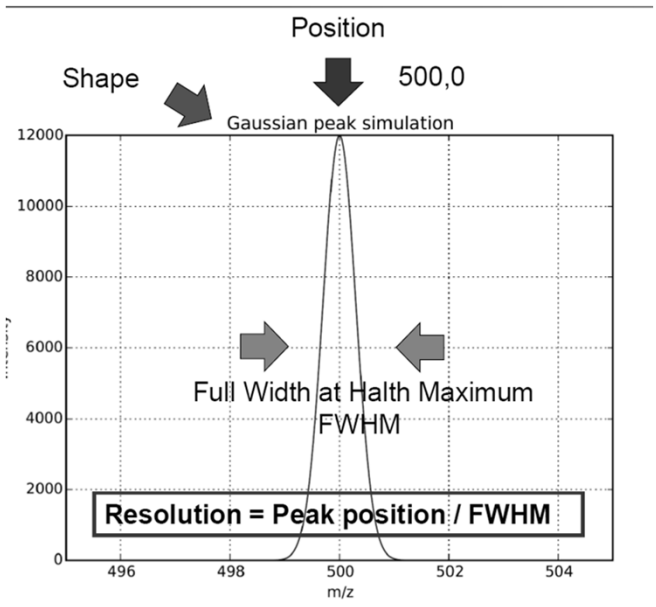
SPETTROMETRIA DI MASSA
Potere Risolutivo, Risoluzione e accuratezza di massa

1



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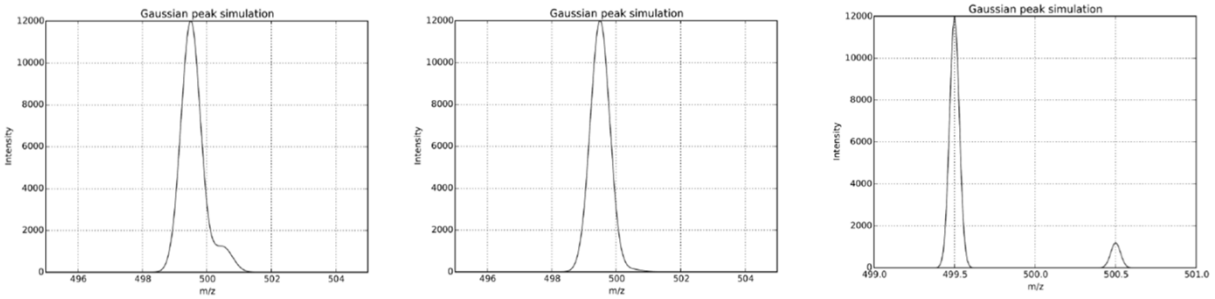

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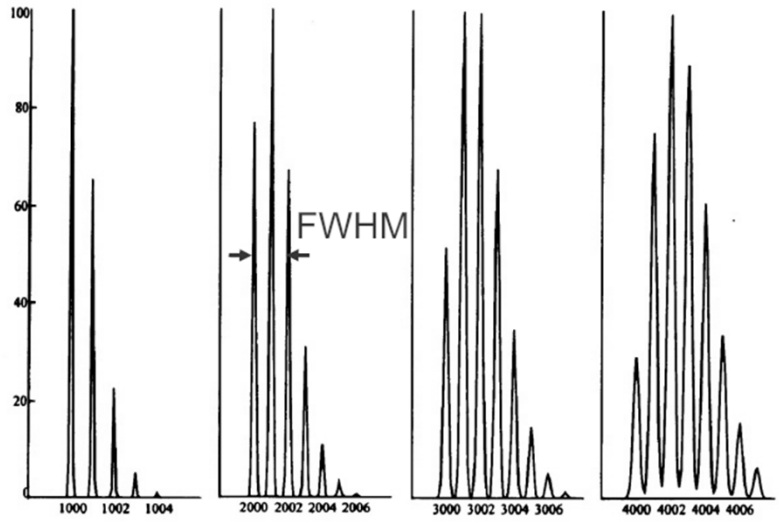


Theoretical isotope distributions of peptides of 1000, 2000, 3000 and 4000 Da.

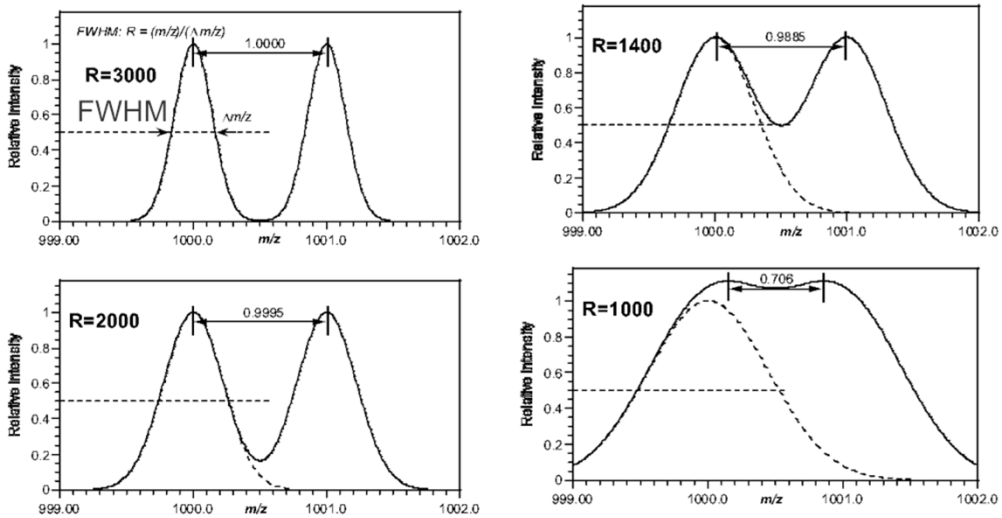
4

Isotope	*Mass*	*Abundance*	*Chemical mass*	*Deviation from the whole number*
^{1}H	1.00782510	99.9852%	1.00794	+0.0079
^{2}H (D)	2.01410222	0.0148%		
^{12}C	12.0(0)	98.892%	12.011	+0.011
^{13}C	13.0033544	1.108%		
^{14}N	14.00307439	99.635%	14.00674	+0.007
^{15}N	15.0001077	0.365%		
^{16}O	15.99491502	99.759%	15.9994	-0.0006
^{17}O	16.9991329	0.037%		
^{18}O	17.99916002	0.204%		
^{31}P	30.9737647	100%	30.9737647	-0.0262
^{32}S	31.9720737	95.0%	32.066	+0.066
^{33}S	32.9714619	0.76%		
^{34}S	33.9678646	4.22%		
^{36}S	35.967090	0.014%		

