Metabolite identification

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&

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Outline

A path to metabolite identification

- Introduction
  - Analytical Technologies
  - Terminology in metabolite identification
- Metabolite identification
  - GC-MS MetID
    - EI spectral deconvolution
    - GC-APCI and exact mass
  - LC-MS MetID
    - Metabolite / Ion annotation
    - Formula calculation
    - Utilization of isotopes for formula calculation
    - MS/MS and RT
- New approaches for MetID
  - Ion mobility
  - *in silico* approaches
- *de novo* identification

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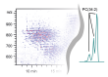
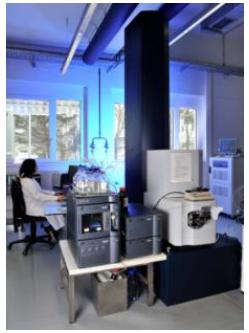


Introduction
Analytical Technologies

- Metabolomics utilizes different analytical chemistry methods for analysis of metabolites
- These methods include
  - **M**ass **S**pectrometry (MS) without or with prior metabolite separation
    - **G**as **C**hromatography (GC)
    - **L**iquid **C**hromatography (LC)
    - **C**apillary **E**lectrophoresis (CE)
  - **N**uclear **M**agnetic **R**esonance (NMR)
- Each method has its specific advantages and disadvantages
- No method can cover all metabolites!
- Certain overlap between the different methods exists



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Introduction
Terminology

- Following cases have to be consider once it comes to metabolite identification

    - *Known metabolites* = known structure, present in matrix, detected by method of choice

    - *Known unknown metabolites* = know structure, shown to be not present in in matrix so far, detected by method of choice, e.g. comparison of MS/MS spectra

    - *Unknown metabolites* = *de novo* identification of truly novel compounds

Introduction
Terminology

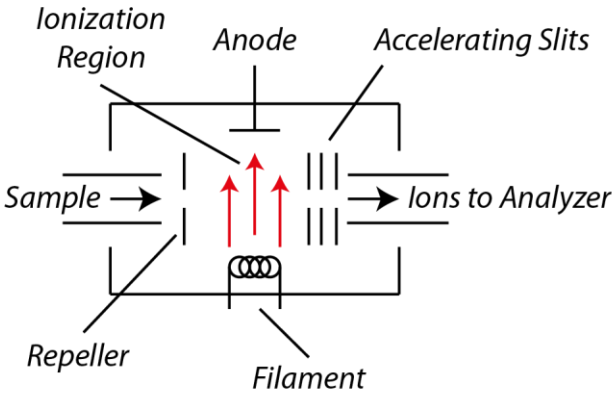
- Not every single metabolite gives a single peak
- GC-MS
  - Different derivatization products
  - In source fragments (EI is a hard ionization technique)
- Although ESI is a soft ionization technique many signals are generated in LC-MS, SFC-MS and CE-MS for a single metabolite
  - Isotopes, adducts and multimers
  - In source fragments
  - Different charge states
- NMR
  - Different shifts and coupling

- It is important to differentiate between
  - Annotation = potential metabolites, e.g. based on comparison with exact masses
  - Identification = proven metabolites

Introduction

Terminology – GC-MS multiple peaks

A

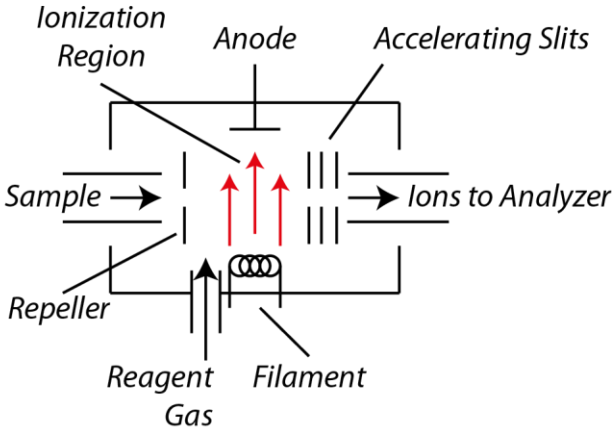


$$M + e^- \rightarrow M^{+\cdot} + 2\,e^-$$

$$M_1^+ + M_2^{\cdot}$$

$$M_3^+ + M_4 \qquad M_5^+ + M_6 \qquad M_7^+ + M_8$$

B



$$CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2\,e^-$$

$$CH_4 + CH_4^{+\cdot} \rightarrow CH_5^+ + CH_3^{\cdot}$$

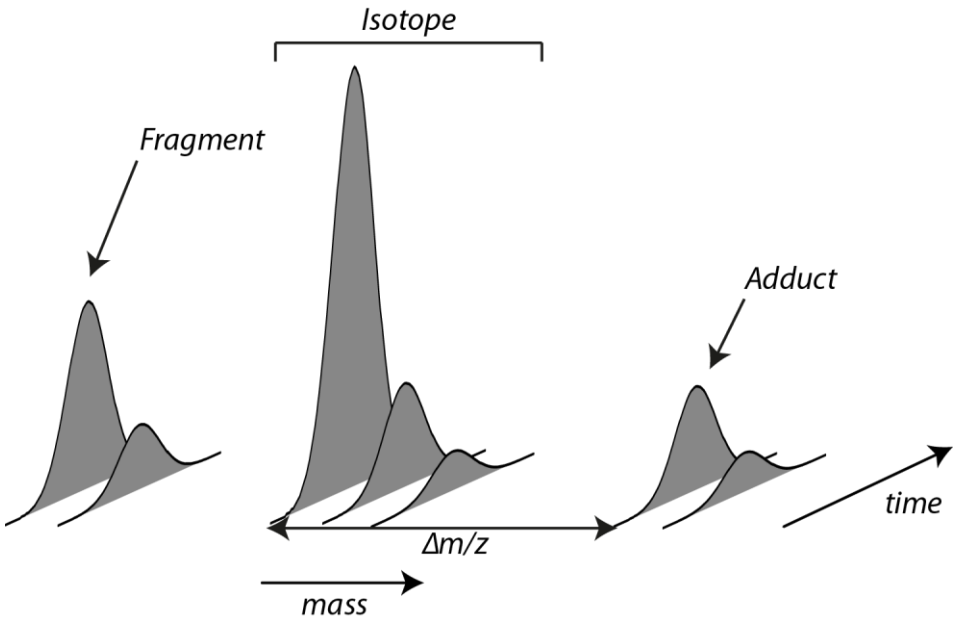
$$CH_4 + CH_3^+ \rightarrow C_2H_5^+ + H_2$$

$$M + CH_5^+ \rightarrow CH_4 + [M+H]^+$$

$$AH + CH_3^+ \rightarrow CH_4 + A^+$$

Introduction

Terminology – LC-MS multiple peaks



Introduction

Terminology – LC-MS multiple peaks

M+3H	M/3 + 1.007276	3+	0.33	1.007276
M+2H+Na	M/3 + 8.334590	3+	0.33	8.334590
M+H+2Na	M/3 + 15.7661904	3+	0.33	15.766190
M+3Na	M/3 + 22.989218	3+	0.33	22.989218
M+2H	M/2 + 1.007276	2+	0.50	1.007276
M+H+NH4	M/2 + 9.520550	2+	0.50	9.520550
M+H+Na	M/2 + 11.998247	2+	0.50	11.998247
M+H+K	M/2 + 19.985217	2+	0.50	19.985217
M+ACN+2H	M/2 + 21.520550	2+	0.50	21.520550
M+2Na	M/2 + 22.989218	2+	0.50	22.989218
M+2ACN+2H	M/2 + 42.033823	2+	0.50	42.033823
M+3ACN+2H	M/2 + 62.547097	2+	0.50	62.547097
M+H	M + 1.007276	1+	1.00	1.007276
M+NH4	M + 18.033823	1+	1.00	18.033823
M+Na	M + 22.989218	1+	1.00	22.989218
M+CH3OH+H	M + 33.033489	1+	1.00	33.033489
M+K	M + 38.963158	1+	1.00	38.963158
M+ACN+H	M + 42.033823	1+	1.00	42.033823
M+2Na-H	M + 44.971160	1+	1.00	44.971160
M+IsoProp+H	M + 61.06534	1+	1.00	61.065340
M+ACN+Na	M + 64.015765	1+	1.00	64.015765
M+2K-H	M + 76.919040	1+	1.00	76.919040
M+DMSO+H	M + 79.02122	1+	1.00	79.021220
M+2ACN+H	M + 83.060370	1+	1.00	83.060370
M+IsoProp+Na+H	M + 84.05511	1+	1.00	84.055110
2M+H	2M + 1.007276	1+	2.00	1.007276
2M+NH4	2M + 18.033823	1+	2.00	18.033823
2M+Na	2M + 22.989218	1+	2.00	22.989218
2M+K	2M + 38.963158	1+	2.00	38.963158
2M+ACN+H	2M + 42.033823	1+	2.00	42.033823
2M+ACN+Na	2M + 64.015765	1+	2.00	64.015765

M-3H	M/3 - 1.007276	3-	0.33	-1.007276
M-2H	M/2 - 1.007276	2-	0.50	-1.007276
M-H2O-H	M- 19.01839	1-	1.00	-19.01839
M-H	M - 1.007276	1-	1.00	-1.007276
M+Na-2H	M + 20.974666	1-	1.00	20.974666
M+Cl	M + 34.969402	1-	1.00	34.969402
M+K-2H	M + 36.948606	1-	1.00	36.948606
M+FA-H	M + 44.998201	1-	1.00	44.998201
M+Hac-H	M + 59.013851	1-	1.00	59.013851
M+Br	M + 78.918885	1-	1.00	78.918885
M+TFA-H	M + 112.985586	1-	1.00	112.985586
2M-H	2M - 1.007276	1-	2.00	-1.007276
2M+FA-H	2M + 44.998201	1-	2.00	44.998201
2M+Hac-H	2M + 59.013851	1-	2.00	59.013851
3M-H	3M - 1.007276	1-	3.00	1.007276
M-3H	M/3 - 1.007276	3-	0.33	-1.007276
M-2H	M/2 - 1.007276	2-	0.50	-1.007276
M-H2O-H	M- 19.01839	1-	1.00	-19.01839
M-H	M - 1.007276	1-	1.00	-1.007276
M+Na-2H	M + 20.974666	1-	1.00	20.974666
M+Cl	M + 34.969402	1-	1.00	34.969402
M+K-2H	M + 36.948606	1-	1.00	36.948606
M+FA-H	M + 44.998201	1-	1.00	44.998201
M+Hac-H	M + 59.013851	1-	1.00	59.013851
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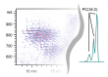
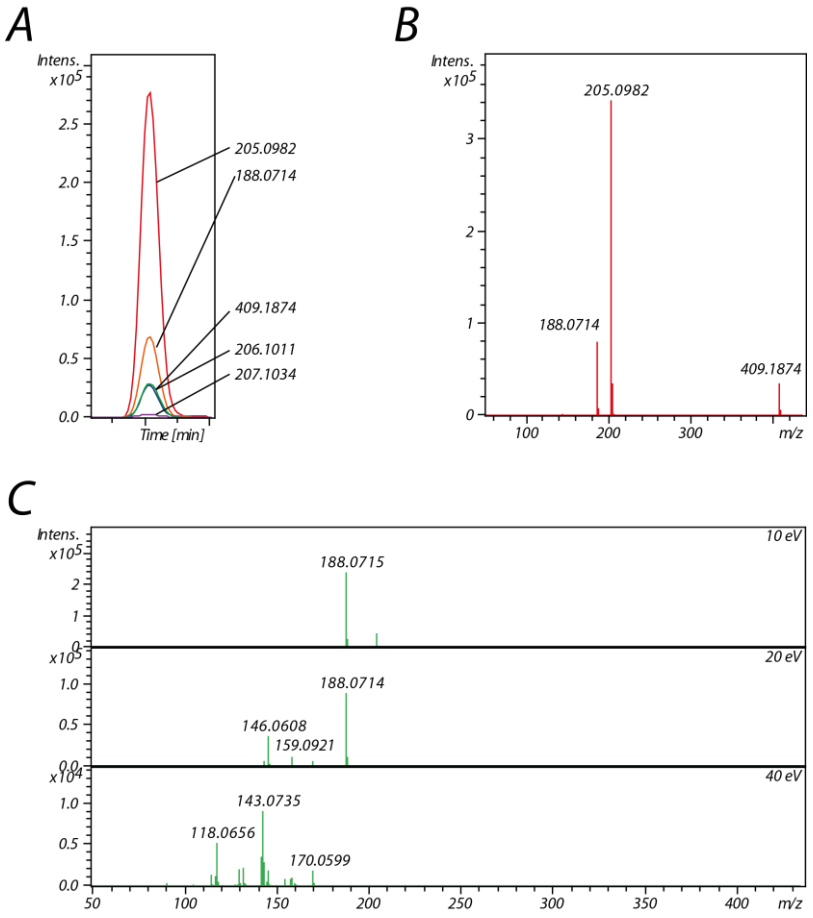
M+TFA-H	M + 112.985586	1-	1.00	112.985586
2M-H	2M - 1.007276	1-	2.00	-1.007276
2M+FA-H	2M + 44.998201	1-	2.00	44.998201
2M+Hac-H	2M + 59.013851	1-	2.00	59.013851



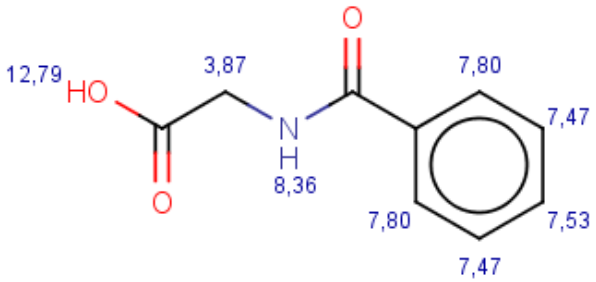
Introduction

Terminology – LC-MS multiple peaks

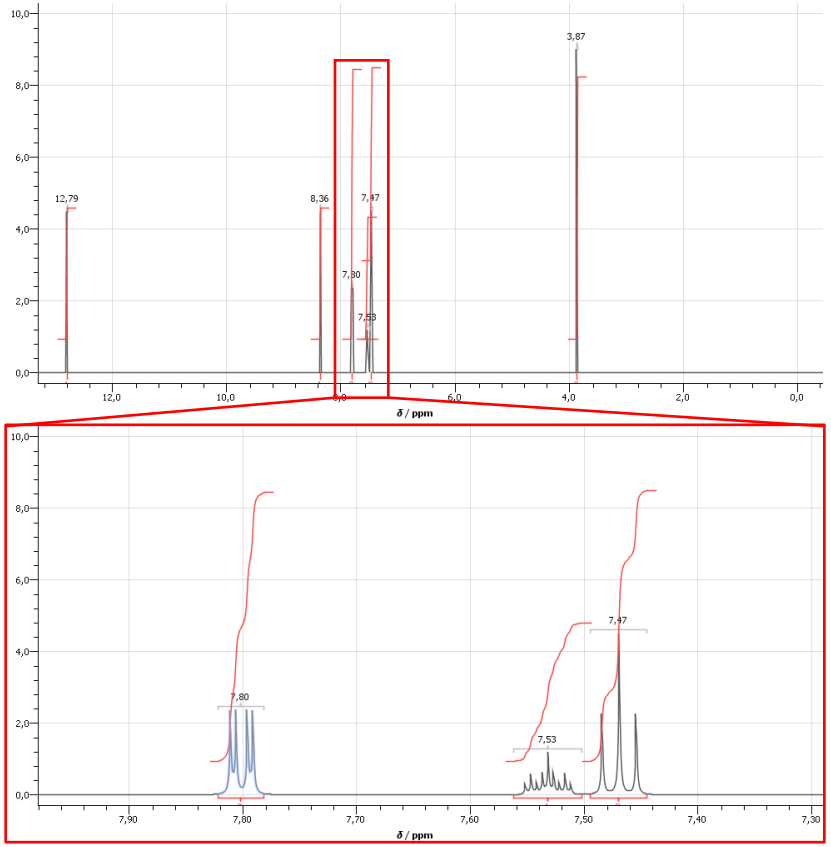


Introduction

Terminology – NMR multiple peaks



Chemical shift	Net intensity	Multiplet information
3.87 ppm	2	s
7.47 ppm	2	t
7.53 ppm	1	n
7.80 ppm	2	q
8.36 ppm	1	s
12.79 ppm	1	s



Introduction

MSI levels of identification

Level	Confidence of identity	Level of evidence
1	Confidently identified compounds	Comparison of two or more orthogonal properties with an authentic standard analyzed under identical analytical conditions
2	Putatively annotated compounds	Based upon physicochemical properties and/or spectral similarity with public/commercial spectral libraries, without reference to authentic chemical standards
3	Putatively annotated compound classes	Based upon characteristic physicochemical properties of a chemical class of compounds or by spectra similarity to known compounds of chemical class
4	Unknown compounds	Although unidentified and unclassified, these metabolites can still be differentiated and quantified based upon spectra data

Sumner et al., Metabolomics . 2007 September ; 3(3): 211–221

Introduction

MSI levels of identification

- Sumner et al. suggested a scoring system for metabolite identification
- Based on different scores for different analytical techniques you „quantitatively“ report your confidence
- The sum of individual scores would give the final score
- If data is compared to an authentic standard the score is multiplied with 2, if compared to a public database the score is multiplied with 1.5
- Example 1: UPLC-MS with accurate mass and comparison with an authentic standard
- (1.5 + 1.0) x 2 = 5.0
- Example 2: GC-MS with high resolution RI, nominal mass and match to NIST library
- (1.5 + 0.5 + 1.5) x 1.5 = 5.25

Table 1 Suggested quantitative scoring system for summarizing a metabolite identification confidence

IR absorbance spectrum	0.5
UV absorbance spectrum	0.5
Retention time (±2.5 %)	1.0
High resolution retention time (±0.5 %, $W_{1/2} < 10$ s)	1.5
High resolution retention index (±0.5 %, RI ± 25, $W_{1/2} < 10$ s)	2.0
Nominal mass of parent ion	0.5
Accurate mass of parent ion (<5 ppm)	1.0
Molecular formula based upon accurate m/z and isotope pattern	1.0
Confident EI Spectral match to commercial library	1.0
Tandem mass spectrum	1.5
Accurate mass tandem mass spectrum	2.0
^{1}H 1D NMR	2.0
^{1}H 2D NMR	3.0
^{1}H × ^{13}C 2D NMR	4.0

IR infrared spectroscopy, *UV* ultraviolet, *Rt* retention time, *RI* retention index, $W_{1/2}$ peak width at half-height, *m/z* mass-to-charge ratio, *EI* electron ionization, *1D* one-dimensional, *2D* two-dimensional, *NMR* nuclear magnetic resonance spectroscopy

Sumner et al., Metabolomics (2014) 10:1047–1049

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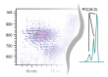

Metabolite Identification

GC-MS MetID – EI spectral deconvolution

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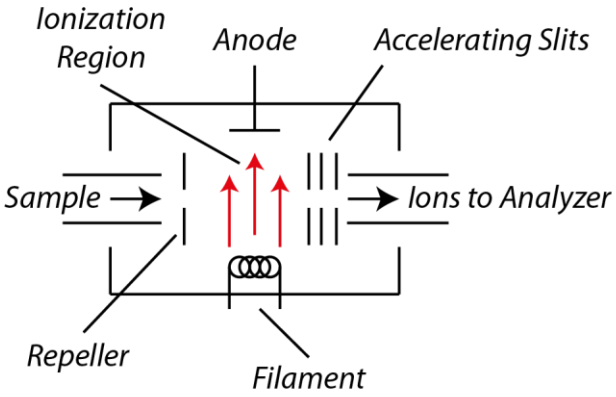

$$M + e^- \rightarrow M^{+\cdot} + 2\,e^-$$

$$M_1^+ + M_2^{\cdot}$$

$$M_3^+ + M_4 \qquad M_5^+ + M_6 \qquad M_7^+ + M_8$$

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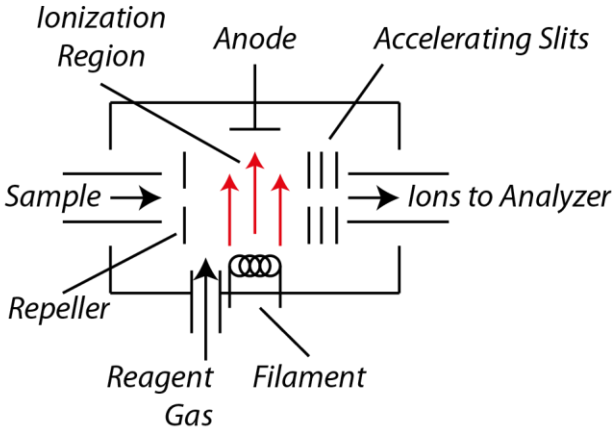


$$CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2\,e^-$$

$$CH_4 + CH_4^{+\cdot} \rightarrow CH_5^+ + CH_3^{\cdot}$$

$$CH_4 + CH_3^+ \rightarrow C_2H_5^+ + H_2$$

$$M + CH_5^+ \rightarrow CH_4 + [M+H]^+$$

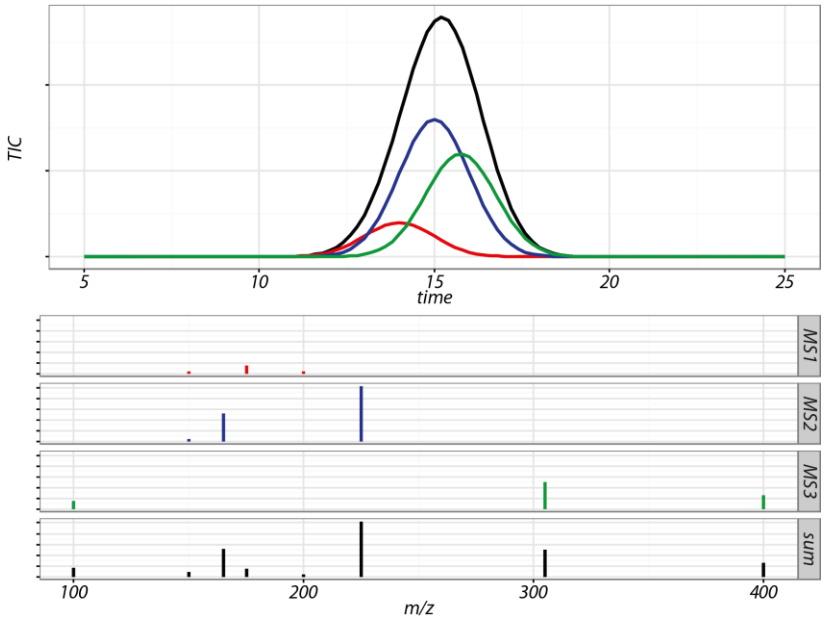
$$AH + CH_3^+ \rightarrow CH_4 + A^+$$

Metabolite Identification

GC-MS MetID – EI spectral deconvolution

- Fragmentation-rich ionization methods like EI lead to mixed mass spectra
- Mixed spectra have to be deconvoluted into individual compound spectra
- AMDIS is a software capable of deconvoluting GC-MS runs

- Deconvoluted compounds and compound spectra can be searched in EI libraries, e.g. NIST, Golm Metabolome Database, Fiehn library, etc..



