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

- Fourier-transform MIR
- Fourier-transform Raman
- GC TOF MS
- GC TOF MS
- DART TOF MS
- Direct Inj. TOF MS
- NMR
- NIR

NON-TARGET SCREENING
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:

- 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

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%

**ANN model**
Recognition ability: 100%
Prediction ability: 98%
DART TOF MS enables fast tracking of beer origin

No problem to distinguish these Budweisers within few minutes!
AMBIENT MASS SPECTROMETRY

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

- Reduced / minimal sample prep
- No chromatographic separation
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
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

He/N₂

Discharge chamber

Heating

Metastable He/N₂

Analyte

Vacuum interface & mass spectrometer

Ion/neutral separation

sample exposure

Autosampler

sample acquisition

AccuTOF
DART Ionization: ANALYTES

**Negative ions**
- Direct (M⁻)
  - 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⁺⁺)
  - 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-mass spectrometry
- Direct Injection mass spectrometry

AMBIENT MASS SPECTROMETRY
Can direct be honey analysed directly?

DART source saturated!

NO!

Honey A

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

Honey B

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

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

DART: positive ion mode
DART temperature: 250°C

DART TOF MS
Head-space SPME profile

Honey A

Honey B

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
AUTHENTIC HONEY SAMPLES

Lime

Acacia

Rape

Sunflower

PHENOLICS

Tectochrysin
Chrysin
Gallangin
Pinocembrin
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–H₂O 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

Extra virgin olive oil

Hazelnut oil

Olive pomace oil

Olive oil
DART–TOFMS [+ ] mass spectra of **POLAR EXTRACTS**

**Extra virgin olive oil**

**Olive oil**

**Hazelnut oil**

**Olive pomace oil**
GROUPING ANALYSIS USING LINEAR DISCRIMINANT ANALYSIS (LDA)

Based on profiles of **TAGs**

- **EVOO**
- **HO**
- **OO**
- **OPO**
- **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

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)
Accepted Manuscript

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
Reference: ACA 229908
Case study # 3: ANIMAL FATS

- Pork mixed with beef?
- Tallow added to lard?
### Classic approach: Fatty acids profile

#### Transesterification (GC-FID)

<table>
<thead>
<tr>
<th>FATTY ACID</th>
<th>TALLOW (%)</th>
<th>LARD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric</td>
<td>1,0</td>
<td>&lt; 1,0</td>
</tr>
<tr>
<td>Myrisic</td>
<td>1,4-7,8</td>
<td>0,5-2,5</td>
</tr>
<tr>
<td>Palmitic</td>
<td>17,0-37,0</td>
<td>20,0-32,0</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>0,7-8,8</td>
<td>1,7-5,0</td>
</tr>
<tr>
<td>Stearic</td>
<td>6,0-40,0</td>
<td>5,0-24,0</td>
</tr>
<tr>
<td>Oleic</td>
<td>26,0-50,0</td>
<td>35,0-62,0</td>
</tr>
<tr>
<td>Linolic</td>
<td>0,5-5,0</td>
<td>3,0-16,0</td>
</tr>
<tr>
<td>Linolenic</td>
<td>&lt; 2,5</td>
<td>&lt; 1,5</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>&lt; 0,5</td>
<td>&lt; 1,0</td>
</tr>
<tr>
<td>Eikosenoic</td>
<td>&lt; 0,5</td>
<td>&lt; 1,0</td>
</tr>
</tbody>
</table>

Typical fatty acid composition of LARD and TALLOW.
Lard and tallow differ in TAGs composition. The table below shows the TAG composition in tallow and lard:

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

Target markers for DART.
DART [+]，m/z 200-925

**LARD**

![LARD spectrum with fragment ions and cholesterol peaks]

**TAGs [M+NH₄]⁺**

**TALLOW**

![Tallow spectrum with fragment ions and cholesterol peaks]
Lard, Tallow, MIX → objects

TAGs: markers → 24 masses → variables

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)

COUNTERFEIT BUTTER

Fragment ions

Plant TAGs [M+NH₄]⁺
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.

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 $^{13}\text{C}_3$-melamine, 0.1μg/ml

Additional identification points for confirmation
Can the sample prep be omitted?

