



# **Analytical approaches for the determination of trace amounts of metal ions released from Ti based alloys**

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# Introduction



Trace elements play a crucial role in maintaining health status of humans and other living beings.

Beside the general constituents **C, H, N, O, P, S**, the *vital biochemical functions of humans depend significantly* on key elements like

**B, Ca, Cl, Co, Cr, Cu, F, Fe, I, K, Mg, Mn, Mo, Na, Se, Si, Sn, V, Zn.**

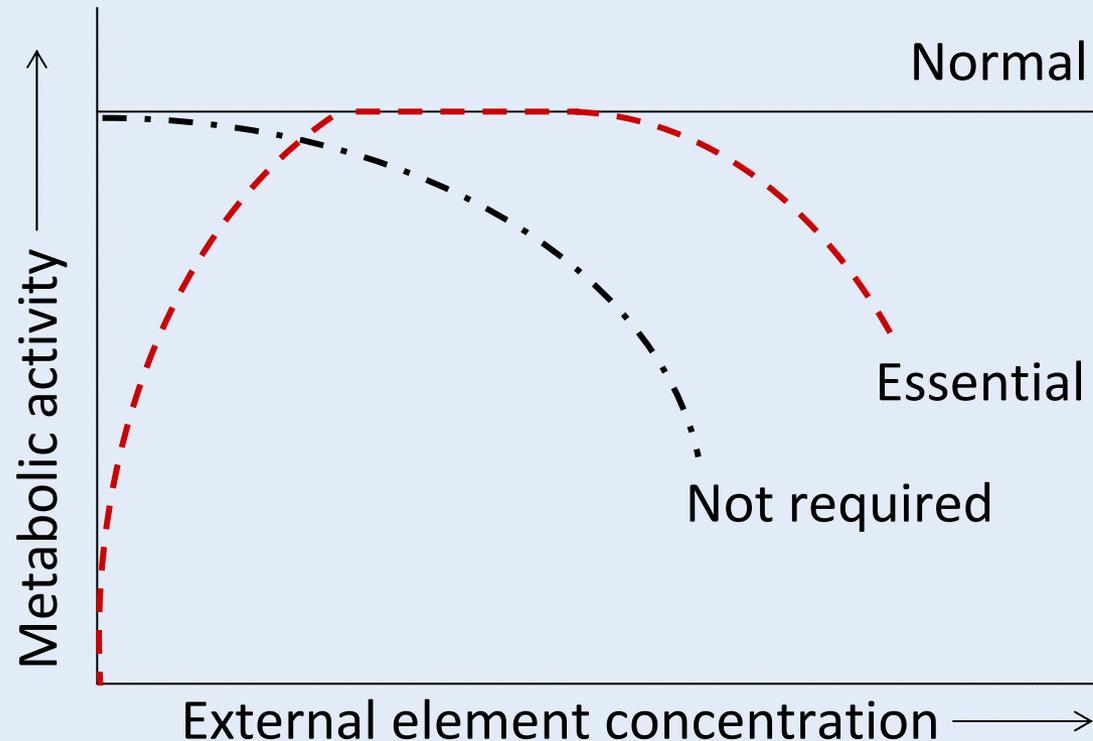
*Caroli et al. Crit. Rev. Anal. Chem., 1994, 14,363-398.*

# Toxic elements for living organisms

Hg, Al, Cd, Pb, Tl and Bi have not known biological function, they are all **TOXIC**.

H																	He
Li	Be	<b>■ Toxic elements</b>										B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Lr	Rf	Db	Sg	Bh	Hs	Mt									
		La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb		
		Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No		

# The effect of external concentration of element on metabolic activity



Essential elements can be **toxic** at high concentration. Not required elements can be **toxic** even at very low concentrations.

<b>Element</b>	<b>Effect of deficiency</b>	<b>Effect of excess</b>	<b>Observations</b>
<b>Si</b>	Growth depression	Silicosis (lungs)	Possible cross-linking of proteins
<b>V</b>	Growth depression; failure of reproduction	Unknown except at high doses	Vanadate complexes: insulin mimic properties
<b>Cr</b>	Cr(III): Impaired glucose tolerance	Toxic as $\text{CrO}_4^{2-}$ , lung cancer, contact dermatitis	Cr(III): Component of a glucose tolerance factor
<b>Fe</b>	Anaemia	Hemochromatosis	Component of haemoglobin
<b>Co</b>	Anaemia; anorexia; growth depression;	Heart failure	Component of vitamin B <sub>12</sub>

<b>Element</b>	<b>Effect of deficiency</b>	<b>Effect of excess</b>	<b>Observations</b>
<b>Ni</b>	Growth depression; impaired reproduction	Lung cancer, contact dermatitis	May replace other transition metals in their sites
<b>Cu</b>	Anaemia; ataxia; defective melanine production;	Liver necrosis e.g. in Wilson's disease	Component of oxidative enzymes involved in haem synthesis
<b>Zn</b>	Anorexia; growth depression; sexual immaturity; skin lesions; depression of immune response; acrodermatitis enteropathica	Relatively non-toxic, except at high doses	Component of many hydrolytic enzymes; component of Zn fingers-affects gene expression
<b>Se</b>	Endemic cardiomyopathy; nutritional muscular dystrophy	Selenosis - hair and nail loss	Component of glutathione peroxidase
<b>As</b>	Impairment of growth	Often poisonous	Blocks sulphdryl groups of

The role of trace elements and their impact to the environment and *living organisms* depend not only on their total concentration but also on chemical forms in which they are actually present.

