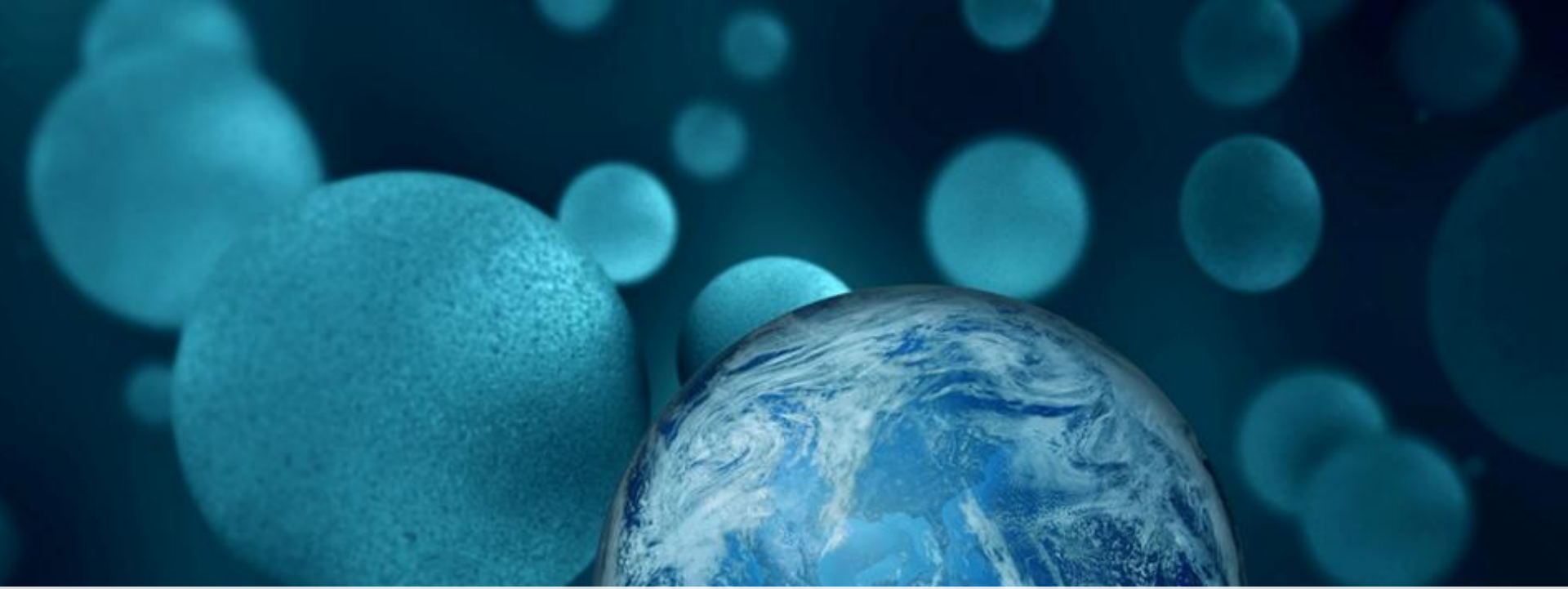

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New Trends in Trace Elemental Speciation Analysis

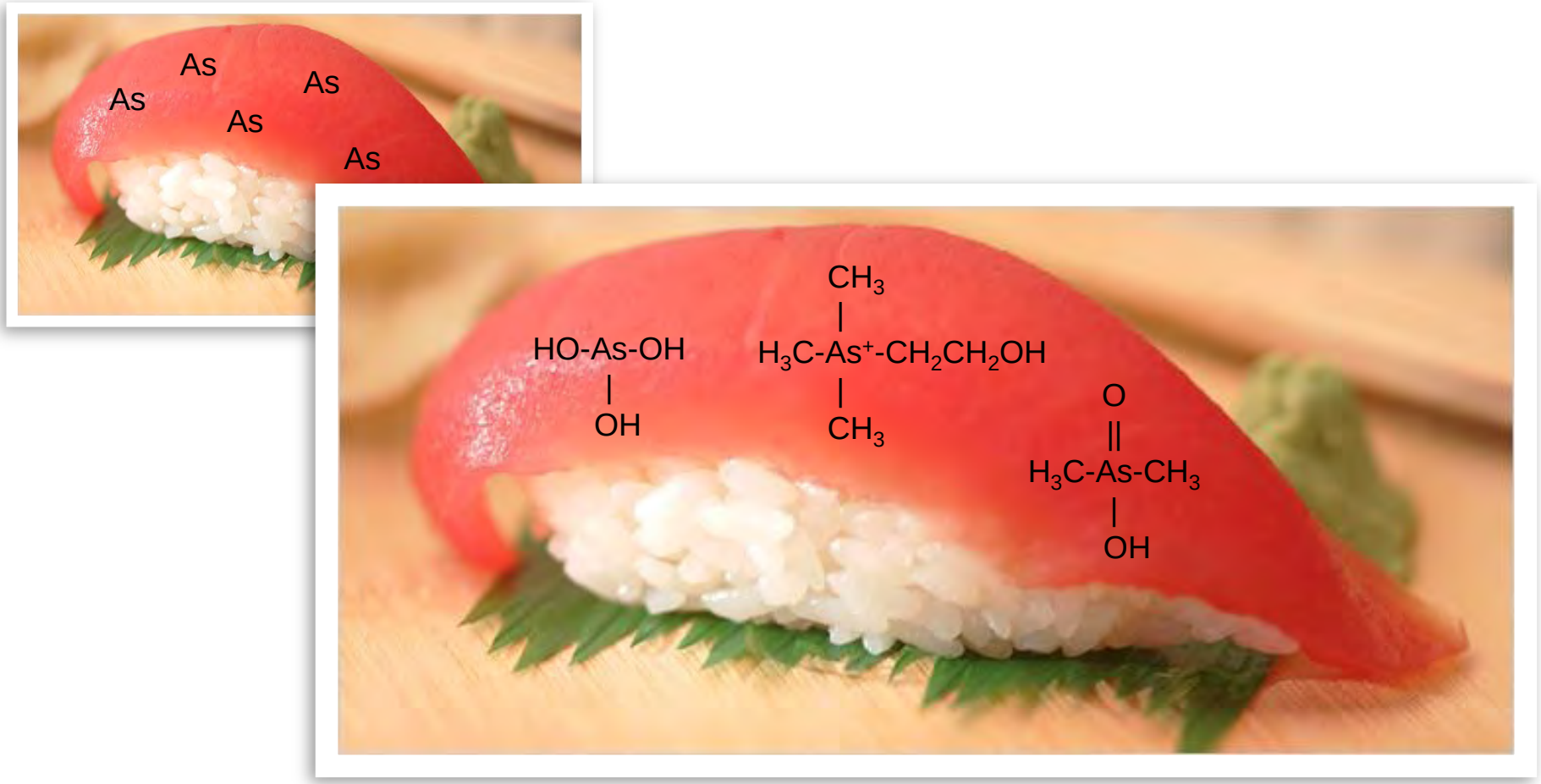
Elsamoul Hamdnalla
Thermo Fisher Scientific

