

A new Concept for *isotope ratio monitoring* LC/MS

Andreas Hilkert, Dieter Juchelka, Michael Krummen, Reinhold Pesch
Thermo Electron (Bremen) GmbH

Content

- Introduction to *irm*-LC/MS
 - *Technology*
 - *Operating Modes*
- New Applications by *irm*-LC/MS
 - ***Authenticity Control***
 - Detection of adulteration by sugars in honey.
 - ***Determination of Origin***
 - Differentiation of analgesic drugs
 - ***Molecular Biology***
 - Carbon isotopic characterization of rRNA.
 - ***Forensic Chemistry***
 - Analysis of aspartic acid in cadaver blood samples.

Why *irm*-LC/MS ?

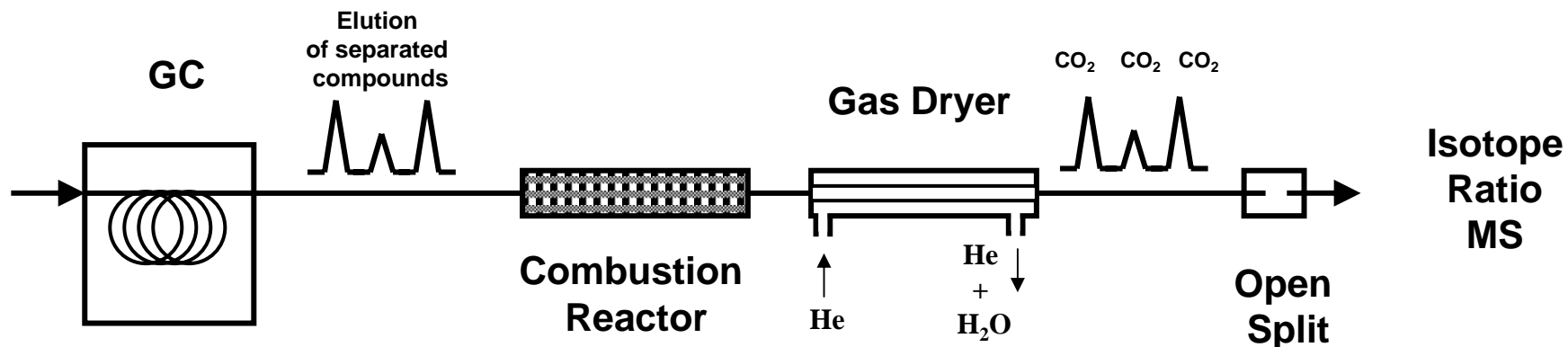
$\delta^{13}\text{C}$ analysis of individual compounds with:

- *High molecular weight*
- *High polarity*
- *Thermal instability*
- *High vapor pressure*

- *Less sample preparation*
- *No derivatization*
- *Direct sample introduction*
- *High sensitivity*

Comparison between *irm*-GC/MS and *irm*-LC/MS

- *irm*-GC/MS

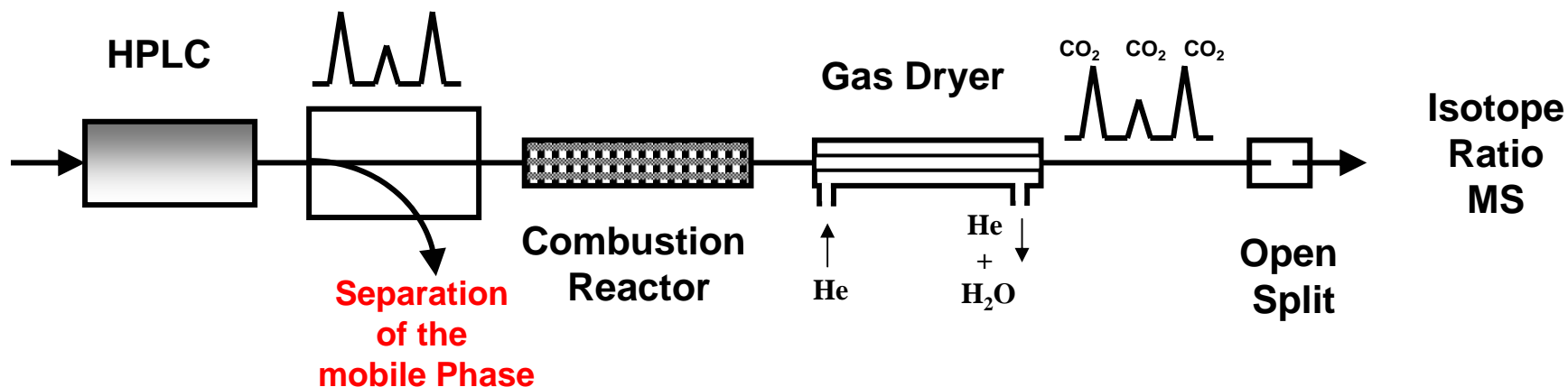


- Helium as carrier for
 - Separation of compounds
 - Transfer to the IRMS
- Helium has
 - No impact on combustion
 - No effects in the IRMS

Dry combustion (oxidation)
in the He phase

Comparison between *irm*-GC/MS and *irm*-LC/MS

- *irm*-LC/MS (first strategy)

