Understanding Small Molecule Biomarker Patterns by Targeted and Non-Targeted Metabolomics using LC/MS/MS

Henri Snijders

Applied Biosystems

Thesssaloniki, 30 October 2008

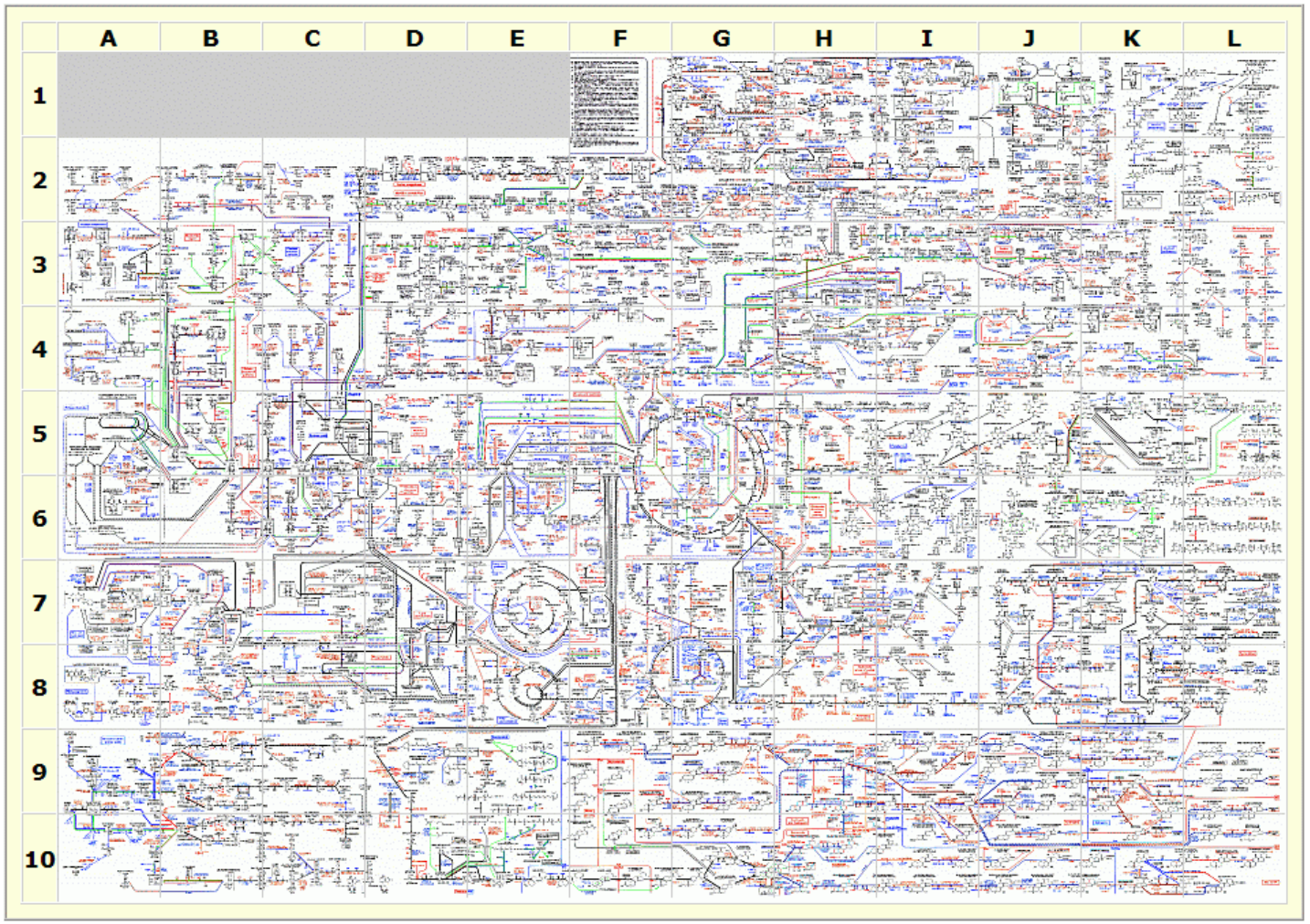
Applied Biosystems | MDS Analytical Technologies

Outline

- Non-targeted Metabolomics
- Targeted Metabolomics
- Novel Workflows for Lipidomics

Applied Biosystems | MDS Analytical Technologies

Human Metabolic Pathways



Metabolomics Workflows

Application

Workflow	Discovery	Pre-Clinical	Clinical
Targeted	**Hypothesis-driven** – have discovered important markers and need to monitor their presence and concentration • Acquire LC/MS data • Monitor for known masses of interest • Determine how these masses varied over multiple samples via stat analyses • Find samples that are different (diseased, etc.)	4000 QTRAP/QTRAP 5500 QUAN	
Non-Targeted	**Non-hypothesis-driven** – not sure what you are looking for, screening large numbers of samples to look for patterns – diseased vs. non-diseased • Acquire LC/MS data – All unknowns or sample vs control (diseased vs nondiseased) • Statistical analysis (PCA, HCA) to look for differences in samples • Find samples that are different • Identify what masses account for differences between samples • Identify compounds	QSTAR/ Qual	

QSTAR® Elite LC MS/MS System

- Latest generation QqTOF platform
- Provides high resolution, accurate mass data (2 ppm and 15,000 resolution)
- 4 orders linear dynamic range in TOF mode
- Capable of acquiring 20 MS spectra/second and MS/MS on up to 7 precursor ions/second
  - Compatible with UPLC and other high throughput chromatography systems

Metabolomics Experiments

- What's really needed to for a metabolomics experiment?
  1. Must be able to find groupings in data (i.e. diseased samples must separate from non-diseased)
  2. Must be able to find potential biomarkers (unique masses that cause groups to separate)
  3. Must be able to determine elemental formula for potential biomarkers
  4. Must be able to identify potential biomarkers (assign name/structure to mass)

- What tools are best for this process?
  - What are the most important capabilities of hardware and software for a metabolomics experiment?
  - How important are scan speed, resolution, mass accuracy, isotope ratios, statistical analysis technique…?

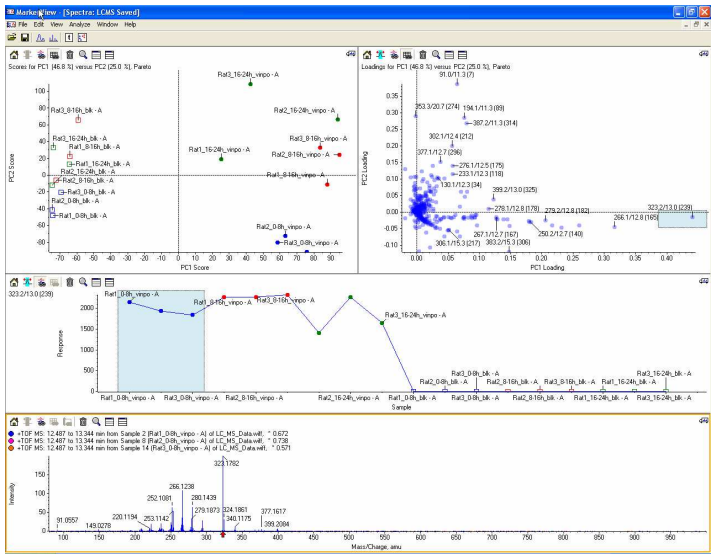
Finding Groupings in the Data

- Typically acquire full-scan MS data and process using vendor-specific or general 3rd party statistical analysis tools

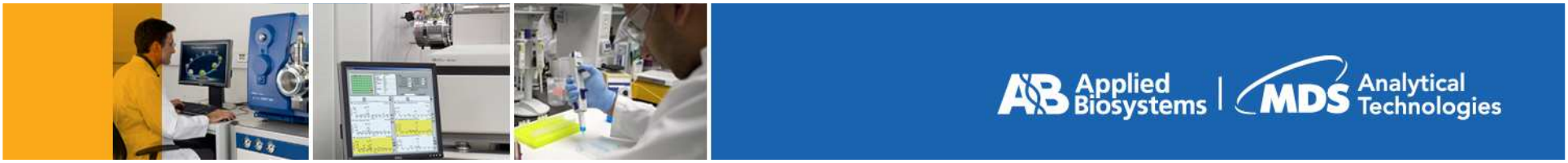
MarkerView™ 1.2 Software

Industry-leading Metabolomics Software

- Software for metabolomics and biomarker profiling experiments
- Finds and aligns peaks
- Performs statistical analysis to find potential biomarkers
  - T-Test
  - PCA
  - PCA-DA



- Can export data to any 3rd party stats package for additional processing
- View trends plots and link back to MS and MS/MS data
- Create IDA inclusions lists to acquire MS/MS data on selected compounds for further analysis
- Generate customized Word reports that can be shared amongst non-MarkerView users



Finding Groupings in the Data

- Assume that if there are differences in the samples, some statistical package will enable you to find these differences
- However, can your data acquisition techniques affect whether you can see correct differences and groupings within data?

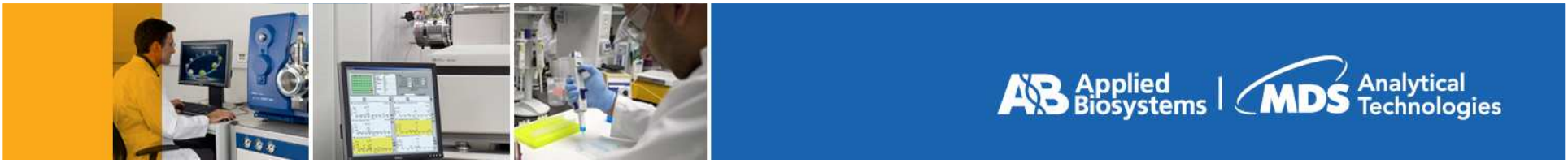
- Many metabolomics researchers moving towards high speed chromatography (UPLC) for faster separations and higher peak capacities
- What is the affect of these narrow LC peaks (1-2 seconds wide) on your data and your ability to correctly interpret data?
  - … well, this depends on your MS scan speed

Finding Groupings in the Data
Importance of Scan Speed

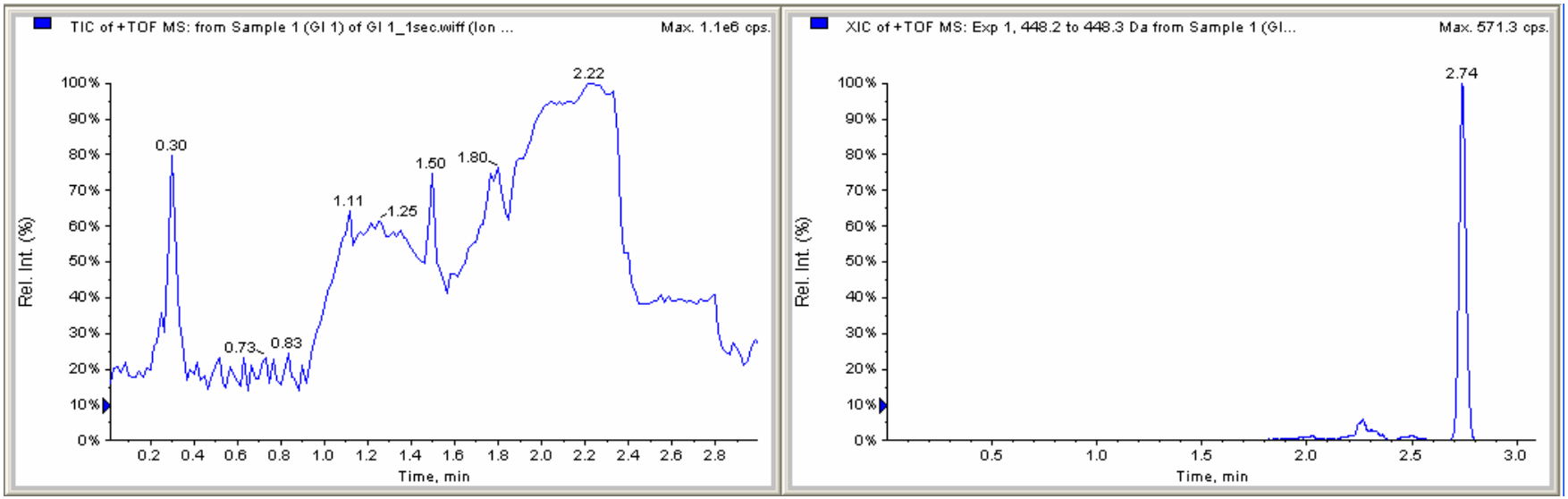
- Can a slow-scanning instrument be used for metabolomics?
  - It depends….
- QSTAR® Elite System can acquire data at a rate of up to 20 spectra/second
- QTRAP® 5500 System has scan speed up to 20,000 amu/s and MRM dwell times down to 2 ms
- What happens to your ability to accurately interpret metabolomics data if you are acquiring only 1 spectrum/second and using high speed chromatography?