Which One Is Safer to Eat?

Total As concentration is not enough to answer!

What is Speciation?

Separation and quantification of different chemical forms of an element to understand environmental or health related impacts associated with a sample



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Who Needs to Performs Speciation Analysis?

Industries	Applications
Environmental	• Hexavalent chromium, arsenic and bromate in drinking waters
Food Safety	• Arsenic in fruit juices and rice grains • Mercury in fish
Occupational Exposure and Consumer Goods	• Hexavalent chromium in toy materials
Pharmaceutical	• Mercury in herbal supplements
Petrochemical	• Sulfur/selenium in produced water

Regulations

More regulations to come with ongoing assessments

Development of Elemental Speciation*

- Rapid development of inorganic elemental analysis in the latter half of 20th century driven by development of atomic spectroscopy
- Enabled analysts to research the role of trace elements in geochemistry, environment, materials science
- Became evident that trace elements play a major role in biological, environmental and materials chemistry
- Realization that distribution, mobility and biological availability of elements depends on chemical form, not just concentration
- E.g. hexavalent chromium [Cr(VI)] is a carcinogenic agent, while trivalent chromium [Cr(III)] is an essential element for humans

* www.speciation.net

How Do You Perform Speciation Analysis?

Separation

Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ System

Detection

Thermo Scientific ™ iCAP Q ICP-MS System

ThermoFisher SCIENTIFIC

Speciation Analysis Workflow

Sample Preparation	Separation	Detection
Total element concentration is the sum of all species ↔ Preservation of original species distribution Loss of species during sample preparation Transformation of species	Different separation mechanisms ↔ Ability to tackle several analytes with same instrumentation **Ion chromatography:** Analyte retention is achieved by interaction of charges with stationary phase	ICP-MS can only detect the element enclosed in a species ↔ Accurate and reliable quantification of different compounds containing the same element!

Relevance of Elemental Speciation*

Environmental Fate

- **Environmental risk analysis**
  - hazard identification, exposure assessment
- **Waste management**
  - information to facilitate remediation

Mobility

- **Occupational health and hygiene**
  - Toxic exposure, e.g. Hexavalent Chromium
- **Toxicology, pharmacy, medicine, clinical chemistry and biology**
  - enzymes (Zn), vitamins (Co), metallo-proteins (Se), metallo-drugs (Pt), toxins (As, Hg, Cr(VI), Cd, Pb) and their metabolic forms.
- **Nutritional sciences**
  - a better understanding of chemical forms of trace elements in food and their subsequent behavior in the digestive tract.

Toxicity

- **Drinking water industry**
  - toxicity of trace elements (e.g. Al, As, Cr) present in the raw water depend strongly on their speciation (e.g. As(III)/As(V)).
- **Food industry**
  - improve the quality of their products.

Process Control Chemistry

- **Chemical industry**
  - chemical activities of reagents, catalysts, products, by-products and impurities are species dependent.
- **Petrochemical industry**
  - metalloporphyrins and other metal species are present in fossil fuels show species dependent behavior in refinery

Reactivity

- **Semiconductor industry**
  - process chemicals used are organometallic compounds or metalloid compounds of high toxicity, calling for strict control

Bioavailability

** www.speciation.net*

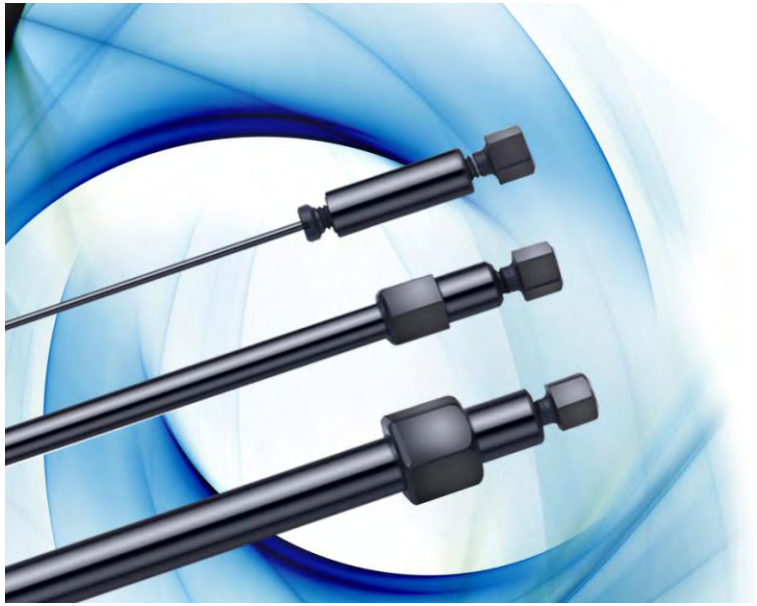
Advantages of ICP-MS

- Measures almost the whole periodic table in any matrix
  - Elemental concentrations
  - High precision isotope ratio determinations
  - **Species information** when coupled to separation devices

