Curent 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 assessement

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

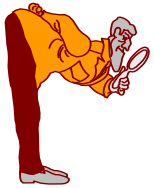
…

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

Official analysis of a food products ⇒ declaration of conformity is issued.

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).

Nature de l'échantillon :
Analyses demandées : Mercure

Informations complémentaires :

RESULTATS

	Analyses effectuées (Méthodes)	Date de début d'analyse	Résultat (et unité)
*	Mercure - ANSES LSAliments LSA-INS-0084 (Technique d'analyse par spectrométrie de masse couplée à un plasma induit - ICP-MS)		

Observation(s) : Le résultat d'analyse après soustraction de l'incertitude de mesure est conforme au seuil défini dans le règlement (CE) 1881/2006 du 19 décembre 2006, modifié par le règlement (CE) 629/2008 de la commission du 2 juillet 2008, modifié par le règlement (UE) 420/2011 de la commission du 29 avril 2011 pour le mercure.

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

Reported result of an official analysis ⇒ $x - U$

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 ⇒ true result ⇒ health risk for the population

U is under-estimated ⇒ true result ⇒ 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…

IUPAC Technical Report: « Harmonized guidelines for single laboratory validation of methods of analysis» , Pure Appl. Chem., 2002, 74(5), 835-855.

***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.*

METHOD VALIDATION IMPLIES A COMPARISON OF THE ANALYTICAL PERFORMANCES WITH THE CUSTOMER'S REQUIREMENTS !

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 oficial analyses in terms of trace metals in food

(1) LOQ and LOD

16.4.2016 EN Official Journal of the European Union L 101/1

REGULATIONS

COMMISSION REGULATION (EU) 2016/582

of 15 April 2016

amending Regulation (EC) No 333/2007 as regards the analysis of inorganic arsenic, lead and polycyclic aromatic hydrocarbons and certain performance criteria for analysis

(Text with EEA relevance)

Parameter (mg/kg)	Pb				As-Cd-Hg	
ML	≤ 0,01	> 0.01 and ≤ 0.02	> 0.02 and < 0.1	≥ 0.1	< 0.10	≥ 0.10
LOQ	≤ ML	≤ 2/3 × ML	≤ 2/5 × ML	≤ 1/5 × ML	≤ 2/5 × ML	≤ 1/5 × ML
LOD	≤ ML	≤ 2/10 × ML	≤ 3/25 × ML	≤ 3/50 × ML	≤ 3/25 × ML	≤ 3/50 × ML

Matrix	LOQ (mg/kg)			
	As_i	Pb	Cd	Hg
Fish/ sea fruits	-	≤ 0.06	≤ 0.02	≤ 0.1-0.2
Meat	-	≤ 0.02-0.1	≤ 0.02-0.2	-
Milk	-	≤ 0.013	-	-
Honey	-	≤ 0.02	-	-
Rice	≤ 0.02-0.06	-	-	-

(2) Uncertainty

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

COMMISSION REGULATION (EC) No 333/2007

of 28 March 2007

laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

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

c (mg·kg^{-1})	α
≤ 0.05	0.20
0.051-0.5	0.18
0.501-1.0	0.15
1.001-10	0.12
> 10	0.10

	Maximum amissible level (mg/kg)			
	Fishery products	Meat	Milk	Honey
Pb	0.3	0.1-0.5	0.02	0.1
Cd	0.05-1.0	0.05-1.0	-	
Hg	0.5-1.0			

Matrix	u_{max} (mg/kg)		
	Pb	Cd	Hg
Fish/ sea fruits	0.056	0.011-0.16	0.093-0.16
Meat	0.019-0.056	0.011-0.16	-
Milk	0.0037	-	-
Honey	0.019		

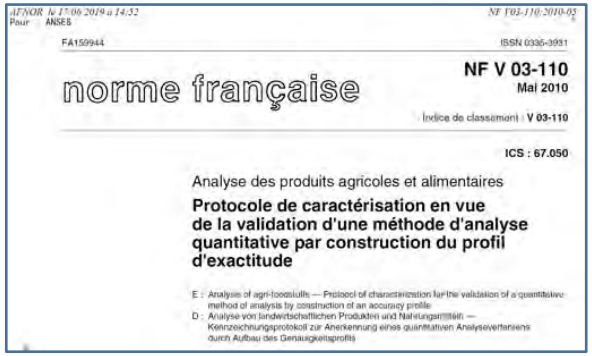
u_{max} = 10 - 20 % (depending on the target concentration)