Experimental

- Samples:
  - Urine samples were collected in the morning from 3 different male and 3 different female subjects and frozen until analysis
  - A 1:10 dilution in mobile phase was done prior to analysis
  - Samples were divided into 9 aliquots, resulting in 9 replicate injections from each of 6 different subjects
- LC:
  - Chromatographic separation was performed using a PE 100 series autosampler and micro pumps
  - A gradient from 95% to 5% aqueous at 250 µL/min through a BetaBasic C18 50 x 2.1 mm, 3 µm column was done over a 3 minute analysis
- MS:
  - A QSTAR® Elite System (Applied Biosystems|MDS Sciex) was used for collection of MS data over a 1000 amu mass range and all detection was performed in positive ion mode using TurboIonSpray® Source
  - Dynamic AutoCalibration was used to maintain mass accuracy throughout the data collection
  - Data acquisition was performed using several different scan speeds, ranging from 1 to 20 spectra/second
- Data Processing:
  - MarkerView™ Software

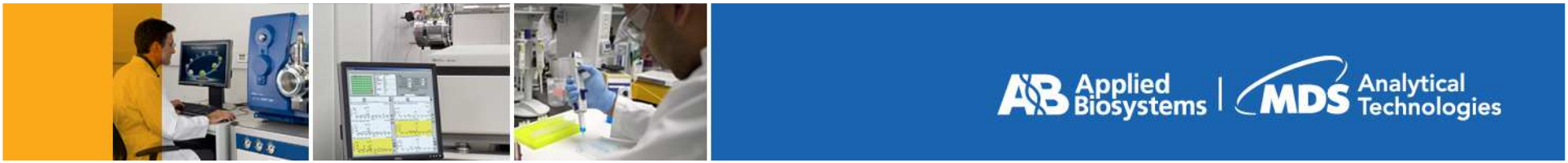


Typical TIC and XIC

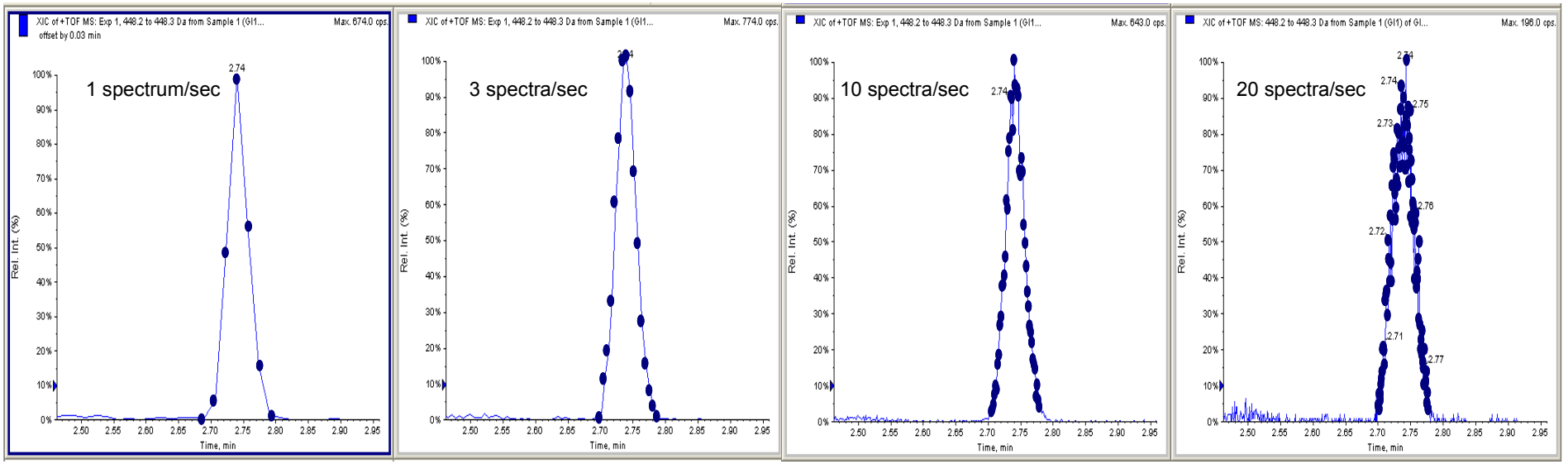


Data acquired at 20 spectra/second

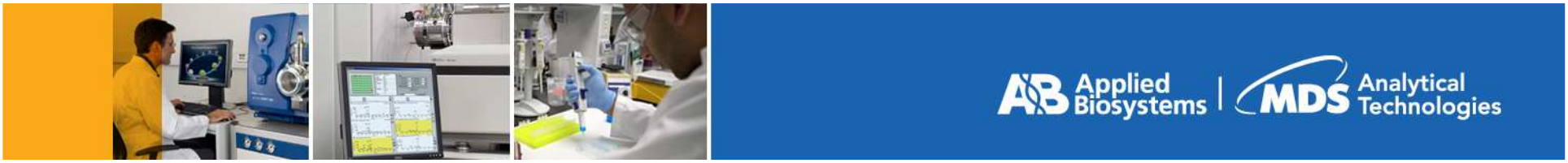
XIC of *m/z* 448 showing the 4-second wide peaks typical from this experiment



Data Points Per Peak at different scan speeds



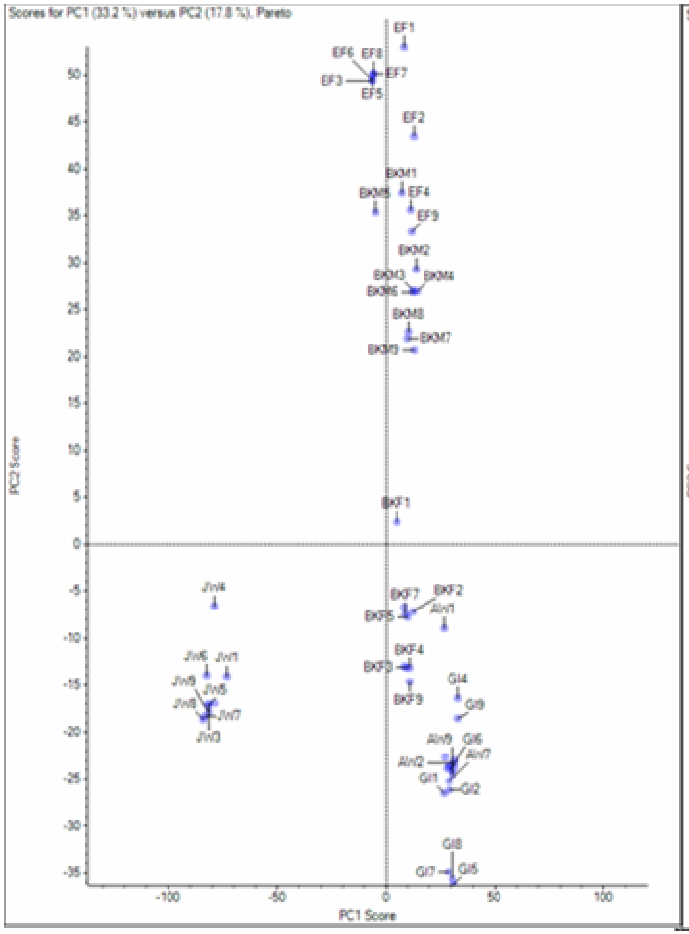
- At an acquisition rate of 1 spectrum/sec, only 3 data points above FWHH are acquired
- At an acquisition rate of 20 spectra/sec, ~40 MS spectra were acquired



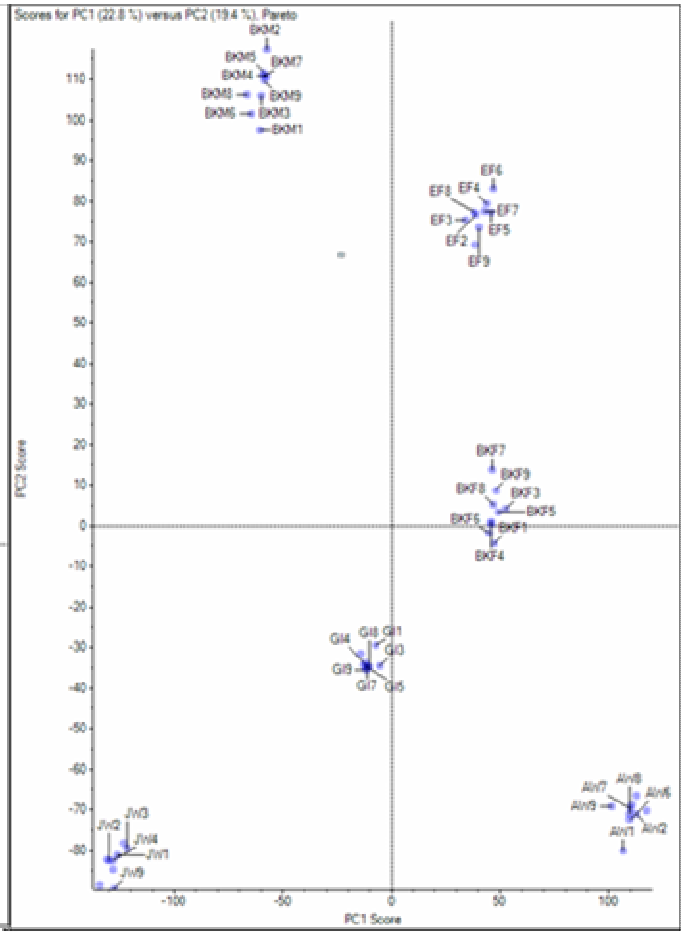
PCA of data acquired at 1 vs 20 spectra/second

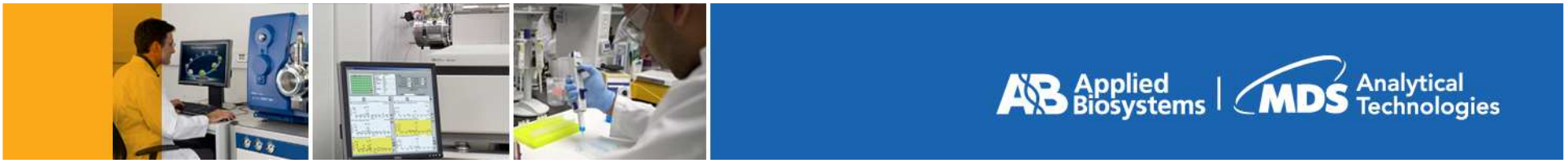
- At 1 spectrum/second, there appear to be 3 distinct groups in the data
- At 20 spectra/second, there appear to be 6 distinct groups in the data
- Which is correct? Are there 6 groups, one for each individual, or 3 groups, one for male, one for female, and one for other?