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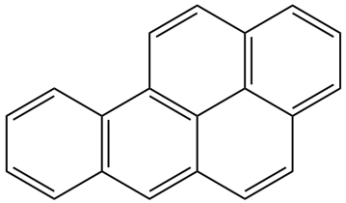
Metabolite Identification

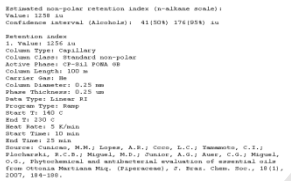
GC-MS MetID – GC-APCI and exact mass

Analytics of LC unamenable compounds



Benzo(a)pyren

Libraries (NIST)



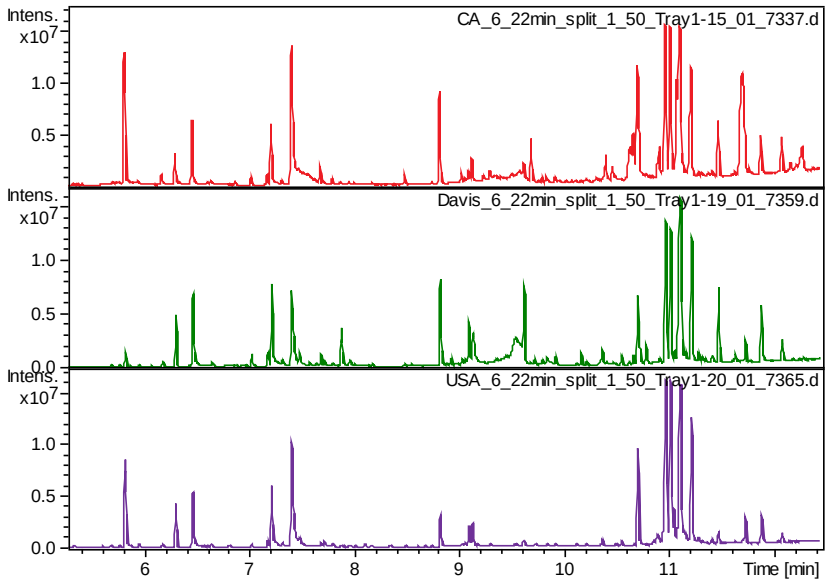
Estimated non-polar retention index (n-alkane scale):
Value: 1238 iu
Confidence interval (Alcohols): 41(SCR) 176(95%) iu

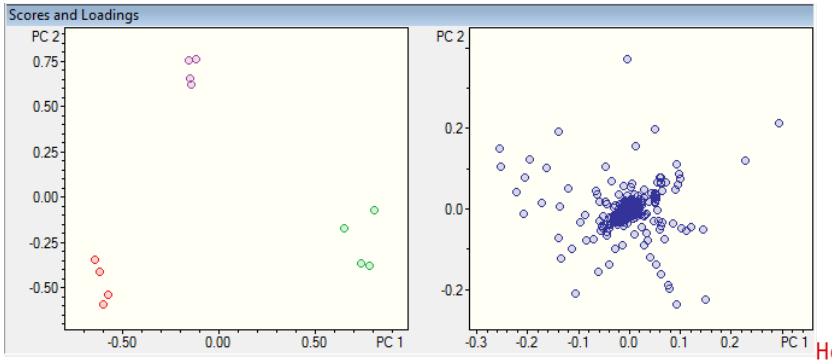
Retention index
1. Value: 1256 iu
Column Type: Capillary
Column Class: Standard non-polar
Active Phase: CP-Sil PONA GB
Column Length: 100 m
Carrier Gas: He
Column Diameter: 0.25 mm
Phase Thickness: 0.25 um
Data Type: Linear RI
Progcmn Type: Ramp
Start T: 140 C
End T: 240 C
Heat Rate: 1 K/min
Start Time: 10 min
End Time: 15 min
Source: Cunleay, M.M.; Lopez, A.E.; Coco, L.C.; Yamamoto, C.I.; Pinchemel, E.C.B.; Miguel, M.D.; Junior, A.G.; Auer, C.G.; Miguel, O.G., Phytochemical and antibacterial evaluation of essential oils from Ocotonia Martiana Miq. (Piperaceae), J. Braz. Chem. Soc., 18(1), 2007, 184-188.

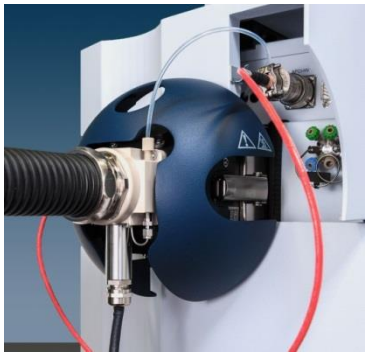
No hit in EI library search?

Metabolite Identification

GC-MS MetID – GC-APCI and exact mass







GC-APCI-TOF/MS coupling

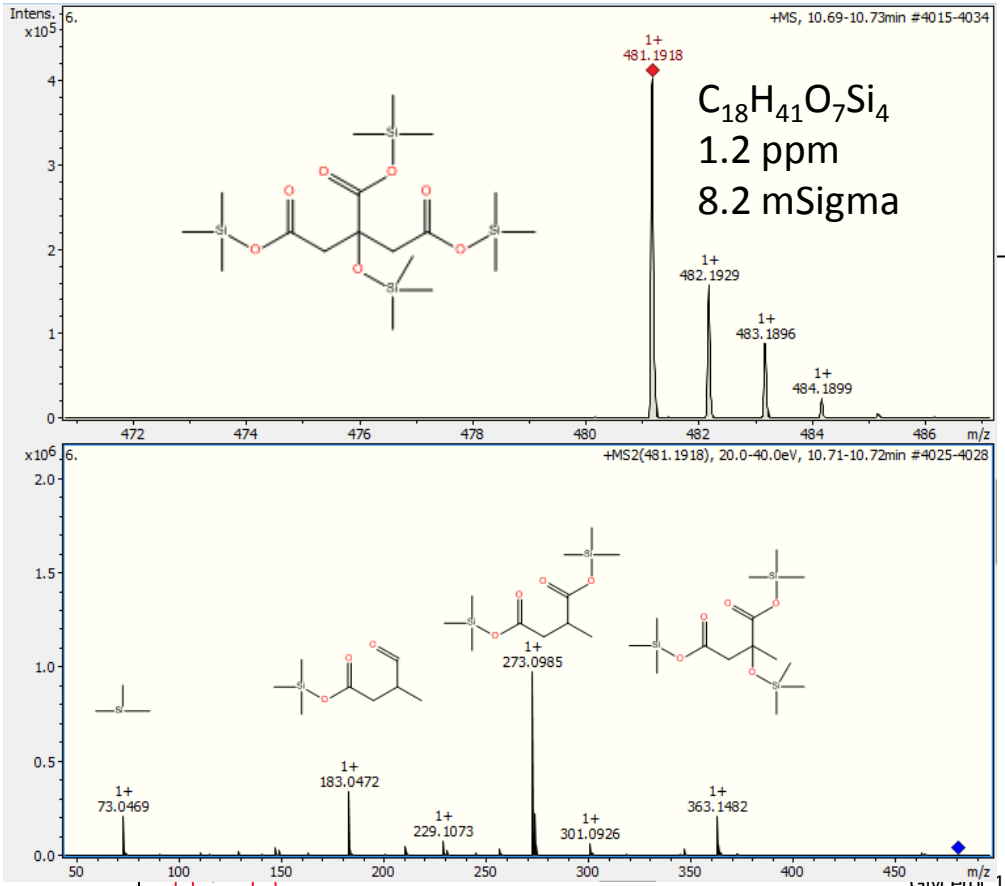
- Soft ionization
- Molecular ion preserved
- High resolution data
- ID by SmartFormula

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

Technische Universität München

Metabolite Identification

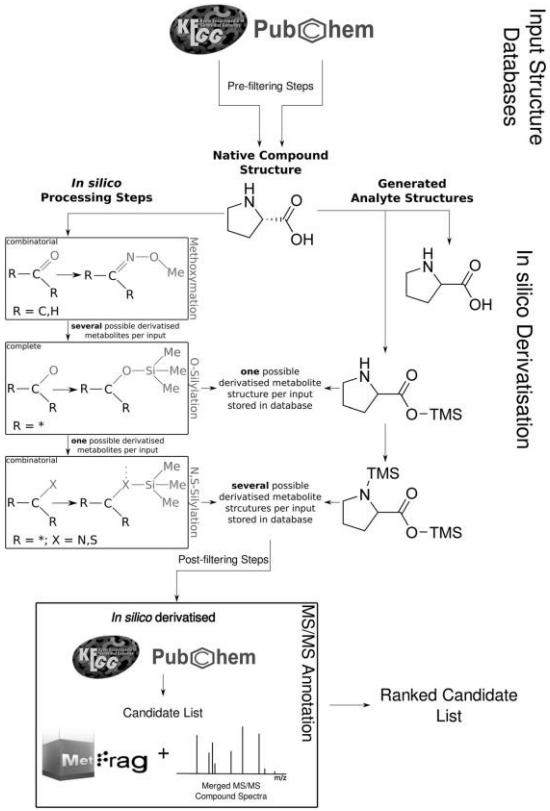
GC-MS MetID – GC-APCI and exact mass

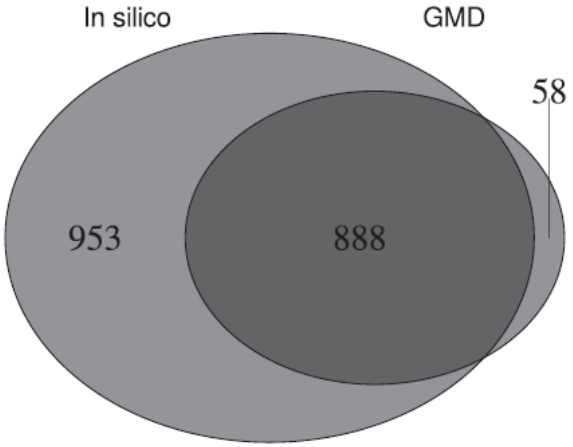


Metabolite Identification

GC-MS MetID – GC-APCI and exact mass

- GC-MS spectral databases still contain only a limited number of APCI-QToF spectra
- Ruttkies et al. developed a workflow based on in silico derivatization of metabolites and in silico fragmentation to identify potential candidate structures





Ruttkies et al., Rapid Commun. Mass Spectrom. 2015, 29, 1521–1529

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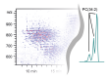
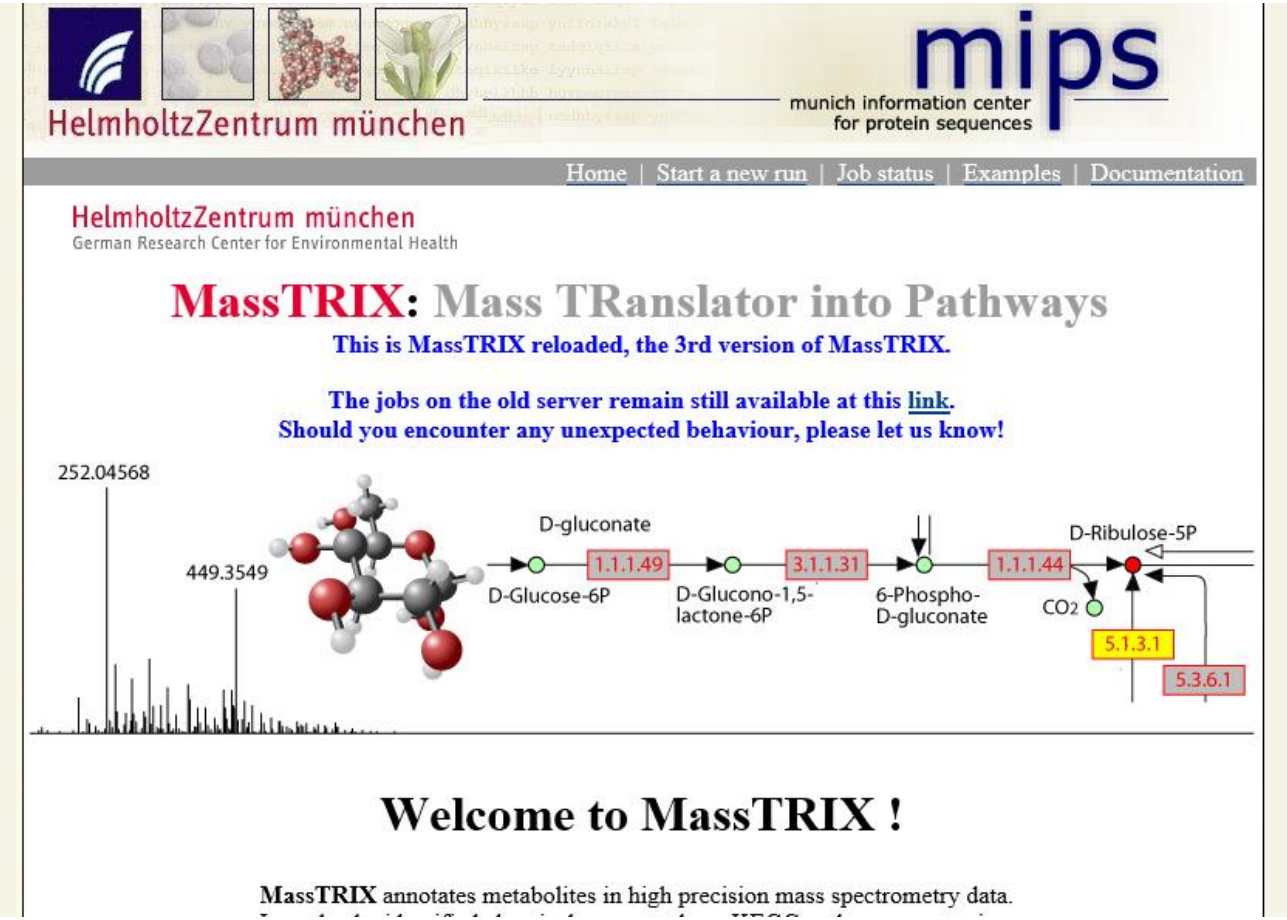


Metabolite Identification

LC-MS MetID - *Metabolite / Ion annotation*



www.masstrix.org

Metabolite Identification

LC-MS MetID - *Metabolite / Ion annotation*

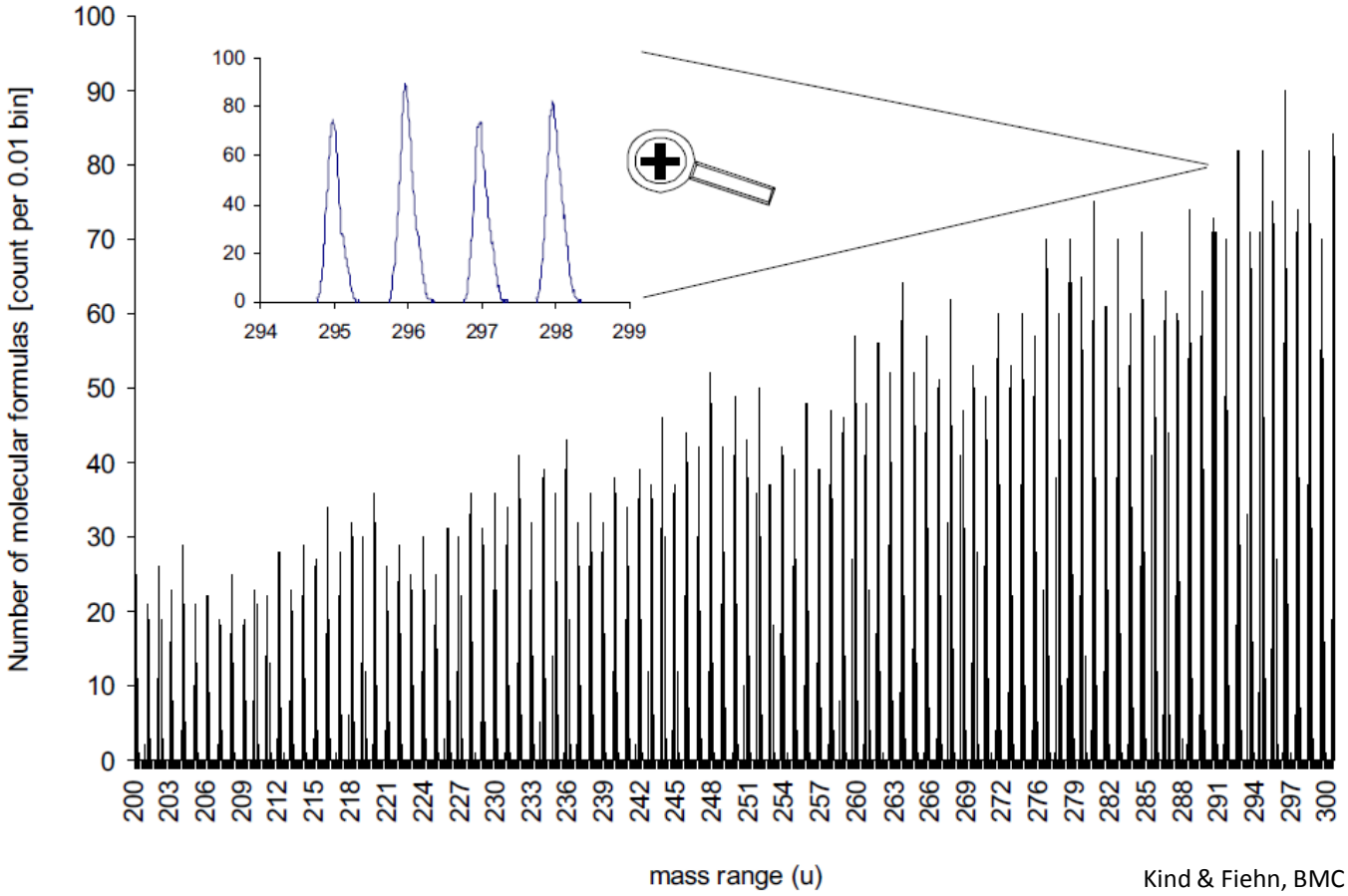
Table 1: Example of a molecular formula search for $C_{15}H_{12}O_7$ in different chemical databases. Search date: July 2007

Database name	Compounds found	Total database entries
Chemical Abstracts (CAS)	181	24,000,000
Beilstein Database (MDL)	166	8,000,000
Dictionary of Natural Products (DNP)	129	170,000
PubChem (NIH)	19	800,000
Available Chemicals Directory (MDL)	6	400,000
ChemIDplus (NIH)	6	370,000
KEGG (Kyoto University)	3	13,000
NIST05 (NIST mass spectral database)	2	163,000
MOLGEN molecular isomer generator (allowing 2 benzene groups; 1 ether group, 1 keto group; 5 hydroxy groups)	788,000	-

Kind & Fiehn, BMC Bioinformatics 2006, 7:234

Metabolite Identification

LC-MS MetID - *Metabolite / Ion annotation*



Kind & Fiehn, BMC Bioinformatics 2006, 7:234

Metabolite Identification

LC-MS MetID - *Metabolite / Ion annotation*

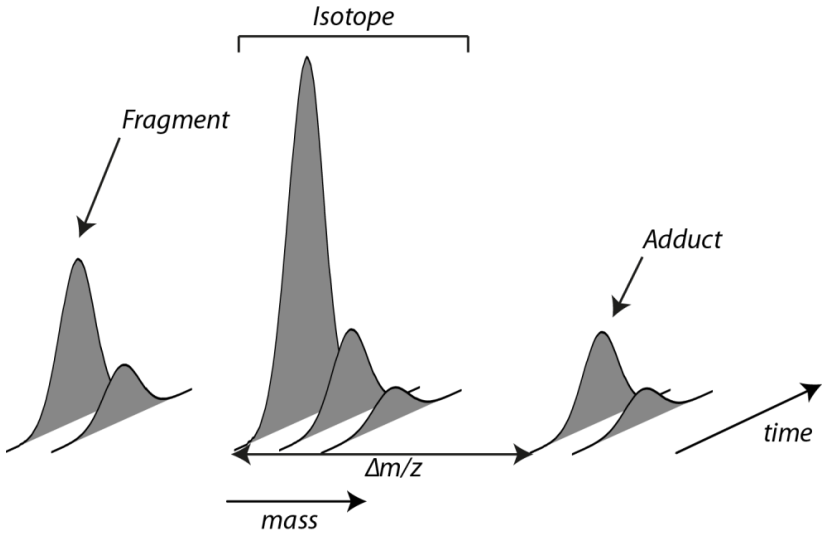
Table 3: Number of possible molecular formulas at different levels of mass accuracy and the impact of isotopic abundance accuracy. A mass spectrometer capable of 3 ppm but with 2% correct isotopic pattern outperforms even a (non-existing) mass spectrometer with 0.1 ppm mass accuracy! The results are computed for randomly selected targets, so single results vary but the trend remains. LEWIS and SENIOR check was applied. Candidates with unrelated high element counts were already excluded

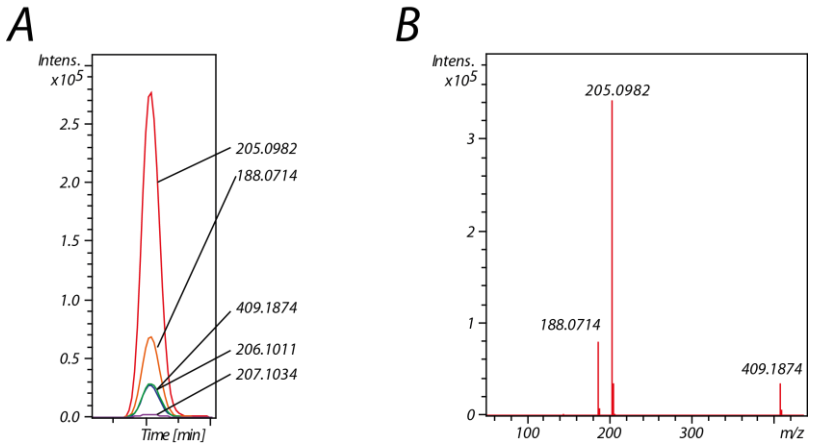
	without isotope abundance information					2% isotopic abundance accuracy	5% isotopic abundance accuracy
molecular mass [Da]	10 ppm	5 ppm	3 ppm	1 ppm	0.1 ppm	3 ppm	5 ppm
150	2	1	1	1	1	1	1
200	3	2	2	1	1	1	1
300	24	11	7	2	1	1	6
400	78	37	23	7	1	2	13
500	266	115	64	21	2	3	33
600	505	257	155	50	5	4	36
700	1046	538	321	108	10	10	97
800	1964	973	599	200	20	13	111
900	3447	1712	1045	345	32	18	196

