

# 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



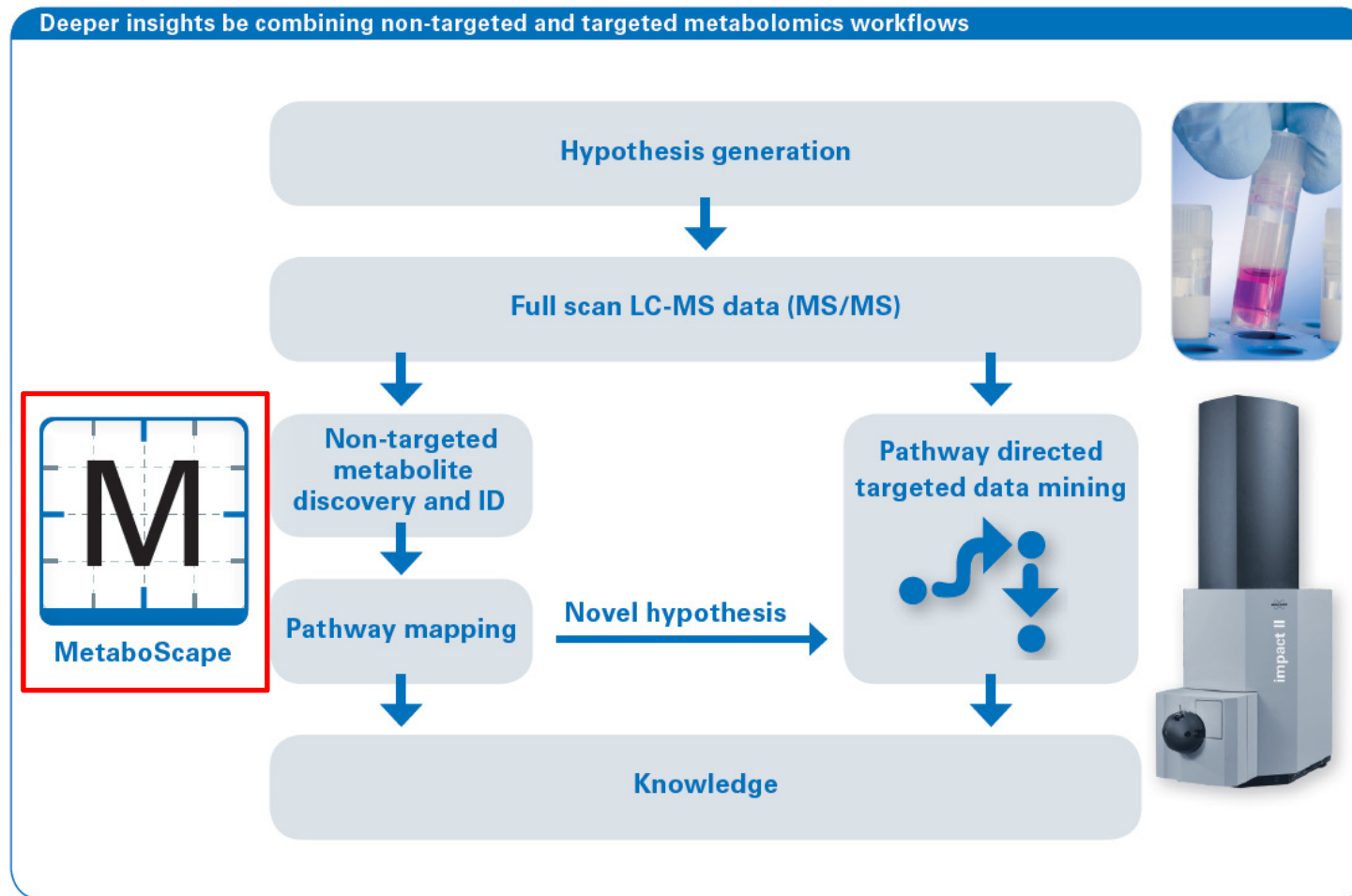
- 13 different coffee capsule types:
  - QC sample: mix of all analytical samples
  - Extracted 2 times each with 35 ml water
  - using XN 3005 Nespresso Pixie espresso machine (Krups)
- Samples centrifuged and diluted 1:50 with water
- Injecting: 5µl on Dionex RSCL (UHPLC) (3 technical replicates each)
- Column: BEH C18, 2.1x50; 1.7µm
- 8 Minute total run time
- MS: **compact** QTOF
- Ionisation: ESI positive