1 spectrum/second



20 spectra/second



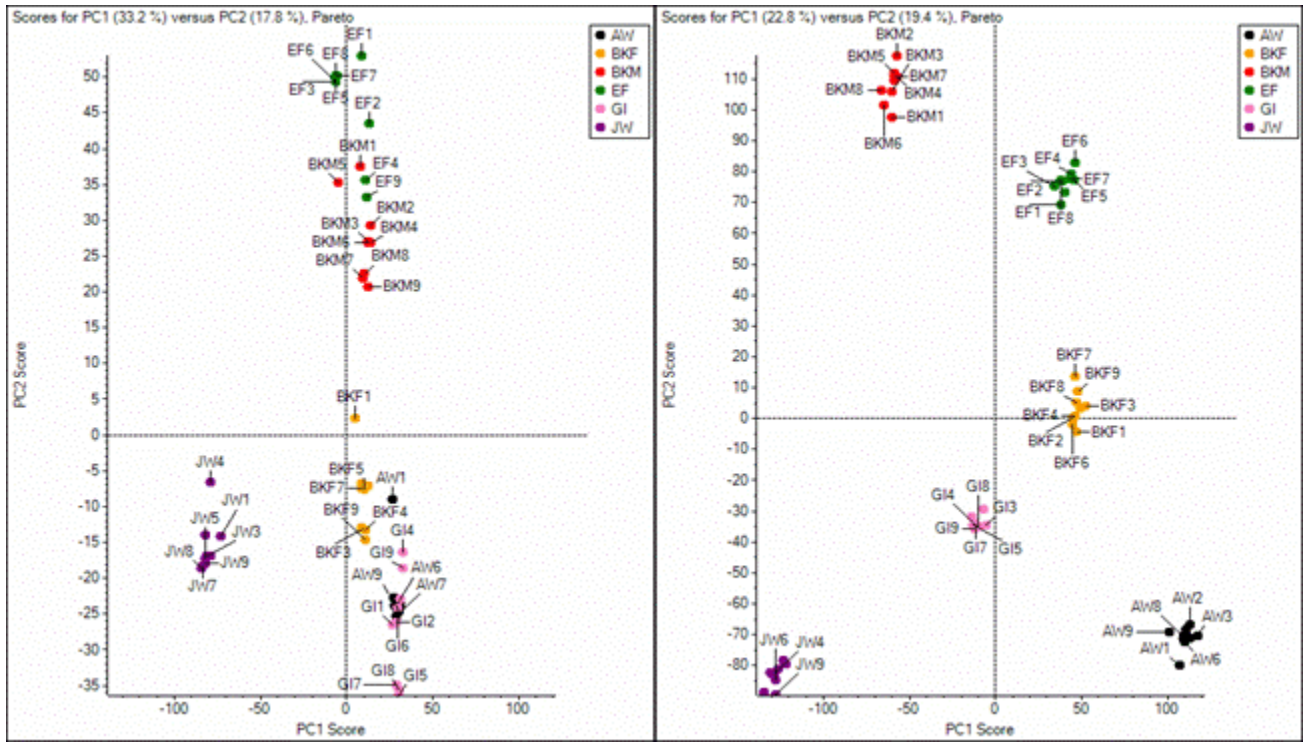


PCA of data acquired at 1 vs 20 spectra/second

1 spectrum/second

20 spectra/second

- Once colors are assigned to correct groups, becomes clear that the data acquired at 1 spectrum/second is very misleading
- Interestingly, at 1 spectrum/second, even the most basic distinction between men and women is incorrect
  - EF, BKF and JW are females
  - GI, AW, BKM are males



Data acquired at 1 spectrum/second is incorrect – shows only 3 groups within the data

Finding Potential Biomarkers

- Assume you have performed experiment correctly and have sufficient mass spec scan speed for the LC system being used and have reproducible data
- You are able to correctly group data and find m/z values that separate samples into groups

Determining Elemental Formula

- You now have a list of m/z values (along with retention times) for potential biomarkers
- Generally, to determine the structure of a compound, the first step is to determine the elemental formula
- Standard elemental calculators have the drawback that they often produce lists of 10's to even 1000's of possible formulae for a given molecular weight, even using a 5 ppm mass tolerance
- What's the solution?
  - Going to instruments with better mass accuracy precision?
  - Maybe not provided you can make more intelligent use of the data that you already have

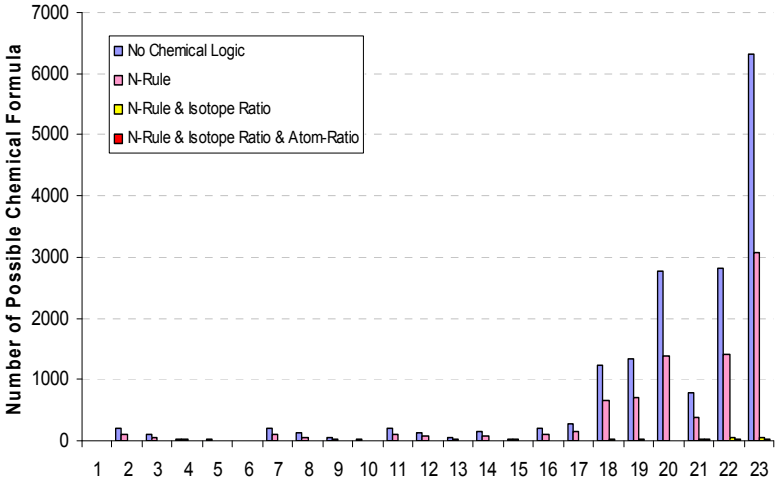
Formula Finder on the QSTAR® Elite

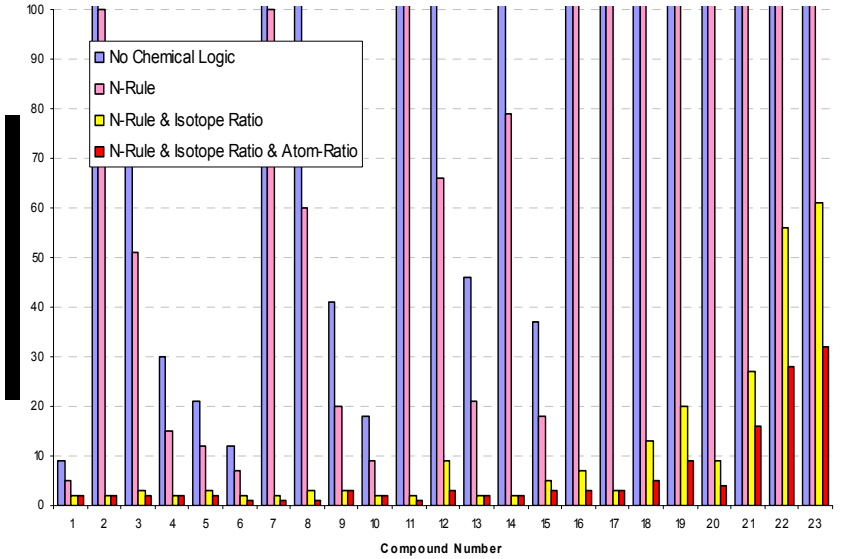
Simplifies compound identification

- Make use of all of the information available to make a better decision
- Uses a compound's molecular weight, isotopic pattern as well as applying 'chemical logic' to determine potential elemental formulae
  - Nitrogen rule (If the protonated pseudo molecular ion gives an odd mass then the number of nitrogen atoms is even)
  - Maximum number of double bond equivalents
  - Number of heteroatoms allowed
- Provides much shorter list of candidate formulae with the correct formula scoring much higher than standard elemental calculators

Reduction of Chemical "Space" using Chemical Sense

Number	Name	Exact Mass MH+	Formula M
1	Detromethorphan	272.2008	C18H25NO
2	Quinoxifen	308.0039	C15H8Cl2FNO
3	Alprazolam	309.0907	C17H13ClN4
4	Phenylbutazone	309.1597	C19H20N2O2
5	Bestatin	309.1808	C16H24N2O4
6	Benoxinate	309.2172	C17H28N2O3
7	Lorazepam	321.0192	C15H10Cl2N2O2
8	Omeprazole	346.1219	C17H19N3O3S
9	Tamoxifen	372.2321	C26H29NO
10	Haloperidole	376.148	C21H23ClFNO2
11	Buspirone	386.2556	C21H31N5O2
12	Clindamicin	425.1877	C18H33ClN2O5S
13	Loperamide	477.2309	C29H33ClN2O2
14	Ketoconazole	531.156	C26H28Cl2N4O4
15	Rescinnamine	635.2969	C35H42N2O9
16	Bromocriptine	654.2291	C32H40BrN5O5
17	Erythromycin - H2O	716.4579	C37H65NO12
18	Roxithromycin	837.5318	C41H76N2O15
19	Tylosin	916.5264	C46H77NO17
20	Bromazepam	316.00790	C14H10BrN3O
21	Oxfendazole	316.07500	C15H13N3O3S
22	Oxycodone	316.15430	C18H21NO4
23	Fendiline	316.20590	C23H25N





- Number of possible chemical formulas within 5ppm when considering
  - **No chemical logic**
  - **N-rule**
  - **N-rule and Isotope ratio**
  - **N-rule, isotope and atom ratio**

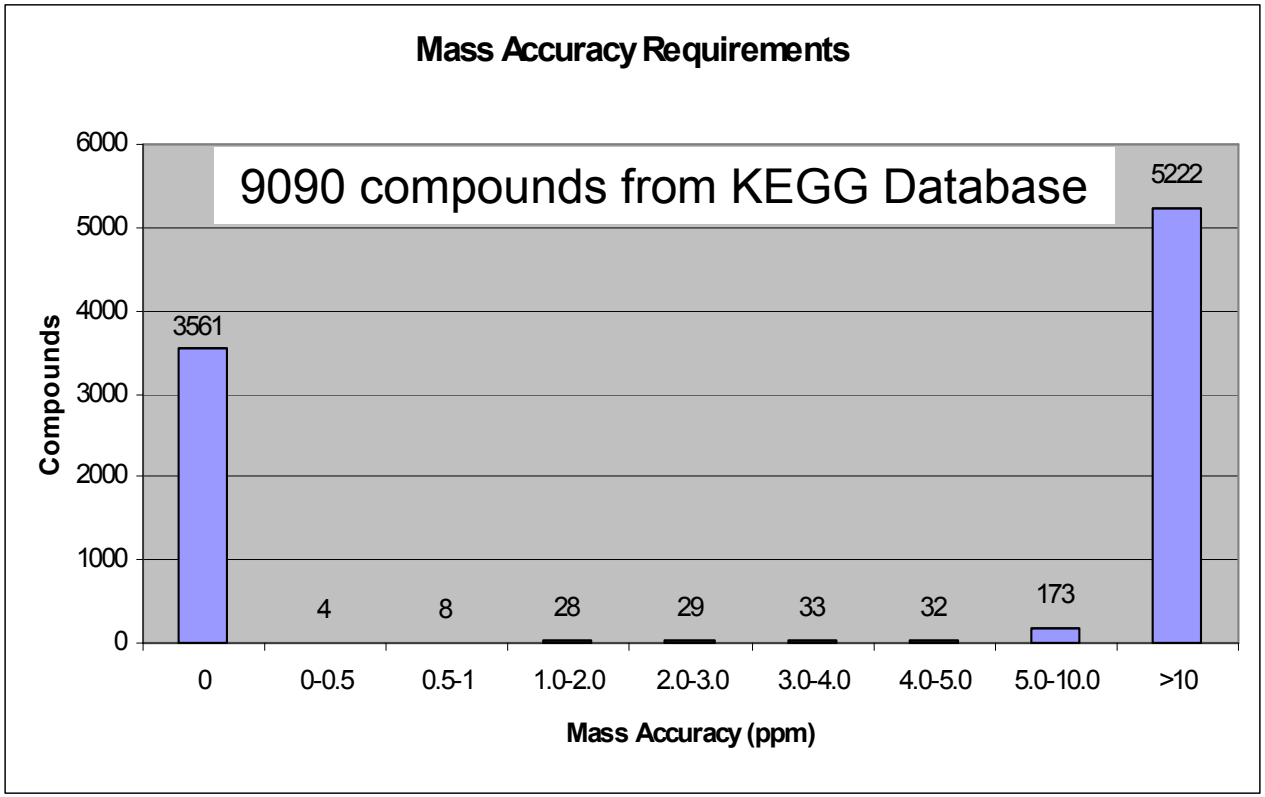
(considering limit of; $C_{500}H_{1000}O_{100}N_{100}P_5S_{10}F_1Cl_2Br_1$)

