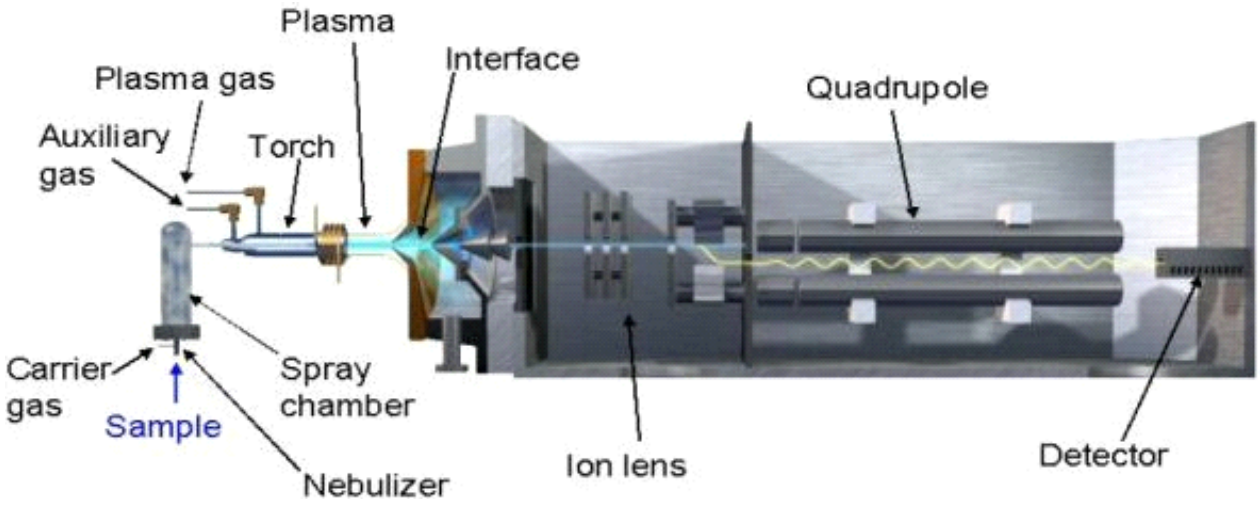
Application of ICP-MS and LC-ICP-MS in Drug Development

Jaap Wieling

Overview

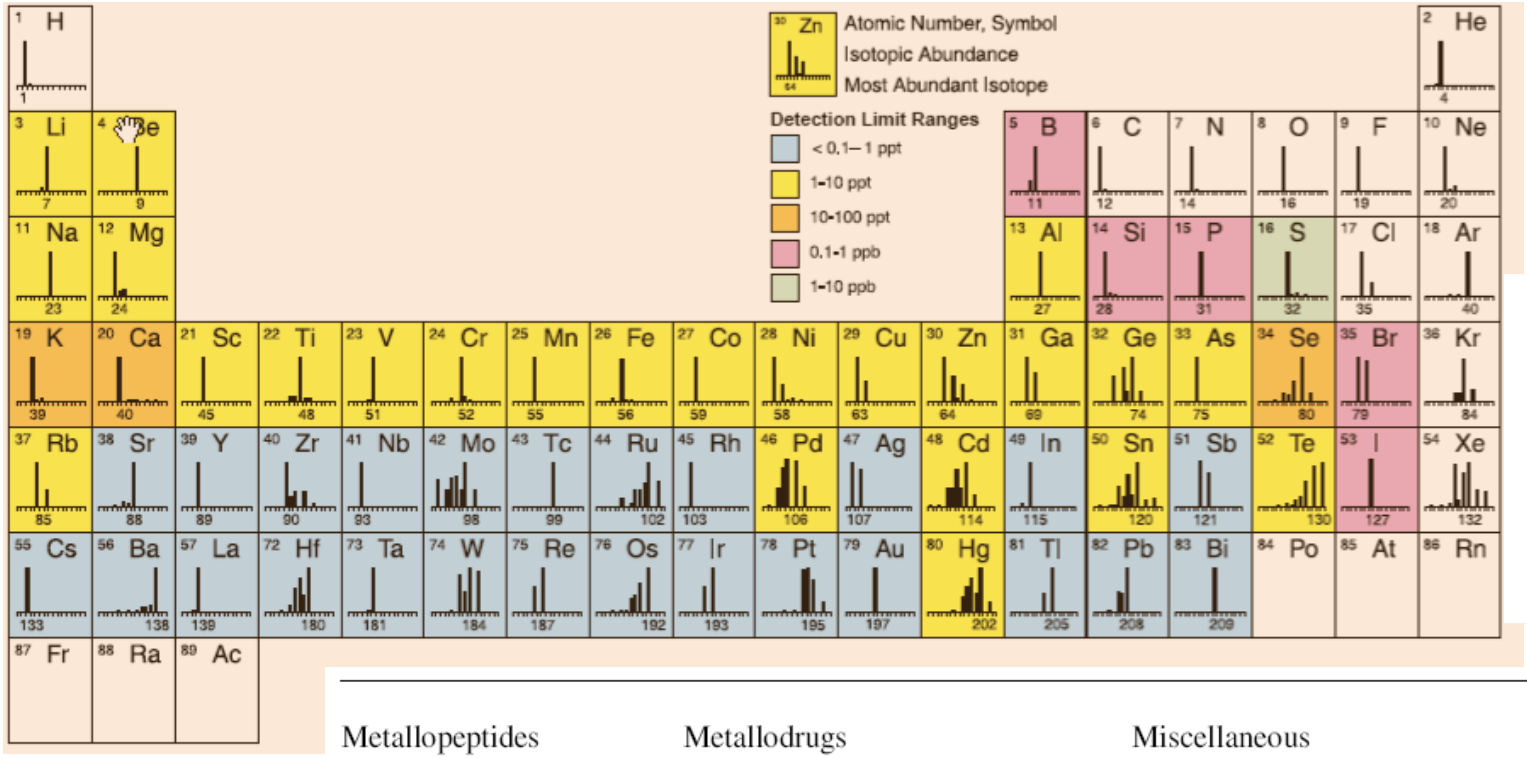
- Introduction
- ICP-MS and LC-ICP-MS
  - Application areas
  - Potential
- Some examples in regulated bioanalysis
- Conclusions

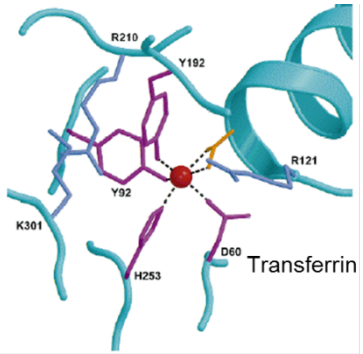
Principle of ICP-MS

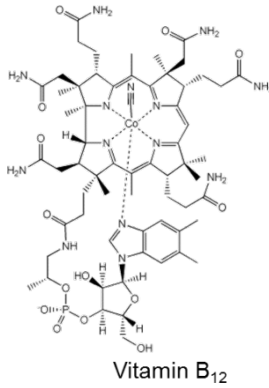


- Liquid samples to formate aerosol in nebulizer
- Introduction of Argon to form the ICP torch, which is located in center of a radio frequency (RF) coil for energy supply
- RF field causes collisions of Ar atoms, generating a high-energy plasma
- Sample aerosol decomposed in plasma (6000 - 10000 K) to form analyte atoms which are simultaneously ionized.
- Ions extracted from the plasma into mass spectrometer region (Quadrupole Mass Analyzer) and detected on an electron multiplier

