Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Application of Ion-Mobility and Advanced Statistical Methods for Small Molecule Profiling

John Shockcor
Director of Metabolic Profiling Business Development
Visiting Fellow, Dept of Biochemistry, University of Cambridge

Five Simple Questions

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- I have 2 sample groups A and B
  - *Are the groups different? If so:*
    - *What has higher in A*
    - *What has lower in A*
    - *What is absent in A*
    - *What is unique to A*

Five Simple Questions

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- I have 2 sample groups A and B
  - *Are the groups different? If so:*
    - *What has higher in A*
    - *What has lower in A*
    - *What is absent in A*
    - *What is unique to A*

These are MARKERS of the difference between A and B

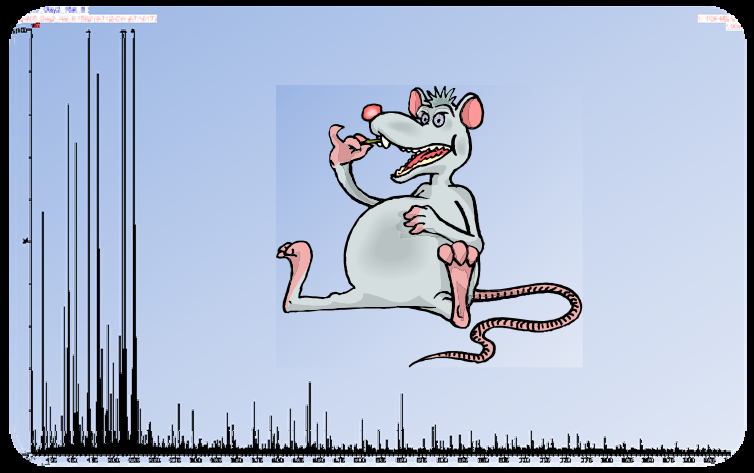
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

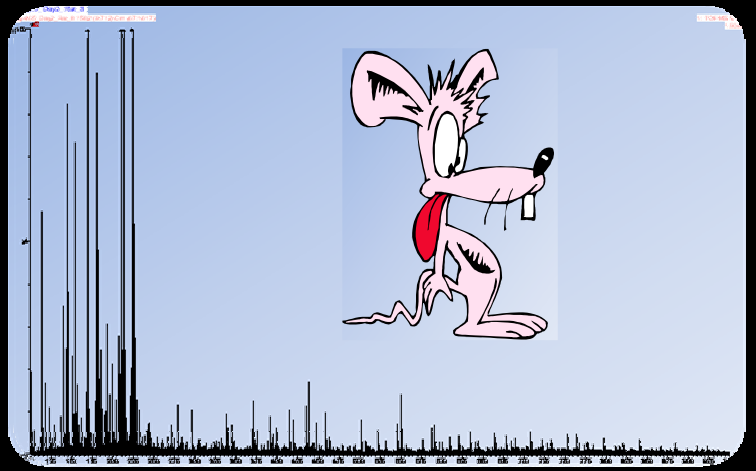
How is Do We Find These Markers?

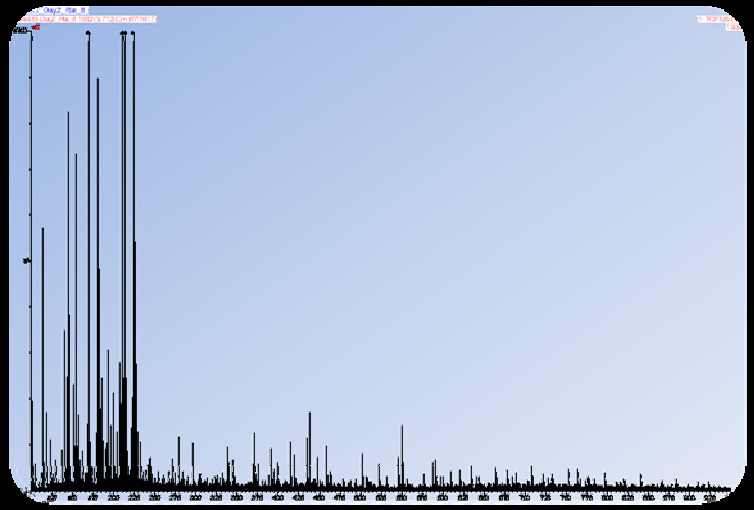
A Manual Approach

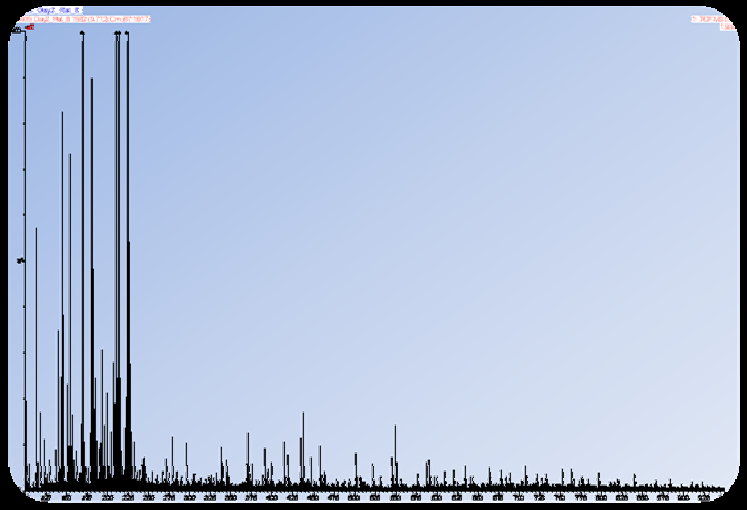
Think about doing a rigorous comparison of just these 4 spectra *(~2,000 masses or metabolites in each).*

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™









A Solution to The Problem

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- It is possible to mine the data using multivariate statistics.
- We first analyze the groups using GC or LC/MS
- We then tabulate all the observed masses and their chromatographic retention times with advanced computational methods.
  - These mass/retention time pairs become the variables used for statistical analysis.

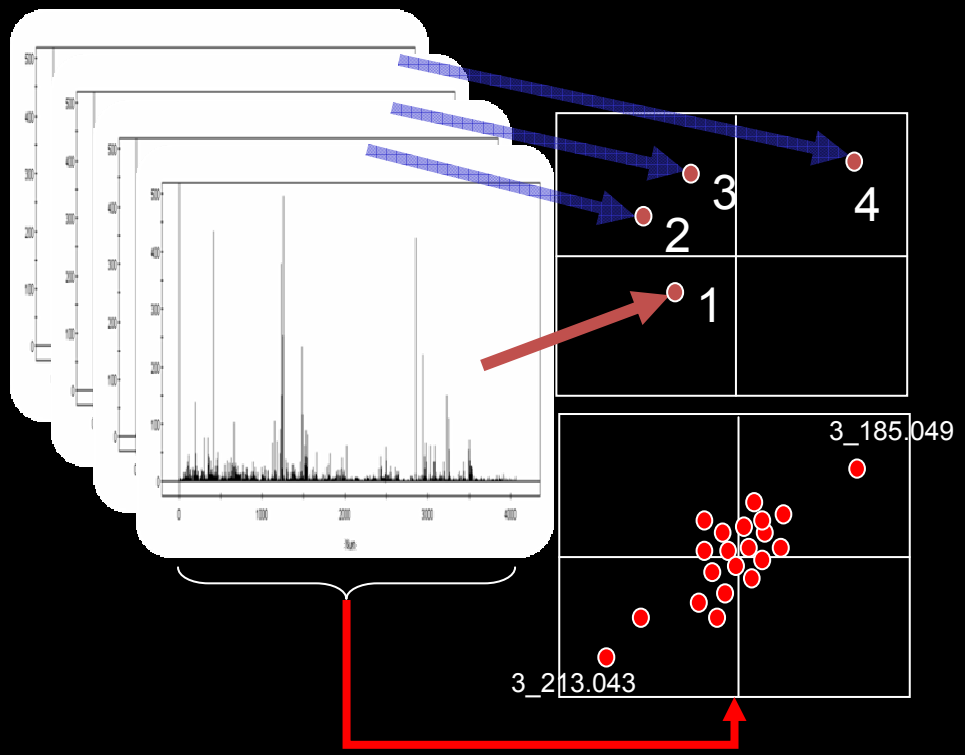
A Solution to The Problem

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Multivariate statistics then allows us to reduce thousands variables (mass/retention time pairs) down to a simple 2 or three dimensional maps.

- These maps shows the difference, if any, between the groups and provides us with a list of the variables which contribute to the difference.

How Spectral Information is displayed



- Spectrum (observation) becomes a point in PCA **Scores plot**
- Variables (m/z_RT) shown in PCA **Loadings Plot**

- Using plots together allows trends in the sample spectra to be *interpreted* in terms of m/z

Why Choose to use Multivariate Statistics?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Why not :

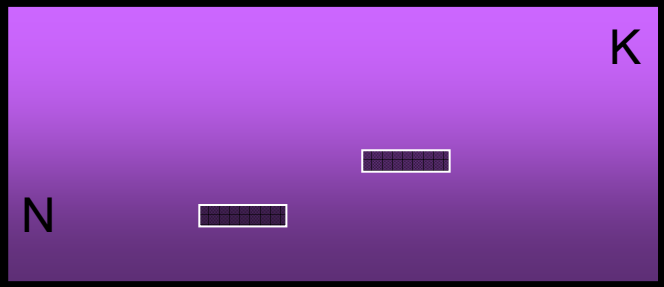
- *Hierarchical Clustering*
- *Heat make*
- *ANOVA*
- *T tests*

- *With MarkerLynx it is possible to export your data to any statistical program you like.*

Why Use Multivariate Analysis?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Short and wide data sets
  - Few observations (N)
  - Many variables (K)
  - Noisy data
  - Missing data/excluded regions
  - Multiple objectives

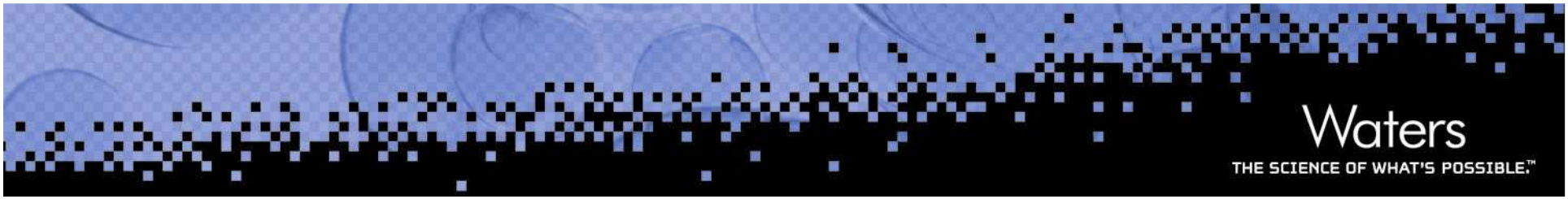


- Implications
  - High degree of correlation (Many variables are related)
  - Difficult to analyse with conventional methods

- Require methods for simplification and visualisation

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Multivariate Statistics
The Basics



Principle Component Analysis
PCA

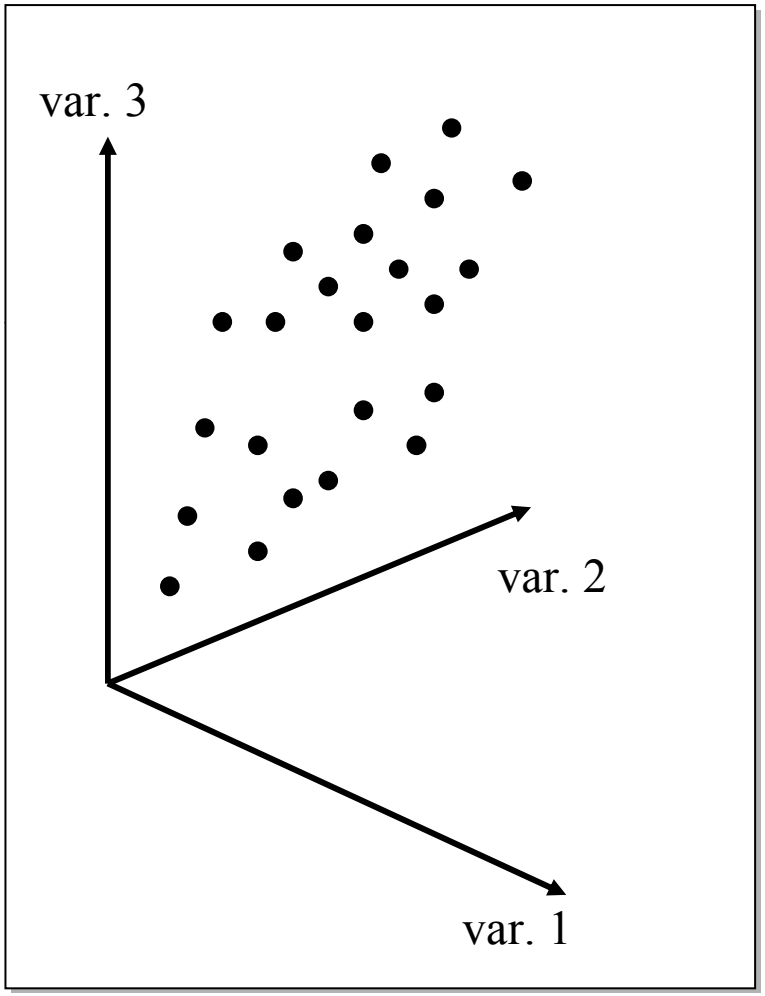
A multivariate statistical approach that facilitates the identification of differences or similarities between groups

Data preparation

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Data table → variable space

	var. 1	var. 2	var. 3
1			
2			
3			
4			
5			
6			
N			

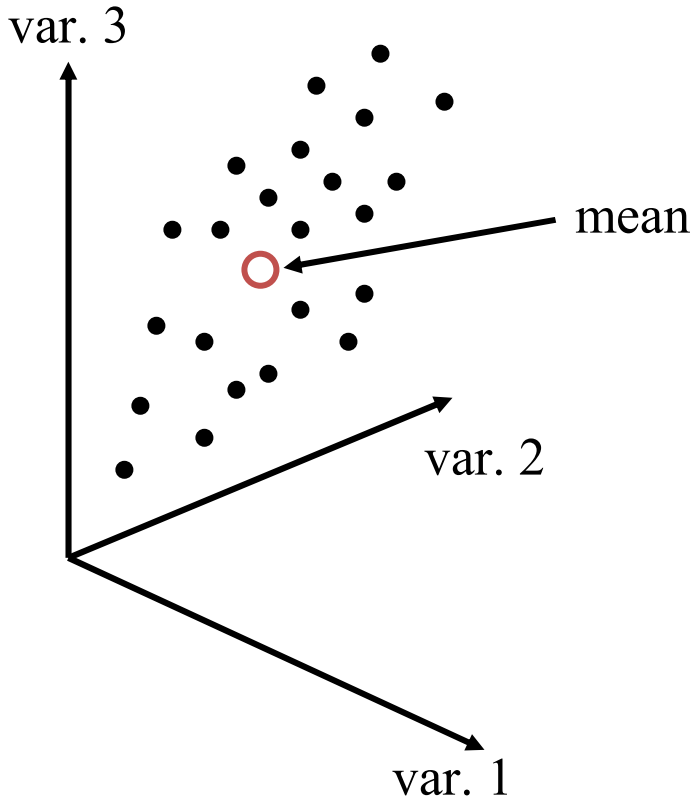


The whole table yields a swarm of points in variable space

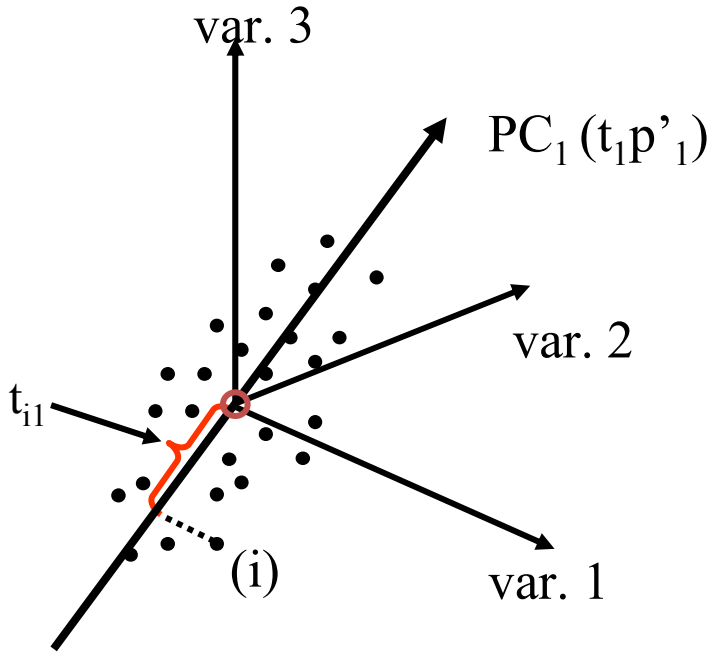
Pre-processing

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

•Centering – move centre of point swarm to the variable origin



PCA theory – step by step

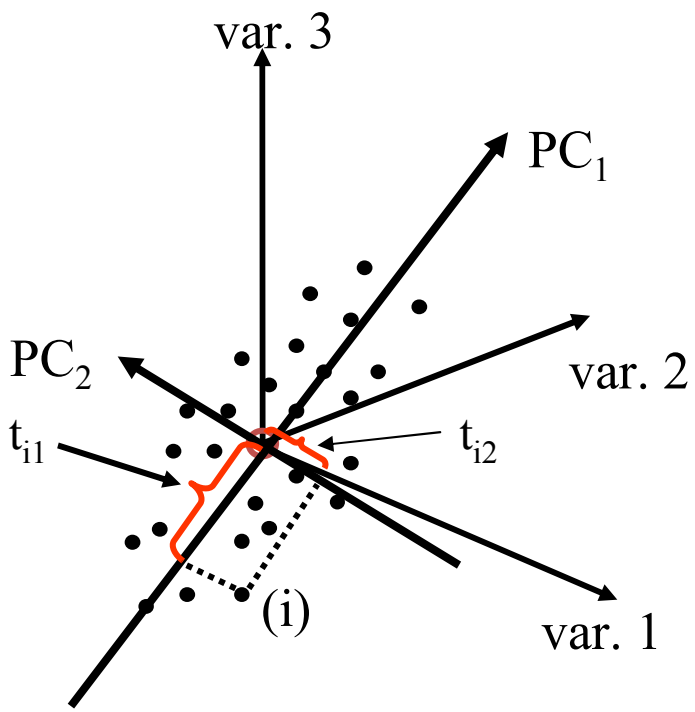


The first principal component (PC_1) is set to describe the largest variation in the data, which is the same as the direction in which the points spread most in the variable space

The Score value (t_{i1}) for the point i is the distance from the projection of the point on the 1:st component to the origin.

PC_1 hence is the first latent variable in a new coordinate system that describes the variation in the data.

PCA theory – step by step



The second principal component (PC_2)
is set to describe the largest variation in the data,
Perpendicular (orthogonal) to the 1:st component

PCA theory – step by step

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

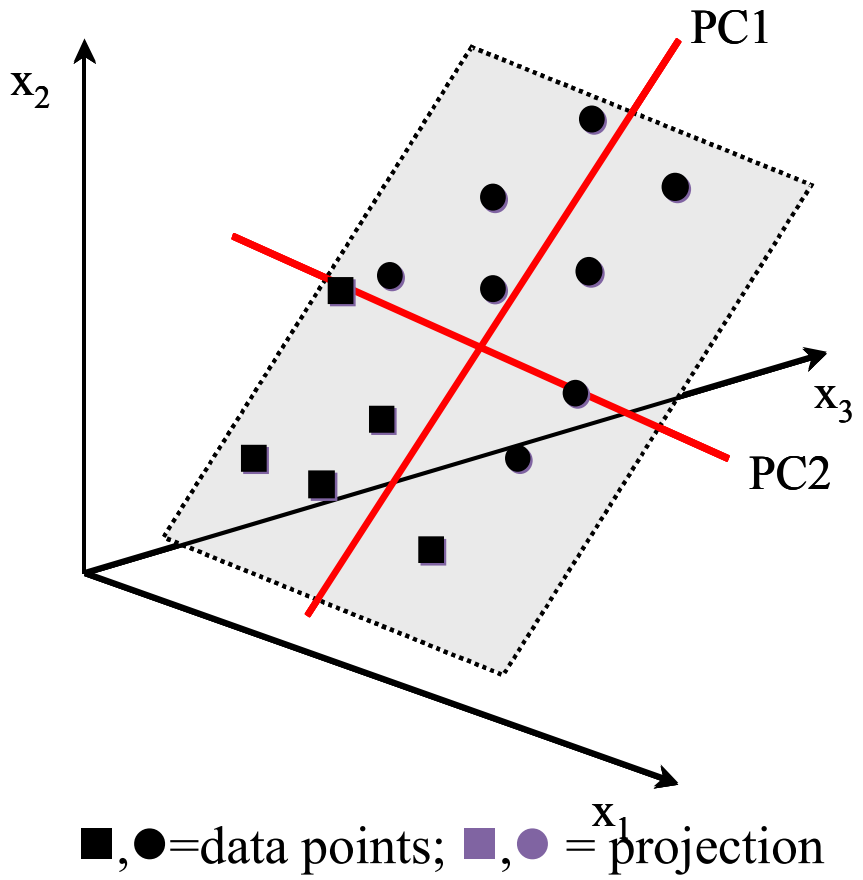
•This is the scores plot similarities or differences between samples can now be seen.

•A corresponding loading plot describes the variables relationships

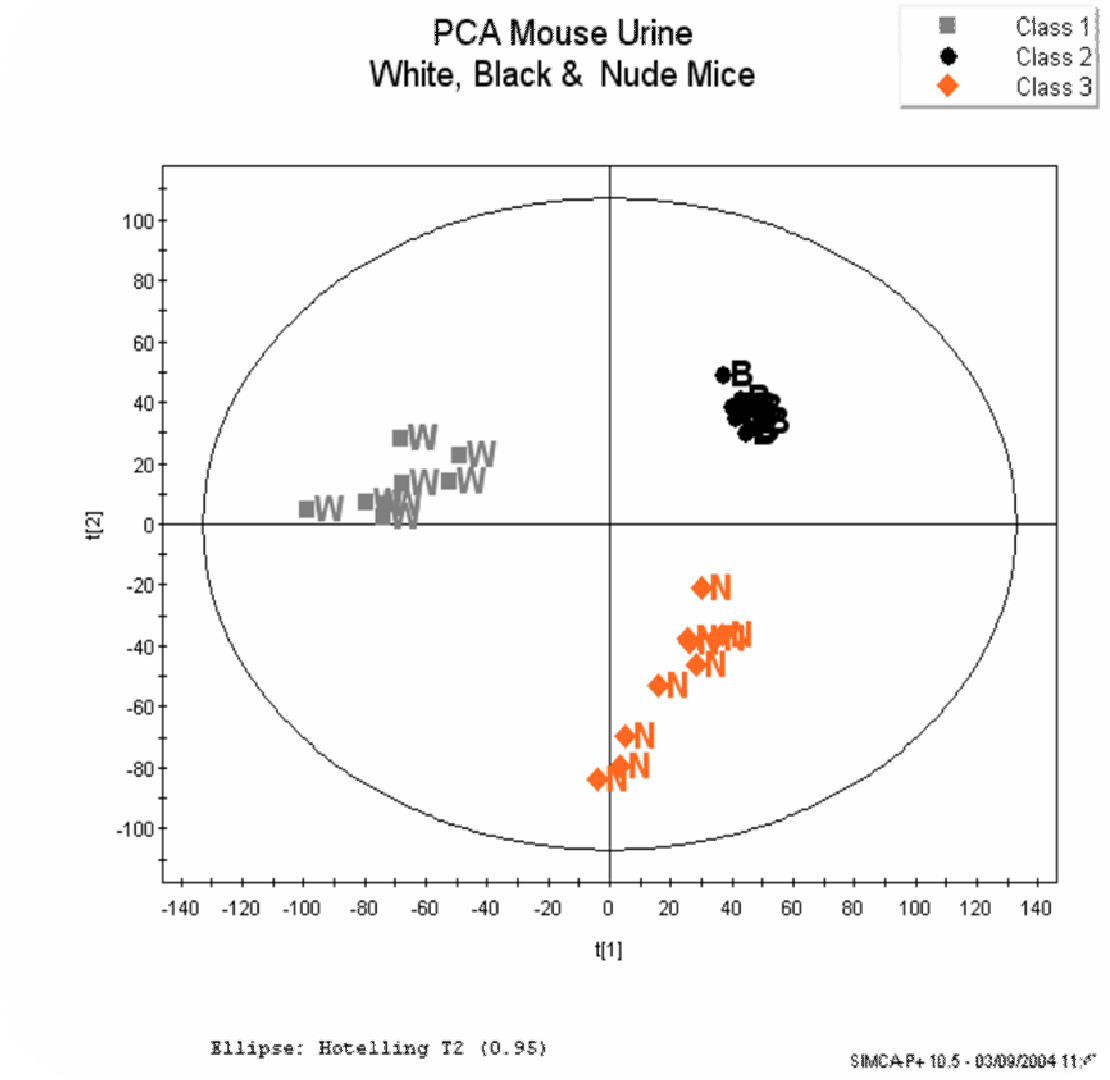
- •allows interpretation of the scores plot by showing which variables are responsible for similarities and differences between samples.

•The perpendicular distance from the object to the projection on the plane is the residual of the two PCs.

•Two PCs make a plane (window) in the K-dimensional variable space. The points are projected down onto the plane which is lifted out and viewed as a two dimensional plot.

