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Development of an on-line isotope dilution technique with HPLC/ICP-MS for the accurate determination of elemental species

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Abstract. An on-line isotope dilution technique has been developed for use with a high performance liquid chromatography system (HPLC) coupled to an inductively coupled plasma mass spectrometer (ICP-MS). With this method it is possible to characterize elemental species at low concentration levels and to quantify them accurately. The possibilities of this method are shown using the examples of the determination of the interactions of different molecular weight fractions of dissolved organic matter (DOM) with copper and molybdenum in a natural water sample.

Introduction

The accuracy of analytical results is a great problem in trace element analysis [1]. This becomes even more significant when elemental species have to be analyzed. However accurate analytical results are an essential precondition for the judgement of the toxicity, bioavailability and environmental behaviour of the different species. Therefore reliable analytical methods are necessary to obtain accurate analytical results for the various species.

By coupling HPLC with an ICP-MS, a powerful and sensitive system for the separation and detection of elemental species in an on-line procedure is available [2–15]. Due to the high sensitivity of this system, the analysis can usually be carried out without sample enrichment steps which can influence the composition of the species in the sample. In addition to the high sensitivity of ICP-MS, the multi-element capability and the feasibility of isotopic ratio determinations are further important features. The rapid determination of isotope ratios in solutions makes ICP-MS an ideal tool for isotope dilution analysis.

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Principles of isotope dilution mass spectrometry (IDMS) for elemental species

IDMS is considered to be one of the most accurate methods in trace element and elemental species analysis [16, 17]. In IDMS, an exactly known quantity of a spike, enriched in one of the isotopes of the element to be analyzed, is added to the sample. After complete mixing of the sample and the spike, the resulting isotope ratio is determined with a mass spectrometer. Since only isotope ratios instead of absolute intensities are used for the calculation of concentrations, calibration drifts, variations in the intensities and matrix effects have less effect on the accuracy of the analytical results compared with conventional calibration strategies. Further detailed descriptions of the IDMS method are given in [18].

ICP-IDMS has been applied in some cases for the determination of different elements with high accuracy and precision [19–24]. It could be shown that the accuracy of analytical results determined by ICP-MS with the isotope dilution technique was better than with conventional calibration methods. An on-line isotope dilution by flow-injection, where the sample and the spike were injected simultaneously into the ICP-MS, was described by Viczián et al. [24]. For speciation using IDMS, two different cases have to be distinguished:

a) IDMS with a species-specific spike

The sample is spiked, prior to the separation of the different species, with the labelled compounds to be determined. This is only possible if the structure and the composition of the species in the sample are exactly known and the compounds labelled with an enriched isotope are available (e.g. Cr^{3+}/CrO_4^{2-}, I^-/IO_3^-, SeO_3^{2-}/SeO_4^{2-}). It must be guaranteed that the different species do not inter-convert and that no isotopic exchange occurs until they are completely separated from each other by the applied separation system. Since the original sample is spiked prior to the separation, one of the main advantages of IDMS, that loss of substance normally has no effect on the analytical result after the isotope dilution step has taken place, can fully be used.

b) IDMS with a species-unspecific spike

In this case the spike must be added after the complete separation of the different species and may exist in a different chemical form than the species to be analyzed. However equilibration between the separated species and the spike, prior to the isotope ratio measurement, must be guaranteed. In the case of an ICP-MS this is achieved by the high temperature of the argon plasma. This type of IDMS technique is necessary if the structure and the composition of the different species to be analyzed are not exactly known, or if a species-specific spike is not available, which is the case, for example, for metal complexes of humic substances (HUS). The structure of HUS cannot be determined in detail and consequently the different species cannot be synthesized. Furthermore, the addition of a spike prior to the chromatographic separation would shift complex equilibrations.

In both cases, separation of the different species has to be carried out prior to the mass spectrometric measurement. For the procedure described under b), a quantitative separation is essential before the spiking step takes place.

