



Tailor made sample preparation for unusual bioanalytical research

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“Usual” bioanalysis

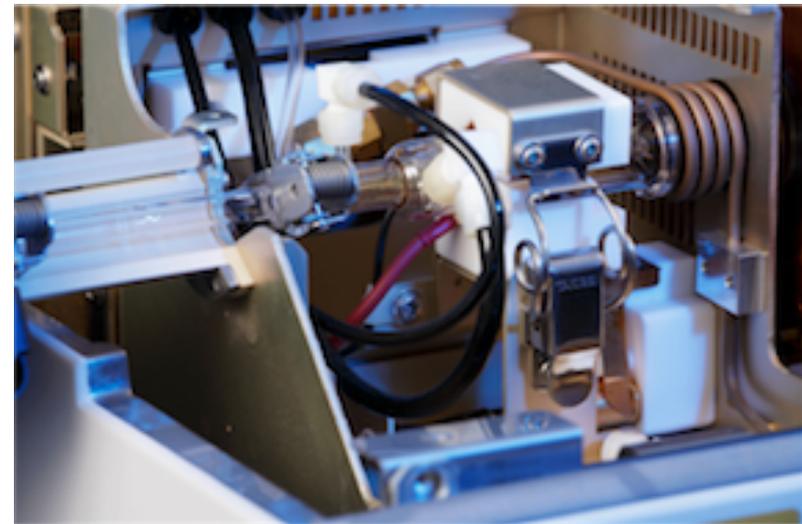
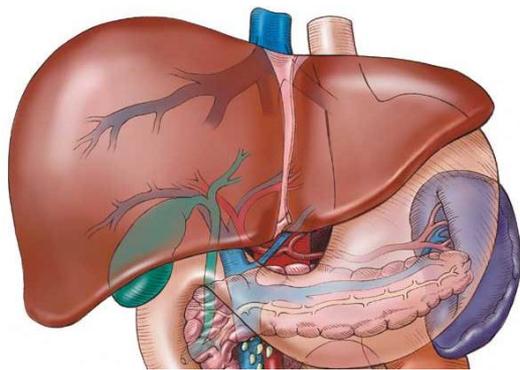
- Regulated BA, usually in compliance to FDA/EMA Guidances and White Papers
- API is organic molecule; for acid/base: non-active counter ion (Cl, Fo, Ac, Na, ..)
- HPLC-MSMS analysis of plasma
- Sample prep limited to Protein Precipitation, SPE or LLE
- Automation and high-throughput



