Commercial and non- commercial software
How do these work together and can benefit form one another?

Some examples

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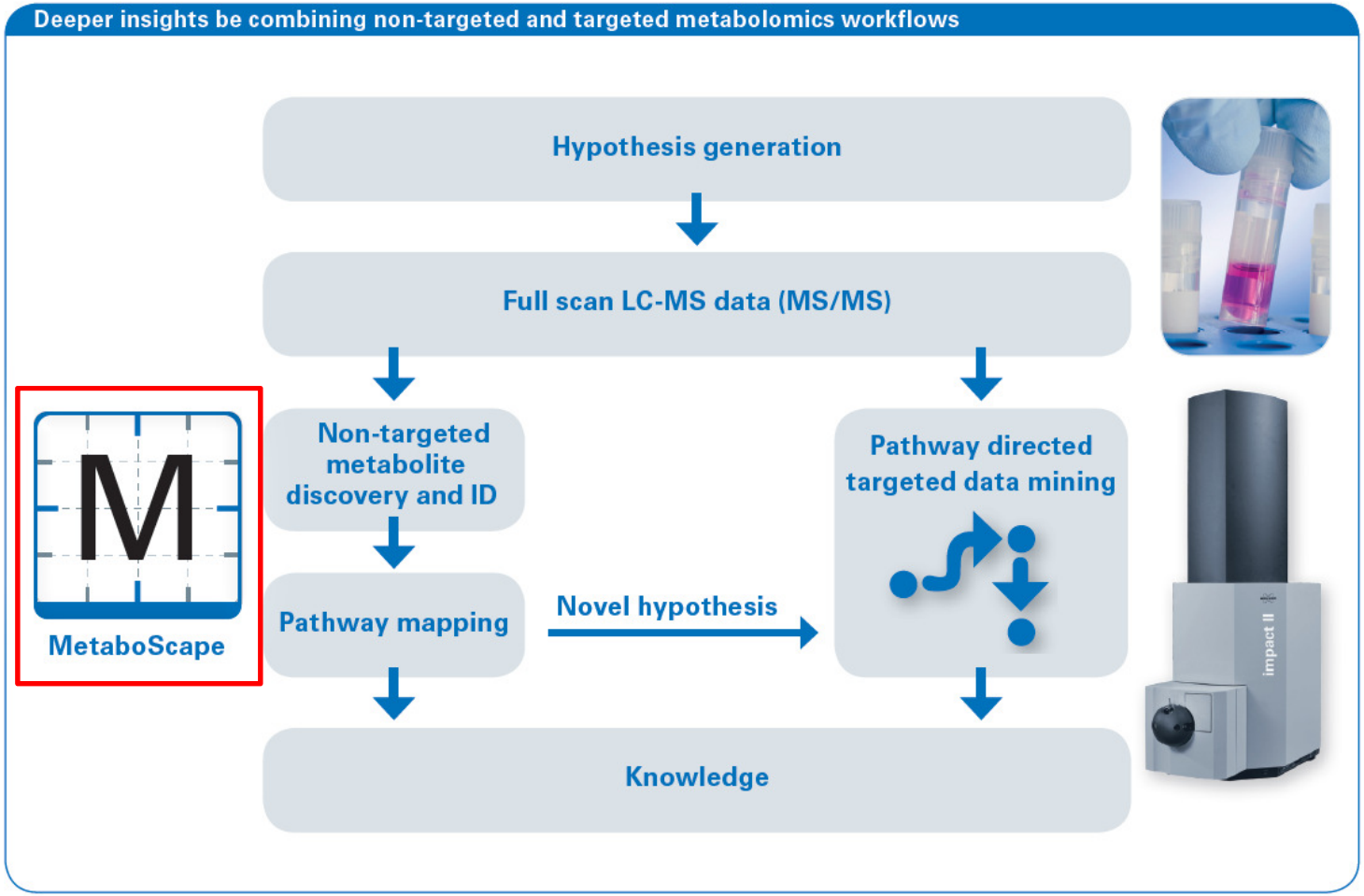
Example: Coffee Metabolomics

- 13 different coffee capsule types:
  - QC sample: mix of all analytical samples
  - Extracted 2 times each with 35 ml water
  - using XN 3005 Nespresso Pixie espresso machine (Krups)
- Samples centrifuged and diluted 1:50 with water

- Injecting: 5µl on Dionex RSCL (UHPLC) (3 technical replicates each)
- Column: BEH C18, 2.1x50; 1.7um
- 8 Minute total run time
- MS: **compact** QTOF
- Ionisation: ESI positive

