GC-MS BASED METABOLOMICS

Pavel Aronov

Stanford Mass Spectrometry
Users' Meeting
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Stanford University
MASS SPECTROMETRY

Outline

- **WHAT** can you achieve with GC/MS based metabolomics?

  Advantages and applications of metabolomics performed using GC/MS

- **HOW** can you organize the workflow for GC/MS based metabolomics?

  Examples of open source platforms and methods

Origin of Metabolomics

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 10, pp. 2374–2376, October 1971

Quantitative Analysis of Urine Vapor and Breath by Gas–Liquid Partition Chromatography

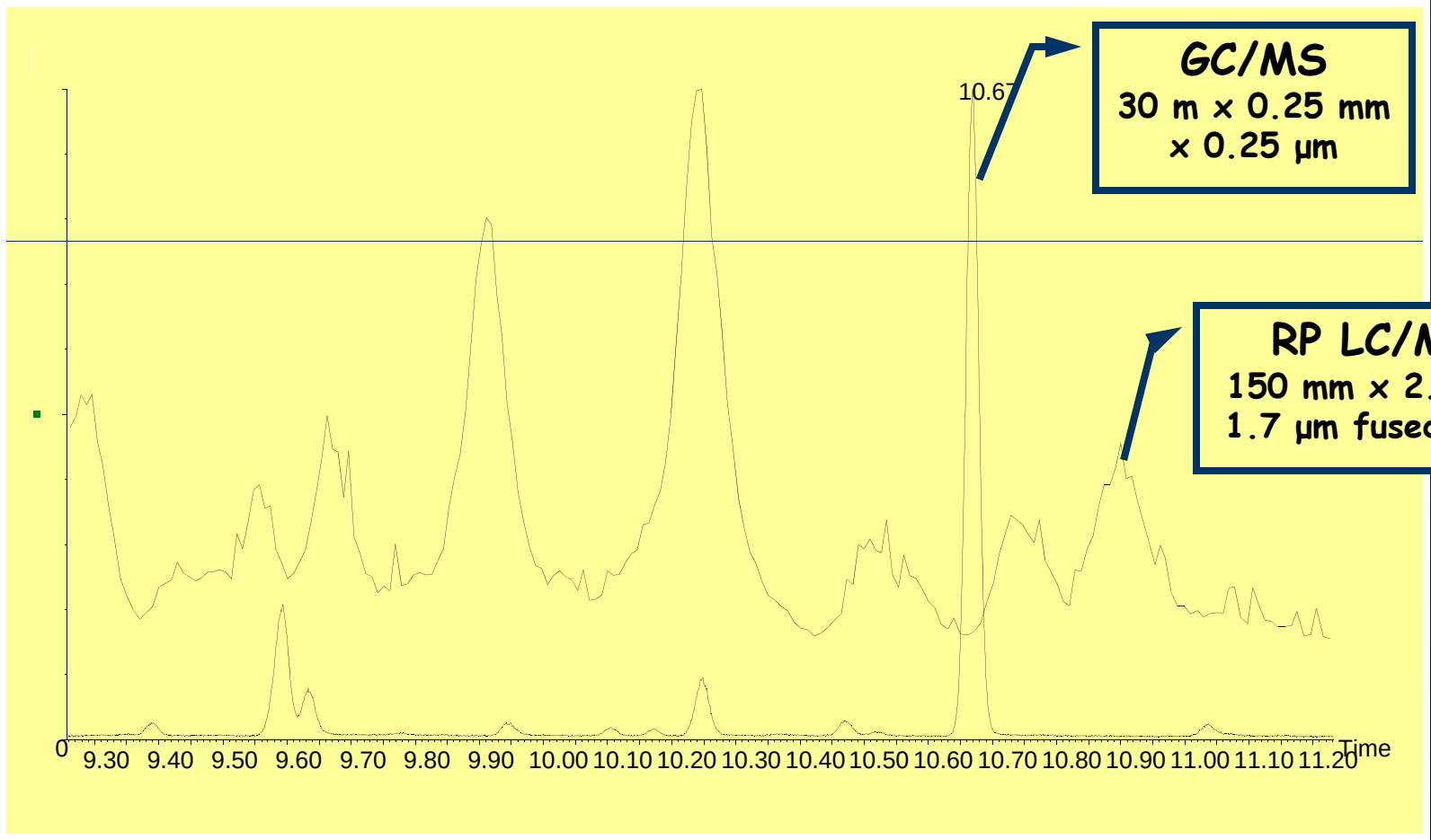
(orthomolecular medicine/vitamins/controlled diet)

