Current status and future perspectives in terms of trace elemental speciation analysis in food products

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PART I. BRIEF OVERVIEW REGARDING OFICIAL ANALYSES OF FOOD PRODUCTS

PART II. SPECIATION ANALYSIS APPLIED TO FOODSTUFF
PART I.

BRIEF OVERVIEW REGARDING OFICIAL ANALYSES OF FOOD PRODUCTS
DIFFERENTS TYPES OF CHEMICAL FOOD ANALYSIS

1) Quality control (raw materials and food products)

(2) Nutrition (biomedical...)

(3) Authenticity / fraud assessment

(4) Control / surveillance plans ⇒ official analyses at EU or national level

European reference laboratory (EURL)

Coordination of the National Reference Laboratories (NRL)

Coordination of the local (routine) laboratories

Network of routine French laboratories for trace metals in food of animal origin
A closer look at official analyses of food products

For declarations of conformity, the expanded uncertainty ($k = 2$) is subtracted from the result.

A lot/sublot is refused if the analytical result of the laboratory sample undoubtedly exceeds the applicable maximum level set by the European Regulation, taking into account the wider measurement uncertainty (EC Regulation N°333/2007).

A closer look at official analyses of food products

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For declarations of conformity, the expanded uncertainty ($k = 2$) is subtracted from the result.

Reported result of an official analysis $\Rightarrow x - U$
The Compliance / non-compliance of an “official” result may be biased by a problem related to:

1) Accuracy

2) Uncertainty (especially in the case of results close to the maximum admissible level / regulated value)

- **U is over-estimated** \(\Rightarrow\) true result \(\Rightarrow\) health risk for the population
- **U is under-estimated** \(\Rightarrow\) true result \(\Rightarrow\) economic impact (destruction of lots of food products, etc.)

Proper validation of an official method (including the assessment of uncertainty) is fundamental to obtain reliable measurements of chemical contaminants in food products.
**Method validation** makes use of a set of tests that both test any assumptions on which the analytical method is based and establish and document the performance characteristics of a method, thereby demonstrating whether the method is fit for a particular analytical purpose.

ISO/IEC 17025:2005 cl. 5.4.5.1 (Validation of methods)

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled.

Main parameters to be validated for the assessment of method’s fitness-for-purpose:

1. **Trueness: accuracy + precision**
2. **Uncertainty**
3. **Detection & quantification limits (LOD, LOQ)**
4. **Selectivity**
5. **Ruggedness**

...
EU (“customer”) requirements for methods to be used for official analyses in terms of trace metals in food

(1) LOQ and LOD

<table>
<thead>
<tr>
<th>Parameter (mg/kg)</th>
<th>Pb</th>
<th>As-Cd-Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ML ( \leq 0.01 )</td>
<td>( &gt; 0.01 ) and ( \leq 0.02 )</td>
</tr>
<tr>
<td>LOQ</td>
<td>( \leq ML )</td>
<td>( \leq 2/3 \times ML )</td>
</tr>
<tr>
<td>LOD</td>
<td>( \leq ML )</td>
<td>( \leq 2/10 \times ML )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Matrix</th>
<th>LOQ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>As&lt;sub&gt;j&lt;/sub&gt;</td>
</tr>
<tr>
<td>Fish/ sea fruits</td>
<td>-</td>
</tr>
<tr>
<td>Meat</td>
<td>-</td>
</tr>
<tr>
<td>Milk</td>
<td>-</td>
</tr>
<tr>
<td>Honey</td>
<td>-</td>
</tr>
<tr>
<td>Rice</td>
<td>( \leq 0.02\text{-}0.06 )</td>
</tr>
</tbody>
</table>
(2) Uncertainty

An official method for food control must provide a combined uncertainty $< u_{max}$