For speciation of compounds with unknown structure, other calibration strategies such as the standard addition method cannot be applied without problems, because the added element in its existing chemical form often shows a different behaviour from the species to be analyzed. In this study an on-line isotope dilution technique with a species-unspecific spike has been developed for use with the coupling of HPLC with an ICP-MS. The possibilities of this method will be shown by the determination of heavy metal interactions with different molecular weight fractions of dissolved organic substances deriving from an aquatic system. However this method should also be transferable to other systems (for example to the interaction of metals with proteins in human body fluids).

Humic substances normally represent the major part of the DOM in natural waters. They appear in nearly all surface waters and their content ranges from 50 to 80% of the dissolved organic carbon (DOC) [25]. Normally metals are bound as complexes by HUS and, therefore, such phenomena as toxicity, transport mechanisms and bioavailability are affected by this complexation [26, 27]. Investigations of the type of metal-HUS interactions are of great ecological and geochemical interest. For separation of different humic substances and other dissolved organic matter of different molecular weight, the HPSEC (high performance size exclusion chromatography) has been applied.

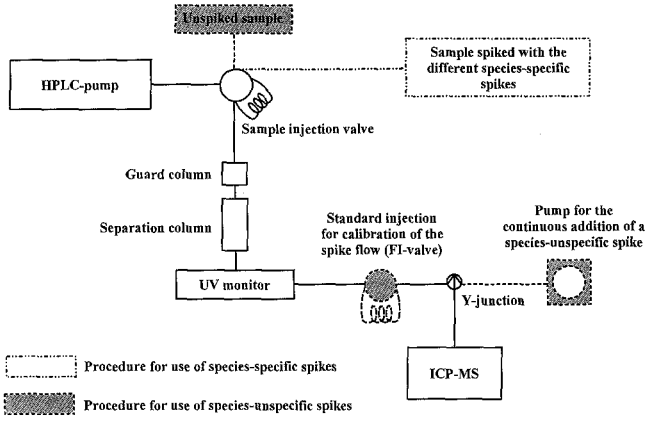

Fig. 1. Schematic diagram of the HPLC/ICP-MS system with simultaneous on-line isotope dilution technique

Table 1. HPSEC and ICP-MS instrumental and operational parameters

HPSEC	
HPLC pump	S 1000 PEEK (SYKAM GmbH, Germany)
UV monitor	LINEAR UVIS 204 with an 8 µl KEL-F cell
Sample injection valve	RHEODYNE Model 9125 fitted with sample loops made of PEEK
Guard column	TosoHaas, glass, 10 mm, 8 mm i.d., filled with TSK Gel Top Off G3PW
Separation column	TSK 3000 PW Glass, 300 mm, 8 mm i.d.
Eluens	bidistilled water
Flow rate	0.55 ml/min
Wavelength of UV absorption	254 nm
On-line IDMS	
Concentrations of the spike solutions	approx. 5 µg/l
Flow rate of spike solution	approx. 0.5 ml/min
ICP-MS (ELAN 5000)	
RF generator	40.68 MHz free running
RF power	1200 W
Nebulizer	Cross-Flow-Nebulizer (PE-SCIEX)
Spray chamber	Scott-Type (PE-SCIEX)
Argon flow rates:	
Plasma	15.0 l/min
Auxiliary	0.80 l/min
Nebulizer	0.9–1.1 l/min
Sampler cone	Platinum
Skimmer cone	Platinum
Measurement parameters:	
Points across peak	1
Dwell time	30 ms
Sweeps per replicate	10

On-line isotope dilution technique coupled with HPLC/ICP-MS

Instrumentation

A schematic diagram of the HPLC/ICP-MS arrangement with the simultaneous on-line isotope dilution technique is shown in Fig. 1. Both isotope dilution techniques (with species-unspecific and species-specific spikes) are indicated in the figure. This arrangement can in principle be used for all separation techniques and, therefore, is not limited to HPSEC. For the chromatographic separation of the DOM, a commercial HPLC system has been used. More detailed descriptions of the different components of this system are given in Table 1.