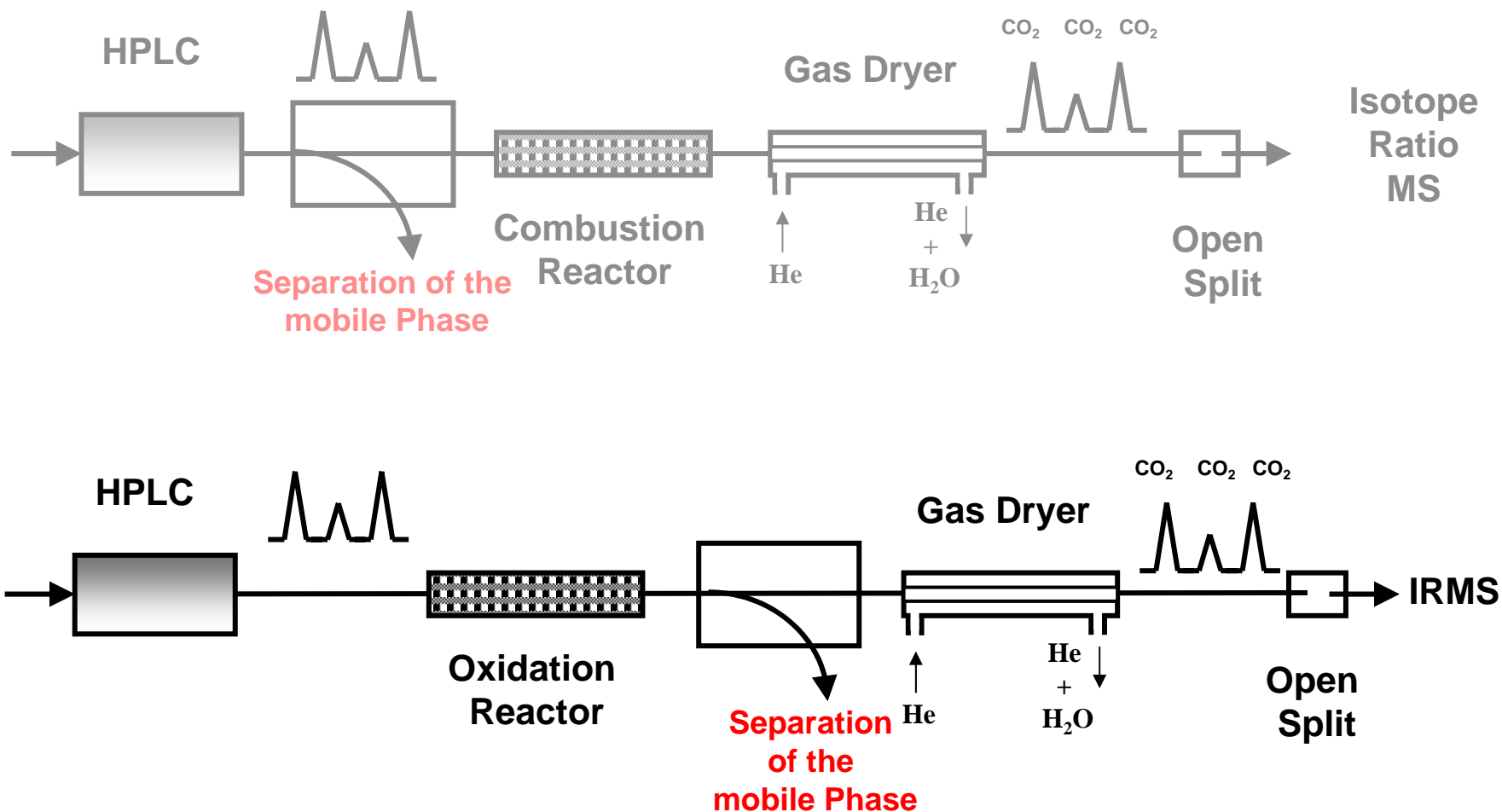


- Solvents as carrier for
 - Separation of compounds
- Solvents are
 - Oxidized
 - Hazardous to IRMS
- No solvents to reactor or IRMS

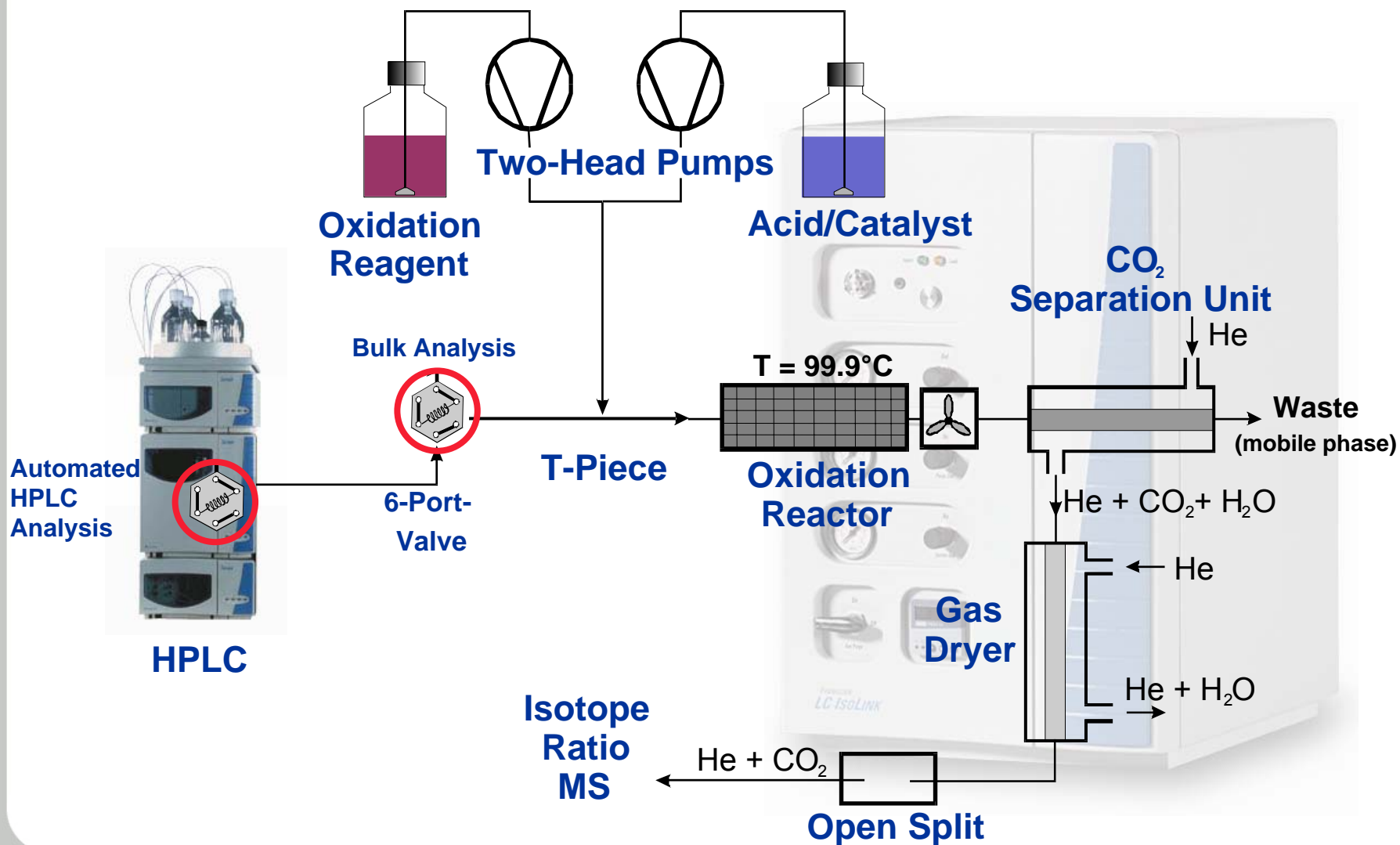
First approaches ('91, '93)

- “Moving Wire” – Drying system (difficult to use)
- “Particle Beam” Separation (low sensitivity, fractionation)