Highly versatile and sensitive detection technique

Unique Advantages of IC Coupled to ICP-MS

- Separation of chemical species
- Completely metal-free flow path
  - Less contamination
  - Lowest chemical noise
  - Better S/N
  - Lower LOD
- Narrower bore columns (2 mm ID)
  - Narrower peak shapes
  - Better S/N



Metal-free flow path best suited for metal speciation applications

Chemical Forms of Metals

REDOX FORMS	ORGANOMETALLIC COMPOUNDS	BIOMOLECULES
As(III)/As(V)	Methyl-Hg	Se amino acids
Se(IV)/Se(VI)	Butyl and Phenyl-Sn	Arsenosugars
Cr(III)/Cr(VI)	Alkyl Pb	Metalloproteins

DIFFERENCE OF

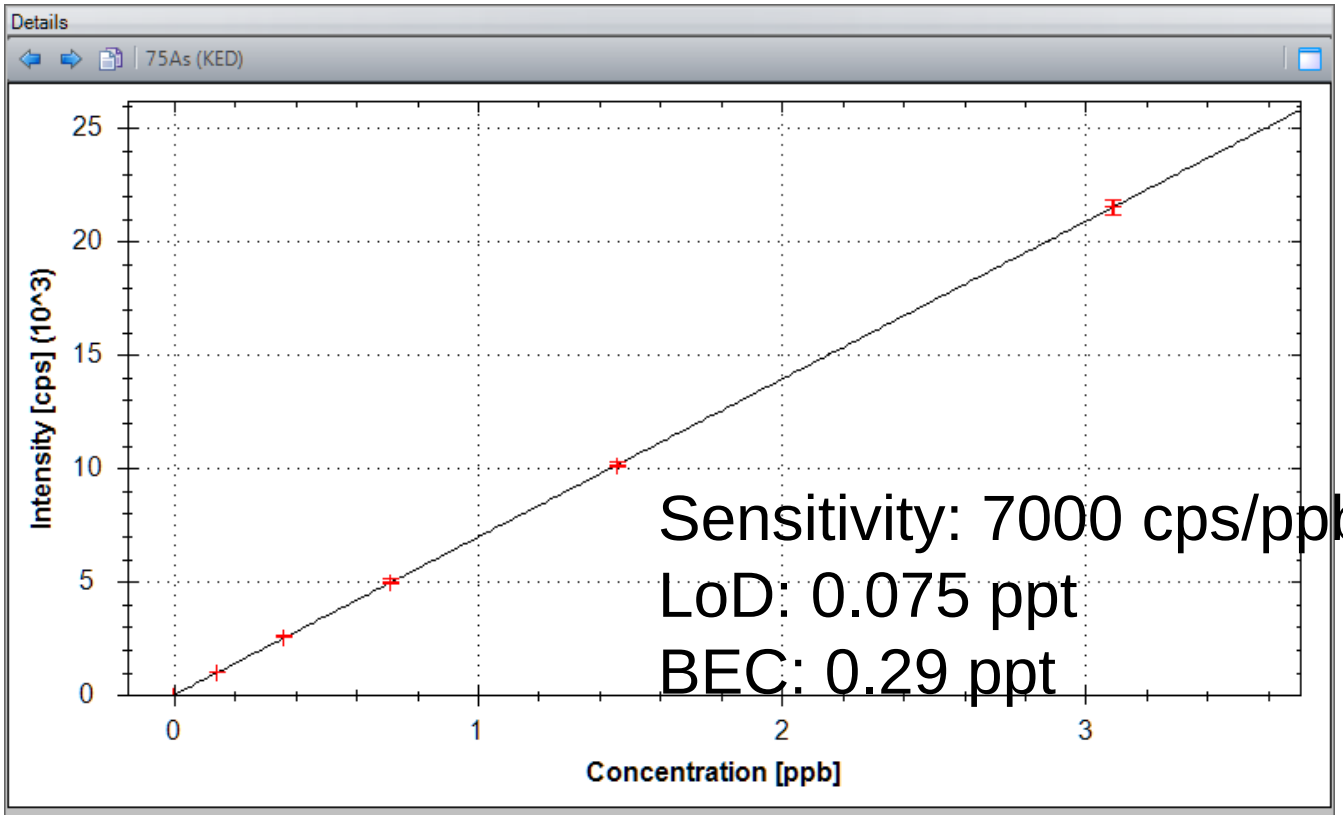
Toxicity, Bioavailability, Bioaccumulation and Mobility

Typical LC- and IC-ICP-MS Applications

Element	*Species*	*Matrices*	*Scientific Sector*
Cr	Chromium III & VI	Water, air particulates, cement	Environment, industry, nutrition, occupational exp.
As	Organoarsenic compounds	Fruit juice, fish, water, sediments, urine, hair	Nutrition, environment, exposure
Se	Organoselenium	Yeast, garlic, urine	Agroindustry, nutrition, pharma, clinical
Hg	Inorganic mercury, organomercury	Fish, sediments, water, urine, blood	Environment, Clinical
Sn	Organotins	Fish, sediments, water, plastics	Environment, industry
Fe	Iron II & III	Bacteria, soils, water	Environment, clinical, nutrition
Cu, Zn, Cd	Phytochelatins, metallothioneins	Plant tissues, brain & kidney tissue	Agroindustry, clinical

