

# Authenticity Testing of Honey

STATE-OF-THE-ART ANALYTICAL METHODOLOGY  
TO DETERMINE ADULTERATION WITH SUGAR SYRUPS

$\delta^{13}\text{C}$ -EA/LC-IRMS Analysis • Foreign Enzyme Analysis

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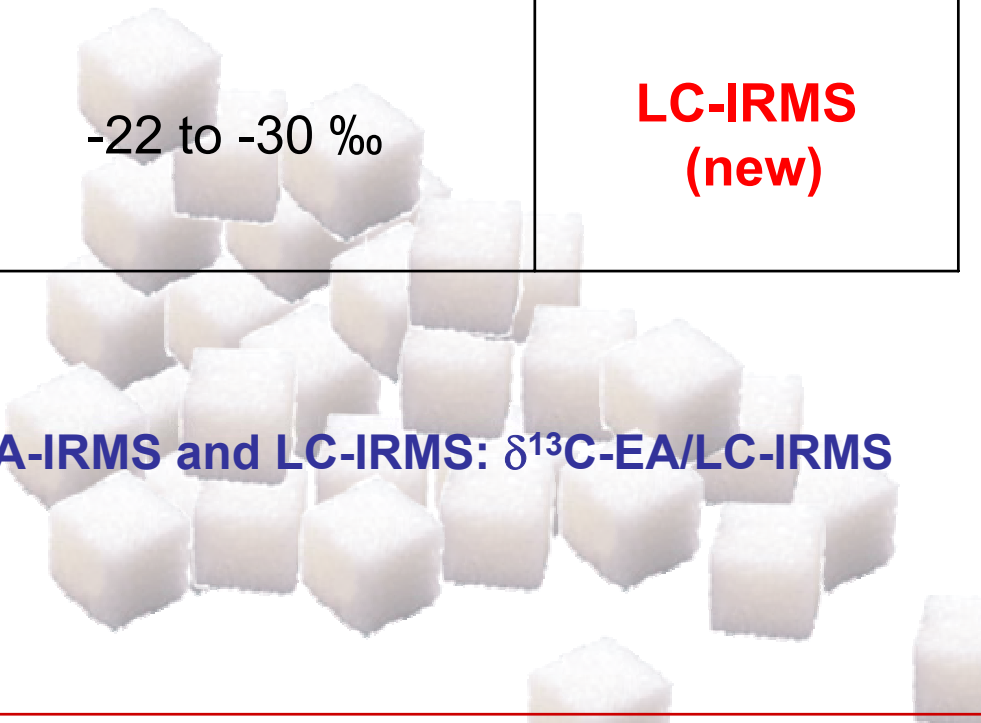
- Principle of EA/LC-IRMS
- $\delta^{13}\text{C}$  values of authentic honeys <sup>1</sup>
- $\Delta\delta^{13}\text{C}$  values as criteria of authenticity <sup>1</sup>
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- EA/LC-IRMS vs. EA/IRMS
- Adulterated honeys: current situation
- Foreign Enzyme Analysis <sup>2</sup>

<sup>1</sup> original paper in press (Apidologie)

<sup>2</sup> patent pending

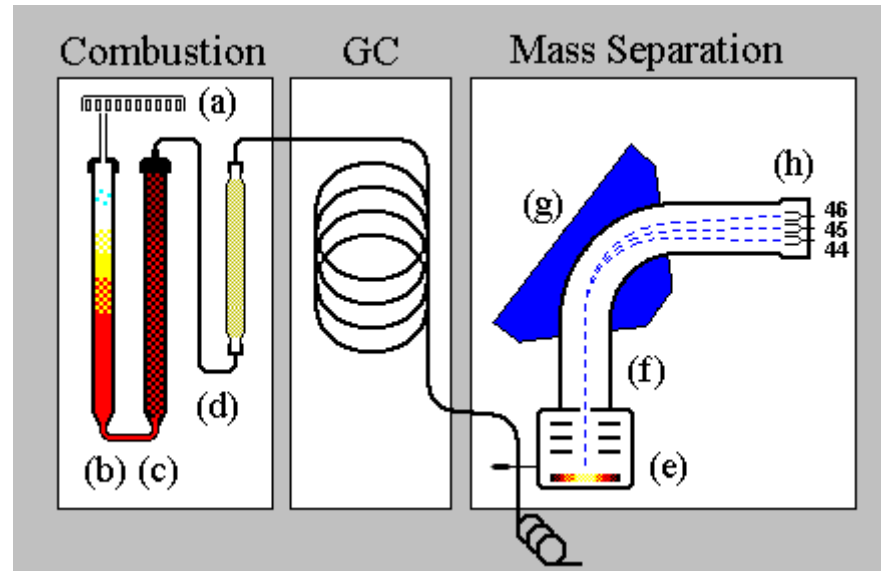
# Sugar Sources for Adulteration

Plant Origin	Examples	Range of $\delta^{13}\text{C}$ values	Method
C4-Plants	Corn Sugar Cane	-8 to -13 ‰	EA-IRMS (AOAC 998.12)
C3-Plants	Beet Rice Wheat Cichory	-22 to -30 ‰	<b>LC-IRMS (new)</b>



**Conclusion: combination of EA-IRMS and LC-IRMS:  $\delta^{13}\text{C}$ -EA/LC-IRMS**

# EA-IRMS



$\delta^{13}\text{C}$  protein  
 $\delta^{13}\text{C}$  honey

- (a) autosampler
- (b) combustion column
- (c) reduction column
- (d) water trap
- (e) ion source
- (f) flight tube
- (g) magnetic beam deflector
- (h) signal detectors