A New Strategy

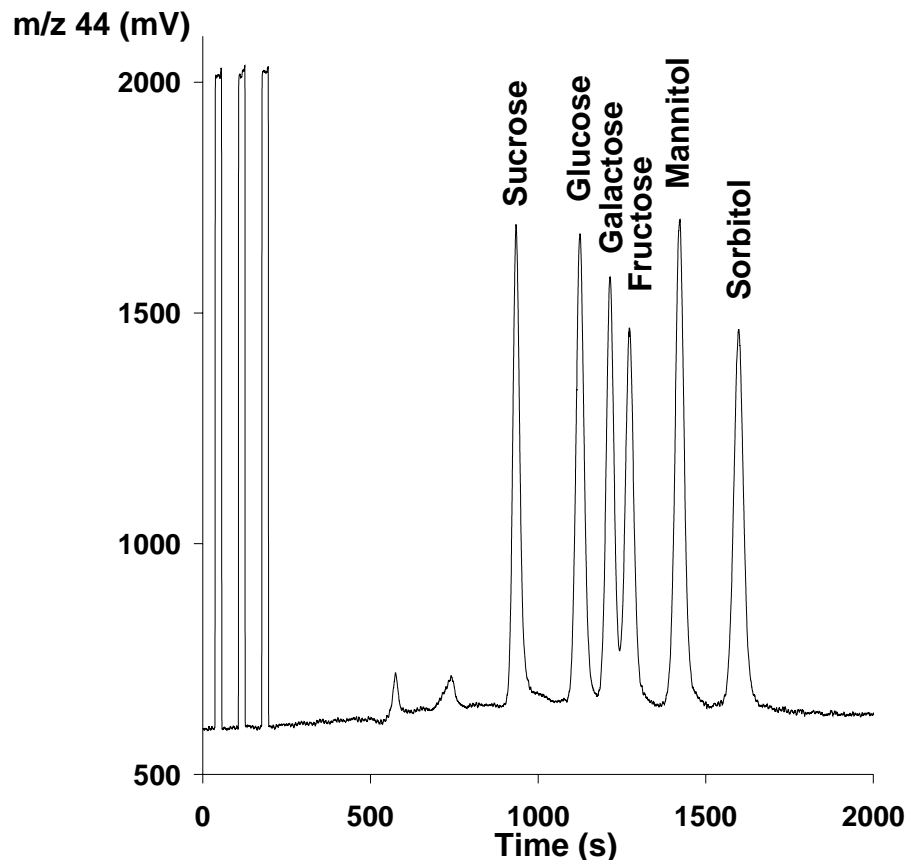


Scheme of the LC IsoLink Interface



HPLC Resolution

➔ HPLC separation of carbohydrates

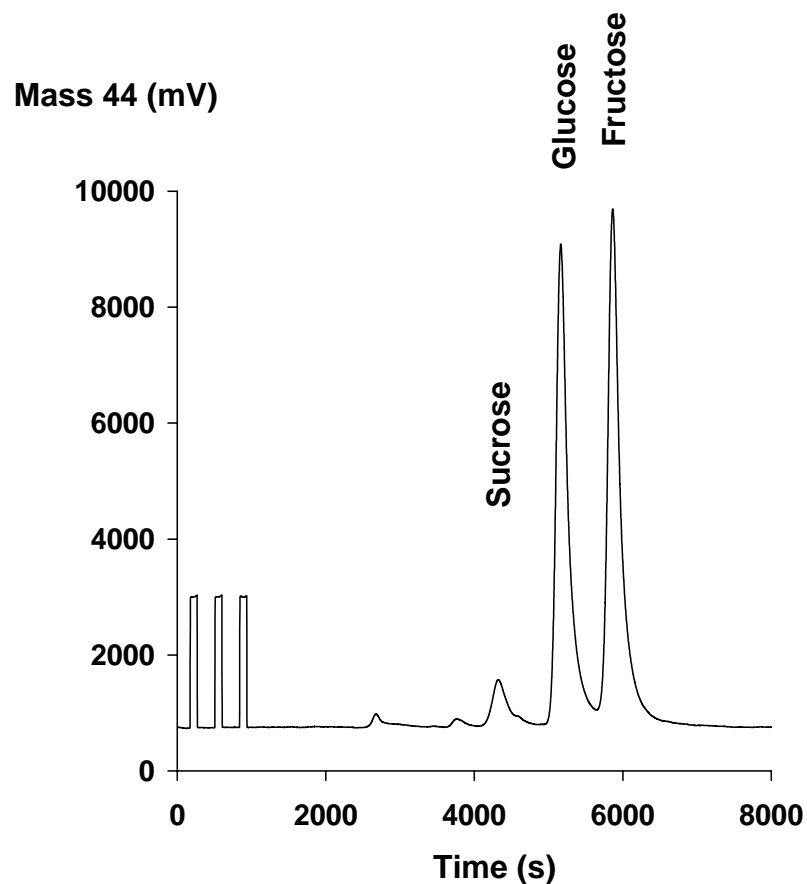


➔ HPLC resolution is maintained

- Parameters:
 - HPLC flow:
 - 300 $\mu\text{l}/\text{min}$
 - Oxidation reagent:
 - 60 $\mu\text{l}/\text{min}$
(NH_4)₂S₂O₈, 100g/l
 - Column:
 - 700 CH
Carbohydrate
Column, 90 °C
 - Reactor:
 - 99.9 °C
 - CO₂ Exchanger:
 - 1 ml/min He flow

Authenticity Control of Honey

Investigation of the adulteration of honey analyzing glucose, sucrose, fructose

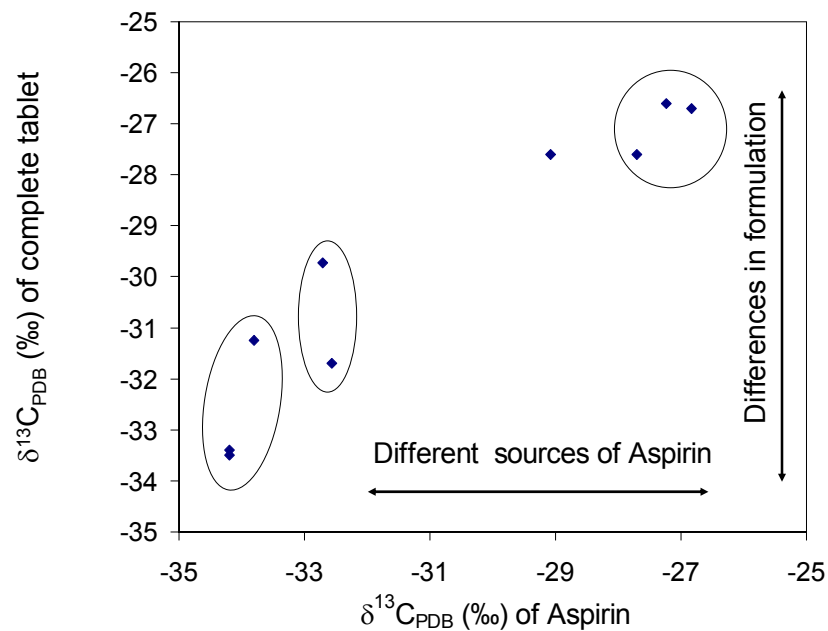
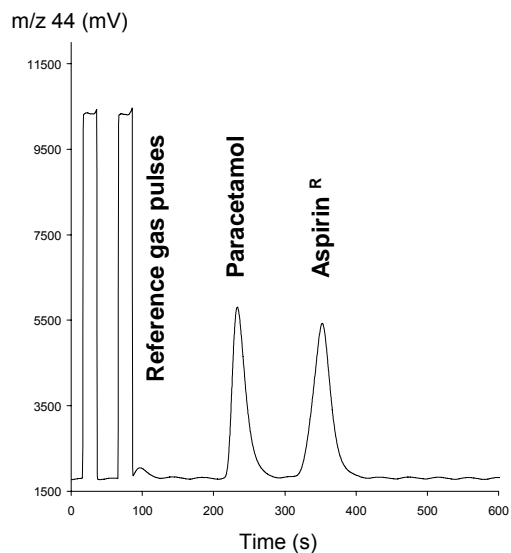


Honey	Glucose $\delta^{13}\text{C}\text{‰}$	Fructose $\delta^{13}\text{C}\text{‰}$	Area Fru/Glu	
A	-27.9	-27.8	1.13	pure
B	-25.1	-26.4	2.17	adulterated
C	-26.5	-26.5	1.35	pure
D	-26.1	-26.0	4.53	adulterated
E	-11.2	-13.9	0.65	adulterated

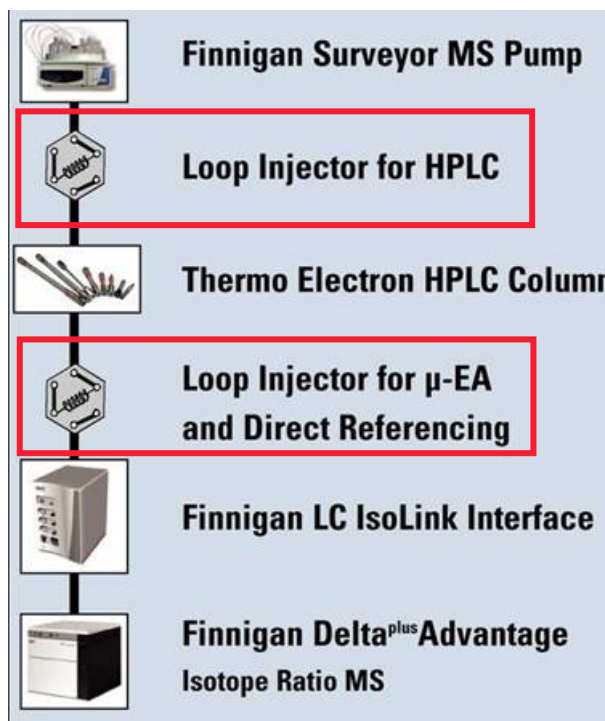
- Absolute $\delta^{13}\text{C}$ value
- $\delta^{13}\text{C}$ difference, Glu – Fru
- Ratio of area, Fru / Glu

