Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Novel Tools for "omics"-based Analysis of Complex Mixtures; Application to a Investigation of the Effect of Prolonged Glucose Stimulation on the Lipid Profile of Mouse Heart Muscle

John P. Shockcor, PhD, FRSC CChem

Director of Strategic Operations, Pharmaceutical and Life Sciences, Waters Corp.
Professor, Dept. Of Surgery and Cancer, Imperial College, London, UK
Visiting Fellow, Dept. of Biochemistry, University of Cambridge, UK

Imperial College London
UNIVERSITY OF CAMBRIDGE

UNIVERSITY OF CAMBRIDGE

MRC Human Nutrition Research

Metabolomic and Lipidomic Research in Diabetes and Obesity

Prof. Jules Griffin,
MRC HNR & University of Cambridge

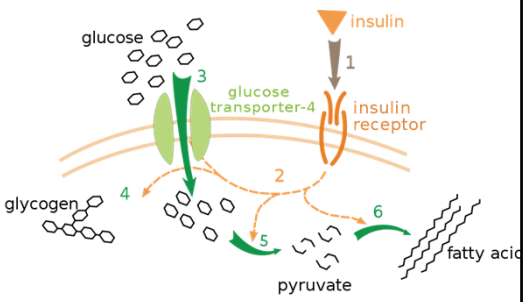
Dr.Helen Atherton
Dr.Melanie Gulston

MRC | Medical Research Council

Effect of insulin on glucose uptake and metabolism.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Insulin binds to its receptor (1) which in turn starts many protein activation cascades (2). These include: translocation of Glut-4 transporter to the plasma membrane and influx of glucose (3), glycogen synthesis (4), glycolysis (5) and fatty acid synthesis (6).



The Diabetic Heart

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Type II diabetes is characterized by insulin resistance, and in the heart this results in a failure to recruit Glucose Transporter 4 (GluT4) into the cell membrane during periods of high blood glucose.
- As a result, metabolism of the diabetic heart has an increased reliance on beta-oxidation of fatty acids. This has been linked to the increased lipid load which may result in lipotoxicity in the diabetic heart.
- In addition the ATP generated by fatty acid oxidation requires more oxygen compared with that derived from the oxidation of glucose, increasing the risk of ischaemic heart disease.

Study Description

- In this study we have examined a mouse model where Glucose Transporter 1 has been increased in expression selectively in the heart. GLUT1 is responsible for the low-level of basal glucose transport. It is a non-insulin dependent glucose transporter and as such it will dramatically increase glucose metabolism in the heart.

Study Description

- Wild-type and transgenic mice were sacrificed at 3 months and the heart tissue rapidly dissected and stored at -80C. A chloroform/methanol extraction was performed and the lipids in the organic layer for all samples were profiled using a UPLC/Synapt G2.

Question?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- **What is the effect of prolonged glucose stimulation on the lipid profile of heart tissue?**

Intact Lipid Analysis:

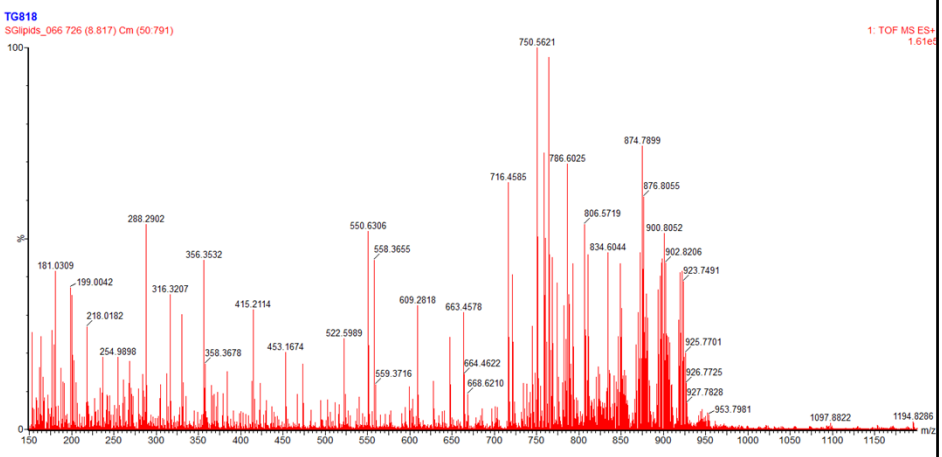
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Chromatography

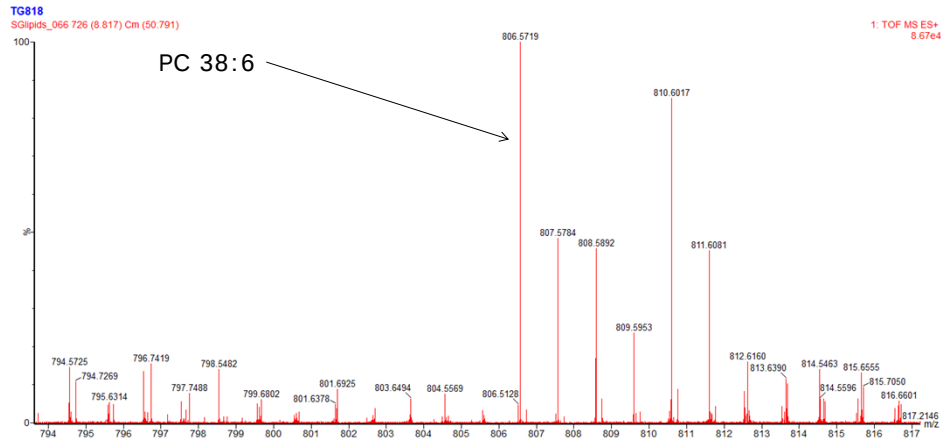
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Direct Infusion of a Tissue Extract