Identifying Biomarkers

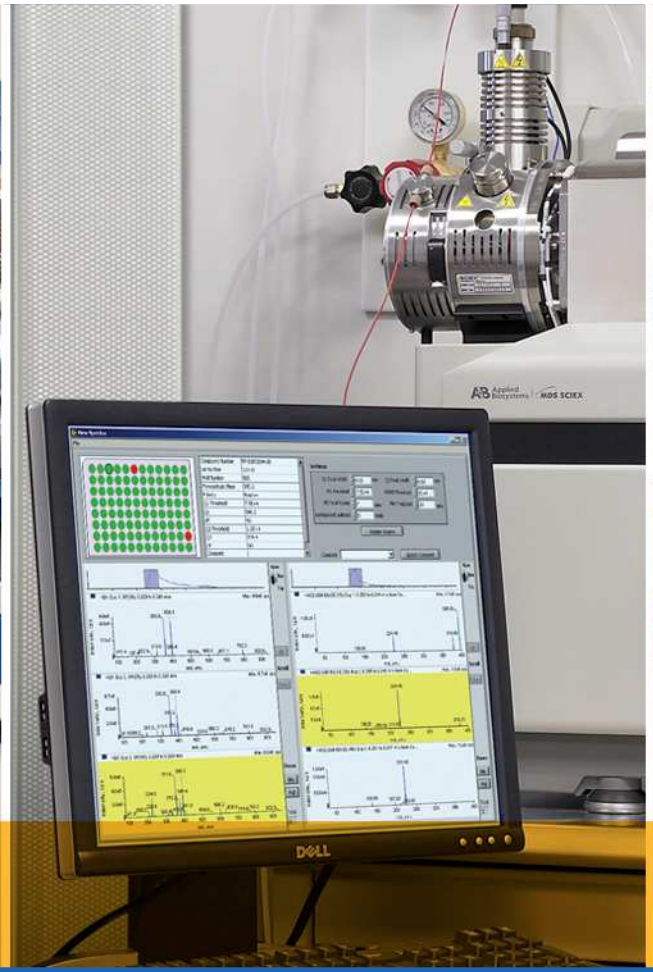
- You have now found groups within the data, determined which masses are responsible for the groupings, and have elemental formulae for these masses
- Now what?
- Need to identify these compounds – many different metabolites can have the same elemental formula – you must determine structure for biomarkers
- Typical place to start is KEGG database, search for mass and elemental formula to try to identify compounds

How Much Mass Accuracy?

- Majority of compounds in database have molecular weights that are at least 10 ppm different from any other compound in database
- 3561 compounds in KEGG database are structural isomers, no amount of MS resolution or mass accuracy can separate these



Sub-5 ppm mass accuracy gives only marginal improvement in compound identification when searching known databases



Targeted Metabolomics using Biocrates Absolute*IDQ*™ kits

Applied Biosystems | MDS Analytical Technologies

BIOCRATES' Approach To Metabolomics

Why Targeted Metabolomics?

- Robust and reproducible technology
- Absolute quantitation, not just relative peak intensities

  Quantitative data is trusted data

  (for you and regulatory authorities)
- Straightforward and rapid data interpretation because of identified and pre-annotated metabolites
- Closer to clinical needs due to resemblance to diagnostically established data formats (comparison to reference ranges)

Absolute*IDQ*™ Kit: Key Facts

- The Absolute***IDQ*** Kit is designed to be used in combination with the API 4000™ or 4000 QTRAP® instrument
- Kit comes in 96-well plate format for both manual and automated operation
- Identifies and quantifies >150 different endogeneous metabolites in blood plasma:
  - Acylcarnitines
  - Amino Acids
  - Glycerophospho- and sphingolipids
  - Hexose
- MS analysis by flow injection method, FIA-MS/MS (Quantification by MRM pairs)

Metabolite Details

163 Metabolites from four analyte classes

Acylcarnitines: 41

Amino acids: 13 proteinogenic + Ornithine

Phospho- and Sphingolipids

- Phosphatidylcholines 77 (Plasmalogene included)
- Lyso-Phosphatidylcholines 15
- Sphingomyelins 15

Hexose: sum of Hexose

MS instrumentation / robotic platform

Applied Biosystems:
Kit designed for Triple-quadrupole mass spectrometer from Applied Biosystems

Alliance with Hamilton Robotics:
Automated preparation possible (optional)
Help with integrating BIOCRATES methods on Hamilton robot will be provided

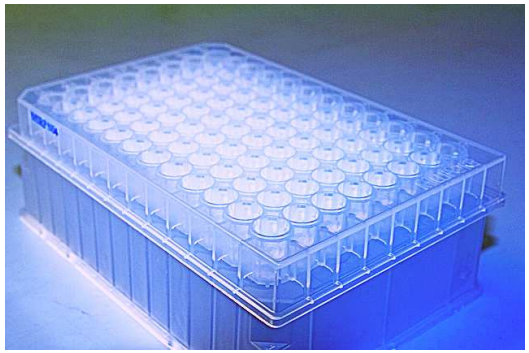
API 4000™ or 4000 QTRAP®

Hamilton Microlab Star

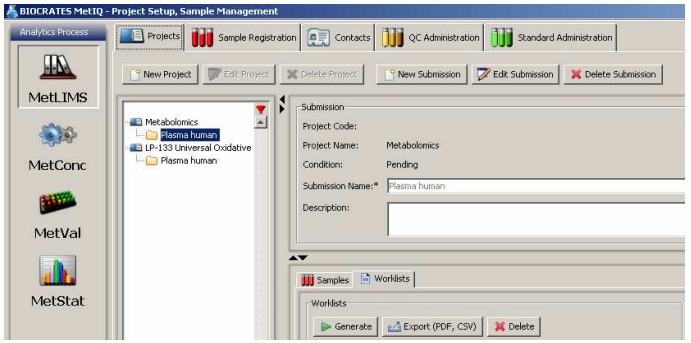
Kit Components

Content	Description
Absolute*IDQ*™ Kit Plate	Consists of a 96-well plate and filter plate attached with sealing tape
96-Deep-Well Plate with Silicone Mat	For Autosampler Adjustment
BIOCRATES Solvent A	250 ml MS running solvent
BIOCRATES Standards (Lyophilized) **BIOCRATES Quality Controls (QC)**	2 vials 3 vials
Kit Manual	
USB Memory Stick	Has MetIQ Software and Oracle Express Database loaded.

Unique Kit-Plate



Proprietary Met*IQ*™ Software



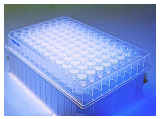


Overview of Workflow

➢ **1. Register the Assay in MetLIMS**
Import and register samples, generate 96-well plate overview and CSV file for Analyst Software

Time Required

30 min



➢ **2. Assay Preparation**
Perform sample preparation in the laboratory

3-4 hours

➢ **3. Process Assay in the Mass Spectrometer**
Analyze extracts in the MS instrument

12 hours

➢ **4. Convert Mass Spectrometer Data**
The MS data (Analyst wiff files) are converted and the concentrations are automatically calculated

15 min

➢ **5.Validate the Kit Plate**
Automated quality assessment of Standards, Quality Controls and Internal Standards

20 min

➢ **6. Evaluate and Export Data**
The results are evaluated and can be exported into other programs for further analysis

20 min

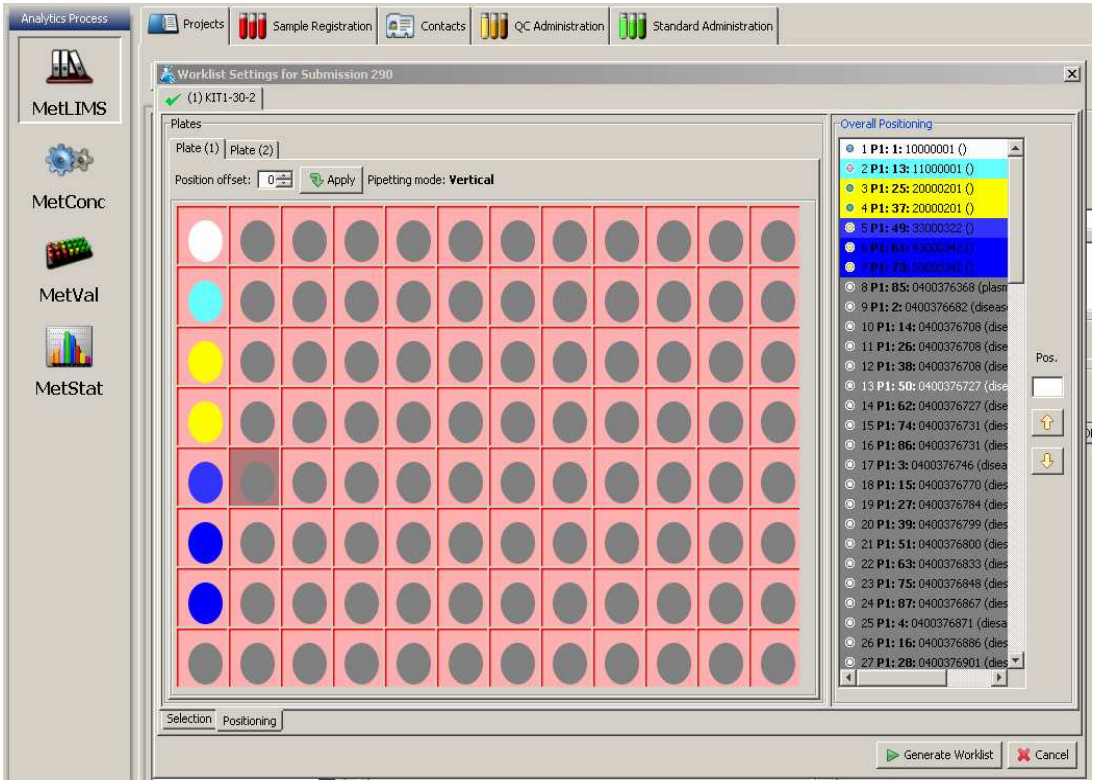
1. MetLIMS: Sample registration

96-well Plate Report and CSV File For Analyst

Two standards, three quality controls, one blank, one zero sample and the registered plasma samples are positioned on the kit plate

A 96-well plate report is printed out to help with the pipetting steps in the lab

A csv file is automatically generated for import into the Analyst acquisition batch

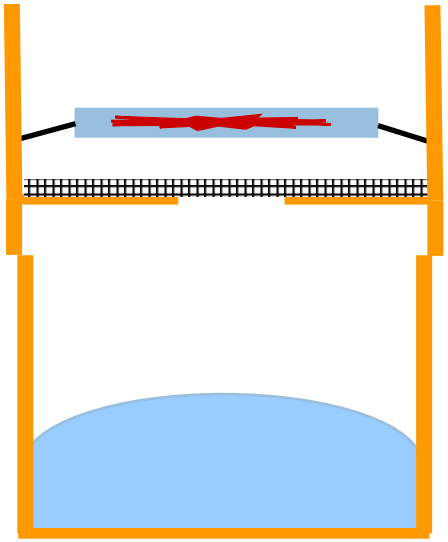


2. Assay Preparation: Unique Design of Kit Plate

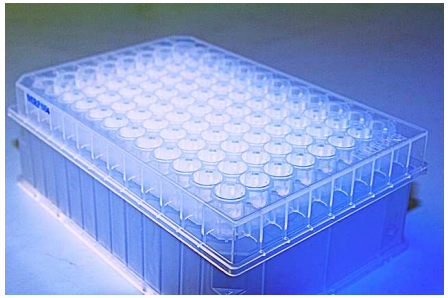
- **Advantages for sample preparation**

Upper Plate
For Extraction

Capture Plate
For Filtrate



- **Internal standards incorporated**
- Protein precipitation and metabolite derivatization on upper filter
- Efficient circulation of nitrogen for drying step
- Efficient solvent extraction step