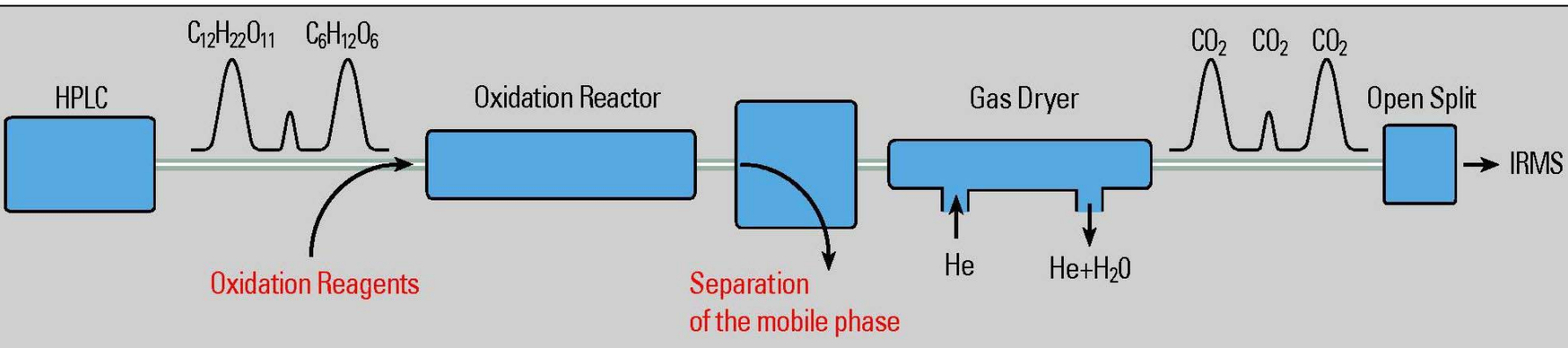
# LC-IRMS

**Step 1:**  
chromatographic separation of sugars

**Step 2:**  
conversion to CO<sub>2</sub>

**Step 3:**  
separation from eluent and transfer to IRMS

**Step 4:**  
<sup>13</sup>/<sup>12</sup>C isotope ratio measurement



Source: Finnigan LC IsoLink Product Brochure



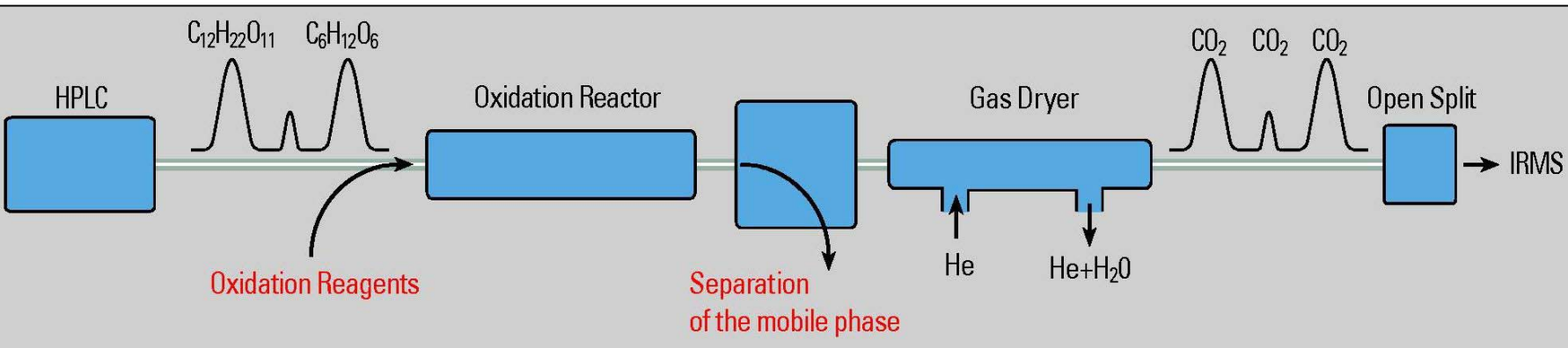
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Source: Finnigan LC IsoLink Product Brochure

... by chemical oxidation



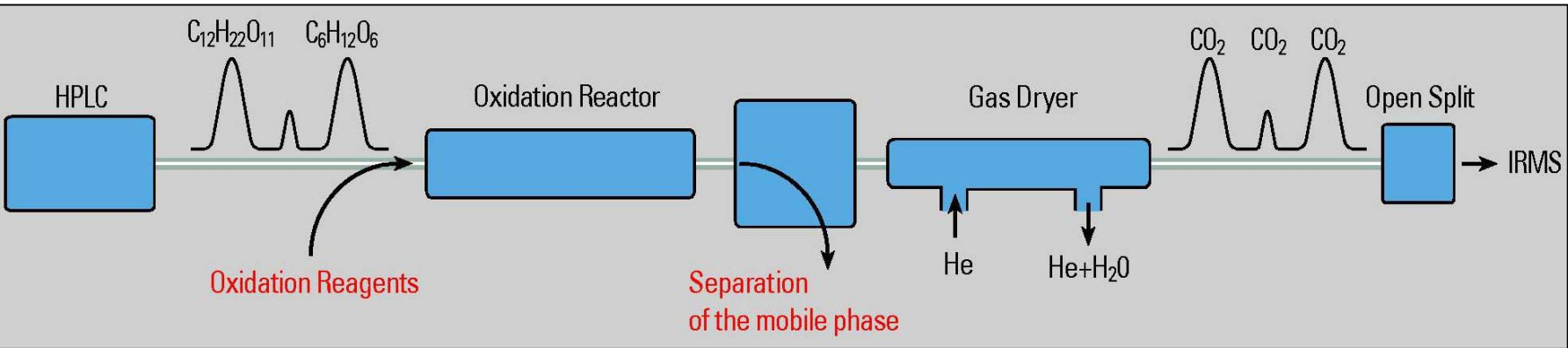
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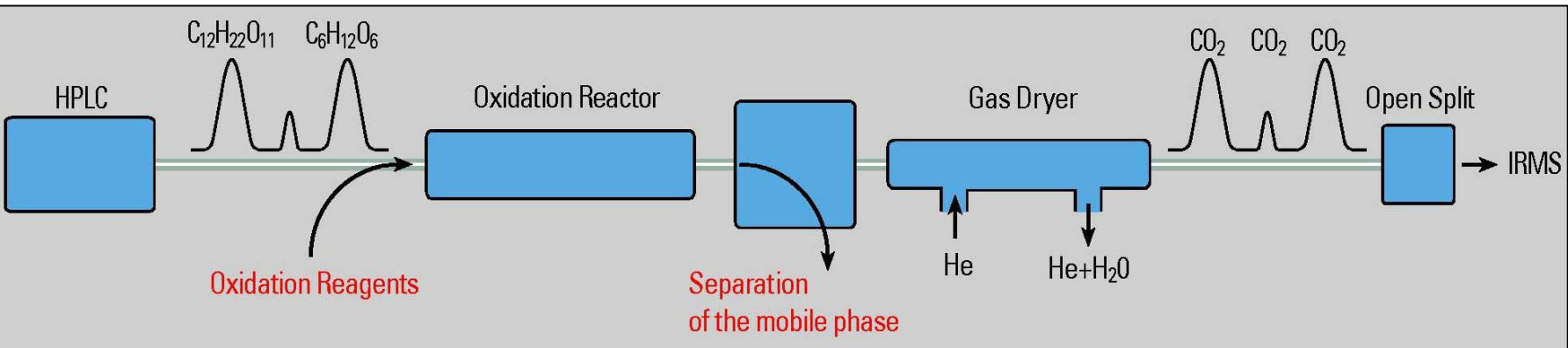
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→  $\delta^{13}C$  of fructose, glucose, di- and trisaccharides



