BfR-Symposium
„Die Rolle der Bioverfügbarkeit im Rahmen der Risikobewertung am Beispiel Spurenelemente“
16. – 17.01.2013

Möglichkeiten zur Bestimmung der Bindungsform von Spurenelementen

Methods for Element Speciation

Bernhard Michalke

Definitions of terms related to speciation according to reference [1].

Chemical species

Specific form of a chemical element defined as molecular or complex structure, or oxidation state.

Speciation of an element

Distribution of defined chemical species of an element in a system.

Speciation analysis.

The analytical activity of identifying and measuring chemical species

⟶ identifying and measuring = a clear identification of the species

+ exact quantification + representative sample + quality controlled.

Operationally and functionally defined species characterization

Operationally = characterization of molecule groups (not single species) according to the similar behaviour during an analytical procedure, such as extraction. The identification of the single species is missing.

Functionally: = characterization of molecule groups concerning their impact on e.g. organisms.

[1] Templeton D M, Ariese F, Cornelis R, Danielsson .-G, Muntau H, van Leeuwen H P, Lobinski R. 2000. Pure Applied Chem 72: 1453 – 1470.

Examples for chemical element species

1. Different oxidation state

2. Metal-organyles:

3. Organo-metal-complexes

4. Ionic species and hydrated ions

5. Metalspecies present as highly disperse colloides

6. colloid-bonded metals

Speciation analysis conceps should be introduced into all steps of an analytical process:

- Sampling
- Storage
- Sample preparation
- Analysis
  - Chosing the most suitable method

⇔

Quality management and quality control
Avoid…

- Contamination
- Losses
- Species conversions
  - -by oxidation
  - - by bacterial activity

Provide…

- Clear species identification
  - -by orthogonal identification concepts

Quality control

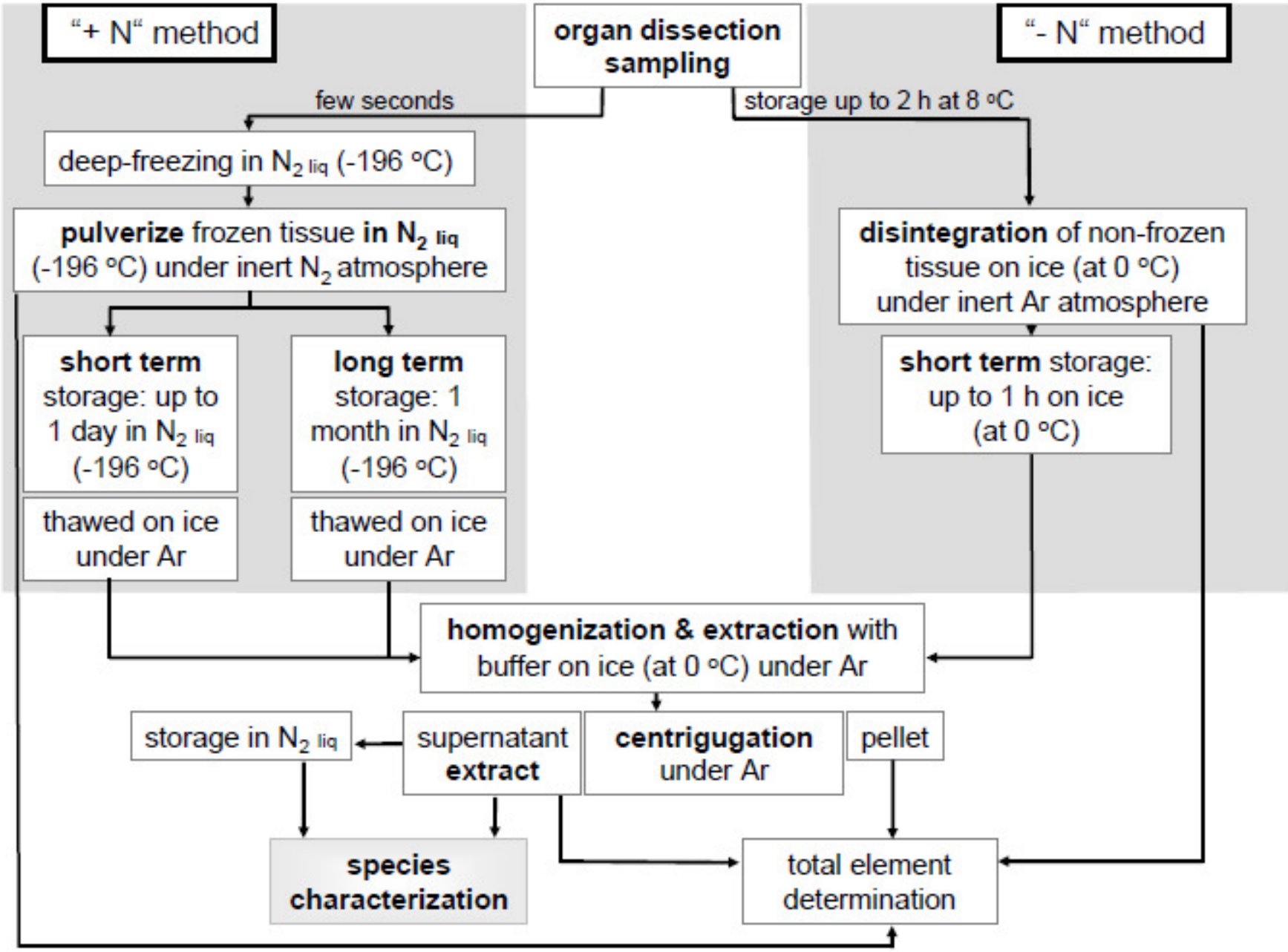
…sampling:	**•Sample respresentativity;** **•Short sampling time;** **•Avoid contamination;** **•Keep volume/surface ratio high: less container wall effects;** **•Use sampling devices from PEEK or quartz:** **Stainless steel devices can cause contamination (Fe, Ni, Cr, Mn) and species transformation;**