Elements and their sensitivity





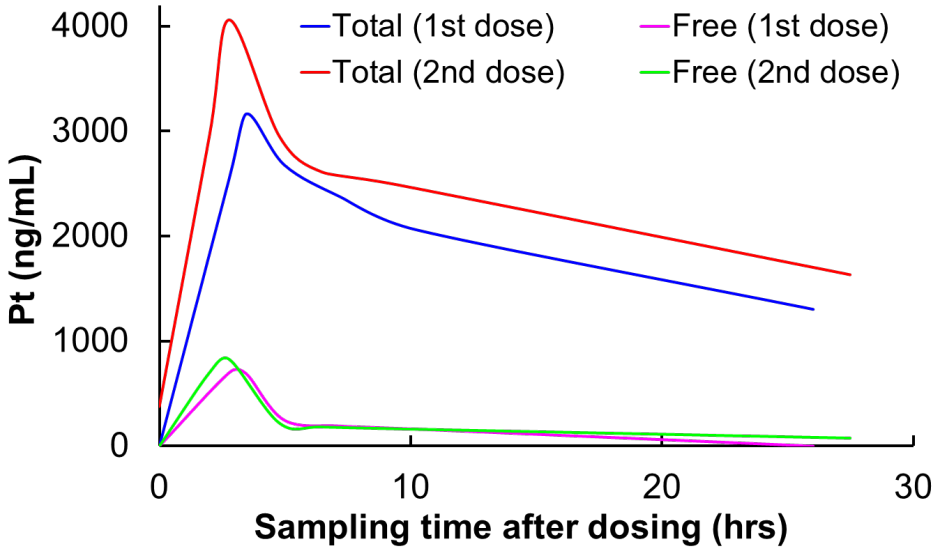


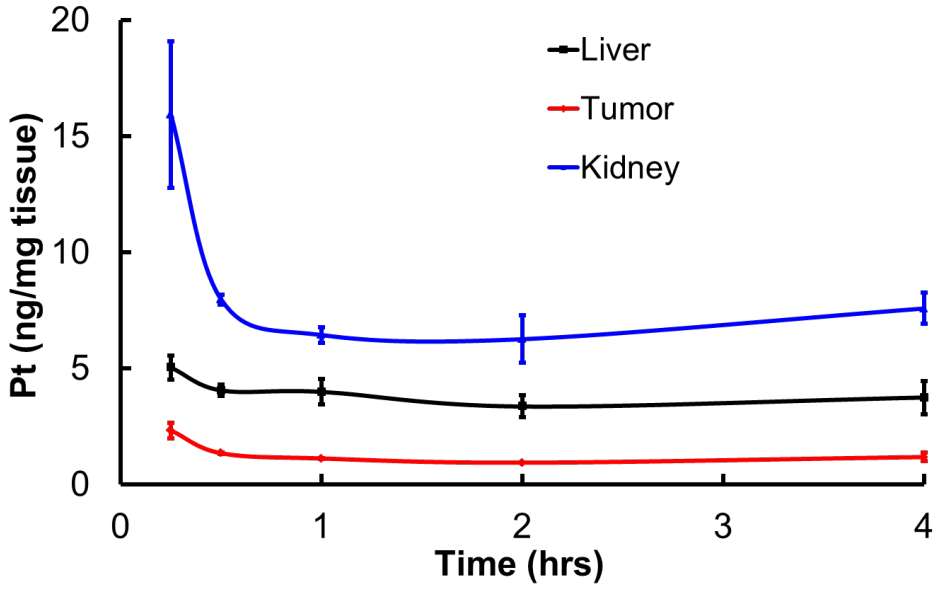
Metallopeptides	Metallodrugs	Miscellaneous
phytochelatins (Cd, Cu, Zn)	cisplatin (Pt)	amino acid-complexed metals
metallothioneins (Cd, Cu, Zn)	carboplatin (Pt)	metalloporphyrines
metalloenzymes	auranofin, aurothiomalate, aurothioglucose	ferrocene derivatives
transport proteins [albumin (Cu, Al), transferrin (Fe, Al]	Ru-imidazole(indazole) complexes	DNA restriction fragments (Fe, Mn, Co, Pb, Cd)
	Tc-imaging agents	cobalamines, cobanamids (Co)
		seleno animoacids (Se)
		organoarsenicals, arsenosugars (As)

ICP-MS - general protocol for a biological sample

- Elemental MS, complementary/orthogonal to molecular MS
- Sample preparation:
  - ultrafiltration
  - extraction
  - combustion / destruction
  - direct plasma introduction
- Inject and nebulize sample and introduce into ICP plasma
- Ionize sample components
- Extract ionized components into mass spectrometer
- Resolve ionized components by mass
- LLOQ <1 pg/mL to >1 µg/mL, element and matrix dependent
- Operation: simple for professionally trained operator

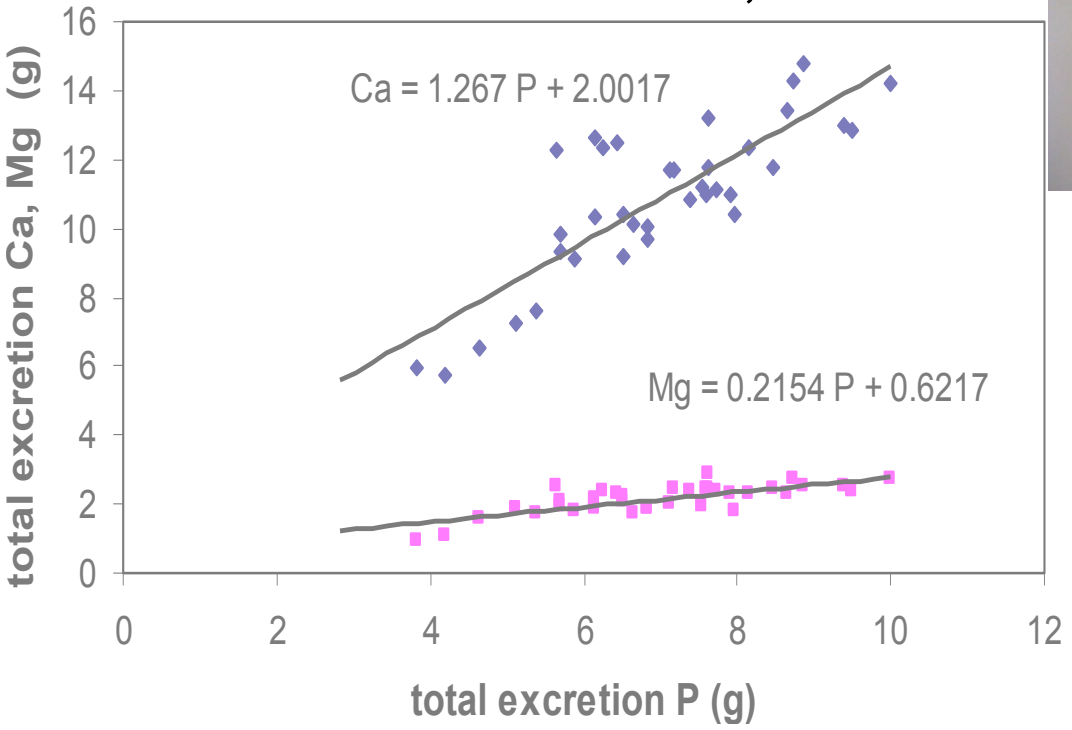
ICP-MS applications (new Pt cmpd, combi-therapy)





