GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

Bundesinstitut für Risikobewertung

IRMS – Part 5 – GC/C-IRMS and LC-IRMS

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International Methods

Year	Method	Product	Fraction	Techniques	Isotope ratios
1987	OIV method OIV-MA-AS311-05	Wine	Ethanol	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$, R
1991	AOAC method 998.12	Honey	Honey, proteins	IRMS	$^{13}C/^{12}C$
1993	CEN (TC174 N108, ENV 12140)	Fruit juice	Sugar	IRMS	$^{13}C/^{12}C$
1995	AOAC method 995.17	Fruit juice	Ethanol (from fermentation)	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$, R
1996	OIV method OIV-MA-AS2-12	Wine	Water	IRMS	$^{18}O/^{16}O$
1997	CEN (TC174 N109, ENV 12141)	Fruit Juice	Water	IRMS	$^{18}O/^{16}O$
2000	AOAC Official method 2000.19	Maple syrup	Ethanol (from fermentation)	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$, R
2001	OIV method OIV-MA-AS312-06	Wine	Ethanol	IRMS	$^{13}C/^{12}C$
2004	AOAC method 2004.01	Fruit juice & maple syrup	Ethanol (from fermentation)	IRMS	$^{13}C/^{12}C$
2006	AOAC method 2006.05	Vanillin	Vanillin	SNIF-NMR	$(D/H)_I$
2010	**OIV method OIV-MA-AS312-07**	**Wine**	**Glycerol**	**GC-C-IRMS, HPLC-IRMS**	$\mathbf{^{13}C/^{12}C}$
2014	OIV method OIV-MA-AS314-03	Sparkling Wine	CO_2	IRMS	$^{13}C/^{12}C$
2017	**OIV method OIV-MA-AS311-09**	**Wine**	**Glucose, Fructose, Glycerol, Ethanol**	**HPLC-IRMS**	$\mathbf{^{13}C/^{12}C}$

Overview

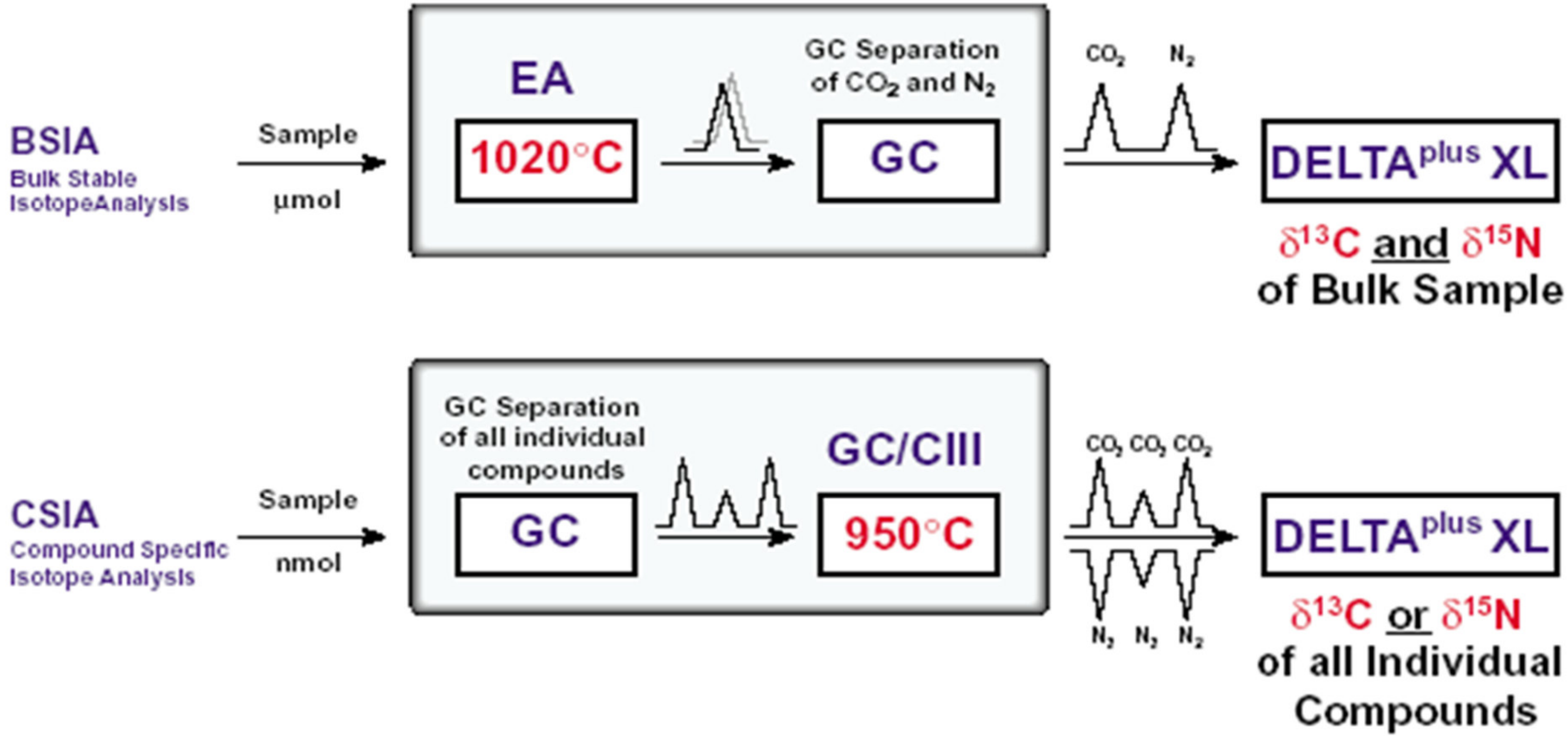
Principals of:

- IRMS measurements ✓
- Elemental Analyzer EA-IRMS ✓
- Equilibration- IRMS ✓
- ^{18}O-Pyrolysis-IRMS and D/H-Pyrolyis-IRMS ✓
- GC/C-IRMS, GC/P-IRMS ☜
- LC-IRMS ☜

Methods:

- $^{13}C/^{12}C$ of wine ethanol ✓
- $^{18}O/^{16}O$ of Wine water ✓
- $^{13}C/^{12}C$ isotope ratio of glycerol in wines by Gas Chromatography Combustion GC/C- IRMS ☜
- $^{13}C/^{12}C$ of glucose, fructose, glycerol and ethanol in products of vitivinicultural origin by HPLC-IRMS ☜

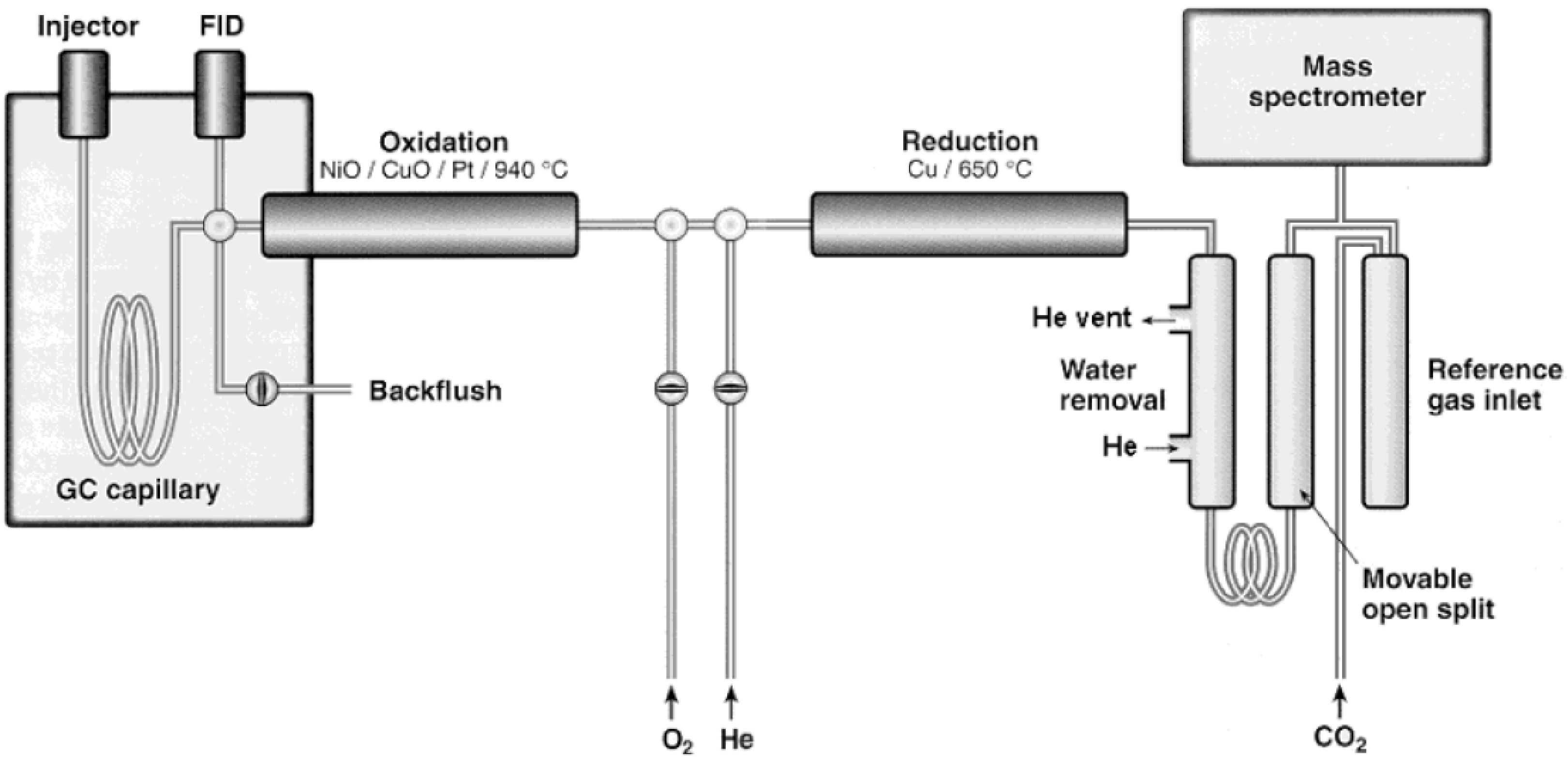
Bulk versus Compound Specific IRMS



Source: Thermo Finnigan

GC-C-IRMS (GC-P-IRMS)