## Example Cr

*Cr(VI) highly toxic*

carcinogenic, cytotoxic, genotoxic, mutagenic, allergic reactions to skin, provoker of dermatitis

*Cr(III) essential*

*required for normal sugar and lipid metabolism, far less toxic than Cr(VI)*

## The pathways of toxic metals entry into the human body

- by consumption of contaminated foodstuffs
- by inhalation
- in contact with skin
- by infusions
- by the release from different implants

# Ti based alloys

- covered with thin layer of  $\text{TiO}_2$
- $\text{TiO}_2$  lowers the direct contact of bone tissue with metals
- $\text{TiO}_2$  reduces the leaching of toxic metal ions

*To prevent possible hazardous health effects it is necessary to estimate the long-term leaching of metals into body tissues and fluids*

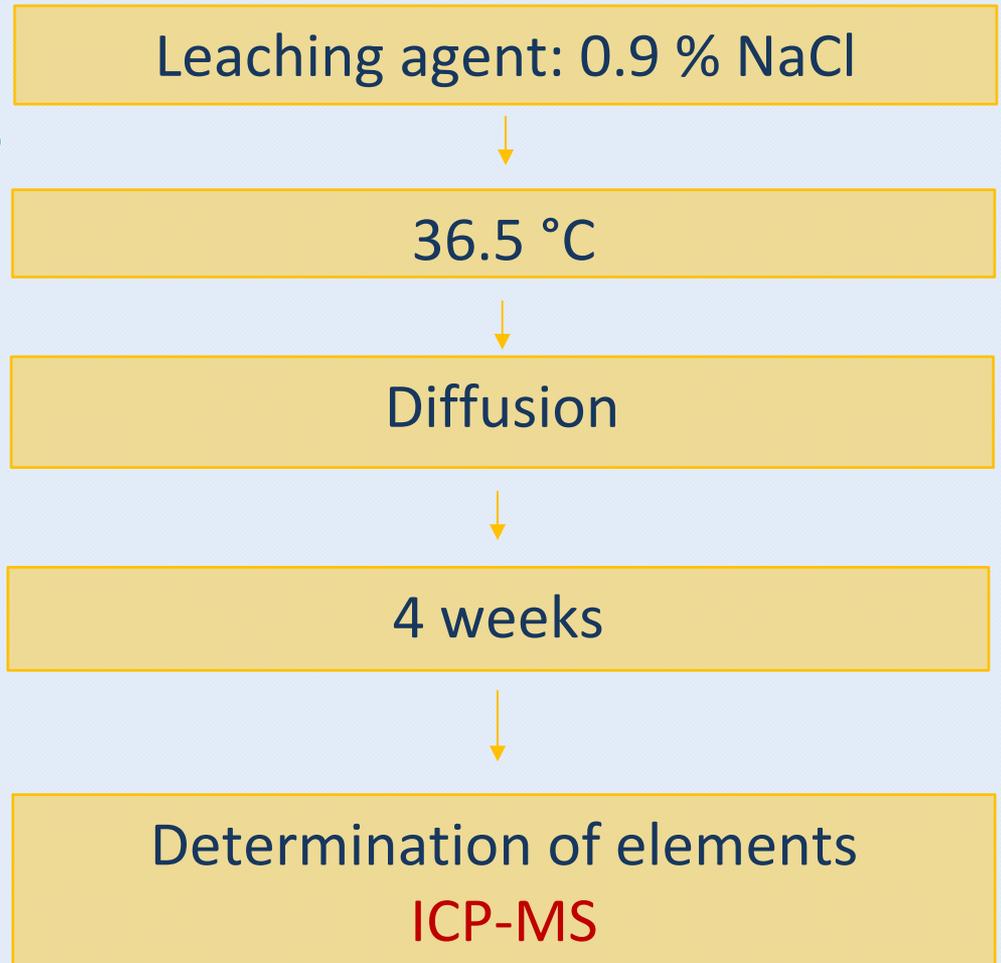
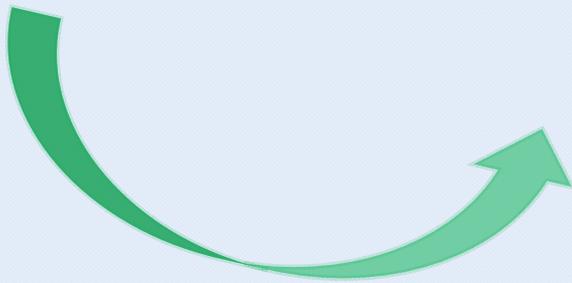
- Adequate leaching tests
- Contamination precautions
- Reliable determination of trace metal ion concentrations by appropriate analytical techniques ( $\text{ng mL}^{-1}$ )

*Efficient cooperation between material scientists and analytical chemists*

# Leaching tests

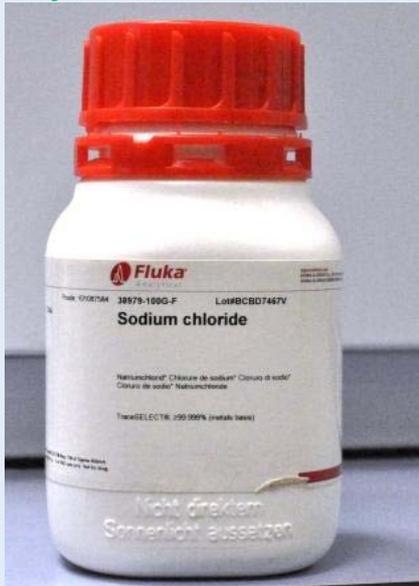
There are no recommended or standardized leaching tests.

One of possible approaches to simulate leaching of metal ions from implants into the body tissues and fluids



# Contamination precautions

Special attention regarding contamination should be addressed when Al or Si are analyzed in leachates



*Sample preparation and leaching test performed under clean room conditions*



Polyethylene or Teflon laboratory ware



Suprapur acids and salts, Ultrapure 18.2 MΩ cm water



Before use, soaking of laboratory ware in 10 % nitric acid for 24 h



Rinsing of sample with, ethanol, 0.9 % NaCl and water before the start of the experiment

# Determination of trace metal ion concentrations

*Reliable determination of trace metal ion concentrations is of crucial importance for interpretation of analytical data and assessment of the potential metal ion release into body tissues and fluids.*

The choice of an appropriate analytical technique depends on the expected concentration range of elements investigated, time of analysis, costs of analysis, availability of instruments.