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

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

abundant interference at the melamine ion
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

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

Sonication 1’ & filtration

dispersive SPE (PSA)

Internal standard $^{13}$C$_3$-melamine

Including sample prep: 8 min per sample

DART–TOFMS
DART TOF MS analysis of dried milk

**SPIKE 1.5ppm**

- **MELAMINE**
- $^{13}$C$_3$-MELAMINE
- MELAMINE fragment ion
- $^{13}$C$_3$-MELAMINE fragment ion

**BLANK**
Ion chromatogram ($m/z$ 127.07): dried milk, spike 1.5 ppm
QUANTIFICATION: isotope dilution technique

CALIBRATION PLOT

SOLVENT STANDARD

Matrix suppression

MATRIX-MATCHED STANDARD (dried milk)

$R^2 = 0.9994$

$R^2 = 0.9984$
## DART TOF MS method performance characteristics

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>WITHOUT VAPUR</th>
<th>WITH VAPUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (S/N 3)</td>
<td>700 - 1000 ppm</td>
<td>100 – 150 ppm</td>
</tr>
<tr>
<td>LOQ (S/N 9)</td>
<td>1500 – 2800 ppm</td>
<td>250 – 280 ppm</td>
</tr>
<tr>
<td>REPEATABILITY (0.5 ppm)</td>
<td>10%</td>
<td>3 – 5%</td>
</tr>
<tr>
<td>RECOVERY (1.0 ppm)</td>
<td>98%</td>
<td></td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>DART - TOFMS</th>
<th>RSD</th>
<th>LC-MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condensed milk</td>
<td>4.04 ppm</td>
<td>3.1%</td>
<td>4.00 ppm</td>
</tr>
<tr>
<td>Dried milk (1)</td>
<td>2.33 ppm</td>
<td>3.5%</td>
<td>2.40 ppm</td>
</tr>
<tr>
<td>Dried milk (2)</td>
<td>0.51 ppm</td>
<td>4.2%</td>
<td>0.57 ppm</td>
</tr>
</tbody>
</table>

Perfect fit !!!
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 in potato chips?
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.)

<table>
<thead>
<tr>
<th>Model</th>
<th>Asparagine (mg/ml)</th>
<th>Glucose (mg/ml)</th>
<th>Fructose (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.3</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>M2</td>
<td>0.3</td>
<td>0.15</td>
<td>-</td>
</tr>
<tr>
<td>M3</td>
<td>0.3</td>
<td>0.30</td>
<td>-</td>
</tr>
<tr>
<td>M4</td>
<td>0.3</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>M5</td>
<td>0.3</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>M6</td>
<td>0.3</td>
<td>-</td>
<td>0.30</td>
</tr>
</tbody>
</table>
MODEL M1 – M3, DART [+] , 250°C

ACRYLAMIDE [M+H]+

ASPARAGINE [M+H]+

QUININE [M+H]+

INCREASING GLUCOSE CONTENT
Intensity of acrylamide was normalized to I.S. intensity (n=6)...

1 min. per sample!
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**

**Diagram:**

1. **Homogenization**
2. **Extraction of potatoes (30g) by shaking (5min) with MeOH:H₂O mixture (1:1, v/v, 200ml)**
3. **Filtration**
4. **Addition of quinine (I.S.) at 10μg/ml**
5. **DART–TOFMS**
POTATO TUBER EXTRACT, DART [+], 250°C

METABOLOME !!!
POTATO EXTRACT, DART [+] , 250°C

ACRYLAMIDE [M+H]^+

ASPARAGINE [M+H]^+

QUININE [M+H]^+

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

\[ R^2 = 0.7981 \]
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

\[ R^2 = 0.9017 \]
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...?

4th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

4–6 November 2009
Prague, Czech Republic

www.rafa2009.eu
- 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
- **Prof. Hans-Gerd Janssen**  Unilever Research and Development, Vlaardingen, NL
- **Prof. Rudolf Krska**  University for Agriculture and Applied Life Sciences, Tulln, A
- **Dr. Steve Lehotay**  United States Department of Agriculture, Wyndmoor, USA
- **Prof. Peter Schieberle**  Technical University of Munich, Garching, D
- **Dr. Richard Stadler**  Nestlé Research Centre, Orbe, CH
- **Prof. Franz Ulberth**  JRC, Institute for Reference Materials and Measurements, Geel, B