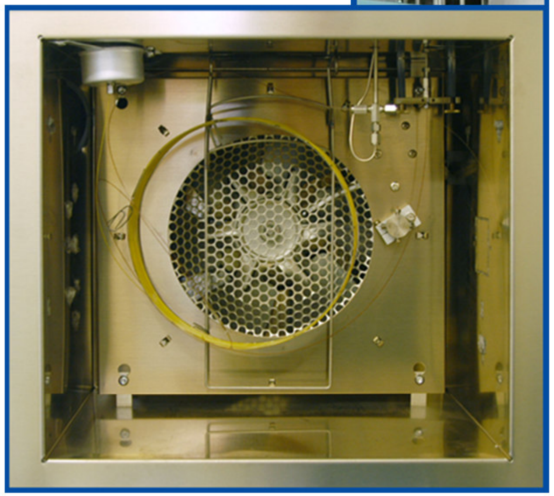
- GC-C-IRMS: determination of the $^{13}C/^{12}C$ isotope ratio or $^{15}N/^{14}N$ isotope ratio
- (GC-P-IRMS: determination of the D/H isotope ratio or $^{18}O/^{16}O$ isotope ratio)

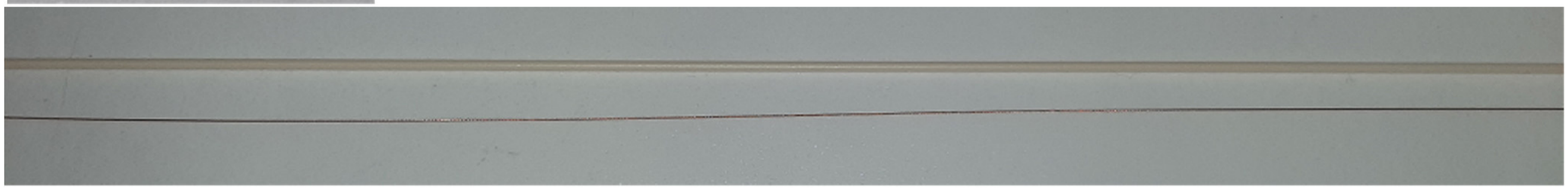


Calderone et al., *J. Agric. Food Chem.*, 52, 5902-5906 (2004)