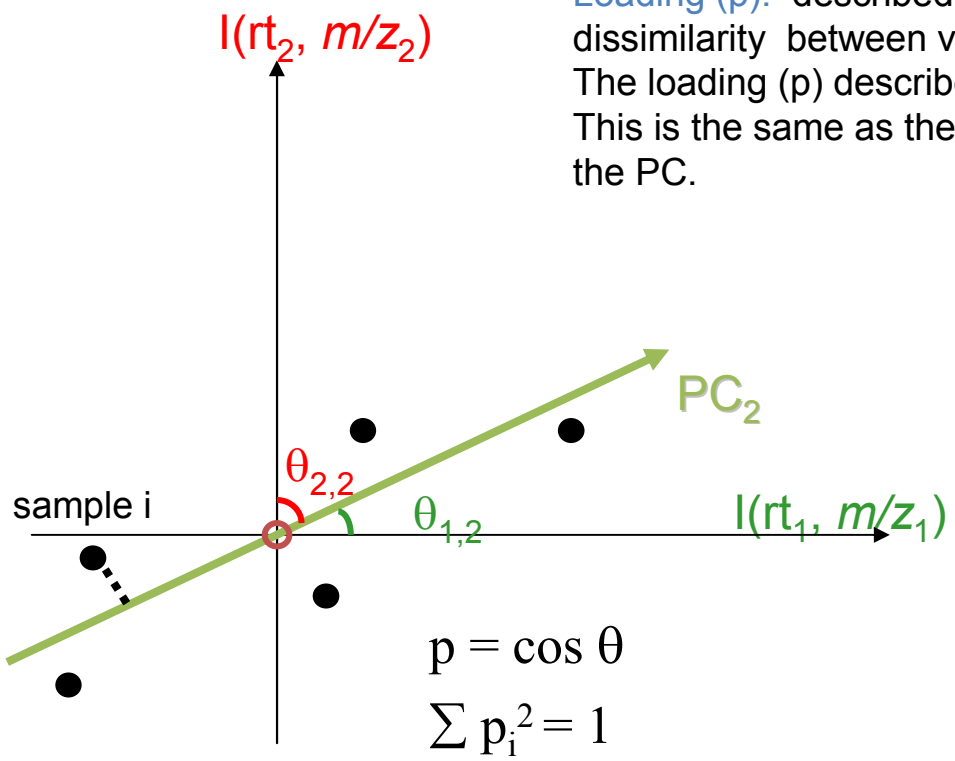


Scores plot example



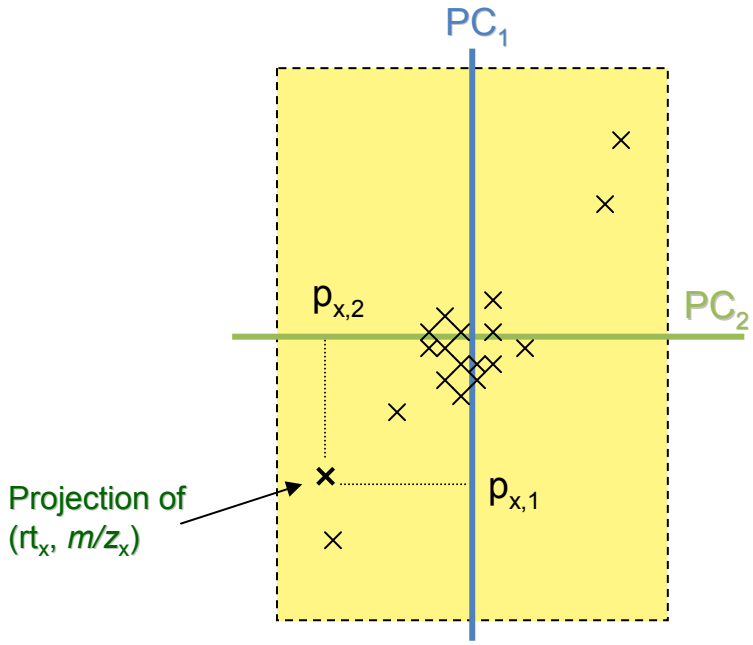
The Loadings Plots

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Loading (p): described the variation in the variable direction i.e. similarity/ dissimilarity between variables, and also explains the variation in scores. The loading (p) describes the original variables importance for respective PC. This is the same as the similarity in direction between the original variable and the PC.

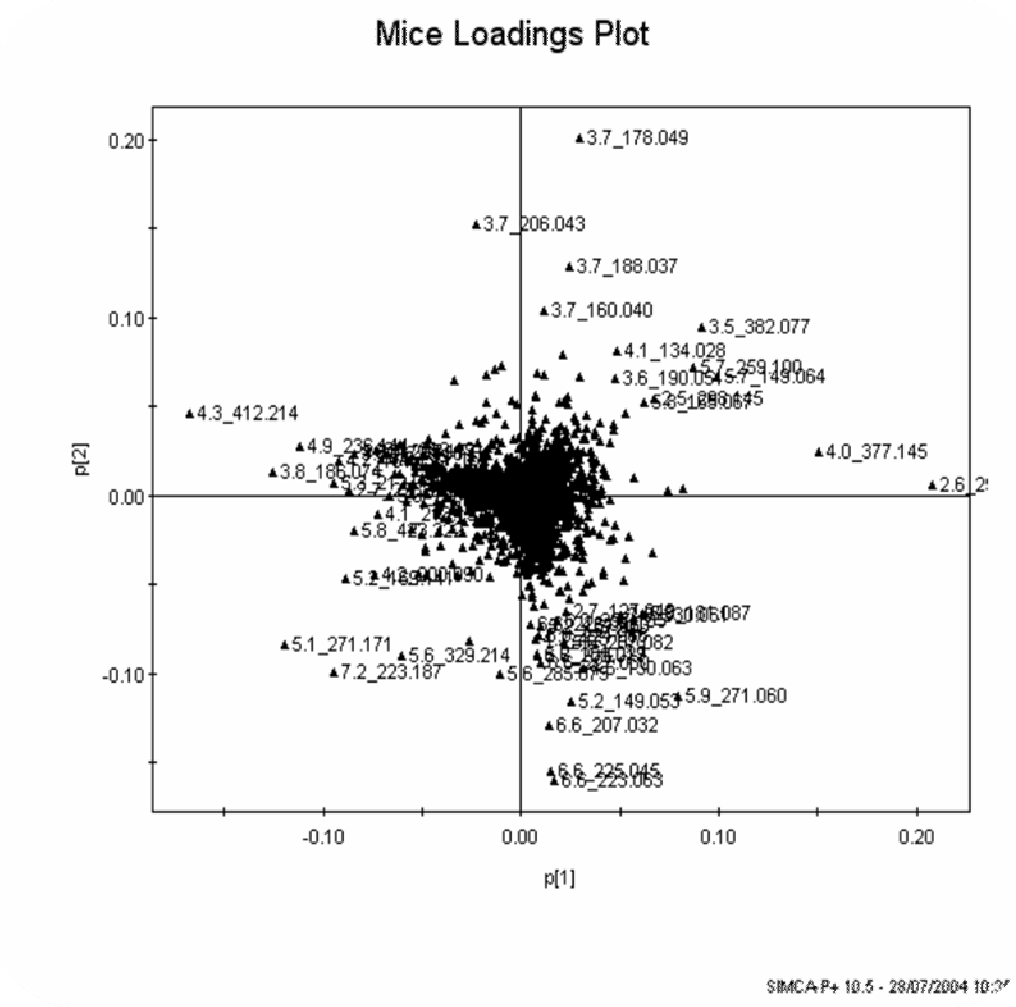
The loading (p) is described as the cosine of the angle between the original variable and the PC.



With $p_{x,1} = \cos(\theta_{x,1})$ and $p_{x,2} = \cos(\theta_{x,2})$
and $\theta_{x,1}$: angle between axe (rt_x, m/z_x) and PC1
and $\theta_{x,2}$: angle between axe (rt_x, m/z_x) and PC2

Example Loadings Plot

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

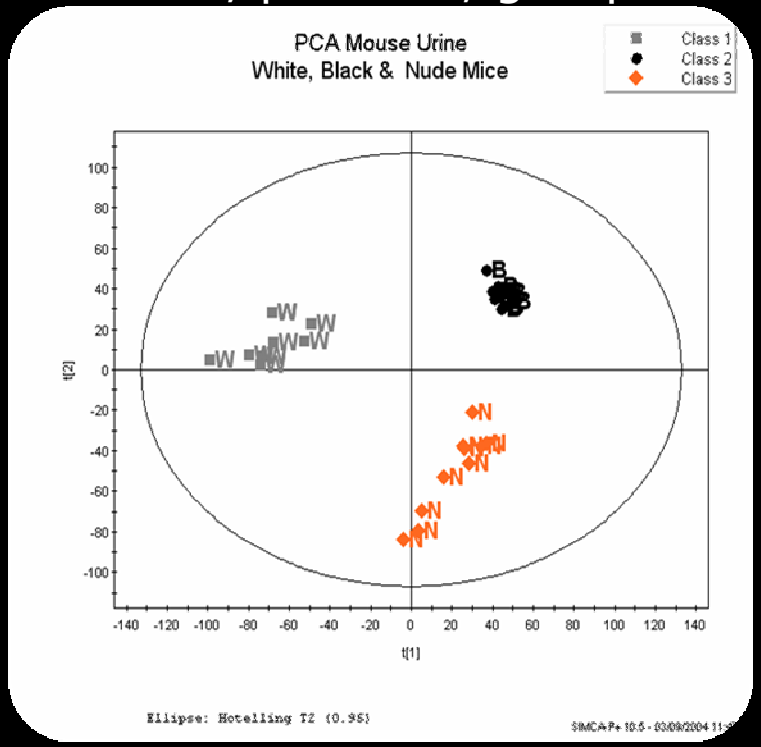


Interpretation of PCA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

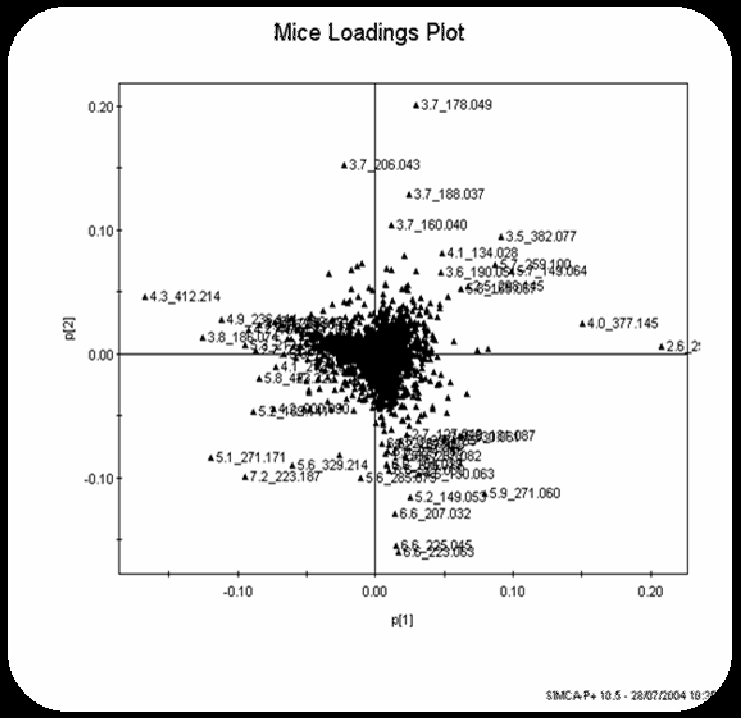
Scores

- Observations (spectra)
- Trends, patterns, groups



Loadings

- Variables (m/z)
- Correlation, influence



Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

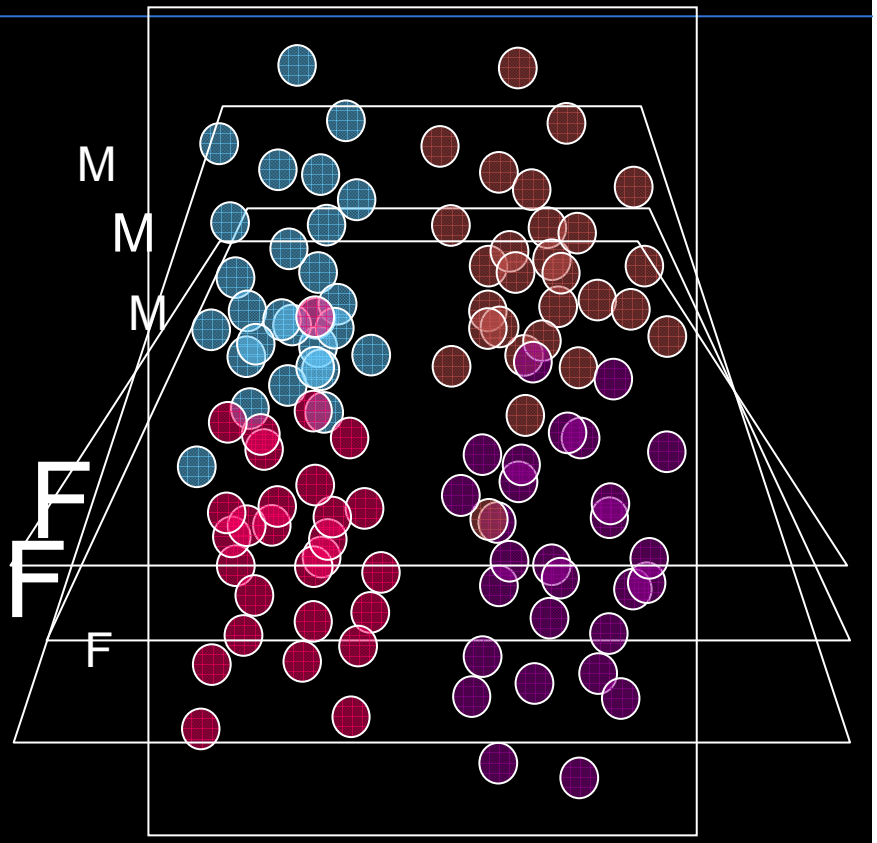
Advanced Methods

OPLS and the S-Plot

Coping with unwanted variation

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Often the effect we are looking for is masked by other unwanted variation
- OPLS is able to rotate the projection so that the model focuses on the effect of interest
- Here we want to focus on **control vs. treated** but **gender** is the bigger influence on X
- OPLS causes a rotation so that the first OPLS component shows the between class difference

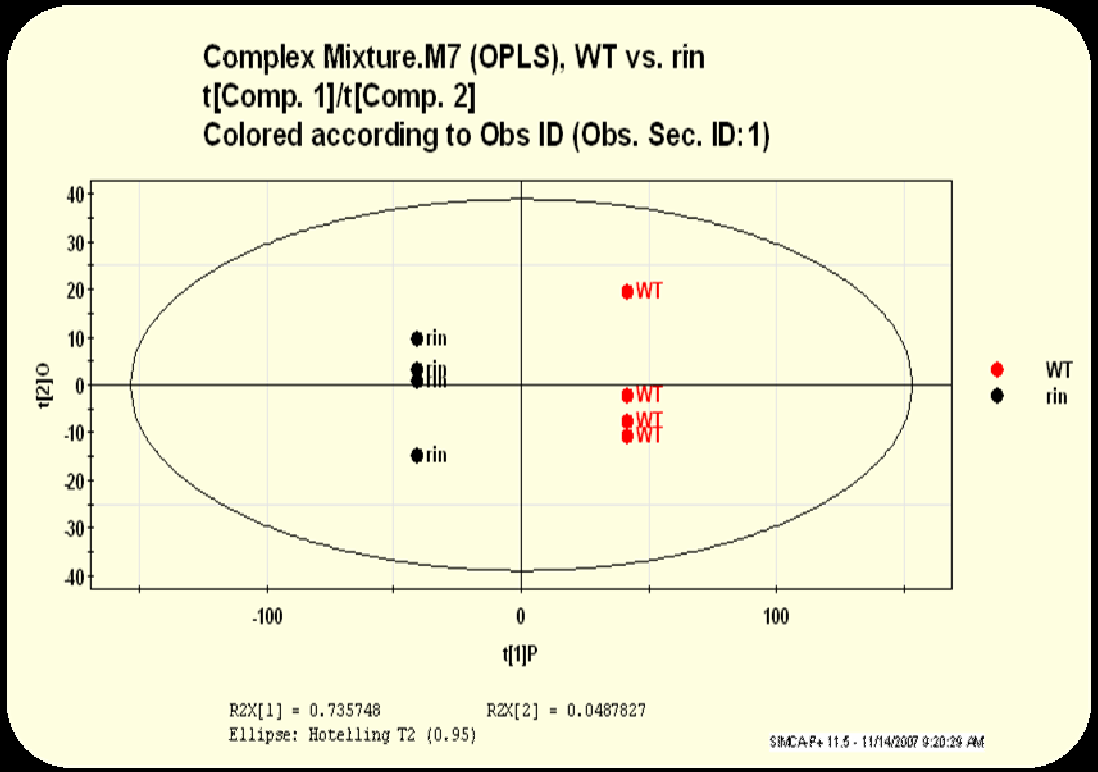


Control vs. Treated

Orthogonal PLS-DA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Within group variation



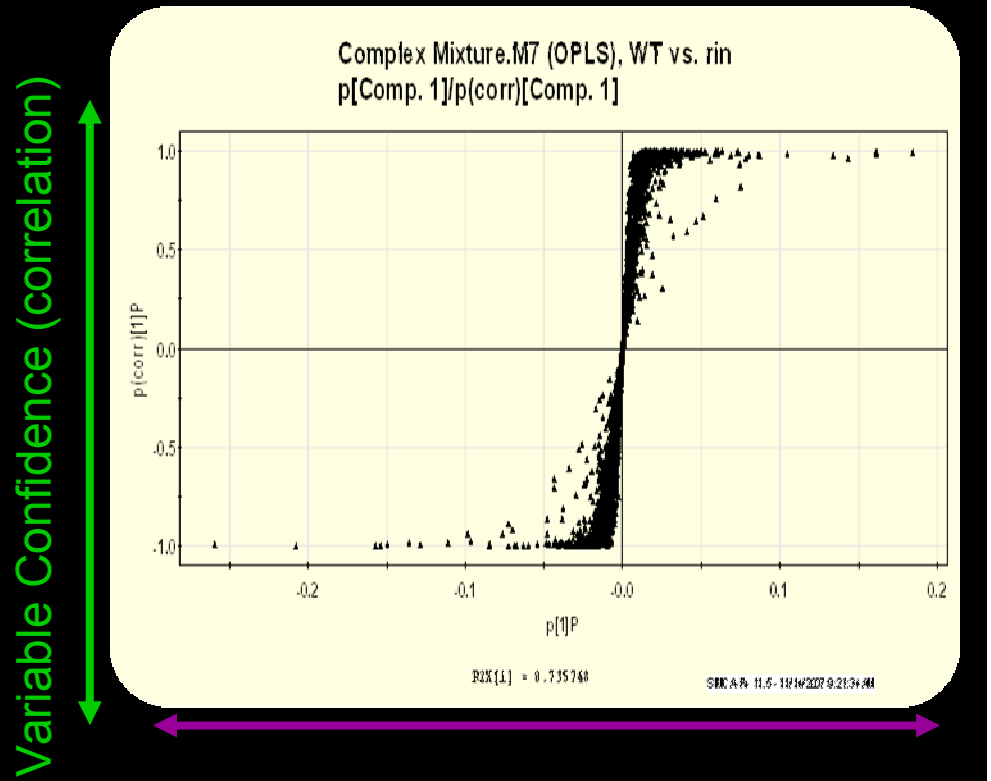
Between group variation

The S-Plot for OPLS-DA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Developed by A team of scientists from:

- The University of Umea
- Umetrics
- Waters

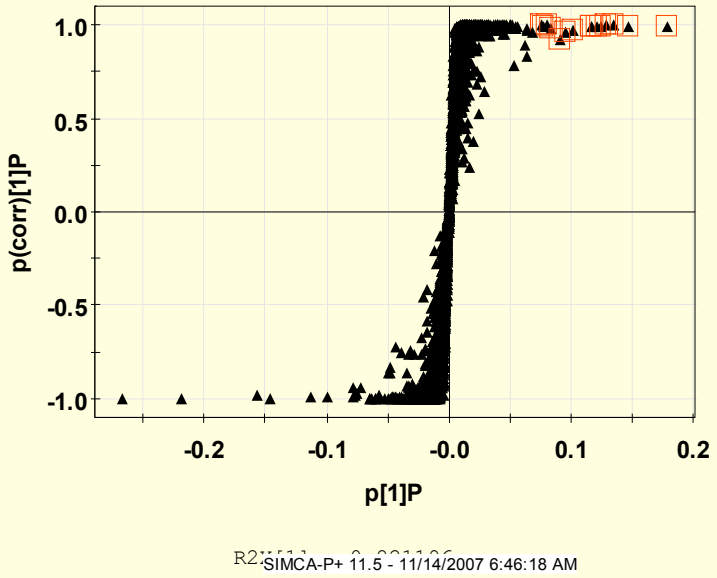


Wiklund S, Johansson E, Sjöström L, Mellerowicz EJ, Edlund U, Shockcor JP, Gottfries J, Moritz T, Trygg J.
Visualization of GC/TOF-MS-Based Metabolomics Data for Identification of Biochemically Interesting Compounds Using OPLS Class Models.
Anal Chem. 2008 Jan 1;80(1):115-122.

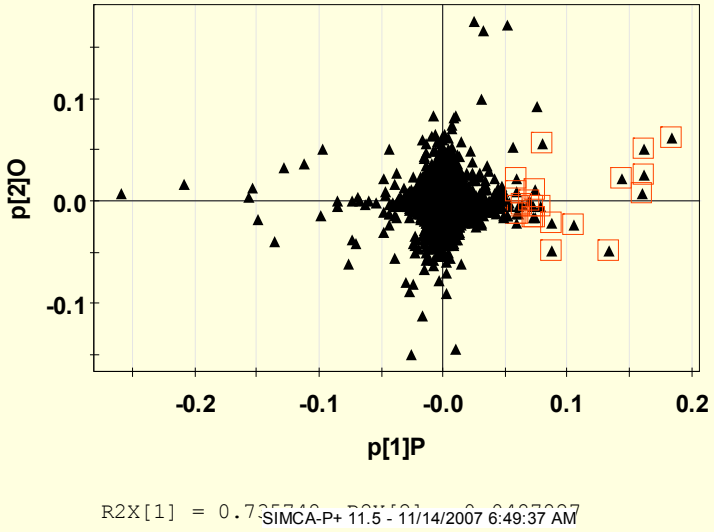
S-Plot vs Loadings. High in Wild Type

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Complex Mixture.M6 (OPLS), WT vs. nor
p[Comp. 1]/p(corr)[Comp. 1]



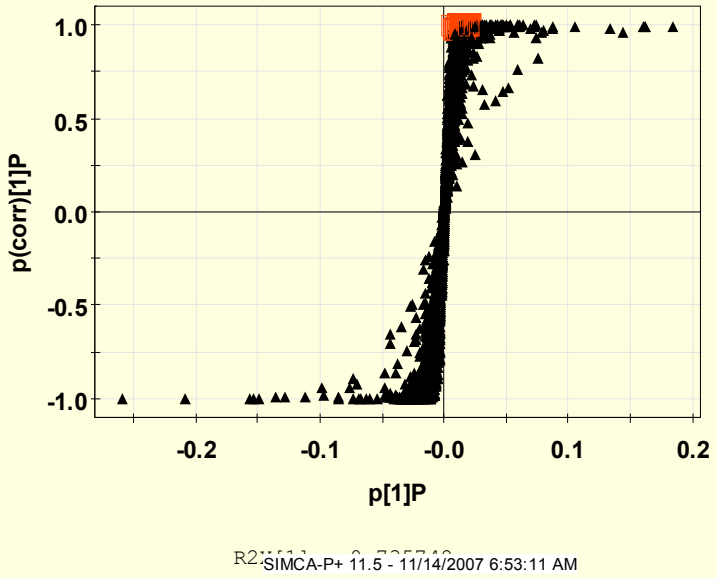
Complex Mixture.M7 (OPLS), WT vs. rin
p[Comp. 1]/p[Comp. 2]
Colored according to model terms



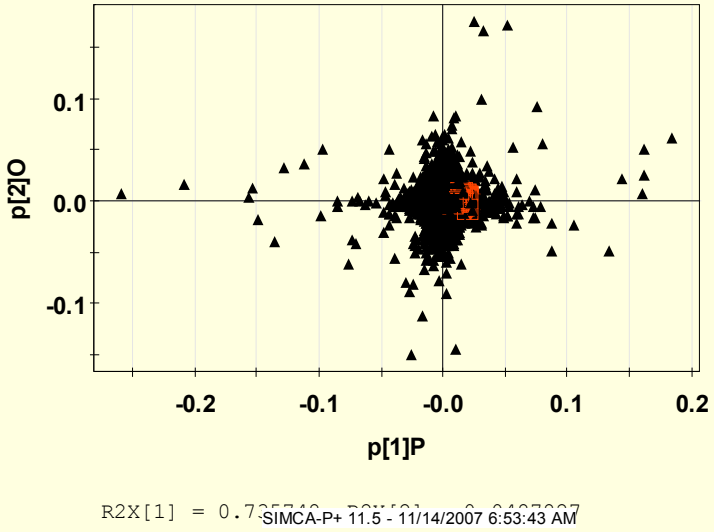
S-Plot vs Loadings. High in Wild Type

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Complex Mixture.M7 (OPLS), WT vs. rin
p[Comp. 1]/p(corr)[Comp. 1]



Complex Mixture.M7 (OPLS), WT vs. rin
p[Comp. 1]/p[Comp. 2]
Colored according to model terms



Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

MarkerLynx XS Software Demo

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Ion-Mobility /oa-TOF Mass Spectrometry And its Application to the Study of Lipids

John Shockcor
Director of Metabolic Profiling Business Development, Waters Corp.
Visiting Fellow, Dept of Biochemistry, University of Cambridge

Ion mobility

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Separation of ions as they drift through a gas under the influence of an electric field
- Rate of drift is dependant on the ion's mobility through the gas
- Mobility is dependant on factors such as,
  - Size
  - Shape
  - Charge

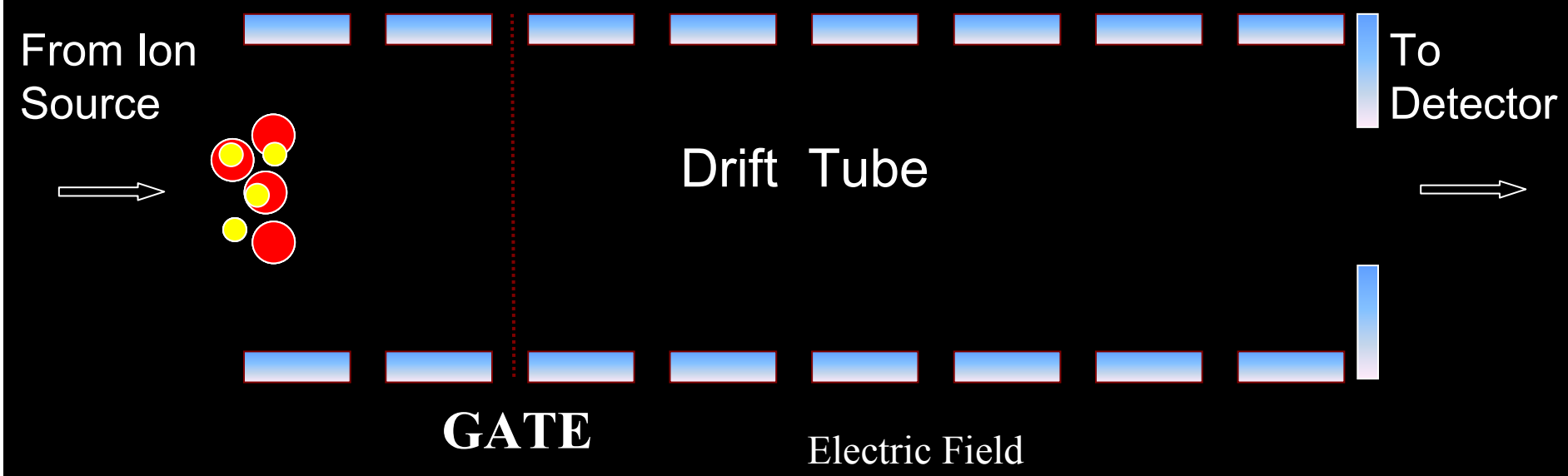
History of IMS

Thomson, J. J.; Rutherford, G. P., "Conduction of Electricity Through Gases", Dover, NY, 1928.