LINUS PAULING*, ARTHUR B. ROBINSON*, ROY TERANISHI†, AND PAUL CARY*

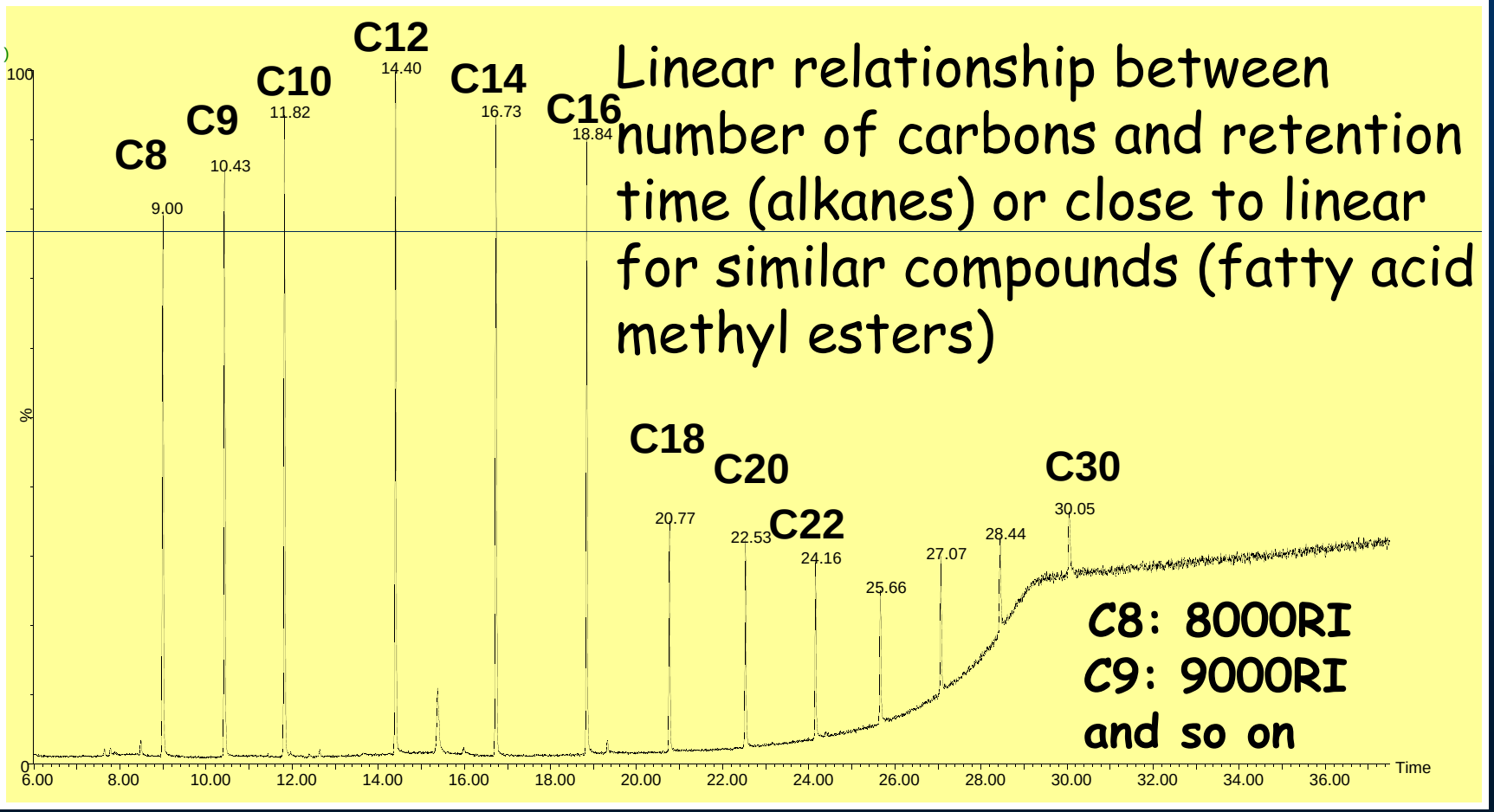
* Department of Chemistry, Stanford University, Stanford, California 94305; and † Western Regional Laboratory, U.S. Department of Agriculture

Contributed by Linus Pauling, July 29, 1971

Comprehensive Separations



Retention (Kovats) Indices (RI)



Linear relationship between number of carbons and retention time (alkanes) or close to linear for similar compounds (fatty acid methyl esters)

C8: 8000RI
C9: 9000RI
and so on

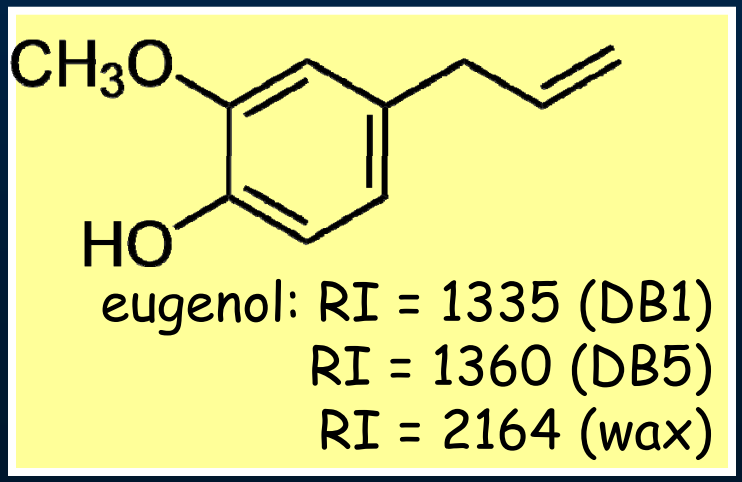
Retention Indices in Identification

GC has fewer variables than LC

RI are independent from:

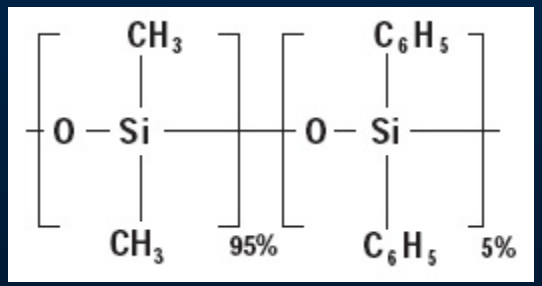
- *Carrier gas pressure and flow rate*
- *Temperature ramp*

RI close for columns of similar chemistries

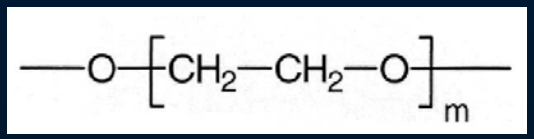


eugenol: RI = 1335 (DB1)
RI = 1360 (DB5)
RI = 2164 (wax)

DB5



wax



RI Libraries

- Collection of experimental RI data began in the 1960s
- Computer prediction of RI indices
- NIST2008 RI library (over 20,000 compounds)
- +/- 5RI interlab deviation for Kovats indices

