Chapter 10

Capillary Electrophoresis-Inductively Coupled Plasma Mass Spectrometry

Bernhard Michalke

Abstract

During the recent years, capillary electrophoresis (CE) has been fully established as a powerful tool in separation sciences as well as in element speciation. This road of success is based on the rapid analysis time, low sample requirements, high separation efficiency, and low operating costs of CE. Inductively coupled plasma mass spectrometry (ICP-MS) is known for superior detection and multielement capability. Consequently, the combination of both instruments is approved for analysis of complex sample types at low element concentrations which require high detection power. Also the diversity of potential applications brings CE–ICP-MS coupling into central focus of element speciation. The key to successful combination of ICP-MS as an (multi-)element selective detector for CE is the availability of a suitable and effective interface.

Therefore, this chapter summarizes the most important and basic principles about coupling of capillary electrophoresis to ICP-MS. Specifically, the major requirements for interfacing are described and technical solutions are given. Such solutions include the closing of the electrical circuit from CE at the nebulization, the adoption of flow rates for efficient nebulization, the reduction of a suction flow through the capillary, caused by the nebulizer, and maintaining the high separation resolution from CE across the interface for ICP-MS detection. Additionally, detailed information is presented to determine and quantify the siphoning suction through the CE capillary by the nebulizer. Finally, two applications, namely, the manganese and selenium speciation in cerebrospinal fluid are shown as examples, providing the relevant operational parameter.

Key words Capillary electrophoresis, Inductively coupled plasma mass spectrometry, Interface, Problem solutions

Abbreviations

CE	Capillary electrophoresis
CZE	Free zone electrophoresis
ICP-MS	Inductively coupled plasma mass spectrometry
IEF	Isoelectric focusing
ITP	Isotachophoresis
LC	Liquid chromatography
LoD	Limit of detection
MEKC	Micellar electrokinetic chromatography
RF	Radiofrequency
USN	Ultrasonic nebulizer

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1 Introduction

Nowadays capillary electrophoresis (CE) is a useful tool with high reproducibility in separation sciences as well as in element speciation. CE provides rapid analysis time, low sample requirements, high separation efficiency, and low operating costs [1, 2]. Specifically the high separation potential of CE [3] combined with the superior multielement detection capability of ICP-MS must be considered as the outstanding potential of CE–ICP-MS. This together with the diversity of potential applications makes the coupling of CE to ICP-MS as a powerful technique for studies in the field of metallomics and element speciation, but also for quantitative proteomics based on elemental tagging. In each of these mentioned fields, the separation of metal-carrying- (organic-) molecules, so-called metal species, is followed by element selective detection resulting in paralleled element-selective electropherograms [1–3].

Consequently, this analytical approach is widely used for speciation analysis in various scientific fields, investigating topics such as lanthanides in humic substances [4] or As, Eu, Hg, Np, Se, U and organo-tins in environmental matrices [5–11], biomedical applications like manganese speciation in paired serum and cerebrospinal fluid samples, the determination of gadolinium-based MRI contrast agents or of phosphorus in DNA organotin compounds and the characterization of metal glycinate- and metal-phytosiderophores complexes [12–17], anticancer drug-related investigations [18–23], and elemental tagging for quantitative proteomics [24–30].

In all of those experiments, the key to a successful CE–ICP-MS hyphenation is an interface which is perfectly adapted to the specific requirements of both techniques. The analytical chemist should properly control the relevant variables for best overall performance [31].

Four major requirements for interfacing both instruments are known [3, 32, 33]: (1) The closing of electrical circuit from CE, (2) an optimized nebulization efficiency and mass transport into ICP-MS, (3) the reduction of suction flow through the capillary caused by the nebulizer, and (4) a low "dead volume."

Several successful approaches were described in literature for setting up such an interface, mostly working along similar technical solutions based on pneumatic nebulization systems [3, 31, 34–37]. Less applied attempts were using an USN device, a direct injection nebulizer with sheath flow or hydride generation.

This chapter summarizes basic principles for coupling CE to ICP-MS. Therefore, a special focus is drawn to interface developments and technical problems, i.e., requirements to the interface setup and respective solutions. Finally, two applications, namely, Mn- and Se-speciation in serum and/or cerebrospinal fluid, are described in detail to enable a simpler startup even for those operators who had not used such an instrumental combination in the past.