$u_{method,\ maximum}$ < 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

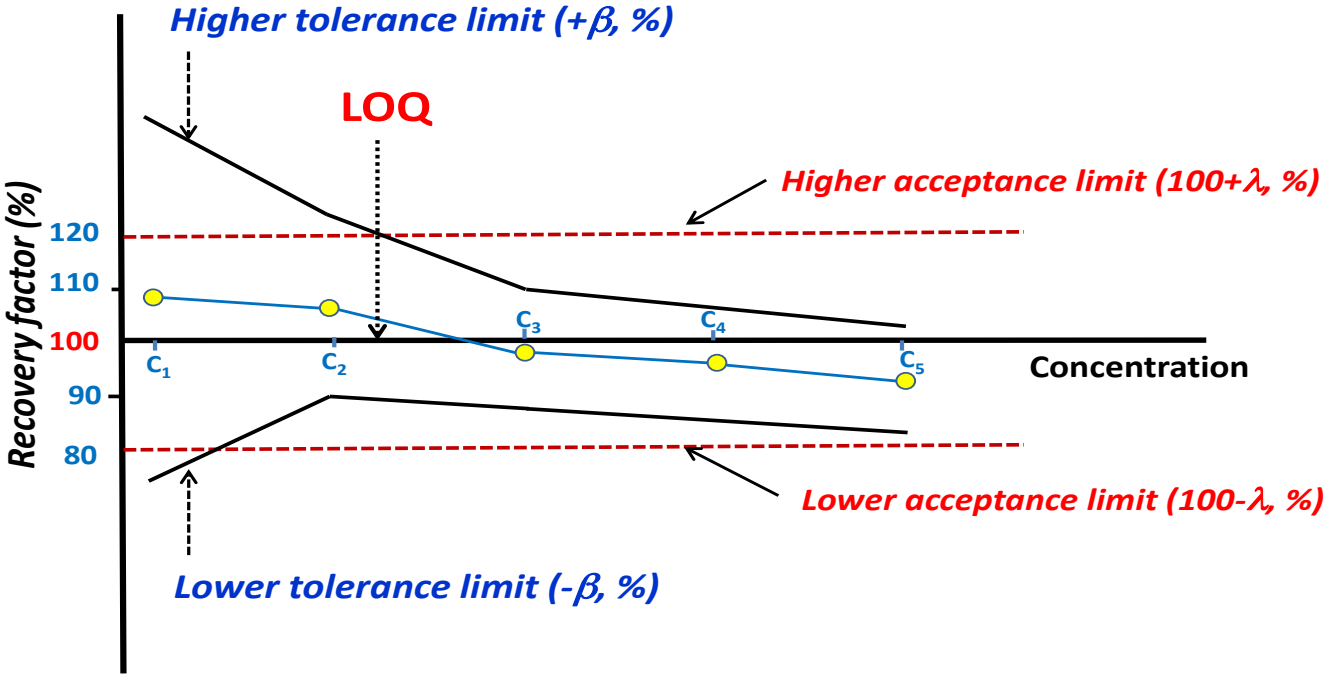


Accuracy profile ⇒ graphical representation of the accuracy generally expressed in terms of recovery factor over a range of analyte levels comprised between the LOQ and an in-house defined concentration imposing two limits:

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

$$\beta_{TI} = k_{TI} \times S_{TI} \qquad k_{TI} = t_{v,\frac{1+\beta}{2}} \qquad S_{TI} = S_R\sqrt{\left(1+\frac{1}{IJB^2}\right)} \qquad B = \sqrt{\frac{A+1}{JA+1}} \qquad A = \frac{S_B^2}{S_r^2}$$

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



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

IUPAC (D. M. Templeton et. al., *Pure Appl. Chem.*, 2000): speciation analysis ⇒

the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample.

Pure Appl. Chem., Vol. 72, No. 8, pp. 1453–1470, 2000.
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GUIDELINES FOR TERMS RELATED TO CHEMICAL SPECIATION AND FRACTIONATION OF ELEMENTS. DEFINITIONS, STRUCTURAL ASPECTS, AND METHODOLOGICAL APPROACHES

(IUPAC Recommendations 2000)

Prepared for publication by
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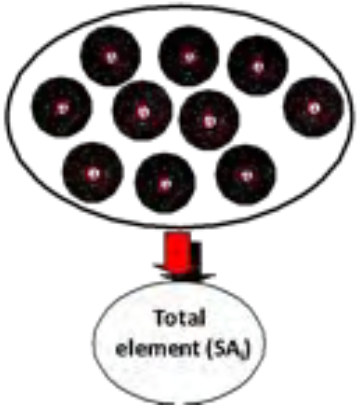
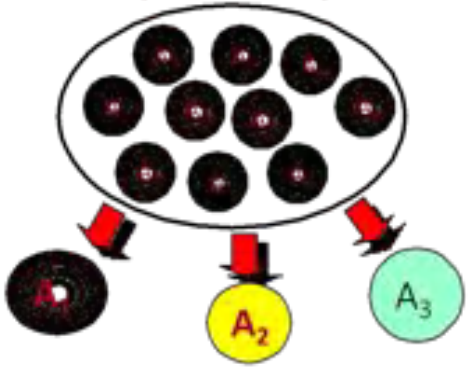
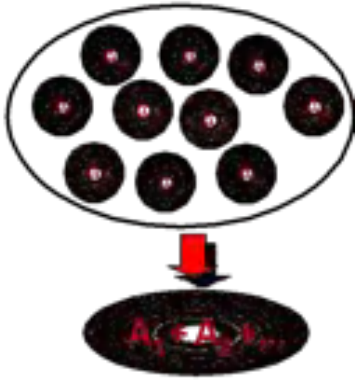
« Conventional » trace analysis



Speciation analysis



Fractionation



« Inorganic arsenic » is a fraction of arsenic (AsIII +AsV) and not a species !

A brief hystory of speciation analysis…

- *Mercury poisoning at Minamata, Japan (1950'): the first large scale poisoning with mercury species (CH_3Hg^+) via fish consumption (CH_3Hg^+ was generated in the process for producing acetaldehyde using mercury as catalyst in a local factory)*
- **2250 people neurologically affected, 1040 died**

"Minamata disease"

- **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**

UNEP
MINAMATA CONVENTION ON MERCURY

(109 countries so far)

entered into force on 16 August 2017.

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.

20.12.2006 EN Official Journal of the European Union L 364/5

COMMISSION REGULATION (EC) No 1881/2006

of 19 December 2006

setting maximum levels for certain contaminants in foodstuffs

(Text with EEA relevance)

Sn(IV)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

food. In the case of contaminants which are considered to be genotoxic carcinogens or in cases where current

- No regulation yet for MeHg (at least in fish) despite its toxicity.
- Regulation exists for inorganic arsenic (AsIII + AsV) ⇒ fraction not species !

12.8.2015 EN Official Journal of the European Union L 213/9

RECOMMENDATIONS

COMMISSION RECOMMENDATION (EU) 2015/1381

of 10 August 2015

on the monitoring of arsenic in food

THE EUROPEAN COMMISSION,

(Inorganic) Arsenic	ML (mg/kg)
Milled rice, not parboiled (polished rice or white rice)	0,2
Parboiled rice and husked rice	0,25
Aglettes of puffed rice, deriz leaves, rice crackers and rice flour cakes	0,30
Rice for the production of foodstuffs for infants and young children	0,10

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 ⇒ $I_i = D_i \times C_i$

Daily exposure: ⇒ $Ei = I\downarrow i / BW$

I_i: daily intake (µg/day)

D_i: daily consumption of the concerned food (g/day)

C_i: average concentration of the element (µg g^{-1})

E_i : 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)

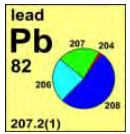
MeHg: 0.19 (JECFA + EFSA)

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



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, $NiCl_2$, $NiSO_4$, $NiCO_3$
Ti species: Ti, $TiCl_4$, TiO_2
Nanoparticles


Pure Appl. Chem., Vol. 72, No. 8, pp. 1453–1470, 2000.
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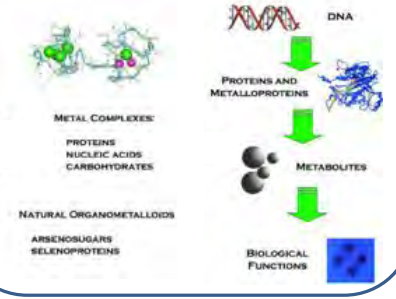
ORGANIC COMPLEXES

