ELECTRONIC SUPPLEMANTARY INFORMATION (ESI)

Cobalamin concentration in fetal liver show gender differences: a result from using an ultra-trace cobalt speciation method

Janine Bosle[1], Sven Goetz[1], Andrea Raab[1], Eva M. Krupp[1], Kirk G. Scheckel[2], Enzo Lombi[3], Andrew A. Meharg[4], Paul A. Fowler[5] and Jörg Feldmann[*1]

Table S1: HPLC-ICP-MS/ESI-MS parameter

HPLC:	
column	Agilent Eclipse XDB-CB (5 µm, 4.6 x 150 mm)
Eluent A	0.1 % Formic Acid in water
Eluent B	0.1 % Formic Acid in methanol
Gradient programme	MeOH gradient linear from 0 to 100 % MeOH from 0 – 20min, then re-equilibration of column.
Injection Volume	0.1 mL
Column temperature	30˚C
FTMS:	
Scan speed	1 Hz
Resolution	MS 30.000, MS/MS 7500 (triggered at intensities above 50.000)
m/z units scanned	200-2000
ESI-MS:	
Instrument	LTQ Orbitrap Discovery (Thermo)
Mode	Positive
Capillary Voltage	4.5 kV
Capillary temperature	320^{o}C
Shealth and aux gas	20 (arb. Units)
Optimization	Standard
ICP-MS:	
Instrument	Element 2 (Thermo)
Sampler	Platinum
Oxygen	20 mL/min
Nebuliser	Micro-concentric
Gas Flow (Argon)	0.65 L/min
Sensitivity	Optimum
Mode	Low Resolution (300)
Injection	200/250 µL/min