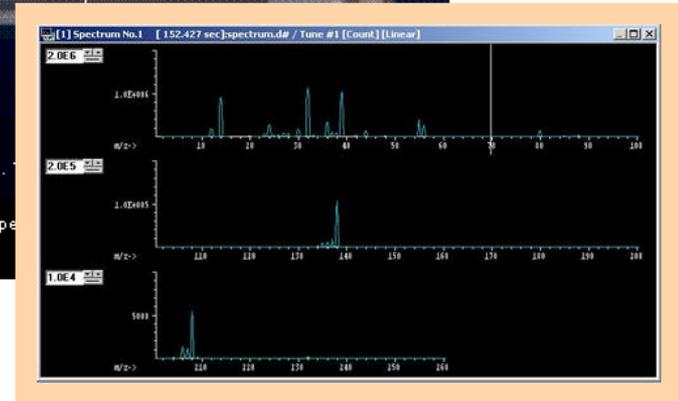
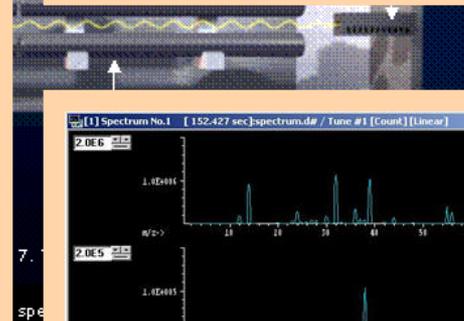
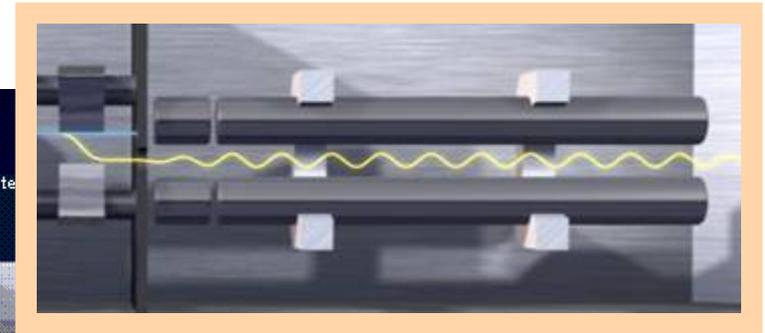
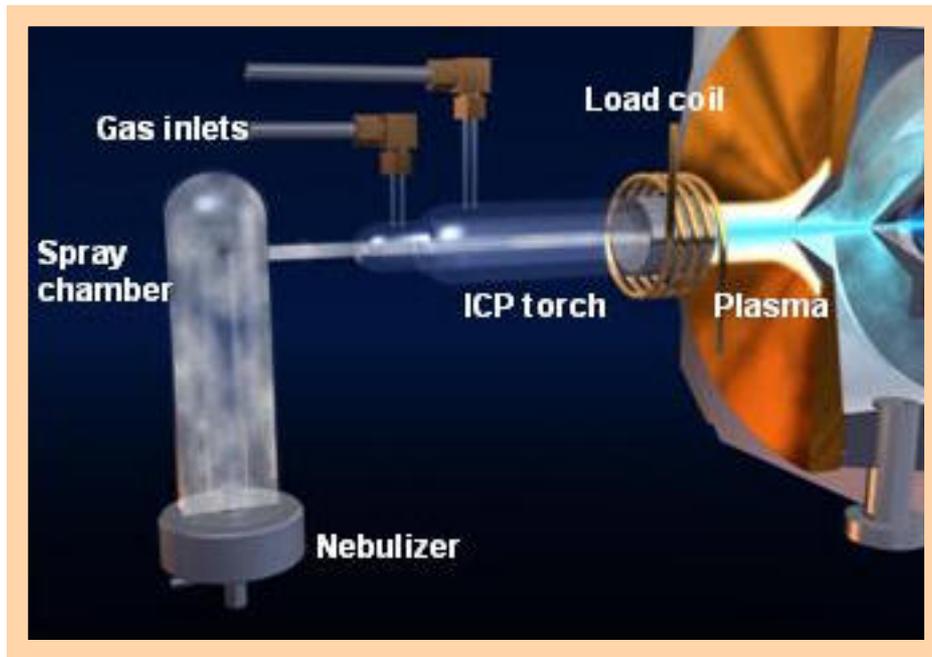
>> at WIL this is >80% of what we do

“Unusual” in today’s presentation

- Inorganic, metallic or specific element instead of -or in addition to- organic molecule
- ICP-MS and HPLC-ICP-MS analysis
- Various tissues: bones, kidney, brain, liver, ...
- Rigorous sample preparation
- Non-classical BA regulations



Principle of ICP-MS



Sample preparation in ICP-MS bioanalysis (1)

- General requirements
 - Low organic load of samples
 - Background interference at low levels
 - Constant viscosity, stable nebulized spray
 - 100% in solution
 - Matrix matched calibrants and QC's



Sample preparation in ICP-MS bioanalysis (2)

- Solubilizing tissue and bones (and analyte)
 - Acid: HNO₃ (65 %)
 - Dissolves everything
 - Degradation of test item, total element detection only
 - Base: TMAH (25 %)
 - Dissolves soft tissues only
 - Test item often stable, allows HPLC-ICP-MS speciation



“This is **not** how we do it ...”

