



VI Bioanalytical School

CHEMICAL SPECIATION ANALYSIS AND ABSOLUTE QUANTIFICATION OF PROTEINS: BIOANALYTICAL APPROACHES USING ICP-MS AND IDA

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Analytical Chemistry: Definition

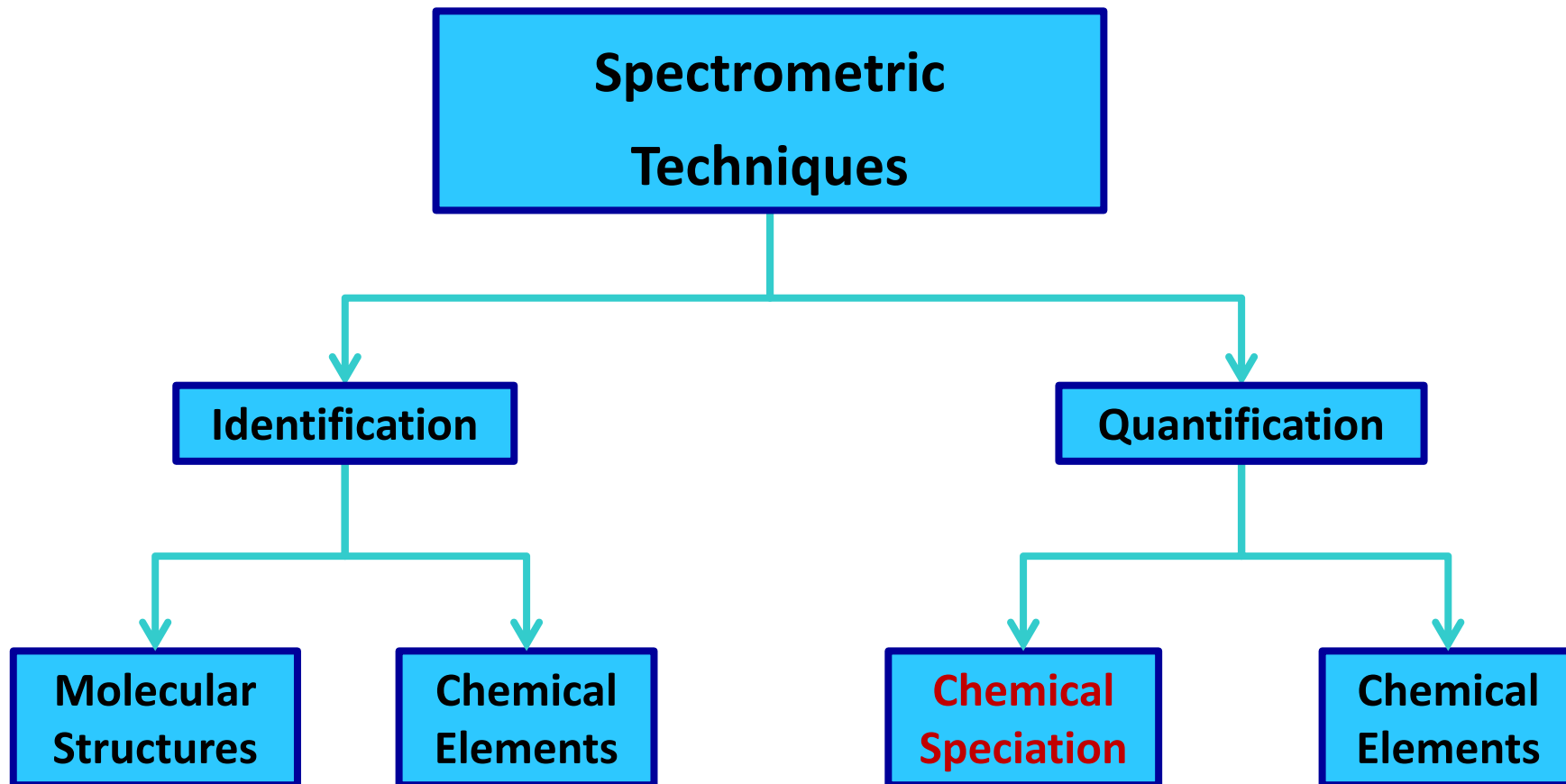


INTERNATIONAL UNION OF
PURE AND APPLIED CHEMISTRY

Analytical chemistry is a scientific discipline that develops and applies methods, instruments, and strategies to obtain information on the composition and nature of matter in space and time, as well as on the value of these measurements, (i.e., their uncertainty, validation, and/or traceability to fundamental standards).



Introduction





Introduction

FRACTIONATION AND SPECIATION – IUPAC Definition

- ✓ **Chemical species:** Specific form of a chemical element;
- ✓ **(Chemical) Speciation Analysis:** Analytical activities to identify or quantify one or more chemical species in a sample;
- ✓ **Speciation of an element:** Distribution of an element amongst defined chemical species in a system;
- ✓ **Fractionation:** Process of classification of an analyte or a group of analytes in a sample according to physical or chemical properties.



Introduction: Omics Sciences

- ✓ Genomics, proteomics, metabolomics.
- ✓ Identify, Characterize and quantify all biological molecules involved in organisms function;
- ✓ Metallomics: interactions between metals and living systems
- ✓ Chemical speciation analysis is fundamental to metallomics.



Introduction

TOTAL QUANTIFICATION



SPECIATION

Do NOT give information about:

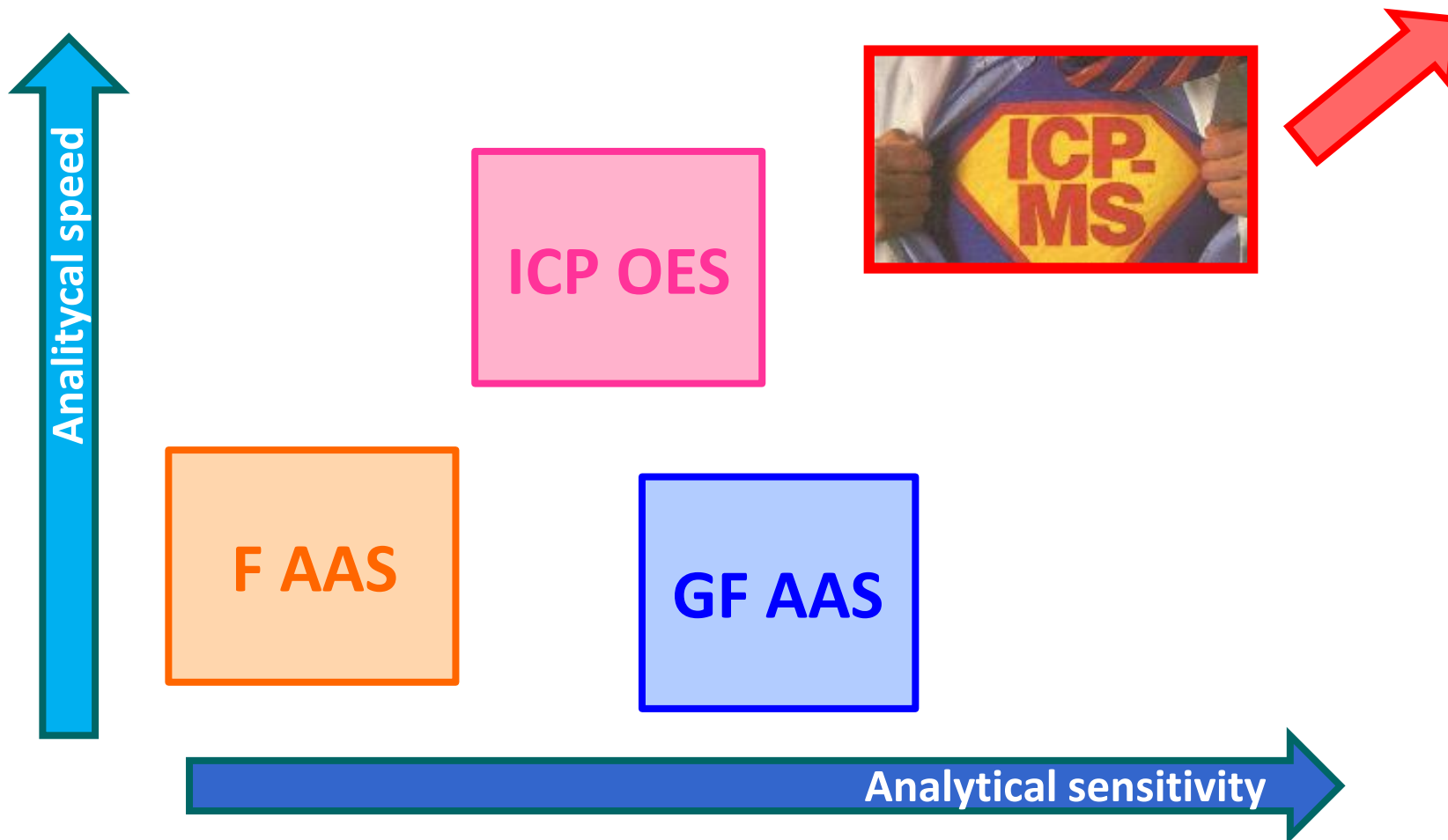
- Mobility;
- Bioavailability;
- Human life impact.