Source Differentiation of Drugs

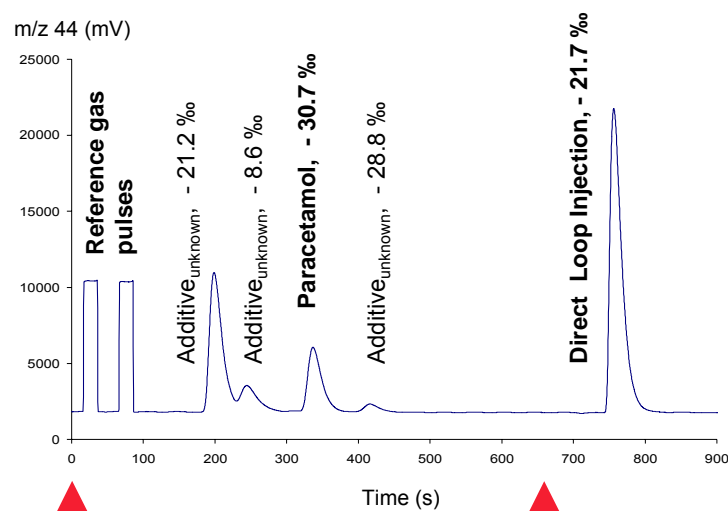
$^{13}\text{C}/^{12}\text{C}$ isotope ratio analysis of analgesic and antipyretic drugs



μ -EA – Bulk Injector



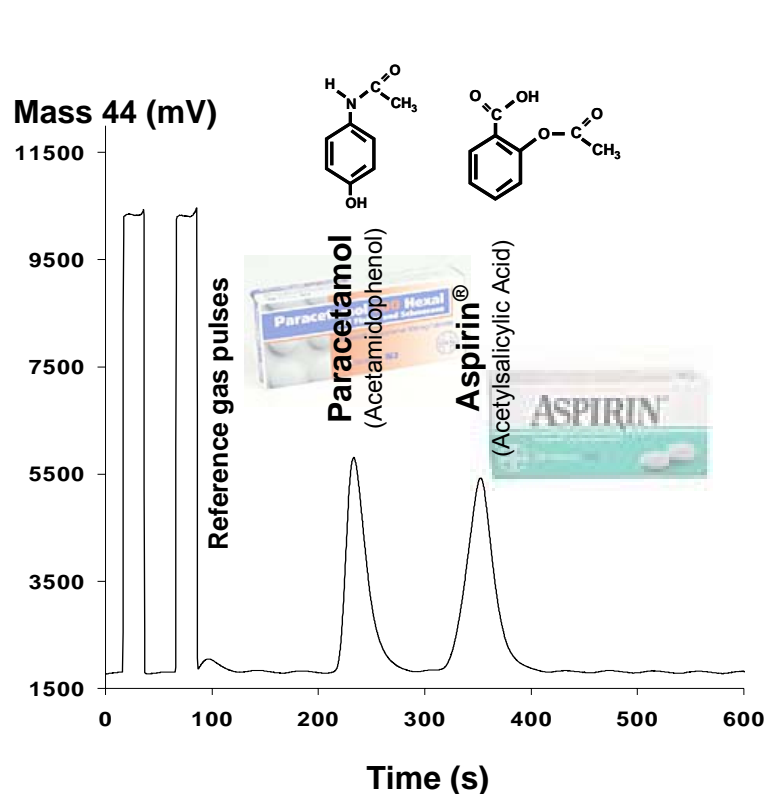
- Fast analysis of all water soluble compounds



Analysis of a tablet followed by direct loop injection (μ -EA). Loop size of the HPLC injector was 5 μ L, the loop size of the μ -EA injector was 10 μ L, which results in two-fold response of the μ -EA peak

Source Differentiation of Drugs

➔ Determination of different analgesic compounds.
 $\delta^{13}\text{C}$ of Paracetamol (Acetamidophenol) and Aspirin[®] (Acetylsalicylic Acid; ASA).



Tablet Type	$\delta^{13}\text{C}$ (‰)		
	Paracetamol	ASA	direct loop
A Country1	-	-34.2	-33.4
A Country2	-	-34.2	-33.5
B	-	-29.1	-27.6
C	-	-27.2	-26.6
D ₁	-	-26.8	-26.7
E	-	-27.7	-27.6
F	-32.3	-32.6	-31.7
D ₂	-28.7	-33.8	-31.3
G	-29.2	-32.7	-29.7

- Tablet type A has the same origin
- 4 sources of ASA
- Producer D use different ASA sources

