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

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Introduction

In clinical diagnostics, multi-element profiling in biological fluids and tissues is becoming increasingly important. A fine balance of trace element concentrations in human body fluids and tissues is essential for maintaining optimal homeostasis to avoid the adverse side effects of excess metal exposure and/or depletion. While for serum and blood, reference levels are fairly well established for most trace elements and serve as important diagnostic parameters, limited data are available for elemental concentrations in the cerebrospinal fluid (CSF). The CSF plays an important role in the homeostasis and metabolism of the central nervous system. Due to the direct connection of the CSF with the extracellular space of brain parenchyma, a misbalance or depletion of trace elements in the brain is likely to be reflected in the CSF. Several studies indicate that metals and metal species seem to play a crucial role in Alzheimer’s disease, Parkinson’s disease and other related diseases, whereas the exact role of metals in these diseases is still under debate. This is mainly due to the fact that currently clinically established methods for reliable and accurate trace element analysis in the CSF are still lacking to get a systematic overview and data pool of metal levels in the CSF. In contrast to serum samples, the CSF is not routinely drawn from patients; it is only taken in the case of neurological indication for diagnostic purposes, resulting in the limited availability of CSF samples from healthy humans for determination of trace element reference values. Moreover, the concentrations of most elements in the CSF are much lower than in serum due to the selective and controlled permeation of metals across the neural barriers. The huge range of trace element concentration values for the CSF reported in the literature can be due to several factors including variations in the nutrition state of patients, contamination during the sampling process and artifacts due to matrix interference. A state-of-the-art technique for multi-element profiling at (ultra-) trace levels in complex biological matrices is inductively coupled plasma-mass spectrometry (ICP-MS) due to its multi-
element characteristics, high sensitivity and wide dynamic range. Previous studies already showed the application of this technique for the elemental quantitation in the CSF using different ICP-MS instruments. The most common instrumental ICP-MS setups in this regard make use of sequential quadrupole7-9 or sector-field mass analyzers10-13 (ICP-QMS and ICP-SFMS), offering interference control either by collision/reaction cell gas technology or by high mass resolving power. The specificity of reactions in the reaction cell in single quadrupole or tandem quadrupole ICP-MS configurations is especially advantageous for the interference-free determination of single elements, as shown by various examples in the literature.14,15 However, this also poses a major limitation when aiming at multi-element profiling due to different sensitivities for the elements in different gas modes, resulting in an increased analysis time and sample volume when different gas modes for different elements are required. Rapid simultaneous multi-element analysis becomes relevant when only a small amount of sample material is available, when there are short transient signals and when aiming at high-throughput analysis. ICP-time-of-flight MS provides pseudo-simultaneous analysis (ions are sampled simultaneously but read out sequentially) of a large part of the entire mass range. Its main application fields are single cell analysis,16 single particle analysis17-19 and high-resolution laser ablation-ICP-MS imaging using low dispersion cell setups,20-22 where in all cases short transient signals are delivered. Of the commercially available ICP-TOF-MS instruments, icpTOF (TOFWERK AG, Thun, Switzerland) provides the analysis of a mass range of \( m/z = 2-256 \) with a mass resolving power \( R = m/\Delta m \) of 3000 and 6000 (FWHM definition).23 In contrast to the ‘CyTOF’ instrument,24 which was specifically designed to target mostly lanthanides for mass cytometry immuno-labeling applications \( (m/z = 75-209) \),25 icpTOF also provides information on biologically relevant elements from the lower mass range. Its performance characteristics were already shown for multi-element analysis of liquid samples with standard direct infusion and a microdroplet sample-introduction system.26

Herein, we evaluate the potential of an icpTOF 2R TOF-based ICP-MS instrument for high-throughput profiling of biologically relevant elements in the CSF material. In order to downscale the volume required for analysis without compromising the sensitivity, flow injection measurements were employed in this study with a sample intake of 5 µL. A comparison of external calibration and isotope dilution approaches for accurate multi-element quantification is made and the results and performance are benchmarked to ICP-SFMS and ICP-MS/MS measurements.

