

Introduction to Mass Spectrometry and Applications Overview

Denis Calnan
Business Development Manager
(Mass Spectrometry)
Waters Ireland






- Mass Spec overview
- Ionisation processes
- Types of instrument
- Areas of application
- Summary

Mass Spectrometry History - Microsoft Internet Explorer

Address: <http://www.dmr.bis.ac.uk/ns/history.html>

The History of Mass Spectrometry

The Five Mass Spectrometry Nobel Prize Pioneers

				
<p>Joseph John Thomson 1906 Nobel Prize for Physics <i>"in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases"</i></p>	<p>Francis William Aston 1922 Nobel Prize for Chemistry <i>"for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule"</i></p>	<p>Wolfgang Paul 1989 Nobel Prize for Physics <i>"for the development of the ion trap technique"</i></p>	<p>John Bennet Fenn 2002 Nobel Prize for Chemistry <i>"for the development of soft desorption ionisation methods (ESI, for mass spectrometric analysis of biological macromolecules"</i></p>	<p>Koichi Tanaka 2002 Nobel Prize for Chemistry <i>"for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analysis of biological macromolecules"</i></p>

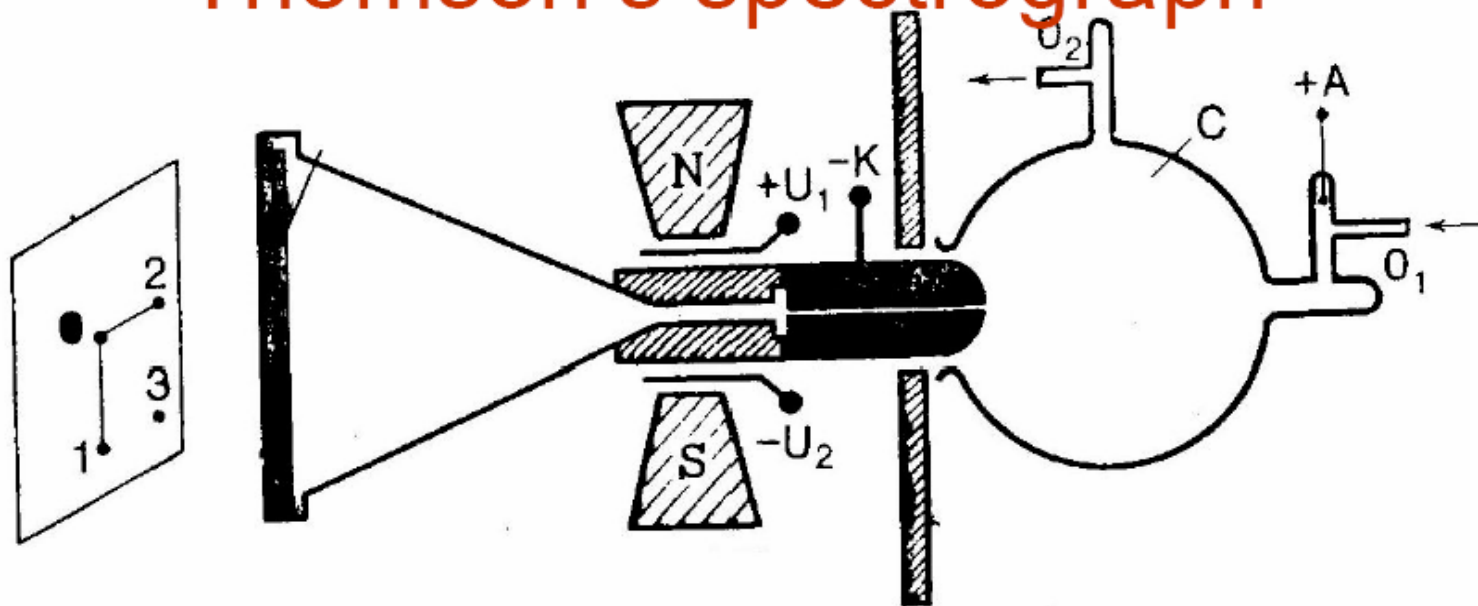
The foundations of mass spectrometry lie in the work of Thomson and Aston at the Cavendish Laboratories, Cambridge University. From 1897, the work carried out by Thomson and his co-workers received Nobel prizes in Physics and Chemistry. Thomson's original work on the existence and properties of canal rays (positive ions) was taken up by Aston and by the end of the First World War he had demonstrated the existence of several isotopes of non-radioactive elements. Aston used electrostatic and magnetic fields to separate isotope ions by their masses and focus them onto a photographic plate.

Internet

Brief History of MS

- The ability to separate molecules based on mass / charge ratio , m/z was described by J.J. Thomson, 1912 (Nobel Prize 1906)
- 1920's Electron Ionisation (EI)
- 1940's First commercial Mass Spectrometers used in the petrochemical industry

Thomson's spectrograph



“I feel sure that there are many basic problems in chemistry which could be solved with far greater ease with this than with any other method. The method is surprisingly sensitive – more so even than the method of spectrum analysis, requiring an infinitesimal amount of material, and does not require this to be specially purified.”

Joseph John Thomson, 1913

The Rays of Positive Electricity and their Applications to Chemical Analysis

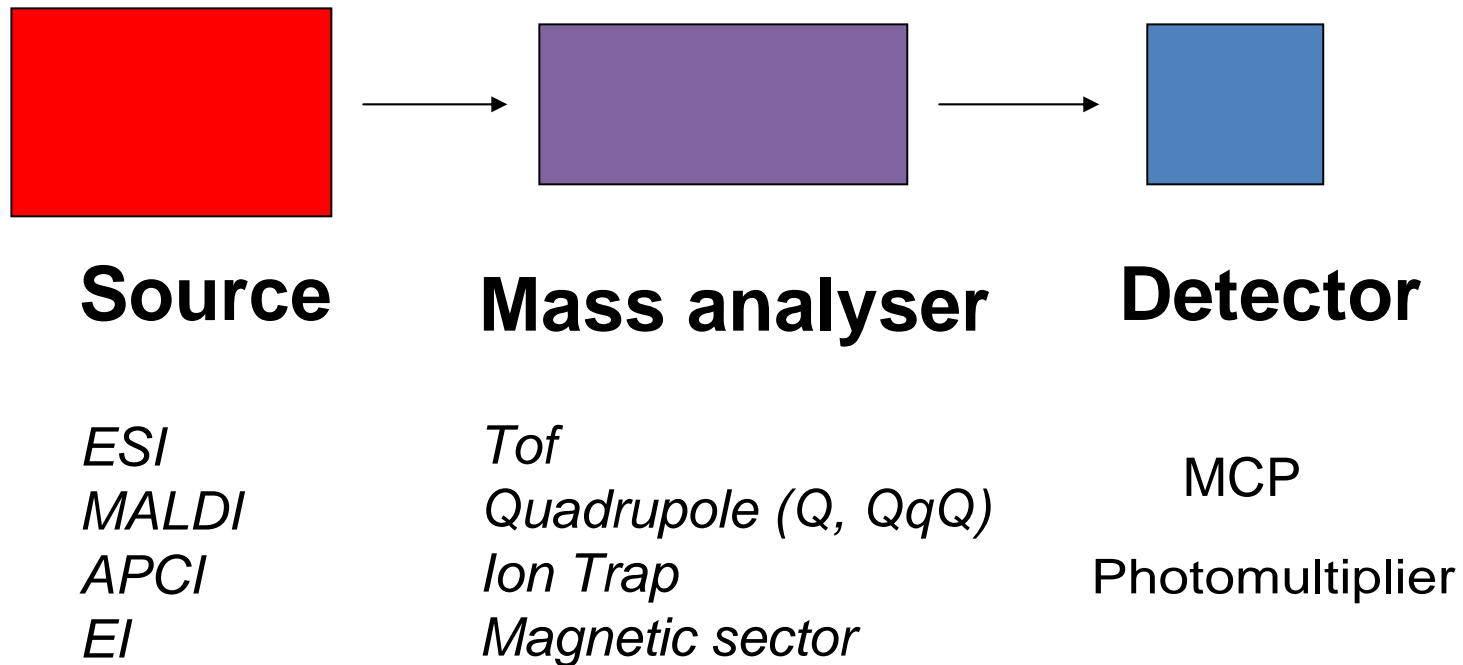
- 1950's first "Time of Flight" (Tof) mass spectrometers
- 1960's Chemical Ionisation introduced
- 1981 Fast Atom Bombardment (FAB) Barber *et al*

Brief History of Mass Spectrometry

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- 1988 “Soft Ionisation” techniques emerged
- Koichi Tanaka –soft laser desorption (leading to MALDI)
- John Fenn – Electrospray Ionisation (ESI)
- This work revolutionised Biological Mass Spectrometry
- Both received the Nobel prize for Chemistry in 2002

“Typical Layout” Mass spectrometer



■ **Biological**

- Proteomics
- Clinical
- Microbes

■ **Pharmaceutical**

- Drug discovery
- QC in production
- Validation

- **Industrial**
- Petrochemicals
- Organic synthesis
- Dyes, perfumes

■ Environmental

- Food contaminants
- Water analysis
- Pesticides
- Dioxins (PCBs)

The significance of Soft Desorption Ionisation development

A fundamental problem in biological mass spectrometry was how to transfer highly polar, non volatile molecules with a mass of tens of kDa into the gas phase without destroying them

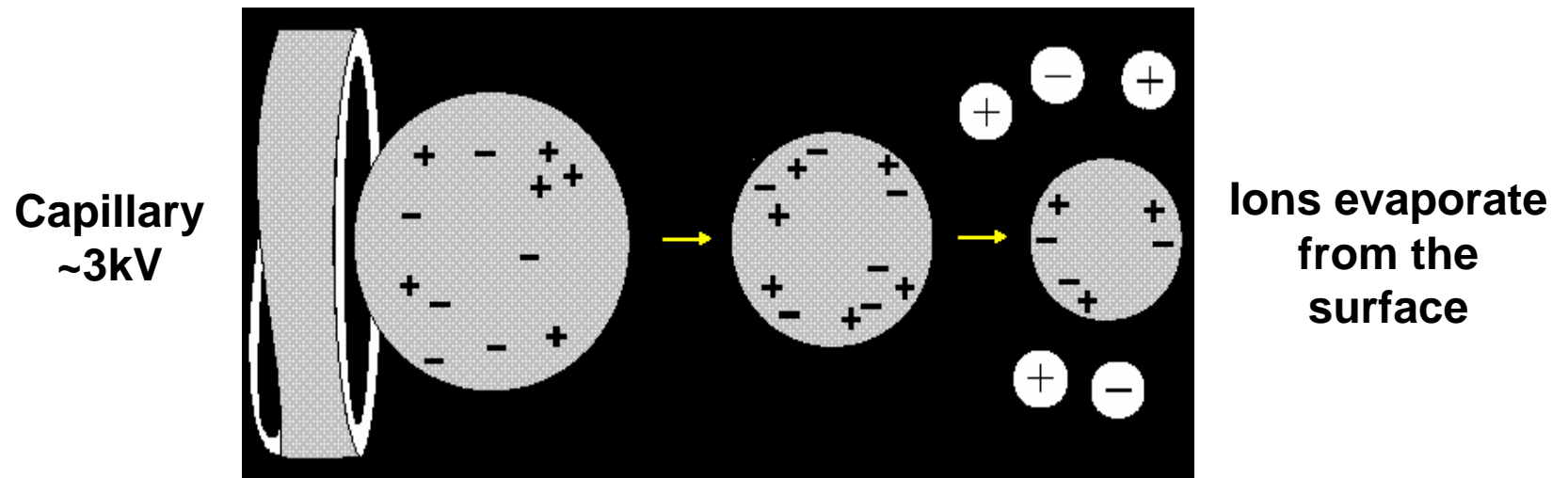
- Fenn and co workers built upon Malcolm Dole's early description of the electrospray principle
- Fenn combined Electrospray Ionisation with quadrupole MS
- This work was presented at the ASMS in 1988 and later published in a landmark paper in 1989 *Science* vol 246
- Multiply charged ions gave rise to spectra identifying proteins >100kDa



Mechanism (*in simple terms*)

- A solution containing sample molecules is electrostatically sprayed through a fine capillary by applying a high electrical potential difference
- Highly charged droplets are produced, the surrounding solvent evaporates and the charge density in the droplet increases
- This leads to the formation of “naked”, highly charged molecules (charge state from +1 up to +40 and beyond for large proteins)

Electrospray Ionisation Theory



As droplet evaporates, the electric field increases and ions move towards the surface.

m/z

- Molecular ions +ve or -ve
- Mass to charge ratio

“m/z value”

