Jochen Vogl · Klaus G. Heumann

Determination of heavy metal complexes with humic substances by HPLC/ICP-MS coupling using on-line isotope dilution technique

Abstract An isotope dilution mass spectrometric (IDMS) method has been developed for the simultaneous determination of the complexes of 11 heavy metals (Ag, Cd, Cu, Mo, Ni, Pb, Tl, U, W, Zn and Zr) with humic substances (HS) by coupling HPLC with ICP-MS and applying the on-line isotope dilution technique. The HPLC separation was carried out with size exclusion chromatography. This HPLC/ICP-IDMS method was applied to samples from a brown water, ground water, sewage and seepage water as well as for a sample containing isolated fulvic acids. The total contents of heavy metals and of their complexes were analyzed in these samples with detection limits in the range of 5–110 ng/L. The analysis of heavy metal/HS complexes from the different waters resulted in characteristic fingerprints of the distribution pattern of heavy metals in the separated HS fractions. A comparison between the total heavy metal concentrations and their portions bound to humic substances showed distinct differences for the various metals. Simultaneous ^{12}C detection was used for the characterization of HS complexes not identified by UV detection and for the determination of relative DOC concentrations of chromatographic peaks.

Introduction

Humic substances (HS) play an important role in water analysis because dissolved organic carbon (DOC) in natural aquatic systems preferably originates from HS. HS show polyfunctional and highly diverse structures, which enables these com-

pounds to form ionic, hydrophobic and electron donor-acceptor interactions, respectively [1]. For example, HS form complexes with heavy metals as well as adducts with pesticides, which strongly influence the mobility of these substances in the environment. HS may therefore distinctly increase the heavy metal content in natural waters. However, determination of these heavy metal/HS complexes result in many analytical problems. Reliable calibrations are often an essential problem with respect to matrix influences, which are due to varying organic composition of separated fractions in HPLC/ICP-MS. However, in combination with the on-line isotope dilution technique [2], HPLC/ICP-MS enables exact calibration and accurate quantification unaffected by the matrix. In addition, this is also the only possibility to obtain real time concentrations of heavy metal complexes during chromatographic separations.

Experimental

Sampling and sample treatment. All samples were taken or prepared by the Engler-Bunte Institute at the University of Karlsruhe (F. H. Frimmel and G. Abbt-Braun) within the research project "Refractory Organic Substances in Waters (ROSIG)" of the Deutsche Forschungsgemeinschaft. The original water samples were stored in pre-cleaned polyethylene bottles in a refrigerator at about 5°C. They were immediately analyzed after filtration using an 0.45 µm pore sized polyvinylidene difluoride filter (Millipore). Fulvic acids (FA) were isolated from the original water samples by using the XAD-8 method [3–5].

HPLC/ICP-IDMS system. All measurements were carried out with a metal-free HPLC system (Sykam) and a quadrupole ICP-MS (Elan 5000, Perkin Elmer) using the on-line isotope dilution technique. Detailed information about this HPLC/ICP-IDMS system is given elsewhere [2, 6, 7]. Detailed descriptions on the principles of isotope dilution mass spectrometry can be obtained from the literature [8–10]. In this work 11 elements (Ag, Cd, Cu, Mo, Ni, Pb, Tl, U, W, Zn and Zr) and their HS complexes were analyzed. Using IDMS, two isotopes must be free of interferences or it must be possible to correct for it. This precondition was experimentally checked for the isotope ratios used for IDMS analyses. Therefore, ^{60}Ni and ^{62}Ni were measured (^{62}Ni was applied as a spike for the isotope dilution technique), because ^{58}Ni is interfered by $^{40}Ar^{18}O$ and ^{58}Fe. Zinc was measured by the ion intensities of ^{66}Zn and ^{68}Zn (spike) because an interference on ^{64}Zn can occur by ^{64}Ni. Tungsten was determined by the isotope ratio $^{182}W/^{183}W$ (^{183}W was used as a spike) because of possible interferences for ^{184}W and ^{186}W by

J. Vogl · K. G. Heumann (✉)
Johannes Gutenberg-University Mainz,
Institute of Inorganic Chemistry and Analytical Chemistry,
Becherweg 24, D-55099 Mainz, Germany

Table 1 Total heavy metal and heavy metal/HS complex concentrations (in µg/L) in a sewage, seepage and ground water sample (errors correspond to 95% confidence limit)