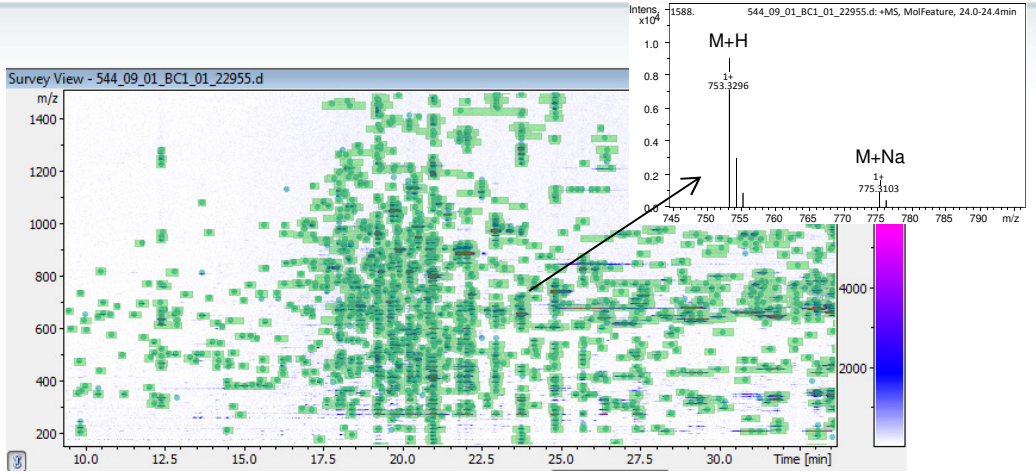
Non- targeted & targeted Metabolomics

Both can be addressed using one ESI-TOF-MS data set



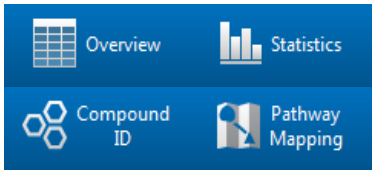
Metabolic profiling

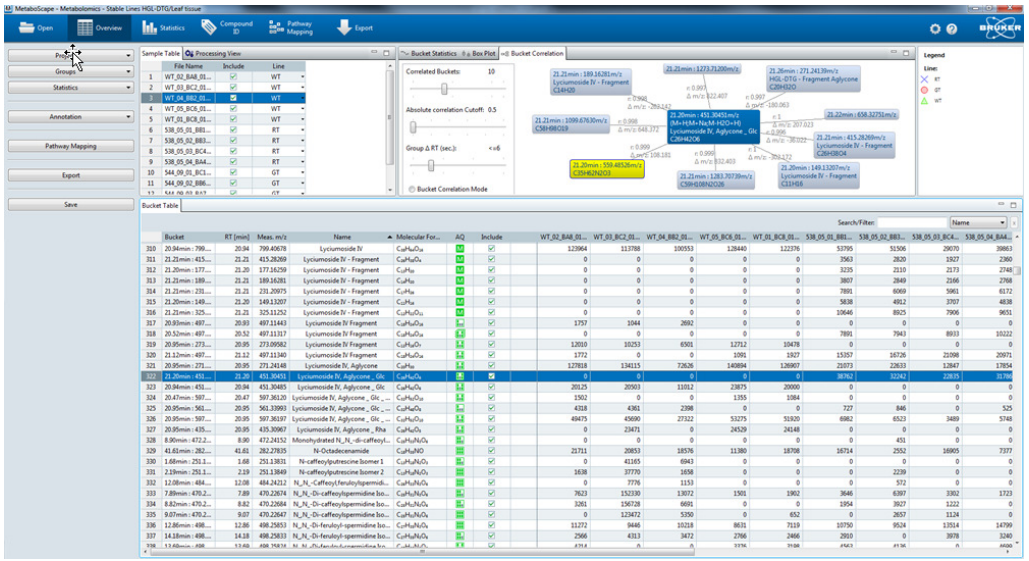
Seamless data evaluation by MetaboScape



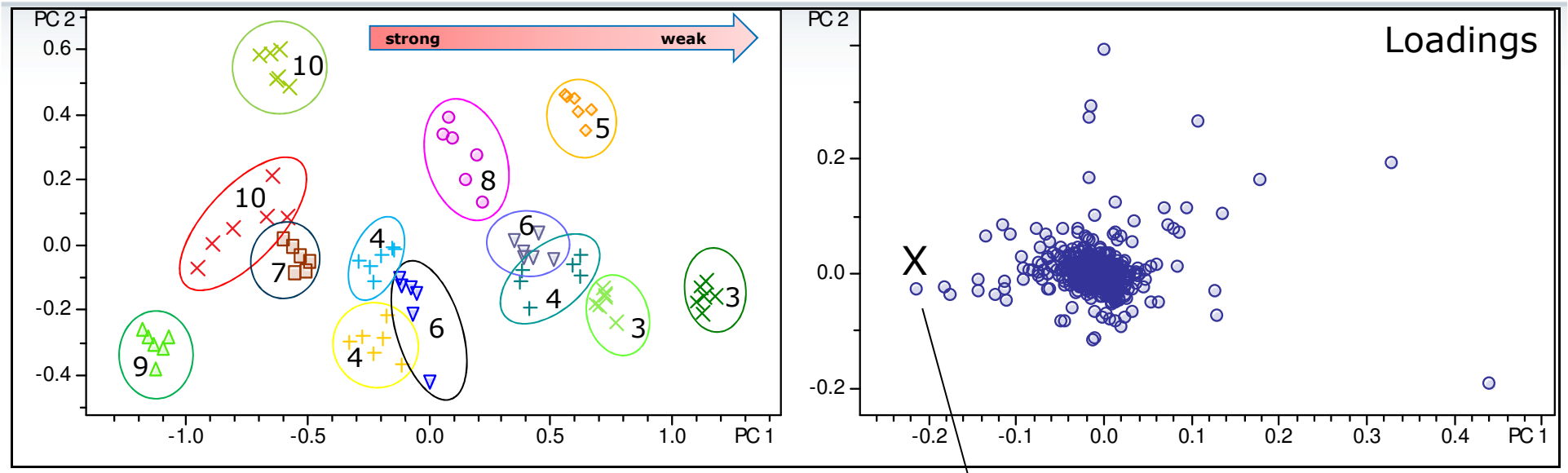
- Comprehensive feature extraction by “Find Molecular Features” algorithm
- RT alignment
- Bucketing
- Normalization Scaling

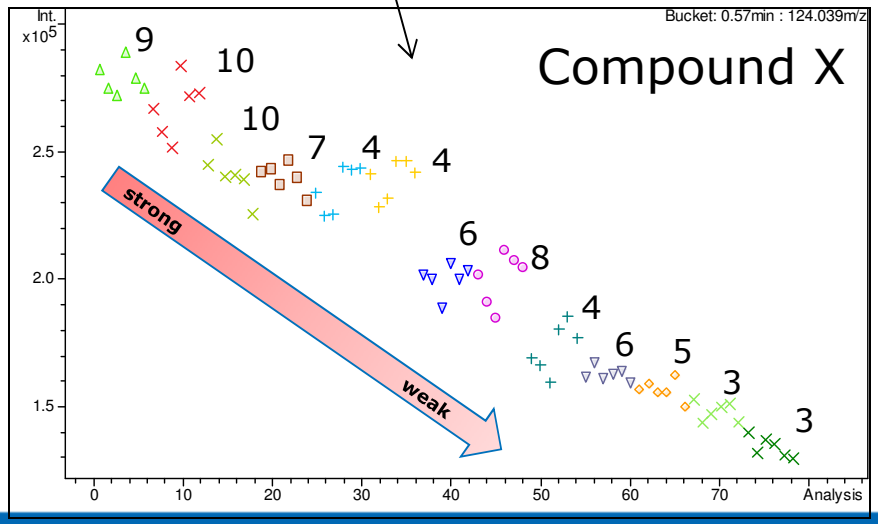
- **Combining** extracted FMF features resulted in **buckets** for further analysis in **MetaboScape** software in this example





Characteristics of strong coffee…

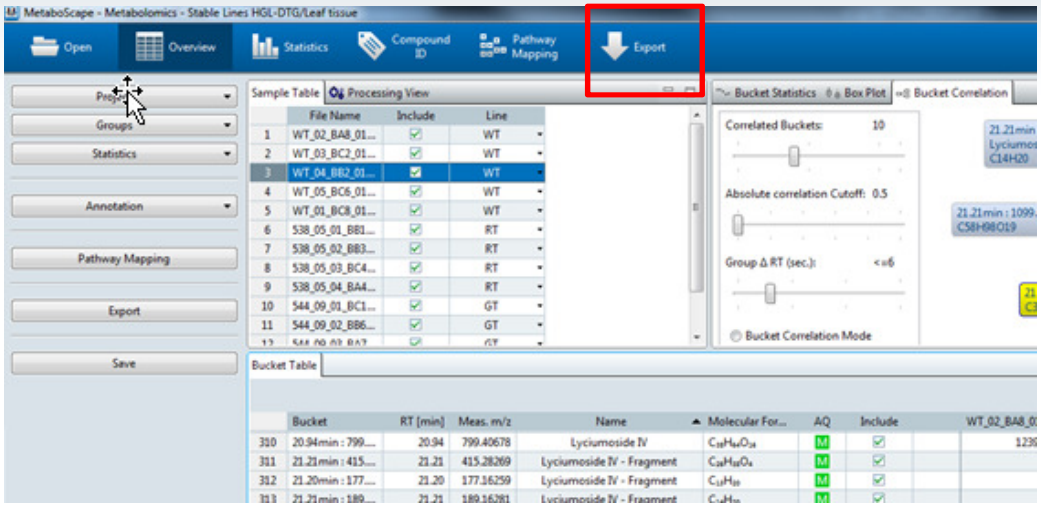




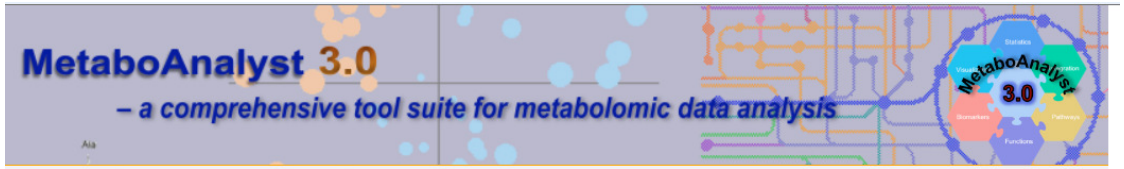
Bucket statistics plot: intensity for compound across all samples

Bucket statistics plot for **0.57 min - 124.039 m/z** reveals higher abundance in strong coffees.