EA/LC-IRMS: Natural  $\delta^{13}\text{C}$  Variations of Pure Honey (1)

parameter	ave.	s. d.	range
$\delta^{13}\text{C}$ (‰) protein (p)	-25.2	0.7	-22.7 to -26.7
$\delta^{13}\text{C}$ (‰) honey (h)	-25.5	0.7	-23.0 to -27.3
$\Delta$ p-h (‰)	0.3	0.4	-0.9 to 1.5
C4 sugar (%) *	0.3	0.9	0 to 5.7
$\delta^{13}\text{C}$ (‰) fructose (fru)	-25.5	0.7	-23.2 to -27.5
$\delta^{13}\text{C}$ (‰) glucose (glu)	-25.5	0.7	-22.7 to -27.2
$\delta^{13}\text{C}$ (‰) disaccharides (ds)	-25.8	1.0	-22.5 to -28.2
$\delta^{13}\text{C}$ (‰) 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	-	-

Number of samples: 451

\* apparent C4 sugar content

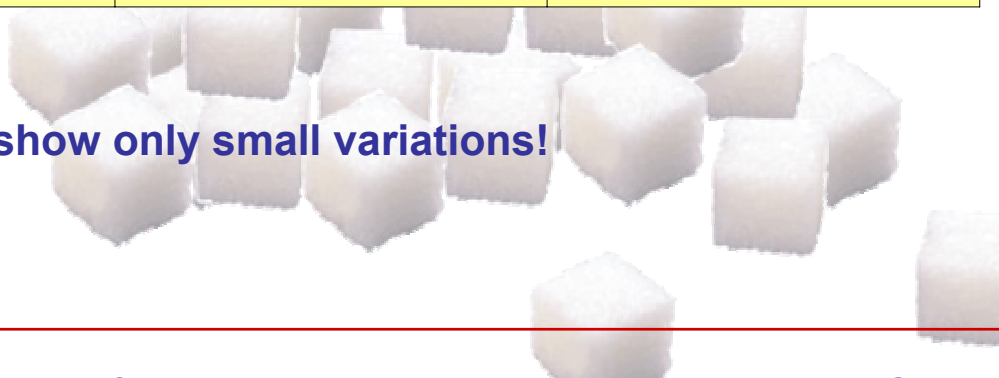
⇒  $\delta^{13}\text{C}$  values of individual sugars in honey are very similar!

## EA/LC-IRMS: Natural $\delta^{13}\text{C}$ Variations of Pure Honey (2)

parameter	ave.	s. d.	max. d. (abs.)
$\Delta\delta^{13}\text{C}$ (‰) fru - glu	0.0	0.3	1.0
$\Delta\delta^{13}\text{C}$ (‰) fru - ds	0.3	0.7	2.0
$\Delta\delta^{13}\text{C}$ (‰) fru - ts	-0.8	0.7	2.0
$\Delta\delta^{13}\text{C}$ (‰) fru - p	-0.3	0.4	1.5
$\Delta\delta^{13}\text{C}$ (‰) glu - ds	0.3	0.7	1.9
$\Delta\delta^{13}\text{C}$ (‰) glu - ts	-0.7	0.7	2.0
$\Delta\delta^{13}\text{C}$ (‰) glu - p	-0.3	0.4	1.6
$\Delta\delta^{13}\text{C}$ (‰) ds - ts	-0.9	0.6	2.1
$\Delta\delta^{13}\text{C}$ (‰) ds - p	-0.6	0.7	2.0
$\Delta\delta^{13}\text{C}$ (‰) ts - p	0.5	0.7	2.1

Number of samples: 451

$\Rightarrow \Delta \delta^{13}\text{C}$  values show only small variations!