$u_{max} = \sqrt{\left(\frac{LOD}{2}\right)^2 + (\alpha c)^2}$

<table>
<thead>
<tr>
<th>Matrix</th>
<th>$u_{max}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish/sea fruits</td>
<td>0.056</td>
</tr>
<tr>
<td>Meats</td>
<td>0.019-0.056</td>
</tr>
<tr>
<td>Milks</td>
<td>0.0037</td>
</tr>
<tr>
<td>Honey</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Maximum amissible level (mg/kg)

<table>
<thead>
<tr>
<th></th>
<th>Fish products</th>
<th>Meat</th>
<th>Milk</th>
<th>Honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>0.3</td>
<td>0.1-0.5</td>
<td>0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Cd</td>
<td>0.05-1.0</td>
<td>0.05-1.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>0.5-1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$u_{max} = 10 - 20\%$ (depending on the target concentration)

$u_{method, maximum} < 20\%$ (depending on the target concentration)

$U_{method, maximum} < 40\%$ (depending on the target concentration / ML)
METHOD VALIDATION BY MEANS OF THE ACCURACY PROFILE:
A REALISTIC APPROACH FOR OFFICIAL CHEMICAL LABORATORIES

Accuracy profile $\Rightarrow$ graphical representation of the accuracy generally expressed in terms of recovery factor over a range of analyte levels comprised between the LOQ and an in-house defined concentration imposing two limits:

- **acceptability interval** ($\lambda$): the maximum accepted deviation of a measurement result compared to a reference value (generally $\lambda \leq 30\%$).
- **tolerance interval** ($\beta$-expectation interval): defines an interval in which a given fraction of the results ($\beta, \%$) will be found.

\[
\beta_{TI} = k_{TI} \times S_{TI}
\]

\[
k_{TI} = \frac{t_{\frac{1+\beta}{2}}}{\sqrt{\frac{1}{IJB^2} + \frac{1}{IJB^2} + \frac{1}{JA + 1}}}
\]

\[
S_{TI} = S_R \sqrt{\left(1 + \frac{1}{IJB^2}\right)}
\]

\[
B = \sqrt{\frac{A + I}{JA + 1}}
\]

\[
A = \frac{S_B^2}{S_r^2}
\]

- $S_r$, standard deviation characterizing the within-laboratory reproducibility;
- $I$, number of series (days);
- $J$, number of measurement replicates per series;
- $B$, parameter depending on intra- and inter-series standard deviations
- $S_r, S_B$, intra- and inter-series standard deviation, respectively
Higher tolerance limit (+\(\beta\), %)  
Lower tolerance limit (-\(\beta\), %)  
Higher acceptance limit (100+\(\lambda\), %)  
Lower acceptance limit (100-\(\lambda\), %)  
LOQ  

Average recovery factor for the analysis of a CRM or a spiked matrix  

Analysis of CRM or spiked samples generally at five concentration levels (at least) is carried out in duplicate in different days during a time span of at least three months.
Part II.

SPECIATION ANALYSIS APPLIED TO FOODSTUFF
the analytical activity of identifying and/or measuring the quantities of **one or more** individual chemical species in a sample.

« Inorganic arsenic » is a fraction of arsenic (AsIII + AsV) and not a species!
A brief history of speciation analysis...

- Mercury poisoning at Minamata, Japan (1950'): the first large scale poisoning with mercury species ($\text{CH}_3\text{Hg}^+$) via fish consumption ($\text{CH}_3\text{Hg}^+$ was generated in the process for producing acetaldehyde using mercury as catalyst in a local factory)
- 2250 people neurologically affected, 1040 died

- Minamata disease » is one of the most significant negative consequences associated with environmental pollution caused by industrial activity in the world
- The fishery products contained 5 to 40 ppm of Hg ⇒ maximum admissible level is nowadays of 1.0 ppm for the predatory species

“Minamata disease”

entered into force on 16 August 2017.

(109 countries so far)

Article 1
Objective
The objective of this Convention is to protect the human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds.

http://www.mercuryconvention.org/
70 years later...