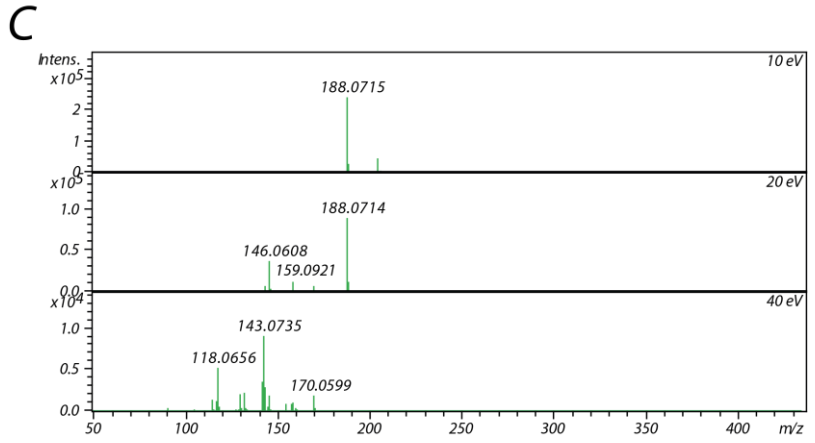
Kind & Fiehn, BMC Bioinformatics 2006, 7:234

Metabolite Identification

LC-MS MetID - *Metabolite / Ion annotation*



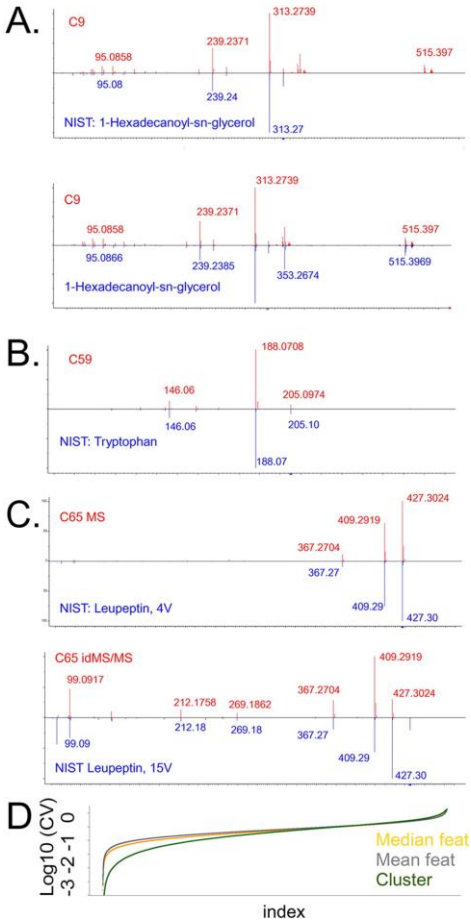




Metabolite Identification

LC-MS MetID - *Metabolite / Ion annotation*

- MS signals from the same metabolite co-elute and co-vary
- Correlation analysis can be used to identify in-source fragments, multimers
- The yielded mass spectra can be used for search in different spectral databases
- Based on the same approach, indiscriminant MS/MS can be used to generate fragmentation for every eluting compound

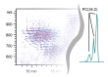


Broeckling et al., Anal. Chem., 2014, 86 (14), pp 6812–6817

Outline

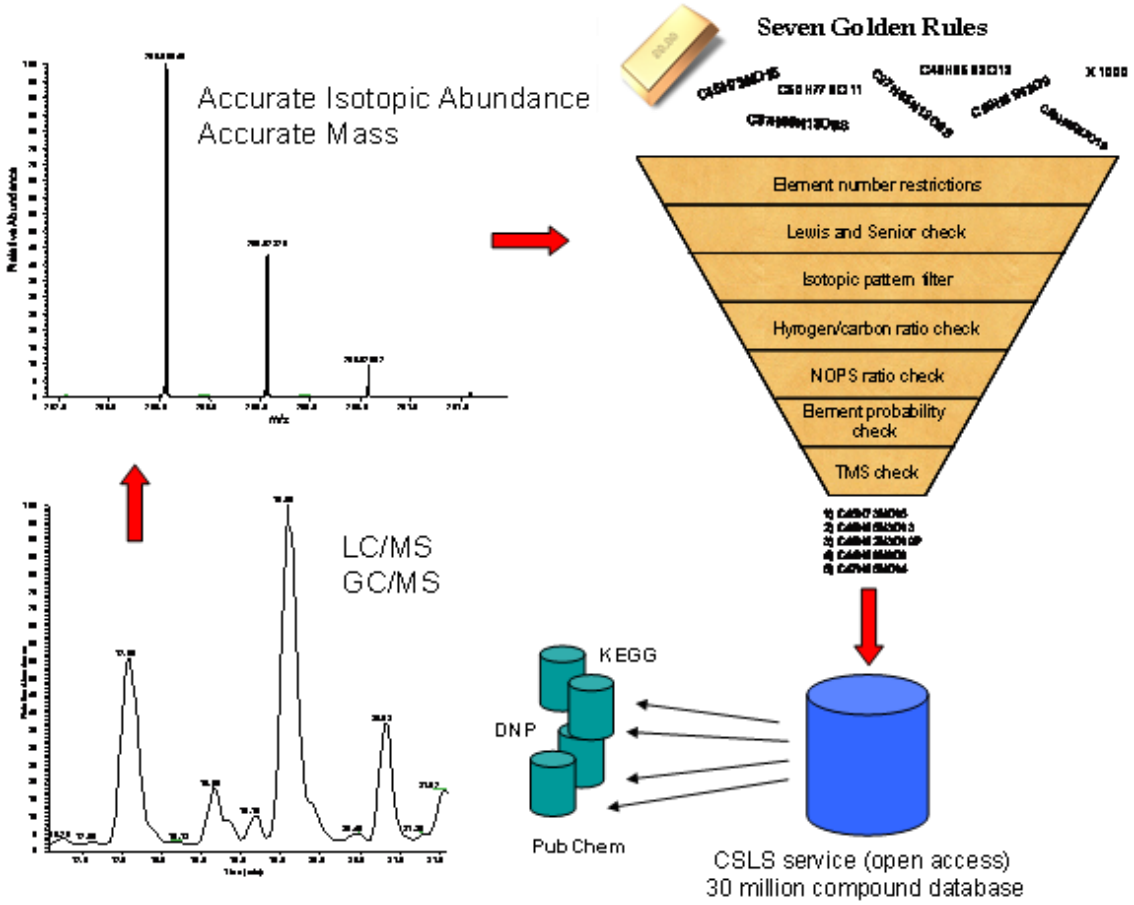
A path to metabolite identification

- Introduction
  - Analytical Technologies
  - Terminology in metabolite identification
- Metabolite identification
  - GC-MS MetID
    - EI spectral deconvolution
    - GC-APCI and exact mass
  - LC-MS MetID
    - Metabolite / Ion annotation
    - Formula calculation
    - Utilization of isotopes for formula calculation
    - MS/MS and RT
- New approaches for MetID
  - Ion mobility
  - *in silico* approaches
- *de novo* identification



Metabolite Identification

LC-MS MetID – Formula calculation



Kind & Fiehn, BMC Bioinformatics 2007, 8:105

Metabolite Identification

LC-MS MetID – Formula calculation

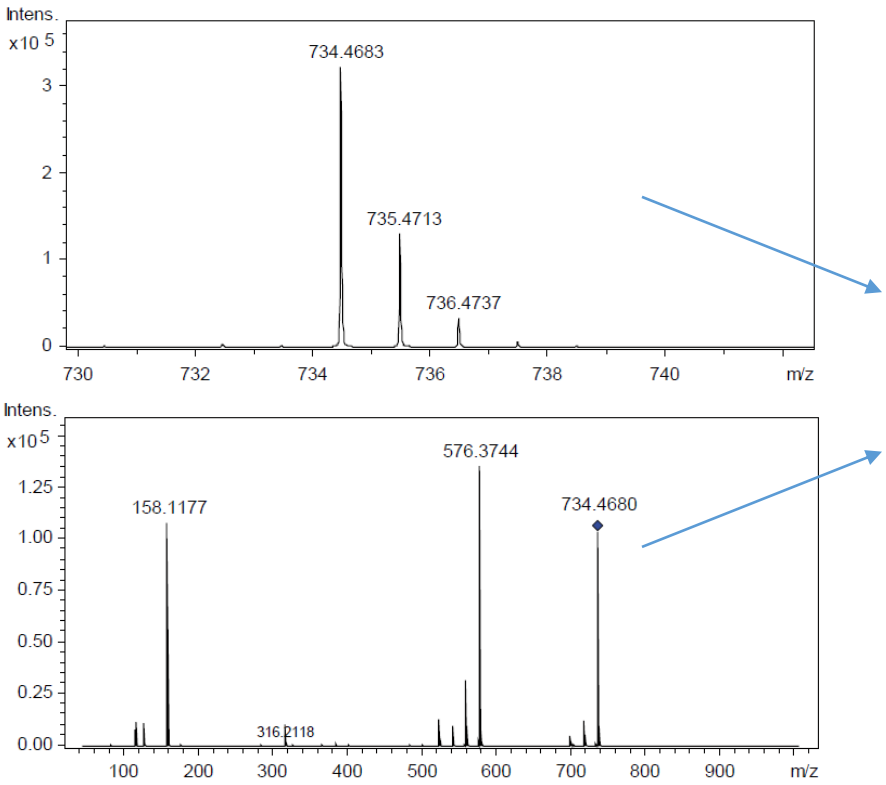
1. Apply heuristic restrictions for number of elements during formula generation
2. Perform LEWIS and SENIOR check
   - LEWIS rule: In its simplest form, the LEWIS rule demands that molecules consisting of main group elements, especially carbon, nitrogen and oxygen, share electrons in a way that all atoms have completely filled s, p-valence shells ('octet rule').
   - SENIOR rule: i) The sum of valences or the total number of atoms having odd valences is even. ii) The sum of valences is greater than or equal to twice the maximum valence. iii) The sum of valences is greater than or equal to twice the number of atoms minus 1.
3. Perform isotopic pattern filter
4. Perform H/C ratio check (hydrogen/carbon ratio)
5. Perform NOPS ratio check (N, O, P, S/C ratios)
6. Perform heuristic HNOPS probability check (H, N, O, P, S/C high probability ratios)
7. Perform –TMS check (for GC-MS if a silylation step is involved)

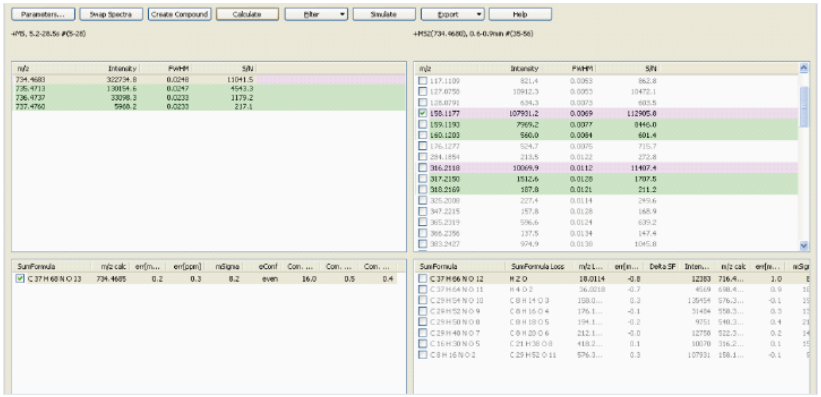
Kind & Fiehn, BMC Bioinformatics 2007, 8:105

Metabolite Identification

LC-MS MetID – Formula calculation

- Formula calculation can be enhanced by using results from MS/MS
- Sum of fragment sum formula +/- neutral losses = parent sum formula





Outline

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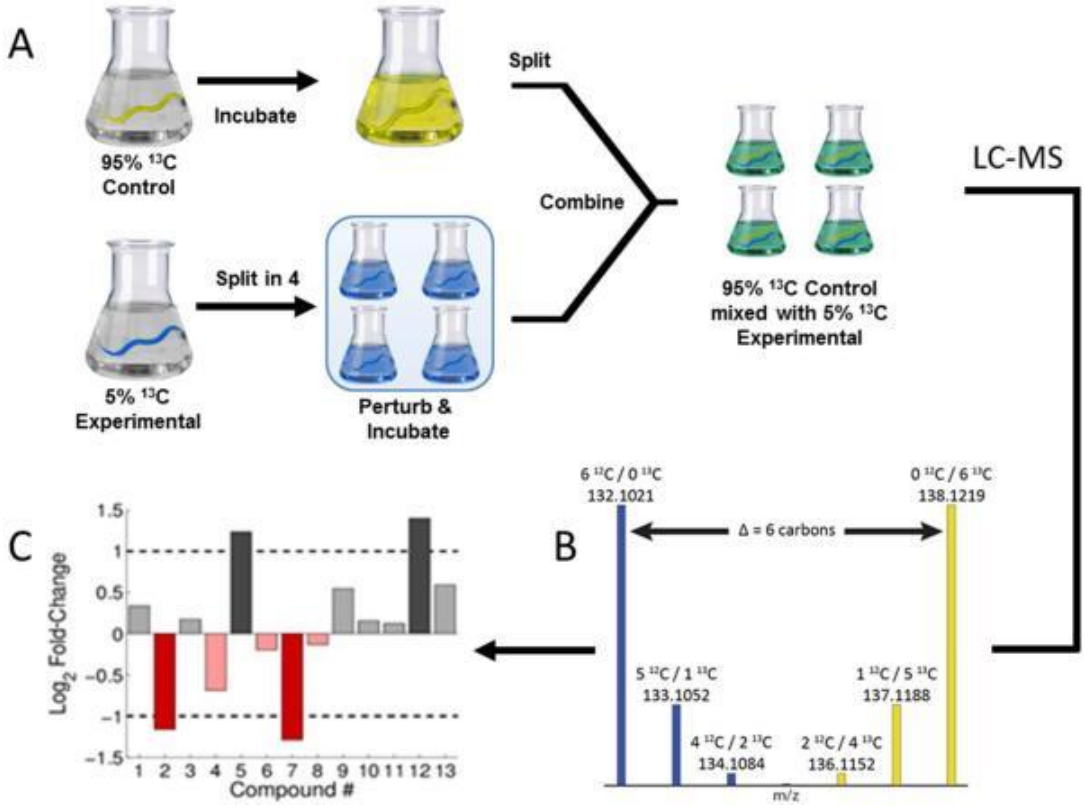
    - GC-APCI and exact mass
  - LC-MS MetID
    - Metabolite / Ion annotation
    - Formula calculation / seven golden rules
    - Utilization of isotopes
    - MS/MS and RT
- New approaches for MetID
  - Ion mobility
  - *in silico* approaches
- *de novo* identification

Metabolite Identification

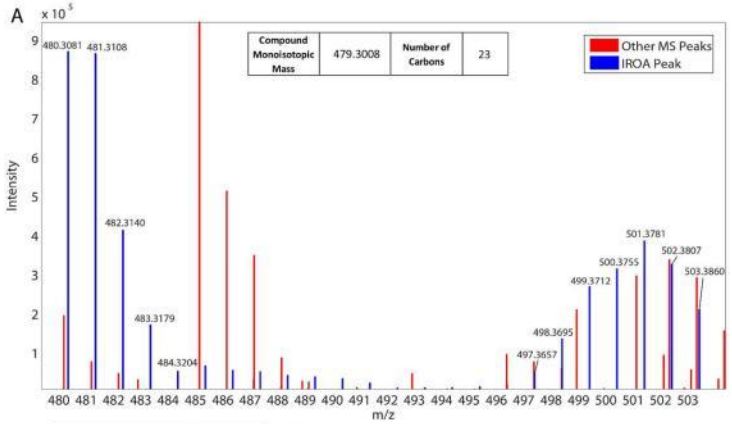
LC-MS MetID – Utilization of isotopes



Stupp et al., Anal. Chem. 2013, 85, 11858−11865

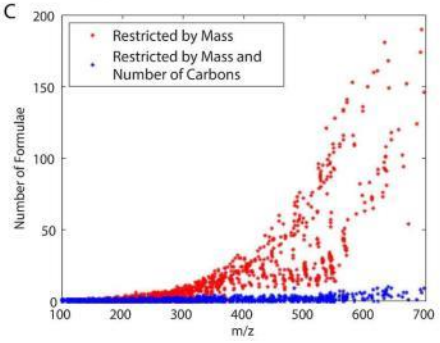
Metabolite Identification

LC-MS MetID – Utilization of isotopes



B

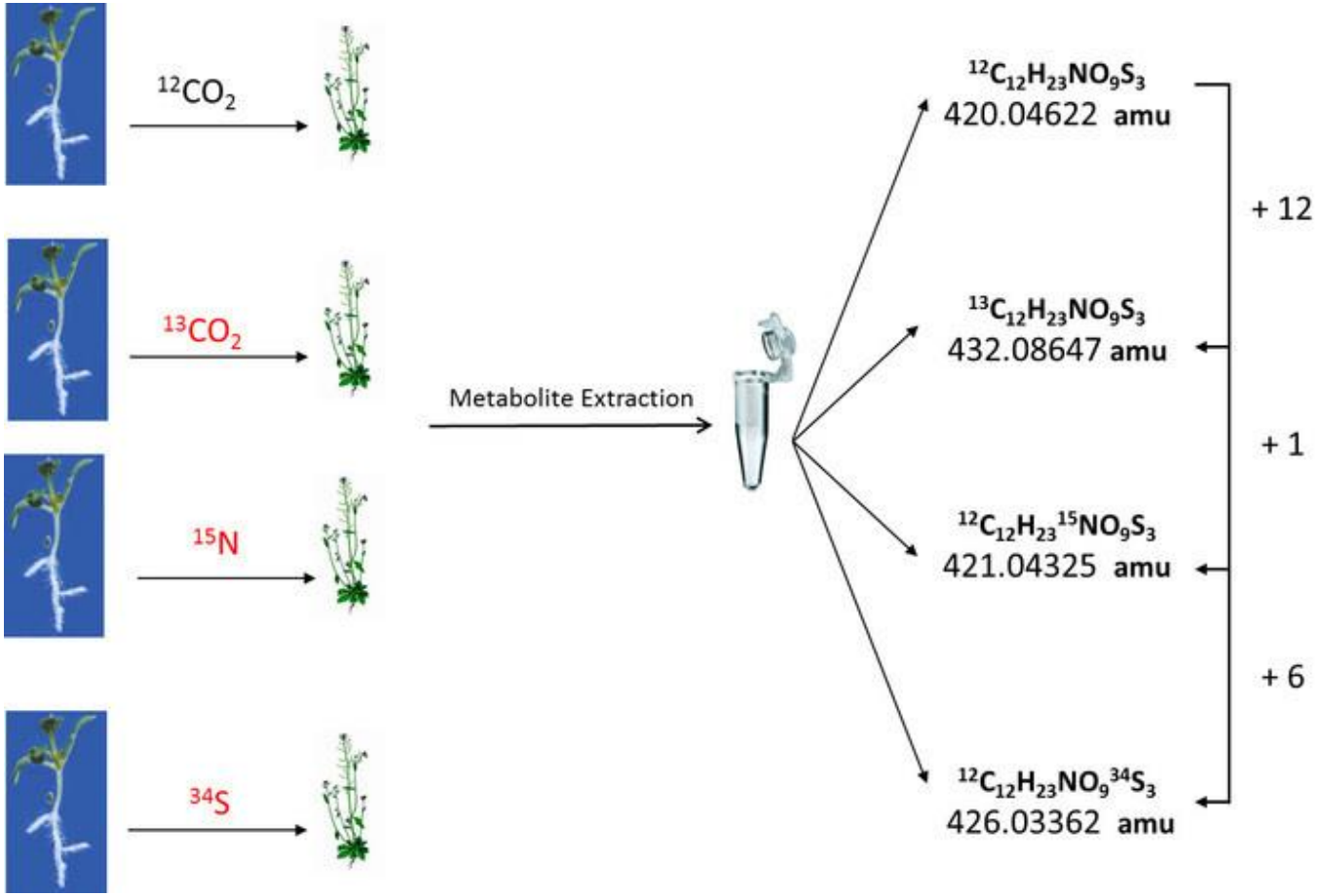
Molecular Formula	Formula Mass	PPM Error
$C_{26}H_{37}N_7O_2$	479.3009	0.17
$C_{17}H_{41}N_{15}O_2S_2$	479.3009	0.17
$C_{18}H_{51}N_9OP_2S_2$	479.3010	0.38
$C_{19}H_{54}N_9P_5$	479.3006	0.46
$C_{18}H_{59}NP_2S_4$	479.3006	0.46
$C_{17}H_{44}N_{13}OP_3$	479.3005	0.67
$C_{23}H_{46}NO_7P$	479.3012	0.79
$C_{26}H_{45}N_9OS_2$	479.3004	0.88
$C_{21}H_{49}N_{13}OS_4$	479.3004	0.88
$C_{11}H_{33}N_{15}O_3$	479.3014	1.21
$C_{17}H_{47}N_9O_6P_2$	479.3002	1.29
$C_{18}H_{43}N_9O_4S$	479.3002	1.29
$C_{18}H_{43}N_9O_3P_2$	479.3015	1.42
$C_{19}H_{37}N_{13}S$	479.3016	1.63
$C_{20}H_{37}N_{15}O_7$	479.3000	1.71
$C_{27}H_{48}NP_3$	479.3000	1.71
$C_{17}H_{12}N_9P_3S_2$	479.3000	1.71



