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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




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MRC | Human
Nutrition
Research



**Metabolomic and
Lipidomic Research in
Diabetes and Obesity**

***Prof. Jules Griffin,
MRC HNR & University of Cambridge***

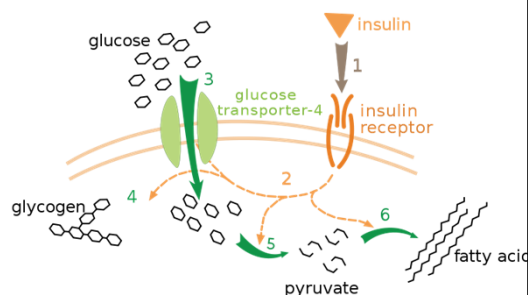
***Dr. Helen Atherton
Dr. Melanie Gulston***

MRC | Medical Research Council

Effect of insulin on glucose uptake and metabolism.

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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).



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The Diabetic Heart

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- 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.

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Study Description

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- 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.

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Study Description

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- 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.

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Question?

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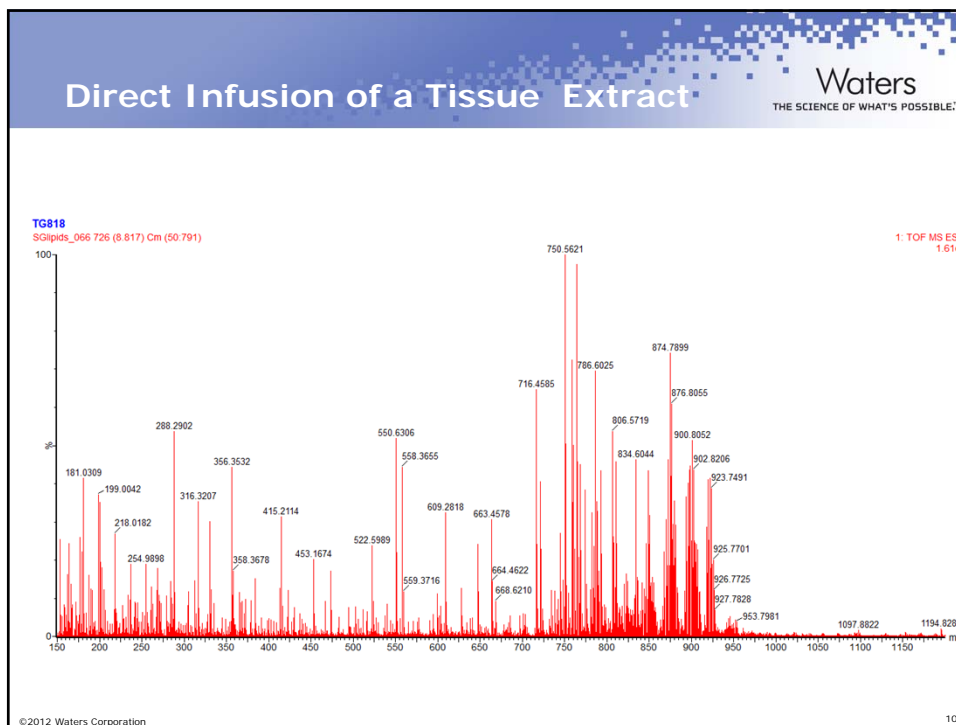
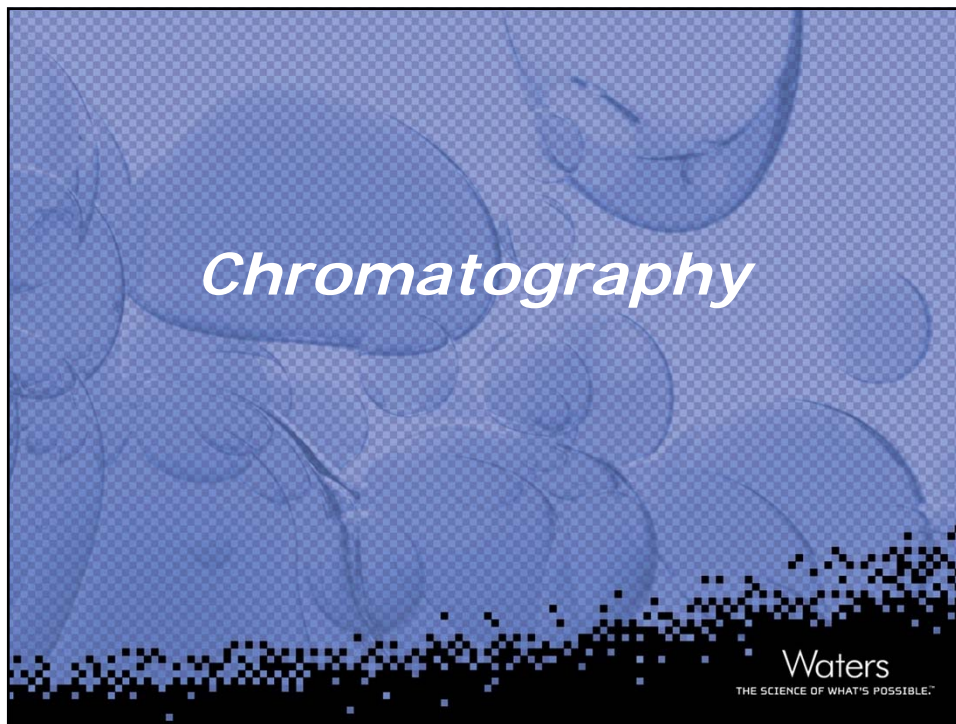
- **What is the effect of prolonged glucose stimulation on the lipid profile of heart tissue?**

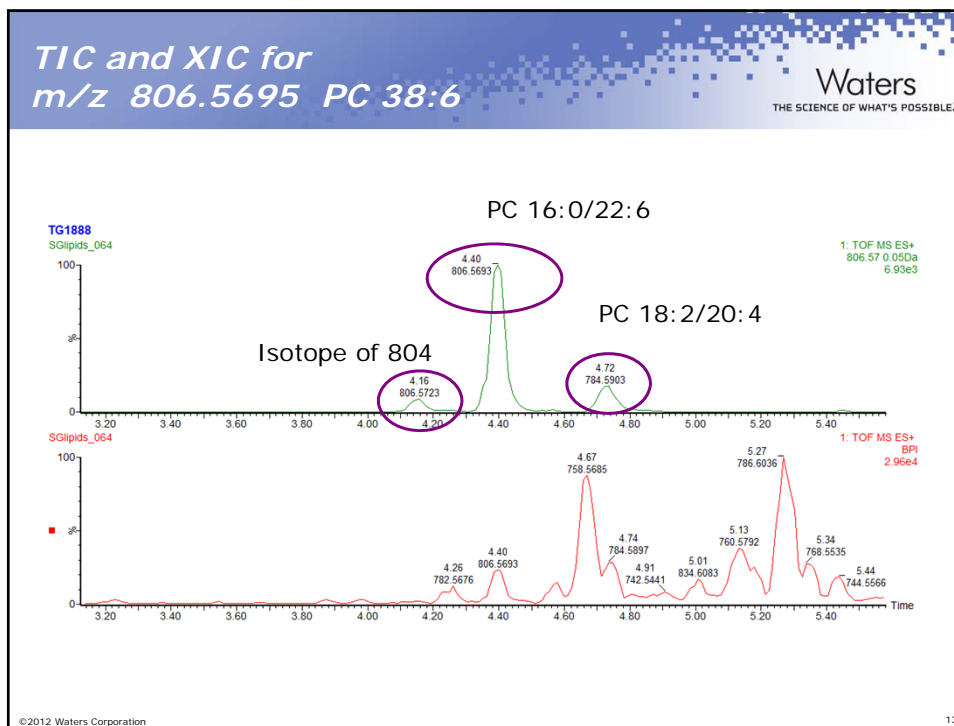
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Intact Lipid Analysis:

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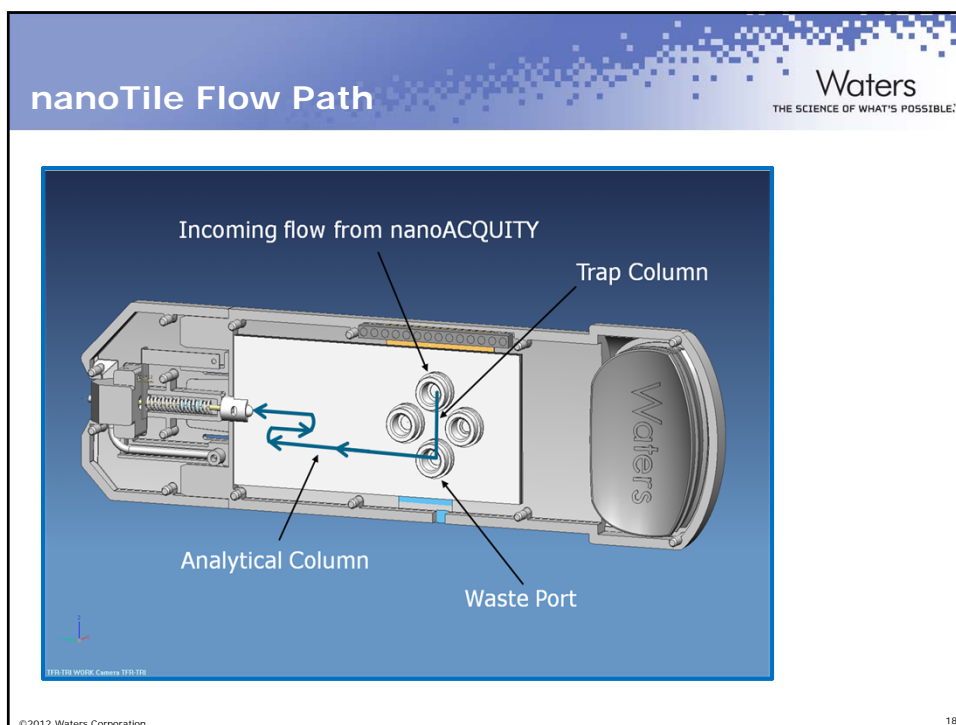


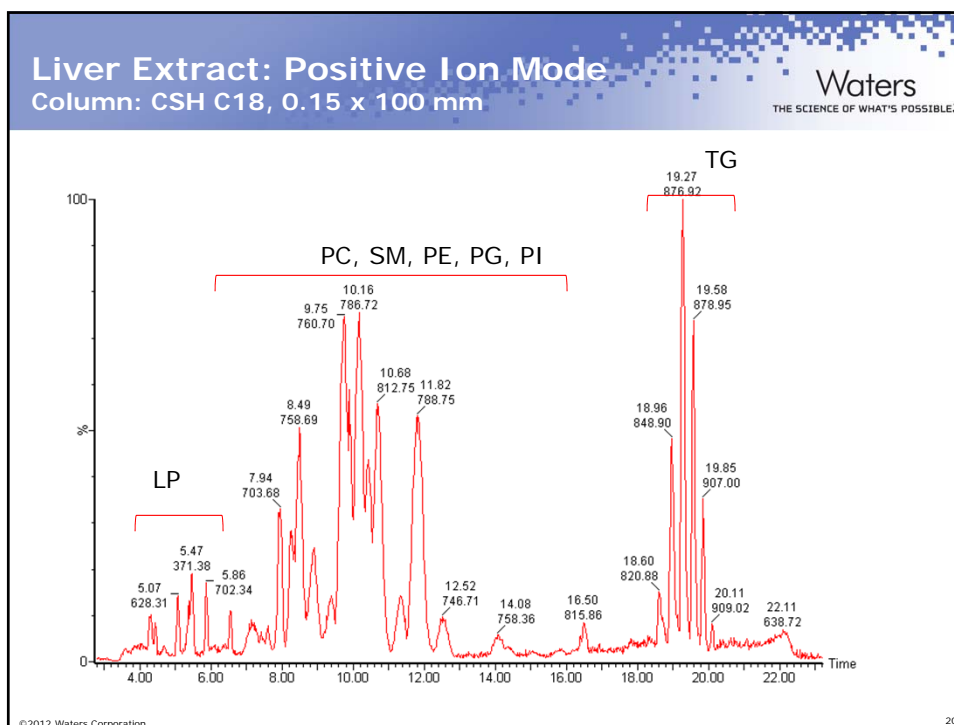
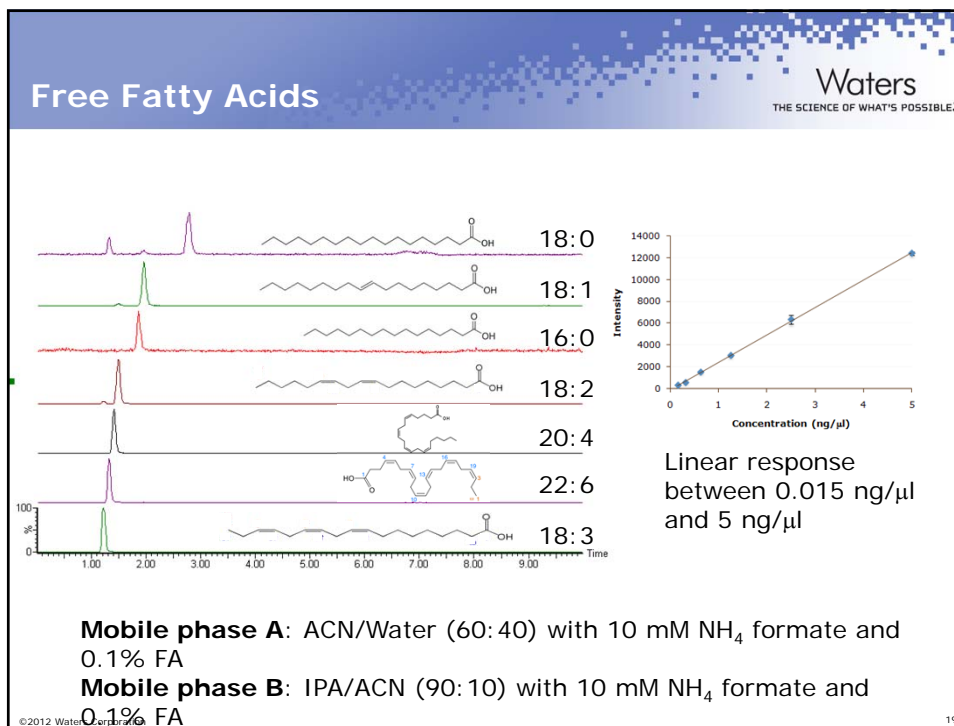
**UPLC and MS Conditions for Global
Lipid Profiling**