ML_n

ORGANOMETALLIC COMPOUNDS

ORGANOARSENIC:
MeAs, Me_2As, Arsenobetain (AsB), Arsenocholin (AsCh)
ORGANOLEAD:
Me, Et, Ph[illegible]
ORGANOMERCURY:
MeHg, Me_2Hg, EtHg, PhHg
ORGANOSELENIUM:
SeMet, SeEt
ORGANOTIN:
$R_xSnX_{(4-x)}$ (X: butyl-, methyl-, phenyl-)

MACROMOLECULAR COMPOUNDS AND COMPLEXES



Increasing molecular mass and complexity

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)

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),
arsenosucres, 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

As_t ⇒ 0.041-0.54 mg/kg

Asi ⇒ 0.025-0.47 mg/kg (organic rice duo)

- The intake varied between 0.18 µg kg^{-1} b.w for As_t and 0.002 and 0.15 µg kg^{-1} b.w for As_i ⇒ 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.

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/jfca

Original research article

Exposure assessment of arsenic speciation in different rice types depending on the cooking mode

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Table 1
Instrumental ICP-MS operating parameters.

Total arsenic determination by Agilent 770 ICP-MS	
Power	1400 W
Nebulizer type	MicroMist
Plasma gas flow rate (Ar)	15 L min^{-1}
Auxiliary gas flow rate (Ar)	1 ± 0.1 L min^{-1} (depending on daily optimization)
Nebulizer argon flow	1 ± 0.1 L min^{-1} (depending on daily optimization)
He gas flow rate (CRC)	4.3 mL min^{-1}
Integration time	3 s
Sampling/skimmer cones	Nickel
Arsenic speciation by AE-HPLC coupled to X-Series® ICP-MS (Thermo Fisher)	
ICP-MS parameters	
Plasma power	1450 W
Plasma gas flow	15 L min^{-1}
Auxiliary gas flow	0.9 ± 0.1 L min^{-1} (depending on daily optimization)
Nebulizer gas flow	0.9 ± 0.1 mL min^{-1} (depending on daily optimization)
Isotopes/masses monitored (m/z)	75 (^{75}As); 77 ($^{40}Ar^{37}Cl$)
Dwell time	500 ms
HPLC parameters	
Analytical column	IonPac AS7 (250 × 4 mm 10 µm particles, Dionex)
Guard column	IonPac AG7 (50 × 4 mm 10 µm particles, Dionex)
Flow rate	1.35 mL min^{-1}
Mobile phase A	0.8 × 10^{-3} mol L^{-1} HNO_3 (0.8 mM) in 1% MeOH (pH = 3.8)
Mobile phase B	500 × 10^{-3} mol L^{-1} HNO_3 (500 mM) in 1% MeOH (pH = 1.4)
Gradient	0–3 min: 99% A 3–5 min: 10% A 5–12 min: 80% A 12–12.5 min 99% A

2.4.4. Arsenic speciation

As speciation analysis was carried out using a method previously developed in our laboratory with slight modifications (Leufroy et al., 2011). Briefly, 0.15 g of freeze-dried sample was mixed with 10 mL of a H_2O_2:H_2O mixture (1:9 ratio, v/v) in the microwave digestion vessels [H_2O_2 was used here to oxidize As(III) to As(V)]. The mixtures were then heated at 80 °C for 6 min. After

(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

	Level (mg/kg)		%Asi (min-max)	%AsB (min-max)
	Mean	min-max		
As_i	0.022	0.004-0.096	0.72 %	81 %
AsB	2.5	0.006-15.1	(0.13-2.7)	(58-96)
As_{total}	3.07	0.013-12.9		

(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

	Level (mg/kg)		%Asi (min-max)	%AsB (min-max)
	Mean	min-max		
As_i	0.16	0.006-1.30	6 %	51 %
AsB	1.34	0.12-3.94	(0.33-10.0)	(41-84)
As_{tota}	2.64	1.30-6.54		

$As_{i\ molluscs} > As_{i\ fish}$ (8 fold in average)

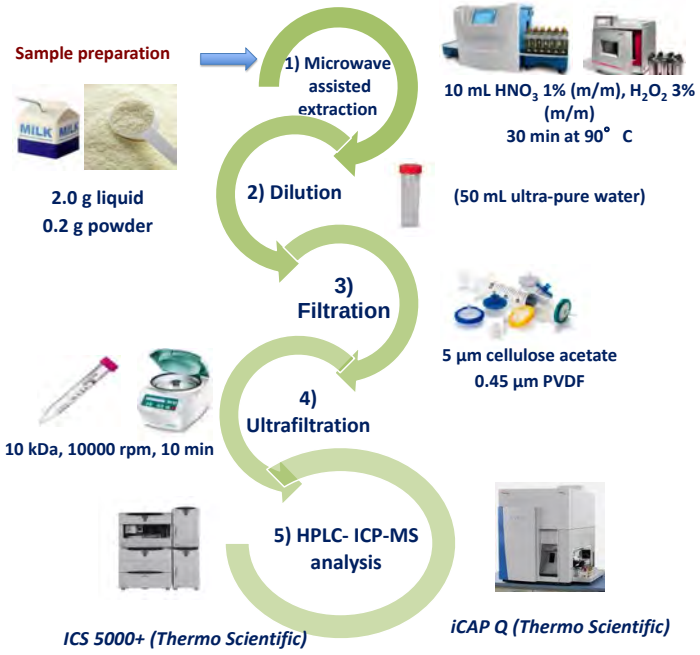
MILK, a potential contributor of As_i?