Arsenic Speciation in Apple Juice -Total Arsenic (As) Quantification

1:10 diluted Apple Juice



Sensitivity: 7000 cps/ppb As

LoD: 0.075 ppt

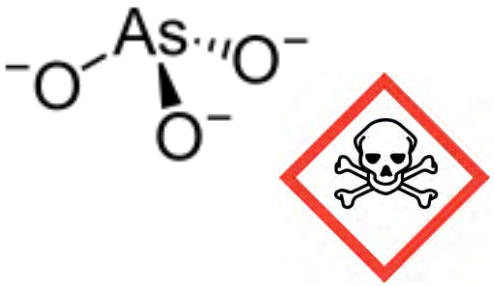
BEC: 0.29 ppt

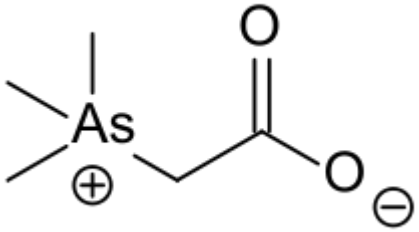
$f(x) = b^*x + a$

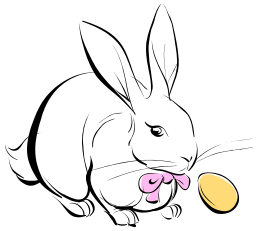
Parameter	Value	Confidence Delta at 90%	StdErr	Rel. StdErr [%]
b	6,961.007	+/-29.209	20.287	0.291
a	2.000	+/-0.000	0.000	0.000
R	1.000			
BEC	0.00029 ppb			
IDL(LOD)	0.00075 ppb			

Speciation of As in Apple Juice

- Differentiation between (toxic) inorganic As(III) & As(V) species and (non-toxic) organic species (MMA etc)







- Requirements:
  - Single run anionic and cationic technique since both positive and negative charged species can be present in a sample
  - Good chromatographic resolution to separate out species
  - Sharp peaks for improved sensitivities

Chemical Forms of As and Their Toxicity

CHEMICAL FORM	LD50 (mg/kg)
Arsenite (As(III))	14
Arsenate (As(V))	20
Arsine (AsH_3)	3
Monomethylarsonic acid (MMA)	700 - 1800
Dimethylarsinic acid (DMA)	700 - 2600
Arsenocholine	> 10000
Arsenobetaine	> 10000
Aspirin	1000 - 1600
Strychnine	16

LD50 rat: Lethal dose 50 (dose for which 50% of a population is dead)

iCAP Q ICP-MS–Dionex ICS-5000^{+} for IC-ICP-MS

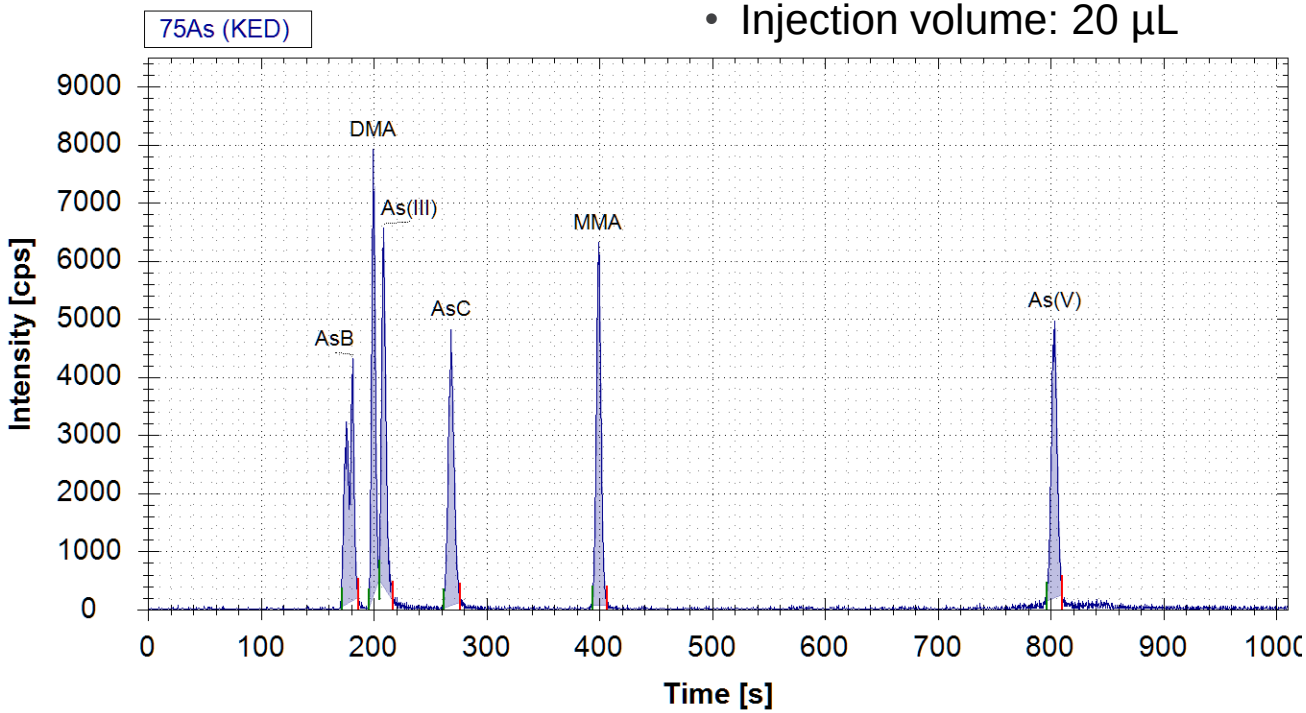
Thermo Scientific™ iCAP™ Q ICP-MS analyzer with
Thermo Scientific™ Dionex™ ICS-5000^{+} Capillary HPIC™ system

ThermoFisher SCIENTIFIC

iCAP Qc ICP-MS with Dionex ICS-5000+

- 0.45 ppb of each As standard
  - 6 species
  - ~8000 cps / ppb
  - ~15 minute analysis