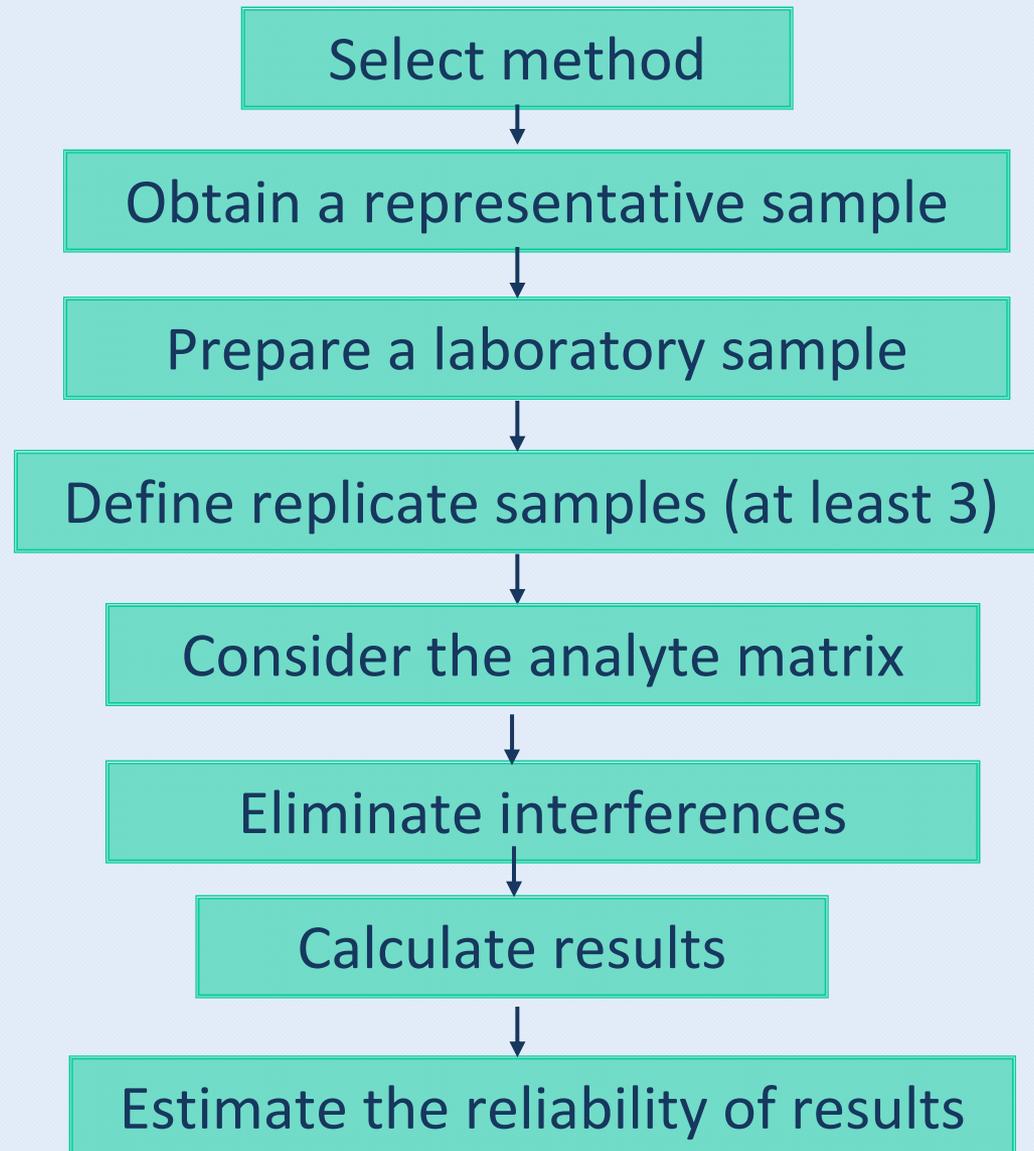
*Flame atomic absorption spectrometry (FAAS)*