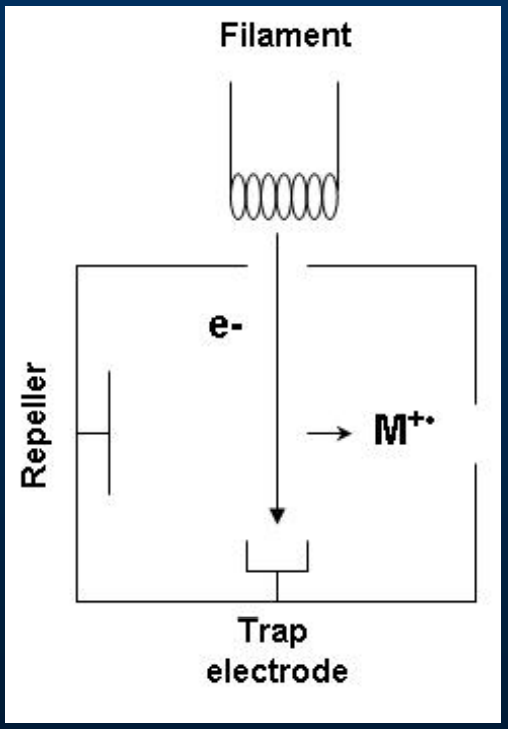
Electron Ionization (EI)

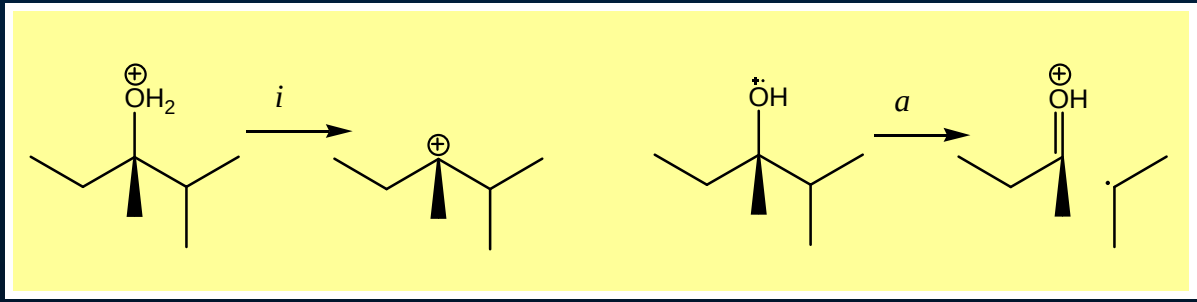
70 eV >> energy of chemical bond

- Highly reproducible
- Extensive fragmentation
- Often no molecular ion observed

EI: alpha-cleavage [*a*] more common

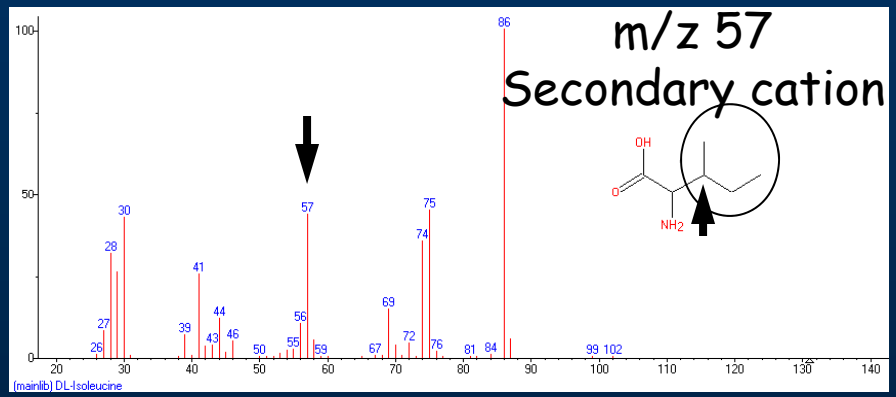
CID MS/MS: inductive cleavage [*i*] common

