Characterization and diversity of plasma origins at fractionator level

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LFB
ETHICAL COMMITMENT

A French major biopharmaceutical company

LFB is a biopharmaceutical group that develops, manufactures and ***markets plasma-derived medicinal products*** and recombinant proteins for the treatment of patients *with* ***serious and often rare diseases.***

Created in France in 1994, LFB is today one of the leading European companies providing plasma-derived and recombinant medicinal products to healthcare professionals. Its mission is to offer patients new treatment options for unmet needs.

3,000 employees:

More than 2,200 in France and 1,800 in Production

3

THERAPEUTIC FIELDS

—

IMMUNOLOGY

HAEMOSTASIS

INTENSIVE CARE

ABOUT FIFTEEN

BIOMEDICINES

—

MARKETED IN ABOUT 30 COUNTIRES

The evolution of plasma sourcing for manufacturing, specifically through the integration of plasma from diverse international origins, introduces significant variability into the production process

TO DEVELOP, MANUFACTURE WITH BETTER PROCESS KNOWLEDGE

PROCESS IMPACT OF PLASMA

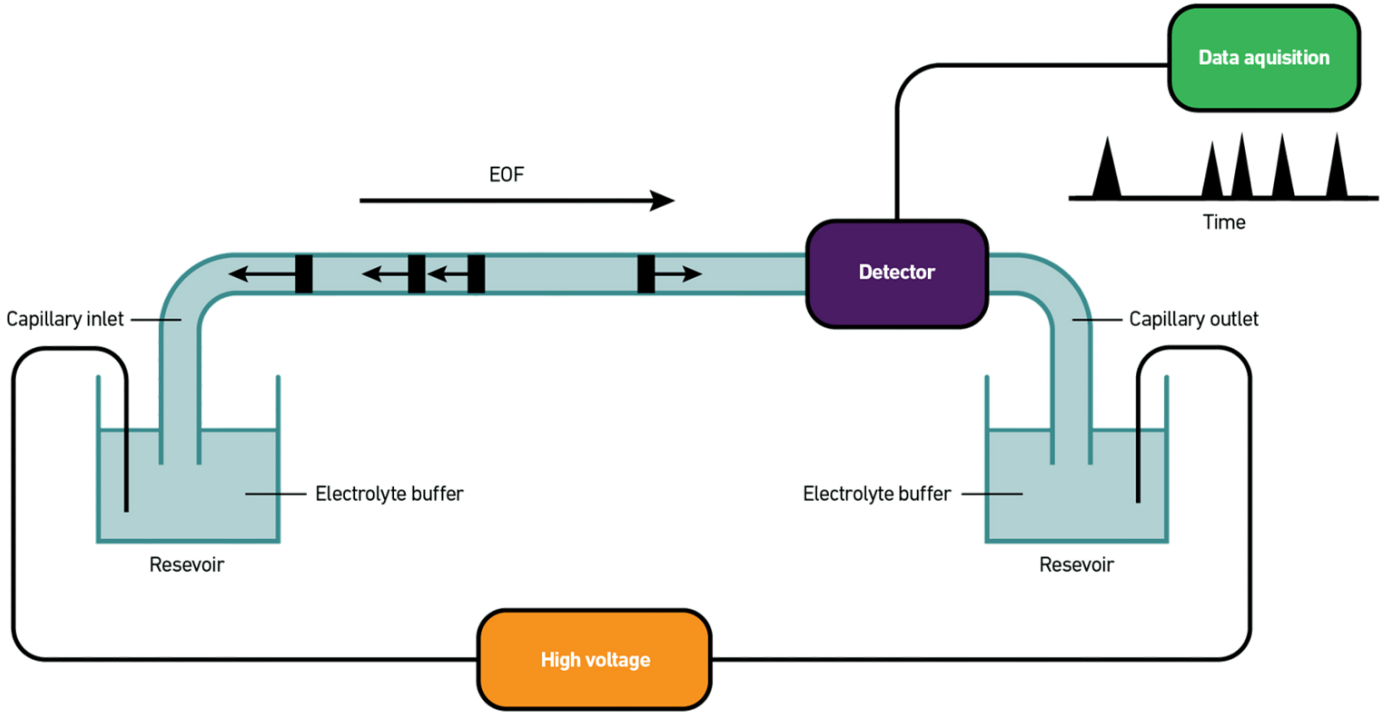
1	2	3
Plasma preparation	**Protein content in plasma**	**Products specific (16)**
Interaction between blood and artificial surfaces (membranes or filters or shear stress)	Donation frequency	Different production routes with different yields

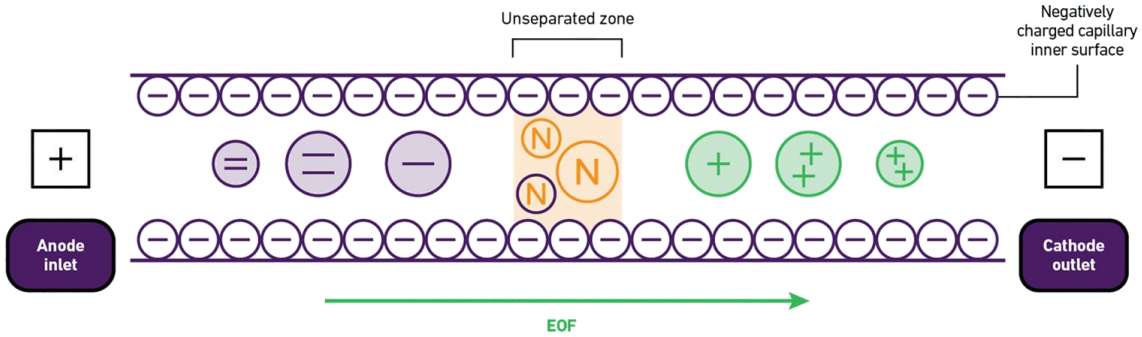
Characterization of plasma

Capillary Zone Electrophoresis (CZE) Rapid Access To A Global Picture Of Plasma

Capillary Zone Electrophoresis (CZE)