All parts of the system which come into contact with the mobile phase are constructed of metal-free materials such as PEEK, KEL-F or PTFE (to avoid heavy-metal

contamination). The outlet of the UV-monitor is connected to a flow-injection valve (ERC 5020, ERC GmbH, Alteglofsheim, Germany) with PTFE tubing (0.3 mm i.d., 25 cm long). The flow-injection valve (FI-valve) is fitted with a 1.5 ml sample loop. This loop is used for the injection of a standard solution which is used for calibration of the spike flow. In this way the standard solution is transported via PTFE tube (0.3 mm i.d., 5 cm long) to the inverse Y-junction under the same flow conditions as the separated species. The continuous addition of the spike is performed by a peristaltic pump (Gilson Minipuls3, Abimed, Langenfeld, Germany) equipped with a tube, made from Isoversinic, (Abimed, Langenfeld, Germany) with an i.d. of 10 mm. When using a species-unspecific spike, the separated species are isotope diluted after the inverse Y-junction and are then delivered directly through a PTFE tube (0.3 mm, 25 cm long) to the pneumatic nebulizer of the ICP-MS. A commercial ICP-MS (ELAN 5000) is employed for the determination of the time resolved isotope ratios. The instrumental and operational parameters of the entire system are summarized in Table 1.

Chemicals and spike solutions

Before coupling the HPLC to the ICP-MS, an optimization of the ICP-MS sensitivity is performed using an ICP-MS multi-element standard solution, prepared by the dilution of a commercially available stock solution (ICP multi-element standard IV, Merck, Darmstadt, Germany). The copper and molybdenum solutions used for calibration of the spike flow are prepared from commercially available ICP standard solutions (Alfa Products, Johnson Mattey GmbH, Karlsruhe, Germany) by dilution with purified water. The spike solutions, with the particular enriched isotopes, are diluted to a concentration of about 5 µg/l with purified water. The isotope abundances of copper and molybdenum in a standard solution and the spike solution determined by ICP-MS are summarized in Table 2.

Matrix effect on the accuracy of results

To investigate the matrix effect of HUS in a water sample on the accuracy of analytical results, model solutions of molybdenum (10.1 ng Mo/g) with different contents of DOC were prepared from a commercially available humic acid (No. 7824, Roth, Germany). One aliquot of each of these HUS solutions was used for the determination of molybdenum by direct injection into the ICP-MS and subsequent calibration with a molybdenum standard solution. Another aliquot of the HUS solutions was mixed with the molybdenum spike solution so that IDMS was also carried out on the same samples. The results are presented in Fig. 2.

As can be seen from Fig. 2, the accuracy of the determined molybdenum concentration decreases with increasing DOC content in the water sample when using the method of external calibration. High DOC contents lead to apparent higher molybdenum concentrations due to changes in ion formation in the plasma caused by the presence of carbon [28].

Using IDMS, such an effect cannot be observed, because here only isotope ratios are determined instead of absolute intensities. Although the ion intensities are enhanced by increasing DOC contents, the measured isotope ratios are independent of the amount of dissolved organic carbon. This investigation shows clearly that, with respect to the accuracy of results, external calibration strategies are not always suitable when coupling methods such as HPLC/ICP-MS are applied. The chromatographic separation system causes a continuous variation of the matrix substance (e.g. of the HUS concentration); this can strongly influence the calibration for the determination of the elemental species.