Example Peptide Glufib
Molecular Mass 1569 Da

$[M+H]^+ = 1570.6 \text{ m/z}$

$[M+2H]^{2+} = 785.8 \text{ m/z}$

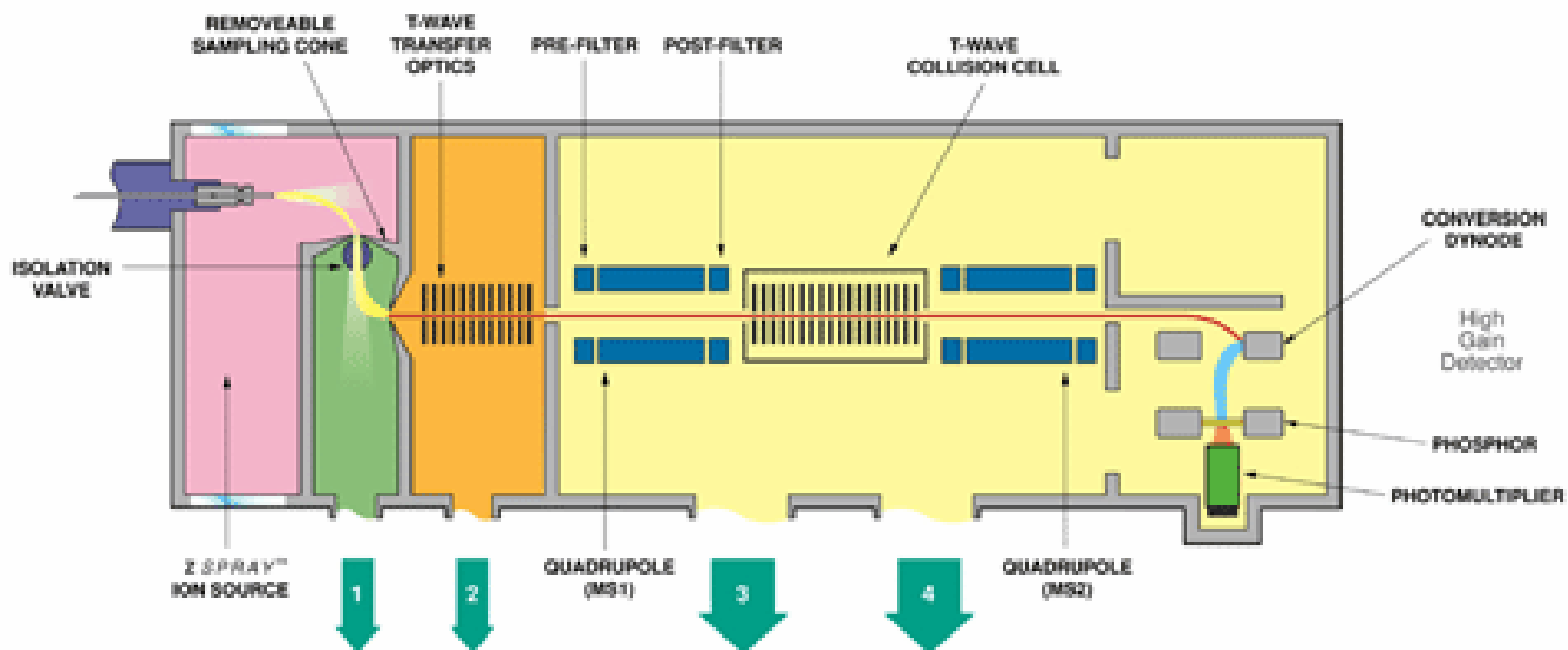
Quattro premier XE

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Tandem Quadrupole

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



AutoSpec Premier

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



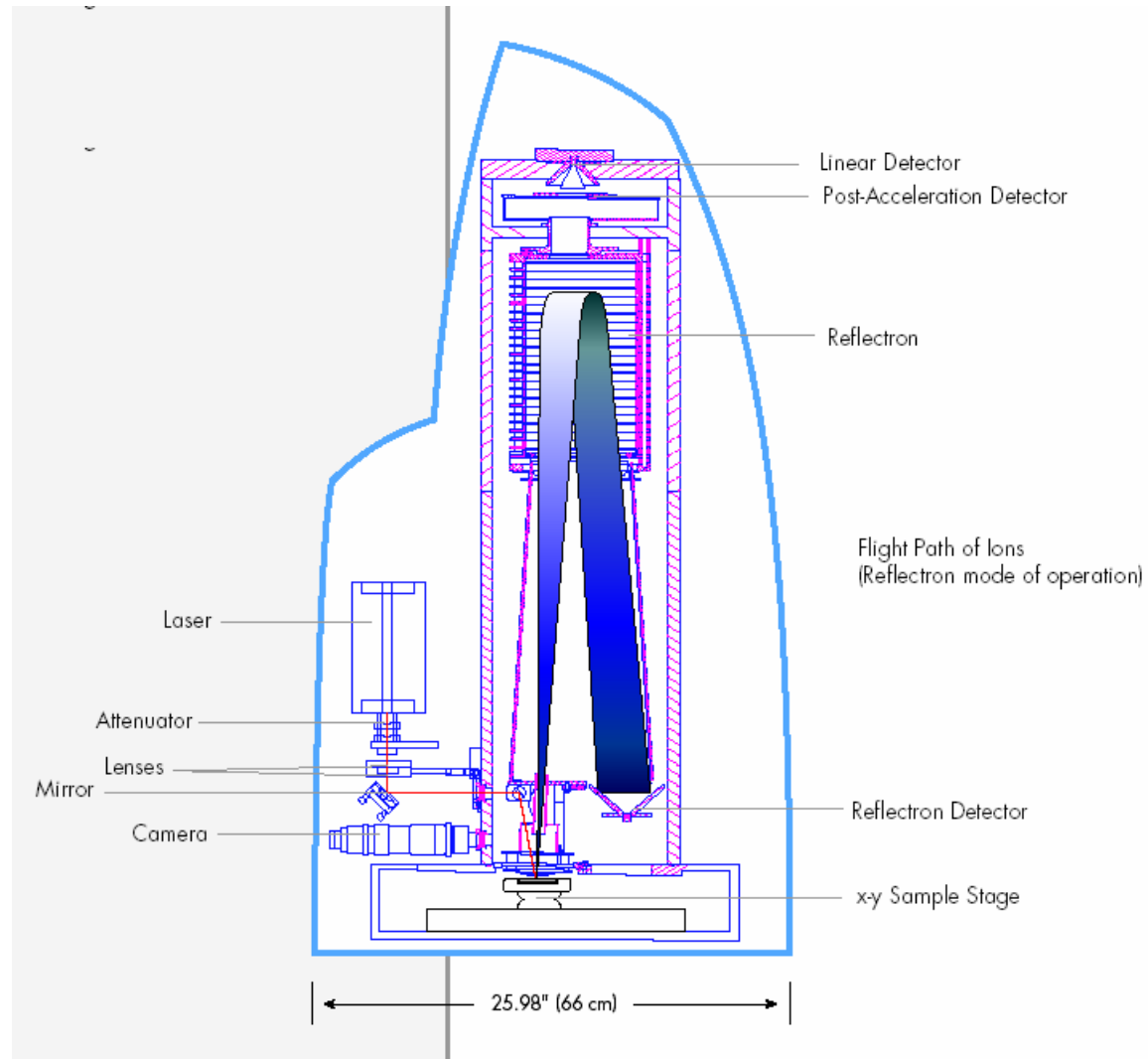
MALDI-ToF MS

MALDI micro MX™



Maldi Micro MX

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



- **Time-of-Flight Mass Spectrometry – TOF MS**
 - The mass of ions is determined by accelerating them and measuring their time-of-flight over a known distance
 - Put simply **heavy** ions fly slowly, **light** ions fly quickly

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

- Nominal mass: The mass of an ion calculated using the integer mass of the most abundant isotope of each element
 - Neglects the mass defect, where H=1, C=12, O=16
- Monoisotopic mass: The mass of an ion calculated using the exact mass of the most abundant isotope of each element
 - Includes the mass defect, where $^1\text{H}=1.0078$, $^{12}\text{C}=12.0000$, $^{16}\text{O}=15.9949$

Why Accurate Mass?

- Confirmation of elemental composition
 - Identification of unknown compounds
 - Patent support and scientific journals
- Additional dimension of specificity
 - Quantitation in accurate mass MS mode rather than MS/MS mode to reduce chemical interferences
 - Differentiation of nominal isobars in combinatorial libraries
 - Improved protein database search results
 - Improved de novo protein sequencing results

Accurate Mass Measurement Theory

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Accurate mass measurements take advantage of the fact that the combination of elements contained in a molecule have a very specific, non-nominal molecular weight

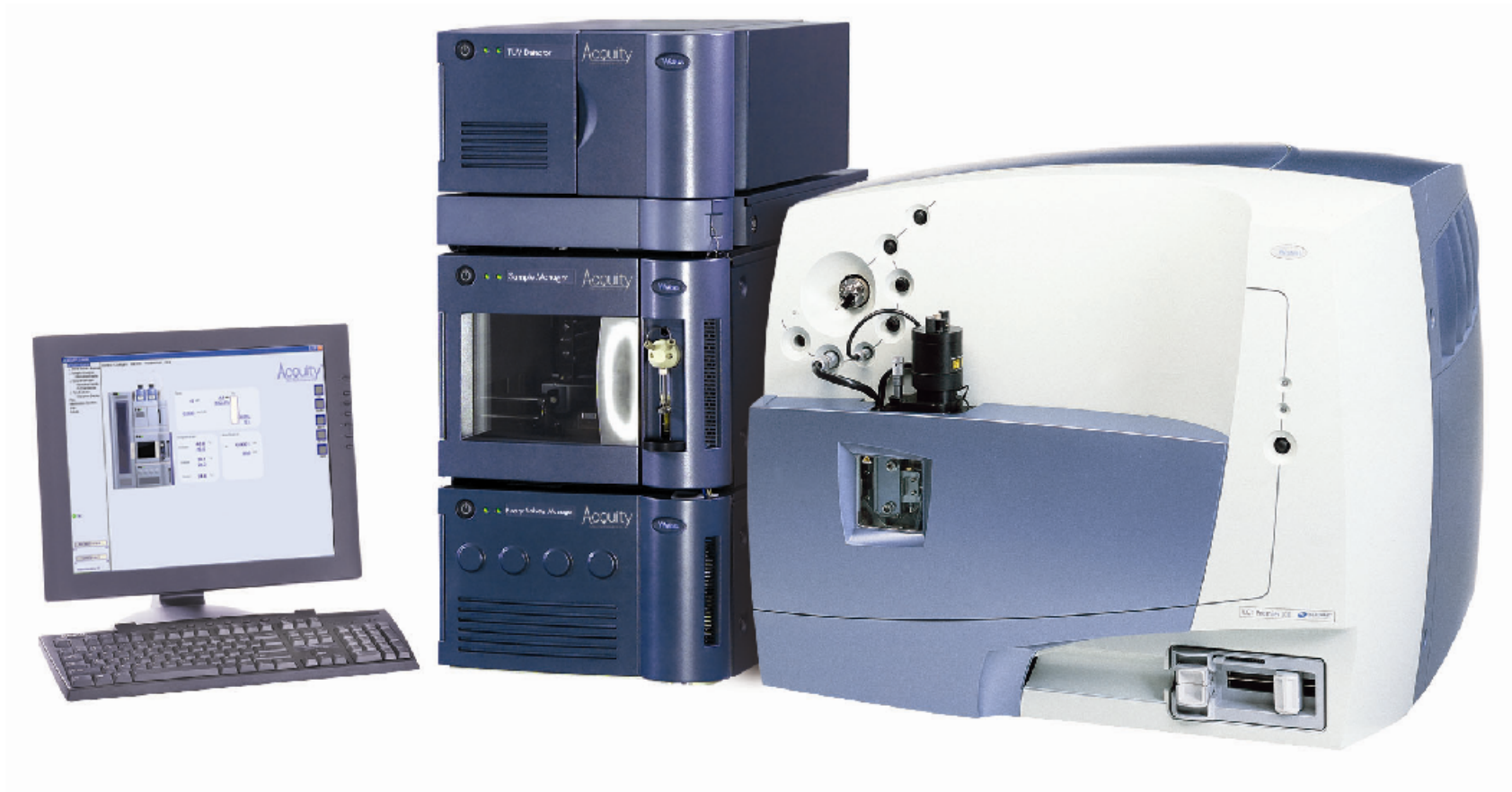
The Fundamentals of Accurate Mass

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Carbon has a mass of 12.0000
- Hydrogen has a mass of 1.0078
- Oxygen has a mass of 15.9949
- Nitrogen has a mass of 14.0031