5

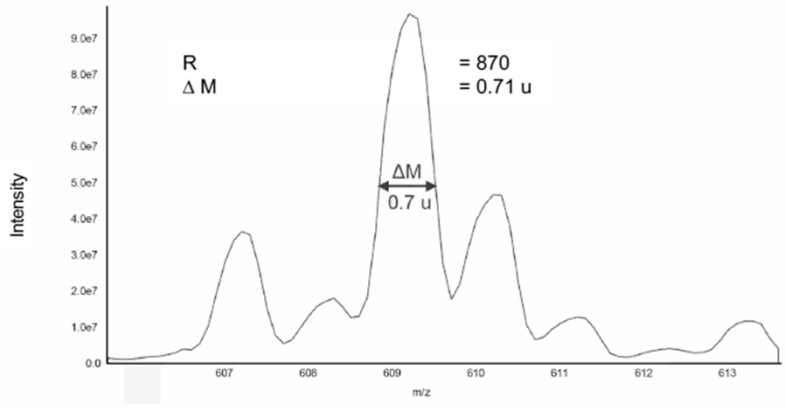
R = $(m/z)_0$/FWHM(m/z)

FWHM: **f**ull **w**idth at **h**alf **m**aximum



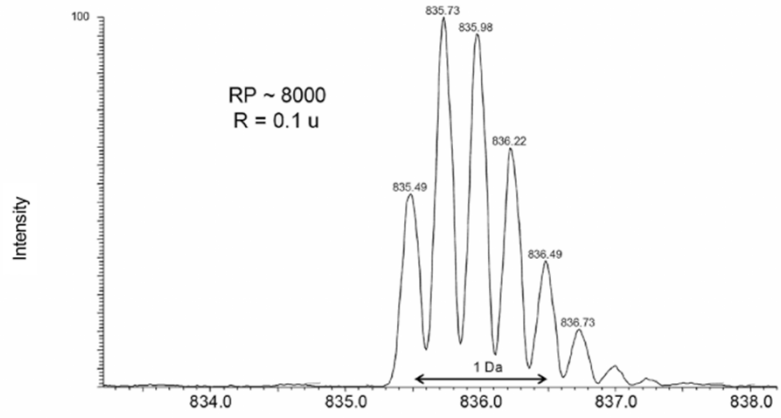
6

low resolution



7

moderate resolution



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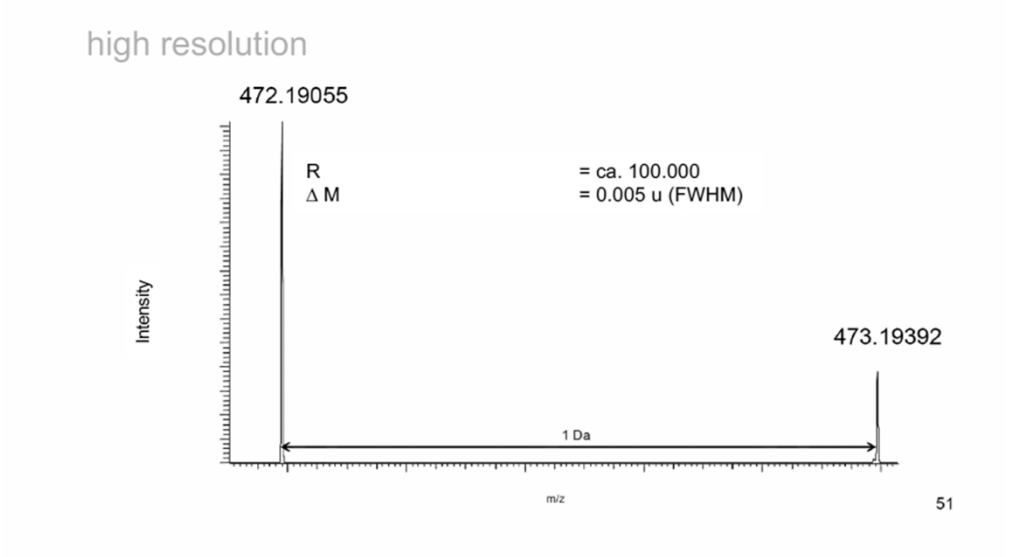


9

Average Mass e.g C=12.01115

Mass of an ion or molecule weighted for its *isotopic composition*.

Monoisotopic Mass (Exact Mass) e.g. C=12.000000

Exact mass of an ion or molecule calculated using the *mass of the most abundant isotope* of each element.

Nominal Mass e.g. C=12

Mass of a molecular ion or molecule calculated using the *isotope mass of the most abundant constituent* (ignoring mass defect) element isotope of each element rounded to the *nearest integer* value and multiplied by the number of atoms of each element (C=12, H=1, N=14, O=16, ….)

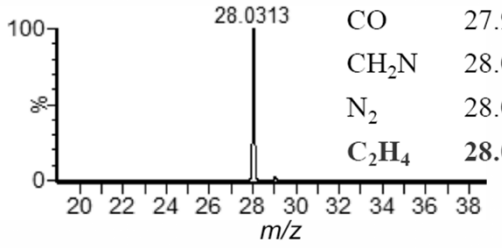
10

	mass	probability	^{1}H	^{2}H	^{12}C	^{13}C	^{14}N	^{15}N	^{16}O	^{17}O	^{18}O	^{32}S	^{33}S	^{34}S	^{36}S
1	5731.6075806688	0.1123023514	377	0	252	2	65	0	75	0	0	6	0	0	0
2	5732.6109355040	0.1028778936	377	0	251	3	65	0	75	0	0	6	0	0	0
3	5730.6042258336	0.0814037470	377	0	253	1	65	0	75	0	0	6	0	0	0
4	5733.6142903392	0.0704027660	377	0	250	4	65	0	75	0	0	6	0	0	0
5	5734.6176451744	0.0383896060	377	0	249	5	65	0	75	0	0	6	0	0	0
7	5733.6033765247	0.0301636876	377	0	252	2	65	0	75	0	0	5	0	1	0
6	5729.6008709984	0.0293871014	377	0	254	0	65	0	75	0	0	6	0	0	0
8	5734.6067313599	0.0276323390	377	0	251	3	65	0	75	0	0	5	0	1	0
9	5732.6046155640	0.0266824062	377	0	252	2	64	1	75	0	0	6	0	0	0

11

e.g. *m/z* 28 can have 4 different elemental compositions with different exact mass values.