2 General Aspects: Limitation and Potential of CE–ICP-MS Coupling

Starting first with some limitations: The most important drawback of this hyphenation technique is the fact, that CE–ICP-MS has worse concentration detection limits than LC–ICP-MS. This is due to the low sample intake to be analyzed. Limits of detection (LoD) are often above environmental or biological relevant species concentrations in real samples. Consequently, many problems can be related to the attempt of decreasing concentration detection limits to real-world concentrations when using (partly inadequate) stacking and separation conditions. Difficulties are often related to chemical interactions of samples, electrolytes, and the capillary or detector interferences [38]. This is not surprising as species stability can be impaired by "wrong" CE conditions, predominantly complexing electrolytes, inadequate pH, etc. [39]. In protein-rich biological samples, a typical problem is a total or partial compound sticking to capillary. Such an outage appears more likely without temperature control of the CE capillary. Most CE systems nowadays provide such a temperature control, however, typically inside the instrument. Since the capillary is managed outside the instrument—at least its terminal part—for being interfaced to ICP-MS it may be advised to install an additional capillary cooling, e.g., in a tubing around the capillary where the coolant is driven by a peristaltic pump (*see* Fig. 1). In case protein sticking appears quantifications are usually wrong and "pseudo-species" may be detected. This is caused by accidentally redissolved protein particles from previously sticking ones. Such peaks may pretend species within a sample, but are only artifacts. If such artifacts appear at specific standard migration time they can be erroneously "identified" as a certain species. The well-known migration time variations caused by differences in ionic strength of buffers or samples are a further problem for species identification [40]. Standard additions help to overcome this uncertainty [41]. However, also the generation of new species during analysis must be considered. As ICP-MS is a sequential detection system, the monitoring of too many isotopes in parallel may result in missing fast migrating peaks of one isotope. Detection limits of the CE–ICP-MS system up to now are just suitable or still too high for several real-world samples. Thus, the demand for coupling to more sensitive detectors, e.g., ICP-sf-MS is recommended and is also realized in literature [17, 42].

Nevertheless, CE–ICP-MS is a powerful tool in metal speciation and elemental tagging approaches and the above-mentioned limitations mainly refer to the capillary electrophoresis side but less to the important interface between the two analytical systems.

It is of paramount importance for this hyphenation technique that the interface is working at its optimum. When the setup of an interface is successfully implementing the specific requirements