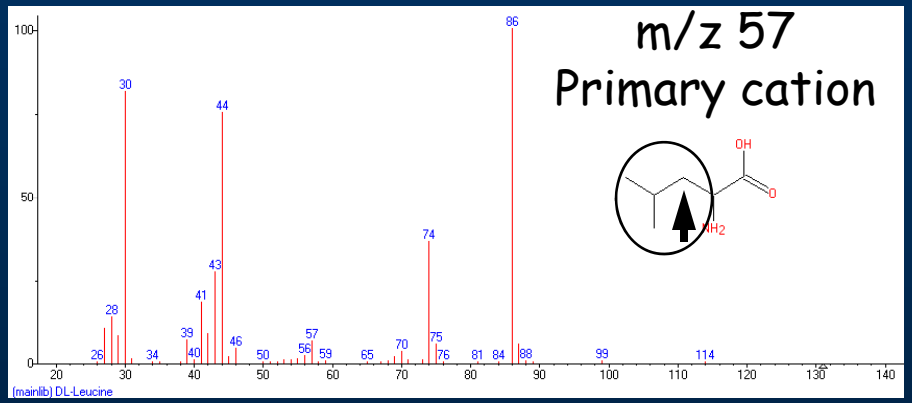




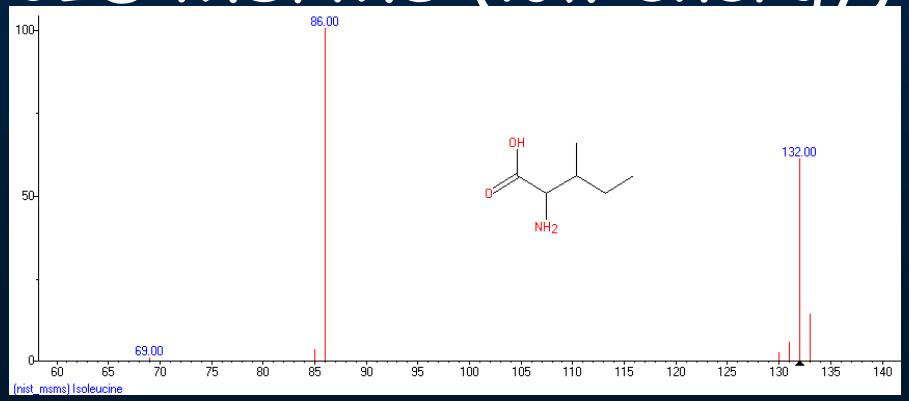
EI and CID MS/MS

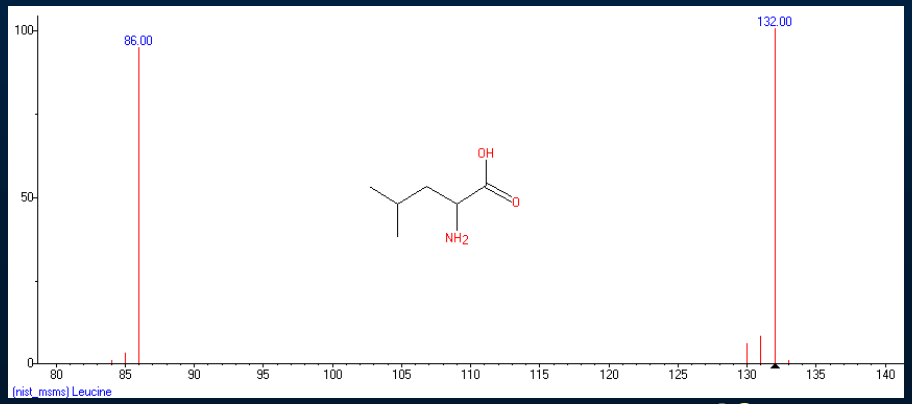
EI (always 70 eV)





CID MS/MS (low energy)





EI MS Libraries

NIST 2008 MS library

~200,000 compounds with EI MS spectra

vs

~5000 ions with API CID MS/MS spectra

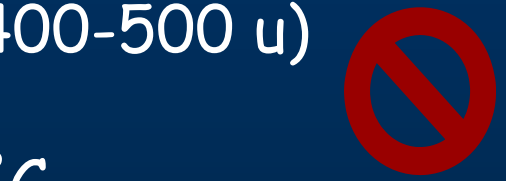
Wiley MS library (9th edition)

~600,000 compounds with EI MS spectra

Limitations of GC-MS

Analytes must be volatile:

- High molecular mass (>400-500 u)
- Ionic nature
- Not stable at 100-300°C



- Drug metabolites, especially phase II (pharma)
- > 3-5 AA peptides (proteomics)

Rise of API sources and LC/MS in 1980-90s

GC/MS was still important in environmental analysis (PAHs, dioxins, PCBs)

Two Typical Sample Prep Protocols

1. Add 10 uL A, spin down, add 100 uL B. Incubate at 55°C for 30 minutes
2. Add 10 uL C, spin down, add 100 uL B and incubate at room temperature for 30 minutes
3. Wash with 250 uL D for 30 minutes.
4. Dry with speedvac
5. Add 5 pmol X. Incubate 1 hour at 50°C
6. Spin down and pull off all liquid. Extract a second time using E, incubate for 10 minutes at 37°C
7. Speedvac total combined extract to dryness and reconstitute in F

1. Take out 30 µL sample aliquots and add 5 uL, vortex for 10 s.
2. Add 0.4 mL of B and vortex vigorously for 20 s. Shake the samples for 5 min in a 4°C.
3. Collect supernatant and speedvac it to dryness
4. Add 10 uL C to dry residue. Shake at 30 C for 90 min
5. Add 90 uL B and incubate at 30°C for 30 min

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SUMS in gel digestion proteomics protocol

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2. Add 0.4 mL of B and vortex vigorously for 20 s. Shake the samples for 5 min in a 4°C.
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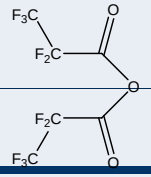
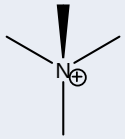
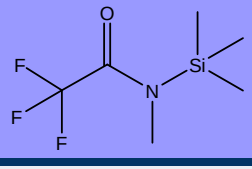
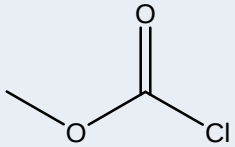
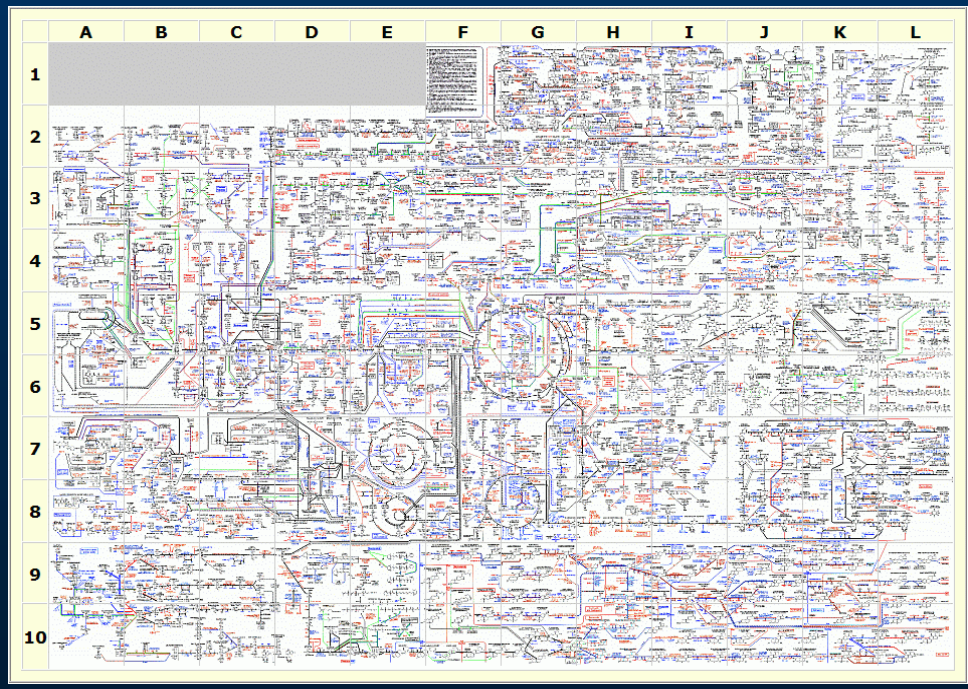
GC/MS FiehnLab metabolomics protocol for blood plasma