# Non- targeted & targeted Metabolomics

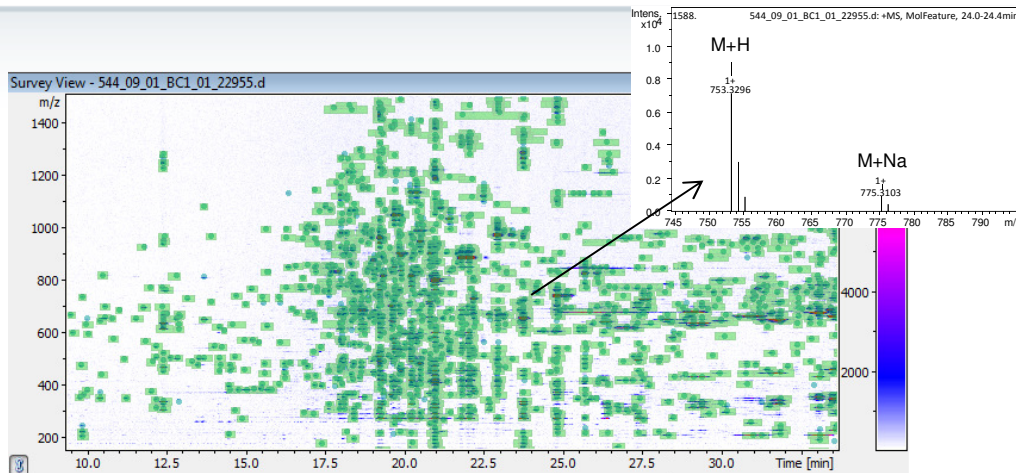


**Both** can be addressed using one ESI-TOF-MS data set



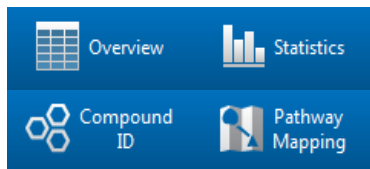
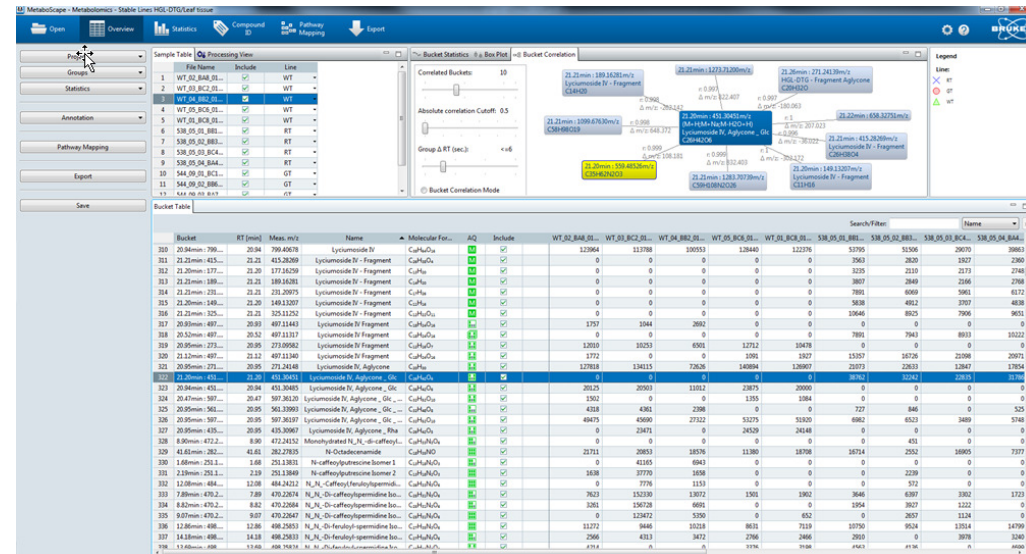
# Metabolic profiling