**Experimental**

**Chemicals and reagents**

Ultrapure water (18.2 MΩ cm, ELGA Water purification system, Purelab Ultra MK 2, UK) and nitric acid (≥69%, Rotipuran Supra, Germany) were used to prepare standards and reagents. Ultrapure water was obtained by double sub-boiling distillation of purified water (18.2 MΩ cm) using an ultra-clear system (SG water GmbH, Barsbüttel, Germany). Sub-boiled HCl (37% w/w, Merck KGaA, Darmstadt, Germany) and double sub-boiled HNO3 (65% w/w) (MLS Duopur, MLS, Leutkirch, Germany) were used to prepare standards and reagents. Multi-element stock solutions were purchased from Labkings (The Netherlands) and Inorganic Ventures (ICP-MS Calibration Standard 6-IV-Stock-6). The serum reference materials Seronorm (Seronorm Trace Elements Serum L-1, Norway) and ClinCheck Serum Control (Recipe Chemicals, Munich, Germany) were reconstituted according to the manufacturer’s protocol. Liquid CSF Control Level-2 was obtained from Randox (Randox Laboratories, UK). The \(^{57}\text{Fe},^{65}\text{Cu} \) and \(^{67}\text{Zn} \) enriched spikes were reconstituted. Briefly, \(^{57}\text{Fe} \) and \(^{65}\text{Cu} \) enriched spike powders were dissolved in 1 mL aqua regia, the solution was evaporated, again dissolved in 1 mL aqua regia and evaporated. The \(^{67}\text{Zn} \) enriched spike was dissolved in 1 mL conc. HNO3, the solution was evaporated, again dissolved in 1 mL conc. HNO3 and evaporated. 1 mL conc. HNO3 was added to the respective dried spike solutions and filled up to 100 mL with sub-boiled H2O. All other reagents and solvents were obtained from commercial sources and were used without further purification. Sample preparation and measurements were carried out in clean room classes 100.000 and 10.000, respectively, with clean benches class 100 with temperature control (20 °C) and overpressure (+5 Pa).

**ICP-SFMS measurements**

**Instrumentation.** An Element2 High Resolution ICP-SFMS instrument (ThermoFisher, Bremen, Germany) specified for three fixed resolution settings \( R = m/\Delta m \) at 5% peak height: low resolution (LR, \( m/\Delta m \) 300), medium resolution (MR, \( m/\Delta m > 4500 \)) and high resolution (HR, \( m/\Delta m > 10 000 \)) was used for multi-element quantification. Measurements were performed in continuous acquisition mode (sample intake via nebulizer self-aspiration) by selecting the appropriate resolution mode for each element for interference-free determination. The interface consisted of a PFA-ST nebulizer (Elemental Scientific Inc., ESI, Omaha, NE, USA), a cooled cyclonic glass spray chamber operated at 4 °C (PC³, ESI), a 2 mm sapphire injector and platinum sample and skimmer cones. Tuning parameters were daily optimized to obtain a sensitivity of approx. 1 000 000 counts per second in self-aspiration mode (0.20 mm i.d. uptake capillary – 50 µL min⁻¹; ESI) for a solution containing 1 µg L⁻¹ indium and to obtain a \( ^{235}\text{U}^{16}\text{O}^{238}\text{U} \) ratio <5%. Masses monitored were \(^{111}\text{Cd}, ^{115}\text{In} \) (internal standard), and \(^{206}\text{pb}, \) at \( m/\Delta m = 300 \) and \(^{25}\text{Mg}, ^{27}\text{Al}, ^{42}\text{Ca}, ^{56}\text{Fe}, ^{57}\text{Fe}, ^{63}\text{Cu}, ^{65}\text{Cu}, ^{66}\text{Zn}, \) and \(^{67}\text{Zn}, \) at \( m/\Delta m > 4500 \). Data acquisition was performed in E-scan mode (3 runs × 3 passes, 30 samples/peak, 100 ms) and instrumental parameters are summarized in Table S1.