Common Derivatization Methods

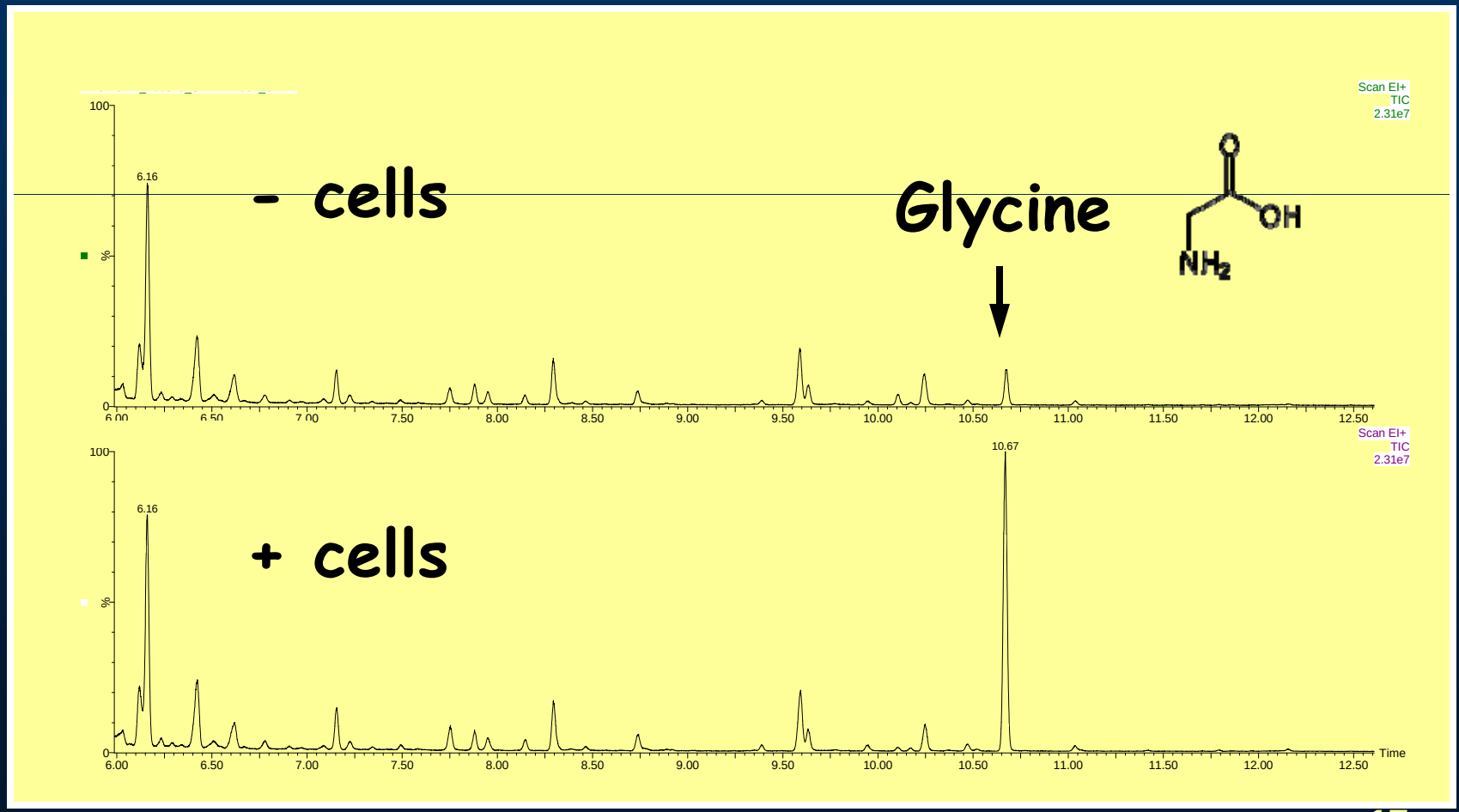
Reaction	Typical Reagents	R-OH	R-NH2	R-SH	R-COOH, Ar-OH
Acylation					
Alkylation	BF_3 MeOH 				
Silylation					
Derivatization with Alkyl Chloroformates					

GC/MS: Primary Metabolism

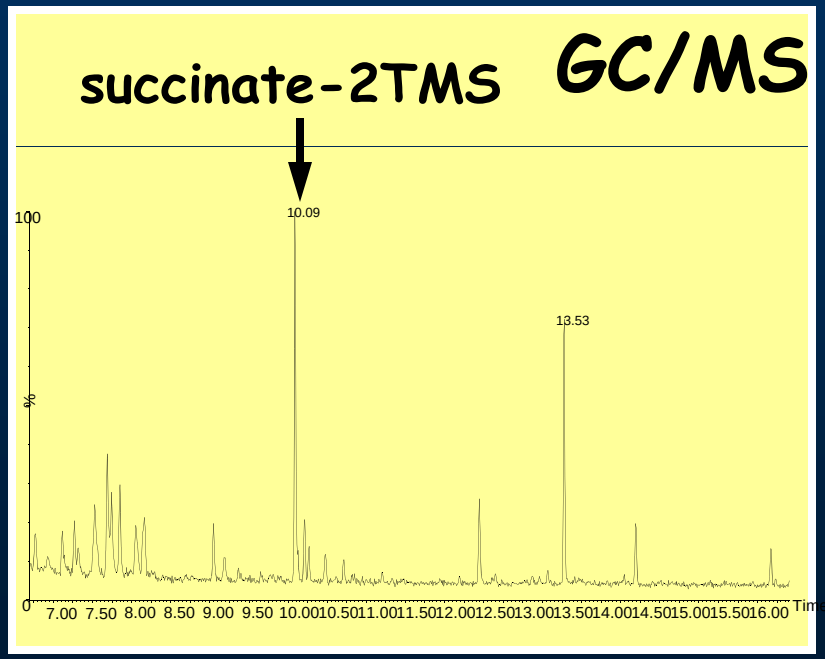
- Amino acids
- Monosaccharides
- Small organic acids
- Fatty acids
- Other small molecules