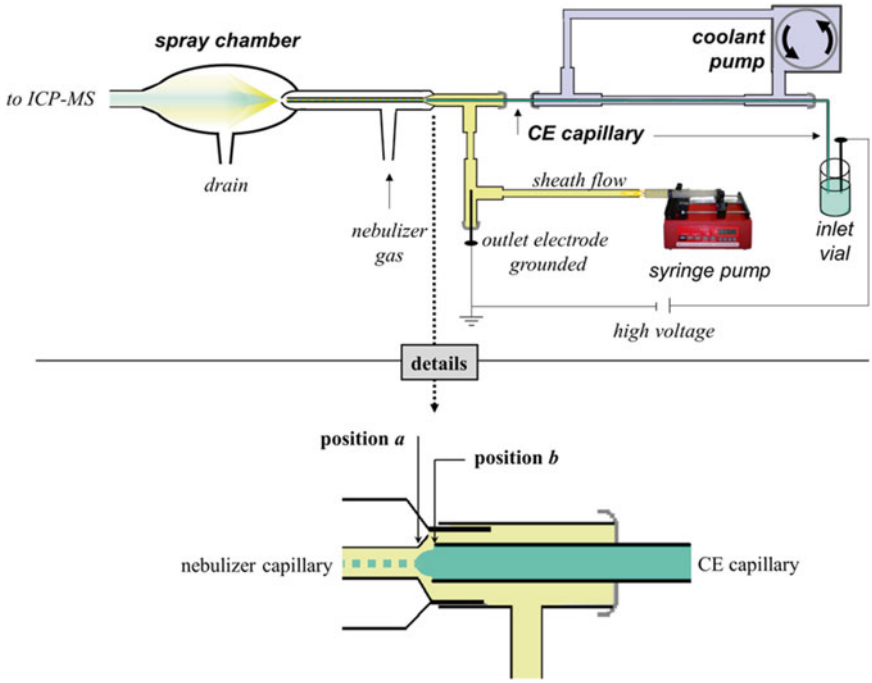

Fig. 1 The scheme shows an overview for mounting an interface between capillary electrophoresis and ICP-mass spectrometry. In the *upper part* the most important issues are demonstrated: Closing of electrical circuit for CE, feeding the sheath flow by a pulse-free syringe pump, temperature control of the capillary outside the CE instrument to ICP-MS, small volume spray chamber. The *lower part* shows the optimal positions of (**a**) the beginning of nebulizer capillary and (**b**) the end of CE capillary. The CE capillary should be moved close to the beginning of the nebulizer capillary, however, leaving sufficient space for the sheath liquid to flow around the capillary end

detailed in this chapter the interface is working reliable and no specific coupling problems occur. Investigations then can concentrate on the broad potential of this technique. The undisputed advantages and potential of CE–ICP-MS are its high separation capability, the short analysis time, and the high selectivity and sensitivity of detection. Since flow rates are low and volumes reaching the plasma are in the nL–μL range only [32] the ICP-MS accepts all buffers and modifiers without any problems. Plasma stability is not affected. Therefore, online preconcentration methods, such as isotachophoresis (ITP) combined with free zone electrophoresis (CZE) are easily possible, providing still acceptable species separation even when sample volumes are drastically increased for improvement of concentration-LoD. Buffer sandwiches or discontinuous buffer systems often result in improvements of separation. Even nearly non-aqueous buffer systems which maintain only little conductivity are accepted by the detector as the sheath flow (e.g., HNO_3) overcompensates the few nl coming from CE capillary. The different separation modes—CZE, ITP, isoelectric focusing (IEF), or micellar electrokinetic chromatography (MEKC)—allow separation solutions

for nearly all element species and stand for a wide characterization of the sample. The powerful ICP-MS detector provides element and isotope information, as well as multielement capability combined with low detection limits. Typical LoDs are in the 0.03–30 μg/l range, depending on species [39]. As with LC–ICP-MS species identification is realized by standard matching. Further, there are no stationary phases that can impair species stability [32]. Several authors already demonstrated applications to real-world samples of very different matrices and very low species concentrations.

3 Method: Important Details About Interfacing CE to ICP-MS

3.1 Requirements and Solutions

Much effort has been devoted (and still is) to interfacing CE with inductively coupled plasma (ICP) mass spectrometry (MS).

Designing an interface for the nebulization of micro-separation technique effluents into a fine aerosol and ensuring efficient transport into the plasma is not an easy task [43]. Thus, the most crucial point in hyphenating CE to ICP-MS first is the interface itself which must fulfill special requirements:

1. The closing of the electrical circuit from CE at the end of the capillary.
2. Adapting the flow rates best suited for CE and nebulization. The low flow rate from CE does not match the flow rate for an efficient nebulization.
3. Minimizing the siphoning suction flow through the capillary.
4. Preserving the high separation resolution from CE while transferring the analytes to ICP-MS.

The requirements 1–3 are solved principally in the same manner in (nearly) all described interfaces, independent on whether they are commercially available or laboratory constructed.

An electrolyte sheath flow, being in contact with the outlet electrode, is mixed with the capillary effluent at the end of the capillary, which is positioned as close as possible to the point of nebulization. When using commercial low-flow nebulizers the CE capillary can be positioned just before the nebulizer capillary (see position “a” and “b” in Fig. 1). Both capillaries should be positioned with minimal distance to each other for avoiding peak broadening, but there must be still a cleft to allow the sheath flow mixing with CE effluent. The task of this coaxial electrolyte flow around the CE capillary is multifacetted: First it must provide the electrical connection from the grounded outlet electrode to the end of the separation capillary. Usually, a current between 10 and 30 μA is determined. Second, the sheath flow is used to adapt the flow rate for suitable nebulization efficiency. It turned out that diluted nitric acid (ca. 0.1 %) was best suited for this task, although the sheath electrolyte also has the function of the outlet electrolyte with respect to suitable pH settings. Potential disadvantages of other

makeup solutions, such as plasma instability, poor precision, and degradation of ICP-MS performance, were avoided when using nitric acid. Further, the use of an inorganic acid instead of a salt solution provides the nebulizer from crusting and blocking.