## Seamless data evaluation by MetaboScape

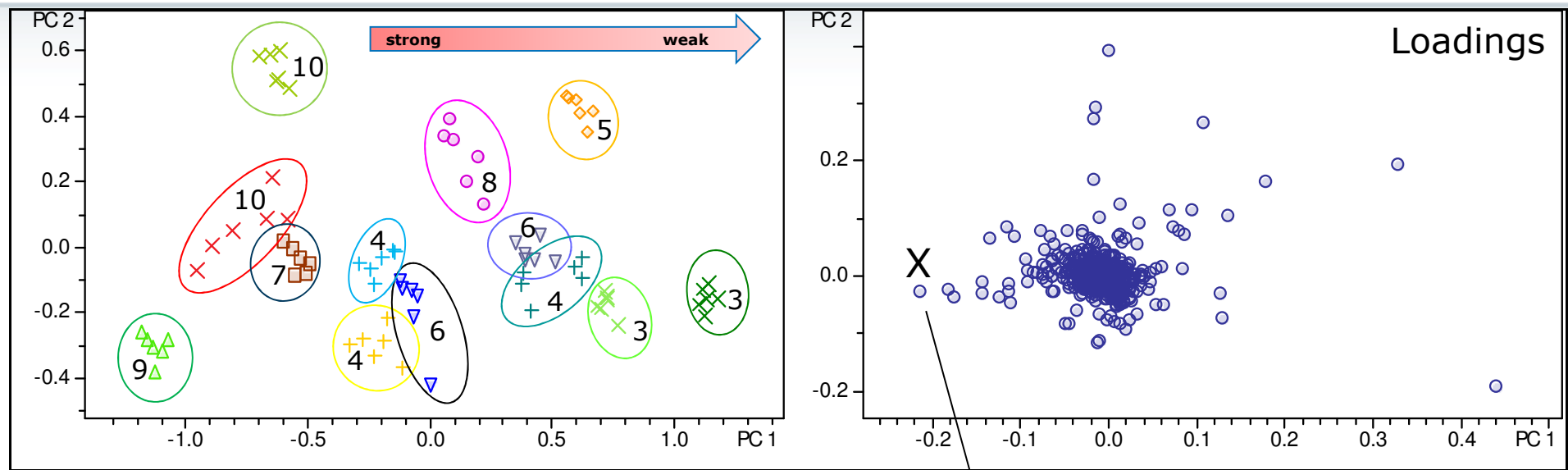


- Comprehensive feature extraction by "Find Molecular Features" algorithm
- RT alignment
- Bucketing
- Normalization Scaling

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

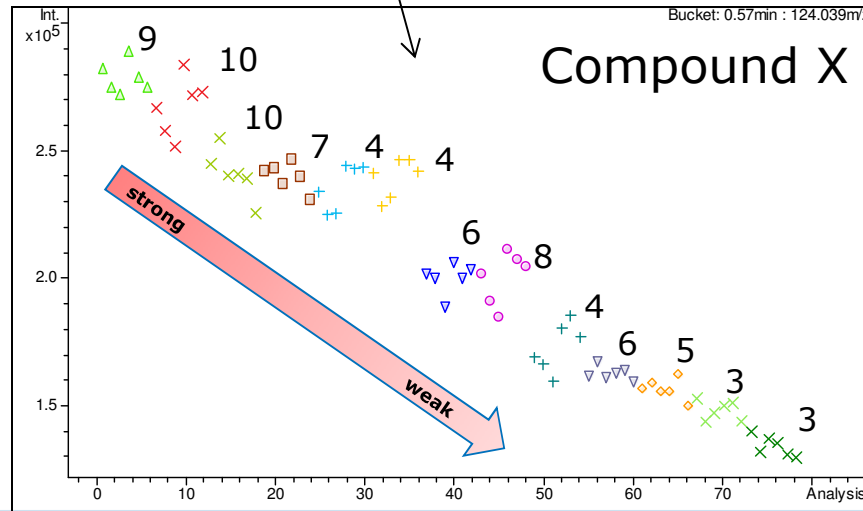


# Characteristics of strong coffee...



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,...



The screenshot shows the Bruker MetaboScape software interface. The 'Export' button in the top right corner is highlighted with a red box. The interface displays a 'Sample Table' with columns for File Name, Include, and Line. Below it, a 'Bucket Table' is visible, showing columns for Bucket, RT [min], Meas. m/z, Name, Molecular For..., AQ, Include, and WT\_02\_BA8\_01. The 'Export' button is located in the top right corner of the software window.

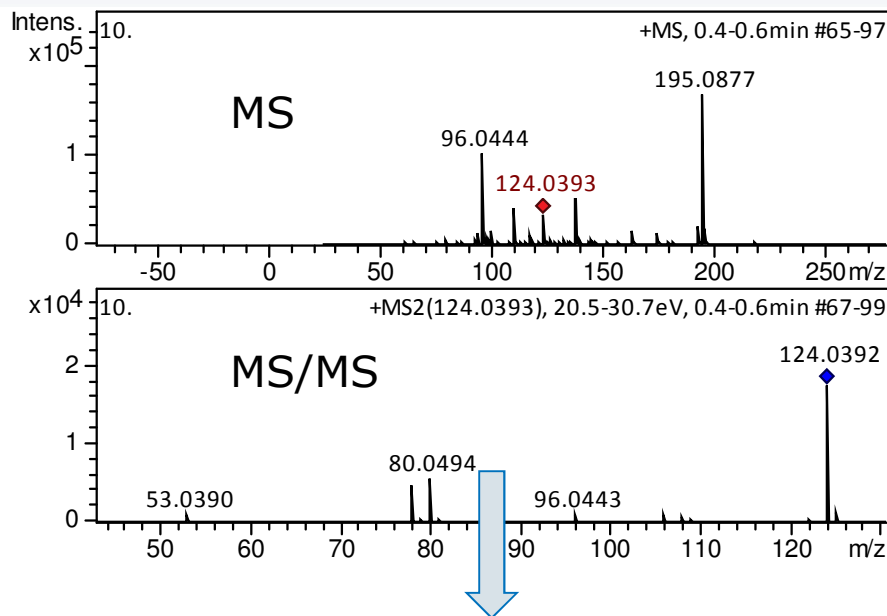
...or optionally you could also export the bucketed data from MetaboScape to other open source statistical tools like **MetaboAnalyst**