GIVES information about:

- Bioavailability;
- Essentiality;
- Toxicity.



Introduction





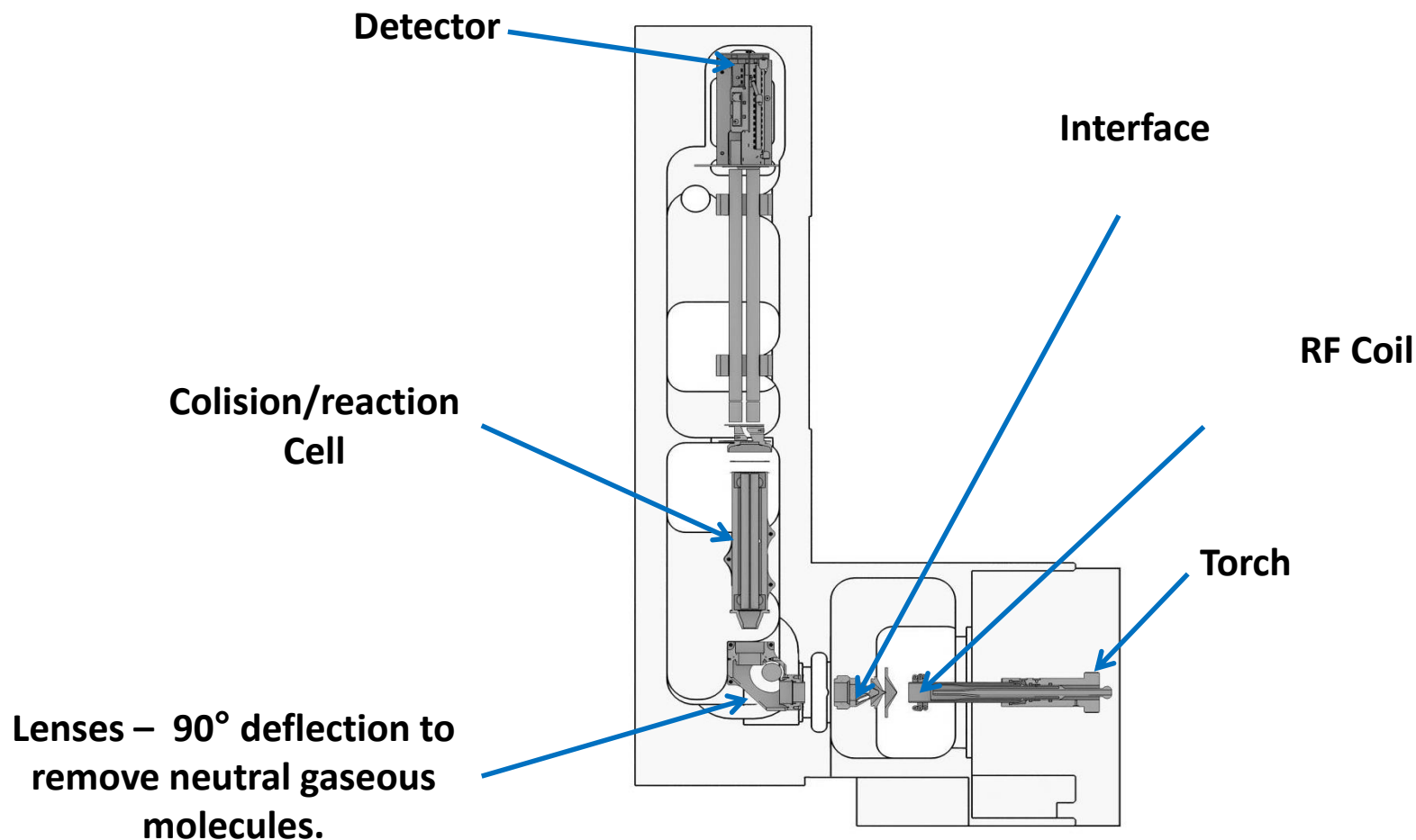
ICP-MS

- Principle:

It is based on the determination of chemical elements (Isotopes and isotope ratios) using the mass spectrometry (MS) of ions produced by an inductively coupled plasma (ICP).



The ICP-MS equipment

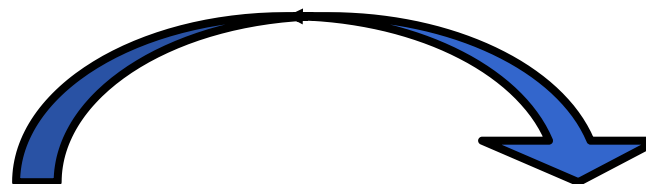


Introduction: Isotopic Dilution Analysis (IDA)

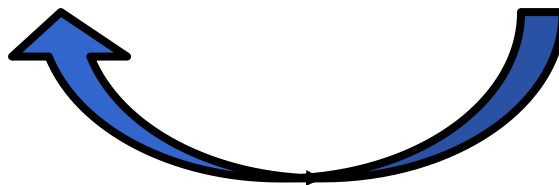
How many fish are there in the lake?



- We can fish all fish in the lake ;



- or we can use the isotope dilution concept



Introduction: Isotopic Dilution Analysis (IDA)

How many fish are there in the lake?

- Using the isotope dilution concept.

$$\frac{N_{Total}}{N_{Adicionados}} = \frac{N_{Natural}}{N_{Marcado}}$$

$$N_{Total} = \frac{N_{Natural} \cdot N_{Adicionados}}{N_{Marcado}}$$



Introduction: Isotopic Dilution Analysis (IDA)

IDA Equations

$$C_s = C_t \cdot \frac{m_s}{m_t} \cdot \frac{M_s}{M_t} \cdot \frac{A_t^b}{A_s^a} \left(\frac{R_m - R_t}{1 - R_m \cdot R_s} \right)$$

$$M_s = C_t \cdot f_t \cdot \frac{M_s}{M_t} \cdot \frac{A_t^b}{A_s^a} \left(\frac{R_m - R_t}{1 - R_m \cdot R_s} \right)$$





Introduction: Isotopic Dilution Analysis (IDA)

How to perform isotope ratio measurement?