In some applications pH stacking occurs resulting in peak sharpening. This is considered as a positive side effect. However, H^+ movement toward the inlet buffer (at −/+ polarity) can result in pH decrease in inlet electrolytes shifting separation conditions out of optimum. For keeping separation conditions defined at optimum, this buffer should be replaced regularly (in case even after each run) when HNO_3 is used as sheath electrolyte. For providing a sheath flow with lowest pulsation a syringe pump is preferred over peristaltic or HPLC pumps. Another task of the sheath flow is preserving an adequate flow rate for efficient nebulization (typically 10–100 μL, depending on the nebulizer used) which is in considerable excess compared to the analyte leaving CE capillary (typically < 2 μL/min). A careful optimization of the flow rate is crucial since a high flow rate often improves nebulization efficiency but contrary results in higher dilution of analytes coming from CE capillary. An additional positive effect of the sheath flow is the reduction of the suction force from nebulizer on the capillary lumen: The more this suction is fed from sheath flow the lower the suction affects the capillary lumen. Therefore, the suction flow usually gets controlled by capillaries with low inner diameter in commercial interfaces and/or by the dimensions of the separation capillary itself. Typically a suction flow is reduced by selection of appropriate column dimensions: a) using a long CE capillary (1.5 m) with a standard inner diameter of 50 μm [33], or b) by a short (2 cm) but narrow interface capillary (10 to ≤25 μm ID) set at the end of the CE capillary [44].

Both problem solutions are based on the law of Hagen–Poiseuille. Some approaches in literature apply a negative pressure at the inlet during separation. However, exactly meeting the point of equilibrium between nebulizer suction and counter suction at inlet is complicated to operate. Finally, self-aspiration of the sheath flow was suggested to overcome the suction flow. Before starting to analyze samples the suction flow should be checked and quantified. Two approaches are described in literature for checking the suction flow (e.g., by Michalke et al. [39, 45] or Schaumlöffel et al. [46, 47]), focusing on whether or not there is a suction flow or, more detailed, even quantifying the amount of the suction flow:

3.2 Method: Determining a Siphoning Suction Flow

The occurrence (yes/no) of the suction flow can be elucidated as described as follows:

The capillary gets first filled with buffer and the electrical current must be determined at high voltage, e.g., at 20 kV. Subsequently, the capillary's inlet should be kept into air while nebulization gas is turned on for 60 min. In case of a suction flow, air will intrude into the capillary. After 1 h the electrical circuit along the capillary must

be checked again when nebulization gas is turned off and the capillary's inlet is dipped again into the inlet electrolyte. In case a suction flow had occurred, an air bubble will now interrupt the electrical circuit and the measured current will be practically zero even at high voltage of 20 kV.

Quantifying a suction flow takes more than double the time compared to getting a simple yes/no answer:

The experiment starts as above with filling the capillary with electrolyte and measuring the current at 20 kV while the nebulizer gas is turned off. Subsequently, the capillary inlet is dipped into a standard solution at higher concentration (~200 μg/L) for 60 min. During this first step, the nebulizer gas remains "off." Therefore, the standard solution can enter the capillary only by diffusion. This "zero-flow–diffusion-value" will be needed later for correction. After 60 min the capillary inlet gets relocated into inlet electrolyte and the nebulizer gas gets turned on. In parallel ICP-MS detection is started while the capillary lumen is purged to ICP-MS. The monitored peak signal which is now detected corresponds to the standard amount which entered the capillary only by diffusion without suction forced flow.

In a second step, this experiment should be exactly repeated except that the nebulizer gas remains "on" during the 60 min period.

The peak signal monitored at the end of the second step corresponds to the standard amount entering the capillary by suction + diffusion.

The final experimental step for suction flow quantification aims for a peak area vs. capillary volume calibration: Therefore, the capillary must be filled completely with standard solution. Subsequently, the monitoring of the baseline by ICP-MS should be started. When now purging the capillary lumen with electrolyte to ICP-MS, the signal will first increase as long as standard from the previously filled capillary is reaching the detector. The signal falls back to baseline when the purging electrolyte has removed the standard solution from capillary lumen and reaches the detector. The area below the resulting broad hump corresponds to the capillary volume (in μL), which in turn is calculated by the equation $r^2 \times \pi \times L$ (r = radius, L = length, both in mm: volume results in (μL)).