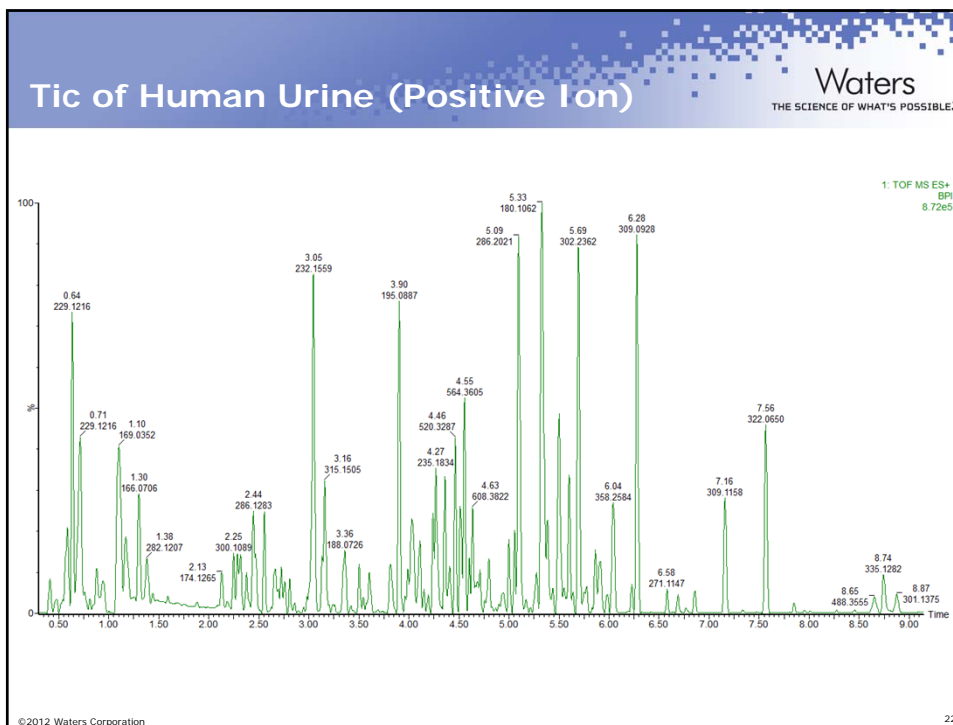
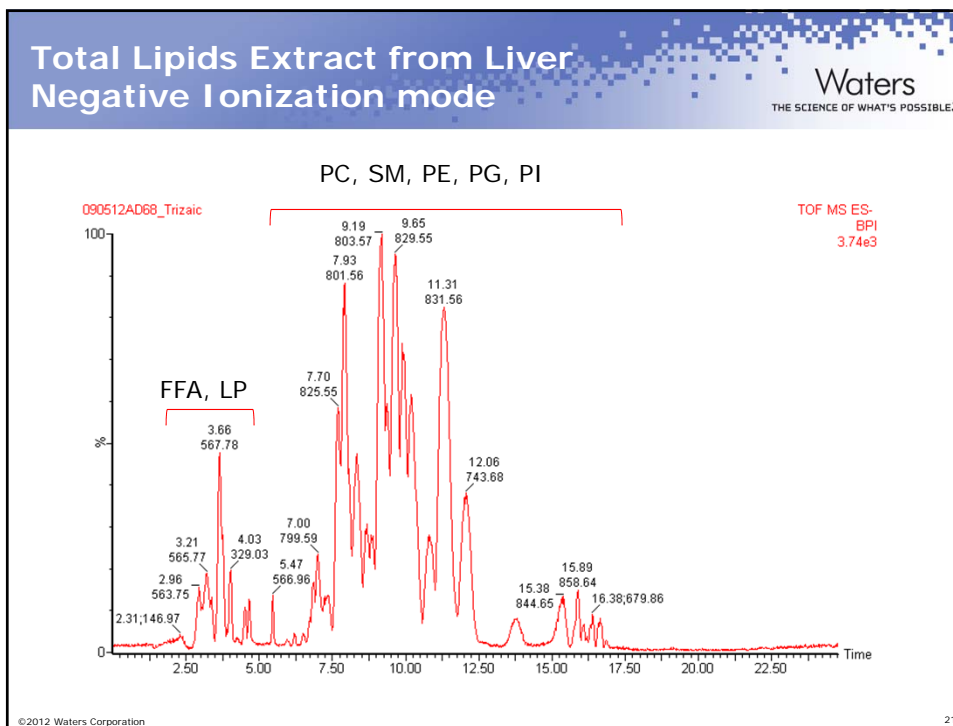
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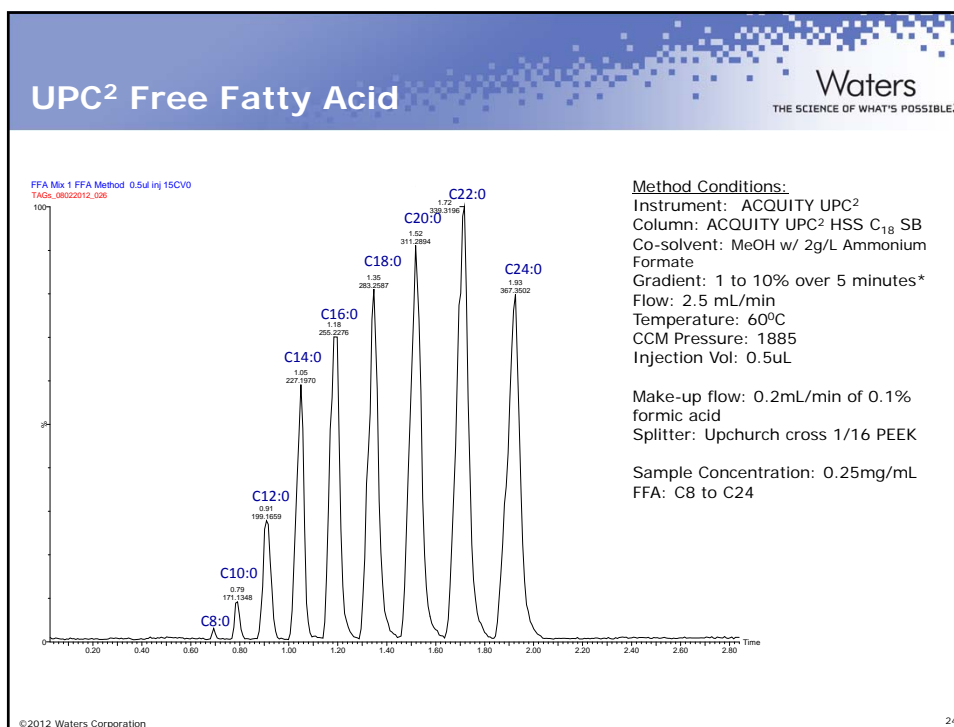
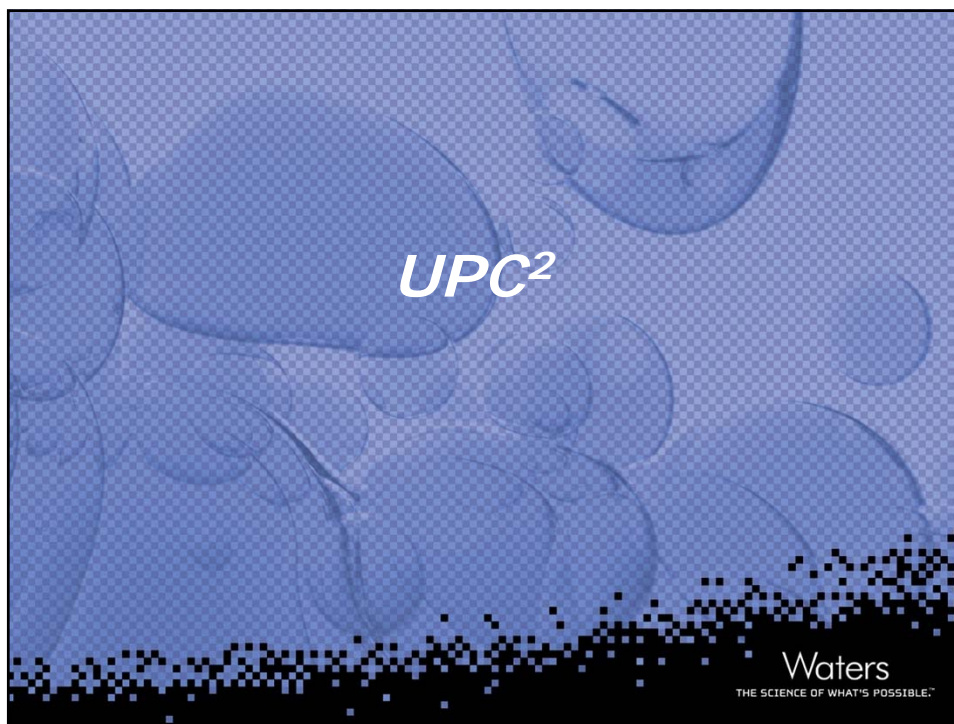
#	Time	Flow	%A	%B	
1	Initial	0.4	60	40	initial
2	2.0	0.4	55	43	6
3	2.1	0.4	50	50	1
4	12.0	0.4	46	54	6
5	12.1	0.4	30	70	1
6	18.0	0.4	1	99	6
7	18.1	0.4	60	40	6
8	20.0	0.4	60	40	1