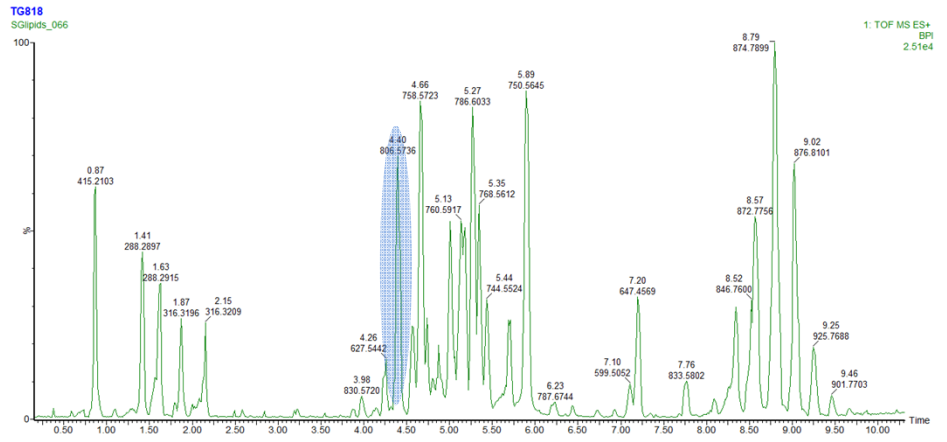
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Exapnded Region around m/z 806

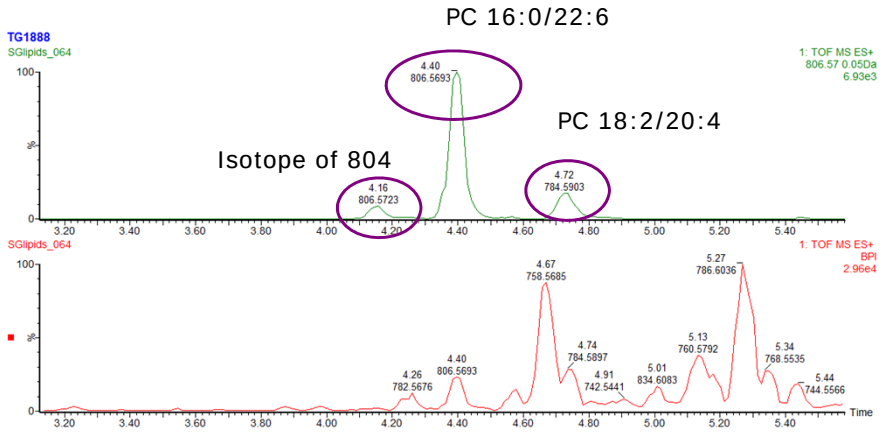


TIC of Tissue Extract



TIC and XIC for m/z 806.5695 PC 38:6

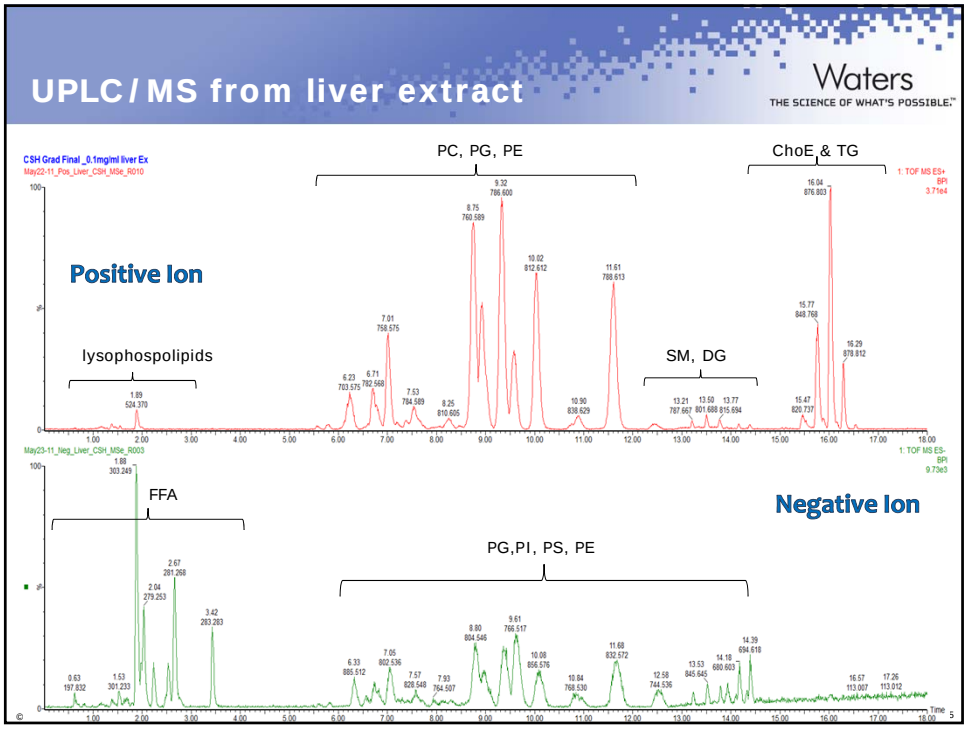
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