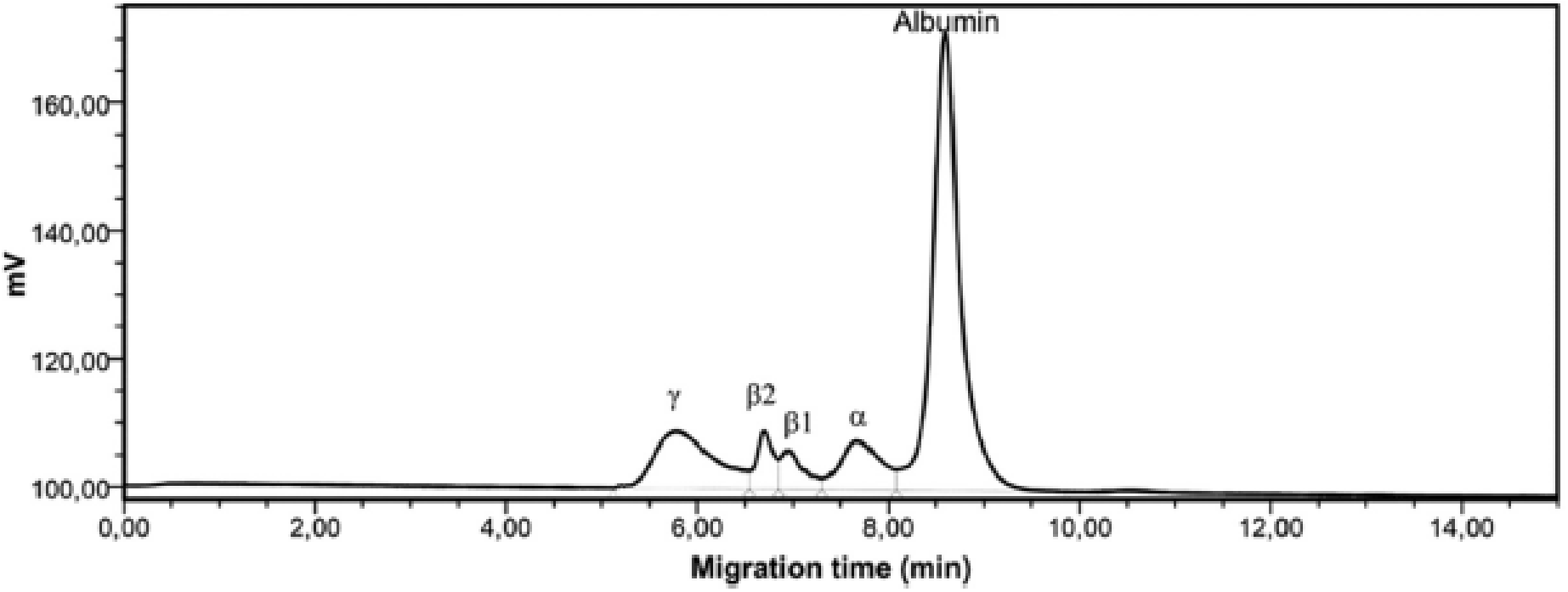
- Global picture, quicker than LC-MS/MS = high throughput possible
- Inconvenient : less resolutive than MS





Zamora Carreras, 2023, *Technology Networks*

Rapid Access To A Global Picture Of Plasma



α Globulines:
α1-Antitrypsin
Haptoglobin
α2-Macroglobulin
Ceruloplasmin
α1-Acid glycoprotein
α1-Lipoprotein
Transcortine
Thyroxine binding protein
α2-Macroglobulin

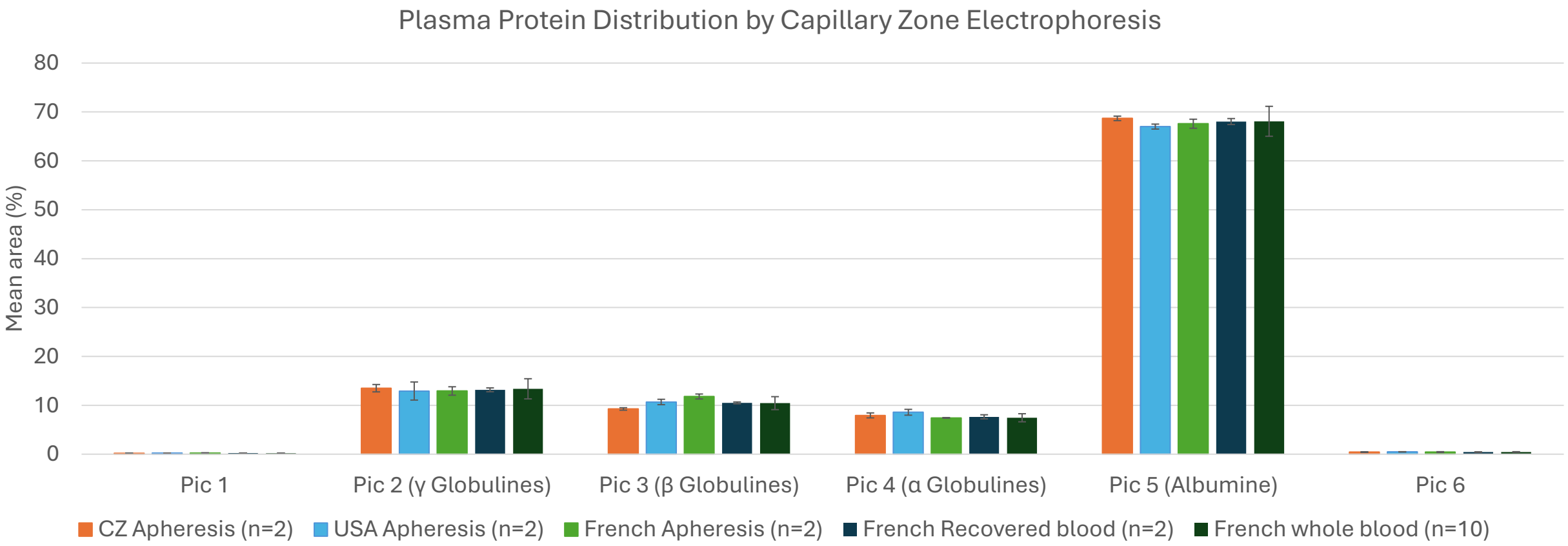
β Globulines:
Transferrine
C3 complement
β-lipoproteins
Hemopexin
C4 complement
β2-microglobuline
Fibrinogen