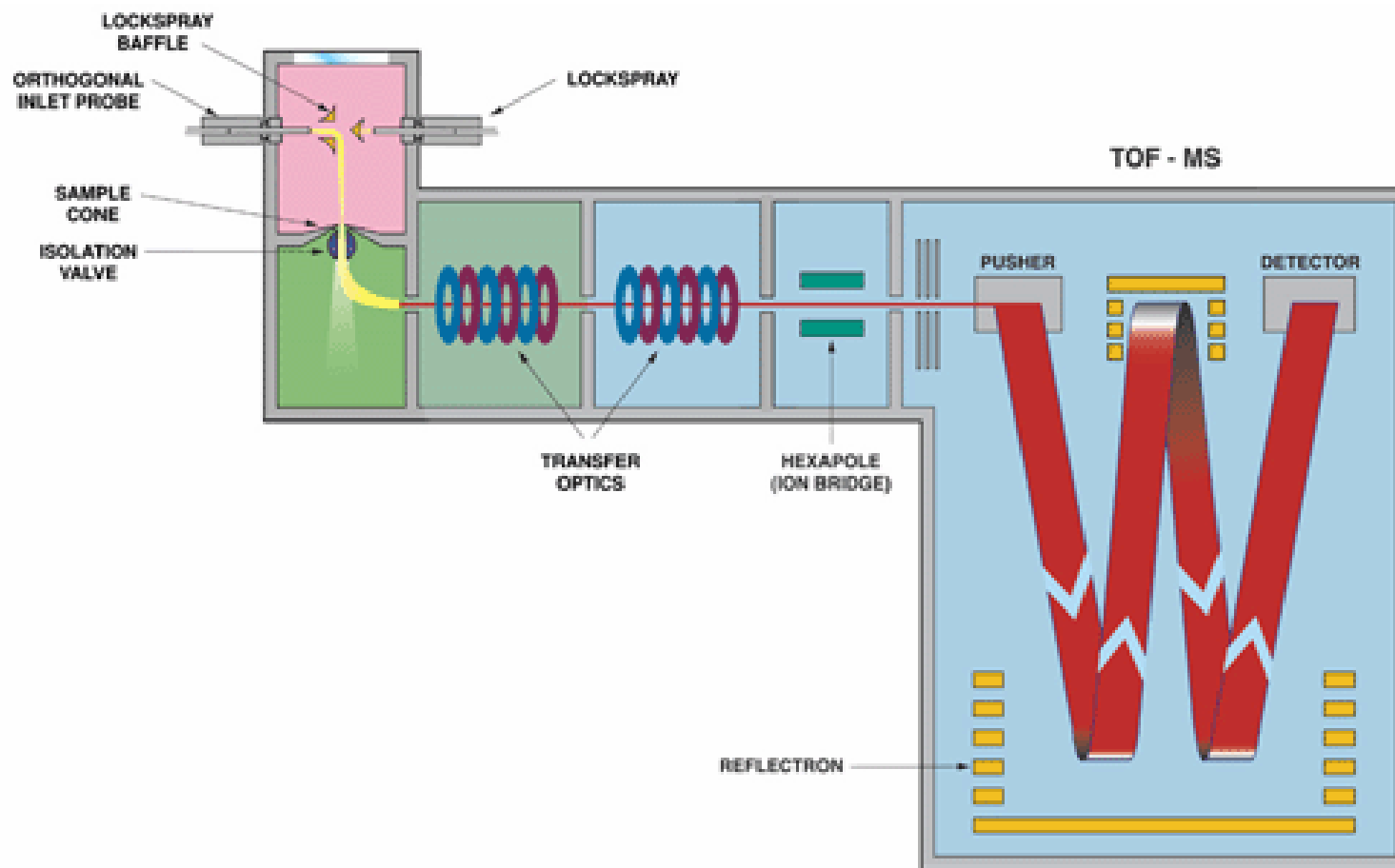
LCT Premier XE

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



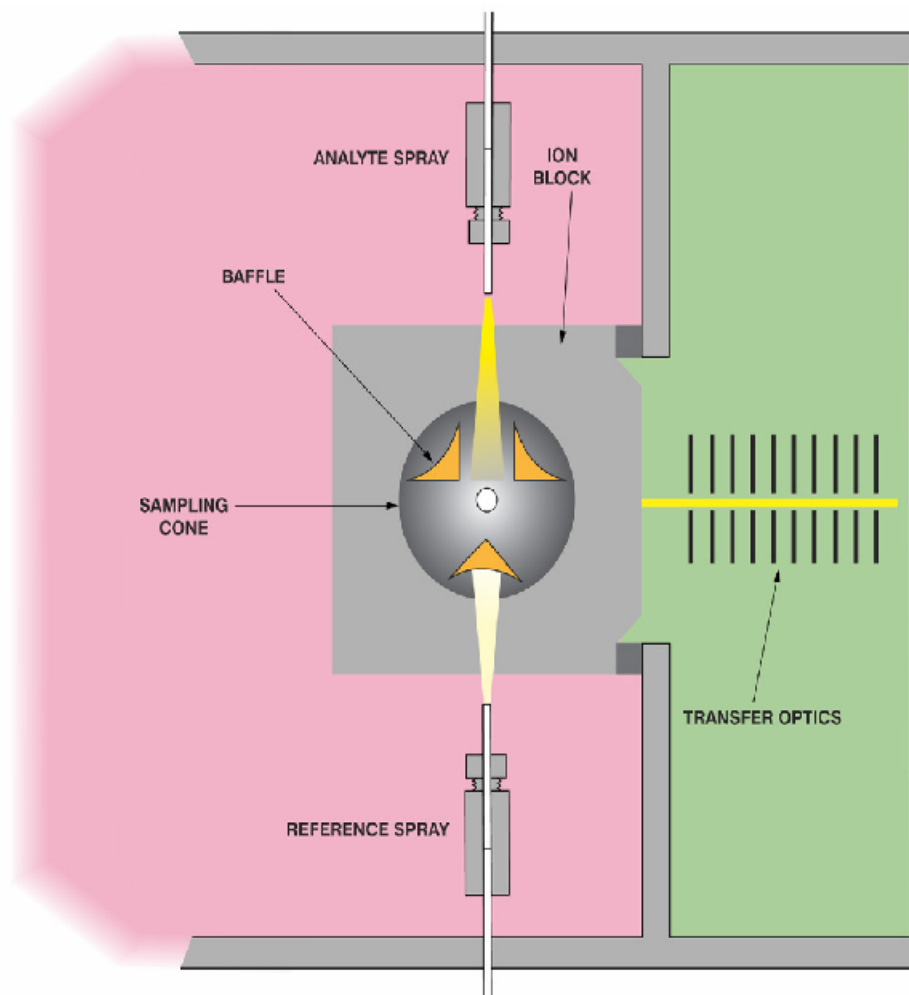
LCT Premier schematics

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



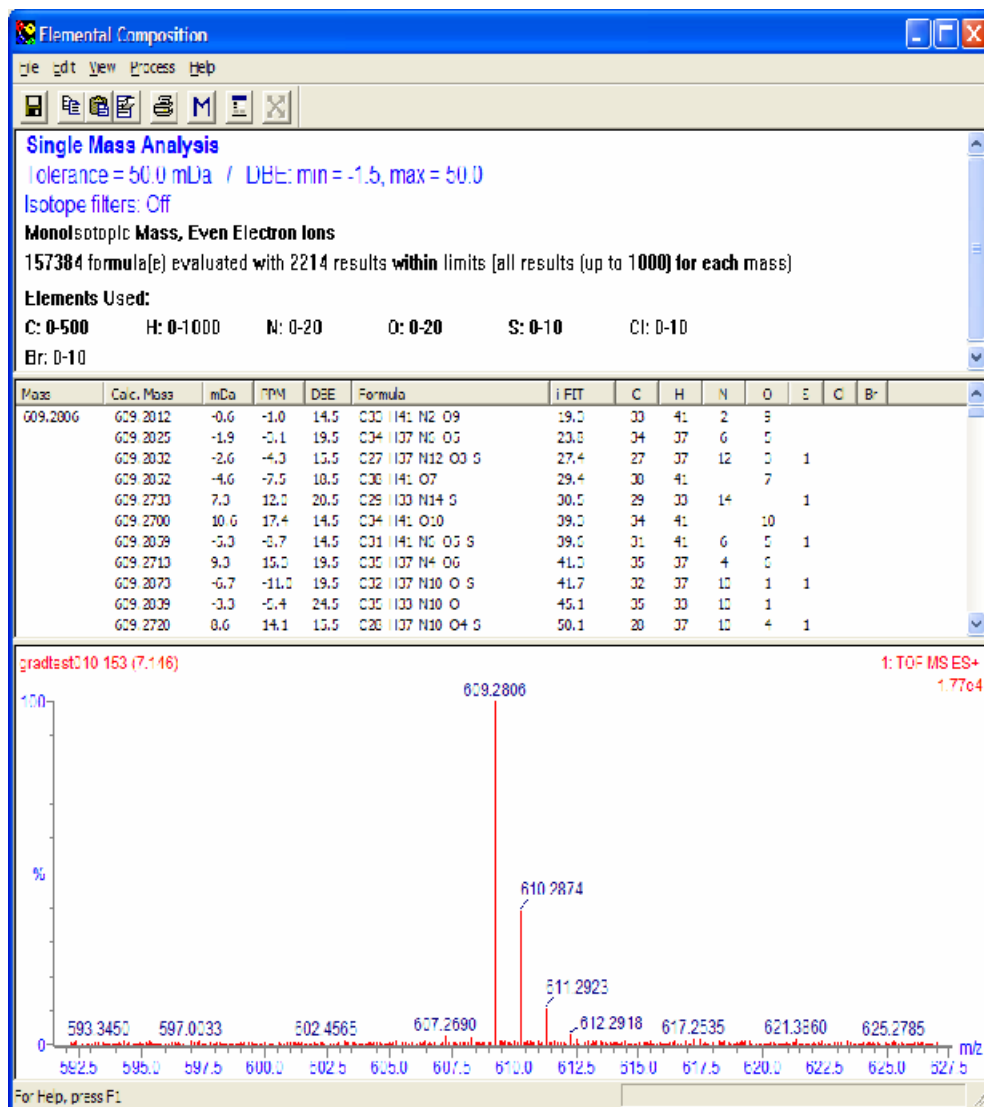
Integrated LockSpray Ion Source

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Example 1 Nominal Mass Measurement

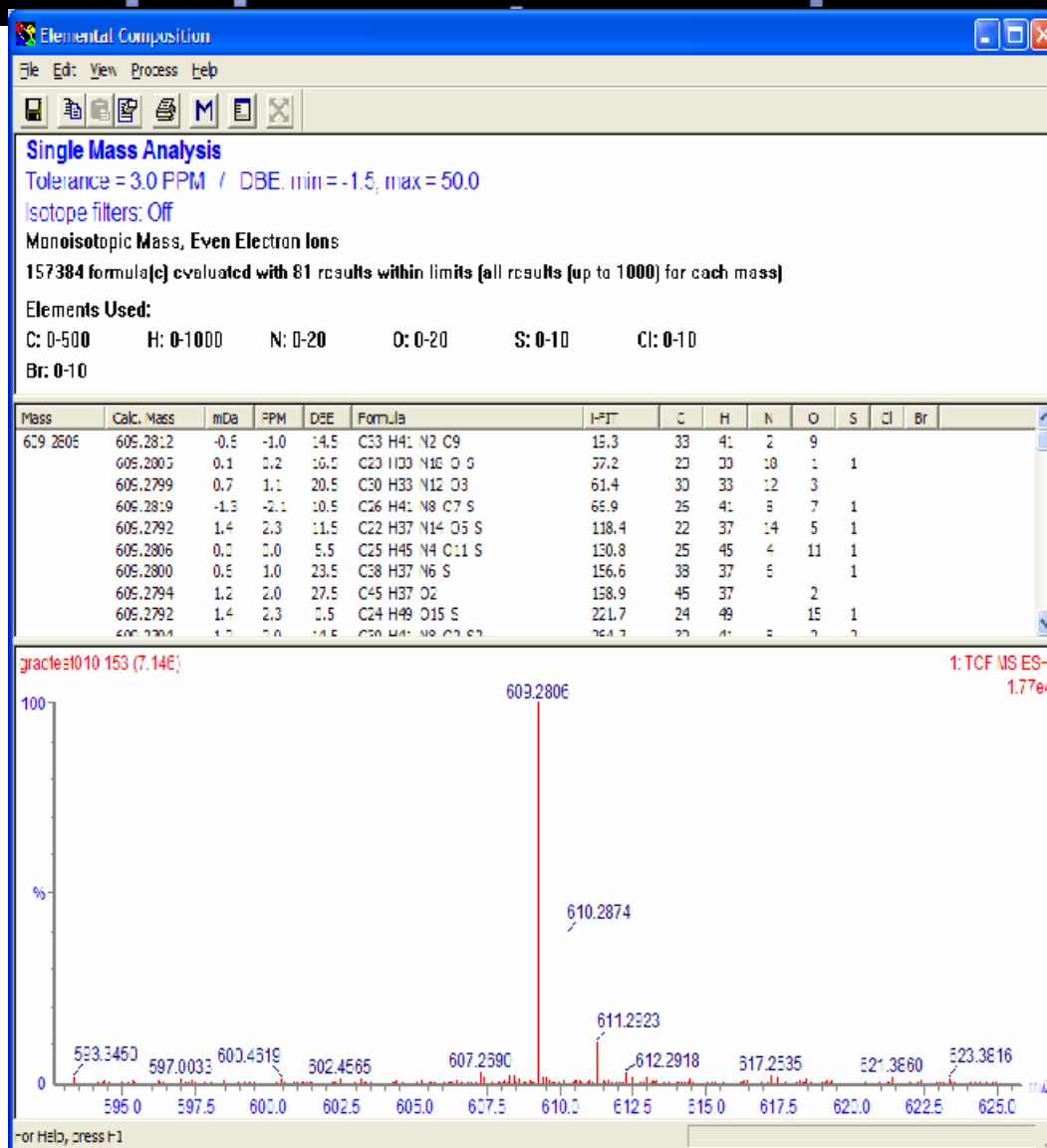
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



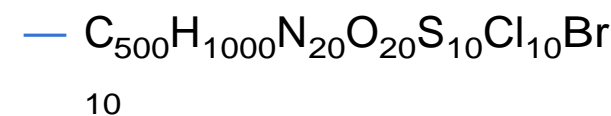
- Nominal mass measurement doesn't provide data specificity
- At m/z609, with 0.1 Da error around mass, **2214** possible combinations
- Using wide range of elements:
 - $C_{500}H_{1000}N_{20}O_{20}S_{10}Cl_{10}Br$
 - 10



Example 1 i-FIT Filters Off (3ppm)