On-line isotope dilution with a species-unspecific spike

Prior to the analysis of different species, the system has to be calibrated. This calibration can be achieved by an inverse isotope dilution analysis. For this purpose a calibration standard, containing the element to be analyzed in any chemical form, is injected via the FI-valve into the mobile phase stream. The constant addition of the spike takes place at the inverse Y-junction (see Fig. 1). The dimension of the sample loop for the injection of the

Table 2. Isotope abundances of copper and molybdenum in the standard and spike solutions measured by ICP-MS

Element	Measured isotope ratio	Abundance [%]	
Cu	$^{65}Cu/^{63}Cu$	$h^{63}_{standard} = 67.91$	$h^{65}_{standard} = 32.09$
		$h^{63}_{spike} = 2.07$	$h^{65}_{spike} = 97.93$
Mo	$^{97}Mo/^{98}Mo$	$h^{97}_{standard} = 9.73$	$h^{98}_{standard} = 24.50$
		$h^{97}_{spike} = 91.88$	$h^{98}_{spike} = 3.75$

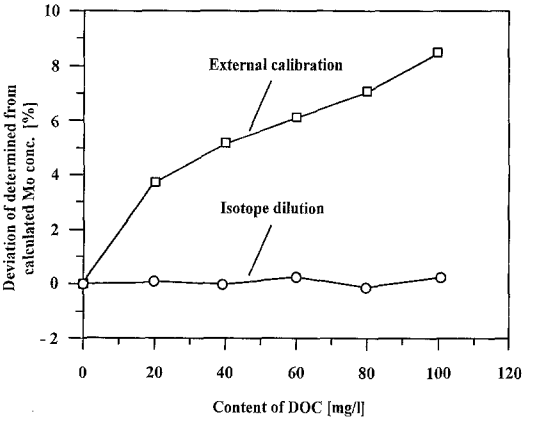

Fig. 2. Matrix effect of HUS on the accuracy of molybdenum determination by IDMS and by external calibration with a standard solution

calibration standard solution is chosen so that a large enough temporary steady-state signal is obtained; from this the isotope ratio of the spike mixed with the calibration standard can be determined (Fig. 3). With a knowledge of this isotope ratio, the entire system is calibrated for constant flow rates. After the respective calibration, the sample is injected through the sample injection valve onto the column. Synchronously with the sample injection, the measurement of the particular isotope ratios and of the UV absorption is started. Subsequently the measured time resolved intensities of the different isotopes are treated by a self-made computer program.

The calculation of the concentrations of the separated species will be described here by using molybdenum as an example. The measured ion intensities of the corresponding isotopes are corrected by the corresponding background intensities and they are then smoothed by a least-squares fit smoothing [29, 30]. From the resulting intensities, $Int^{isotope}$, the isotope ratios R(t) for every measured time are calculated. Every isotope ratio R(t) agrees with a certain number of molybdenum atoms, $N_{total}(t)$, which consists of the amount of molybdenum in the sample, $N_{sample}(t)$, and the blank of the system, N_{blank}.

$$N_{total}(t) = N_{sample}(t) + N_{blank} \tag{1}$$

From the pre-run of the chromatographic separation (up to the void volume) the isotope ratio caused by the blank, R_{blank}, can be determined and from that N_{blank} can be calculated,

$$R_{blank} = \frac{Int^{97}_{blank}}{Int^{98}_{blank}} = \frac{N_{blank} \cdot h^{97}_{sample} + N_{spike} \cdot h^{97}_{spike}}{N_{blank} \cdot h^{98}_{sample} + N_{spike} \cdot h^{98}_{spike}} \tag{2}$$

$$N_{blank} = N_{spike} \cdot \frac{h^{97}_{spike} - R_{blank} \cdot h^{98}_{spike}}{R_{blank} \cdot h^{98}_{sample} - h^{97}_{sample}} = N_{spike} \cdot K_{blank} \tag{3}$$

where $h^{isotope}_{sample}$ is the isotope abundance of the respective isotope in the sample and $h^{isotope}_{spike}$ of the respective isotope in the spike. Together with the isotope ratio R_{cal} obtained from the calibration procedure and the amount of molybdenum in the calibration standard N_{cal} the amount of the added spike N_{spike} can be calculated using Eqs. (4) to (6):

$$N_{cal} = N_{spike} \cdot \frac{h^{97}_{spike} - R_{cal} \cdot h^{98}_{spike}}{R_{cal} \cdot h^{98}_{standard} - h^{97}_{standard}} - N_{blank} \tag{4}$$