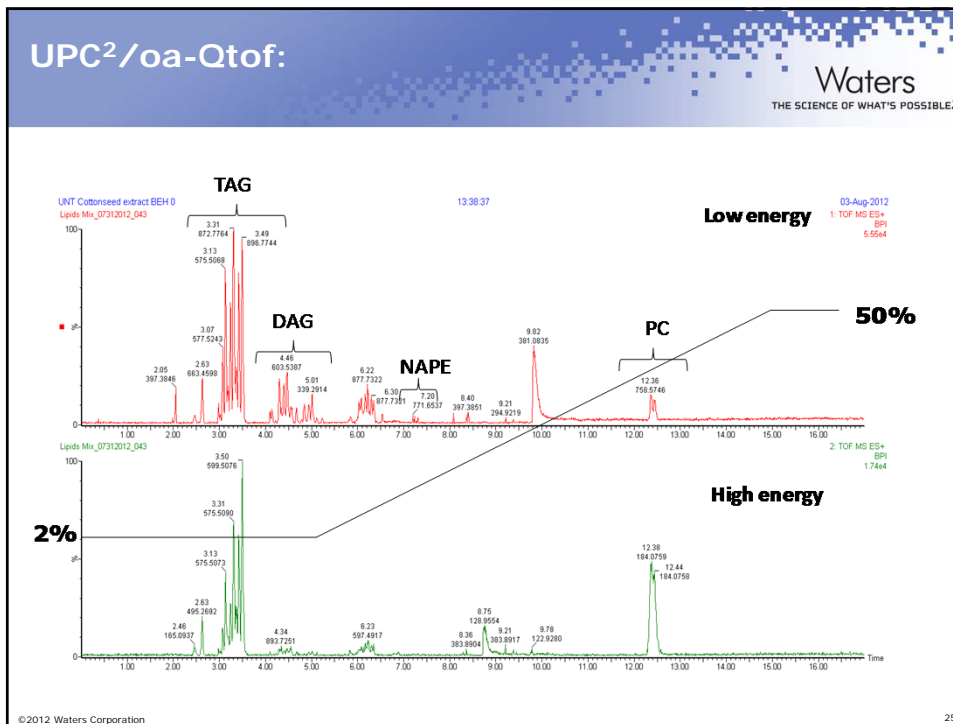
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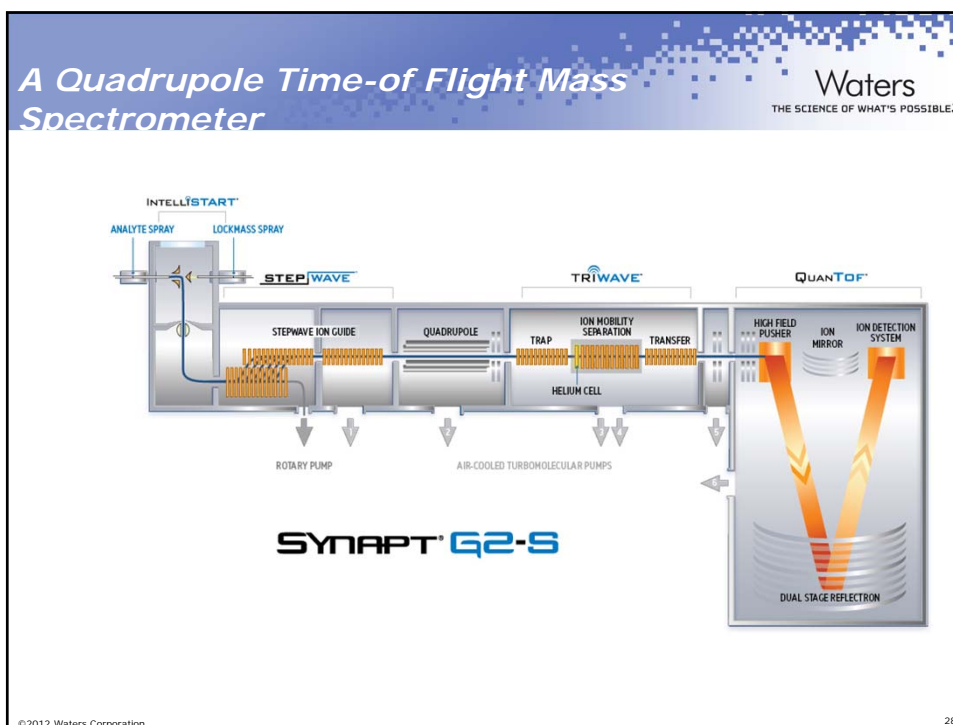
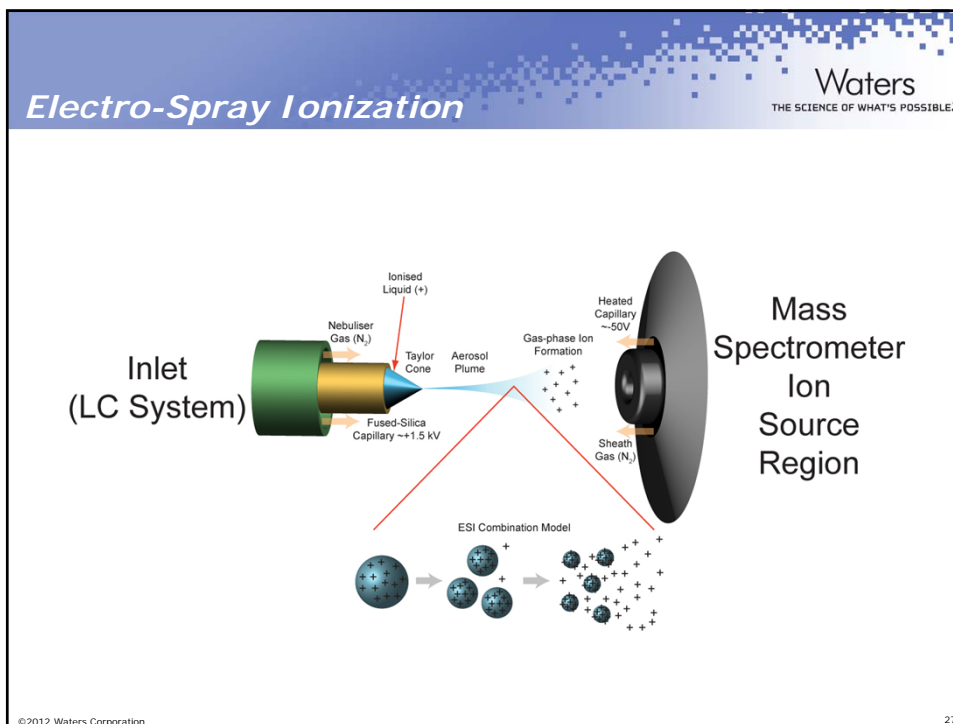