Mass balance study

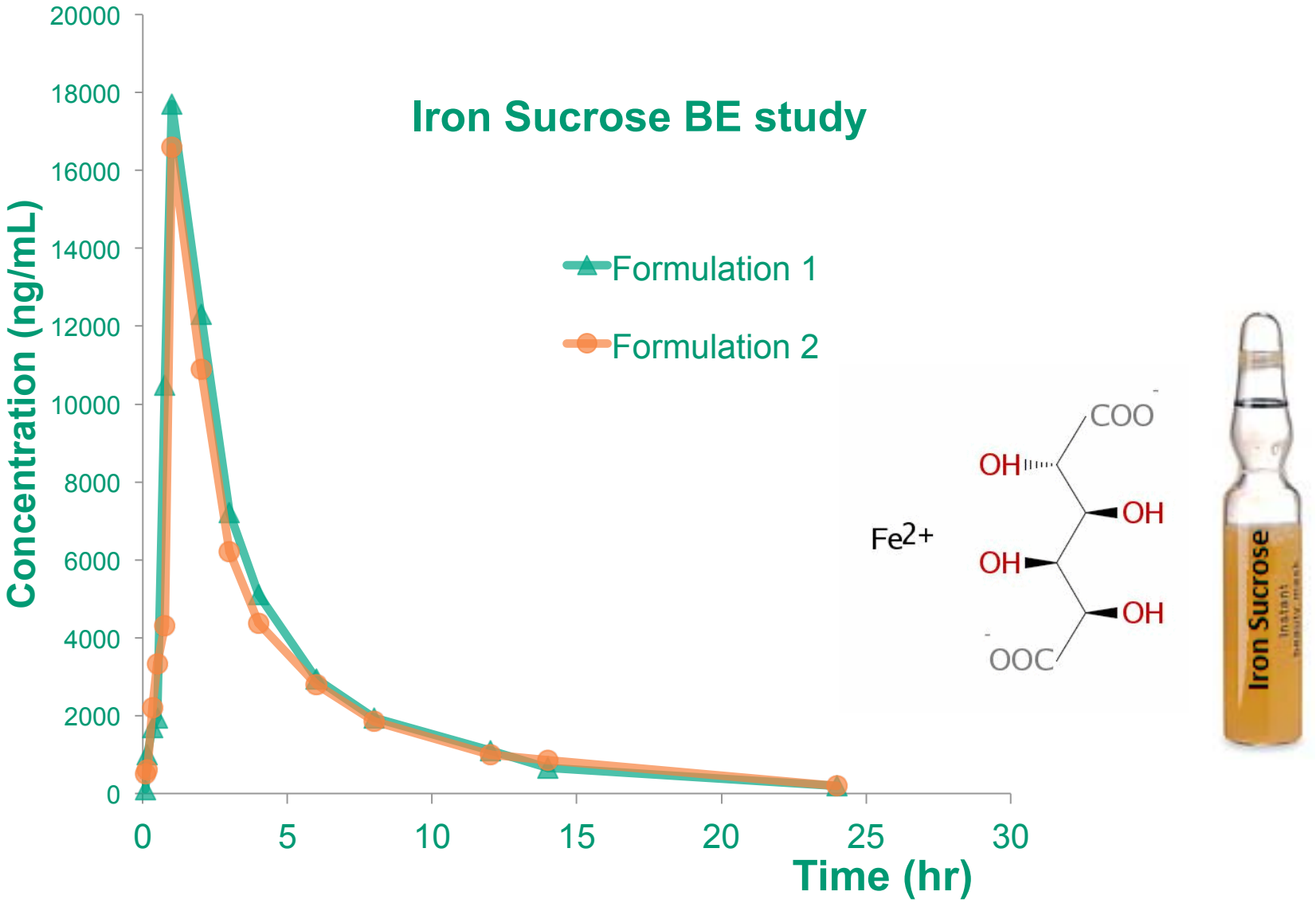
- Phosphate binder for renal insufficient patients
- P, Ca and Mg:
  - Contents in food
  - Excretion in faeces, urine

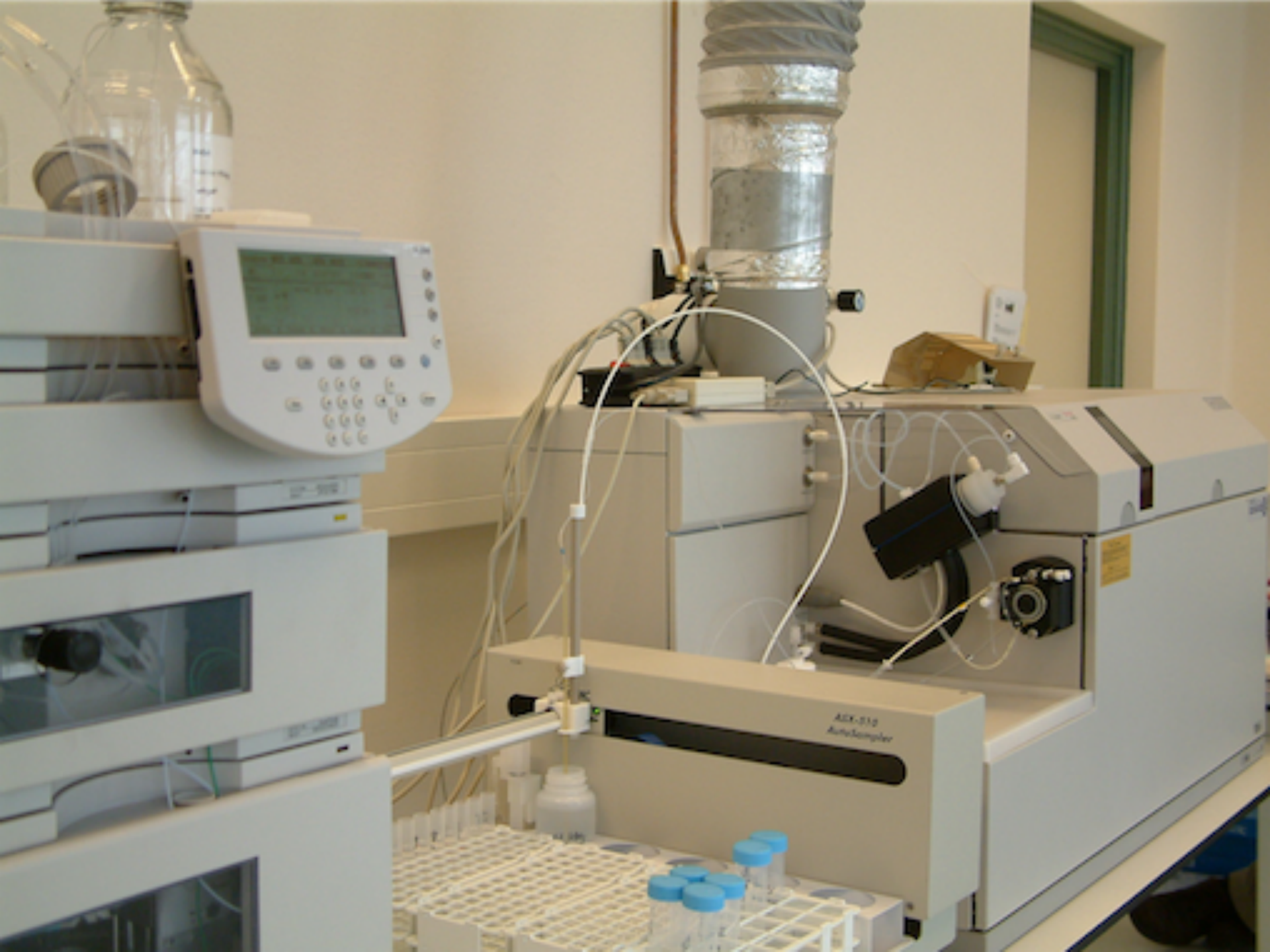


- Excellent data (CV% < 4.0)
- Efficacy of phosphate binder demonstrated
- Also for drug substance (e.g. cumulating metab's)

Iron sucrose BE study

(iron deficiency anemia in hemodialysis patients)

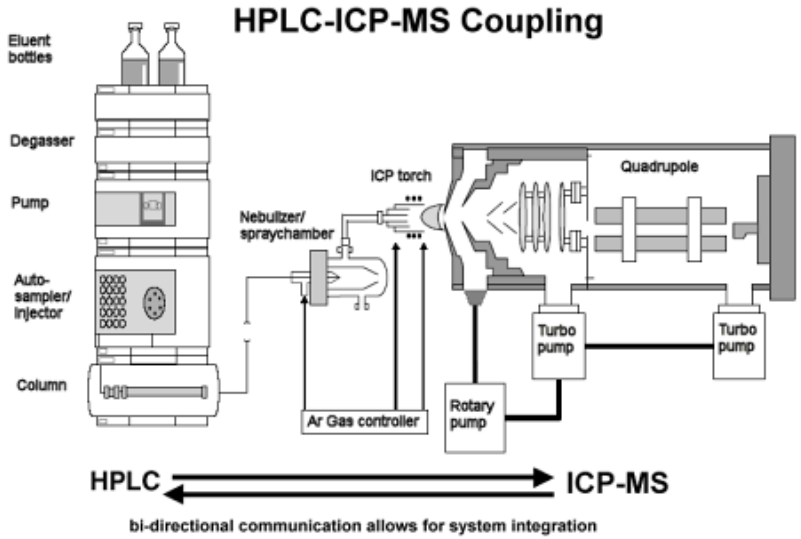



ASX-510
AutoSampler

LC-ICP-MS power

Advantages of HPLC

- Wide applicability
- High resolution
- Rapid analysis
- High sensitivity
- High reproducibility
- Quantitative
- Easily automated



ICP-MS as detector

- Selective for the element
- Provide isotopic information
- Determination of multiple elements simultaneously
- Universal – regardless the mode of chromatography
- Extremely sensitive
- Detection limits in ppt range

Simple connection - compatibility of LC flow rates with ICP-MS sample uptake, typical flows of 1-1.5 ml/min or lower

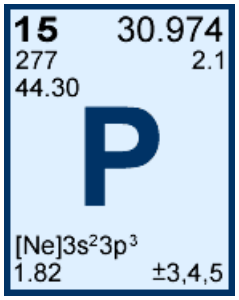
Interfacing LC to ICP-MS

- Challenges
  - destabilisation of Argon plasma (gentle gradient steps)
  - peak broadening from plasma
  - Polyatomic interferences
- Various HPLC modes possible (GPC, IEX, RPC, IAC, ..)