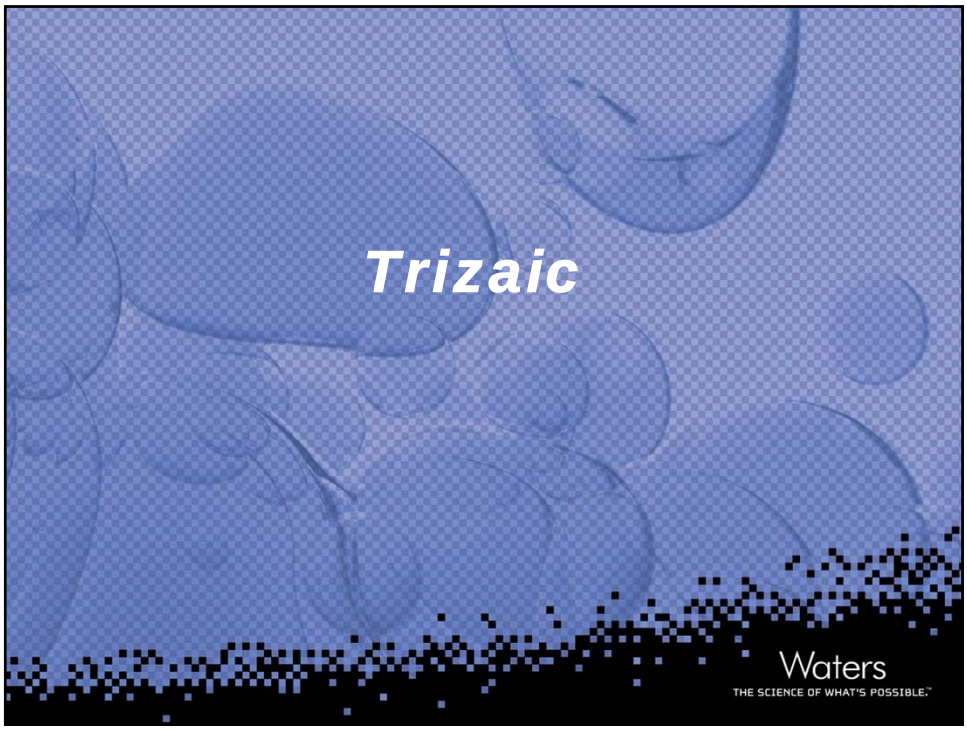


UPLC and MS Conditions for Global Lipid Profiling

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

#	Time	Flow	%A	%B	
1	Initial	0.4	60	40	initial
2	2.0	0.4	55	43	6
3	2.1	0.4	50	50	1
4	12.0	0.4	46	54	6
5	12.1	0.4	30	70	1
6	18.0	0.4	1	99	6
7	18.1	0.4	60	40	6
8	20.0	0.4	60	40	1


UPLC / MS from liver extract
Waters
THE SCIENCE OF WHAT'S POSSIBLE.
PC, PG, PE
ChoE & TG
Positive Ion
lysophospolipids
SM, DG
FFA
Negative Ion
PG,PI, PS, PE

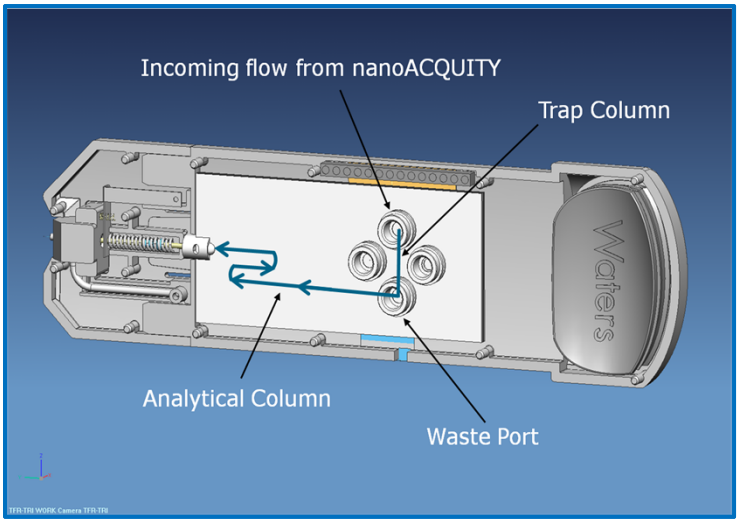

Trizaic
Waters
THE SCIENCE OF WHAT'S POSSIBLE.

TRIZAIC™ UPLC® System

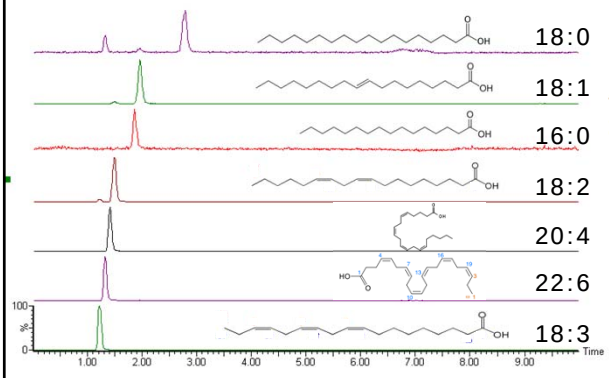
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

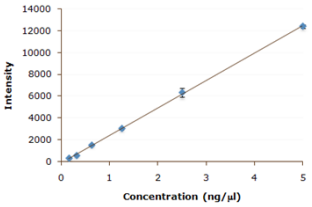
nanoTile Flow Path

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Free Fatty Acids





Linear response between 0.015 ng/µl and 5 ng/µl

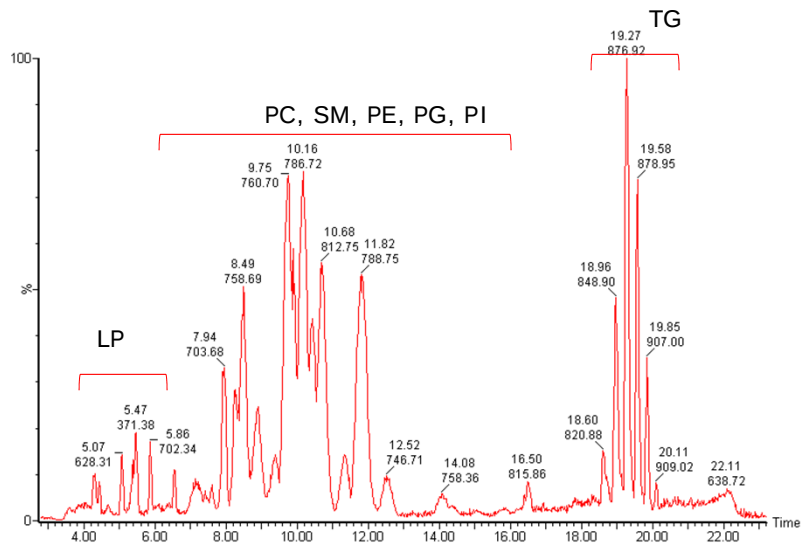
Mobile phase A: ACN/Water (60:40) with 10 mM NH_4 formate and 0.1% FA