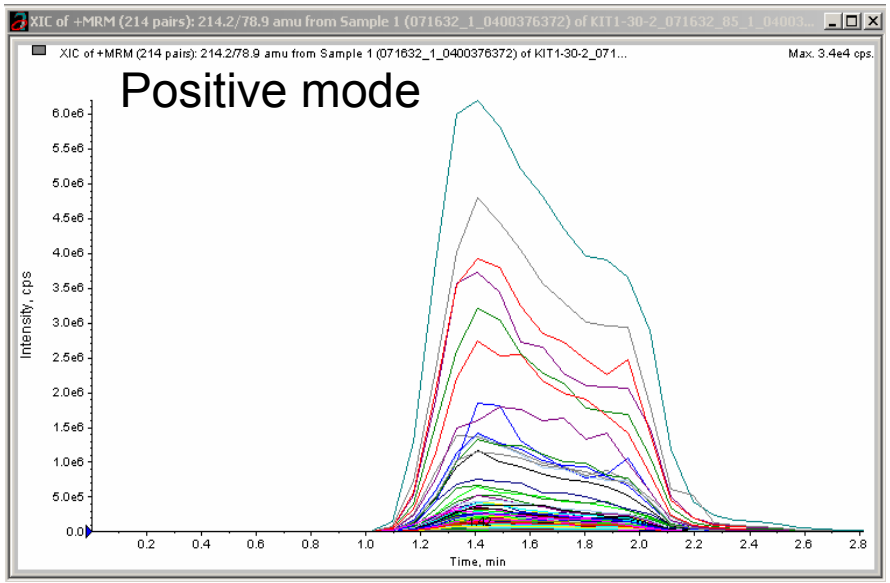


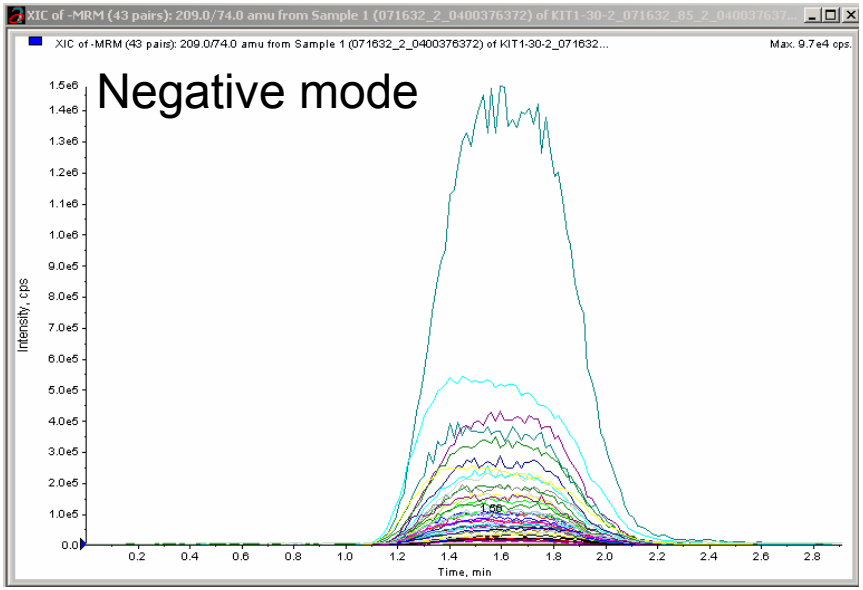
Very few sample preparations steps:
1) add 10 µL plasma (dry with nitrogen)
2) derivatize with PITC (dry with nitrogen)

3) extract metabolites (300 µL methanol)
4) dilute with MS running solvent

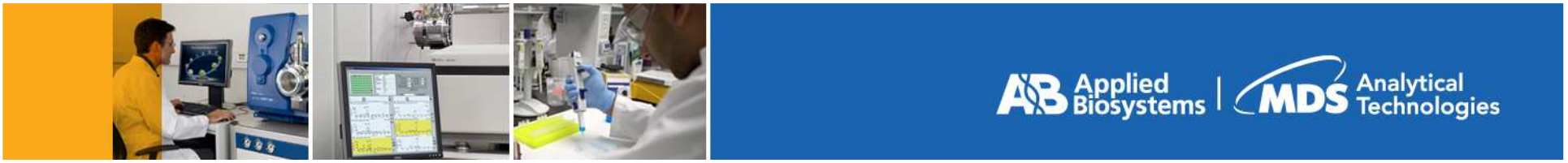
3. Process Assay in the Mass Spectrometer

175 MRM are measured in positive mode (22 IS)
15 MRM are measured in negative mode (7 IS)
2x 20 µl injection, 30 µl/min flow rate
3 min run time, 7 min total time per sample



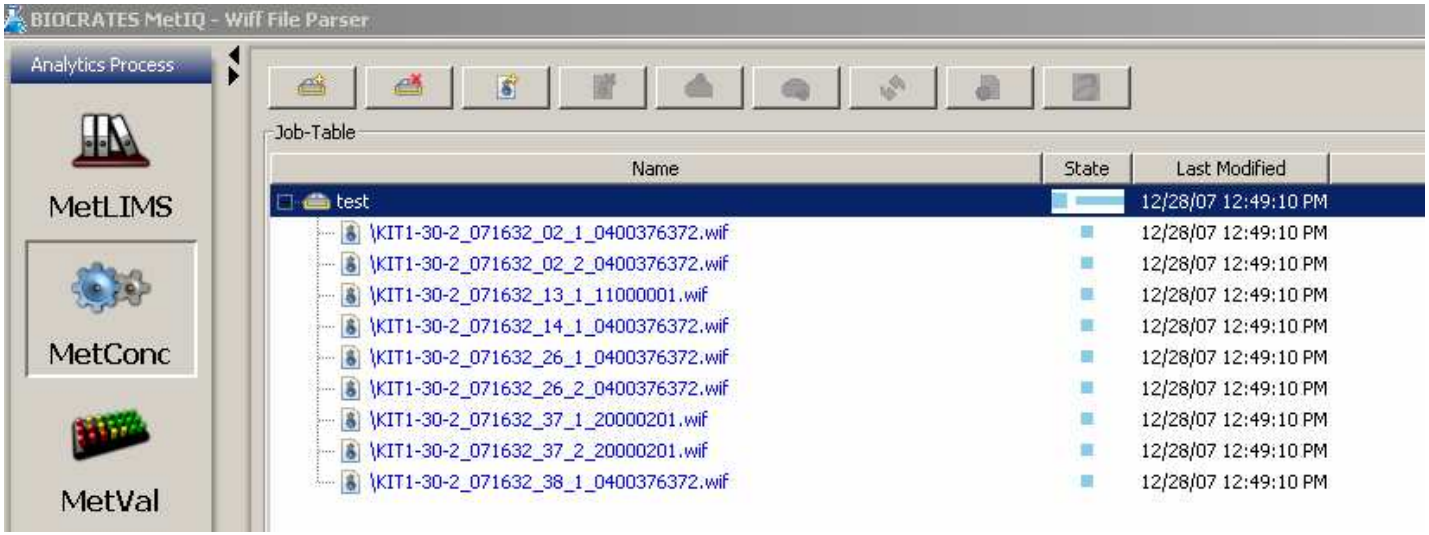


Calculation of concentrations is automated by Met*IQ* software



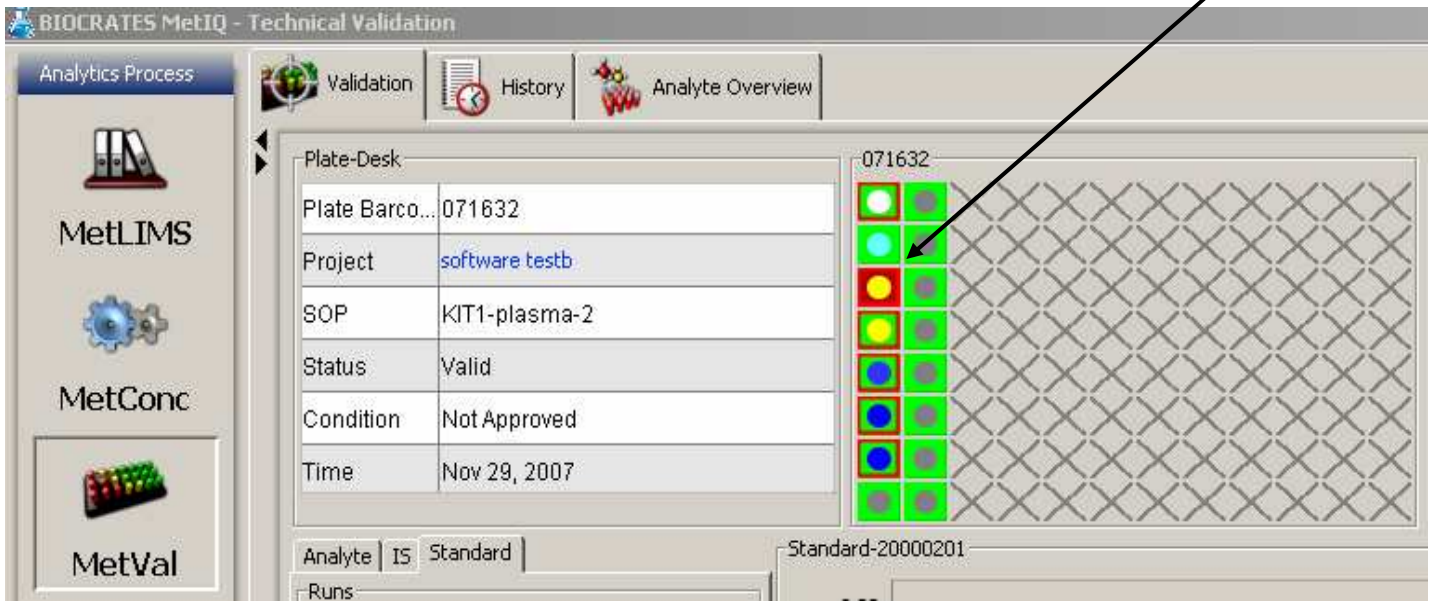
4. Convert Mass Spectrometric Data

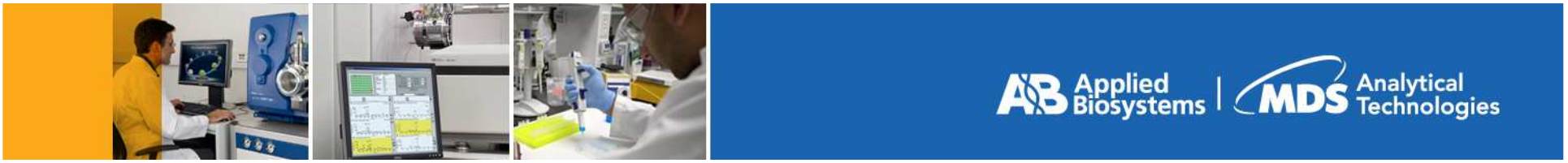
- In the MetConc module the Analyst wiff files are converted and imported into the Met*IQ* Databank
- The concentrations of the metabolites are automatically calculated
- Direct link to Analyst software to open wiff files from this module



5. Validate the Kit Plate

- In the MetVal module an automated quality assessment of the Kit is performed
- Specifically, it is checked if the internal standards, standards, blank and quality control samples are within the specified ranges
- In case a value is out of range this will be indicated in red