- Anion Exchange:
  - Thermo Scientific™ Dionex™ IonPac™ AS7 (2 x 250 mm)
  - Gradient elution with 20–200 mM ammonium carbonate
  - Flow rate: 0.3 mL/min
  - Injection volume: 20 µL



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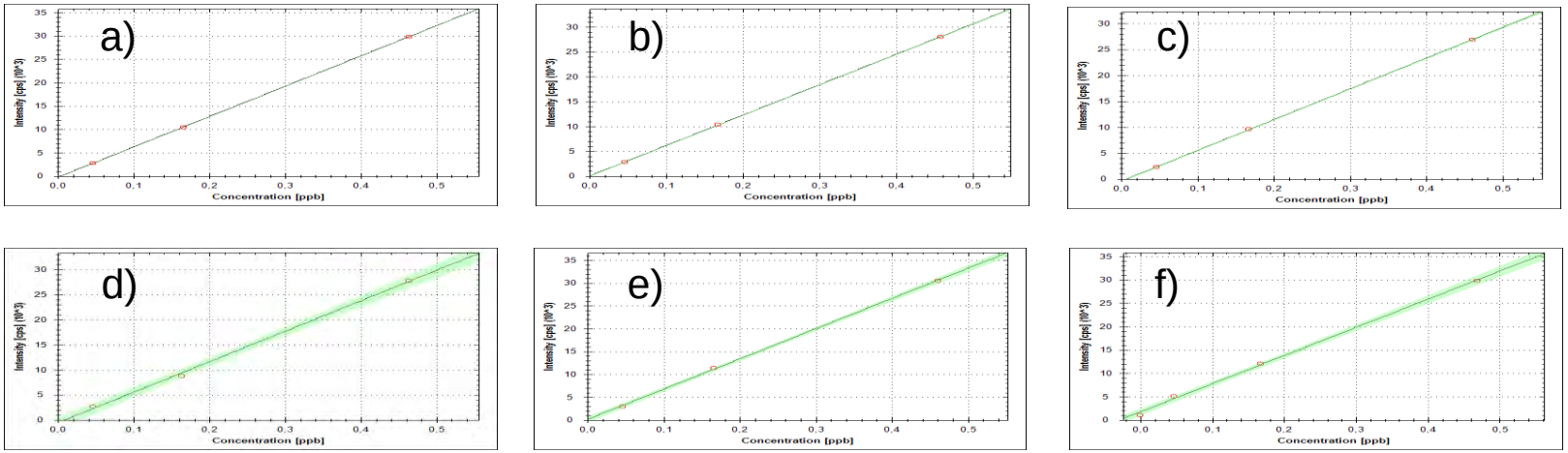
As Species Calibrations

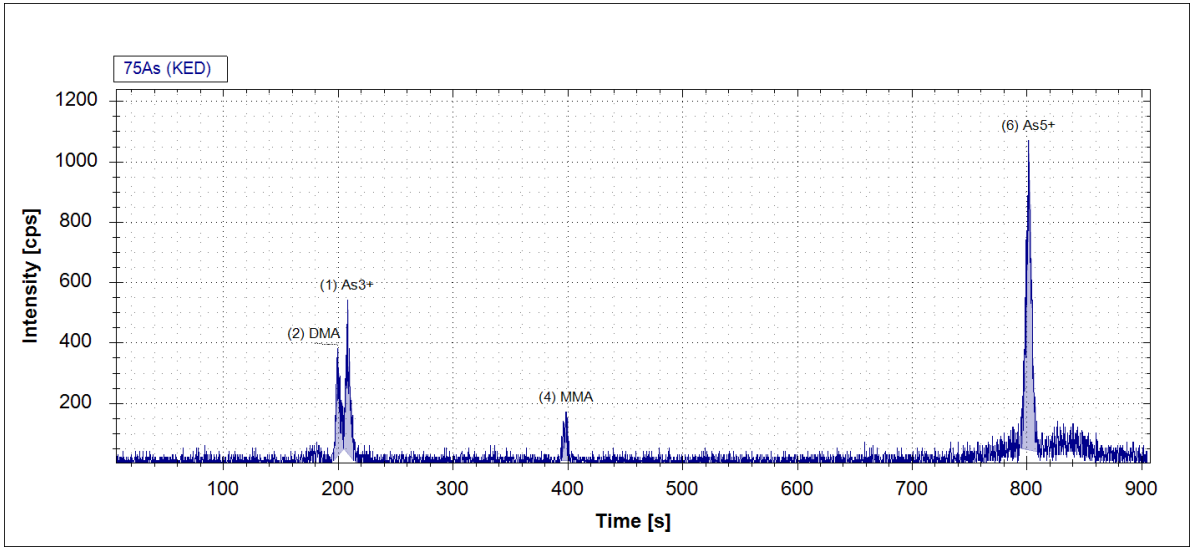


Fully quantitative calibration graphs for AsB (a), DMA (b), As^{3+} (c), AsC (d), MMA (e) and As^{5+} (f)

Compound	Detection limit pg g^{-1}
AsB	2.3
DMA	3.8
As^{3+}	4.6
AsC	4.4
MMA	11.4
As^{5+}	1.2

As Species Detection Limits by IC-ICP-MS

As Species in Apple Juice



Species	Expected [ng g-1]	Found [ng g-1]	Recovery [%]
AsB	2.19	2.27	104
DMA	1.40	1.15	82
As3+	1.35	1.38	102
AsC	1.94	1.87	94
MMA	1.09	1.13	104
As5+	1.10	1.07	98

As Species – Spike Recoveries in Apple Juice

Public Health Issue: As in Apple Juice

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FDA examines level of arsenic in apple juice

By Amanda Gardner, HealthDay

Updated 11/30/2011 6:41 PM

Comment 31 | Recommend 1k | Tweet 135

WASHINGTON (AP) – The Food and Drug Administration is considering lowering its standards for the levels of arsenic allowed in apple juice after consumer groups pushed the agency to crack down on the contaminant.