Mobile phase B: IPA/ACN (90:10) with 10 mM NH_4 formate and 0.1% FA

19

Liver Extract: Positive Ion Mode

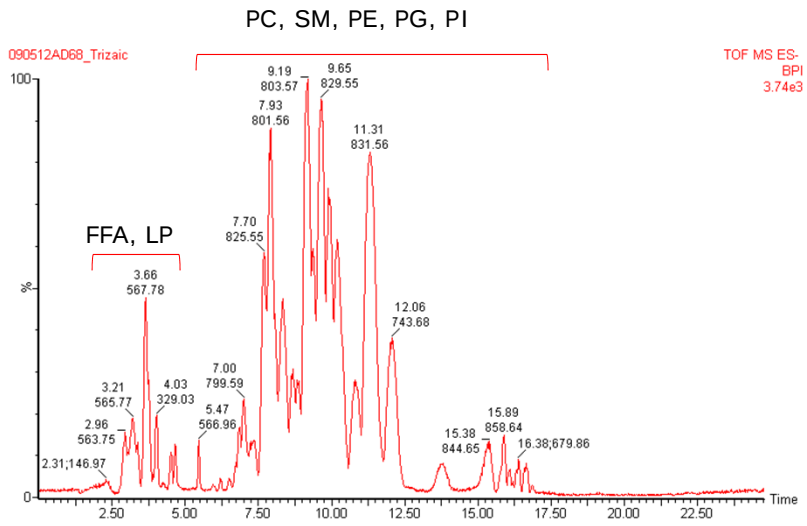
Column: CSH C18, 0.15 x 100 mm



20

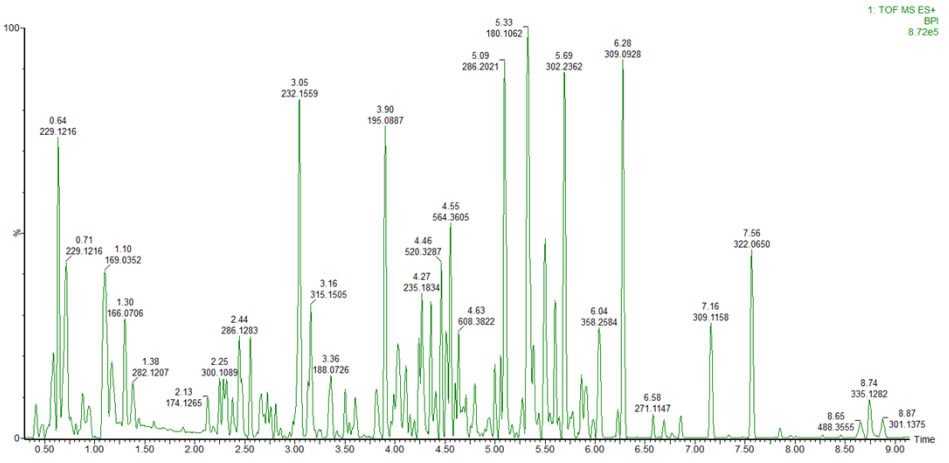
Total Lipids Extract from Liver Negative Ionization mode

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Tic of Human Urine (Positive Ion)

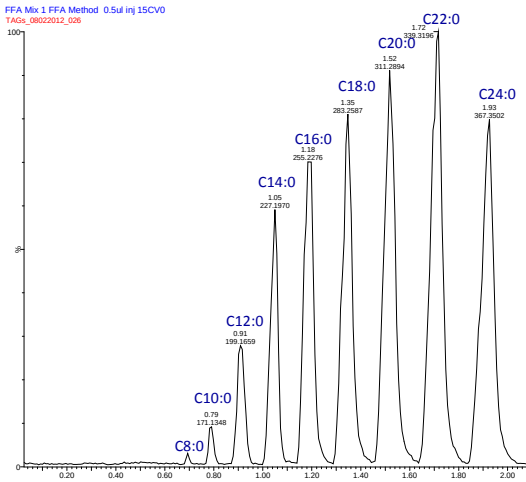
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



UPC²

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UPC² Free Fatty Acid



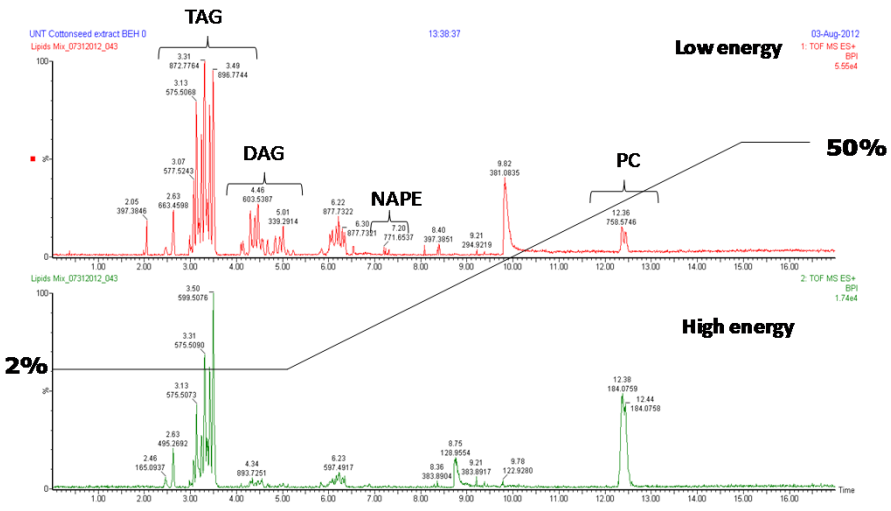
Method Conditions:
Instrument: ACQUITY UPC²
Column: ACQUITY UPC² HSS C_{18} SB
Co-solvent: MeOH w/ 2g/L Ammonium Formate
Gradient: 1 to 10% over 5 minutes*
Flow: 2.5 mL/min
Temperature: 60°C
CCM Pressure: 1885
Injection Vol: 0.5uL

Make-up flow: 0.2mL/min of 0.1% formic acid
Splitter: Upchurch cross 1/16 PEEK

Sample Concentration: 0.25mg/mL
FFA: C8 to C24

UPC² / oa-Qtof:

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



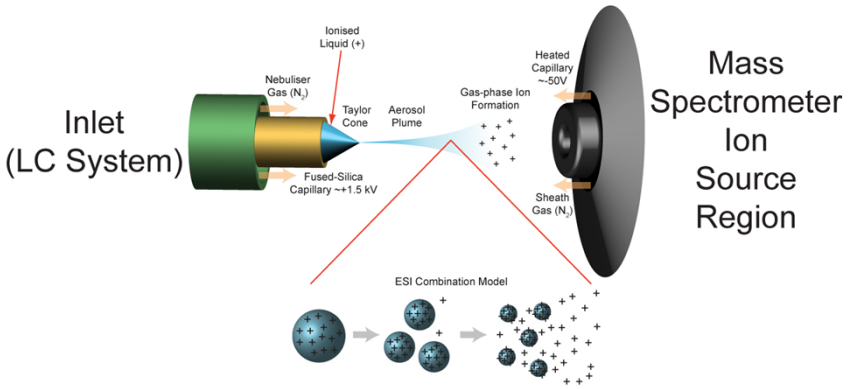
25

MS Analysis

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

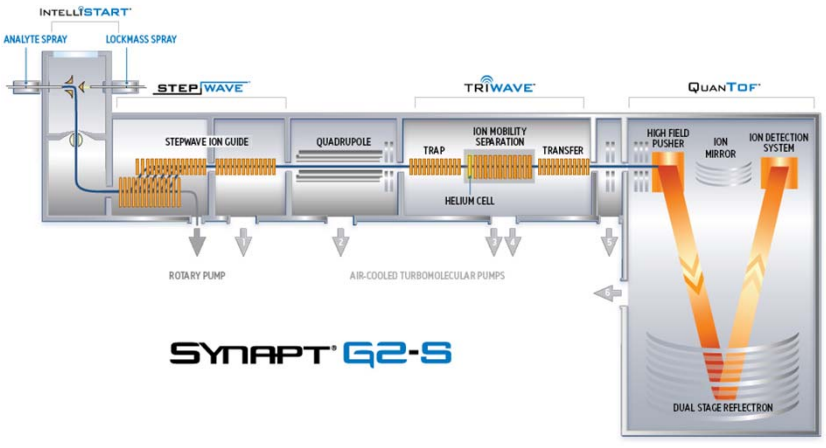
Electro-Spray Ionization

Waters
THE SCIENCE OF WHAT'S POSSIBLE.



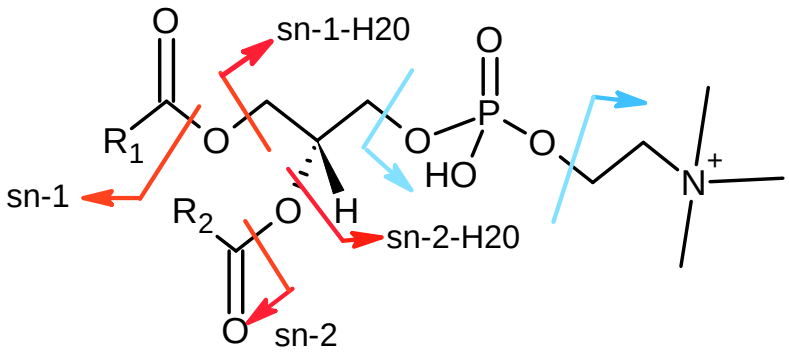
A Quadrupole Time-of Flight Mass Spectrometer

Waters
THE SCIENCE OF WHAT'S POSSIBLE.



Fragmentation of a Typical Glycerophospholipid

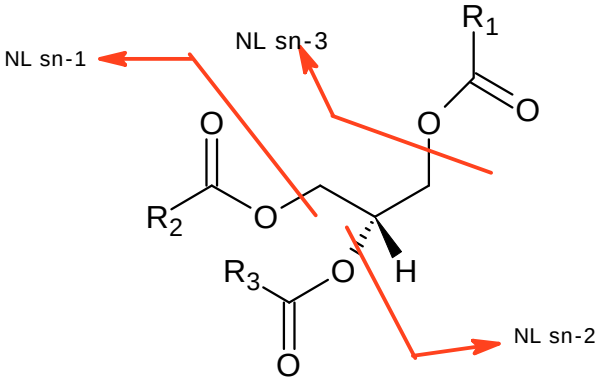
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Phosphotadylcholine (PC)

Fragmentation of Triglycerides

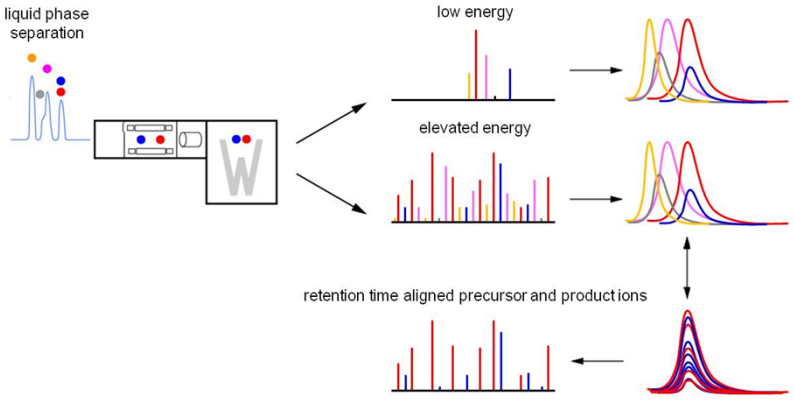
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Note: The fragmentation for Mono and Di Glycerides cleaves the same bonds.