Quality control

…storage:	**•Short storage at 4 °C, long term storage : $N_{2\ liq}$ shock freezing and storage in $N_{2\ liq}$ or at -80 °C;**
…sample preparation:	**•Extraction procedures result mostly in „operationally defined“ species characterization:** **•Chose carefully extraction parameter and/or use species preserving extraction schemes:** **E.g. aiming for:** **Water soluble species: (hot) water extraction** **Digestion (stomach, intestine): extraction with simulated gastric juice** **extraction with proteases** **extraction with lysozyme** **native species (unchanged): pH adopted, low temperature, no O_2** **•Mass balances and recovery rates should be determined (species spikes).**

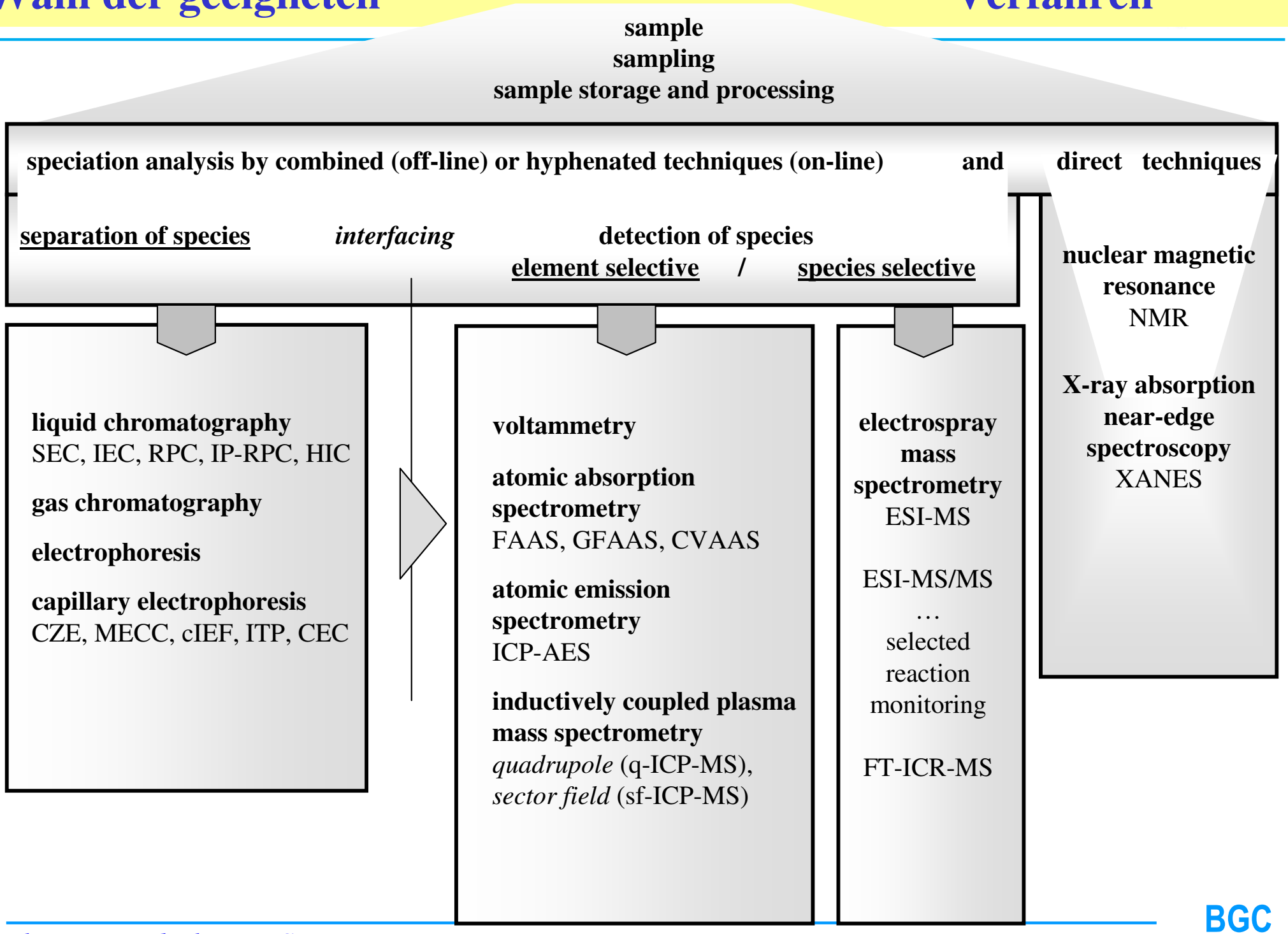
Extraction schemes

Diederich & Michalke 2010 | Nischwitz, Michalke, Kettrup 2003



Literature: Nischwitz, V., Michalke, B., Kettrup, A.Investigations on species-preserving extraction from liver samples, Analytical and Bioanalytical Chemistry, **(2003)** 375: 145 - 156
Diederich, J., Michalke B..Enhanced extract preparation for manganese and iron speciation in brain and liver tissue, Anal Bioanal Chem., **2010,** 399:1799–1806.

Wahl der geeigneten Verfahren



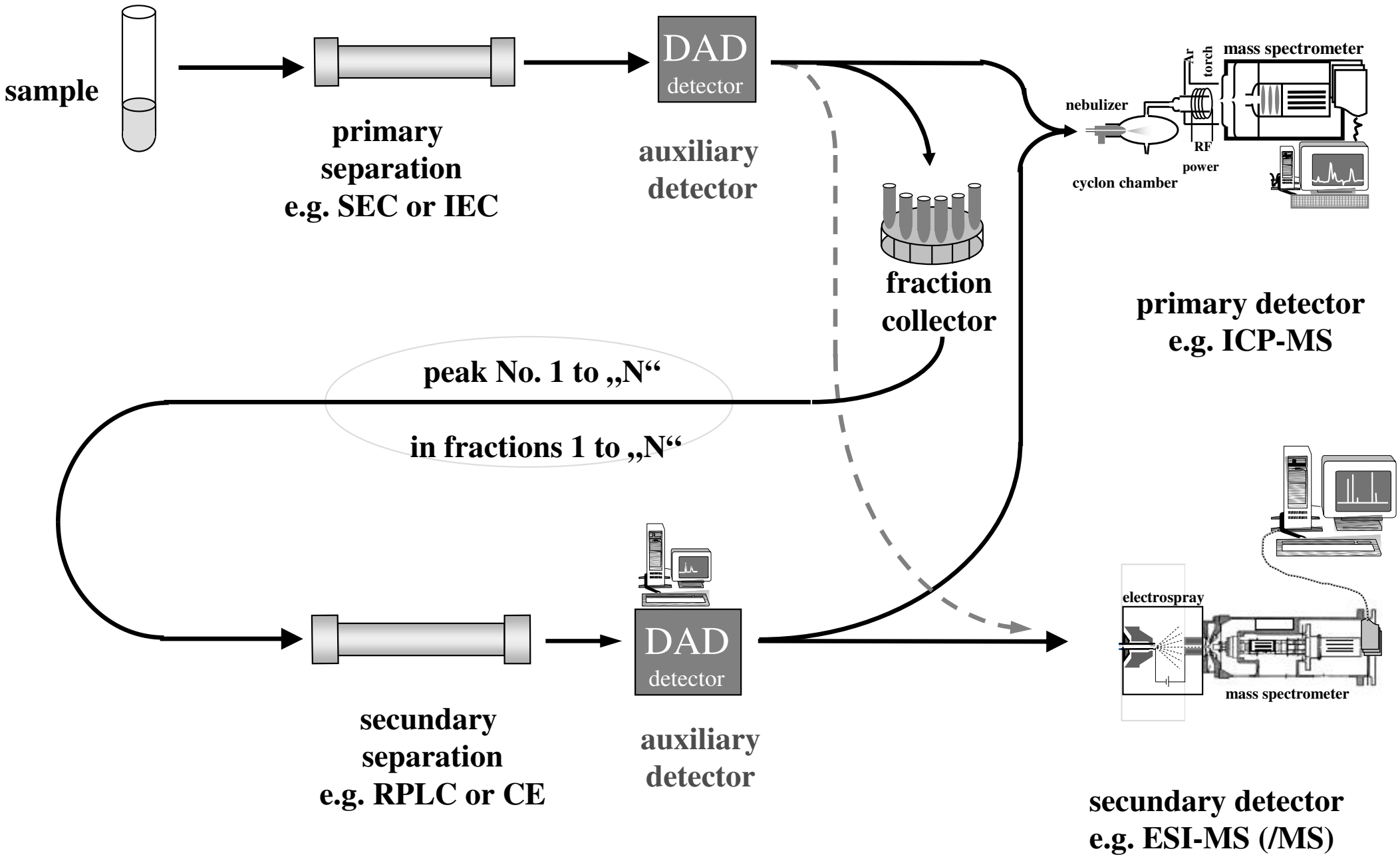
advantages and limitations of separation technologies in speciation