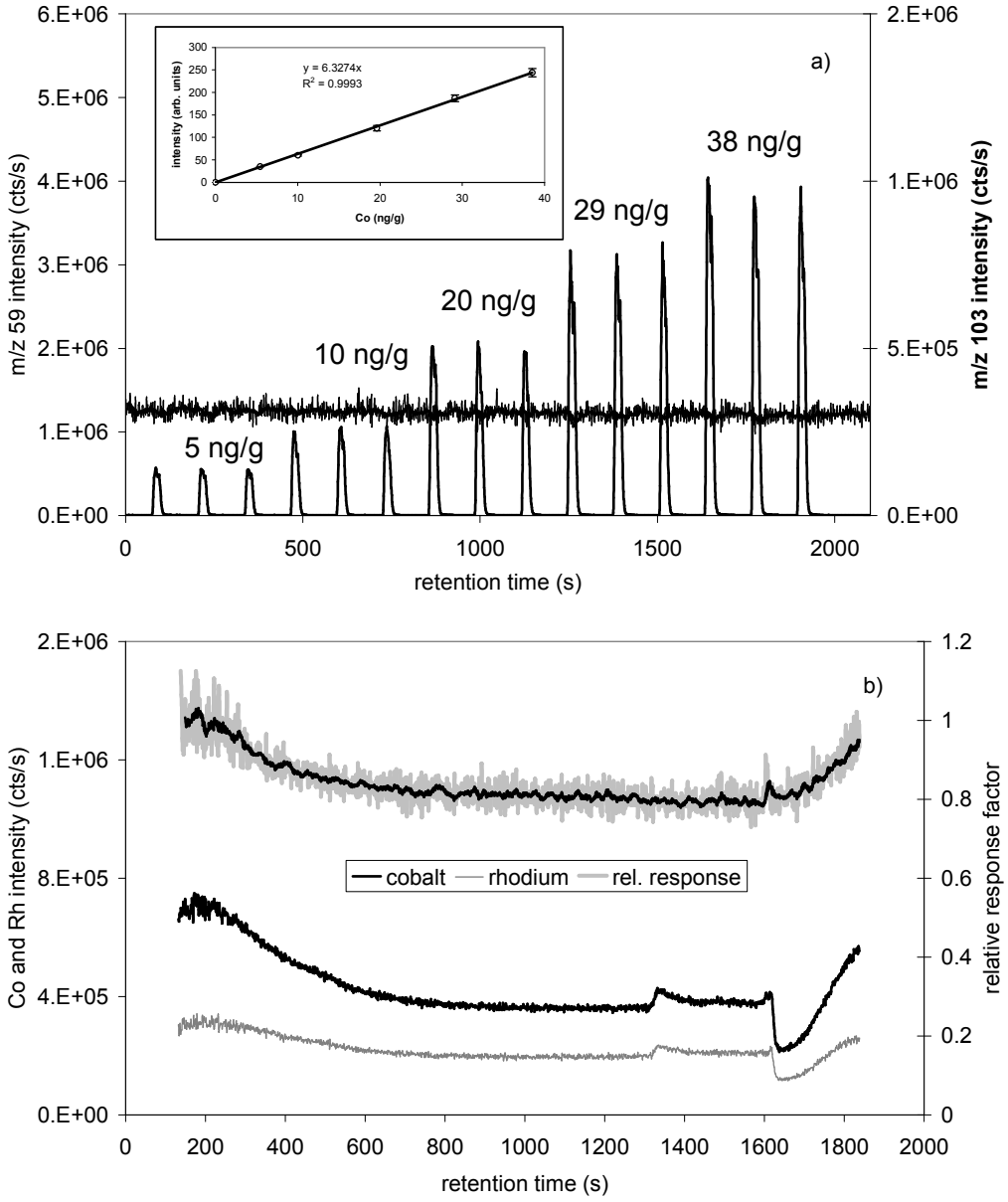

Figure S1: Calibration of cobalt: flow injection of calibration standards with starting conditions for speciation and online post-column rhodium addition (a), while adding online post-column cobalt (m/z 59) and rhodium (m/z 103) together and run the gradient programme of the HPLC separation the behaviour of cobalt and rhodium could be monitored. The relative response factor of cobalt is shown with the reference to the starting conditions.

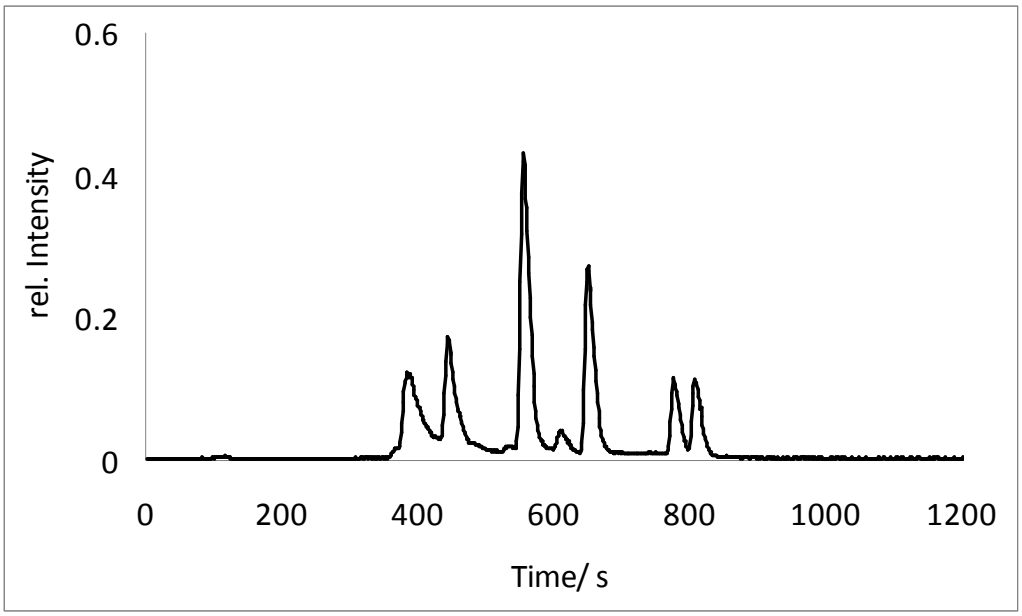

Figure S2: chromatogram of four cobalamin species spiked to liver. The extraction with water is the most gentle extraction method because water does not help to precipitate the proteins. The measurement of the cobalamin standards showed that there is no unbound cobalt (100-200s), but the characteristic Cbl species have changed. ESI-MS data show that HO-Cbl has doubled (two peaks at 400s), CN-Cbl at 600 s and Ado-Cbl at 650 s, while Me-Cbl showed also two peaks (around 800 s)..