γ Globulines:
IgG
IgA
IgM
IgD
IgE
Complement proteins
Reactive C protein

Fig. 1. Specificity: Detection of serum protein fractions in human serum. Capillary zone electrophoresis of a normal human serum ultrafiltrated in borate buffer. Shown is the electropherogram from sample #31 from Instand eV, Germany. The absorbance signal is expressed in mV.

Capillary Zone Electrophoresis

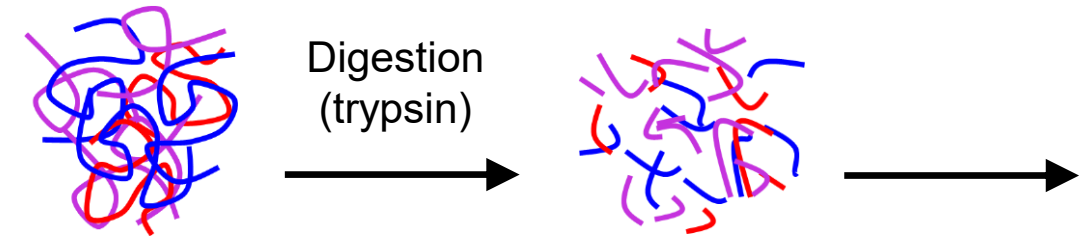
COMPARISON OF PLASMA ORIGINS USA / CZ / FR WITH APHERESIS COLLECTION



- Method gives results quickly, however the resolution is too low to detect differences

Liquid Chromatography Coupled To Tandem Mass Spectrometry (LC-MS/MS (Hi3)) : Relative Quantification Of Plasma Proteins

Liquid Chromatography Coupled To Tandem Mass Spectrometry (LC-MS/MS (Hi3))

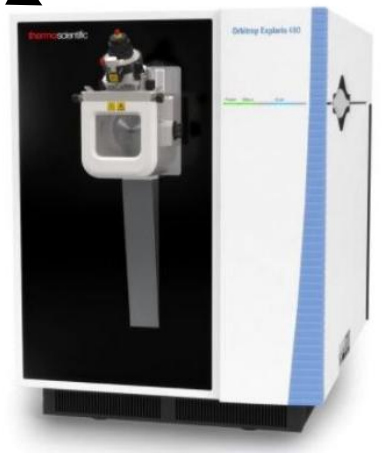


Protein mixture

Digestion
(trypsin)

Peptides mixture

Separation by liquid chromatography

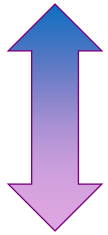


MS/MS analysis

List of mass spectra

Database search

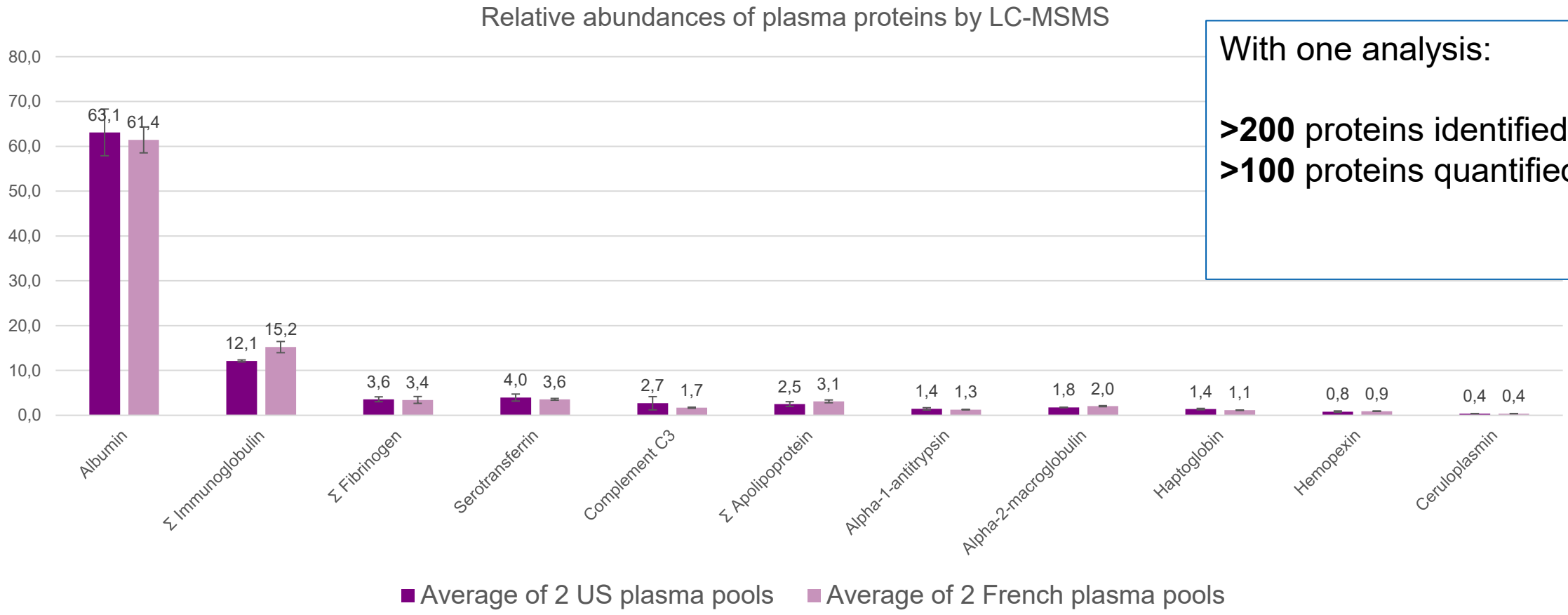
Protein identification



Analysis of peptide intensity:
Relative quantification (Hi3)

