

(LC)-ICP-MS FOR ELEMENTAL ANALYSIS IN **DRUG DEVELOPMENT STUDIES**



ASSAY VALIDATION ACCORDING TO FDA, EMA AND ICH GUIDELINES

(LC)-ICP-MS AT QPS

- ▶ ICP-MS detects elements instead of molecules. With the exception of a few elements (such as C, H, N, O and the noble gasses), all 'pharmaceutical-important' elements can be detected. A specific element serves as a tag for the molecule of interest, thus enabling quantitation of the drug molecule in a particular matrix. The technique is highly linear and can be used quantitatively for a broad concentration range. Sample processing is relatively straightforward and with high throughput, allowing fast turn-around times (Fig. 1).

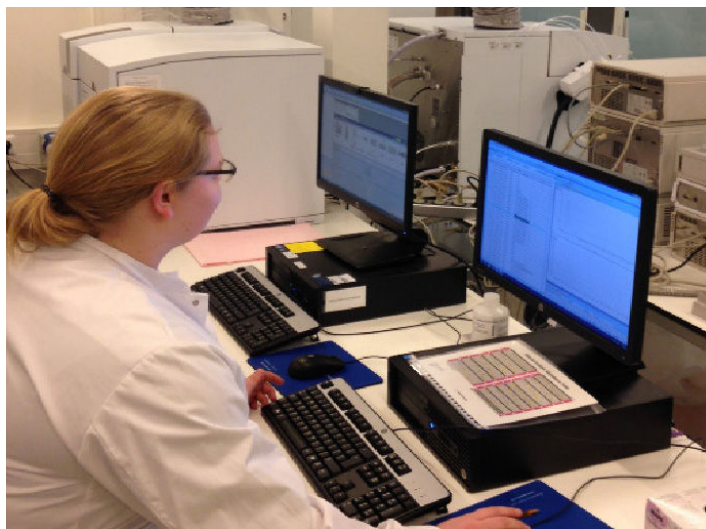


Figure 1: Two of the three LC-ICP-MS Agilent systems at the QPS lab

- ▶ ICP-MS measures total and free concentrations. Typical applications are for pharmacokinetics, pharmacodynamics assessments, toxicology, mass balance, imaging, and metabolite profiling. Furthermore, ICP-MS is frequently used for limit testing of elements, trace elemental analysis, and formulation analysis (Fig. 2 and Fig. 5).

- ▶ LC-ICP-MS measures molecule specific concentration. ICP-MS coupled with HPLC measures the concentrations of all compounds in the matrix and contains the element of interest that are chromatographically separated. This combination enables metabolite profiling and determination of biotransformation and/or degradation products, and different valences or species of the element (Fig. 3).

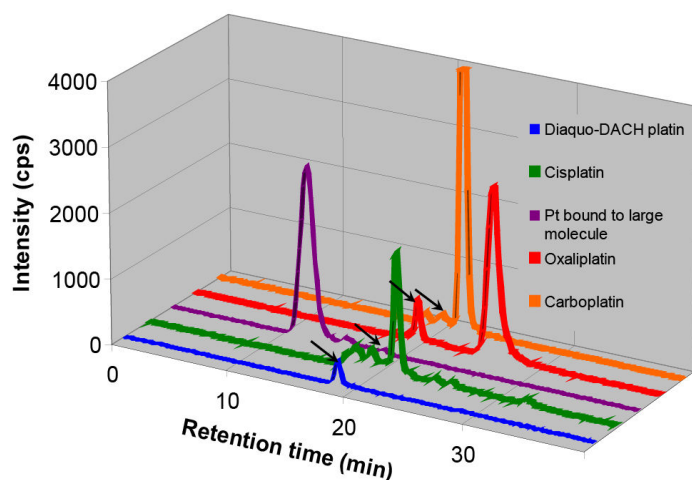


Figure 2: Human serum PK curves of total (protein bound + free) and free iron concentrations after dosage of an iron sucrose compound for screening purposes. Free iron was obtained using ultra-filtration (10 kDa) prior to quantitation by ICP-MS.