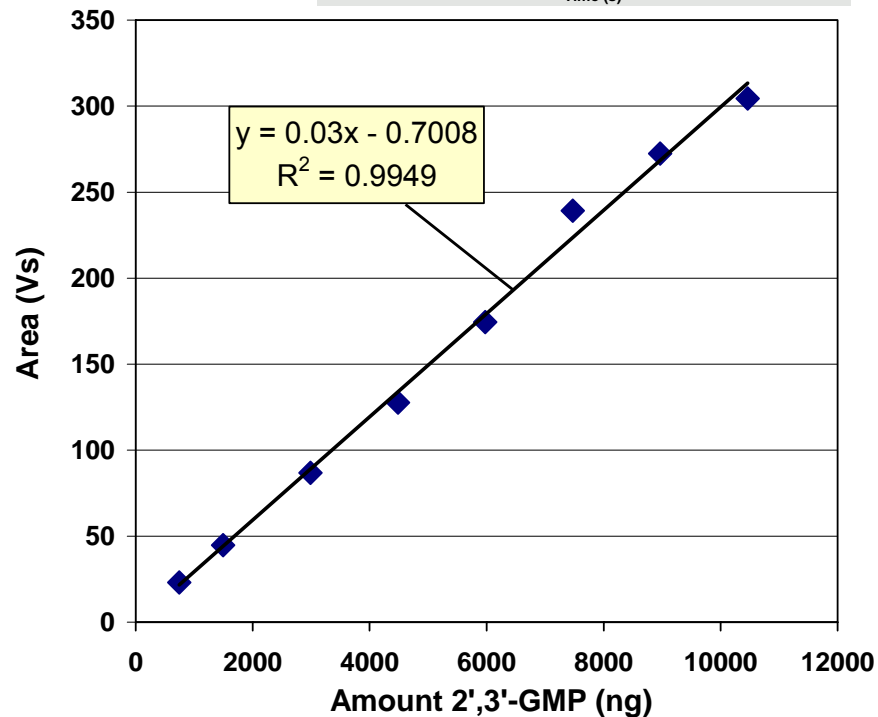
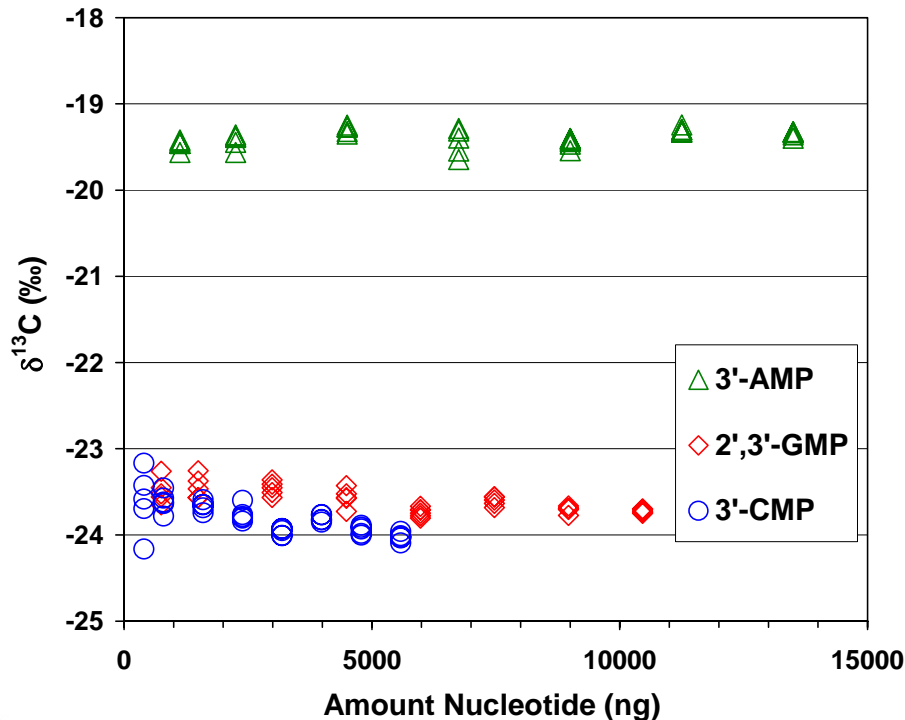
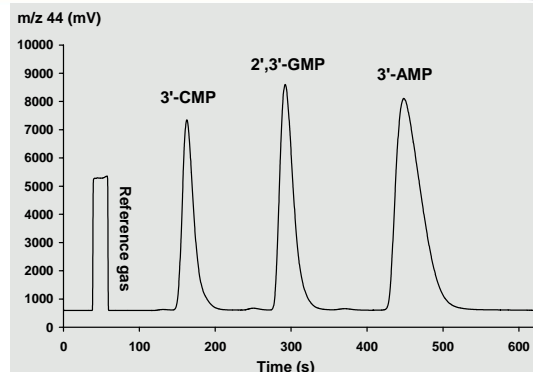
$\delta^{13}\text{C}$ Analysis of RNA by *irm*-LC/MS

- **Molecular biological approach**

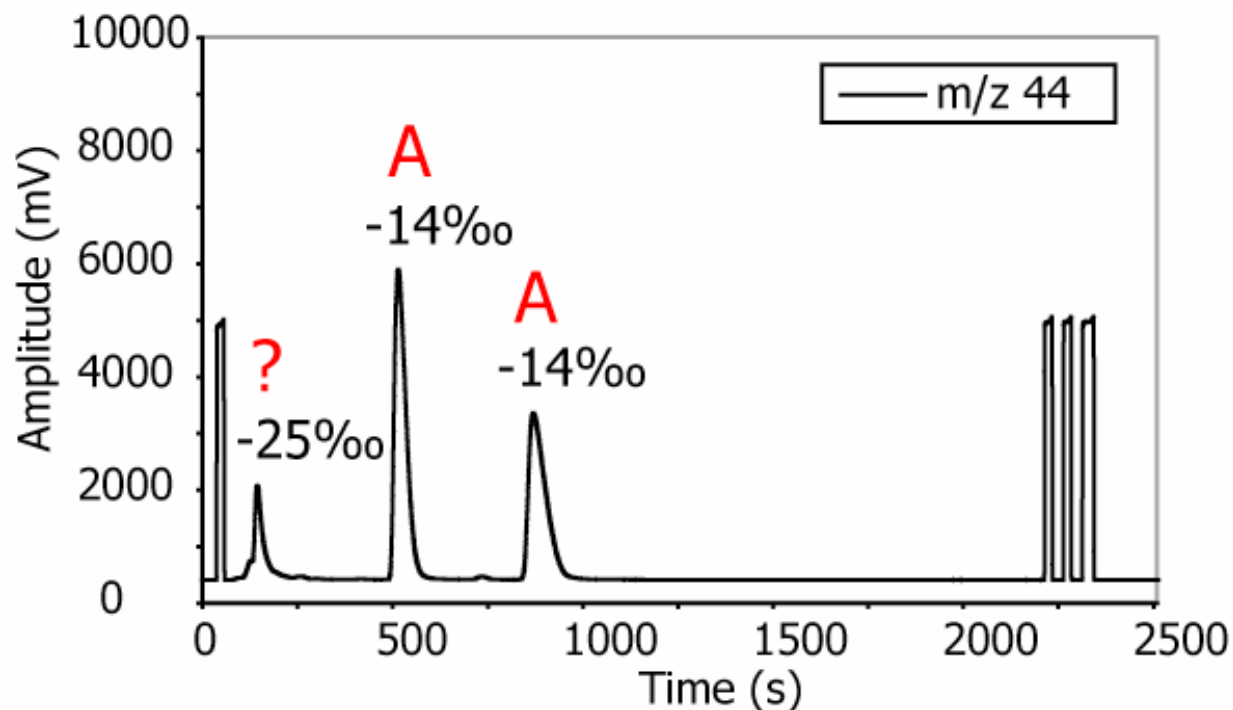
Linkage of microbiological species identity with carbon source utilization by carbon isotopic characterization of rRNA.

$\delta^{13}\text{C}$ Analysis of Nucleotides by *irm*-LC/MS

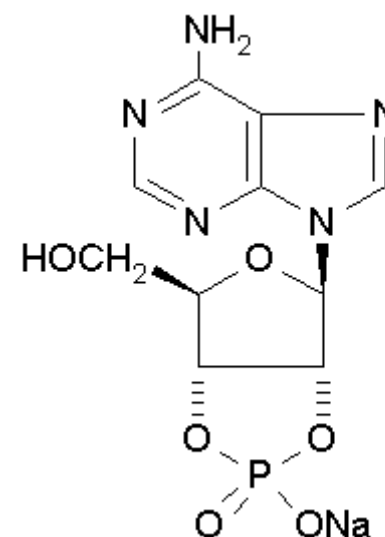
- Cytidine-3'-monophosphate (3'-CMP)
- Guanosine-2',3'-cyclic monophosphate (2',3'-GMP)
- Adenosine-3'-monophosphate (3'-AMP)