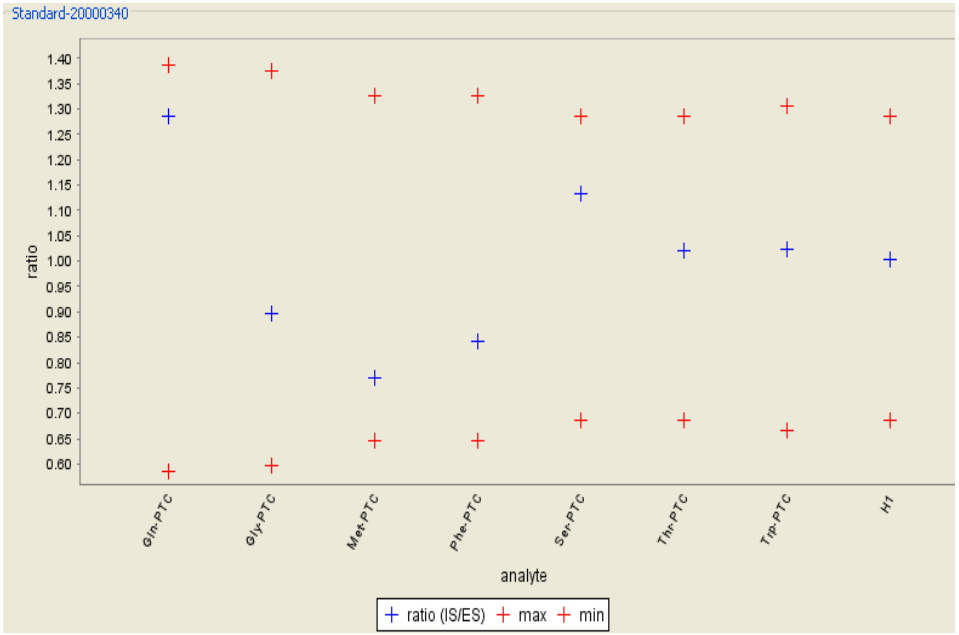




5. Graphical display of data in MetVal

Different graphs for easy visualization of validation results:
Standard graph: The BIOCRATES Standards (ES) are a mixture of analytes in identical concentrations as the Internal Standards (IS)

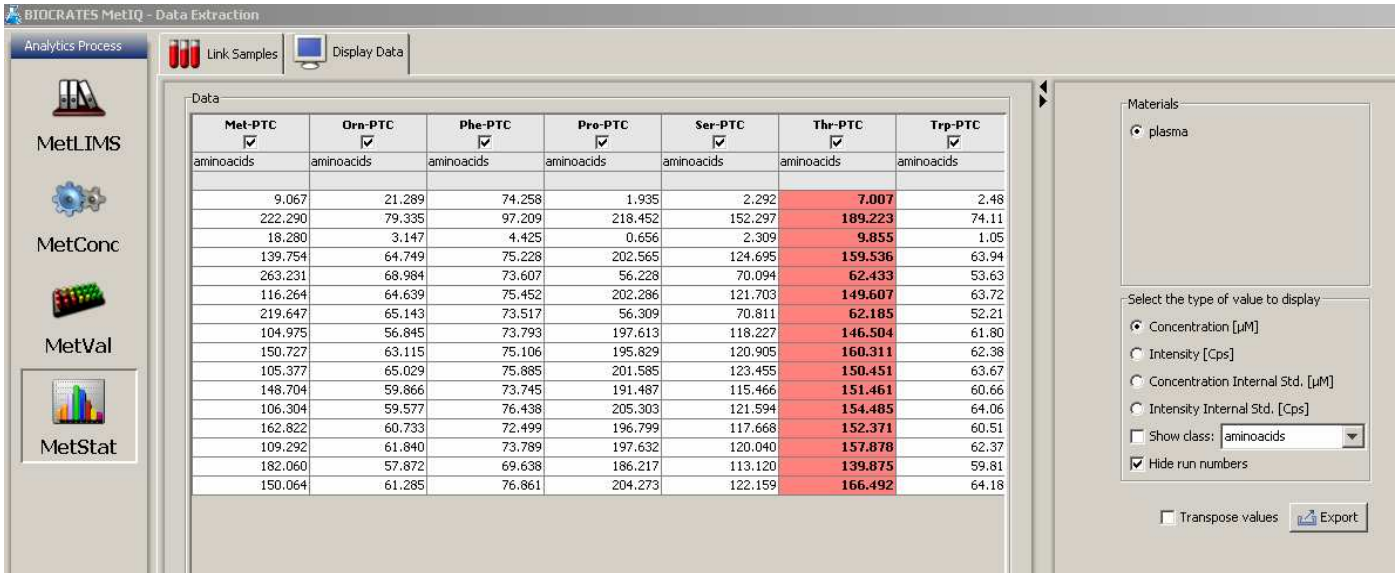
The graph shows the ratio of IS to ES – it should be close to 1



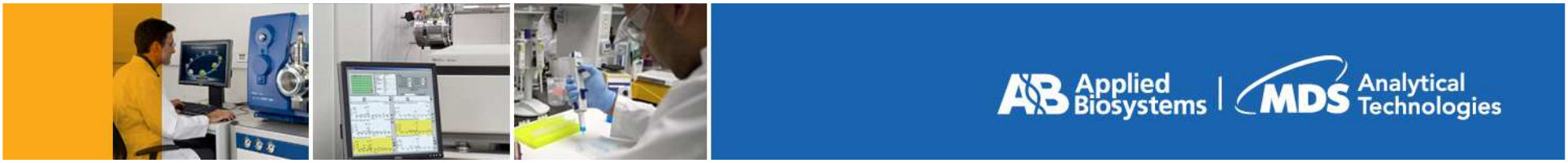
Red upper and lower range
Blue : measured values

6. Evaluate and Export Results

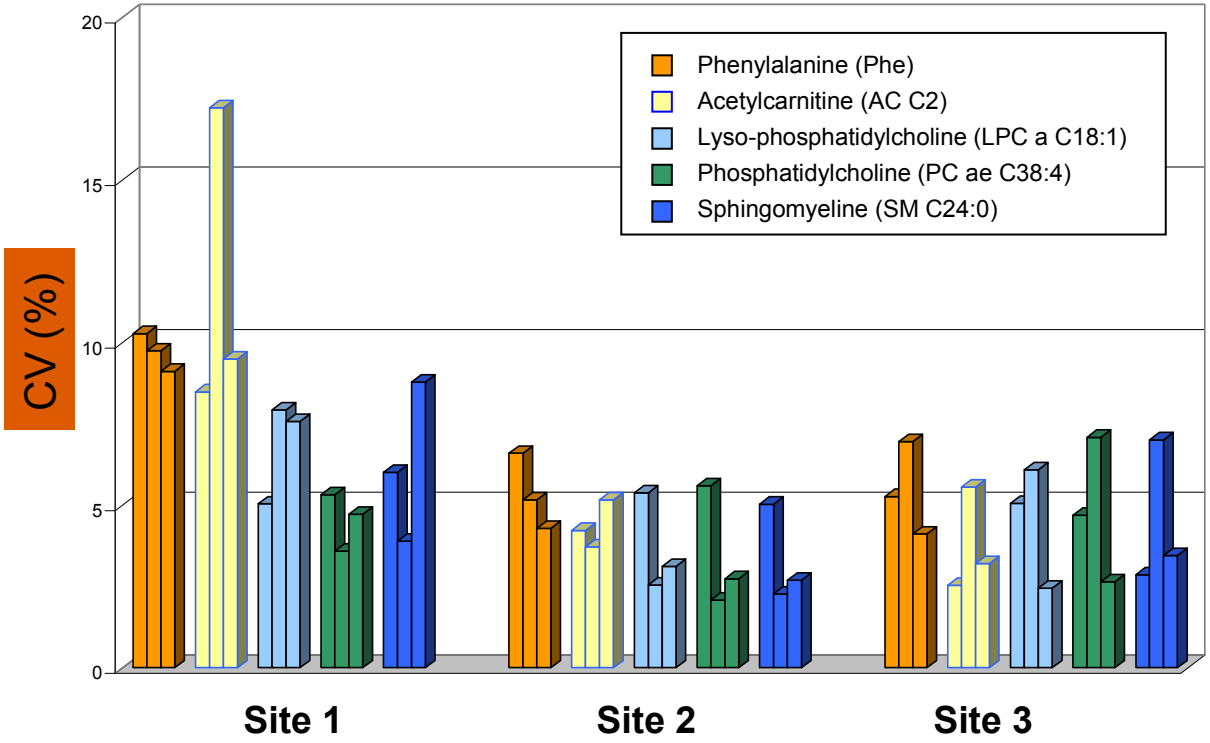
- The data are presented in tables sorted by options (analyte class, concentration, intensities)
- The information from the validation is transferred into the tables: In this example the blank value for the amino acid Thr was out of range as indicated by the red color
- The data can be exported from MetStat as a .txt or .csv file for import into other programs



Met-PTC	Orn-PTC	Phe-PTC	Pro-PTC	Ser-PTC	Thr-PTC	Trp-PTC
aminoacids	aminoacids	aminoacids	aminoacids	aminoacids	aminoacids	aminoacids
9.067	21.289	74.258	1.935	2.292	**7.007**	2.48
222.290	79.335	97.209	218.452	152.297	**189.223**	74.11
18.280	3.147	4.425	0.656	2.309	**9.855**	1.05
139.754	64.749	75.228	202.565	124.695	**159.536**	63.94
263.231	68.984	73.607	56.228	70.094	**62.433**	53.63
116.264	64.639	75.452	202.286	121.703	**149.607**	63.72
219.647	65.143	73.517	56.309	70.811	**62.185**	52.21
104.975	56.845	73.793	197.613	118.227	**146.504**	61.80
150.727	63.115	75.106	195.829	120.905	**160.311**	62.38
105.377	65.029	75.885	201.585	123.455	**150.451**	63.67
148.704	59.866	73.745	191.487	115.466	**151.461**	60.66
106.304	59.577	76.438	205.303	121.594	**154.485**	64.06
162.822	60.733	72.499	196.799	117.668	**152.371**	60.51
109.292	61.840	73.789	197.632	120.040	**157.878**	62.37
182.060	57.872	69.638	186.217	113.120	**139.875**	59.81
150.064	61.285	76.861	204.273	122.159	**166.492**	64.18



Variability in different laboratories



Representative results of test in three different laboratories: Coefficient of Variation (CV) of 5 metabolites at 3 different days and in 3 different sites are shown. In general the CVs were significantly below 15 % and comparable in the different labs.

Main Application Areas

Biomarker Research & Diagnostics Disease Phenotyping Metabolic Biomarker Discovery Early Disease Diagnosis	**Pharmaceutical R&D** **-Clinical & Pre-clinical studies** Drug metabolism / Pharmacokinetics Pharmacodynamics / Efficacy Toxicity / Safety Quantitative comparison of drug candidates
Nutritional & Functional Food Analysis Health effects of functional food Effects of environment & excercise	

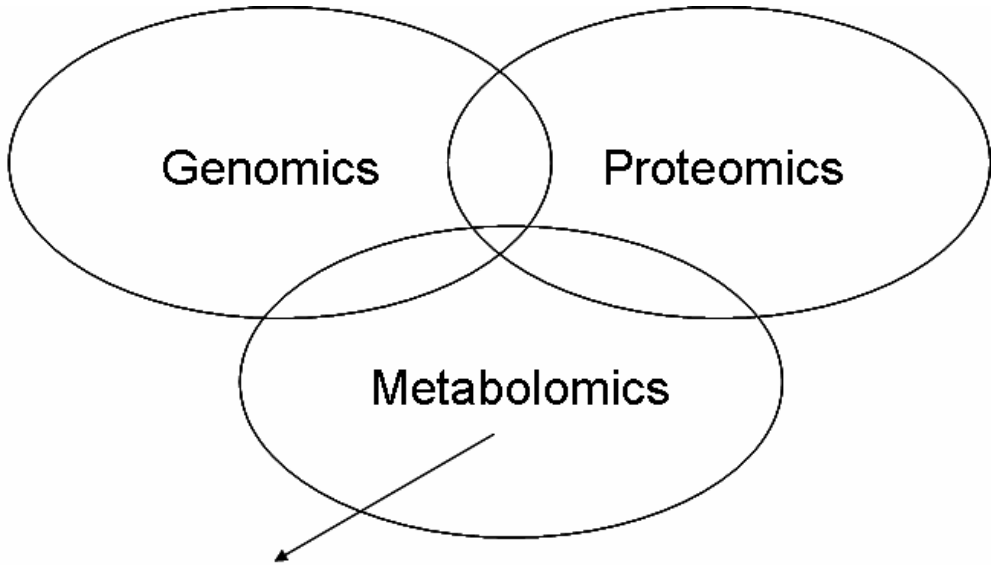
Lipidomics: *Applications & Workflows*

Applied Biosystems | MDS Analytical Technologies

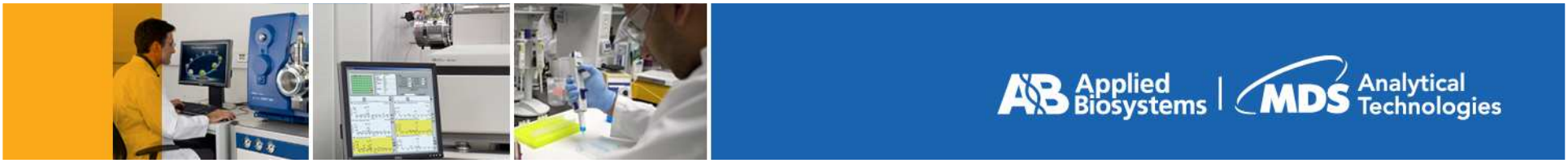
Lipidomics Overview: the "omics" era

Where does Lipidomics fit into the "omics" era?