Heavy metal	Sewage water		Seepage water from soil		Ground water	
	Total	HS complexes	Total	HS complexes	Total	HS complexes
Ag	0.09 ± 0.03	< 0.014	0.18 ± 0.05	0.21 ± 0.06	< 0.08	< 0.014
Cd	0.03 ± 0.01	< 0.012	0.20 ± 0.03	0.07 ± 0.07	< 0.03	< 0.012
Cu	3.9 ± 0.2	5.4 ± 1.5	4.3 ± 0.2	2.92 ± 0.08	0.46 ± 0.08	0.20 ± 0.12
Mo	3.0 ± 0.1	3.8 ± 1.7	0.22 ± 0.02	0.19 ± 0.02	0.21 ± 0.06	0.17 ± 0.03
Ni	14.7 ± 1.5	2.81 ± 0.07	1.02 ± 0.07	0.32 ± 0.09	1.7 ± 0.1	0.77 ± 0.13
Pb	0.11 ± 0.05	0.13 ± 0.12	11.5 ± 0.2	0.6 ± 0.4	0.04 ± 0.02	0.04 ± 0.03
Tl	0.005 ± 0.007	< 0.005	0.139 ± 0.003	< 0.005	< 0.009	< 0.005
U	0.79 ± 0.07	0.03 ± 0.02	0.08 ± 0.01	< 0.007	0.04 ± 0.02	< 0.007
W	0.18 ± 0.02	0.06 ± 0.02	0.74 ± 0.15	0.5 ± 0.3	0.03 ± 0.02	< 0.010
Zn	22.3 ± 0.5	9.7 ± 0.5	90 ± 2	1.6 ± 0.4	3.5 ± 0.3	0.52 ± 0.21
Zr	0.06 ± 0.05	< 0.028	1.07 ± 0.07	1.25 ± 0.10	1.302 ± 0.004	0.71 ± 0.05

Table 2 Total heavy metal and heavy metal/HS complex concentrations in a brown water sample and in a sample prepared by fulvic acids isolated from the original brown water (errors correspond to 95% confidence limit)

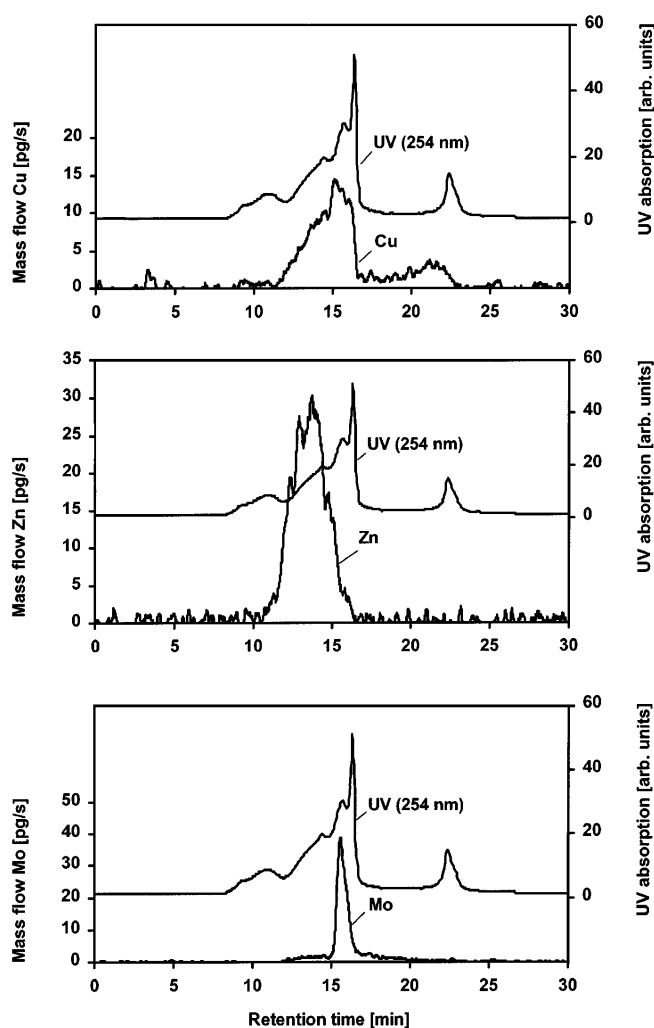| Heavy metal	Total concentration		HS complex concentration	
	Original sample [μg/L]	Isolated FA [ng/mg DOC]	Original sample [μg/L]	Isolated FA [ng/mg DOC]
Ag	0.17 ± 0.02	< 0.4	0.15 ± 0.02	< 0.6
Cd	0.06 ± 0.02	< 0.8	< 0.012	< 0.5
Cu	3.4 ± 0.1	16.3 ± 2.5	1.33 ± 0.17	18.0 ± 1.6
Mo	< 0.024	145 ± 20	< 0.024	117 ± 24
Ni	0.77 ± 0.12	1.3 ± 1.0	0.13 ± 0.07	–[a]
Pb	3.11 ± 0.07	3.6 ± 2.2	0.09 ± 0.05	2.0 ± 1.9
Tl	0.023 ± 0.005	0.4 ± 0.3	< 0.005	< 0.2
U	0.008 ± 0.002	< 0.2	< 0.007	< 0.3
W	0.016 ± 0.017	0.9 ± 0.8	< 0.010	< 0.4
Zn	37.0 ± 1.2	32.6 ± 5.5	< 0.11	12 ± 1
Zr	0.09 ± 0.02	6.1 ± 1.7	< 0.028	–[a]