The final equation $F\left[\frac{\mu L}{h}\right] = \frac{As - Ad}{Ac} \times Vc$ calculates the net suction flow (without diffusion) during 60 min (1 h), where As = peak area while nebulizer gas was turned on (suction + diffusion), Ad = peak area while nebulizer gas was turned off (diffusion), Ac = peak area while purging the filled capillary, and Vc = the calculated volume of the capillary.

Finally, requirement 4 (see earlier: preserving the separation of CE at the interface) is achieved by a low-volume spray chamber. An advantageous design for immediate peak response is given

when the chamber volume is minimal and spray direction is in direct line to the ICP entrance. Mostly such spray chamber designs are laboratory constructed.

Based on the earlier designs and rules for an interface the following two short sections give hints for Se and Mn speciation in biological samples.

4 Examples of Applications for CE Interfacing to ICP-MS

4.1 Selenium Speciation

4.1.1 Analytes

The chemical list consists of certified selenium stock standard (1000 mg/L, CPI, Santa Rosa, USA). Selenite, selenate, Selenomethionine, Selenocystine, Thioredoxinreductase (EC 1.8.1.9.), Glutathioneperoxidase (EC 232-749-6), human serum albumin (HSA), and TRIS buffer (Sigma-Aldrich, Deisenhofen, Germany). Ammonium acetate and acetic acid (Merck, Darmstadt, Germany). Ar_{liq} and methane (99.999% purity, Air Liquide, Gröbenzell, Germany).

4.1.2 Samples

Serum (and CSF if required) sample pairs should be drawn at a medical station or hospital. After ethical aspects are cleared and patients consented to the use of their samples for scientific investigations, the previously aliquoted, frozen-stored samples can be used for Se speciation. The samples should be thawed at 4 °C in the refrigerator, vortexed (and only for serum samples: diluted 1/5 with Milli-Q water), and subjected to sample vials of the CE device.

Working standards of Mnf species should be prepared daily from their stock standard solutions by appropriate dilution with Milli-Q water.

4.1.3 Capillary Electrophoresis (CE)-ICP-DRC-MS

"Biofocus 3000" (Bio-Rad, Munich, Germany) or PrinCe CEC 760 (Prince Technologies, Emmen, Netherlands) capillary electrophoresis system, equipped with an uncoated capillary (CS-Chromatographie Service GmbH, Langerwehe, Germany) 120 cm × 50 μm ID. Hyphenation is detailed earlier in this chapter. Analytical preparation: Before each run, purge the capillary with NH_4-acetate/acetic acid buffer, 10 mM, pH 3.0 (70 s, 8 bar).

Pressurized sample injection for 2 s, followed from 1 s buffer injection. The separation voltage is set to +25 kV. Sheath flow (diluted running buffer 1/25) around outlet electrode and capillary end: 80 μL/min

4.1.4 Parameter for Inductively Coupled Plasma Mass Spectrometry

Table 1 shows typical experimental settings chosen for ICP-DRC-MS after optimization.

4.1.5 Data Processing

Export Se data files from the NexIon software and process the files with a suitable chromatography software, e.g., "Clarity" from Data

Table 1

Typical experimental settings for ICP-DRC-MS regarding Se speciation

Instrument	Perkin Elmer Nexlon DRC
Plasma conditions	
RF power (W)	1250
Plasma gas flow (L/min)	15
Auxiliary gas flow (L/min)	1.05
Nebulizer gas flow (L/min)	0.98 Daily optimized
Nebulizer (optimal flow rate according to provider)	Meinhard low flow (100 μL/min)
Mass spectrometer settings	
Dwell time (ms)	100
Sweeps per reading	1
Readings per replicate	1600
Autolens	On
Ions monitored	^{78}Se, ^{80}Se
Reaction gas	CH_4
Reaction gas flow rate (mL/min)	0.6
Rejection parameter *q*	0.6
Rejection parameter *a*	0

Apex for peak area integration. Peak areas can be used for the calibration curve (standards) or for calculating the concentration according to the calibration curve (samples).

An example of separation is given in Fig. 2.