irm-LC/MS of NaOH-hydrolyzed Polynucleotide A

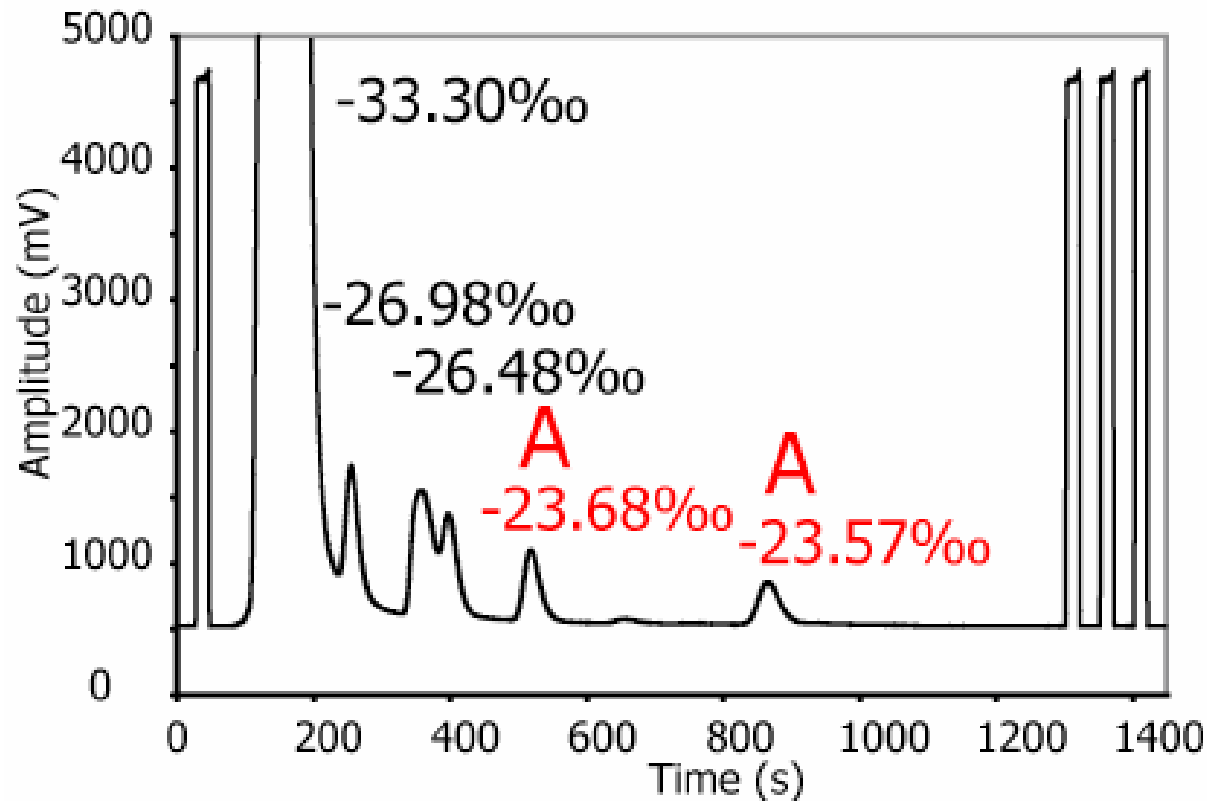


Adenosine 2':3'-
cyclic
monophosphate
sodium salt



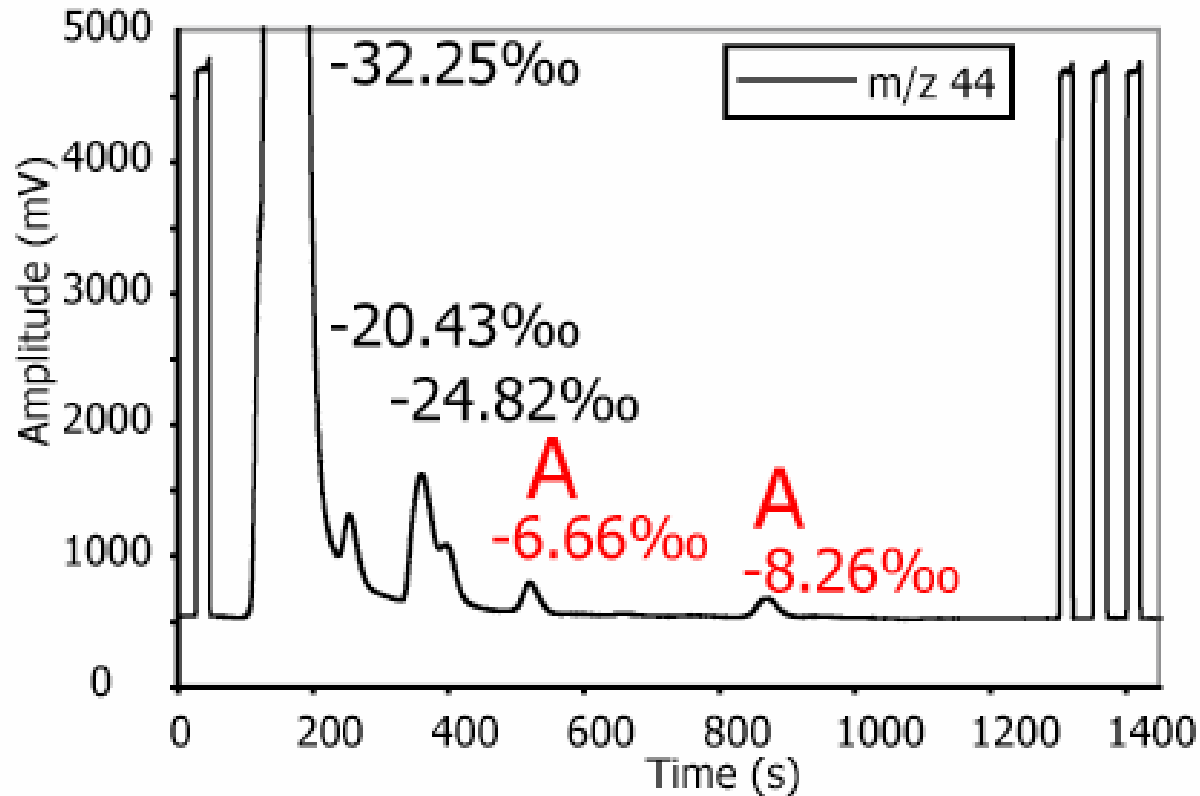
- Mobile Phase: 100 mM KH_2PO_4 , pH 5, Flow: 400 $\mu\text{l}/\text{min}$
- HPLC Column: HyPURITY AQUASTAR (Thermo Electron)
150 mm x 2.1 mm, particle size: 5 μm spherical
- Hydrolyse: 0.2 N NaOH for 15 min at 50 °C