**Sample preparation**

**External calibration.** Reconstituted serum reference material, CSF and blanks (50 µL sample and 50 µL sub-boiled conc. HNO3) were mineralized by open vessel digestion using a pro-
grannable metal-free, acid resistant graphite hotplate coated with PTFE and a graphite rack for 1.5 mL PFA vials (AHF Analysetechnik AG, Tuebingen, Germany). In the case of the Seronorm reference material, 20 µL H2O2 (31%, ultrapure; Merck) was added to enable complete digestion due to the higher protein content compared to the serum ClinCheck reference material. The hotplate was heated up to 105 °C and samples were digested at 105 °C for 40 min. Samples were taken off the hotplate, cooled to room temperature and 50 µL internal standard solution (indium) was added. The samples were filled up with 470 µL 2% (v/v) HNO3 to a total volume of approx. 550 µL. Quantification was performed by external calibration with multi-element standard solutions and ICP-SFMS analysis with the instrumental parameters shown in Table S1.†

**Isotope dilution analysis.** For quantification of Fe, Cu and Zn by means of isotope dilution, two spike solutions containing different concentrations of isotopically enriched 57Fe, 65Cu and 67Zn were prepared. The spike concentrations added to CSF and blanks were 250 µg L\(^{-1}\), 50 µg L\(^{-1}\) and 1250 µg L\(^{-1}\) for Fe, Cu and Zn, respectively. The spike concentrations added to the serum Seronorm reference material were 1250 µg L\(^{-1}\), 1000 µg L\(^{-1}\) and 1250 µg L\(^{-1}\) for Fe, Cu and Zn, respectively. The exact spike concentration was assessed each day by reverse isotope dilution using a standard solution containing the three elements. Blends were prepared using 50 µL sample and 50 µL mixed spike solutions and digestion was performed by adding 50 µL sub-boiled conc. HNO3 and 20 µL H2O2 using open vessel digestion as described above. Samples were digested for 40 min at 105 °C and filled up with 470 µL of 2% HNO3 to a total volume of ~500 µL. Isotope ratios were determined by ICP-SFMS using the instrumental parameters summarized in Table S1.†

**ICP-TOFMS measurements**

**Instrumentation.** Measurements were performed with an icpTOF 2R (TOFWERK AG, Thun, Switzerland) TOF-based ICP-MS instrument with a mass resolution (R = m/Δm) of 6000 (FWHM definition). The standard operation mode was used which balances mass resolving power, sensitivity and ion transmission across the entire measured mass range and which allows the analysis of ions from m/z = 14–256. The sample introduction system consisted of a PFA MicroFlow pneumatic nebulizer (MicroMist 200 µL min\(^{-1}\) nominal sample uptake, Elemental Scientific Inc., Omaha, USA) and a quartz cyclonic spray chamber (Elemental Scientific Inc., Omaha, USA) that was Peltier-cooled to 2 °C. The optimized nebulizer Ar gas flow rate of ~1.18 L min\(^{-1}\) resulted in a net sample uptake rate of ~100 µL min\(^{-1}\). The instrument was equipped with a torch injector of 2.5 mm inner diameter and nickel sample and skimmer cones with a skimmer cone insert of 2.8 mm inner diameter. The instrument was tuned daily in order to achieve the maximum intensity based on the 57Co, 115In and 238U signal, low oxide formation ([\(^{140}\)Ce\(^{16O}/^{140}\)Ce\(^{18O}\) < 1.5%]) and a doubly charged ratio of [\(^{138}\)Ba\(^{2+}/^{138}\)Ba\(^{+}\) < 2%. The instrumental parameters for ICP-TOFMS measurements are summarized in Table S1.†

For flow injection and on-line isotope dilution measurements, a Dionex ICS-5000+ Ion Chromatography system (Thermo Fisher Scientific, Bremen, Germany) was coupled directly to the nebulizer of the ICP-TOFMS instrument using a PEEK capillary (0.127 mm i.d., length approx. 0.8 m). The following chromatographic conditions were used: injection volume: 5 µL; flow rate: 0.30 mL min\(^{-1}\); isocratic elution; CH\(_3\)COONH\(_4\) (50 mM, pH = 6.8) was employed as the mobile phase.