CO	27.9949 u	CH_2N	28.0187 u
N_2	28.0061 u	C_2H_4	28.0313 u



CO 27.9949 u
CH_2N 28.0187 u
N_2 28.0061 u
C_2H_4 28.0313 u

12

- Mass error in millimass units (mmu)

  [measured mass (u) – theoretical mass (u)] x 1000

- Mass error in parts per million (ppm)

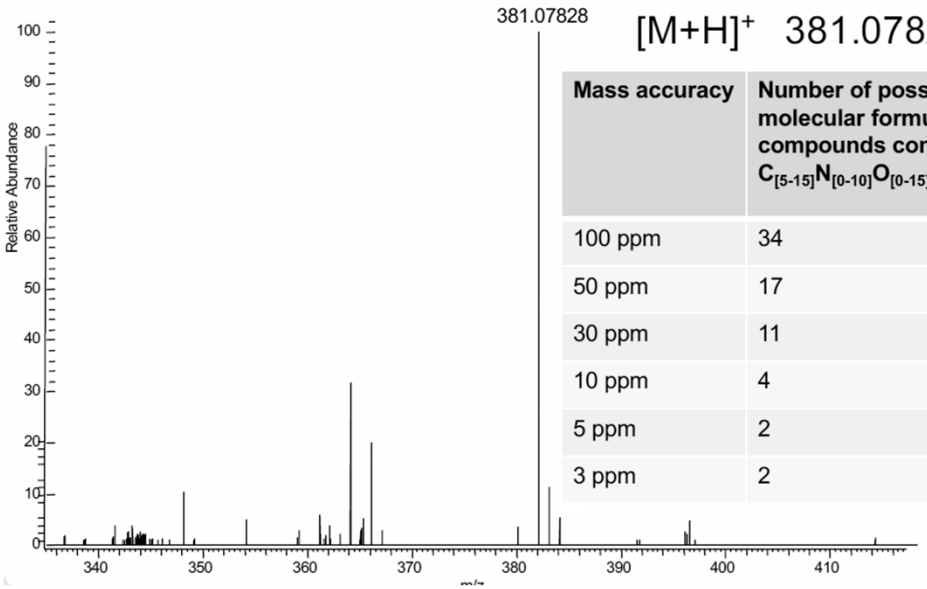
$$\frac{[\text{measured mass (u)} - \text{theoretical mass (u)}]}{\text{theoretical mass (u)}} \times 1000000$$

Measured Mass	Theoretical Mass	Mass Difference (u)	ppm error
200.0020	200.0000	0.002	$(0.002/200)1e^6 = 10$ ppm
400.0020	400.0000	0.002	$(0.002/400)1e^6 = 5$ ppm
800.0020	800.0000	0.002	$(0.002/800)1e^6 = 2.5$ ppm
1000.0020	1000.0000	0.002	$(0.002/1000)1e^6 = 2$ ppm
		Constant with mass	Varies with mass

13

Mass Analyzer	Mass Range (u)	Mass Resolution	Mass Accuracy (ppm)
Fourier Transform Ion Cyclotron Resonance (FT ICR)	30.000	1.000.000+	< 1 (@ 400 u)
Orbitrap	50.000	500.000	< 2 (@ 100-2000)
Magnetic Sector (BE)	20.000	100.000	< 10
Time-of-Flight (TOF, RTOF)	> 1.000.000	5.000-20.000	200; 5-10
Quadrupole (Q)	4.000	2.000	100
Iontrap (IT)	6.000	4.000	100

14



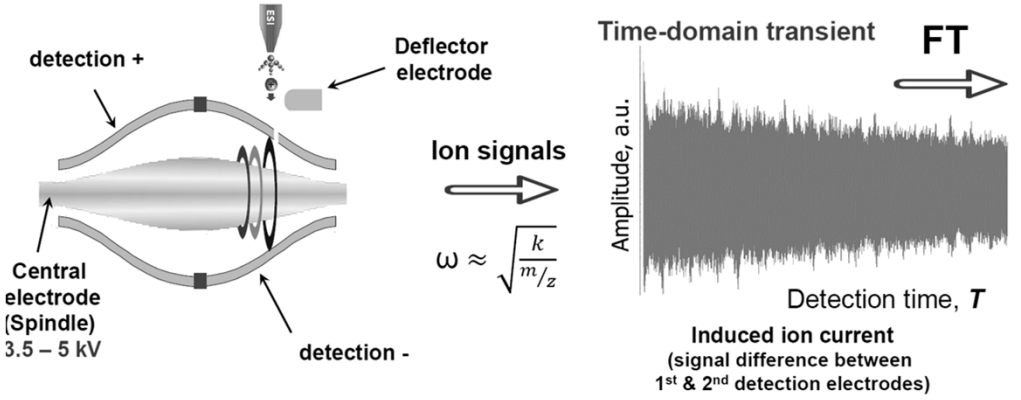
Mass accuracy	Number of possible molecular formulae for compounds containing $C_{[5-15]}N_{[0-10]}O_{[0-15]}H_{[0-40]}$
100 ppm	34
50 ppm	17
30 ppm	11
10 ppm	4
5 ppm	2
3 ppm	2

15

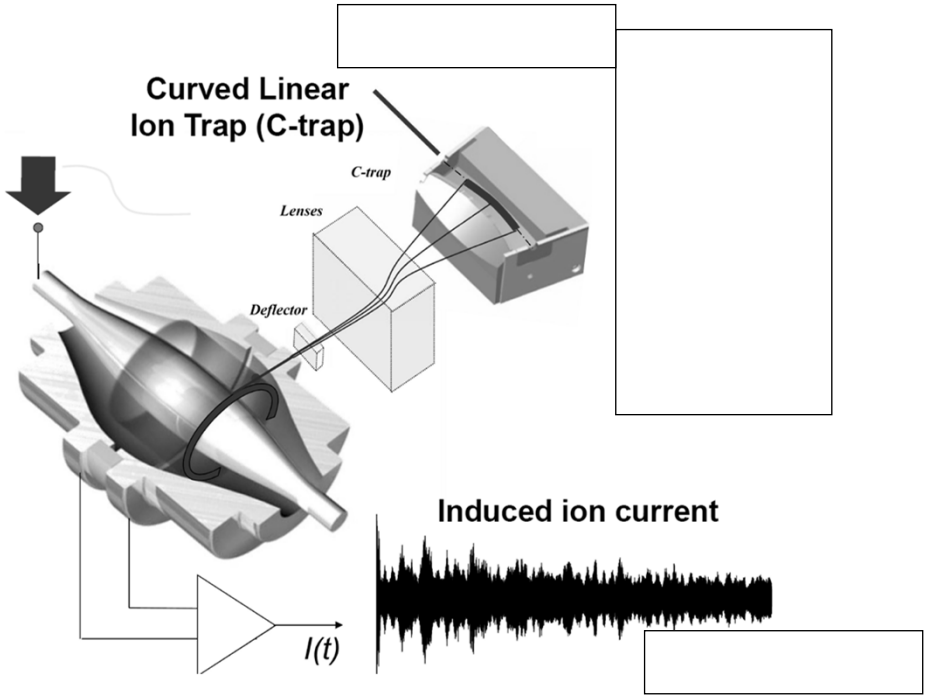
ANALIZZATORI DI MASSA AD ALTA RISOLUZIONE