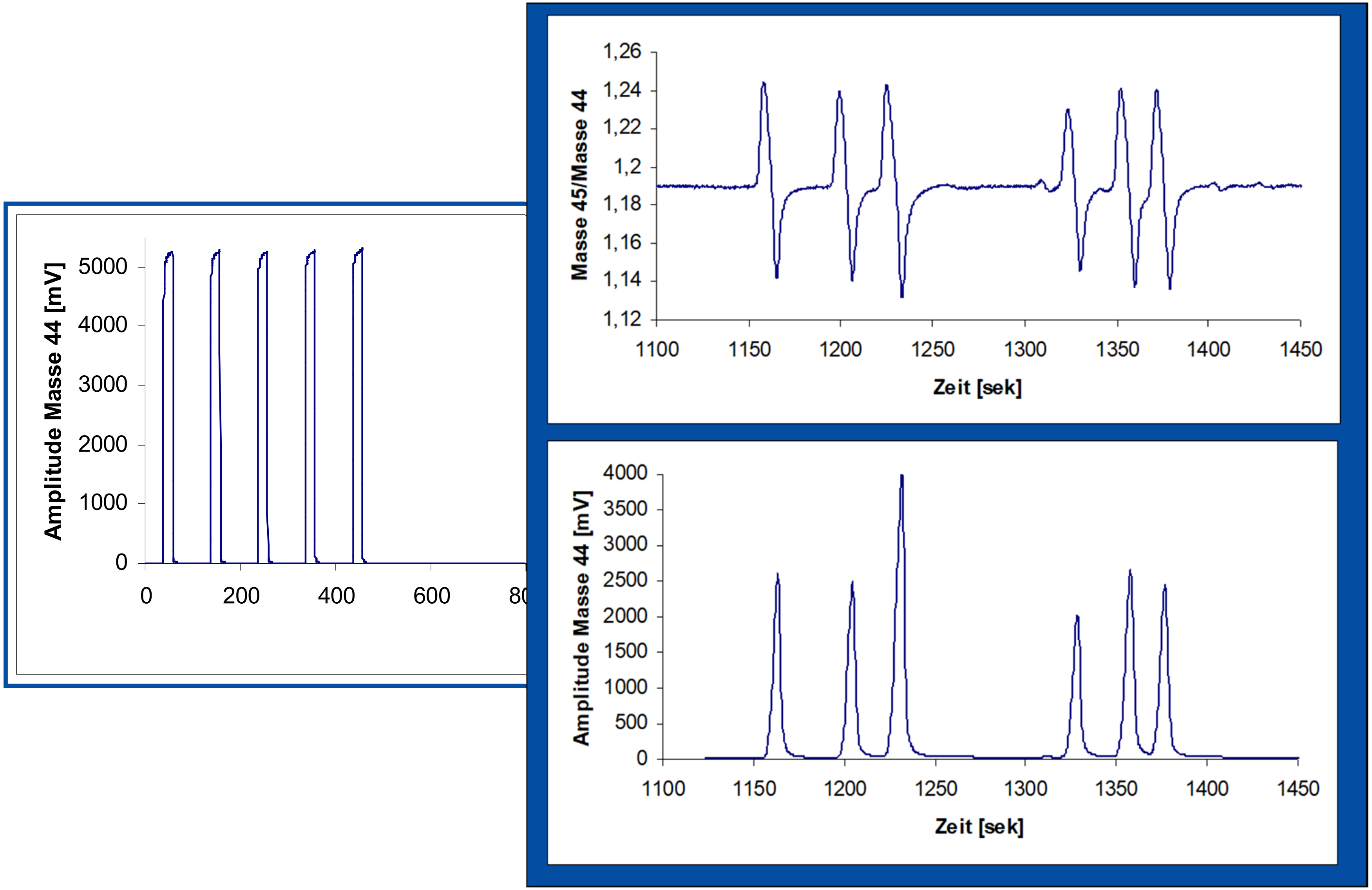
GC-C-IRMS: Combustion reactor

- Combustion reactor: CuO / NiO / Pt or CuO / Pt in a quartz or ceramic tube
- Reoxidation on a regular basis
- 30 cm long
- Wires 0.125 mm diameter
- Temperature approx. 850 to 950 °C





GC-C-IRMS: Chromatogram



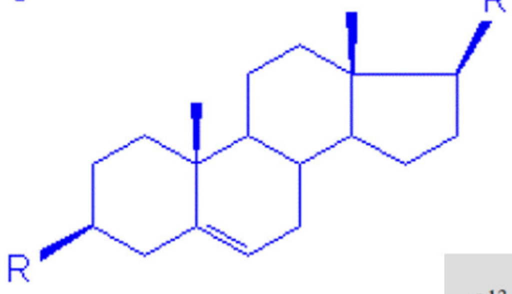
GC-C-IRMS: Derivatisation

Derivatisation, if compounds are not volatile or too polar for GC.

Most common: Acetylation, Esterification, Silylyation

- Addition of the analyte element: e.g. methylation or acetylation
- Adulteration caused by non-quantitativ sample conversion
- Isotopic fractionation due to kinetic isotope effects

R = CH_3COO



$$\delta^{13}C[Steroidacetat] = \frac{\delta^{13}C[Steroid]*n}{(n+m)} + \frac{\delta^{13}C[Essigsäureanhydrid]*m}{(n+m)}$$

- AND kinetic isotope effect has to be considerd !

GC-C-IRMS: important points

- non-discriminating sample processing required
- derivatization of analytes under constant conditions
- higher concentrations required than for GC/MS
- baseline separated peaks, clean samples
- GC parameters: Column, Injection, Flow rate, temperature program,
- reoxidation cycle for the oxidation oven
- background correction (important for less clean samples)

$^{13}C/^{12}C$ isotope ratio of glycerol in wines by Gas Chromatography Combustion GC/C- IRMS

OIV

Method OIV-AS312-07 Type of the method **IV**

Method for the determination of the $^{13}C/^{12}C$ isotope ratio of glycerol in wines by Gas Chromatography Combustion or High performance Liquid Chromatography coupled to Isotopic Ratio Mass Spectrometry (GC-C-IRMS or HPLC-IRMS)

(OIV-Oeno 343-2010)

Principle: The measurement of the 13/12C ratio of glycerol may enable possible detection of addition of gylcerol from maize (C4 plant) or from synthesis (fossil sources) to wine.

GC/C-IRMS: $^{13}C/^{12}C$ isotope ratio of glycerol in wines

Wine Sample

↓

Filtration on 0,2 µm filter, dilution 1:4 with ethanol, transfer to a GC vial

OR

Alternative: for quantification dilution with an 1,5-pentanediol solution

↓

Injection alternating with Quality Control Samples, each sample at least 3 times and with at least 2 Reference gas pulses in each analyis

