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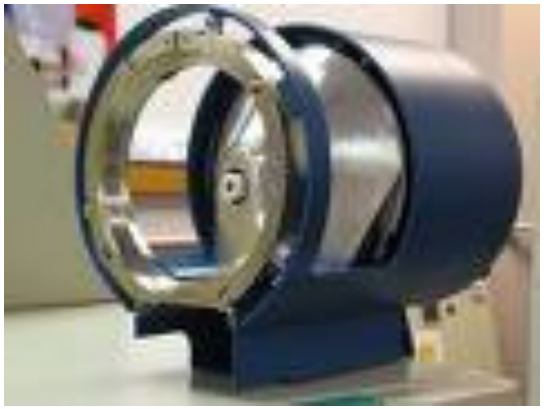
The Use of DART MS for Characterizing Food

Jana Hajslova



10th joint CSL / JIFSAN symposium on Food Safety and Nutrition, Methods and Systems for Tracking, Tracing, and Verifying Foods

May 13 – 15, 2009, Greenbelt Marriot Hotel, MD, USA

OVERVIEW

Setting the scene:

- A lot of requirements for fast food analysis

Ambient mass spectrometry:

- What is the principle and scope

- DART TOF MS - a challenging alternative

Examples of DART -TOF MS use:

- Tracking / authentication

- Food safety control - traceability

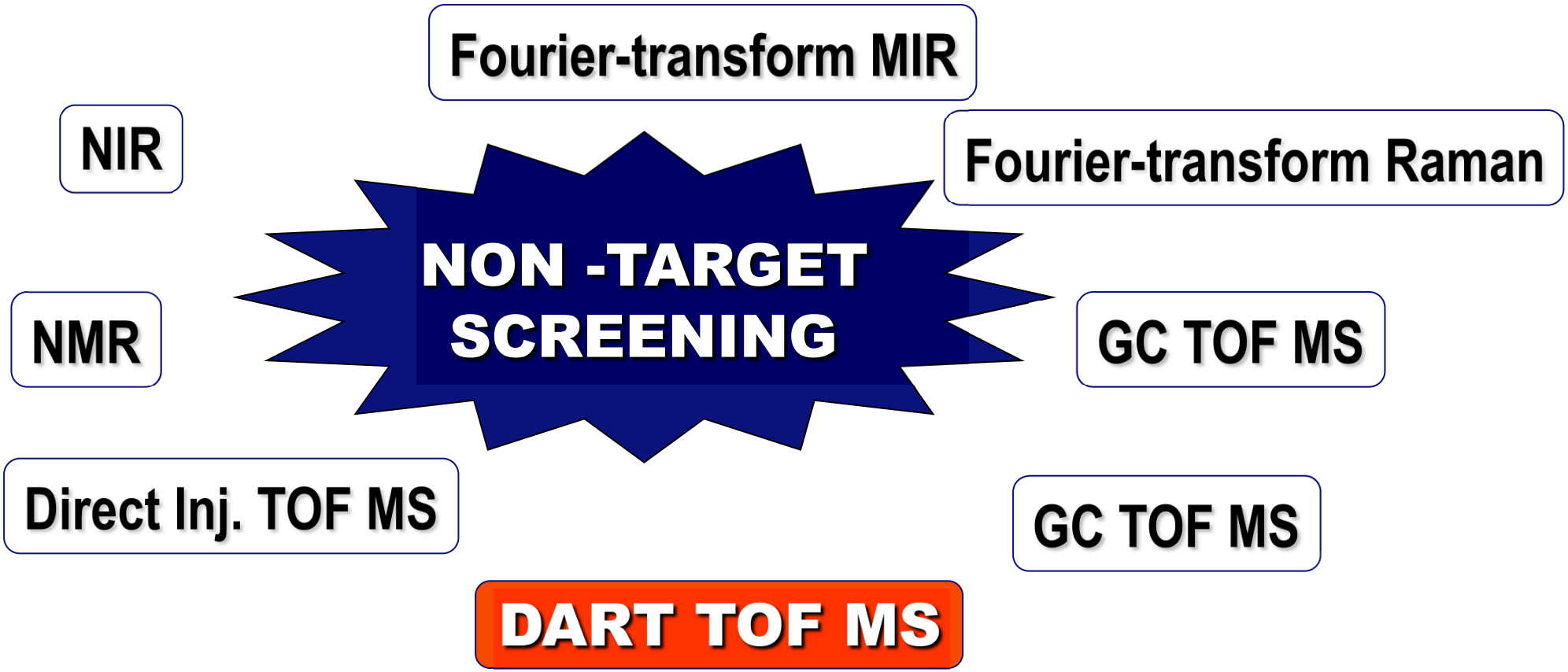
A lot of analytical work on food quality / safety

- Proteins, lipids, saccharides
- Vitamins, minerals
- Flavours and odours
- Food additives, supplements
- Residues and contaminants
- Processing and packaging contaminants
- Mycotoxins, marine and plant toxins
- Genetically modified organisms (GMO´s)
- Allergens
- Nanoparticles

- Compliance with legislation
- Label declarations
- Authenticity, traceability, fraud

Setting the scene:

DART- TOF MS JOINS SPECTROSCOPIC TRACKING TOOLS FAMILY



Outline of the TRACE study

Trappist beer is a Belgium (very nice but rather exoensive) speciality produced by monkies

400 beer samples involved in study:

AUTHENTIC TRAPPIST PRODUCT

Rochefort
other Trappist
non-Trappist

►**The aim of study:**

DISTINGUISH ROCHEFORT 8° FROM OTHER TRAPPIST AND NON-TRAPPIST BEERS

CHEMOMETRIC ANALYSIS OF DART-TOF MS DATA

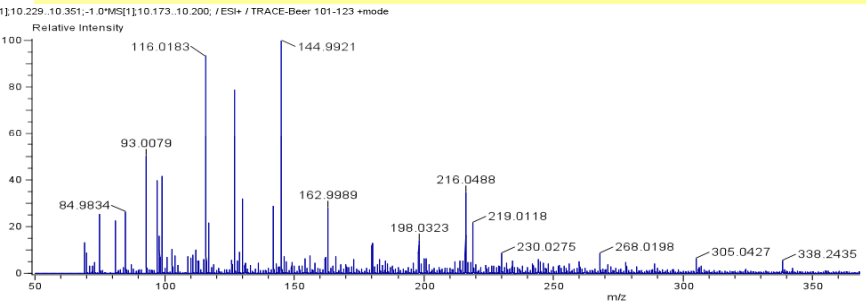
tools ► Linear Discriminant Analysis (**LDA**)
Artificial Neural Networks (**ANN**)

Trappist Rochefort 6°, 8°, 10° beers vs. rest of beers

LDA model

Recognition ability: **98%**

Prediction ability: **97%**



m/z

ANN model

Recognition ability: **100%**

Prediction ability: **98%**

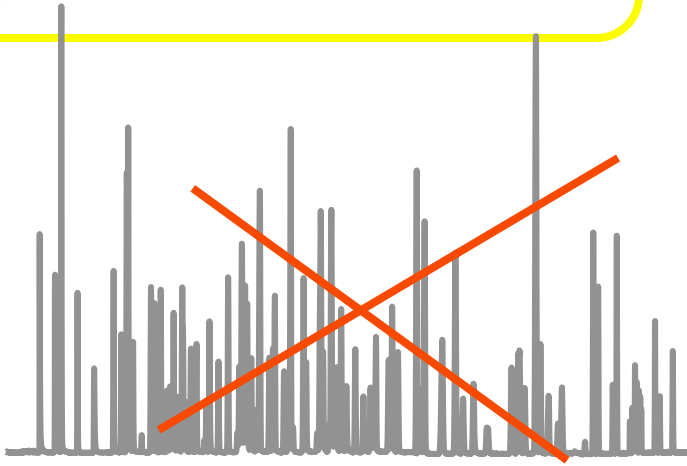
DART TOF MS enables fast tracking of beer origin

NEW

AMBIENT MASS SPECTROMETRY

…opens the doors to many challenging applications in various areas including food / feed control

- Reduced / minimal sample prep
- No chromatographic separation



TRAC, Volume 27, Issue 4, April 2008, Pages 284-290

Recent review

Ambient desorption ionization mass spectrometry

Andre Venter, Marcela Nefliu, R. Graham Cooks

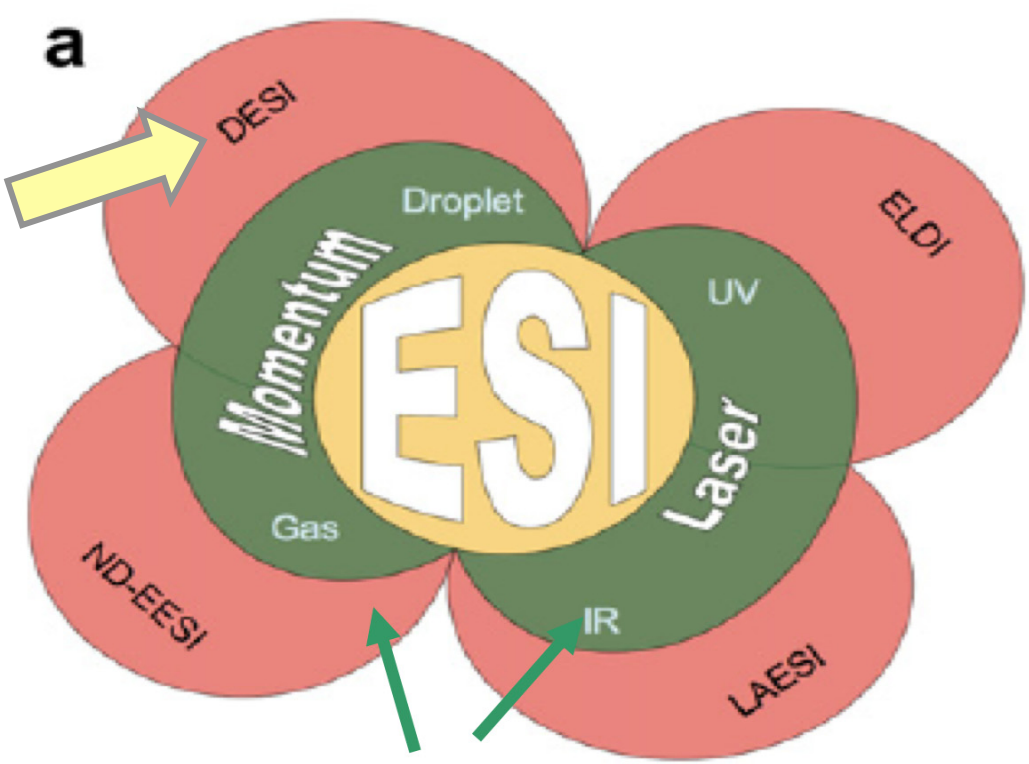
The ambient ionization methods retain the signature advantages of MS

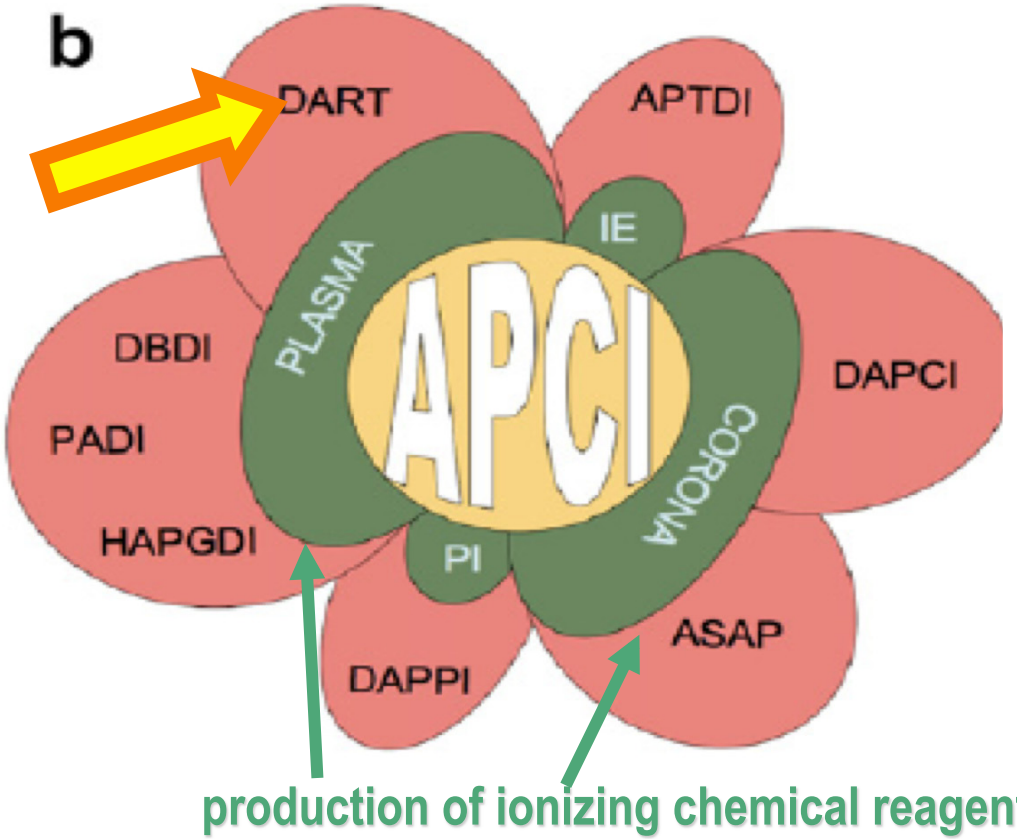
➡ **speed**

➡ **chemical specificity**

➡ **low detection limits**

AMBIENT DESORPTION IONIZATION METHODS

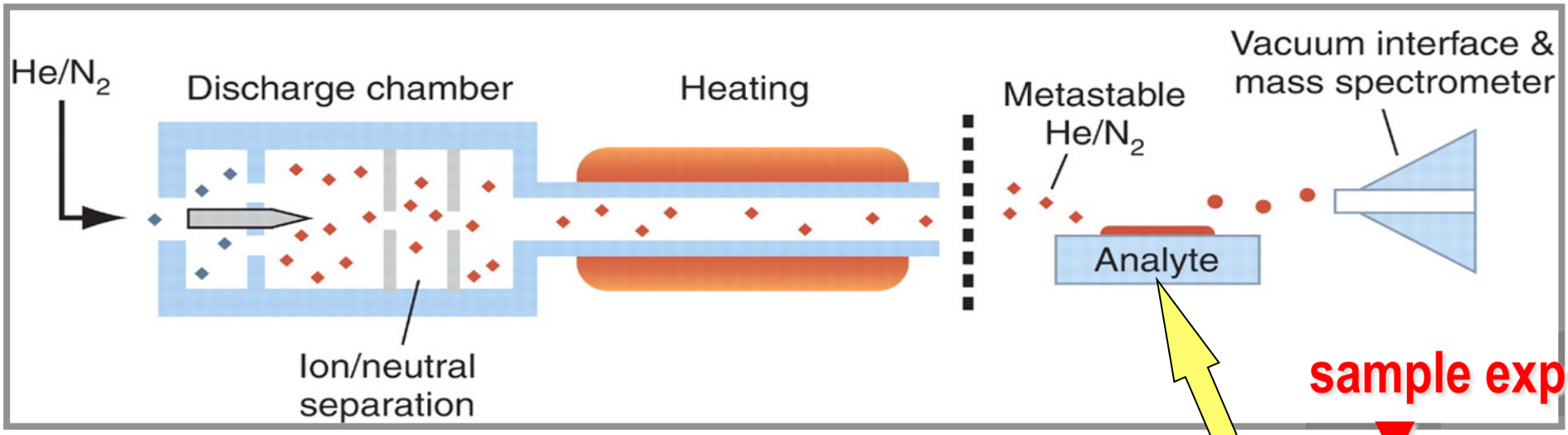




a Techniques where **ESI** mechanisms are mainly responsible for ionization.