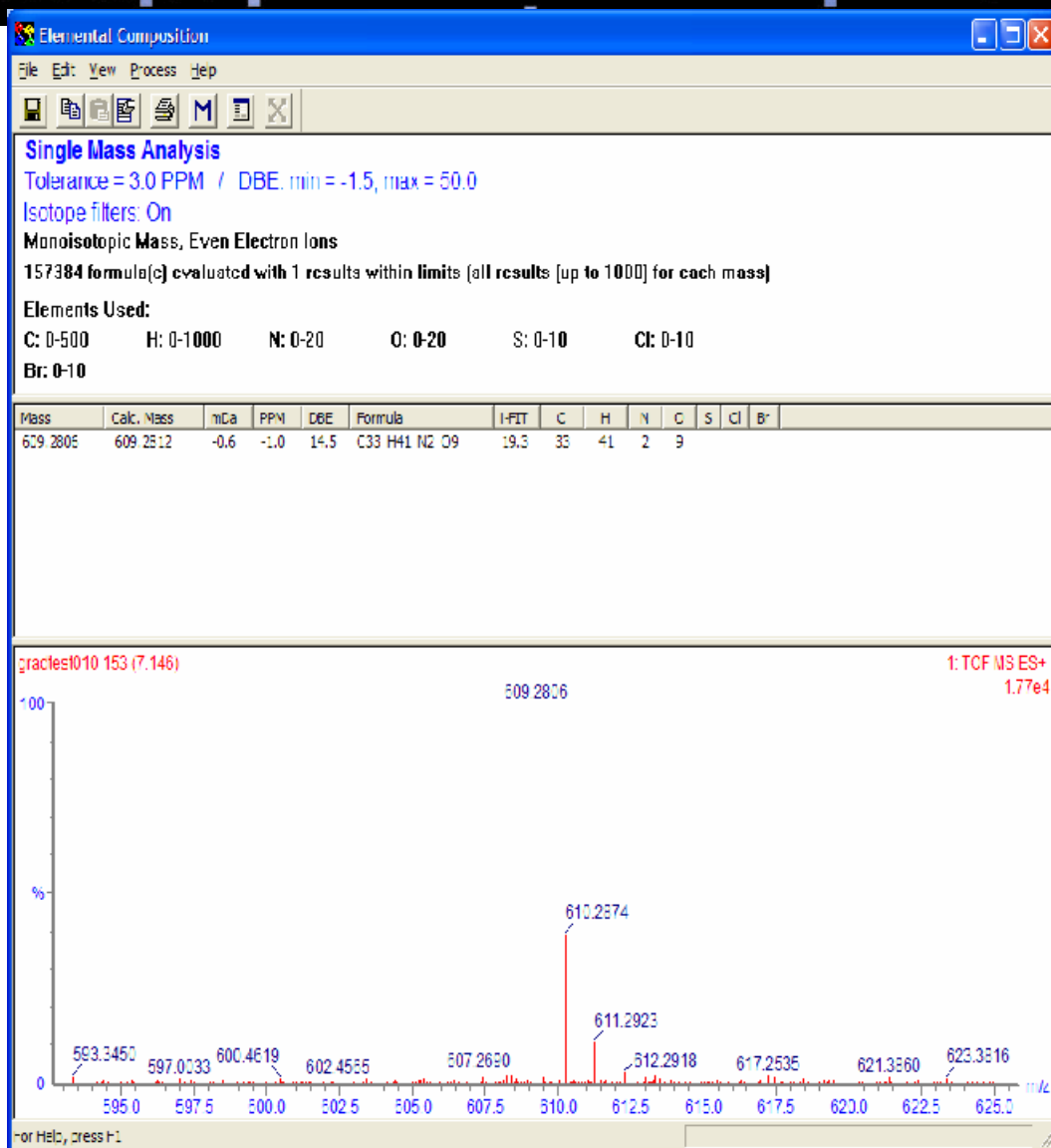


- At m/z609, with 3ppm error around mass, **81** possible combinations
- Using wide range of elements:



Example 1 i-FIT Filters On (3ppm)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



- At m/z609, with 3ppm error around mass, only 1 possible combination
- Using wide range of elements:
 - $C_{500}H_{1000}N_{20}O_{20}S_{10}Cl_{10}Br_{10}$
- Isotope Filters turned on
 - 3% instrument error
 - Carbon range = +/- 3



- LCT Premier XE - simplified exact mass with high sensitivity over a wide dynamic range
 - Ideal for screening applications
- Wizard driven set up procedures for easy operation
- Complete exact mass ionisation coverage with dedicated LockSpray for ESI, ESCi and APci
- i-FIT – realisation of exact mass measurement
 - Simplification of elemental composition results through isotope interrogation and data interpretation
- System solutions with OpenLynx, ChromaLynx, MarkerLynx and MetaboLynx

GCT Premier

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



ACQUITY UPLC™ QTOF Premier™ System

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



MALDI-TOF

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



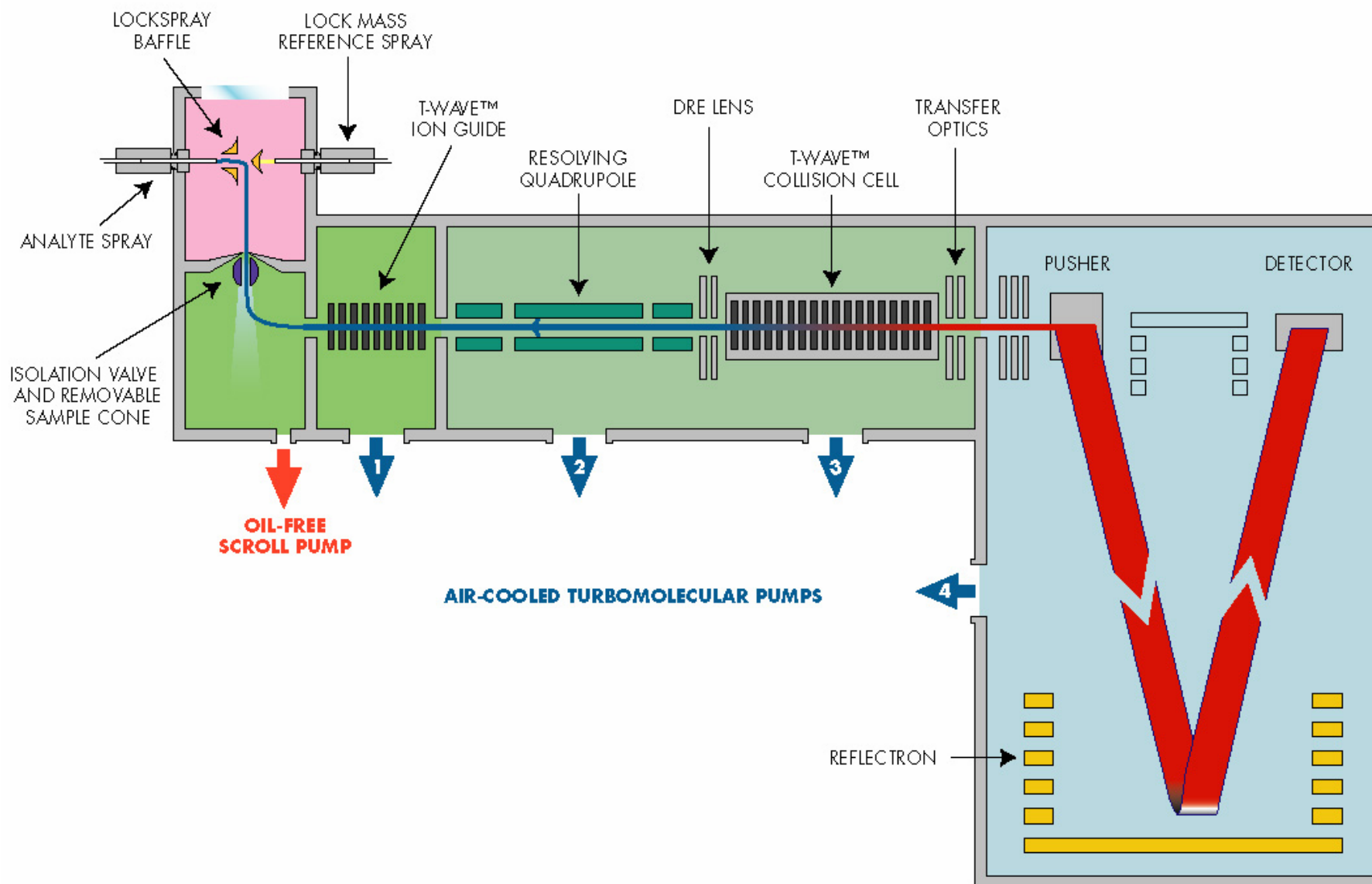
MALDI

+

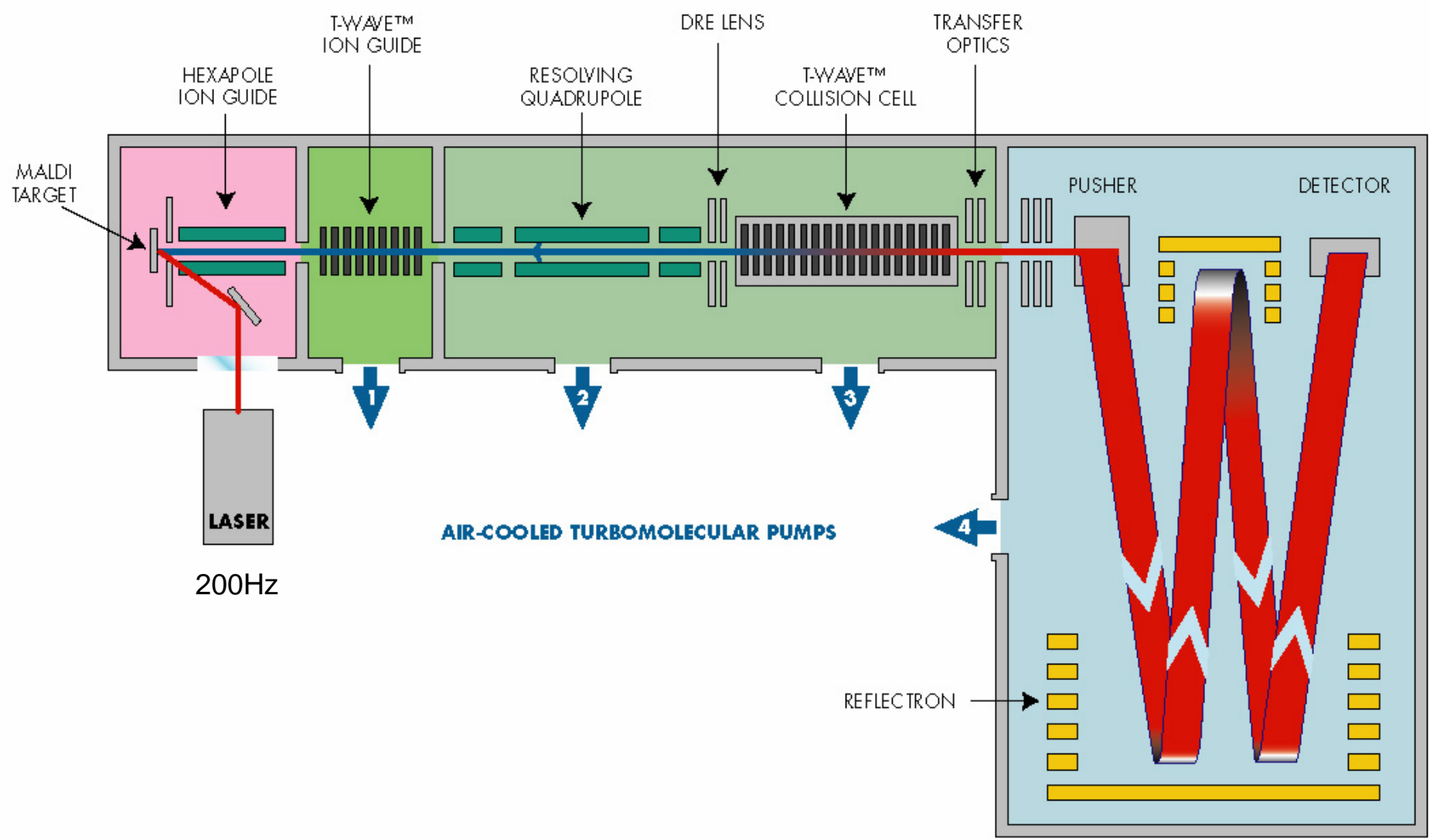


ESI

ESI-QToF Premier schematic



MALDI-QToF Premier schematic



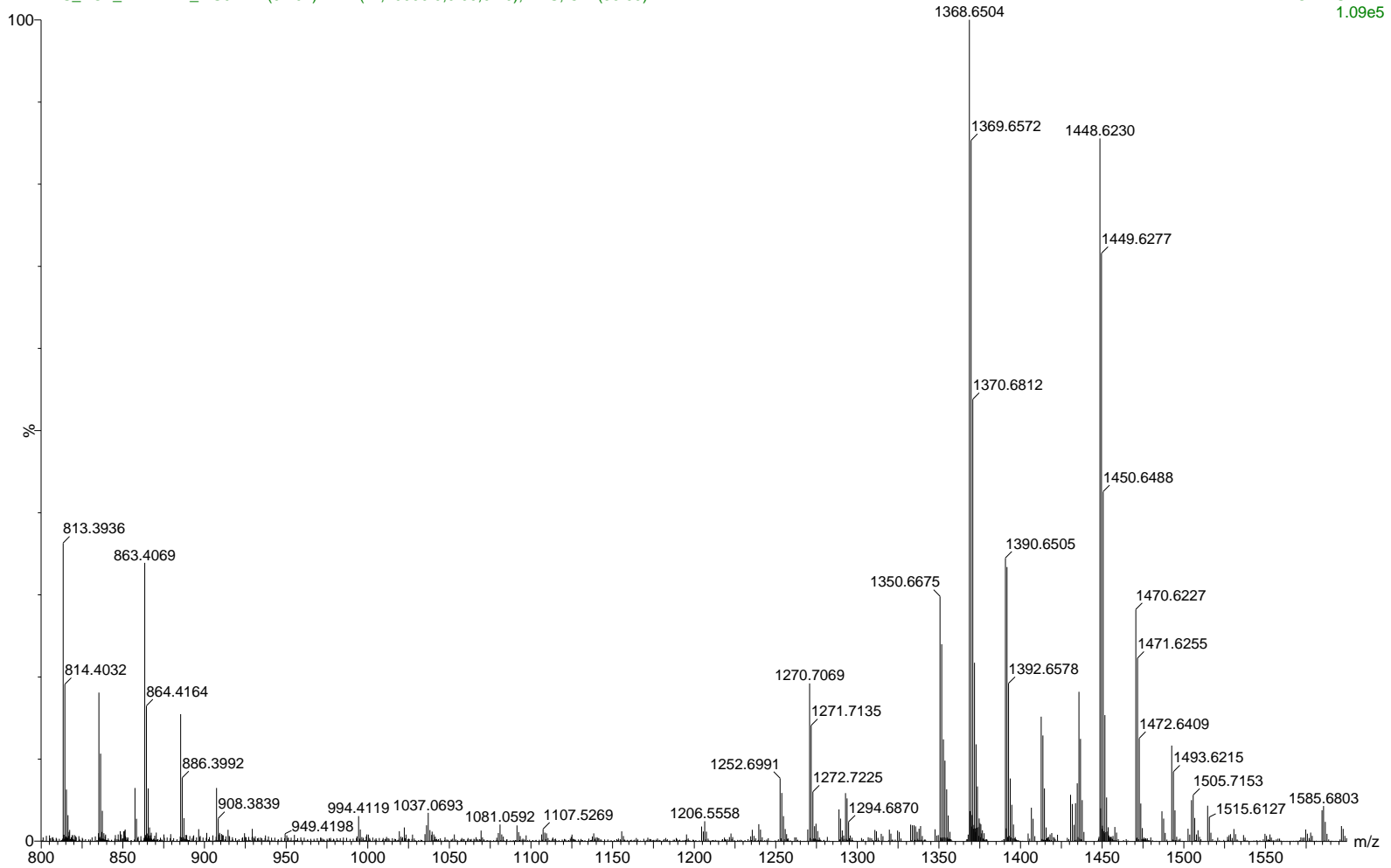
Waters phosphopeptide mixture MALDI MS



waters std Phosphopeptide alpha

WATERS_PO4_PEPTIDE_MS01 41 (0.701) AM2 (Ar,10000.0,0.00,0.70); ABS; Cm (36:69)