ICP-TOFMS data were saved in the open-source hierarchical data format (HDF5, http://www.hdfgroup.org). Post-acquisition data processing was performed with Tofware, which is a TOFWERK data analysis package and used as an add-on on IgorPro (WaveMetrics Inc., Oregon, USA). The data processing comprised the following steps: (1) drift correction of the mass peak position in the spectra over time via mass calibration (2) determining the peak shape (3) fitting and subtracting the mass spectral base-line and (4) calculating high-resolution peak fits for peak deconvolution. For flow injection measurements, the chromatographic workflow implemented in Tofware was used for data evaluation. Following post-processing in Tofware, integrated mass-spectral signals were exported as csv files and further processed with Microsoft Excel 2016 (Microsoft Corporation, California, USA).

**Sample preparation**

**External calibration.** Matrix-matched standards were prepared using serial dilutions of serum Seronorm reference material in NaCl solution (150 mM) and external calibration was performed using the target values to set up calibration curves. CSF samples and serum ClinCheck reference material were measured undiluted using flow injection with a sample intake of 5 µL in combination with ICP-TOFMS and for cross-validation with ICP-MS/MS detection with the instrumental parameters summarized in Table S1.† Details of the ICP-MS/MS measurements are given in the ESI.†

**On-line isotope dilution analysis.** A spike solution containing isotopically enriched 57Fe, 65Cu, and 67Zn was prepared gravimetrically using 3% HNO3 yielding concentrations of around 200 µg L\(^{-1}\) Fe, 50 µg L\(^{-1}\) Cu and 1200 µg L\(^{-1}\) Zn. The exact spike concentration was assessed each day by reverse isotope dilution using a standard solution containing the three elements. The spike solution was added into the mobile phase flow of the ion chromatography system via the on-line internal standard kit of the ICP-MS system. The mass flow of the standard/spike solutions was determined gravimetrically in each measurement session. The isotope abundances and ratios were determined experimentally by measuring three blanks respectively after each sample and used to calculate the mass flow of the sample as a function of time (eqn (1), ESI†). The resulting peaks were integrated and the area was divided by the injection volume. The accuracy of the methodology was validated using serum Seronorm reference material and the CSF samples were measured undiluted using flow injection with a sample intake of 5 µL and ICP-TOFMS analysis with the instrumental parameters summarized in Table S1.†
Results and discussion

ICP-SFMS analysis as the gold standard for multi-element quantification in CSF

One of the main challenges for accurate multi-element quantification in the CSF by ICP-MS is the lack of certified reference materials. In our study, we used commercially available liquid CSF quality control material, which was designed as an assay control for different proteins and should therefore match the matrix and trace element content of the CSF samples. Multi-element quantification in CSF quality control was first carried out by ICP-SFMS measurements to establish a reference dataset for the flow injection ICP-TOFMS and ICP-MS/MS methods. Elements were chosen according to literature data based on their possible relevance for Alzheimer’s disease and include magnesium, aluminium, calcium, iron, copper, zinc, cadmium and lead. Commonly, sample preparation for biological material is based on microwave-assisted acid digestion which is accompanied by high dilution factors. Consequently, multi-element quantification at (ultra-)trace levels in a limited sample volume is very challenging. Therefore, the sample preparation for the CSF was adapted accordingly and mineralization was performed using an open vessel acid digestion procedure with a sample intake of 50 µL and further dilution by a factor of 10 for SF-ICPMS analysis. Quantification was performed by external calibration and internal standardization using indium; in addition, isotope dilution analysis has been applied to the quantification of Fe, Cu and Zn (Table 1). The serum Seronorm reference material was analyzed by the ICP-SFMS method and the analyte concentrations were in agreement with the target values (Table S2†), proving that the developed method is fit for purpose. Therefore, the CSF results obtained by ICP-SFMS can be used as a reference dataset for the flow injection ICP-TOFMS and ICP-MS/MS methods and the target values of the serum Seronorm reference material can be used to set up calibration curves for the flow injection methods. Only Zn shows higher values than the target range of the serum reference material, as measured by ICP-SFMS both with external calibration and isotope dilution analysis. For CSF analysis, the concentrations of Fe, Cu and Zn are in agreement within the uncertainty when applying quantification by external calibration and isotope dilution analysis.