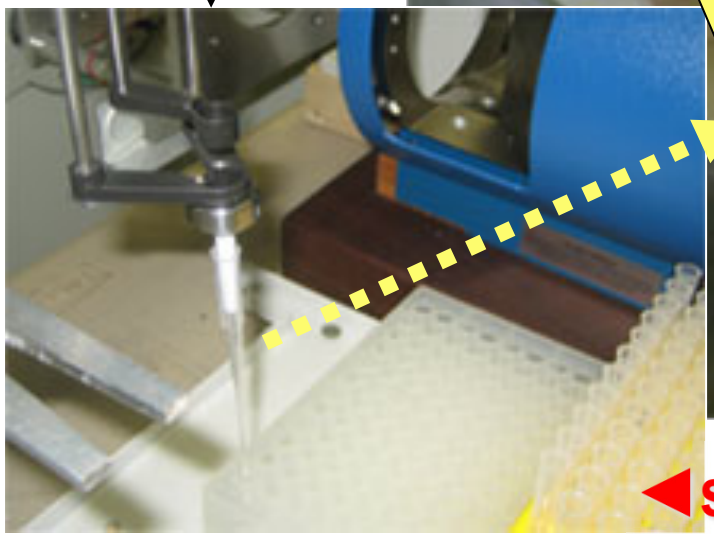
b Methods where **chemical ionization** is responsible for ionization
(photoionization - PI, ion evaporation - IE and electrical discharge)

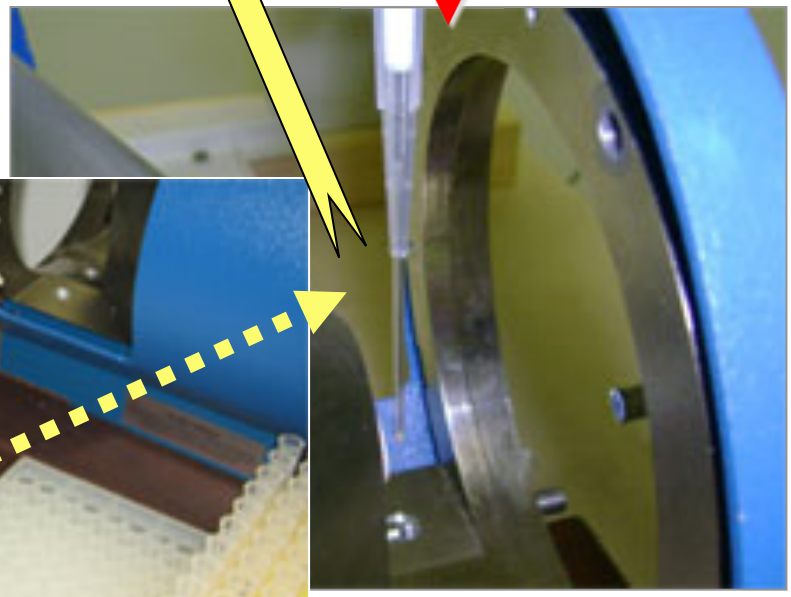
DART ion source: → → → Direct Analysis in Real Time



sample exposure

Autosampler





sample acquisition

DART Ionization: ANALYTES

Negative ions

- Direct ($M^{-\cdot}$)
  - *ionic compounds, some electrophiles*
- Proton abstraction $[M-H]^-$
  - *acidic compounds, nitroaromatics*
- Adduct formation $[M+X]^-$
  - *Unstable nitro compounds, some halocarbons*

Positive ions

- Direct (M^+, $M^{+\cdot}$)
  - *ionic compounds, low-IP organics*
- Proton transfer $[M+H]^+$
  - *Bases, alkenes, small alcohols, ethers, ketones, aldehydes*
  - *H/D exchange*
- Other adducts $[M+Z]^+$
  - *Polar compounds, ethers, ketones, acids, peroxides*

When interfaced to a high-resolution TOFMS → measurement of exact masses and accurate isotopic abundances → identification of unknowns

Case study # 1:

HONEY

- *What is the origin?*
- *Is it authentic?*

STRATEGIES IN ASSESSMENT OF HONEY QUALITY AND AUTHENTICITY

Target analysis of physico-chemical parameters / components / enzymes

Molecular biology, PCR (pollen)

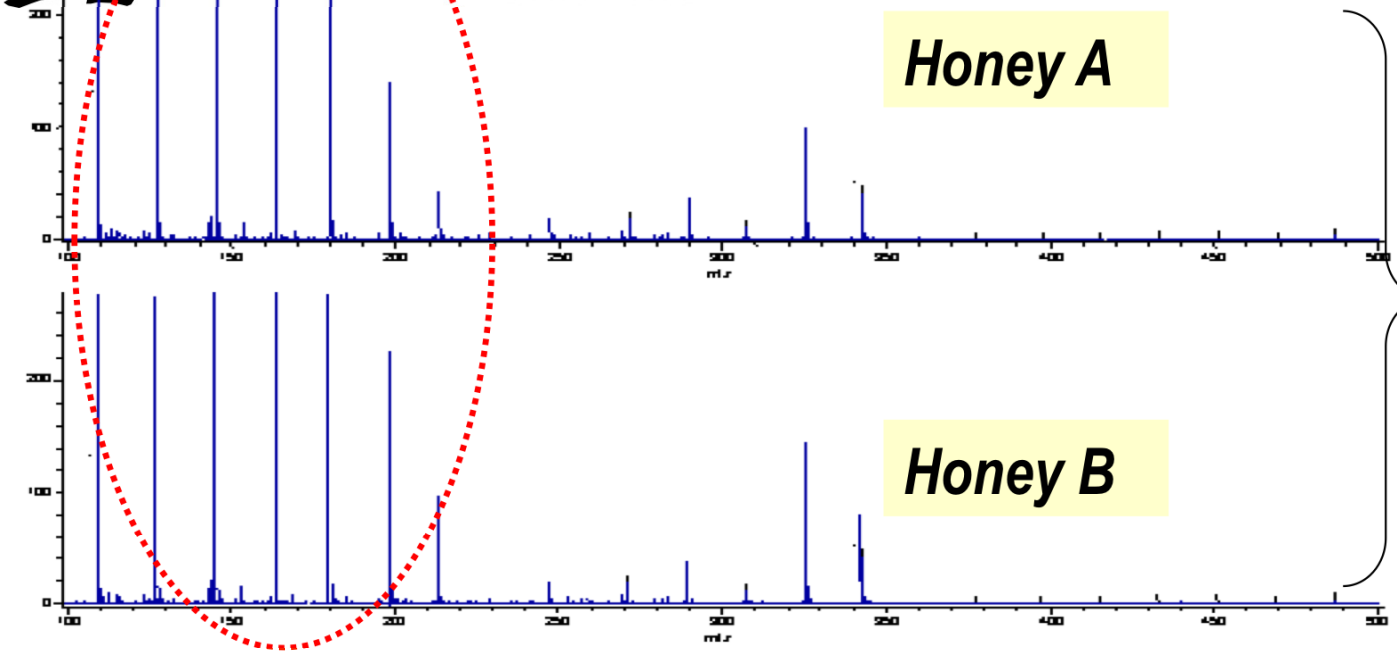
NON-TARGET PROFILING

- Near infrared spectroscopy
- Fourier-transform mid-infrared spectroscopy
- Fourier-transform Raman spectroscopy
- Nuclear magnetic resonance spectroscopy
- Gas chromatography-massspectrometry
- Direct Injection mass spectrometry

AMBIENT MASS SPECTROMETRY

Can direct be honey analysed directly?

◀ DART source saturated !



NO !

High intensity of non-characteristic ions - mostly degradation products of sugars (carameliation)

DART: positive ion mode, DART temperature: 250°C

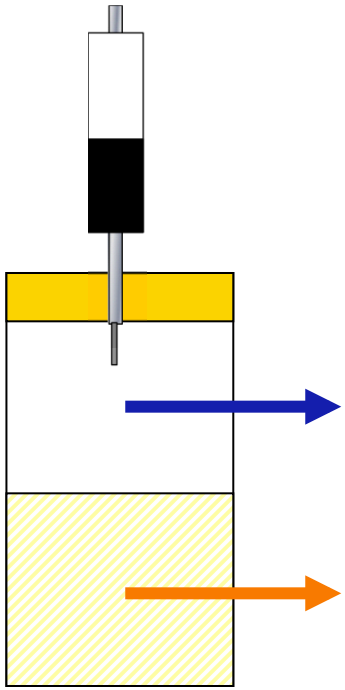
Honey + water (2 g + 2 mL)

PRE-CONCENTRATION USING SPME

- *isolation of volatile compounds in the presence of (abundant) non-target compounds (e.g. sugars)*

or

- *absorbtion also less volatile (more polar) fraction by immersion of a fibre into sample solution?*



HEADSPACE: honey + water (2 g + 2 mL)

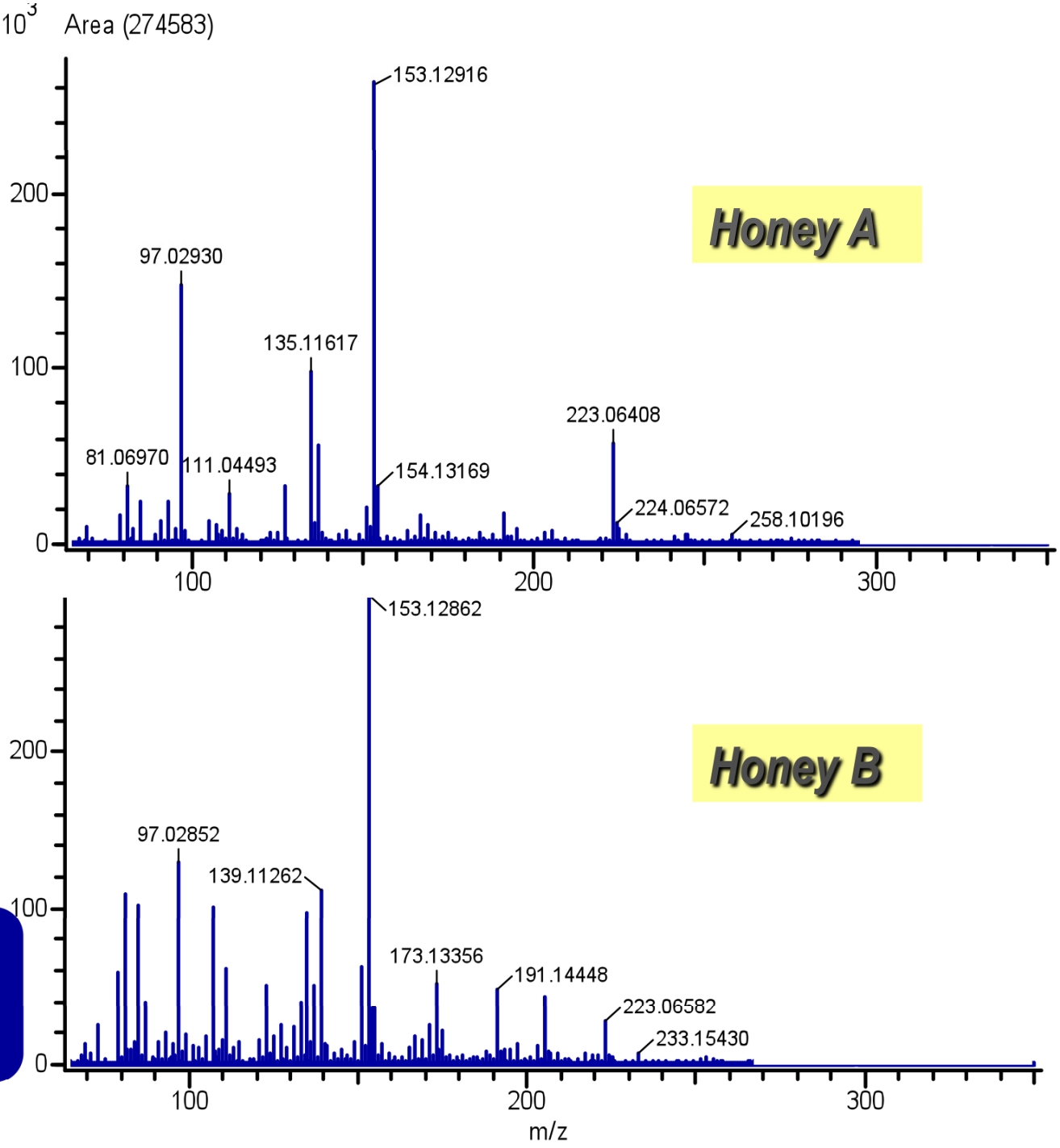
DIRECT IMMERSION: honey + water (2 g + 6 mL)

DART TOF MS
Head-space SPME profile

Fiber:
DVB/CAR/PDMS 50/30 µm
(divinylbenzene/Carboxen/ polydimethylsiloxane)

DART: positive ion mode
DART temperature: 250°C

lower MW compounds extracted

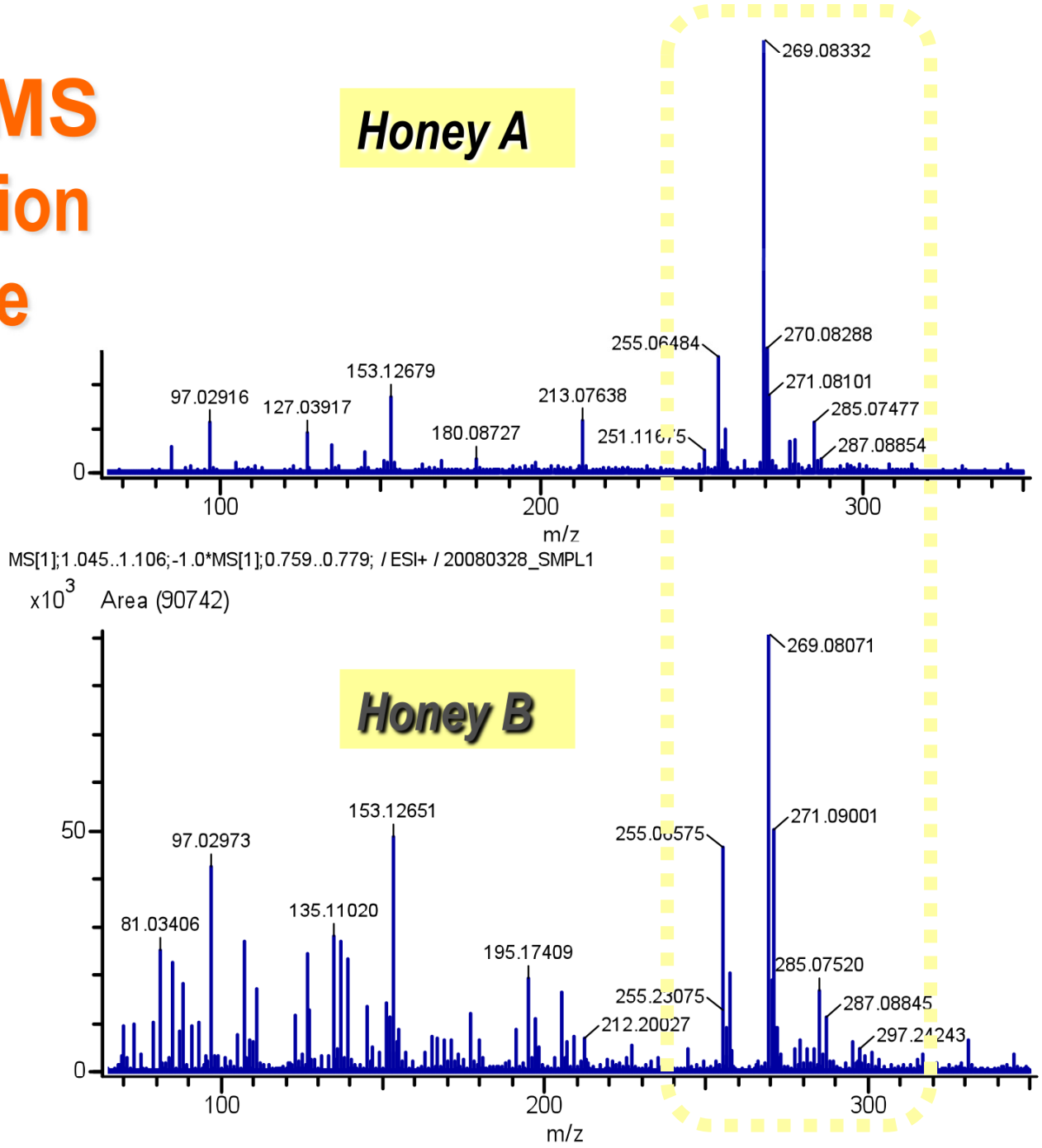


DART TOF MS direct immersion SPME profile

Fiber: DVB/CAR/PDMS 50/30 µm (divinylbenzene/Carboxen/ polydimethylsiloxane)

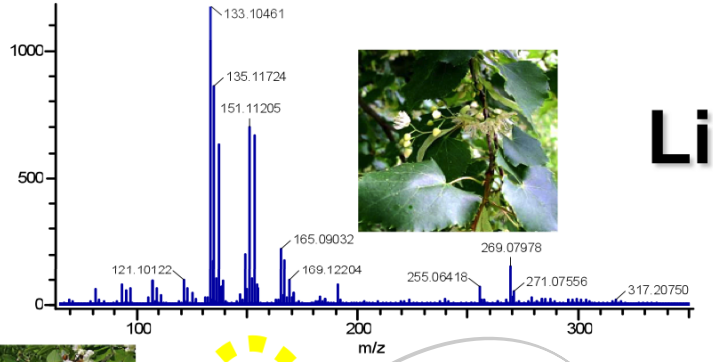
DART: positive ion mode
DART temperature: 250°C