*Electrothermal atomic absorption spectrometry (ETAAS)*

*Inductively coupled plasma atomic emission spectrometry (ICP-AES)*

*Inductively coupled plasma mass spectrometry (ICP-MS)*

# *Steps in a quantitative analysis of trace elements concentrations*

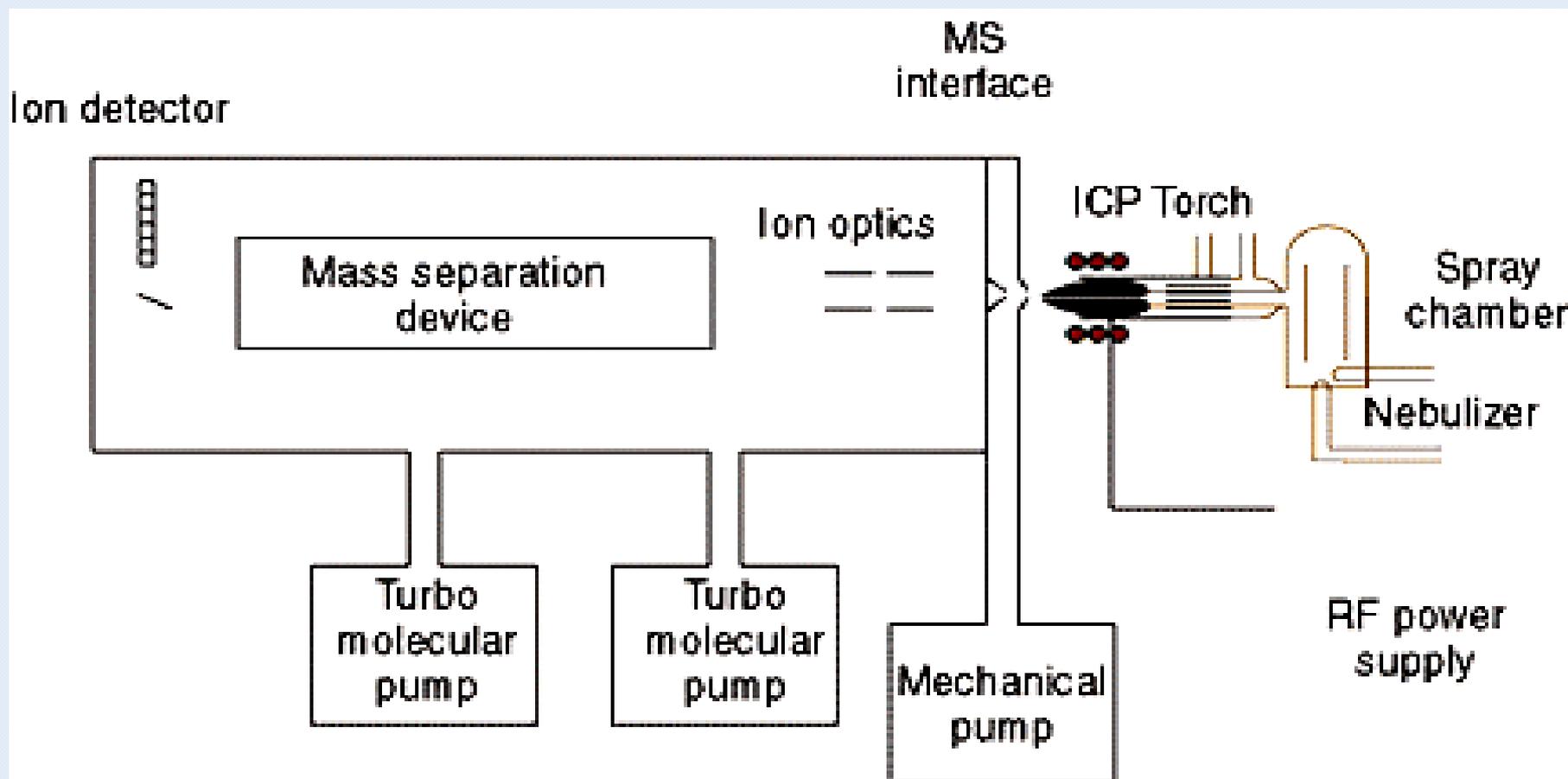


# Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

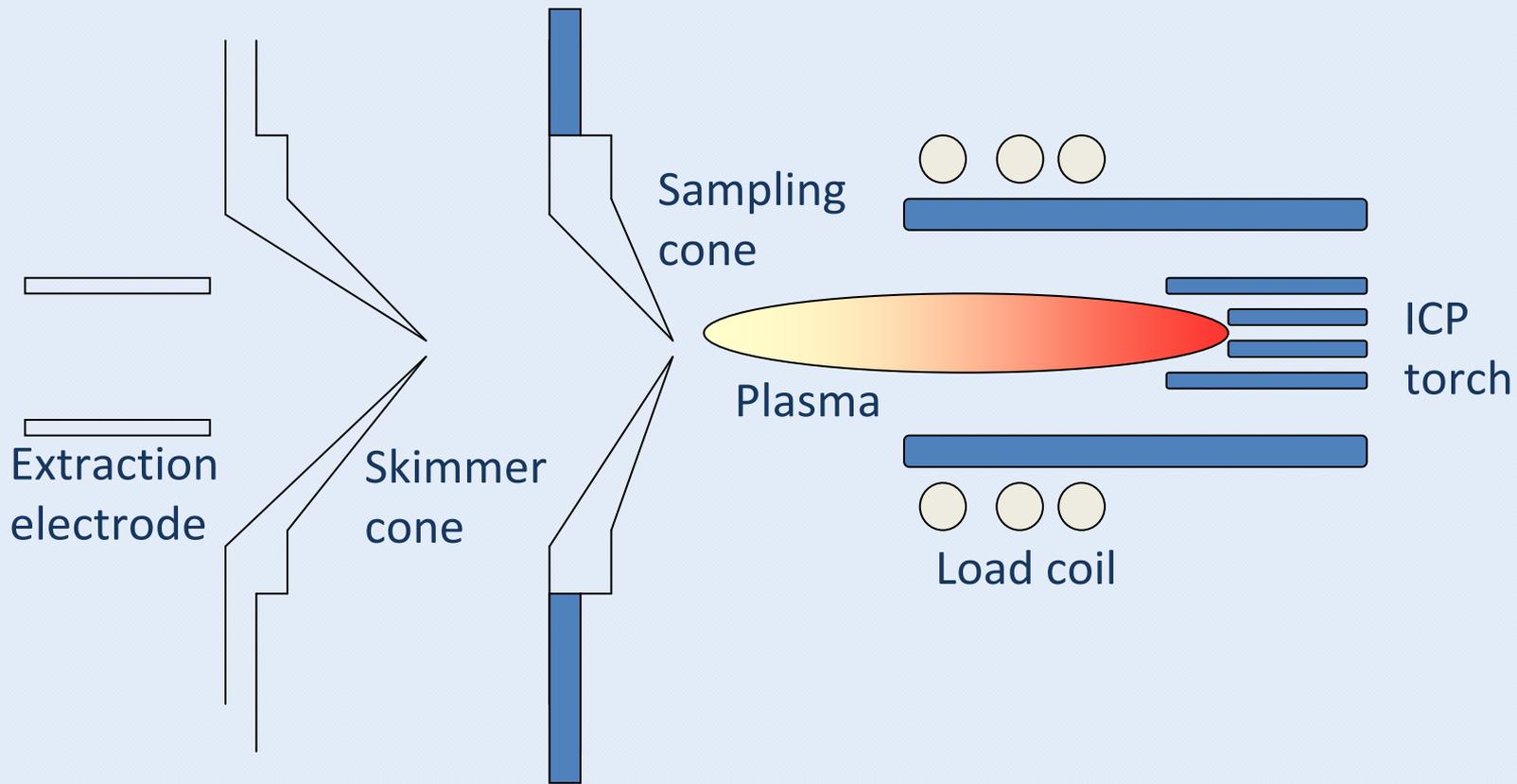
highly sensitive multielemental technique, determination of elements at  $10^{-12}$  concentration levels.