Flow injection ICP-TOFMS analysis for multi-element quantification in CSF

The combination of different calibration approaches and ICP-TOFMS was explored by injecting small volumes of CSF via flow injection into the mobile phase of an HPLC system followed by ICP-TOFMS detection. The used metal-free ion chromatography system and the employed buffer as the mobile phase provided the low backgrounds necessary for trace element analysis, whereas the TOF analyser enabled the simultaneous measurement of elements from m/z = 14–256 over the transient signal of the flow injection peak. The flow injection peaks of different isotopes with a duration of around 6–8 s are shown in Fig. 1. One significant improvement achieved by this approach is the downscaling of the CSF sample intake to 5 µL compared to 50 µL for ICP-SFMS analysis. In the case of CSF, where ultra-trace element analysis is required in a limited sample volume, any further dilution of the sample material could potentially lead to concentrations below the limit of quantification. A recent study by injecting small volumes of undiluted CSF evaluated the use of a total sample consumption system operated at high temperature followed by ICP-QMS detection. The major advantages of these methods are the direct injection and analysis of undiluted CSF material, without the need for sample mineralization, thereby minimizing the sample preparation steps compared to ICP-SFMS analysis. This is of particular importance when aiming at high-throughput analysis, an essential requirement in clinics where hundreds to thousands of samples need to be analyzed in a short time frame.

The *icpTOF 2R* ICP-MS instrument was characterized in terms of sensitivity for biologically relevant elements typically analyzed in the CSF. Instrumental limits of detection (LOD) and quantification (LOQ) were compared between ICP-SFMS, ICP-MS/MS and ICP-TOFMS and are summarized in Table 2. LODs were in general lower for ICP-SFMS and ICP-MS/MS than for ICP-TOFMS using a standard liquid introduction system. For the flow injection measurements, a metal-free HPLC system was used in order to keep the background from

Table 1  Multi-element quantification in CSF quality control by open vessel acid digestion using external calibration and isotope dilution analysis with ICP-SFMS detection, measured in low resolution mode (m/Δm = 300) and medium resolution mode (m/Δm > 4500), for n = 5 independently prepared samples

| Isotope      | Concentration [µg L⁻¹] | RSD [%] | Concentration [µg L⁻¹] | RSD [%] |
|--------------|------------------------|---------|------------------------|---------|
| **Isotope**  | **External calibration** |         | **Isotope dilution analysis** |         |
| ²⁵Mg(MR)     | 4.06 ± 0.1 X 10⁴       | 2.6     | —                      | —       |
| ²⁷Al (MR)    | 11.2 ± 1.3             | 11      | —                      | —       |
| ⁴⁴Ca(MR)     | 1.89 ± 0.03 X 10³      | 1.6     | —                      | —       |
| ⁵⁶Fe(MR)     | 188 ± 5                | 2.4     | 198 ± 8                | 1.5     |
| ⁶⁵Cu(MR)     | 28.4 ± 0.3             | 0.95    | 28.1 ± 0.4             | 1.5     |
| ⁶⁶Zn(MR)     | 2.26 ± 0.09 X 10³      | 4.1     | 2.11 ± 0.03 X 10³      | 1.3     |
| ¹¹¹Cd(LR)    | 0.15 ± 0.05            | 33      | —                      | —       |
| ²⁰⁶Pb(LR)    | 0.80 ± 0.03            | 3.5     | —                      | —       |
the sample introduction system as low as possible. An increase in LOQs was observed when the flow injection method was used which might be critical for CSF analysis where elemental concentrations are low. However, for the elements Fe, Cu, Zn and Cd with major biological functions, LOQs are well below the expected concentrations in the CSF material, whereas the LOQs for Al and Pb are close to the concentrations for the CSF reported in the literature.6,10,12

For flow injection experiments, multi-element quantification in the CSF was performed by external calibration using matrix-matched standards based on the serial dilutions of serum Seronorm reference material in sodium chloride solution. The sodium chloride concentration (∼150 mM) was chosen according to the one certified for the CSF quality control material to match the sample matrix and thus the resulting interference for CSF samples. The target values of the serum Seronorm reference material were used to set up calibration curves. Samples were analyzed using flow injection with a sample intake of 5 µL and ICP-TOFMS detection and for additional cross-validation with ICP-MS/MS detection, in standard mode and in oxygen gas mode in mass shift modality (Tables 3 and S3†).