Amy Sancetta, AP

The Consumer Reports research also discovered that 25 percent of apple juice samples had lead levels higher than that recommended by the FDA for bottled water.

Studies show that apple juice has generally low levels of arsenic, and the government says it is safe to drink. But consumer advocates say the FDA is allowing too much of the chemical — which is sometimes natural, sometimes man made — into apple juices favored by thirsty kids.

There is little consensus on whether these low levels could eventually be harmful, especially to children. Michael Taylor, FDA's deputy commissioner for foods, said Wednesday the agency has already stepped up testing and research on arsenic in apple and other juices and is seriously considering lowering the FDA's so-called "level of concern" for the contaminant.

MORE: FDA disputes TV suggestion of apple juice risk

Source: http://yourlife.usatoday.com

- Interest was triggered in the USA as some apple juices were reported to contain high arsenic levels:
  - Total As concentration only
  - No information on the type (species) of As

As Speciation in Apple Juice

- iCAP Qc ICP-MS with Dionex ICS-5000^{+} HPIC system:
  - Anion exchange chromatography

	AsB	DMA	As(III)	AsC	MMA	As(V)	Sum of Species	Total As
MDL	0.002	0.004	0.005	0.004	0.011	0.001	-	0.005
Juice 3	ND	ND	0.5 ± 0.01	ND	ND	0.7 ± 0.01	1.2	1.7 ± 0.05
Juice 4	ND	0.4 ± 0.05	0.3 ± 0.01	ND	0.1 ± 0.05	0.7 ± 0.01	1.5	1.8 ± 0.05

- iCAP Qc ICP-MS benefits:
  - Low method detection limits: 0.001 and 0.01 ng/g per species, 0.005 ng/g total As vs current EPA MCL (maximum contaminant level) is 10 ng/g in drinking water

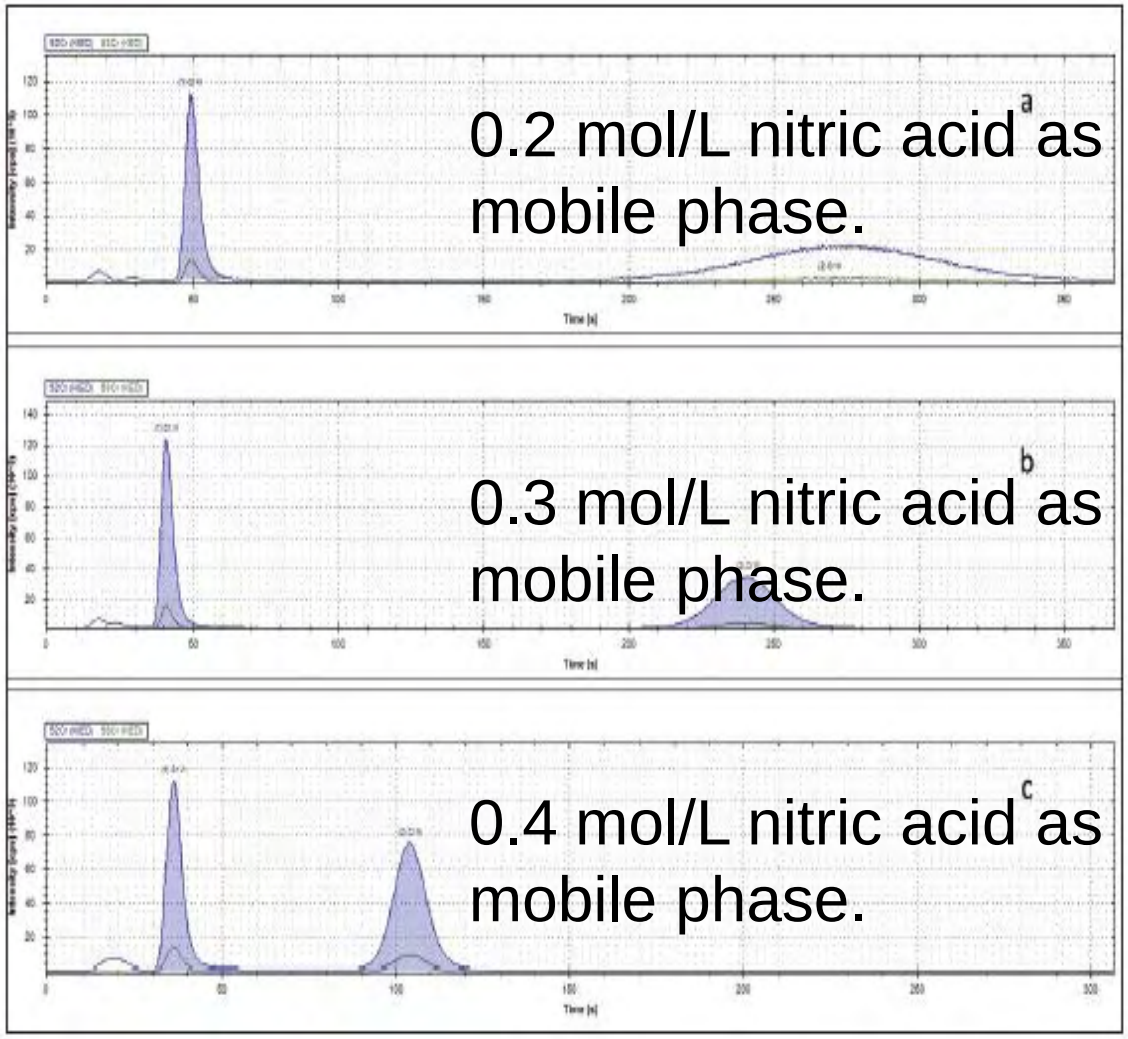
Why Speciation Analysis of Cr (III) & Cr (VI) in Drinking Waters