Larger range of compounds extracted

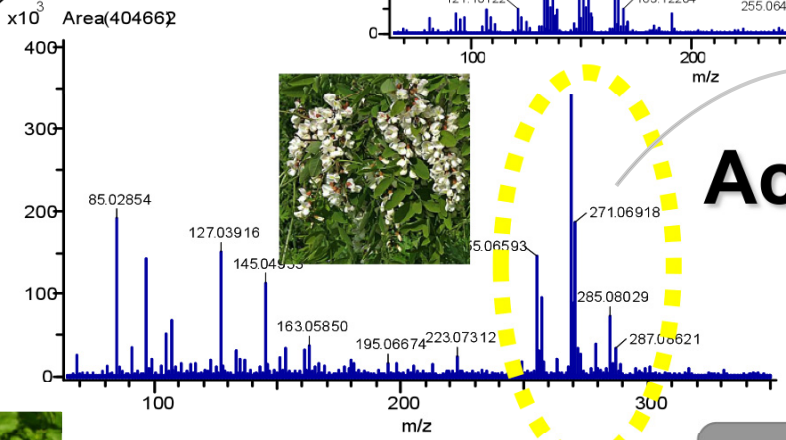


in 10 min

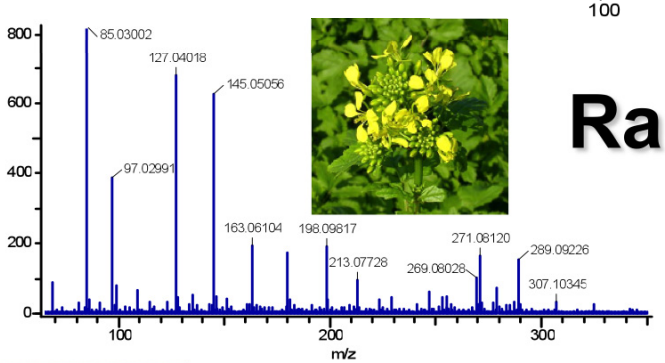
AUTHENTIC HONEY SAMPLES



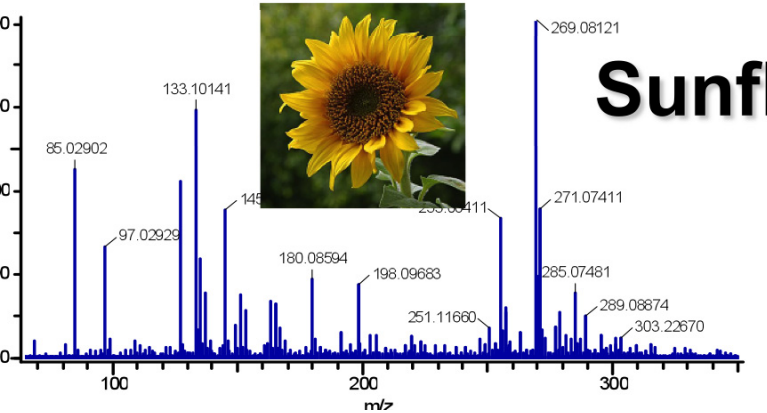
Lime



Acacia

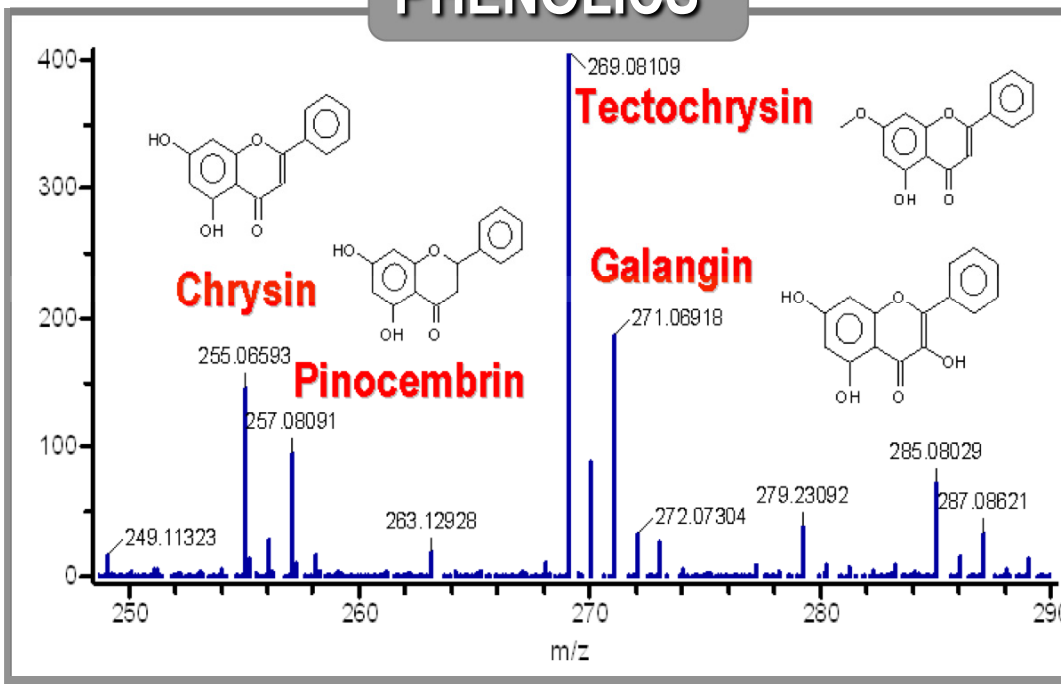


Rape

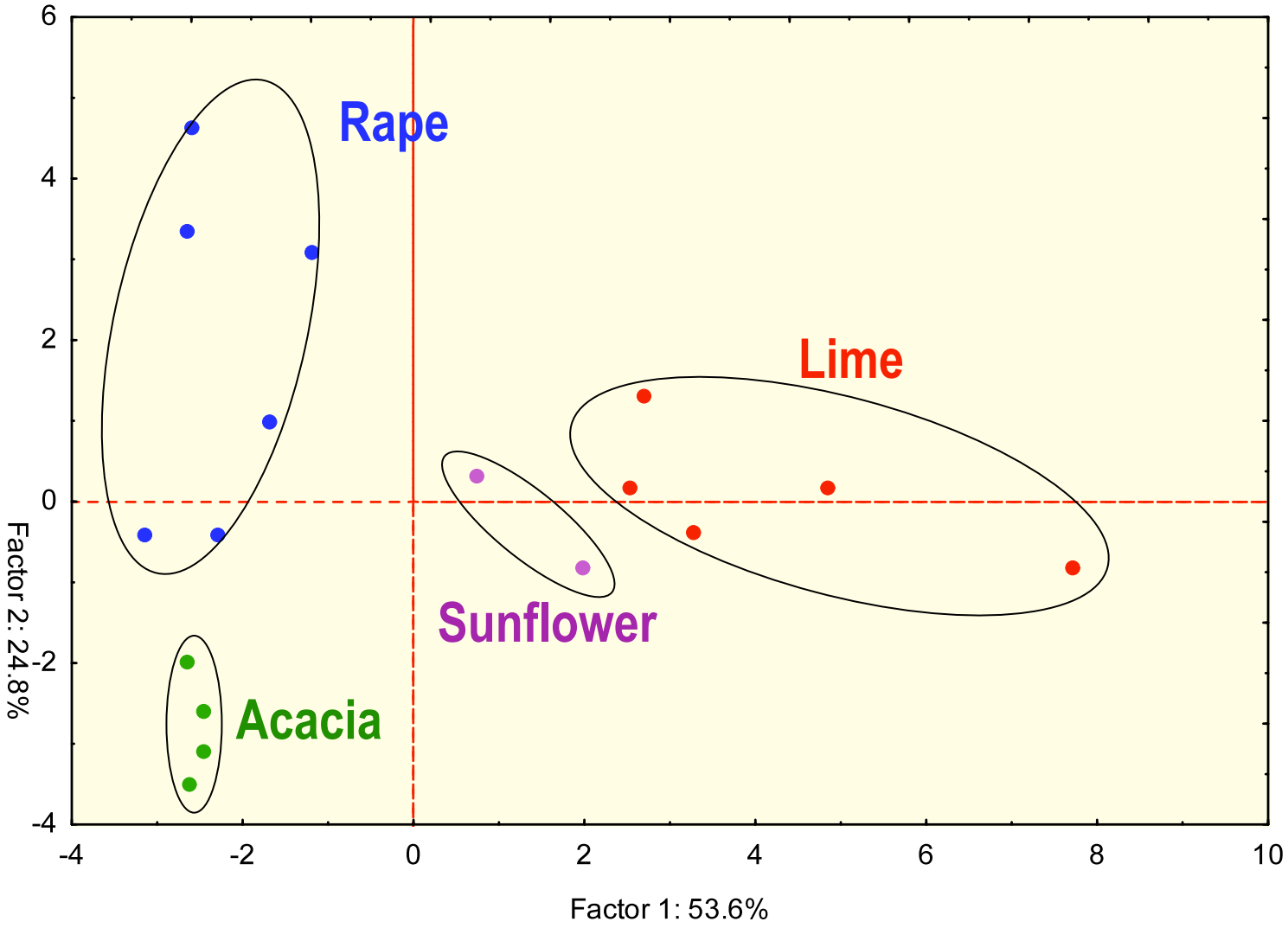


Sunflower

PHENOLICS



CLASSIFICATION USING PCA
(20 marker masses selected)



Case study # 2:

OLIVE OIL

- *What is its origin?*
- *Is it authentic?*

Examined samples

various quality grade and botanical origin:

- Extra virgin olive oil (EVOO)
- Olive oil (OO)
- Olive pomace oil (OPO)
- Hazelnut oil (HO)

EXTRA VIRGIN OLIVE OIL

The oil obtained from the fruit of the olive tree solely by mechanical or other physical means under the conditions. particularly thermal conditions, that do not lead to alternations in the oil, and which has not undergone any treatment other than washing, decantation, centrifugation and filtration.

SAMPLE PREPARATION

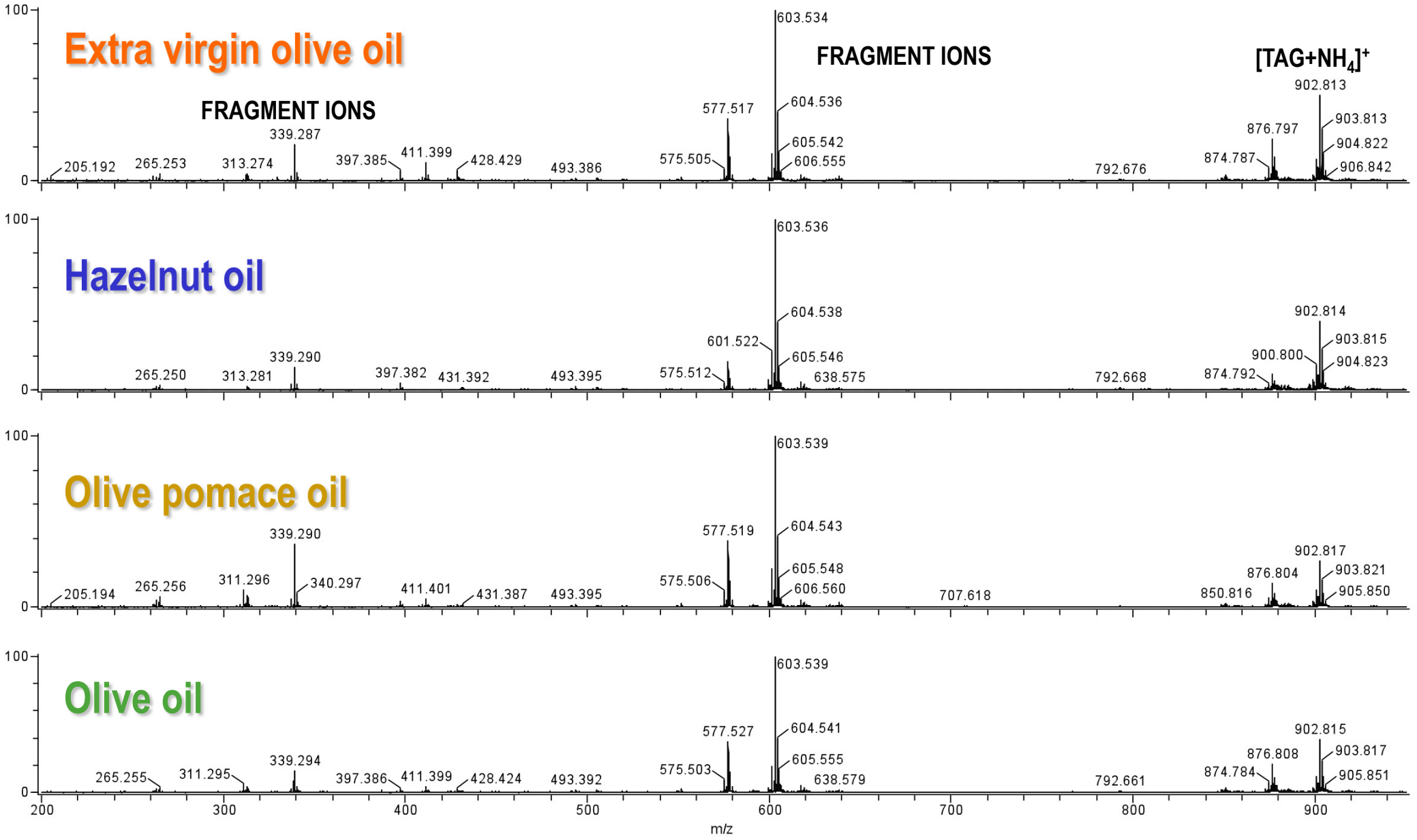
- **TAGs analysis:** oil dilution with toluene (1:50, *v/v*)

- **Polar compounds analysis:** 2 min shaking of oil with MeOH–H_2O mixture (80:20, *v/v*)

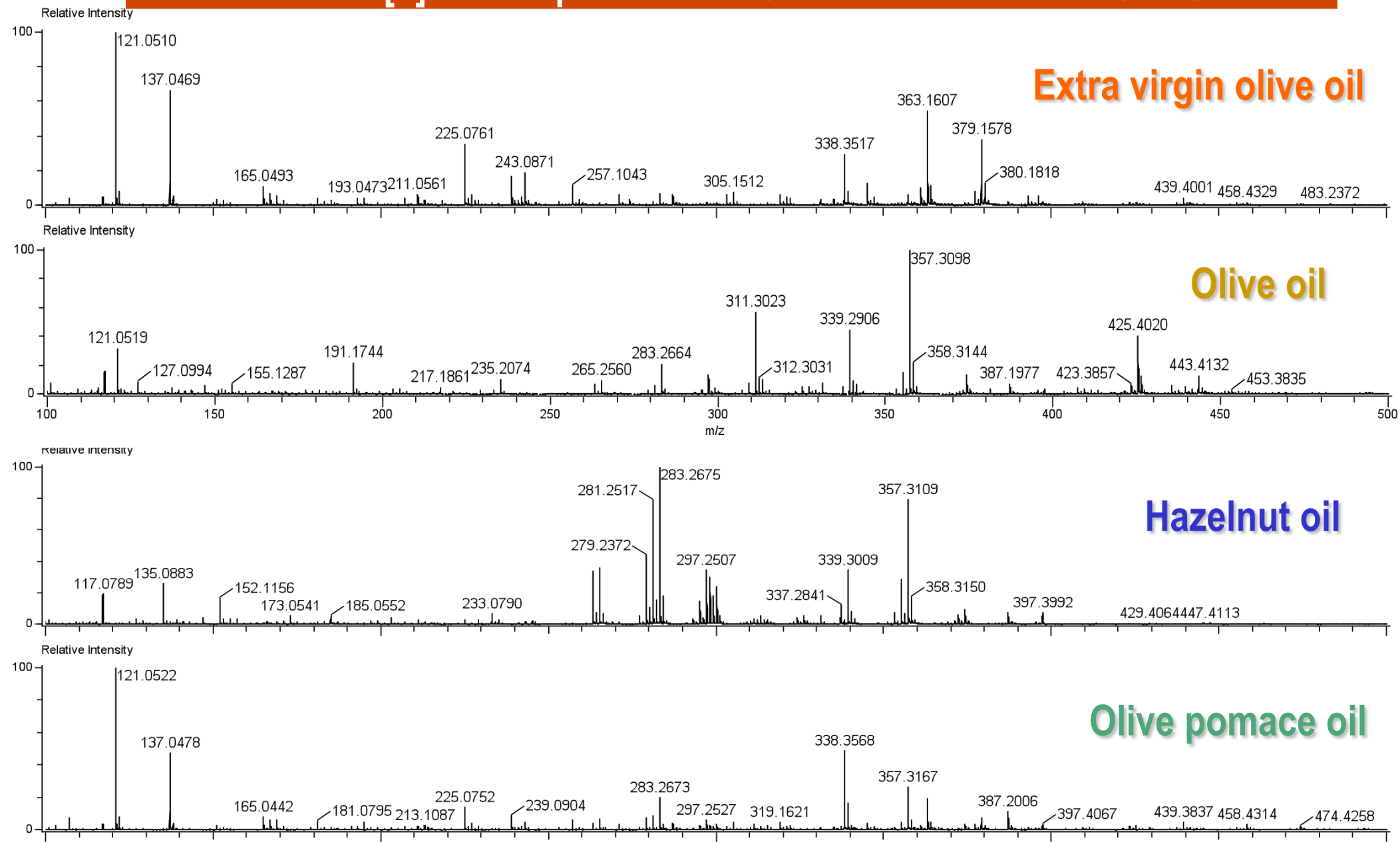
DART–TOFMS method

- **IONZATION MODE: positive**
- **ANALYSIS TIME: 1 min**
- **GAS BEAM TEMPERATURE**
  - **(i) 350°C,**
  - **(ii) 220°C**
- **For TAGs analysis ammonia solution was employed as dopant**

DART–TOFMS [+] mass spectra of DILUTED OILS

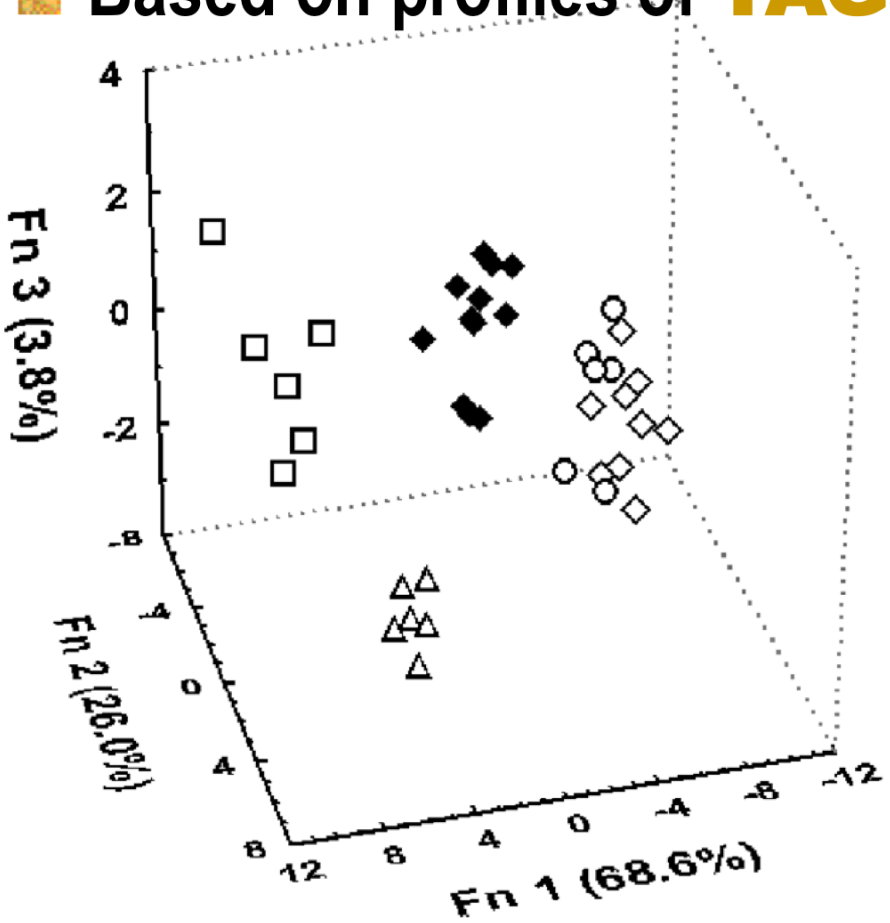


DART–TOFMS [+] mass spectra of POLAR EXTRACTS



GROUPING ANALYSIS USING LINEAR DISCRIMINANT ANALYSIS (LDA)