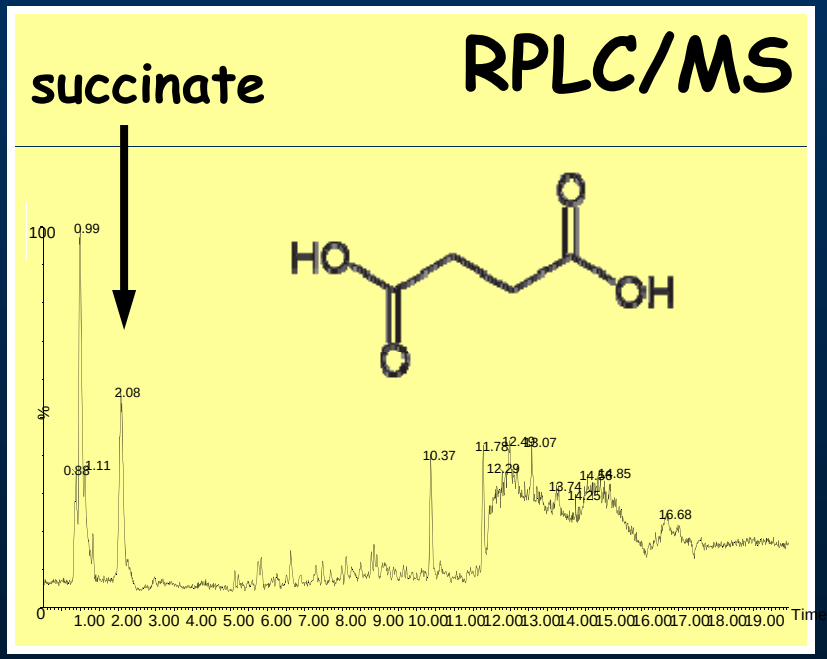
Application Examples: Cell Culture



RPLC-MS and GC-MS: Succinate



DB5 helium
tR = 10.08 min
k′ > 10



C18 100 % aqueous
tR = 2.08 min
k′ ~ 1.5

Two Complete Commercial GC/MS Platforms for Metabolomics

- Agilent

  GC-MS (single and triple quads), software
- Leco

  GC-TOF, software
- Gerstel

  Autosamplers for automated sample prep and derivatization

Sample Extraction

- Lipophilic metabolites (fatty acids, sterols) included

  Extraction with cold methanol, or acetone. Supernatant is dried and analyzed.

- Lipophilic metabolites excluded (more robust)

  Methanol-chloroform extraction (Bligh-Dyer), aqueous phase dried and analyzed.

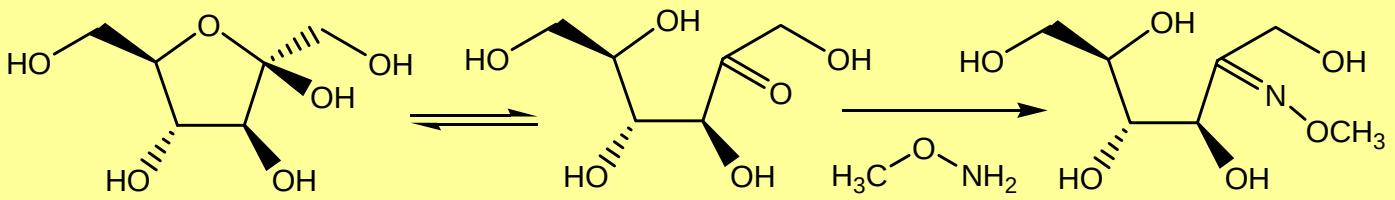
Internal Standards

- Use one or several isotopically labeled metabolites to measure recovery and QC
- [optional] use RI calibration standards (alkanes, FAMEs) to build RI system

Oximation

40 mg/mL methoxiamine in pyridine at 30°C for 90 min

- Prevents a-ketoacids from thermal decarboxylation
- Keeps sugars in open conformation to minimize number of conformations and relieve steric hindrances for next step