For magnesium, the results of ICP-MS/MS measurements were in agreement with those of ICP-SFMS analysis, for both standard and oxygen gas modes. Using ICP-TOF-MS analysis, the magnesium concentration found in the CSF was around 25% lower compared to that found by using the other two methods, which can be attributed to the high sodium signal

Fig. 1  Flow injection profiles of different isotopes measured by FI-ICP-TOFMS.

Table 2  Overview of the limits of detection and quantification for multi-element analysis using ICP-SFMS, ICP-MS/MS and ICP-TOFMS detection. Instrumental LODs and LOQs were calculated using three and ten times the standard deviation of blank measurements, respectively. The LODs and LOQs for flow injection measurements were calculated using three and ten times the standard deviation of a low concentration standard.

| Isotope | ICP-SFMS | ICP-MS/MS | ICP-TOFMS |
|---------|----------|-----------|-----------|
|         | LOD [µg L⁻¹] | LOQ [µg L⁻¹] | LOD [µg L⁻¹] | LOQ [µg L⁻¹] | LOD [µg L⁻¹] | LOQ [µg L⁻¹] |
| ²⁵Mg    | 0.35      | 1.2       | —          | —          | —          | —          |
| ²⁷Al    | 0.11      | 0.38      | 0.08       | 0.27       | 0.55       | 1.8        |
| ⁴¹Ca    | 1.3       | 4.4       | —          | —          | —          | —          |
| ⁵⁶Fe    | 0.20      | 0.68      | —          | —          | 0.04       | 0.14       |
| ⁵⁷Fe    | —         | —         | 0.47       | 1.6        | —          | —          |
| ⁶⁵Cu    | 0.02      | 0.06      | 0.02       | 0.06       | —          | —          |
| ⁶⁶Zn    | 0.15      | 0.51      | —          | —          | —          | —          |
| ¹¹¹Cd   | 0.001     | 0.002     | 0.001      | 0.003      | —          | —          |
| ²⁰⁶Pb   | 0.001     | 0.002     | 0.004      | 0.01       | —          | —          |

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due to the high sodium concentration present in the CSF. The proximity of sodium with mass m/z = 23 to magnesium with m/z = 25 leads to a high Na peak in the TOF spectrum and thus to an underestimation of the Mg concentration. Aluminium with m/z = 27 was not affected by the sodium background and its concentration was in good agreement with the one obtained by ICP-SFMS analysis. For ICP-MS/MS measurements, two times higher Al concentrations were found in the CSF in both gas modes, which might be due to the presence of interference such as MgH+ resulting from the matrix. The isotope 43Ca+ was used for quantification, as it was not influenced in the TOF spectrum by the argon background, and the value was in agreement with the results obtained by ICP-SFMS analysis. Using ICP-MS/MS measurements, significantly higher values for 43Ca+ were obtained in both gas modes, indicating inefficient interference removal under the conditions used. Cadmium levels of ∼0.15 µg L<sup>−1</sup> were found in the CSF, as determined by both ICP-SFMS and ICP-TOF-MS using external calibration. The Cd levels were also determined using ICP-MS/MS measurements in no gas and oxygen gas modes, and they were found to be under the limit of quantification. For lead, concentrations in CSF ranged between 0.45 and 0.80 µg L<sup>−1</sup>, as determined by the three different ICP-MS methods.