- Based on profiles of **TAGs**



△ EVOO
□ HO
◇ OPO
○ OO
◆ MIX

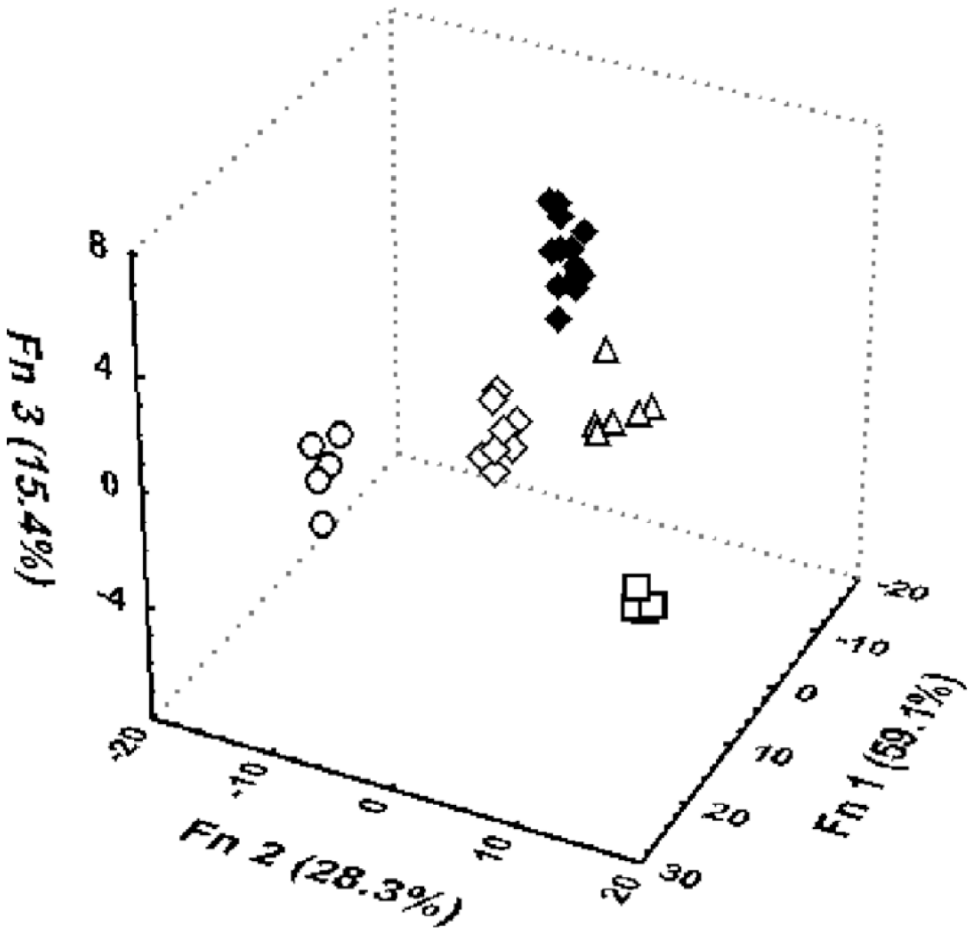
- EVOO, HO, OO, OPO, MIX → *objects*
- TAGs: markers → 11 masses → *variables*

Prediction ability 100% for the EVOO/HO mixtures in the range 50:50 – 85:15 (v/v)

Software statistiXL 1.8

GROUPING ANALYSIS USING LDA

- Based on profiles of **POLAR COMPOUNDS**



△ EVOO
□ HO
◇ OPO
○ OO
◆ MIX

- EVOO, HO, OO, OPO, MIX → *objects*
- Polar compounds: markers → 12 masses → *variables*

Software statistiXL 1.8

Prediction ability 100% for the EVOO/HO mixtures in the range 50:50 – 94:6 (v/v)

Note: **Prediction ability was obtained on the basis of leave-one-out cross validation (LOOCV)**

Title: Ambient mass spectrometry employing direct analysis in real time (DART) ion source for olive oil quality and authenticity assessment

Authors: Lukas Vaclavik, Tomas Cajka, Vojtech Hrbek, Jana Hajslova

PII: S0003-2670(09)00602-3
DOI: doi:10.1016/j.aca.2009.04.043
Reference: ACA 229908

Case study # 3:

ANIMAL FATS

- *Pork mixed with beef?*
- *Tallow added to lard?*

Classic approach: Fatty acids profile

Transesterification

GC-FID

Typical fatty acid composition of LARD and TALLOW

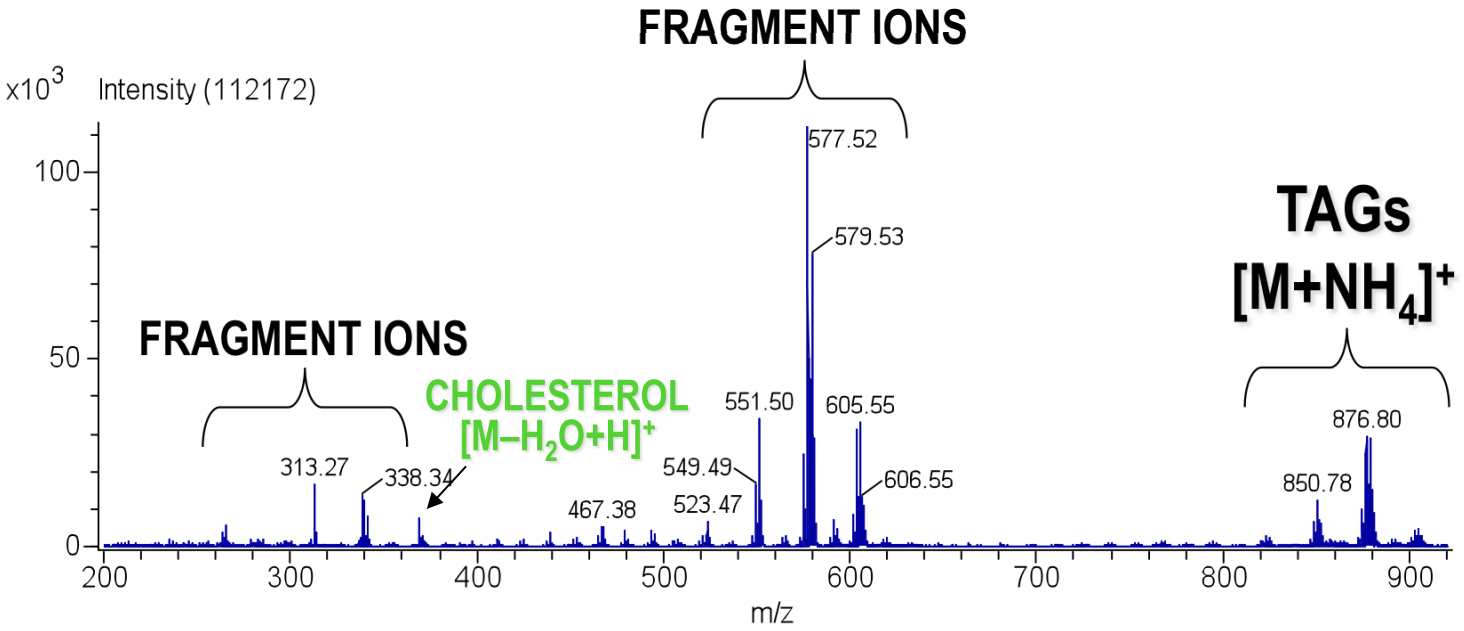
FATTY ACID	TALLOW (%)	LARD (%)
Lauric	1,0	< 1,0
Myrisic	1,4-7,8	0,5-2,5
Palmitic	17,0-37,0	20,0-32,0
Palmitoleic	0,7-8,8	1,7-5,0
Stearic	6,0-40,0	5,0-24,0
Oleic	26,0-50,0	35,0-62,0
Linolic	0,5-5,0	3,0-16,0
Linolenic	< 2,5	< 1,5
Arachidonic	< 0,5	< 1.0
Eikosenoic	< 0,5	< 1,0

Lard and tallow differ in TAGs composition ▶

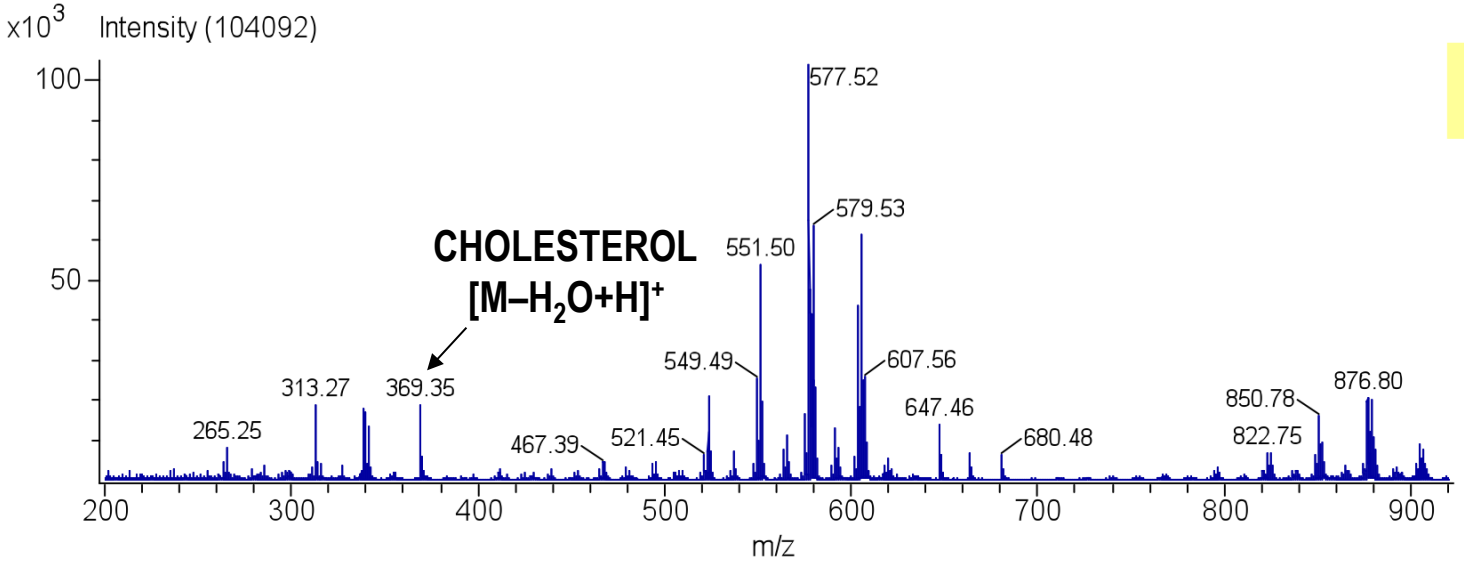
Target markers for DART

TAG	TALLOW (%)	LARD (%)
LLO	0,0	2,6
PoOL	0,0	1,6
LLP	0,0	2,2
MOL	0,0	1,1
OOL	0,0	5,0
PoOO	2,1	2,0
POL	3,8	10,0
PoPO	5,9	2,9
MOP	5,1	0,0
PLP	0,0	2,6
OOO	3,4	5,7
POO	23,0	20,8
PLS	0,0	6,1
POP	10,8	8,0
MPS	3,7	0,0
SOO	11,4	4,6
POS	14,9	15,5
PPS	5,7	3,6
SOS	6,6	2,1
PSS	3,7	3,7

DART [+], *m/z* 200-925

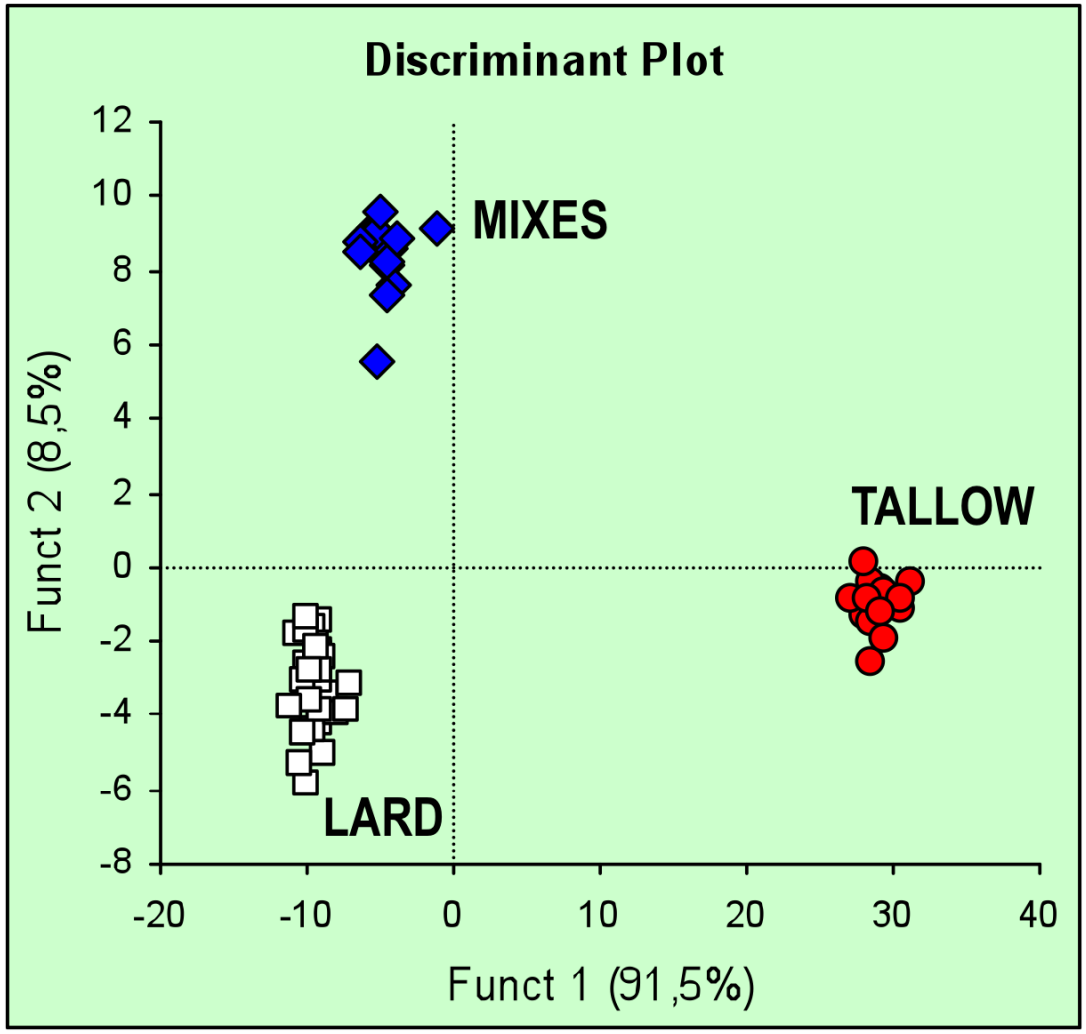


LARD



TALLOW

CHEMOMETRIC ANALYSIS - LDA



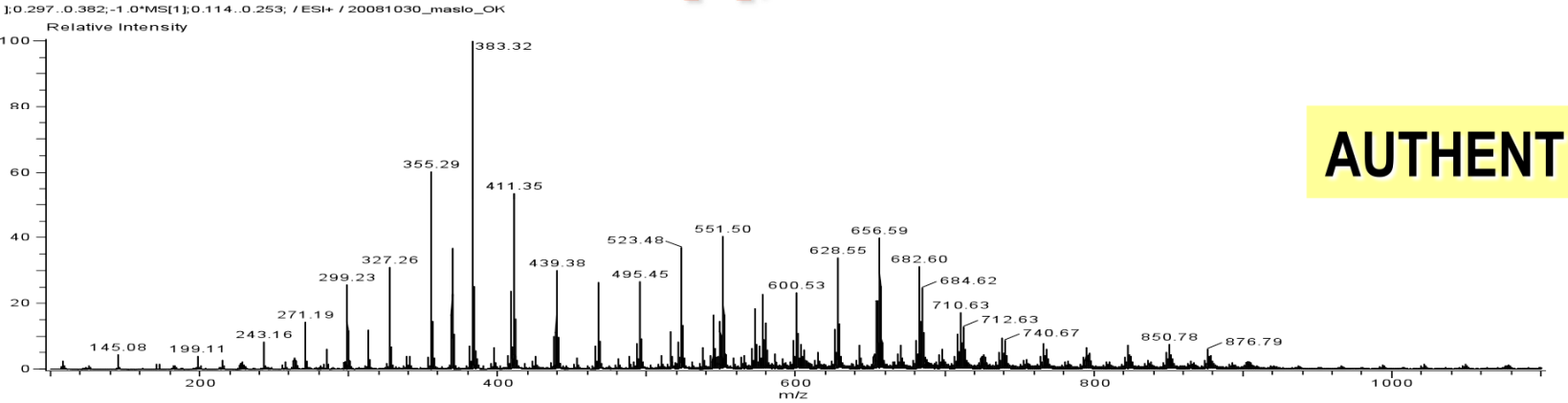
- Lard, Tallow, MIX → *objects*
- TAGs: markers → 24 masses → *variables*

Software statistiXL 1.8

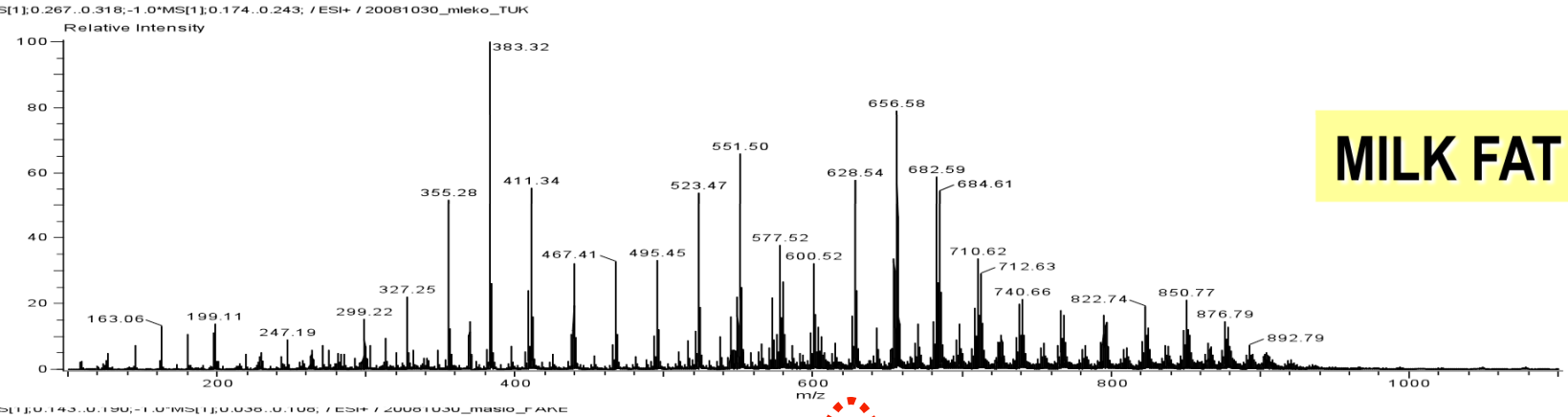
Model prediction ability 100% for the L / T mixtures in the range 50:50 – 95:5 (v/v)