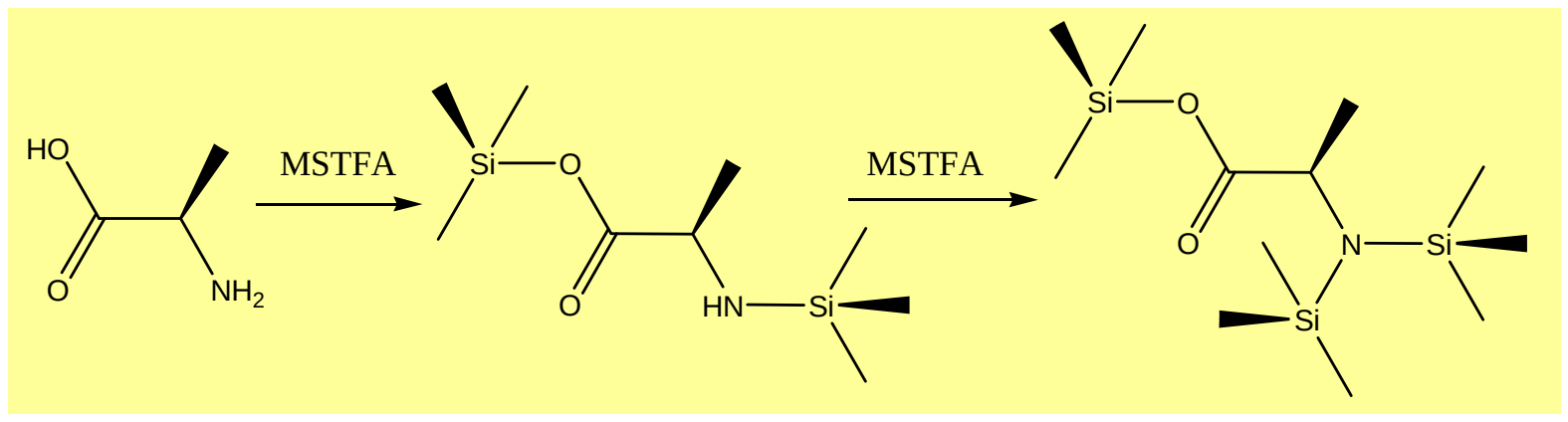
α/β epimers

Syn/anti isomers

Silylation

MSTFA, 1% TMCS at 37°C for 30 min

- Substitution of active hydrogens
- Sample must be dry, no water or other protic solvents
- Incomplete derivatization is possible



GC/MS

- 10 m guard column and 30 m length; 0.25 mm i.d.; 0.25 µm film 95% dimethyl/ 5% diphenyl polysiloxane analytical column (e.g. DB5)
- injection at 250 °C and split ratio of 1:5 to 1:10 into glasswool-packed split liner
- 1 mL/min constant flow helium; oven ramp 60 °C (1 min hold) to 325 at 10 °C/min, 10 min hold before cool-down; 37.5 min run time.
- scan range 50–600 u at 2-20 spectra/s

(based on Fiehn Lab method)

CDF Data File Standard

- Universal file format supported by the majority of mass spec manufacturers
  *.cdf

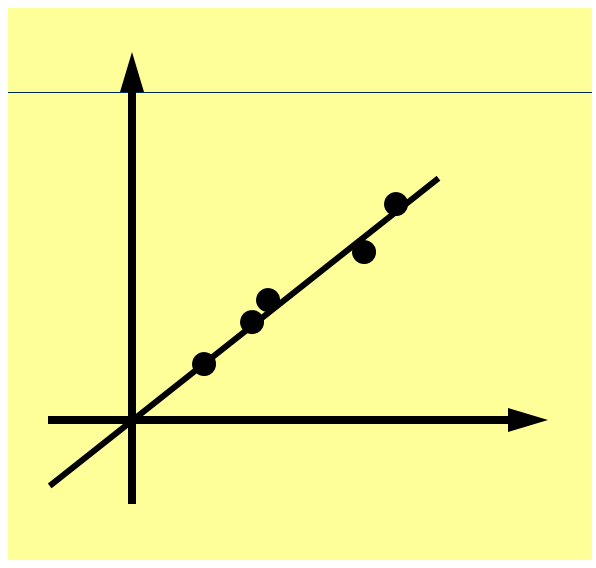
Agilent ChemStation:	AIA format
Thermo Xcalibur:	ANDY format

Peak Picking

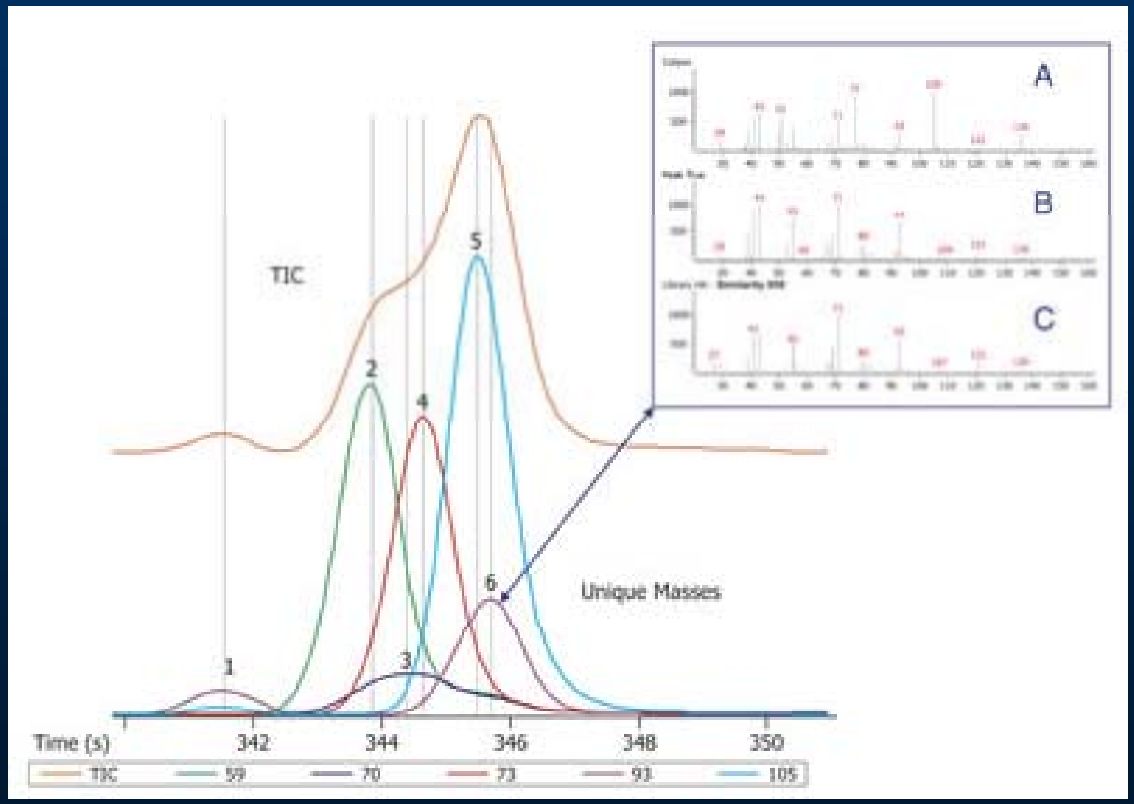
- e.g. use XCMS or MZmine

  Problem: one compound is represented with multiple MS peaks due to EI fragmentation