Mass spectrometry relative quantification of proteins

COMPARISON OF PLASMA ORIGINS USA / FR WITH APHERESIS COLLECTION



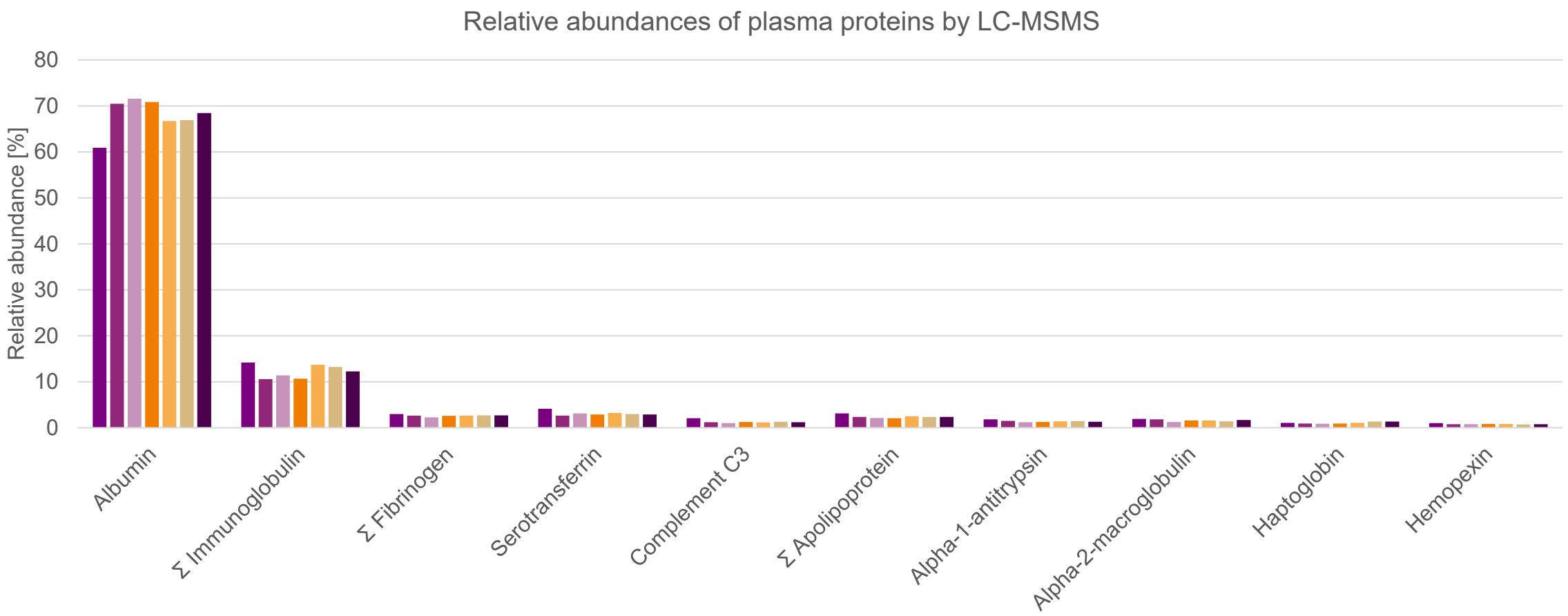
With one analysis:

>200 proteins identified
>100 proteins quantified

- Method detects difference in levels of Immunoglobulins, lower in US plasma pools as observed by others using nephelometry (Laub *et al.* 2010)

Mass spectrometry relative quantification of proteins

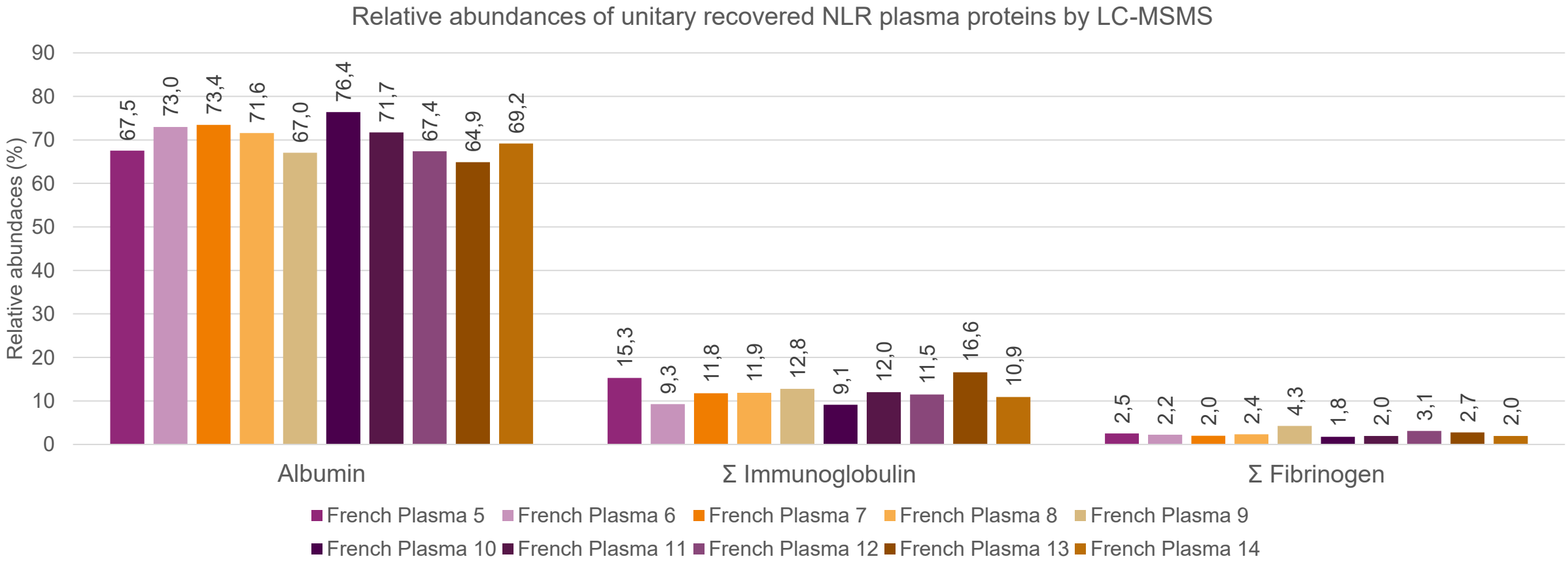
COMPARISON OF PLASMA POOLS FROM CZECH REPUBLIC