4.2 Manganese Speciation

4.2.1 Analytes

The chemical list consists of certified manganese stock standard (1000 mg/L, CPI, Santa Rosa, USA). $MnCl_2$, human serum albumin (HSA), transferrin, α2-macroglobuline, arginase, citrate, and TRIS buffer (Sigma-Aldrich, Deisenhofen, Germany). Ammonium acetate, sodium acetate, and acetic acid (Merck, Darmstadt, Germany). Ar_{liq} and methane (99.999% purity, Air Liquide, Gröbenzell, Germany).

Mn—citrate stock solution: mixing a solution of 1 g/L citrate with a $MnCl_2$ solution (5 mg/L) using a ratio of 4 + 1 (v:v), resulting in a Mn-citrate stock concentration of 1 mg Mn/L. Mn-albumin and Mn-transferrin stock solutions: in analogy by mixing 1 g/L protein solution with 5 mg/L $MnCl_2$ solution (4 + 1, each), resulting in 1 mg Mn/L for each compound. Working solutions should be prepared daily by appropriate dilution with Tris–HCl, 10 mM, pH 7.4.

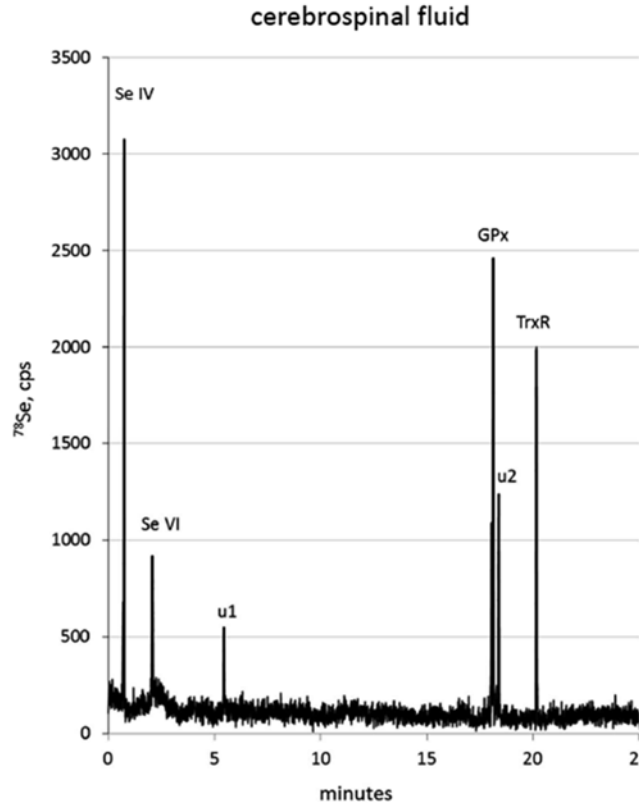

Fig. 2 This figure shows an electropherogram of Se species from a cerebrospinal fluid sample monitored at the isotope ^{78}Se. Se compounds u1 and u2 showed no standard match and were not identified. *GPx* glutathione peroxidase, *TrxR* thioredoxin reductase

4.2.2 Samples

Serum (and CSF if required) sample pairs, should be drawn at a medical station or hospital. After ethical aspects are cleared and patients consented to the use of their samples for scientific investigations, the previously aliquoted, frozen-stored samples can be used for Mn speciation. The samples should be thawed at 4 °C in the refrigerator, vortexed (and only for serum samples: diluted 1/5 with Milli-Q water), and subjected to sample vials of the CE device.

Working standards of Se species should be prepared daily from their stock standard solutions by appropriate dilution with Milli-Q water.

4.2.3 Capillary Electrophoresis (CE)–ICP-DRC-MS

"Biofocus 3000" (Bio-Rad, Munich, Germany) or PrinCe CEC 760 (Prince Technologies, Emmen, Netherlands) capillary electrophoresis system, equipped with an uncoated capillary (CS-Chromatographie Service GmbH, Langerwehe, Germany) 120 cm × 50 μm ID. Hyphenation is detailed earlier in this chapter. Analytical preparation: Before each run, the capillary should be

purged with Milli-Q H_2O (180 s, 8 bar) and TRIS (10 mM, adjusted to pH 8.0 with HAc) buffer = background electrolyte ("BE," 180 s, 8 bar).

