

## Supporting Information

### FI-ICP-TOFMS for quantification of biologically essential trace elements in cerebrospinal fluid - high-throughput at low sample volume

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#### ICP-MS/MS measurements

Measurements were performed with an Agilent 8800 ICP-MS/MS unit (Agilent Technologies, Tokyo, Japan). The ICP-MS instrument was equipped with a MicroMist nebulizer (200  $\mu\text{L min}^{-1}$  nominal sample uptake, AHF Analysentechnik AG, Tuebingen, Germany) and a quartz cyclonic spray chamber (Elemental Scientific Inc., Omaha, USA) that was Peltier-cooled to 2 °C. The instrument was tuned on a daily basis in order to achieve maximum sensitivity, low oxide formation ( $^{140}\text{Ce}^{16}\text{O}^+ / ^{140}\text{Ce}^+ < 1.5\%$ ) and a doubly charged ratio of  $^{140}\text{Ce}^{2+} / ^{140}\text{Ce}^+ < 2\%$ . Elements were monitored in standard mode and using oxygen as reaction gas with a flow rate of 0.32  $\text{mL min}^{-1}$  and mass selection steps on both quadrupoles. The following isotopes with a respective integration time of 0.05 s were monitored in standard mode:  $^{25}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{27}\text{Al}$ ,  $^{56}\text{Fe}$ ,  $^{65}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{111}\text{Cd}$  and  $^{206}\text{Pb}$ . The following isotopes with a respective integration time of 0.05 s were monitored in oxygen gas mode using mass shift modality:  $^{25}\text{Mg} \rightarrow ^{41}\text{Mg}$ ,  $^{27}\text{Al} \rightarrow ^{43}\text{Al}$ ,  $^{43}\text{Ca} \rightarrow ^{59}\text{Ca}$ ,  $^{56}\text{Fe} \rightarrow ^{72}\text{Fe}$ ,  $^{65}\text{Cu} \rightarrow ^{81}\text{Cu}$ ,  $^{66}\text{Zn} \rightarrow ^{82}\text{Zn}$ ,  $^{111}\text{Cd} \rightarrow ^{127}\text{Cd}$  and  $^{206}\text{Pb} \rightarrow ^{222}\text{Pb}$ .

A bio-inert Agilent 1260 HPLC system (Agilent Technologies, Waldbronn, Germany) was used for flow injection measurements. The HPLC was directly connected to the nebulizer of the ICP-MS/MS instrument by a PEEK capillary tubing with an inner diameter of 0.127 mm and a length of approx. 0.8 m. The following chromatographic conditions were used: injection volume: 5  $\mu\text{L}$ ; flow rate: 0.30  $\text{mL min}^{-1}$ ; isocratic elution;  $\text{CH}_3\text{COONH}_4$  (50 mM, pH=6.8) was employed as mobile phase. The data were recorded and evaluated with the Agilent MassHunter Chromatography software package, MassHunter 4.1 Version C.01.01, 2014. Instrumental parameters of ICP-MS/MS measurements are summarized in Table S1.

**Table S1.** Instrumental parameters for ICP-MS measurements.

	ICP-SFMS	ICP-MS/MS	ICP-TOFMS
Plasma power [W]	1250	1550	1550
Cone materials	Pt	Ni	Ni
Plasma gas flow [L min <sup>-1</sup> ]	16.0	15.0	14.0
Auxiliary gas flow [L min <sup>-1</sup> ]	0.80	0.80	0.80
Nebulizer gas flow [L min <sup>-1</sup> ]	1.18	1.10	1.18
Masses notched	-	-	<sup>16</sup> O <sup>+</sup> , <sup>37</sup> Cl <sup>+</sup> , <sup>40</sup> Ar <sup>+</sup>

**Table S2.** Multi-element quantification in serum Seronorm reference material by an open vessel acid digestion procedure with a sample intake of 50  $\mu\text{L}$  using external calibration and isotope dilution analysis followed by ICP-SFMS detection, measured in low resolution mode ( $m/\Delta m$  300) and medium resolution mode ( $m/\Delta m >4500$ ), for  $n=5$  independently prepared samples, respectively.

isotope	external calibration				isotope dilution analysis		
	certified value [ $\mu\text{g L}^{-1}$ ]	concentration [ $\mu\text{g L}^{-1}$ ]	RSD [%]	recovery [%]	concentration [ $\mu\text{g L}^{-1}$ ]	RSD [%]	recovery [%]
$^{25}\text{Mg}(\text{MR})$	$1.68 \times 10^4 \pm 0.34 \times 10^4$	$1.72 \times 10^4 \pm 0.069 \times 10^4$	4	102	-	-	-
$^{27}\text{Al}(\text{MR})$	$50 \pm 25$	$66 \pm 2.3$	3	124	-	-	-
$^{43}\text{Ca}(\text{MR})$	$8.6 \times 10^4 \pm 1.75 \times 10^4$	$9.53 \times 10^4 \pm 0.41 \times 10^4$	4	110	-	-	-
$^{56}\text{Fe}(\text{MR})$	$1.47 \times 10^3 \pm 0.3 \times 10^3$	$1.4 \times 10^3 \pm 0.05 \times 10^3$	4	95	$1.46 \times 10^3 \pm 0.018 \times 10^3$	1.3	99
$^{65}\text{Cu}(\text{MR})$	$1.07 \times 10^3 \pm 0.22 \times 10^3$	$1.08 \times 10^3 \pm 0.023 \times 10^3$	2	102	$1.13 \times 10^3 \pm 0.016 \times 10^3$	1.4	106
$^{66}\text{Zn}(\text{MR})$	$1.06 \times 10^3 \pm 0.21 \times 10^3$	$1.91 \times 10^3 \pm 0.004 \times 10^3$	2	181	$1.86 \times 10^3 \pm 0.031 \times 10^3$	1.7	176
$^{111}\text{Cd}(\text{LR})$	0.13*	$0.18 \pm 0.008$	4	128	-	-	-
$^{206}\text{Pb}(\text{LR})$	0.40*	$0.52 \pm 0.04$	7	123	-	-	-