- ***Genomics & Proteomics*** – mapping of genes, gene products and their interaction networks in a functioning cell
- ***Metabolomics*** – mapping of endogenous metabolites and how they're affected by environmental conditions, including disease & toxicity, etc…
  - Metabolome is enormous and complex
  - Can only be approached in sections, e.g Lipids
  - ***Lipidomics*** – characterization of the global changes in lipid metabolites ("lipidomics")

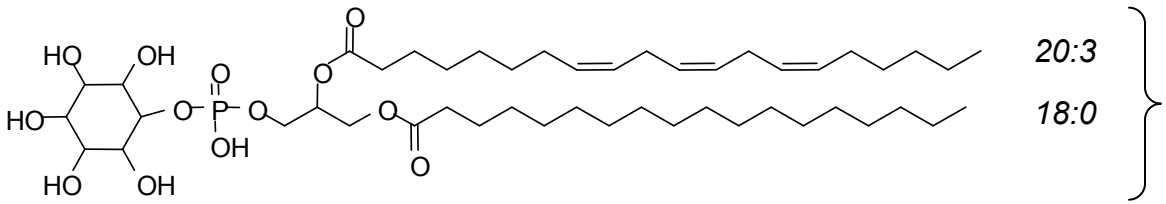


Approach in sections: eg. ***Lipids***



Lipidomics Overview: What are they?

- Lipids are subdivided into classes:
  - Fatty Acids
  - Glycerolipids
  - Glycerophospholipids
  - Sphingolipids
  - Sterol Lipids
  - Prenol Lipids
  - Saccharolipids
- Nomenclature based on lipid class, head group and fatty acid chain length (with designations for number of double bonds – and location is potentially very important also)



Glycerophospholipid linositol (GPIns)

Lipidomics Overview: Application Areas?

- Pharma
  - Better drug design (e.g. COX inhibitors)
  - Better drug delivery
  - Focus on obesity
- Nutritional science
  - Better health markers
    - Omega-3 fatty acids
- Clinical – huge implications in a wide range of diseases
    - Heart disease
    - Stroke
    - Cancer
    - Alzheimer's
  - Possible to have a diagnostic tool around specific lipid monitoring (lipid biomarkers)

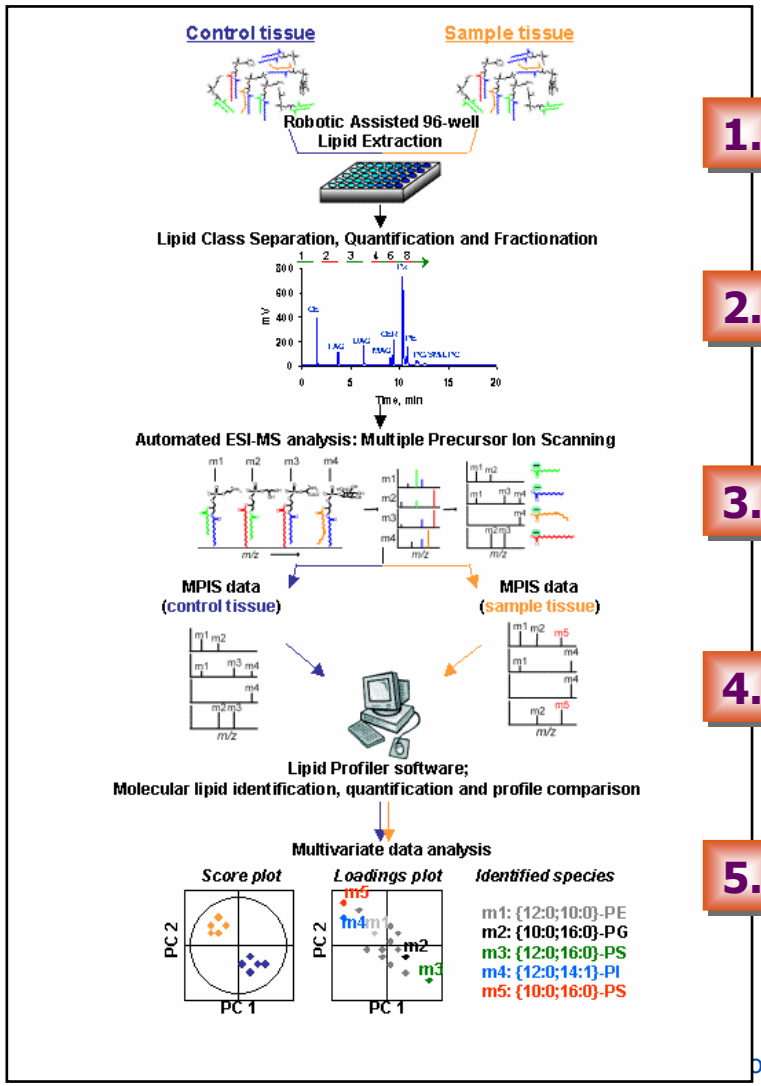
Software Enabling Lipidomics Workflows

LipidProfiler™ *(Prototype Research Grade Software)*

- *Lipid Profiler* is a data processing tool which identifies and semi-quantitates lipids from electrospray MS and MS/MS data.
- Three modes of data processing:
  1. information for all *m/z* ion peaks
  2. *m/z* ion peaks for a set of target lipid species
  3. *m/z* masses for characteristic lipid masses (Identify species mode)
- Qualitative profiling of lipid MS data is validated by links to the information in the database for characteristic lipid headgroup, fatty acid, or long chain base fragments and neutral losses.
- The peak profiles, lipid profiles, and fatty acid profiles are displayed in a graphical interface which can be exported from the application along with all numerical quantitative data.
- Compatible with Analyst® QS and Analyst® Software

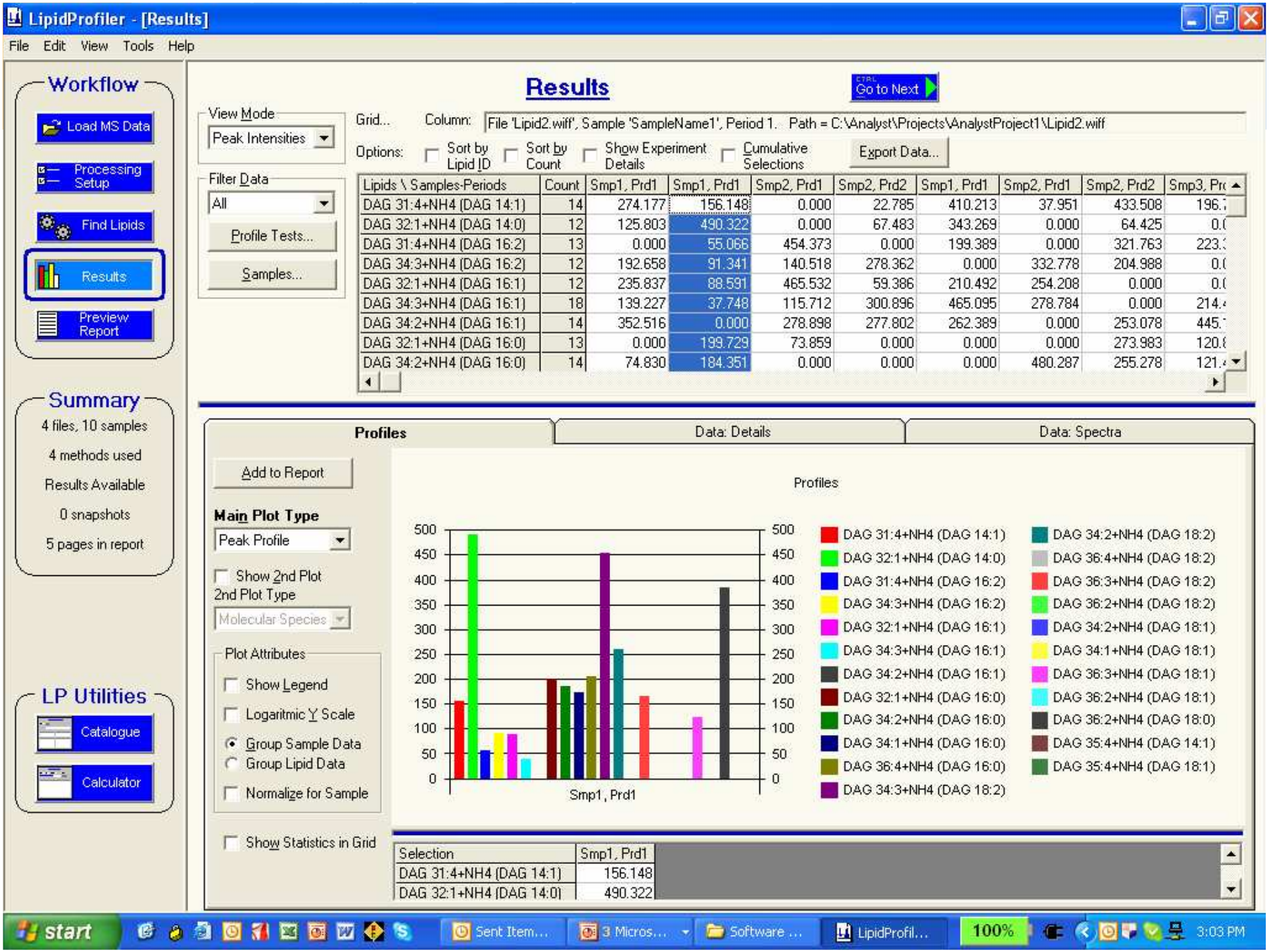
Applied Biosystems | MDS Analytical Technologies

MarkerView™ and LipidProfiler™ Workflow:



1. Robotic Assisted 96-well Total Lipid Extraction
2. Lipid Class Separation, Quantification & Fractionation
3. Robotic Sampling and Automated MS Acquisition
4. Computer-assisted Identification & Quantification of Molecular Lipids *(LipidProfiler)*
5. Multivariate & Univariate Data Analysis *(MarkerView)*