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 <sub>2</sub>	122.0237	0.6
$C_6H_4NO$	H <sub>2</sub> O	106.0287	0.5
$C_5H_5NO$	CO	96.0444	0.1
$C_6H_5N$	CO	105.0393	0.1
$C_6H_5NO$	CO	122.0343	0.5
$C_6H_6NO$	CO	138.0393	0.5
$C_6H_6NO_2$	CO	154.0393	0.5
$C_6H_6NO_3$	CO	170.0393	0.5
$C_6H_6NO_4$	CO	186.0393	0.5
$C_6H_6NO_5$	CO	202.0393	0.5

**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>



**METHODOLOGY ARTICLE**

**Open Access**

# In silico fragmentation for computer assisted identification of metabolite mass spectra

Sebastian Wolf<sup>1\*</sup>, Stephan Schmidt<sup>1</sup>, Matthias Müller-Hannemann<sup>2</sup>, Steffen Neumann<sup>1</sup>



Use KEGG, PubChem, ChemSpider or Upload likely structure for in silico fragmentation in MetFrag:

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



MetFrag MzAnnotate Viewer About / News

Database Settings

Database:  KEGG  PubChem  ChemSpider  Local SDF

Upload SDF:  Browse... Upload

594mz.sdf

Limit # of structures:

Search upstream DB 1 hits!

MetFrag Settings

Mode:  [M+H]  [M-H]  [M]

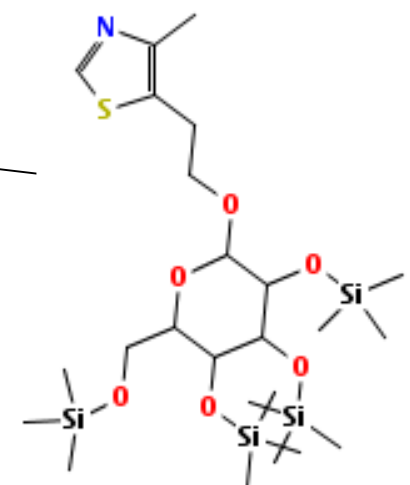
Charge:  pos.  neg.

Mzabs (e.g. 0.01):

Mzppm (e.g. 10):

0 of 1 compounds processed

Process all 1 compounds! START Stop



# In silico fragments are matched against measured fragment ions

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

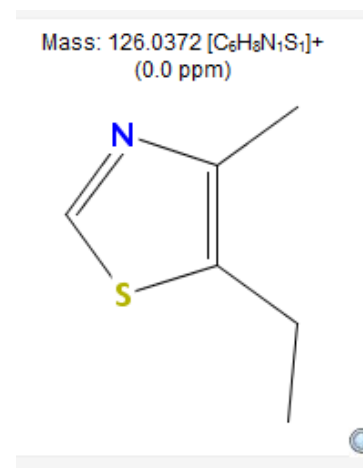
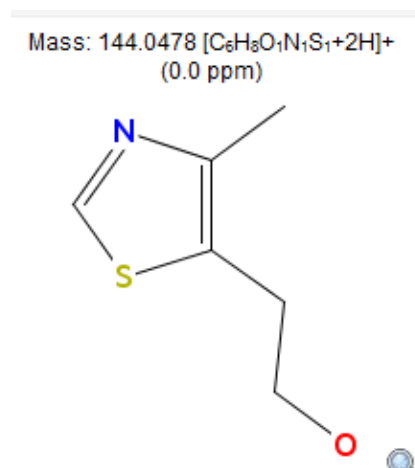
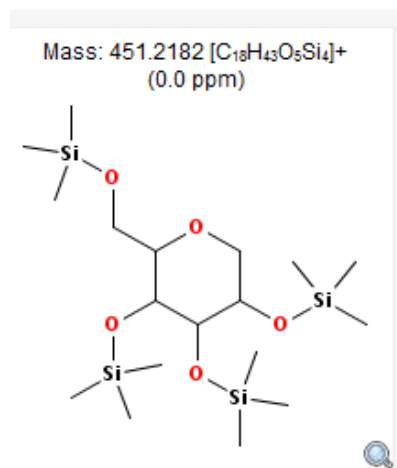