- Despite the advanced analytical methodologies nowadays available, there is not a single EU regulation in terms of speciation analysis in food products ⇒ exception: Sn(IV) in canned foods (since 2006) ⇒ related to contamination via food contact materials.

- No regulation yet for MeHg (at least in fish) despite its toxicity.

- Regulation exists for inorganic arsenic (AsIII + AsV) ⇒ fraction not species!

<table>
<thead>
<tr>
<th>(Inorganic) Arsenic</th>
<th>ML (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milled rice, not parboiled (polished rice or white rice)</td>
<td>0,2</td>
</tr>
<tr>
<td>Parboiled rice and husked rice</td>
<td>0,25</td>
</tr>
<tr>
<td>Aglettes of puffed rice, deriz leaves, rice crackers and</td>
<td>0,30</td>
</tr>
<tr>
<td>rice flour cakes</td>
<td></td>
</tr>
<tr>
<td>Rice for the production of foodstuffs for infants and</td>
<td>0,10</td>
</tr>
<tr>
<td>young children</td>
<td></td>
</tr>
</tbody>
</table>
Why there is a lack in regulation in terms of speciation analysis when reference analytical methodologies are available?

The basis of regulation of chemical contaminants in foodstuffs is a compromise between the risk assessment and the economical impact.

**Daily nutritional intake**

\[ I_i = D_i \times C_i \]

- **I**<sub>i</sub>: daily intake (µg/day)
- **D**<sub>i</sub>: daily consumption of the concerned food (g/day)
- **C**<sub>i</sub>: average concentration of the element (µg g<sup>-1</sup>)

**Daily exposure**

\[ E_i = \frac{I_i}{BW} \]

- **E**<sub>i</sub>: daily exposure (µg/kg bw/day) ; **BW**: body weight (kg)

In brief, risk assessment implies the comparison of the daily (weekly or yearly) exposure with the provisional tolerable daily (weekly or weekly or yearly) intake (µg/kg bw/day) (toxicological reference value).

**Exemples of PTDI (µg/kg bw/ day)**

- **MeHg**: 0.19 (JECFA + EFSA)
- **Hg(II)**: 0.57 (JECFA)

**Total Diet Studies (TDS):** the most comprehensive tool for risk assessment of the general population
Main chemical species of interest in terms of food analysis

- **Stable Isotopes**
  - Lead (Pb)

- **Electronic and Redox States**
  - Se (IV) / Se (VI)
  - As (III) / As (V)
  - Cr (III) / Cr (VI)
  - Fe (II) / Fe (III), ...

- **Inorganic Compounds and Complexes**
  - Ni species: Ni^0, NiO, NiCl₂, NiSO₄, NiCO₃
  - Ti species: Ti, TiCl₄, TiO₂
  - Nanoparticles

- **Organometallic Compounds**
  - ORGANOSBISMUTH: Me₃Bi, Me₃Bi₂, Me₅Bismuth (AsB)
  - ORGANOSILICON: Me₃SiCl, Me₃SiCH₂Cl
  - ORGANOMERCURY: MeHg, Me₂Hg, EtHgCl
  - ORGANOCOPPER: Cu₂(CN)₃, Cu₃(CN)₄
  - ORGANOSTANNANE: R₃SnMe₃, X: butyl, methyl, phenyl

- **Organic Complexes**
  - MLₙ

- **Macromolecular Compounds and Complexes**
  - DNA
  - Proteins and Metalloproteins
  - Metal Complexes
  - Proteins
  - Multifunctional Carbohydrates
  - Natural Organoselenium Argesociogalactelinosides

**To resume:**
- As(III)-As(V) (to define the inorganic fraction...)
- Cr(III)-Cr(VI) (not regulated)
- MeHg (not regulated)
- Organotin (not regulated)
- Organic molecules containing heteroatoms (Cl, Br, S, metals) (partially regulated)

Increasing molecular mass and complexity
I. Speciation defined from the point of view of the oxidation state

I.1. Inorganic arsenic: As(III) and As(V)

- Arsenite: As(III)
- Arsenate: As(V)  
  (cancerigen)

Acide monomethylarsonic (MMA); 
Acide dimethylarsonic (DMA)  
(Cancer promoter?)