Kim Ekroos, VTT, Finland


LipidProfiler - [Results]
File Edit View Tools Help
Workflow
Load MS Data
Processing Setup
Find Lipids
Results
Preview Report
Summary
4 files, 10 samples
4 methods used
Results Available
0 snapshots
5 pages in report
LP Utilities
Catalogue
Calculator
Results
Go to Next
View Mode
Peak Intensities
Filter Data
All
Profile Tests...
Samples...
Grid...
Column:
File 'Lipid2.wiff', Sample 'SampleName1', Period 1. Path = C:\Analyst\Projects\AnalystProject1\Lipid2.wiff
Options:
Sort by Lipid ID
Sort by Count
Show Experiment Details
Cumulative Selections
Export Data...
Lipids \ Samples-Periods | Count | Smp1, Prd1 | Smp1, Prd1 | Smp2, Prd1 | Smp2, Prd2 | Smp1, Prd1 | Smp2, Prd1 | Smp2, Prd2 | Smp3, Pr
DAG 31:4+NH4 (DAG 14:1) | 14 | 274.177 | 156.148 | 0.000 | 22.785 | 410.213 | 37.951 | 433.508 | 196.
DAG 32:1+NH4 (DAG 14:0) | 12 | 125.803 | 490.322 | 0.000 | 67.483 | 343.269 | 0.000 | 64.425 | 0.
DAG 31:4+NH4 (DAG 16:2) | 13 | 0.000 | 55.066 | 454.373 | 0.000 | 199.389 | 0.000 | 321.763 | 223.
DAG 34:3+NH4 (DAG 16:2) | 12 | 192.658 | 91.341 | 140.518 | 278.362 | 0.000 | 332.778 | 204.988 | 0.
DAG 32:1+NH4 (DAG 16:1) | 12 | 235.837 | 88.591 | 465.532 | 59.386 | 210.492 | 254.208 | 0.000 | 0.
DAG 34:3+NH4 (DAG 16:1) | 18 | 139.227 | 37.748 | 115.712 | 300.896 | 465.095 | 278.784 | 0.000 | 214.
DAG 34:2+NH4 (DAG 16:1) | 14 | 352.516 | 0.000 | 278.898 | 277.802 | 262.389 | 0.000 | 253.078 | 445.
DAG 32:1+NH4 (DAG 16:0) | 13 | 0.000 | 199.729 | 73.859 | 0.000 | 0.000 | 0.000 | 273.983 | 120.
DAG 34:2+NH4 (DAG 16:0) | 14 | 74.830 | 184.351 | 0.000 | 0.000 | 0.000 | 480.287 | 255.278 | 121.
Profiles
Data: Details
Data: Spectra
Add to Report
Main Plot Type
Peak Profile
Show 2nd Plot
2nd Plot Type
Molecular Species
Plot Attributes
Show Legend
Logaritmic Y Scale
Group Sample Data
Group Lipid Data
Normalize for Sample
Show Statistics in Grid
Profiles
500
450
400
350
300
250
200
150
100
50
0
Smp1, Prd1
DAG 31:4+NH4 (DAG 14:1)
DAG 32:1+NH4 (DAG 14:0)
DAG 31:4+NH4 (DAG 16:2)
DAG 34:3+NH4 (DAG 16:2)
DAG 32:1+NH4 (DAG 16:1)
DAG 34:3+NH4 (DAG 16:1)
DAG 34:2+NH4 (DAG 16:1)
DAG 32:1+NH4 (DAG 16:0)
DAG 34:2+NH4 (DAG 16:0)
DAG 34:1+NH4 (DAG 16:0)
DAG 36:4+NH4 (DAG 16:0)
DAG 34:3+NH4 (DAG 18:2)
DAG 34:2+NH4 (DAG 18:2)
DAG 36:4+NH4 (DAG 18:2)
DAG 36:3+NH4 (DAG 18:2)
DAG 36:2+NH4 (DAG 18:2)
DAG 34:2+NH4 (DAG 18:1)
DAG 34:1+NH4 (DAG 18:1)
DAG 36:3+NH4 (DAG 18:1)
DAG 36:2+NH4 (DAG 18:1)
DAG 36:2+NH4 (DAG 18:0)
DAG 35:4+NH4 (DAG 14:1)
DAG 35:4+NH4 (DAG 18:1)
Selection | Smp1, Prd1
DAG 31:4+NH4 (DAG 14:1) | 156.148
DAG 32:1+NH4 (DAG 14:0) | 490.322
start
Sent Item...
3 Micros...
Software ...
LipidProfil...
100%
3:03 PM

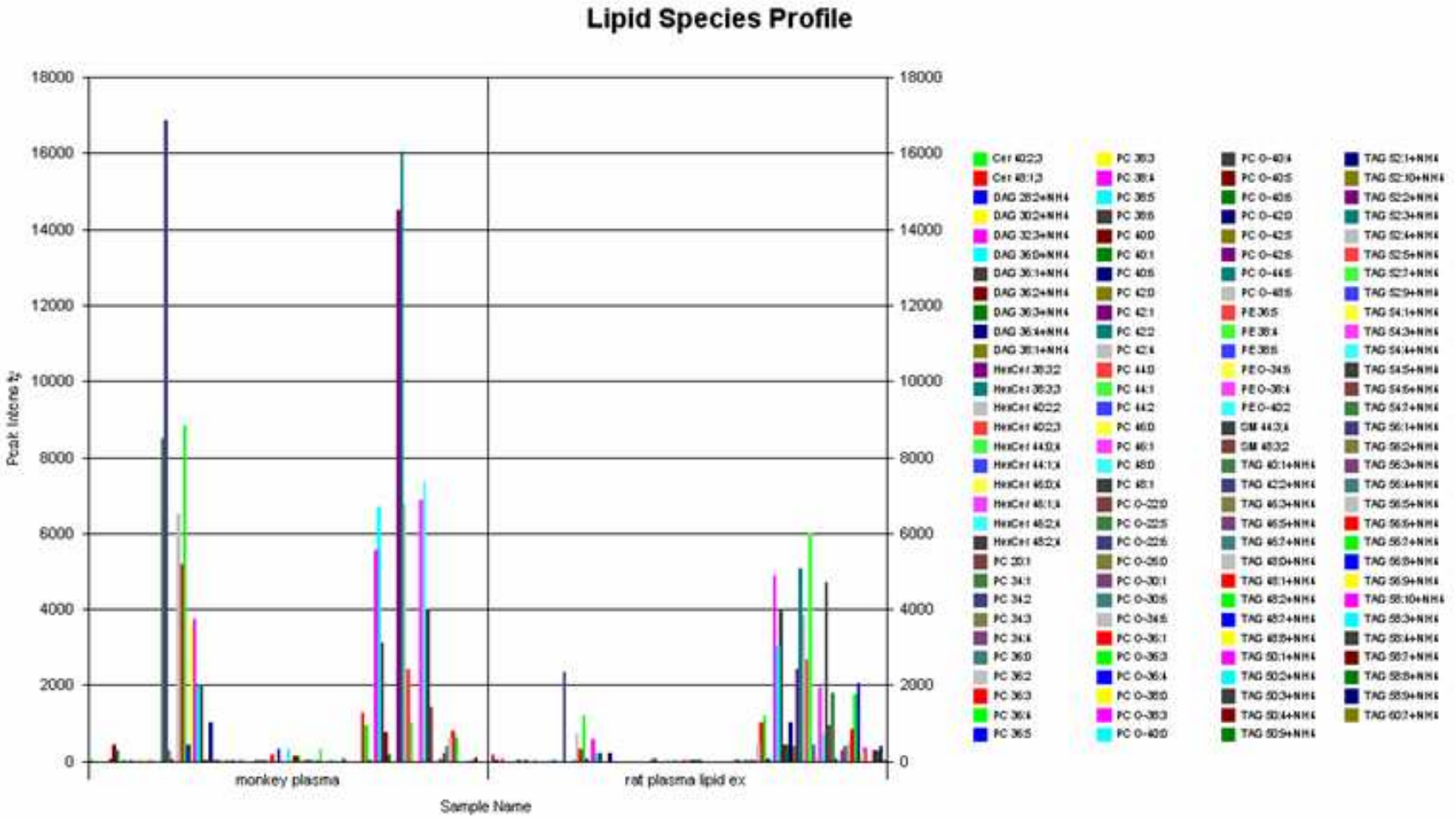
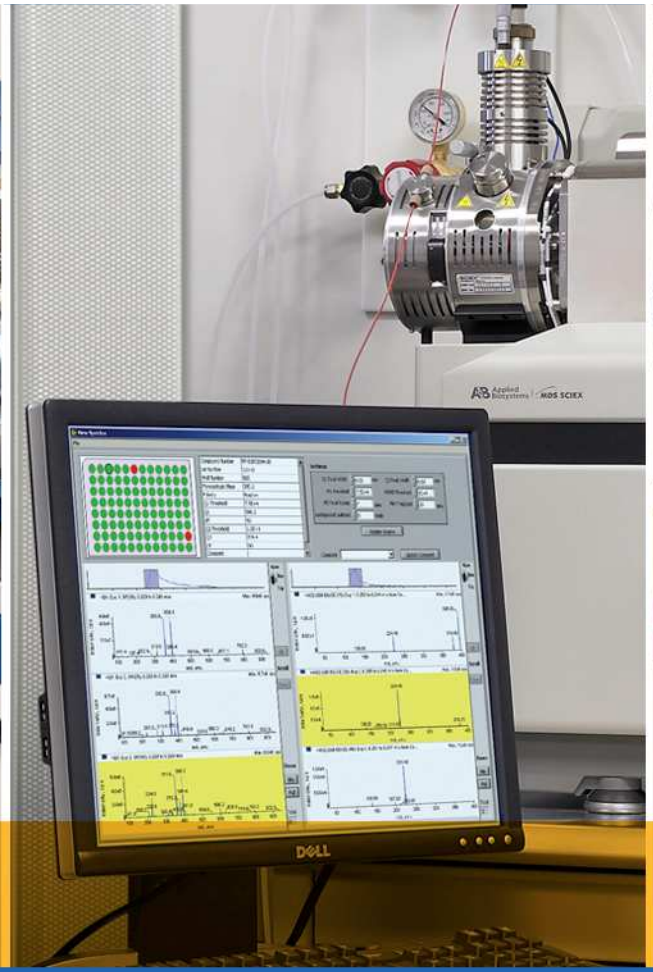


Figure 3. Lipid species profile comparisons of monkey and rat plasma lipid extracts generated by processing QSTAR® Elite +TOF MS and IDA product ion data through LIPID PROFILER. Over 120 lipid species were identified through a glycerophospholipid database targeted analysis.



Thank You!

Questions?

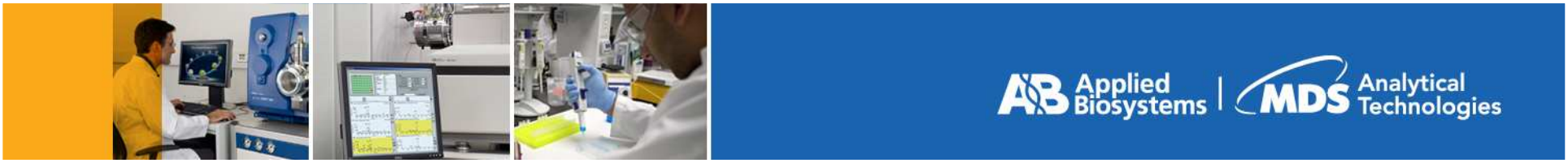
Applied Biosystems | MDS Analytical Technologies

Trademarks/Licensing

For research use only. Not for use in diagnostic procedures.

AB (Design) and Applied Biosystems and are registered trademarks of Applied Biosystems Inc. or its subsidiaries in the US and/or certain other countries.

MIDAS, API 5000, AcQuRate, eQ, DiscoveryQuant, iMethods, MRMPilot, ProteinPilot, MultiQuant and AB SCIEX Triple Quad are trademarks and QTRAP, Cliquid, LightSight, and LINAC are registered trademark of Applied Biosystems/MDS Analytical Technologies, a joint venture between Applied Biosystems Inc and MDS Inc.



For Research Use Only. Not for use in diagnostic procedures.

Applera, Applied Biosystems and AB (design) are registered trademarks of Applera Corporation or its subsidiaries in the US and/or certain other countries.

MarkerView is a trademark and Analyst, QTRAP and QSTAR are registered trademarks of Applied Biosystems/MDS Analytical Technologies, a joint venture between Applera Corporation and MDS Inc.

All other trademarks are the sole property of their respective owners.