Stupp et al., Anal. Chem. 2013, 85, 11858−11865

Metabolite Identification

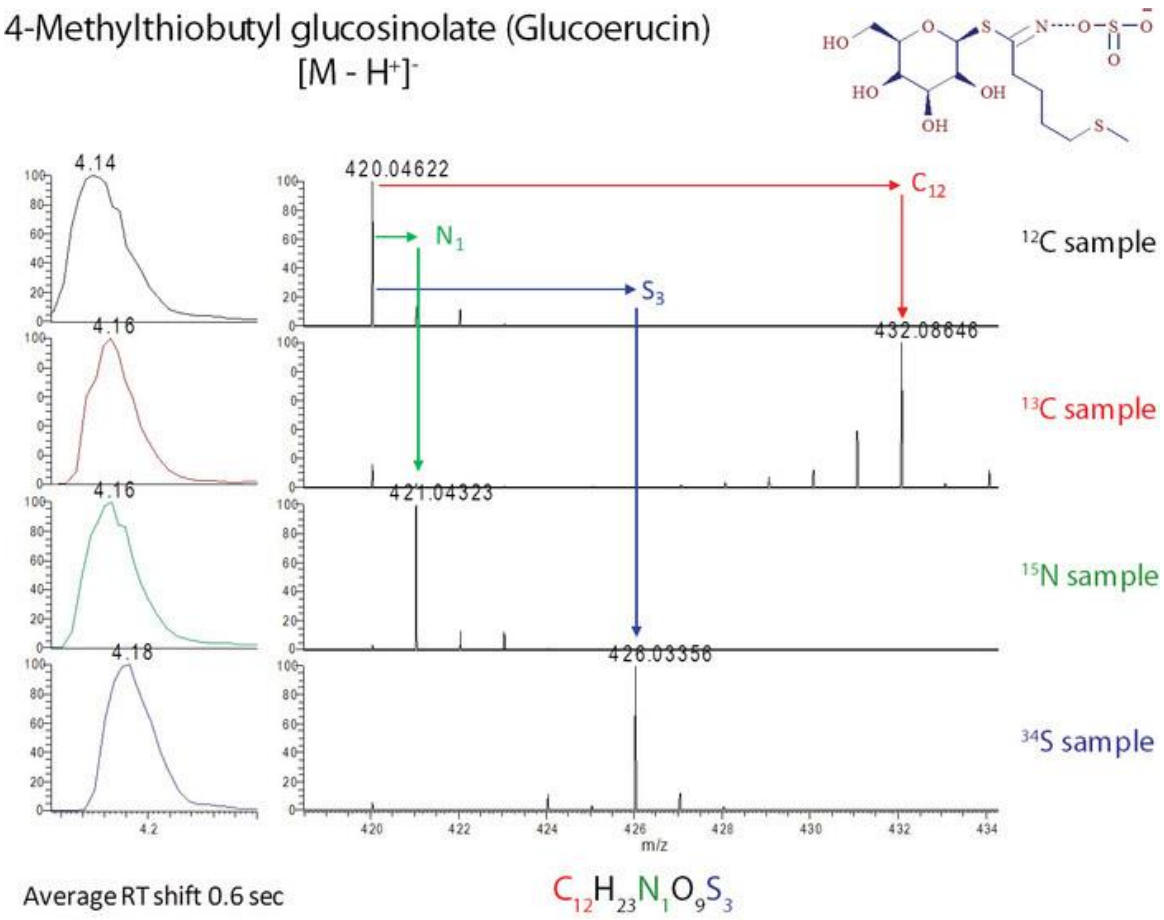
LC-MS MetID – Utilization of isotopes



Patrick Giavalisco et al., The Plant Journal (2011) 68, 364–376

Metabolite Identification

LC-MS MetID – Utilization of isotopes



Patrick Giavalisco et al., The Plant Journal (2011) 68, 364–376

Metabolite Identification

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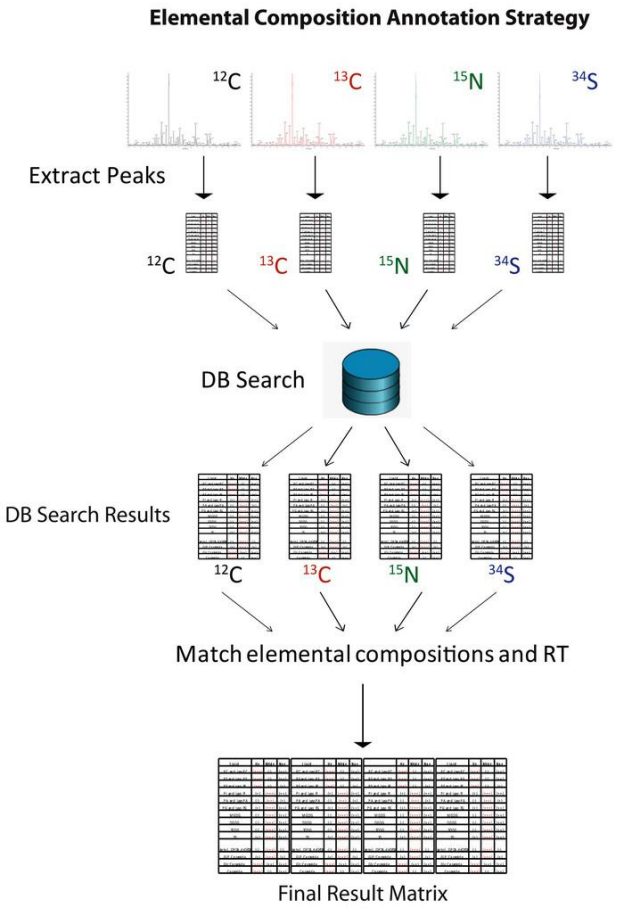

Patrick Giavalisco et al., The Plant Journal (2011) 68, 364–376

Metabolite Identification

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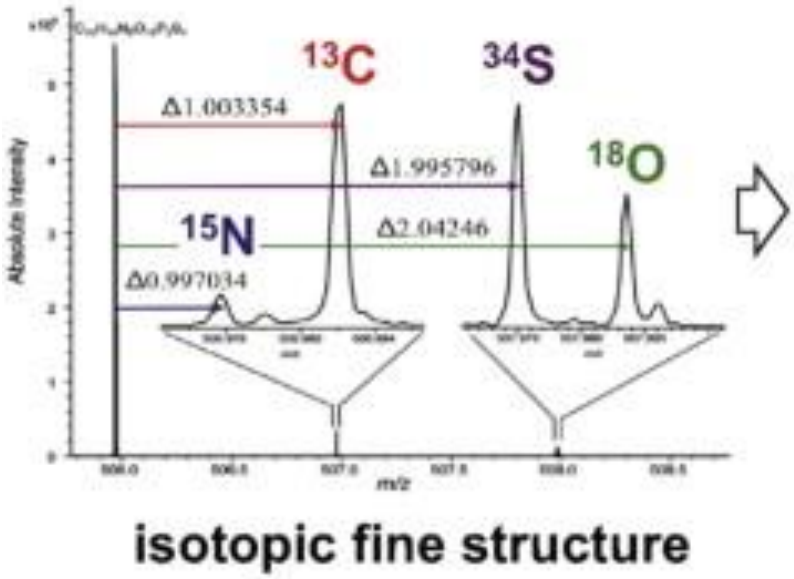
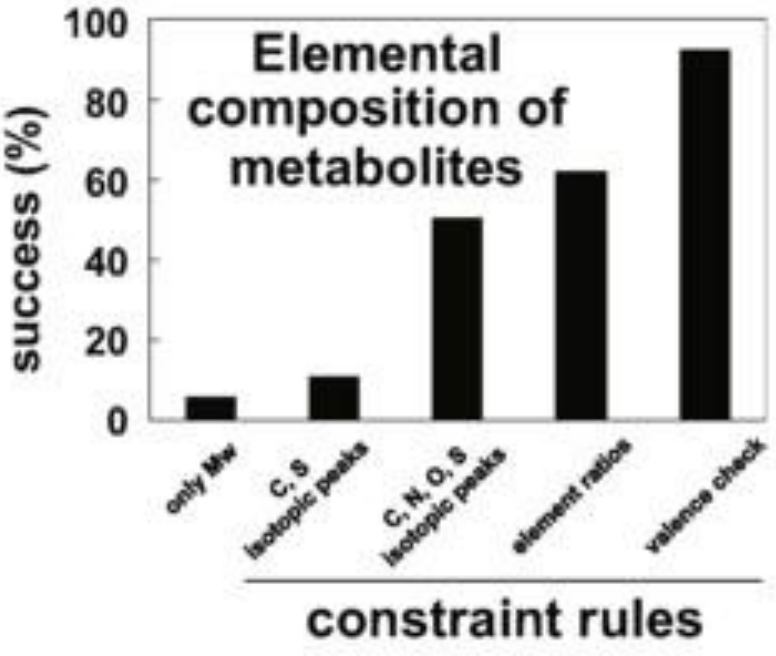


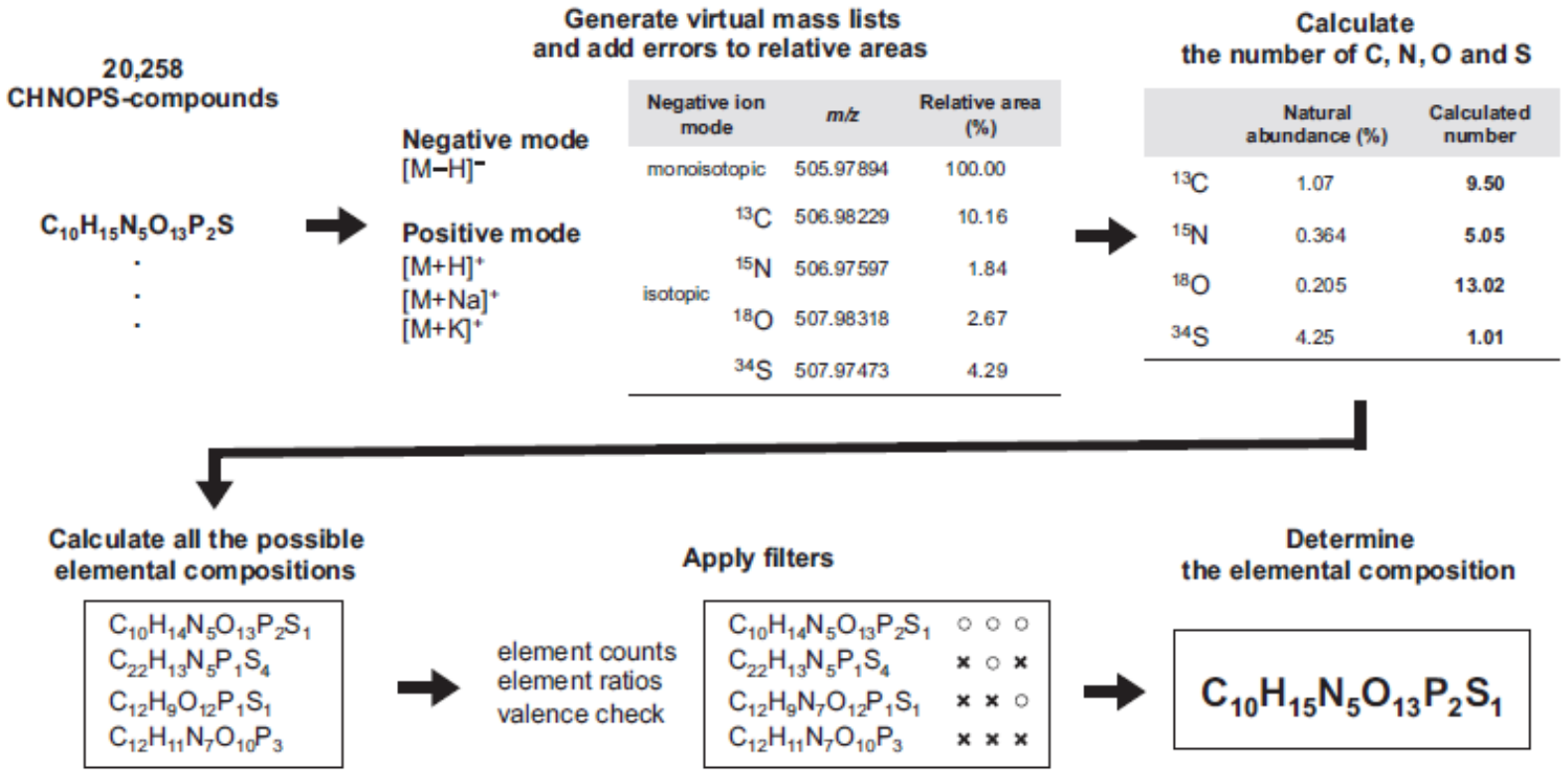
isotopic fine structure



T. Nagao et al., Analytica Chimica Acta 813 (2014) 70–76

Metabolite Identification

LC-MS MetID – Utilization of isotopes



T. Nagao et al., Analytica Chimica Acta 813 (2014) 70–76

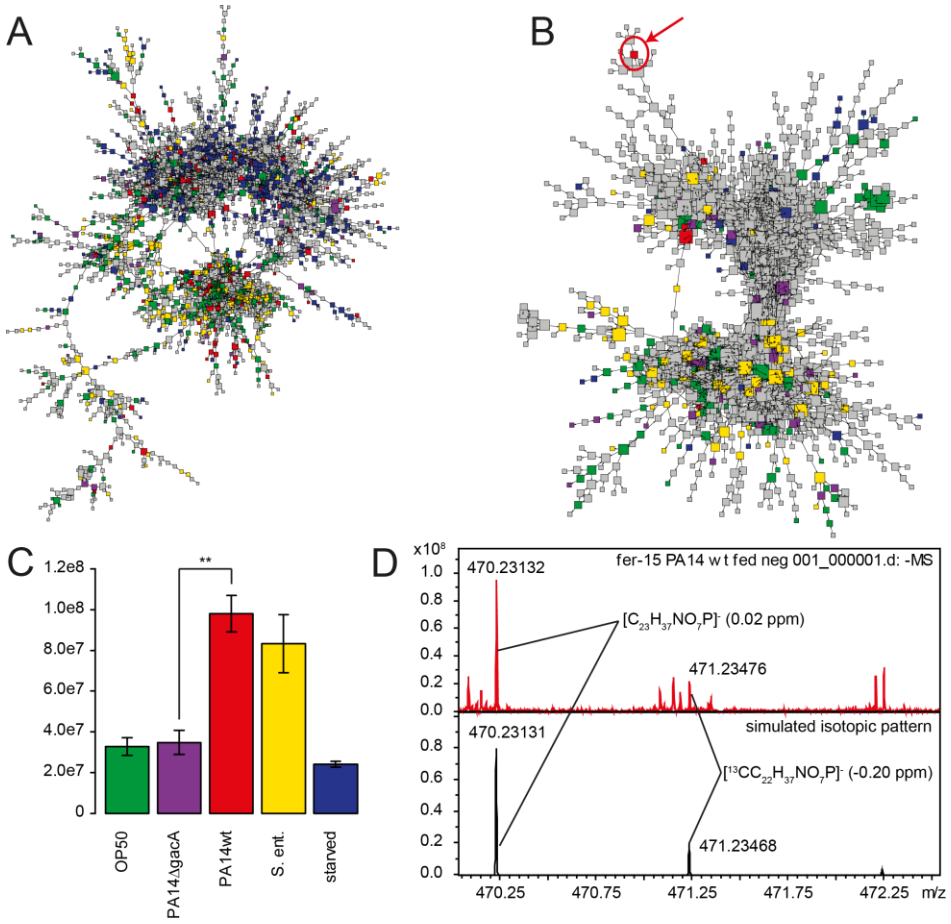
Metabolite Identification

LC-MS MetID – Utilization of isotopes

Element	Isotope	Abundance (%)	Mass	Massdifference
H, hydrogen	^{1}H	99.985	1.007825	
C, carbon	^{12}C ^{13}C	98.93 1.07	12.000000 13.003355	 1.003355
N, nitrogen	^{14}N ^{15}N	99.632 0.368	14.003074 15.000109	 0.997035
O, oxygen	^{16}O ^{17}O ^{18}O	99.757 0.038 0.205	15.994915 16.999132 17.999160	 1.004217 2.004245
P, phosphorus	^{31}P	100	30.973762	
S, sulfur	^{32}S ^{33}S ^{34}S	94.93 0.76 4.29	31.972071 32.971459 33.967867	 0.999388 1.995796
Cl, chlorine	^{35}Cl ^{37}Cl	75.78 24.22	34.968853 36.965903	 1.99705

Metabolite Identification

LC-MS MetID – Utilization of isotopes



Witting et al., Anal Bioanal Chem (2015) 407:1059–1073

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

Technische Universität München

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    - MS/MS and RT
- New approaches for MetID
  - Ion mobility
  - *in silico* approaches
- *de novo* identification

Metabolite Identification

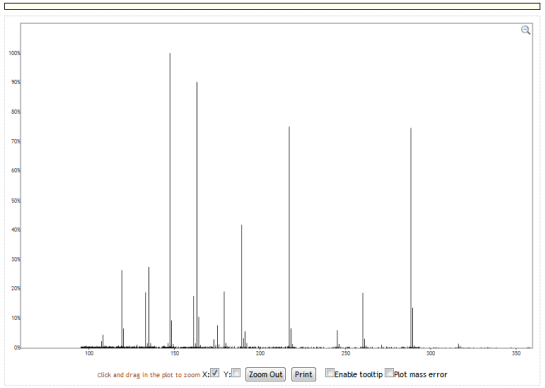
LC-MS MetID – MS/MS and RT

- Metabolomics Tandem MS databases
  - Metlin
  - MassBank of Japan
  - MassBank of Europe
  - MassBank of North America (Mona)
  - Global Natural Products Social Molecular Networking (GNPS)
  - Human Metabolome Database (HMDB)
  - RIKEN MSn spectral database (Respect)
- Overlap between all spectra (Splash http://splash.fiehnlab.ucdavis.edu/)
- *in silico tandem* solutions
  - MetFrag / MetFusion
  - CSI:FingerID
  - CFM-ID
  - MAGMa

Metabolite Identification

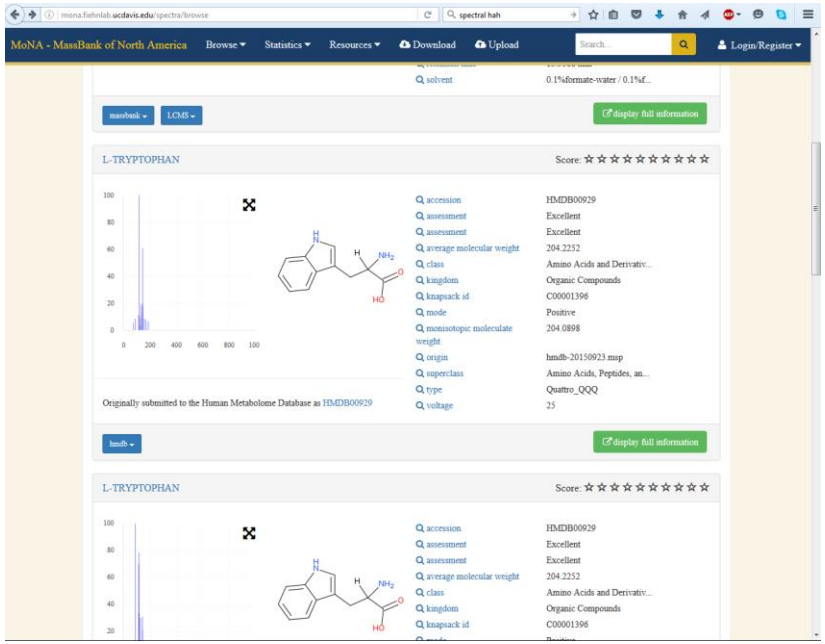
LC-MS MetID – MS/MS and RT

GNPS Library Spectrum CCMSLIB00000079354



Latest Library Spectrum Information

Spectrum ID	CCMSLIB00000079354
Compound Name	"MLS001158449-01!(2S)-2-[[(2S)-3-methyl-2-[[(2S)-3-oxo-2-propan-2-yl-2,4-dihydroquinoxaline-1-carbonyl]amino]butanoyl]amino]-3-phenylpropanoic acid"
PI	Dorrestein
Data Collector	VVP/LMS
CAS Number	N/A
Original Submitter	vphelan
Most Recent Revisor	vphelan
Library Quality	Gold Spectrum
Smiles	CC(C)[C@H](NC(=O)N1[C@@H](C(C)C)C(=O)Nc2ccccc12)C(=O)N[C@@H](Cc3ccccc3)C(=O)O
InChI	N/A
Structure	Chiral
Precursor M/Z	481.245
Exact Mass	480.237

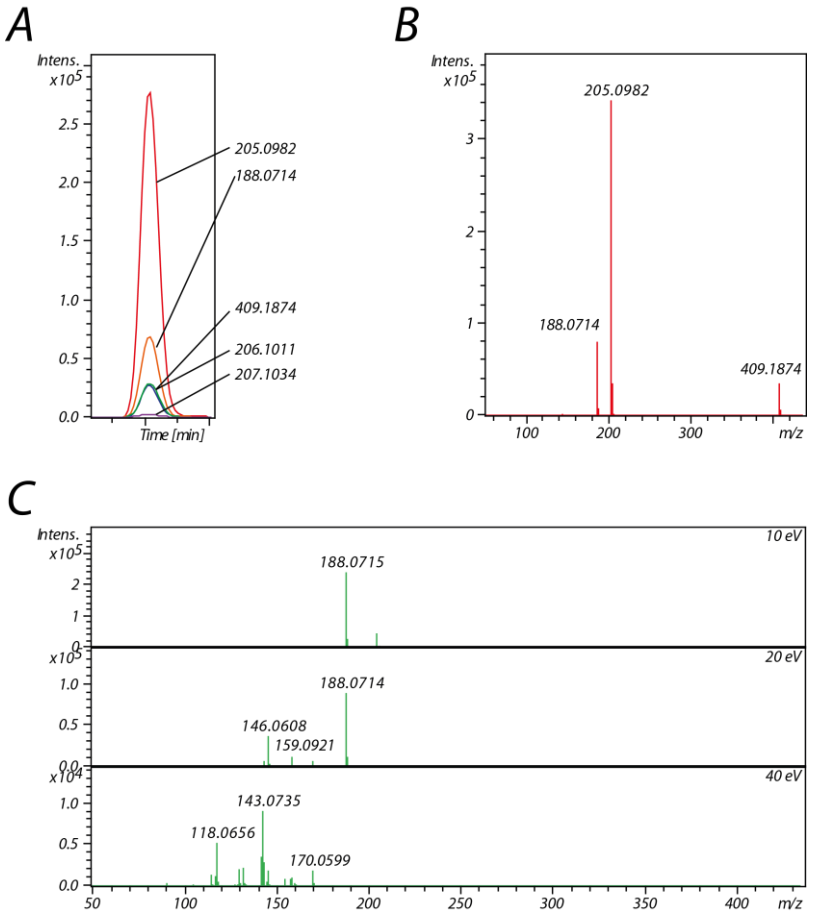


https://gnps.ucsd.edu/ProteoSAFe/gnpslibraryspectrum.jsp?SpectrumID=CCMSLIB00000079354&#{}

http://mona.fiehnlab.ucdavis.edu/spectra/browse

Metabolite Identification

LC-MS MetID – MS/MS and RT



Metabolite Identification

LC-MS MetID – MS/MS and RT

Trypto10eVpos.mgf | Trypto20eVpos.mgf | Trypto40eVpos.mgf

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CHARGE=1+, 2+ and 3+
### D:\Users\michael.witting\Desktop\Trypto10eVpos.mgf
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### MRM_1A5_1-A,5_01_5202.d
### Instrument: maXis
###    MRM_1A5
###    S/N 3.0, RelIntensity 0.0, MinimumIntensity 100, Centroid 80.0, UseCent
###    Retain Residuals no, Create neutrally no,
###    FullScan Parameters: AdductIon (+)+H (-)-H, LowMass 250, HighMass 4000,
###    Perf. isotope decon yes, Max Charge Istopic decon 0, MW agreement 0.00,
###    MaxRes   Parameters: AdductIon (+)+H (-)-H, LowMass 250, HighMass 4000
###    Perf. isotope decon yes, Max Charge Istopic decon 0, Abund. cutoff 2.00
###    MSn      Parameters: AdductIon (+)+H (-)-H, LowMass 100, HighMass 3000
###    Perf. isotope decon yes, Max Charge Istopic decon 0, Abund. cutoff 1.00
###    Global Charge Limit: yes 3, Prefer FullScan Result: yes, Export what: 1
### +MS2(205.0985), 10.0eV, 2.42min #1057

###FS:    #m/z: 205.09846 #charge   1
###MaxRes:#m/z:  0.00000 #charge=128
####MS:
###MSMS:
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RTINSECONDS=0
SCANS=MS:  MSMS:
PEPMASS=205.09846