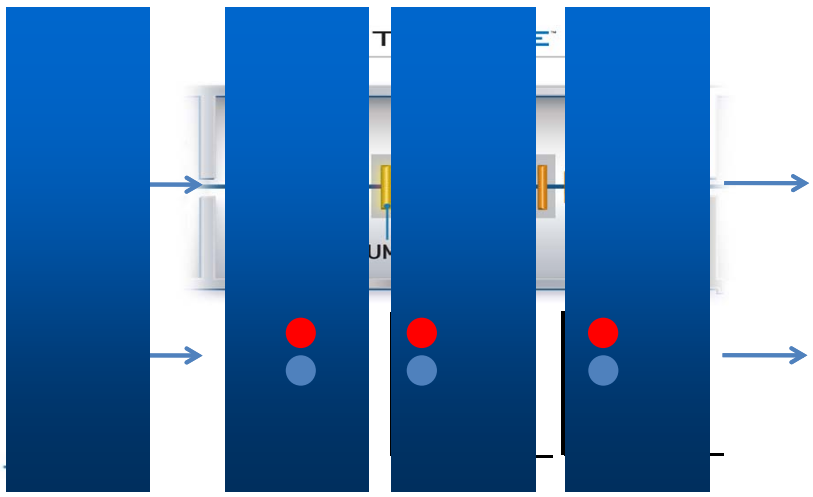
UPLC / MS^E (Step 1)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



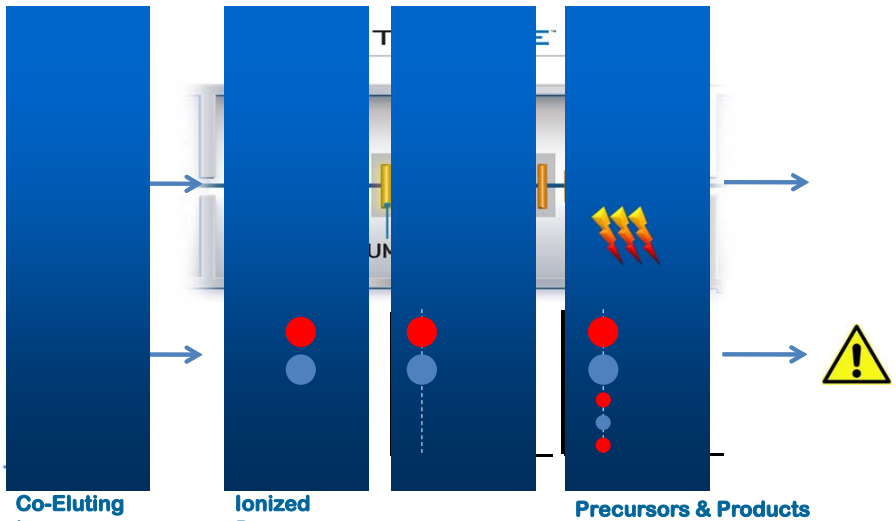
UPLC / MS^E (low energy)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



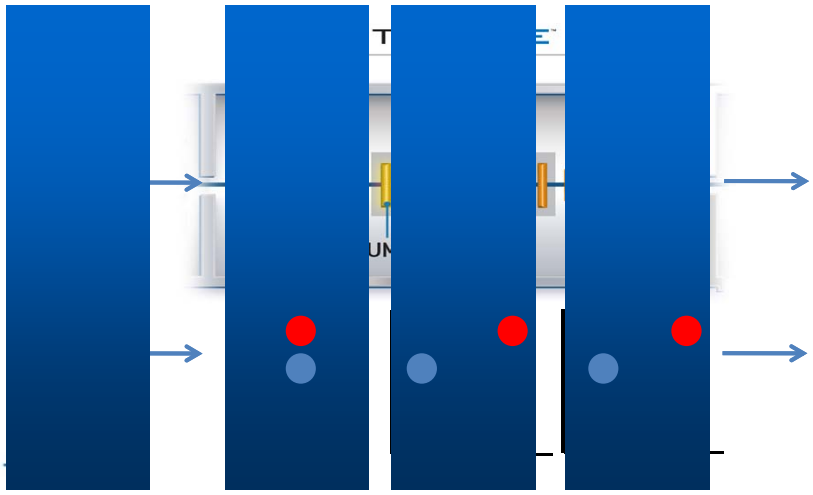
UPLC / MS^E (high energy)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



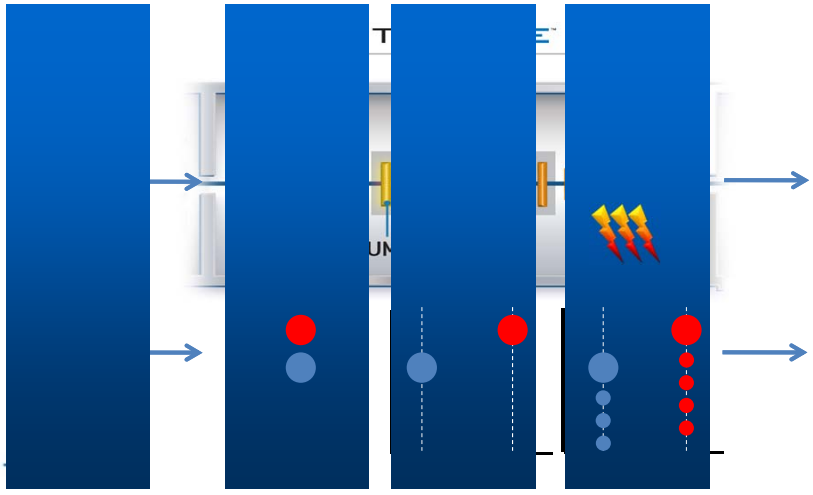
UPLC / HDMS^E (low energy)
...deconvoluting chimericy

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



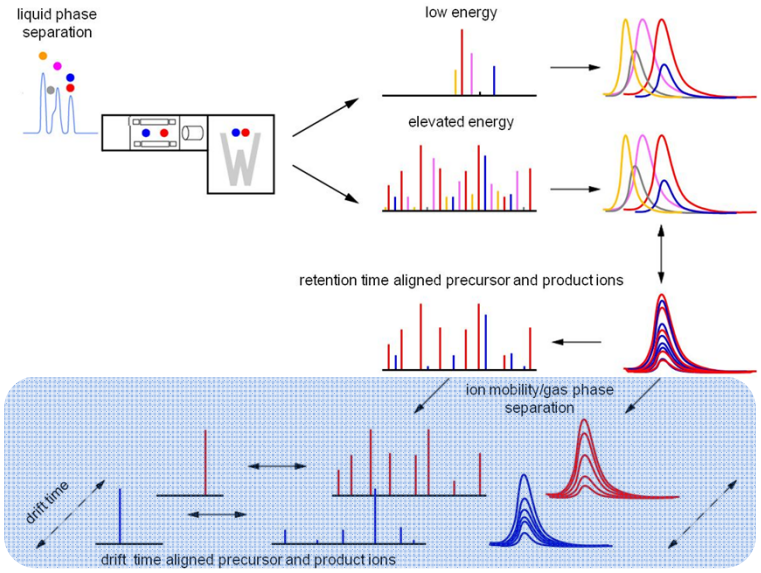
UPLC / HDMS^E (high energy)
...deconvoluting chimericy

Waters
THE SCIENCE OF WHAT'S POSSIBLE.