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

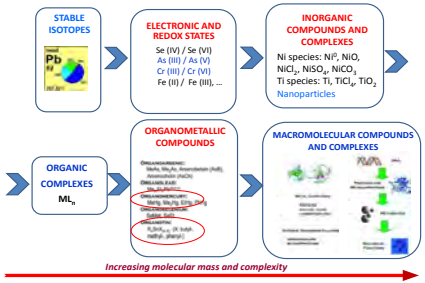
50 samples distributed across the whole country

Concentration (mg/kg)	
Total As	0.00040 (LOD) – 0.0019
Inorganic As	0.00010 (LOD) - 0.00030
As_i /As_t	≤ 16%

Speciation analysis of As at ultra-trace levels



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).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Chromate in food samples: an artefact of wrongly applied analytical methodology?†

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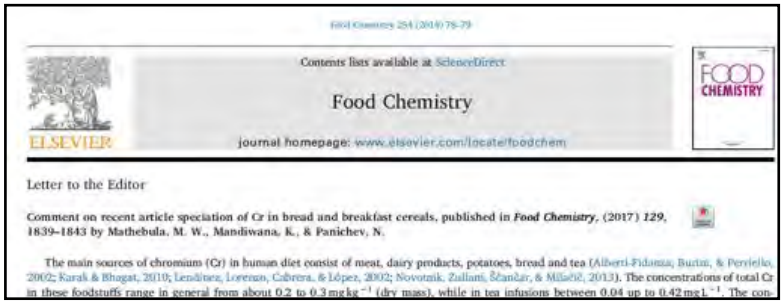
Recently several papers were published in highly ranked journals on the presence of Cr(vi) in tea infusions, bread samples and plants. These statements were made on the basis of determination of total Cr concentrations in alkaline and aqueous sample extracts by ETAAS, without applying any speciation analysis. If Cr(vi) really exists in bread samples and tea infusions, consumption of bread and tea would


Food Chemistry

Analytical Methods

Speciation of chromium in bread and breakfast cereals

Mpho Wendy Mathebula, Khakhathi Mandiwana *, Nikolai Panichev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Letter to the Editor

Comment on recent article speciation of Cr in bread and breakfast cereals, published in *Food Chemistry*, (2017) *129*, 1839–1843 by Mathebula, M. W., Mandiwana, K., & Panichev, N.

The main sources of chromium (Cr) in human diet consist of meat, dairy products, potatoes, bread and tea (Alberti-Fidanza, Burini, & Perriello, 2002; Kurak & Bhagat, 2010; Lenäinez, Lorenzo, Cabrera, & López, 2002; Novotnik, Zuliani, Ščančar, & Milačič, 2013). The concentrations of total Cr in these foodstuffs range in general from about 0.2 to 0.3 mg kg^{-1} (dry mass), while in tea infusions between 0.04 up to 0.42 mg L^{-1}. The con-

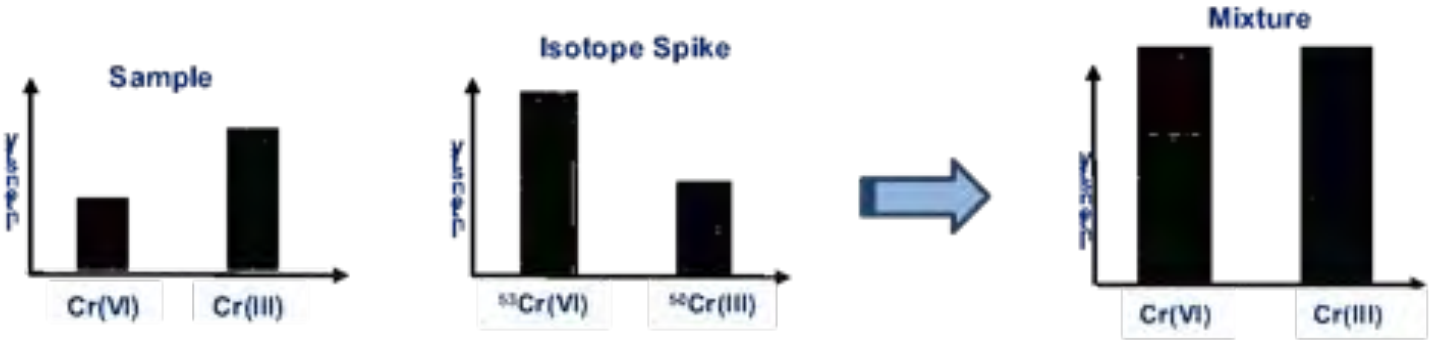
isotopically enriched Cr(VI) and Cr(III) spike solutions, which are used as tracers, importantly contribute to the trueness of the results obtained. In this critical review, the significance of the use of adequate analytical methodologies and speciation analysis in the determination of Cr(VI) was emphasized, in order to prevent erroneous conclusions made on the basis of artefacts of the wrongly applied analytical methodologies.

With this letter to the Editor, we want to warn Food Chemistry readers against wrongly interpreted data on Cr speciation in foodstuffs, which were based on total Cr determination without performing adequate speciation analysis.

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

$$R^{III}_{50/52} = \frac{\left(C_x^{III}\cdot^{50}A_x W_x + C_{spike}^{III}\cdot^{50}A_{spike}^{III}\cdot W_{spike}^{III}\right)(1-\alpha) + \left(C_x^{VI}\cdot^{50}A_x\cdot W_x + C_{spike}^{VI}\cdot^{50}A_{spike}^{VI}\cdot W_{spike}^{VI}\right)\beta}{\left(C_x^{III}\cdot^{52}A_x\cdot W_x + C_{spike}^{III}\cdot^{52}A_{spike}^{III}\cdot W_{spike}^{III}\right)(1-\alpha) + \left(C_x^{VI}\cdot^{52}A_x\cdot W_x + C_{spike}^{VI}\cdot^{52}A_{spike}^{VI}\cdot W_{spike}^{VI}\right)\beta} \qquad \frac{m}{M} = W$$

$$R^{III}_{53/52} = \frac{\left(C_x^{III}\cdot^{53}A_x W_x + C_{spike}^{III}\cdot^{53}A_{spike}^{III}\cdot W_{spike}^{III}\right)(1-\alpha) + \left(C_x^{VI}\cdot^{53}A_x\cdot W_x + C_{spike}^{VI}\cdot^{53}A_{spike}^{VI}\cdot W_{spike}^{VI}\right)\beta}{\left(C_x^{III}\cdot^{52}A_x\cdot W_x + C_{spike}^{III}\cdot^{52}A_{spike}^{III}\cdot W_{spike}^{III}\right)(1-\alpha) + \left(C_x^{VI}\cdot^{52}A_x\cdot W_x + C_{spike}^{VI}\cdot^{52}A_{spike}^{VI}\cdot W_{spike}^{VI}\right)\beta}$$