↓

Reference gas CO_2 is previously calibrated against V-PDB-calibrated standards

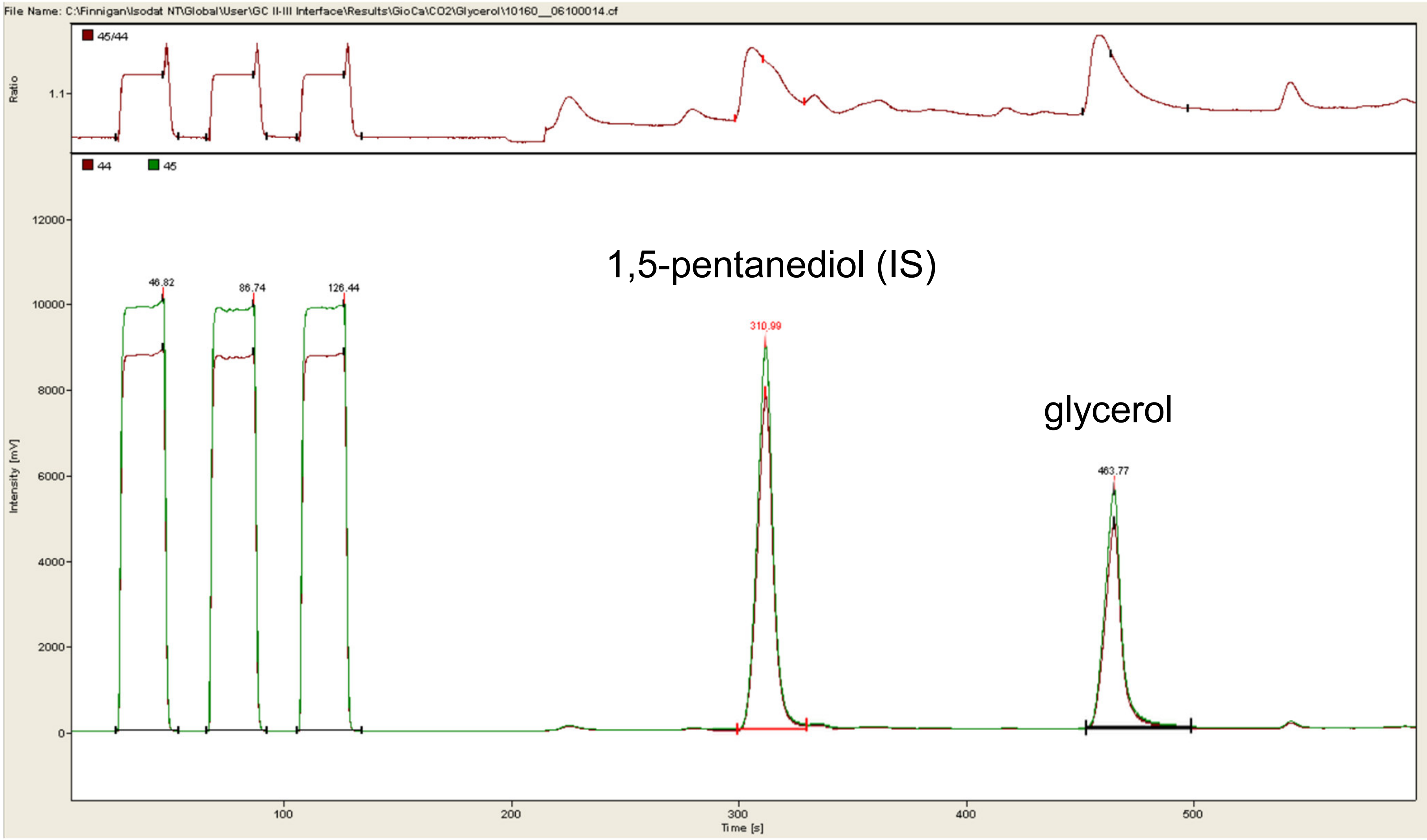
GC/C/IRMS: $^{13}C/^{12}C$ isotope ratio of glycerol in wines – sequence example

Injection	Sample
1	Quality Control Sample 1
2	Quality Control Sample 1
3	Quality Control Sample 1
4	Sample 1
5	Sample 1
6	Sample 1
7	Sample 2
8	Sample 2
9	Sample 2
10	Quality Control Sample 1
11	Quality Control Sample 1
12	Quality Control Sample 2
13	Quality Control Sample 2
14	Sample 3
15	Sample 3
16	Sample 3
17	Sample 4
18	Sample 4
19	Sample 4
20	Quality Control Sample 1
21	Quality Control Sample 1
22	Quality Control Sample 2
23	Quality Control Sample 2
24	Sample 5
25	Sample 5
26	Sample 5
27	Sample 6
28	Sample 6
…	
x	Quality Control Sample 1
y	Quality Control Sample 1
z	Quality Control Sample 1

GC/C/IRMS: $^{13}C/^{12}C$ isotope ratio of glycerol in wines – GC conditions example

- carrier gas: helium
- injector temperature 270°C
- injection of 0.3 µl in split mode (split flow 120 ml/min)
- polar capillary column, 25 m, 0.25 mm ID, 0.20 µm film thickness
- temperature program: 120°C (2 min), increase at a rate of 10°C/min up to 220°C (2 min)
- combustion at 950°C, reduction temperature 640°C
- reoxidation at regular intervals depending on the total amount of samples
- Note: correct values for volumes, temperatures, flows and times are indicative. Working conditions should be optimized individually.

GC/C-IRMS: $^{13}C/^{12}C$ isotope ratio of glycerol in wines - chromatogram



Source: Method OIV-AS312-07

GC/C/IRMS: $^{13}C/^{12}C$ isotope ratio of glycerol in wines – calculation

- The $^{13}C/^{12}C$ isotope ratio can be expressed by its deviation from a working reference.

$$\boxed{\delta^{13}C_{Sample}\,(‰) = [(R_{Sample} / R_{reference}) - 1\,] * 10^{3}}$$

- Small variations may occur while measuring on-line due to changes in the instrumental conditions.
- A drift of the $^{13}C/^{12}C$ isotope ratio may occur within the sequence.
- A correction can then be calculated for each sample using linear interpolation.
- Quantification possible via the internal standard.