159.09264   3888    1+
188.07206   264768  1+
205.09838   51752   1+
END IONS
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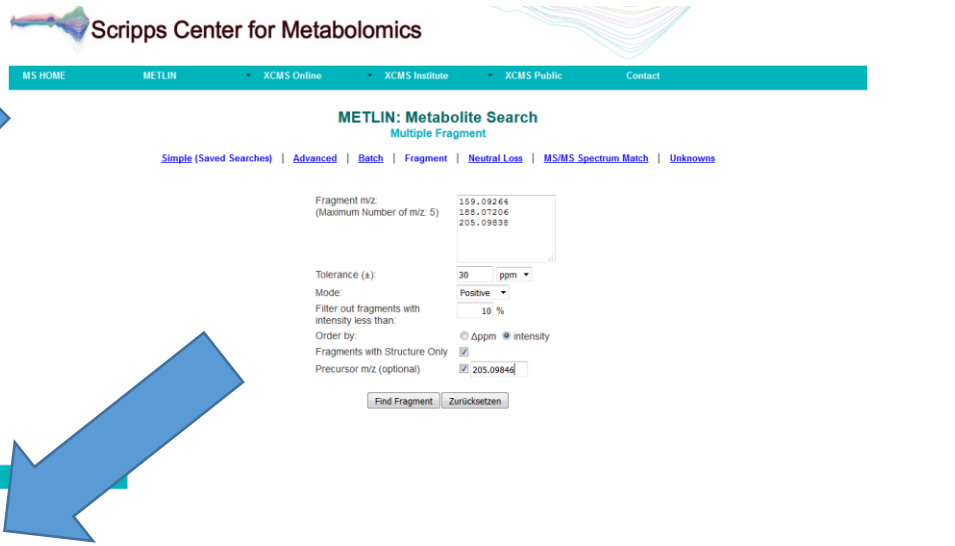
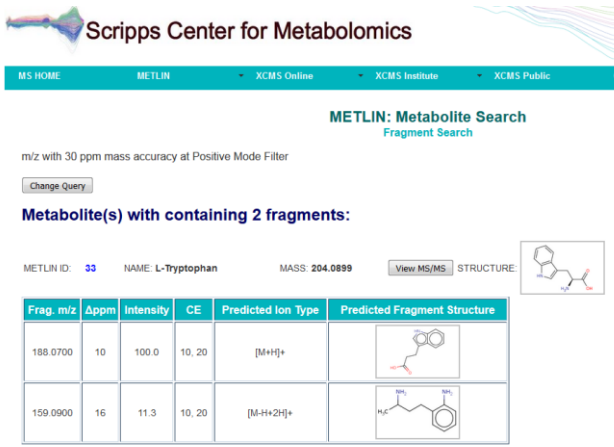
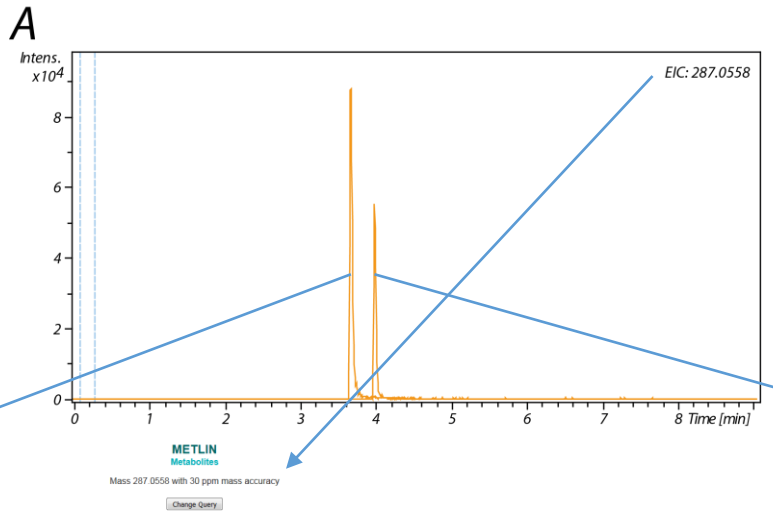
https://metlin.scripps.edu/index.php
https://metlin.scripps.edu/fragment_search_multi.php





Metabolite Identification

LC-MS MetID – MS/MS and RT

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###MSMS:
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RTINSECONDS=0
SCANS=MS:  MSMS:
PEPMASS=287.05577

135.04405   1788    1+
137.02522   676 2+
153.01855   8412    1+
161.02339   1480    1+
179.03415   816 1+
185.05838   1012    1+
203.03516   772 1+
213.05480   1352    1+
241.04922   4240    1+
245.04233   564 1+
259.06004   944 1+
269.04502   2540    1+
287.05611   34712   10+
287.11843   984 8+
288.05840   6092    7+
289.05988   1100    1+
END IONS
```

Total: 38 Metabolites

METLIN ID	MASS	Appm	NAME	MS/MS	STRUCTURE
69545	[M+Na]+ m/z 287.0558 M 264.0666	0	Ozagrel hydrochloride Formula: C13H13ClN2O2 CAS: 78712-43-3	NO	
50019	[M+H]+ m/z 287.0550 M 286.0477	2	3,7,8,4'-Tetrahydroxyflavone Formula: C15H10O6 CAS:	NO	
50040	[M+H]+ m/z 287.0550 M 286.0477	2	Datiscetin Formula: C15H10O6 CAS:	NO	
49785	[M+H]+ m/z 287.0550 M 286.0477	2	Isoscutellarein Formula: C15H10O6 CAS:	NO	
49369	[M+H]+ m/z 287.0550 M 286.0477	2	Norartocarpetin Formula: C15H10O6 CAS:	NO	

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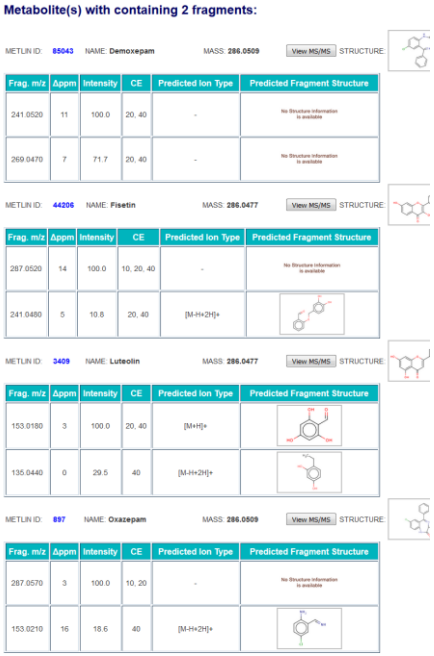
RTINSECONDS=0
SCANS=MS:  MSMS:
PEPMASS=287.05566

136.01759   716 1+
140.06141   332 1+
147.04301   456 1+
153.01913   4052    1+
153.06592   316 4+
154.04158   312 1+
157.06510   1060    1+
165.01940   2452    1+
178.02877   276 1+
185.06013   1268    1+
188.04982   392 1+
195.04595   280 1+
197.06170   280 1+
201.05693   384 1+
213.05481   2080    11+
229.04913   500 1+
231.06539   1144    1+
241.05134   2036    1+
244.03836   308 1+
258.05319   2328    1+
269.04640   676 1+
285.04744   292 1+
287.05561   13408   1+
287.17779   448 11+
END IONS
```

Metabolite Identification

LC-MS MetID – MS/MS and RT

3.69 minutes



Metabolite(s) with containing 2 fragments:

METLIN ID: 85043 NAME: Demoxepam MASS: 286.0509 View MS/MS STRUCTURE:

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
241.0520	11	100.0	20, 40	-	No Structure Information is available
269.0470	7	71.7	20, 40	-	No Structure Information is available

METLIN ID: 44206 NAME: Fisetin MASS: 286.0477 View MS/MS STRUCTURE:

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
287.0520	14	100.0	10, 20, 40	-	No Structure Information is available
241.0480	5	10.8	20, 40	[M-H+2H]+	

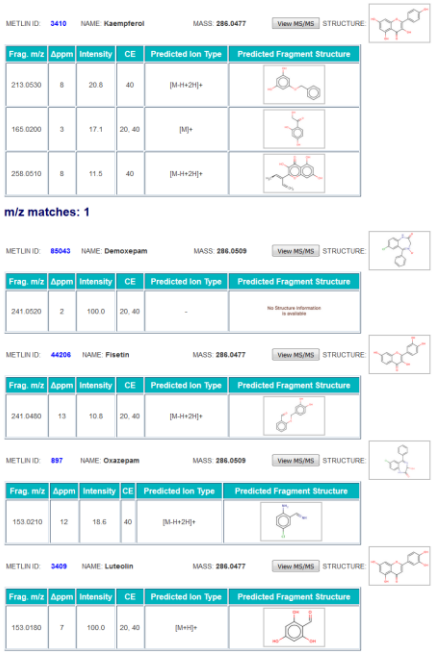
METLIN ID: 3409 NAME: Luteolin MASS: 286.0477 View MS/MS STRUCTURE:

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
153.0180	3	100.0	20, 40	[M+H]+	
135.0440	0	29.5	40	[M-H+2H]+	

METLIN ID: 897 NAME: Oxazepam MASS: 286.0509 View MS/MS STRUCTURE:

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
287.0570	3	100.0	10, 20	-	No Structure Information is available
153.0210	16	18.6	40	[M-H+2H]+	

4.01 minutes



METLIN ID: 3410 NAME: Kaempferol MASS: 286.0477 View MS/MS STRUCTURE:

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
213.0530	8	20.6	40	[M-H+2H]+	
165.0200	3	17.1	20, 40	[M]+	
258.0510	8	11.5	40	[M-H+2H]+	

m/z matches: 1

METLIN ID: 85043 NAME: Demoxepam MASS: 286.0509 View MS/MS STRUCTURE:

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
241.0520	2	100.0	20, 40	-	No Structure Information is available

METLIN ID: 44206 NAME: Fisetin MASS: 286.0477 View MS/MS STRUCTURE:

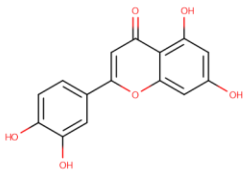
Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
241.0480	13	10.8	20, 40	[M-H+2H]+	

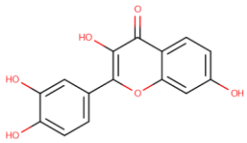
METLIN ID: 897 NAME: Oxazepam MASS: 286.0509 View MS/MS STRUCTURE:

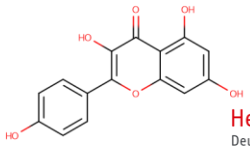
Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
153.0210	12	18.6	40	[M-H+2H]+	

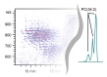
METLIN ID: 3409 NAME: Luteolin MASS: 286.0477 View MS/MS STRUCTURE:

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
153.0180	7	100.0	20, 40	[M+H]+	









Metabolite Identification

LC-MS MetID – MS/MS and RT

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

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###FS:    #m/z: 287.05577 #charge  10
###MaxRes:#m/z:  0.00000 #charge-128
###MS:
###MSMS:
BEGIN IONS
TITLE=+MS2(287.0558), 40.0eV, 3.69min #1672
RTINSECONDS=0
SCANS=MS:  MSMS:
PEPMASS=287.05577

135.04405   1788    1+
137.02522   676 2+
153.01855   8412    1+
161.02339   1480    1+
179.03415   816 1+
185.05838   1012    1+
203.03516   772 1+
213.05480   1352    1+
241.04922   4240    1+
245.04233   564 1+
259.06004   944 1+
269.04502   2540    1+
287.05611   34712   10+
287.11843   984 8+
288.05840   6092    7+
289.05988   1100    1+
END IONS
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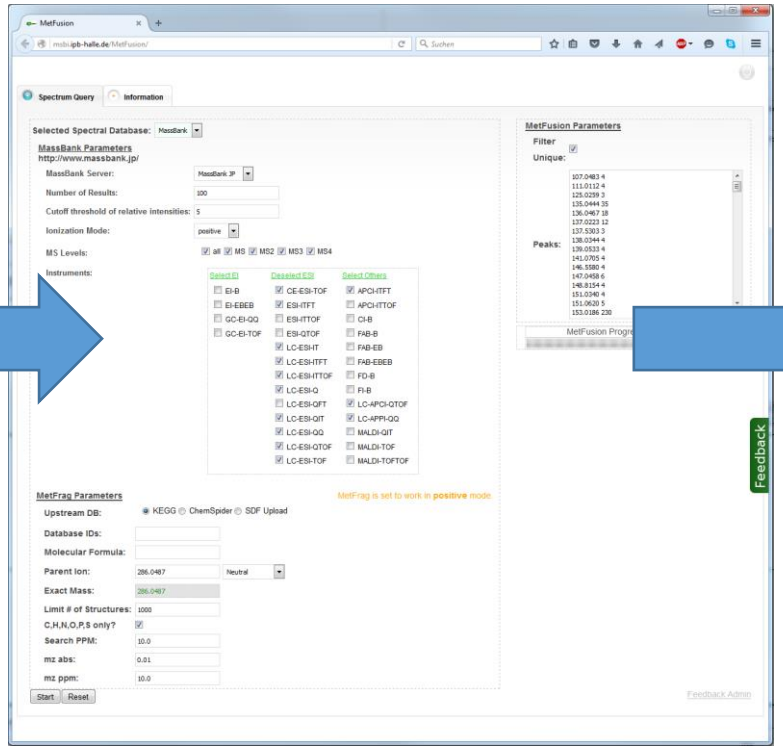
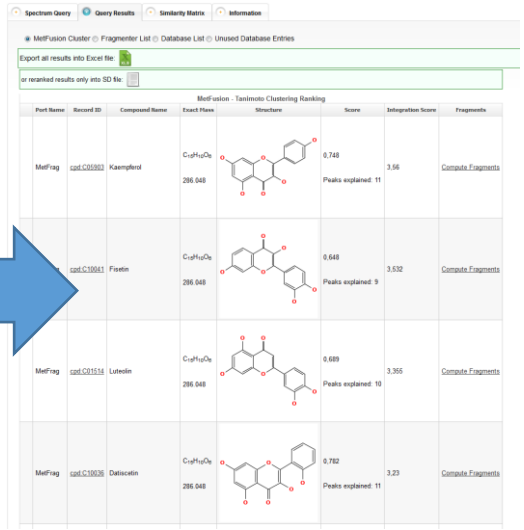
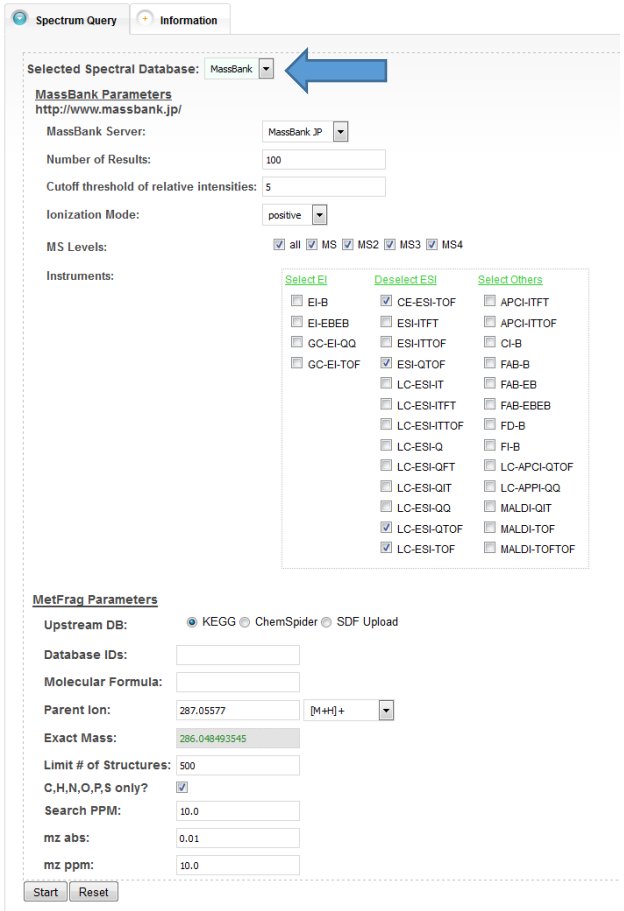


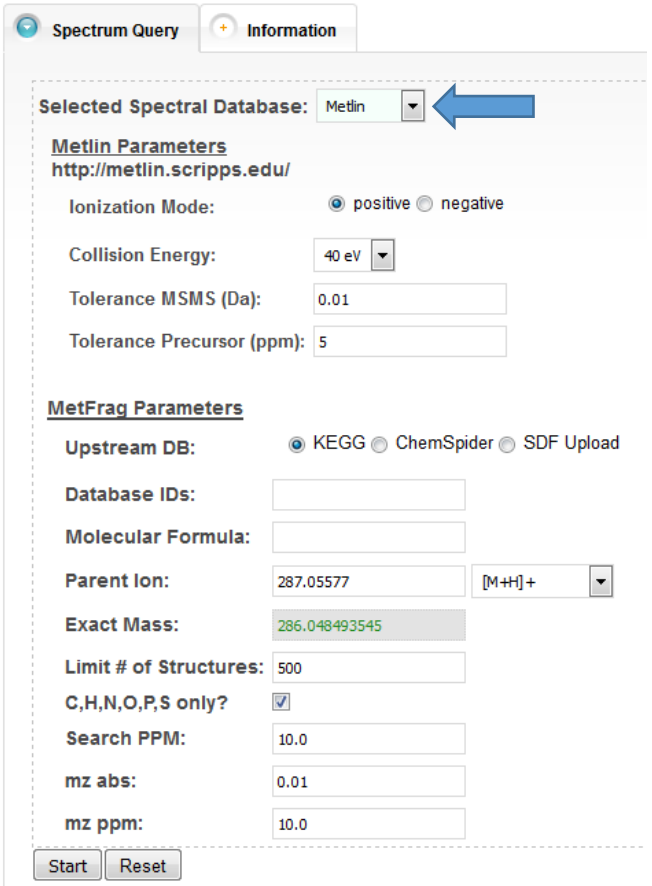


Gerlich et al., J. Mass Spectrom. 2013, 48, 291–298

Metabolite Identification

LC-MS MetID – MS/MS and RT



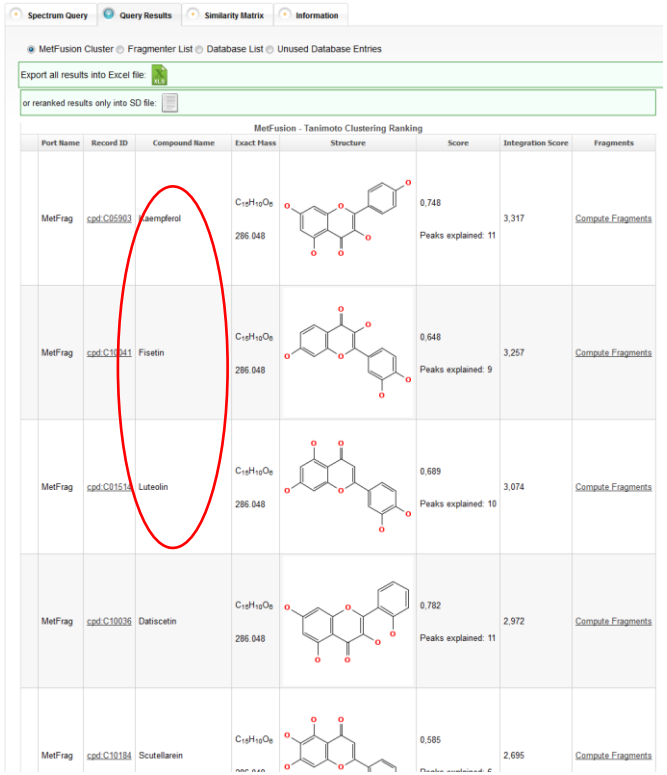


Gerlich et al., J. Mass Spectrom. 2013, 48, 291–298

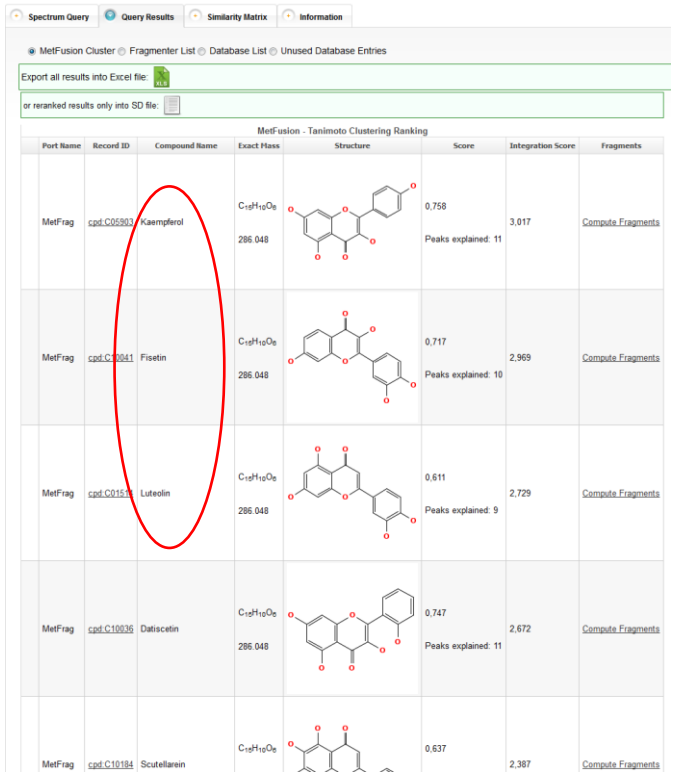
Metabolite Identification

LC-MS MetID – MS/MS and RT

3.69 minutes



4.01 minutes



Gerlich et al., J. Mass Spectrom. 2013, 48, 291–298

Metabolite Identification

LC-MS MetID – MS/MS and RT

3.69 minutes

Luteolin
Apigenin
Kaempferol

Datiscetin
Kaempferol
Luteolin

Spectrum Query | Query Results | Similarity Matrix | Information

Original Similarity Matrix | Reranked Similarity Matrix

MetFrag \ MassBank Compounds	PB000743	FIO00087	PB005705	FIO00762	PR020007	FIO00173	PR100243
cpd:C10036	0.673	0.783	0.885	0.23	0.258	0.597	0.569
cpd:C05903	0.752	0.872	1.0	0.225	0.253	0.616	0.587
cpd:C17786	0.509	0.415	0.434	0.199	0.259	0.365	0.347
cpd:C10510	0.528	0.494	0.492	0.218	0.268	0.407	0.424
cpd:C12134	0.501	0.456	0.49	0.213	0.248	0.391	0.417
cpd:C08720	0.516	0.549	0.495	0.221	0.237	0.428	0.446
cpd:C01514	1.0	0.742	0.752	0.23	0.256	0.511	0.541
cpd:C08576	0.576	0.549	0.553	0.22	0.237	0.443	0.467
cpd:C10041	0.742	1.0	0.872	0.232	0.26	0.593	0.571
cpd:C10184	0.881	0.664	0.714	0.222	0.262	0.497	0.524
cpd:C10097	0.85	0.647	0.694	0.224	0.264	0.492	0.518
cpd:C17810	0.304	0.291	0.282	0.206	0.231	0.273	0.27