- Method detects differences between plasma pools from Czech collection centers

Mass spectrometry relative quantification of proteins

COMPARISON OF RELATIVE QUANTITIES OF 3 MAIN PROTEINS IN UNITARY RECOVERED NLR PLASMAS



- Method detects considerable variability in the relative abundance of Albumin, Immunoglobulins and Fibrinogen between different donors

Ion Exchange Chromatography (IEX) : Albumin as a marker for plasma integrity

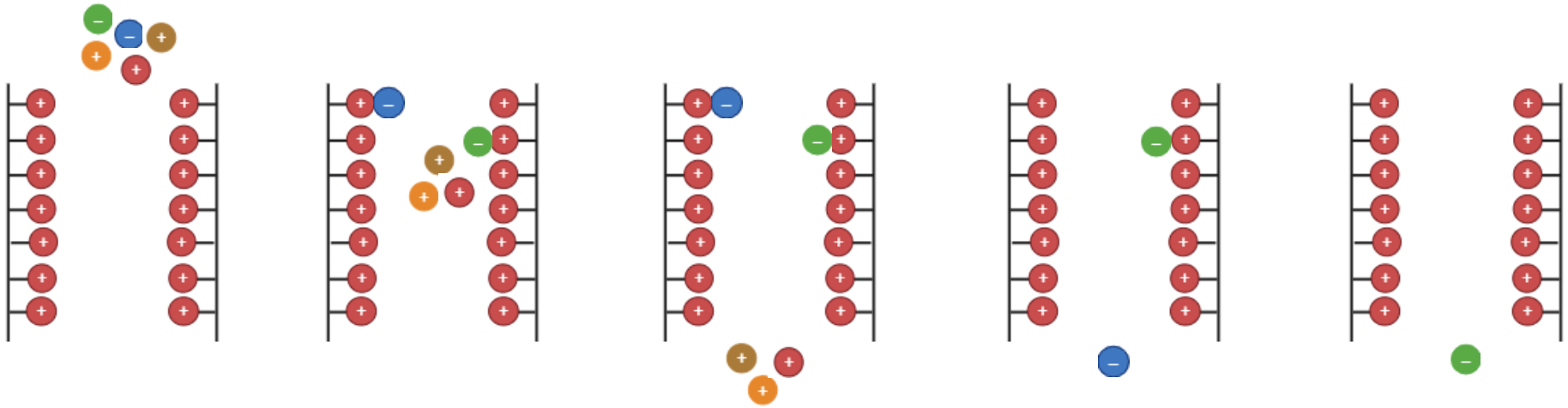
IEX - Experimental conditions

INSTRUMENTS :

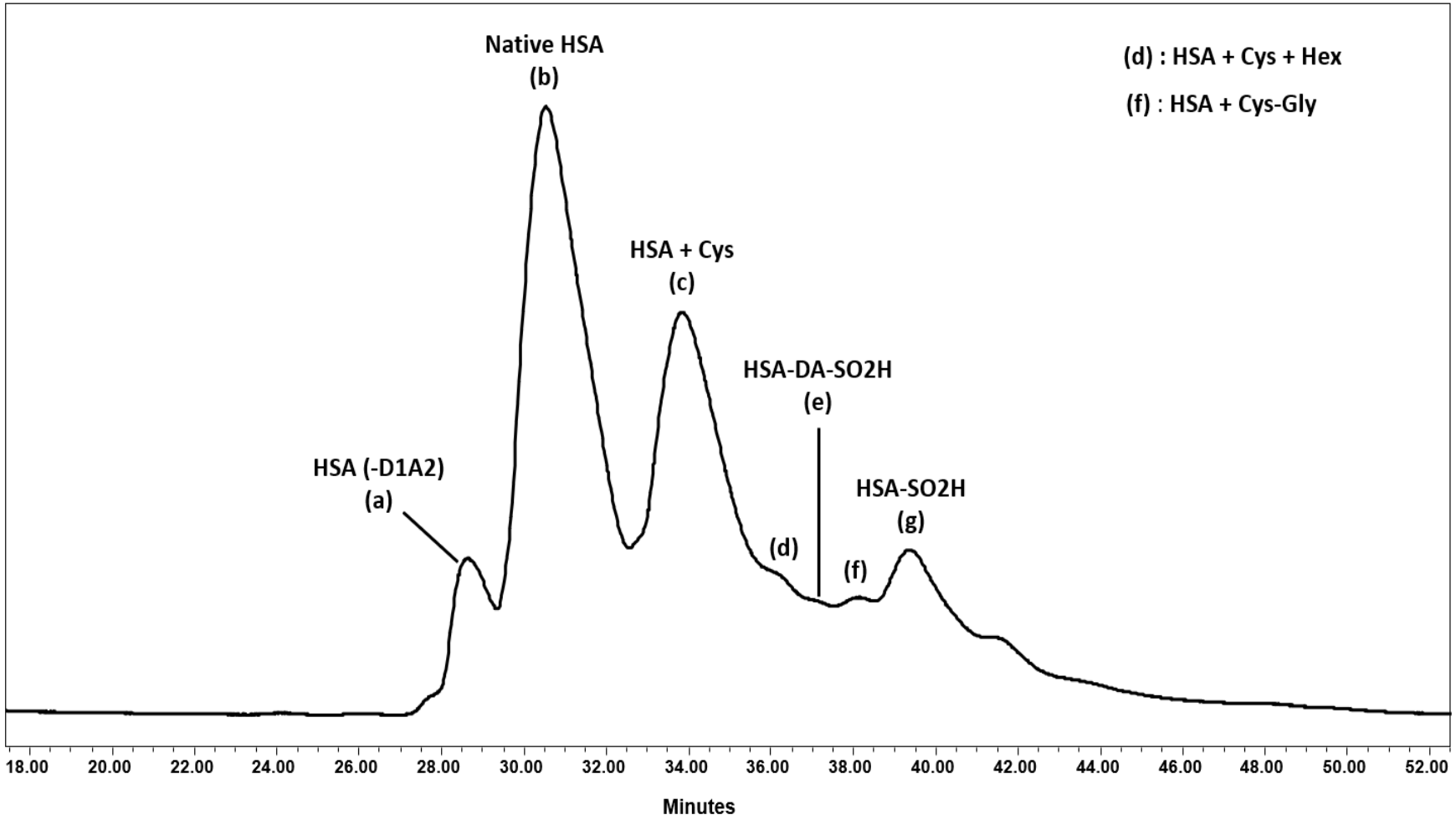
- UPLC System : Acquity HClassBio UHPLC (Waters, Milford, MA, USA)
- SAX column: Proteomix SAX NP5 2,1 mm x 150 mm , 5µm, 300 Å