- *In quadrupole ICP-MS, are required some corrections;*

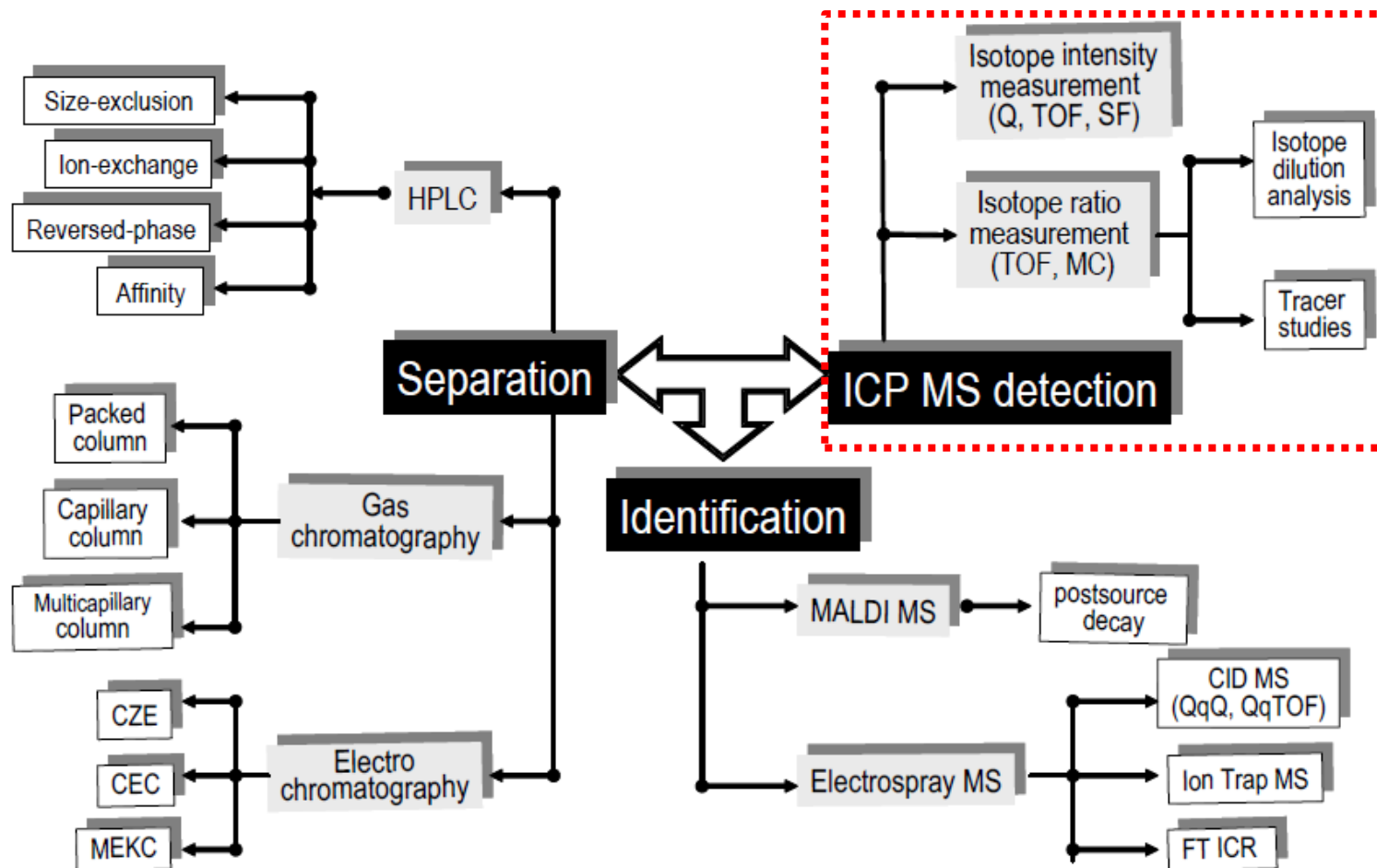
- *Dead time correction;*
$$I_{\text{corrigida}} = \frac{I_{\text{medida}}}{1 - I_{\text{medida}} \cdot T(s)}$$

- *Mass discrimination factor;*
$$R_{\text{corrigida}} = \frac{R_{\text{medida}}}{1 + F \cdot \Delta m}$$