TOF MS LD+
1.09e5



Isolation of phosphopeptide ion 1368 m/z

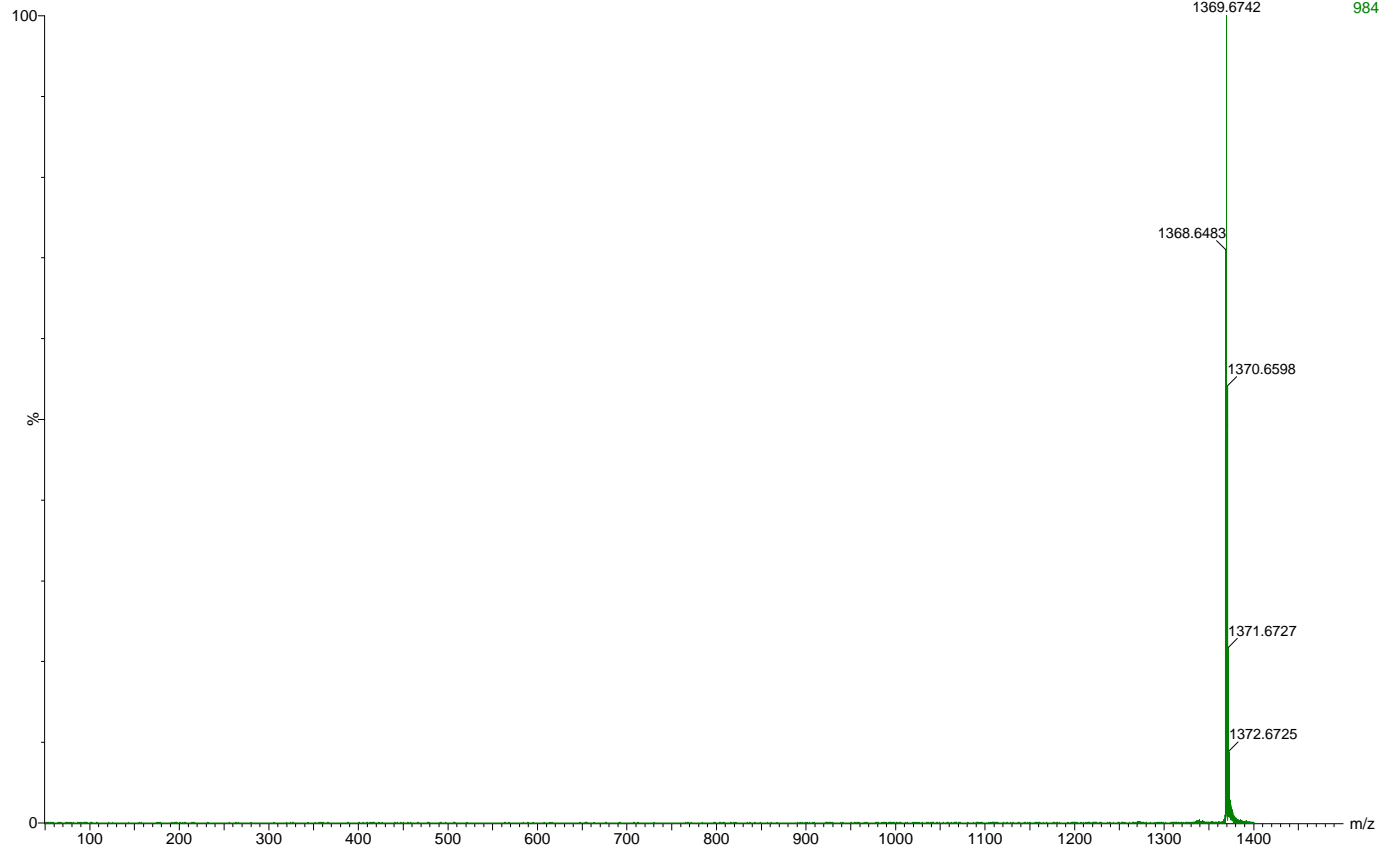
Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

waters std Phosphopeptide alpha MSMS of 1368.6776
WATERS_PO4_PEPTIDE_MSMS02 28 (0.479) Cm (28:32)

HBB170

02-May-2007 12:12:42
TOF MSMS 1368.68LD+
1369.6742 984



De novo sequencing of phosphopeptide VNQIGpTLSESIK, 1368 m/z (using MaxEnt3 and BioLynx)

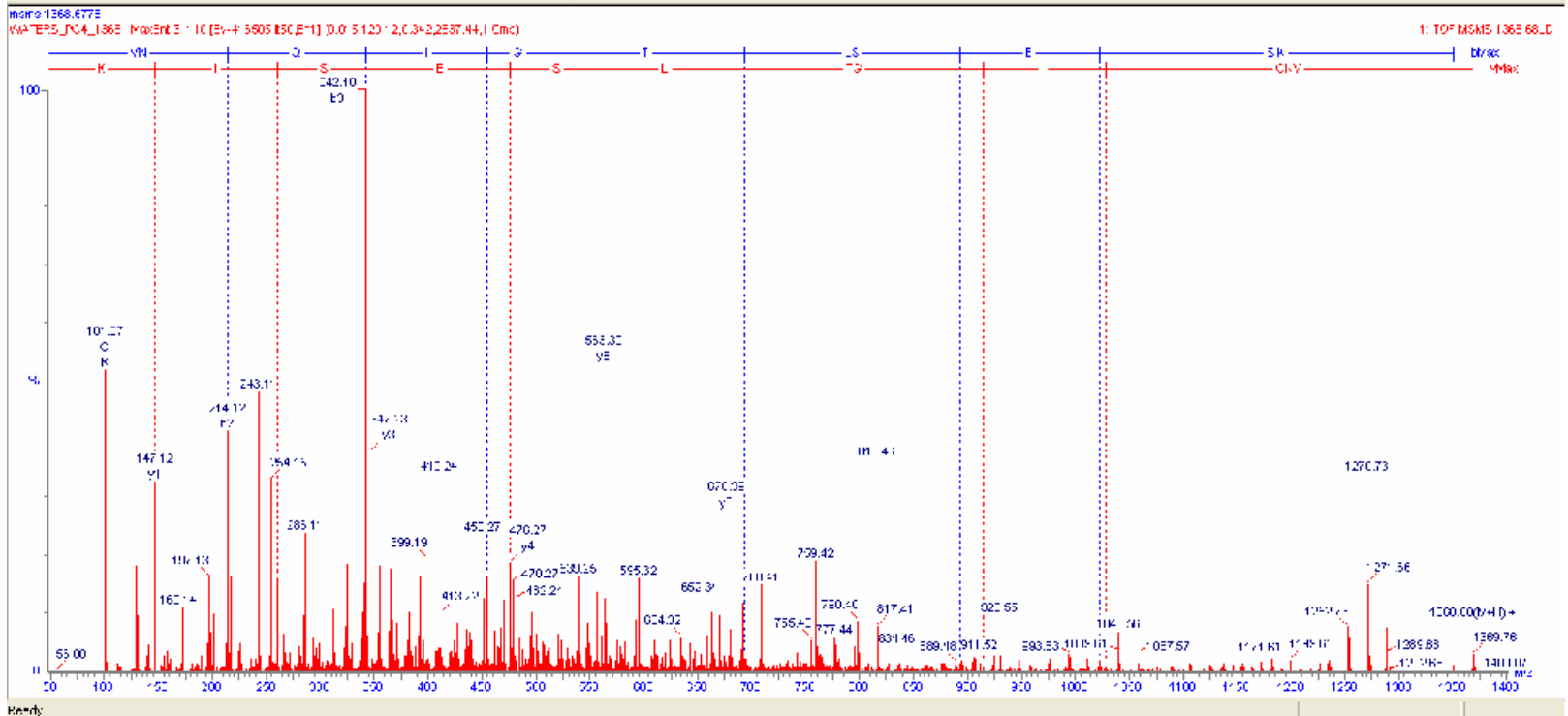
PepSeq - [laetiaphos.psq]

File Edit View Process Options Tools Window Help

Seq name	#	Score	Ident (%)	Prob (%)	Identified MS
VNQIGpTLSESIK	1	546	100	100.00	1367.6297

Observed MW: 1367.6297 Precursor Ion Charge state: 1
 Max tolerance: 0.50 Identity threshold: 100 (1.750%)
 Modifications: Phospho (+)

	Val	Arg	Gln	His	Gly	Pro	Ileu	Ser	Glu	Thr	Met	Lys
a	72.08	186.12	184.18	427.27	481.29	665.30	778.33	869.42	894.46	1081.49	1194.58	1322.6
b	160.08	214.12	349.18	455.24	512.28	693.30	806.33	893.41	1022.46	1169.49	1222.51	1350.6
	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.10			
r	1360.60	1269.61	1355.67	1027.61	918.62	857.63	646.69	569.69	476.27	347.23	260.20	147.11
				0.02	0.01		-0.01	0.00	0.00	-0.00	-0.00	-0.00
c	1362.65	1252.58	1338.64	1018.68	897.69	810.67	659.66	616.27	469.21	330.20	234.11	130.09
		-0.13	0.00				0.02	-0.05	0.02	0.03	0.03	-0.01



MS^e data analysis (▶ 70% sensitivity)

Waters

THE SCIENCE OF WHAT'S POSSIBLE™

ProteinDataBrowser

File Edit View Windows Options Tools

Projects: 3258_EPFL

Container Manager

MS^e SP_200 (digests: 04:02)

Cont.	Accession	Defn.	Descriptor	PK1	P	PL3C Score	Probab. (%)	Tag. des.	Coverage (%)	Mean Zncr.	RFD Zncr.	Ms
1	P02707	TPST1_HUMAN	Carotransferin precursor	76999	0.0	674.9	100.00	30	65.6	0.1	4.6	
2	P00924	CD4211_MOUSE	Endlease 1 CD4211 29 kDa glycoprotein	46342	0.2	475.0	100.00	25	64.0	0.3	6.9	
3	P02794	LACE_BONIN	Beta lactoglobulin precursor	18574	4.7	432.7	100.00	19	61.0	0.0	7.1	
4	P00922	CD4211_MOUSE	Endlease 2 CD4211 29 kDa glycoprotein	20962	0.0	390.9	100.00	11	57.5	0.0	5.2	
5	P48166	CD4211_MOUSE	Endlease 2 CD4211 29 kDa glycoprotein	47143	0.1	375.2	75.00	18	45.7	0.3	5.8	
6	P00330	ALDH1YE8	Alcohol dehydrogenase 1 EC 1.1.1.1	36968	0.3	322.9	100.00	19	43.6	0.0	4.7	
7	P00925	CD4211_MOUSE	Endlease 2 CD4211 29 kDa glycoprotein	46754	0.5	325.4	100.00	16	40.0	0.0	7.0	
8	P00928	CD4211_MOUSE	Endlease 2 CD4211 29 kDa glycoprotein	18228	0.0	340.7	100.00	10	35.7	0.0	4.7	

Cont.	msc	z	Tag. des. (%)	Protein m/z	Delta (Da)	Delta (ppm)	Probab. (%)	Log Likelihood	Start	End	Sequence
1	1253.1063	2	122.6429	1225.6439	-3.0067	-4.7	100.00	32.7672	35	44	(S)PPTTPTKPPK(T)
2	951.4403	2	950.4329	950.4345	-0.0077	-1.9	100.00	7.3027	37	44	(S)DQREKPPK(S)
3	5661.5767	2	5681.5767	5681.5773	-1.1765	-2.4	100.00	80.6834	67	75	(S)K(L)E(L)A(L)K(L)LPK(S)D(L)
4	1143.1159	2	1148.1159	1148.1163	-0.0029	-1.7	100.00	8.3636	36	45	(S)DQVPTTPTK(T)
5	1453.5732	2	1457.5732	1457.5733	-0.0029	-1.9	100.00	53.3035	76	80	(S)DQVQVSSQDQK(S)
6	1117.8351	2	1118.8351	1118.8351	0.0000	0.0	100.00	10.2455	36	40	(S)LPLDQK(S)
7	4541.214	2	4541.214	4541.214	0.0000	0.0	100.00	16.274	48	51	(S)EYKATK(S)