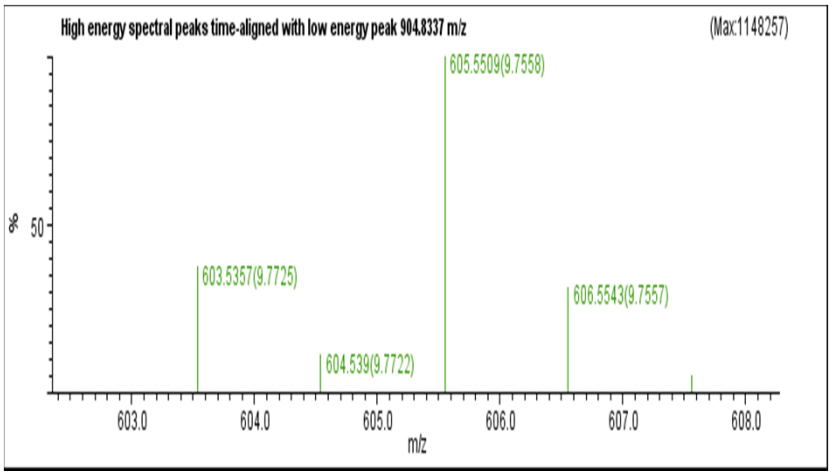
UPLC / HDMS^E

Waters
THE SCIENCE OF WHAT'S POSSIBLE.



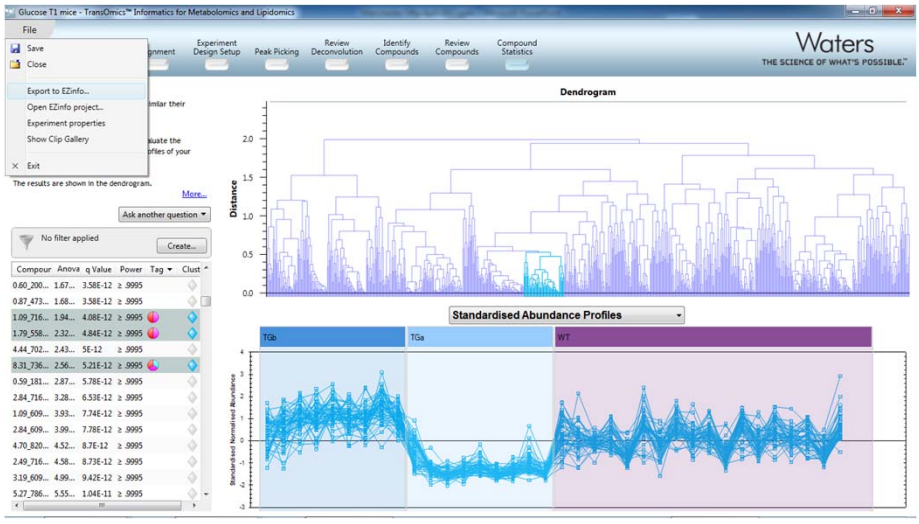
Fragmentation Spectrum of TG (18:0/18:1/18:1) m/z 904.8333