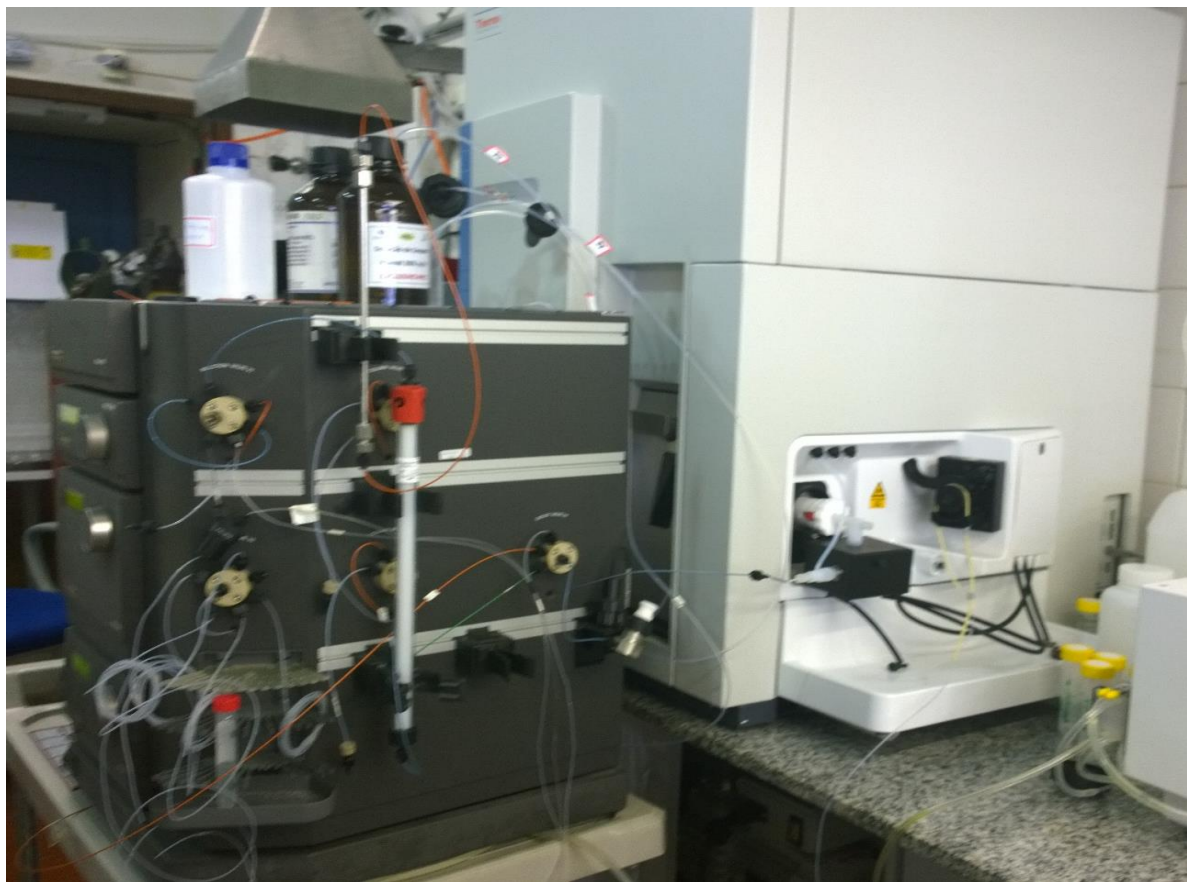
- *Isobaric interferences corrections.*



Principal techniques for chemical speciation



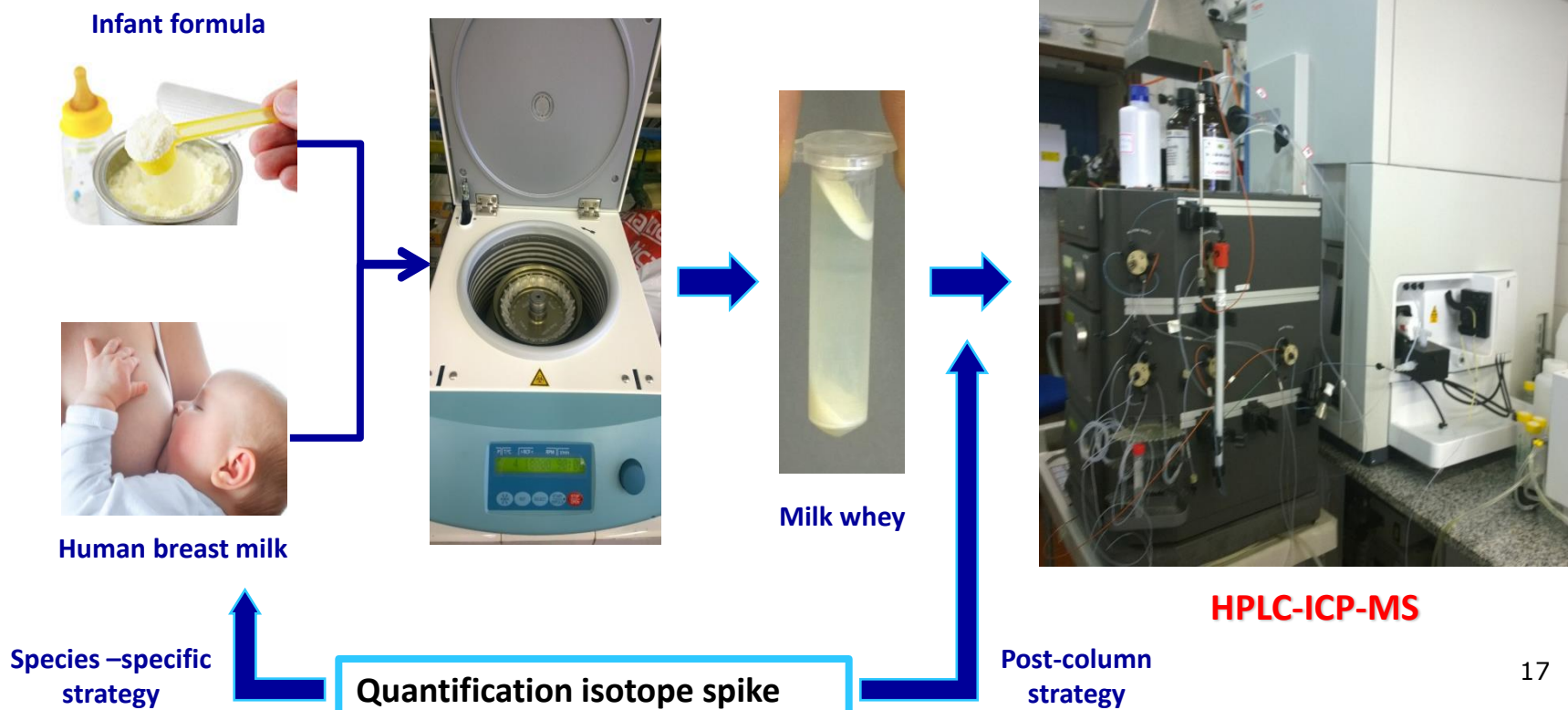
Introduction



HPLC-ICP-MS Speciation system (LaDA/IC/UFRJ)

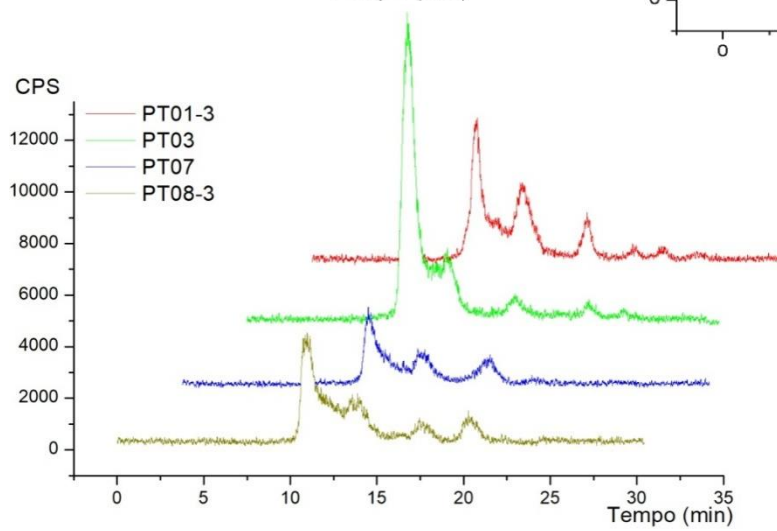
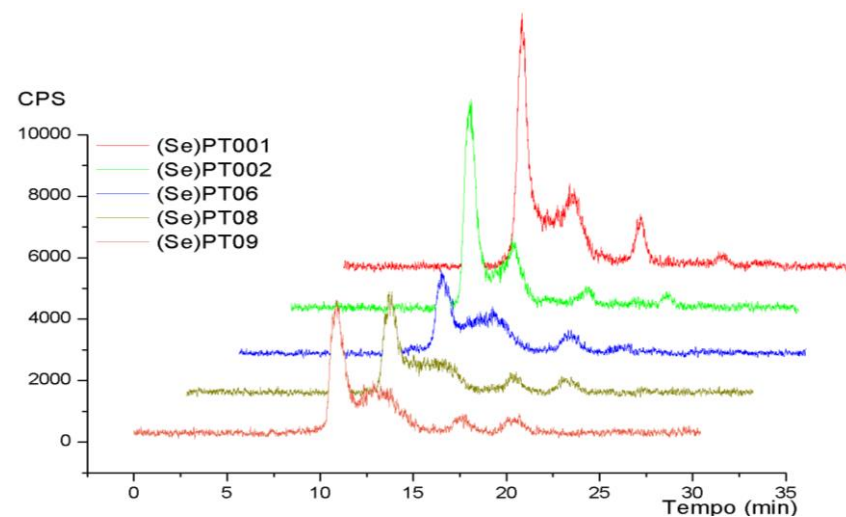
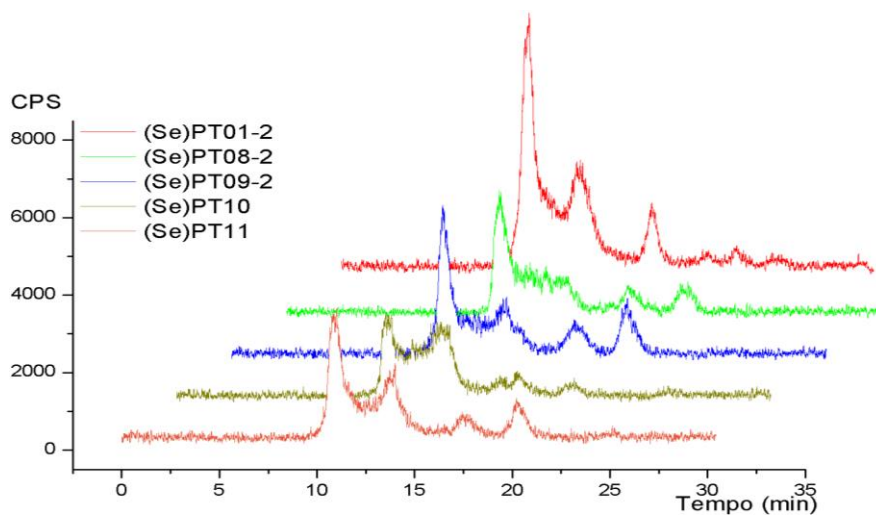
Speciation of biometals in milk