Thomson, J. J.; "Rays of Positive Electricity", Green and Co., London, England, 1933.

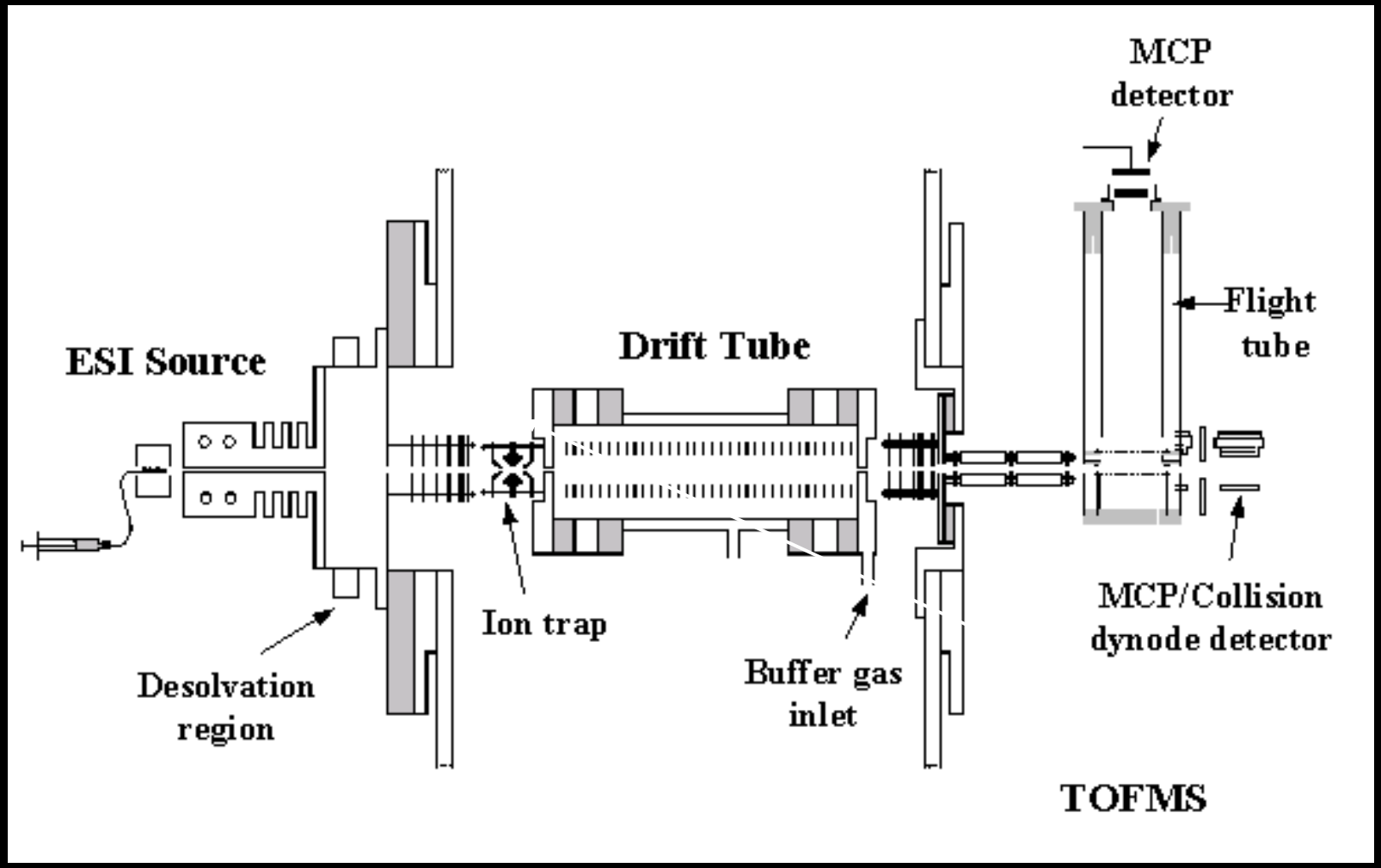
Conventional ion mobility spectrometers

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



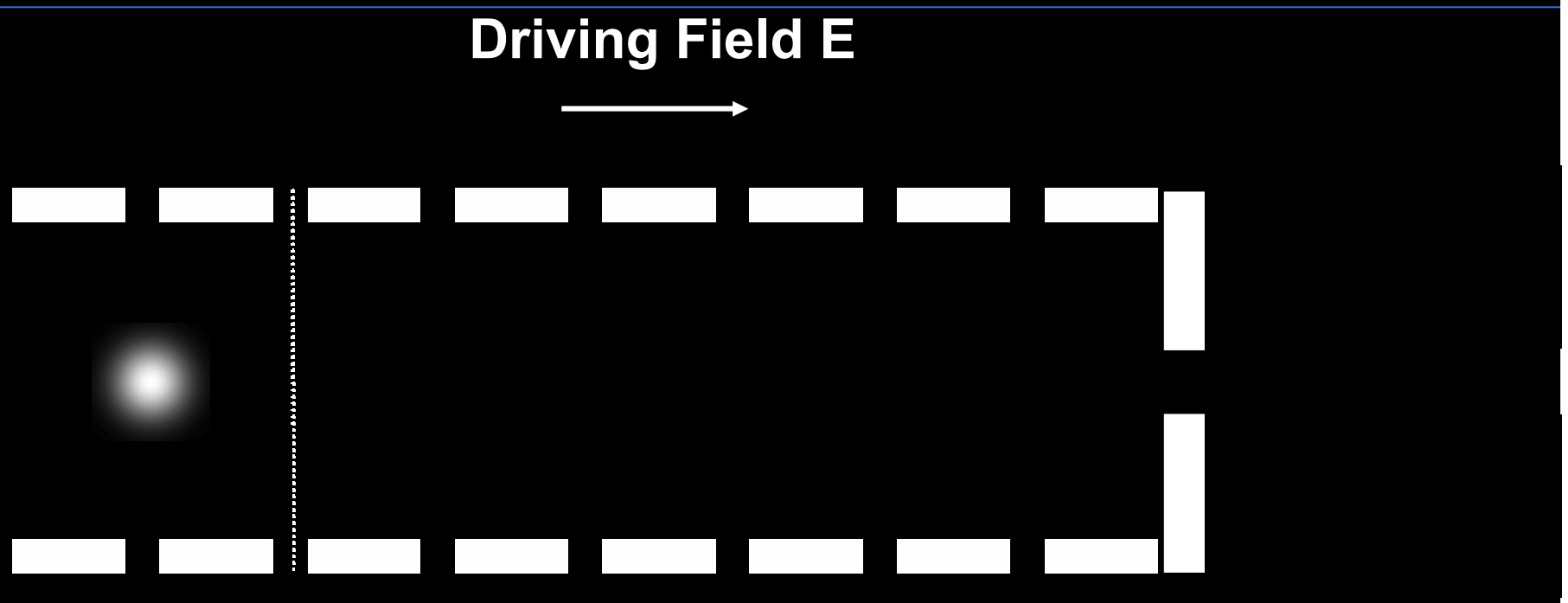
Clemmer et al. introduced an accumulating ion trap upstream to increase duty cycle [1997]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



However: Drift tubes suffer from diffusion losses

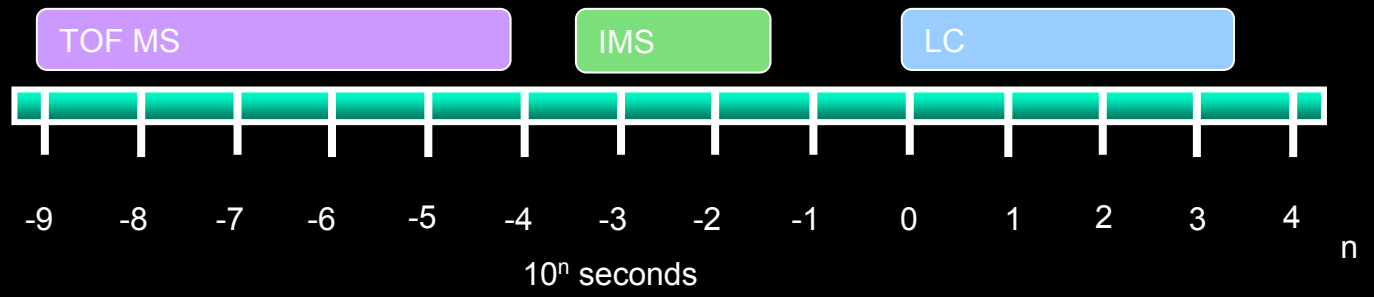
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Ion Mobility Spectrometry Perspective:- The Benefits

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

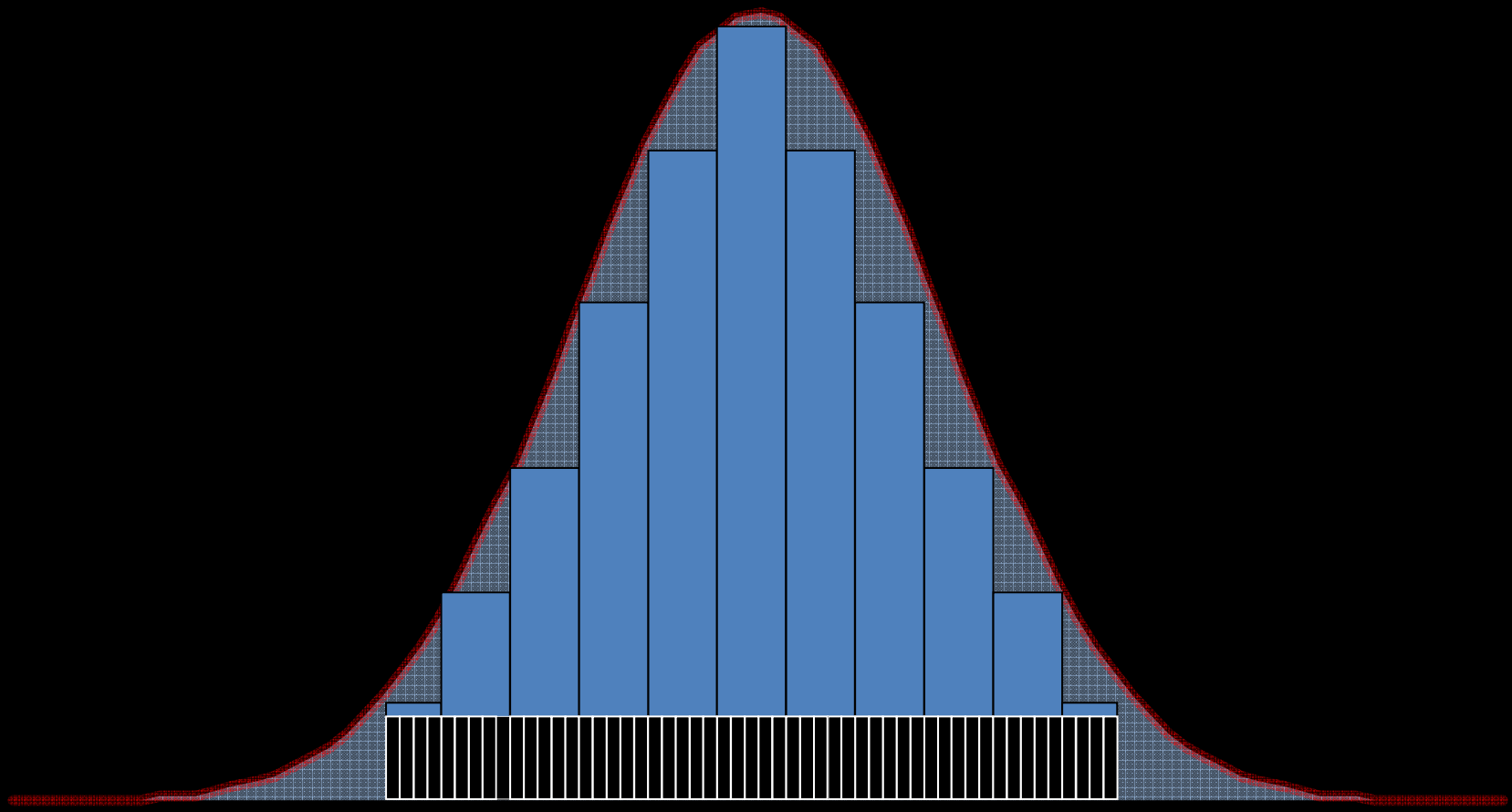
- Rapid orthogonal separation capability



- Improve peak capacity
- Provide novel functionality
- Enhance performance of the mass spectrometer
- IMS is an orthogonal separation
- How can T-Wave technology be implemented ?

IMS Timing

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



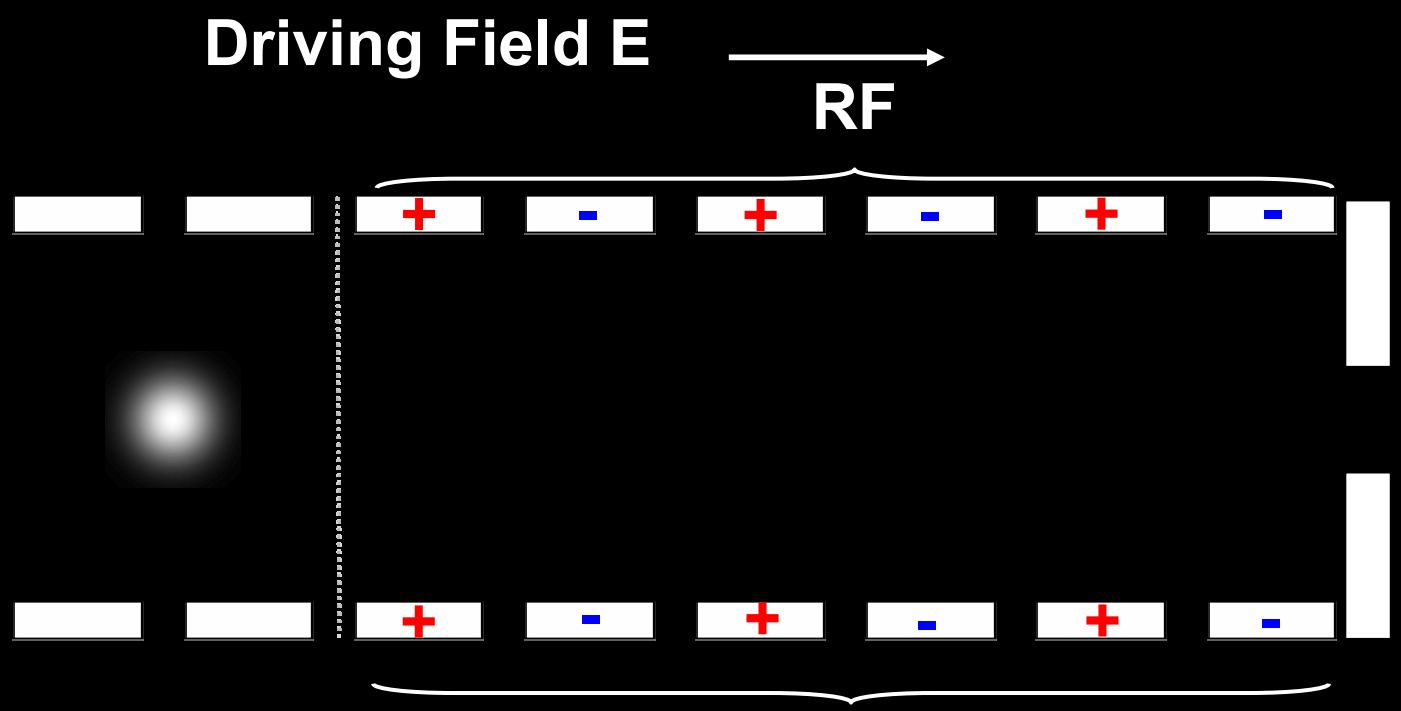
→ MS: µsec

→ IMS run: msec

Chromatographic peak: sec

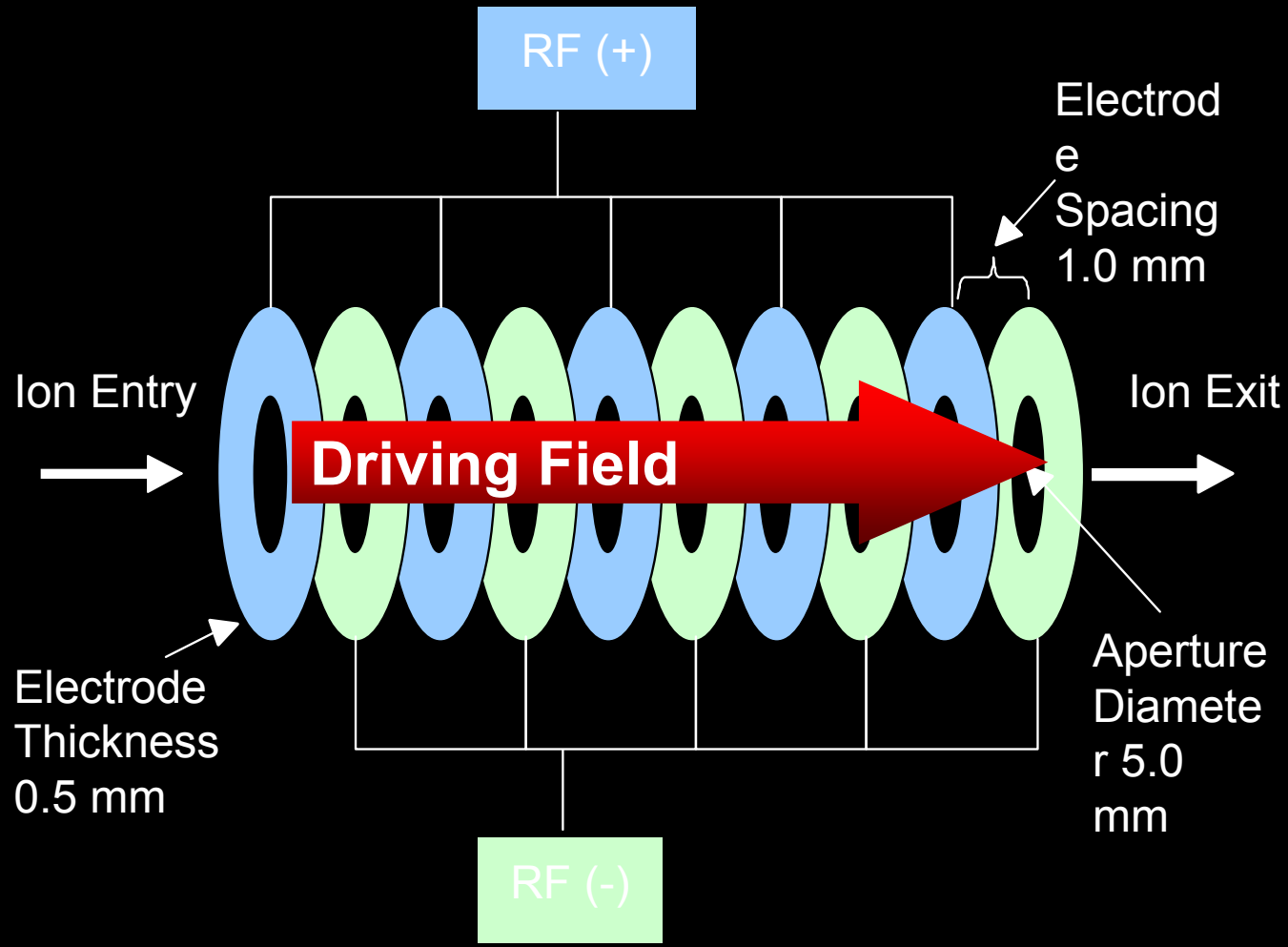
Adding RF gives radial confinement and greater sensitivity

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



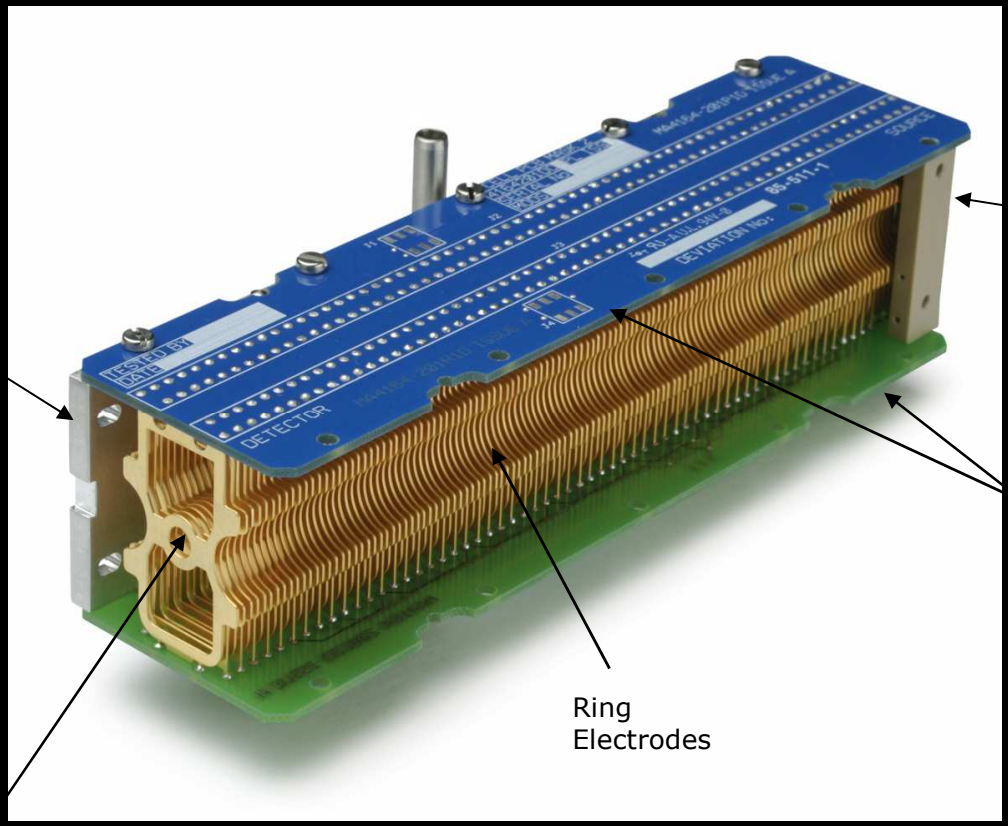
An RF-Only Stacked Ring Ion Guide transports ions with 100% efficiency