SAX SETTINGS

- Mobile phase A: 50 mM Ammonium Acetate (Binding)
- Mobile phase B: 500 mM Ammonium Acetate (Elution)
- Temperature: 30°C
- Flowrate: 0.2 mL/min
- Detection: UV 280 nm



Charge Isoform Distribution Profile of purified Albumin



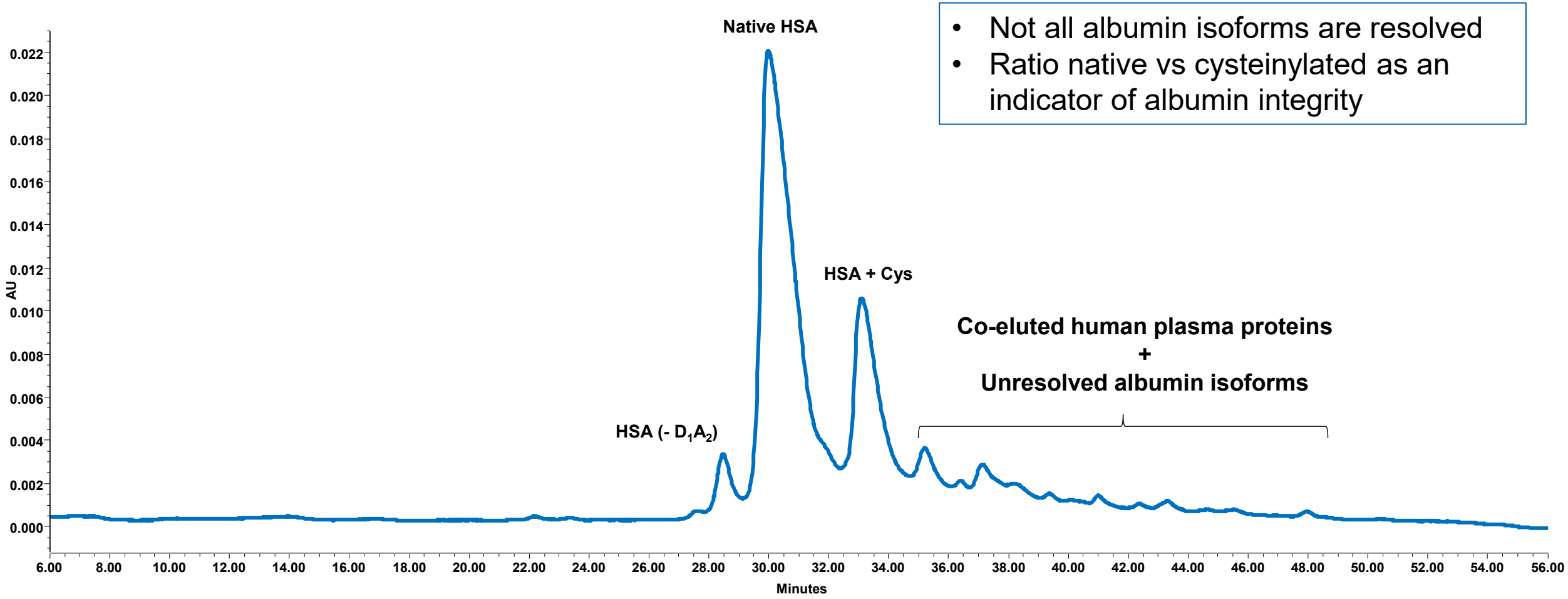
Method previously developed at LFB for albumin isoforms :

- 7 different forms of albumin resolved (*)
- Native HSA possesses a reduced cysteine 34 important for its antioxidant potential (**)

*Leblanc *et al.: Characterization of human serum albumin isoforms by ion exchange chromatography coupled on-line to native mass spectrometry*. J CHROMATOGR. B, 2018