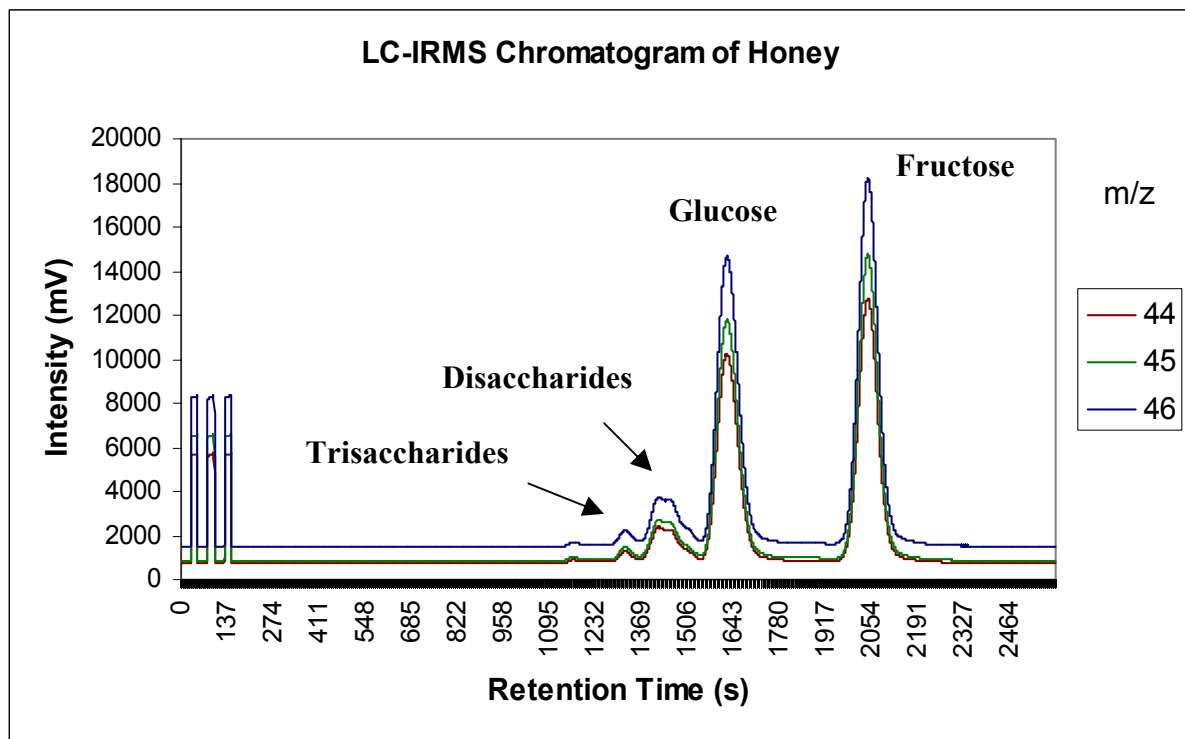


EA/LC-IRMS:  $\Delta\delta^{13}\text{C}$ , Proposed Limits for Pure Honey

parameter		proposed limit
$\Delta\delta^{13}\text{C}$ (‰) fru - glu		$\pm 1.0$
$\Delta\delta^{13}\text{C}$ (‰) fru - ds	} = $\Delta\delta^{13}\text{C}$ (‰) max.	$\pm 2.1$
$\Delta\delta^{13}\text{C}$ (‰) fru - ts		
$\Delta\delta^{13}\text{C}$ (‰) fru - p		
$\Delta\delta^{13}\text{C}$ (‰) glu - ds		
$\Delta\delta^{13}\text{C}$ (‰) glu - ts		
$\Delta\delta^{13}\text{C}$ (‰) glu - p		
$\Delta\delta^{13}\text{C}$ (‰) ds - ts		
$\Delta\delta^{13}\text{C}$ (‰) ds - p		
$\Delta\delta^{13}\text{C}$ (‰) ts - p		
$\Delta\delta^{13}\text{C}$ (‰) p - h		$\geq -1.0$

statistical certainty (confidence level): 99.7 % (3 × s.d.)

# LC-IRMS Chromatogram of Pure Honey



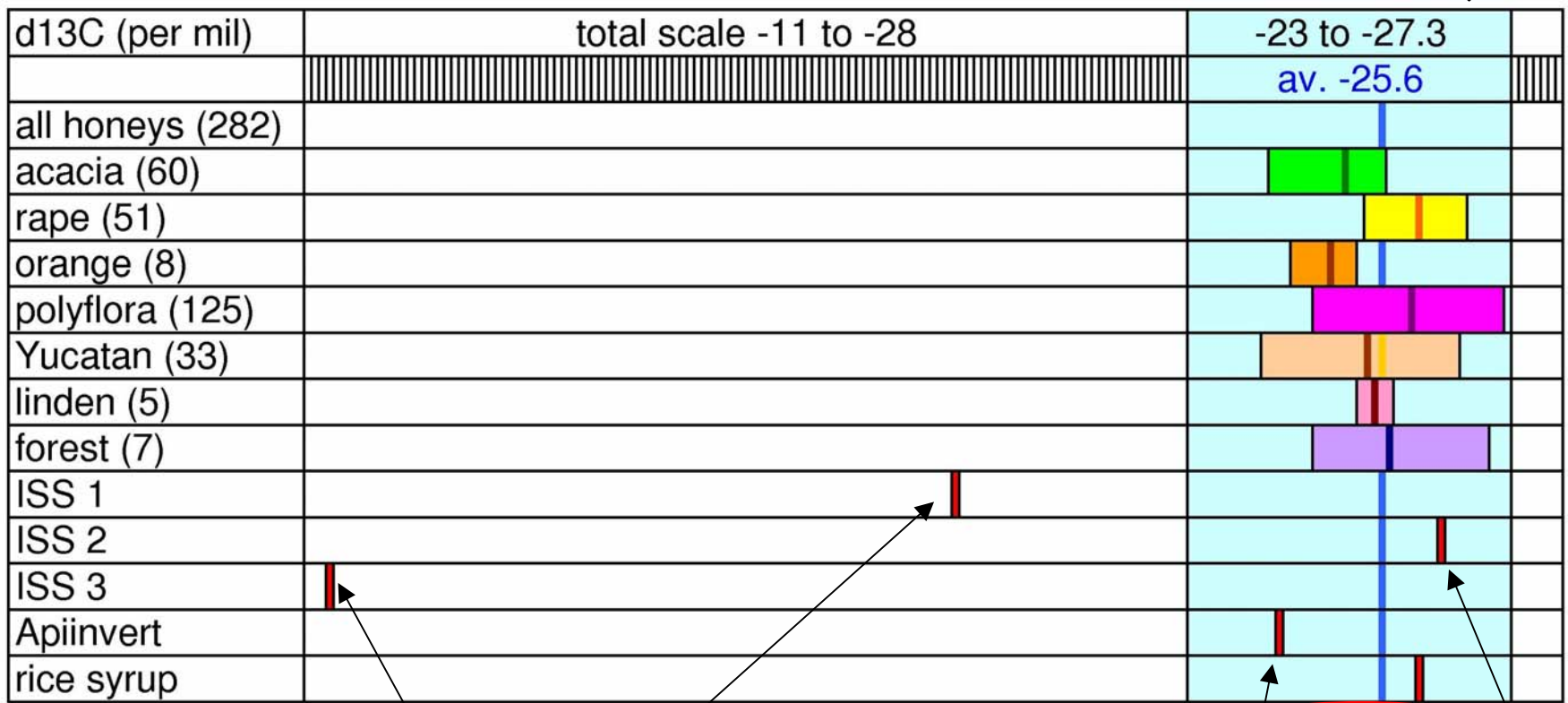
	RT (s)	Area (Vs)	$\delta^{13}\text{C}$ (‰) vs. VPDB
trisaccharides	1167	61	-26,05
disaccharides	1393	253	-26,78
glucose	1479	2300	-25,88
fructose	1864	2687	-26,01