GC/C/IRMS: Troubleshooting

- High background O_2 (mass 32)

- Combustion reactor is not conditioned
- Reactor will bleed after reoxidation

- Switch the system into backflush-mode for some hours
- See also „System Tests and Trouble Shooting“

GC/C/IRMS: Troubleshooting

- High background H_2O (mass 18)

- Nafion® old or damaged
- GC-column bleed

- Inspect Nafion®-tube for damage and colour, replace if necessary
- Condition the GC-column
- See also „System Tests and Trouble Shooting“

GC/C/IRMS: Troubleshooting

- High background Ar (mass 40)

- Atmospheric leak

- Inspect GC-injector
- Inspect all connections, all pressfits between the GC-column, the reactors and other capillarys
- See also „System Tests and Trouble Shooting“

GC/C/IRMS: Troubleshooting

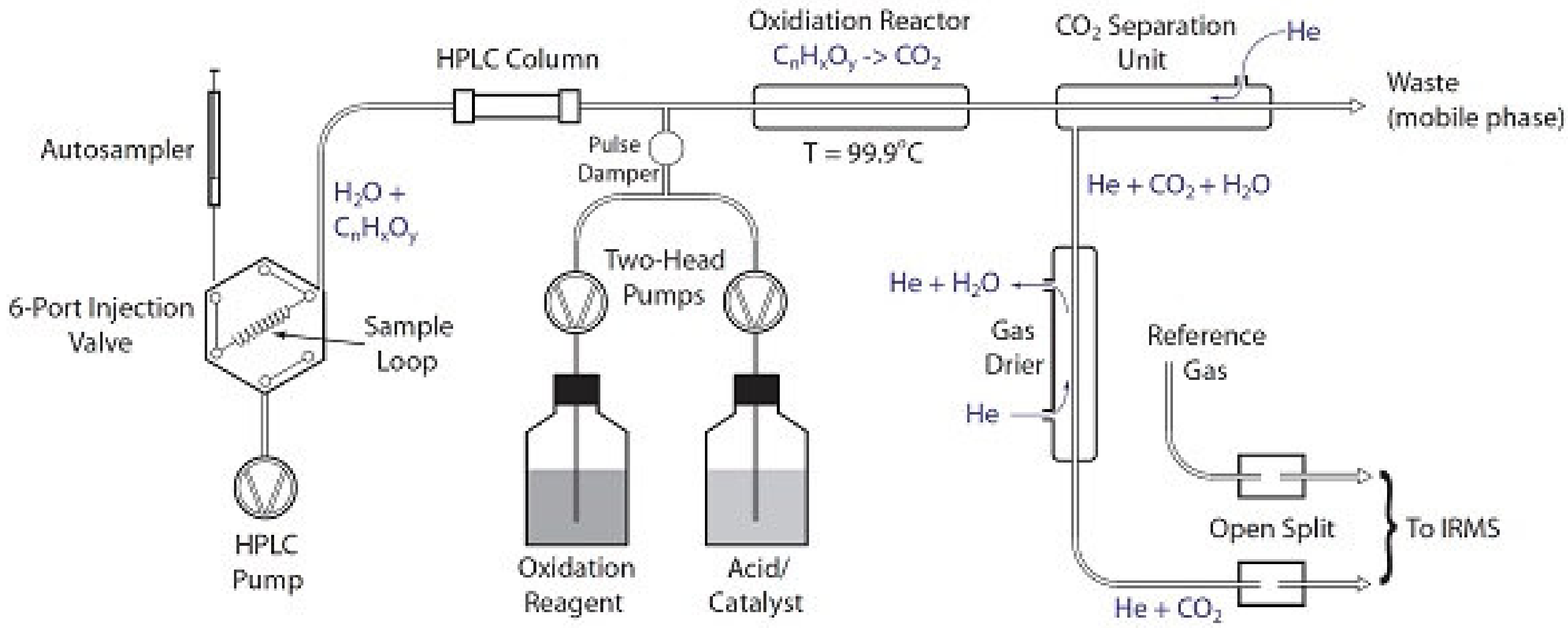
- Variable delta values

- Atmospheric leaks
- Reactor depleted
- Reactor not conditioned

- Inspect all connections, all pressfits for leaks
- Re-oxidize the reactor or replace it
- Check 32 Background
- See also „System Tests and Trouble Shooting"

LC-IRMS: Principle

- Chemical oxidation after aquous liquid chromatography



$Na_2S_2O_8$ H_3PO_4

Source: https://www.bristol.ac.uk/chemistry/facilities/nerc-lsmsf/techniques/lcirms/

LC-IRMS: $^{13}C/^{12}C$ of Glucose, Fructose, Glycerol and Ethanol by HPLC-IRMS

Method OIV-MA-AS311-09 Type II and III method

Determination of the $^{13}C/^{12}C$ isotope ratios of glucose, fructose, glycerol, ethanol in production of vitivinicultural origin by high-performance liquid chromatography coupled to isotope ratio mass spectrometry

(Resolution Oeno 479/2017)

LC-IRMS: $^{13}C/^{12}C$ of Glucose, Fructose, Glycerol and Ethanol by HPLC-IRMS

Sample (wine, grape juice, concentrated must)