Statistics can be done in Bruker MetaboScape software,…



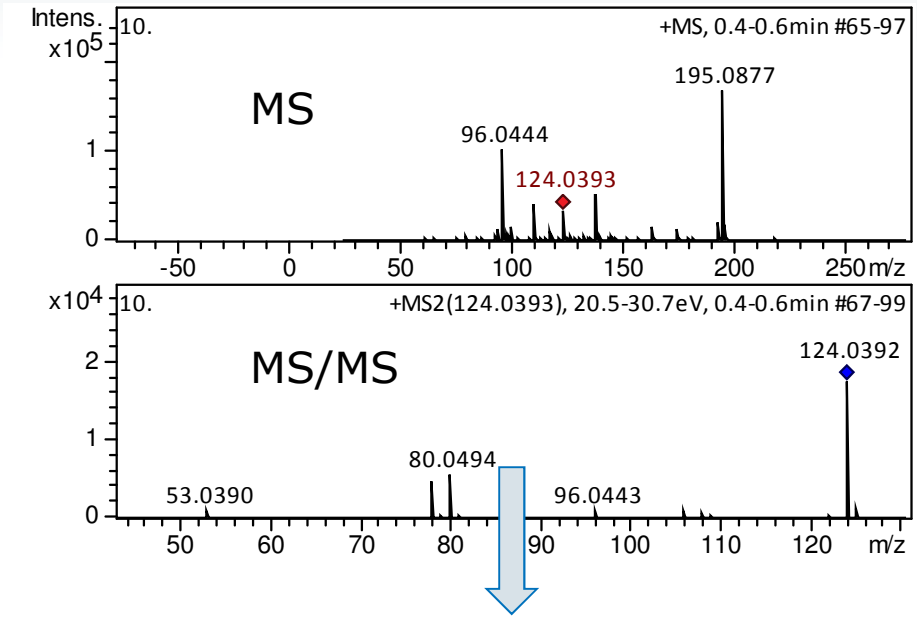
…or optionally you could also export the bucketed data from MetaboScape to other open source statistical tools like **MetaboAnalyst**



Xia, J., Sinelnikov, I., Han, B., and Wishart, D.S. (2015) Nucl. Acids Res. (DOI: 10.1093/nar/gkv380).

… or commercial software like Simca-P

SmartFormula3D delivers a unique molecular formula for Compound X: $C_6H_6NO_2$



SmartFormula3D combines accurate mass and isotopic pattern information in MS and MS/MS spectra for *de novo* molecular formula generation.

Unique molecular formula hit with -0.4ppm generated by **SmartFormula3D**

SumFormula	m/z calc	err [mDa]	err [ppm]	mSigma
$C_6H_6NO_2$	124.0393	-0.1	-0.4	3.8

proposed molecular formula of precursor ion after combination of MS and MS/MS information

MS

SumFormula	SumFormula Loss	m/z Calc.	err [mDa]
$C_6H_4NO_2$	H_2	122.0237	0.6
C_6H_4NO	H_2O	106.0287	0.5
C_5H_6NO	CO	96.0444	0.1

molecular formulae of fragment ions

MS/MS

Link from SmartFormula3D to MetFrag:

http://msbi.ipb-halle.de/MetFrag/

Wolf *et al. BMC Bioinformatics* 2010, **11**:148
http://www.biomedcentral.com/1471-2105/11/148