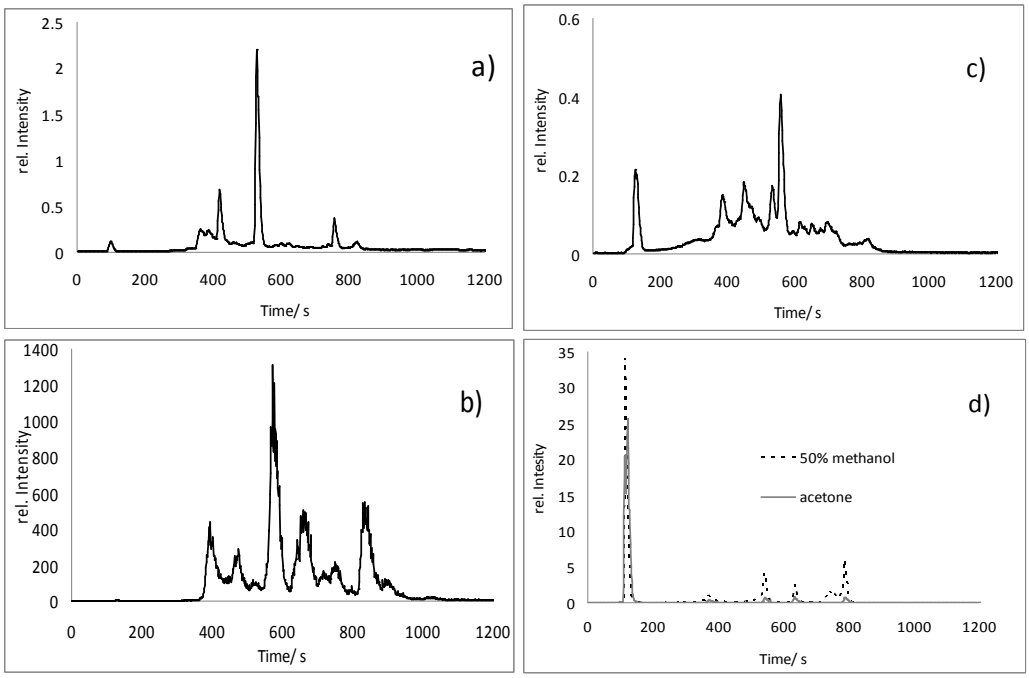

Figure S3a-d: Chromatograms of cobalt species after spiking Cbl-species (OH-Cbl, CN-Cbl, Ado-Cbl, Me-Cbl) in different extraction solution without cyanide. Papain extraction (a); nitric acid extraction (b); methanol extraction (100%) (c); and acetone extraction in comparison to 50 % methanol (d). ESI-MS data show that peaks <200 s are unbound inorganic cobalt species (no characteristic Cbl masses), HO-Cbl has doubled (two peaks at 400-450 s), CN-Cbl at 600 s and Ado-Cbl at 650 s, while Me-Cbl showed also two peaks (around 800 s). All other peaks are unknown Co peaks without a charcertistic ESI-MS spectrum.

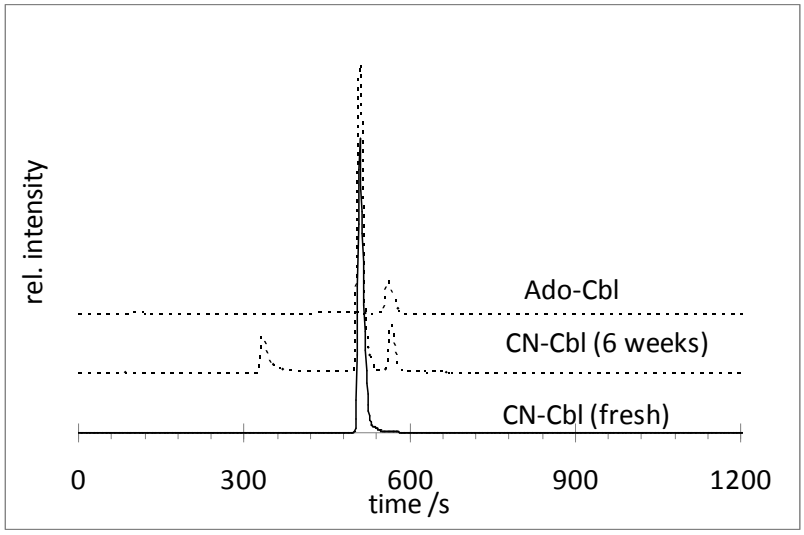

Figure S4: Chromatograms (HPLC-ICPMS, m/z 59) of CN-Cbl as a freshly made standard compared to one stored six weeks in the fridge and an Ado-Cbl/ Ho-Cbl standard (a) and Papein extract of a CN-Cbl standard under optimised conditions (b), mass spectrum of ESI-Orbitrap MS of the peak 4 at 560s (c).