Note: **Prediction ability was obtained on the basis of leave-one-out cross validation (LOOCV)**

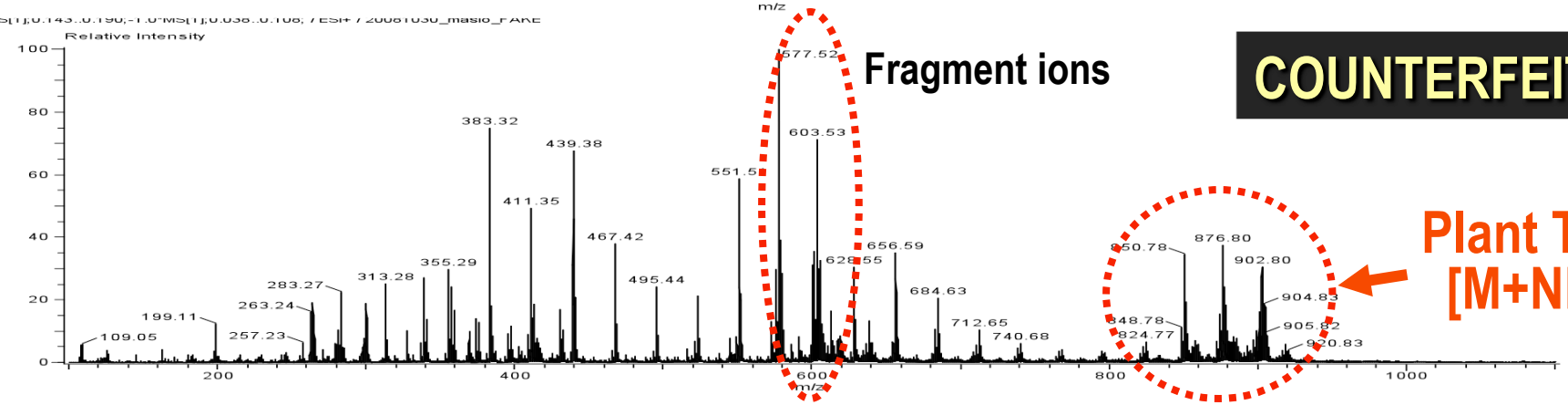
DART [+], *m/z* 100-1100



AUTHENTIC BUTTER



MILK FAT (control)



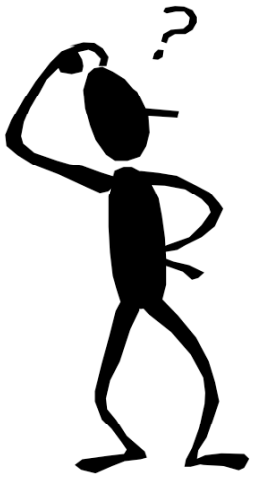
Fragment ions

COUNTERFEIT BUTTER

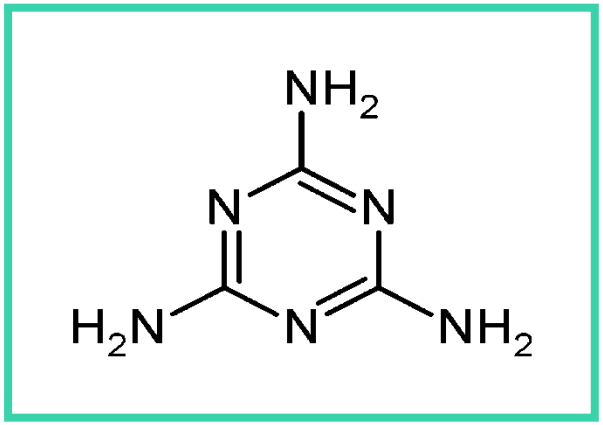
Plant TAGs
$[M+NH_4]^+$

Case study # 3:

MELAMINE

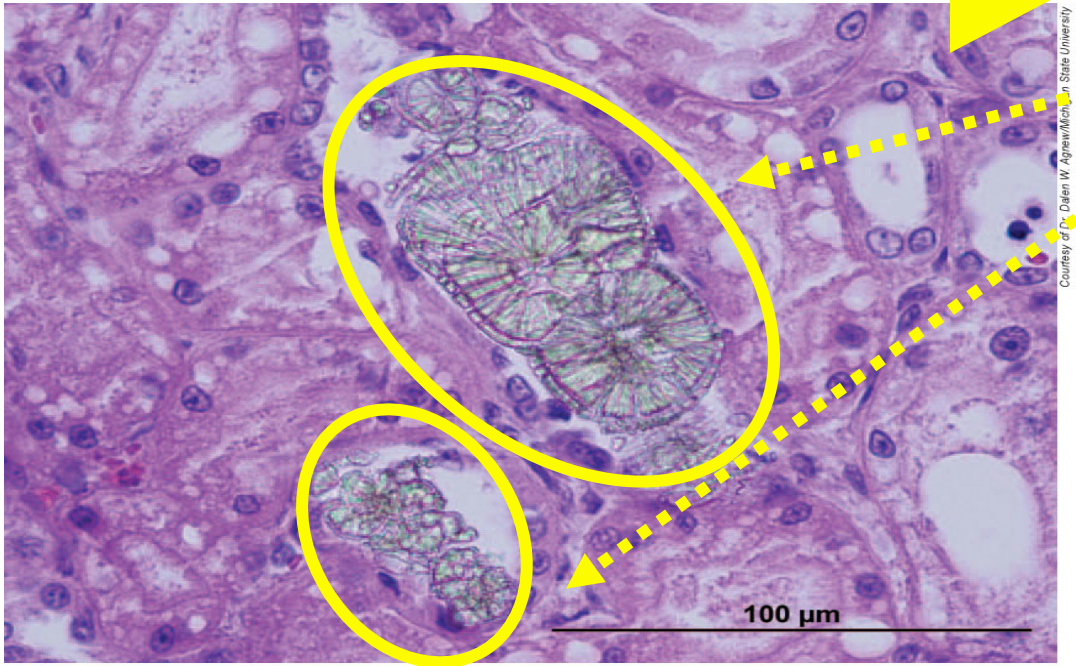


- *How fast can be the baby food formula checked ?*
- *Can be the melamine content determined accurately?*



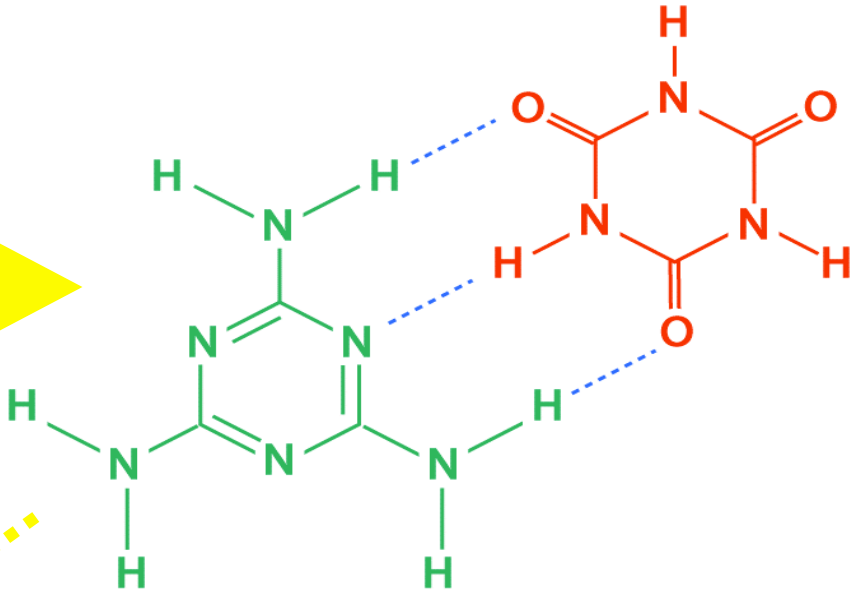
▶ **Addition of melamine with high content of nitrogen in molecule increases apparent protein content**

Crystals with cyanuric acid
➔kidney damage, renal failure



Crystals are visible in this histologic section of kidney from a cat with a history of eating some of the pet food that manufacturers recalled because of adulteration with melamine and co-contaminants.Toxicologic analysis of the kidney found cyanuric acid, melamine, and ammelide. Melamine and cyanuric acid can combine to form crystals.

Courtesy of Dr. Dalen W. Agnew/Michigan State University



World Health Organization

TDI 0.5 mg/kg body weight

Current analytical tools employed for determination of melamine in foods / feeds

LC–MS/MS

extraction
SPE clean-up
microfiltration/centrifugation
chromatogr. separation (HILIC)

LOQs: 5 – 250 ppb

GC–MS(/MS)

extraction
SPE clean-up
derivatization
chromatogr. separation

LOQs: 100 - 2000 ppb

ELISA

(AgraQuant®, Romer Labs)
extraction
incubation

LOQs: 0.1 – 250 ppm

MALDI–TOFMS

extraction
matrix preparation

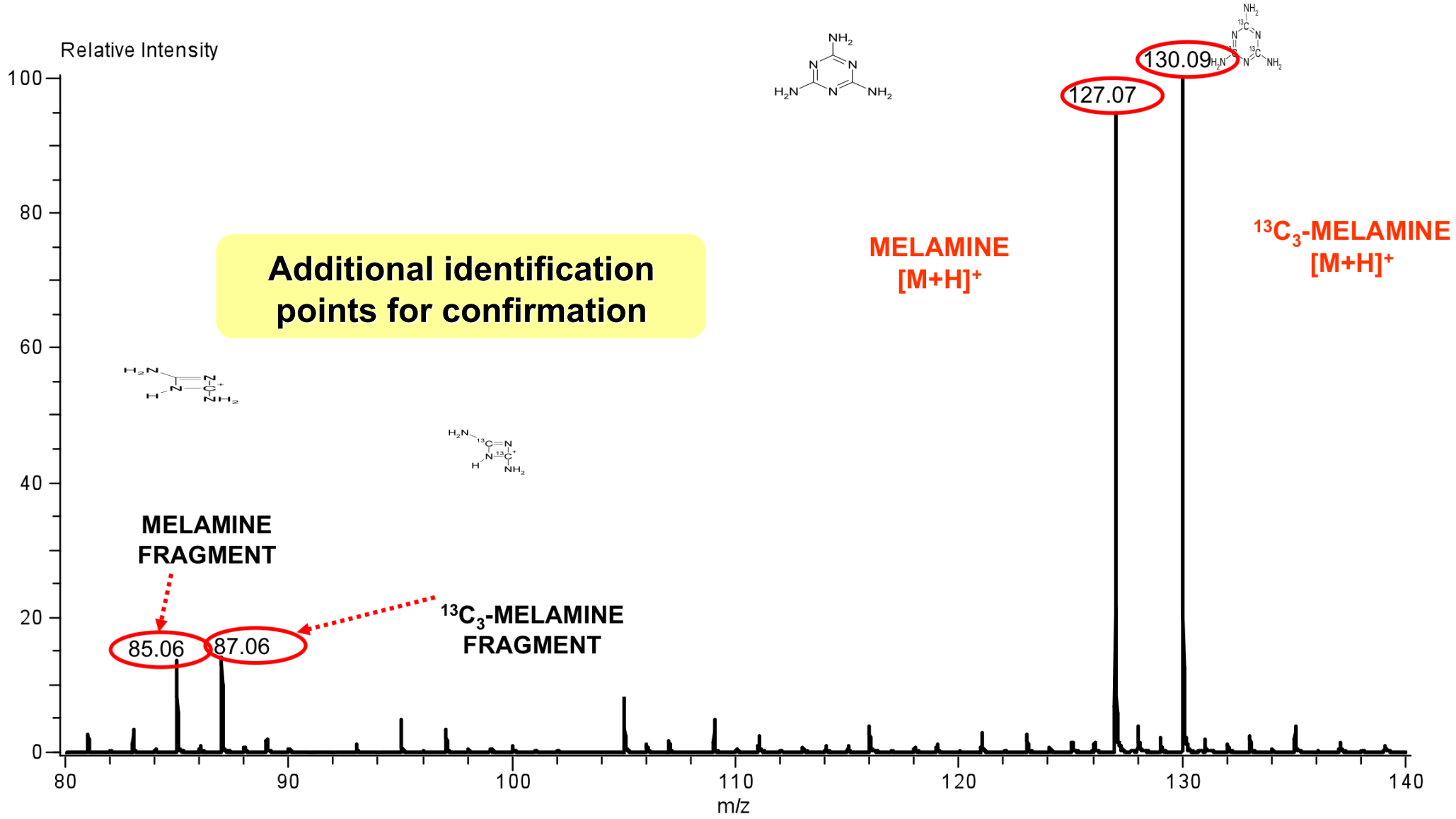
SCREENING

RAMAN SPECTROSCOPY

extraction
LODs: 0.05–0.1%

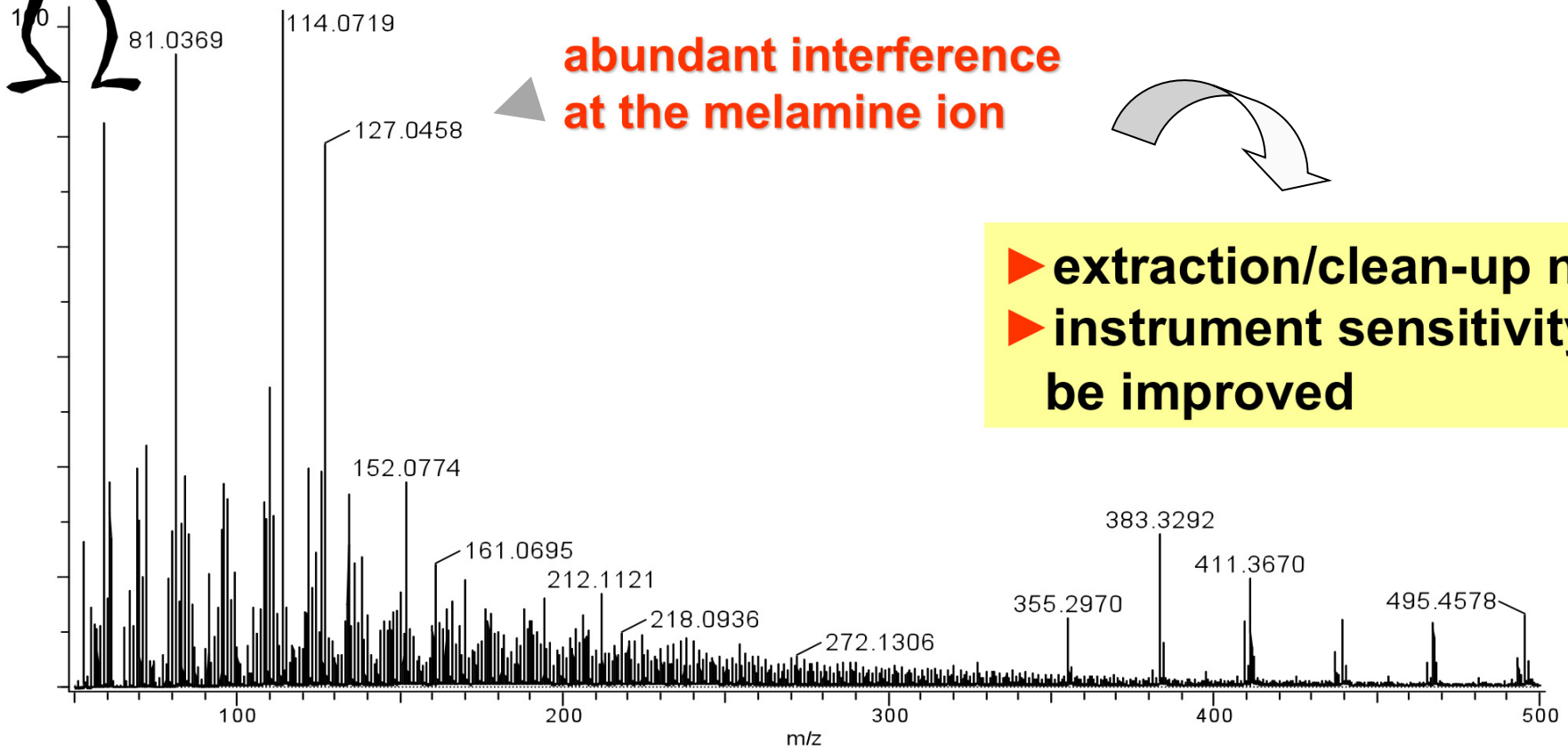
http://www.who.int/foodsafety/fs_management/Melamine_methods.pdf

DART -TOF MS positive mass spectra: solvent standard and $^{13}C_3$-melamine, 0.1µg/ml



Can the sample prep be omitted?