The three major biological transition metals supposed to be involved in many neurodegenerative diseases are iron, copper and zinc. Analogous to the ICP-SFMS measurements, online isotope dilution analysis was applied to the quantification of Fe, Cu and Zn in the CSF material using ICP-TOFMS detection. For this purpose, a mixed spike solution containing the isotopically enriched isotopes of all three elements (57Fe, 65Cu, 67Zn) was continuously added as a make-up flow to the carrier flow of the flow injection system. The concentration of the spike was determined by reverse online-ID and the make-up flow was assessed gravimetrically in each measurement session. Due to the simultaneous measurement of all isotopes by ICP-TOFMS, no mass bias correction was required and the measured isotope abundances/ratios were used for calculation of the mass flow as a function of time (eqn (1), ESI†). An overview of the precision of isotopic ratios as determined by ICP-TOFMS is given in Table S5.† For Cu, isotope ratios were determined with a precision of ∼0.5% and for Fe and Zn with a precision of ∼1.5%. The accuracy of the online isotope dilution method was validated by the measurement of serum Seronorm reference material. For all three elements, the concentration values were in good agreement with the target values of the serum reference material (Table S4†) proving that the developed method is fit for purpose. The performance in terms of intermediate repeatability (n = 6) over a measurement day is for Cu and Zn ∼2.5% and for Fe slightly higher with ∼3.5%. The online isotope dilution ICP-TOFMS method was applied to the CSF quality control material with a sample intake of 5 µL (Table 3). One advantage of the used approach is the capability of ICP-TOFMS to provide information on all isotopes of all three elements in a single flow injection signal with a duration of around 6–8 s. For iron, the concentration of around 190 µg L<sup>−1</sup> obtained by online isotope dilution and ICP-TOFMS detection was in excellent agreement with the values measured by ICP-SFMS. Particularly for Fe, the concentration range in the CSF found in the literature varies strongly, with reported values between five and several hundred µg L<sup>−1</sup>. This strong variation in Fe levels can be due to several factors including contamination during the sampling process when using steel syringes for lumbar puncture or sample preparation as well as due to interference during ICP-MS analysis. In our study, Fe concentrations ranged between 170 and 200 µg L<sup>−1</sup> with a concentration of 180 µg L<sup>−1</sup> as the reference value from the ICP-SFMS experiments. For Cu, the results of ICP-TOFMS analysis were in excellent agreement with those obtained by ICP-SFMS and ICP-MS/MS in standard mode showing an average value of ∼32 µg L<sup>−1</sup> in CSF. For Cu, isotope 65Cu was subjected to interference from 23Na40Ar and high saline concentrations as in the CSF matrix can lead to potentially higher concentrations. Using the ICP-MS/MS tandem configuration, no signal was obtained for Cu in oxygen gas shift modality and other cell gases as for example hydrogen or helium gas should be used to remove the interference. The Cu concentrations obtained in our study are in the range of those reported in the literature for control samples from healthy patients. For patients suffering from neurodegenerative diseases, higher Cu values ranging from 70 to 100 µg L<sup>−1</sup> were reported. The Zn concentrations obtained by online isotope dilution ICP-TOFMS analysis are slightly higher than the values obtained by ICP-SFMS and ICP-MS/MS. In general, Zn concentrations in our study ranged between around 2200 and 2400 µg L<sup>−1</sup>, which is in agreement with the concentration values of 1700–3300 µg L<sup>−1</sup> found in the literature for CSF samples from healthy patients.
Conclusions

This work demonstrated for the first time the capability of ICP-TOFMS for accurate multi-element quantification in CSF material using the flow injection of undiluted CSF with a required sample volume of only 5 µL. Compared to ICP-MS/MS analysis, ICP-TOFMS is advantageous in terms of sample throughput and measurement time due to its multi-element capabilities for the analysis of transient signals. Whereas for ICP-MS/MS detection, different gas modes are required to cope with the removal of interference on different elements, ICP-TOFMS provides information on all analytes of interest. For the biologically relevant elements analyzed, the values of Al, Ca, Fe, Cu, Zn, Cd and Pb were in agreement with each other using the different ICP-MS methods and in the range of values reported in the literature. Online-isotope dilution in combination with ICP-TOFMS detection proved to be suitable for accurate, interference-free quantification of Fe, Cu and Zn in the CSF material. The present study developed and compared different ICP-MS methods using a small sample volume to establish reliable values for the metal concentrations in the CSF material of the control population and these methods can be further applied for the analysis of CSF samples from patients.

Conflicts of interest

There are no conflicts of interest to declare.

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