$$R^{VI}_{50/52} = \frac{\left(C_x^{III}\cdot^{50}A_x\cdot W_x + C_s^{III}\cdot^{50}A_s^{III}\cdot W_s^{III}\right)\alpha + \left(C_x^{VI}\cdot^{50}A_x\cdot W_x + C_{spike}^{VI}\cdot^{50}A_{spike}^{VI}\cdot W_{spike}^{VI}\right)(1-\beta)}{\left(C_x^{III}\cdot^{52}A_x\cdot W_x + C_{spike}^{III}\cdot^{52}A_{spike}^{III}\cdot W_{spike}^{III}\right)\alpha + \left(C_x^{VI}\cdot^{52}A_x\cdot W_x + C_{spike}^{VI}\cdot^{52}A_{spike}^{VI}\cdot W_{spike}^{VI}\right)(1-\beta)}$$

$$R^{VI}_{53/52} = \frac{\left(C_x^{III}\cdot^{53}A_x\cdot W_x + C_{spike}^{III}\cdot^{53}A_{spike}^{III}\cdot W_s^{III}\right)\alpha + \left(C_x^{VI}\cdot^{53}A_x\cdot W_x + C_{spike}^{VI}\cdot^{53}A_{spike}^{VI}\cdot W_{spike}^{VI}\right)(1-\beta)}{\left(C_x^{III}\cdot^{52}A_x\cdot W_x + C_{spike}^{III}\cdot^{52}A_{spike}^{III}\cdot W_{spike}^{III}\right)\alpha + \left(C_x^{VI}\cdot^{52}A_x\cdot W_x + C_{spike}^{VI}\cdot^{52}A_{spike}^{VI}\cdot W_s^{VI}\right)(1-\beta)}$$

- **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)

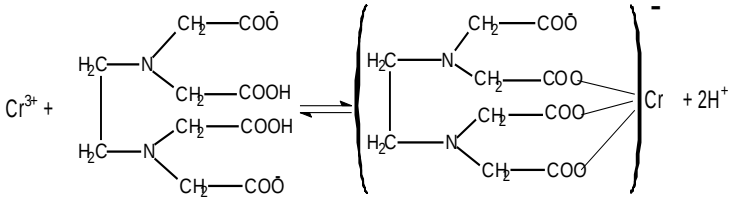
Simultaneous complexation of Cr (III) and Cr (VI)

⇒ **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)**



CrO_4^{2-} + Cr^{3+} +

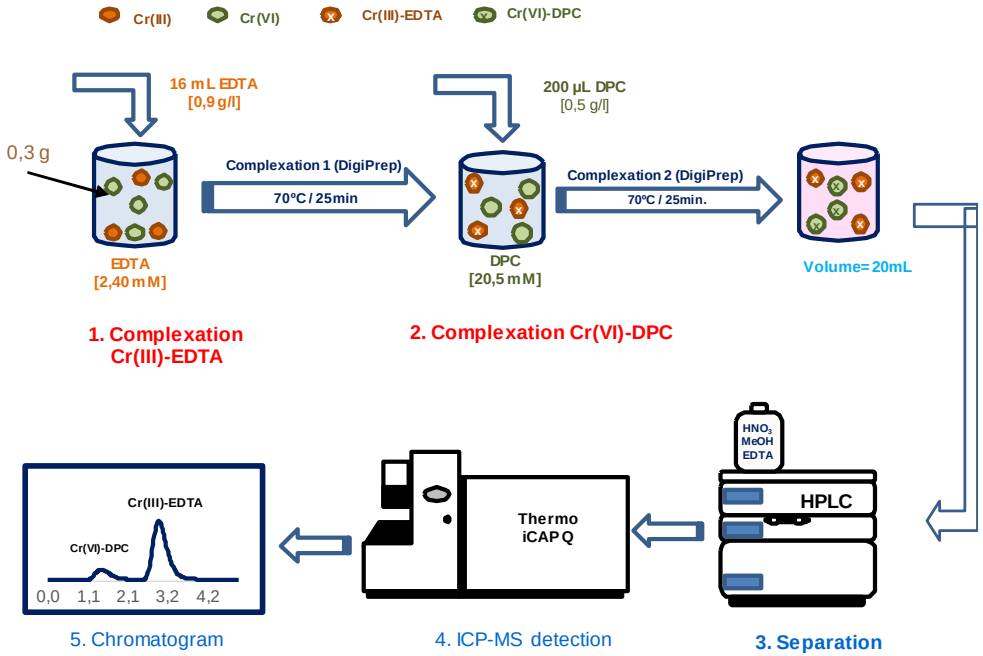
HPLC separation (anion exchange)

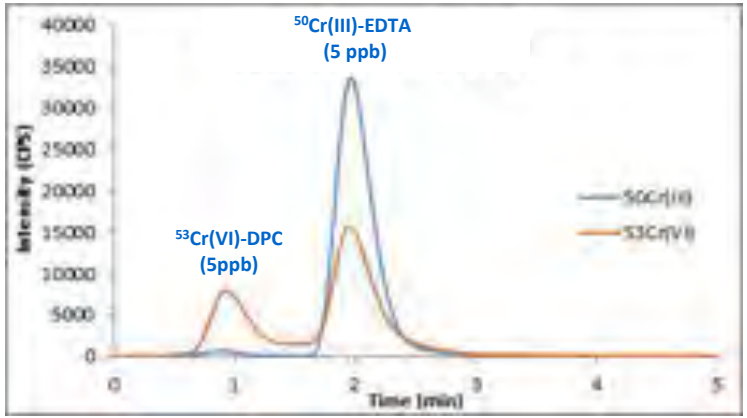
⇒ **Column Dionex IonPac™ AG7 : 2 mm x 50 mm (10 µm)**

⇒ **Mobile phase : 10 mM HNO_3 + 2.5% Methanol + 0.32 M EDTA (isocratic) (pH=2)**

⇒ **Column Temperature : 30°C**

⇒ **Flow : 0.20 mL/min**


Cr(II)
Cr(VI)
Cr(III)-EDTA
Cr(VI)-DPC
16 mL EDTA
[0,9 g/l]
0,3 g
EDTA
[2,40 mM]
Complexation 1 (DigiPrep)
70°C / 25min
200 µL DPC
[0,5 g/l]
DPC
[20,5 mM]
Complexation 2 (DigiPrep)
70°C / 25min.
Volume= 20mL
1. Complexation Cr(III)-EDTA
2. Complexation Cr(VI)-DPC
HNO3
MeOH
EDTA
HPLC
Thermo
iCAP Q
Cr(III)-EDTA
Cr(VI)-DPC
0,0 1,1 2,1 3,2 4,2
3. Separation
4. ICP-MS detection
5. Chromatogram