Sample preparation for CSA



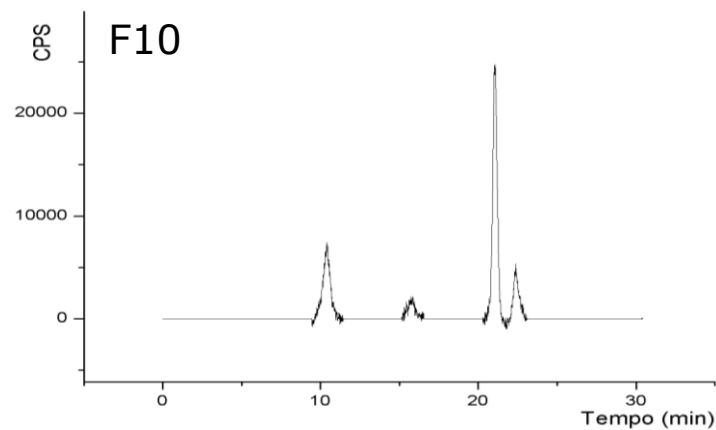
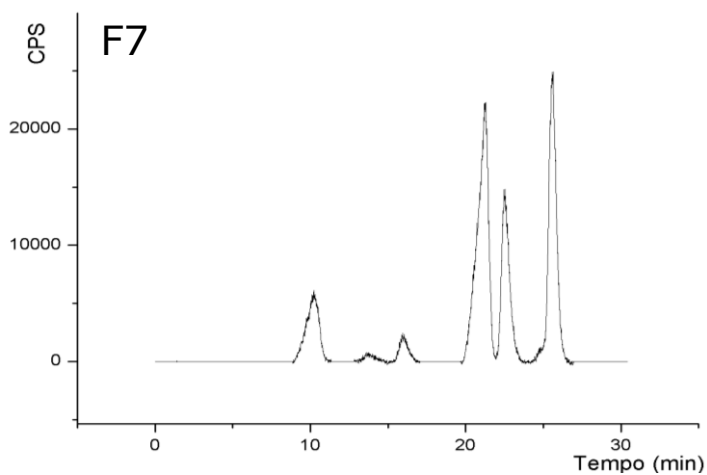
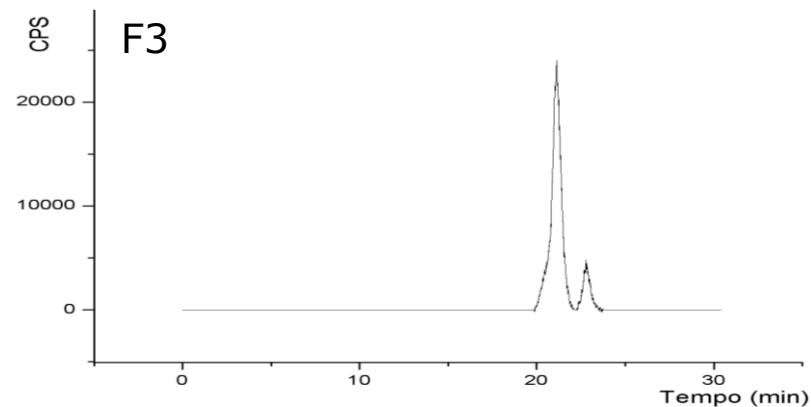
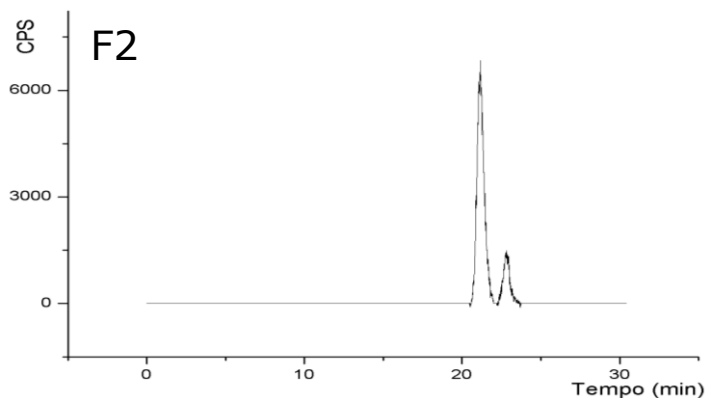
Speciation of biometals in milk

Selenium speciation in human milk (full-term birth)



