores de **Suscetibilidade**

Figura 26 – Gráfico dispersão (S-plot) para os extratos de folhas do clone 4 (saudável) relacionados aos dos clone 4 (doente).

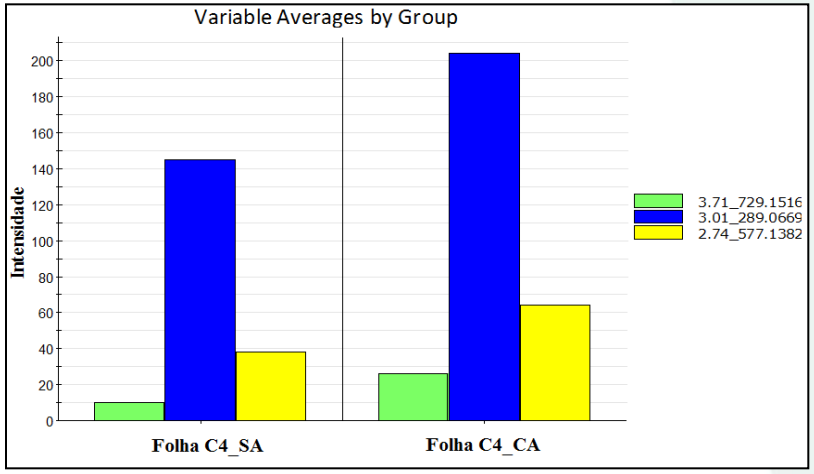


Compostos	t_r	*m/z*
Monogalato de dímero de procianidina	3,71	729,1516
Catequina	3,01	289,0669
Procianidina tipo B-dímero	2,74	577,1382

Fonte: Elaborada pelo autor

Total de quatro biomarcadores de suscetibilidade ligados às comparações entre a planta doente do clone 4 e as demais plantas saudáveis.

Figura 27 –Variação média dos discriminantes do extrato etanólico do clone 4 (saudável) em relação ao clone 4 (doente).



Fonte: Elaborada pelo autor