	$\delta^{13}\text{C}$ (‰) vs. VPDB
protein	-25,6
honey	-26,0

$\delta^{13}\text{C}$  (F-G): - 0,13 ‰  
 $\delta^{13}\text{C}$  (max.): 1,18 ‰

### Range of $\delta^{13}\text{C}$ Values for Honey and Syrups

not all sugar syrups are outside the naturally occurring range of honey



ISS: invert sugar syrup

EA-IRMS ☺

-11.3  
C4 sugar

-19.8  
C4/C3 sugar mix

EA-IRMS ☹

LC-IRMS ☺

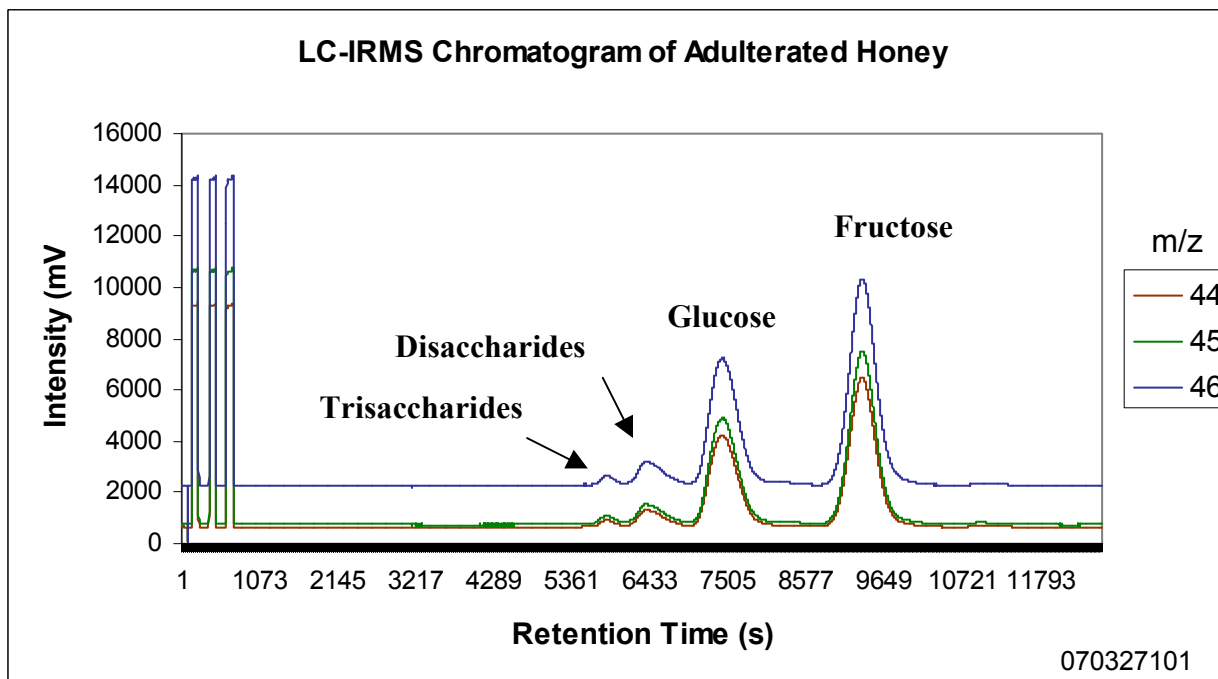
-24.2 -26.1 -26.4  
C3 sugars

## EA-IRMS ↔ LC-IRMS: Real Life Examples

HONEY TYPE	EA-IRMS (AOAC 998.12)			LC-IRMS (APPLICA)					
	d <sup>13</sup> C (‰) Protein	d <sup>13</sup> C (‰) Honey	C4 Sugar (%)	d <sup>13</sup> C (‰) Fructose	d <sup>13</sup> C (‰) Glucose	d <sup>13</sup> C (‰) Disaccharides	d <sup>13</sup> C (‰) Trisaccharides	d <sup>13</sup> C (‰) max.	F/G-Ratio
Acacia pure	-24.9	-25.1	0	-25.1	-25.3	-25.3	-24.4	0.9	1.57
Acacia adulterated	-25.2	-24.6	3.9	-23.8	-26.0	-25.7	-25.0	2.2	1.60
Linden pure	-25.7	-25.6	0.6	-25.5	-25.4	-25.9	-25.5	0.5	1.35
Linden adulterated	-25.6	-25.7	0	-26.0	-25.7	-23.9	-24.4	2.1	1.27
Honeydew pure	-25.6	-25.8	0	-25.7	-25.5	-26.5	-26.1	1.0	1.33
Honeydew adulterated	-25.3	-25.6	0	-26.0	-25.7	-23.8	-23.8	2.2	1.37
Polyflora pure	-25.6	-25.8	0	-25.7	-25.5	-26.5	-26.1	1.0	1.33
Polyflora adulterated	-24.7	-24.0	4.7	-24.8	-24.4	-19.2	-20.1	5.6	1.23
Polyflora adulterated	-25.2	-25.0	1.3	-24.4	-25.7	-24.8	-24.5	1.3	1.27

# Adulterated Acacia Honey (Real Life Example 1)

Adulteration with fructose syrup !



Ca<sup>2+</sup> column  
water eluent

	RT (s)	Area (Vs)	$\delta^{13}\text{C}$ (‰) vs. VPDB
trisaccharides	1219	12	-24,96
disaccharides	1334	48	-25,71
glucose	1552	331	-25,96
fructose	1953	529	-23,79