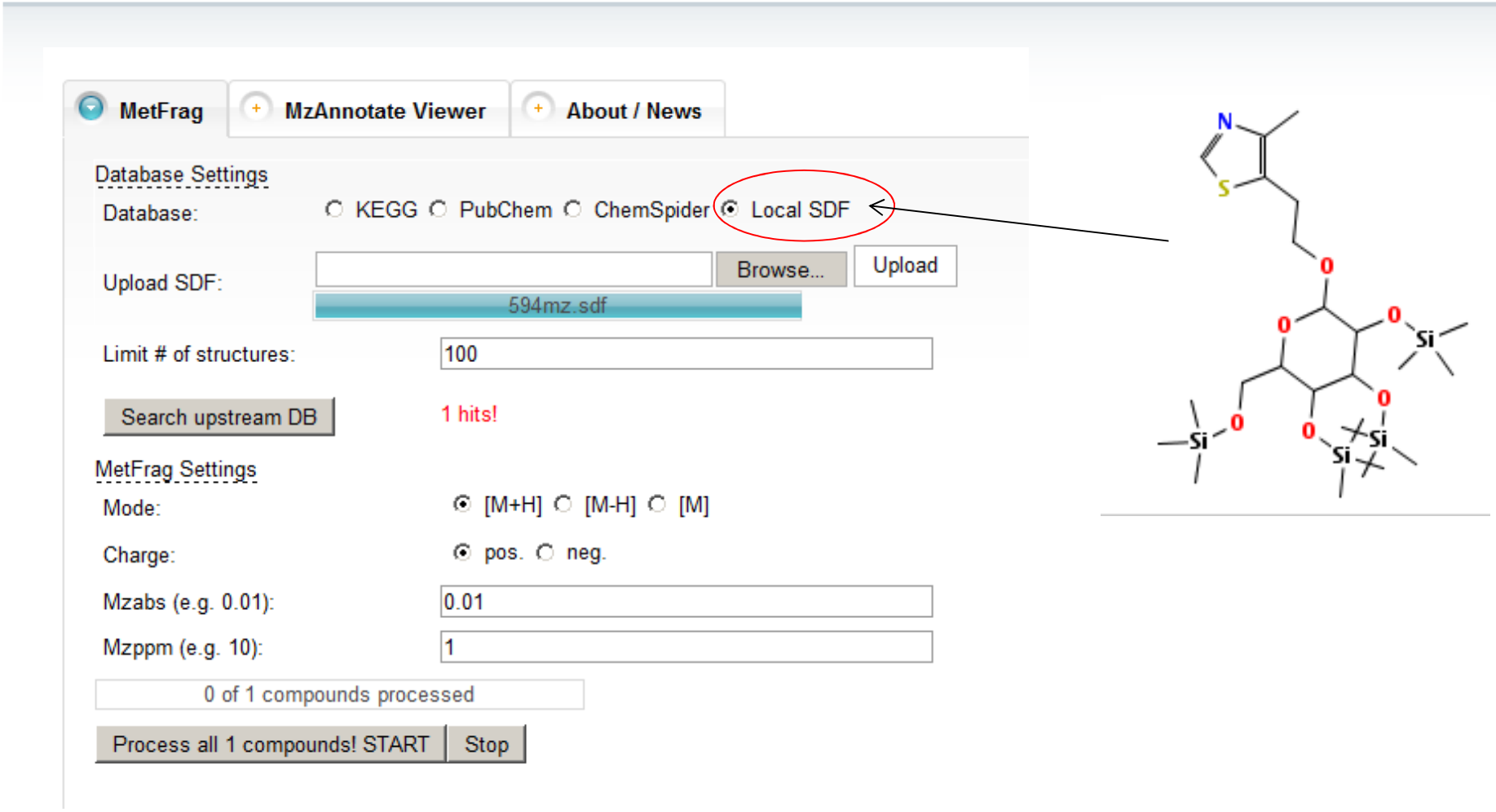
BMC Bioinformatics

METHODOLOGY ARTICLE Open Access

In silico fragmentation for computer assisted identification of metabolite mass spectra

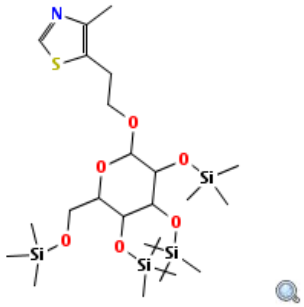
Sebastian Wolf[1*], Stephan Schmidt[1], Matthias Müller-Hannemann[2], Steffen Neumann[1]

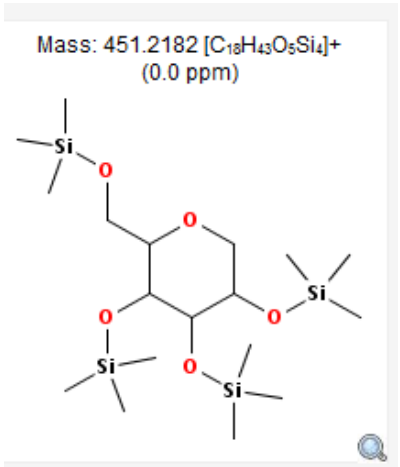
Use KEGG, PubChem, ChemSpider or Upload likely structure for in silico fragmentation in MetFrag: http://msbi.ipb-halle.de/MetFrag/

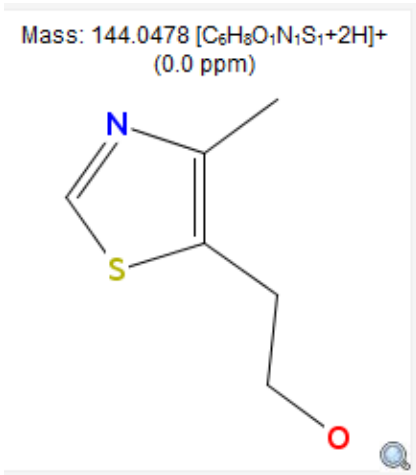


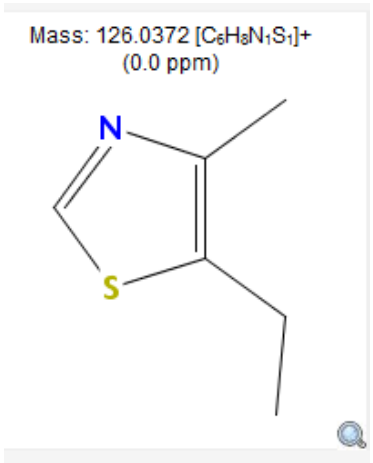
In silico fragments are matched against measured fragment ions

http://msbi.ipb-halle.de/MetFrag/

	Score	# Explained Peaks	Trivial Name	Exact Mass	Structure	Database ID	Actions
	1.0	11	Untitled Document	$C_{24}H_{51}O_6N_1Si_4S_1$ 593.2514		0	Fragments Download





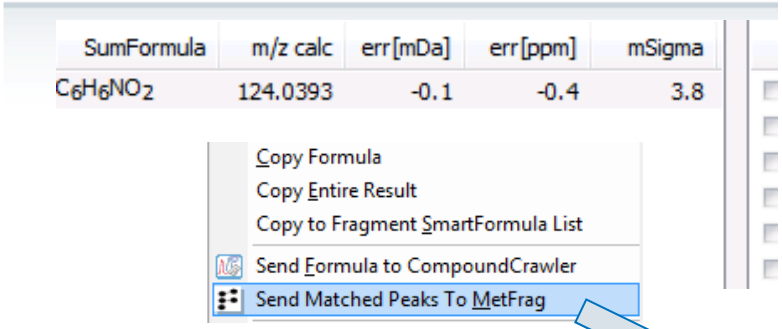


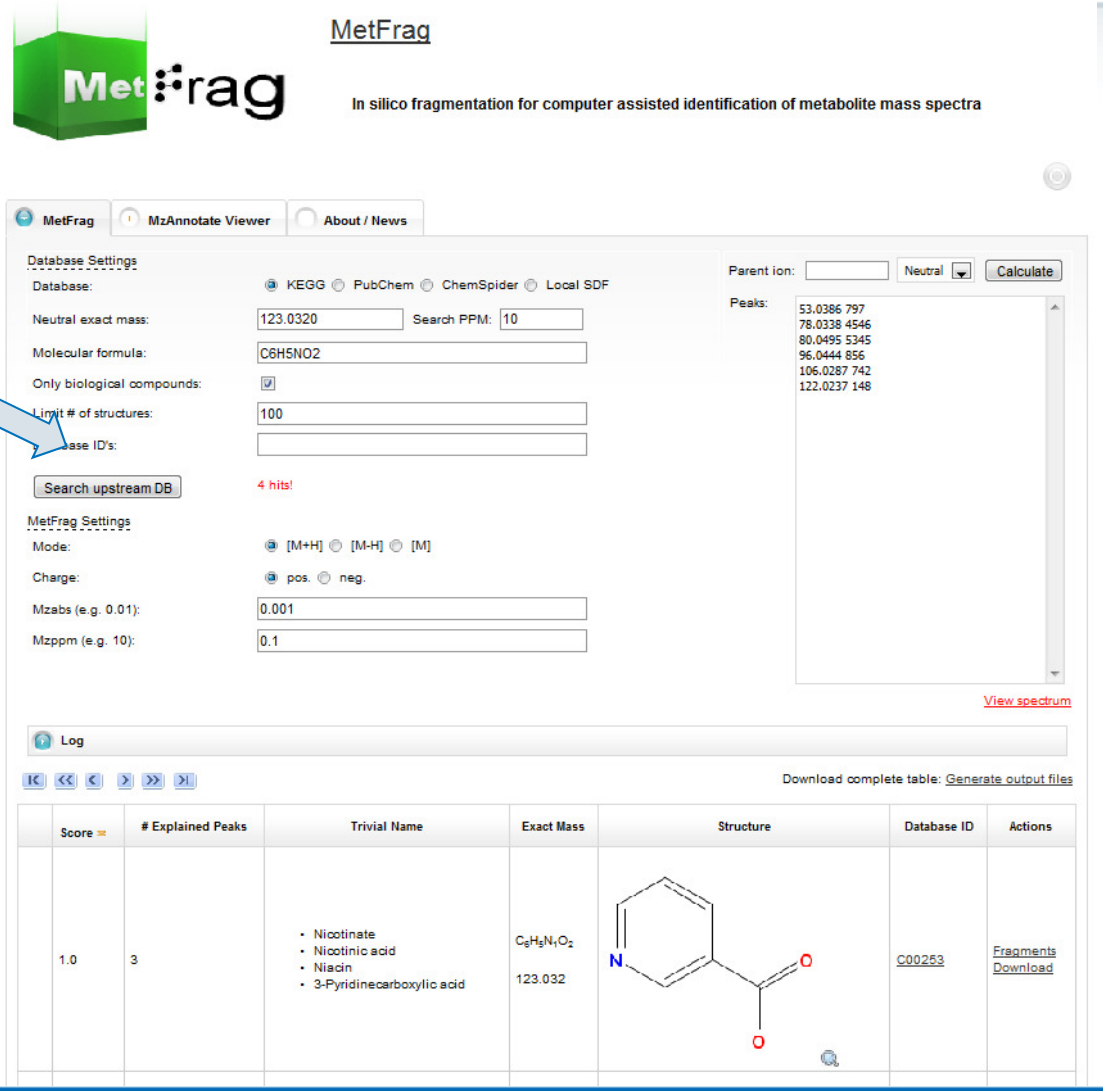
"Fragments-link"