- Enabling technology
  - Isotope analysis capability
  - Ability to support tracer experiments with enriched stable isotopes as tracers (metabolism, mechanism)
  - Mass balance studies

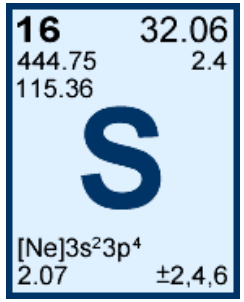
Challenges of LC-ICP-MS

- Interferences, polyatomic, esp. in high matrix samples
- Also from isotopes
- C, O and N are principal limitations for detection of pharmaceutically relevant elements such as P and S in quadrupole ICP-MS
  (→ high-res ICP-MS)



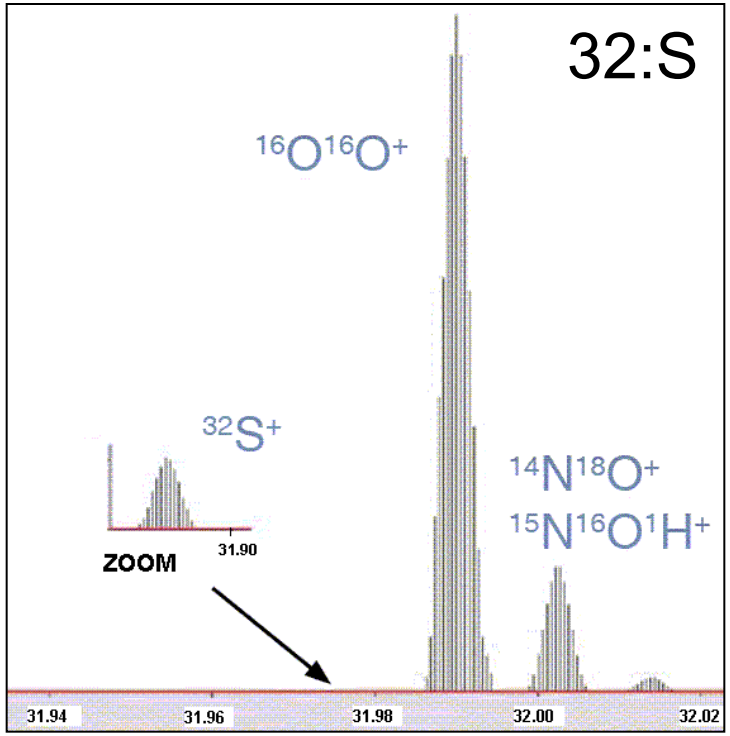
$^{15}N^{16}O$
$^{14}N^{17}O$
$^{13}C^{18}O$
$^{12}C^{18}O^{1}H$
$^{62}Ni^{2+}$

31



$^{16}O_2$
$^{14}N^{18}O$
$^{15}N^{17}O$
$^{14}N^{17}O^{1}H$
$^{15}N^{16}O^{1}H$

32

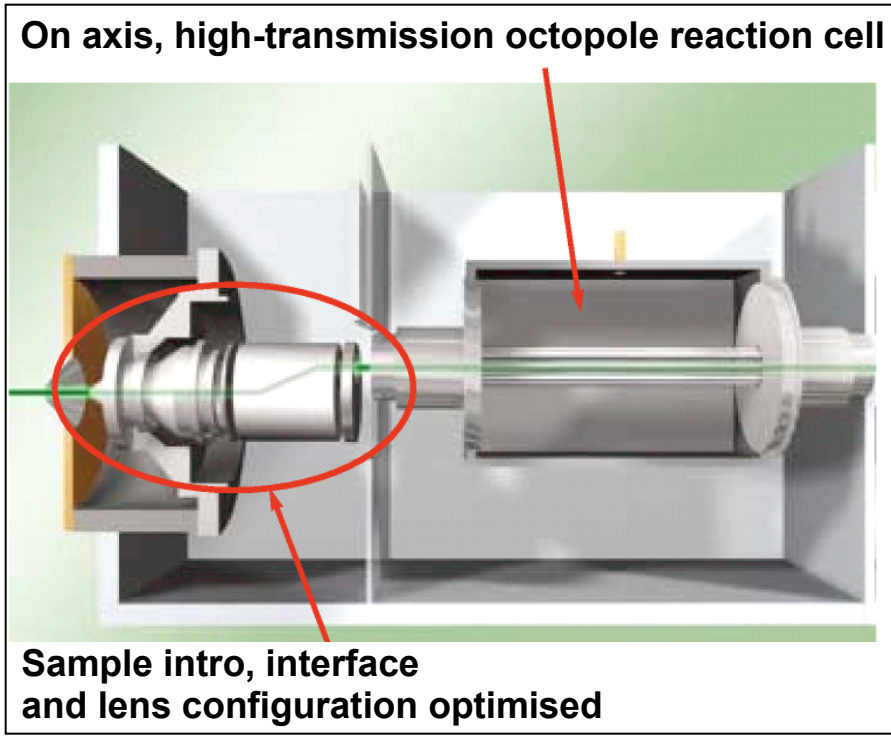


Answers to polyatomic interferences (1)

- Get rid of interferences, make ‘derivative’: use O_2 as reaction gas to transfer all S, P ions to S=O or P=O

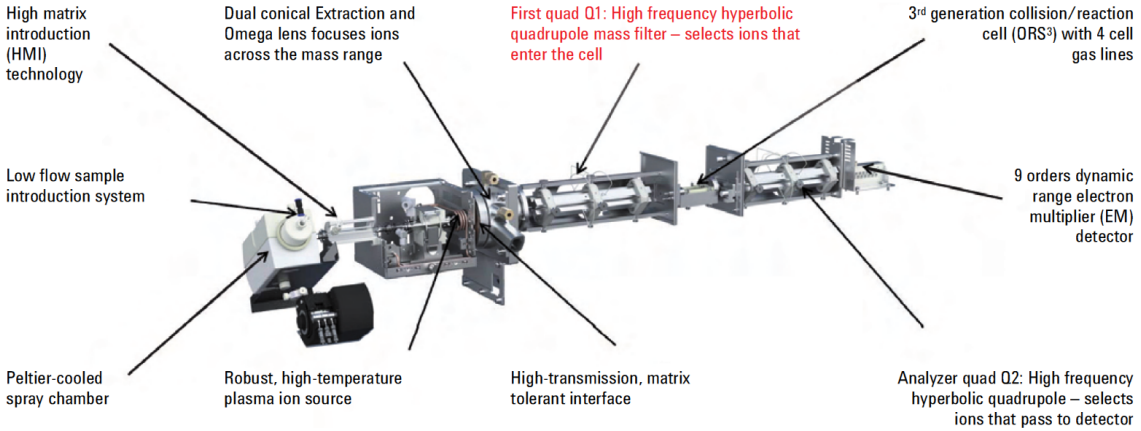
- Increase sensitivity: use Xenon as collision gas: release S=O or P=O polyatoms to single S and P ions