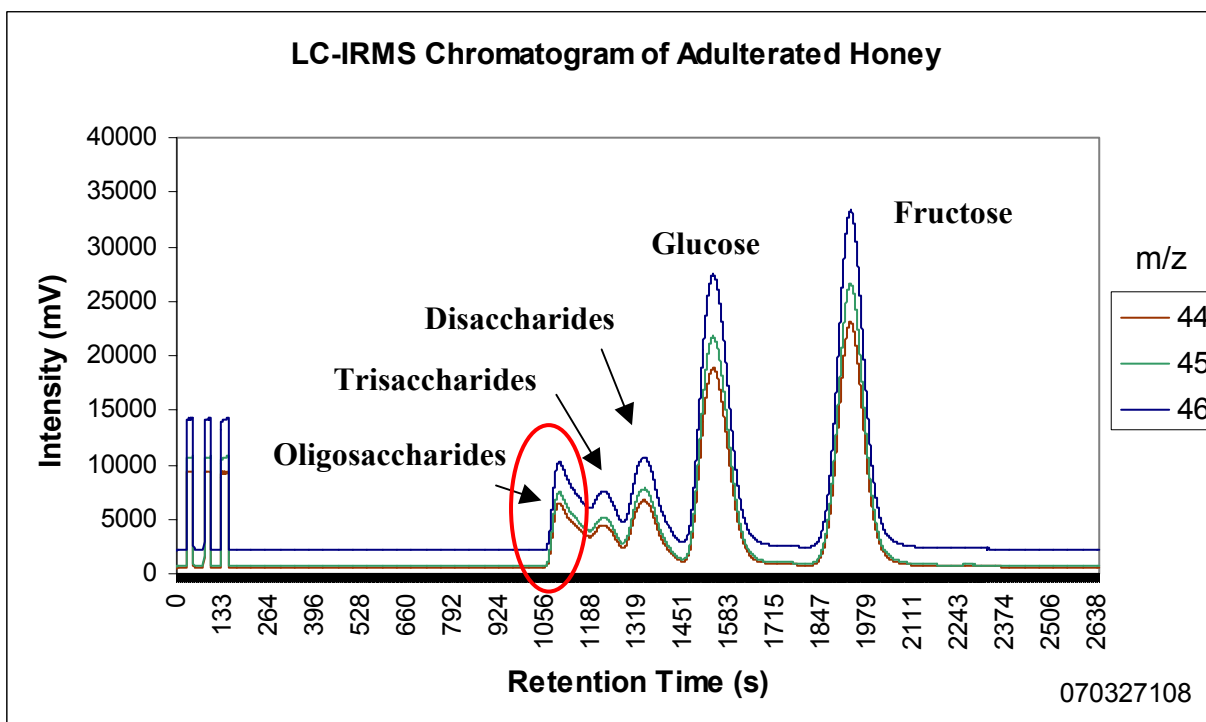
	$\delta^{13}\text{C}$ (‰) vs. VPDB
protein	-25,2
honey	-24,6

$\delta^{13}\text{C}$  (F-G): 2,17 ‰  
 $\delta^{13}\text{C}$  (max.): 2,17 ‰

EA-IRMS ok!

## Adulterated Acacia Honey (Real Life Example 2)

**Adulteration with ca. 50 % invert sugar syrup containing higher saccharides!**



Ca<sup>2+</sup> column  
water eluent

	RT (s)	Area (Vs)	$\delta^{13}\text{C}$ (‰) vs. VPDB
oligosaccharides	1095	479	-23,47
trisaccharides	1221	303	-23,02
disaccharides	1339	573	-23,24
glucose	1538	1765	-24,98
fructose	1930	2060	-24,91

	$\delta^{13}\text{C}$ (‰) vs. VPDB
protein	-25,0
honey	-24,5

**F/G = 1.17**

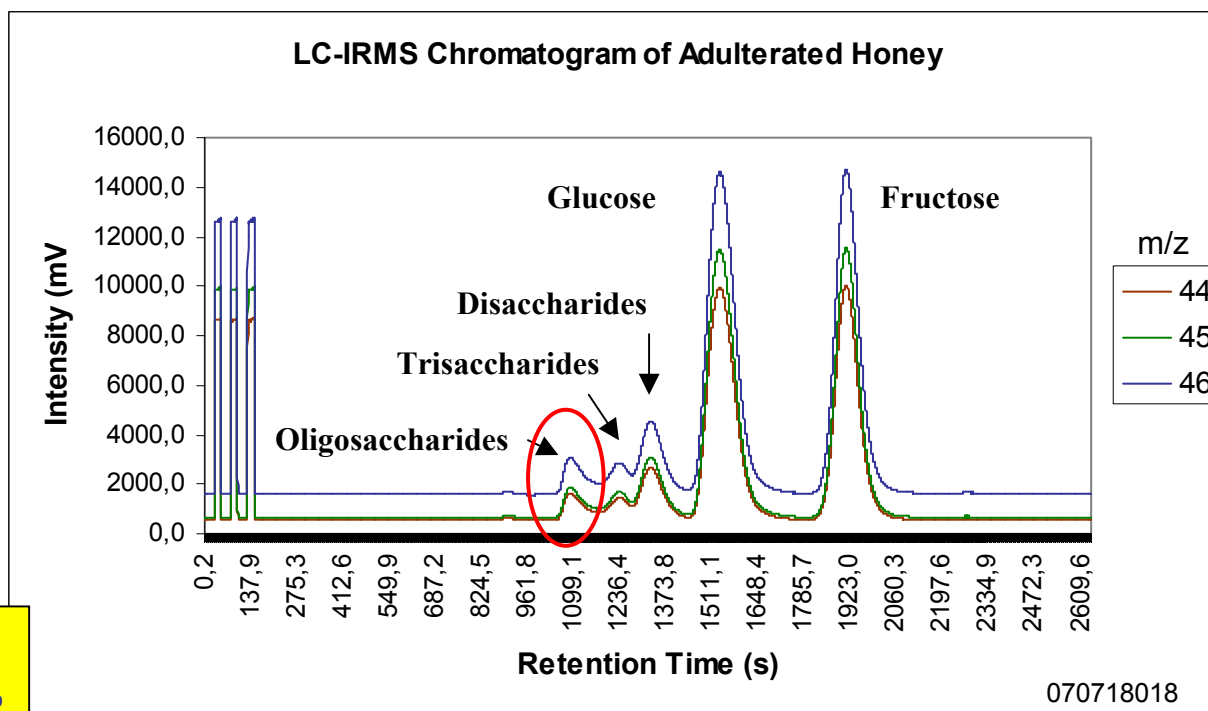
**EA-IRMS ok!**



## Adulterated Polyflora Honey (Real Life Example 3)

Adulteration with ca. 13 % rice syrup containing higher saccharides!

