Supplementary Material

Speciation of copper in human serum using conjoint liquid chromatography on short-bed monolithic disks with UV and post column ID-ICP-MS detection

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Table 1S ICP-MS operating parameters

Parameter	Type/Value Speciation analysis	Type/Value Analysis of total Cu content
Sample introduction		
Nebuliser	Miramist	Miramist
Spray chamber	Scott	Scott
Skimmer and sampler	Ni	Ni
Plasma conditions		
Forward power	1550 W	1550 W
Plasma gas flow (Ar)	15.0 L min^{-1}	15.0 L min^{-1}
Carrier gas flow (Ar)	0.80 L min^{-1}	1.00 L min^{-1}
Dilution gas flow (Ar)	0.30 L min^{-1}	/
Make up gas flow (Ar)	/	0.20 L min^{-1}
He flow	9.5	4.5
Data acquisition parameters		
m/z of isotopes monitored	^{63}Cu, ^{65}Cu	^{63}Cu
m/z of internal standards*	^{103}Rh*	^{103}Rh
Total acquisition time	960 s	/

*In speciation analysis, internal standards were not used when the ID-ICP-MS procedure was applied.

Table 2S Chromatographic program for separation of Cu species on the CLC monolithic column

Time (min)	Eluent				Flow rate (mL min^{-1})	Steps in the chromatographic procedure
	A (%)	B (%)	C (%)	D (%)		
0-3	100				0.3	separation
3-12	50	50			0.6	
12-13.5	50	50			0.6	
			100		0.6	
13.5-16			100		1.0	
16-20				100	1.5	regeneration
20-25		100			1.5	
25-26				100	1.5	
26-30	100				1.5	equilibration
30-30.5	100				0.3	

Buffer A: 50 mmol L^{-1} MOPS, pH 7.4
Buffer B: buffer A + 2 mol L^{-1} of ammonium chloride (NH_4Cl), pH 7.4
Eluent C: 0.5 mol L^{-1} acetic acid, pH 2.45
Buffer D: 2 mol L^{-1} MOPS, 2 g L^{-1} EDTA and 1% Tween 20, pH 7.4