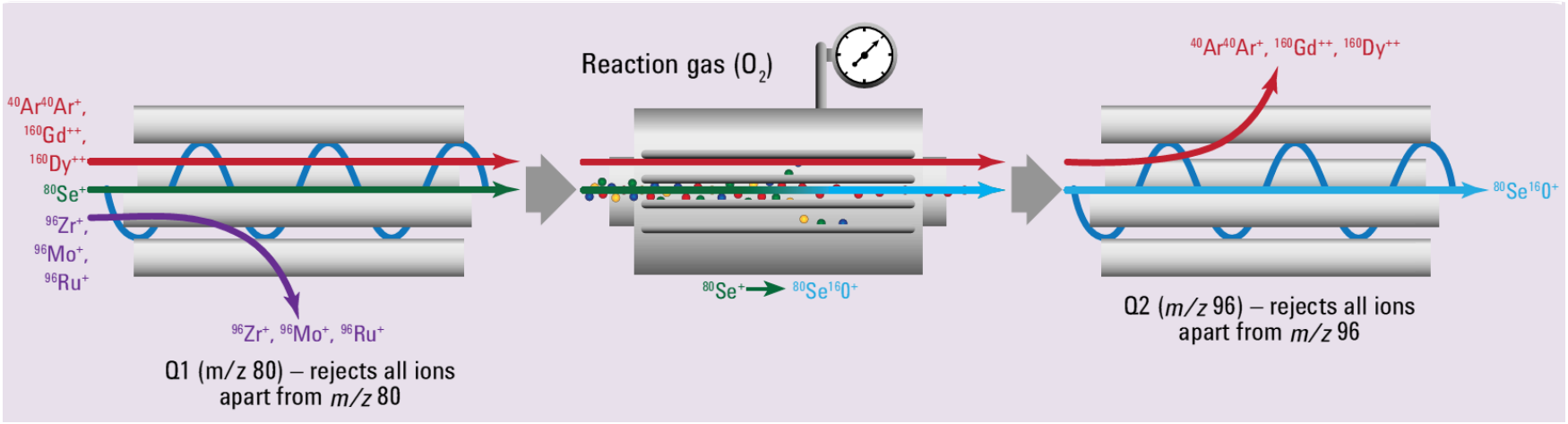
  Inert gas avoids formation of new interferences,
  no analytes lost



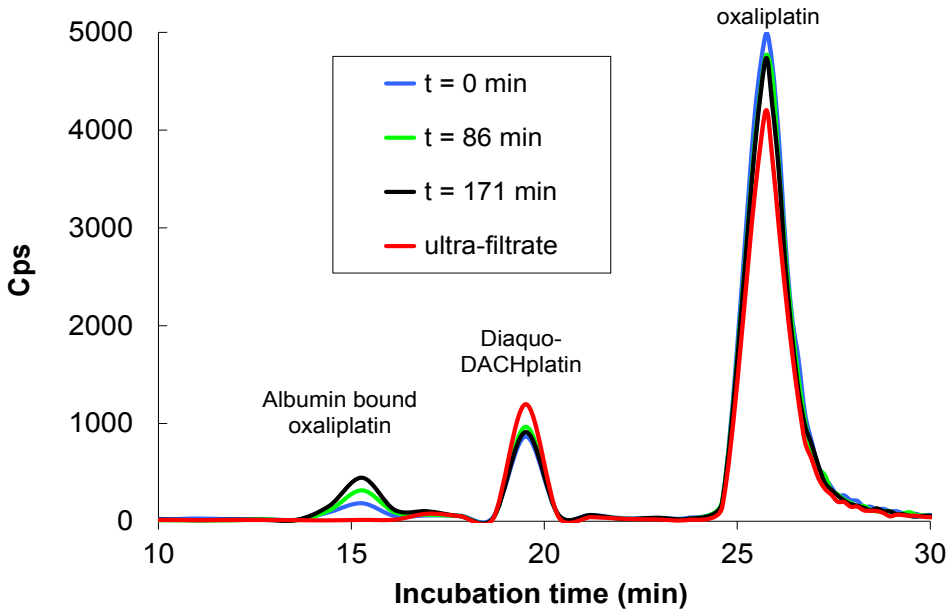
Answers to polyatomic interferences (2)

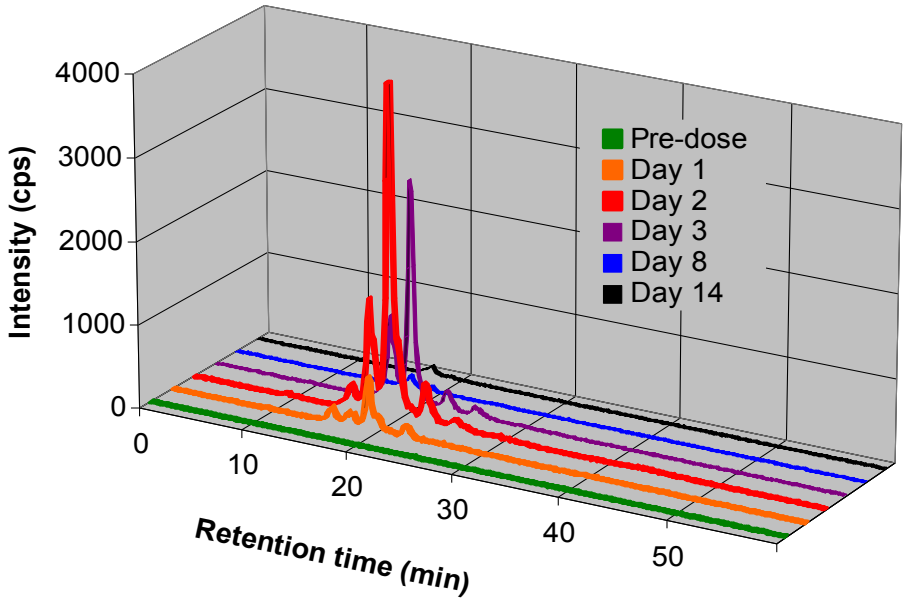
- New: Triple Quad ICP-MS





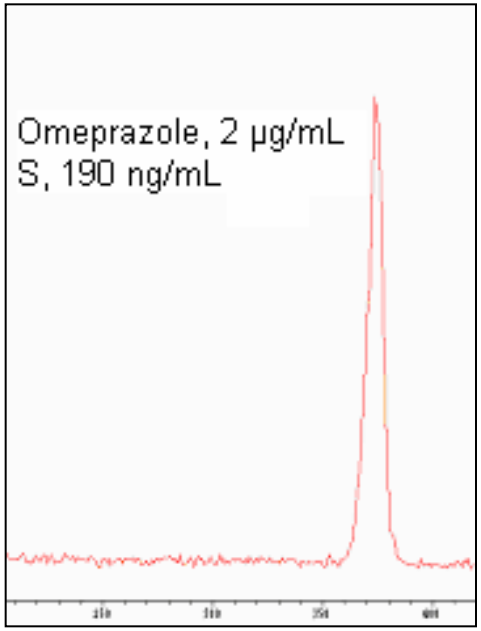
LC-ICP-MS - metabolic profiling





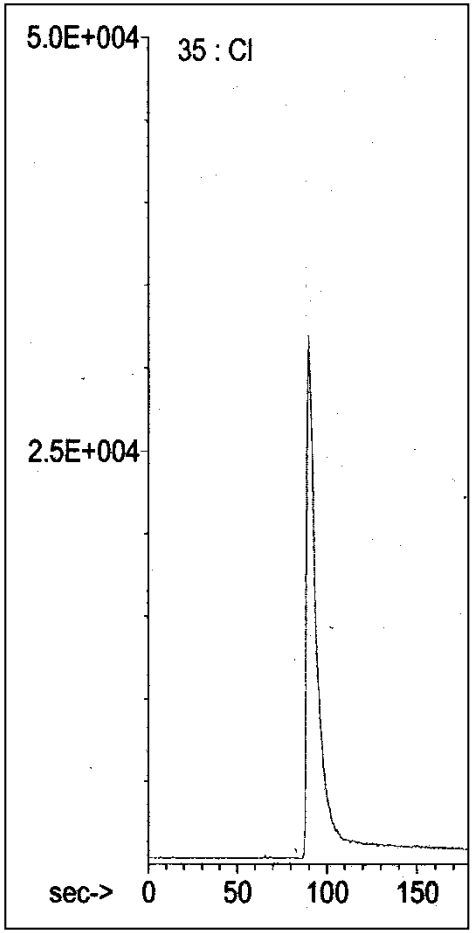
Omeprazole (S)