Organic As: 
arsenobetaine (AsB) 
arsenocholine (AsC) 
trimethylarsineoxyde (TMAO), 
arsenosuccres, arsenolipides, etc.  
(non-toxic)

Main As contributors via food

(1) Rice and cereals

- main contributor after drinking water
- mainly contaminated with inorganic As
- Despite the relatively large panel of As species present in food, rice accumulates mostly arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA),
- Arsenic levels in rice depend on the geographical location, growing/soil conditions and also on the level of contamination of the irrigation water.
Overview of arsenic levels in rice sampled in France

- 37 composite samples
- 7 groups of rice types mostly consumed in France: Basmati, Thai, White, White for risotto, Organic semi-wholegrain duo, Three-rice mix and Wholegrain rice

$\text{As}_t \Rightarrow 0.041-0.54 \text{ mg/kg}$

$\text{As}_i \Rightarrow 0.025-0.47 \text{ mg/kg (organic rice duo)}$

- The intake varied between $0.18 \mu g \text{ kg}^{-1} \text{ b.w}$ for $\text{As}_t$ and $0.002$ and $0.15 \mu g \text{ kg}^{-1} \text{ b.w}$ for $\text{As}_i \Rightarrow$ well below that providing a minimal risk of chronic toxicity.

- Organic wholegrain rice may entail a significant risk for children in case of sole consumption at the expenses of polished rice.
(2) Fish as As contributor

- primarily organic As species (non-toxic)
- no regulation

Overview of As species levels in fish

- PS / PC fish 2011/2012: 85 fish samples
- 25 different fish species collected from 22 French regions

<table>
<thead>
<tr>
<th>Level (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>As_i</td>
</tr>
<tr>
<td>AsB</td>
</tr>
<tr>
<td>As_total</td>
</tr>
</tbody>
</table>

(3) Bivalve molluscs as As contributor

Overview of As species levels in bivalve molluscs

- PS / PC bivalve molluscs 2017: 54 samples (mussels and oysters)
- 27 samples of oysters and 27 samples of mussels collected from 6 French regions

<table>
<thead>
<tr>
<th>Level (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>As_i</td>
</tr>
<tr>
<td>AsB</td>
</tr>
<tr>
<td>As_total</td>
</tr>
</tbody>
</table>

As_i molluscs > As_i fish (8 fold in average)
MILK, a potential contributor of As$_i$?

Surveillance plan (2016) concerning the total and inorganic As in bovine milk

<table>
<thead>
<tr>
<th>Concentration (mg/kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total As</td>
<td>0.00040 (LOD) – 0.0019</td>
</tr>
<tr>
<td>Inorganic As</td>
<td>0.00010 (LOD) - 0.00030</td>
</tr>
<tr>
<td>As$_i$ / As$_t$</td>
<td>≤ 16%</td>
</tr>
</tbody>
</table>

Speciation analysis of As at ultra-trace levels

1) Microwave assisted extraction
2) Dilution
3) Filtration
4) Ultrafiltration
5) HPLC-ICP-MS analysis

Sample preparation
- 10 mL HNO$_3$, 1% (m/m), H$_2$O$_2$, 3% (m/m)
- 30 min at 90°C

Instrumentation:
- ICS 5000+ (Thermo Scientific)
- iCAP Q (Thermo Scientific)

50 samples distributed across the whole country
I.2. Speciation analysis of chromium (CrIII-CrVI)