N Glycan MALDI MS

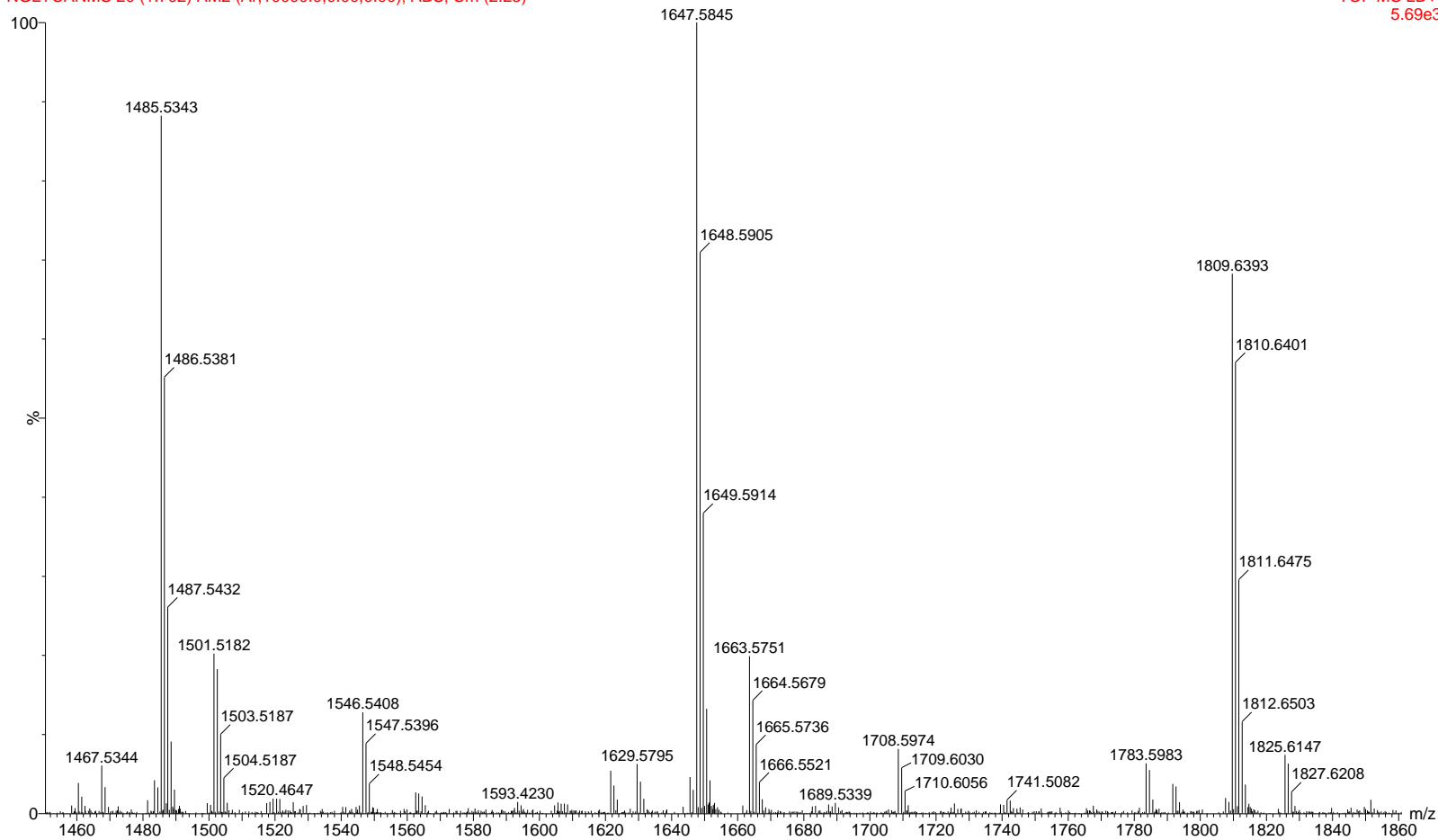
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

N Glycan sample sanofi Aventis

NGLYCANMS 20 (1.702) AM2 (Ar,10000.0,0.00,0.00); ABS; Cm (2:25)

CE = 5.00

TOF MS LD+
5.69e3

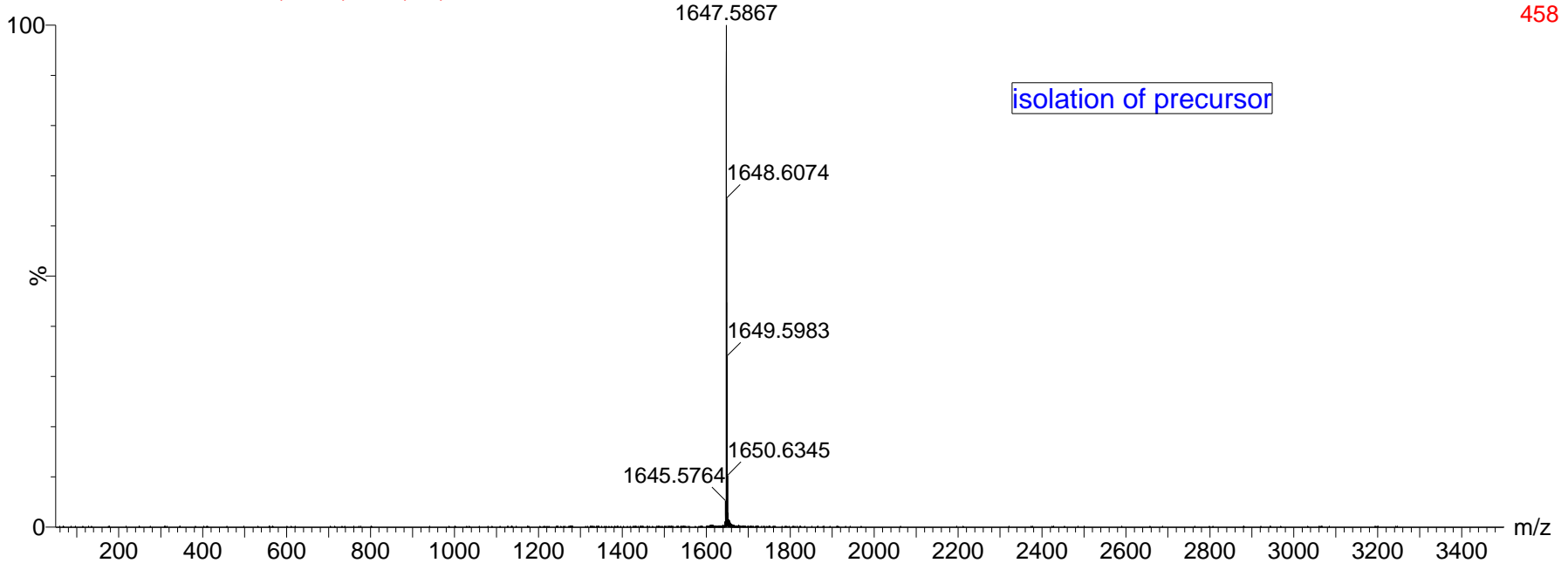


Isolation of glycan m/z 1647

N Glycan sample sanofi Aventis msms m/z 1647.58 **CE = 5.00**
NGLYCAN_MSMS1647 4 (0.341) Cm (2:4)

TOF MSMS 1647.58LD+
458

isolation of precursor



MALDI MSMS of m/z 1647

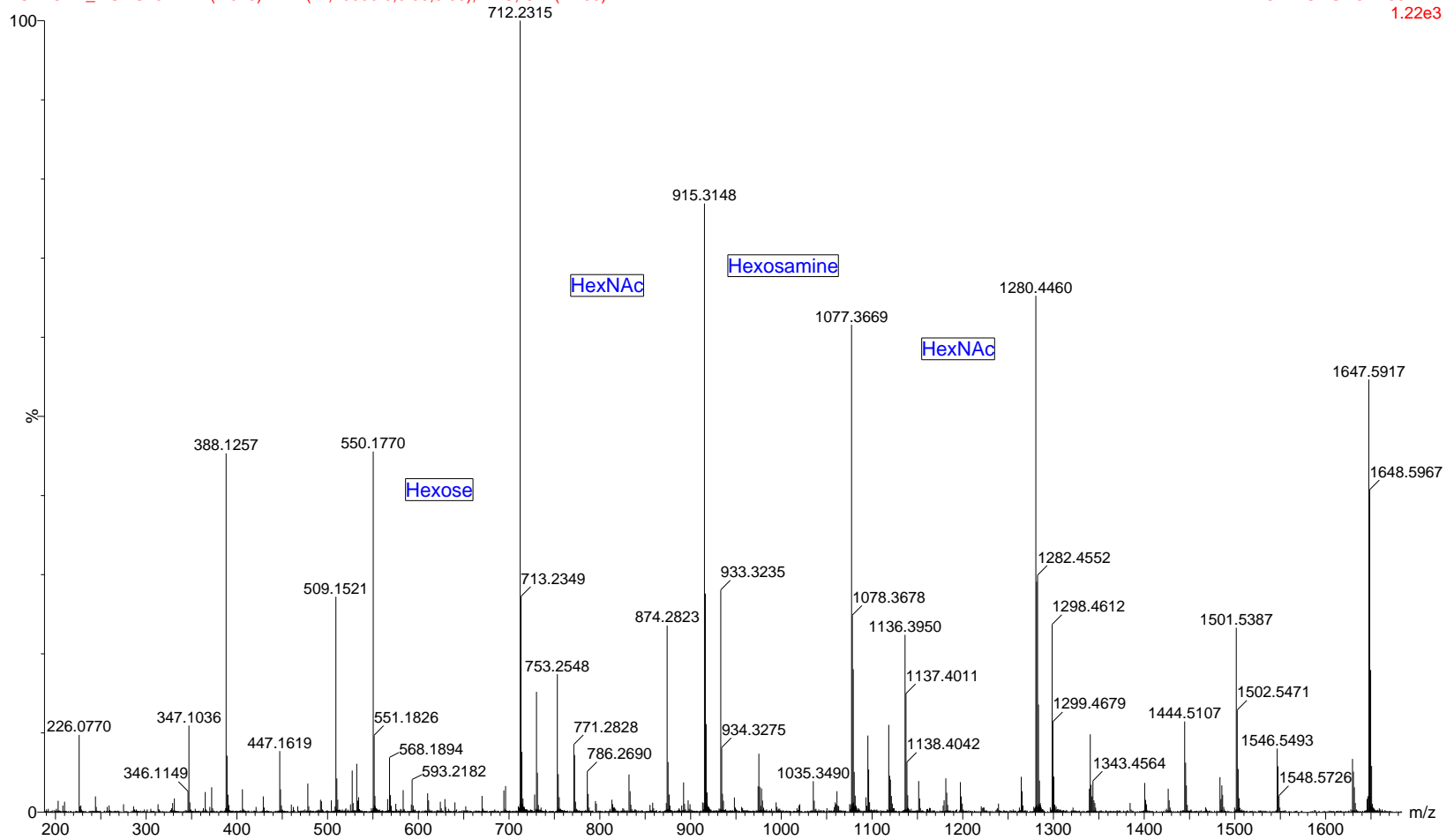
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

N Glycan sample sanofi Aventis msms m/z 1647.58

CE = 95.00

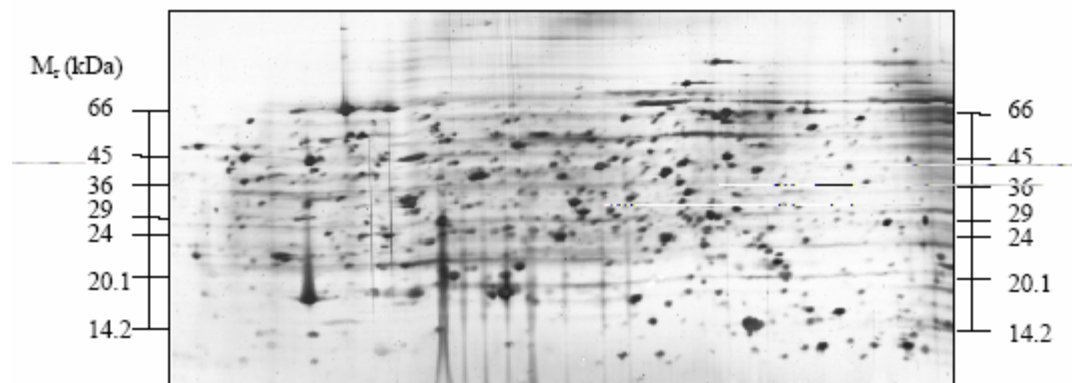
NGLYCAN_MSMS1647 24 (2.045) AM2 (Ar,10000.0,0.00,0.00); ABS; Cm (12:35)

TOF MSMS 1647.58LD+
1.22e3

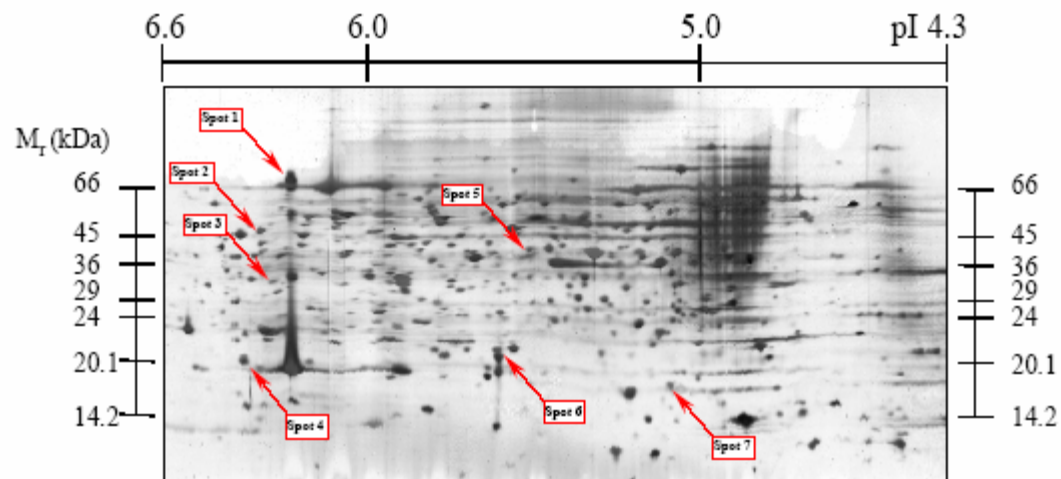


- Example of “Proteomic analysis”
- Protein identification may involve:
 - MALDI ToF - Micro mx (peptide mass finger print)
 - LC MSMS – QToF Premier with nano ACQUITY UPLC (protein sequencing)