- Contains 1 S atom
- Collision cell mode with Xenon
- According to literature,
  1 to 10 ng/mL is feasible
- In O_2 mode (reaction gas):
  20 ng/mL
- Proteomics applications



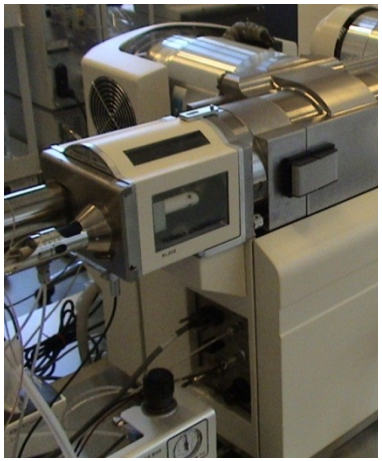
Benzodiazepine (Cl)

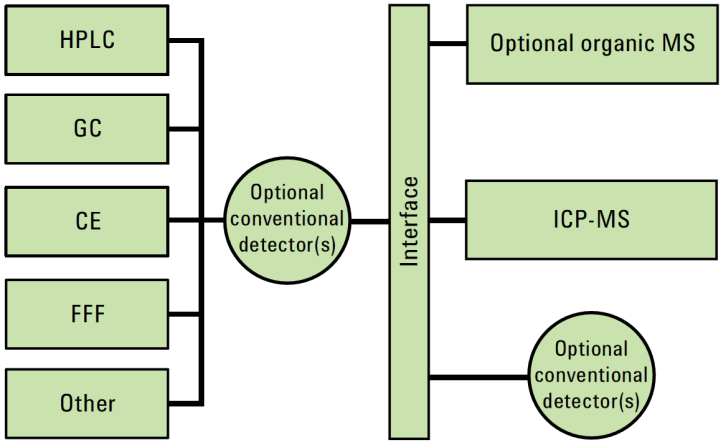
- Contains 1 Cl atom
- Interference of $^{36}ArH^+$, giving incorrect $^{35}Cl/^{37}Cl$-ratio
  Application of He/H_2 reaction gases: no improvement
- Different cones used (Pt, Ni)
- Maximum sensitivity: 50 to 100 ng/mL
  - too electronegative
  - -ve ion mode required ?



Combined elemental & molecular MS detection

QPS helps you navigate





ICP-MS

MS/MS

splitter in tubing synchronizing retention times

HPLC

Sample processing

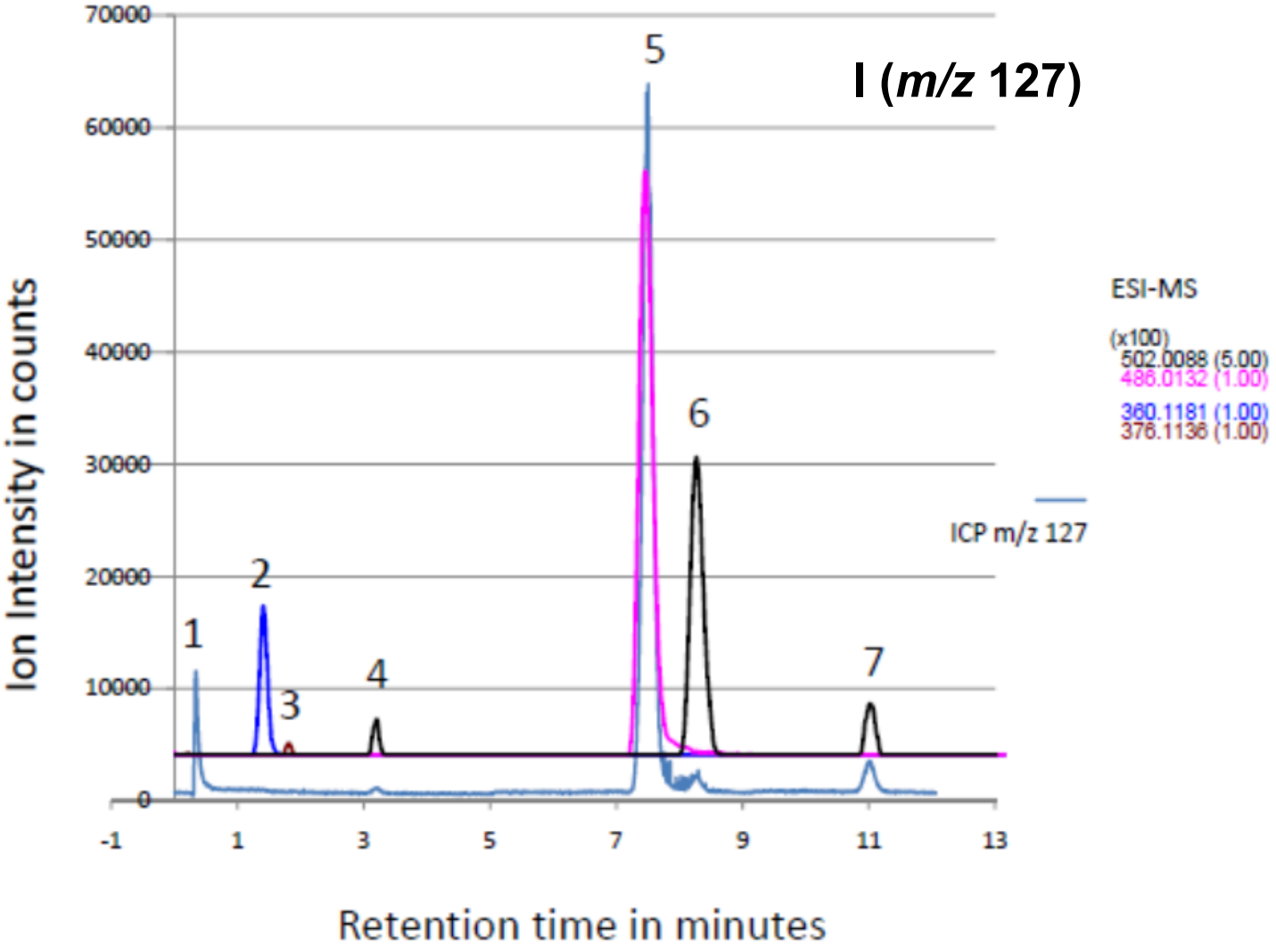
ICP-MS:

- tracing of elements / elemental tags

MS/MS:

- molecular mass
- structural elucidation

Drug discovery – metabolic stability, Iodine



Metabolic stability study of I (*m/z* 127) compound
(p38 kinase inhibitor screening programme)