- Food is main source of Cr exposure
- There are no maximum levels (MLs) for chromium in food
- Cr(VI) has been classified for a long time carcinogenic to humans by inhalation (IARC, 2012);
- Cr(III) has long been considered an essential nutrient for human health but EFSA Panel in 2014 considered that there is no convincing evidence of beneficial effects associated with chromium intake in healthy subjects

Challenges in terms of chromium speciation in foodstuff

- Both chromium species are very labile ⇒ they may oxidase (CrIII → CrVI) or reduce (CrVI → CrIII) depending on the redox properties of the (foodstuff) matrix
- Each species is stable in different media (pH) hence making their simultaneously determination a truly analytical challenge

Speciation analysis of chromium in food is generally carried out sequentially by focusing on one species at a time.

The main disadvantage of conventional speciation analysis methods is the impossibility to assess the speciation degradation (if the case).
Controversy related to chromium speciation in foodstuffs

EFSA states that in (most) foodstuff Cr is present as Cr(III) (EFSA, 2014).

Some authors reported the presence of Cr(VI) in food (Soares et al., J. Agric. Food Chem., 2010).

33-73% of Cr(VI) (of Crtotal) in toast bread → Cr(III) oxidation during toasting? (Mathebula; Food Chem., 2017).
Sate of the art (simultaneous) speciation analysis of chromium ⇒ species specific isotope dilution

PhD project (2018-2021): Ultra-trace speciation analysis of chromium in foodstuff by HPLC-ICP-MS using species specific isotope dilution (CHROSPID)

Principle of isotope dilution

- IDMS is the only primary method nowadays available that can be applied to trace and ultra-trace analysis.

- The equilibration of the analyte with the spike is primordial for achieving the maximum accuracy ⇒ partial loss of the analyte after equilibration of the spike with the sample will not influence the accuracy of the determination.

*The main disadvantage of conventional speciation analysis methods is the impossibility to assess and correct the speciation degradation (if the case).*
Principle of specis-specific isotope dilution applied to Cr(III) and Cr(VI) determination

\[
R_{\text{III}^{50/52}} = \left( \frac{C_{\text{III}^{50}} A_x W_x + C_{\text{spike}^{50}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{III}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) (1 - \alpha) + \left( \frac{C_{\text{VI}^{50}} A_x W_x + C_{\text{spike}^{50}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{VI}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) \beta
\]

\[
R_{\text{III}^{53/52}} = \left( \frac{C_{\text{III}^{53}} A_x W_x + C_{\text{spike}^{53}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{III}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) (1 - \alpha) + \left( \frac{C_{\text{VI}^{53}} A_x W_x + C_{\text{spike}^{53}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{VI}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) \beta
\]

\[
R_{\text{VI}^{50/52}} = \left( \frac{C_{\text{III}^{50}} A_x W_x + C_{\text{spike}^{50}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{III}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) \alpha + \left( \frac{C_{\text{VI}^{50}} A_x W_x + C_{\text{spike}^{50}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{VI}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) (1 - \beta)
\]

\[
R_{\text{VI}^{53/52}} = \left( \frac{C_{\text{III}^{53}} A_x W_x + C_{\text{spike}^{53}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{III}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) \alpha + \left( \frac{C_{\text{VI}^{53}} A_x W_x + C_{\text{spike}^{53}} A_{\text{spike}} W_{\text{spike}}}{C_{\text{VI}^{52}} A_x W_x + C_{\text{spike}^{52}} A_{\text{spike}} W_{\text{spike}}} \right) (1 - \beta)
\]

- SS-IDMS is the only analytical approach capable to evaluate the species inter-conversion and to correct mathematically for such transformations.
- SS-IDMS permit species quantification and assessment of their interconversion in the same analytical run.
- In addition, partial loss of the analyte after equilibration of the spike and the sample will not influence the accuracy of the determination.
Analytical procedure for simultaneous speciation analysis of Cr(III) and Cr(VI)