50Cr(III)-EDTA
(5 ppb)
53Cr(VI)-DPC
(5ppb)
Intensity (CPS)
Time [min]
50Cr(III)
53Cr(VI)

Method validation ⇒ spike recovery experiments

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

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

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

analysis

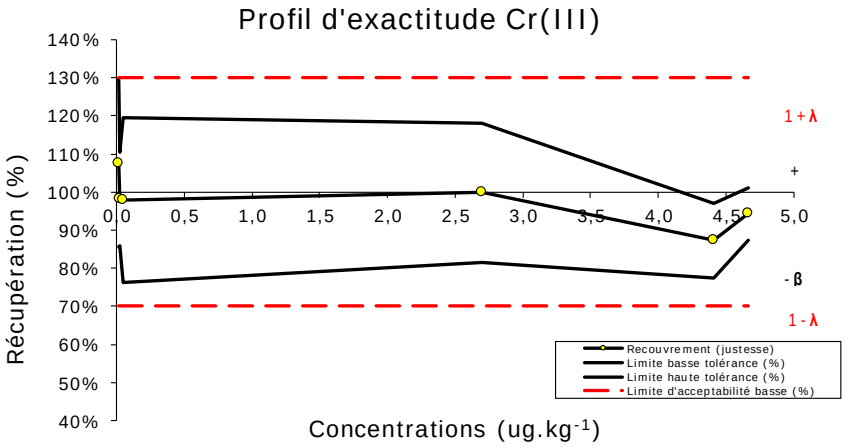
$Cr(III)_{natural}$ = 0 ppb ; Cr(VI) = 32 ppb (27 + 5)

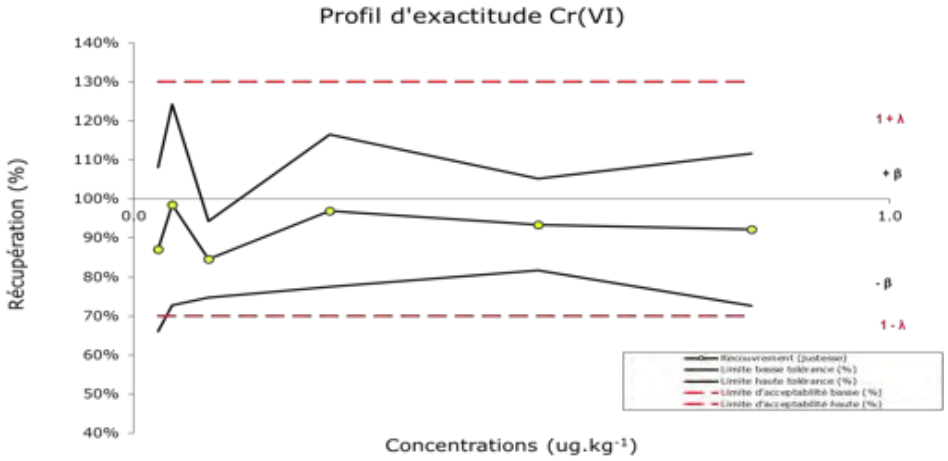
In water with the addition of Cr species, interconversion occurs in both directions, while in food matrices there is the conversion of Cr(III) to Cr(VI).

Cr(III) and Cr(VI) in a water standard solution
α = 31%
β = 16%

Cr(III) and $Cr(VI)_{added}$ in foodstuff
α = 0%
β = 100-150%

Accuracy profiles





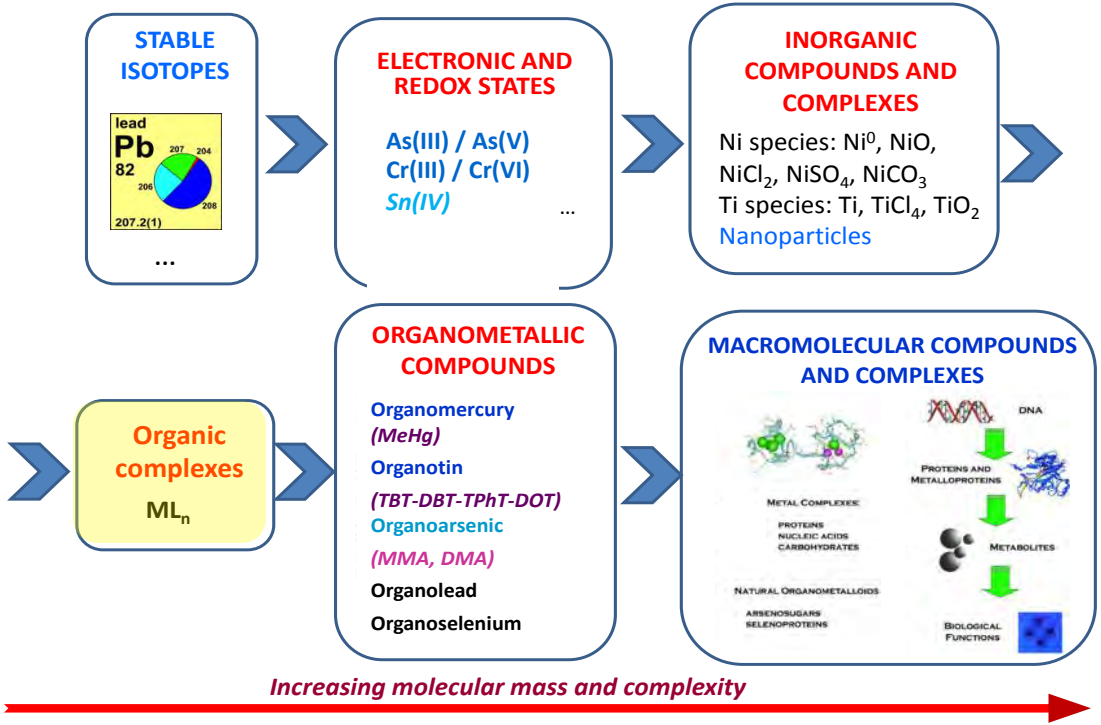
Analytical figures of merit

Cr(III)						
Parameter	1	2	3	4	5	6
Spiking level (µg/kg)	0,013	0,024	0,048	2,70	4,41	4,67
Recovery (%)	108%	98%	98%	100%	87%	94%
RSD(%)*	12%	8%	10%	10%	7%	3%
LOQ (ppb)	0.035					

Cr(VI)						
Parameter	1	2	3	4	5	6
Spiking level (µg/kg)	0,032	0,051	0,10	0,26	0,54	0,82
Recovery (%)	87%	93%	85%	95%	93%	92%
RSD(%)*	9%	8%	7%	16%	8%	13%
LOQ (ppb)	0.049					

*** 5 non-consecutivedays (1 month)**

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



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

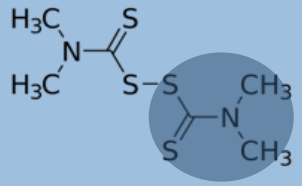
(H2020 – MSCA-IF MET-PEST)

- **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**).