ICP: a source of ions ( $M^+$ )

Mass spectrometer: separates and detects ions

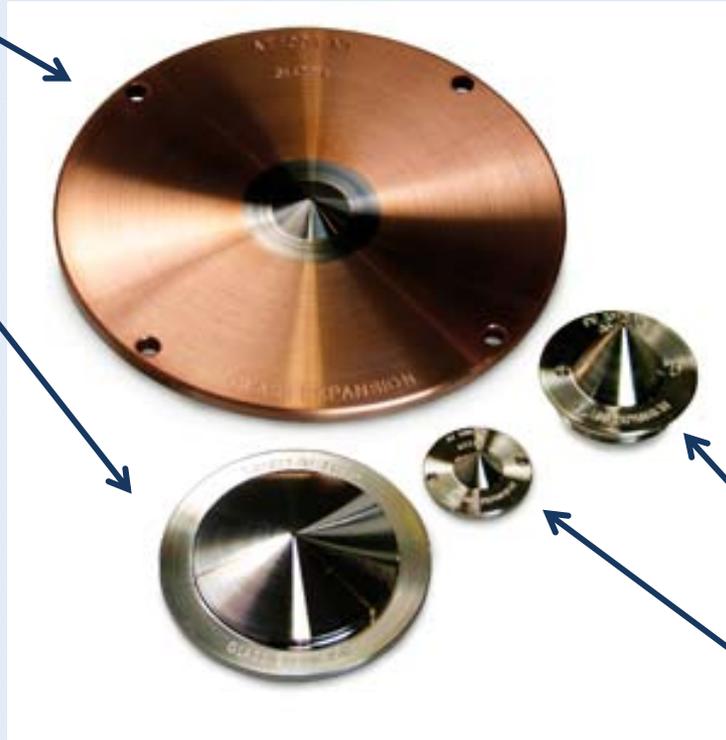


A special set of metal cones and ion-focusing elements are used to extract the charged atoms from the plasma, which operates at atmospheric pressure, into the mass analyzer, which operates at a pressure of  $1 \times 10^{-7}$  mBar.



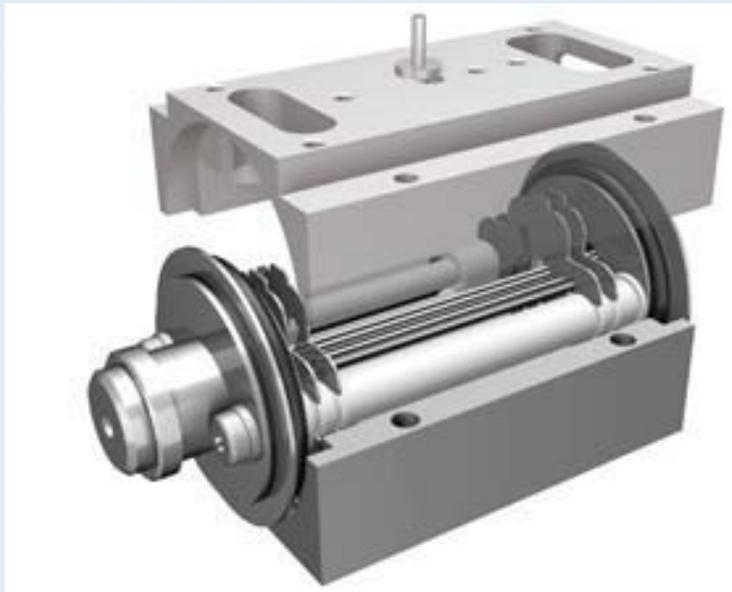
ICP-MS: Sampling the ions

Sampler cones



Skimmer cones

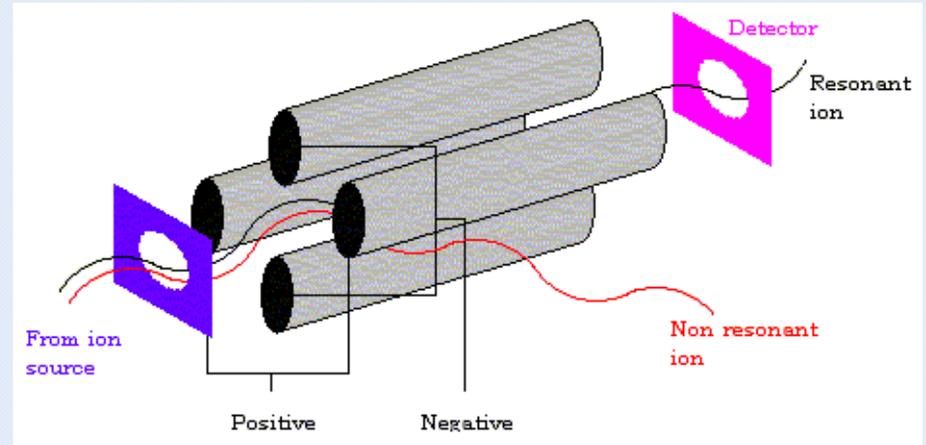
The collision/reaction cell is located before the quadropole in the ICP-MS device and is used to remove interfering ions.