DART MS+ SPECTRUM: MILK SPIKED WITH MELAMINE (5ppm)



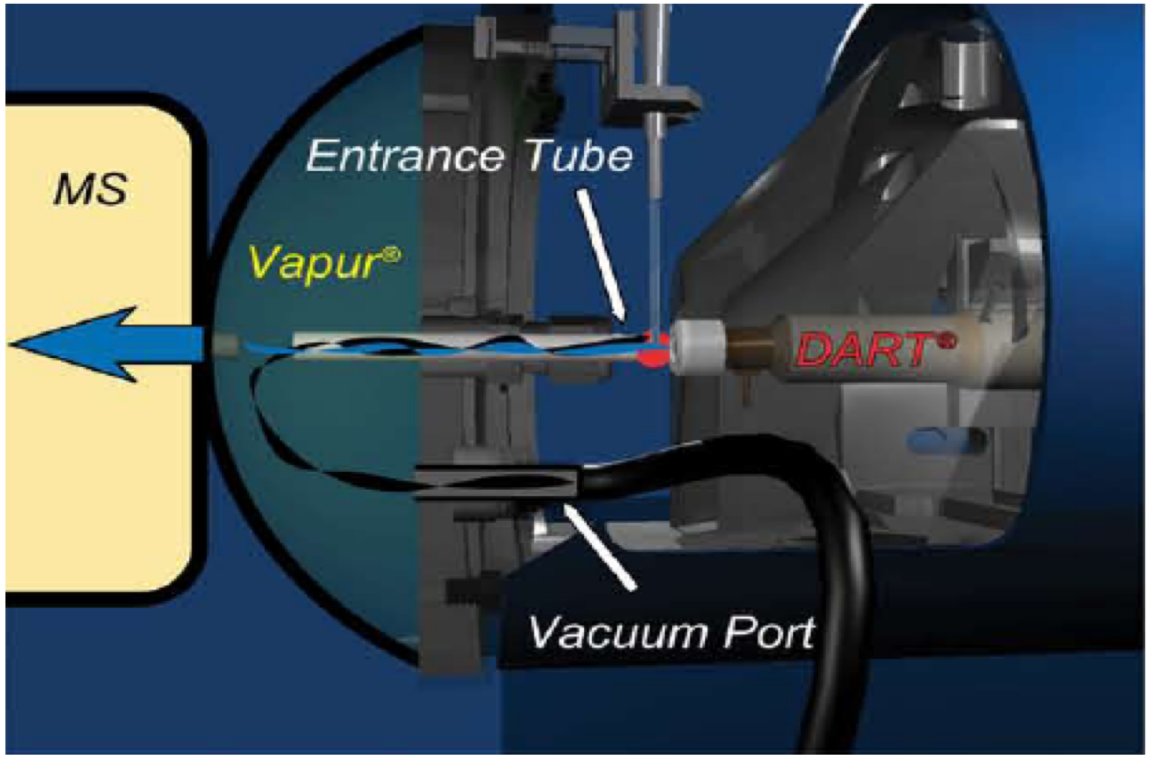
abundant interference at the melamine ion

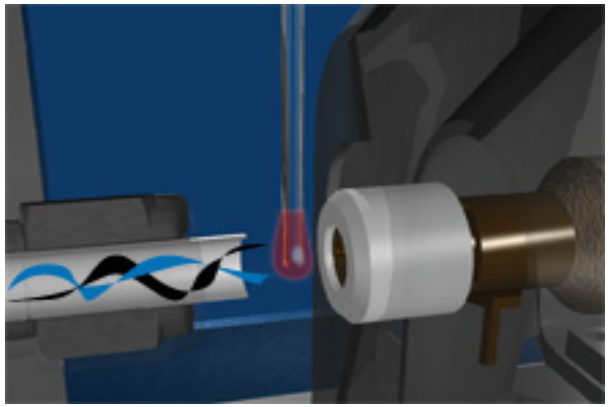
- **extraction/clean-up needed**
- **instrument sensitivity should be improved**

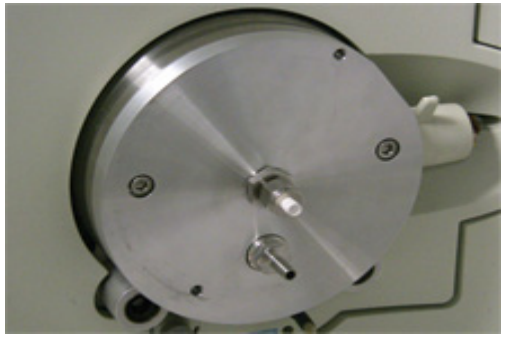
DART–TOFMS PERFORMANCE IMPROVEMENT
by VAPUR™ API INTERFACE (IonSense, USA)

Principle: ions formed during DART ionization process are collected and transferred through the ceramic tube into the MS inlet

➔ **effective ion transport** ➔ **BETTER SENSITIVITY AND REPEATABILITY**







SIMPLIFIED SAMPLE PREPARATION PROCEDURE

1g of dried milk

Internal standard
$^{13}C_3$-melamine

9 ml of acetonitrile: 0.3% formic acid mixture (1:1,*v/v*) added

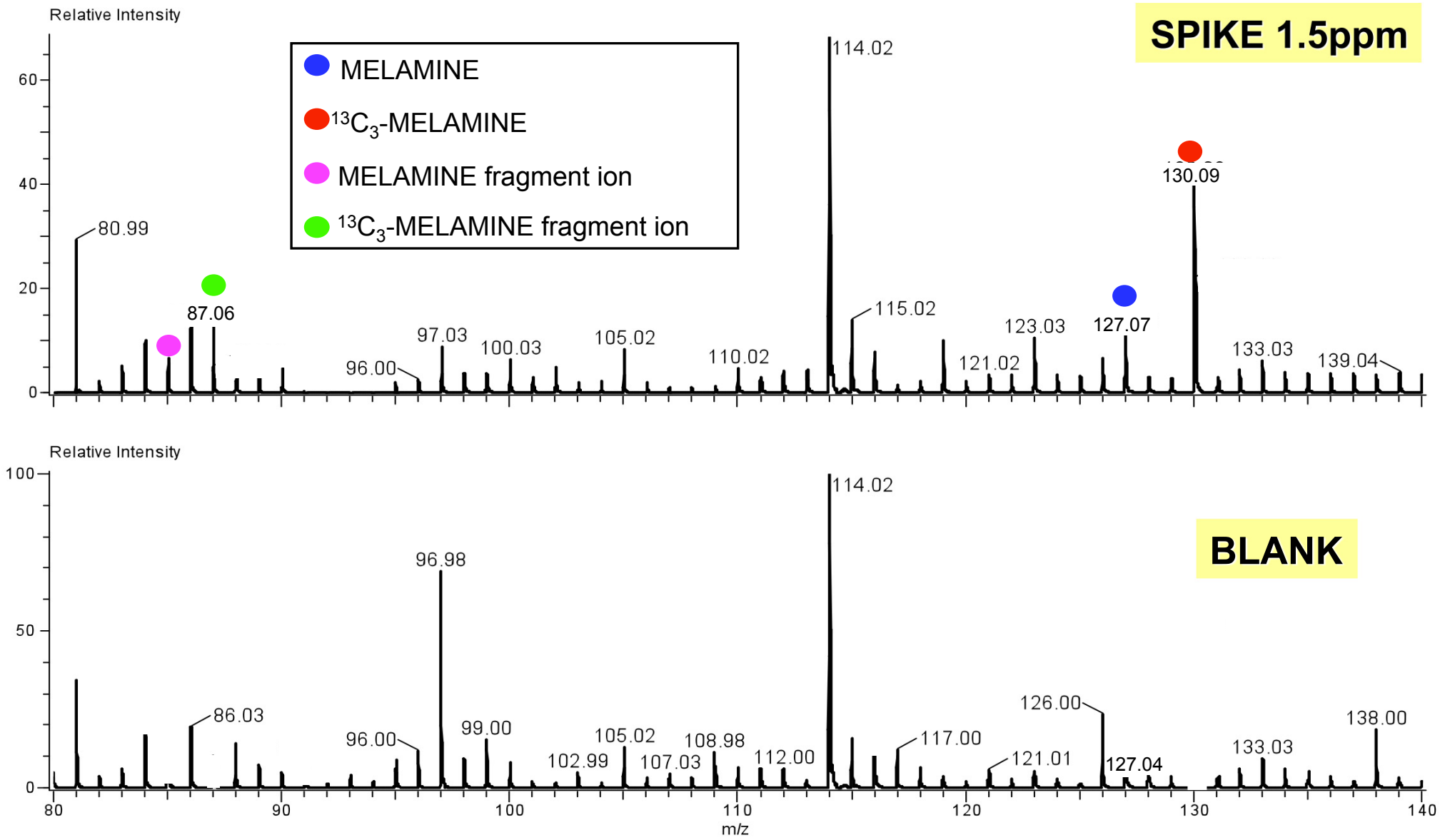
Including sample prep: 8 min per sample

Sonication 1´ & filtration

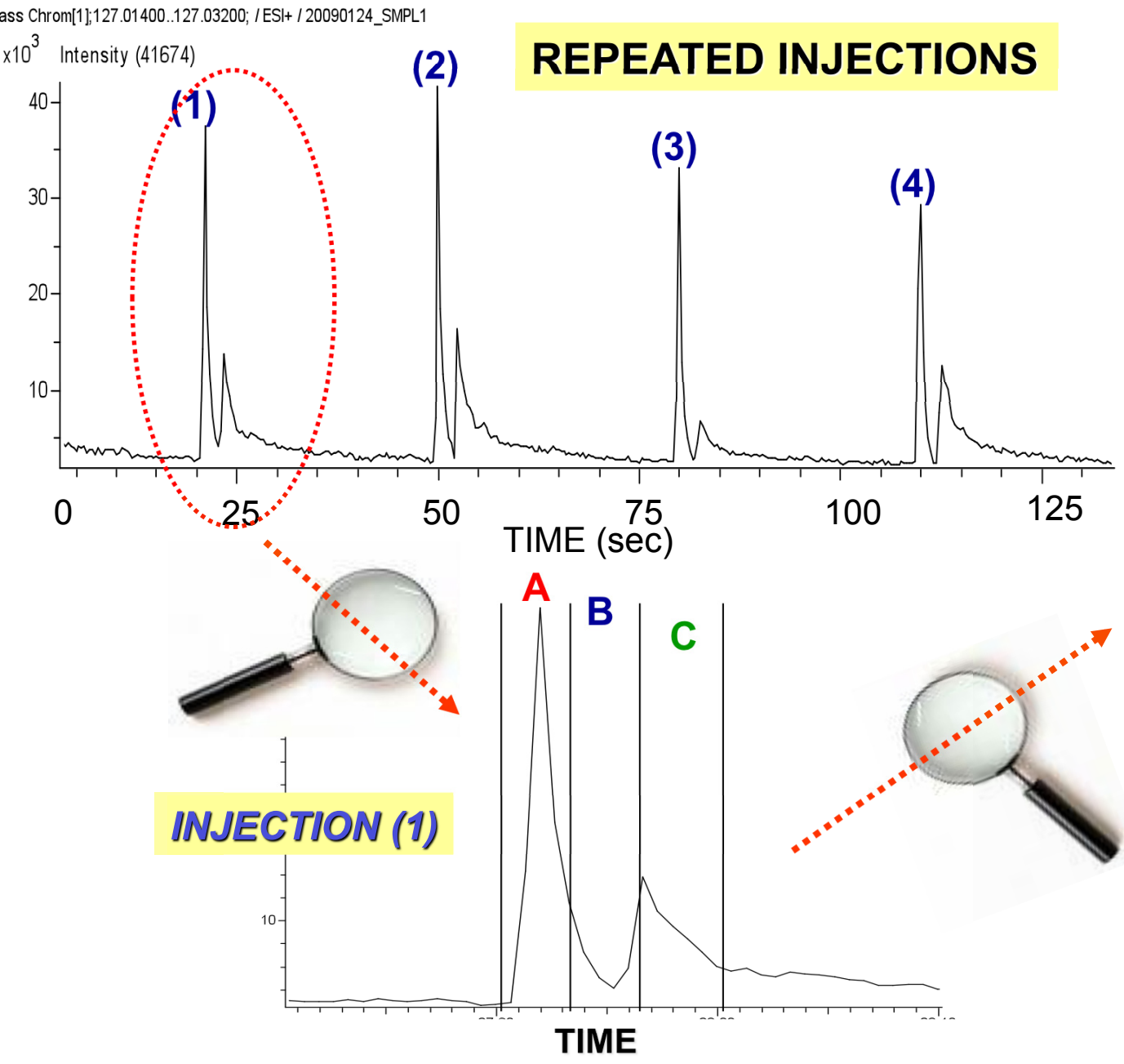
dispersive SPE (PSA)

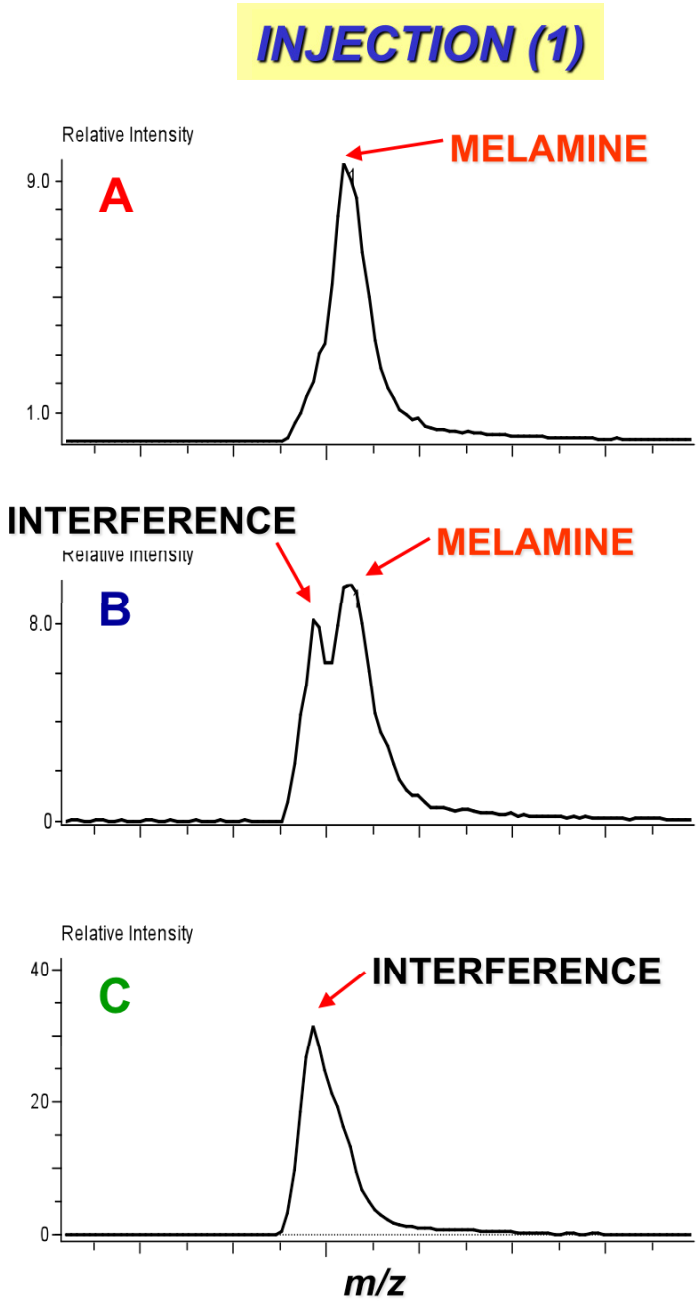
DART–TOFMS

DART TOF MS analysis of dried milk



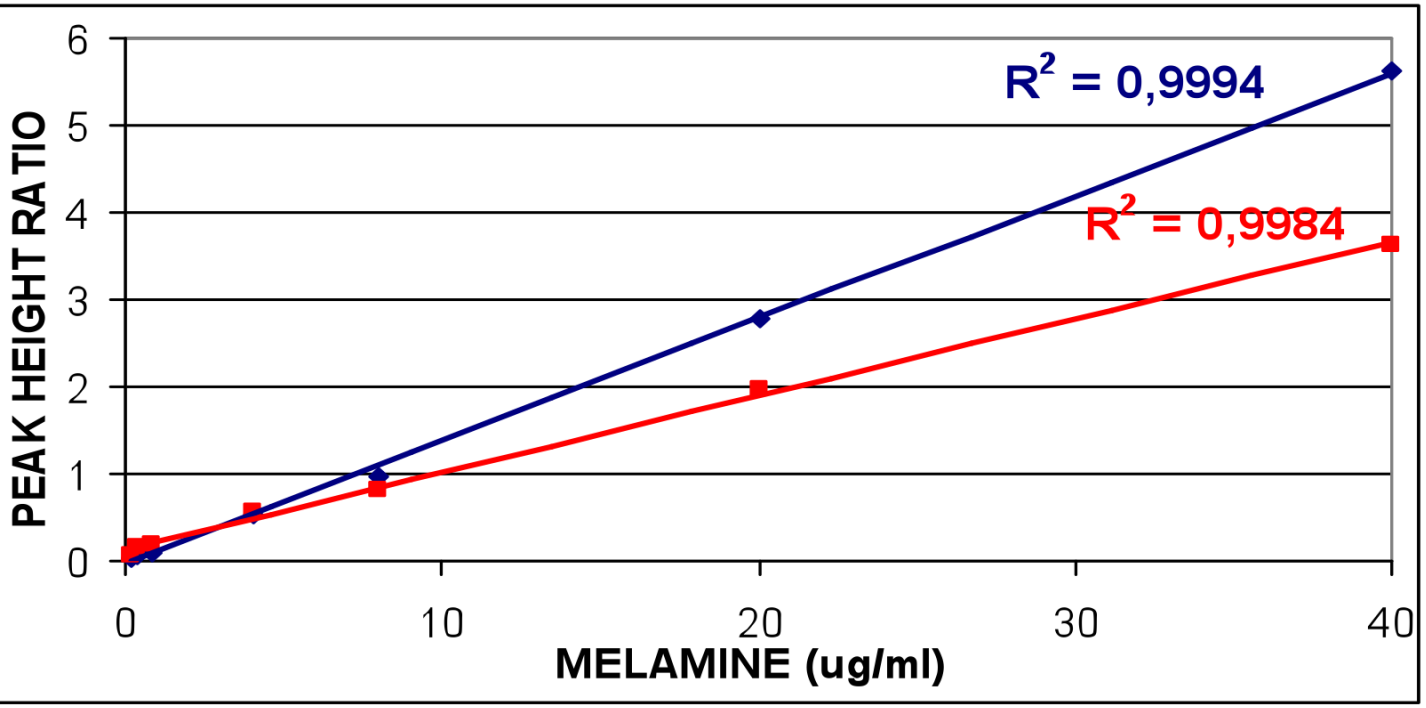
Ion chromatogram (*m/z* 127.07): dried milk, spike 1.5 ppm





QUANTIFICATION: isotope dilution technique

CALIBRATION PLOT



SOLVENT STANDARD

Matrix suppresion

MATRIX-MATCHED STANDARD (dried milk)

DART TOF MS method performance characteristics

PARAMETER	WITHOUT VAPUR	WITH VAPUR
LOD (S/N 3)	700 - 1000 ppm	**100 – 150 ppm**
LOQ (S/N 9)	1500 – 2800 ppm	**250 – 280 ppm**
REPEATABILITY (0.5 ppm)	10%	**3 – 5%**
RECOVERY (1.0 ppm)	98%	