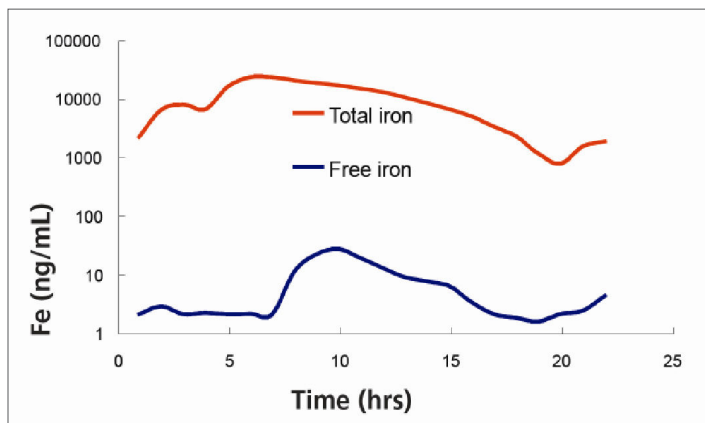


Figure 3: Chromatographic separation of platinum compounds, both free and bound to large molecules in a single run. Arrows indicate the Diaquo-DACH platin present as degradation product in different platinum compounds.

SAMPLE PREPARATION

- ▶ Acidic dilution (e.g., plasma, blood, serum, urine, formulation).
- ▶ Digestion of samples (e.g., feces, tissue, bone) by two DigiPREP MS systems (Fig. 4).
- ▶ Ultrafiltration and equilibrium dialysis for determination of free (unbound) drug concentrations.
- ▶ Solid-phase and liquid extraction if required.

ANALYTICAL POSSIBILITIES

- ▶ HPLC separation for quantitation of parent compound and metabolites.
- ▶ Serial detection using ICP-MS and UV (diode array).
- ▶ Parallel detection using ICP-MS and LC-MS/MS.
- ▶ ELISA-ICP-MS.



Figure 4: 2x DigiPREP for digestion of 216 samples (tissue, feces or bone) per run using nitric acid, hydrogen peroxide and controlled heating.

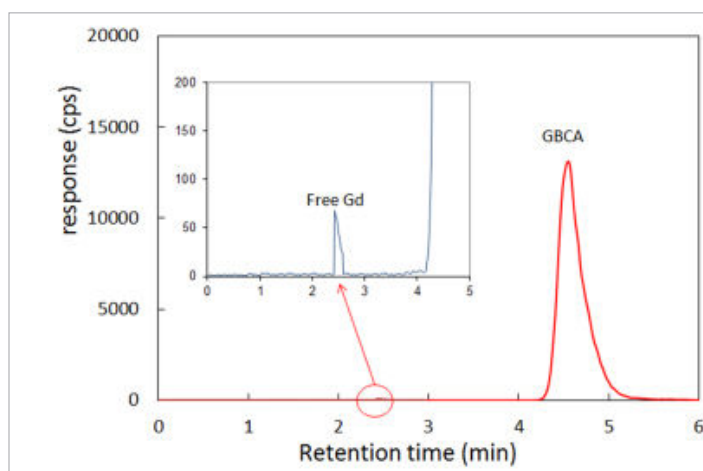


Figure 5: Example of an LC-ICP-MS chromatogram showing the peaks of free Gd (gadolinium), as originated from the degradation of the chelated Gd molecule, (see insert) and the intact chelated Gd, depicted by GBCA analyzed from a human plasma sample. LC-ICP-MS assays were developed to determine free Gd³⁺ and chelated for Dotarem, Primovist and several potential new Gd based contrast agents (GBCA's). A ratio of free to chelated up to 1 : 20,000 can be obtained with an LLOQ of 10 ng/ml. The LLOQ can vary and depends on the matrix. In addition, an ICP-MS assay was developed in parallel for the determination of total Gd in human plasma with precision and accuracy within 5%.

EXAMPLES OF DIFFERENT ELEMENTS OF INTEREST FOR ICP-MS APPLICATIONS:

METALLOPEPTIDES	METALLODRUGS	MISCELLANEOUS
Metalloenzymes: Cu, Fe, Mg, Mn, Mo, Ni, Se, Zn	Indazolium, imidazolium: Ru	DNA restriction fragments: Fe, Mn, Co, Pb, Cd
Metallothioneins	Chemotherapeutics: Pt, Ru, Rh, Ti, Ga, As, Au	Metalloporphyrines: As(III)/As(V), Ge, Sb, Se, Co(II)/Co(III)
Physiological: Zn, Cu, Se	Bone resorption: La, Eu, Gd, Tb, Yb	Ferrocene derivatives: Fe
Xenobiotic: Cd, Hg, Ag, ...	Anti-arthritic therapeutics: Au	Cobalamines, cobanamids: Co
Phytochelators: Cd, Ag, Cu, Pb, Zn	Anti-diabetes therapeutics: V, Cr, Cu, Zn, Mn, Mo	Imaging agents: Tc, Fe, Gd, Mn, I, Ba
Transport Proteins	Gastrointestinal disorders, stomach ulcer: Bi, Al	Metal-complexes amino acids: Zn, Cu, Mn, Ni
Albumin: Cu, Al; Transferrin: Fe, Al		
CorA: Mg, Co, Fe, Ni		

SOME EXAMPLES OF (LC)-ICP-MS ASSAYS DEVELOPED AT QPS

- ▶ Determination of free and total platinum in urine, whole blood, red blood cells or plasma from new or existing platinum drug formulations used in oncolytic studies, e.g., oxaliplatin, cisplatin and carboplatin.
- ▶ Development of a new assay to analyze micelle and/or protein bound and free platinum in one analytical run using LC-ICP-MS and size exclusion chromatography.
- ▶ Simultaneous determination of free and chelated Gd (up to 1 : 20,000) in MRI Gadolinium Based Contrast Agents (GBCA) which are in development for having lower free Gd concentration (Fig. 6).
- ▶ Determination of platinum in mouse plasma and mouse organs by LC-ICP-MS.
- ▶ Determination of total and non-ceruloplasmin bound copper in human serum and copper in human urine for Wilson disease.
- ▶ Determination of copper, zinc, aluminum, and iron in human blood. These elements play a role in Alzheimer and Parkinson disease and are considered as biomarker.
- ▶ Determination of total and free iron in iron sucrose studies (Fig. 4), and transferrin bound copper in human serum by LC-ICP-MS (Fig. 6).

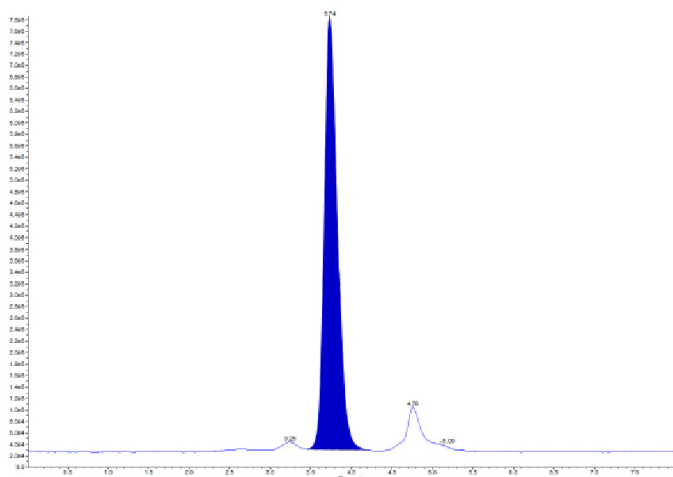


Figure 6: Chromatogram of iron in human serum determined as Transferrin bound iron (TBI) by LC-ICP-MS after dosing of iron sucrose. The TBI is chromatographically separated from other iron sources in human serum. This method to determine TBI by LC-ICP-MS and total iron by ICP-MS is much more reliable than the classical spectrophotometric method.

WHY QPS?

- ▶ We have built up vast experience since 2016 developing and validating hybrid LC-MS/MS methods for the quantitation of many biotherapeutics in various animal species and matrices.
- ▶ We have fully GLP compliant laboratories in USA and Europe.
- ▶ We have 5 dedicated hybrid LC-MS/MS systems and thus sufficient capacity for supporting large (preclinical and clinical) studies.
- ▶ We are very flexible, cooperative and have a broad experience in biochemistry, enzymology, molecular biology, mass spectrometry, and bioanalytical chemistry, including regulatory aspects.
- ▶ We are used to conducting complex studies.
- ▶ We value face-to-face meetings. You are welcome to visit us and view our laboratories.

Whether your focus is:

- ▶ Small molecules
- ▶ Protein biotherapeutics
- ▶ Vaccines
- ▶ Gene therapy

QPS provides a full range of bioanalytical solutions to support drug development from discovery through clinical development and filing.