separation	positive aspects	negative aspects
SEC	Mass characterization of unknown compounds Gentle method, mostly preserving even labile biomolecules	•Limited peak capacity •Incomplete resolution of peaks in natural samples •Electrostatic effects, adsorption, hydrophobic interaction observed
IEC	• High separation efficiency and • wide applicability • Relative retention canbe governed by three variables • (pH, ionic strength, nature of ion exchanger) • IEC is predestined for separation of kovalently bound element species	•Pore size must be adopted to species •Changes in column temperature may result in changed column efficiency and selectivity •Looslely bound metal ions get lost or replaced
RPLC and IP-RPLC	Wide analyte spectrum Very efficient separation High flexibility	•Undesired adsorption effects •Eluents may change species/destabilization •Loosely bound elements get released •Species transfer reactions •Use of organic solvents: changes in ionzation characteristics, destabilization of plasma or extinction, polyatomic C-interferences, C precipitation, flash over
CE	Very efficient separation Different separation principles available (CZE, IEF, MEKC, ITP, CEC) Short analysis time	•Worse concentration detection limits •Need for interface designs for hyphenation •Suction flow during hyphenation

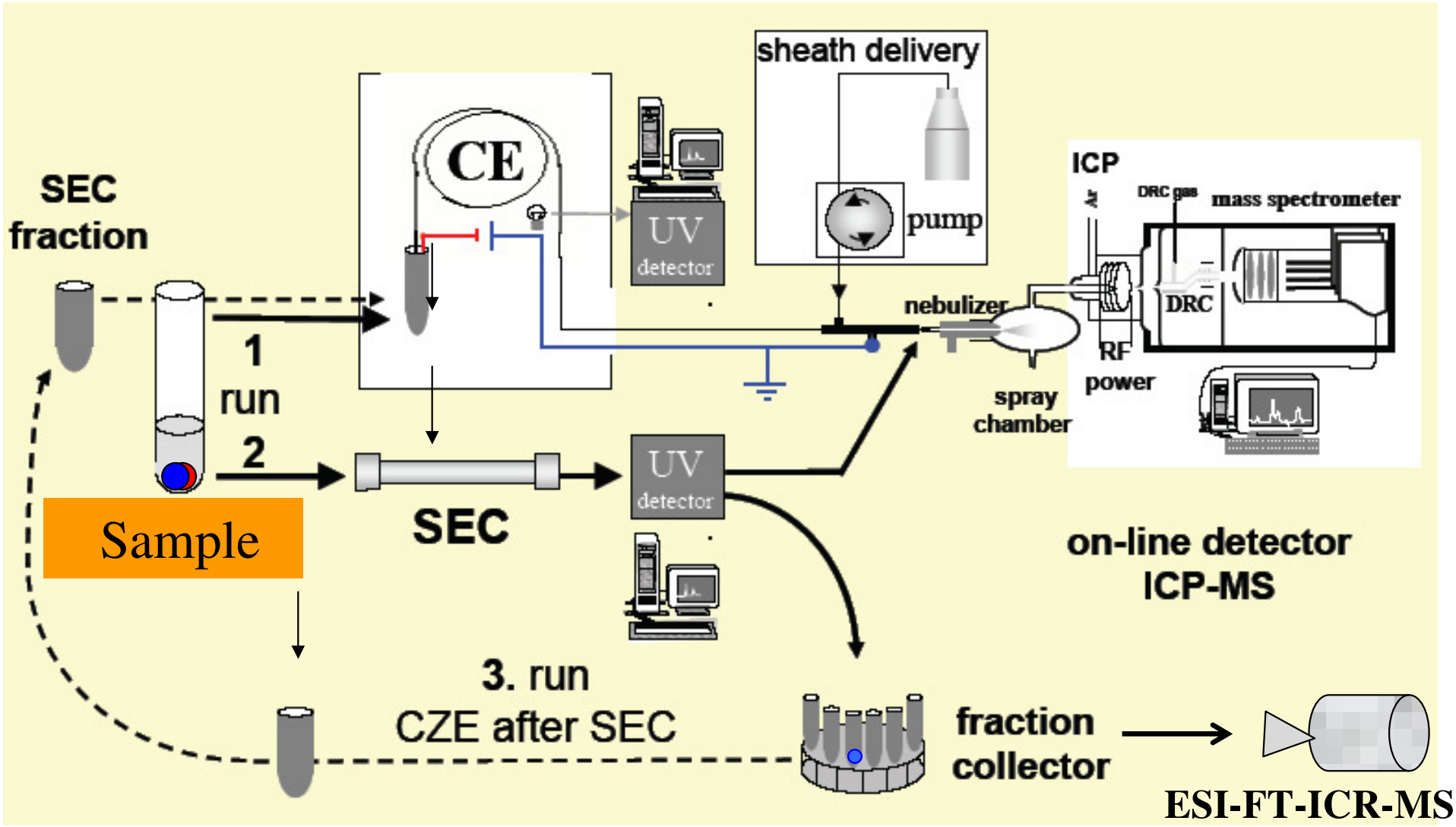
advantages and limitations of detection technologies in speciation

method	positive aspects	negative aspects