Structures for predicted and matched fragment ions are shown

...

A direct link from SmartFormula3D to MetFrag indicates nicotinic acid as likely structure for Compound X

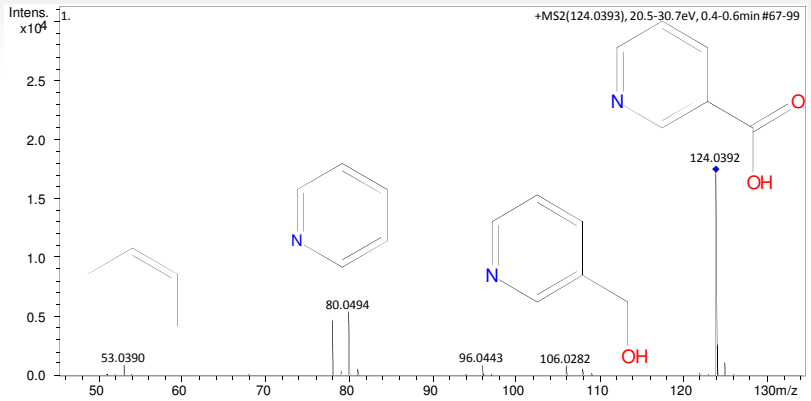




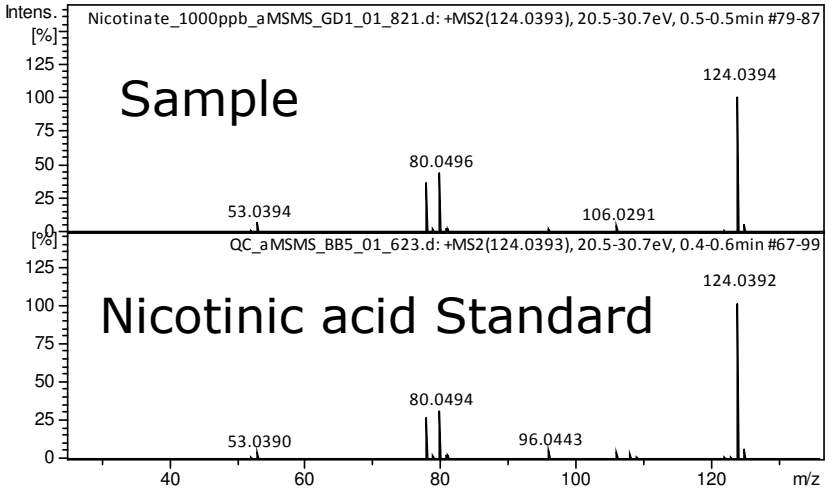
Nicotinic acid returned as likely compound by in-silico fragmentation in the open source **MetFrag** tool

MetFrag:
http://msbi.ipb-halle.de/MetFrag/
Wolf, S. et al.:
BMC Bioinformatics 2010, 11:148

Nicotinic acid ID verified by FragmentExplorer & proven by comparison to authentic standard

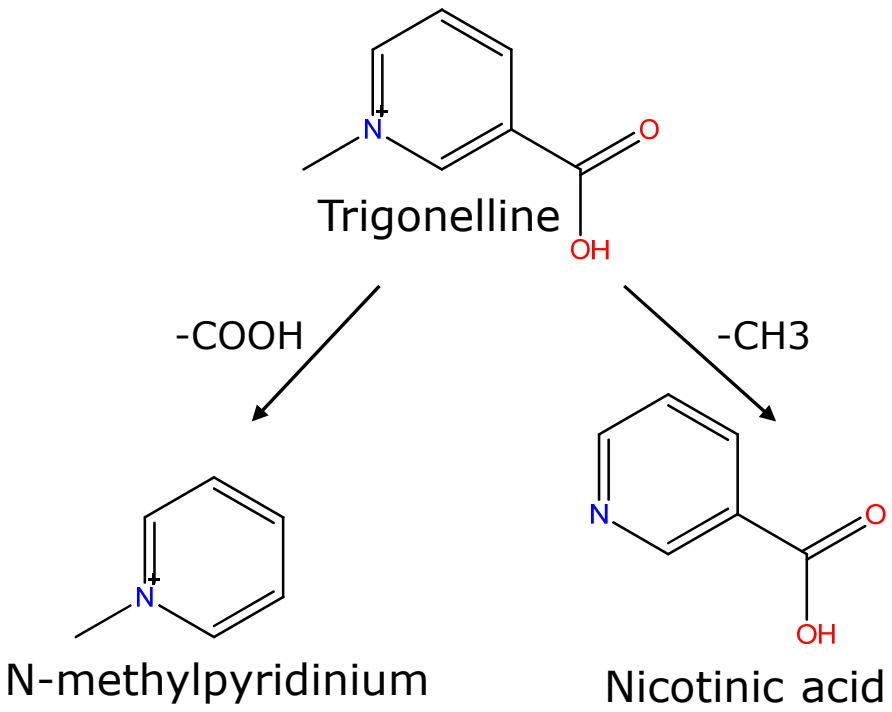


Fragments assigned using FragmentExplorer in Bruker DataAnalysis software



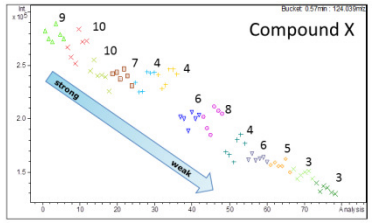
Comparison of MS/MS spectra

Compound ID fits to chemical knowledge: Nicotinic acid is a known degradation product from Trigonelline contibuting to a roasty coffee aroma



"Trigonelline degradation is proportional to roast degree. Its byproducts include pyridines, which are said to contribute a roasty aroma to the coffee."