[a] Not determined

osmium isotopes. The isotope abundances of ^{114}Cd and ^{116}Cd (spike) were corrected for ^{114}Sn and ^{116}Sn interferences by measuring other tin isotopes. For all other heavy metals the following non-interfered isotope ratios were detected using always the isotope with minor natural abundance as a spike isotope: $^{63}Cu/^{65}Cu$, $^{90}Zr/^{91}Zr$, $^{97}Mo/^{98}Mo$, $^{107}Ag/^{109}Ag$, $^{203}Tl/^{205}Tl$, $^{206}Pb/^{208}Pb$ and $^{235}U/^{238}U$. Taking into account that only low precision can be obtained with a quadrupole ICP-MS for the determination of extreme isotope ratios, the corresponding ratios in the spike solution should not exceed a value of about 25. With respect to this restriction an HNO_3 acidic (5% by weight) multi-element spike solution was prepared from single-element spike solutions. This multi-element spike solution contained concentrations of the different isotopically enriched heavy metals in the range of 0.5–5 μg/L, which were accurately quantified by inverse IDMS using a multi-element standard solution of natural isotopic composition.

The detection limit was mainly influenced by the uncertainty of the isotope ratio measurement by ICP-MS. In this case, the precision of the spike isotope ratio determination is the limiting factor for the detection limit. Because this precision is largely independent whether the isotope ratio is measured in the original sample or in a HS fraction, detection limits are in the same range for total heavy metal as well as for their HS complex determinations. The corresponding detection limits (3xs definition) for most of the heavy metals and their HS complexes in the original water samples can be drawn from Tables 1 and 2 (values indicated as "<"). However, detection limits lie always in the range of 5–110 ng/L.
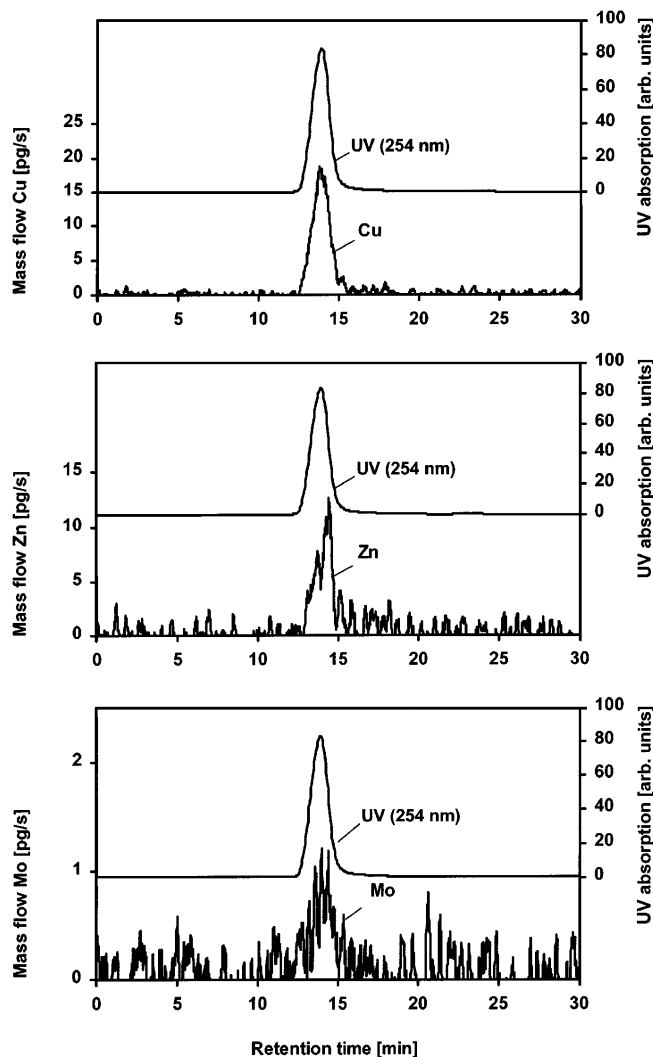
Results and discussion