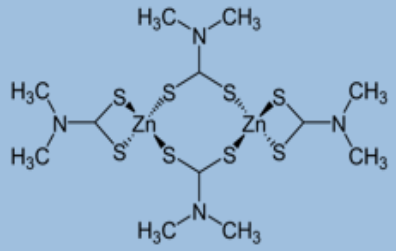
- The development of a method with increased selectivity for the determination of DTCs and of their degradation products in food (by a multi-approach strategy) is highly needed.

Dithiocarbamate Fungicides

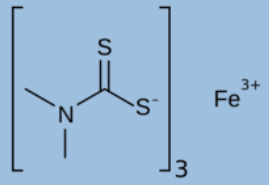
Thiram



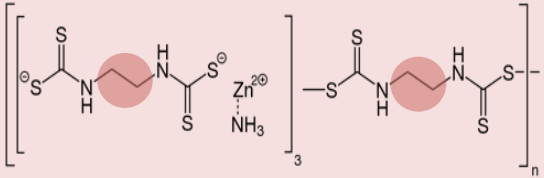
Ziram



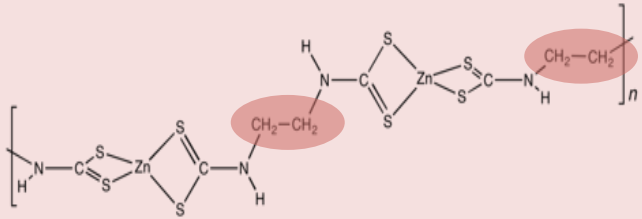
Ferbam



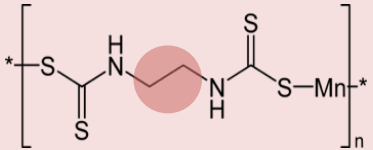
Metiram



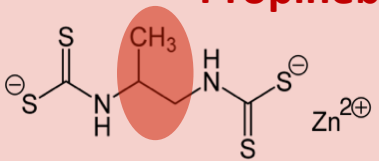
Zineb



Maneb

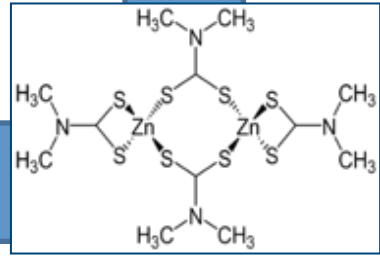


Propineb



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

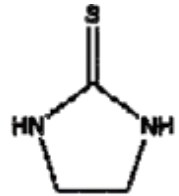
The presence of sulphur in the DTCs structure ⇒ HPLC-ICP-QQQMS

Main analytical challenge of DTCs determination: low stability and solubility

Determination of their degradation products ⇒ very stable

Metiram
Zineb
Maneb
Nabam
Mancozeb

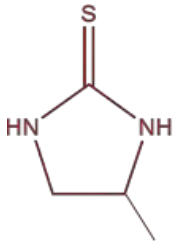
degradation



Ethylene thiourea (ETU)

Propineb

degradation



Propylene thiourea (PTU)

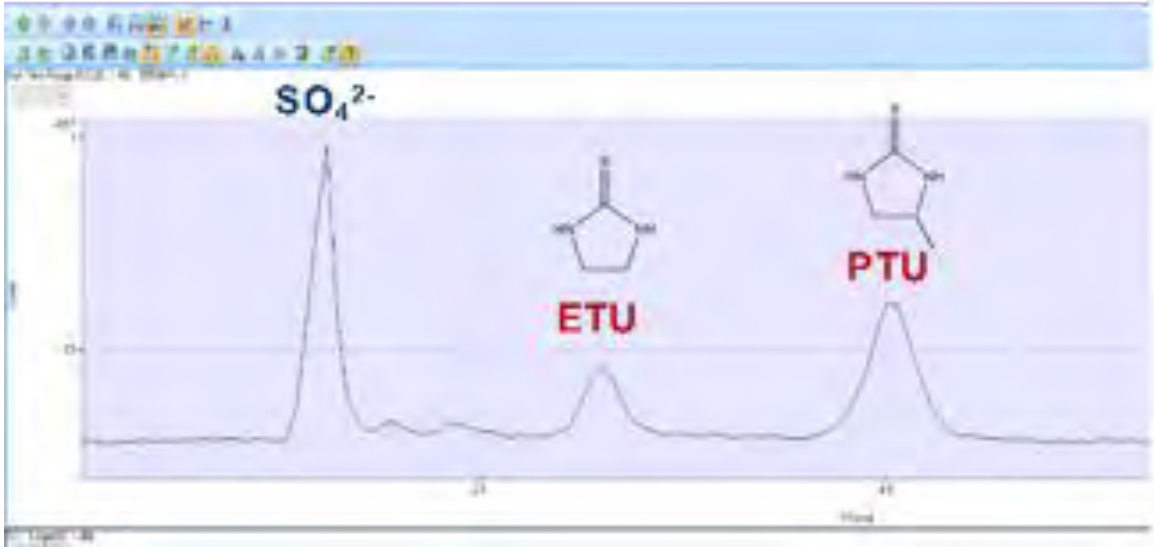
Main advantage:

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 inrganic sulfur by RP-HPLC coupled to ICP-QQQMS

(first ICP-MS based method…)

HPLC column	Aqua® 3µm C18, 125 Å; 150 × 2 mm
Mobile Phase	5% MeOH, isocratic elution
Flow (mL/min)	0,25
Monitored signals	[32]S → [48]SO; [34]S → [50]SO