For sample stacking a buffer sandwich gets injected consisting of 160 nL Na-acetate (200 mM, high conductivity), acting as leading electrolyte (LE), 60 nL sample, and 235 nL terminating electrolyte (TE), consisting of BE/H_2O (1:100; low conductivity). The inlet vial gets filled with BE adjusted to pH 6, the sheath flow at capillary end is BE/methanol (1:1). The applied voltage is set to +28 kV.

4.2.4 Parameter for Inductively Coupled Plasma Mass Spectrometry

Table 2 shows typical experimental settings chosen for ICP-DRC-MS after optimization.

4.2.5 Data Processing

Export Mn data files from the NexIon software and process the files with a suitable chromatography software, e.g., "Clarity" from

Table 2

Typical experimental settings for ICP-DRC-MS regarding Mn speciation

Instrument	Perkin Elmer NexIon DRC,
Plasma conditions	
RF power (W)	1250
Plasma gas flow (L/min)	15
Auxiliary gas flow (L/min)	1.05
Nebulizer gas flow (L/min)	1.02 Daily optimized
Nebulizer (optimal flow rate according to provider)	MicroMist low flow (50 μL/min)
Mass spectrometer settings	
Dwell time (ms)	100
Sweeps per reading	1
Readings per replicate	1000
Autolens	On
Ions monitored	^{55}Mn, ^{56}Fe
Reaction gas	NH_3
Reaction gas flow rate (mL/min)	0.58
Rejection parameter q	0.45
Rejection parameter a	0

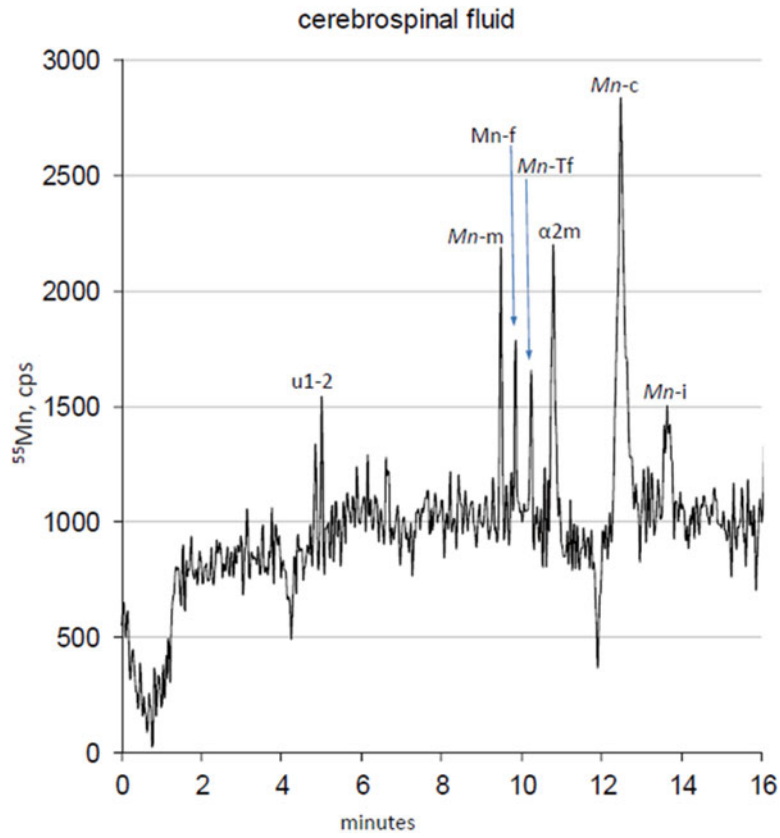

Fig. 3 This figure shows an electropherogram of Mn species from a cerebrospinal fluid sample monitored at the isotope ^{55}Mn. Mn species concentrations are rather low and noise of baseline is already clearly monitored. Mn compounds u1 and u2 showed no standard match and were not identified. Mn-m = Mn-malate, Mn-f = Mn-fumarate, Mn-Tf = Mn carrying transferrin, α2m = α-2-macroglobulin, Mn-c = Mn-citrate, Mn-i = inorganic Mn

Data Apex for peak area integration. Peak areas can be used for the calibration curve (standards) or for calculating the concentration according to the calibration curve (samples).

An example of separation is given in Fig. 3.

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