*Energy loss from each collision with a He atom is the same for analyte and polyatomic ion, but polyatomics are bigger and so collide more often.*

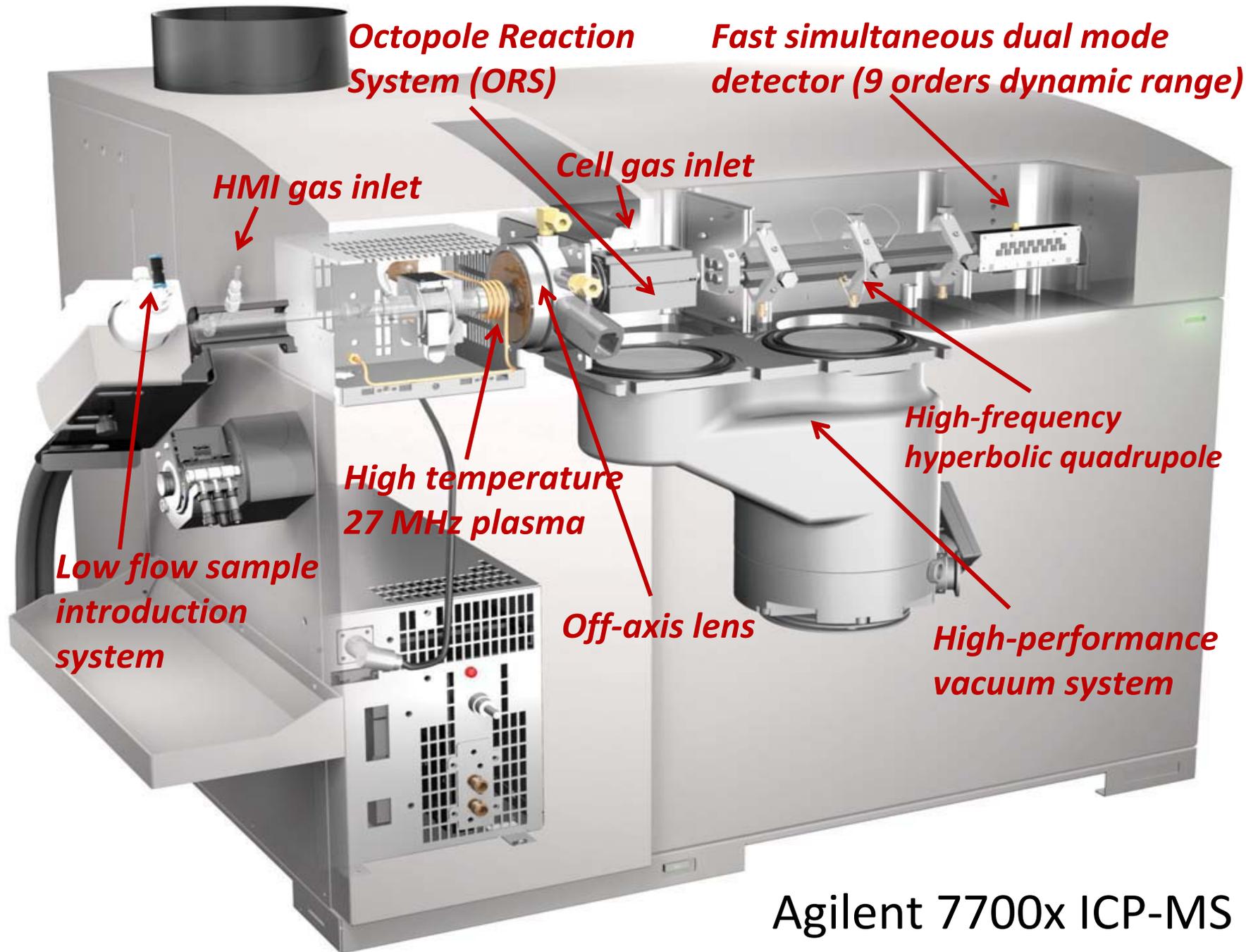
The Agilent octopole reaction system uses only **helium** or **hydrogen** but is based only on collision reactions and not on chemical reactions.

The ions are separated on the basis of their mass-to-charge ( $m/z$ ) ratio and a detector receives an ion signal proportional to the concentration.



## The Quadrupole Mass Analyzer

The quadrupole mass filter is made up of four metal rods. A combined DC and AC electrical potential is applied to the rods. Opposite rods have a net negative or positive potential. By adjusting DC and AC voltages the ion with a specific  $m/z$  ratio reaches a detector.



**Octopole Reaction System (ORS)**

**Fast simultaneous dual mode detector (9 orders dynamic range)**

**HMI gas inlet**

**Cell gas inlet**

**High temperature 27 MHz plasma**

**High-frequency hyperbolic quadrupole**

**Low flow sample introduction system**

**Off-axis lens**

**High-performance vacuum system**

**Agilent 7700x ICP-MS**



ICP-MS Agilent 7700x with GC and HPLC separation system  
*Department of Environmental Sciences, Jožef Stefan Institute*