Score	# Explained Peaks	Trivial Name	Exact Mass	Structure	Database ID	Actions
1.0	11	Untitled Document	C <sub>24</sub> H <sub>51</sub> O <sub>8</sub> N <sub>1</sub> Si <sub>4</sub> S <sub>1</sub> 593.2514		0	<a href="#">Fragments Download</a>

Navigation icons: Home, Previous, Next, First, Last

“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**



SumFormula	m/z calc	err[mDa]	err[ppm]	mSigma
C <sub>6</sub> H <sub>6</sub> NO <sub>2</sub>	124.0393	-0.1	-0.4	3.8

[Copy Formula](#)  
[Copy Entire Result](#)  
[Copy to Fragment SmartFormula List](#)  
[Send Formula to CompoundCrawler](#)  
[Send Matched Peaks To MetFrag](#)

**MetFrag**  
 In silico fragmentation for computer assisted identification of metabolite mass spectra

Database Settings  
 Database:  KEGG  PubChem  ChemSpider  Local SDF  
 Neutral exact mass:  Search PPM:   
 Molecular formula:   
 Only biological compounds:   
 Limit # of structures:   
 Database ID's:

MetFrag Settings  
 Mode:  [M+H]  [M-H]  [M]  
 Charge:  pos.  neg.  
 Mzabs (e.g. 0.01):   
 Mzppm (e.g. 10):

Parent ion:  Neutral   
 Peaks:
 

- 53.0386 797
- 78.0338 4546
- 80.0495 5345
- 96.0444 856
- 106.0287 742
- 122.0237 148

[View spectrum](#)

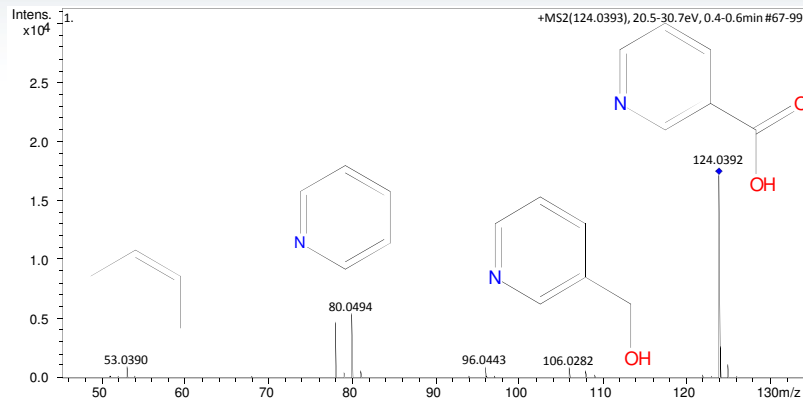
[Download complete table: Generate output files](#)

Score	# Explained Peaks	Trivial Name	Exact Mass	Structure	Database ID	Actions
1.0	3	<ul style="list-style-type: none"> <li>Nicotinate</li> <li>Nicotinic acid</li> <li>Niacin</li> <li>3-Pyridinecarboxylic acid</li> </ul>	C <sub>6</sub> H <sub>5</sub> N <sub>1</sub> O <sub>2</sub> 123.032		C00253	<a href="#">Fragments Download</a>

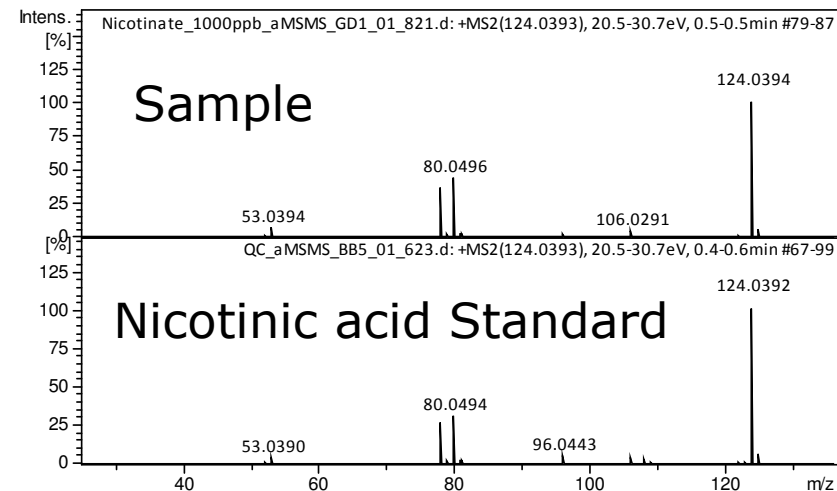
*Nicotinic acid* returned as likely compound by in-silico fragmentation in the open source **MetFrag** tool