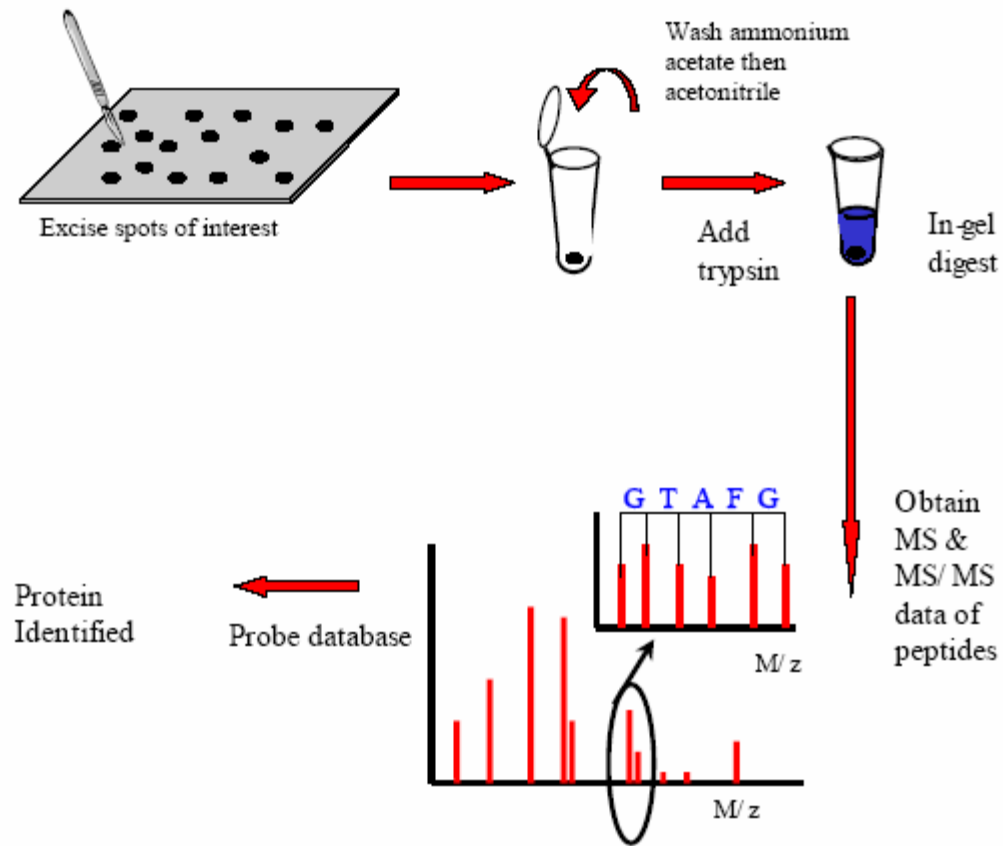
Typical 2-D Gel showing normal and disease state



B



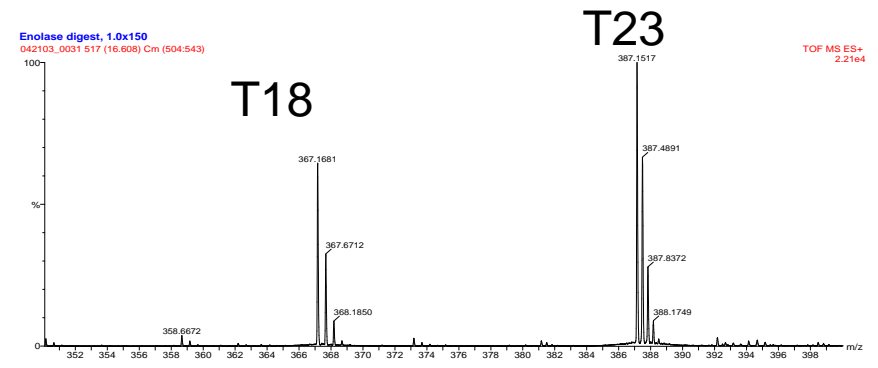
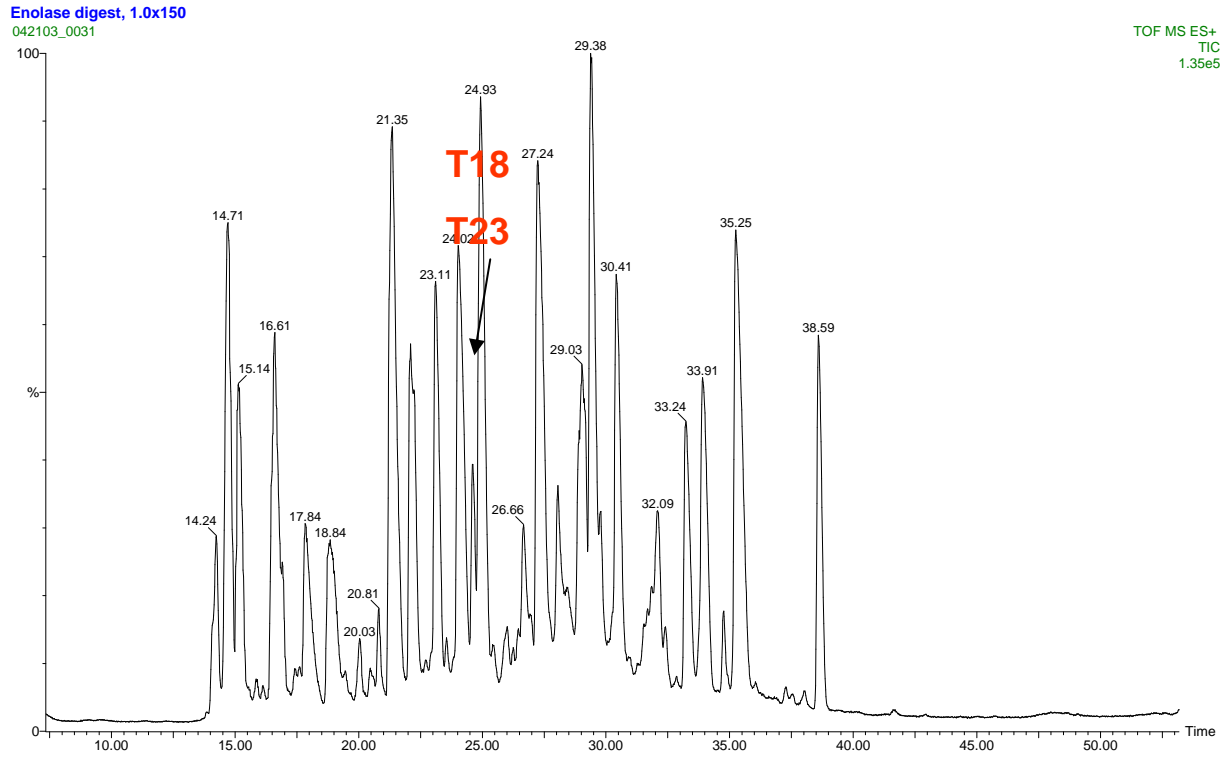
Identification of proteins from gels



LC MS of Peptides

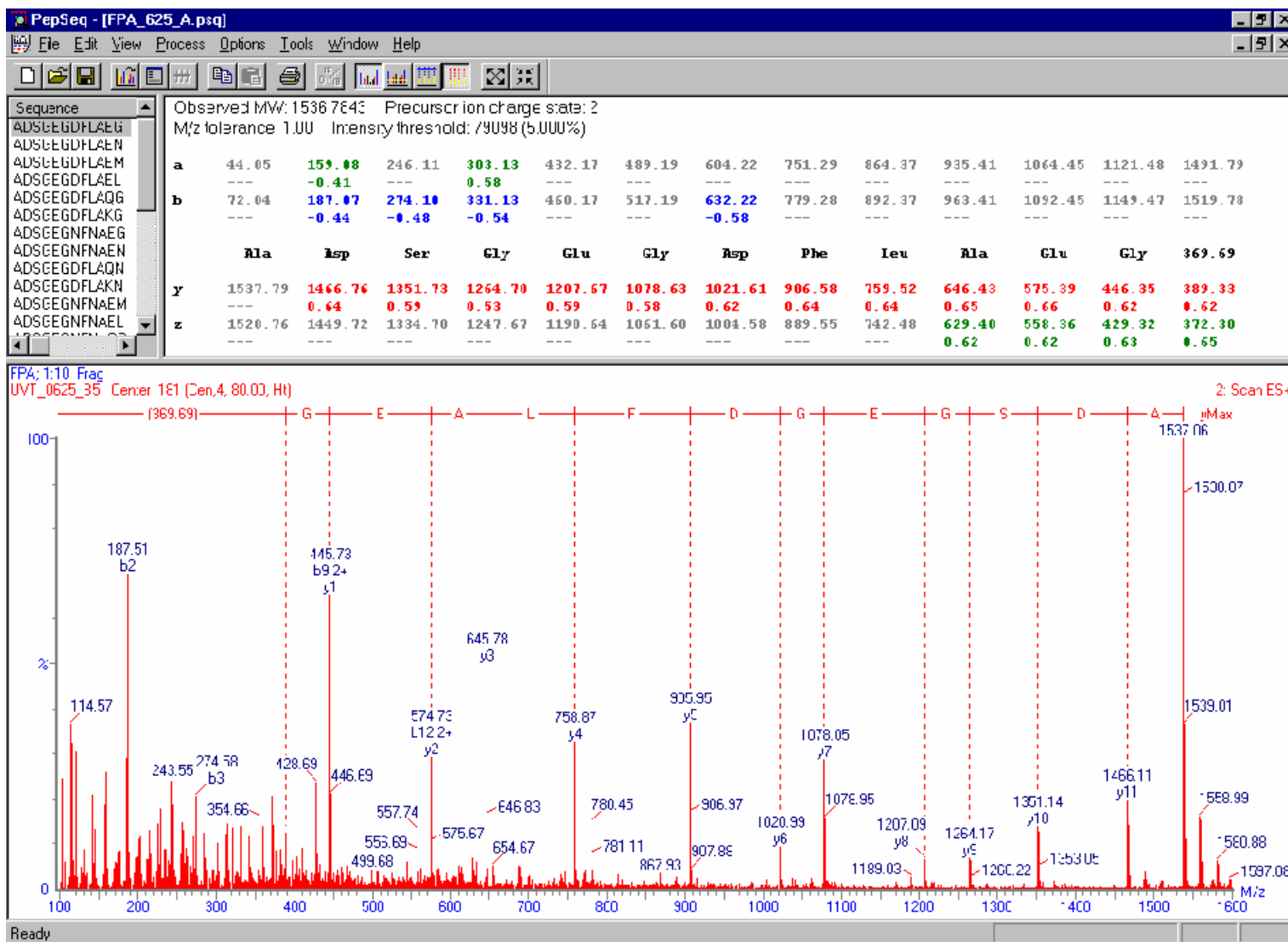
Q-Tof micro™

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Peptide Sequencing ESI MS/MS data

Waters
THE SCIENCE OF WHAT'S POSSIBLE™



What does it do?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

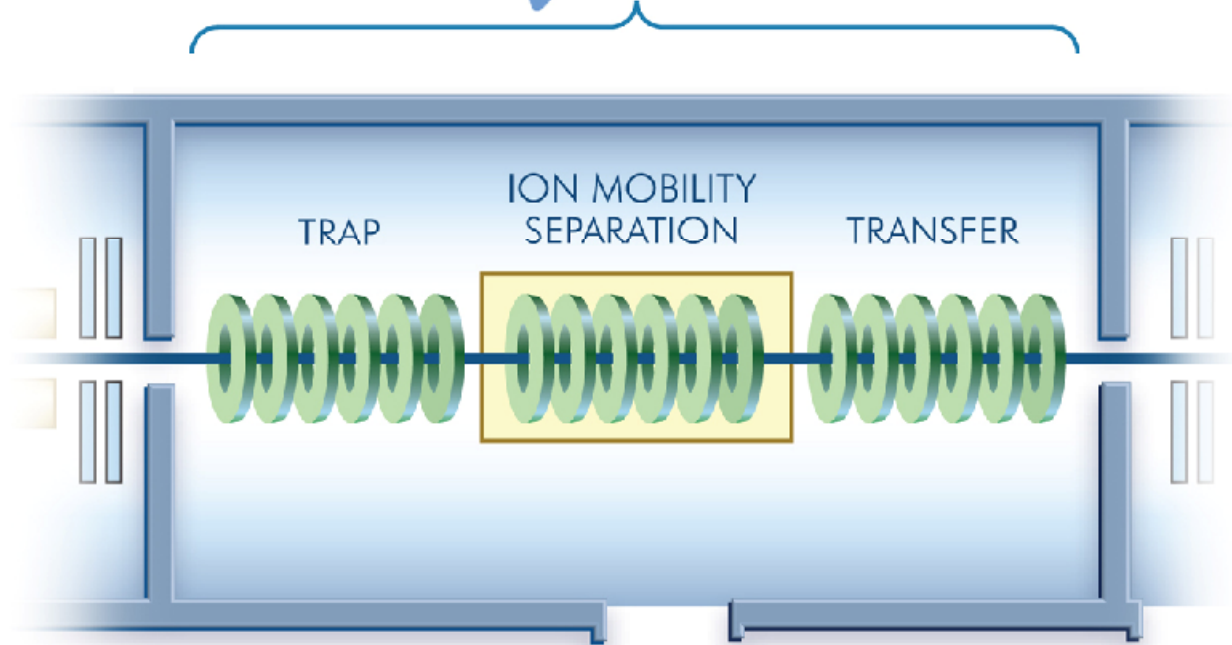
SYNAPT™

High Definition **Mass Spectrometry**™

This enables the analysis of samples differentiated by size and shape and charge, as well as mass, to deliver increased specificity and sample definition