**Simultaneous complexation of Cr (III) and Cr (VI)**

⇒ Cr(III) complexation with EDTA (0.60 mM)
⇒ Cr(VI) complexation with dyphenylcarbazide (0.02 mM)
⇒ pH = 4
⇒ Heating at 70 °C (25 min + 25 min)

**HPLC separation (anion exchange)**

⇒ Column Dionex IonPac™ AG7 : 2 mm x 50 mm (10 μm)
⇒ Mobile phase : 10 mM HNO₃ + 2.5% Methanol + 0.32 M EDTA (isocratic) (pH=2)
⇒ Column Temperature : 30°C
⇒ Flow : 0.20 mL/min
1. Complexation Cr(III)-EDTA

2. Complexation Cr(VI)-DPC

3. Separation

4. ICP-MS detection

5. Chromatogram

$^{53}$Cr(VI)-DPC (5 ppb)

$^{50}$Cr(III)-EDTA (5 ppb)
Method validation ⇒ spike recovery experiments

→ Infant formula milk : Cr(III)$_{\text{natural}}$ = 3 ppb ; Cr(VI)$_{\text{spiked}}$ : 0.25 ppb
→ Milk half-fat : Cr(III)$_{\text{natural}}$ = 5 ppb ; Cr(VI)$_{\text{spiked}}$ : 1 ppb
→ Steak beef : Cr(III)$_{\text{natural}}$ = 5 ppb ; Cr(VI)$_{\text{spiked}}$ : 0.5 ppb
→ Bread : Cr(III)$_{\text{natural}}$ = 27 ppb ; Cr(VI)$_{\text{spiked}}$ : 5.0 ppb

Problem with bread spiked with Cr(VI): complete conversion of Cr(III) to Cr(VI) ?

\[
\text{Bread : Cr(III)$_{\text{natural}}$ = 27 ppb ; Cr(VI)$_{\text{spiked}}$ : 5 ppb}
\]

\[
\text{analysis}
\]

\[
\text{Cr(III)$_{\text{natural}}$ = 0 ppb ; Cr(VI) = 32 ppb (27 + 5)}
\]

In water with the addition of Cr species, interconversion occurs in both directions, while in food matrices there is the conversion of Cr(III) to Cr(VI).

<table>
<thead>
<tr>
<th>Cr(III) and Cr(VI) in a water standard solution</th>
<th>Cr(III) and Cr(VI)$_{\text{added}}$ in foodstuff</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha = 31%$</td>
<td>$\alpha = 0%$</td>
</tr>
<tr>
<td>$\beta = 16%$</td>
<td>$\beta = 100-150%$</td>
</tr>
</tbody>
</table>
Analytical figures of merit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cr(III)</th>
<th>Cr(VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Spiking level (µg/kg)</td>
<td>0.013</td>
<td>0.024</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>108%</td>
<td>98%</td>
</tr>
<tr>
<td>RSD(%)*</td>
<td>12%</td>
<td>8%</td>
</tr>
<tr>
<td>LOQ (ppb)</td>
<td>0.035</td>
<td></td>
</tr>
</tbody>
</table>

* 5 non-consecutivedays (1 month)
Increasing molecular mass and complexity

III.3. Speciation analysis of organic complexes: mission (im)possible?

- **STABLE ISOTOPES**
- **ELECTRONIC AND REDOX STATES**
  - As(III) / As(V)
  - Cr(III) / Cr(VI)
  - Sn(IV)
- **INORGANIC COMPOUNDS AND COMPLEXES**
  - Ni species: Ni⁰, NiO, NiCl₂, NiSO₄, NiCO₃
  - Ti species: Ti, TiCl₄, TiO₂
  - Nanoparticles
- **ORGANOMETALLIC COMPOUNDS**
  - Organomercury (MeHg)
  - Organotin (TBT-DBT-TPhT-DOT)
  - Organoaarsenic (MMA, DMA)
  - Organolead
  - Organoselenium
- **MACROMOLECULAR COMPOUNDS AND COMPLEXES**

*MLₙ*
Multi-approach determination of dithiocarbamate fungicides and their degradation products in food

(H2020 – MSCA-IF MET-PEST)

- **Dithiocarbamates (DTCs)** - relatively intense used as fungicides being effective against a broad spectrum of plant diseases.