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Traveling Wave Device

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



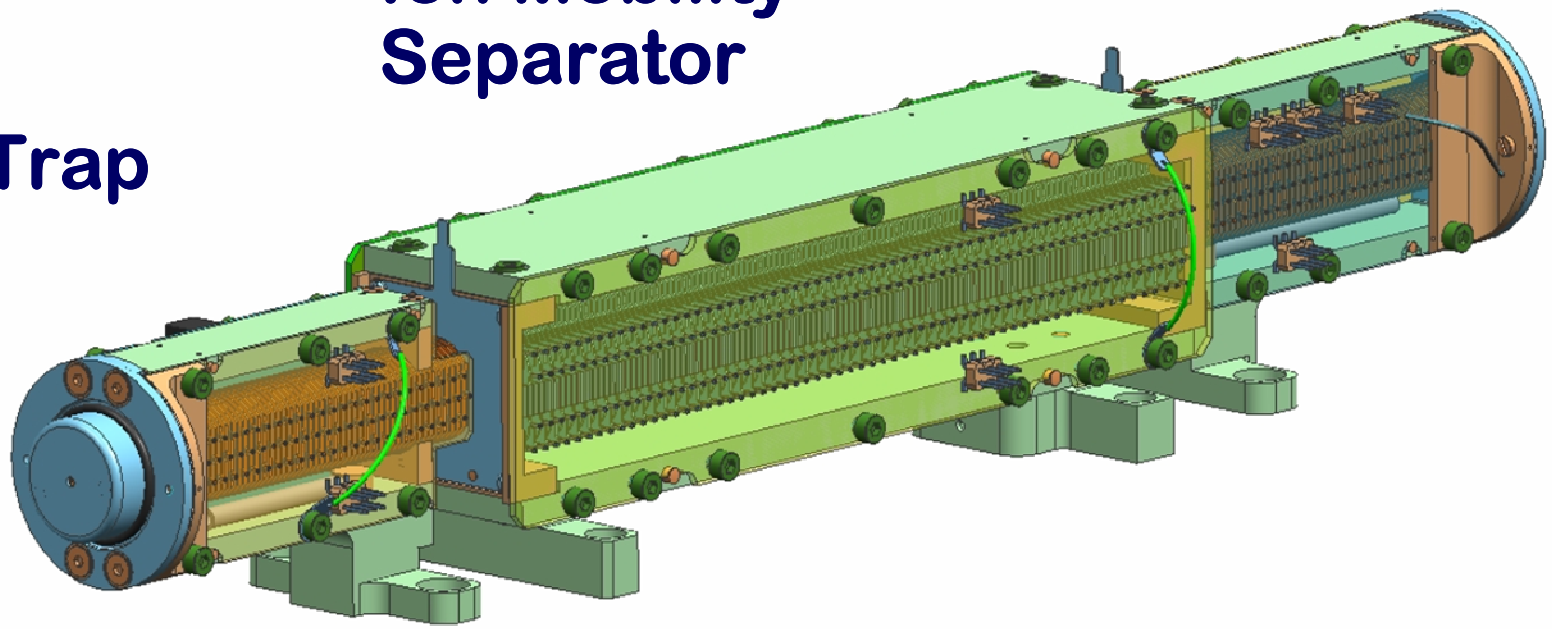
The TriWave

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Transfer

Ion Mobility Separator

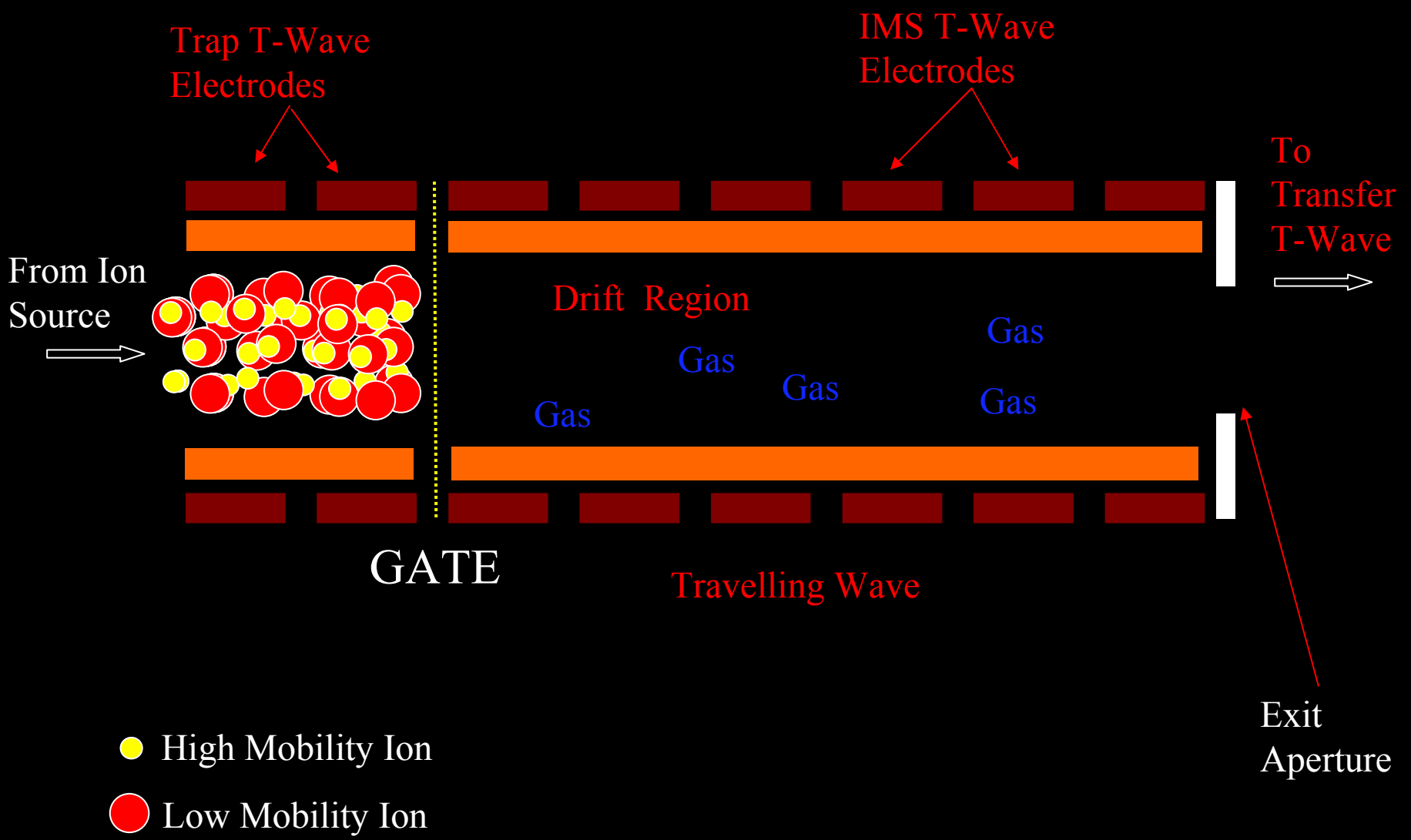
Trap

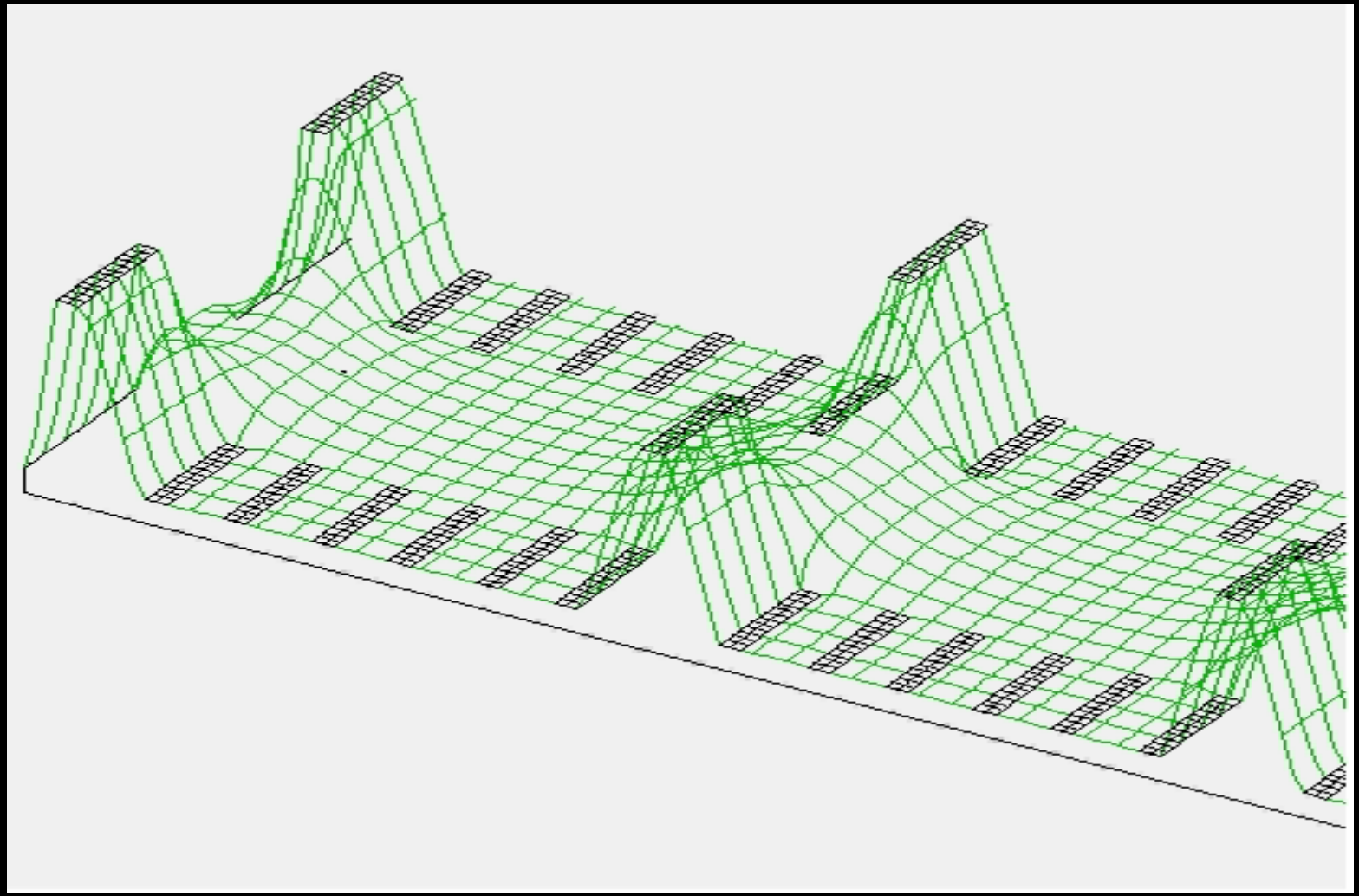


Fragmentation (CID) can be induced in both the TRAP and TRANSFER regions (~10^{-3} mbar). For IMS analysis helium is admitted into the Ion Mobility Separator (~0.5 mbar)

Synapt HDMS System
TriWave - High Duty Cycle High Transmission

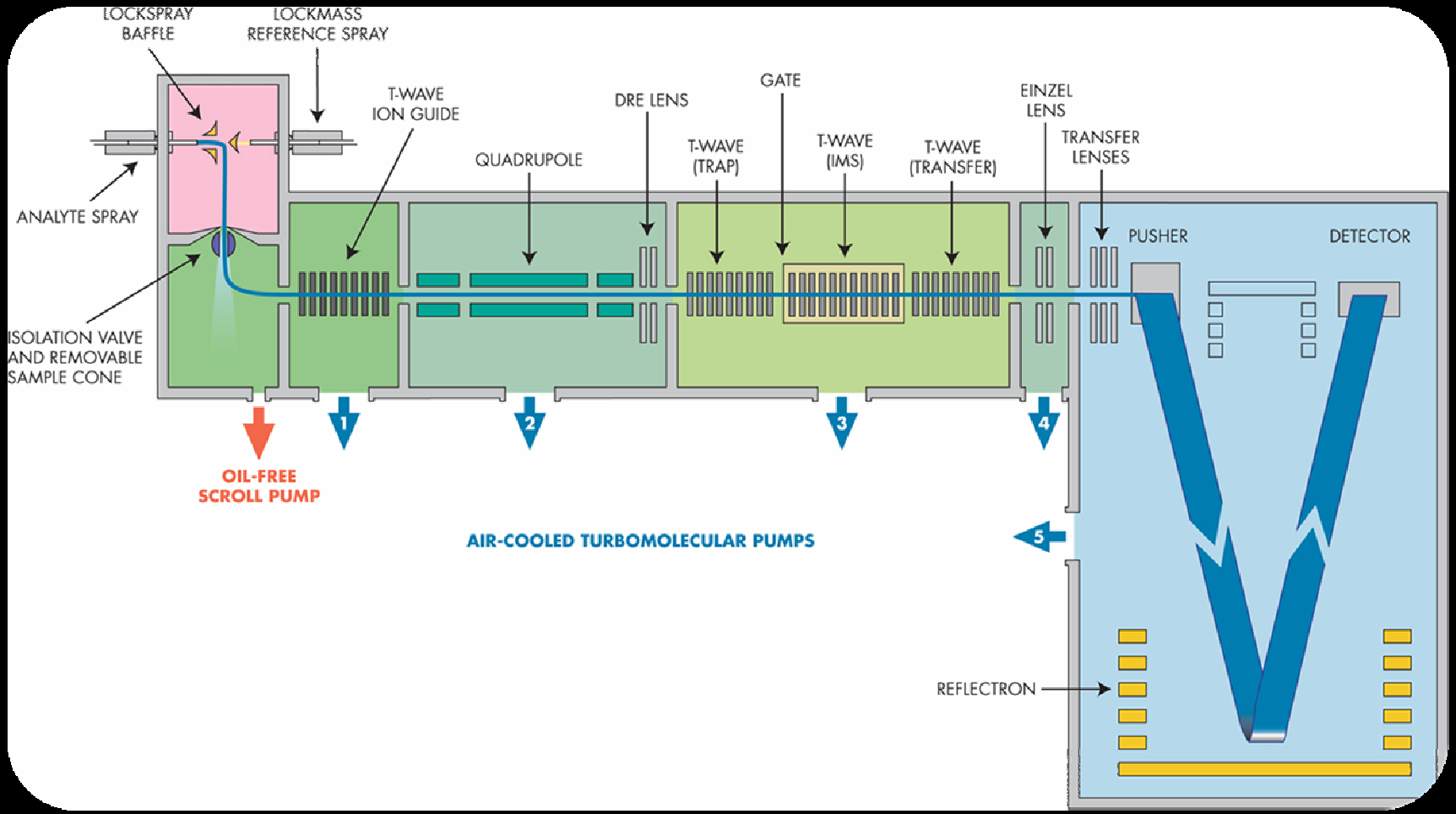
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™





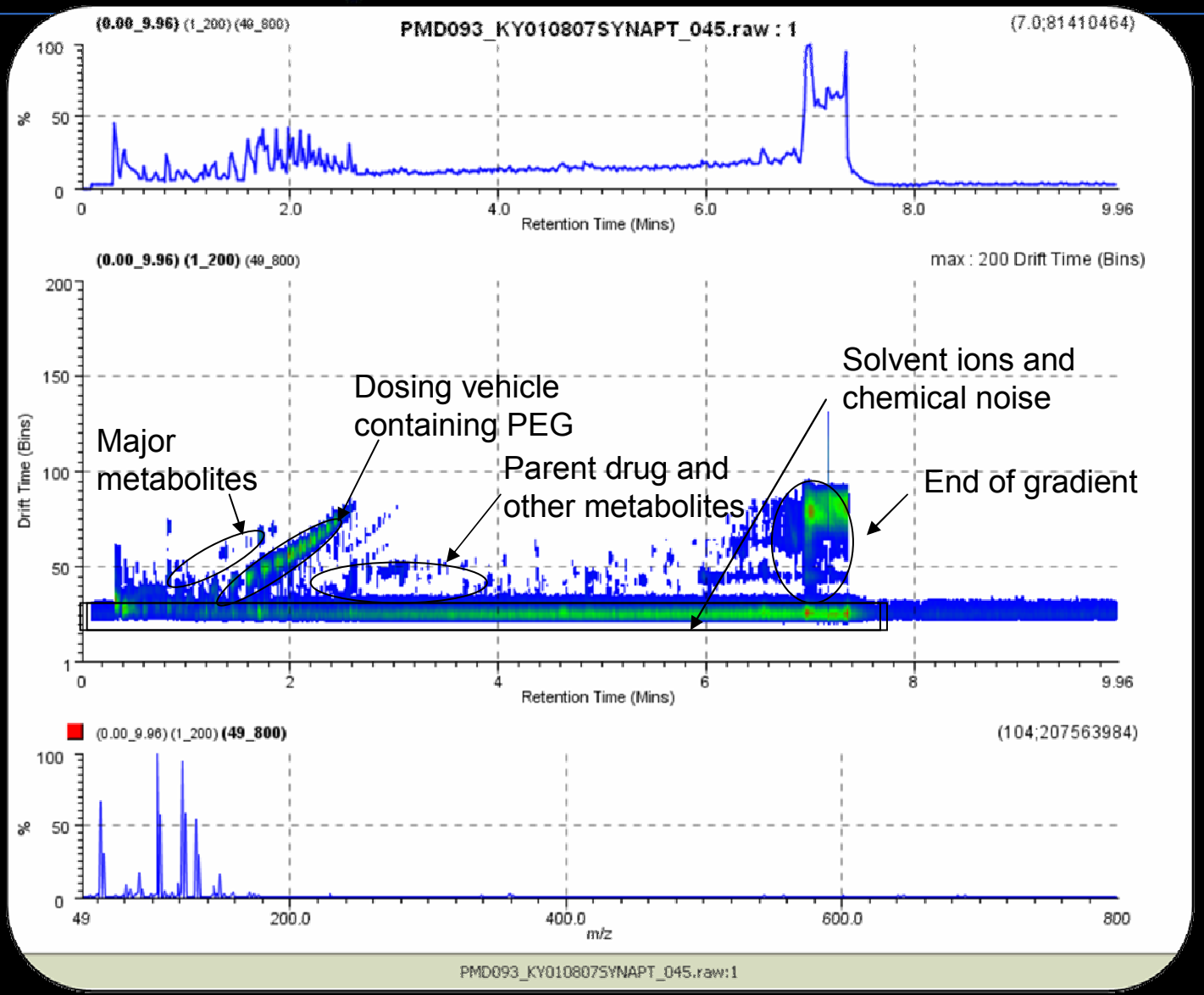
Hybrid Quadrapole / IMS / oa-ToF

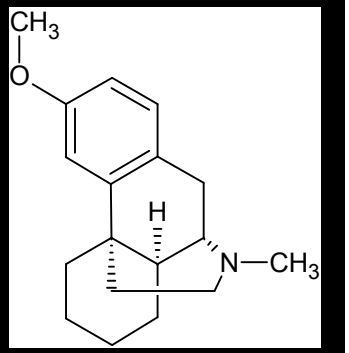
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Removing Interferences

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

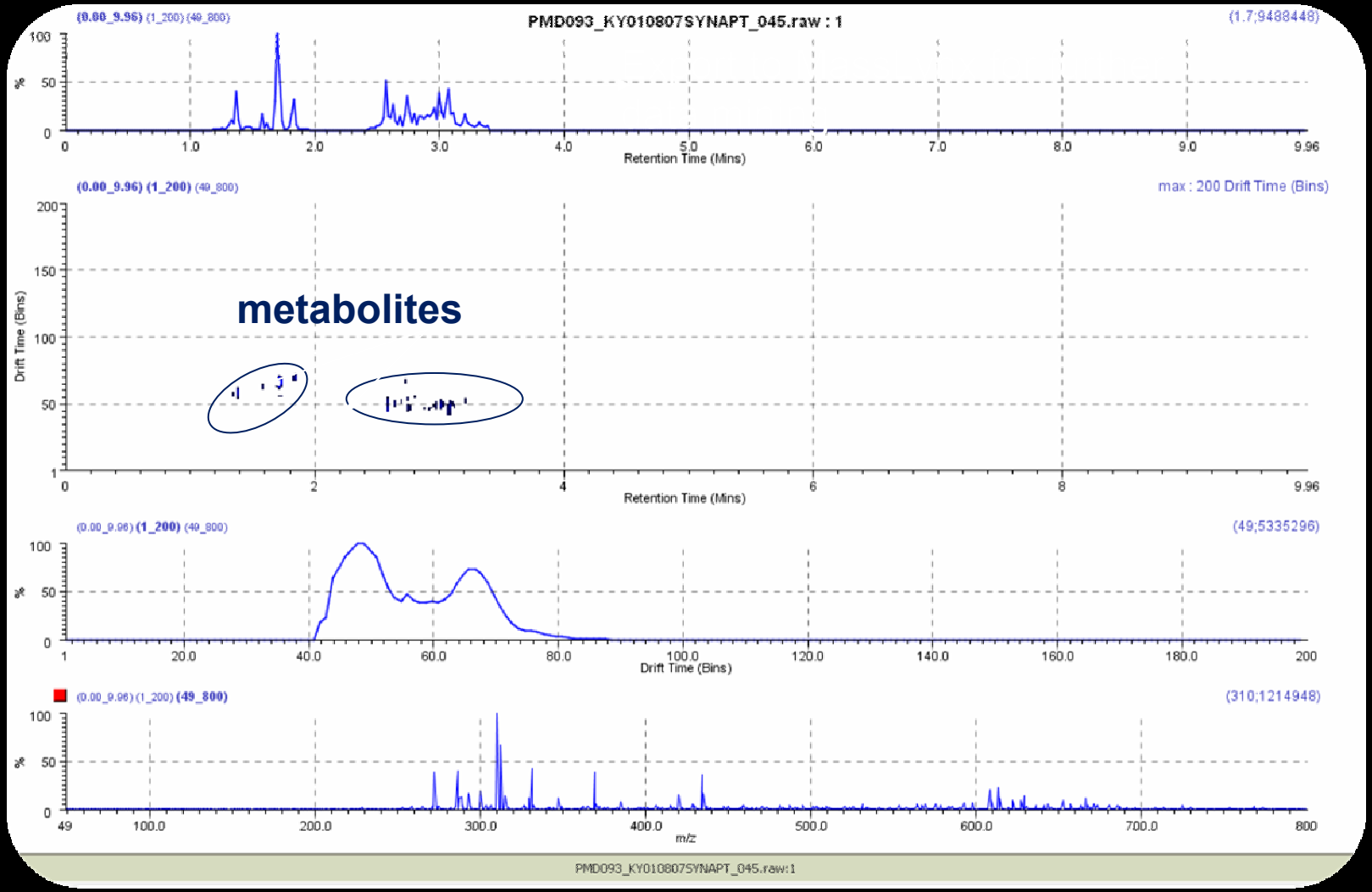




Dextromethorphan

Data clean-up step using the drift time function

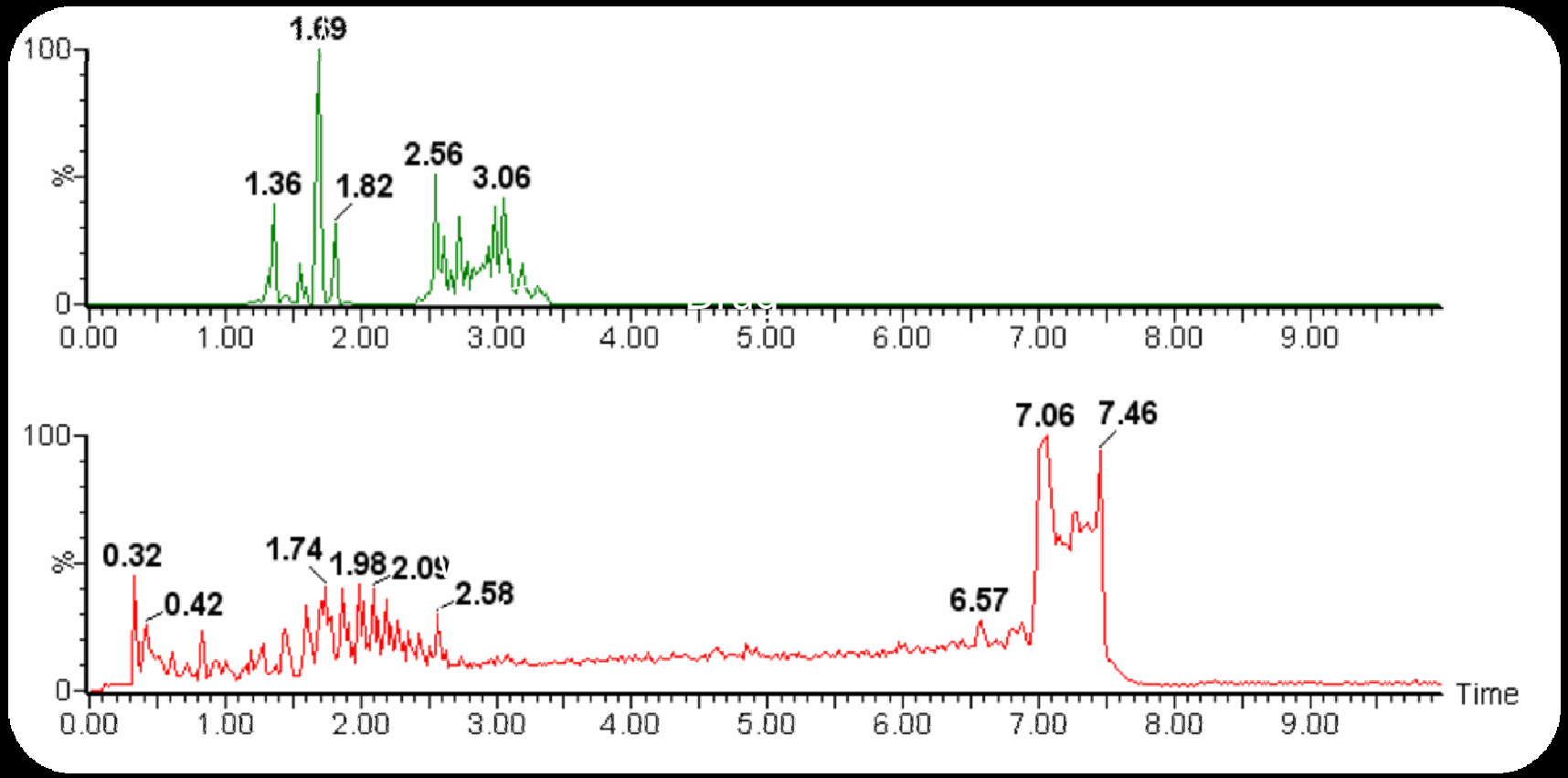
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Metabolite search by UPLC-IMS-TOF