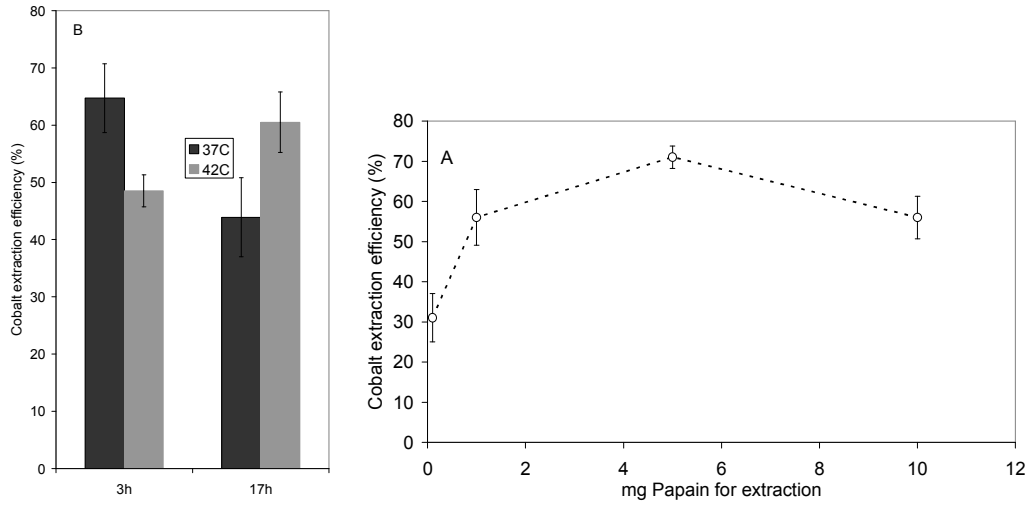

Figure S5: The extraction efficiencies of cobalt from a pig liver containing 43.3 ± 2.3 ng Co/g (n=4). (a) shows the variation of papein, while (b) shows the variation of temperature and incubation time. The error bars are standard deviations for three or four replicates.

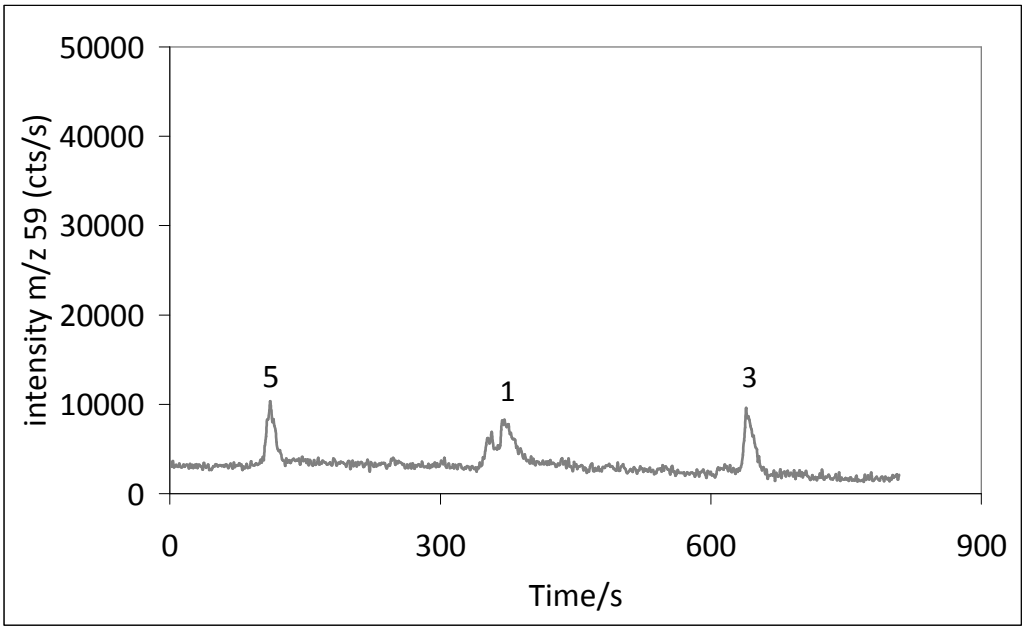

Figure S6: HPLC-ICPMS chromatograms (monitored on m/z 59 for cobalt) of an unspiked pig-liver extracted with heated water. In addition to OH-Cbl (peak 1), only Ado-Cbl (Peak 3) and hydrophilic cobalt (peak 5) could be detected.