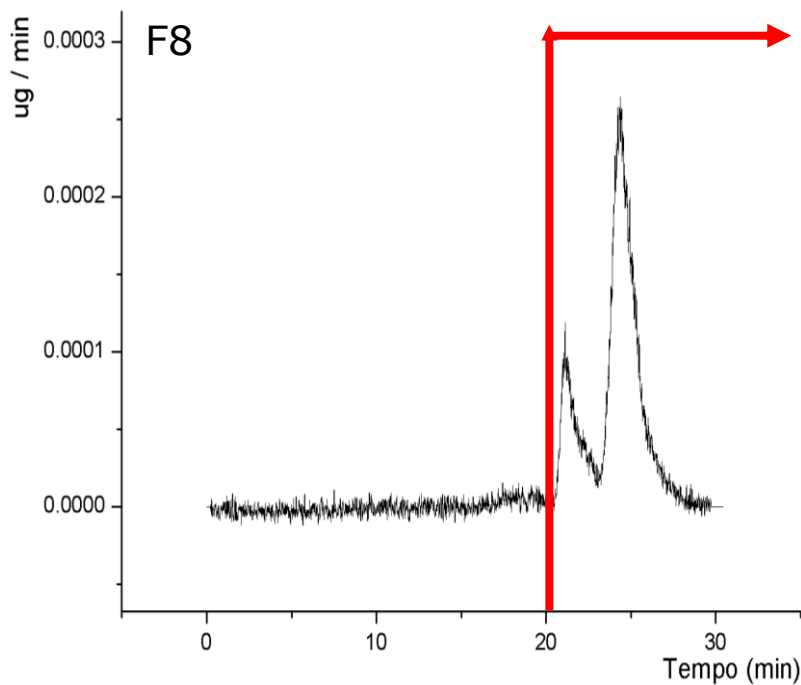


Speciation of biometals in milk

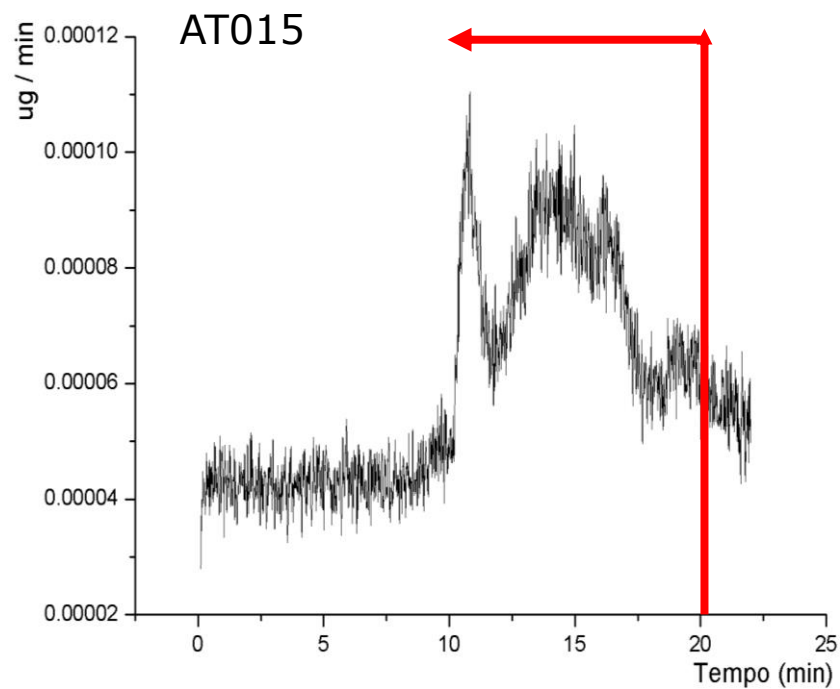


Speciation of biometals in milk

Infant Formula



Human breast milk





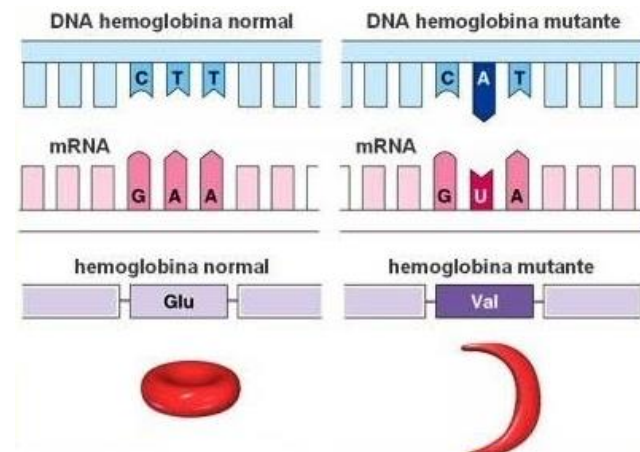
Absolute quantification of proteins

- ✓ Direct quantification of proteins through any element except of C, N, H or O.
- ✓ **Heteroatoms**: any other elements which are part of the structure of proteins (**S, Se, P and metals**);
- ✓ Any other element intentionally tagged (heteroatom tagged-target proteomics) present in protein (**Ru**).

Absolute quantification of proteins

• Sickle cell disease

- ✓ Hemoglobinopathy due the polymerization of deoxygenated hemoglobin, sickle cell;
- ✓ Characterized by chronic hemolysis and inflammation;
- ✓ May be asymptomatic or severe;
- ✓ It is the hereditary disease with the highest occurrence in brazil (and in the world);
- ✓ 14-years (1970) and 48-years (2018) life expectancy.



PLATT, O. S. et al. New Eng. J. Med. 330 (1993) 1639.

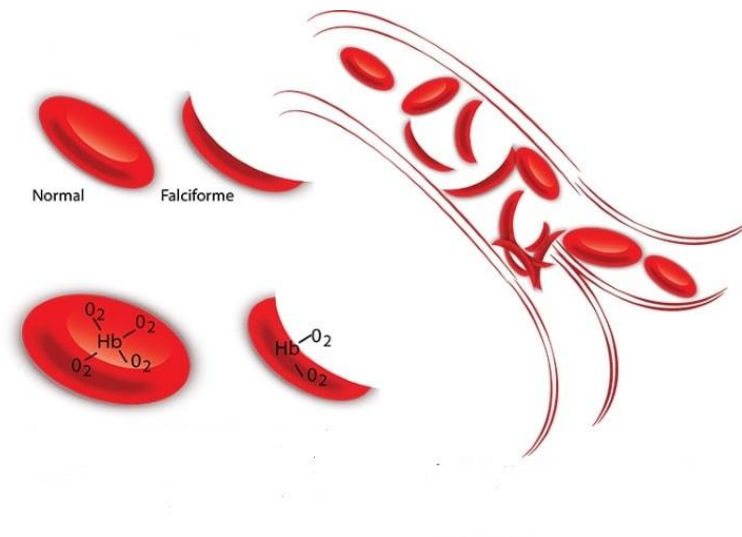
BRASIL. ANVISA. Manual de Diagnóstico e Tratamento de Doenças Falciformes. Brasília, 2002, 142 p.

REES, D. C.; WILLIAMS, T. N.; GLADWIN, M. T. Lancet 376 (2010) 2018.

Absolute quantification of proteins

• How to treat?

- ✓ Almost (always) PALLIATIVE;
- ✓ Hydroxyurea;
- ✓ Blood transfusion.

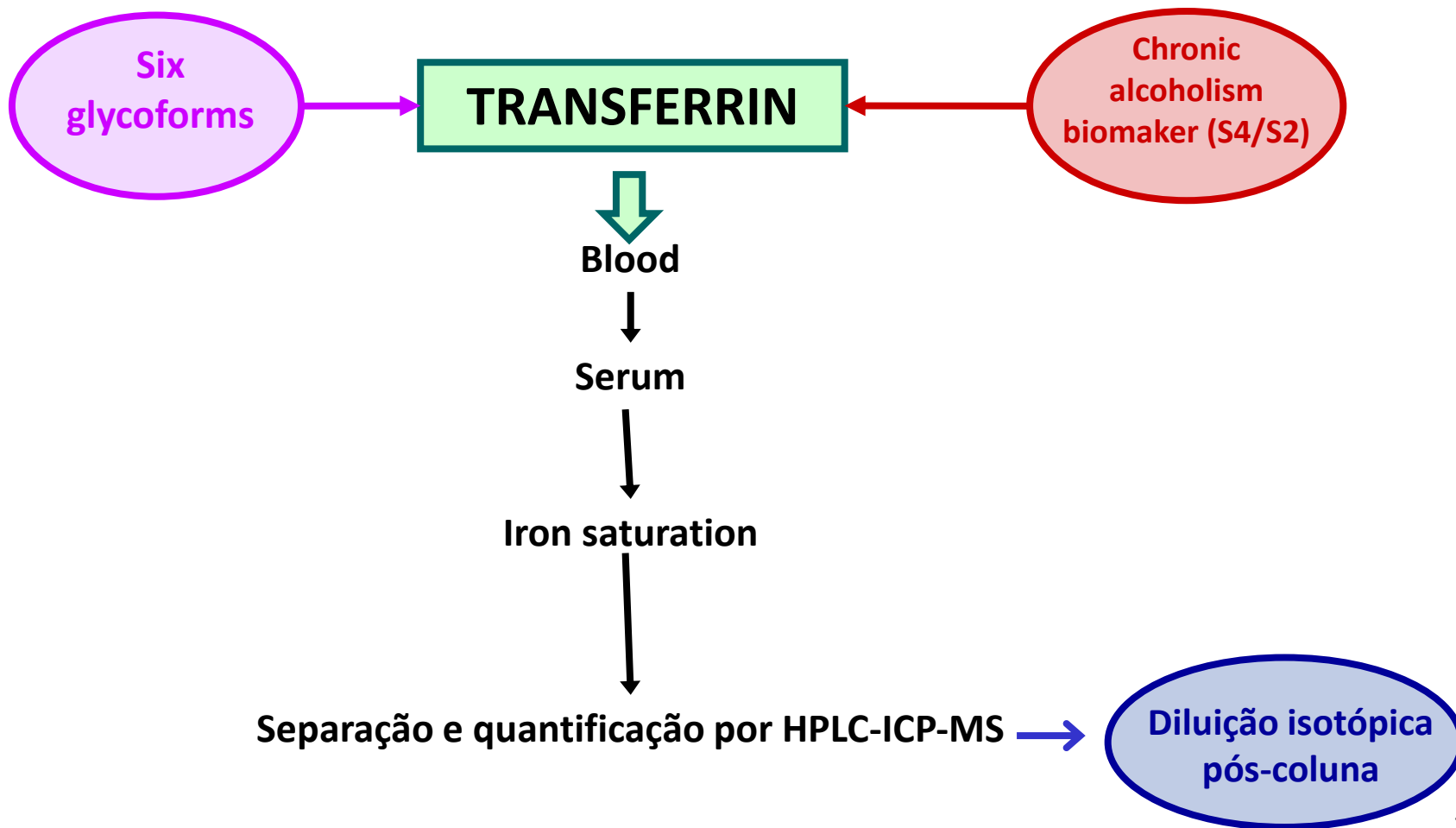


• Treatment consequences

- ✓ Hydroxyurea is a chemotherapeutic agent;
- ✓ Approximately 250 mg of Fe per transfusion unit;
- ✓ Risk of iron overload → Reliable diagnosis → Iron chelating.