$$N_{cal} = N_{spike} \cdot K_{cal} - N_{spike} \cdot K_{blank} = N_{spike} \cdot (K_{cal} - K_{blank}) \tag{5}$$

$$N_{spike} = \cdot \frac{N_{cal}}{(K_{cal} - K_{blank})} \tag{6}$$

The element amount N can be substituted in Eq. (6) by the mass flow M, e.g. for M_{cal}:

$$M_{cal}\left[\frac{pg}{s}\right] = FR_{HPLC}\left[\frac{ml}{min}\right] \cdot \frac{1}{60}\left[\frac{min}{s}\right] \cdot c_{cal}\left[\frac{pg}{ml}\right] \tag{7}$$

where FR_{HPLC} is the flow rate of the mobile phase and c_{cal} the concentration of the calibration standard. The mass flow of the spike can be obtained from the insertion of M_{cal} into Eq. (6). For every measured isotope ratio, R(t), a corresponding mass flow of the analyzed species, $M_{sample}(t)$, can be calculated now:

$$M_{sample}(t) = M_{spike} \cdot \frac{h^{97}_{spike} - R(t) \cdot h^{98}_{spike}}{h^{98}_{sample} \cdot R(t) - h^{97}_{sample}} - M_{blank}\left[\frac{pg}{s}\right] \tag{8}$$

Plotting $M_{sample}(t)$ versus the retention time, a mass flow profile is obtained. To obtain the concentration of the different species in the sample, the various peaks detected have to be integrated and then normalized to the injection volume. The evaluation of the chromatograms is carried out by the above scheme built into a home made computer program.

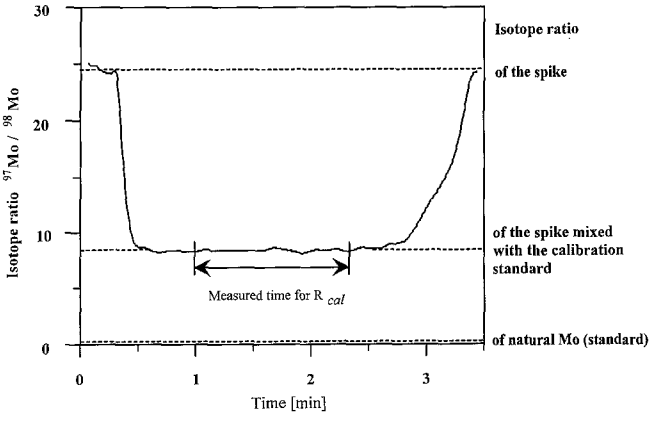

Fig. 3. Calibration of the molybdenum spike flow by a standard solution

Verification of the developed method by standard solutions

The verification of the above method is carried out by injection of molybdenum standard solutions. For this, the guard and separation column are placed between the HPLC pump and the sample injection valve. This arrangement is necessary to get the required back pressure for stable operation of the HPLC pump and to guarantee equal flow conditions for the subsequent analysis of real water samples. The outlet of the sample injection valve is now directly connected to the inlet of the UV monitor via a PEEK capillary. The calibration of this system is carried out with a 2.01 µg/l Mo standard solution.

107 µl of other molybdenum standard solutions of different concentrations are injected by the sample injection valve and the time resolved signals of the ^{97}Mo and ^{98}Mo intensities are detected by the ICP-MS. The time-dependent and background-corrected ^{97}Mo/^{98}Mo isotope ratio and the corresponding molybdenum mass flow are shown in Fig. 4 for the injection of a 5.06 µg/l standard solution. The amount of analyzed molybdenum is