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

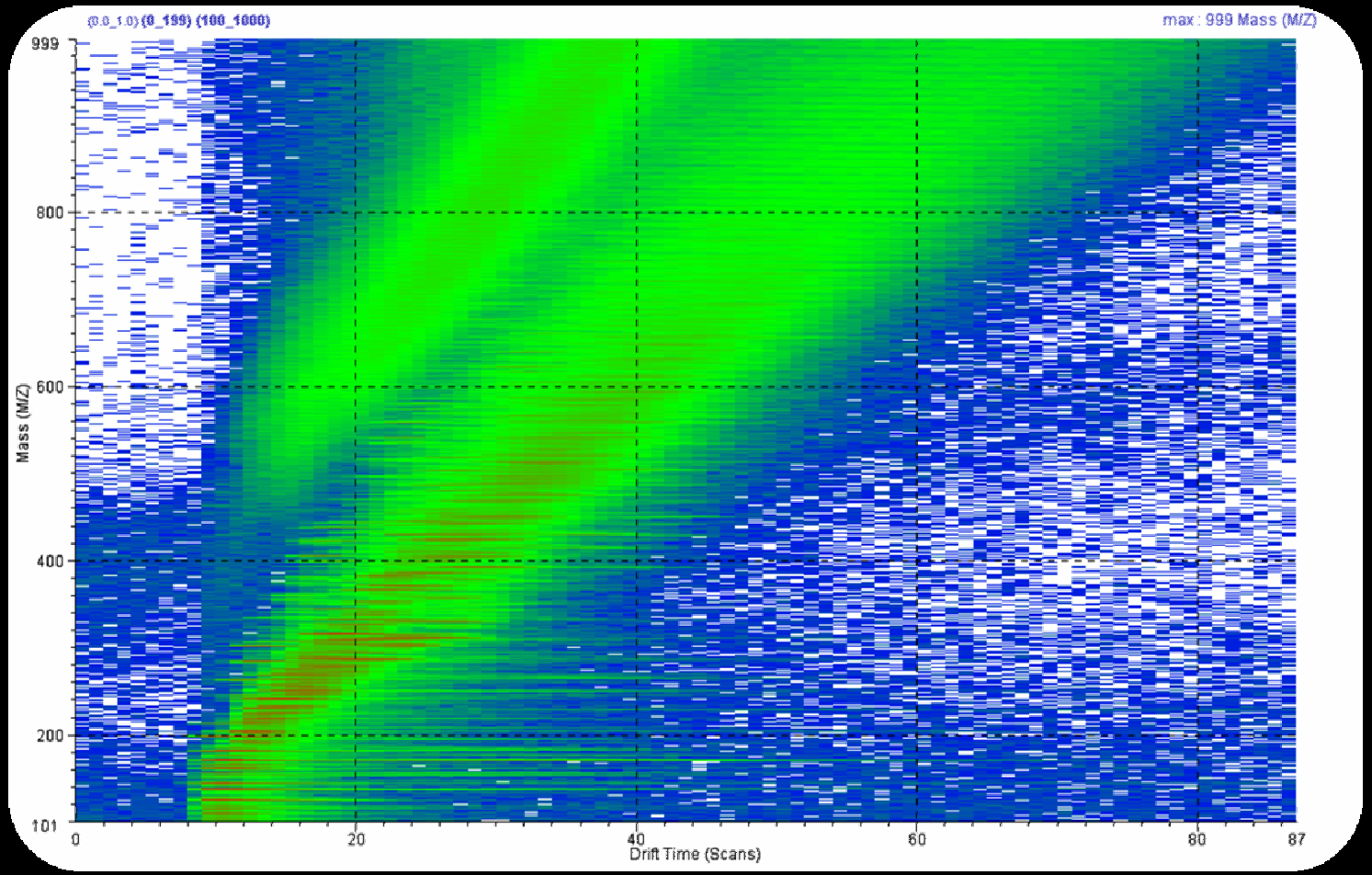
Metabolites



Urine Infusion on SYNAPT™ HDMS

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

m/z



Drift Time

IMS Data Review in Drift Scope

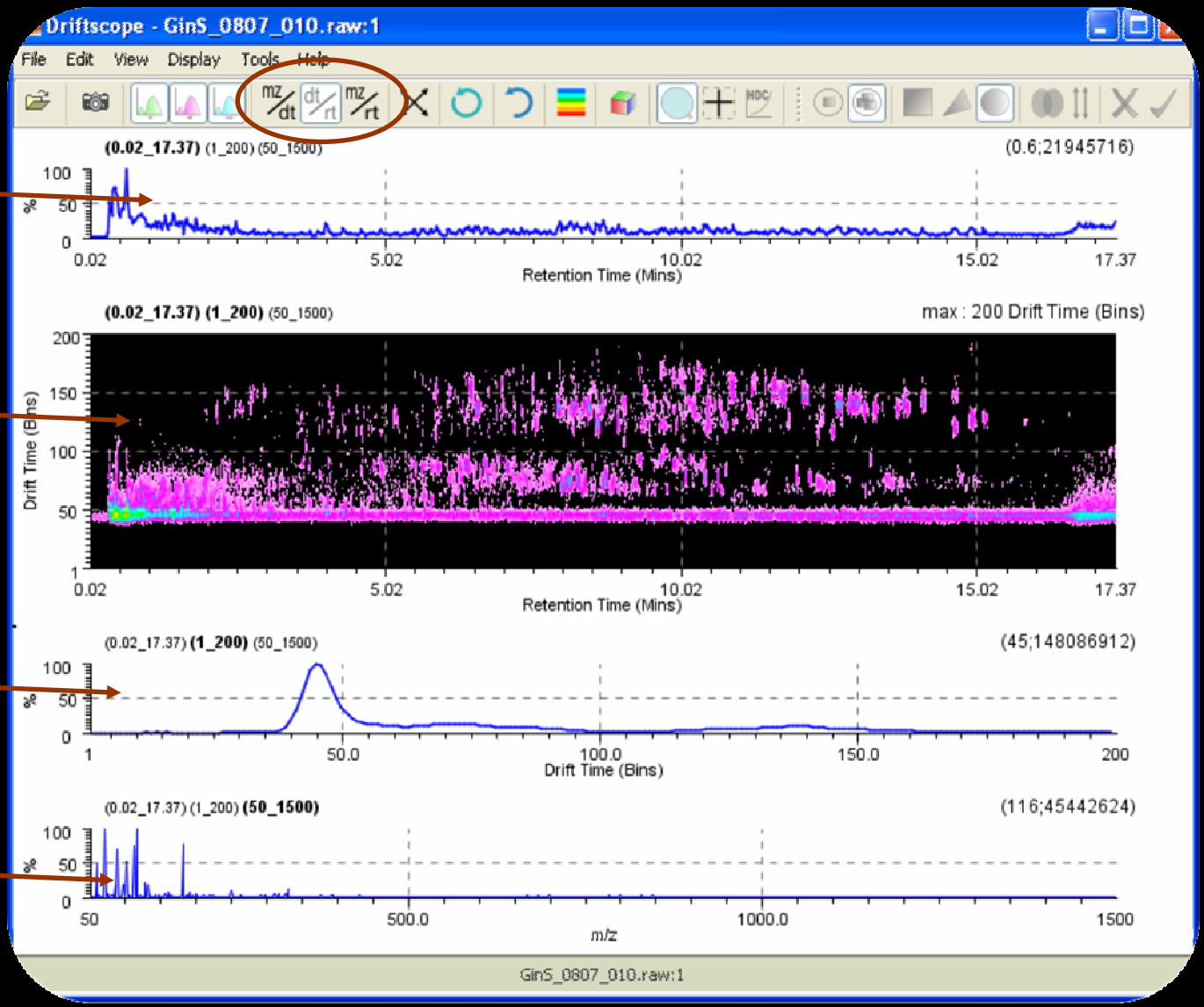
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

LC Chromatogram

IMS Drift Scope

Drift Time Plot

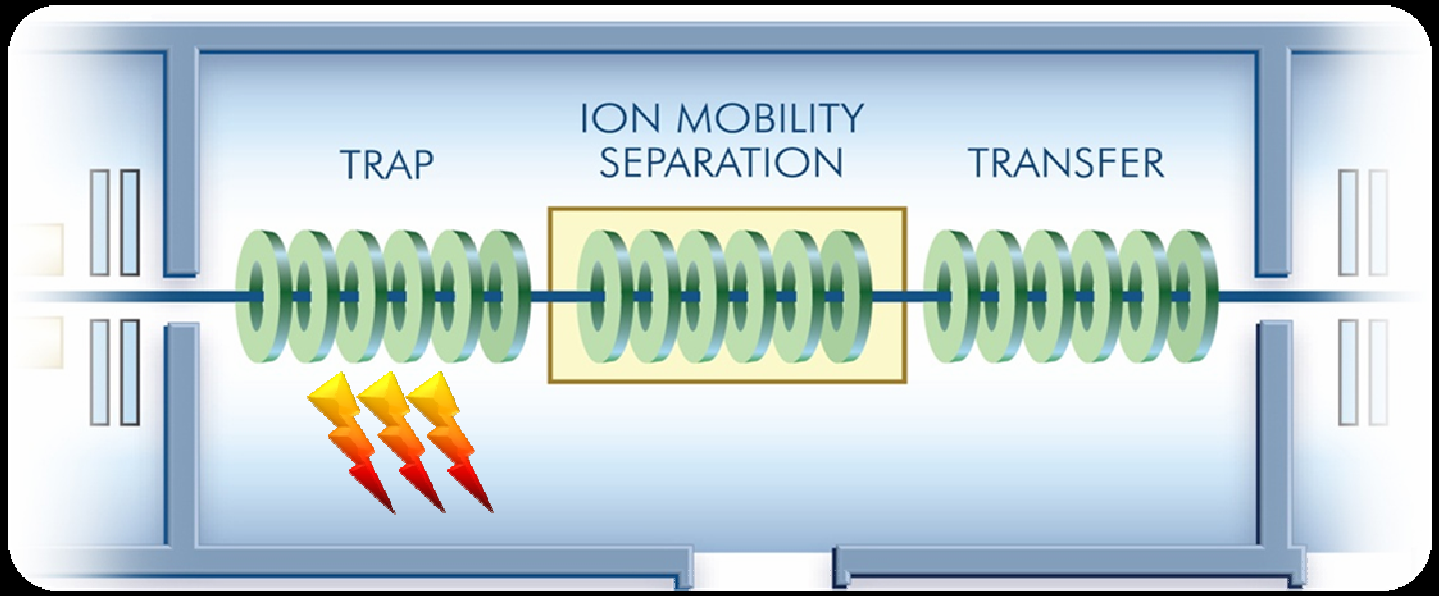
Mass Spectrum

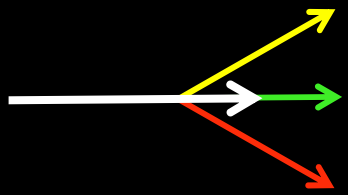


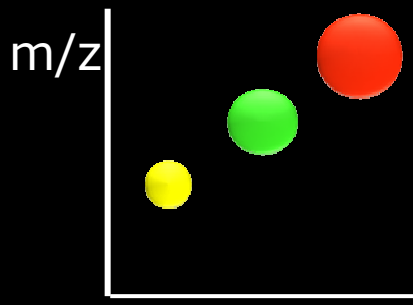
Trap fragmentation (CID-IMS)

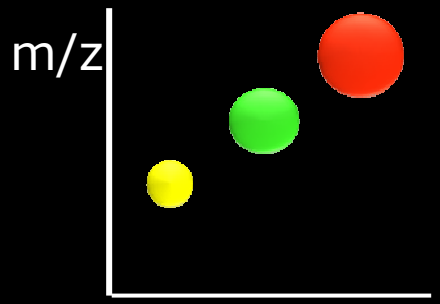
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Single ion









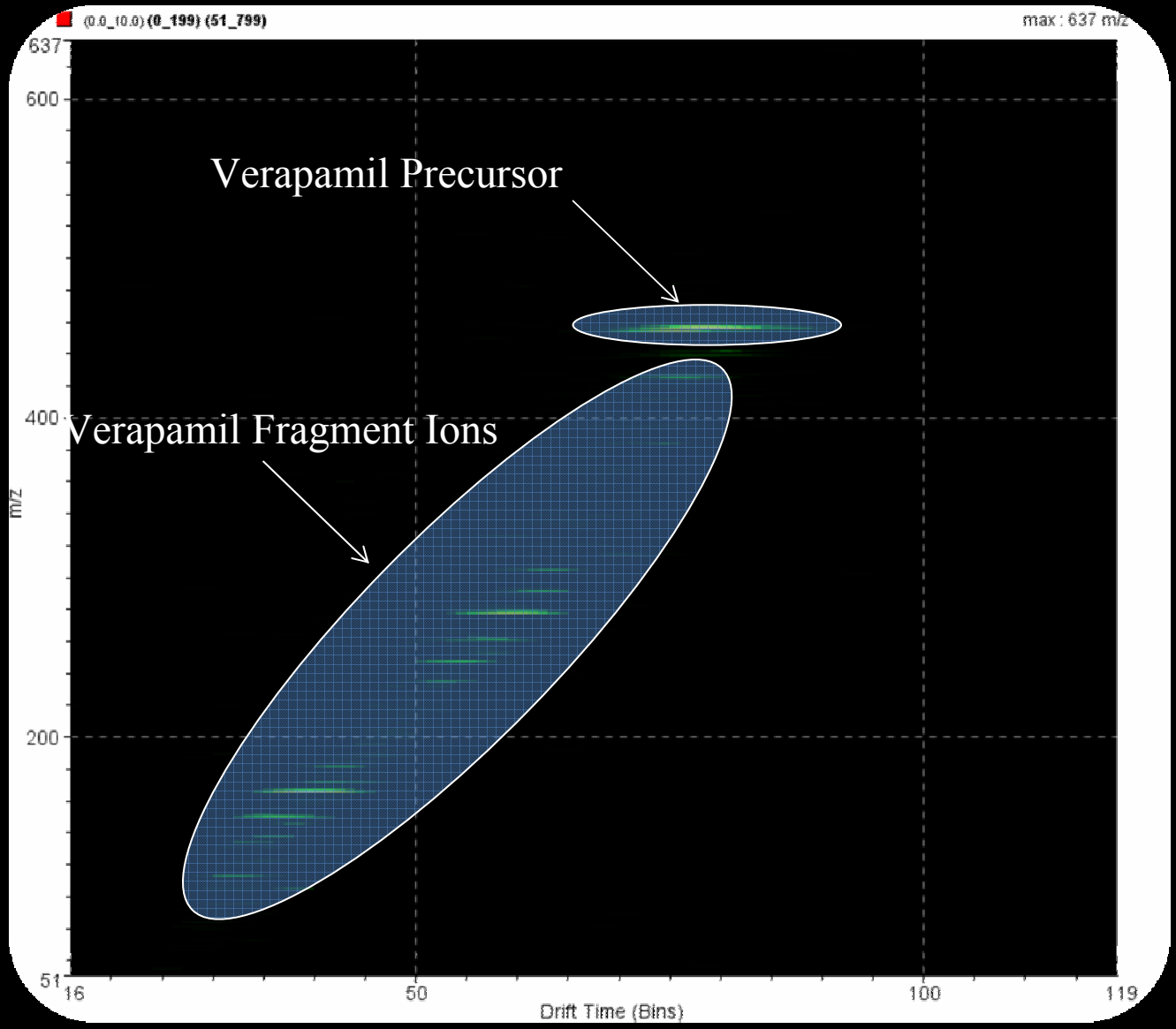
Precursor ion fragmented

Product ions separated by IMS

Transferred to oa-Tof

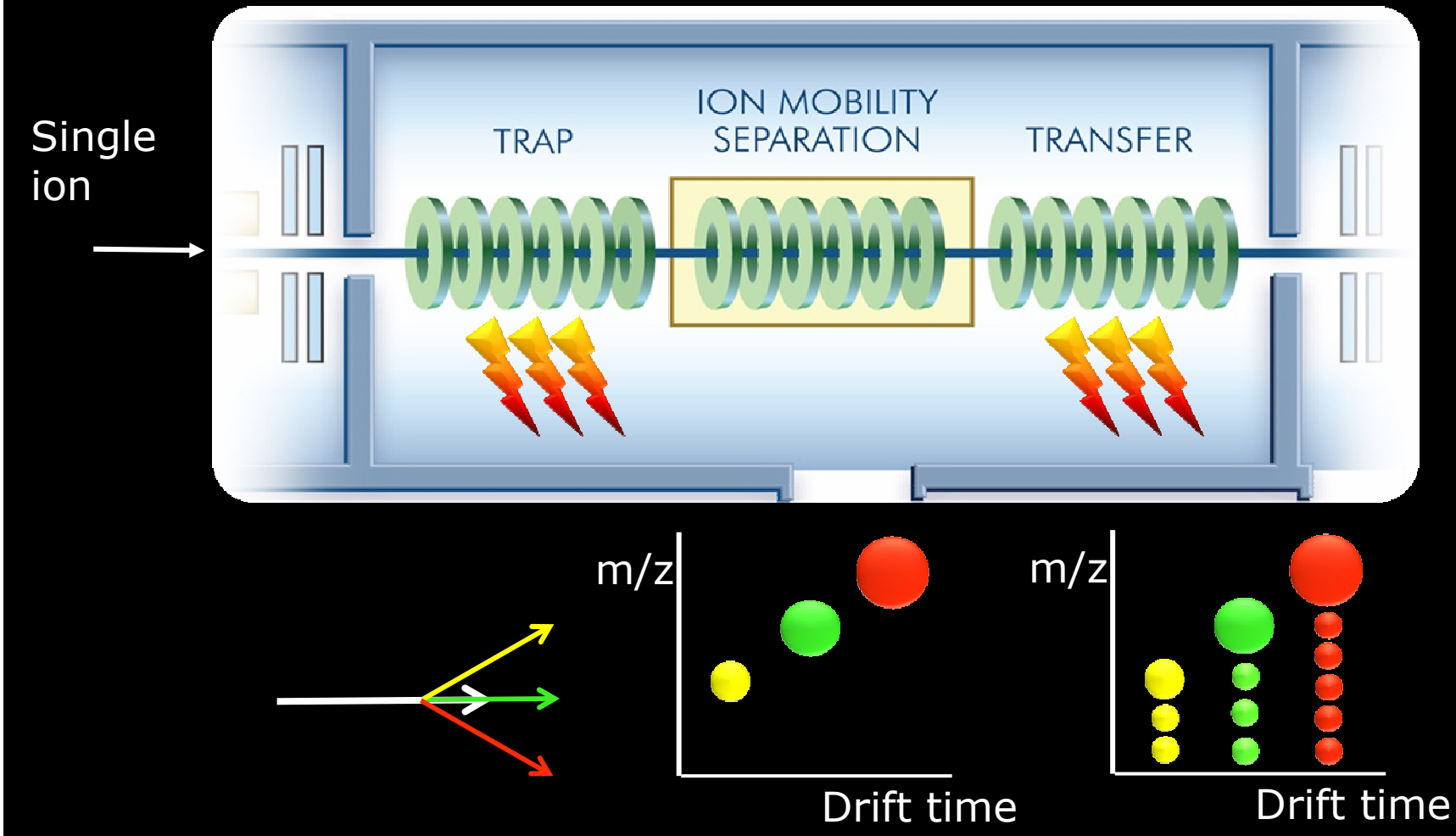
Trap fragmentation (CID-IMS)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Time Aligned Parallel Fragmentation (TAP) CID-IMS-CID

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



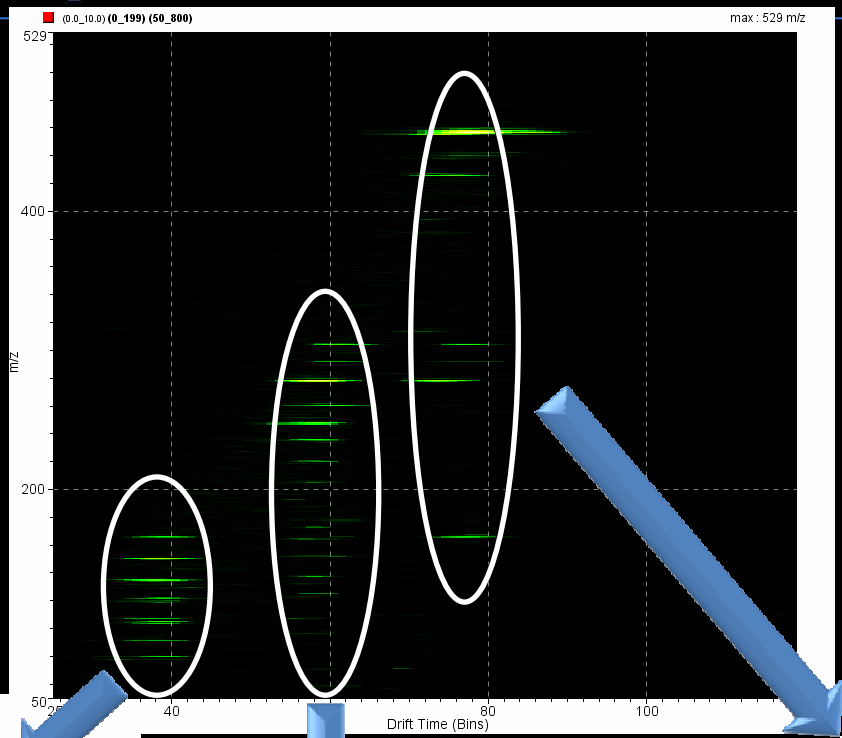
Precursor ion fragmented

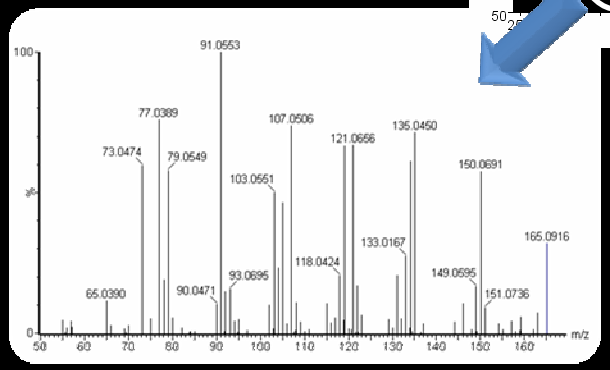
Product ions separated by IMS

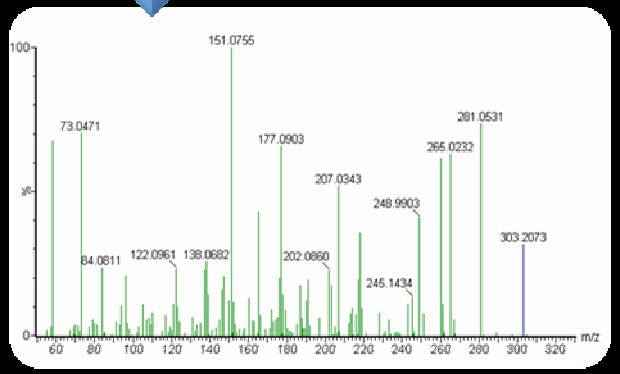
Precursor and products share same drift time

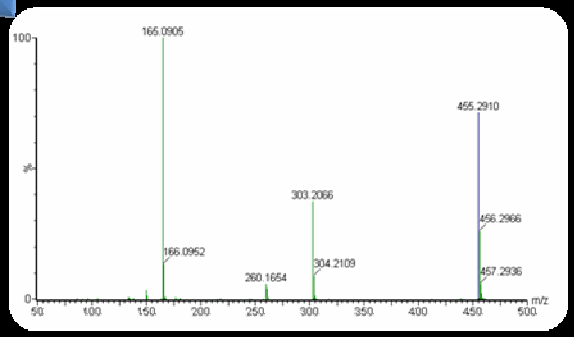
TAP fragmentation

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™





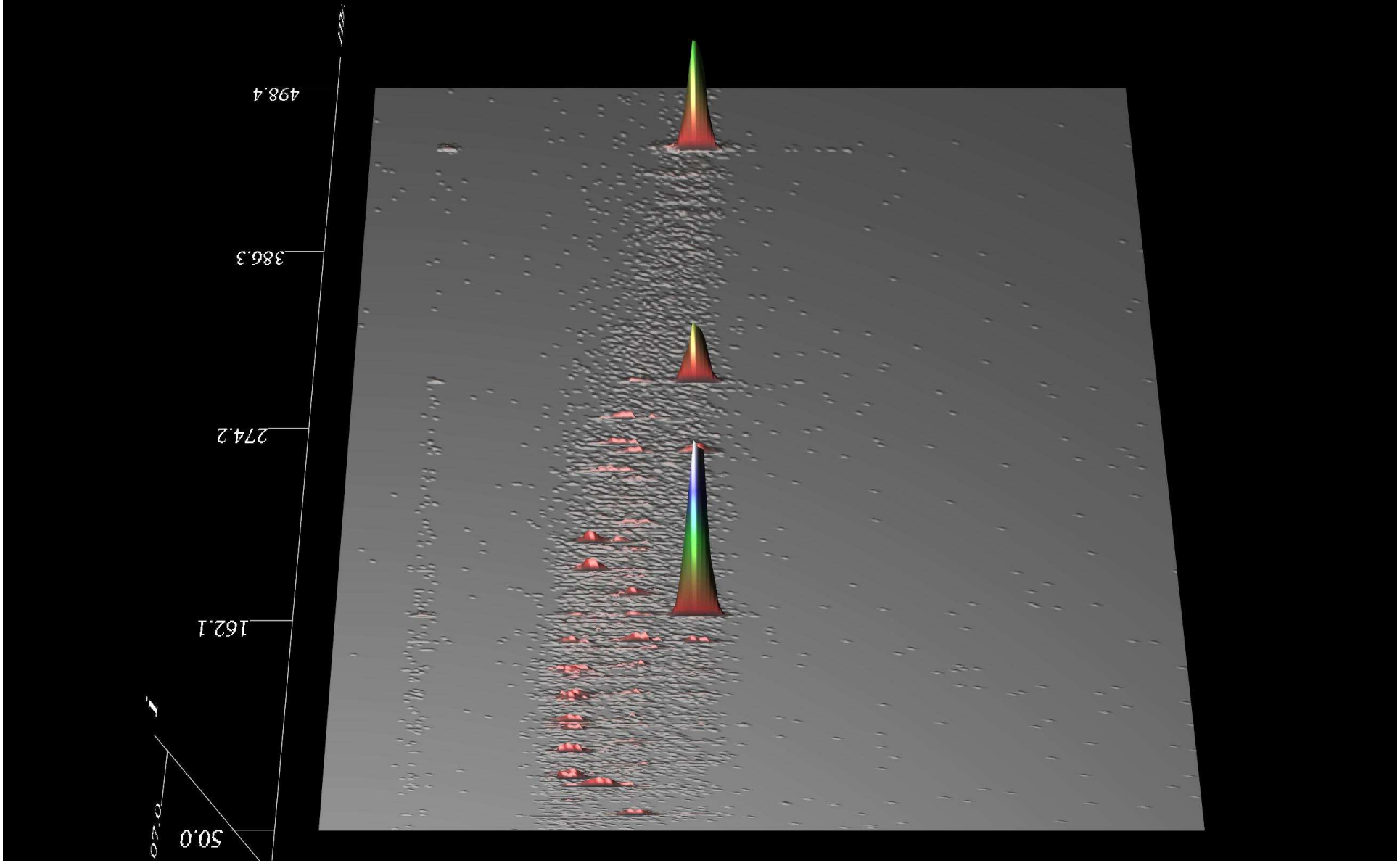




Ability to interrogate all drift time areas independently

3D representation of TAP for Verapamil

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



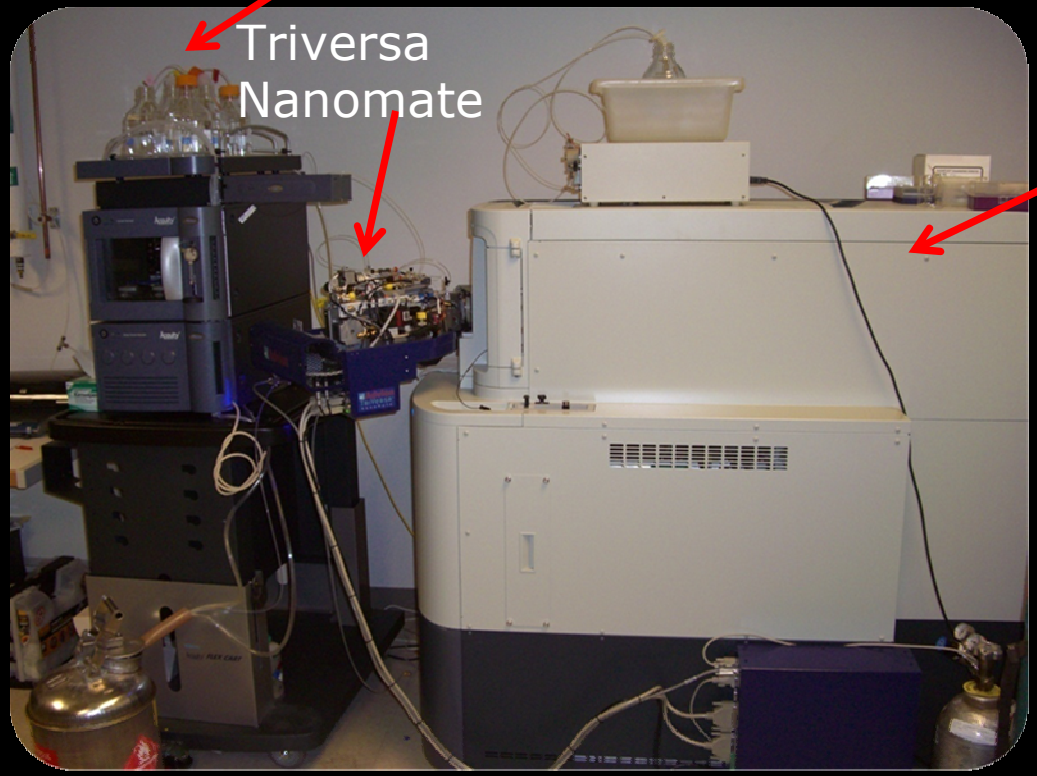
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Lipidomics using Ion Mobility