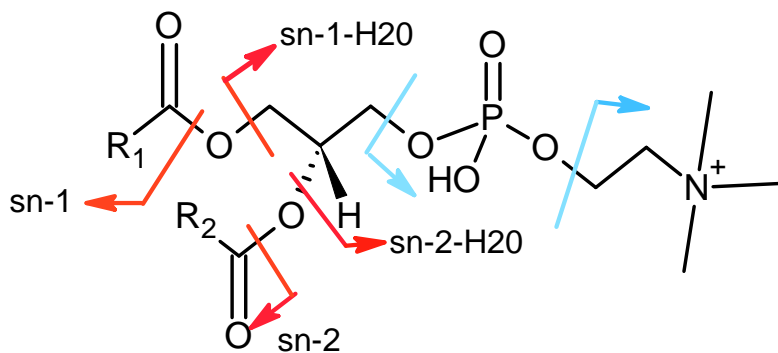
MS Analysis

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Fragmentation of a Typical Glycerophospholipid

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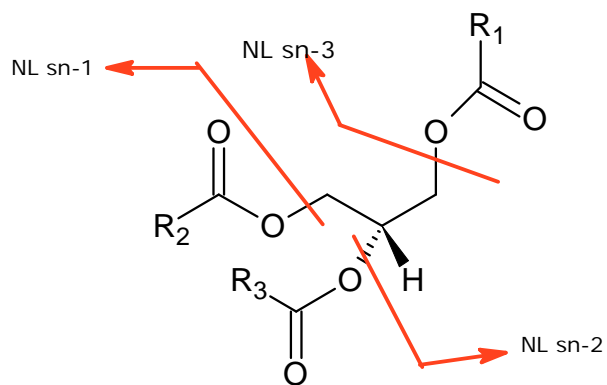
Phosphatidylcholine (PC)

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Fragmentation of Triglycerides