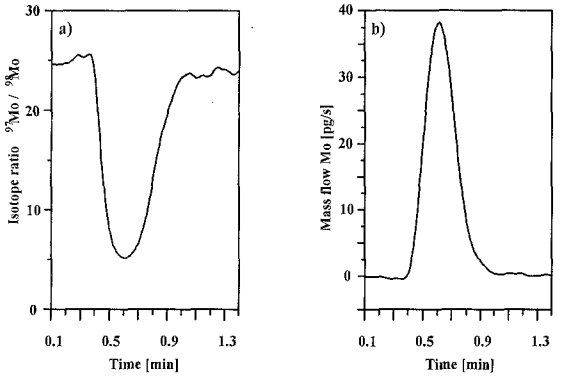

Fig. 4a, b. Determination of the time-dependent $^{97}Mo/^{98}Mo$ isotope ratio (**a**) and molybdenum mass flow (**b**) after injection of 107 µl of a molybdenum standard solution (5.06 µg/l)

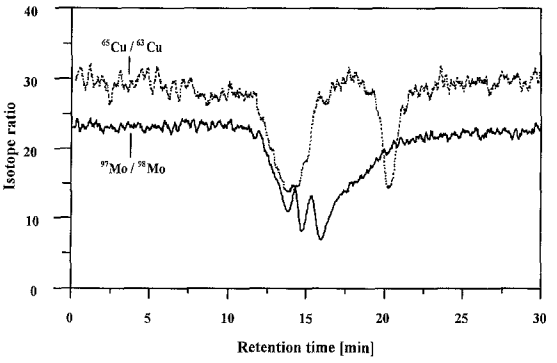

Fig. 5. $^{65}Cu/^{63}Cu$ and $^{97}Mo/^{98}Mo$ isotope ratios determined during HPSEC separation of a natural water sample (Vils, 494 µl sample volume)

Table 3. Verification of the on-line IDMS method with a species-unspecific spike by injection of different standards

Analysis No.	Detected amount of Mo [pg]	
	Standard A (2.01 µg/l)	Standard B (5.06 µg/l)
1	224	536
2	212	560
3	215	563
Analyzed mean	217 ± 6 ($s_{rel} = 3\%$)	553 ± 15 ($s_{rel} = 3\%$)
Calculated amount by concentration of the standard	214	542

obtained by integration of the mass flow peak. The results of three independent injections of two different standards are summarized in Table 3.

The analyzed means of both standard solutions agree well, within the limits of error, with the calculated amount from the known standard concentrations. This verifies the accuracy of the developed on-line IDMS method with HPLC/ICP-MS.

Quantification of copper and molybdenum interactions with dissolved organic substances and their characterization by HPSEC

The HPLC/ICP-MS method with on-line isotope dilution with a species-unspecific spike has been applied to the quantification of copper and molybdenum interactions with dissolved organic substances (preferably HUS) and their characterization by size exclusion chromatography. In this connection the corresponding complexes from a natural river sample were separated by HPSEC using bidistilled water as eluent. The distribution of copper and molybdenum in the resulting fractions of the humic substances was also investigated. The sample was taken from the Vils, near Amberg, a heavily polluted river in Bavaria [31]. Directly after sampling, the sample was filtered through a 0.45 µm PTFE-filter in order to separate suspended particles from the liquid phase and, therefore, to determine only the dissolved fractions. The filtered sample can be directly injected onto the column without further sample preconcentration or preparation steps because of the high sensitivity of the employed system. In this way conversion of the different species, which can occur during sample preconcentration or preparation steps, is avoided as far as possible. For the same reason, a buffer solution was not used as the mobile phase. A buffer solution certainly improves the separation efficiency but it also influences the interaction of heavy metals with HUS much more than bidistilled water, so that it is difficult to obtain a true analysis of the species in the sample.

For the on-line IDMS investigation 494 µl of the filtered sample were injected. The detection of the separated DOM fractions was carried out at a wavelength of 254 nm. For the determination of the different Mo and Cu species, a mixed spike of ^{65}Cu and ^{97}Mo with about 3.6 µg/l Mo and 8.7 µg/l Cu was used. The calibration of the spike flow was carried out using a mixed standard solution of both elements (2.63 µg/l Cu and 2.59 µg/l Mo). The dependence of the isotope ratios $^{65}Cu/^{63}Cu$ and $^{97}Mo/^{98}Mo$ on the retention time of the chromatographic separation of the sample are shown in Fig. 5. From this chromatogram, the corresponding mass flow profiles are calculated by the procedure described above and then correlated with the UV chromatogram (Fig. 6).