12 candidates found, displaying 12 candidates from 1 to 12. Page 1 / 1

4.01 minutes

Kaempferol
Maritemein
Fisetin

Kaempferol
Datiscetin
Fisetin
Luteolin

Spectrum Query | Query Results | Similarity Matrix | Information

Original Similarity Matrix | Reranked Similarity Matrix

MetFrag \ MassBank Compounds	PB005706	PR100360	FIO00087	PB000743	PR100243	PR101007	PR040005
cpd:C05903	1.0	0.42	0.872	0.752	0.587	0.592	0.253
cpd:C10036	0.885	0.418	0.783	0.673	0.569	0.575	0.258
cpd:C10041	0.872	0.461	1.0	0.742	0.571	0.576	0.26
cpd:C10184	0.714	0.419	0.664	0.881	0.524	0.526	0.262
cpd:C01514	0.752	0.424	0.742	1.0	0.541	0.543	0.256
cpd:C08576	0.553	0.564	0.549	0.576	0.467	0.471	0.237
cpd:C08720	0.495	0.635	0.549	0.516	0.446	0.45	0.237
cpd:C10097	0.694	0.432	0.647	0.85	0.518	0.52	0.264
cpd:C17786	0.434	0.339	0.415	0.509	0.347	0.35	0.259
cpd:C10510	0.492	0.418	0.494	0.528	0.424	0.426	0.268
cpd:C12134	0.49	0.384	0.456	0.501	0.417	0.418	0.248
cpd:C17810	0.282	0.27	0.291	0.304	0.27	0.27	0.231

12 candidates found, displaying 12 candidates from 1 to 12. Page 1 / 1

Gerlich et al., J. Mass Spectrom. 2013, 48, 291–298

Metabolite Identification

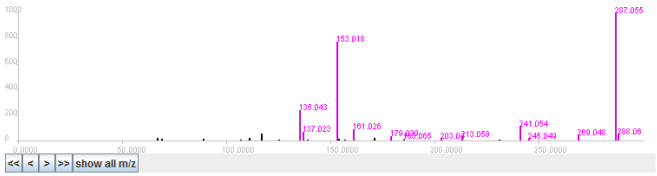
LC-MS MetID – MS/MS and RT

MassBank Record: PB000743

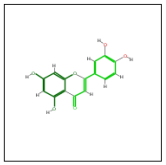
Home | Spectrum | Quick | Peak | Substructure | Prediction | Browser | Batch | Browse | Index | MassBank ID: Go

Luteolin; LC-ESI-QTOF; MS2; CE:40 eV; [M+H]+

Mass Spectrum



Chemical Structure



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ACCESSION: PB000743
RECORD_TITLE: Luteolin; LC-ESI-QTOF; MS2; CE:40 eV; [M+H]+
DATE: 2016.01.19 (Created 2008.03.23, modified 2013.06.04)
AUTHORS: Boettcher C, Institute of Plant Biochemistry, Halle, Germany
LICENSE: CC BY-SA
COMMENT: IPB_RECORD: 361
COMMENT: CONFIDENCE: confident structure

CH$NAME: Luteolin
CH$NAME: 5,7,3',4'-tetrahydroxy-flavone
CH$COMPOUND_CLASS: Natural Product; Flavone
CH$FORMULA: C15H10O6
CH$EXACT_MASS: 286.04774
CH$SMILES: C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O
CH$IUPAC: InChI=1S/C15H10O6/c16-8-4-11(19)15-12(20)6-13(21-14(15)5-8)7-1-2-9(17)10(18)3-7/h1-6,16-19H
CH$LINK: INCHIKEY IQPNAANSBPBGFQ-UHFFFAOYSA-N
CH$LINK: KEGG C01514
CH$LINK: PUBCHEM CID:5280445

AC$INSTRUMENT: API QSTAR Pulsar i
AC$INSTRUMENT_TYPE: LC-ESI-QTOF
AC$MASS_SPECTROMETRY: MS_TYPE MS2
AC$MASS_SPECTROMETRY: ION_MODE POSITIVE
AC$MASS_SPECTROMETRY: COLLISION_ENERGY 40 eV
AC$MASS_SPECTROMETRY: IONIZATION ESI