## Cr : four naturally occurring isotopes

$^{50}\text{Cr}$  - 4.351 %

$^{52}\text{Cr}$  - 83.789 %

$^{53}\text{Cr}$  - 9.501 %

$^{54}\text{Cr}$  - 2.365 %

0.9 % NaCl matrix

HMI

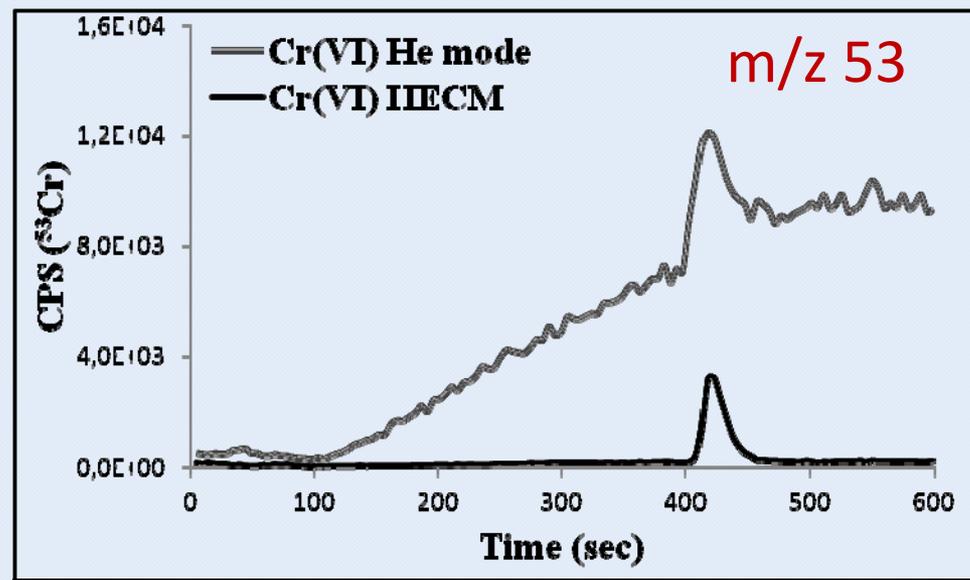
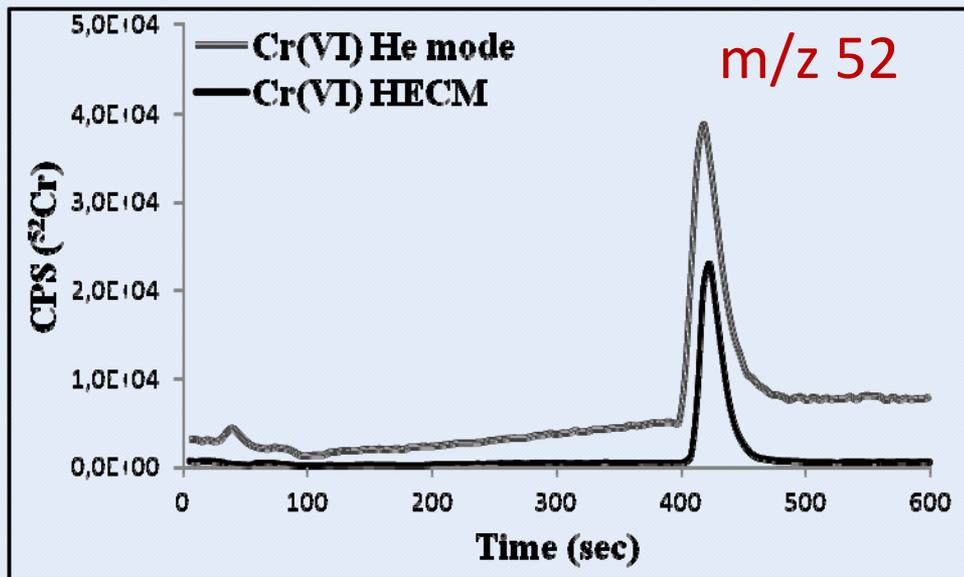
He mode  
HECM

High salt content

Chlorine polyatomic interferences:

$^{35}\text{Cl}^{16}\text{O}^{1}\text{H}^+$ ,  $^{35}\text{Cl}^{17}\text{O}^+$  interfere with  $^{52}\text{Cr}^+$

$^{37}\text{Cl}^{16}\text{O}^+$ ,  $^{35}\text{Cl}^{18}\text{O}^+$  interfere with  $^{53}\text{Cr}^+$



Determination of Cr(VI) by FPLC-ICP-MS using NaCl as eluent. HMI along with He mode or HECM.

## Isobaric interferences

Occur for equal mass isotopes of different elements.

Examples:

$^{58}\text{Fe}$  on  $^{58}\text{Ni}$

$^{64}\text{Ni}$  on  $^{64}\text{Zn}$

$^{48}\text{Ca}$  on  $^{48}\text{Ti}$

Mathematical corrections:

$^{50}\text{Ti}$  and  $^{50}\text{V}$  on  $^{50}\text{Cr}$

$^{46}\text{Ti}$  – 8.25 %

$^{47}\text{Ti}$  – 7.44 %

$^{48}\text{Ti}$  – 73.72 %

$^{49}\text{Ti}$  – 5.41 %

$^{50}\text{Ti}$  – 5.18 %

$^{50}\text{V}$  – 0.250 %

$^{51}\text{V}$  – 99.75 %

$$50 = (50) * 1 - (47) * 0.6962 - (51) * 0.0025$$

## Doubly-charged ion interferences

are due to relatively rare doubly-charged sample ions with twice the mass of the analyte and hence the same mass/charge ratio.

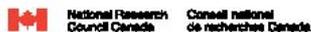
An example:

$^{90}\text{Zr}^{++}$  on  $^{45}\text{Sc}^+$

The formation of doubly-charged species can generally be minimized by optimizing instrument operating conditions.

# Quality control

Certified Reference Material



## CASS-4

### Nearshore Seawater Reference Material for Trace Metals

The following table shows the twelve metals for which certified values have been established. Certified values are based on the results of determinations by at least two independent methods of analysis. Each uncertainty is based on a 95 percent confidence limit for the mean. That is, 95 percent of samples from any bottle would be expected to have concentrations within the specified range 95 percent of the time.