Use of Internal Standard in ICP-MS

- No stable isotopes available
- Analogue IS with comparable ionization energy as test item element
- Low-abundant element: Y, Sc, ...
- Compensate for:
 - Sample-to-sample matrix variations
 - Irregular spray
 - MS sensitivity variability
- In LC-ICPMS, analyte with same element



Three BA ICP-MS experiences

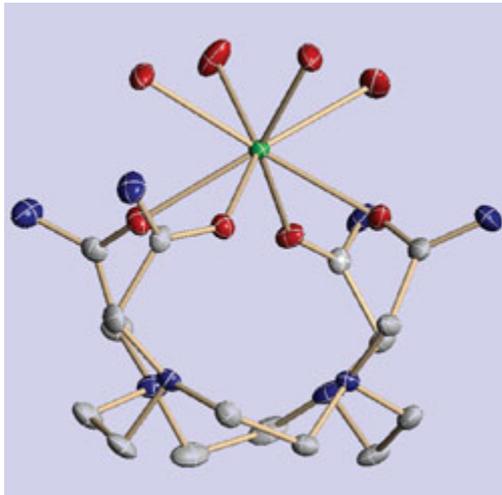
- Org-Gd as MRI contrast agent
- Se-compounds as source of essential element in feed
- Sr as alternative counter ion in API

1 H																	2 He													
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne													
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar													
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr													
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe													
55 Cs	56 Ba	57 La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn													
87 Fr	88 Ra	89 Ac	104 Unq	105 Unp	106 Unh	107 Uns	108 Uno	109 Une	110 Uun																					
																	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
																	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

(sponsor names and compound structure not to be disclosed)

1. Org-Gd by HPLC-ICP-MS

- Gadolinium (Gd) in use as MRI contrast agent
- Free Gd is toxic, hence organic complex
- Evaluate metabolism of Org-Gd complex in ADME study



Org-Gd: Bioanalytical study design

- Usual approach: ^{14}C labelling to detect and identify metabolites by HPLC-MS
- Here: HPLC-ICP-MS to detect Gd containing metabolites
- Benefit: also free Gd^{2+} detectable

Challenges:

- HPLC analysis of Gd^{2+} and Org-Gd and metabolite
- Free metal ion extremely difficult to chromatograph