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*



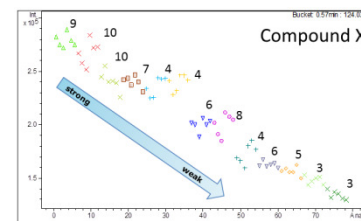
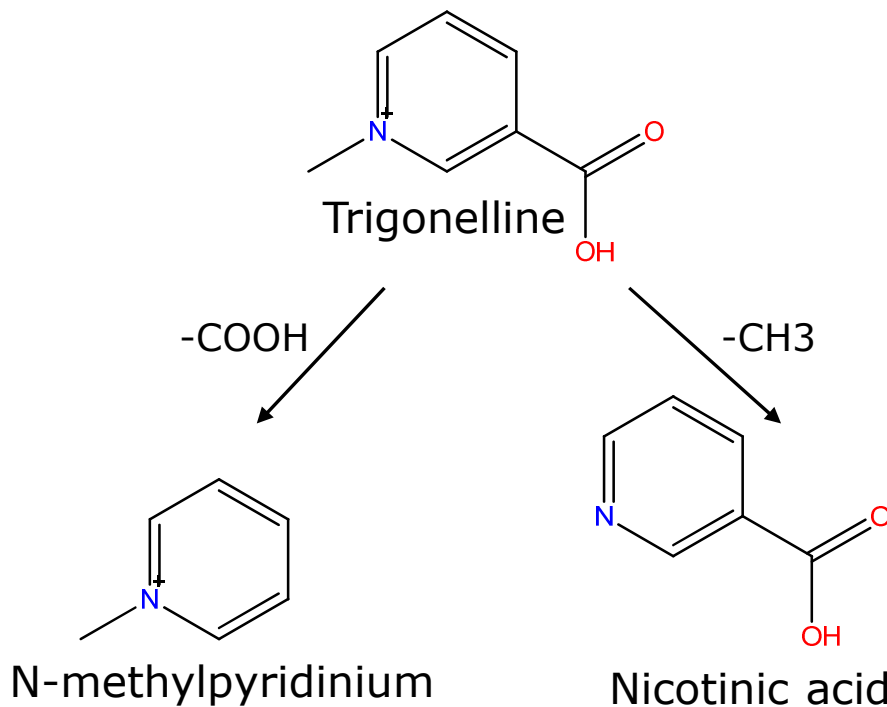
*Comparison of MS/MS spectra*

Compound ID fits to chemical knowledge:  
Nicotinic acid is a known degradation product from  
Trigonelline contributing 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

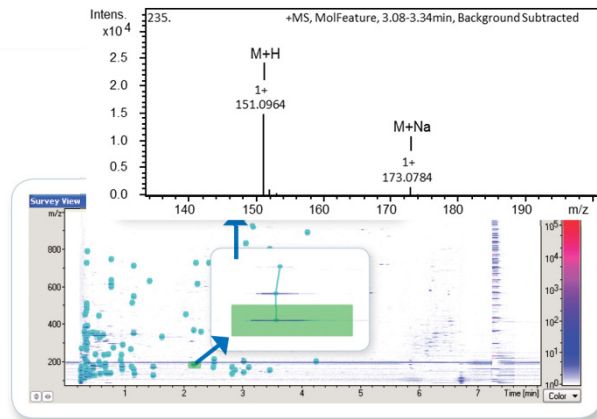
# Non- targeted & targeted Metabolomics



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

## Non-targeted Metabolomics:

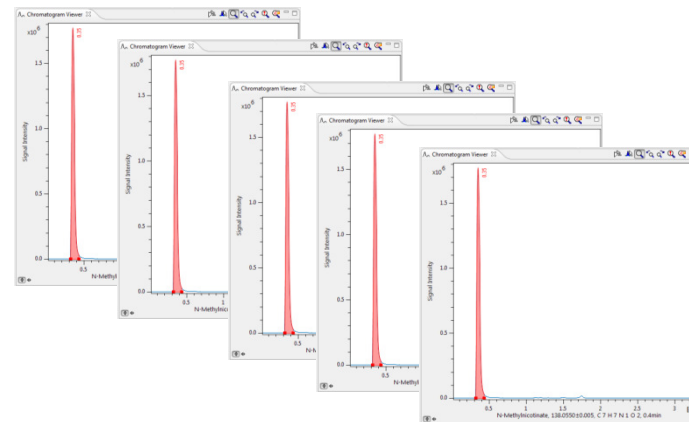
- “Think” extract all **Features** first