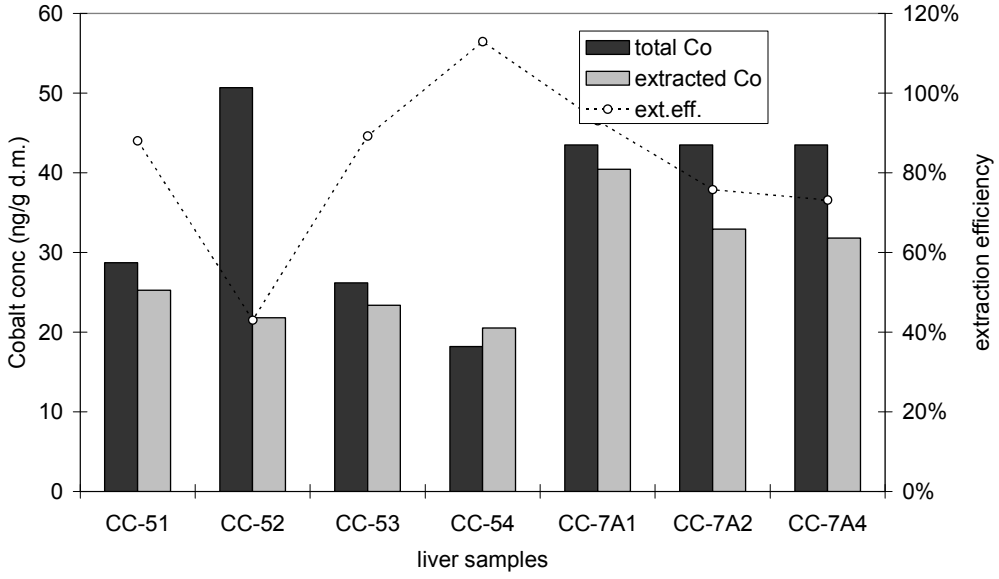

Figure S7: Comparison of total and extractable cobalt in pig liver samples (CC-5 and CC-7A) samples and calculated extraction efficiency. CC-52 is an outlier.

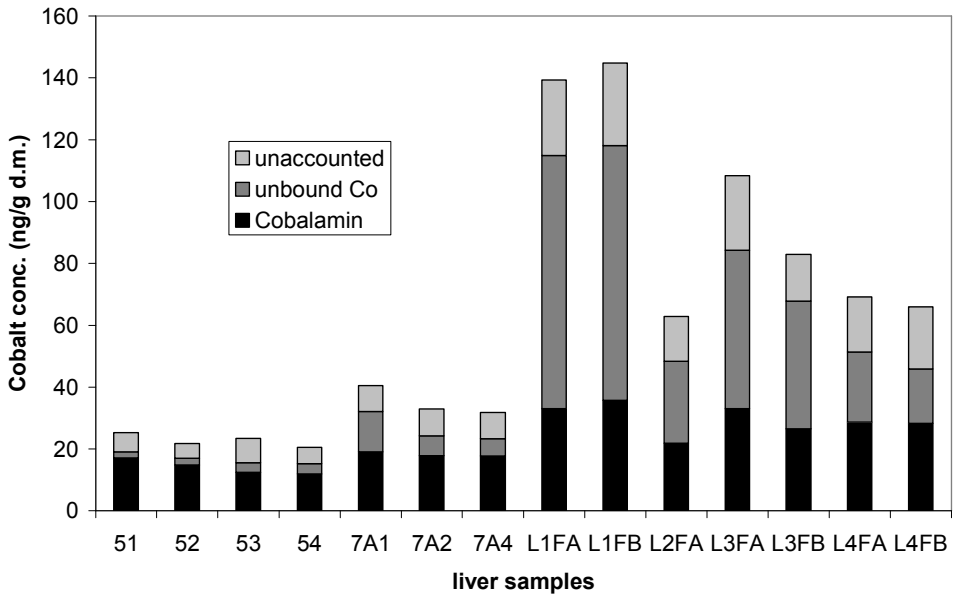

Figure S8: Cobalt speciation in pig liver samples. Cobalamin measured as CN-Cbl, unbound Co eluted in the void and unaccounted Co was calculated as the difference between total Co and extracted Co.