16

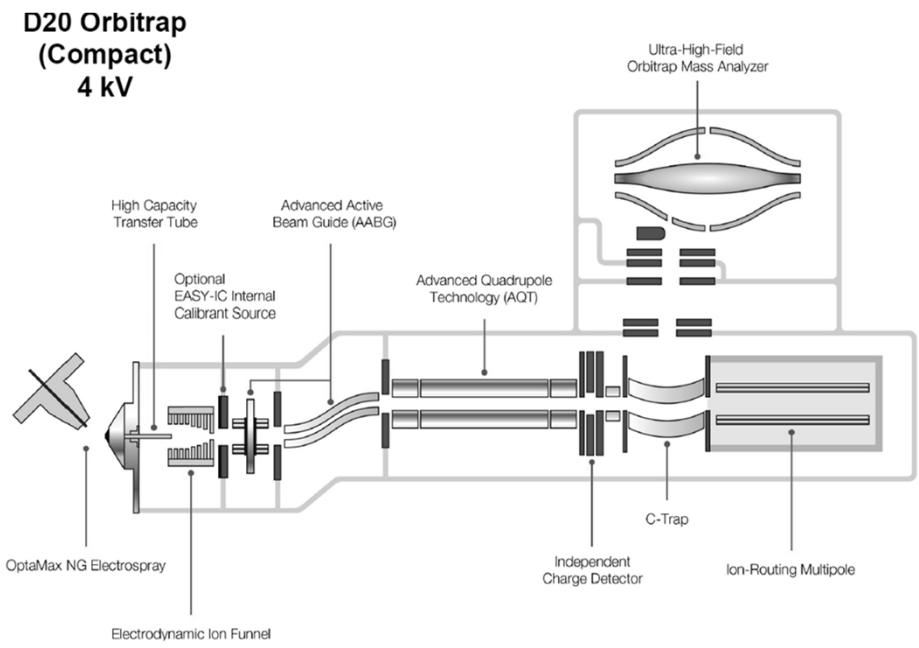
ORBITRAP



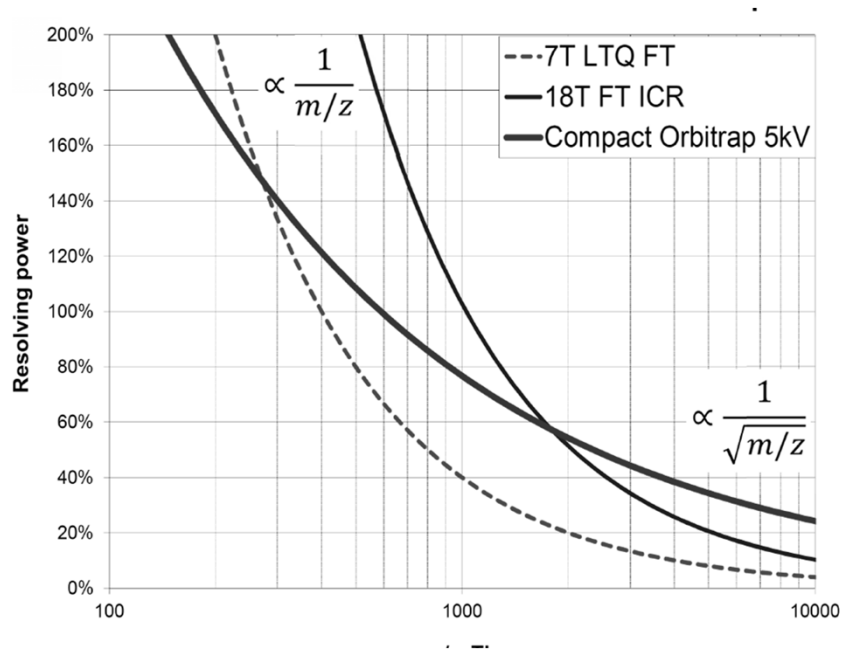
17



18


D20 Orbitrap
(Compact)
4 kV
Ultra-High-Field
Orbitrap Mass Analyzer
High Capacity
Transfer Tube
Advanced Active
Beam Guide (AABG)
Optional
EASY-IC Internal
Calibrant Source
Advanced Quadrupole
Technology (AQT)
C-Trap
OptaMax NG Electrospray
Electrodynamic Ion Funnel
Independent
Charge Detector
Ion-Routing Multipole

19


Resolving power
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
100
1000
10000
$\propto \frac{1}{m/z}$
$\propto \frac{1}{\sqrt{m/z}}$
7T LTQ FT
18T FT ICR
Compact Orbitrap 5kV