MS$FOCUSED_ION: PRECURSOR_TYPE [M+H]+

PK$SPLASH: splash10-1zw0000000-e245bb6cbc97af71cd5e
PK$NUM_PEAK: 25
PK$PEAK: m/z int. rel.int.
  67.018 257.309 24
  68.996 198.486 18
  89.038 161.870 15
  107.048 123.989 11
  111.007 233.672 22
  117.032 564.586 55
  125.020 104.437 9
```

Horai et al., J. Mass. Spectrom. 2010, 45, 703–714.

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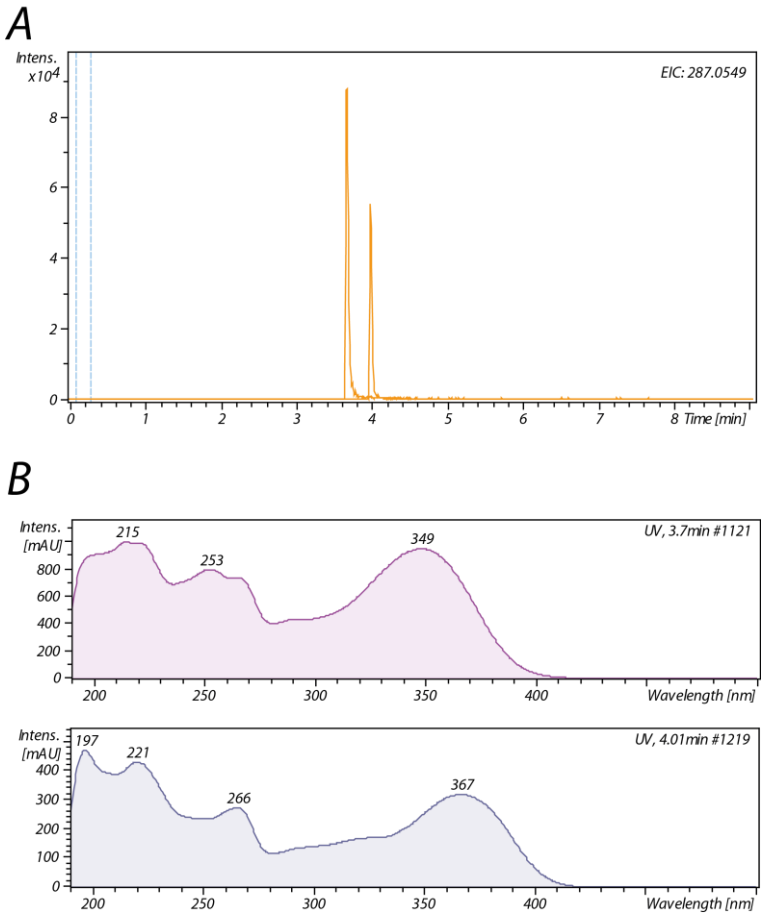
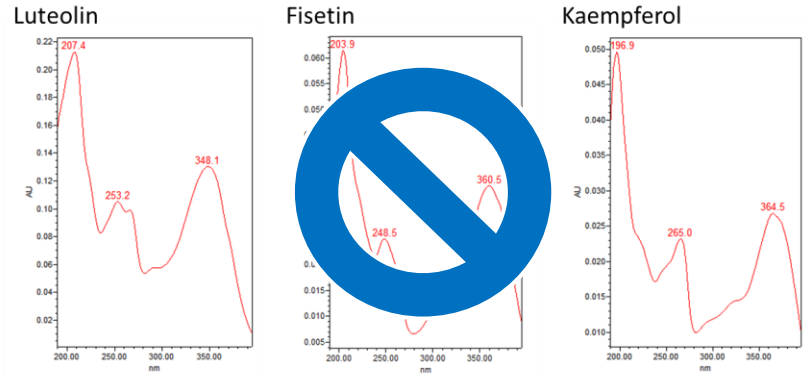
Deutsches Forschungszentrum für Gesundheit und Umwelt

Technische Universität München

Metabolite Identification

LC-MS MetID – MS/MS and RT

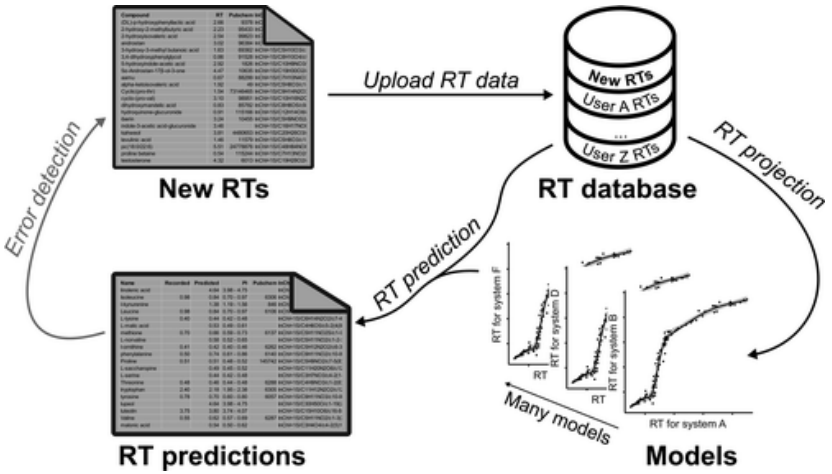


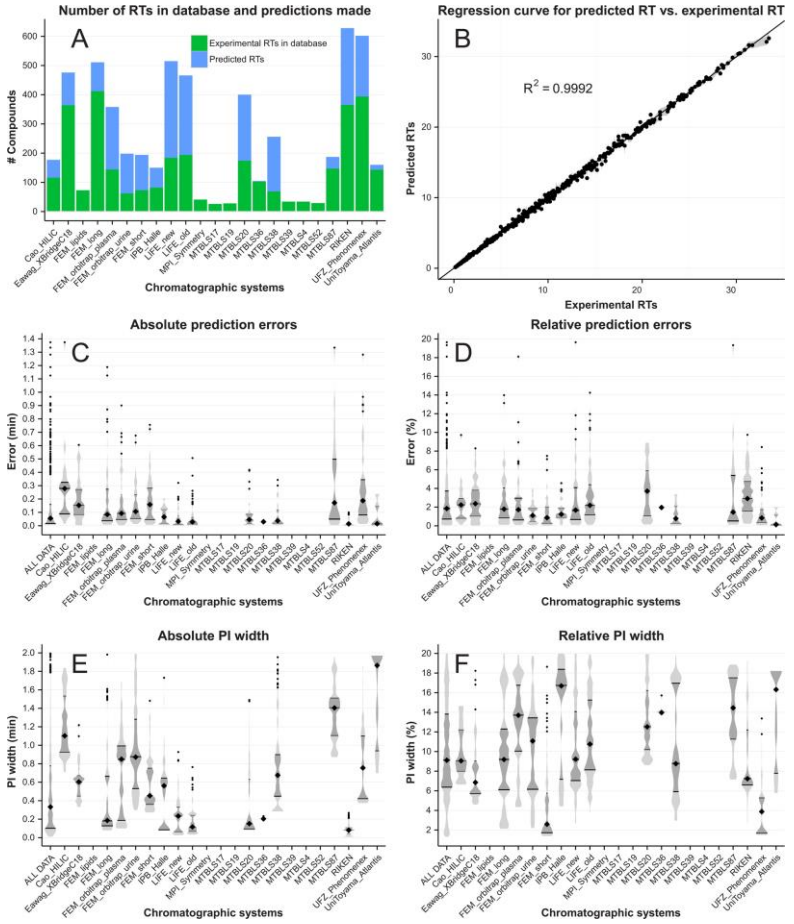


Metabolite Identification

LC-MS MetID – MS/MS and RT

- RTs can projected between different (comparable) LC-MS setups
- If the same compounds have been measured on both systems a function for projection can be calculated allowing to predict RT of compound measured on one system to another

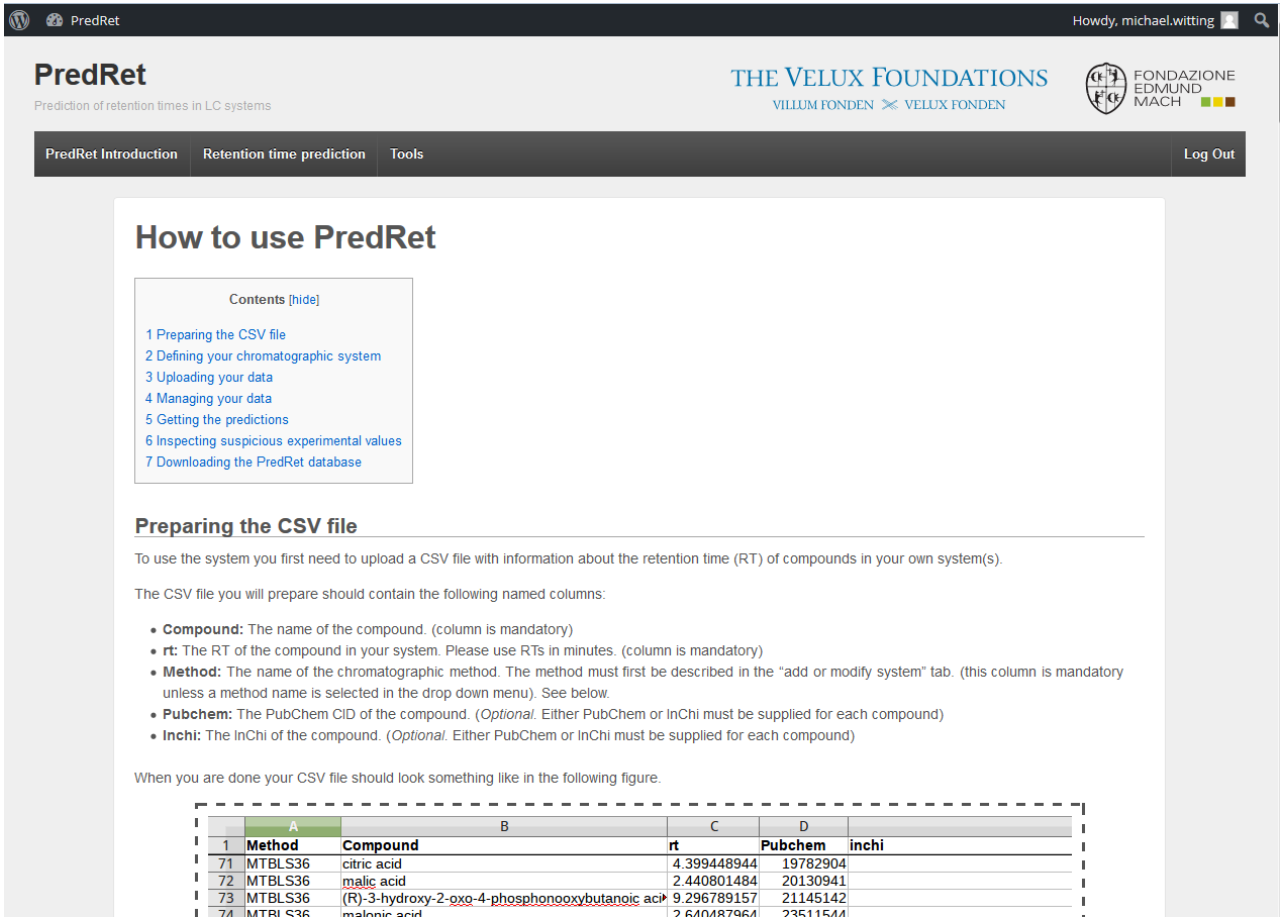




Stanstrup et al., Anal. Chem., 2015, 87 (18), pp 9421–9428

Metabolite Identification

LC-MS MetID – MS/MS and RT



http://predret.org/how-to-use-predret/

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Technische Universität München

Outline

A path to metabolite identification

- Introduction
  - Analytical Technologies
  - Terminology in metabolite identification
- Metabolite identification
  - GC-MS MetID
    - EI spectral deconvolution
    - GC-APCI and exact mass
  - LC-MS MetID
    - Metabolite / Ion annotation
    - Formula calculation
    - Utilization of isotopes for formula calculation
    - MS/MS and RT
- New approaches for MetID
  - Ion mobility
  - *in silico* approaches
- *de novo* identification

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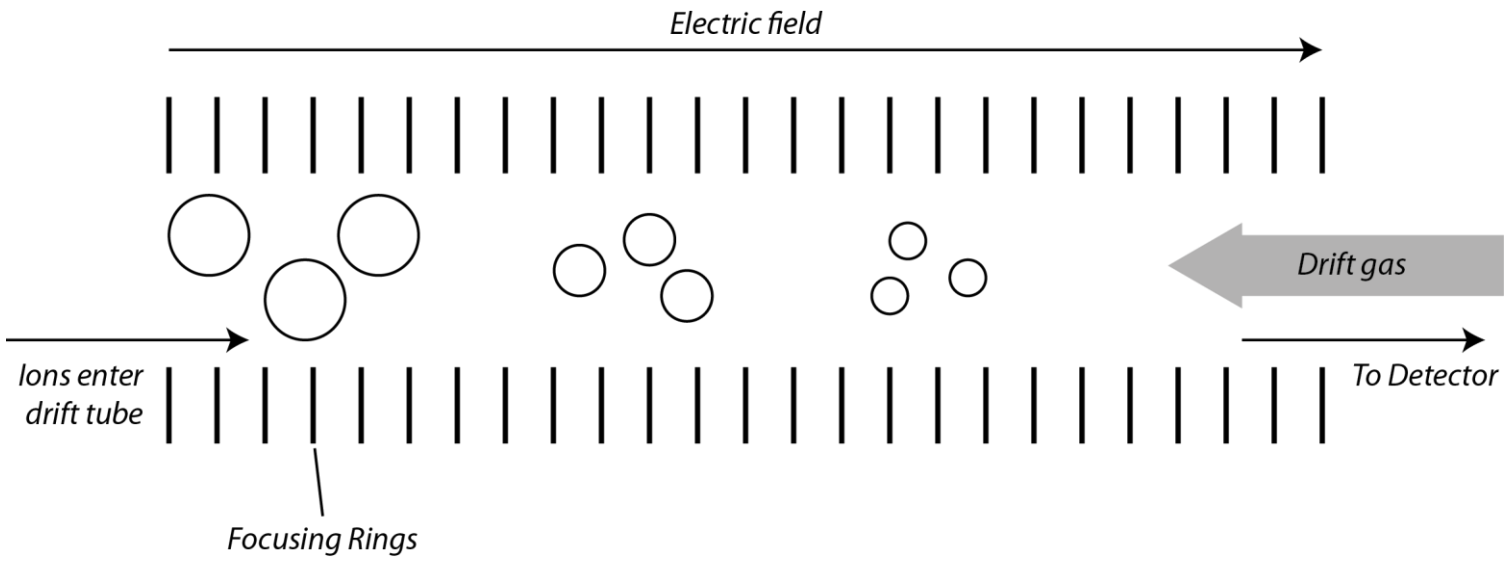
- *de novo* identification

New approaches for MetID

Ion mobility

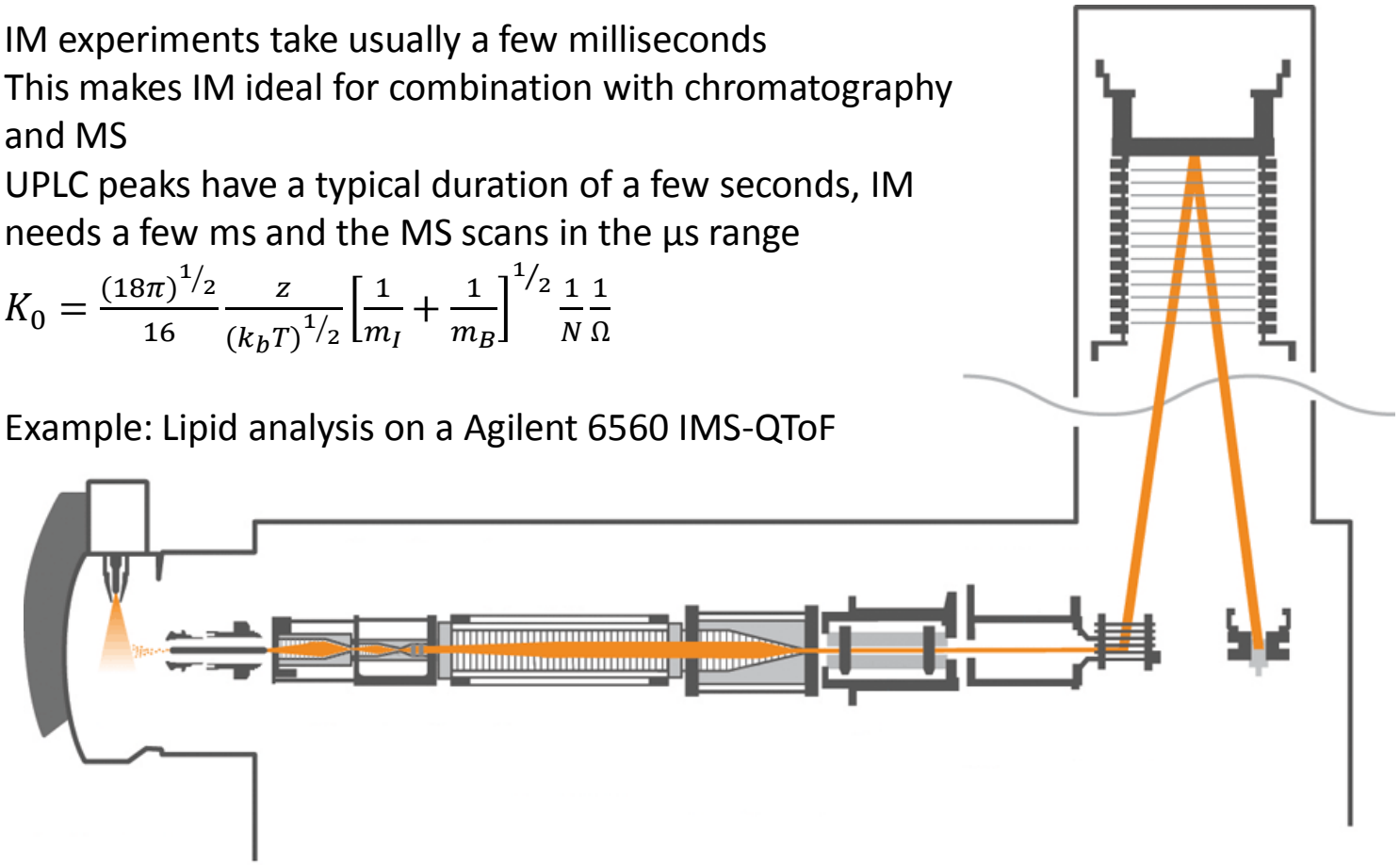


New approaches for MetID

Ion mobility

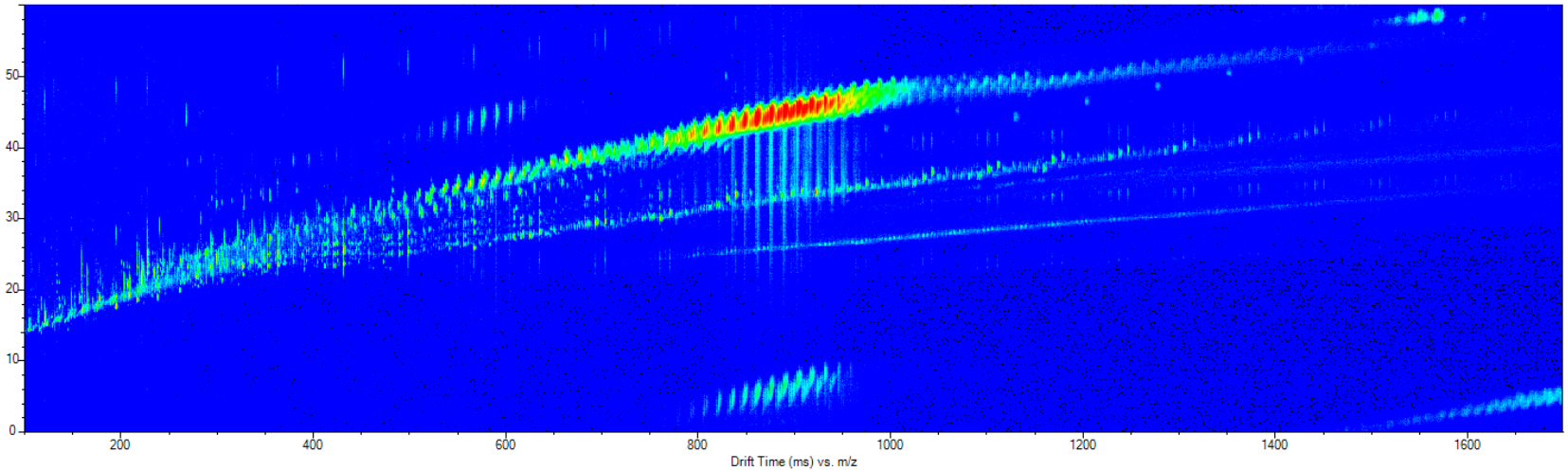
- IM experiments take usually a few milliseconds
- This makes IM ideal for combination with chromatography and MS
- UPLC peaks have a typical duration of a few seconds, IM needs a few ms and the MS scans in the µs range
- $K_0 = \frac{(18\pi)^{1/2}}{16} \frac{z}{(k_b T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_B}\right]^{1/2} \frac{1}{N} \frac{1}{\Omega}$

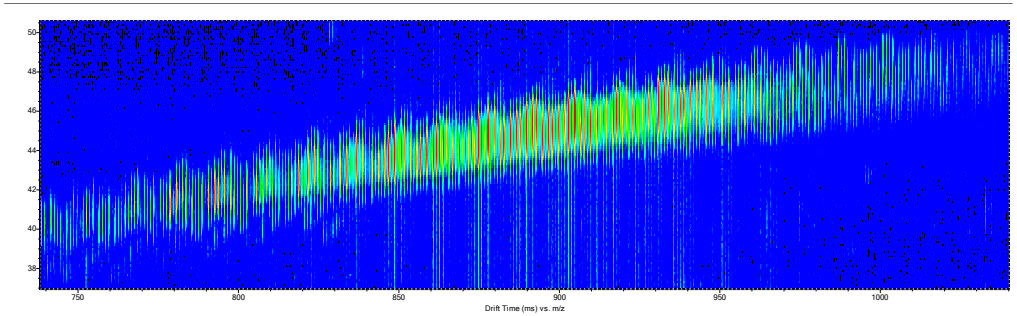
- Example: Lipid analysis on a Agilent 6560 IMS-QToF

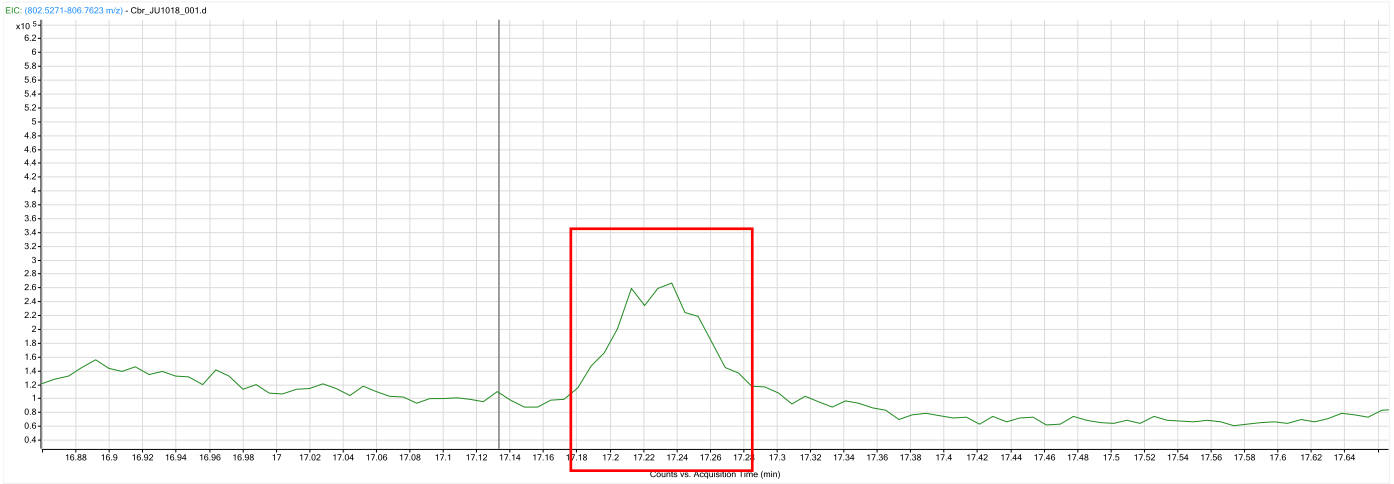


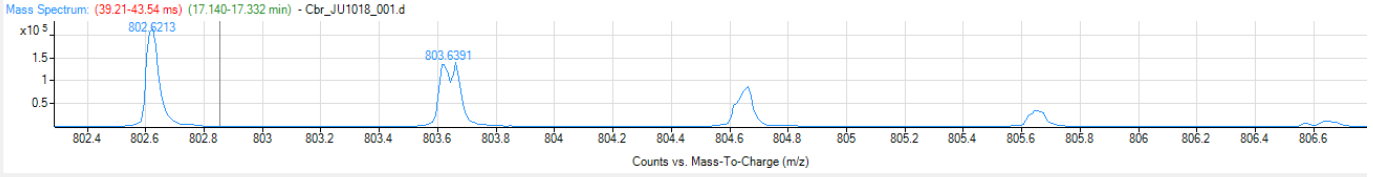
New approaches for MetID

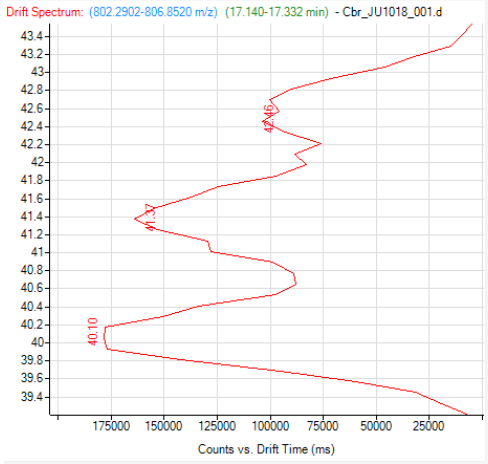
Ion mobility

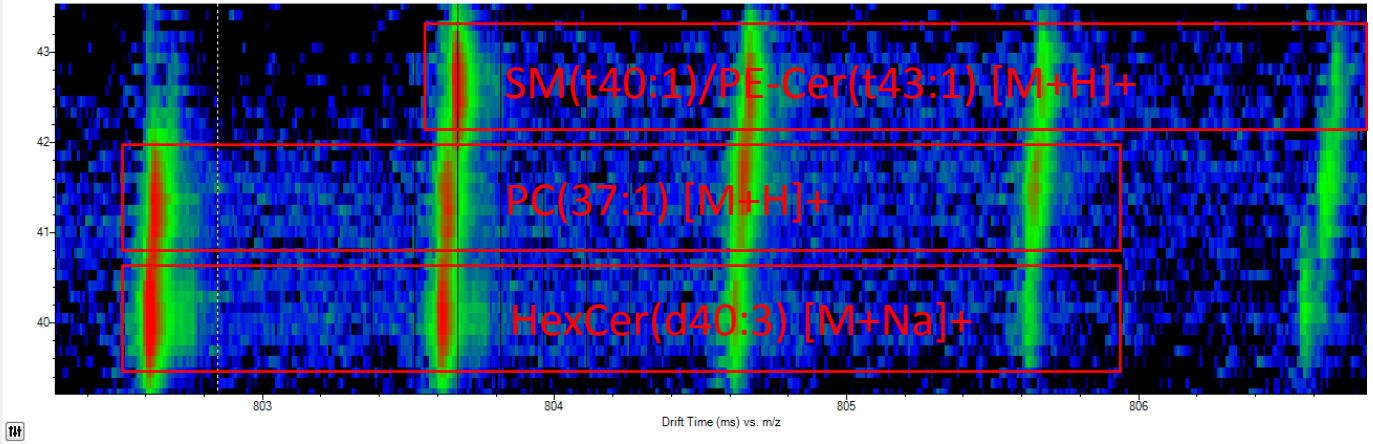












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- New approaches for MetID
  - Ion mobility
  - *in silico* approaches
- *de novo* identification

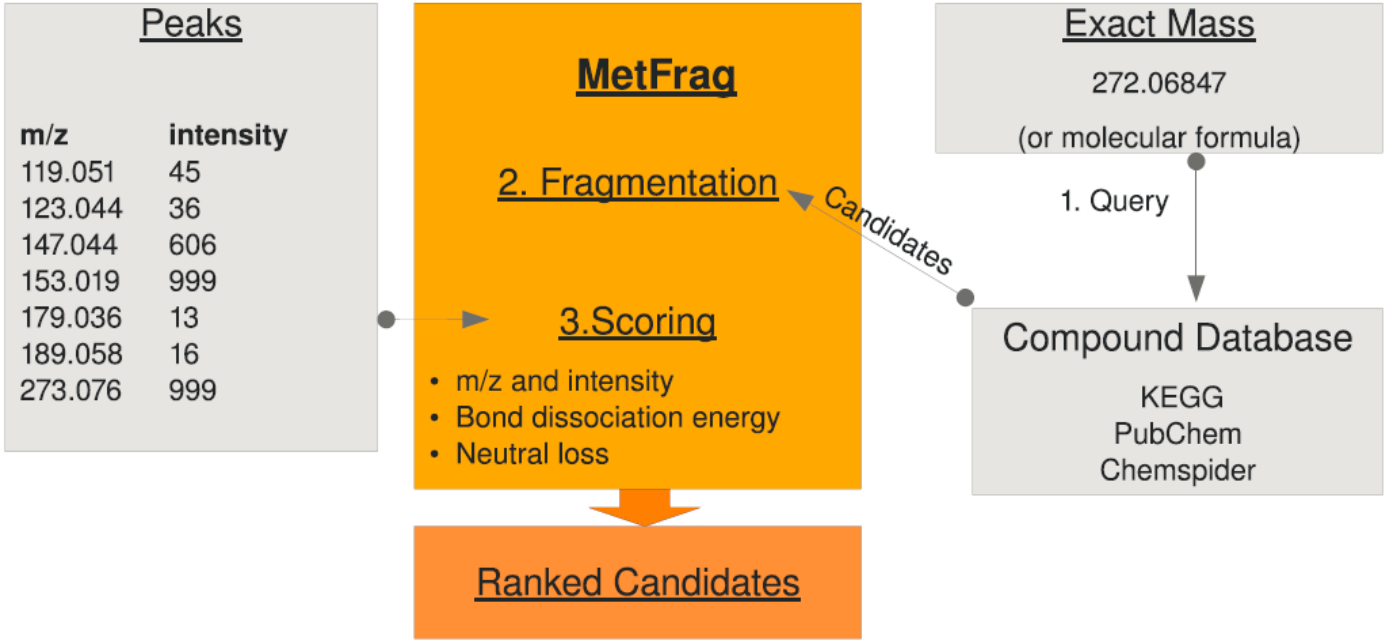
New approaches for MetID
in silico approaches

- *in silico* approaches do not rely on previously acquired MS/MS for the exact molecule, but either matches predicted fragments from simple bond breaking or rule based fragmentation

- A selection of *in silico* tandem solutions are
  - MetFrag / MetFusion (IPB Halle)
  - CSI:FingerID (University of Jena)
  - CFM-ID (Wishart Lab)
  - MAGMa (Netherlands Metabolomics Center)

New approaches for MetID

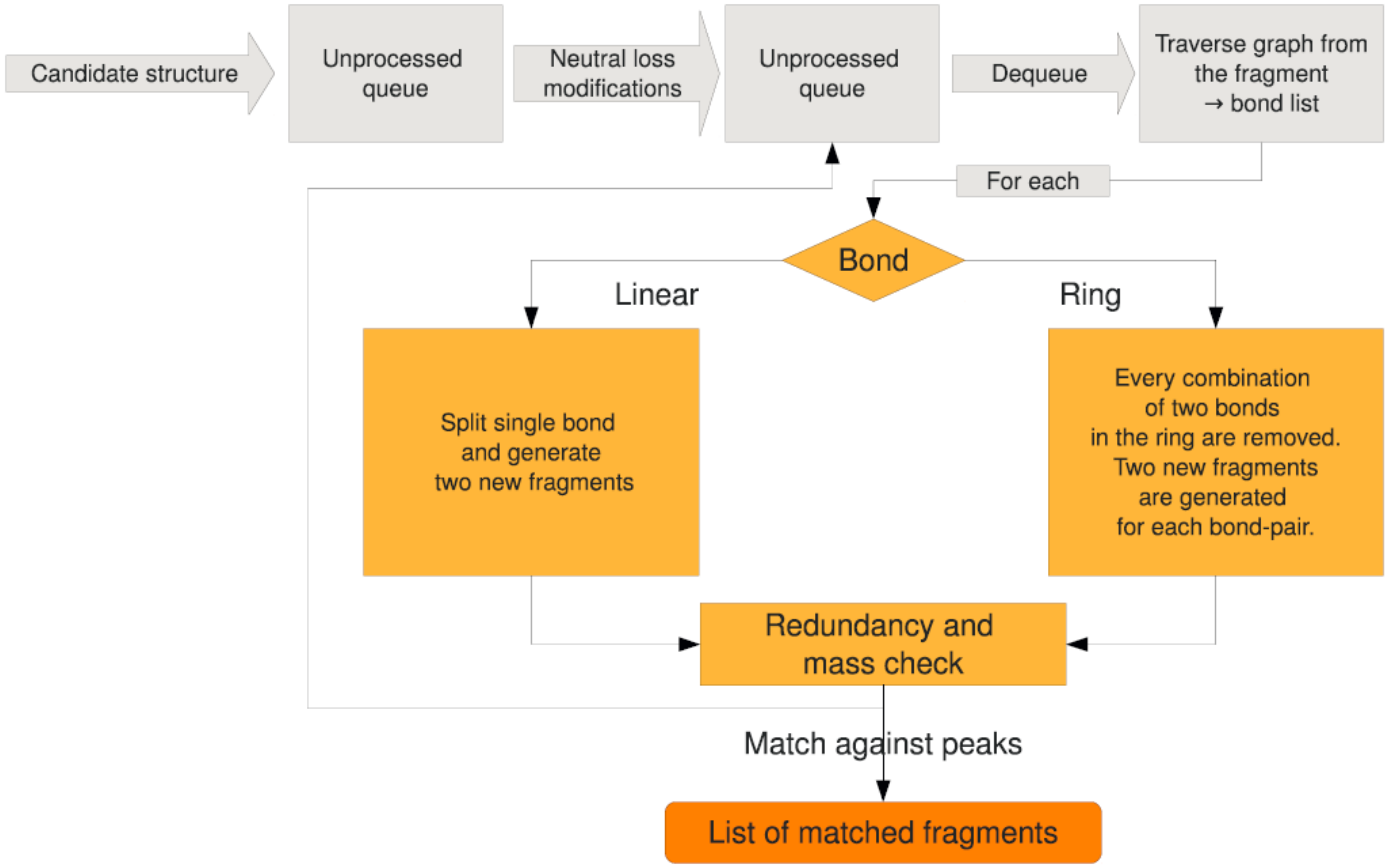
in silico approaches - MetFrag



Wolf et al. BMC Bioinformatics 2010, 11:148

New approaches for MetID