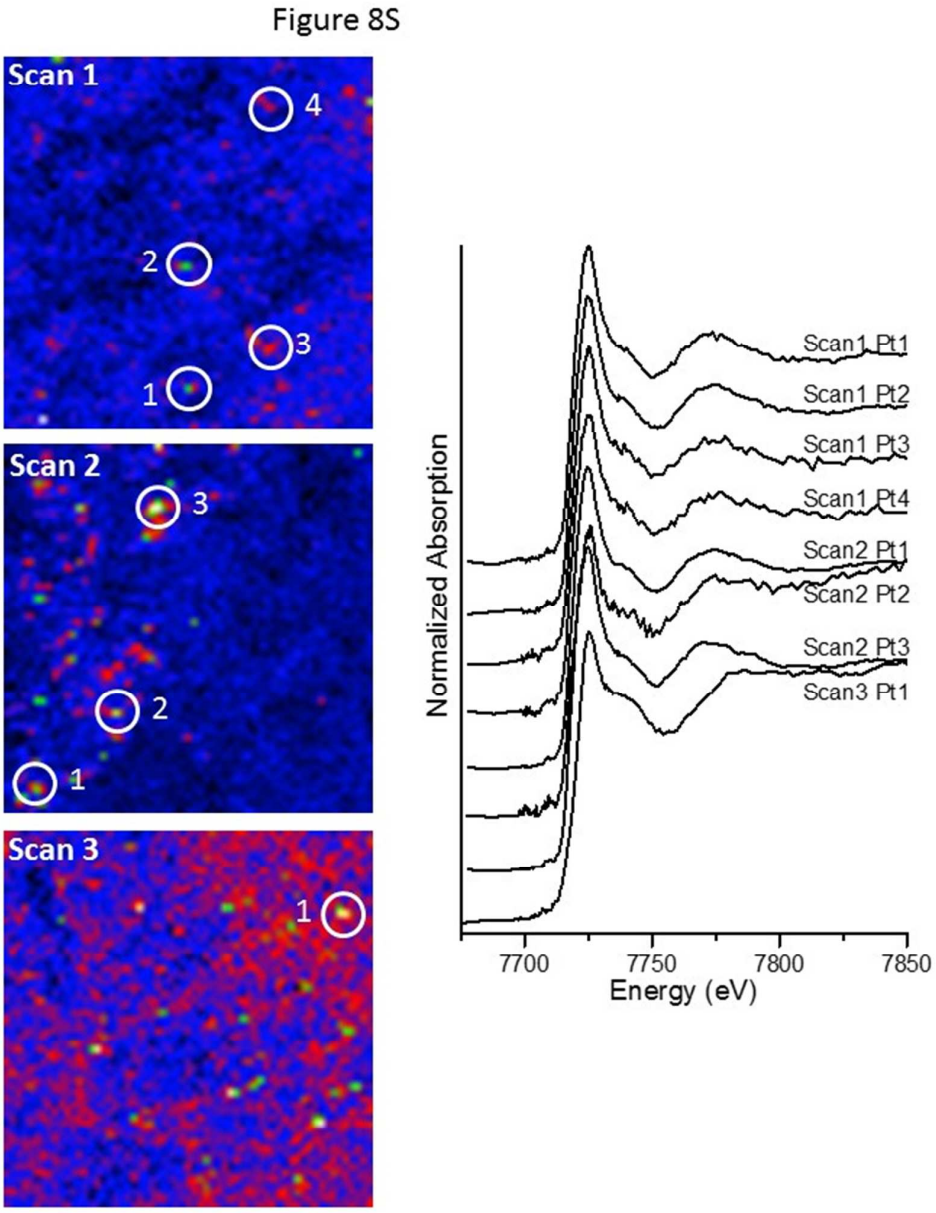

Figure S9: Element distribution of cobalt in pig liver using micro-XANES.