20

TIME OF FLIGHT

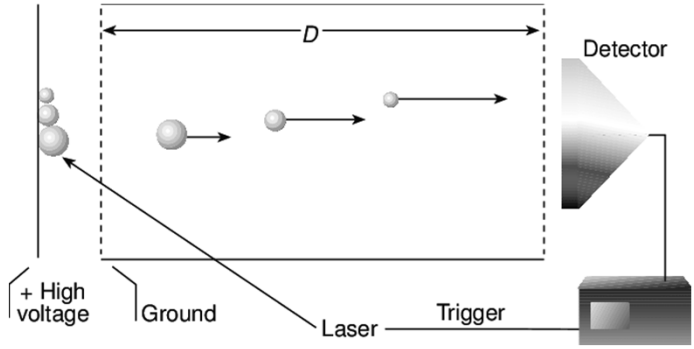
$E_p = qU$

$E_k = \frac{1}{2}mv^2$

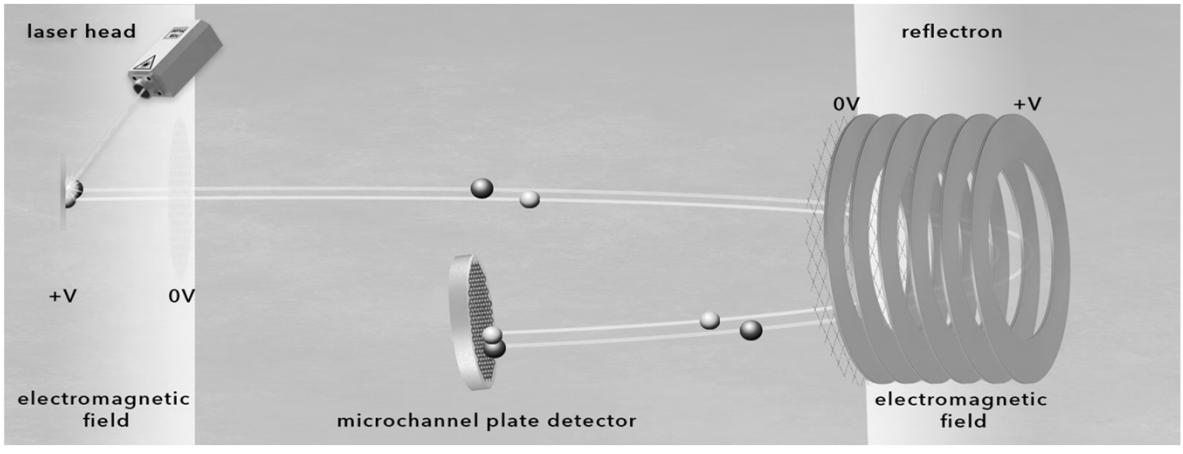
$E_p = E_k$

$qU = \frac{1}{2}mv^2 \qquad v = \frac{d}{t}$

$t = k\sqrt{\frac{m}{q}}$

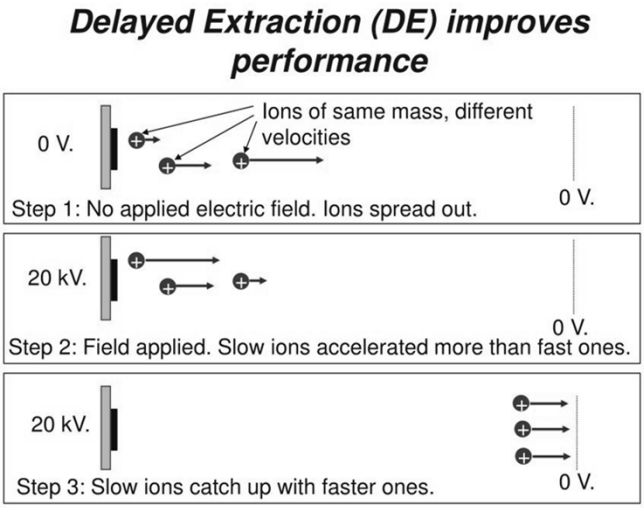


21



22

DELAYED EXTRACTION



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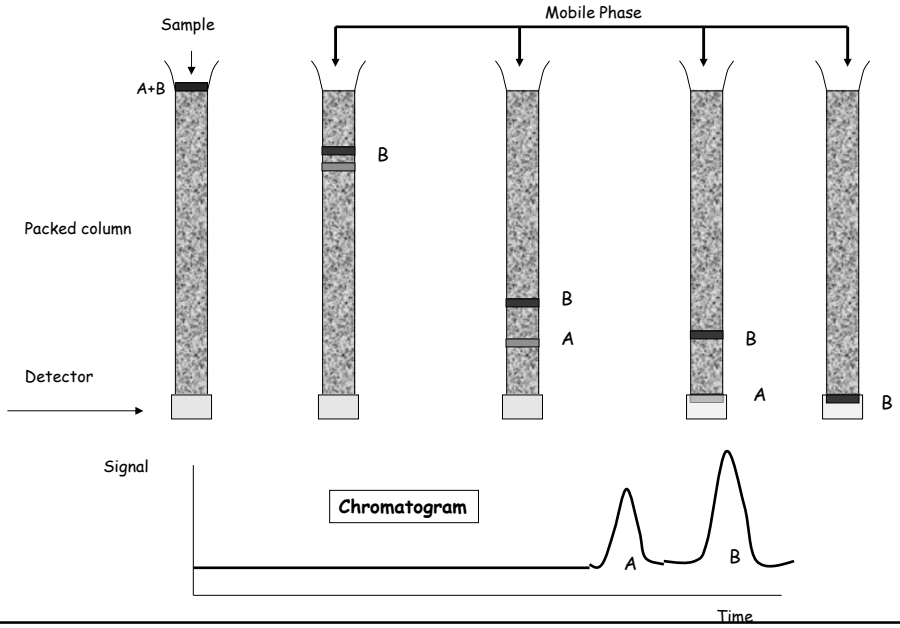
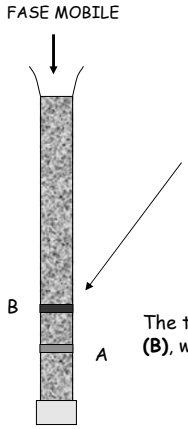


24



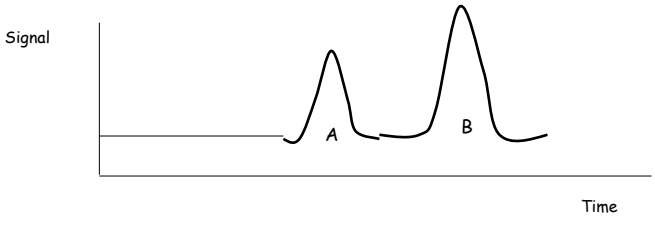
1. Movement along the column occurs only when the solute is in the mobile phase
2. Thus, the velocity depends on the time the solute spends in the mobile phase

The time fraction is small for **highly retained compounds (B)**, while it is large for **poorly retained compounds (A)**.

The partitioning of a solute between the stationary phase and mobile phase then determines elution times, and whether complex mixtures can be separated.

25

Chromatogram

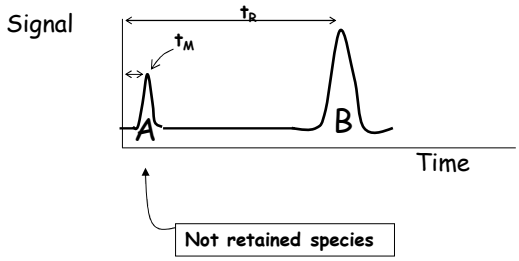


QUALITATIVE Information → PEAK POSITION

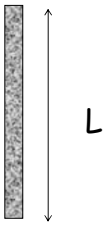
QUANTITATIVE Information → PEAK DIMENSION (HEIGHT AND WIDTH)

NEED TO HAVE WELL-DEFINED PEAKS

26



t_R: Time from the injection of a sample into a column to the appearance of a peak on the chromatogram

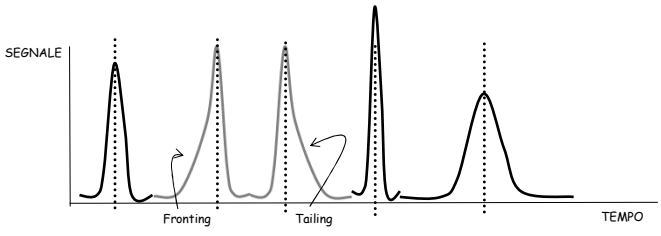


Linear average velocity of solute and mobile phase

$$v = L/T_R$$

$$u = L/T_M$$

Peak geometry and column efficiency

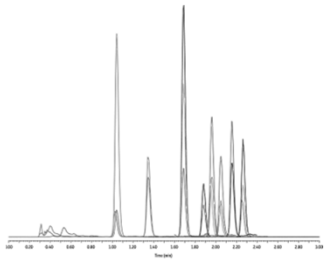


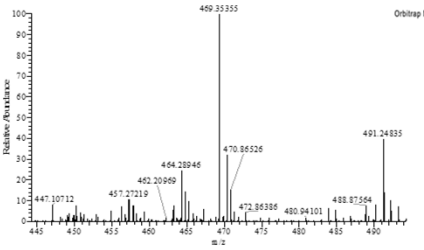
Tailing and fronting are produced by a nonlinear distribution coefficient