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



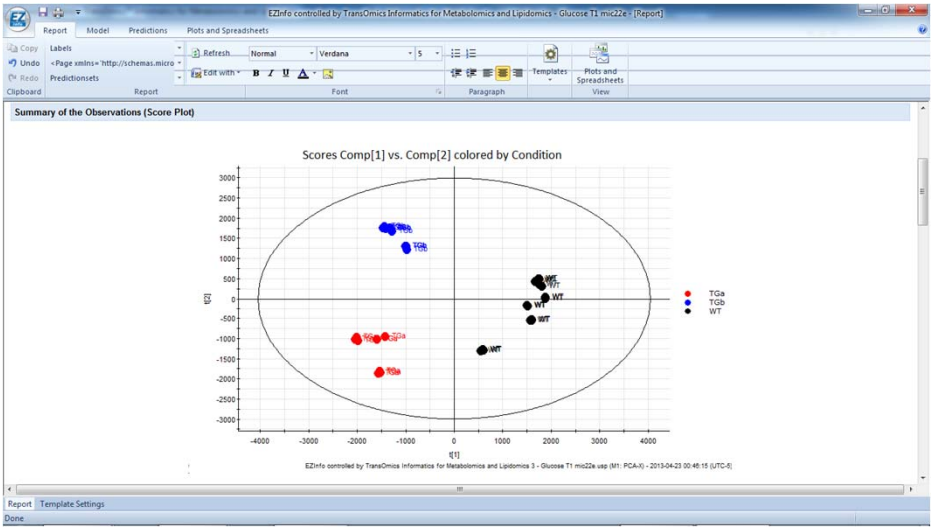
Export to EZinfo

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



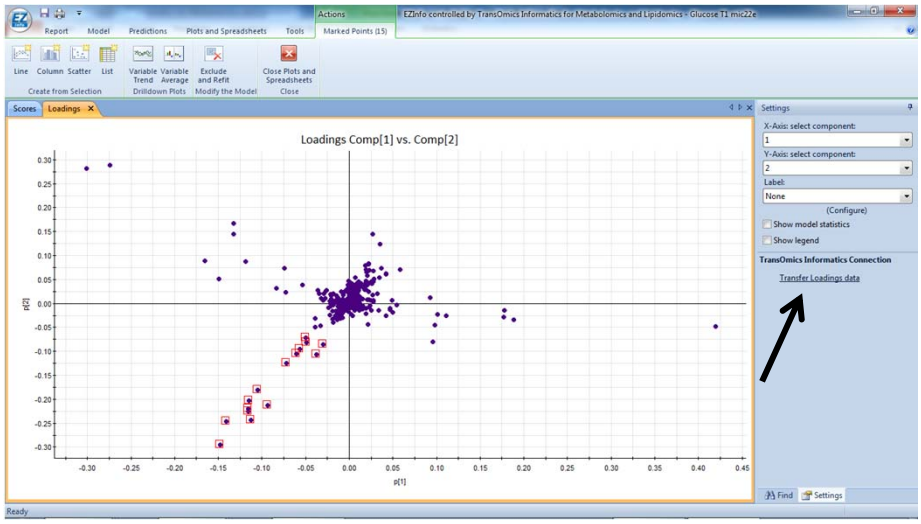
PLS-DA of All Groups

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



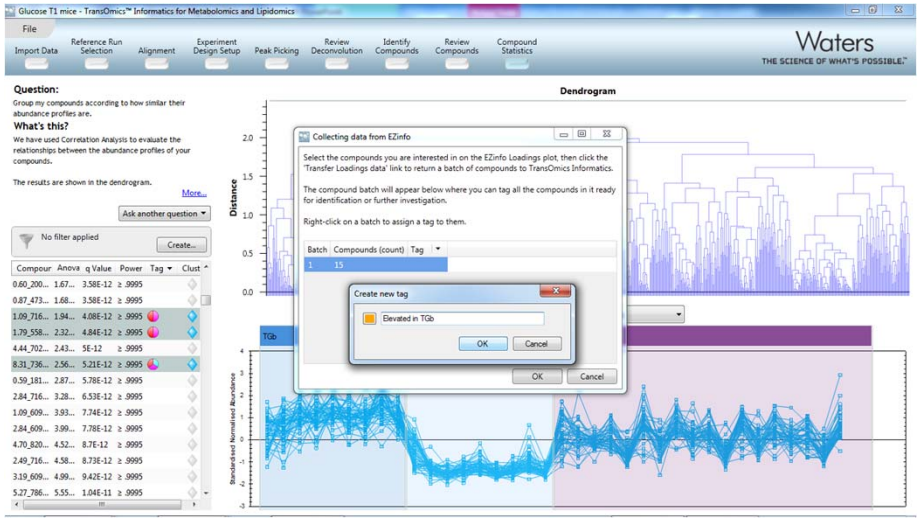
Transfer of Loadings

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



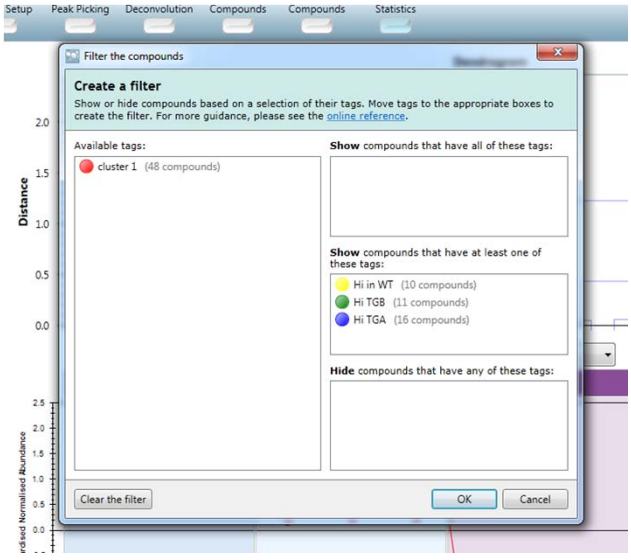
Tagging Imported Loadings

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

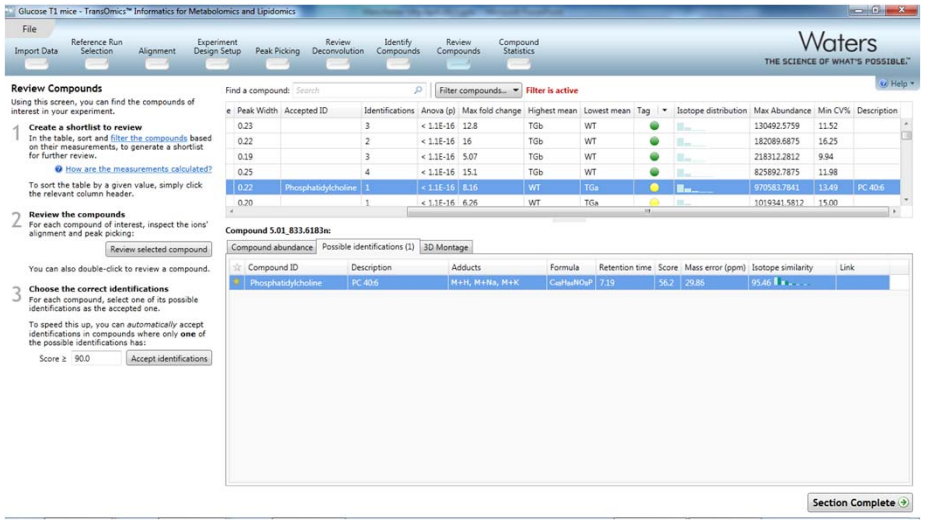


Creating a Filter

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Working with Selected Compounds Mined from MVA



Acknowledgements

Waters Corporation
Milford, USA

Scot Germanos
Giorgis Issac
Andy Baker

Waters Corporation
Manchester, UK

James Langridge
Mike Morris
John Hoyes
Martin Green
Jason Wildgoose
Kevin Giles
Steve Pringle
Ronan O'Malley
Alistair Wallace
Jeff Goshawk
Richard Gilpin

Acknowledgements

University Of Cambridge
Cambridge, UK

Jules Griffin
Helen Atherton
Melanie Gulston

Imperial College
London, UK

Jeremy Nicholson
Elaine Holmes
Liz Want
Matt Lewis

Merck
USA

Jose Castro Perez

Acknowledgements

nonlinear DYNAMICS