↓

Dilution with pure water
grape juice ~1:200
Concentrated must ~1:500

↓

Injection alternating with Quality Control Samples, each sample at least 3 times and with at least 2 Reference gas pulses in each analyis

↓

Reference gas CO_2 is previously calibrated against V-PDB-calibrated standards

LC-IRMS: $^{13}C/^{12}C$ of Glucose, Fructose, Glycerol and Ethanol by HPLC-IRMS: conditions

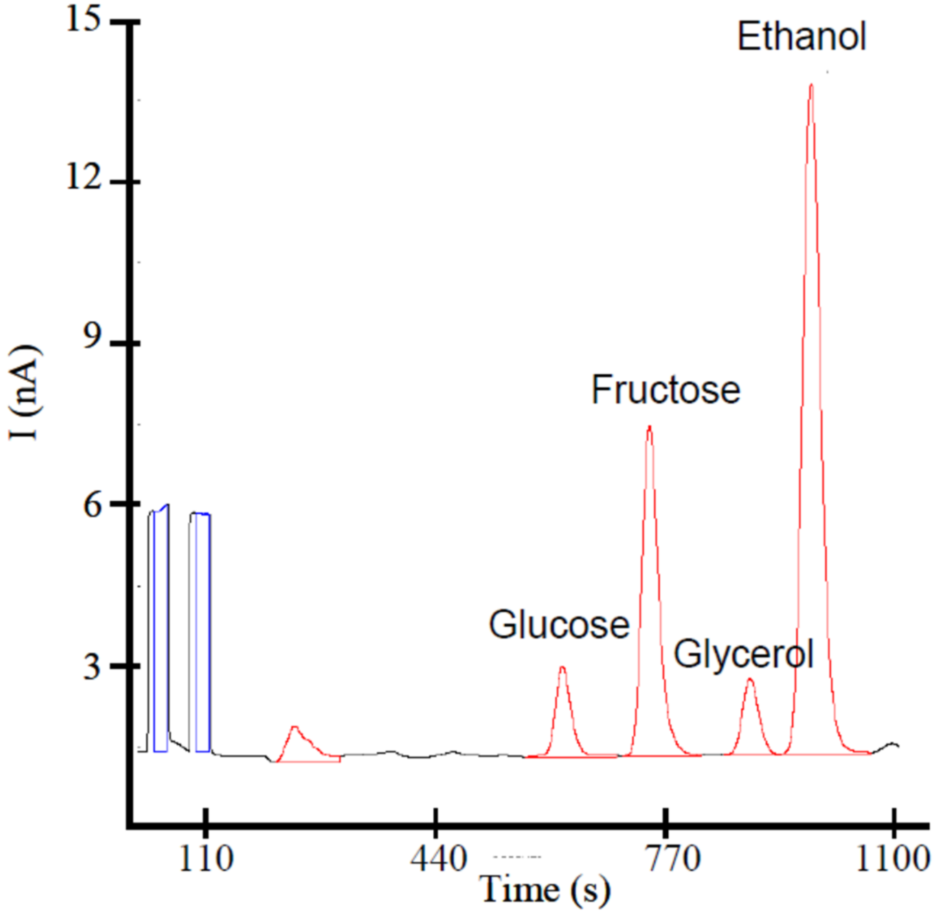
HPLC:

- injection volume 25 µl
- column: carbohydrate column
- mobile phase: pure water
- flow rate 0.4 mL/min
- column temperatute 80 °C

Liquid Interface:

- Solution of ammonium persulfate and Orthophosphoric acid
- Peristaltic pump flow: 0.6 mL/min
- Heater: 93 °C
- Flow Helium carrier gas: 15 mL/min
- Helium flow for drying: 50 mL/min

LC-IRMS: $^{13}C/^{12}C$ of Glucose, Fructose, Glycerol and Ethanol by HPLC-IRMS: chromatogram and calculation