Improved parameters by VAPUR

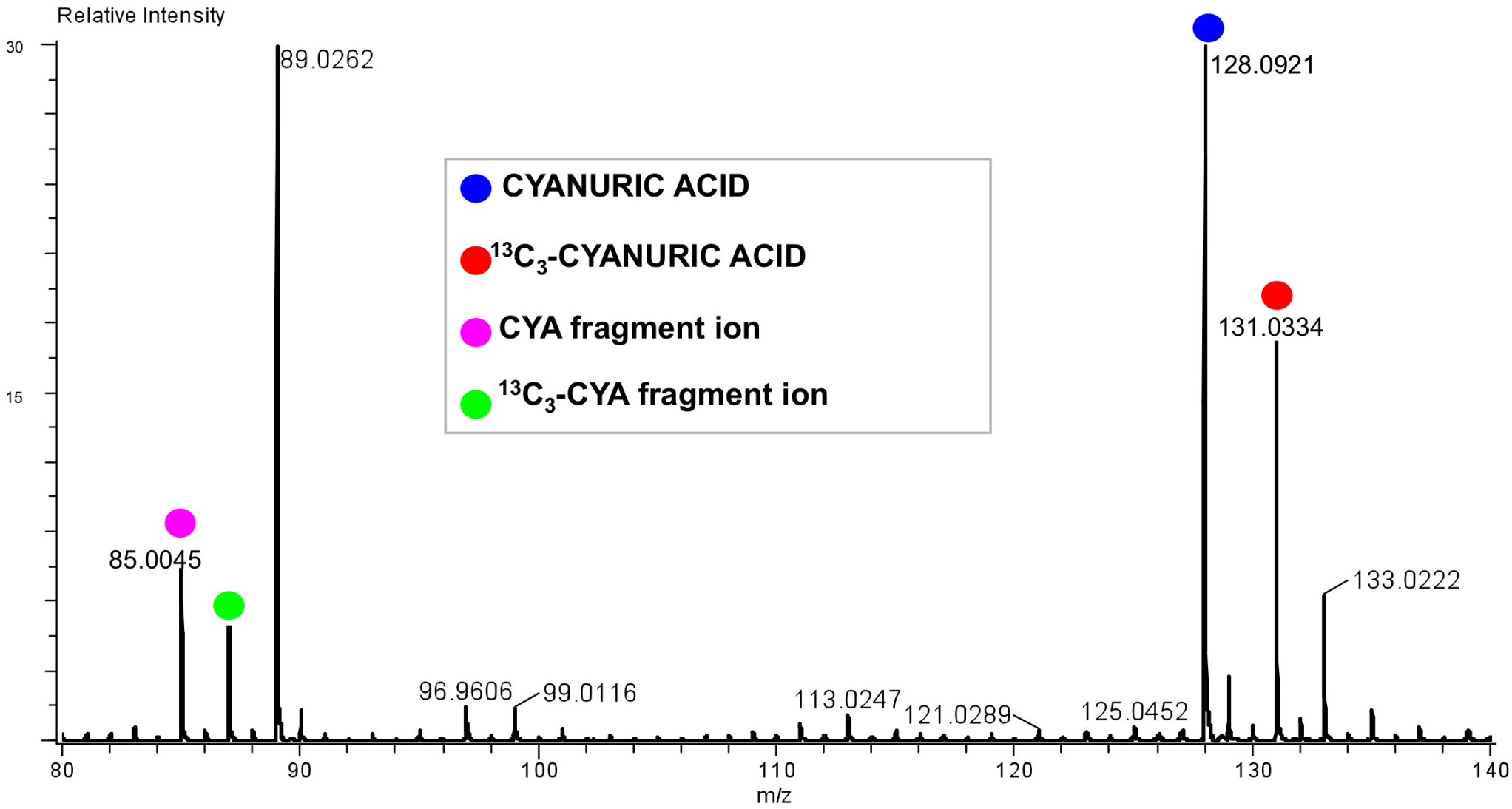
Real life samples: INTERLABORATORY COMPARISON

- **Results of DART–TOFMS analyses were compared with those obtained by LC-MS/MS method (results provided by Eurofins)**

SAMPLE	DART - TOFMS	RSD	LC-MS/MS
Condensed milk	4.04 ppm	3.1%	4.00 ppm
Dried milk (1)	2.33 ppm	3.5%	2.40 ppm
Dried milk (2)	0.51 ppm	4.2%	0.57 ppm

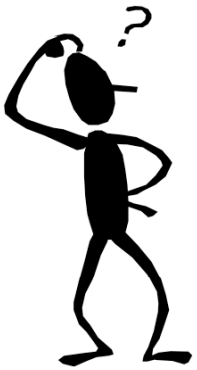
CYANURIC ACID can be determined in the same sample

DART negative mass spectrum: spike 2 ppm

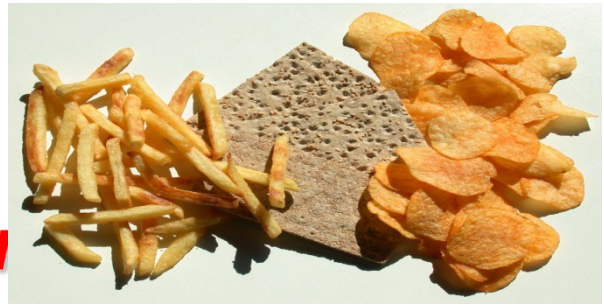


Case study # 4:

ACRYLAMIDE



- *Is it possible to determine acrylamide precursors?*
- *Can be predicted acrylamide formation extent i potato chips?*



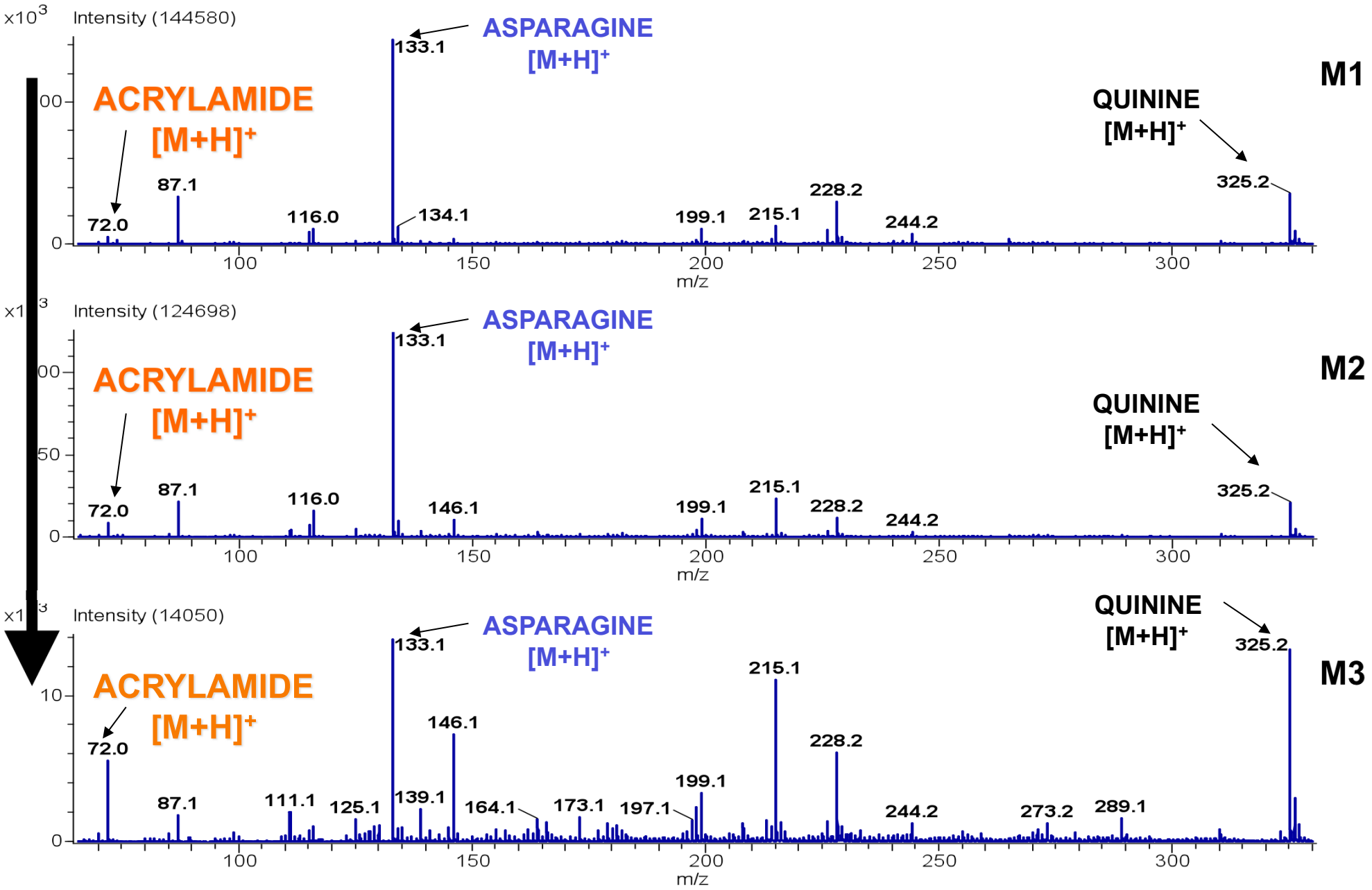
MODEL SYSTEMS

- Solutions of glucose or fructose with asparagine in methanol:water (1:1, *v/v*)
- Quinine was added to each model mixture prior analysis (10µg/ml) as internal standard (I.S.)

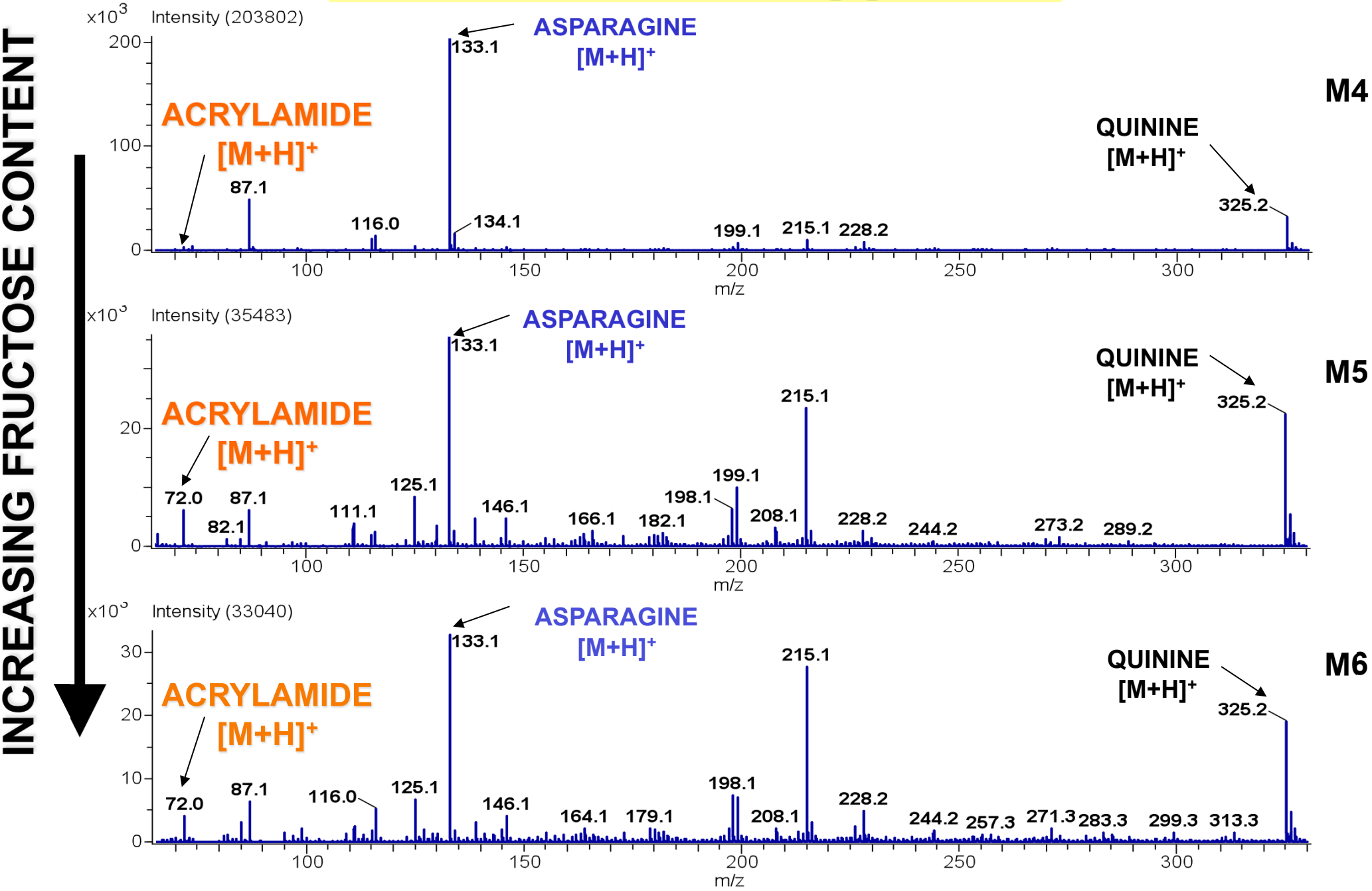
Model	Asparagine (mg/ml)	Glucose (mg/ml)	Fructose (mg/ml)
M1	0.3	0.05	-
M2	0.3	0.15	-
M3	0.3	0.30	-
M4	0.3	-	0.05
M5	0.3	-	0.15
M6	0.3	-	0.30

MODEL M1 – M3, DART [+] , 250°C

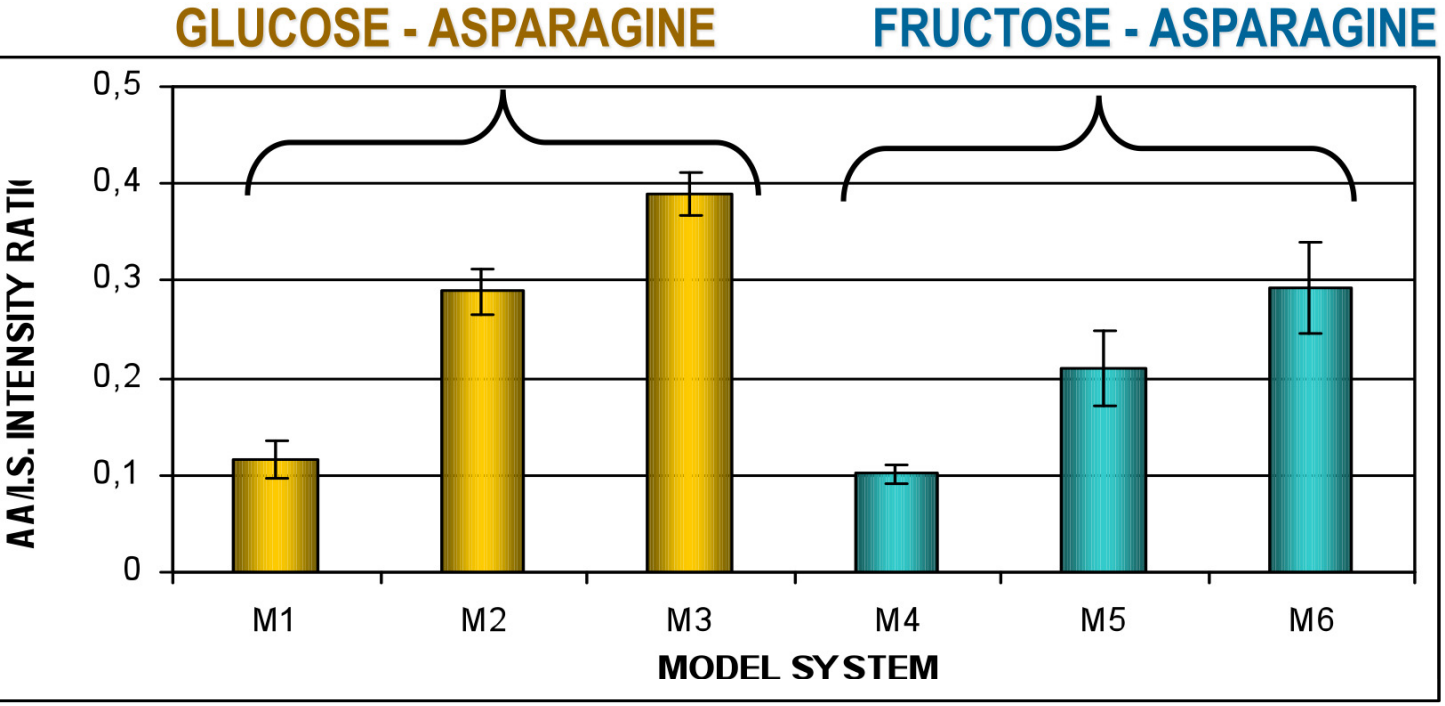
INCREASING GLUCOSE CONTENT



MODEL M4 – M6, DART [+] , 250°C



ACRYLAMIDE FOMATION IN MODEL SYSTEMS

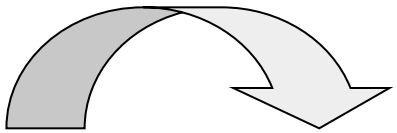


➔ *Intensity of acrylamide was normalized to I.S. intensity (n=6)…*

1 min. per sample !

Model	Asparagine (mg/ml)	Glucose (mg/ml)	Fructose (mg/ml)
M1	0.3	0.05	0.00
M2	0.3	0.15	0.00
M3	0.3	0.30	0.00
M4	0.3	0.00	0.05
M5	0.3	0.00	0.15
M6	0.3	0.00	0.30

OK, it works in model , but what about real-life samples?