AAS	comparatively cheap element selective detectors **HGAAS: selective derivatization for matrix separation and detectibility of species** GFAAS shows best (AAS) detection sensitivity	FAAS shows insufficient detection sensitivity **The detector response is strongly species dependent** GFAAS: unsuitable for on-line coupling due to discontinuous measurement
ICP-AES	multi-element capability and high sensitivity **on-time multi element monitoring in hyphenated systems possible**	for ultra-trace levels insufficient
ICP-MS 	detection selectivity, multi-element capability best sensitivity **suitable for ultra-trace levels (especially sf-ICP-MS)** isotope information	polyatomic interferences (q-ICP-MS !) **monitoring of several isotopes of one element necessary** sequential detector: if too much isotopes in parallel the detector gets too slow for highly resolved, fast appearing peaks
ESI-MS 	suitable for extremely low flow rates **the whole species is detected** capability to produce multi-charged ions: analysis up to MW = 200 000 possible **MS/MS mode :structural information**	ion-solvent clusters **electrolytic processes: generating new species, species transformation** gas phase ligand exchange **gas phase intra molecular charge transfer**

orthogonal speciation scheme

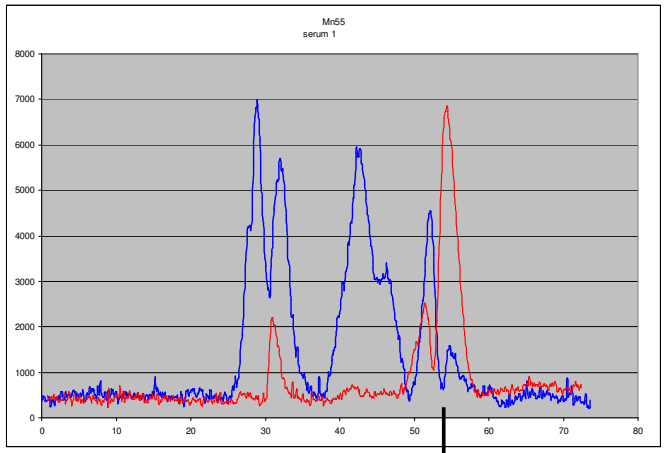


Bioavailability	⟶ barrier: intestine
Example for speciation analysis at	⟶ blood brain barrier: Mn speciation in paired serum / CSF samples