From the representation of the mass flow profiles and the UV chromatogram in Fig. 6, three molybdenum species, which interact with HUS fractions of different molecular weight, can be detected. All three species correspond to humic substances of higher molecular weight as shown by a low retention time in HPSEC. In the case of copper, two species can be recognized; one of these copper species is different from the detected molybdenum species. Copper species 1 (Fig. 6) interacts with high molecular weight components of the humic substances, whereas copper species 2 interacts with the low molecular fractions. The result of the integration of the different peaks and subsequent normalization to the injected volume, as

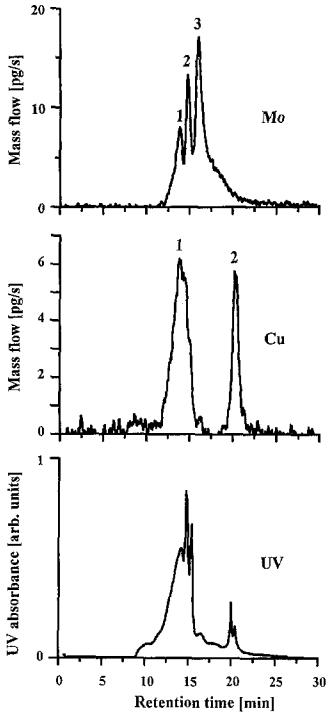

Fig. 6. Cu and Mo mass flow diagrams calculated from the results in Fig. 5 with the corresponding UV absorption curve

Table 4. Amounts and concentrations of the different copper and molybdenum species determined by HPLC/ICP-IDMS and total element concentrations determined by ICP-IDMS

	Detected amount [pg]	Concentration [μg/l]
Cu species 1	899	1.82
Cu species 2	341	0.69
Total Cu by HPLC/ICP-IDMS	1240	2.51
Total Cu by ICP-IDMS		2.55 ± 0.05
Mo species 1	562	1.14
Mo species 2	537	1.09
Mo species 3	1785	3.61
Total Mo by HPLC/ICP-IDMS	2884	5.84
Total Mo by ICP-IDMS		5.69 ± 0.04

well as the total concentration of copper and molybdenum in the sample determined by ICP-IDMS without species separation, are summarized in Table 4. The sum of the concentrations of the different species of each element agrees well with the total concentration of the corresponding element in the sample; this emphasizes the reliability of the developed HPLC/ICP-IDMS technique for elemental speciation.

Conclusion

By coupling HPLC with an ICP-MS and the simultaneous application of the described on-line isotope dilution technique, a powerful, sensitive and reliable method for elemental speciation is available. Using this method, fast and accurate analyses of elemental species, even with unknown structure and composition in complex matrices such as aqueous systems of humic substances and other organic substances, are possible down to the pg/ml level without time-consuming sample preparation steps which can easily be a source of error. Nevertheless, preconditions for this type of analytical method are a quantitative chromatographic separation of the species to be analyzed and the existence of at least two stable or long-lived radioactive isotopes of the element to be analyzed; these should be free from spectroscopic interferences.

The decisive advantages of this new on-line HPLC/ICP-IDMS method are the following:
- application of a reliable method with high sensitivity;
- ideal internal standardization – and, therefore, normally good accuracy – by the use of enriched spike isotopes of the same element;
- elimination of matrix effects and calibration drifts by the determination of isotope ratios;
- easy method of calibration in an on-line procedure;
- analysis of species with unknown structure and composition are also possible;
- better characterization of species by the employment of different chromatographic separation methods, including gradient elution techniques, is possible;
- this analytical method can be transferred to other systems (e.g. elemental speciation in human body fluids).

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