Instrument configuration

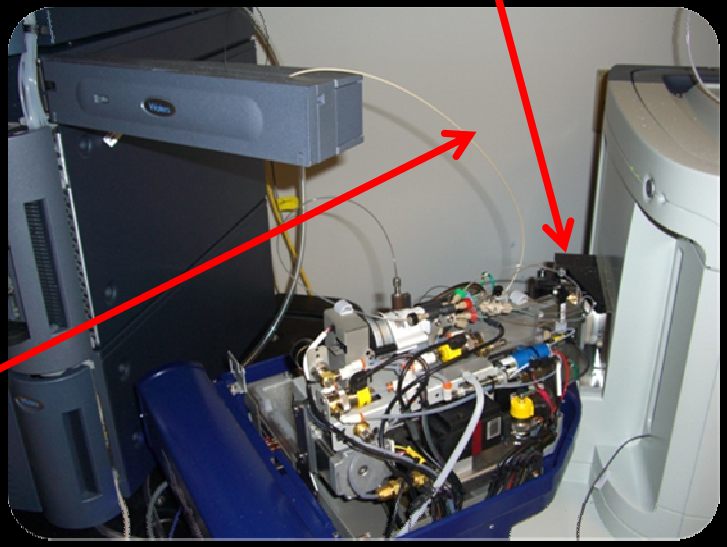
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UPLC flow rate 600 µL/min



Synapt HDMS

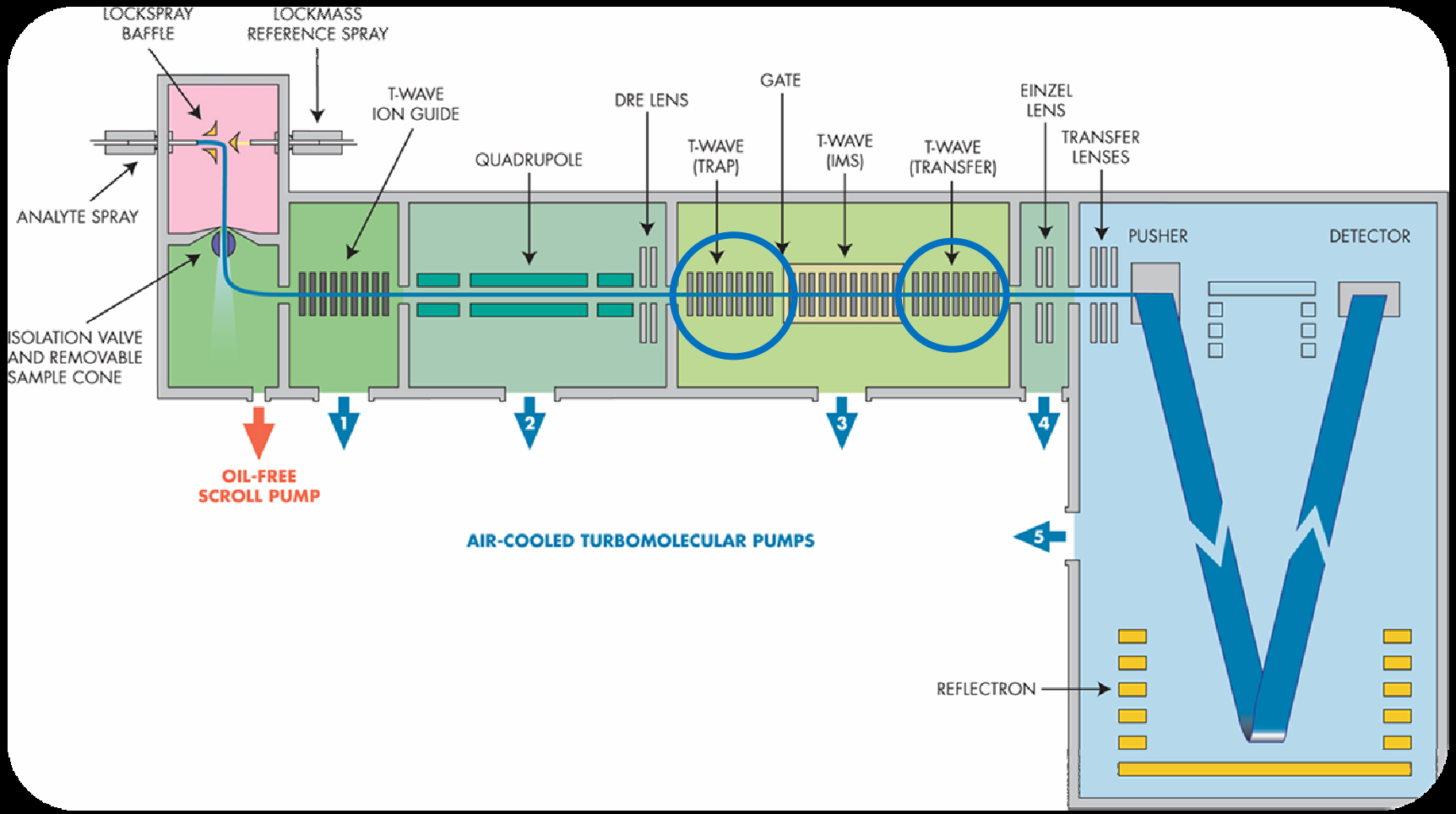
Flow rate into the Synapt
289nL/min



UPLC Flow connected to
Nanomate for fraction
collection 599.7µL/min

Hybrid Quadrapole / IMS / oa-ToF

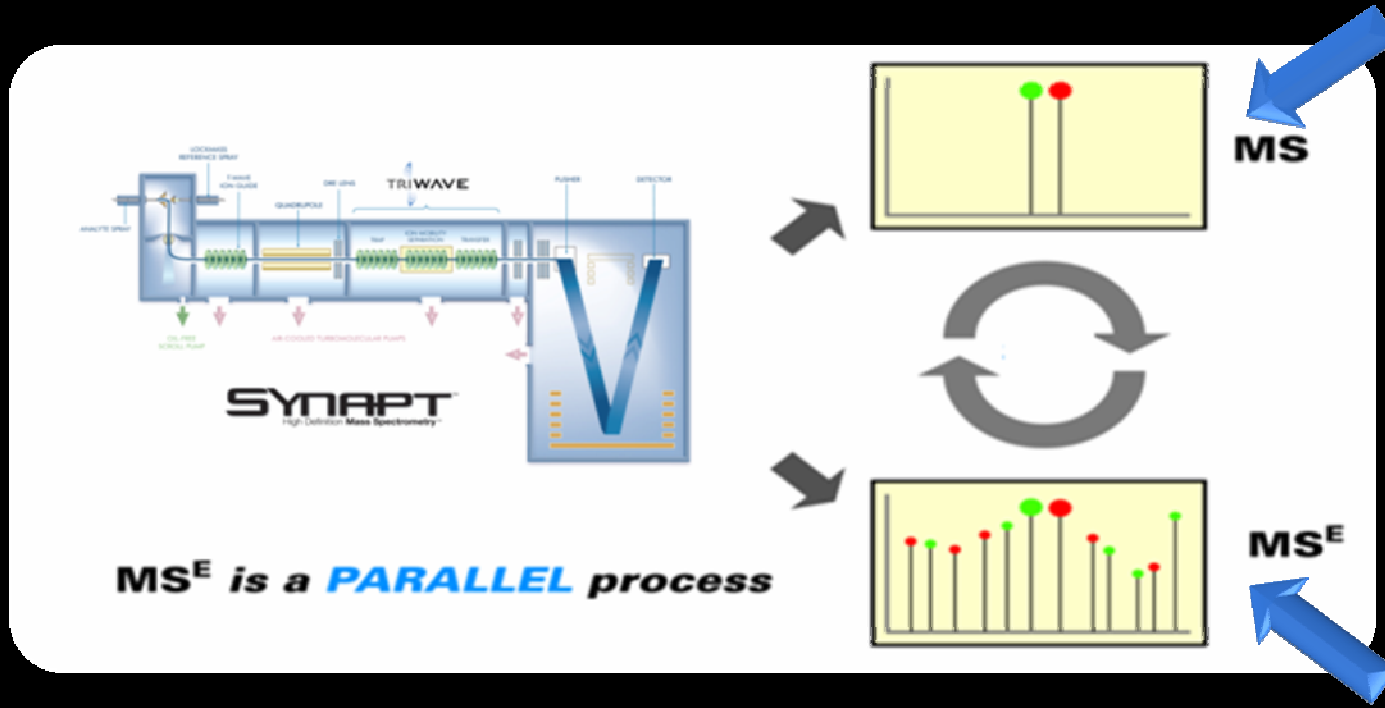
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



What is MS^E?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Low collision energy mode to produce precursor ion data



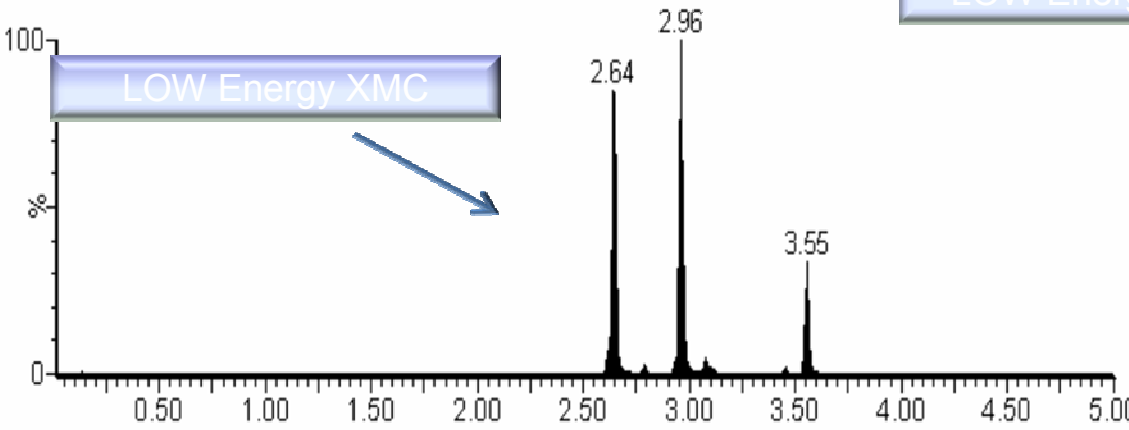
High collision energy mode to produce fragment ion data

MS^E provides data rich information for Metabolite ID assays- in a single injection

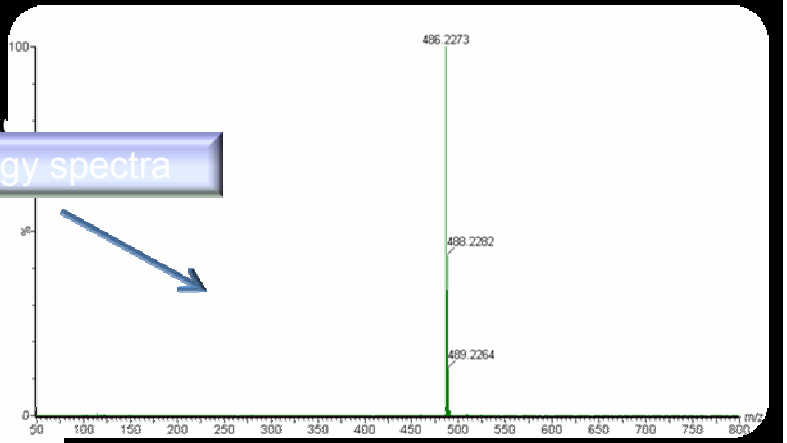
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Nefazadone *in vitro* +*OH* metabolites

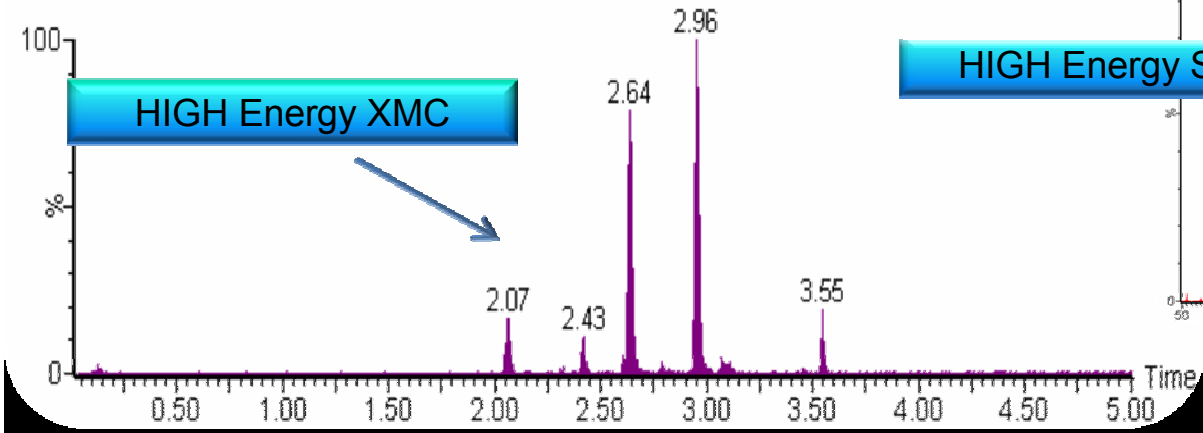
LOW Energy XMC



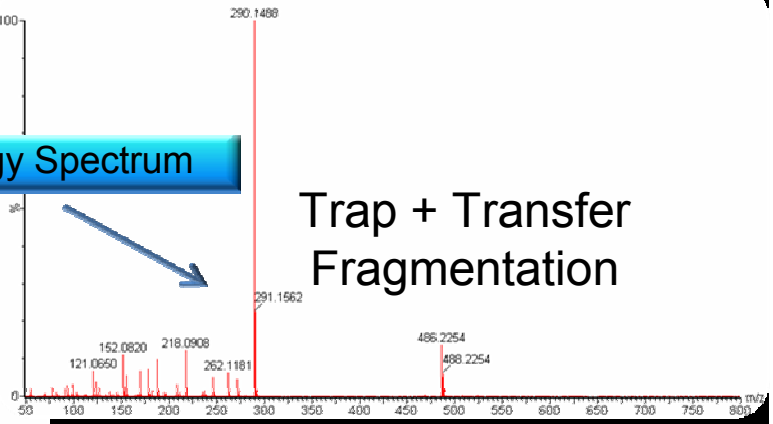
LOW Energy spectra



HIGH Energy XMC



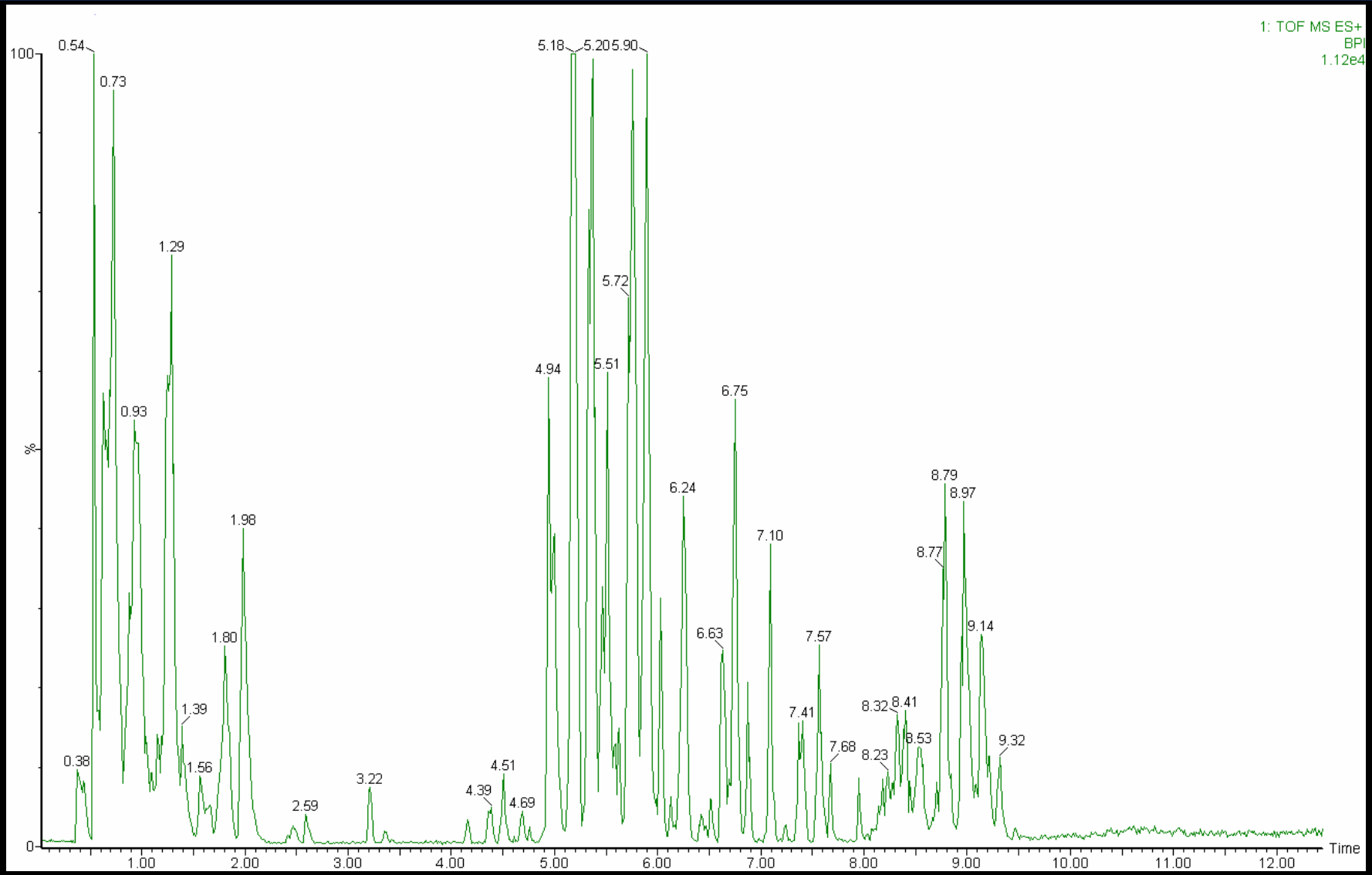
HIGH Energy Spectrum



Trap + Transfer Fragmentation

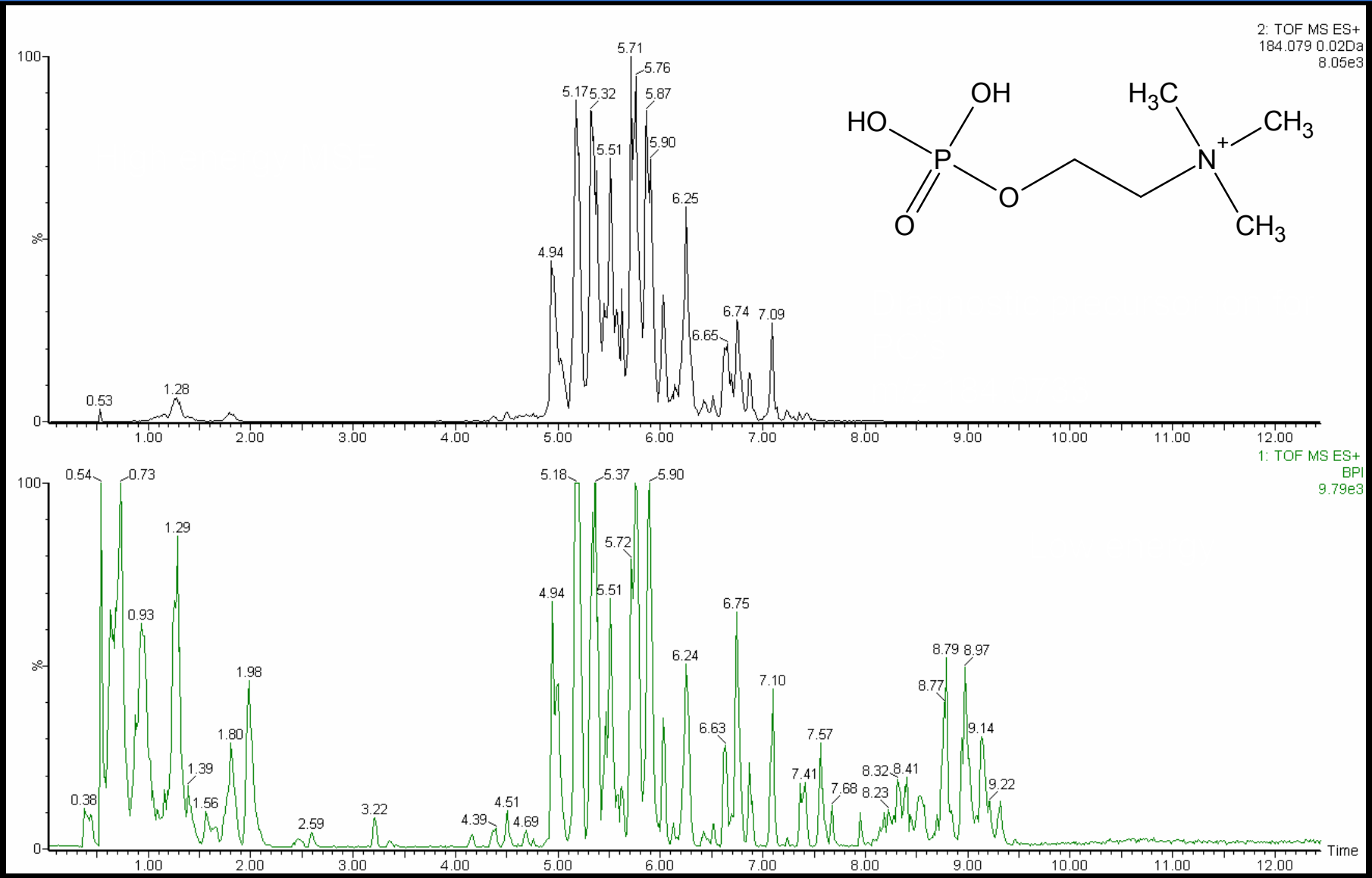
UPLC-TOF MS Analysis for Lipids and TAG's

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



MS^E analysis for Lipids and TAG's showing polar head group for PC's

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

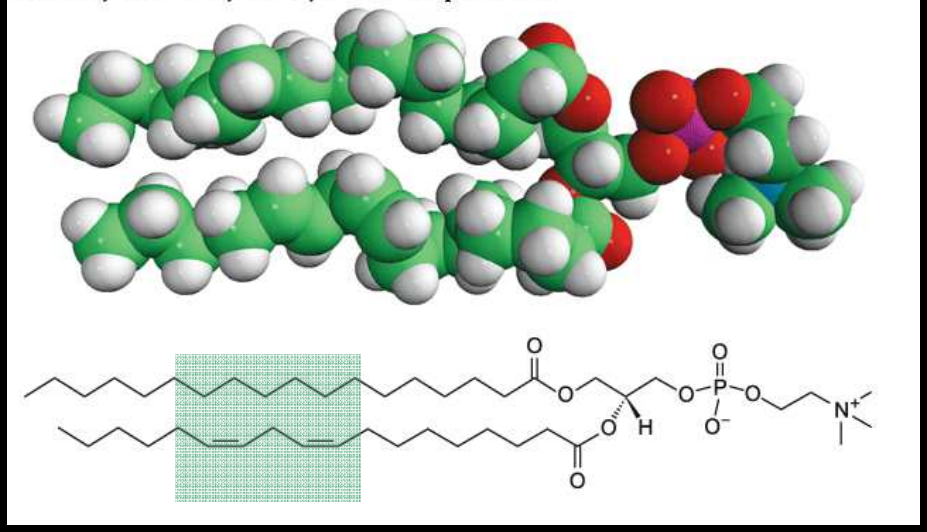


Phosphocholine Structures

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Molecular Weight: 786.130
Molecular Weight (Exact Mass): 785.593
Molecular Formula: $C_{44}H_{84}NO_8P$

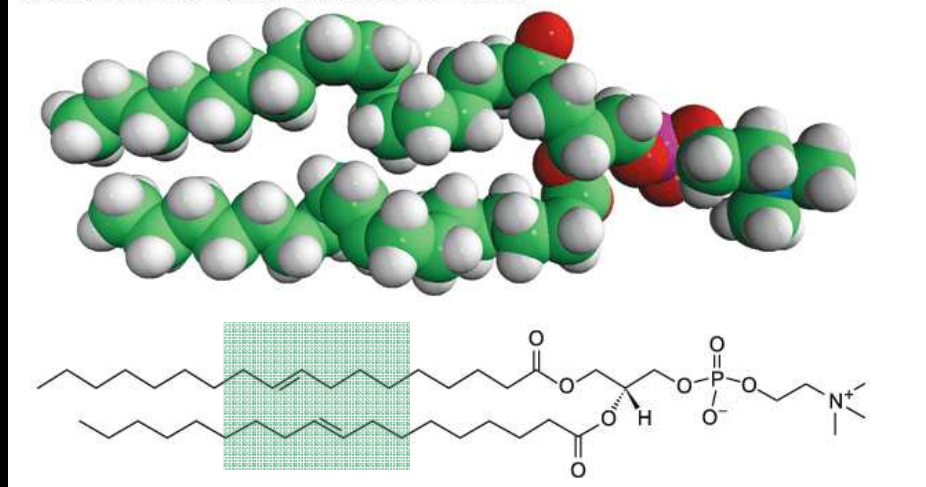
18:0-18:2 PC
1-Stearoyl-2-Linoleoyl-*sn*-Glycero-3-Phosphocholine



18:0/18:2

Molecular Weight: 786.130
Molecular Weight (Exact Mass): 785.593
Molecular Formula: $C_{44}H_{84}NO_8P$