**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



1) **Trigonelline**

2) Query formula or name in KEGG\* Pathway database

3) Select relevant Metabolic Pathway(s)

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



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

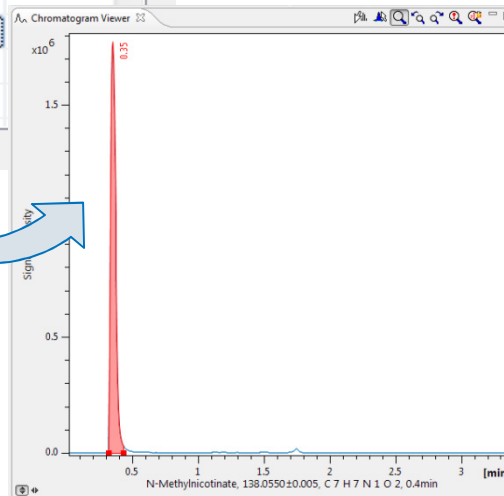


4) Target list of analytes derived from Metabolic Pathway automatically created:

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
<b>N-Methylnicotinate</b>	<b>C7H7NO2</b>	<b>137.0477</b>
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:

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# 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**

The screenshot displays the QCube software interface. The main window shows a 'Batch Summary' table with columns for Row, Sample, Analyte, SampleType, Status, Found, Man.,  $\Delta$  RT [min], m/z meas., Err [ppm], Err [mDa], and mSigma. A list of analytes is shown on the left, including neochlorogenic acid, N-formylmaleamic acid, Nicotinamide, Nicotinate, N-Methylnicotinate, p-Coumaroyl quinic acid,  $\gamma$ -irapoyl isoenoye, Succinate semialdehyde, trans-2-Hydroxycinnamate, and Vanillin. A chromatogram viewer shows a single sharp peak at 0.35 minutes. A spectrum viewer shows a plot of Intensity vs Analysis time (min) with a red box highlighting the data points and a text box stating 'Tailored views enable straight forward data review: e.g. Peak Intensity'.

Row	Sample	Analyte	SampleType	Status	Found	Man.	$\Delta$ RT [min]	m/z meas.	Err [ppm]	Err [mDa]	mSigma
4	9_1_GB3_01_416	N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0550	0.001	0.000	1
5		N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0550	0.141	0.019	1
6		N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0548	-0.839	-0.116	1
7		N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0549	-0.444	-0.061	0
8		N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0549	-0.488	-0.067	0
9		N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0548	-1.237	-0.171	0
10	8_1_GD3_01_434	N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.00	138.0547	-1.736	-0.240	1
11	8_1_GD3_01_385	N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0550	0.612	0.085	0
12	8_1_GD3_01_337	N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0547	-1.805	-0.249	1
13	7_2_GB2_01_415	N-Methylnicotinate	sample	initialized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	0.01	138.0548	-0.954	-0.132	1