in silico approaches - MetFrag



Wolf et al. BMC Bioinformatics 2010, 11:148

New approaches for MetID

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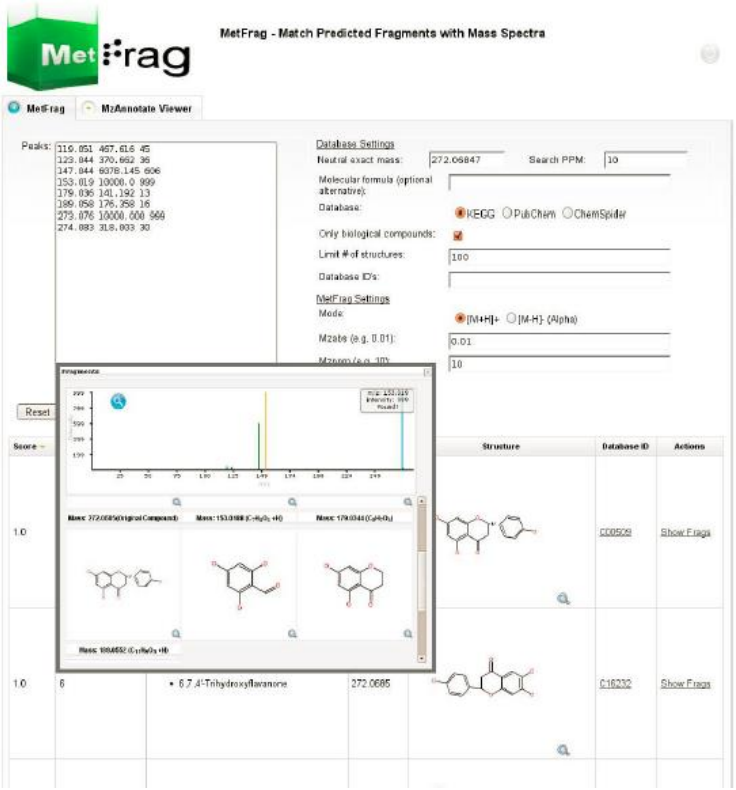


Wolf et al. BMC Bioinformatics 2010, 11:148

Outline

A path to metabolite identification

- Introduction
  - Analytical Technologies
  - Terminology in metabolite identification
- Metabolite identification
  - GC-MS MetID
    - EI spectral deconvolution
    - GC-APCI and exact mass
  - LC-MS MetID
    - Metabolite / Ion annotation
    - Formula calculation
    - Utilization of isotopes for formula calculation
    - MS/MS and RT
- New approaches for MetID
  - Ion mobility
  - *in silico* approaches
- *de novo* identification

Metabolite Identification
de novo *identification of unknowns*

- Complete *de novo* identification is a tedious process and involves several steps

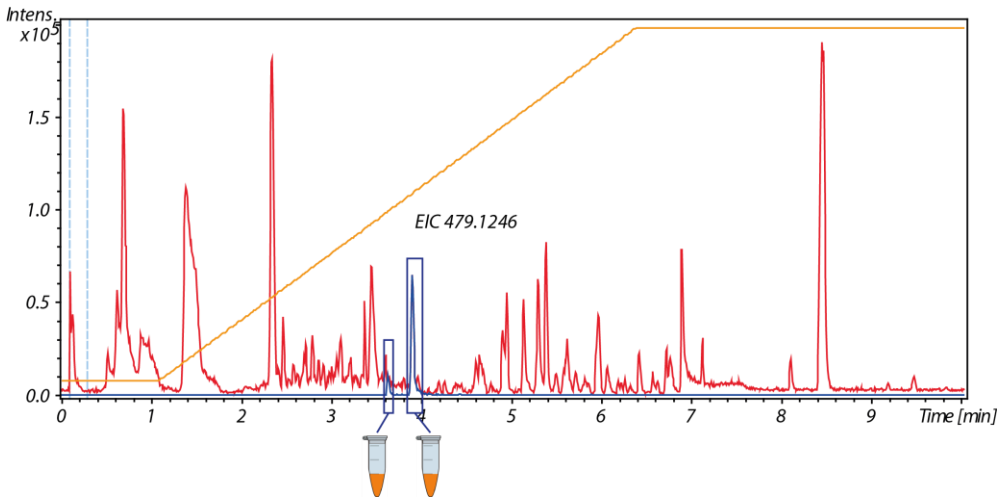
- *de novo* identification should be only conducted if metabolite cannot be identified by any other means

- Complete *de novo* identification requires
  - Full structural elucidation
  - Synthesis of a pure reference substance

de novo identification

Fractionation strategies

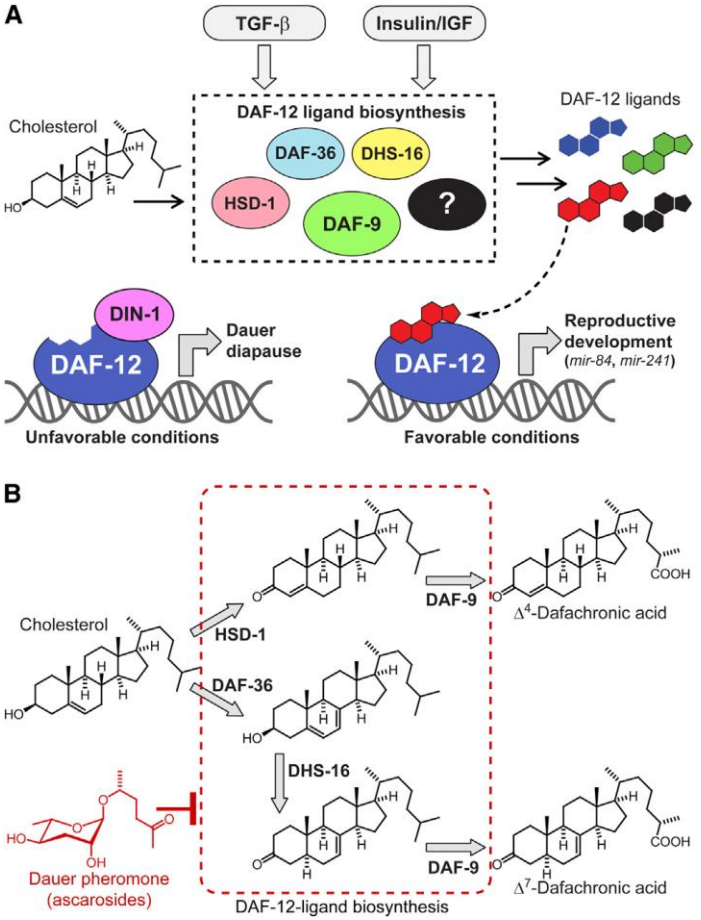
- Enough biomass available?
- Potential activity of metabolite, e.g. hormone?
- Test system available?
- Simple or activity guided fractionation



de novo identification

Fractionation strategies

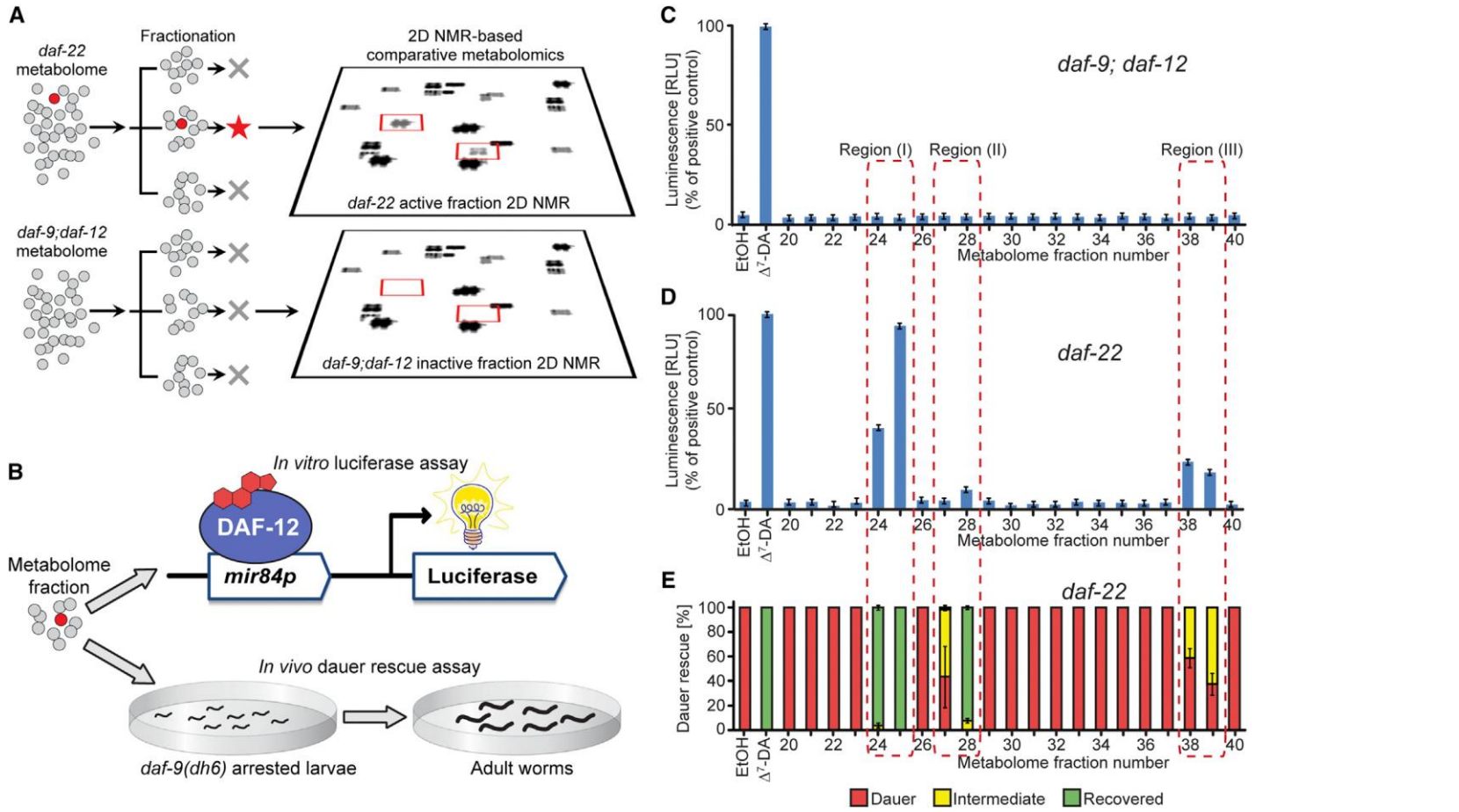
- Mahanti et al. searched for novel ligands of the nuclear hormone receptor DAF-12 in *C. elegans*
- Activity guided fractionation was used in combination with 2D-NMR based metabolomics to find bioactive molecules
- Activity was checked *in vivo* and *in vitro*
- Previously known molecules Δ4- and Δ7-Dafachronic acid were re-identified together with novel variants
- Synthesis of chemical reference standards confirmed identity



Mahanti et al., Cell Metabolism 19, 73–83, January 7, 2014

de novo identification

Fractionation strategies



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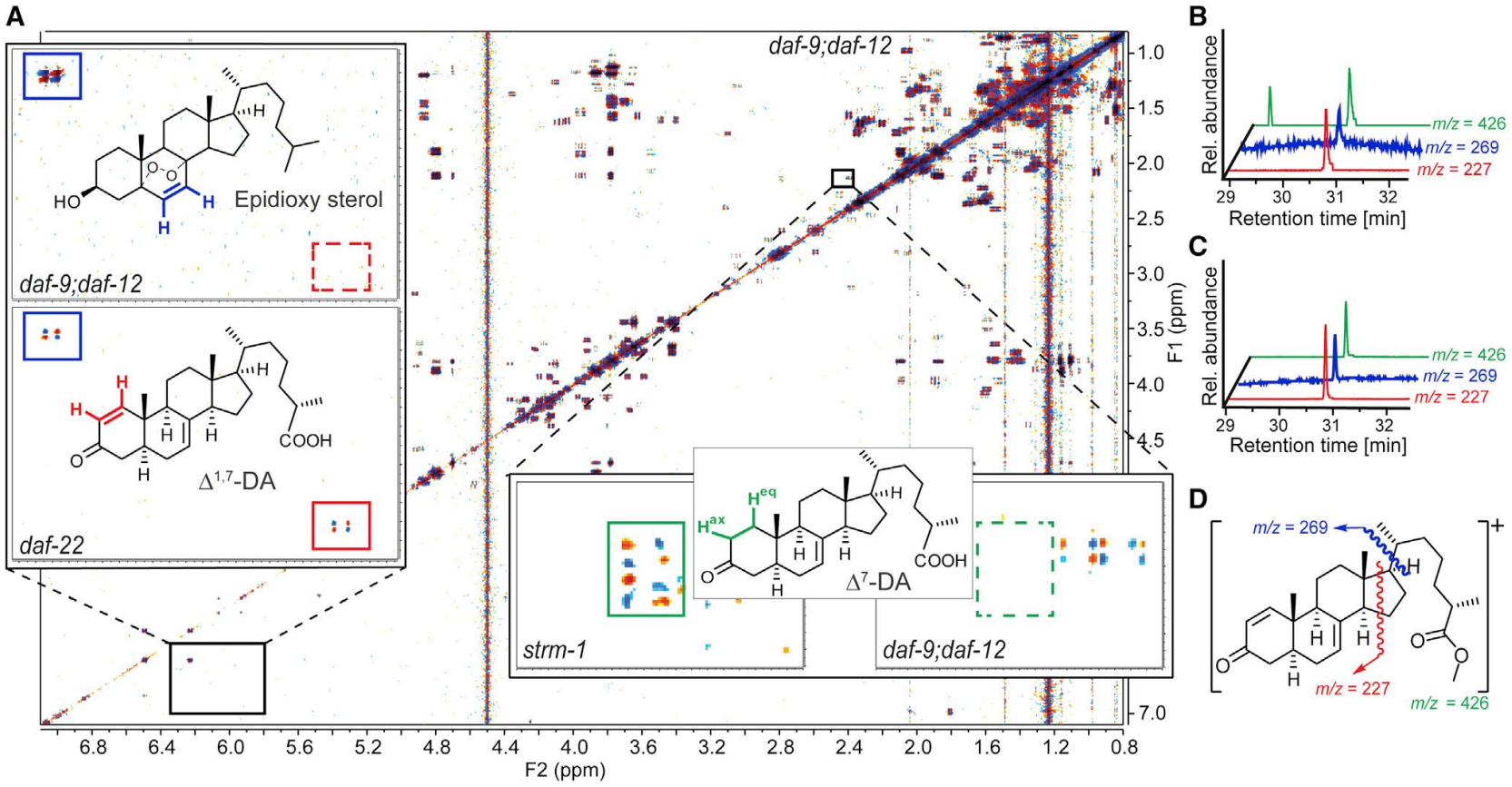
de novo identification

Fractionation strategies



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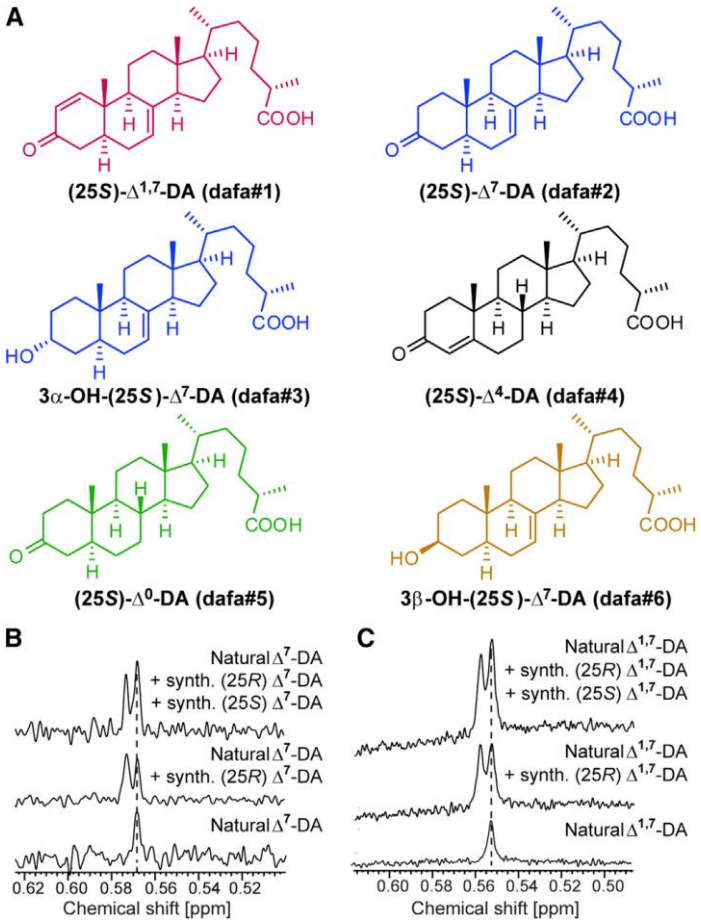
de novo identification

Fractionation strategies



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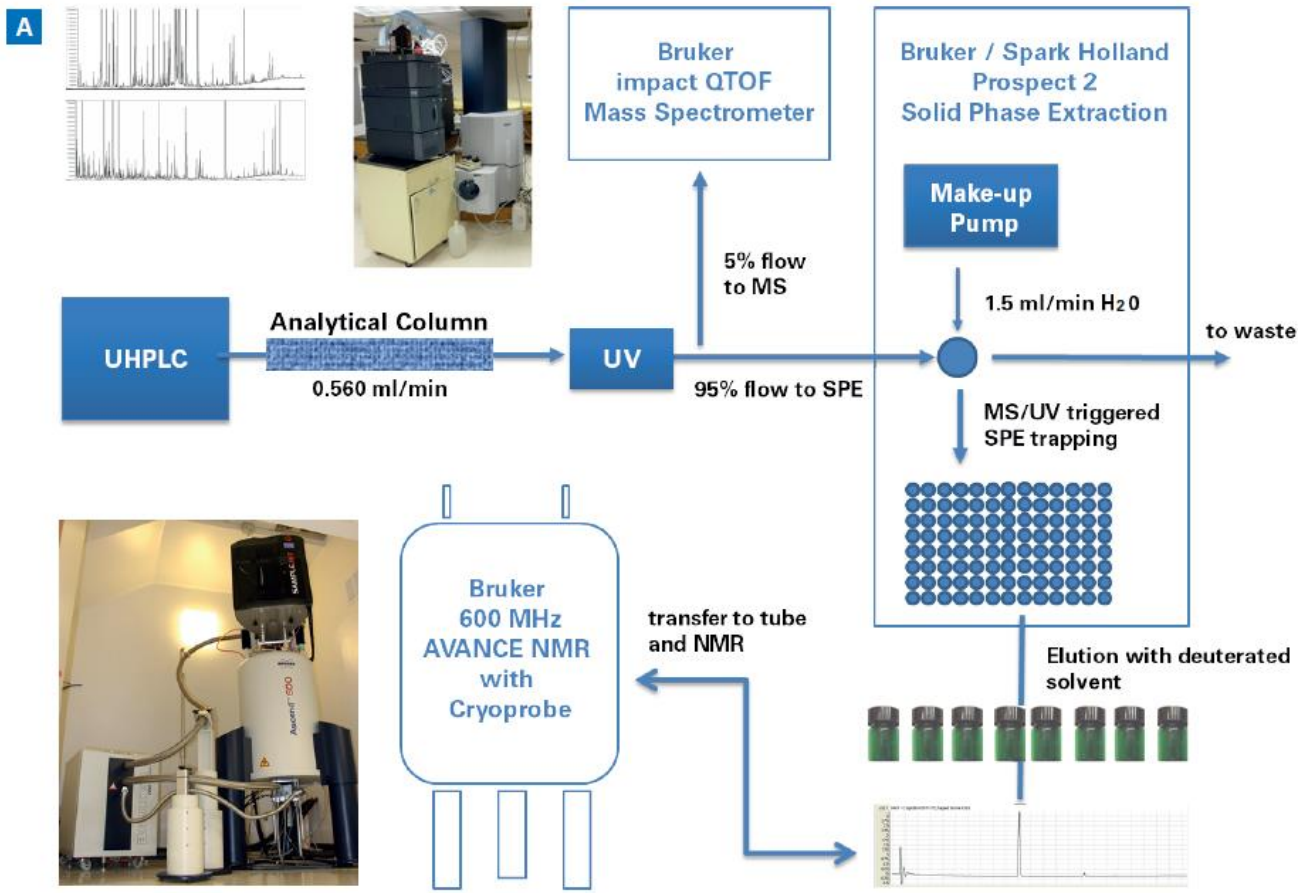
de novo identification

Fractionation strategies

Bruker Metabolic Profiler

de novo identification

Fractionation strategies



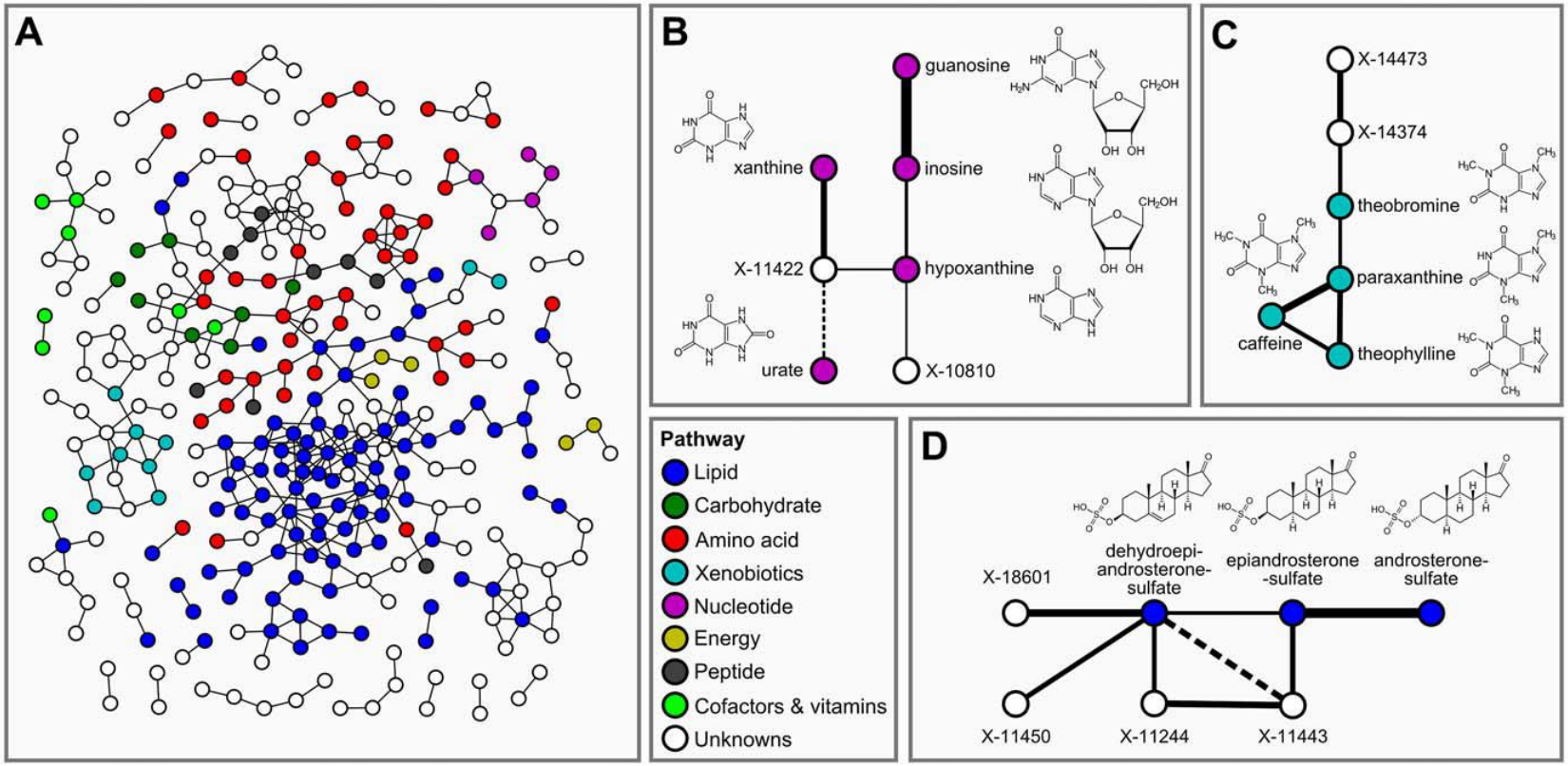
Sumner et al. & Bruker

de novo identification

Fractionation strategies

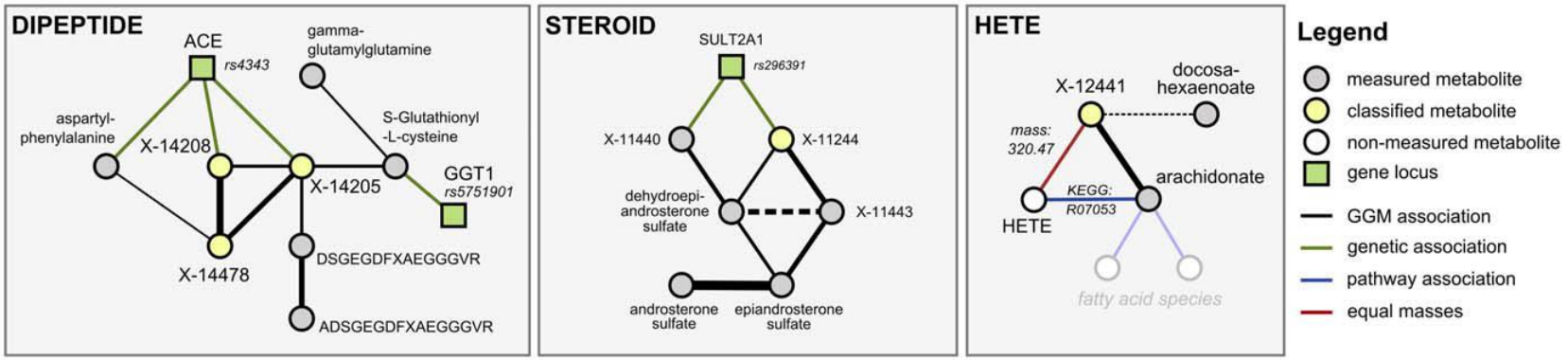
Sumner et al. & Bruker

de novo identification
in silico strategies



Krumsiek et al., PLoS Genet 8(10): e1003005

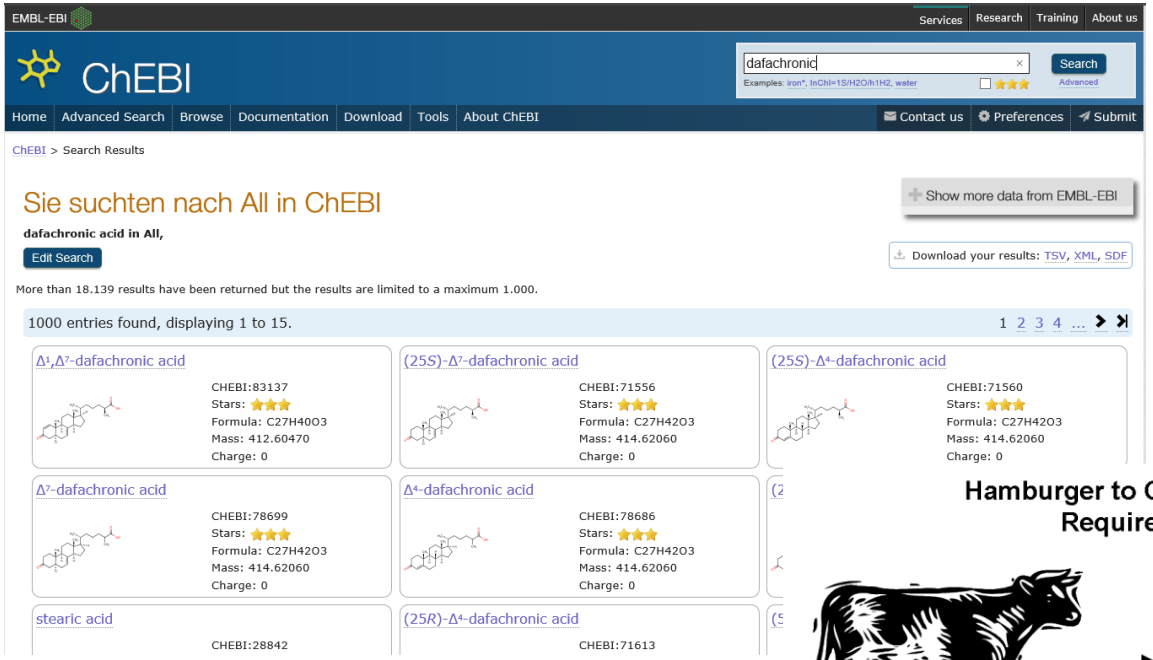
de novo identification
in silico strategies

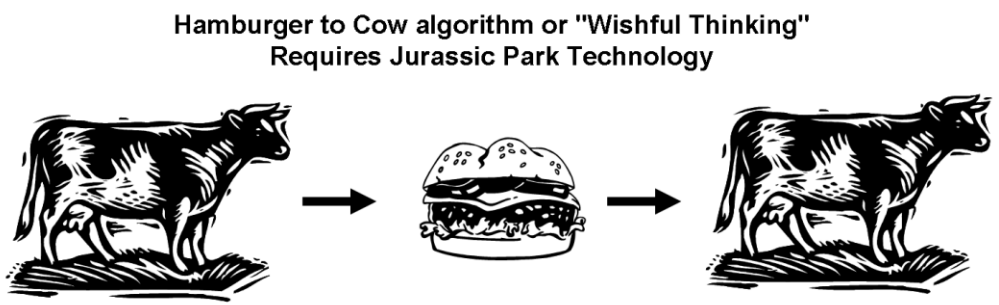


Krumsiek et al., PLoS Genet 8(10): e1003005

de novo identification
Reporting

- Please report your de novo identified metabolite in (electronically) searchable manner





http://fiehnlab.ucdavis.edu/projects/rice_metabolome/hamburger-to-cow-algorithm.png

HelmholtzZentrum münchen
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Technische Universität München

Thank you! Questions?

PFP Postdoctoral Fellowship Program

DAAD

DFG Deutsche Forschungsgemeinschaft

Habilitationsfachmentorat:

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Technische Universität München