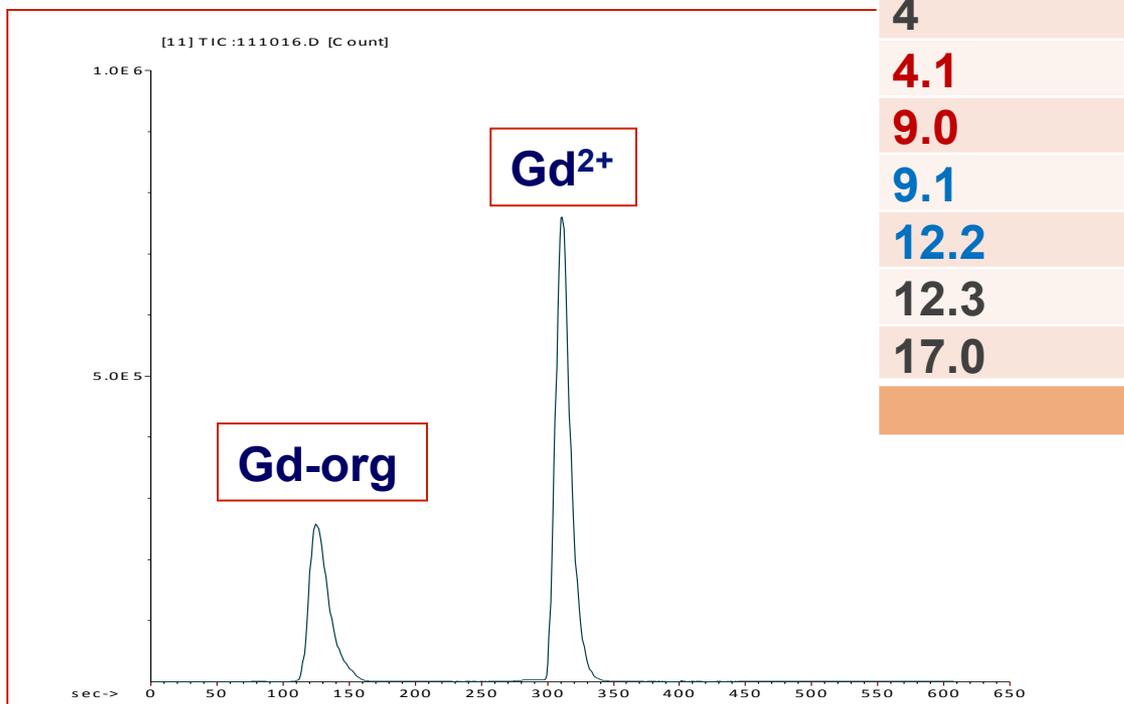
Analytical conditions

Column	PRP-1, 100 x 2.1 mm; dp = 5 μ m; 30°C
Injection	10 μ l
Flow	0.3 ml/min

A	5/95 acetonitrile/water
B	5/5/90 nitric acid (65%)/acetonitrile/water
C	10/5/85 ammonia (25%)/acetonitrile/water

- neutral conditions at which Org-Gd is eluted without release of free Gd
- acidic conditions to elute free Gd
- basic conditions to neutralize column

Gd LC-ICPMS



Time [min]	%A	%B	%C
0	100	0	0
4	100	0	0
4.1	0	100	0
9.0	0	100	0
9.1	0	0	100
12.2	0	0	100
12.3	100	0	0
17.0	100	0	0

2. Selenium as feed-additive

- Organic-Se is less toxic than inorganic forms (selenite and selenate)
- Cost of production and bio-availability were driving forces to search for alternative Org-Se compounds
- Focus:
 - Bioavailability of Se
 - Se uptake in tissues (liver, muscle, kidney)
 - Metabolism of Org-Se



Org-Se: Bioanalytical study design

- **Total-Se by ICP-MS** of plasma and tissues to evaluate transport and uptake of Se
- **HPLC-ICP-MS** to evaluate Selenium metabolism of Org-Se
- HPLC-MS to evaluate behaviour of parent Org-Se compound

Challenges:

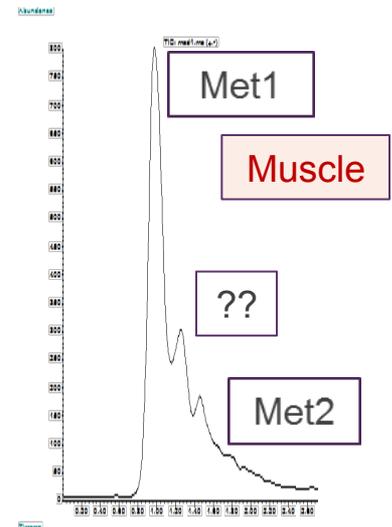
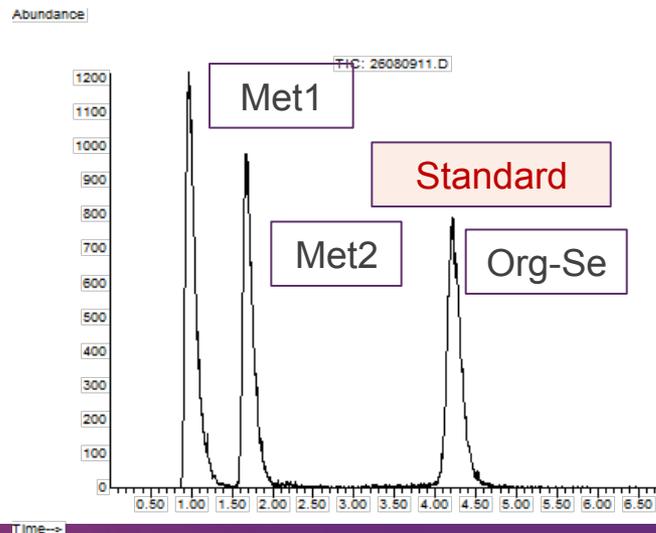
- Solubilize tissues and trouble-free injection onto ICP-MS
- HPLC separation of Org-Se compounds
- Low Se content tissues for method validation and QCs