irm-LC/MS of NaOH-hydrolyzed *E. coli* RNA - Grown on LB Medium -



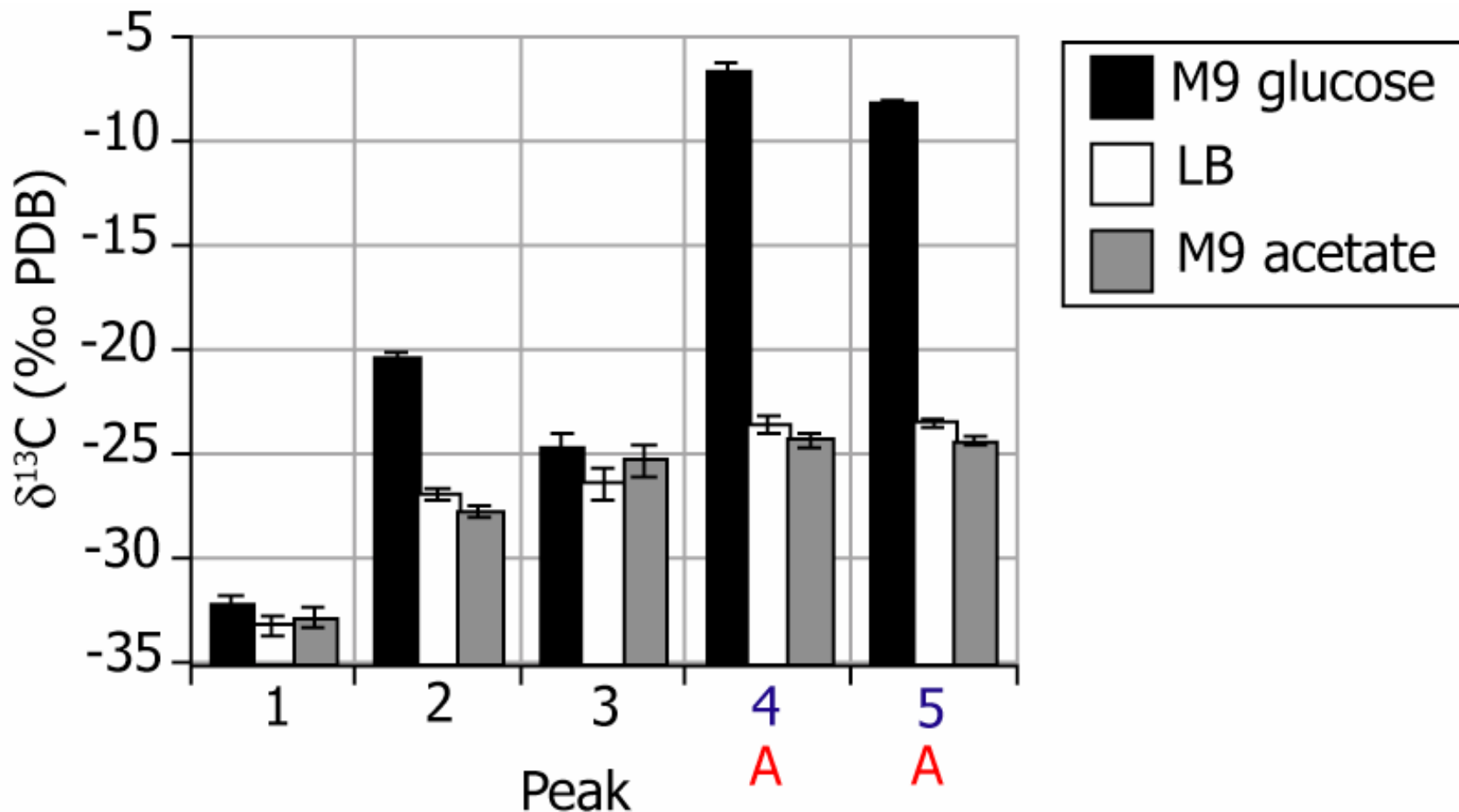
- Cell growth and Isolation: *RNA was extracted from overnight cultures of E. coli grown on LB medium.*
- Hydrolyse: *0.2 N NaOH for 15 min at 50 °C*

irm-LC/MS of NaOH-hydrolyzed *E. coli* RNA - Glucose grown -



- Cell growth and Isolation: *RNA was extracted from overnight cultures of E. coli grown on M9 minimal salts with glucose as sole carbon source Medium.*
- Hydrolyse: *0.2 N NaOH for 15 min at 50 °C*

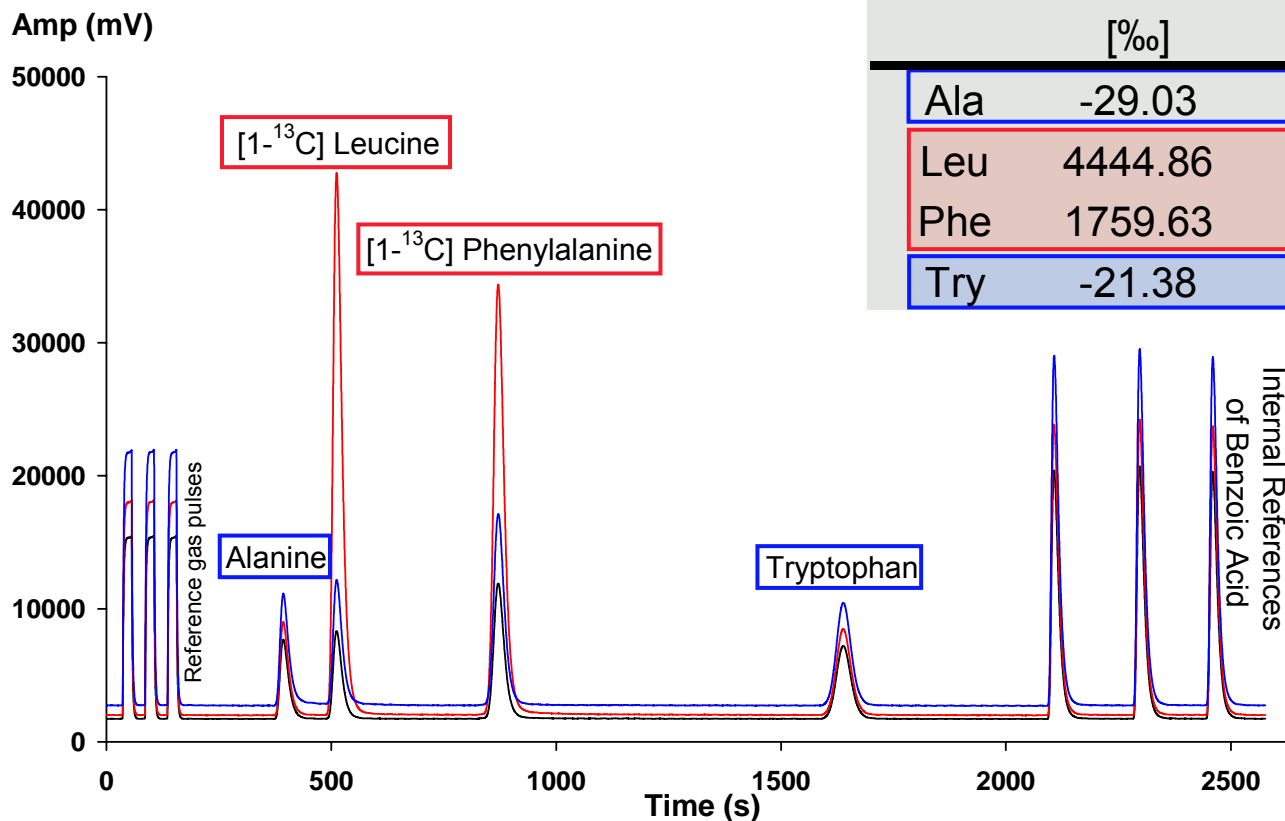
Carbon Isotopic Composition of Individual Peaks from NaOH-hydrolyzed E. coli RNA