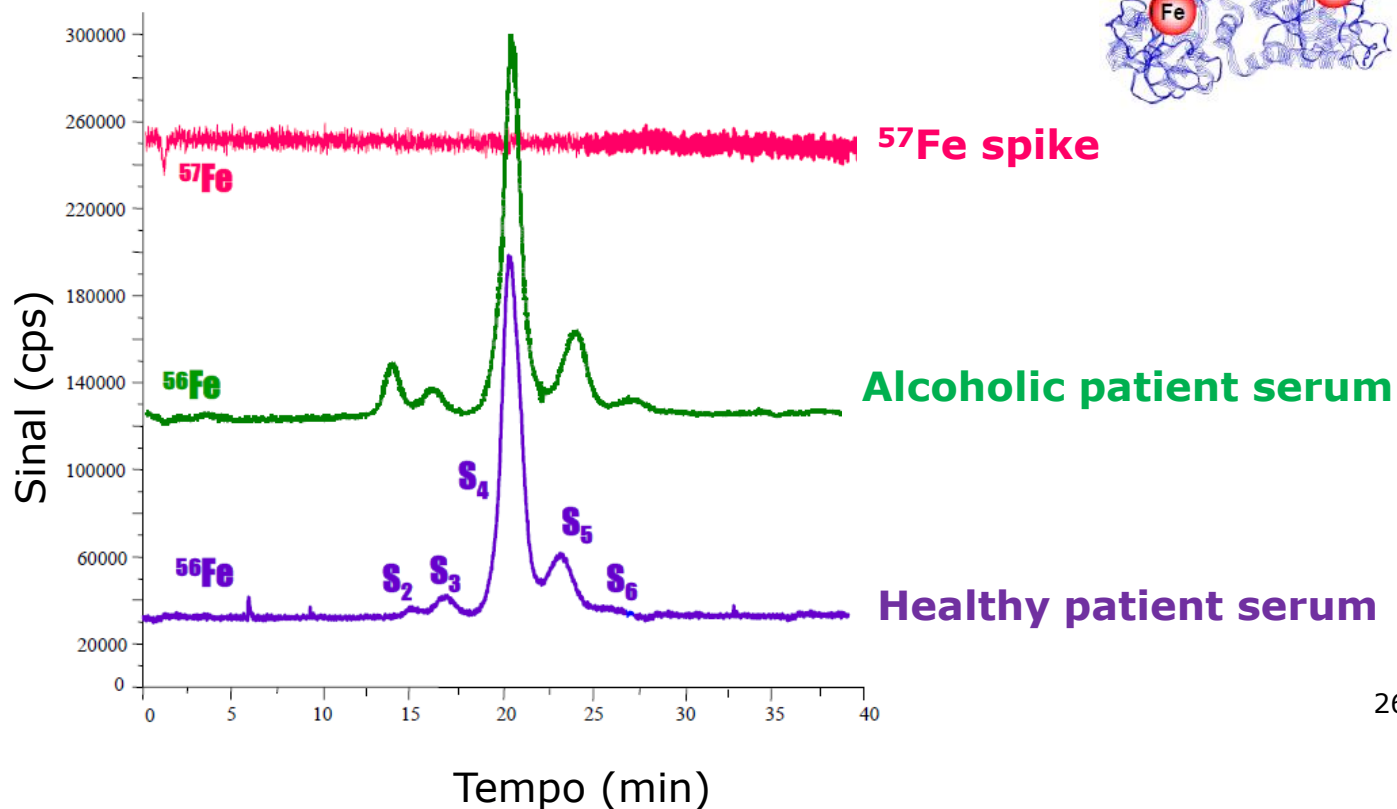
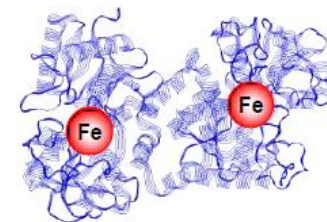
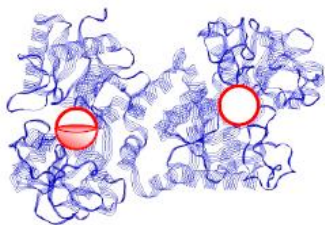