$\delta^{13}\text{C}$  (F-G): - 0,47 ‰  
 $\delta^{13}\text{C}$  (max.): - 3,13 ‰



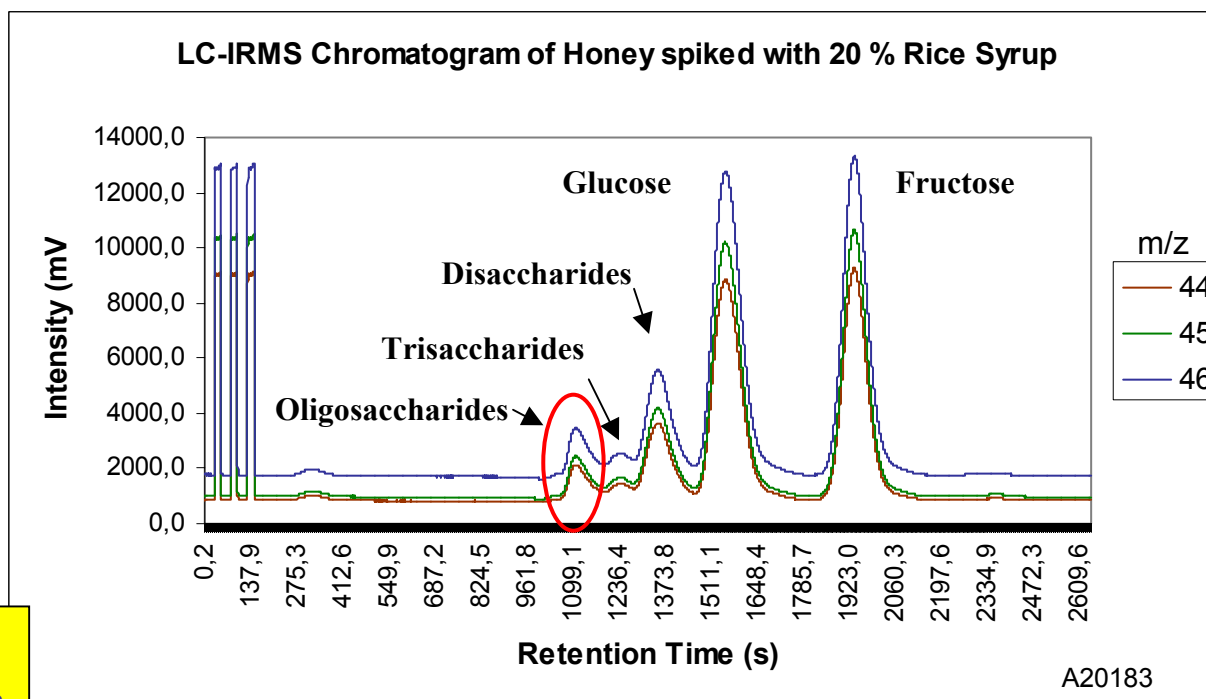
	RT (s)	Area (Vs)	$\delta^{13}\text{C}$ (‰) vs. VPDB
oligosaccharides	1088	73	-26,68
trisaccharides	1237	62	-24,30
disaccharides	1331	169	-26,35
glucose	1534	914	-26,96
fructose	1912	789	-27,43

	$\delta^{13}\text{C}$ (‰) vs. VPDB
protein	-26,4
honey	-27,0

F/G = 0.86

EA-IRMS ok!

## Polyflora Honey spiked with 20 % Rice Syrup



$\delta^{13}\text{C}$  (F-G): 0,27 ‰

$\delta^{13}\text{C}$  (max.): - 2,18 ‰

	RT (s)	Area (Vs)	$\delta^{13}\text{C}$ (‰) vs. VPDB
oligosaccharides	1106	102	-26,16
trisaccharides	1238	49	-24,39
disaccharides	1351	270	-26,46
glucose	1553	832	-26,57
fructose	1936	735	-26,30

	$\delta^{13}\text{C}$ (‰) vs. VPDB
protein	-26,2
honey	-26,4

F/G = 0.88

EA-IRMS ok!

## Acacia Honey: Addition of Invert Sugar Syrup (ISS 1)

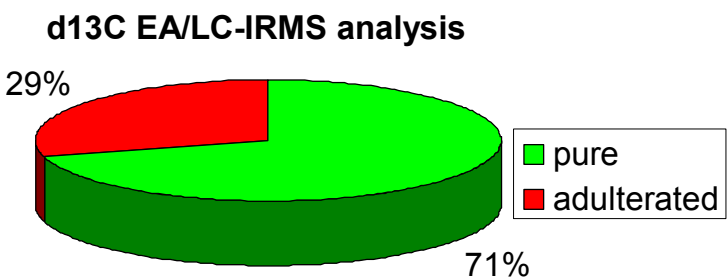
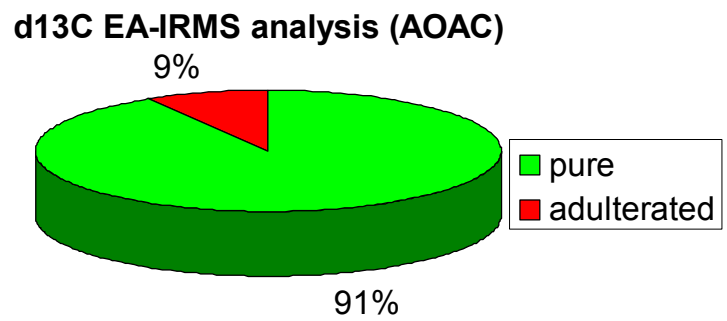
Parameter	0 % ISS	5 % ISS	10 % ISS	20 % ISS	50 % ISS	100 % ISS
d13C protein (‰)	-24.30	-24.30	-24.30	-24.30	-24.30	n.a.
d13C honey (‰)	-24.40	-24.30	-24.10	-23.50	-22.30	-19.80
% C4 sugar (spec. < 7 %)	0	0	1.4	5.5	<b>13.7</b>	n.a.
<b>EA-IRMS (AOAC)</b>	<b>ok</b>	<b>ok</b>	<b>ok</b>	<b>ok</b>	<b>adulterated</b>	
d13C fructose (‰)	-24.40	-24.19	-24.26	-24.20	-23.66	-22.78
d13C glucose (‰)	-24.54	-24.57	-24.08	<b>-23.02</b>	<b>-20.80</b>	-10.87
d13C disaccharides (‰)	-24.21	-24.13	-23.52	<b>-22.26</b>	<b>-19.70</b>	-12.40
d13C trisaccharides (‰)	-23.64	-23.05	<b>-22.11</b>	<b>-20.62</b>	<b>-19.12</b>	-11.36
d13C oligosaccharides (‰)	n.a.	n.a.	n.a.	<b>-12.31</b>	<b>-12.96</b>	-12.05
F/G ratio	1.48	1.48	1.67	<b>1.87</b>	<b>2.46</b>	5.61
disaccharides (% DM)	8.15	9.23	9.75	9.68	8.85	4.14
trisaccharides (% DM)	2.81	2.89	3.23	3.40	2.86	2.18
oligosaccharides (% DM)	n.d.	n.d.	n.d.	<b>0.75</b>	<b>1.65</b>	3.15
<b>LC-IRMS</b>	<b>ok</b>	<b>ok</b>	<b>adulterated</b>	<b>adulterated</b>	<b>adulterated</b>	

n.a. – not analysable; n.d. – not detected < 0.7 % DM; DM – dry matter; ISS 1 – invert sugar syrup (80 % fructose)