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



**Export ProfileAnalysis**

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

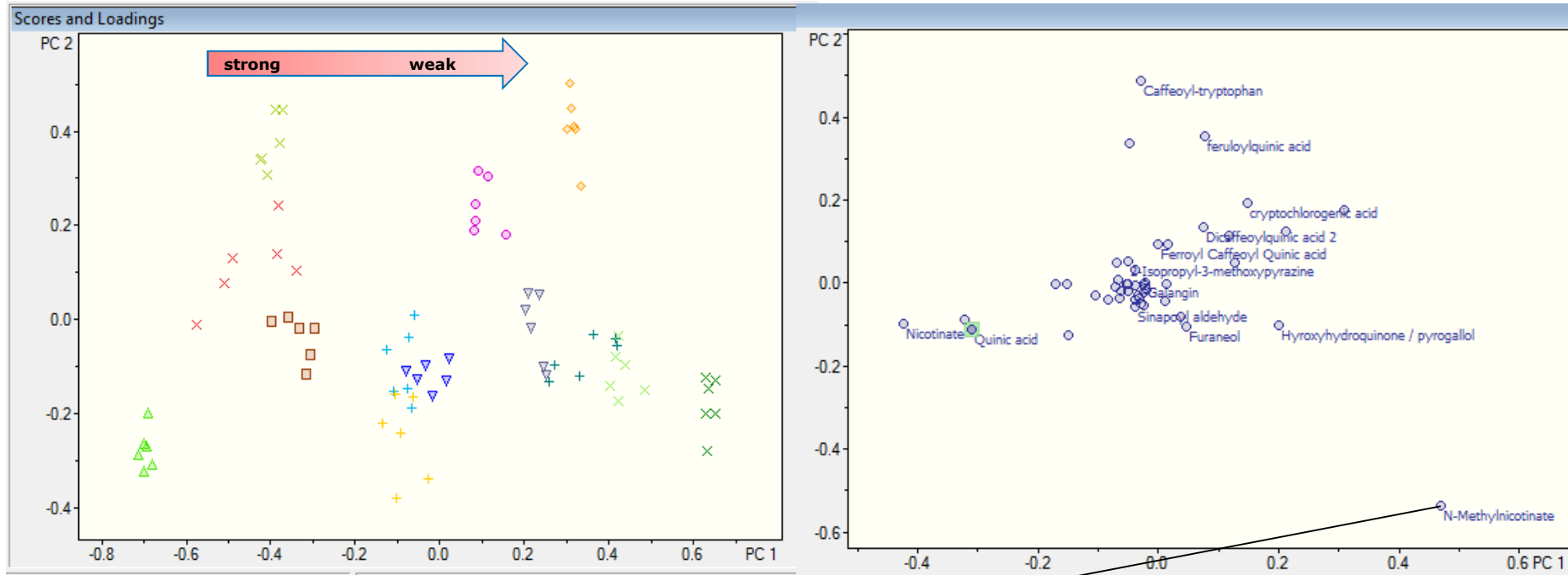
Bucket	Include	2_1_GC2_01_327	2_1_GC2_01_375
5	<input checked="" type="checkbox"/>	87402.67	89126.0
6	<input checked="" type="checkbox"/>	31898.05	32970.9
7	<input checked="" type="checkbox"/>	4404.63	3778.6
8	<input checked="" type="checkbox"/>	1949632.14	1922946.6
9	<input checked="" type="checkbox"/>	20963.22	20667.0
10	<input checked="" type="checkbox"/>	10421.37	10935.0
11	<input checked="" type="checkbox"/>	2925.90	3737.7
12	<input checked="" type="checkbox"/>	3826.62	3966.9
13	<input checked="" type="checkbox"/>	24683.70	22422.0
14	<input checked="" type="checkbox"/>	49571.06	48441.6

## Workflow:

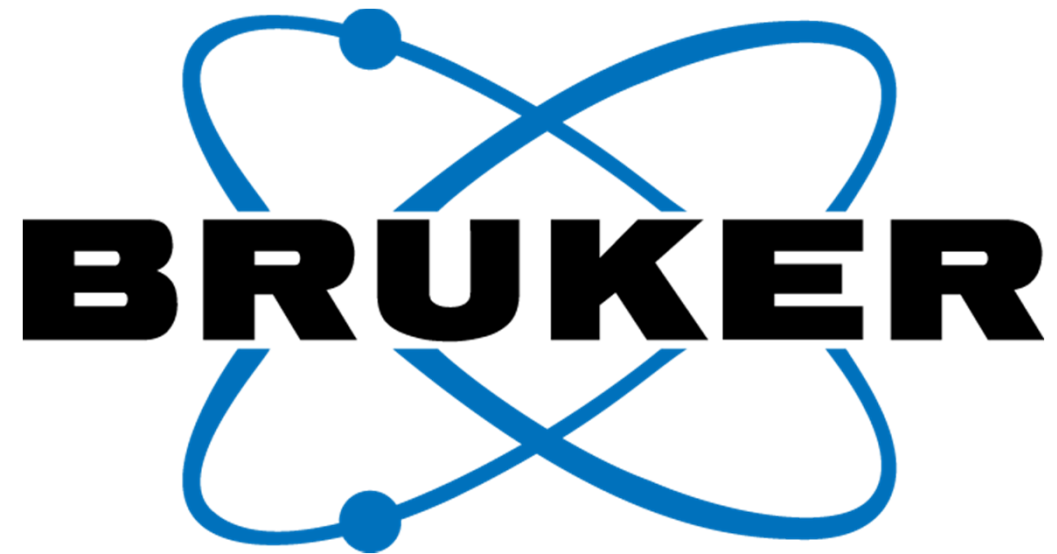
- 1) Non-targeted QTOF Metabolomics -> one Biomarker identified
- 2) Hypothesis: there are other biochemically related metabolites changed in the samples as well  
-> Query known target in Metabolic Pathway Database
- 3) selected Pathway
- 4) retrieve name and formula of all metabolites
- 5) Targeted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

All compounds have a tentative ID!

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](http://www.bruker.com)