Absolute quantification of proteins



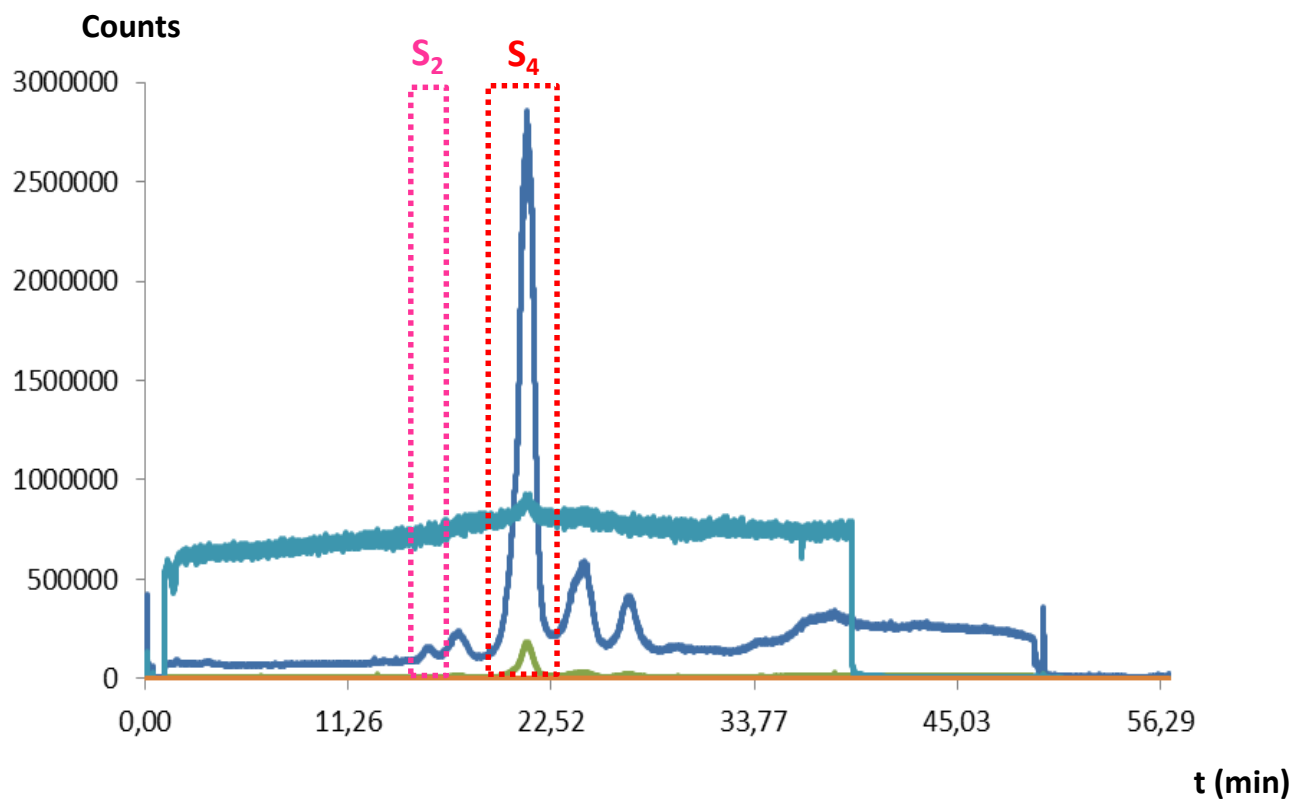
Absolute quantification of proteins

Transferrin absolute quantification

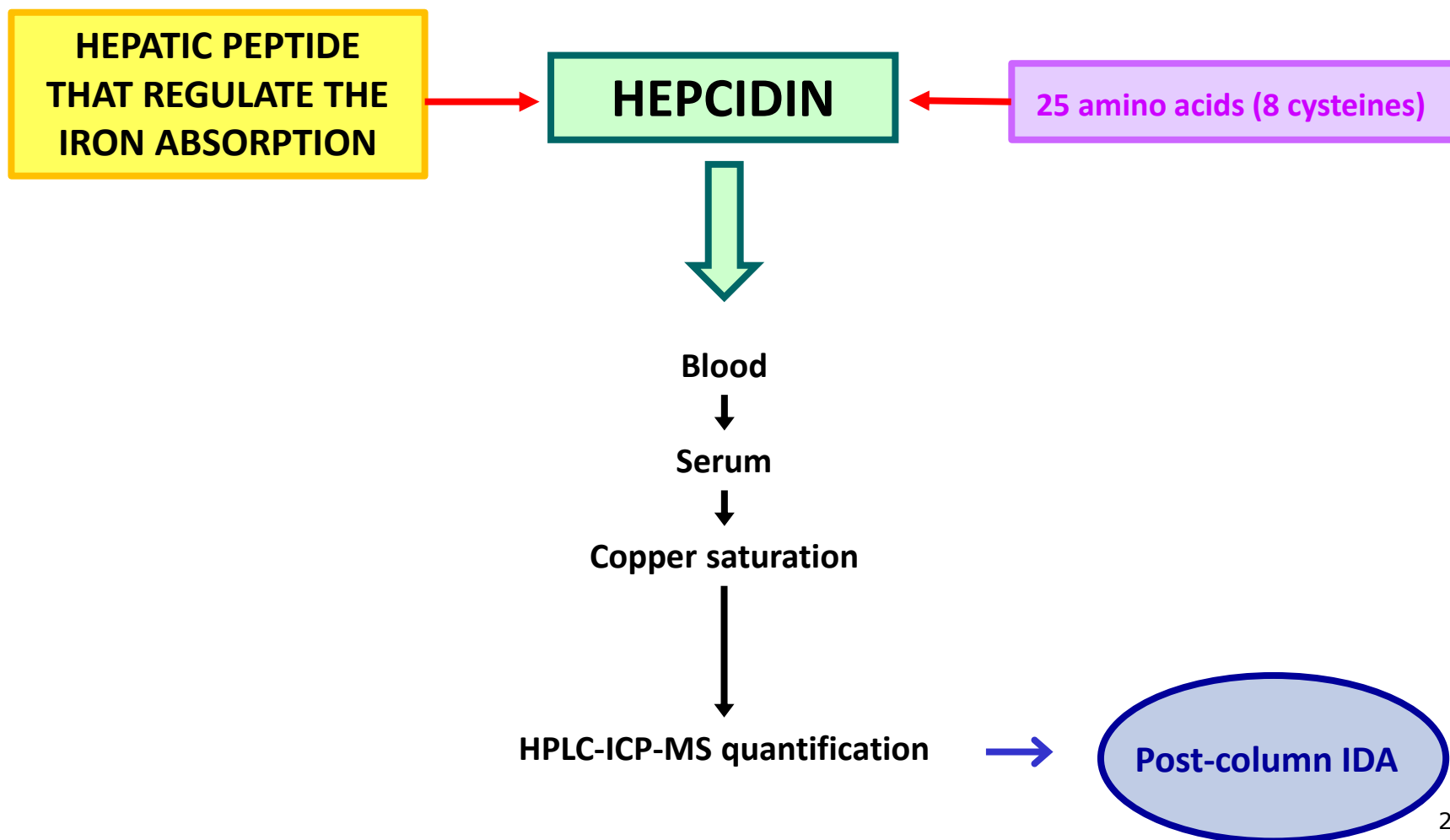


Absolute quantification of proteins

TRANSFERRIN

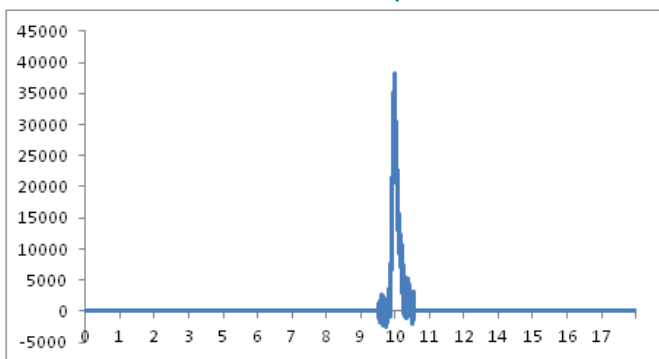
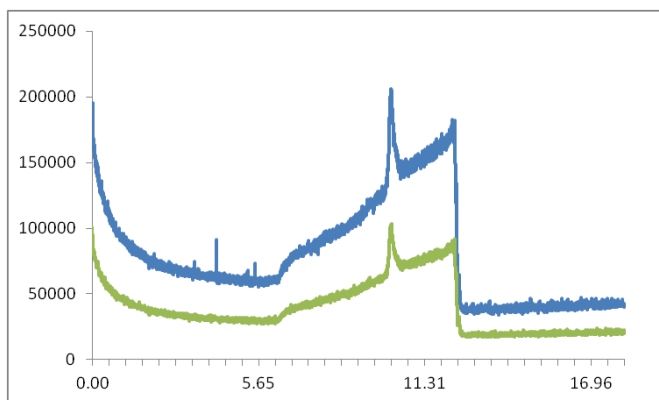


Absolute quantification of proteins



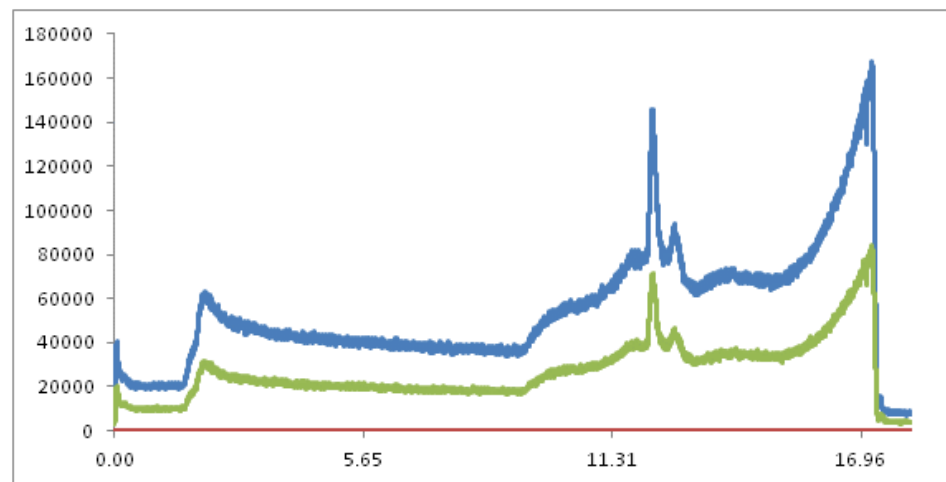
Absolute quantification of proteins

Hepcidin standard: ^{63}Cu e ^{65}Cu



Human serum saturated with Copper

^{63}Cu e ^{65}Cu





Agradecimentos



Fim

I'd like to thank the organizing comitee of the VI Bioanalytical school and congratulate them for the wonderful event, THANKS A LOT!



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UFRJ**

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