Regulated bioanalysis - cases

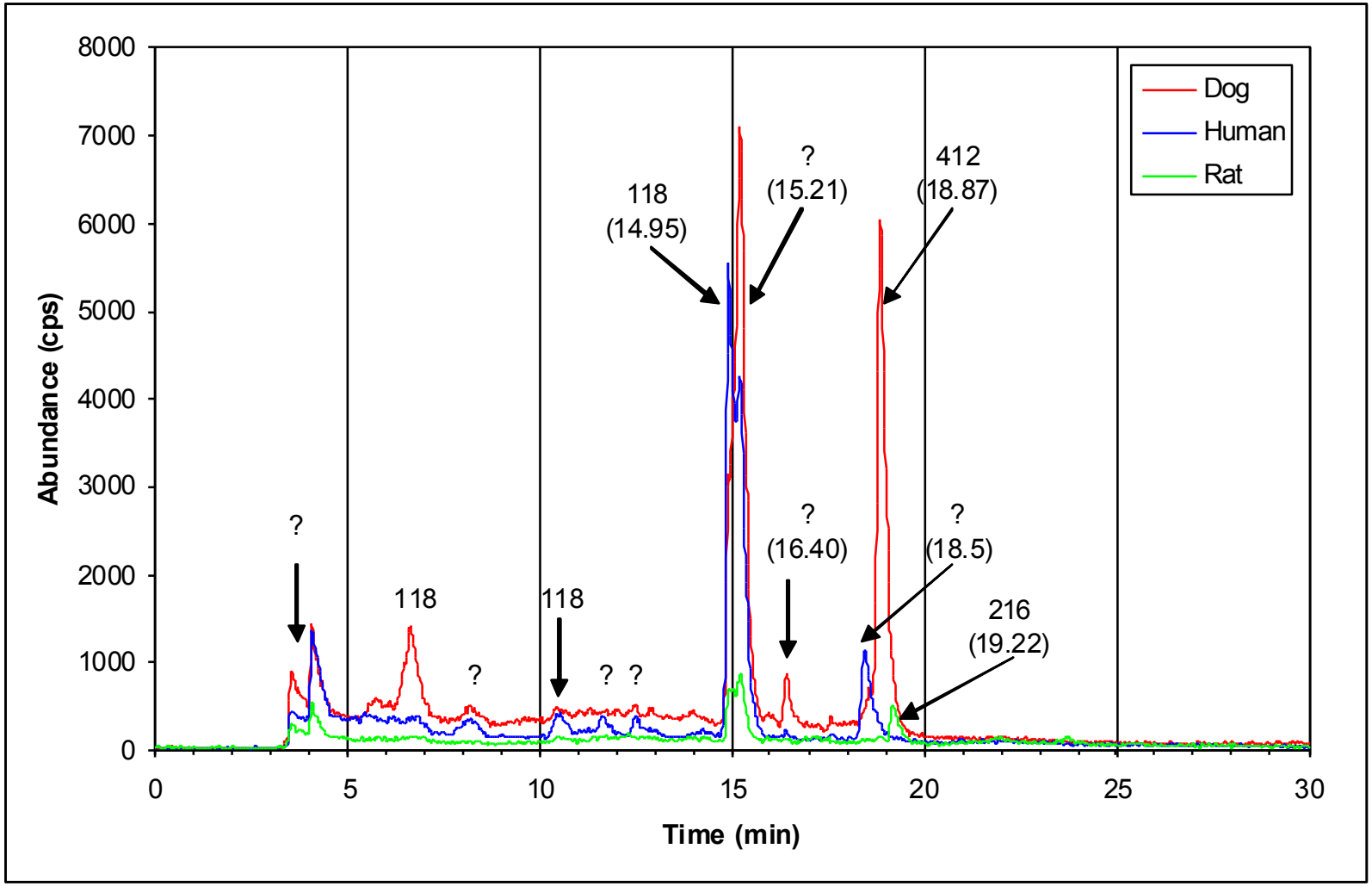
Development of new Platin compounds

- Platin compounds used in cancer chemotherapy
- Dose limited due to toxic side effects
  - Nephrotoxicity
  - Severe nausea
- Toxicity believed to be mainly linked to metabolites
- For new product and formulation studies (new administration routes) comparison of metabolism required → focus on less side effects
- For new entities extensive metabolism studies required
- Assay developed for platin compound and metabolites
- Separation of all metabolites
- LLQ: 0.1 – 1.0 ng/mL

Dog/rat/human PUF sample dosed with new Pt compound (IV), metabolite profiling study

helps you navigate

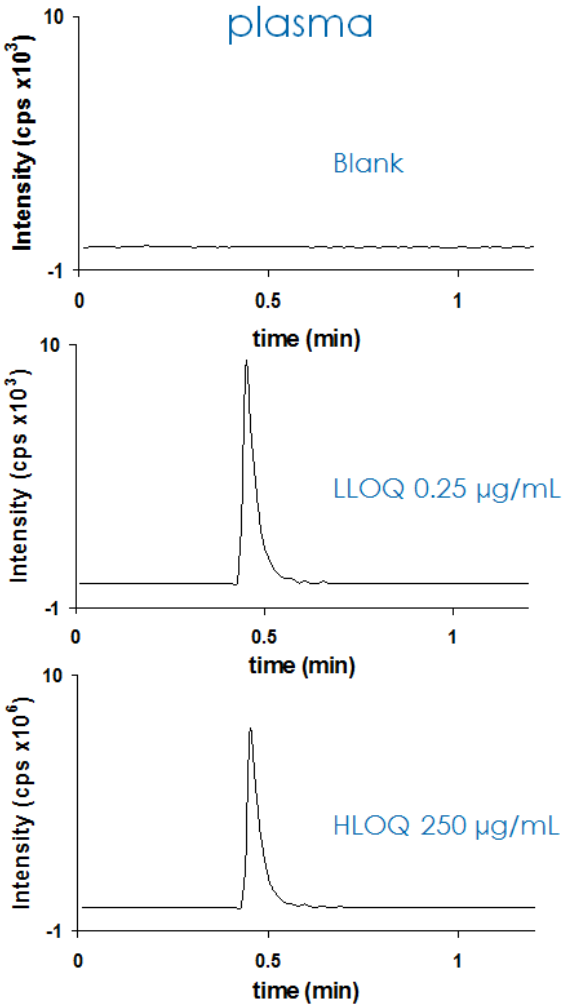
LC-ICP-MS



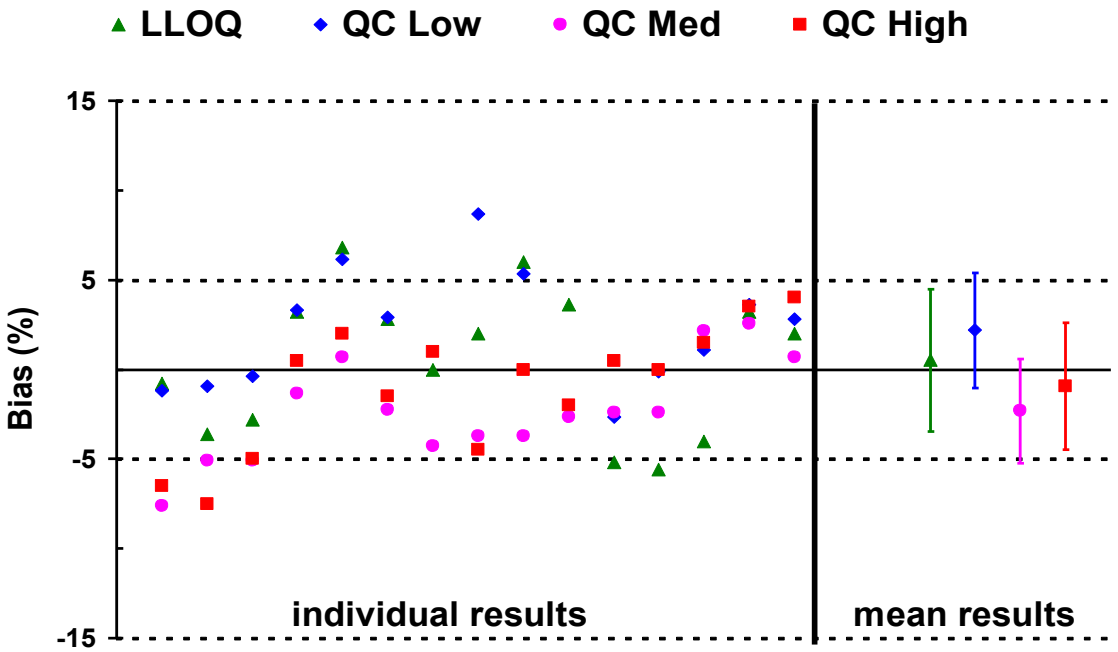
Gadolinium in plasma

- Gd based contrast agents (GBCA's) widely used in MRI (large paramagnetic moment)
- Free Gd (Gd3+) may lead to serious side effects, i.e. nephrogenic systemic fibrosis (NSF)
- Gd, commonly administered as chelating complex, e.g. Gd-DPTA.
- Recently, recognition of toxic potential of all Gd contrast agents due to degradion in-vivo into toxic Gd3+.
- Toxicity determined by amount of formated free Gd3+, i.e. the stability properties of the Gd-complex in-vivo.
- Highly reliable assays required to support clinical studies with (new) stable contrast agents and formulations
  Objective: accuracy and precision < 5%

Results Gd in plasma



Selectivity: highly selective for Gd, no polyatomic interf.
Calibration curves: linear model with 1/xx weighting
CV%: ≤4.0% at all concentrations
Accuracy: 97.7% - 100.5%
Dilution: at least 10-fold dilutions
Carry over: not observed
Matrix effect: not observed (nor polyatomic interferences)