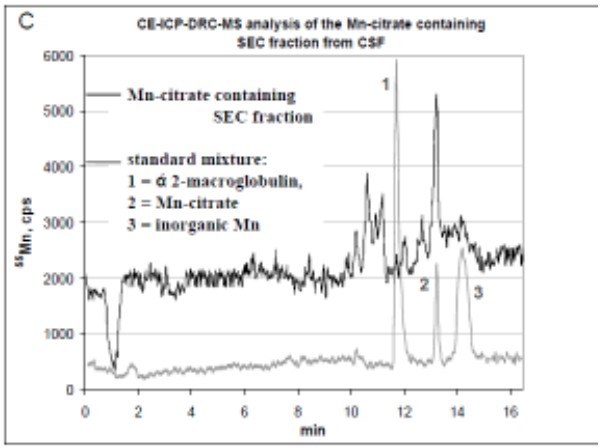


Example for 2D- orthogonal identification: Mn-speciation

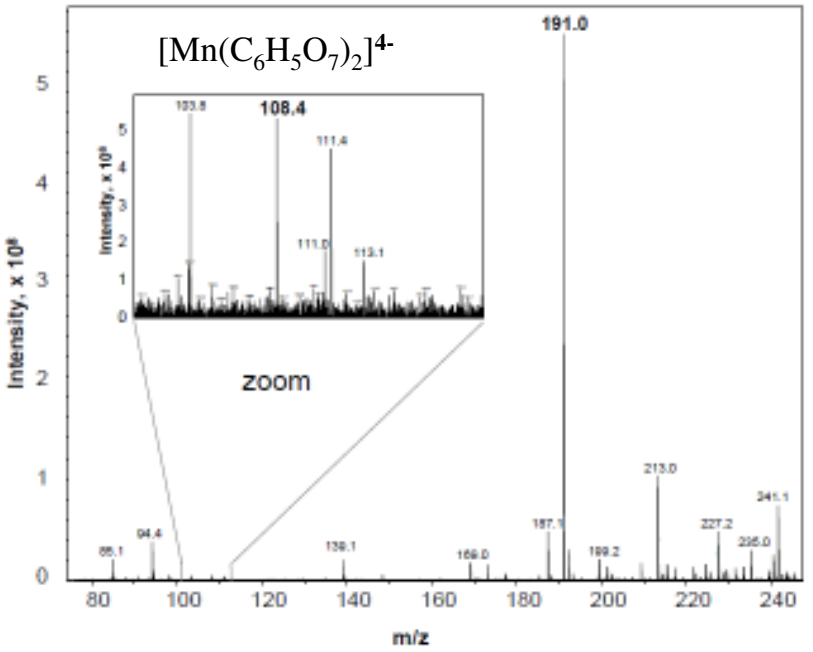
SEC-ICP-DRC-MS



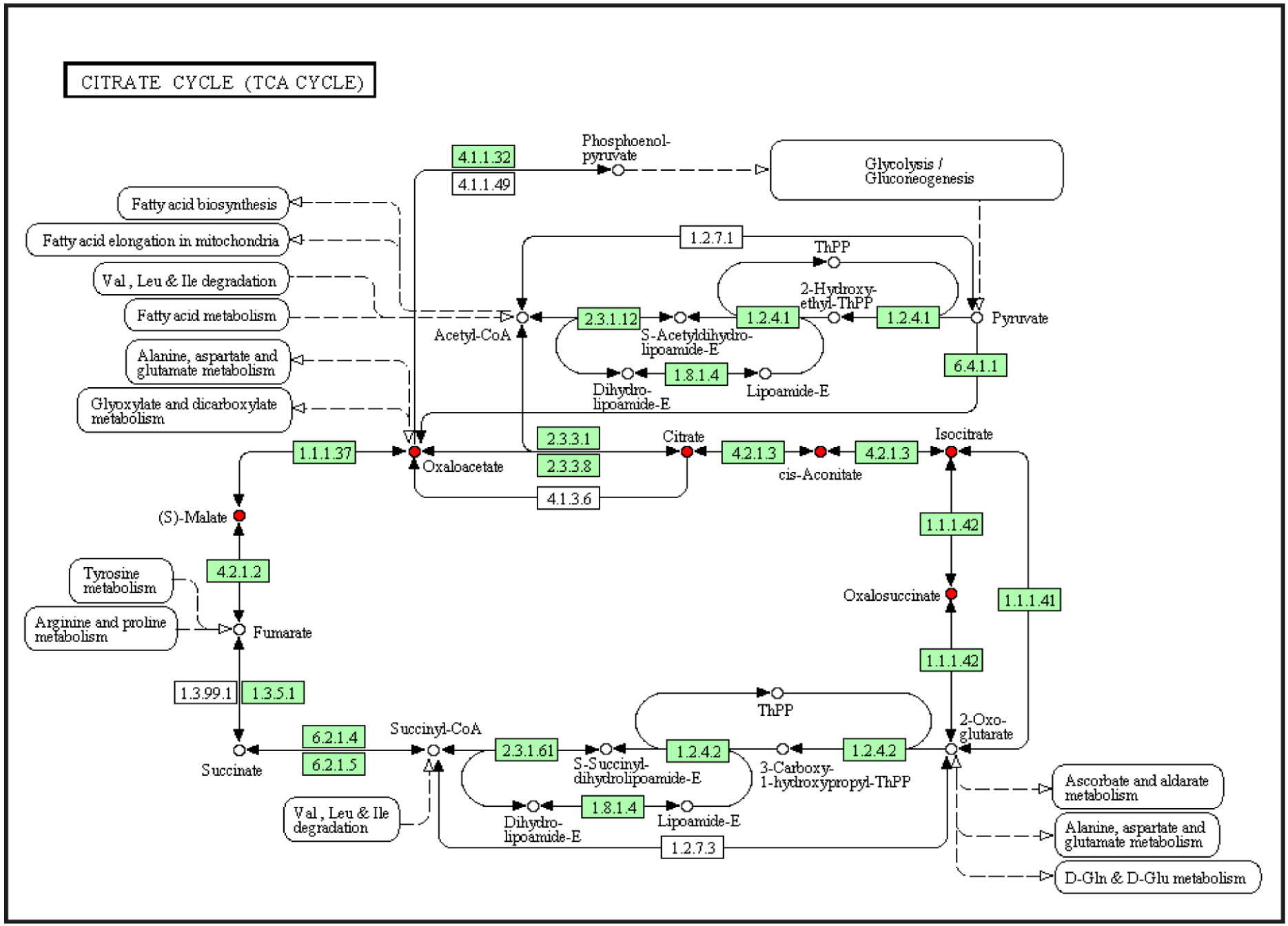
CE-ICP-DRC-MS



ESI-FT-ICR-MS

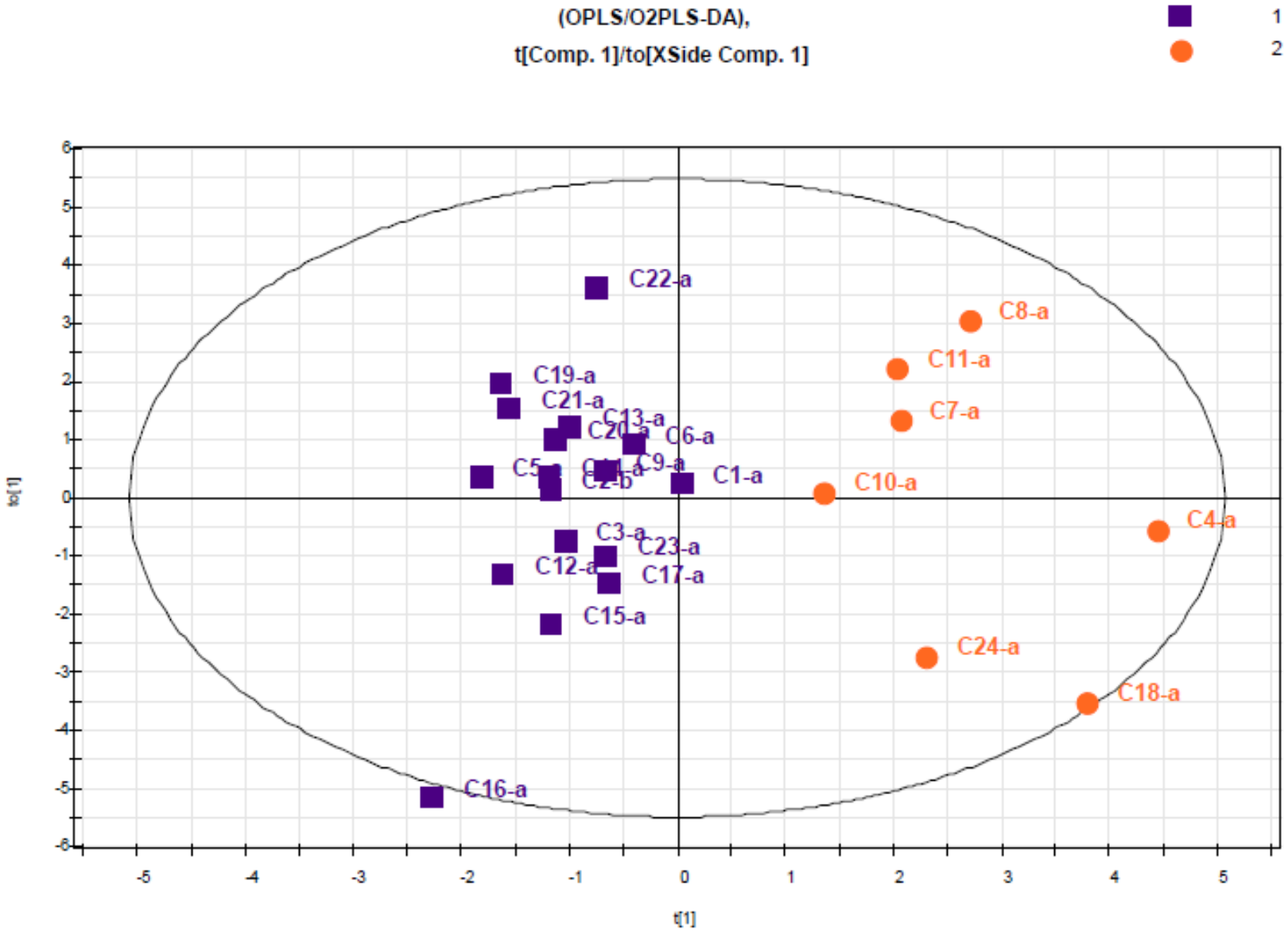


Analysis with ESI-FT-ICR-MS and processing with MassTrix



BGC

Suhre K, Schmitt-Kopplin Ph, (2008) MassTrix: Mass translator into pathways. Nucleic Acid Research 36/2: W481 –W484.
Michalke, B., Lucio, M., Berthele, A., Kanawati, B. Manganese speciation in paired serum and CSF samples using SEC-DRC-ICP-MS