Trace Metal Concentrations (micrograms/litre)

Arsenic (f,h,j,r)	1.11 ± 0.16
Cadmium (c,i,p,r,s)	0.026 ± 0.003
Chromium (i,p)	0.144 ± 0.029
Cobalt (f,i,q)	0.026 ± 0.003
Copper (i,p,r,s)	0.592 ± 0.055
Iron (i,p,s)	0.713 ± 0.058
Lead (c,i,p,s)	0.0098 ± 0.0036
Manganese (f,i,q,s)	2.78 ± 0.19
Molybdenum (d,p)	8.78 ± 0.86
Nickel (c,i,p,r,s)	0.314 ± 0.030
Uranium (p)	(3.0)*
Vanadium (f,q)	1.18 ± 0.16
Zinc (f,i,p,s)	0.381 ± 0.057

\*information value only

- c - Chelation-solvent extraction separation, inductively coupled plasma mass spectrometry (ICPMS)
- d - Direct determination by graphite furnace atomic absorption spectrometry (GFAAS)
- f - Reductive precipitation separation, ICPMS determination
- h - Hydride generation, electrothermal vaporization ICPMS determination
- i - Immobilized ligand separation, GFAAS determination
- j - Hydride generation, atomic absorption determination
- p - Immobilized ligand separation, isotope dilution ICPMS
- q - Immobilized ligand separation, ICPMS
- r - Reductive precipitation separation, GFAAS determination
- s - Chelation-solvent extraction separation, GFAAS determination

Analysis of standard reference materials, spiking of samples

CASS-4, Nearshore Seawater for Trace Elements (National Research Council Canada)

Trace Elements in Surface Waters SPS-SW1 (Spectrapure Standards As, Oslo, Norway)

Recoveries for determined and calculated concentrations of elements by ICP-MS in standard reference material **sea water CASS-4**. Results are given as mean concentration of three parallel determinations  $\pm$  standard deviation.

<b>Element</b>	<b>Determined concentration (<math>\mu\text{g/L}</math>)</b>	<b>Calculated value after spiking with 5 <math>\mu\text{g/L}</math> of particular element (<math>\mu\text{g/L}</math>)</b>	<b>Recovery (%)</b>
Cr	5.32 $\pm$ 0.17	5.17 $\pm$ 0.03	103
Co	4.96 $\pm$ 0.14	5.023 $\pm$ 0.003	99
Ni	5.55 $\pm$ 0.21	5.34 $\pm$ 0.03	104
Cu	5.70 $\pm$ 0.17	5.65 $\pm$ 0.06	101
Zn	5.60 $\pm$ 0.25	5.44 $\pm$ 0.06	103
As	6.58 $\pm$ 0.34	6.27 $\pm$ 0.16	105
Se	5.88 $\pm$ 0.33	5.55 $\pm$ 0.19	106
Mo	14.78 $\pm$ 0.48	14.64 $\pm$ 0.86	101
Cd	5.13 $\pm$ 0.24	5.029 $\pm$ 0.003	102
Sb	5.10 $\pm$ 0.15	5.23 $\pm$ 0.10	98
Pb	5.03 $\pm$ 0.14	5.006 $\pm$ 0.004	101
Ag	4.88 $\pm$ 0.13	5.004 $\pm$ 0.001	98

Recoveries for determined and calculated concentrations of elements by ICP-MS in quality control material for **surface waters SPS-SW1**. Results are given as mean concentration of three parallel determinations  $\pm$  standard deviation.

Element	Determined concentration ( $\mu\text{g L}^{-1}$ )	Certified concentration ( $\mu\text{g L}^{-1}$ )	Recovery (%)
Al	49 $\pm$ 1	50 $\pm$ 1	98
Cd	0.48 $\pm$ 0.04	0.50 $\pm$ 0.01	96
Pb	4.91 $\pm$ 0.04	5.0 $\pm$ 0.1	98
Ni	9.79 $\pm$ 0.13	10.0 $\pm$ 0.1	98
As	10.30 $\pm$ 0.22	10.0 $\pm$ 0.1	103
Cu	19.46 $\pm$ 0.32	20 $\pm$ 1	97
Zn	20.42 $\pm$ 0.53	20 $\pm$ 1	102
Fe	21 $\pm$ 1	20 $\pm$ 1	105
Cr	1.96 $\pm$ 0.03	2.00 $\pm$ 0.02	98
Mo	9.67 $\pm$ 0.1	10.0 $\pm$ 0.1	97
Co	1.91 $\pm$ 0.03	2.00 $\pm$ 0.02	96
Se	2.05 $\pm$ 0.10	2.00 $\pm$ 0.02	103

Concentrations of elements in control samples and titanium chloride leachates determined by ICP-MS. Standard deviation of measurement is better than  $\pm 3\%$  for all measured elements.

Element	Control ( $\mu\text{g L}^{-1}$ )	Titanium chloride ( $\mu\text{g L}^{-1}$ )
$^{56}\text{Fe}$	0.112	18.0
	0.021	16.0
	0.082	21.8
$^{60}\text{Ni}$	1.953	4.98
	1.941	4.56
	1.879	6.30
$^{75}\text{As}$	0.023	0.05
	0.026	0.03
	0.021	0.03
$^{208}\text{Pb}$	0.44	0.43
	0.32	0.67
	0.26	0.32

Element	Control ( $\mu\text{g L}^{-1}$ )	Titanium chloride ( $\mu\text{g L}^{-1}$ )
$^{27}\text{Al}$	0.252	50.4
	1.092	35.8
	0.003	63.2
$^{47}\text{Ti}$	0.035	9.40
	0.051	80.0
	1.487	105
$^{51}\text{V}$	0.028	0.08
	0.028	0.07
	0.025	0.09
$^{52}\text{Cr}$	0.015	0.17
	0.012	1.15
	0.008	0.23

## Conclusion

ICP-MS enables reliable determinations of metal ions released from implants.

Leaching experiments should be planned together with material scientists and analytical chemists.

Interpretation of analytical data should be made together with medical doctors.