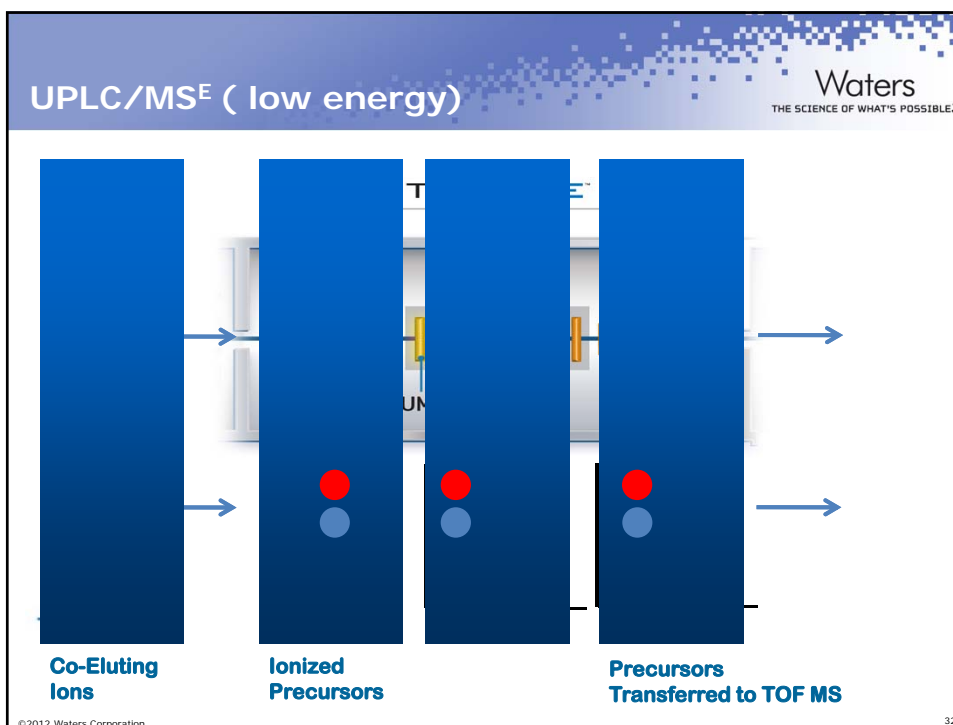
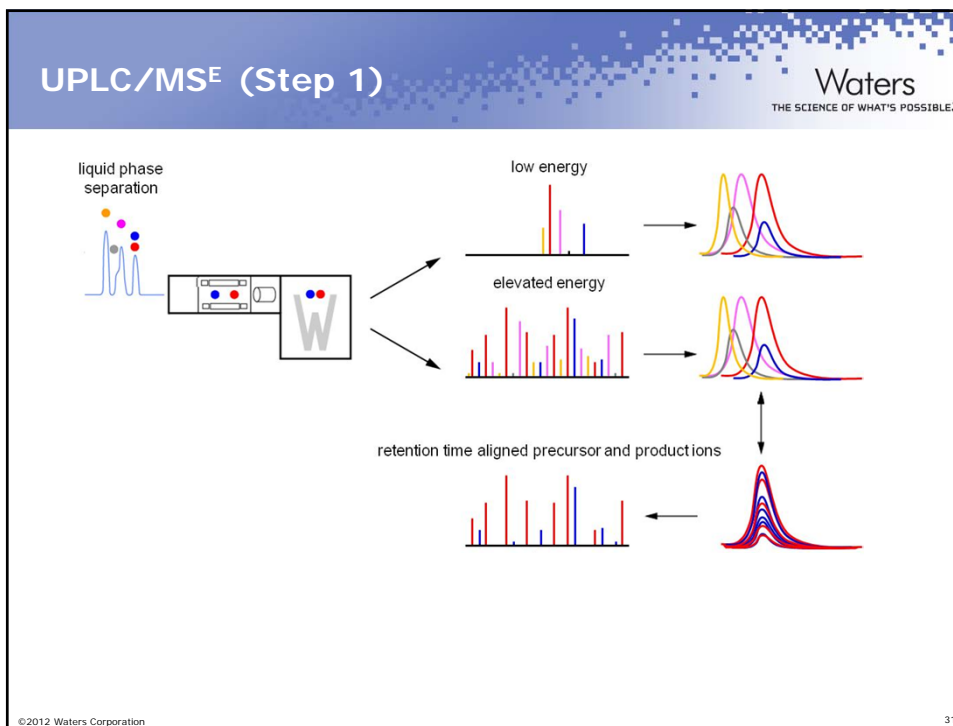
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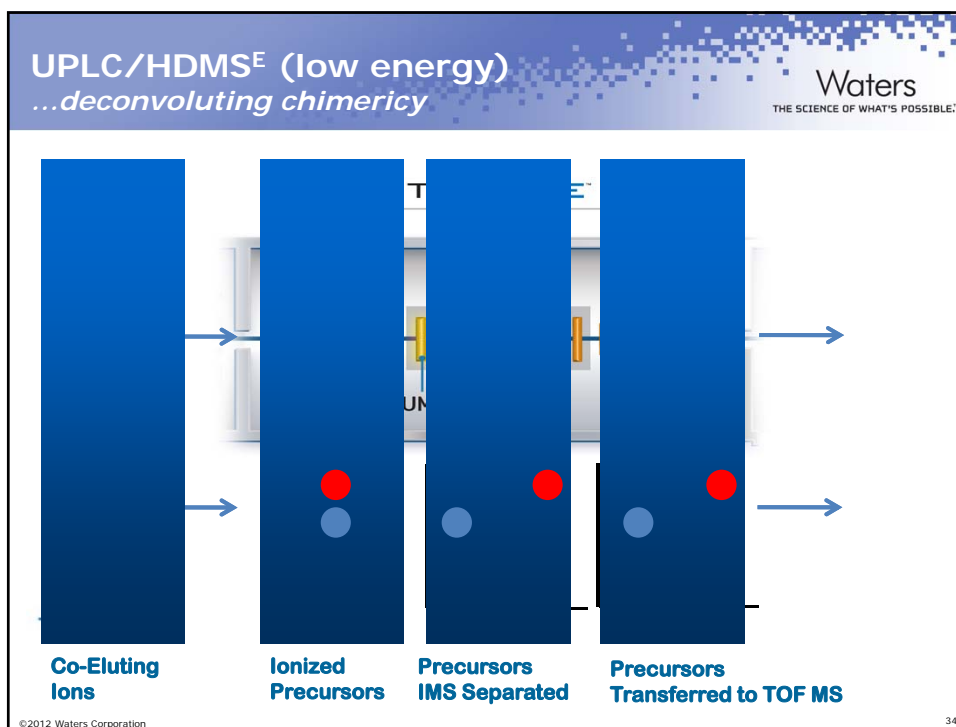
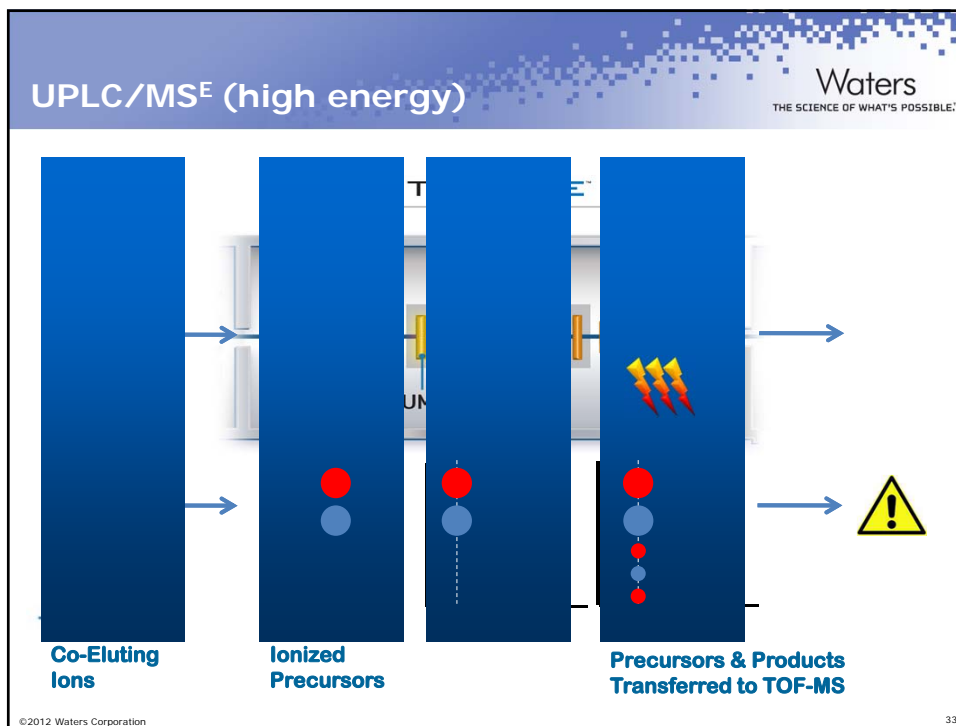