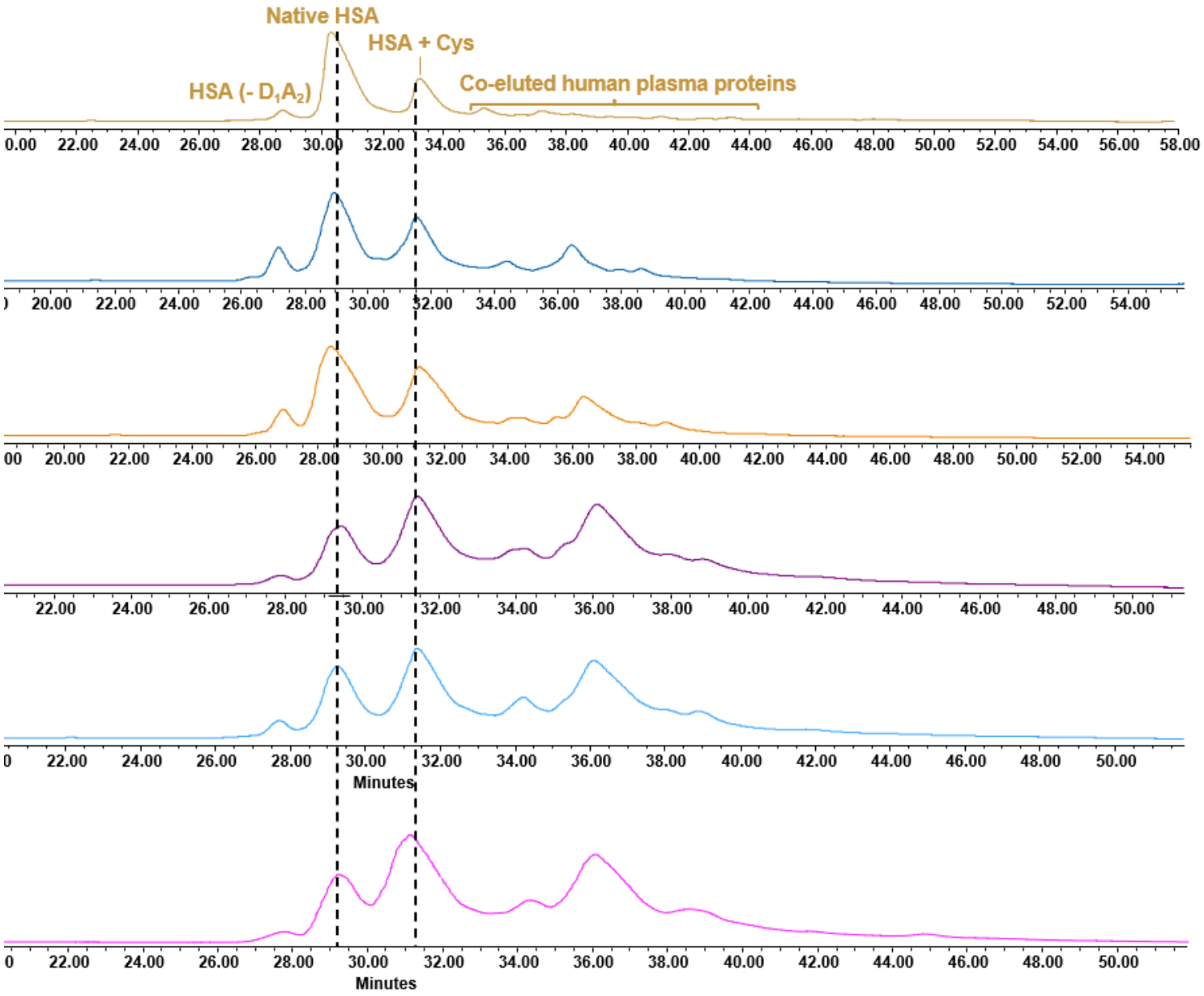
**Plantier *et al.*: *Comparison of antioxidant properties of different therapeutic albumin preparations.* BIOLOGICALS, 2016

IEX Profiles of Plasma a pool



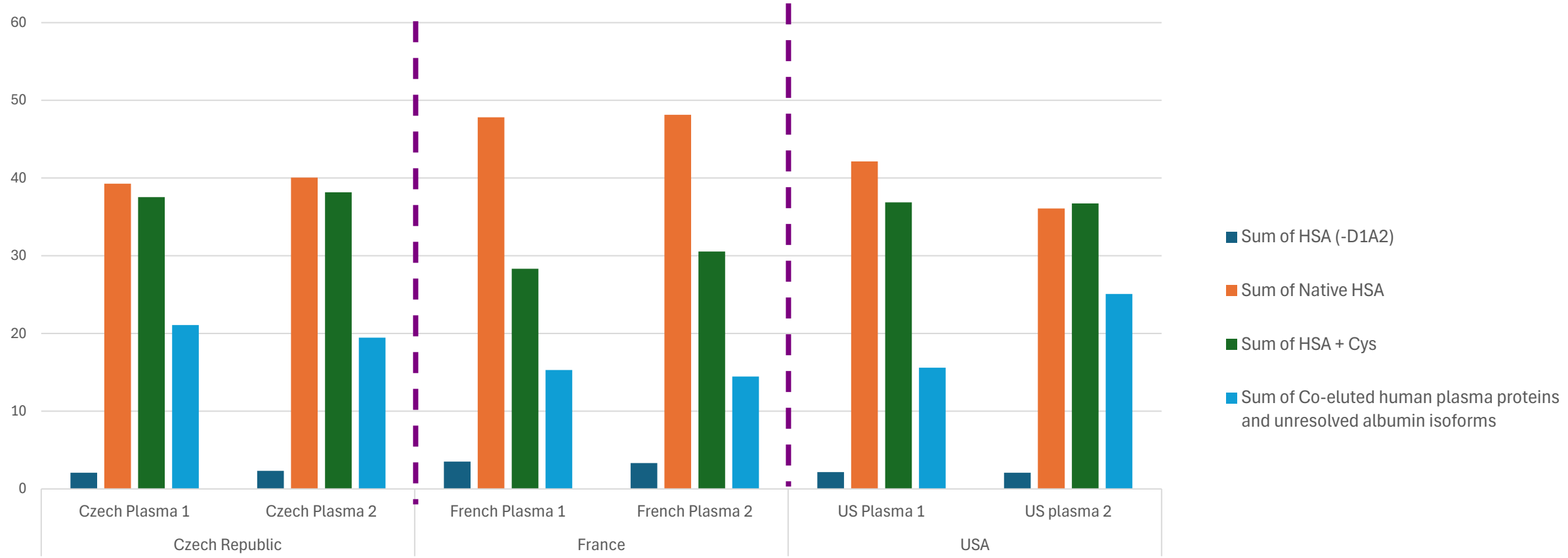
- Not all albumin isoforms are resolved
- Ratio native vs cysteinylated as an indicator of albumin integrity