Heavy metal/HS complexes in original water samples

The HPLC/ICP-IDMS chromatograms of Cu, Zn and Mo of a sewage water sample (500 μL) after separation by size exclusion chromatography (SEC) using bidistilled water as an eluent, together with the simultaneously detected UV absorption curve at a wavelength of 254 nm, are represented in Fig. 1. The UV absorption curve shows different fractions of HS over a broad range of molecular sizes, where low retention times correspond with high molecular sizes and high retention times correspond with low molecular sizes. Specific interactions of single HS fractions occur with the different metal ions. Copper forms complexes not only with HS fractions of high molecular size (at retention times of 12–16 min) but also with those of lower molecular size at retention times up to 23 min. Contrary to copper, zinc shows more specific interactions with the HS
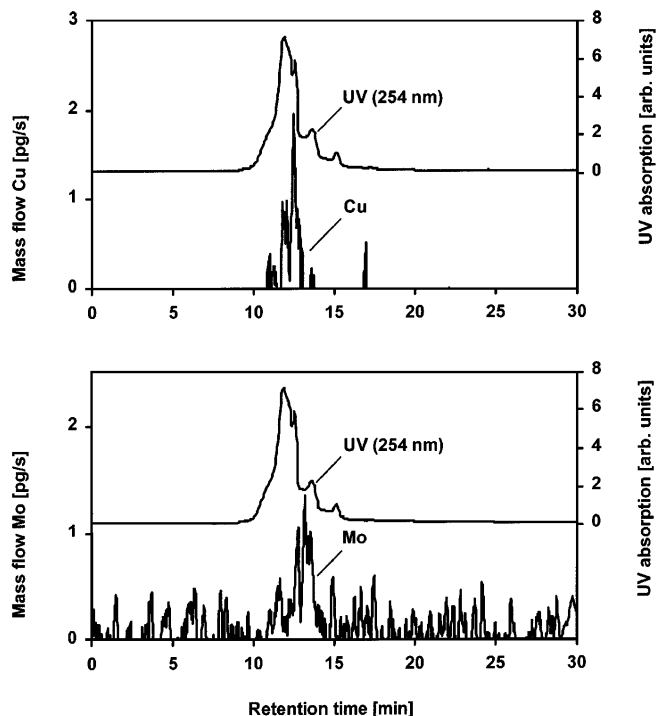

Fig. 1 HPLC/ICP-IDMS chromatograms of copper, zinc and molybdenum, together with the UV absorption curve, of a sewage water sample using SEC separation (column: TSK Gel 3000 PW)