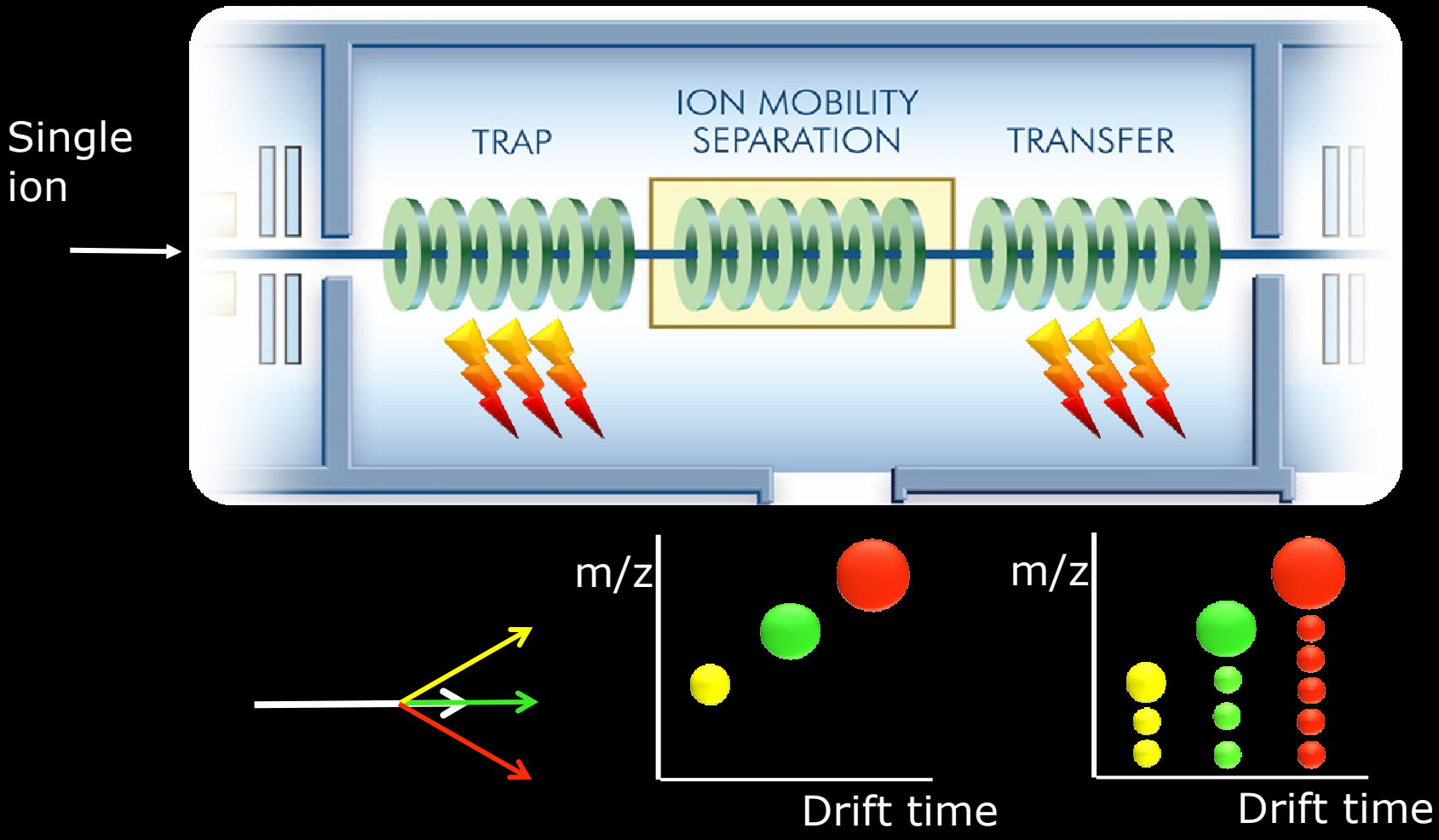
18:1 Trans PC
1,2-Dielaidoyl-*sn*-Glycero-3-Phosphocholine (Formerly 85T375)
HYGROSCOPIC AS LYOPHILIZED POWDER



18:1/18:1

Time Aligned Parallel Fragmentation (TAP) CID-IMS-CID

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



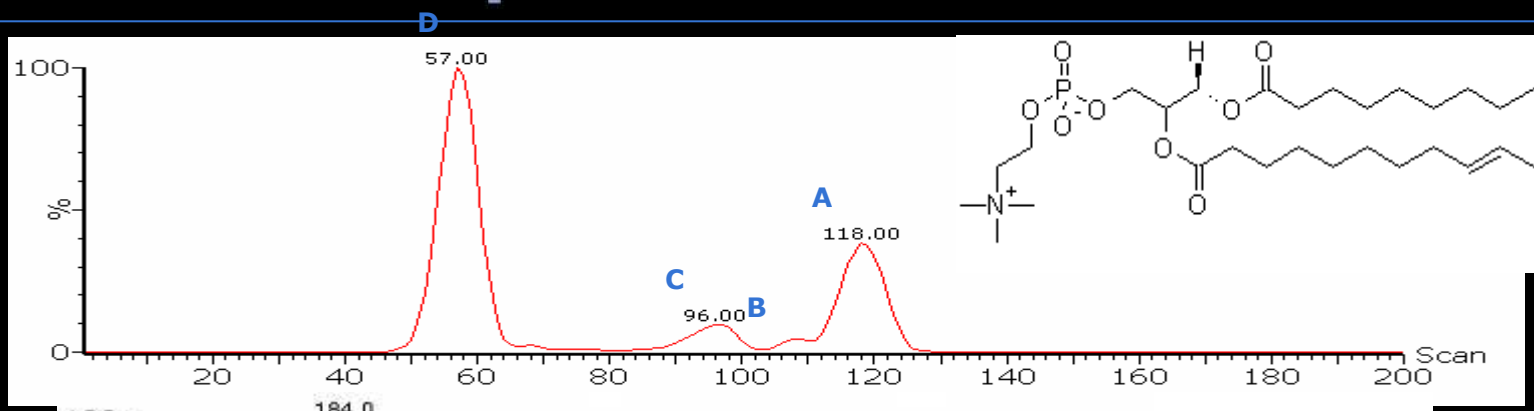
Precursor ion fragmented

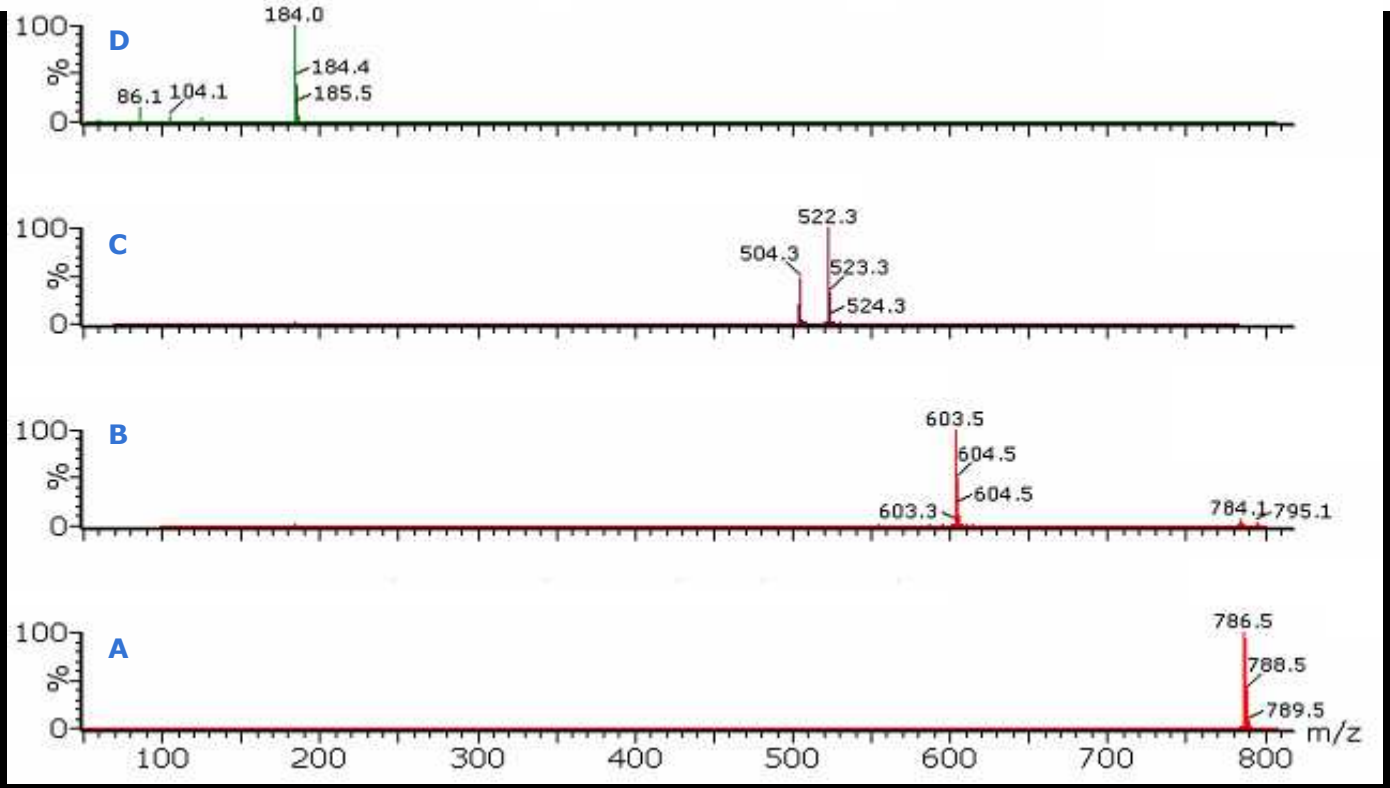
Product ions separated by IMS

Precursor and products share same drift time

Individual drift time spectra PC 18:1/18:1

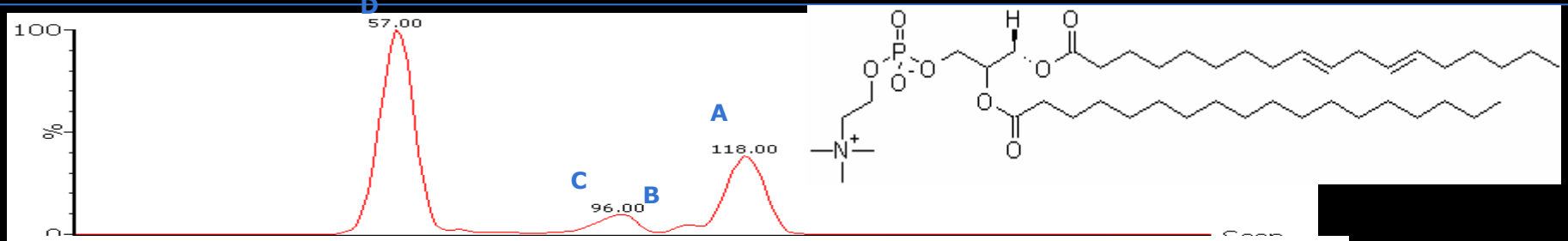
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

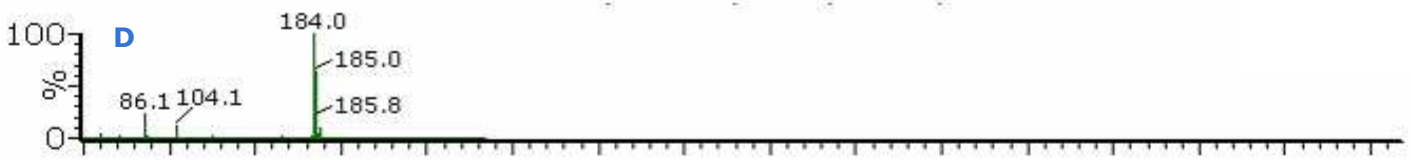


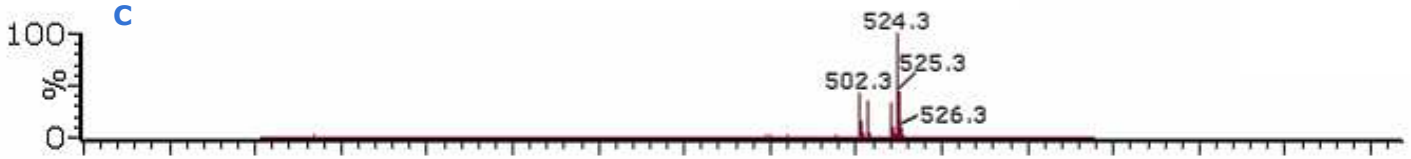


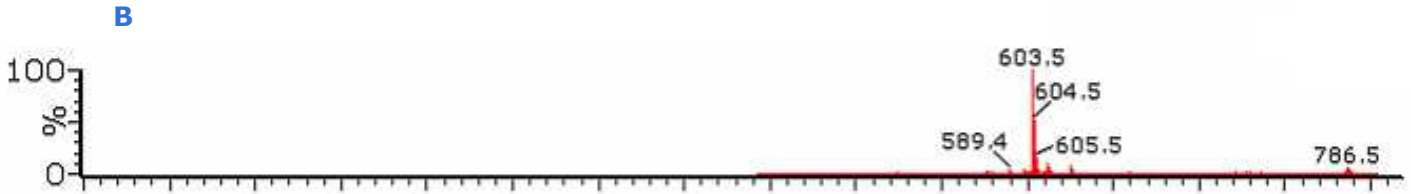
Individual Drift time traces PC 18:0/18:2

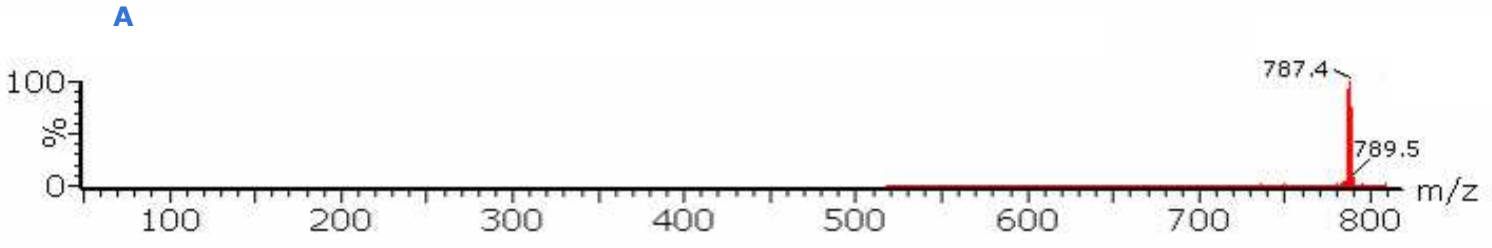
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™





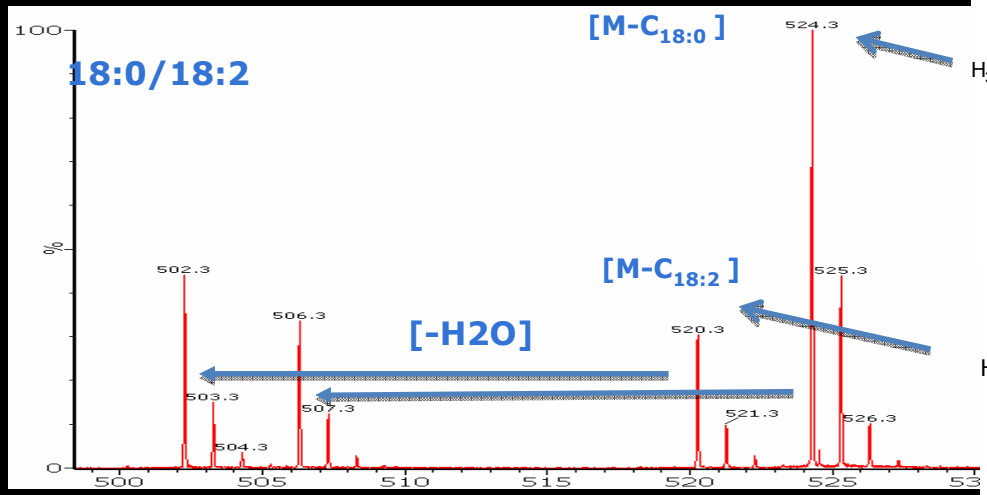


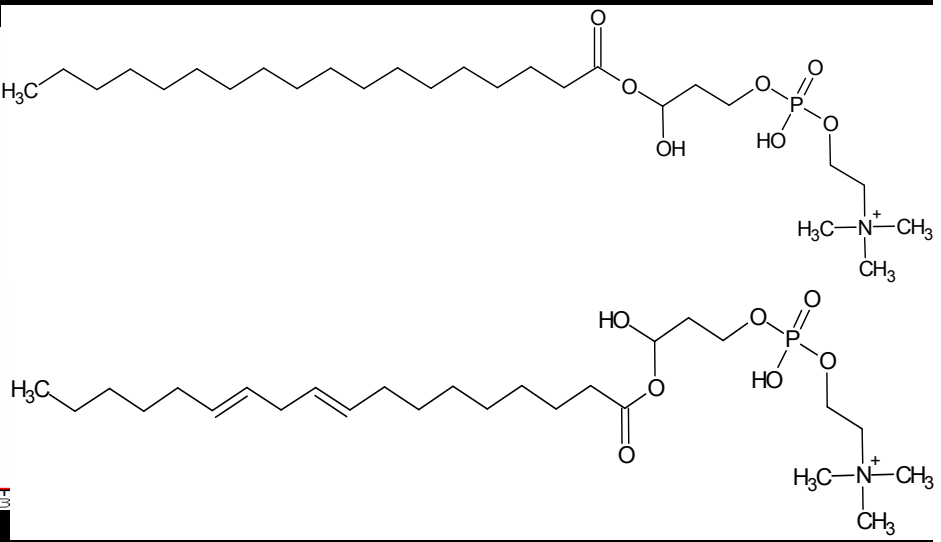


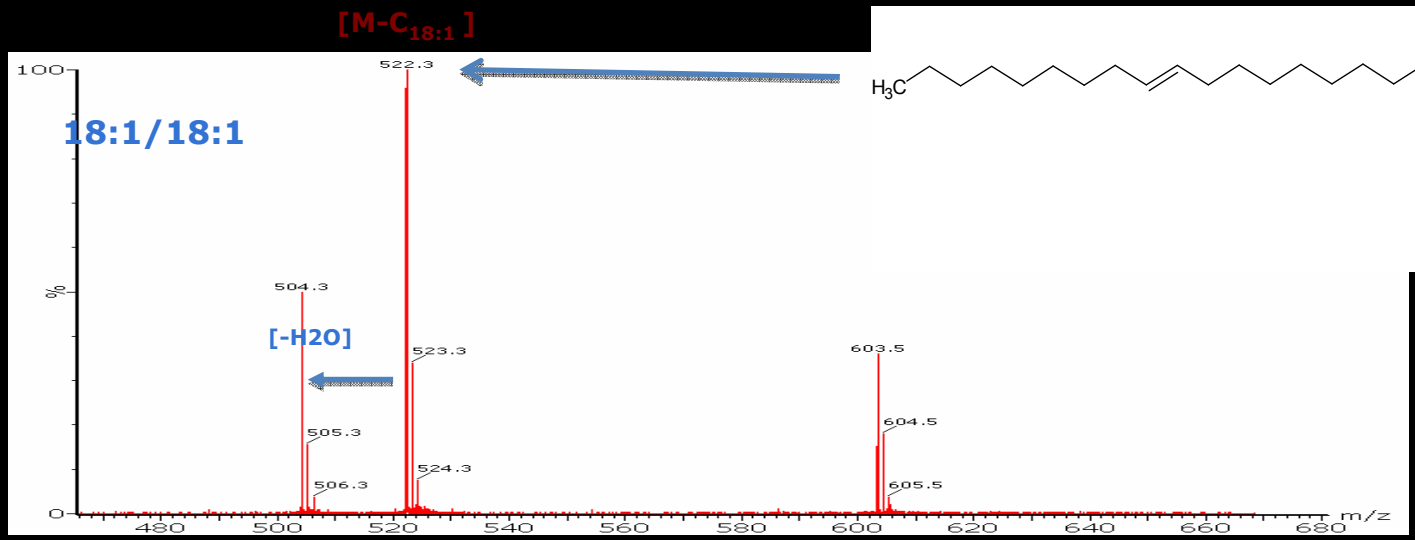


PC 18:0/18:2 vs. PC 18:1/18:1 Fragmentation Differences

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

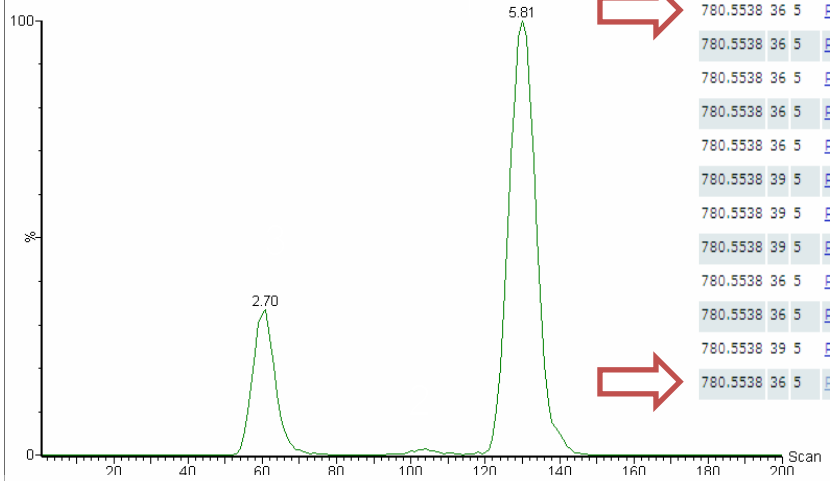




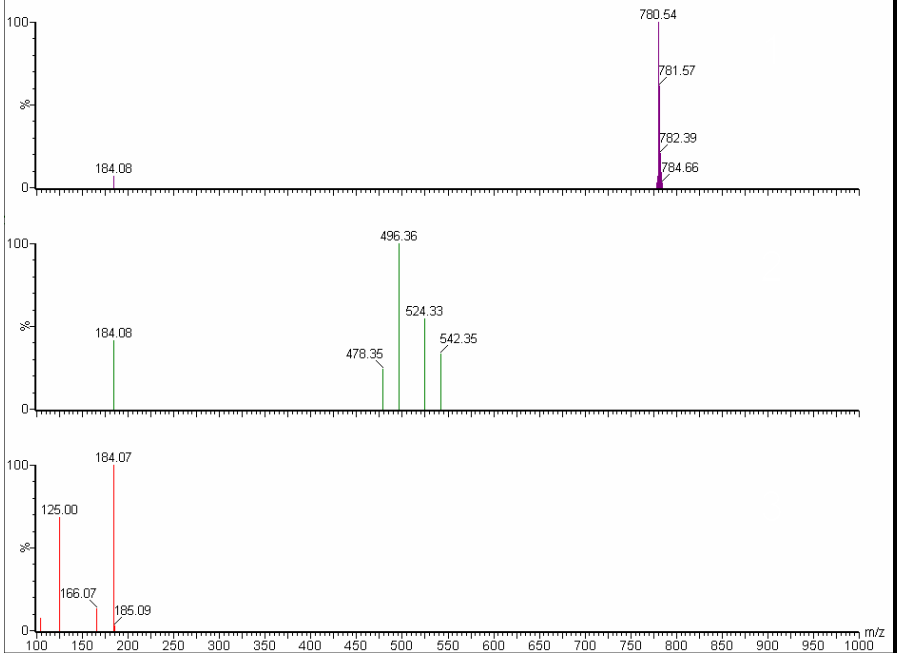


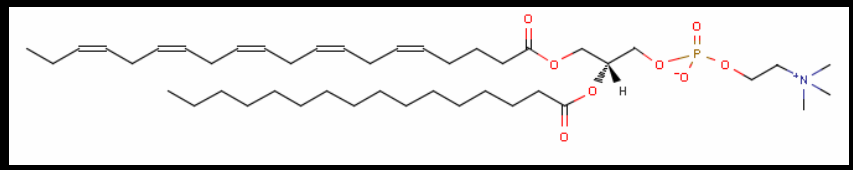
TAP fragmentation results for m/z 780.5 (16:0/20:5)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

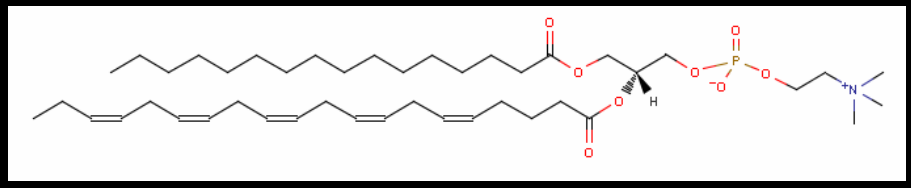


Mass	C	DB	Abbreviation	M-sn1[M+H]+	M-sn1-H2O[M+H]+	M-sn2[M+H]+	M-sn2-H2O[M+H]+	sn1 acid(-)	sn2 acid(-)	HG	Formula	Ion
780.5538	36	5	PC(16:0/20:5(5Z,8Z,11Z,14Z,17Z))	542.3241	524.3135	496.3398	478.3292	255.2324	301.2168	PC	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	36	5	PC(16:1(9Z)/20:4(5Z,8Z,11Z,14Z))	544.3398	526.3292	494.3241	476.3135	253.2168	303.2324	PC	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	36	5	PC(18:2(9Z,12Z)/18:3(9Z,12Z,15Z))	518.3241	500.3135	520.3398	502.3292	279.2324	277.2168	PC	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	36	5	PC(18:3(9Z,12Z,15Z)/18:2(9Z,12Z))	520.3398	502.3292	518.3241	500.3135	277.2168	279.2324	PC	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	36	5	PC(20:4(5Z,8Z,11Z,14Z)/16:1(9Z))	494.3241	476.3135	544.3398	526.3292	303.2324	253.2168	PC	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	39	5	PE(22:4(7Z,10Z,13Z,16Z)/17:1(9Z))	466.2928	448.2822	530.3241	512.3135	331.2637	267.2324	PE	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	39	5	PE(19:0/20:5(5Z,8Z,11Z,14Z,17Z))	500.2772	482.2666	496.3398	478.3292	297.2794	301.2168	PE	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	39	5	PE(20:5(5Z,8Z,11Z,14Z,17Z)/19:0)	496.3398	478.3292	500.2772	482.2666	301.2168	297.2794	PE	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	36	5	PC(22:4(7Z,10Z,13Z,16Z)/14:1(9Z))	466.2928	448.2822	572.3711	554.3605	331.2637	225.1855	PC	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	36	5	PC(14:1(9Z)/22:4(7Z,10Z,13Z,16Z))	572.3711	554.3605	466.2928	448.2822	225.1855	331.2637	PC	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	39	5	PE(17:1(9Z)/22:4(7Z,10Z,13Z,16Z))	530.3241	512.3135	466.2928	448.2822	267.2324	331.2637	PE	$C_{44}H_{78}NO_8P$	[M+H]+
780.5538	36	5	PC(20:5(5Z,8Z,11Z,14Z,17Z)/16:0)	496.3398	478.3292	542.3241	524.3135	301.2168	255.2324	PC	$C_{44}H_{78}NO_8P$	[M+H]+