http://www.coffeeresearch.org/science/bittermain.htm



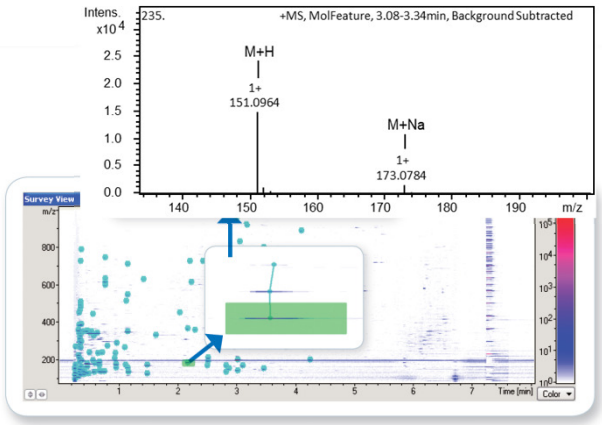
Adepted from:
Boettler U. et al 2011, The Journal of Nutritional Biochemistry Vol. 22 (5), p.426-440

Non- targeted & targeted Metabolomics

Both can be addressed using one ESI-TOF-MS data set

Non-targeted Metabolomics:

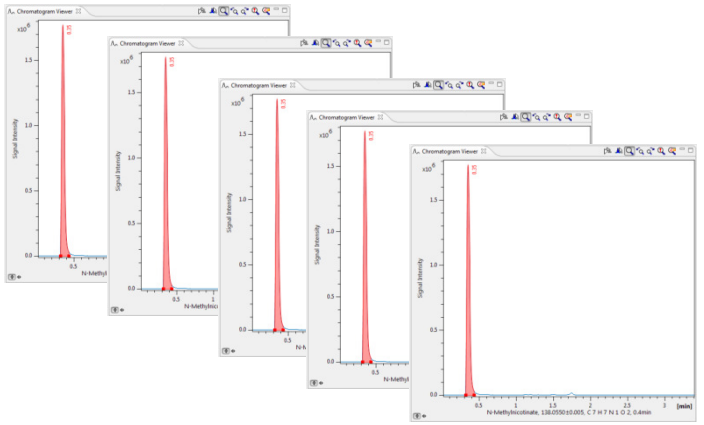
- "Think" extract all **Features** first



MetaboScape

Targeted Metabolomics:

- "Think" **hrEICs –** if you know what you are looking for



PathwayScreener

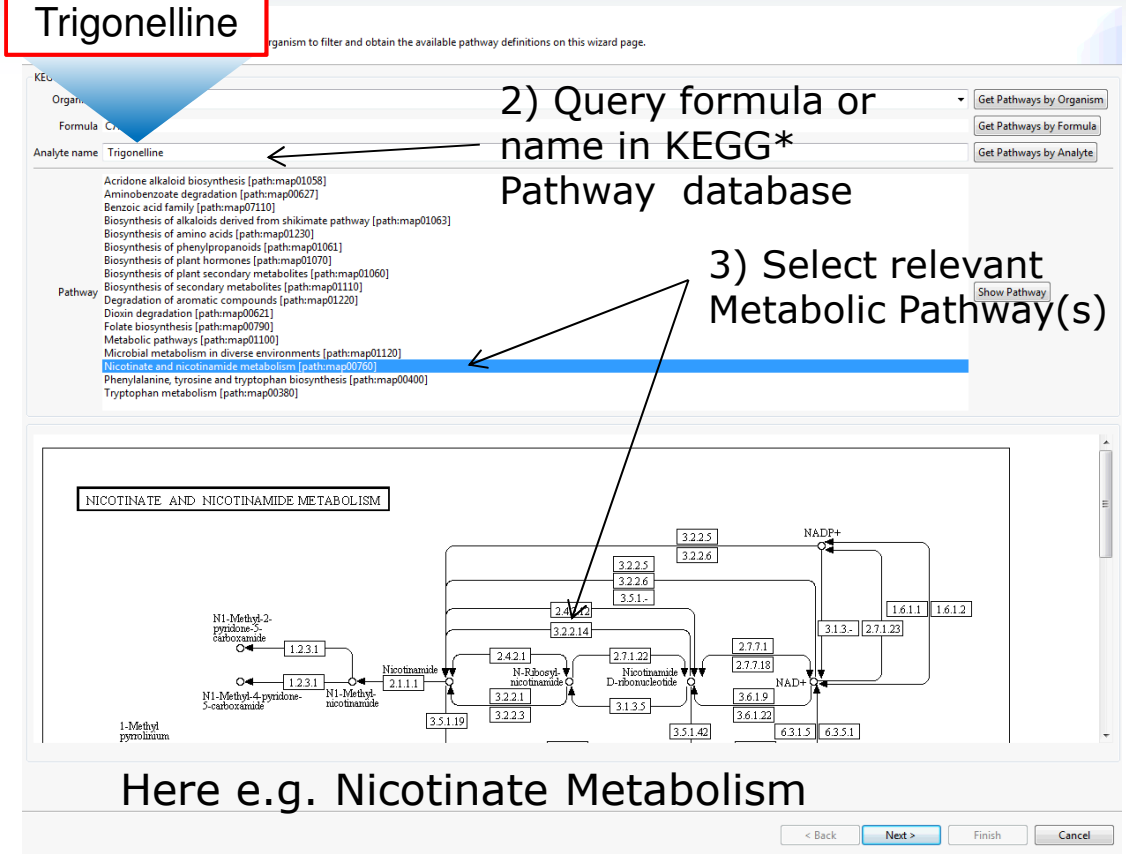
Metabolic **Pathway driven targeted Metabolomics**

using same high resolution full scan QTOF data

PathwayScreener

BRUKER

1) Trigonelline



Here e.g. Nicotinate Metabolism

Workflow:

1) Non-targeted QTOF Metabolomics -> one Biomarker identified
2) Hypothesis: there are other biochemically related metabolites changed in the samples as well ->Query known target in Metabolic Pathway Database
3) selected Pathway
4) retrieve name and formula of all metabolites
5) Targeted screening for these compounds by hrEICs in QTOF data
6) Optional statistical analysis

* KEGG Authorized Service Provider

Note: restrictions apply to use KEGG for commercial purposes for details see: http://www.kegg.jp/kegg/legal.html

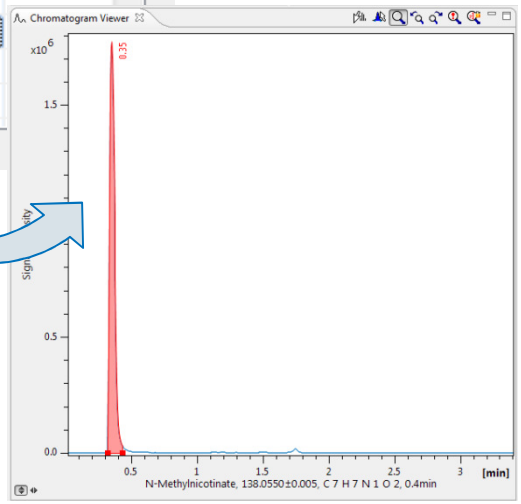
Metabolic **Pathway driven targeted Metabolomics** using same high resolution full scan QTOF data