Fig. 2 HPLC/ICP-IDMS chromatograms of copper, zinc and molybdenum, together with the UV absorption curve, of a seepage water sample using SEC separation (column: TSK Gel 3000 PW)
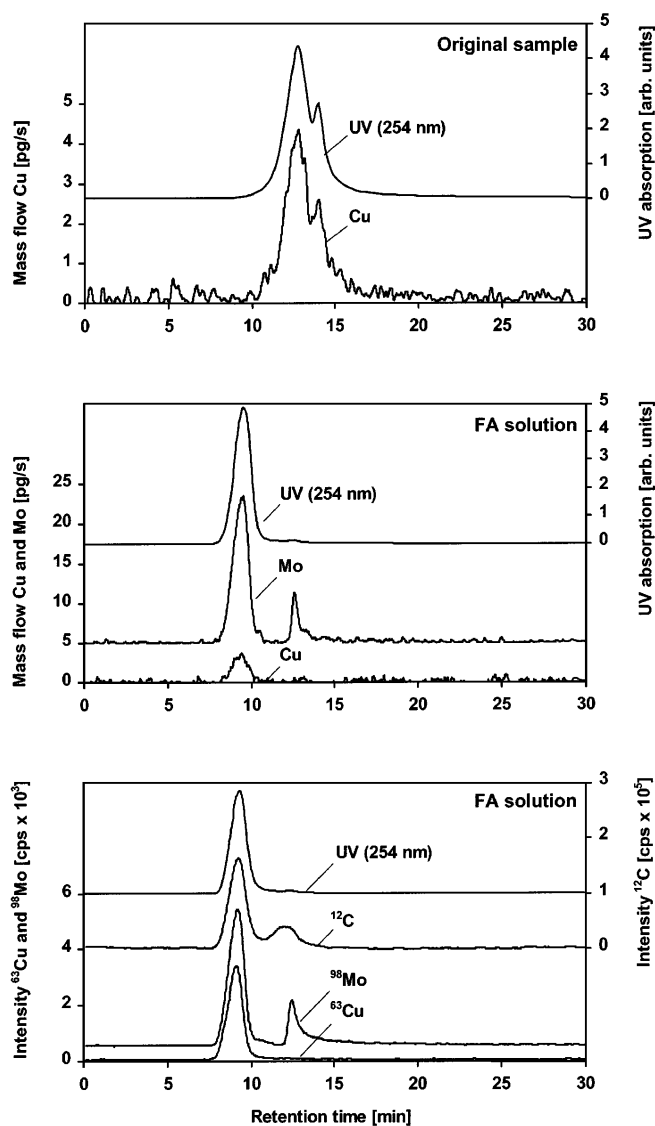

Fig. 3 HPLC/ICP-IDMS chromatograms of copper and molybdenum, together with the UV absorption curve, of a ground water sample using SEC separation (column: HEMA BIO 300)

fraction at retention times of 11–13 min, molybdenum with one of the minor abundant HS fractions of lower molecular size.

In Tables 1 and 2 the total heavy metal and the heavy metal/HS complex concentrations are listed for the different samples (sewage water, DOC = 8.7 mg/L; seepage water from a soil, DOC = 73 mg/L; ground water, DOC = 11.6 mg/L; brown water, DOC = 29.4 mg/L). The total heavy metal concentration was determined by direct ICP-IDMS without any separation process. The data for the HS complexes represent the sum of the corresponding heavy metal contents in all HS fractions. From these results much detailed and improved information can be obtained. In the sewage water, for example, heavy metals of typical anthropogenic origin such as Cu, Ni and Zn were found to have the highest content of HS complexes. The portion complexed by HS differs strongly from one heavy metal to the other and also from sample to sample. For example, silver is totally complexed by HS in the brown water sample, whereas this is true for about 40% of the total copper and only for less than 1% of the zinc in the same aquatic system. On the other hand, copper, molybdenum and lead are totally complexed by HS in the sewage water sample.

Heavy metal/HS complexes with fulvic acids

Fulvic acids (FA), isolated from a brown water sample, were diluted by bidistilled water so that a DOC concentration was achieved, which was comparable with the original sample. The corresponding total heavy metal and heavy metal/HS complex concentrations in this FA solution are summarized in Table 2. Because this FA sample is synthetic, the concentrations were correlated to the amount of DOC present in this solution, instead of correlating the content to the sample volume. As can be seen, for most of the heavy metals a few nanograms have been isolated per milligram of organic material by the FA separation process. In contrast to the other heavy metals, the

fraction eluted at retention times of 12–15 min. The most specific interaction, however, takes place between molybdenum and a small HS fraction at 16 min retention time. Other heavy metals, not presented in Fig. 1, can be compared with the behaviour of one of the elements shown in this figure. For example, lead fits best the behaviour of zinc, nickel that one of copper.