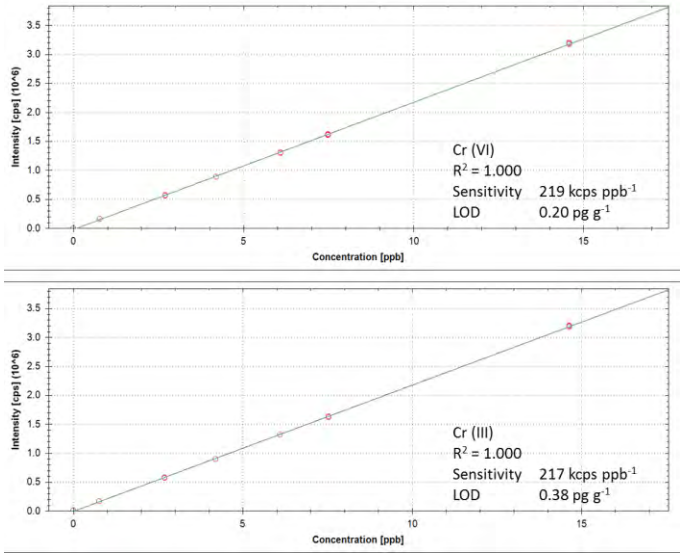
- Chromium (Cr) concentrations in environmental samples are monitored due to widespread use in industrial applications
- US EPA and the EU have specify maximum chromium concentrations in their drinking water directives
- Cr is found in more than one chemical form, each with different chemical properties and behavior such as bioavailability and toxicity
- Cr (III) is essential to humans while Cr (VI) is highly toxic
- Speciation analysis is challenging as stability of Cr species is easily affected during collection and treatment.
  - low pH values may lead to the degradation of Cr (VI) to Cr (III) due to the increased redox potential,
  - high pH values may lead to the precipitation of Cr (III) as Cr(OH)3
- An additional difficulty in the analysis of Cr by ICP-MS are the numerous spectral interferences (e.g. $^{35}Cl^{16}O^{1}H^{+}$ or $^{40}Ar^{12}C^{+}$) on the most abundant chromium isotope, ^{52}Cr
  - Collision Cell Technology is required for accurate determination

Instrument Configuration

- Separations were carried out using the Dionex ICS-5000^{+} ion chromatography system.
  - Its metal-free solvent pathway is non-contaminating and thus perfectly suited for elemental speciation studies
- A Dionex AG-7 anion exchange column (2 x 50 mm) was used throughout this study.
  - This column is a guard column but its highly effective separation medium is able to completely separate both Cr species in less than three minutes.
- An iCAP Qc ICP-MS was used as a high performing elemental detector of the Cr species eluted from the Dionex ICS-5000+ system:
  - With the use of flatapole technology in the QCell collision cell, the system offers the selectivity to suppress spectral interferences while maintaining the high sensitivity for trace metal detection using IC-ICP-MS.

Method Development





Calibration

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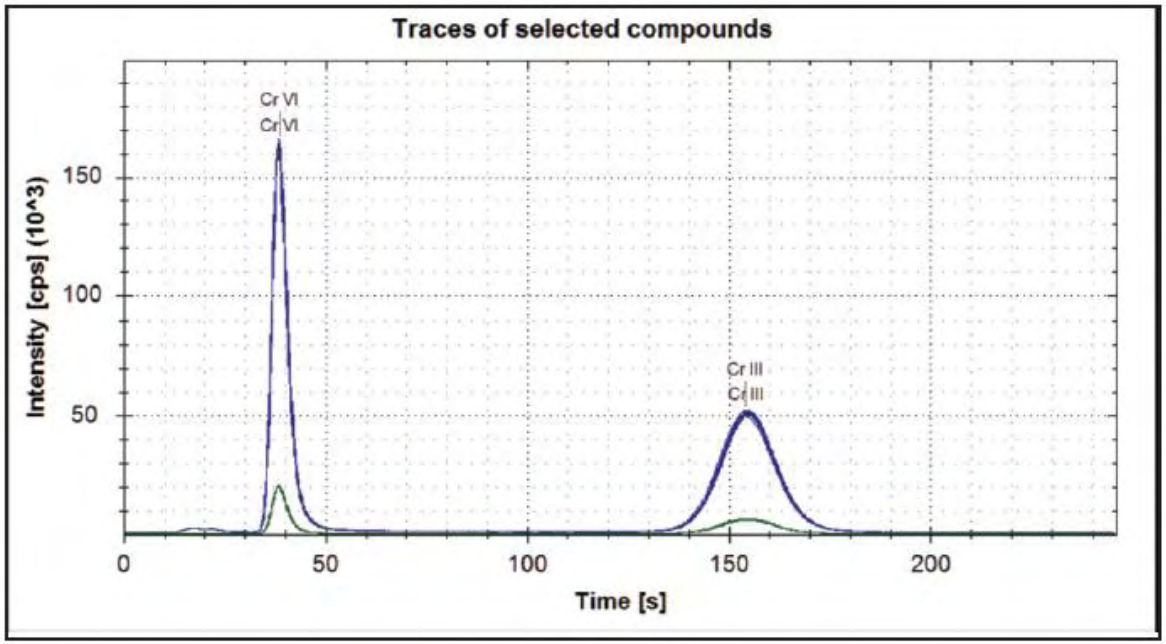
Duplicate Analysis and Spike Recovery