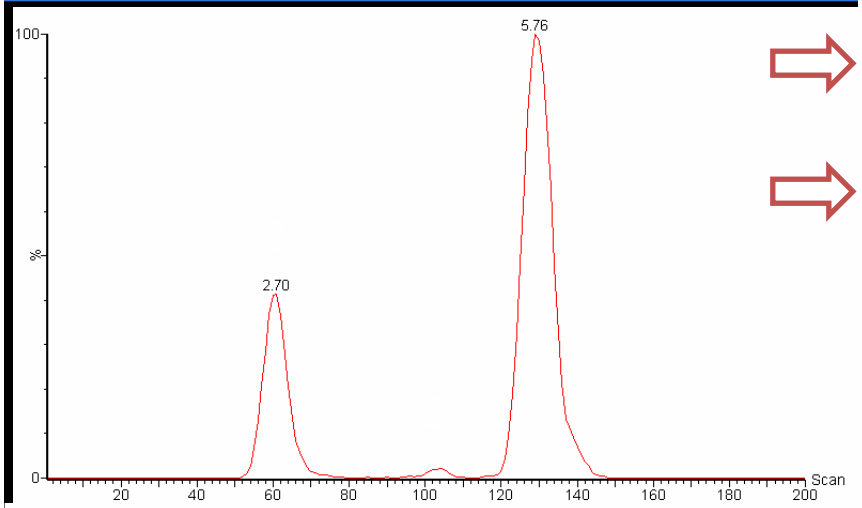


or

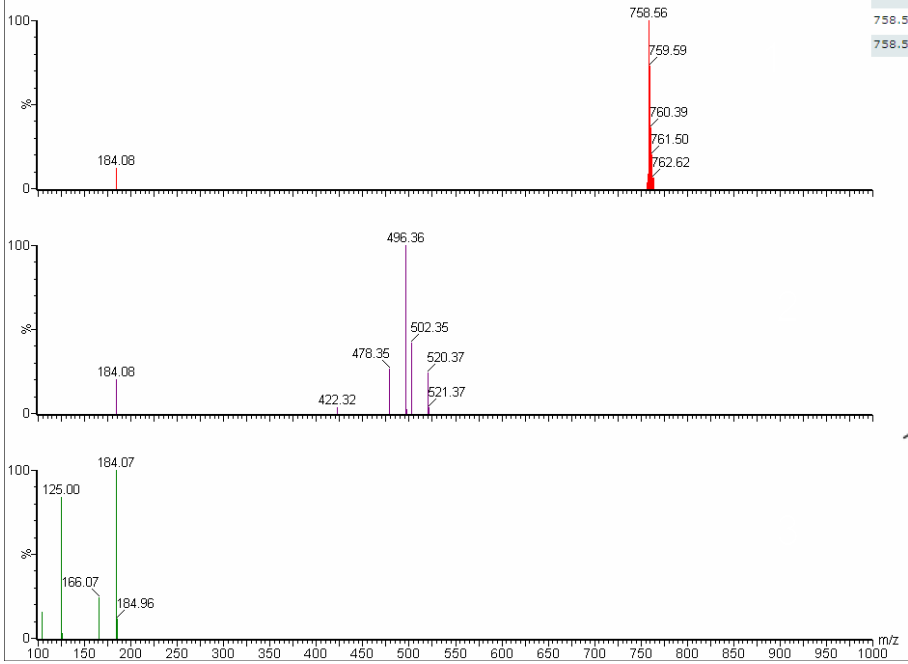


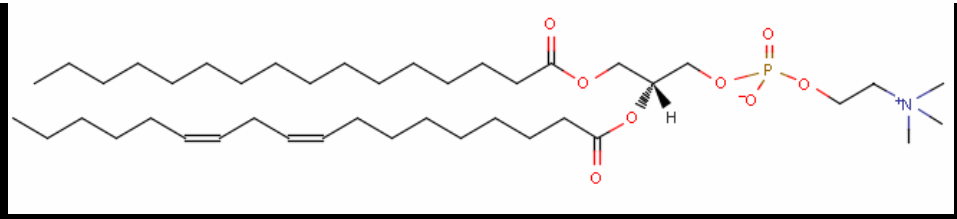
TAP fragmentation results for m/z 758.5 PC(18:2/16:0)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

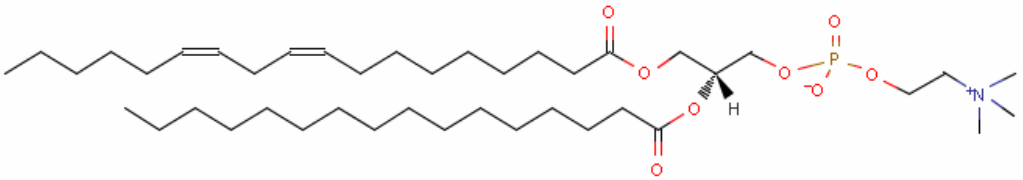


Mass	C	DB	Abbreviation	M-sn1[M+H]+	M-sn1-H2O[M+H]+	M-sn2[M+H]+	M-sn2-H2O[M+H]+	sn1 acid(-)	sn2 acid(-)	HG	Formula	Ion
758.5695	34	2	PC(12:0/22:2(13Z,16Z))	576.4024	558.3918	440.2772	422.2666	199.1698	335.295	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(20:1(11Z)/17:1(9Z))	466.2929	448.2823	508.3398	490.3292	309.2794	267.2324	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(16:0/18:2(9Z,12Z))	520.3398	502.3292	496.3398	478.3292	255.2324	279.2324	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(16:1(9Z)/18:1(9Z))	522.3555	504.3449	494.3242	476.3136	253.2168	281.2481	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(17:0/17:2(9Z,12Z))	506.3242	488.3136	510.3555	492.3449	269.2481	265.2168	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(17:2(9Z,12Z)/17:0)	510.3555	492.3449	506.3242	488.3136	265.2168	269.2481	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(18:1(9Z)/16:1(9Z))	494.3242	476.3136	522.3555	504.3449	281.2481	253.2168	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(18:2(9Z,12Z)/16:0)	496.3398	478.3292	520.3398	502.3292	279.2324	255.2324	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(20:2(11Z,14Z)/14:0)	468.3085	450.2979	548.3711	530.3605	307.2637	227.2011	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(22:2(13Z,16Z)/12:0)	440.2772	422.2666	576.4024	558.3918	335.295	199.1698	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(15:0/22:2(13Z,16Z))	534.3555	516.3449	440.2772	422.2666	241.2168	335.295	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(17:0/20:2(11Z,14Z))	506.3242	488.3136	468.3085	450.2979	269.2481	307.2637	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(17:2(9Z,12Z)/20:0)	510.3555	492.3449	464.2772	446.2666	265.2168	311.295	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(18:2(9Z,12Z)/19:0)	496.3398	478.3292	478.2929	460.2823	279.2324	297.2794	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(19:0/18:2(9Z,12Z))	478.2929	460.2823	496.3398	478.3292	297.2794	279.2324	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(20:0/17:2(9Z,12Z))	464.2772	446.2666	510.3555	492.3449	311.295	265.2168	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(20:2(11Z,14Z)/17:0)	468.3085	450.2979	506.3242	488.3136	307.2637	269.2481	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(22:2(13Z,16Z)/15:0)	440.2772	422.2666	534.3555	516.3449	335.295	241.2168	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(20:1(11Z)/14:1(9Z))	466.2929	448.2823	550.3868	532.3762	309.2794	225.1855	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(14:1(9Z)/20:1(11Z))	550.3868	532.3762	466.2929	448.2823	225.1855	309.2794	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(17:1(9Z)/17:1(9Z))	508.3398	490.3292	508.3398	490.3292	267.2324	267.2324	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	37	2	PE(17:1(9Z)/20:1(11Z))	508.3398	490.3292	466.2929	448.2823	267.2324	309.2794	PE	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(17:1(9Z)/17:1(9Z))	508.3398	490.3292	508.3398	490.3292	267.2324	267.2324	PC	$C_{42}H_{80}NO_8P$	[M+H]+
758.5695	34	2	PC(14:0/20:2(11Z,14Z))	548.3711	530.3605	468.3085	450.2979	227.2011	307.2637	PC	$C_{42}H_{80}NO_8P$	[M+H]+



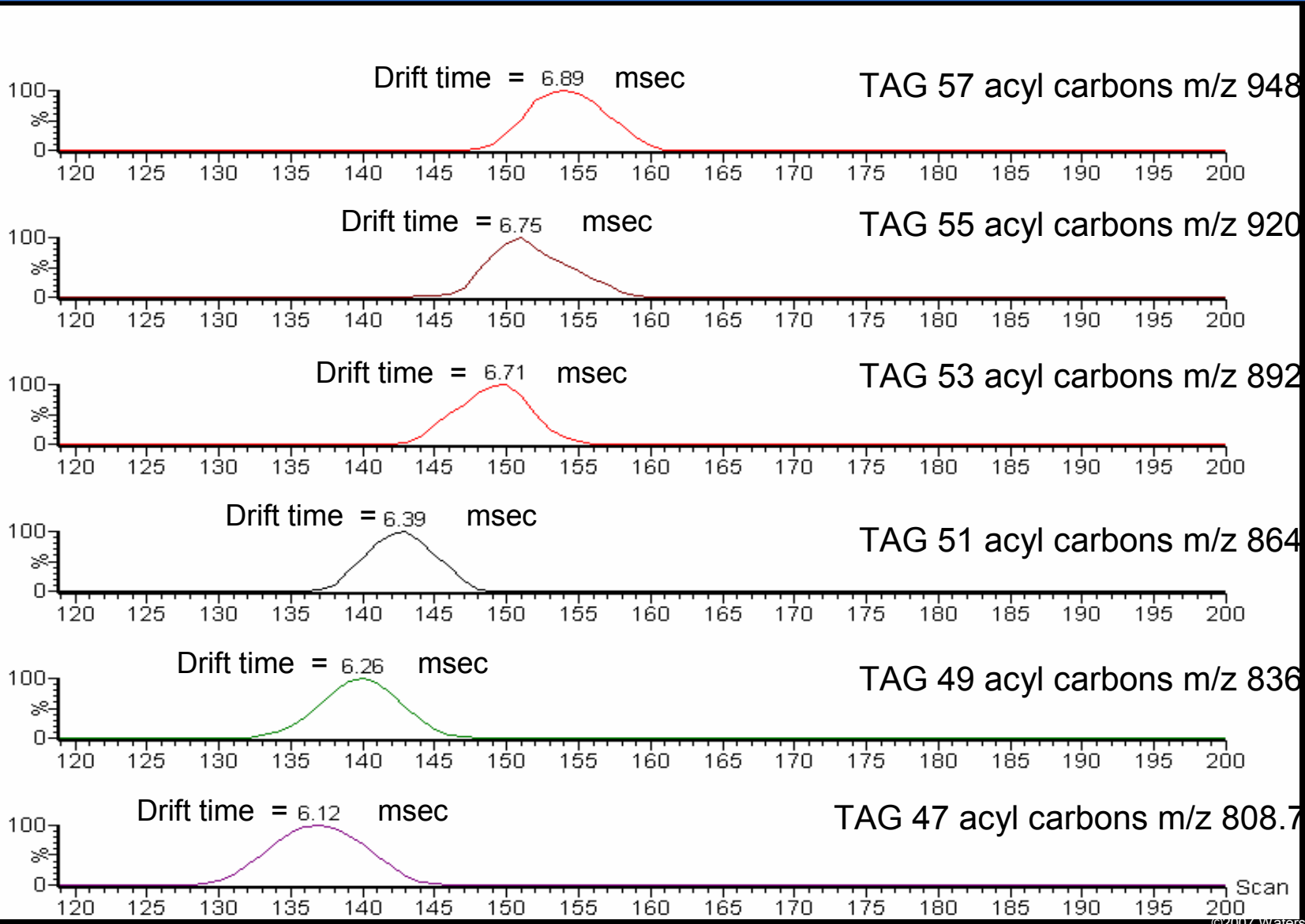


or



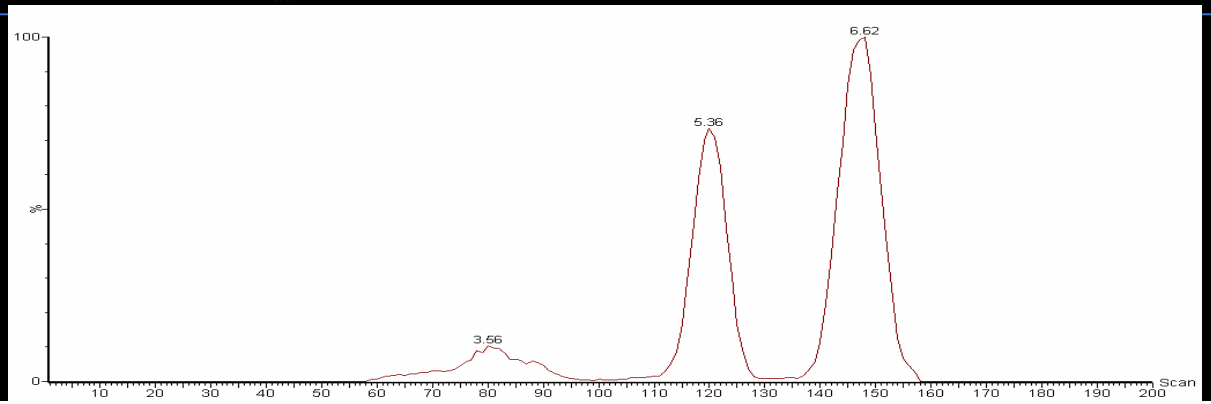
Drift times for different TAG acyl carbon numbers

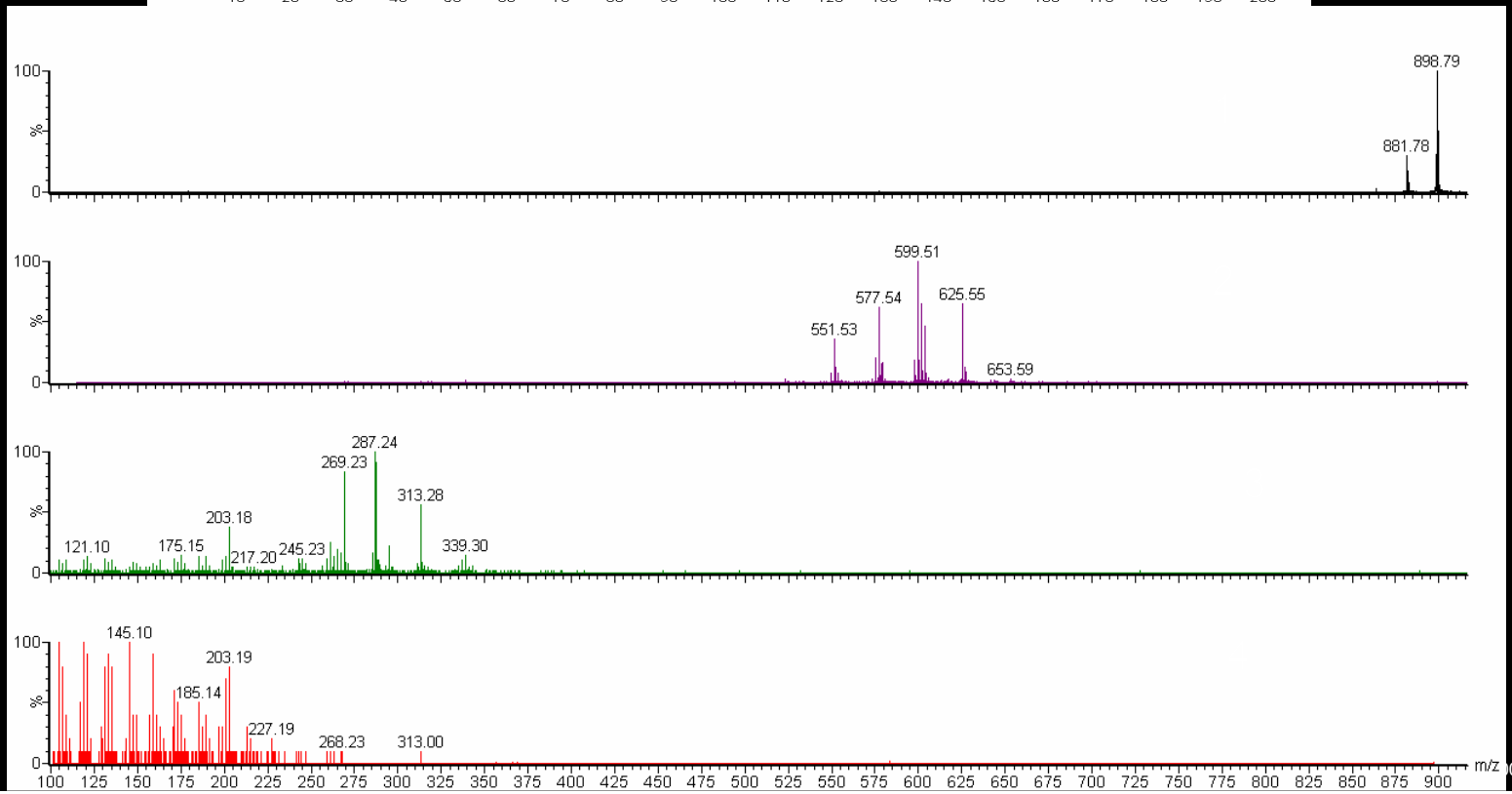
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



TAP fragmentation results for m/z 898.8 TAG(54:5)

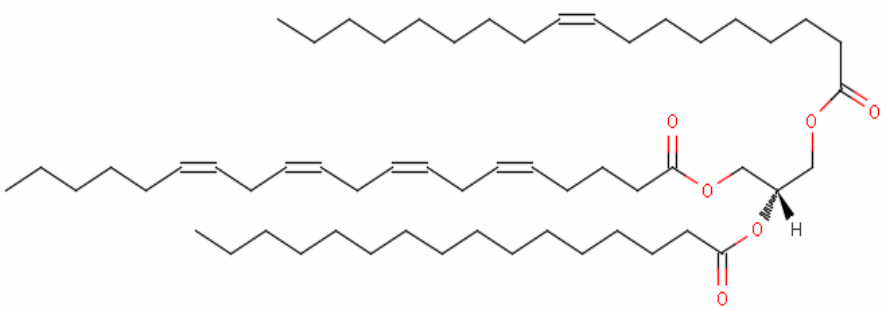
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



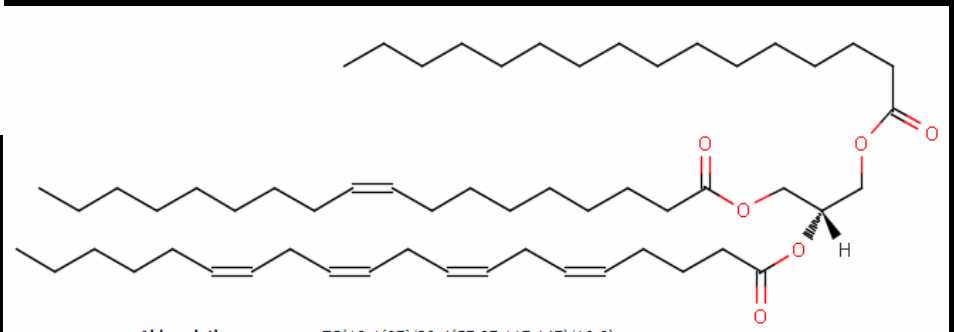


3 Possible structures for the TAG (54:5) 16:0/18:1/20:4

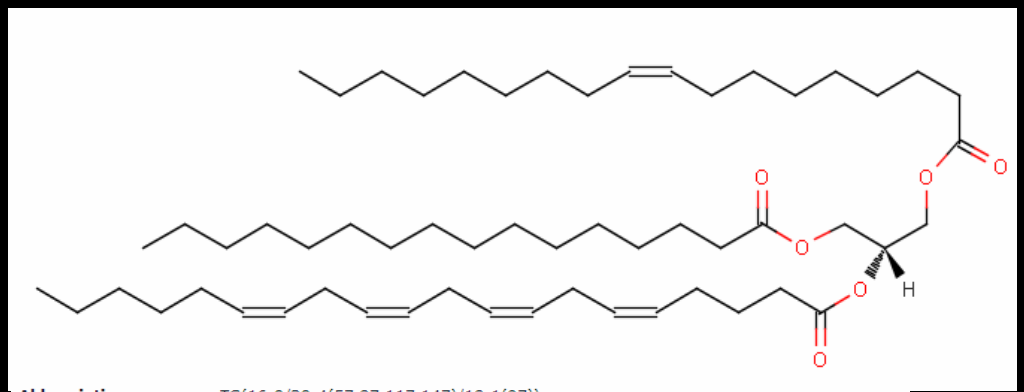
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Abbreviation	TG(20:4(5Z,8Z,11Z,14Z)/16:0/18:1(9Z))
Systematic Name	1-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-2-hexadecanoyl-3-(9Z-octadecenoyl)-sn-glycerol
Formula (Neutral)	$C_{57}H_{100}O_6$
m/z	881.7593
Ion	M+H



Abbreviation	TG(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)/16:0)
Systematic Name	1-(9Z-octadecenoyl)-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-3-hexadecanoyl-sn-glycerol
Formula (Neutral)	$C_{57}H_{100}O_6$
m/z	881.7593
Ion	M+H



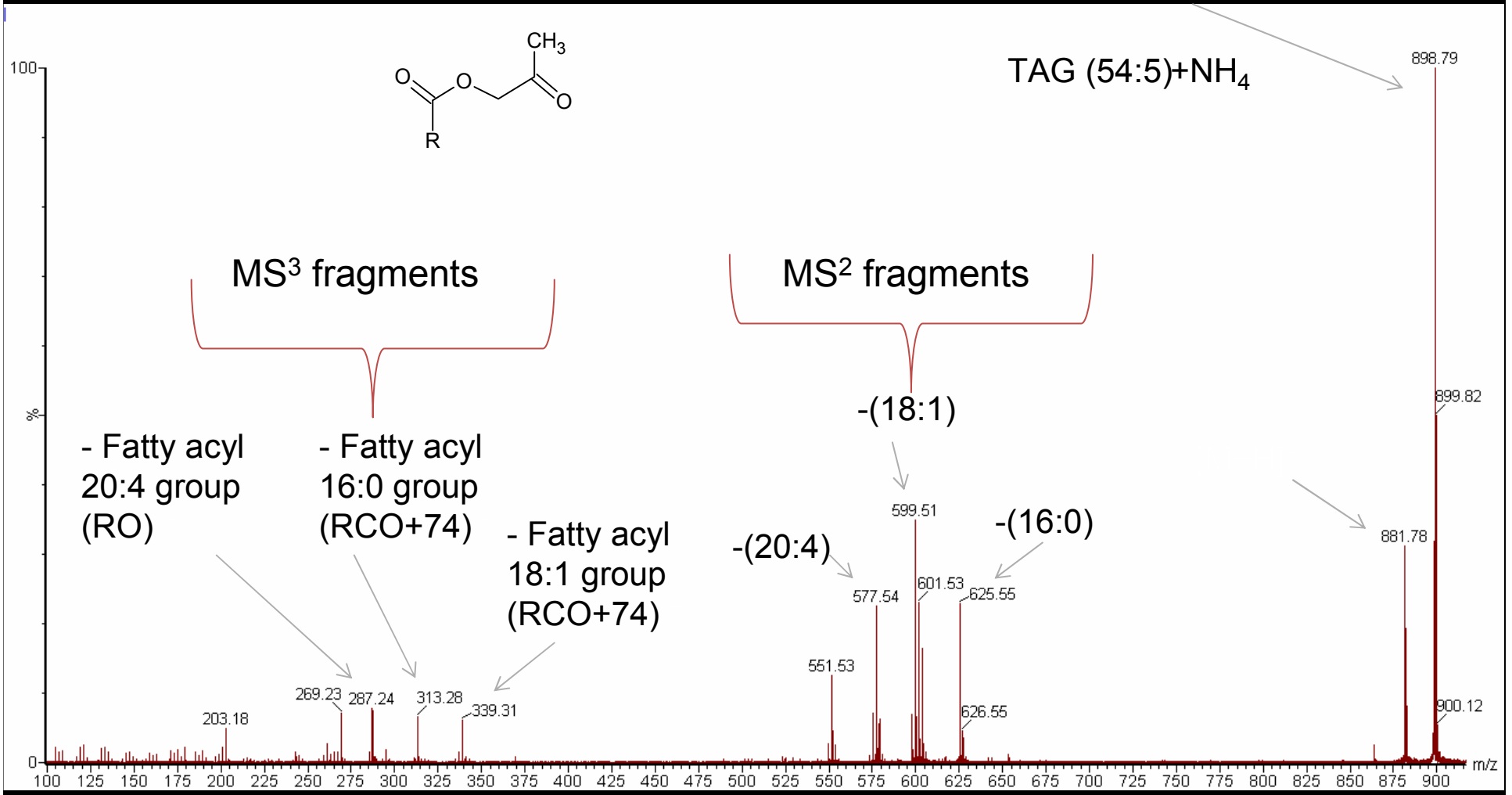
Abbreviation	TG(16:0/20:4(5Z,8Z,11Z,14Z)/18:1(9Z))
Systematic Name	1-hexadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-3-(9Z-octadecenoyl)-sn-glycerol
Formula (Neutral)	$C_{57}H_{100}O_6$
m/z	881.7593
Ion	M+H

TAP fragmentation results for m/z 898.8 TAG(54:5)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

(RCO+74) $^{+}$ MS^3 fragment ion

$[M+NH_4]^+$



Acknowledgements

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

The Metabolic Profiling Group

- Jose Castro-Perez
- Kate Yu
- Emma Marsden-Edwards
- Henry Shion

Our Colleagues

- Andy Baker
- Richard Lock
- Steve McDonald
- Tim Riley
- Richard Gilpin
- Jeff Goshawk
- Iggy Kass

A Few of Our Collaborators

- Johan Trygg U. of Umea
- Erik Johansson Umetrics
- **Jules Griffin U. Of Cambridge**
- Kathie Knights Flinders U.
- John Miners Flinders U.
- **Andrew Tyler Novartis**
- United States Department of Agriculture
- Jeremy Nicholson Imperial College
- Elaine Holmes Imperial College
- Liz Want Imperial College
- The MetaPhor Project (EU)
- Gerhard Wagner Harvard Medical Ctr.
- Colorado State U.