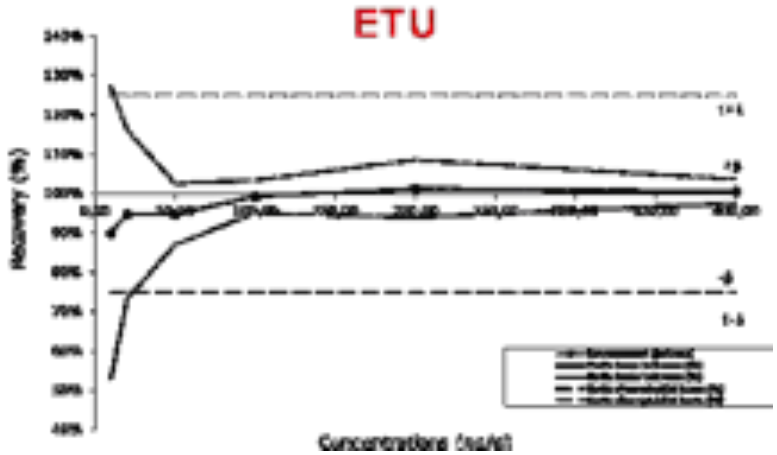


Method Validation ⇒ acuracy profile approach

Matrices tested (spiking)	Tomatoes, Grapes, Strawberries, Cherries
Spiking levels (ppb)	10 – 400 (6 different levels)
Extraction solvent	Aqueous 5% MeOH
Extraction procedure	Surface extraction (whole fruit) (30 min)
Recovery (%)	71 - 110

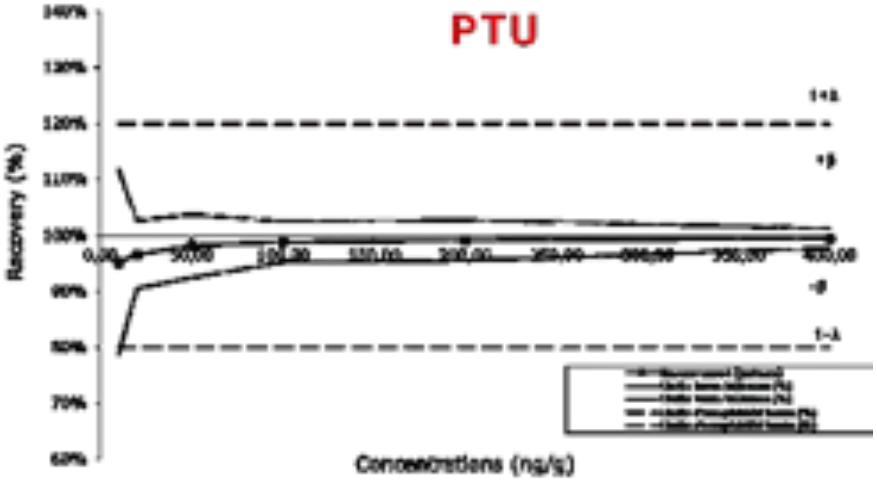
Analytical figures of merit

Linear range tested (ng/mL)	10 – 1000
Linearity (r^2)	0,9999 – 1,000
Back calculated concentrations	95 – 105 %
Instrumental precision (% RSD)	< 2%



LOQ = 15 ppb

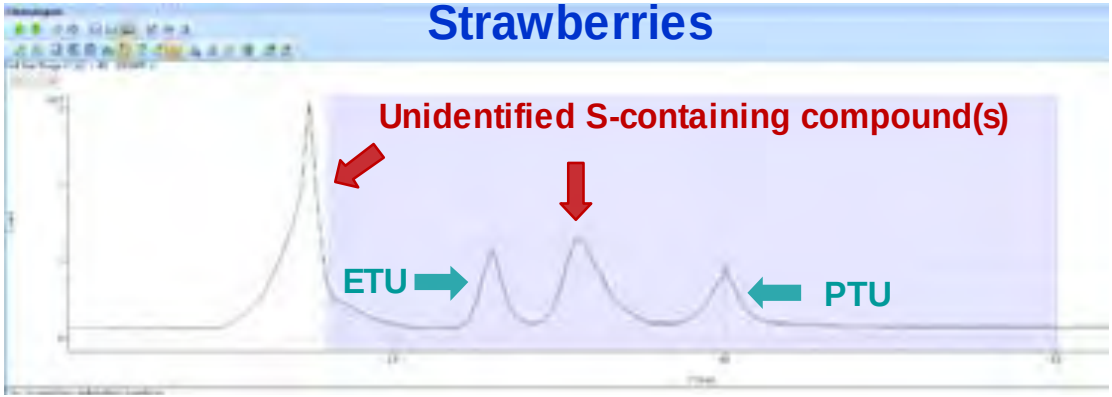
Intermediate precision: 2-10% (depending on the spiking level)

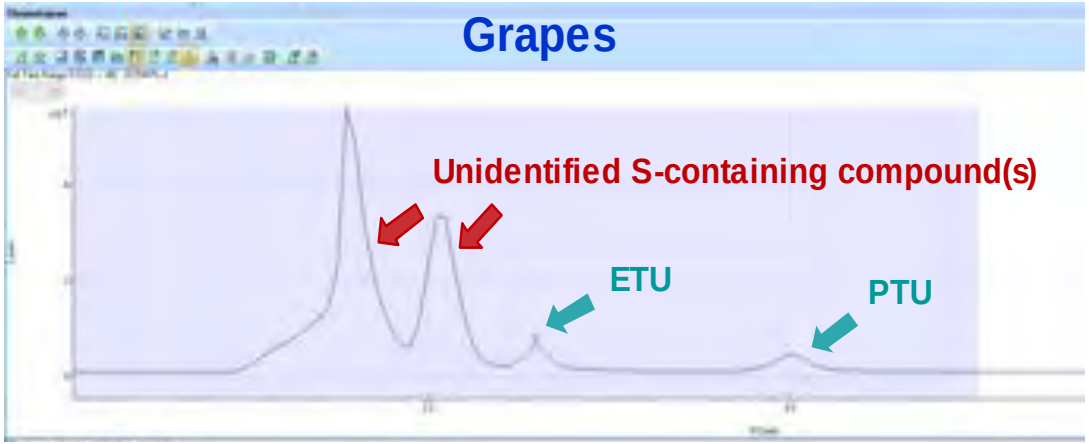


LOQ = 10 ppb

Intermediate precision: 1-8% (depending on the spiking level)

Chromatograms obtained for the analysis of real-life spiked samples (whole fruits)





FUTURES PERSECTIVES IN TERMS OF SPECIATION ANALYSIS IN FOOD PRODUCTS

- **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 Asi 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 dilutio in foodstuffs**

- **An intersting 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 attentione !