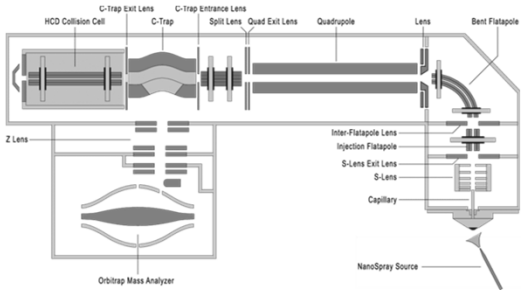
UHPLC-HRMS(/MS)

Identification by:

- ✓ Retention Time
- ✓ Mass Spectrum
  - Accurate Mass
  - Parent Ion
  - Fragment Ions
  - Fragment intensity ratio







Acquisition Mode:

- ✓ Full Scan
- ✓ HRMS/MS
- ✓ Data-Dependent Scan (FS-ddA)
- ✓ Selected Ion Monitoring

29

Omics sciences

The omics sciences study pools of biological molecules (e.g., ions, nucleic acids, proteins, enzymes) with various functions within living organisms. These functions are related to the abilities, inherent in such molecules, to be able to transform (translation process) their structures and chemical and/or electrostatic bonds into energetic/biochemical processes aimed at creating other structures or interacting with other structures, with the ultimate goal of modifying/creating structures or functions different from the original ones.

The omics sciences thus have the primary goal of analyzing as a whole:

1. the genes contained in DNA (genomics) and their multiple functions (functional genomics);
2. the product of DNA transcription: the RNA (transcriptomics);
3. the proteins encoded by DNA through RNA (proteomics);
4. the molecules that interact within an organism (metabolites: metabolomics).

30

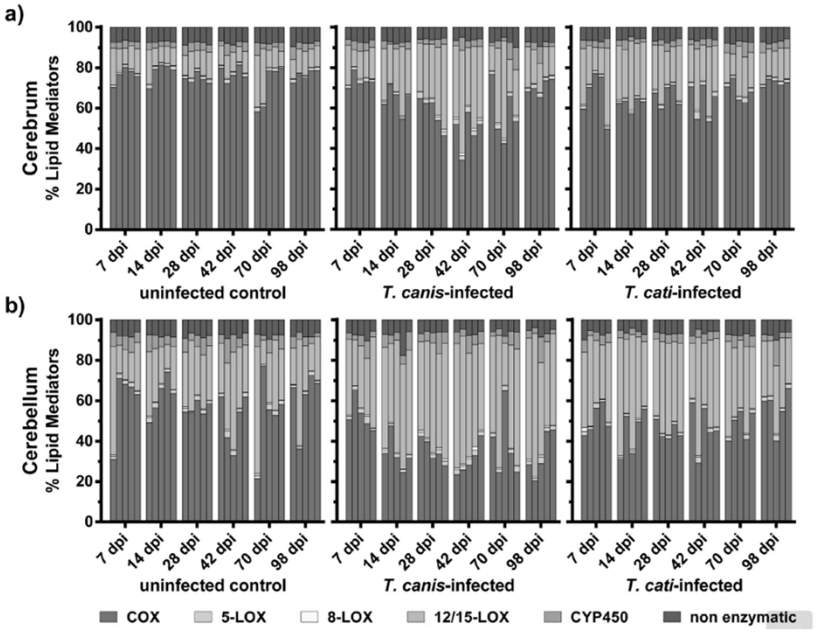
PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

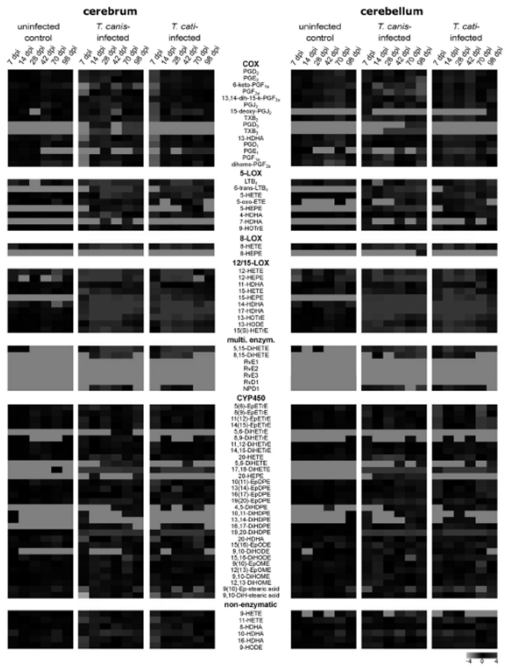
Multiplex profiling of inflammation-related bioactive lipid mediators in *Toxocara canis*- and *Toxocara cati*-induced neurotoxocarosis

Patrick Waindok[1], Elisabeth Janecek-Erfurth[1¤a], Dimitri Lindenwald[1¤b], Esther Wilk[2], Klaus Schughart[2,3], Robert Geffers[4], Laurence Balas[5], Thierry Durand[5], Katharina Maria Rund[6,7], Nils Helge Schebb[6,7], Christina Strube[1]*

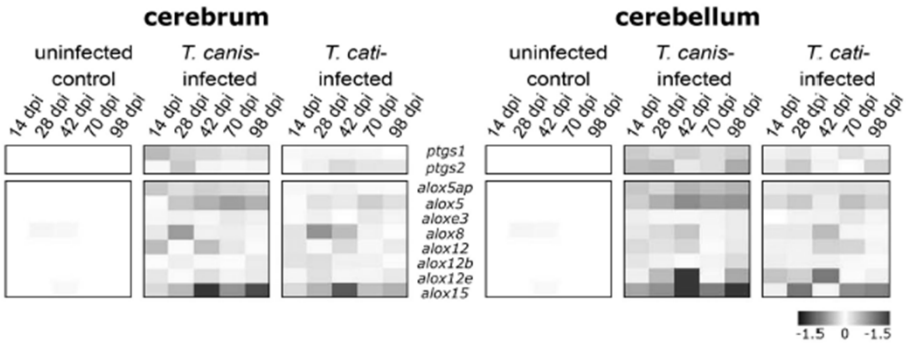
31



32


cerebrum
cerebellum
uninfected control
T. canis-infected
T. cati-infected
COX
5-LOX
8-LOX
12/15-LOX
multi. enzym.
CYP450
non-enzymatic

33


cerebrum
cerebellum
uninfected control
T. canis-infected
T. cati-infected
14 dpi
28 dpi
42 dpi
70 dpi
98 dpi
ptgs1
ptgs2
alox5ap
alox5
aloxe3
alox8
alox12
alox12b
alox12e
alox15
-1.5
0
-1.5