POTENTIAL OF POTATOES TO YIELD ACRYLAMIDE

- *Potatoes (n=14), various varieties*
- *Glucose, fructose, sucrose and asparagine were determined in methanol:water extracts using HPLC–RID and HPLC–FLD, respectively*
- *Formation of acrylamide during desorption/ionization process was monitored with DART–TOFMS*

HOMOGENIZATION

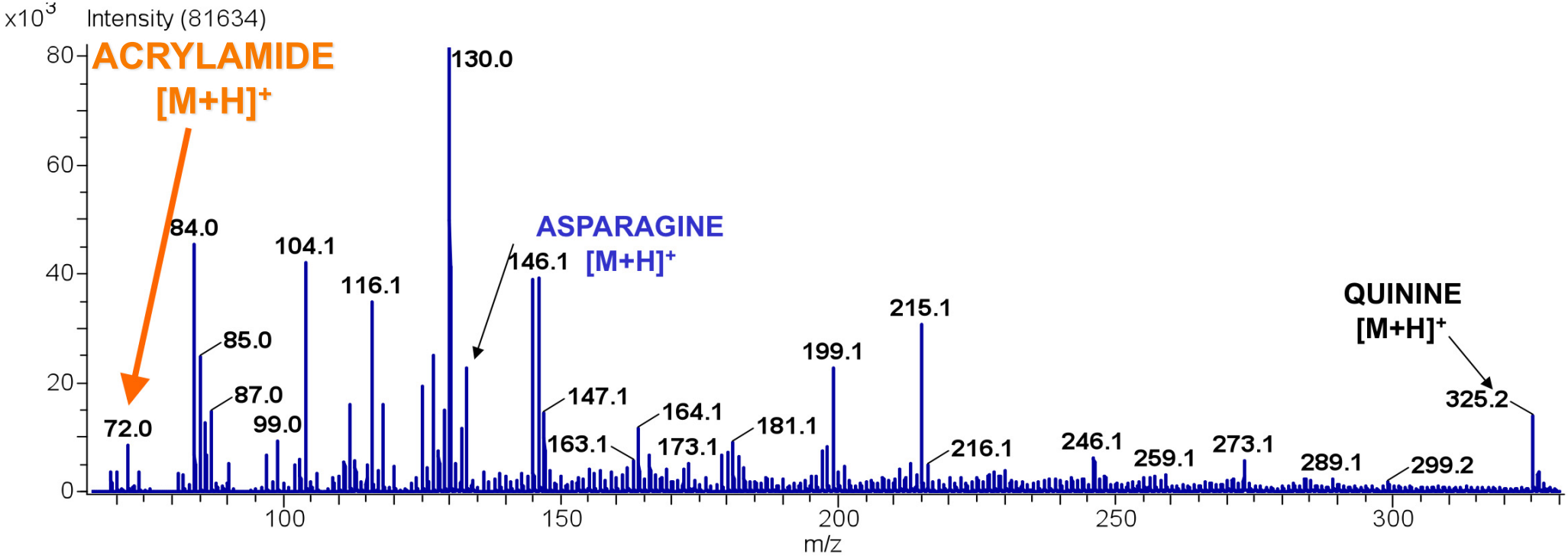
EXTRACTION OF POTATOES (30g) BY SHAKING (5min)WITH MeOH:H_2O MIXTURE (1:1, *v/v*, 200ml)

FILTRATION

ADDITION OF QUININE (I.S.) AT 10µg/ml

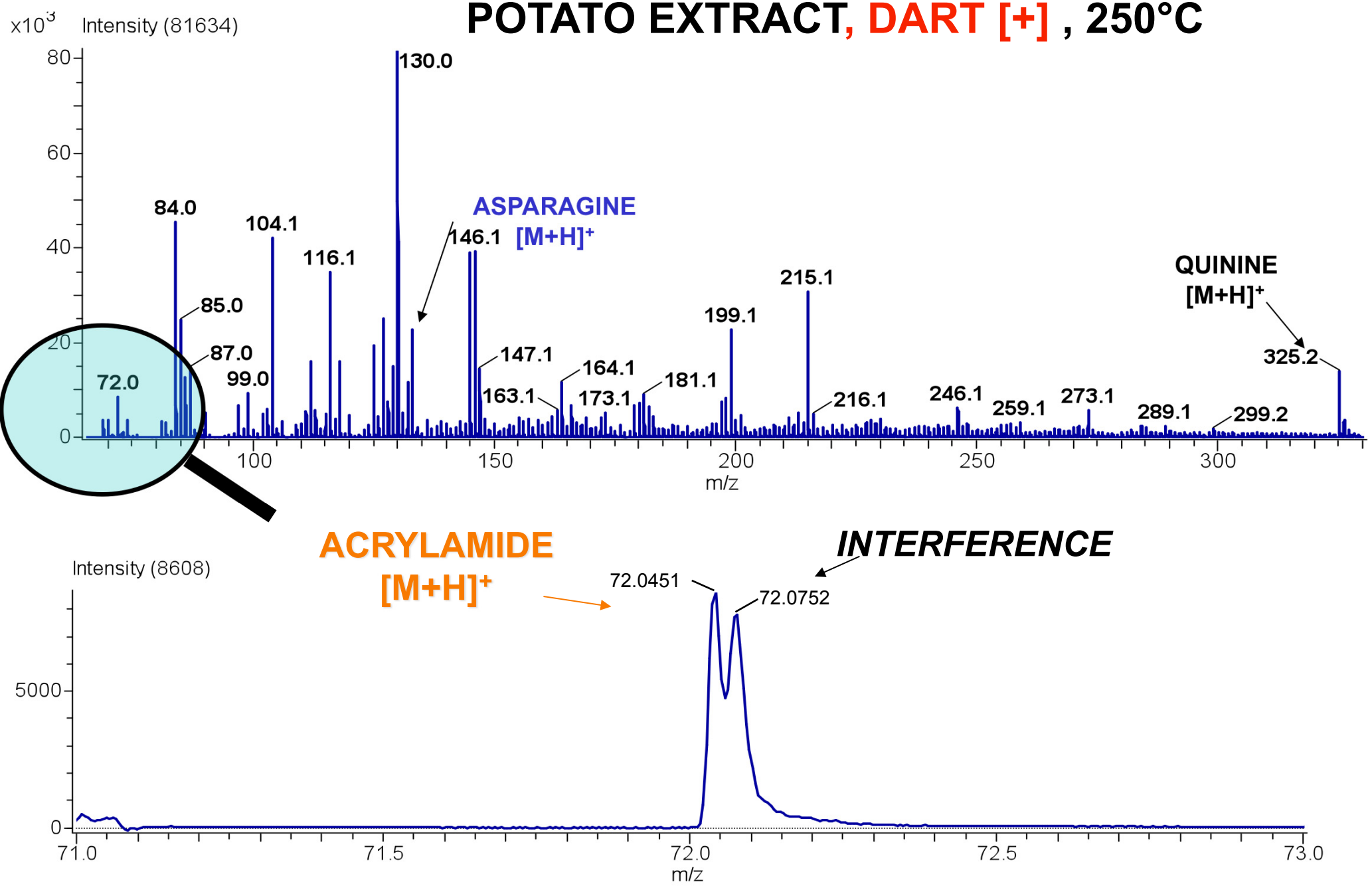
DART–TOFMS

POTATO TUBER EXTRACT, DART [+] , 250°C

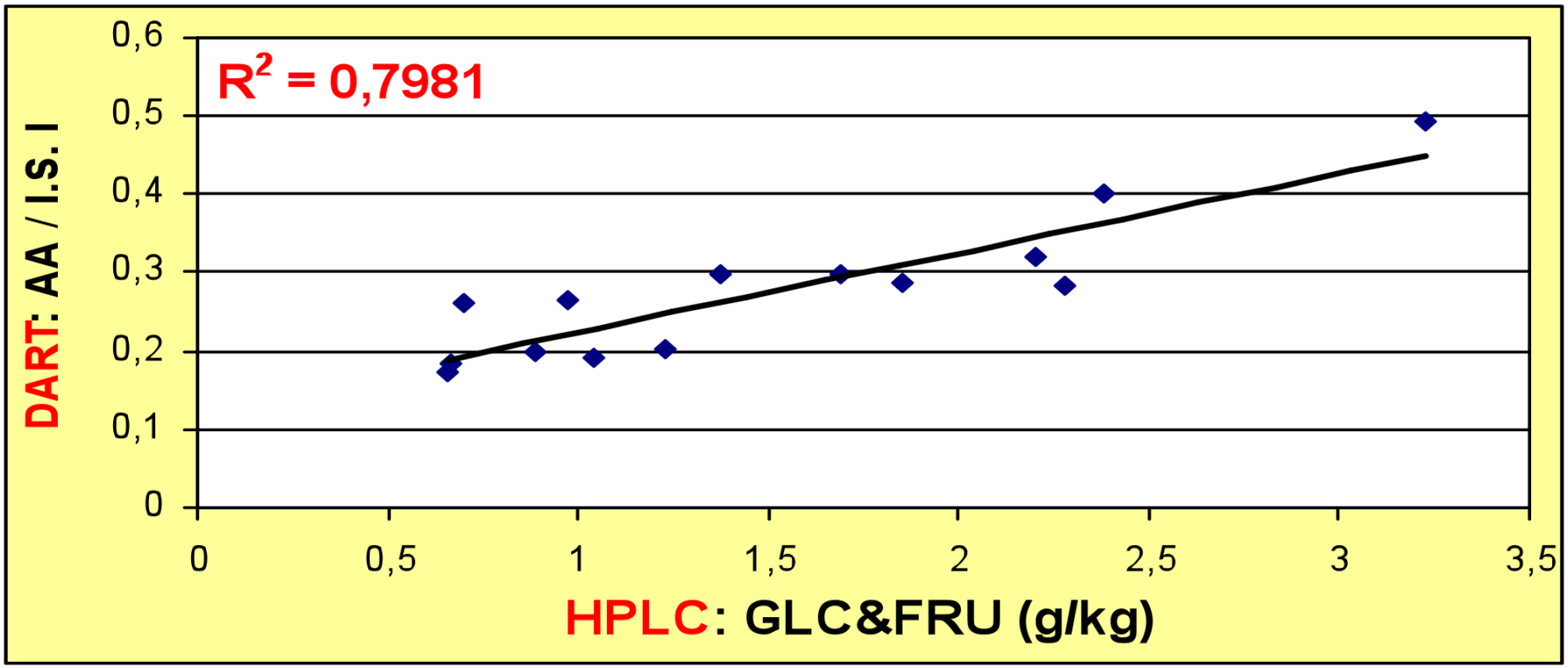


METABOLOME !!!

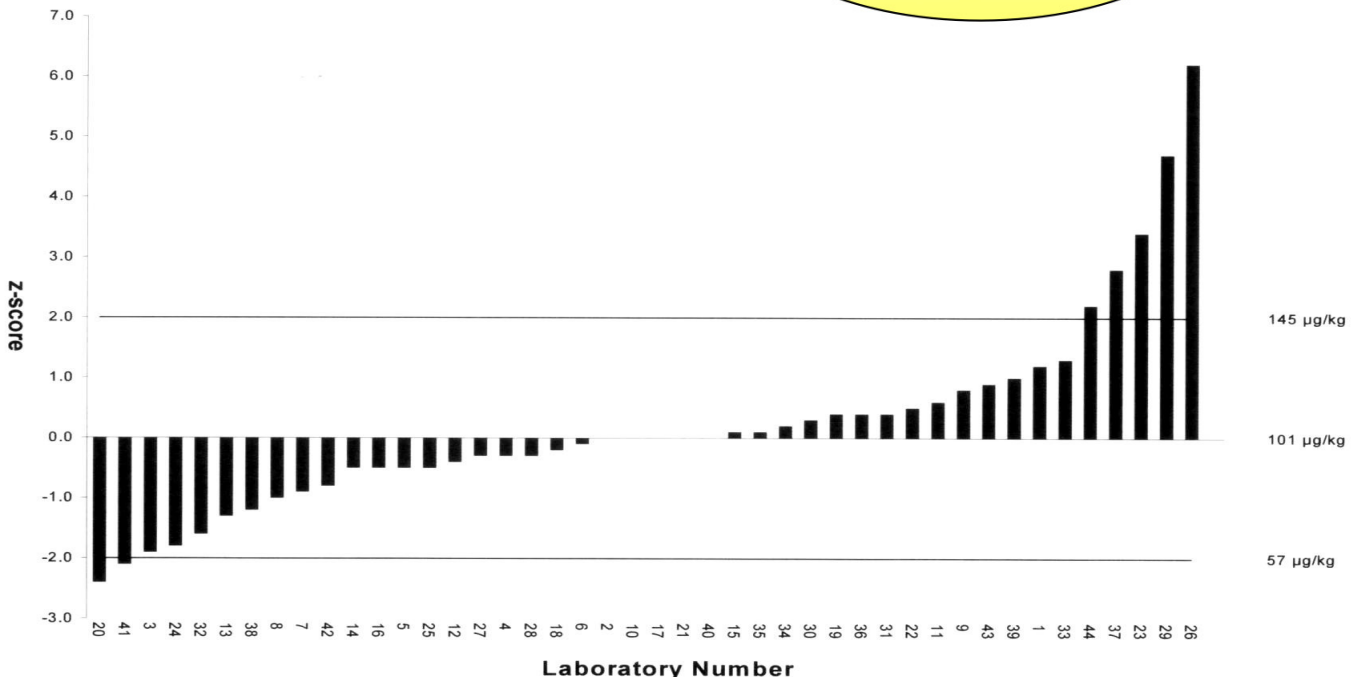
POTATO EXTRACT, DART [+] , 250°C



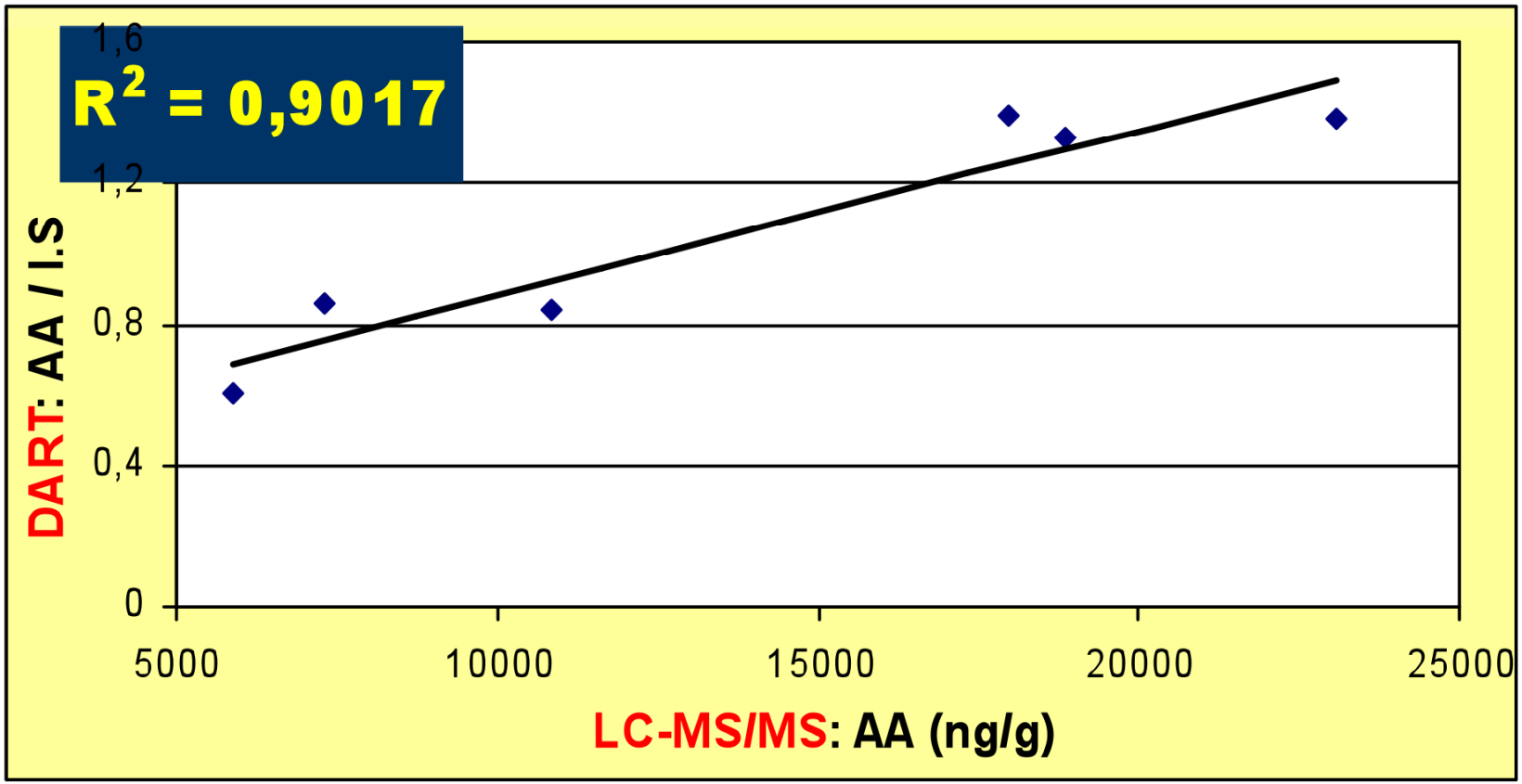
Normalized signal of acrylamide (*m/z* 72.045) versus glucose and fructose content in potatoes



OK, but confirmation of observed trends is needed using established method



Correlation of DART normalized acrylamide signal with acrylamide content in fried potato crisps determined by LC-MS/MS



SUMMARY

DART is primarily small-molecules analysis technique (with some exceptions) allowing rapid (real-time) METABOLOMIC PROFILING (FINGERPRINTING)

sample handling minimised / eliminated → workload decreased → analyses throughput increased

When DART coupled with HR TOF MS, identification of unknowns possible

DART IS A REAL CHALLENGE IN RAPID FOOD / FEED ANALYSIS

Interested in recent food analysis innovations…?

RAFA 2009

4th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

4–6 November 2009

Prague, Czech Republic

www.rafa2009.eu

INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

International Association of Environmental Analytical Chemistry

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

- Residues and contaminants
- Authenticity, traceability, fraud
- Flavours and odours
- Processing and packaging contaminants
- Mycotoxins, marine and plant toxins
- Allergens
- Genetically modified organisms (GMO´s)
- Nanoparticles
- Novel foods, nutritional supplements, organic food

SCIENTIFIC COMMITTEE (tentative list)

Prof. Jana Hajslova	*Institute of Chemical Technology, Prague, CZ* ***(chair)***
Prof. Michel Nielen	*RIKILT-Institute of Food Safety, Wageningen, NL* ***(co-chair)***
Prof. John Gilbert	*Central Science Laboratory, York, UK*
Dr. Samuel Godefroy	*Health Canada, Ottawa, Canada*
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