*Peaks are numbered from first- to last-eluting

irm-LC/MS: $\delta^{13}\text{C}$ Analysis of Amino Acids

➔ Mixture of two **natural** and two **labeled** amino acids

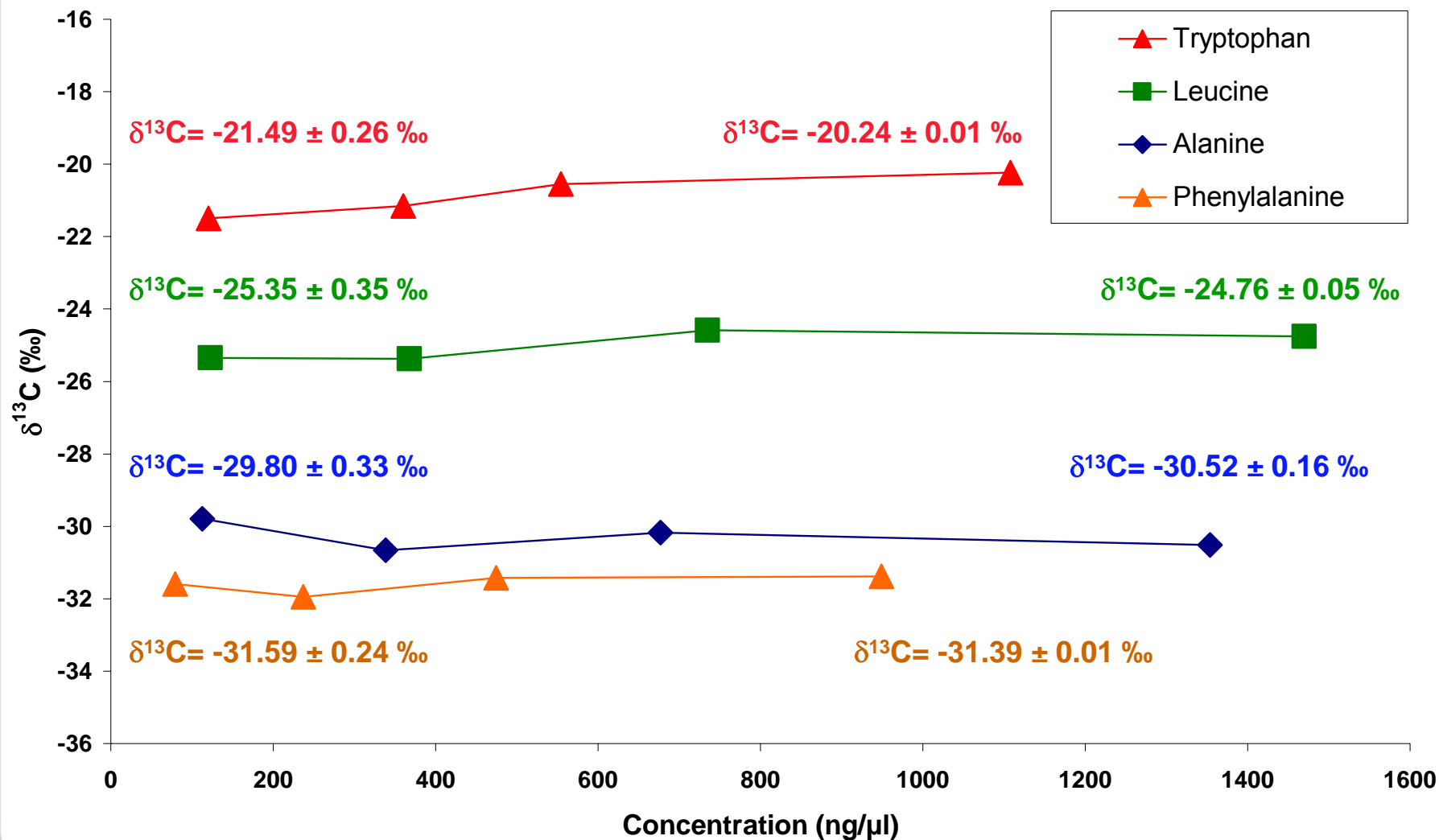


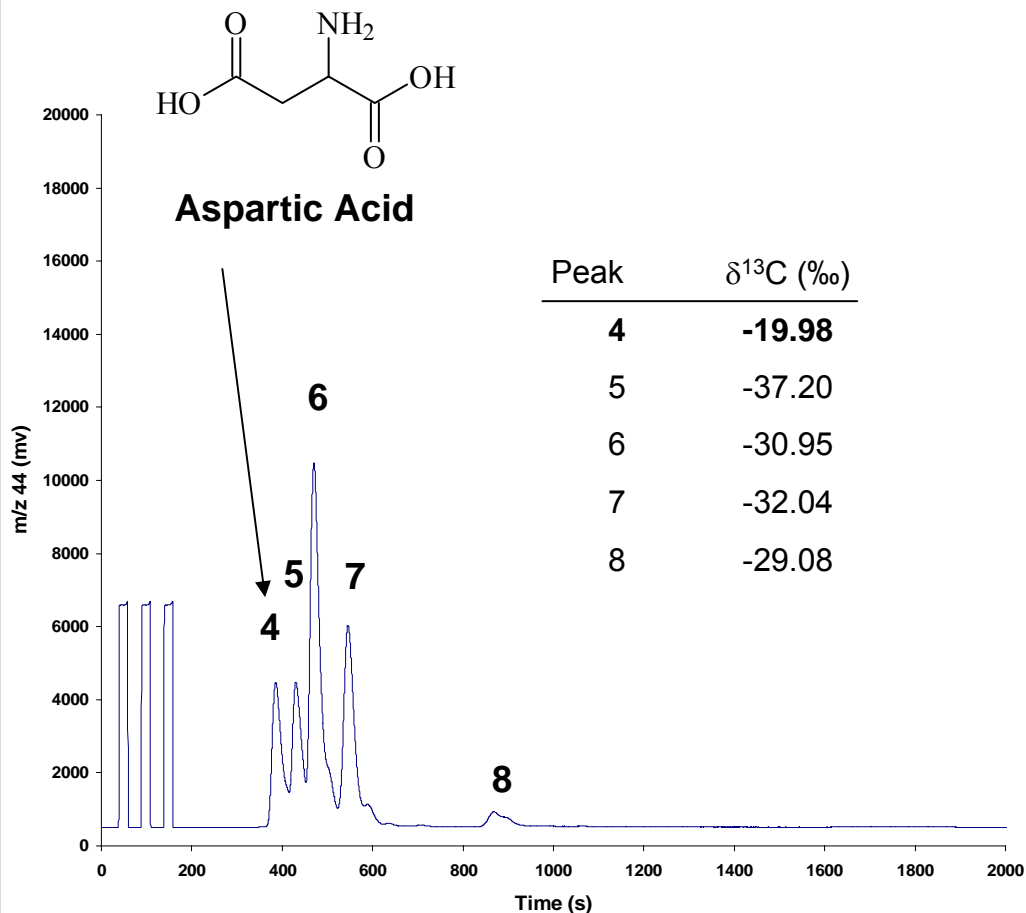
	$\delta^{13}\text{C}$ [‰]	S.D. [‰]	SDM [%]	n
Ala	-29.03	0.3	0.04	5
Leu	4444.86	14	0.25	5
Phe	1759.63	13	0.47	5
Try	-21.38	0.2	0.03	5

— m/z 44
— m/z 45
— m/z 46

➔ Good reproducibility; no memory effects by the labeled amino acids

irm-LC/MS: $\delta^{13}\text{C}$ Analysis of Amino Acids



$\delta^{13}\text{C}$ Analysis of Aspartic Acid in Cadaver Blood**Aspartic Acid**

Sample	$\delta^{13}\text{C}$ (‰)	Std. Dev.
A	-32.12	0.08
B	-32.66	0.11
C	-16.18	0.03
D	-18.76	0.14
E	-19.98	0.06
F	-16.62	0.12
Standard		
G	-24.79	0.05
H	-26.19	0.11

- Mobile Phase: 10mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, pH 4.7, Flow: 300 $\mu\text{l}/\text{min}$
- HPLC Column: Nova-Pack® C18, 60 Å (4 μm , 3.9 mm x 300 mm).

Summary

- A new approach to *irm*-LC/MS opens a wide range of interesting applications in various fields.
- Macromolecules, non-volatile components and components which tend to decompose are directly accessible for precise isotopic analysis.

Acknowledgement

- Barbara J. MacGregor and Adam Friedman, Department of Marine Sciences, University of North Carolina, Chapel Hill, N.C.
- Kurt-Peter Raezke, APPLICA GmbH, Bremen
- Jean-Philippe Godin and Jörg Hau, Nestlé Research Center, Lausanne