Figure 2 represents corresponding chromatograms for a seepage water sample from soil after SEC separation. With respect to the UV absorption curve, the organic material is concentrated in a relatively small fraction (retention times 13–15 min). As a consequence, all heavy metals form complexes with this HS fraction. Similar results were obtained for heavy metal/HS complexes in brown water samples, so that the HS complex concentrations listed in Table 2 for the original brown water sample always result from a small HS fraction. The results of copper and molybdenum for a ground water sample are shown in the chromatograms of Figure 3. Here, the UV active humic substances cover a larger range of molecular sizes compared with seepage water. Because of the relatively low heavy metal concentrations in ground water, HS complexes could only be determined for six of the metals investigated (Table 1). However, copper preferably forms complexes with the main HS

Fig. 4 HPLC/ICP-IDMS chromatogram of copper, together with the UV absorption curve, of a brown water sample (SEC column: TSK Gel 3000 PW) compared with the copper and molybdenum chromatograms of a fulvic acid solution prepared by FAs isolated from the same original brown water (SEC column: HEMA BIO 300). For better presentation the Mo curves of the FA solution are parallely shifted upwards with respect to the scale on the y-axis

forms complexes of similar stability with all UV active (at an absorption wavelength of 254 nm) HS and FA fractions, which follows from the similar shape of the Cu and UV curves. In contrast to copper, molybdenum shows two distinctly separated peaks in the chromatograms of the FA solution. The second molybdenum peak could not be identified by UV absorption as a complex compound of the FA material. Simultaneous ^{12}C detection was therefore carried out, which definitely also identified this molybdenum peak as a Mo/FA complex. It is well known that preferably organic compounds with π electron systems have absorption bands at wavelengths of 254 nm. It can therefore be followed that the second Mo/FA complex was formed by aliphatic organic material. Following the ^{12}C$^+$ signal in HPLC/ICP-MS chromatograms allows simultaneous DOC detection of chromatographic peaks. Because of the high plasma temperature, the C$^+$ signal should be largely independent on the organic compound. This could be confirmed by measuring the C$^+$ ion intensity of standard solutions containing substances of very different molecular weight, such as phthalate, β-dextrine and polyethylene glycol. This enables direct estimation of relative DOC concentrations in chromatographic fractions by the signal intensity of carbon. From this it follows that the second molybdenum fraction of the chromatogram in Fig. 4 (chromatogram at the bottem) contains about one third of the DOC content compared with the first fraction containing copper and molybdenum.

Conclusion

HPLC/ICP-MS coupling is a sensitive and fast method for the characterization of heavy metal/HS complexes in aquatic systems. Significant distribution patterns were found depending on the origin of the humic substance, which confirm our first results published elsewhere [6]. Such information cannot easily be obtained with other analytical methods and reliable real time quantification of separated metal/HS complexes is only possible by using the on-line isotope dilution technique. In addition, the simultaneous detection of the carbon intensity allows a sensitive DOC determination of chromatographic peaks, which is another important improvement in the analytical chemistry of humic substances.

molybdenum content is relatively high for both the total and the FA complex concentration, which means that the XAD-8 method [3–5] does not separate molybdenum very well from fulvic acids. The results also show that copper is totally complexed in the FA solution, whereas for most of the other heavy metals complexation is distinctly lower than 100%, except for molybdenum with a complexation grade of about 80%.

Figure 4 represents a comparison of the HPLC/ICP-IDMS chromatograms for copper after SEC separation of an original brown water sample and of the FA solution prepared by the fulvic acids isolated from this original sample. The molybdenum chromatogram is added in the two graphs for the FA solution, which was not possible for the original sample because of its low Mo concentration (see Table 2). Because two different SEC columns were used for the original sample and the FA solution, respectively, the absolute retention times are not directly comparable. However, the results clearly show that copper

References

1. Ziechmann W (1980) Huminstoffe. Verlag Chemie, Weinheim, p 13–57
2. Rottmann L, Heumann KG (1994) Fresenius J Anal Chem 350:221–227
3. Aiken GR (1985) In: Aiken GR, McKnight DM, Wershaw RL, MacCarthy P (eds) Humic substances in soil, sediment and water. Wiley, New York, p 363–387
4. Abbt-Braun G, Frimmel FH, Lipp P (1991) Z Wasser-Abwasser-Forsch 24:285–292
5. Malcolm RL, MacCarthy P (1992) Environm Int 18:597–607
6. Rottmann L, Heumann KG (1994) Anal Chem 66:3709–3715
7. Heumann KG, Rottmann L, Vogl J (1994) J Anal At Spectrom 9:1351–1355
8. Heumann KG (1992) Mass Spectrom Rev 11:41–67
9. De Bièvre P (1994) Fresenius J Anal Chem 350:227–283
10. Fassett JP, Paulsen PJ (1989) Anal Chem 61:643A–649A