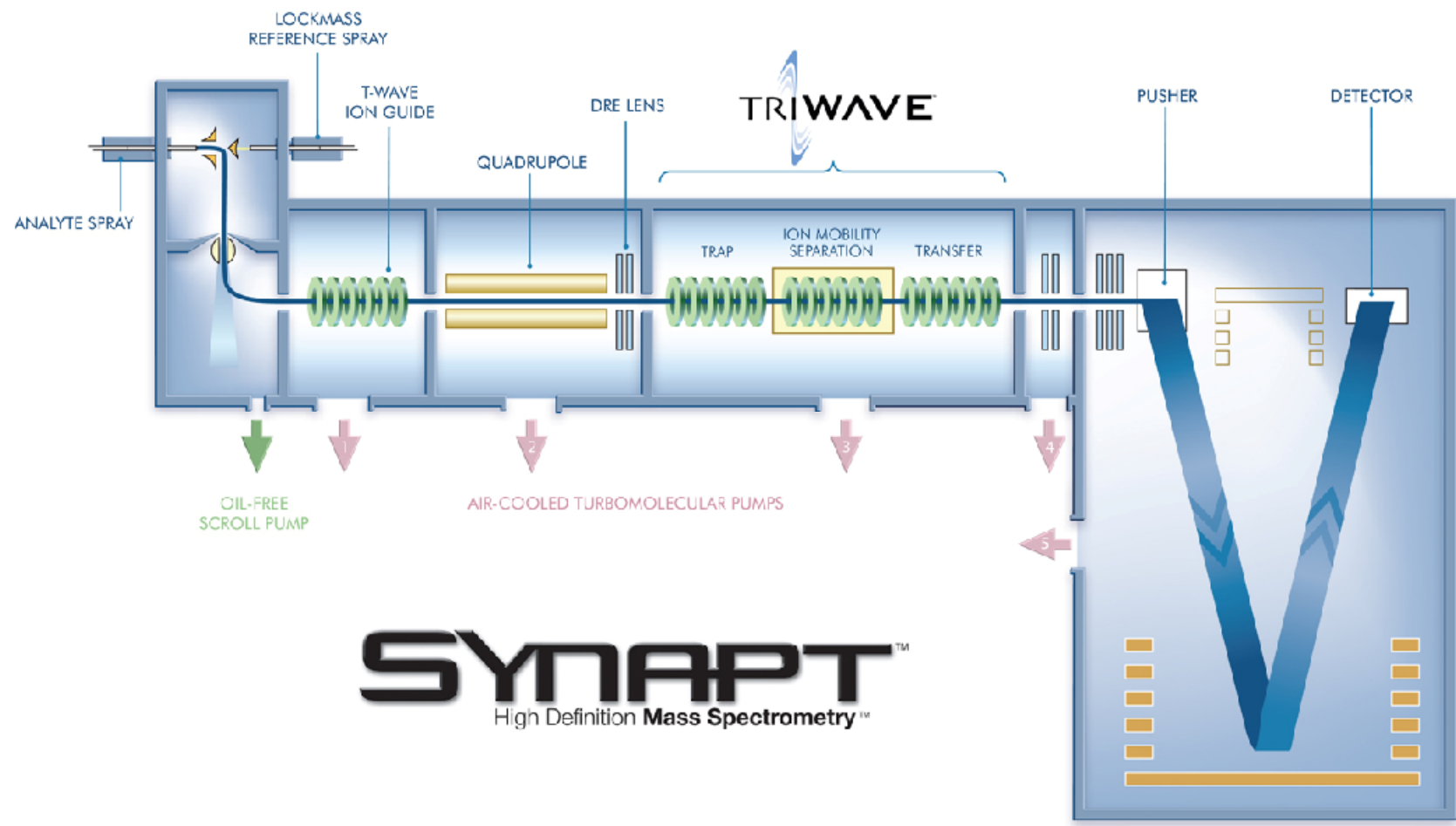


TRIWAVE



Synapt HDMS system

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



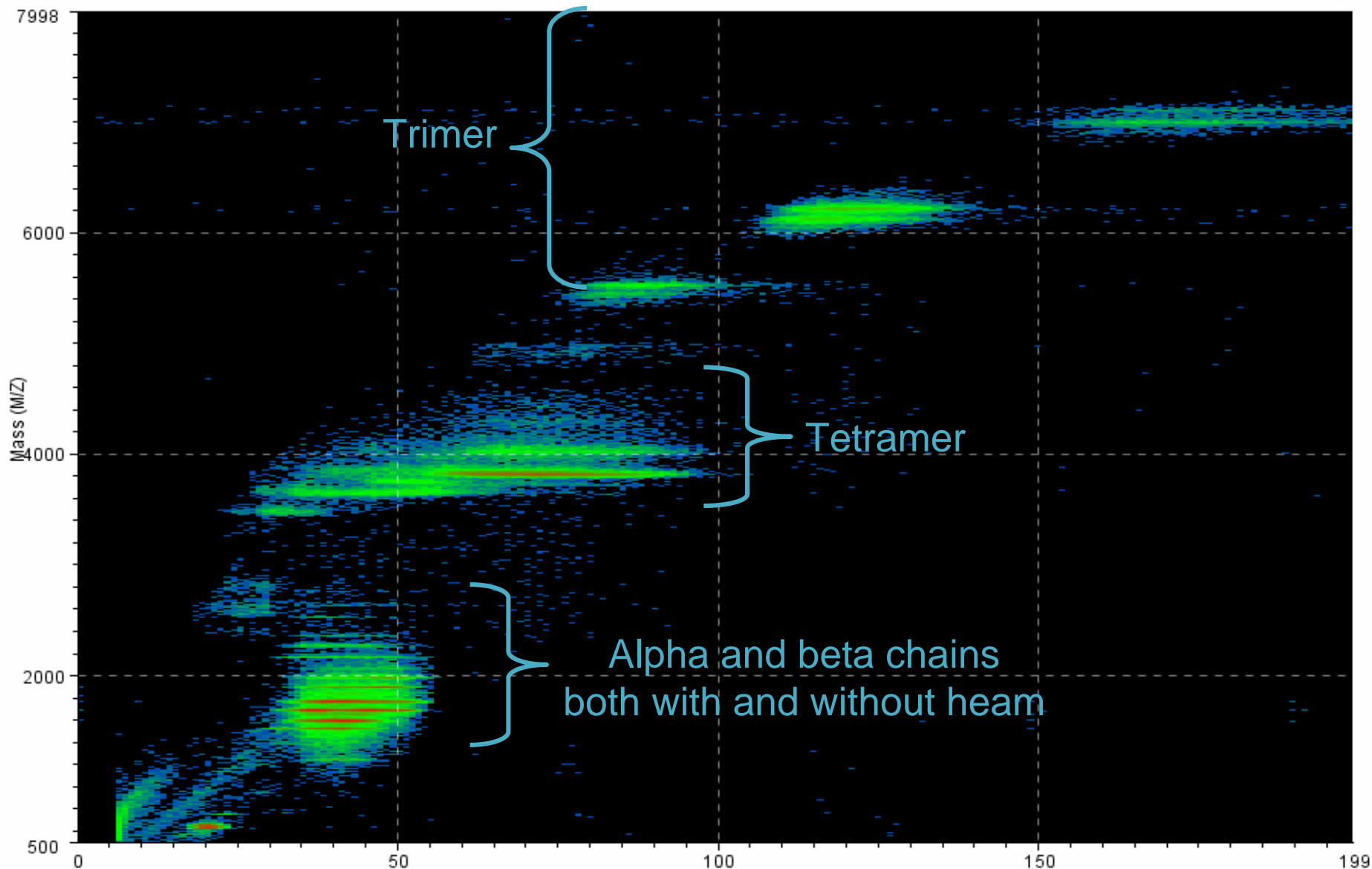
SYNAPT
High Definition Mass Spectrometry™

Haemoglobin drift time analysis

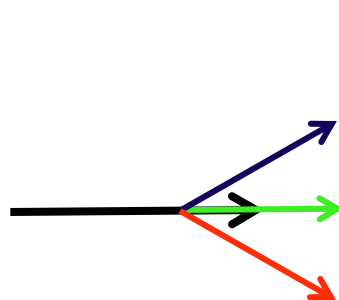
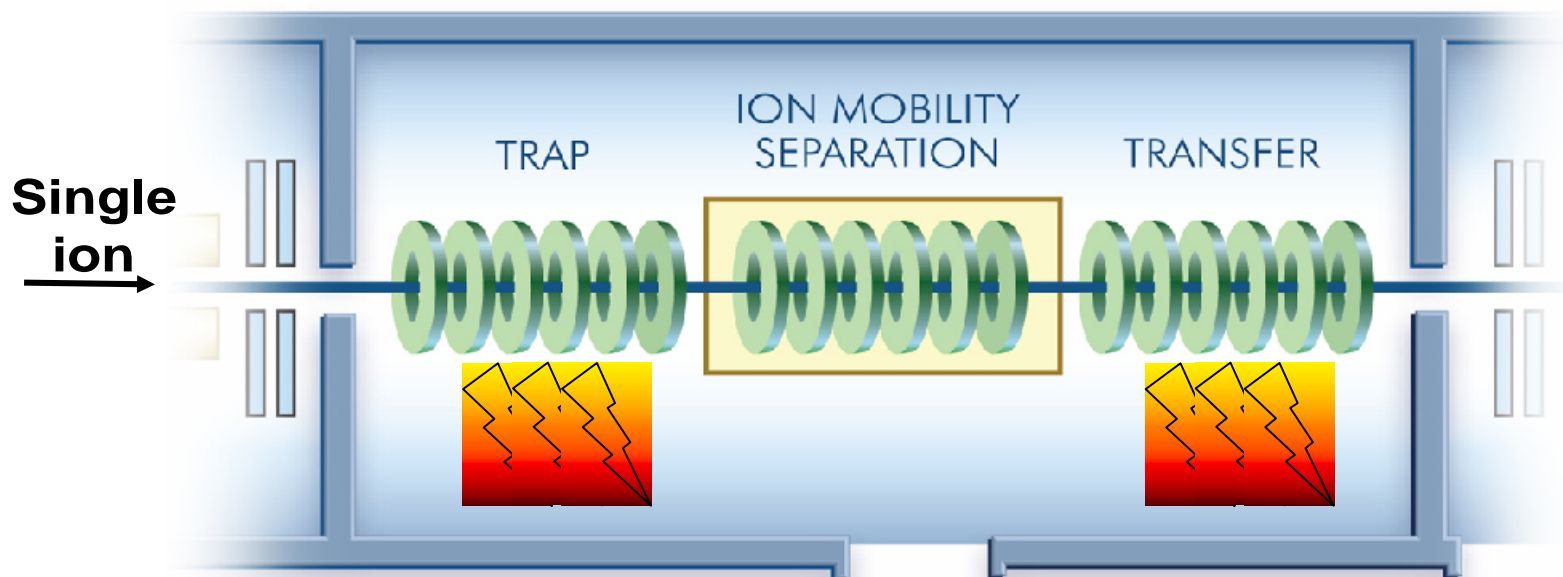
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

RT 0.0 1.0 DT 0 199 MZ 500.0000 7998.0000

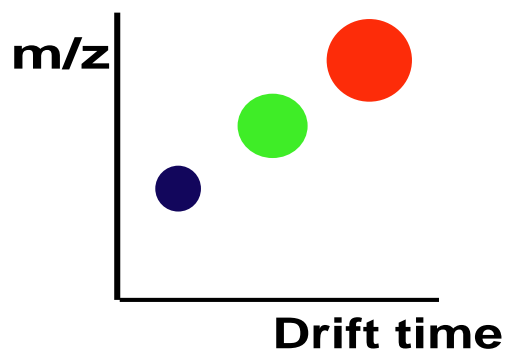
max: 7998 Mass (M/Z)



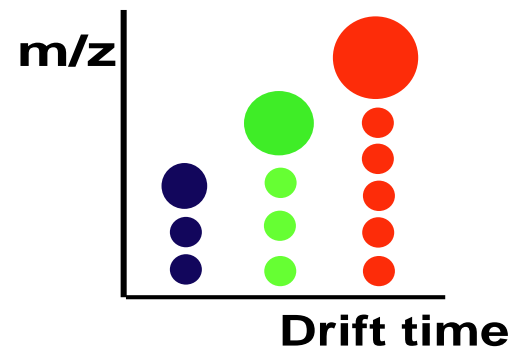
Time Aligned Parallel Fragmentation (TAP)



Precursor ion fragmented



Product ions separated by IMS



Precursor and products share same drift time



**ACQUITY TQD featuring the
Waters TQ Detector**

SUMMARY

- **In Chemistry at Queens**
- LCT P , GCT P , MALDI micro MX
- Variety of ionisation methods
- Flexible sample preparation and sample type
- TOF accurate mass measurement, elemental composition and structural analysis
- Mass Spectrometry continues to evolve and will become more important in routine analysis