\*approximate values given in the serum Seronorm reference material certificate, no control range available

**Table S3.** Multi-element quantification in CSF quality control using external calibration and flow injection experiments with ICP-MS/MS detection, using either standard mode or oxygen gas mode in mass shift modality, for  $n=5$  independently prepared samples, respectively.

isotope	standard mode		isotope	oxygen gas mode	
	concentration [ $\mu\text{g L}^{-1}$ ]	RSD [%]		concentration [ $\mu\text{g L}^{-1}$ ]	RSD [%]
$^{25}\text{Mg}$	$3.96 \times 10^4 \pm 0.008 \times 10^4$	1.9	$^{25}\text{Mg} \rightarrow ^{41}\text{Mg}$	$4.11 \times 10^4 \pm 0.18 \times 10^4$	0.4
$^{27}\text{Al}$	$24.2 \pm 1.6$	6.8	$^{27}\text{Al} \rightarrow ^{43}\text{Al}$	$27.2 \pm 5.1$	19
$^{43}\text{Ca}$	$7.28 \times 10^3 \pm 0.14 \times 10^3$	19	$^{44}\text{Ca} \rightarrow ^{60}\text{Ca}$	$2.82 \times 10^3 \pm 0.13 \times 10^3$	3.6
$^{56}\text{Fe}$	$164 \pm 18$	11	$^{56}\text{Fe} \rightarrow ^{72}\text{Fe}$	$162 \pm 1$	0.6
$^{65}\text{Cu}$	$30.2 \pm 0.82$	2.7	$^{65}\text{Cu} \rightarrow ^{81}\text{Cu}$	<LOQ	-
$^{66}\text{Zn}$	$2.17 \times 10^3 \pm 0.079 \times 10^3$	3.6	$^{66}\text{Zn} \rightarrow ^{82}\text{Zn}$	$2.12 \times 10^3 \pm 0.088 \times 10^3$	4.2
$^{111}\text{Cd}$	< LOQ	-	$^{111}\text{Cd} \rightarrow ^{127}\text{Cd}$	< LOQ	-
$^{206}\text{Pb}$	$0.44 \pm 0.02$	5.5	$^{206}\text{Pb} \rightarrow ^{222}\text{Pb}$	< LOQ	-

**Table S4.** Multi-element quantification in serum Seronorm reference material by flow injection with a sample intake of 5  $\mu\text{L}$  using online isotope dilution analysis and ICP-TOFMS detection, for  $n=5$  independently prepared samples.

serum Seronorm reference material				
isotope	target value [ $\mu\text{g L}^{-1}$ ]	concentration [ $\mu\text{g L}^{-1}$ ]	RSD [%]	recovery [%]
Fe	$1.47 \times 10^3 \pm 0.3 \times 10^3$	$1.51 \times 10^3 \pm 0.057 \times 10^3$	3.8	102
Cu	$1.07 \times 10^3 \pm 0.22 \times 10^3$	$1.07 \times 10^3 \pm 0.029 \times 10^3$	2.7	100
Zn	$1.06 \times 10^3 \pm 0.21 \times 10^3$	$9.97 \times 10^2 \pm 0.22 \times 10^2$	2.2	94

**Table S5.** The precision of Fe, Cu and Zn isotope ratios is determined from blank measurements ( $n=6$ ) using online isotope dilution analysis and ICP-TOFMS detection.

isotope ratio	average	stdev	RSD [%]
$^{56}\text{Fe}/^{57}\text{Fe}$	70.9	1.1	1.6
$^{63}\text{Cu}/^{65}\text{Cu}$	2.02	0.01	0.47
$^{66}\text{Zn}/^{67}\text{Zn}$	6.33	0.08	1.3
$^{64}\text{Zn}/^{67}\text{Zn}$	10.7	0.15	1.4
$^{68}\text{Zn}/^{67}\text{Zn}$	4.67	0.06	1.4
$^{70}\text{Zn}/^{67}\text{Zn}$	0.17	0.002	1.4

### Equations used for online isotope dilution analysis

$$M_x(t) = M_y(t) \times \frac{R_y - M_b R_b(t)}{M_b R_b(t) - R_x} \times \frac{h_{iy}}{h_{ix}} \quad M_y(t) = M_x(t) \times \frac{R_x - M_b R_b(t)}{M_b R_b(t) - R_y} \times \frac{h_{ix}}{h_{iy}} \quad \text{Equation (1)}$$

$M_x(t)$	mass flow of the sample
$M_y(t)$	mass flow of the spike
$R_x$	ratio of the isotopes A/B in the sample
$R_y$	ratio of the isotopes A/B in the spike
$R_b(t)$	ratio of the isotopes A/B in the blend
$h_{ix}$	abundance of the spike isotope in the sample
$h_{iy}$	abundance of the spike isotope in the spike