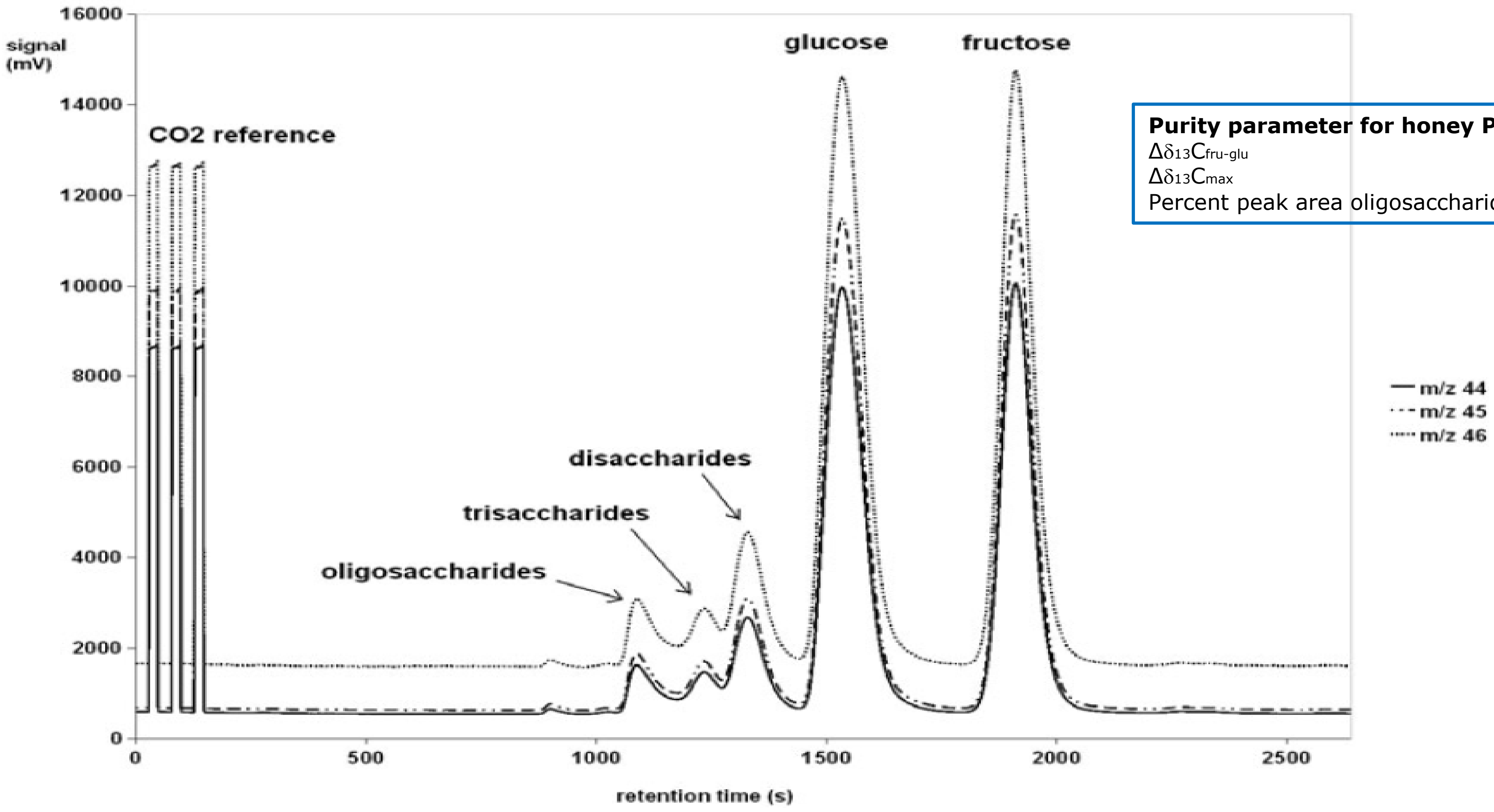
$$\delta^{13}C_{Sam}\ (‰) = [(R_{Sam} / R_{St}) - 1\ ] * 10^3$$

with:
Sam = sample
St = standard
R = $^{13}C/^{12}C$ isotope ratio

Source: OIV-MA-AS311-09

LC-IRMS: δ^{13}C of sugar compounds of honey

- Detection of adulteration with C_4 sugars (sugarcane, maize, sensitivity 1%) and C3 sugars (sugar beet, sensitivity 10%)



Purity parameter for honey	Proposed limit
$\Delta\delta^{13}C_{fru\text{-}glu}$	± 1.0‰
$\Delta\delta^{13}C_{max}$	± 2.1‰
Percent peak area oligosaccharides	< 0.7%

Elflein, Raezke, *Adipologie*, 39, 574-587 (2008): Adulterated polyfloral honey

LC-IRMS: $\delta^{13}C$ of sugar compounds of honey

JRC
EUROPEAN COMMISSION

Scientific support to the implementation of a Coordinated Control Plan with a view to establishing the prevalence of fraudulent practices in the marketing of honey

Results of honey authenticity testing by liquid chromatography-isotope ratio mass spectrometry

- all EU Member states, Norway, Switzerland
- 893 honeys analyzed by LC-IRMS
- 14,2 % found suspicious

Parameter	ave.	s. d.	range
$\delta^{13}C$ (‰) protein (p)	–25.2	0.7	–22.7 to –26.7
$\delta^{13}C$ (‰) honey (h)	–25.5	0.7	–23.0 to –27.3
$\Delta\delta^{13}C$ (‰) p - h	0.3	0.4	–0.9 to 1.5
C4 sugar (%) *	0.3	0.9	0 to 5.7
$\delta^{13}C$ (‰) fructose (fru)	–25.5	0.7	–23.2 to –27.5
$\delta^{13}C$ (‰) glucose (glu)	–25.5	0.7	–22.7 to –27.2
$\delta^{13}C$ (‰) disaccharides (ds)	–25.8	1.0	–22.5 to –28.2
$\delta^{13}C$ (‰) trisaccharides (ts)	–24.7	1.0	–22.6 to –27.5
fru/glu ratio	1.30	0.21	0.92 to 1.82
ds (area %)	6.8	2.4	1.2 to 14.1
ts (area %)	1.8	1.1	0.0 to 8.0
oligosaccharides (area %)	< 0.7	–	–

Elflein, Raezke, *Adipologie*, 39, 574-587 (2008): Adulterated polyfloral honey

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Thank you for your attention

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