and CE-DRC-ICP-MS, Anal Bioanal Chem, 2013, DOI 10.1007/s00216-012-6662-7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C4-a
C3-a
C23-a
C12-a
C17-a
C15-a
C24-a
C18-a
C16-a
to[1]
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in paired samples

Total Mn concentration in CSF versus Mn-total, Mn-Tf or Mn-Citrate, each in serum:

Mn-total in CSF is related to

Mn-transferrin in serum for $\mathbf{Mn_{total(serum)}}$ < 1.55 µg/L

but is related to

Mn-citrate in serum for $\mathbf{Mn_{total\ (serum)}}$ > 1.55 µg/L

⇨ **Potential for *future methods for biomonitoring***

Michalke, B., Lucio, M., Berthele, A., Kanawati, B. Manganese speciation in paired serum and CSF samples using SEC-DRC-ICP-MS and CE-DRC-ICP-MS, Anal Bioanal Chem, 2013, DOI 10.1007/s00216-012-6662-7

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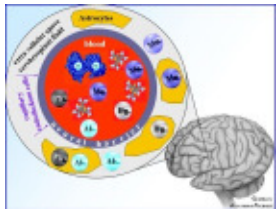
Thank you for your attention

Group: Metallomics – Elements and Element Speciation

Metallomics - Element Speciation

- specifically related to neurodegenerative diseases



Central Inorganic Analytics Service

Consultation
Sample Preparation
Sample Analysis
Analytical Quality Control