  Partial solution: filter correlations statistically. In PCA loading plot, fragment ions from the same precursor align along one line drown through origin

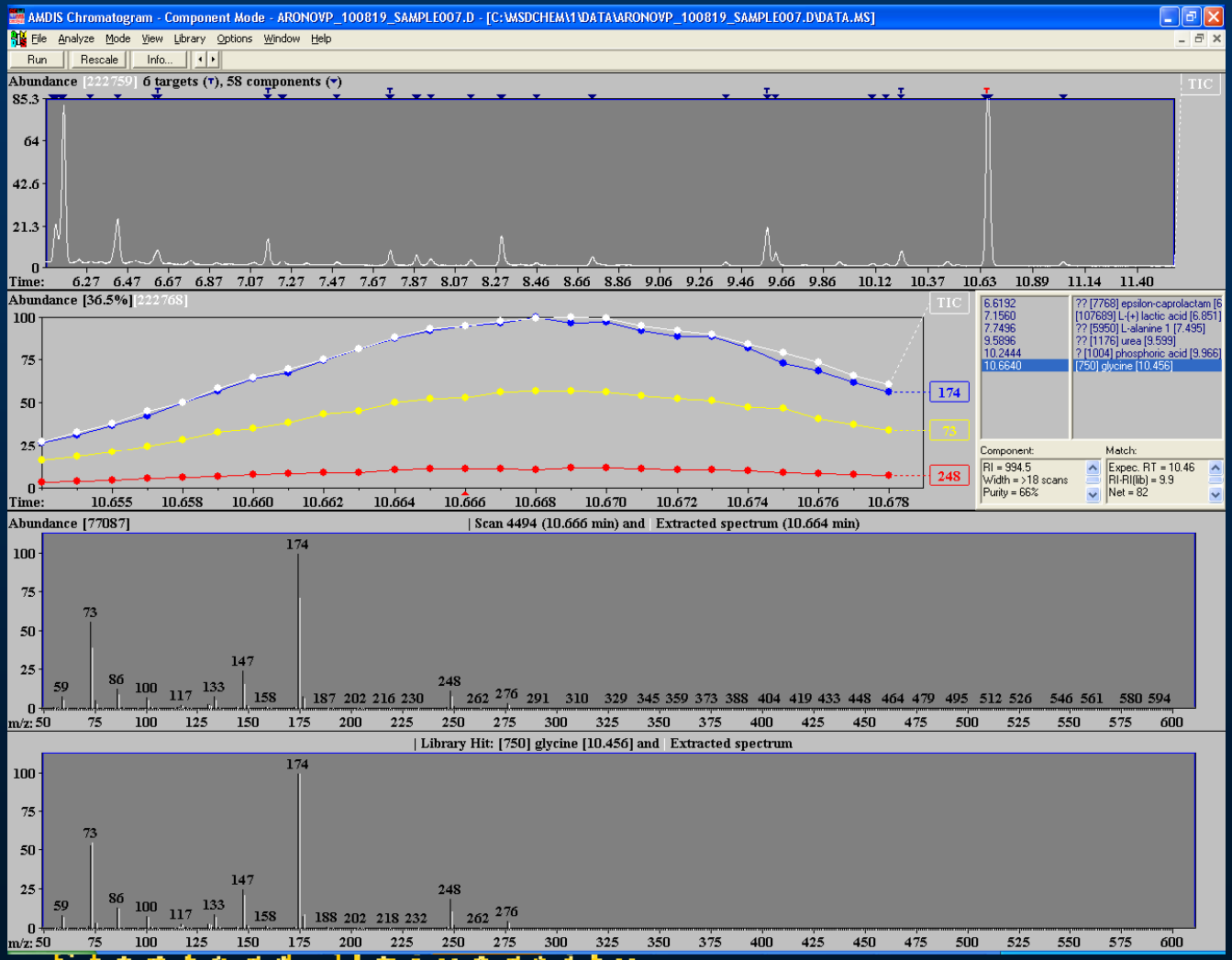


Data Analysis: Deconvolution



From www.leco.com

MSRI Identification: AMDIS



MSRI Libraries for AMDIS

- Free GOLM database

  306 compounds (link)

- Fiehn database from Agilent and Leco*

  ~1000 compounds (link)

  Now available at SUMS!

- Build your own library in AMDIS

  NIST AMDIS download page , info page

*Leco uses own deconvolution software

Alignment of Multiple Samples

Free web-based services:

www.metaboanalyst.ca

No deconvolution, XCMS based peak finding

www.metabolome-express.org

Deconvolution, MSRI libraries, data depository

References

Derivatization:

- Bulletin 909A: Guide for derivatization reagents
- D. Knapp. Handbook of Analytical Derivatization Reactions(1979).
- J.L. Little. Artifacts in Trimethylsilyl Derivatization Reactions

GC/MS metabolomics:

- O. Fiehn. (2008). Extending the breadth of metabolite profiling by gas chromatography coupled to mass spectrometry (review)
- T. Kind et al (2009). FiehnLib: Mass Spectral and Retention Index Libraries for Metabolomics (methodology)

Acknowledgements

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-Stephan Bauman