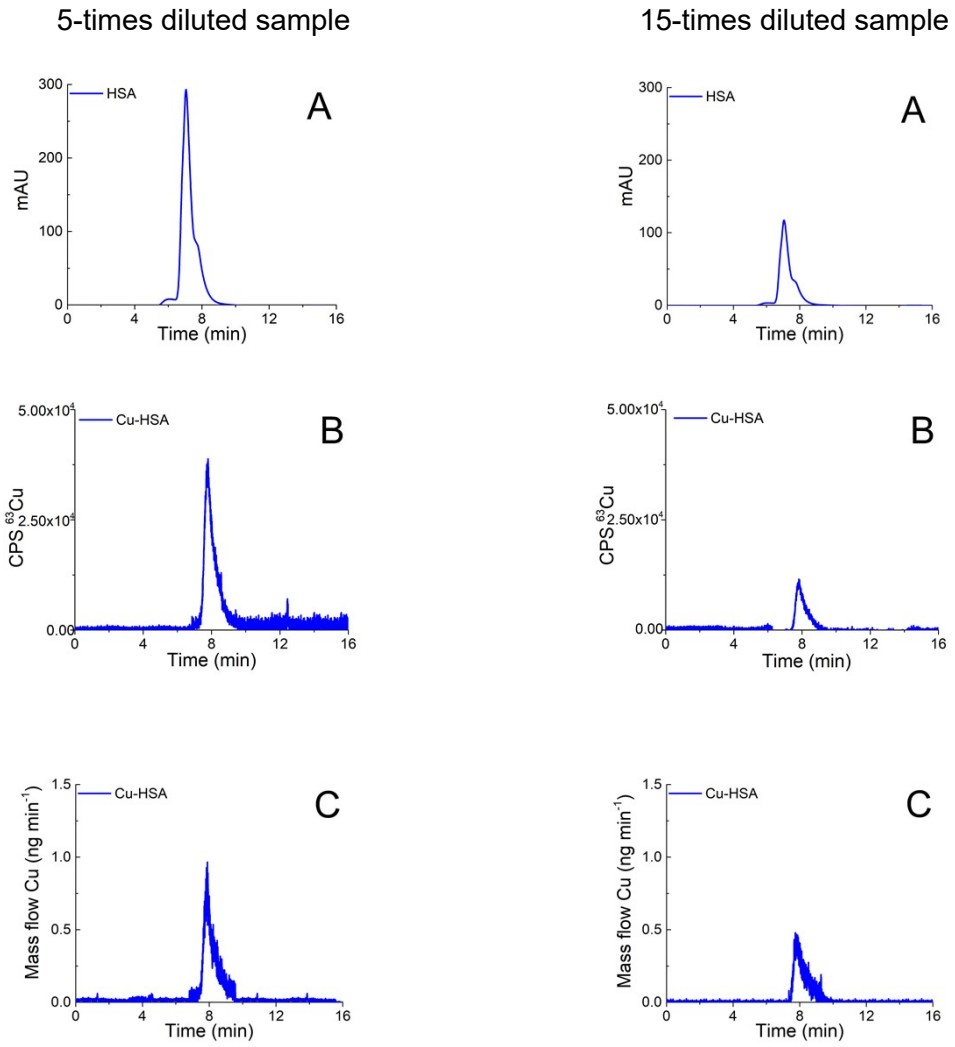

Fig. 1S Chromatograms of separation of 5-times and 15-times diluted sample of standard serum protein HSA (25 g L^{-1}) on the DEAE disk monitored by (A) UV detection (278 nm), (B) ICP-MS detection at *m/z* 63 and (C) ICP-MS detection. Cu mass flow is based on measurements of isotope ratios *m/z* 63 and 65.

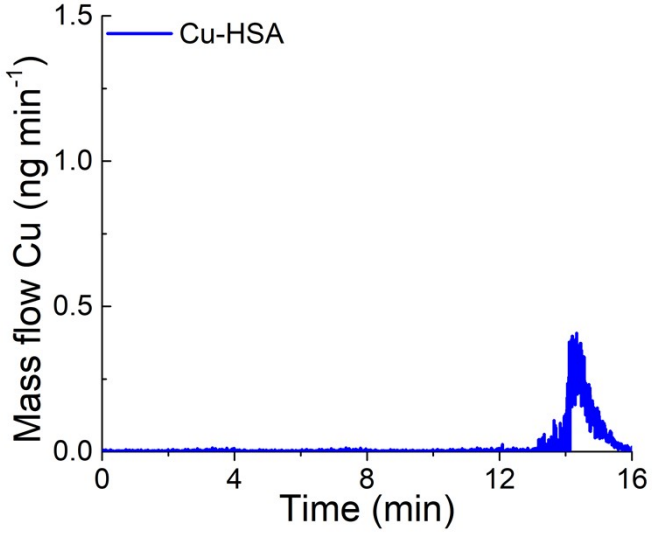

Fig. 2S Chromatogram of separation of 15-times diluted sample of standard serum protein HSA (25 g L^{-1}) on the CLC monolithic column monitored by ICP-MS detection. Cu mass flow is based on measurements of isotope ratios *m/z* 63 and 65.