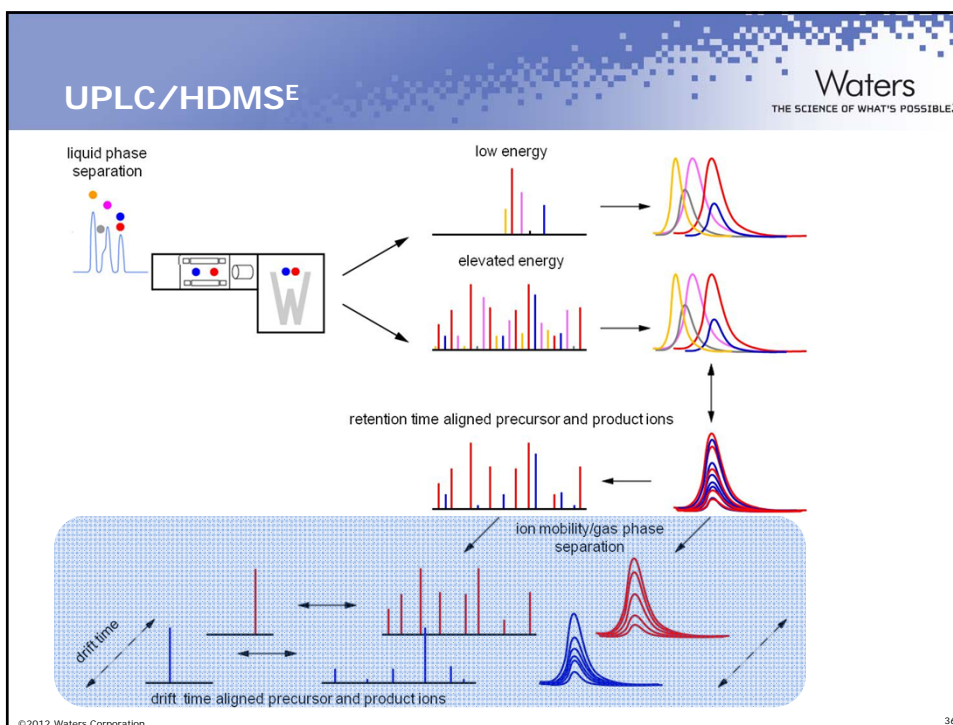
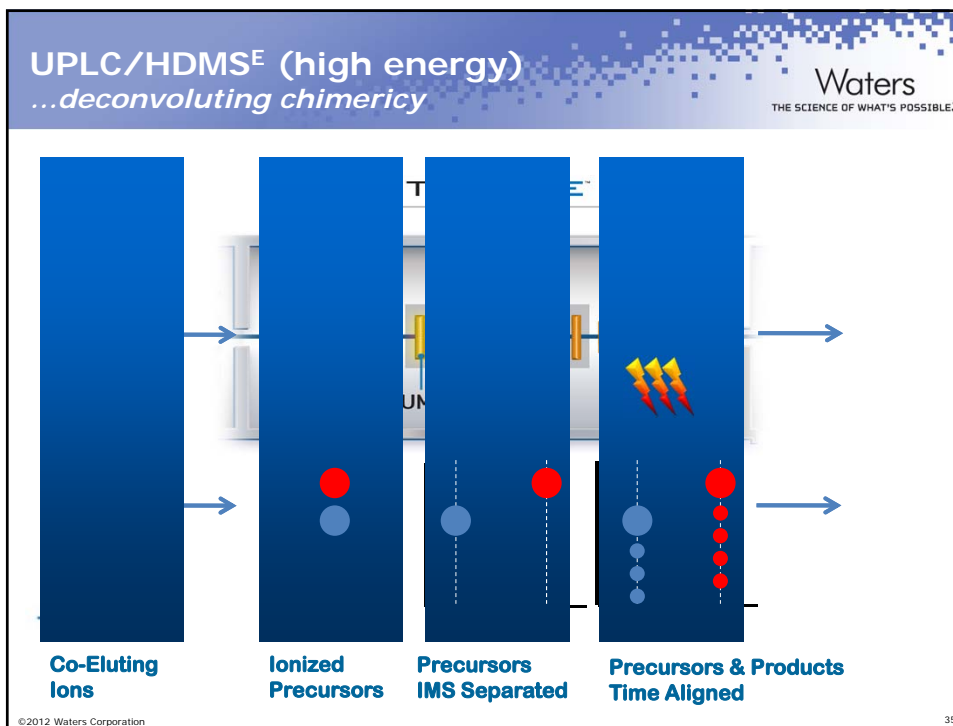
Note: The fragmentation for Mono and Di Glycerides cleaves the same bonds.

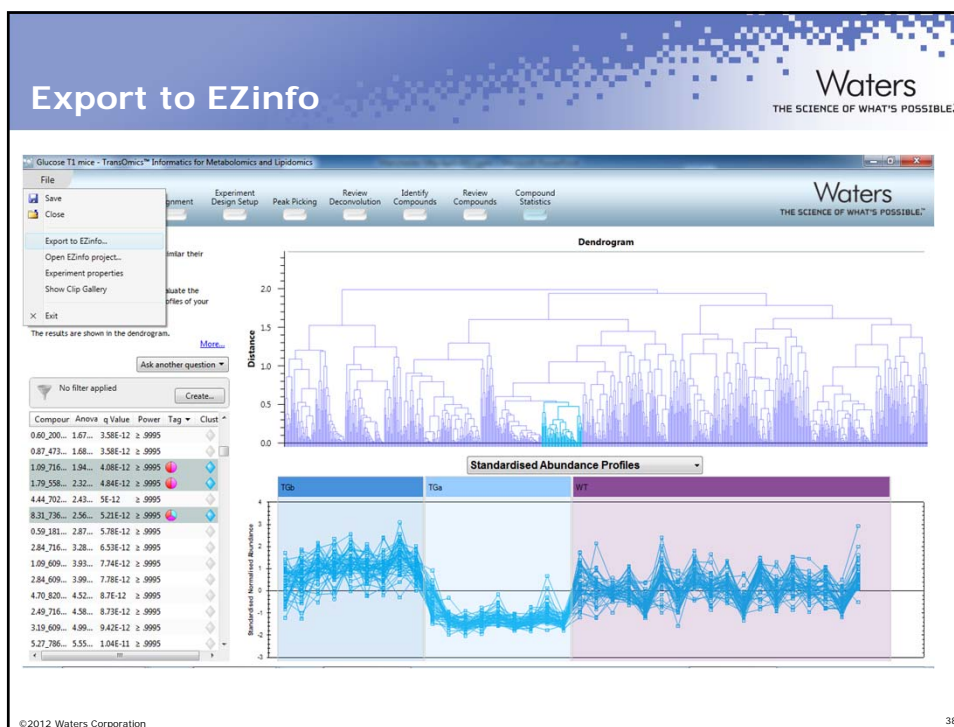
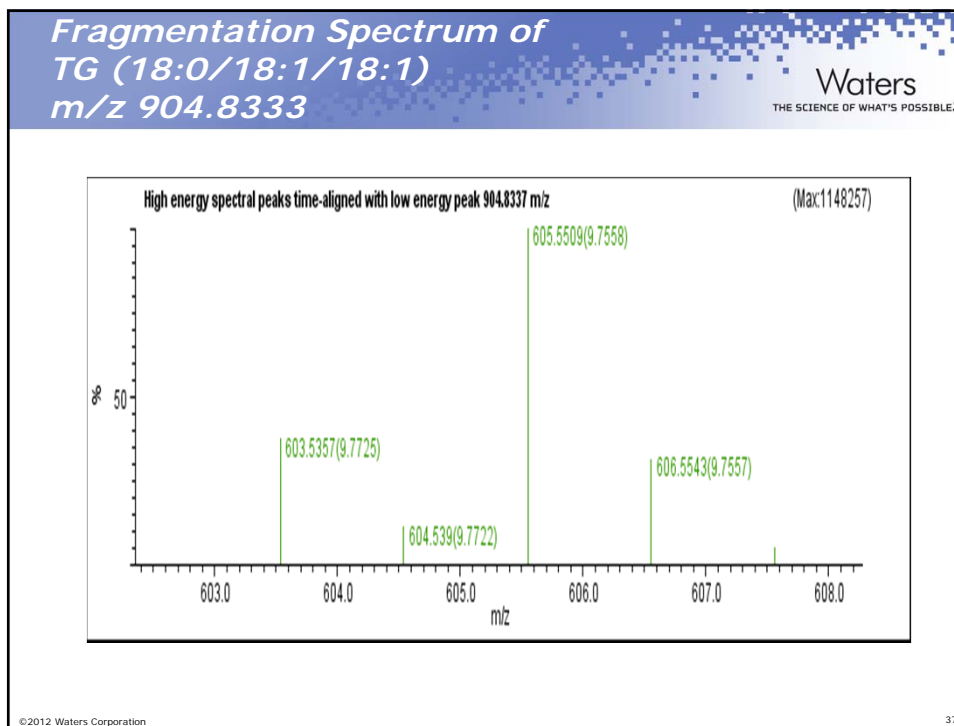
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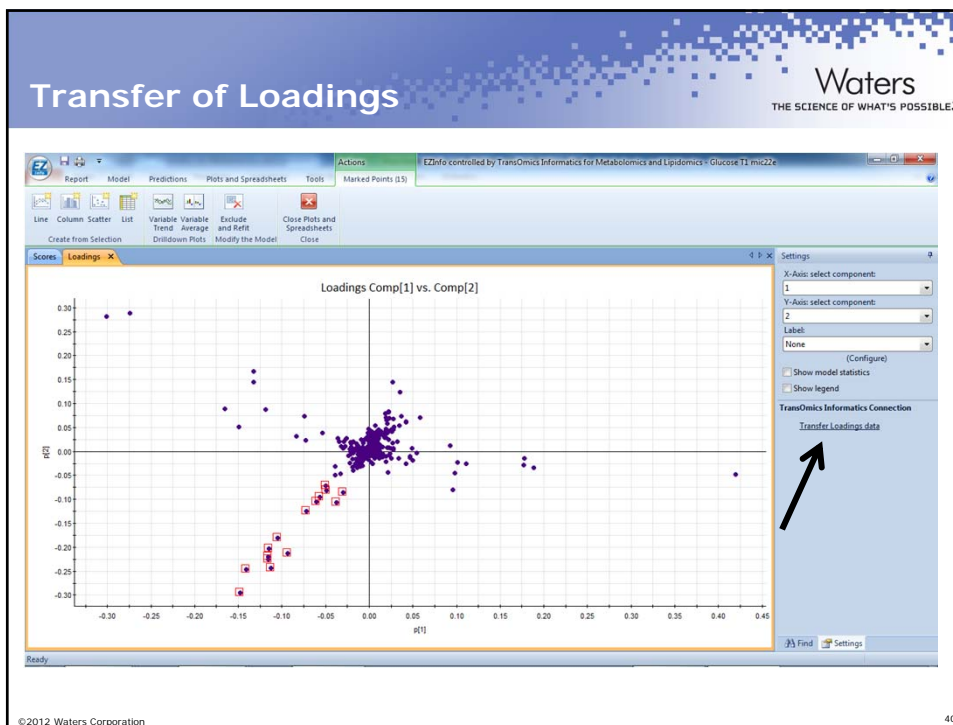
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Tagging Imported Loadings