4) Target list of analytes derived from Metabolic Pathway automatically created:

Analytes in Group:

Analyte	Formula	Mass
Deamino-NAD+	C21H27N6O15P2	665.1010
Fumarate	C4H4O4	116.0110
Glycerone phosphate	C3H7O6P	169.9980
Iminoaspartate	C4H5NO4	131.0219
L-Aspartate	C4H7NO4	133.0375
Maleamate	C4H5NO3	115.0269
Maleic acid	C4H4O4	116.0110
Methylitaconate	C6H8O4	144.0423
N-formylmaleamic acid	C5H5NO4	143.0219
N-Methylnicotinate	C7H7NO2	137.0477
N1-Methyl-2-pyridone-5-carboxam...	C7H8N2O2	152.0586
N1-Methyl-4-pyridone-5-carboxam...	C7H8N2O2	152.0586

5) create "hrEIC" to screen for target compound in full scan high resolution data -> analog to TargetScreening



Workflow:

1) Non-targeted QTOF Metabolomics -> one Biomarker identified

2) Hypothesis: there are other biochemically related metabolites changed in the samples as well
->Query known target in Metabolic Pathway Database

3) selected Pathway

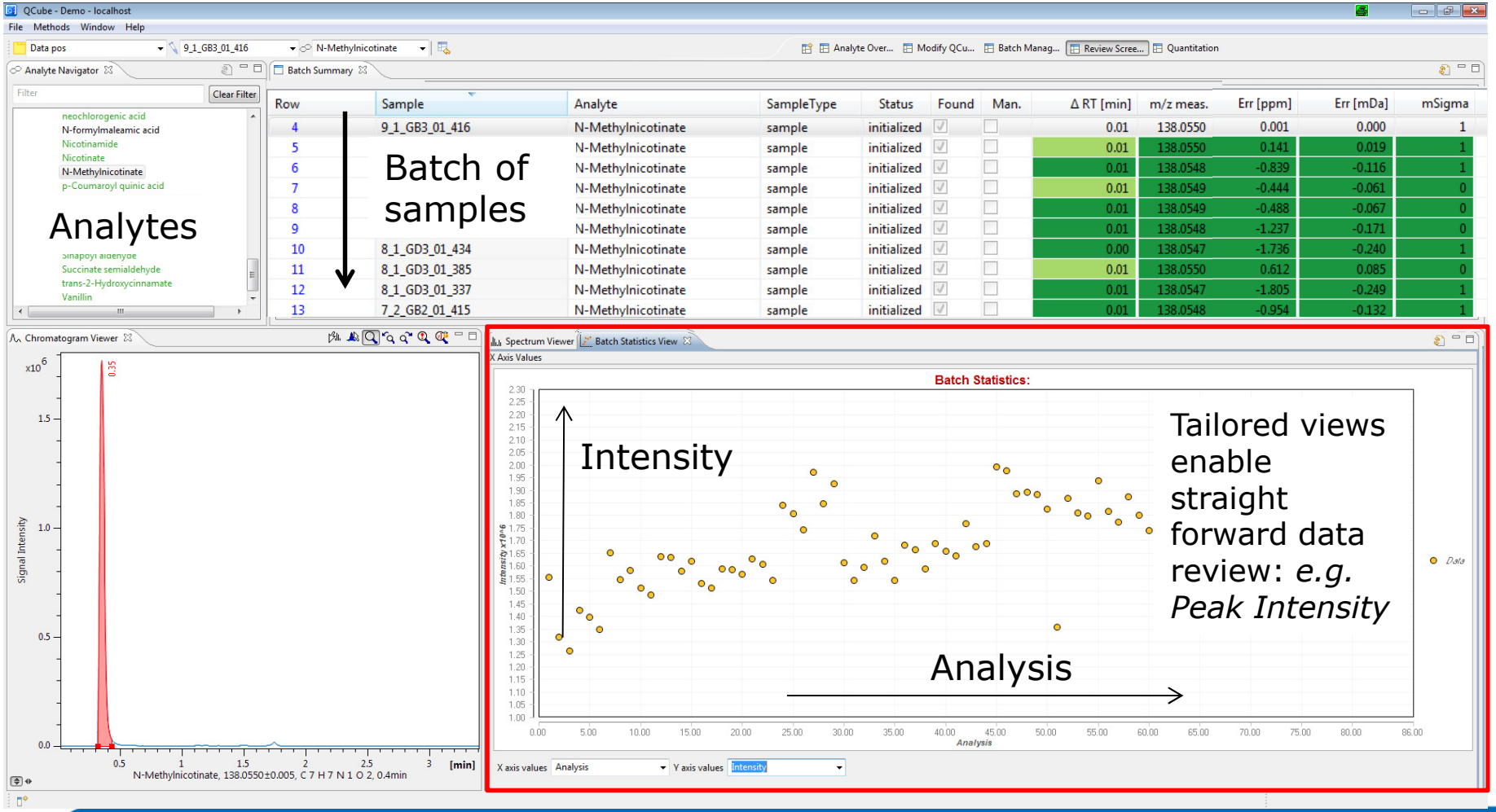
4) retrieve name and formula of all metabolites

5) Targeted screening for these compounds by hrEICs in QTOF data

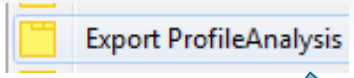
6) Optional statistical analysis

Metabolic **Pathway driven targeted Metabolomics** using same high resolution full scan QTOF data

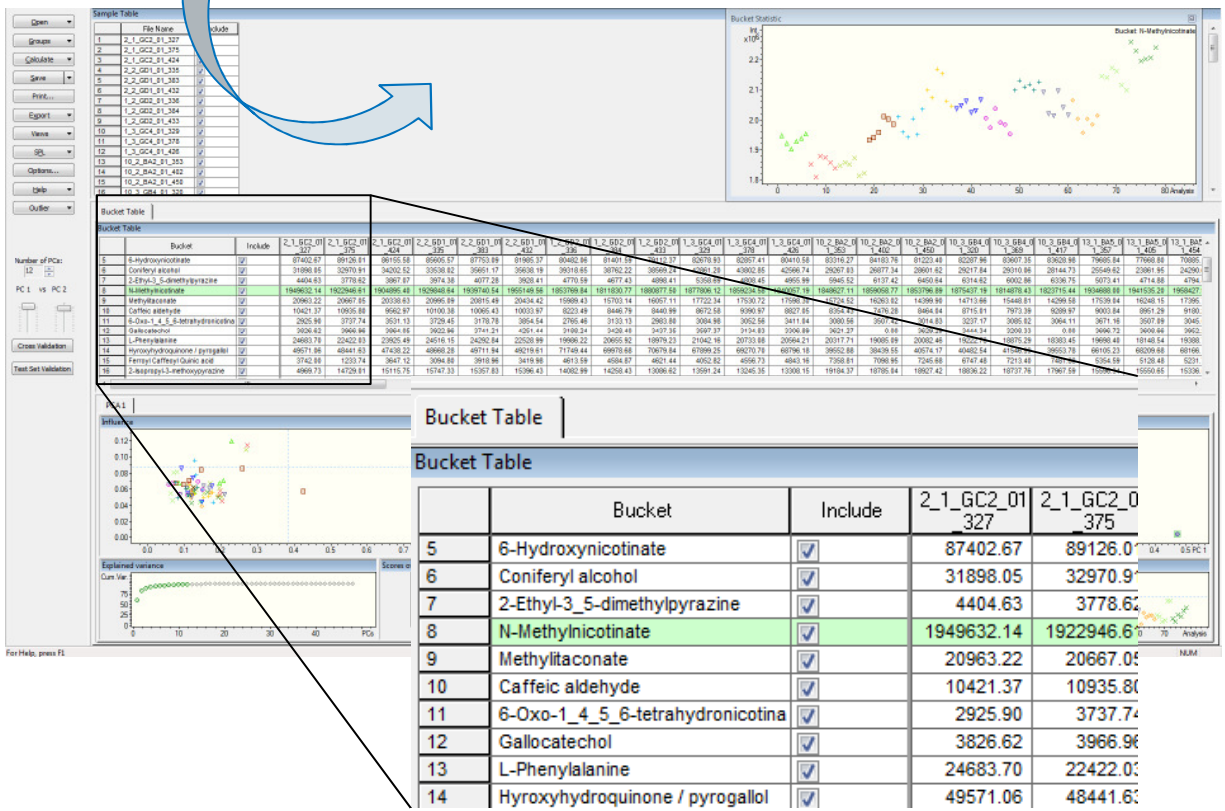
5) Targeted screening for compounds can be applied to ***entire sample batch***