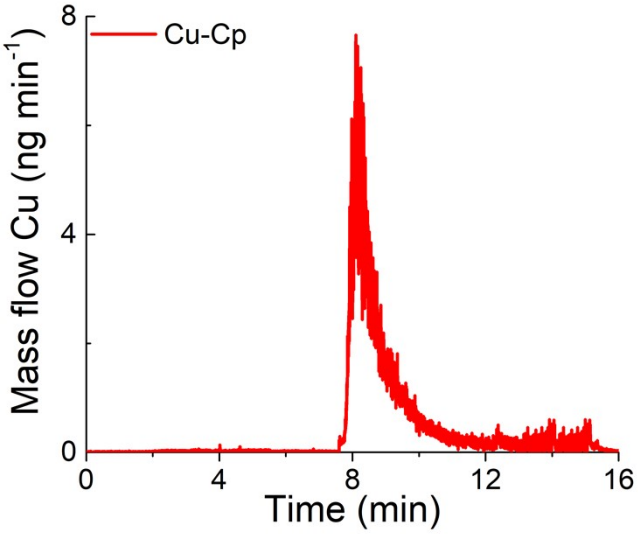

Fig. 3S Chromatogram of separation of 15-times diluted sample of standard serum protein Cp (3 g L^{-1}) on the CLC monolithic column monitored by ICP-MS detection. Cu mass flow is based on measurements of isotope ratios *m/z* 63 and 65.

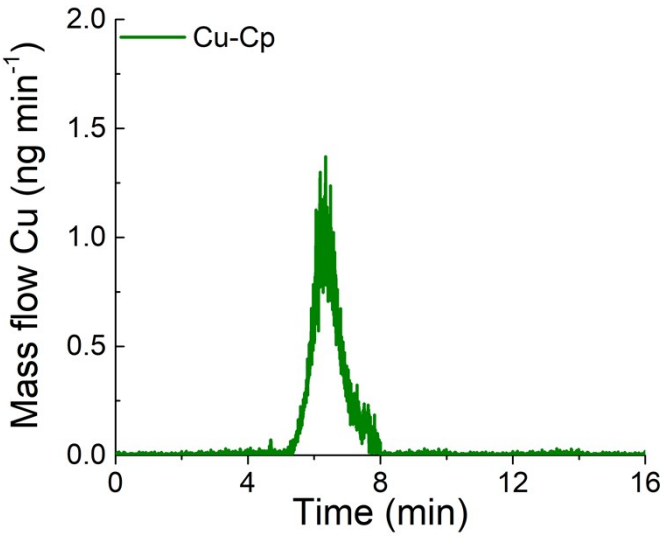

Fig. 4S Chromatogram of separation of synthetically prepared Cu-glycine (47.0 ng mL^{-1} Cu) on the CLC monolithic column monitored by ICP-MS detection. Cu mass flow is based on measurements of isotope ratios *m/z* 63 and 65.

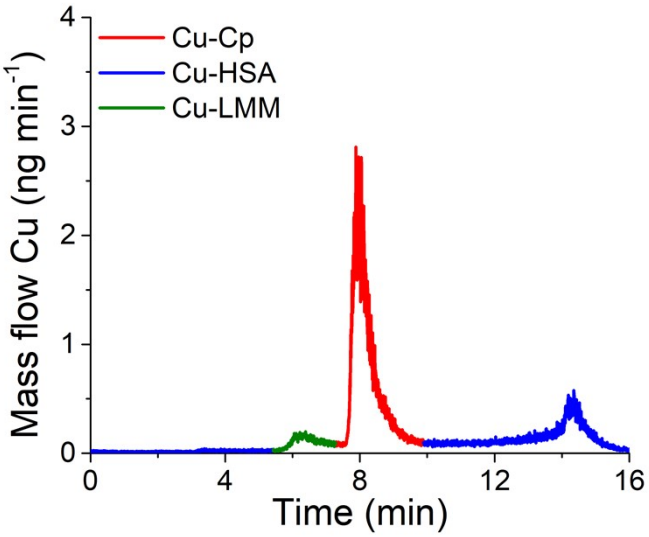

Fig. 5S Chromatogram of separation of Cu species in 15-times diluted human serum sample H1 on the CLC monolithic column. Cu mass flow is based on measurements of isotope ratios *m/z* 63 and 65.