34

Journal of proteome research

Subscriber access provided by University of Newcastle, Australia

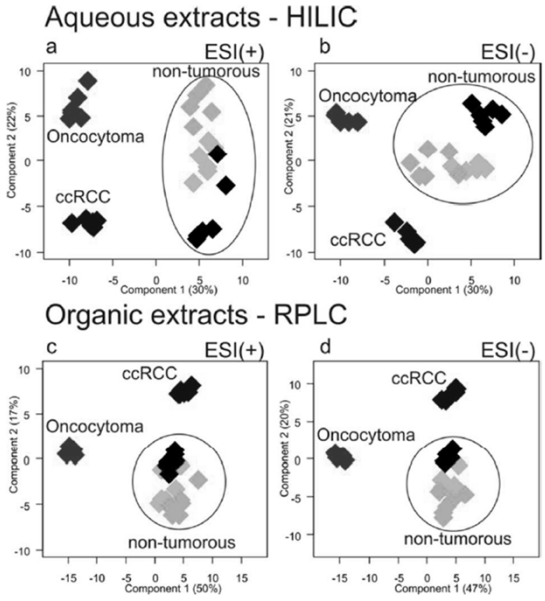
Article

Comprehensive metabolomic and lipidomic profiling of human kidney tissue: a platform comparison

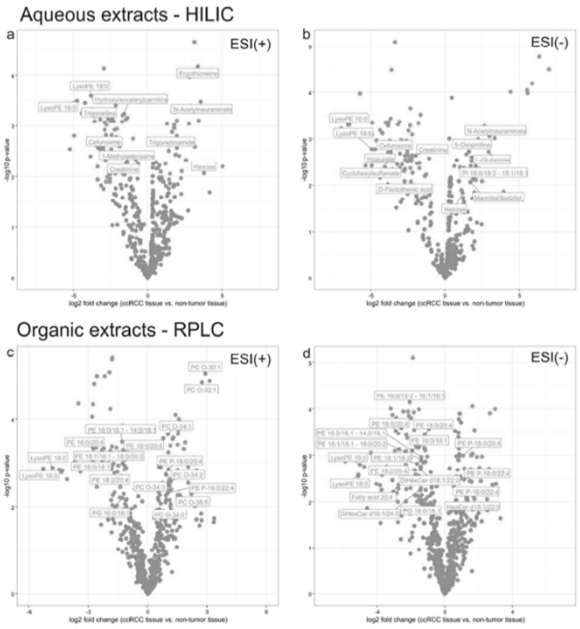
Patrick Leuthold, Elke Schaeffeler, Stefan Winter, Florian Büttner, Ute Hofmann, Thomas E Mürdter, Steffen Rausch, Denise Sonntag, Judith Wahrheit, Falko Fend, Jörg Hennenlotter, Jens Bedke, Matthias Schwab, and Mathias Haag

J. Proteome Res., **Just Accepted Manuscript** • DOI: 10.1021/acs.jproteome.6b00875 • Publication Date (Web): 19 Dec 2016

35



36



37

SCIENTIFIC REPORTS

nature research

OPEN

LC-HRMS based approach to identify novel sphingolipid biomarkers in breast cancer patients

Priyanka Bhadwal[1], Divya Dahiya[2], Dhananjay Shinde[3], Kim Vaiphei[4], Raviswamy G. H. Math[5], Vinay Randhawa[1] & Navneet Agnihotri[1*]

38

Characteristics	
Number of patients	31
Age (Years)	
Median (Range)	50 (32–76)
≤50	16
>50	15
Tumor Type	
Ductal carcinoma *in situ* (DCIS)	1
Invasive ductal carcinoma	28
Metastatic carcinoma	2
Tumor subtypes	
ER+/PR+	20
ER+/PR−	6
Her2neu+	4
Triple Negative	1
Nodal Status	
Positive	15
Negative	16
Ki67 (%)	
≤30 (%)	25
>30 (%)	6
TNM Staging	
IA	8
IIA	10
IIB	3
IIIA	5
IIIC	3
IV	2

Table 1. Clinicopathological characteristics of patients with breast cancer.

Class	Fatty acid	Retention Time(min)	Molecular Formula	Observed Mass	Error (ppm)	Calculated Mass	MS/MS fragments (m/z)
Cer	(d18:1/12:0) **(IS)**	3.0	C30 H58 O3 N1	526.4484292	−1.76	481.4495	283,270,88
	(d18:1/16:0)	3.0	C35 H68 O5 N1	582.5112221	−1.93	537.5121	311,298,88
	(d18:1/18:1)	2.9	C37 H70 O5 N1	608.5272988	−2.63	563.5277	102
	(d18:1/23:2)	2.9	C43 H80 O5 N1	690.6053293	−1.49	631.5903	390,347,235
	(d18:1/24:1)	2.9	C43 H82 O5 N1	692.6212052	−2.32	647.6216	546,390,237
	(d18:2/16:0)	3.0	C35 H66 O5 N1	580.4956225	−2.11	535.4964	256,104
	(d18:2/23:0)	2.9	C42 H80 O5 N1	678.6055060	−2.22	633.6060	102
	(d18:2/24:1)	2.9	C43 H80 O5 N1	690.6053805	−2.00	645.6060	316,168
	(d18:1/25:0) **(IS)**	2.9	C44 H86 O5 N1	708.6525276	−2.30	663.6529	495,439,102
DHCer	(d18:0/16:0)	3.0	C34 H70 O3 N1	584.5269029	−2.06	539.5277	280,255,237
	(d18:0/18:1)	2.9	C37 H72 O5 N1	610.5425634	−1.91	565.5434	102
	(d18:0/21:2)	2.9	C41 H78 O5 N1	664.5900060	−1.97	605.5747	618,364
LacCer	(d18:1/12:0) **(IS)**	8.0	C42 H80 O13 N1	806.5628915	0.26	805.5551	190,146,102
SM	(d18:1/21:1)	7.2	C46 H90 O8 N2 P1	829.646159	−2.36	770.6302	392,168,78
	(d18:2/22:0)	7.2	C45 H90 O6 N2 P1	785.6525117	1.64	784.6458	184,86
	(d18:1/12:0) **(IS)**	7.3	C35 H72 O6 N2 P1	647.5118463	1.78	646.5050	184,102
DHSM	(d18:0/18:1)	7.2	C41 H84 O6 N2 P1	731.6055663	1.82	730.5989	184,102
	(d18:0/24:2)	7.1	C47 H94 O6 N2 P1	813.6838264	1.57	812.6771	184,86
	(d18:0/22:1)	7.2	C45 H91 O6 N2 P1	787.6683	1.27	786.6615	102

Table 3. Identification of sphingolipids in breast tissue using UHPLC in "Organic Phase Extract". IS- Internal Standard; CerP- Ceramide 1-Phosphate; So-Sphingosine/Sphinganine; S1P- Sphingosine 1-Phosphate; Cer-Ceramide, DHCer- Dihydro Ceramide; LacCer- Lactosyl Ceramide; SM-Sphingomyelin; DHSM-DihydroSphingomyelin.