Metabolic **Pathway driven targeted Metabolomics** using same high resolution full scan QTOF data



6) Exported data from **targeted approach** can be readily imported for further statistical evaluation.

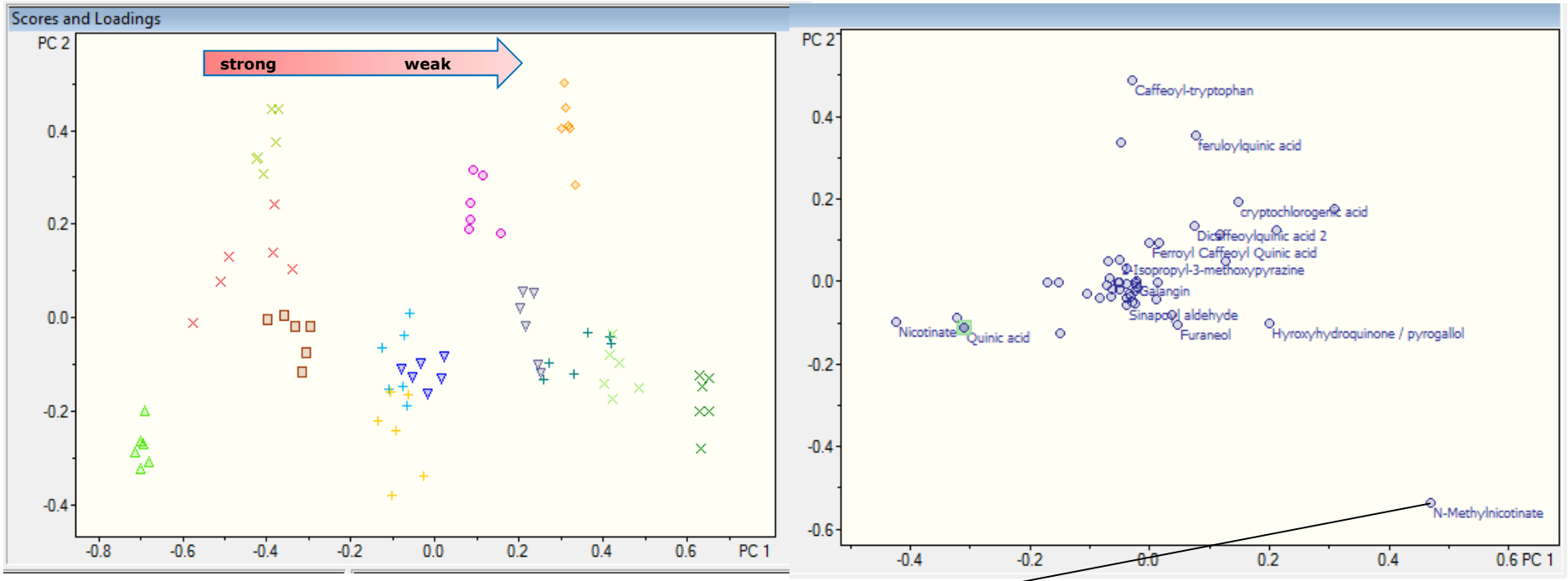


All compounds have a tentative ID!

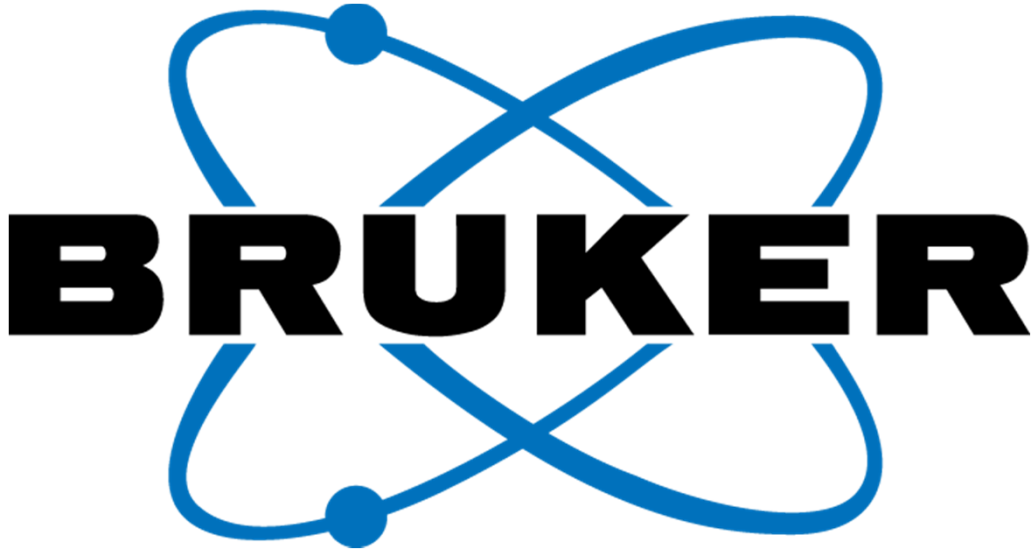
Workflow:

1) Non-targeted QTOF Metabolomics -> one Biomarker identified
2) Hypothesis: there are other biochemically related metabolites changed in the samples as well ->Query known target in Metabolic Pathway Database
3) selected Pathway
4) retrieve name and formula of all metabolites
5) Targeted screening for these compounds by hrEICs in QTOF data
6) Optional statistical analysis

Pathway driven targeted Metabolomics data evaluated in ProfileAnalysis: PCA reveals similar separation according to Coffee Intensity like untargeted approach



ID directly accessible from loading plot.



www.bruker.com