Sample prep of tissues for Se analysis by ICP-MS

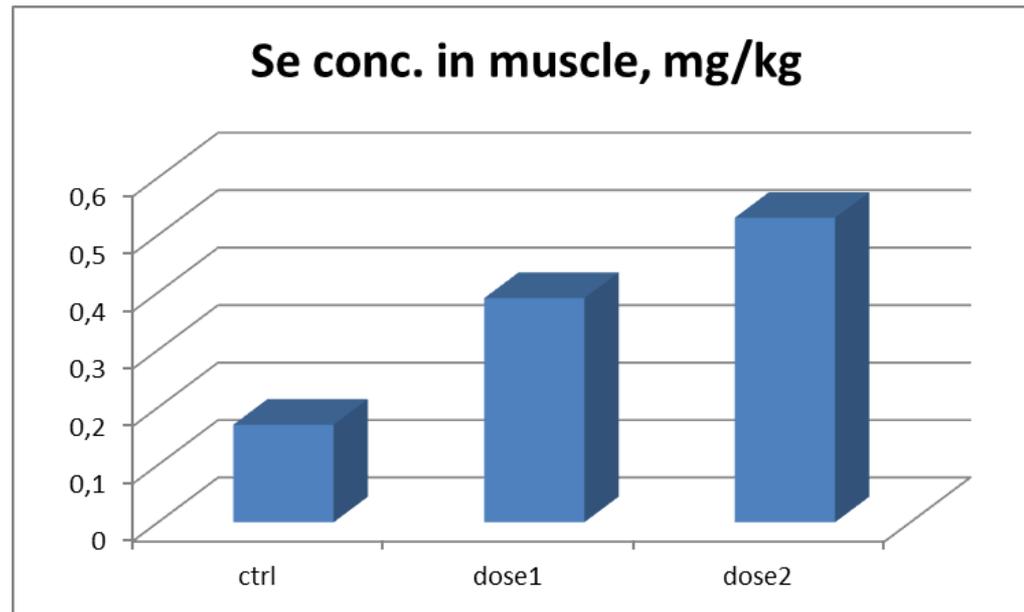
■ Procedure:

tot-Se	<ol style="list-style-type: none"> 1. 250 mg tissue + 1.5 ml TMAH over night at RT 2. 1:9 dilution in Triton-X100/HNO₃ 3. centrifuge 5 min at 12,000 g 4. 2 ml supernatant + 20 μl IS solution (Y)
Org-Se + Metab	<ol style="list-style-type: none"> 1. Ultraturrax in 1:3 PBS 2. Centrifuge 5 min at 12,000 g 3. PP by 4.5% sulfosalicylic acid 4. Evap + reconstitute

HPLC-ICP-MS:



Dose-effect relationship for total-Se



- Efficient uptake of Se into tissue

3. Strontium as alternative counter ion

- No literature data on effect of Sr during early development
- Focus:
 - Demonstrate exposure to Sr
 - Evaluate dose relationship for tissues
 - Evaluate barriers during pregnancy
 - Evaluate efficacy and tox compared to other salts



Org-Sr: Bioanalytical study design

- **Total-Sr by ICP-MS** of plasma, tissues and bones to evaluate exposure and uptake of Sr

Challenges:

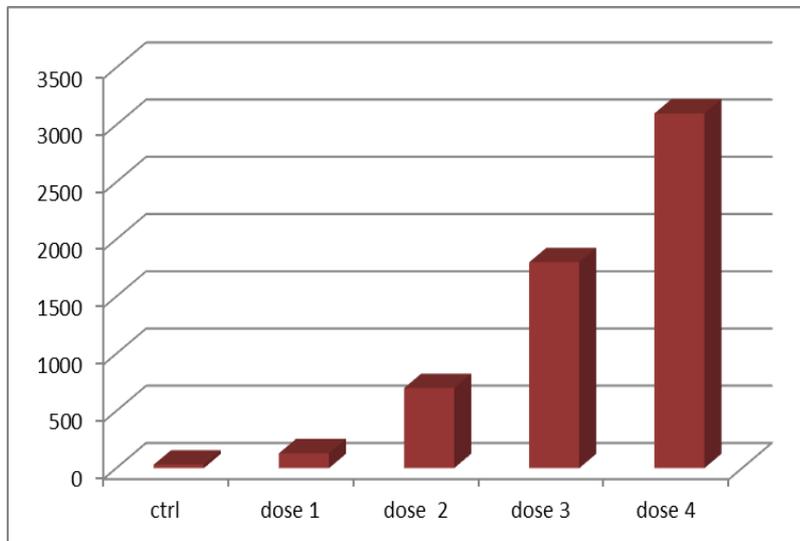
- solubilize tissues/bones and trouble-free injection onto ICP-MS
- low-content Sr for method validation and QCs
- simultaneous measurement of Ca in bones (Ca standards always contain ca 0.1% Sr)

Sample prep of tissues/bones for ICP-MS

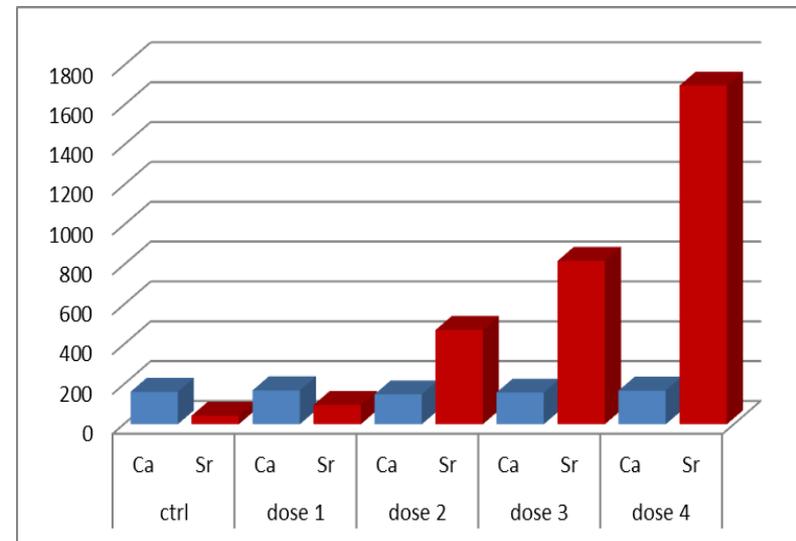
- Procedure:
 - 100 – 400 mg tissue/bone
 - Add 2.5 ml/g or 25 ml/g (tissue/bone) HNO_3
 - Incubate at 60 °C overnight
 - Take subsample, add IS, dilute
- Parallel processing of up to 300 samples in one batch
- Tedious, high pressure/temp MW digestion can be avoided

Exposure to Sr – plasma and bone

Sr (ng/ml) in plasma



Ca (mg/g) and Sr (μ g/g) in bone



Ca levels in bone unaffected

After expose Sr/Ca ratio increases from 0.025% to 1%

Conclusions

- ICP-MS and HPLC-ICP-MS well suitable for bioanalysis
- Matrices include plasma, blood, soft tissues, bones
- Sample preparation needs special attention
- Digestion by Nitric acid, TMAH
- LLOQ depends on element and background levels, but can be as low as ng/ml in plasma