39

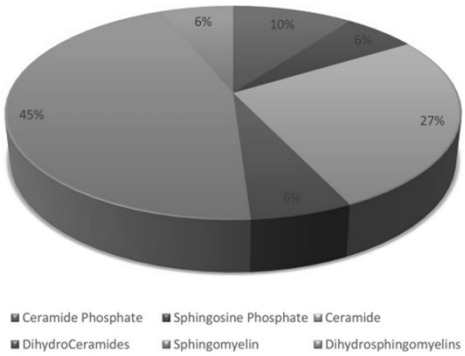


Figure 1. Pie chart representing the abundance of sphingolipid metabolites in breast tissue.

40

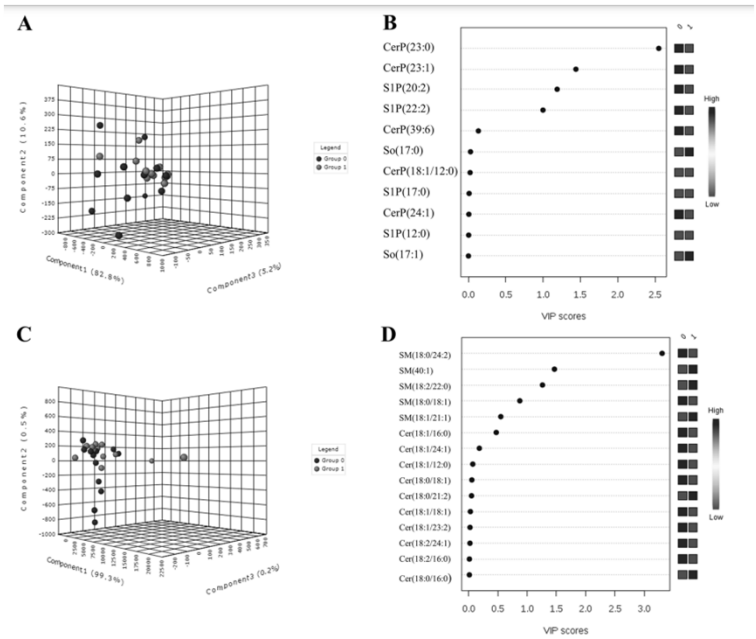


Figure 2. (**A**) 3D score plot (**B**) VIP score plot for sphingosine and ceramide phosphates and (**C**) 3D score plot (**D**) VIP score plot for ceramides and sphingomyelins. Group 0 (red) indicates tumor tissues and Group 1(green) indicates adjacent normal breast tissues.

41

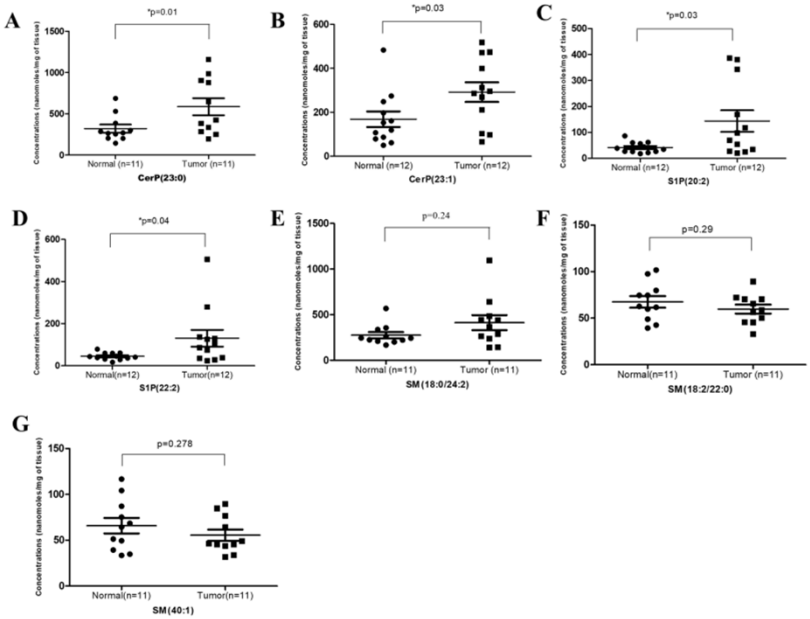


Figure 3. Levels of sphingolipid metabolites in breast tumor and adjacent normal tissue samples (**A**) CerP(23:0), (**B**) CerP(23:1), (**C**) S1P(20:2), (**D**) S1P(22:2), (**E**) SM(18:0/24:2), (**F**) SM(18:2/22:0), (**G**) SM(40:1).

42

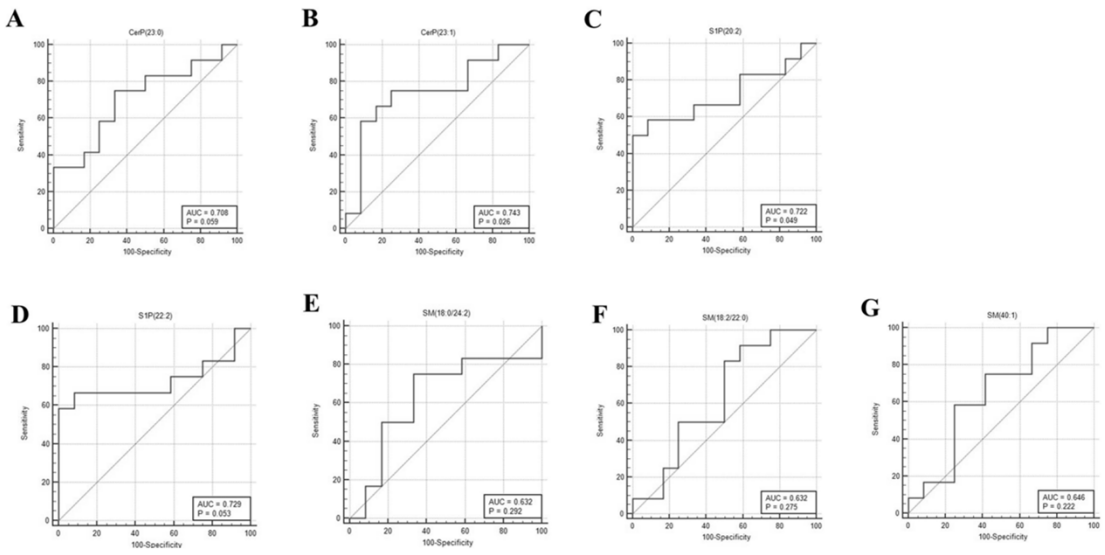


Figure 4. ROC curves for evaluating the diagnostic potential of sphingolipids. (**A**) CerP(23:0), (**B**) CerP(23:1), (**C**) S1P(20:2), (**D**) S1P(22:2), (**E**) SM(18:0/24:2), (**F**) SM(18:2/22:0), (**G**) SM(40:1).