- Despite the significant environmental and food chain impact of DTCs, the current analytical approaches for their determination suffer from serious drawbacks.

- The European reference method for this purpose relies on non-selective quantification by indirect determination of the sum of DTC species (single residue method).

![Diagram](image-url)

- The development of a method with increased selectivity for the determination of DTCs and of their degradation products in food (by a multi-approach strategy) is highly needed.
Dithiocarbamate Fungicides

- Thiram
- Ziram
- Ferbam
- Metiram
- Zineb
- Maneb
- Propineb
Multi-approach analytical strategies for DTCs determination

HPLC-MS/MS ⇒ targeting DTCs

- Presence of a **metal** moiety (Fe, Zn and/or Mn) in the DTCs structure ⇒ HPLC ICP-MS
- The presence of **sulphur** in the DTCs structure ⇒ HPLC-ICP-QQQMS
Main analytical challenge of DTCs determination: low stability and solubility

Determination of their degradation products ⇒ very stable

Main advantage:

- use of RP-HPLC-ICP-QQQMS instead of HILIC
  (as for the DTCs)
Main analytical parameters for the determination of DTCs degradation products (ETU, PTU) and inorganic sulfur by RP-HPLC coupled to ICP-QQQMS

(first ICP-MS based method...)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC column</td>
<td>Aqua® 3µm C18, 125 Å; 150 × 2 mm</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>5% MeOH, isocratic elution</td>
</tr>
<tr>
<td>Flow (mL/min)</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Method Validation ⇒ accuracy profile approach

<table>
<thead>
<tr>
<th>Matrices tested (spiking)</th>
<th>Tomatoes, Grapes, Strawberries, Cherries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiking levels (ppb)</td>
<td>10 – 400 (6 different levels)</td>
</tr>
<tr>
<td>Extraction solvent</td>
<td>Aqueous 5% MeOH</td>
</tr>
<tr>
<td>Extraction procedure</td>
<td>Surface extraction (whole fruit) (30 min)</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>71 - 110</td>
</tr>
</tbody>
</table>

**Analytical figures of merit**

<table>
<thead>
<tr>
<th>Linear range tested (ng/mL)</th>
<th>10 – 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity ($r^2$)</td>
<td>0.9999 – 1.000</td>
</tr>
<tr>
<td>Back calculated concentrations</td>
<td>95 – 105 %</td>
</tr>
<tr>
<td>Instrumental precision (% RSD)</td>
<td>&lt; 2%</td>
</tr>
</tbody>
</table>

**LOQ = 15 ppb**
Intermediate precision: 2-10% (depending on the spiking level)

**LOQ = 10 ppb**
Intermediate precision: 1-8% (depending on the spiking level)
Chromatograms obtained for the analysis of real-life spiked samples (whole fruits)

**Strawberries**

Unidentified S-containing compound(s)

ETU ➔
PTU ←

**Grapes**

Unidentified S-containing compound(s)

ETU ➔
PTU ←
The species which (still) pose an “official” interest in terms of food are: MeHg, As(III)-As(V) and Cr(III)-Cr(VI)

The accurate determination of inorganic As is still challenging in fishery products (because of the low levels of As and the matrix complexity)

More analytical developments must be carried for the simultaneous speciation analysis of Cr(III) and Cr(VI) by species specific isotope dilution in foodstuffs

An interesting perspective is the speciation analysis of organic contaminants containing heteroatoms (S, Cl, Br, ...) by exploiting the new analytical features provided by ICP-QQQMS.
Grazie per la vostra attenzione!

"Chemists love it. It goes very well with most food additives we use."