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Question:
Group my compounds according to how similar their abundance profiles are.
What's this?
We have used Correlation Analysis to evaluate the relationships between the abundance profiles of your compounds.
The results are shown in the dendrogram.

Dendrogram

Distance

Collecting data from EZinfo

Select the compounds you are interested in on the EZinfo Loadings plot, then click the 'Transfer Loadings data' link to return a batch of compounds to TransOmics Informatics.
The compound batch will appear below where you can tag all the compounds in it ready for identification or further investigation.
Right-click on a batch to assign a tag to them.

Batch	Compounds (count)	Tag
1	15	

Create new tag

Elevated in TGB

OK Cancel

OK Cancel

Compounds

Compound	Area	q Value	Power	Tag	Clust
0.60_200...	1.67...	3.58E-12	> 9995		
0.87_473...	1.68...	3.58E-12	> 9995		
1.09_716...	1.94...	4.08E-12	> 9995		
1.79_558...	2.32...	4.84E-12	> 9995		
4.44_702...	2.43...	5E-12	> 9995		
8.31_736...	2.56...	5.21E-12	> 9995		
0.59_181...	2.87...	5.78E-12	> 9995		
2.84_716...	3.28...	6.53E-12	> 9995		
1.09_609...	3.93...	7.74E-12	> 9995		
2.84_609...	3.99...	7.78E-12	> 9995		
4.70_820...	4.52...	8.7E-12	> 9995		
2.49_716...	4.58...	8.73E-12	> 9995		
3.19_609...	4.99...	9.42E-12	> 9995		
5.27_286...	5.55...	1.04E-11	> 9995		

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Creating a Filter

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Setup Peak Picking Deconvolution Compounds Compounds Statistics

Filter the compounds

Create a filter

Show or hide compounds based on a selection of their tags. Move tags to the appropriate boxes to create the filter. For more guidance, please see the [online reference](#).

Available tags:

- cluster 1 (48 compounds)

Show compounds that have all of these tags:

Show compounds that have at least one of these tags:

- Hi in WT (10 compounds)
- Hi TGB (11 compounds)
- Hi TGA (16 compounds)

Hide compounds that have any of these tags:

Clear the filter

OK Cancel

Distance

Adjusted Normalized Abundance

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Working with Selected Compounds Mined from MVA

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Review Compounds

Using this screen, you can find the compounds of interest in your experiment.

- Create a shortlist to review**
In the table, sort and filter the compounds based on their measurements, to generate a shortlist for further review.
[How are the measurements calculated?](#)
To sort the table by a given value, simply click the relevant column header.
- Review the compounds**
For each compound of interest, inspect the ions' alignment and peak picking:
[Review selected compound.](#)
You can also double-click to review a compound.
- Choose the correct identifications**
For each compound, select one of its possible identifications as the accepted one.
To speed this up, you can automatically accept identifications in compounds where only one of the possible identifications has:
Score [Accept identifications](#)

Peak Width	Accepted ID	Identifications	Anova (p)	Max fold change	Highest mean	Lowest mean	Tag	Isotope distribution	Max Abundance	Min CV%	Description
0.23		3	< 1.1E-16	12.8	TGb	WT			130492.5759	11.52	
0.22		2	< 1.1E-16	16	TGb	WT			182089.6875	16.25	
0.19		3	< 1.1E-16	5.07	TGb	WT			218312.2812	9.94	
0.25		4	< 1.1E-16	15.1	TGb	WT			825892.7875	11.98	
0.22	Phosphatidylcholine	1	< 1.1E-16	6.16	WT	TGb			970583.7841	13.49	PC 406
0.20		1	< 1.1E-16	6.26	WT	TGb			1019341.5812	15.00	

Compound 5.01_833.6183a:

Compound ID	Description	Adducts	Formula	Retention time	Score	Mass error (ppm)	Isotope similarity	Link
Phosphatidylcholine	PC 406	M+H, M+Na, M+K	C ₄₈ H ₉₈ O ₈ P	7.19	56.2	29.86	95.46	

Section Complete →

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Acknowledgements

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nonlinear
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