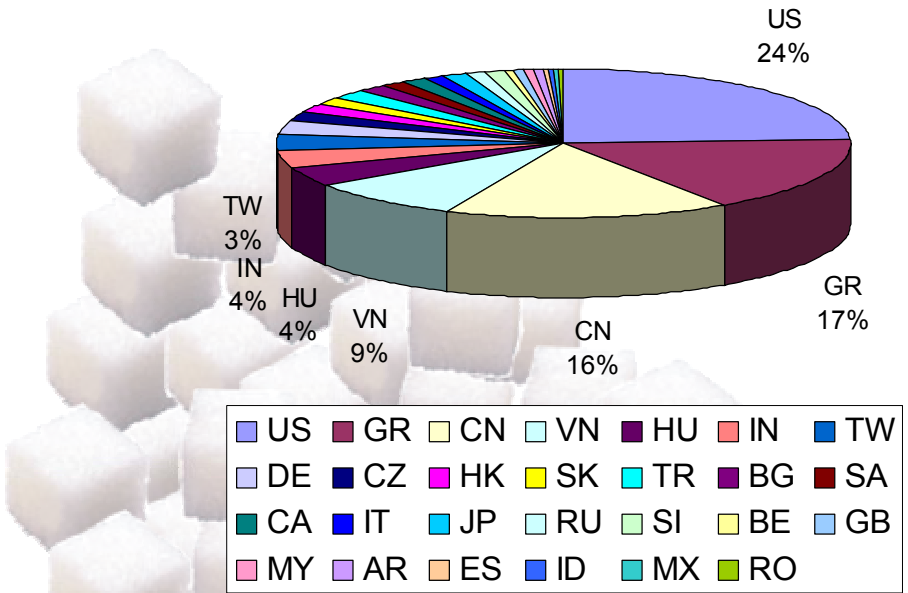
**Limit of detection: EA-IRMS ~ 27 % ⇔ EA/LC-IRMS ~ 8 %**

**misleading results causing a wrong result interpretation !**

### Honey: Detection of Adulteration, EA-IRMS vs. EA/LC-IRMS

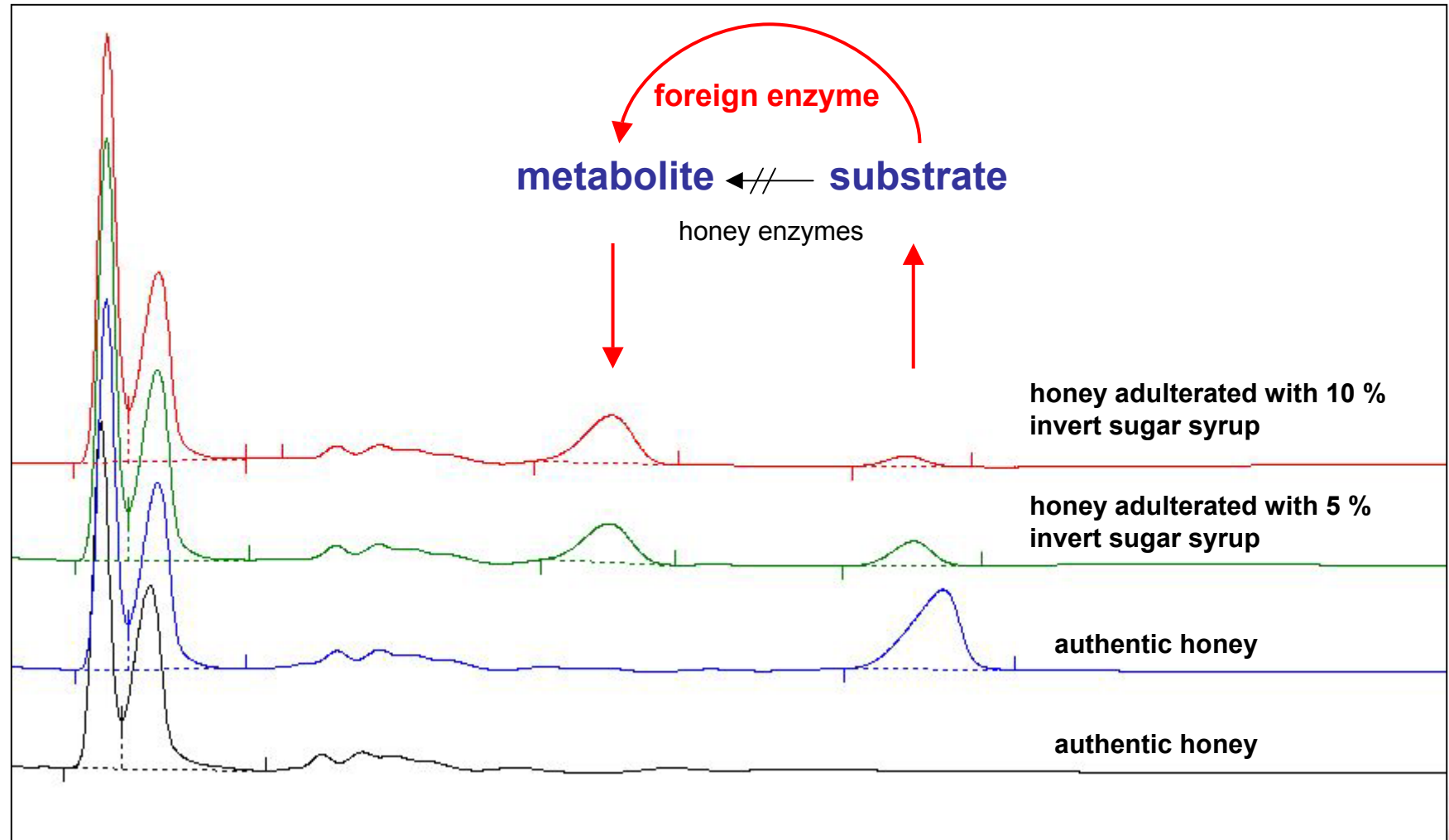


**d13C EA/LC-IRMS honeys not corresponding to purity criteria**



**1086 samples suspected of being adulterated (08/2007 – 06/2008)**

# Foreign Enzyme Test



Thank you!