Free Gd3+ present in excess (1:10000) of complex by LC-ICP-MS

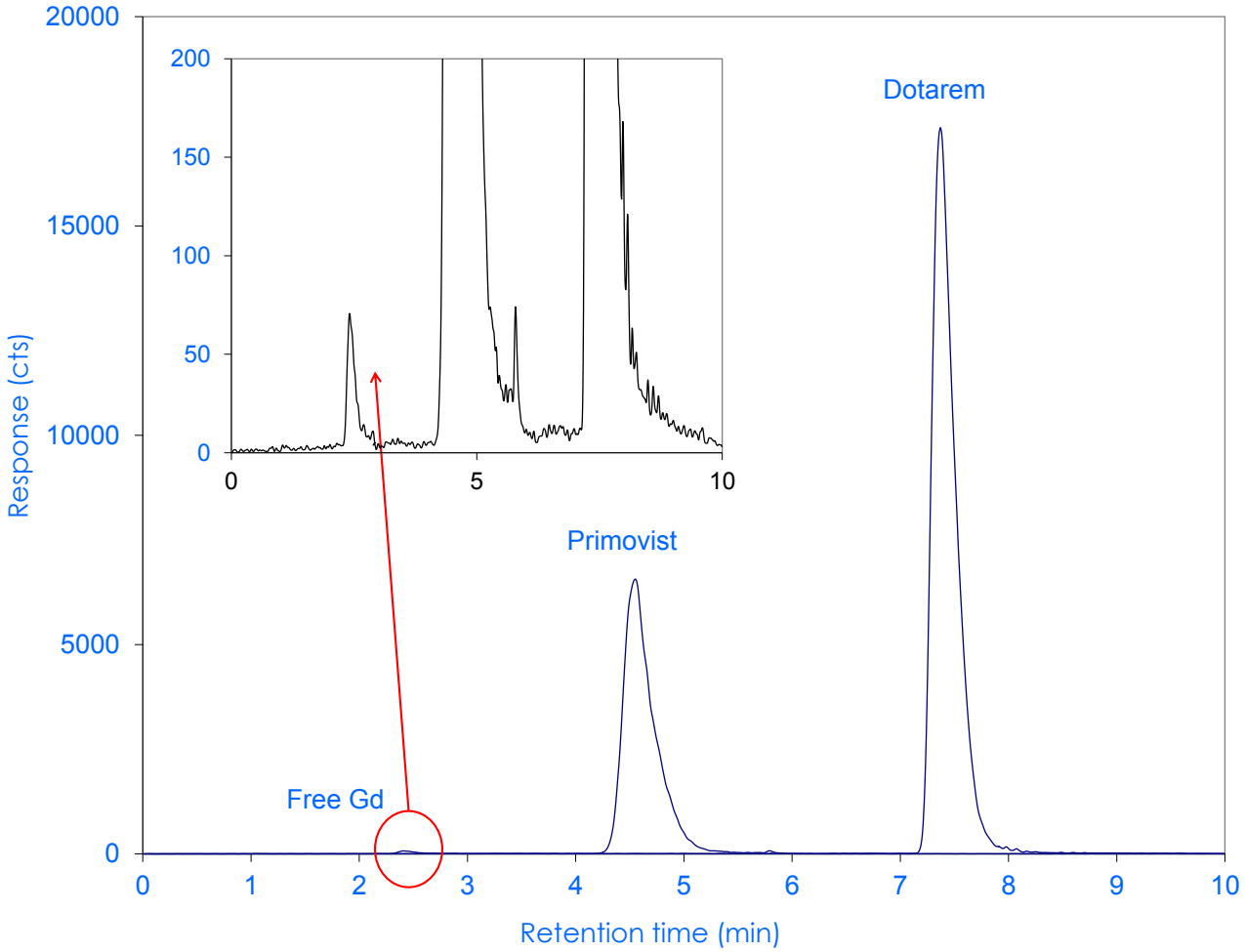
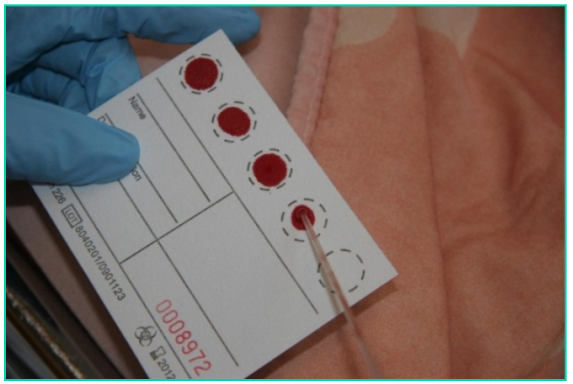
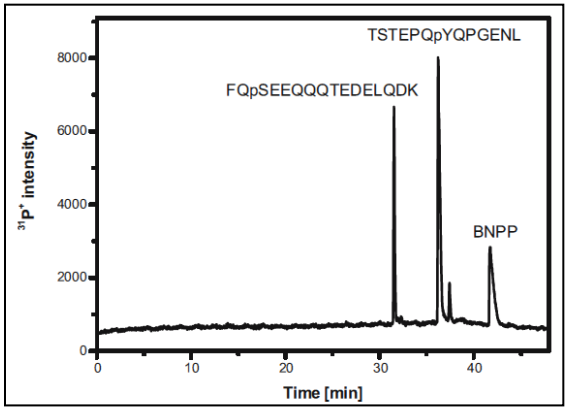


Figure 4: LC-ICP-MS of gadolinium in human plasma. The chromatogram shows the separation Dotarem, Primovist (both at 250 µg/mL) and free Gd (50 ng/mL) spiked in human plasma. A concentration ratio of free to bound 1 : 10000 with an LLOQ of 10 ng/mL can be obtained in this way.

Future Potential

- Dried Blood Spots
  - Essential element analysis, imaging
  - Metallomics, transferrins
  - Toxic elements
- Proteomics
  - Using S or P as ‘internal standard’
  - Transferrins
- Alternative detection technique for immunoassays





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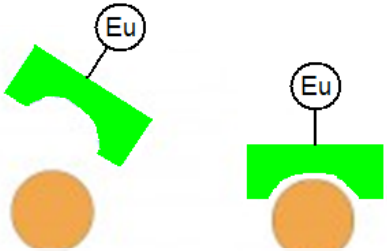
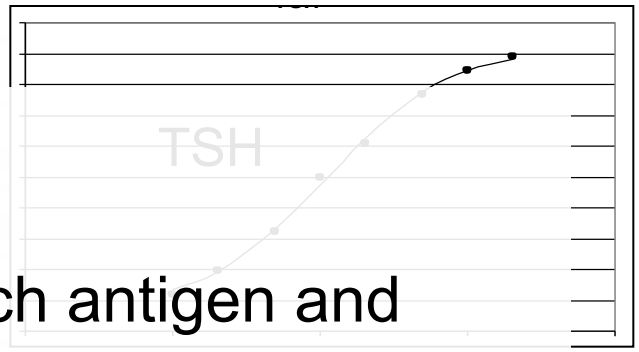
Principle – ICP-MS





- High Precision
- Low detection limits
- Large dynamic range, both for each antigen and between antigens
- Lower matrix effects from other components of biological sample
- Lower background from plastic containers and plates
- Independence of non-specific background and analytical response from incubation or storage times
- Large Multiplexing Potential
- Better Spectral Resolution

Conclusions

- ICP-MS in Drug Development, huge potential, limited recognition in DD
- Excellent detector for HPLC in bioanalysis - orthogonal to other detectors
- Enables simple quantification in all kind of matrices, little to no clean up
- Rapid and efficient technique for PK and metabolism studies / speciation, with or without combination with other detection methods
- (semi-)quantitative for unknowns
- Large potential in quantitative work => more than 40 different elements, mainly metals but also non-metal based compounds, different options assay principles
- Large potential in qualitative work => metabolic profiling studiesLarge potential in other bioanalytical applications (immunoassays, proteomics, imaging)

Acknowledgements

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- *jaap.wieling@qps.com*