Integrity of human native Albumin products on the market



Relative Quantification of Albumin's Isoforms

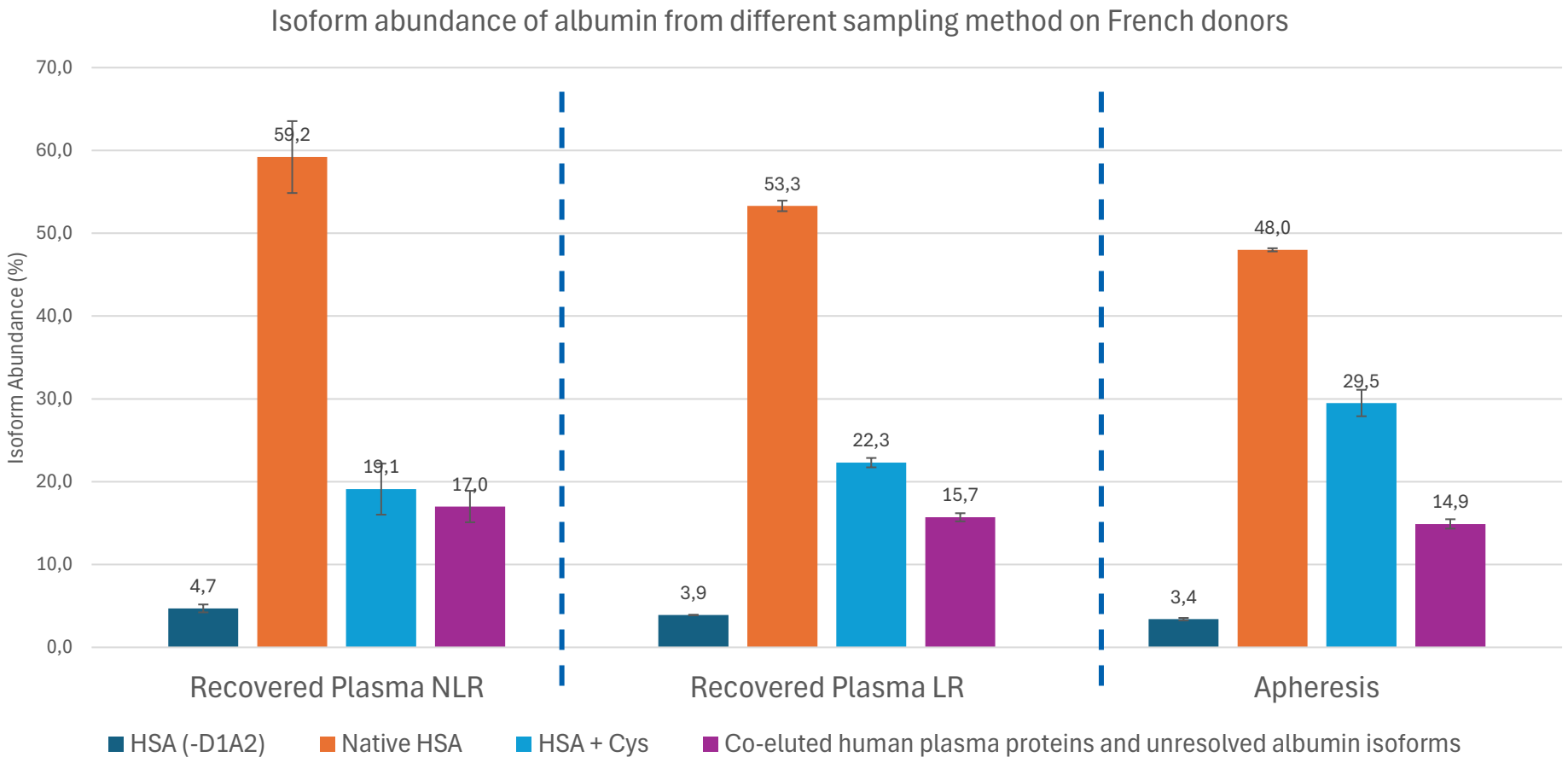
COMPARISON OF PLASMA ORIGINS USA / CZ / FR WITH APHERESIS COLLECTION



- The method detects differences between different centers with respect to the levels of native albumin

Relative Quantification of Albumin's Isoforms

COMPARISON OF COLLECTION METHODS ON FRENCH PLASMA : APHERESIS AND RECOVERED PLASMA LEUKOCYTE REDUCED (N=2 POOLS OF 10 DONORS) VS RECOVERED PLASMA NON-LEUCOCYTE REDUCED (N= AVERAGE OF 10 UNITARY PLASMAS)



The blood collection technique seems to have an impact on the level of native albumin:

(Recov. Plasma NLR > Recov. Plasma LR > Apheresis)

Results need to be confirmed with additional plasma pools

Conclusion

This characterization allows for the comparison of new plasmas with historical standards (recovered plasma or apheresis)

The use of these three analytical approaches adds to the control strategy before introducing a new plasma source to the process.

Anticipation of process risks: production yields etc.

Decision support for development: decision for initiation of smaller-scale studies

THANK YOU FOR YOUR ATTENTION

LFB BIOTECHNOLOGIES

LFB BIOMÉDICAMENTS

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