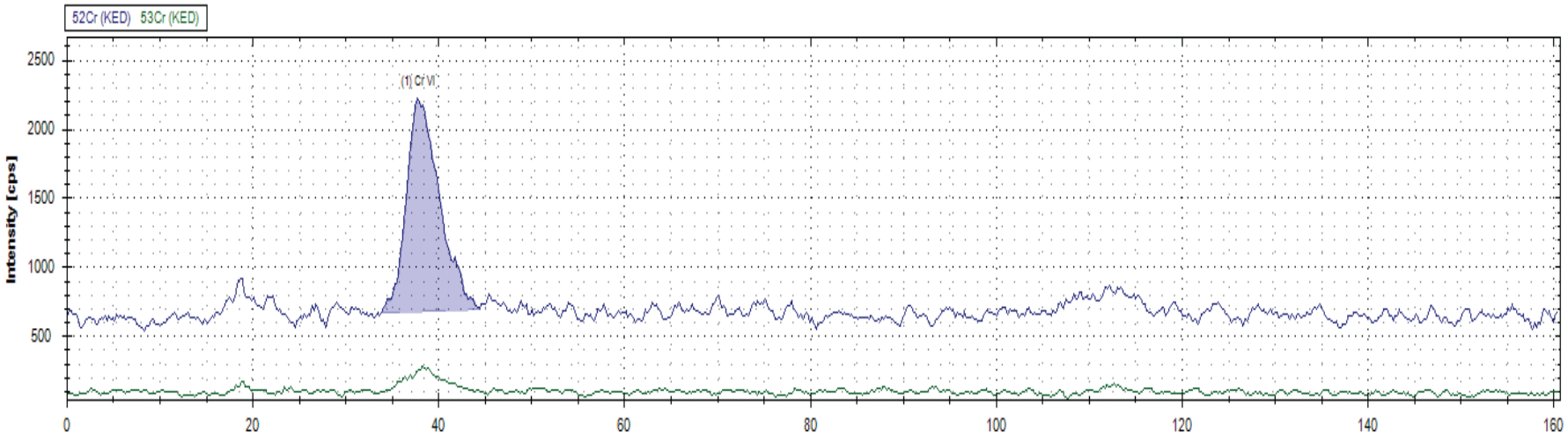


Overlay of 20 repeated injections of Cr(VI) and Cr(III)

Conc. spiked [ng/g]	Cr (VI)		Cr (III)	
	Found (ng/g)	Recovery (%)	Found (ng/g)	Recovery (%)
2.34 of each	2.31 ± 0.01	99 ± 1	2.35 ± 0.02	100 ± 1
6.03 Cr (VI); 1.90 Cr (III)	6.01 ± 0.02	100 ± 1	2.00 ± 0.01	105 ± 1
1.87 Cr (VI); 6.20 Cr (III)	1.85 ± 0.01	99 ± 1	6.15 ± 0.03	99 ± 1

Quantification of Cr (III) and Cr (VI) in Tap Water

- Potable water was collected locally and analyzed using the proposed method.



- Only trace amounts of Cr (VI) could be detected in this sample
- The amount of Cr (VI) observed was found to be 42.5 ± 1 pg/g
- To confirm the peak is Cr and not affected co-eluting compounds causing spectral interferences (e.g. chlorine or carbon based polyatomic species), the isotope ratio $^{52}Cr+/^{53}Cr+$ was calculated and corresponds well to the theoretical value of 8.81

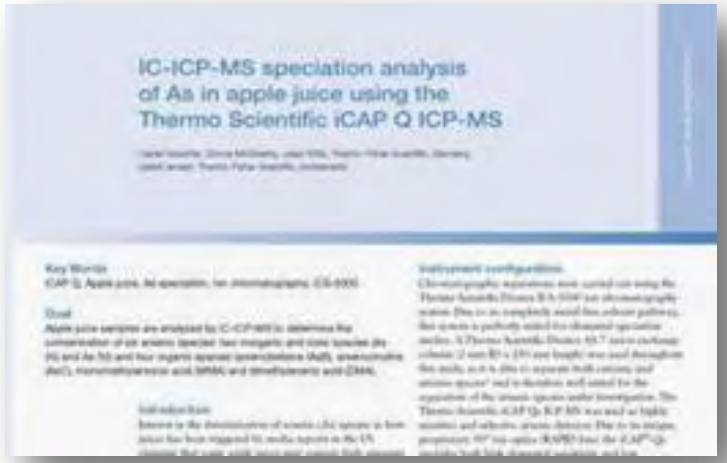
Conclusions

- The combination of the Dionex ICS-5000^{+} system with the iCAP Qc ICP-MS system yields a sensitive, robust IC-ICP-MS for the speciation analysis of trace levels of Cr (III) and Cr (VI) in natural waters
- This enables fast and reliable speciation analysis of Cr species in water samples without prior incubation steps and with high purity nitric acid as mobile phase
- The short, but highly efficient Dionex AG-7 column, provides complete separation of both species in under 150 s, enabling high sample throughput for the routine analysis of water samples
- The new flatapole QCell in the iCAP Q ICP-MS provides interference-free detection of the ^{52}Cr and ^{53}Cr ions
- Sub-ppt detection limits are achievable due to the completely metal free pathway of the Dionex ICS-5000^{+} system and the high instrumental sensitivity offered by the iCAP Q ICP-MS system's He KED mode
- A highly sensitive and specific method for the speciation analysis of arsenic in apple juice samples has been developed and applied to the analysis of different juices after a simple ten-fold dilution

Conclusions (continued)

- The Dionex AS-7 anion exchange column used in this study was not only able to efficiently separate six different arsenic species and two chromium species, but also helped to improve the sensitivity of the technique by concentrating each eluted species into a narrow peak. The low flow rate of 0.3 mL min-1 helps to reduce both sample and mobile phase consumption.

- iCAP Q ICP-MS application notes available at www.thermofisher.com/ICP-MS



AN43099-IC-ICP-MS
Speciation of As in
Apple Juice

AN43098-IC-ICP-MS
Speciation of CR(III) and CR(VI)

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Thank you!

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