THE INFLUENCE OF COLUMN PROPERTIES ON METAL SPECIATION USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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Abstract

This paper describes an investigation into the presence of artefacts associated with the column properties on metal speciation in chromatographic systems and identifies possible limitations of columns used. The analytical procedure developed consisted of a High Performance Liquid Chromatography coupled Inductively Coupled Plasma-Mass Spectrometry system. The separation characteristics of two reversed-phase high-performance liquid chromatography columns, Hamilton C_{18}-PRP-1 poly-(styrene divinylbenzene) co-polymer and a conventional silica based C_{18}-ODS, were assessed based on the separation of metal species present at trace levels as soluble low molecular weight organic acid species. A synthetic digestive system was also employed in this study based on an enzymolysis procedure to generate dietary available metal species in the presence of fibre and multivitamins. In general, the C_{18}-ODS column was found to give a better separation of the organic species present compared to the C_{18}-PRP-1 column due to the attraction of highly polar species to uncapped silanol groups in the C_{18}-ODS column. The study illustrated that samples may interact with the residual silanol groups and as a result produce artefacts of the chromatographic retention mechanism, peak tailing and loss of chromatographic resolution.

Key words: metal speciation; HPLC; inductively coupled plasma-mass spectrometry (ICP-MS).

Introduction

In dietary studies the measurement of the total concentration of a trace element provides little information about its bioavailability, as the complex mechanisms of intestinal absorption and cell anatomy vary from unicellular to mammalian species.[1] A high degree of chemical speciation can exist for any trace element in biological systems,[2] for example not all forms of iron are effective in the treatment of iron deficiency. In addition it may be necessary to reduce iron(III) to iron(II) before it can be absorbed by intestinal mucous, with the reduction being carried out by reducing agents

such as ascorbic acid. The valence of an element also greatly affects its biological action, chromium (IV) is much more toxic than chromium (III), which is an essential form of chromium.[3] The use of HPLC coupled ICP-MS systems for the identification of elemental species in foods has proved a very useful tool[4-8] offering high resolution separations and very sensitive elemental determinations,[9-12] for example organotin species in marine muscle samples,[13] arsenobetaine in fish,[14] and dimethylarsinate in human urine.[15] The coupling of HPLC to ICP-MS can however compromise the operating performance of the separation and detection processes. For example in achieving the appropriate separation of elemental species one may require a chromatographic eluent that will subsequently cause salt deposition in the nebulizer of the ICP-MS.[16] Chromatographic systems are commonly used in speciation studies but little is known about their potential to induce for example metastable species during the separation process thus leading to the possibility of producing artefacts associated with the methodology. Consequently, column and eluent selection may seriously compromise the ideal chromatographic conditions selected in speciation studies. Reversed-phase stationary phases have been widely used in many liquid chromatographic applications.[17-20] As samples become more chemically complicated more involved interactions between sorbent and sample are likely to occur and the complexity of the analysis will undoubtedly increase. In addition many columns operate with a narrow pH range e.g. C_{18}-ODS, which may also compromise the column performance. In order to avoid these limitations, polymer based, silanol-free, more stable reversed-phase C_{18}-PRP-1 column has also been used in speciation studies.[21]

Experimental

Instrumentation

A Perkin-Elmer Model Series 2 (Norwalk, Connecticut, USA) HPLC (High Performance Liquid Chromatography) pump, and Hewlett-Packard 85 8-bit computer, Model HP7470A plotter and Model 9122 disc drive (Waldbronn, Germany) were used for chromatography and data processing. UV detection was carried out using HP1040A model diode-array UV system (Hewlett-Packard) operating at four wavelengths, 210

nm, 230 nm, 254 nm and 280 nm. A Rheodyne (Berkeley, California, USA) model 7125 injector with a 20 µl sample loop was used to introduce samples into the chromatographic system. The column after the UV/diode-array detector was paired with an FIA manifold, which consisted of, a Rheodyne injection valve (Model 5020 Anachem, Luton, Bedfordshire, UK) fitted with a 20 µl sample loop and then to a De Galan nebulizer by means of 0.5 mm i.d. Teflon® tubing. The ICP-MS instrument used was a VG PlasmaQuad (VG instruments, Winsford, Cheshire, UK), tuned using a 10 ng ml^{-1} standard solution of Be, Mg, Y, U, Bi, Eu, Co, La and In. The analytical columns were a styrene based reversed-phase C_{18}-PRP-1 column (Hamilton, Reno, USA) and conventional silica based reversed-phase C_{18}-ODS column (Hichrom, Reading, UK). Swinnek filters (0.45 µm) used were obtained from Millipore Water Associates (Harrow, UK) and a temperature-controlled waterbath used in the experiments was purchased from Grant instruments (Cambridge, UK). A Decon FS Minor model ultrasonic bath (Hove, East Sussex, UK) was used to remove any dissolved oxygen in the mobile phase. The optimum operating parameters for the HPLC, ICP-MS and FI system are shown in table 1 and the interfacing system used for the coupling of HPLC to ICP-MS is shown schematically in figure 1. The FIA manifold containing a 20 µl sample loop was also incorporated in the system to simply achieve direct analysis into the ICP-MS for calibration purposes.[17]

Reagents and Standards

All glassware and storage bottles used were soaked in 10 % nitric acid (v/v) overnight and rinsed with fresh high-purity ultra high quality (UHQ) water prior to use. All the enzymes (Pepsin porcine Cat. No. P7000, Pancreatin porcine Cat. No. P1750, Amylase porcine Cat. No. A6880) and bile salts (Cat. No. B8756) were purchased from Sigma Chemicals (Poole, Dorset, UK). All other reagents were of AnalaR grade as supplied by BDH (Poole, Dorset, UK). High-purity UHQ standard water (specific resistivity 18 $M\Omega\ cm^{-1}$) was used in all experiments. A 1 mg ml^{-1} standards of Copper(II), Zinc(II) and Iron(III) as the nitrate (BDH, Dorset, UK) in 5% nitric acid, was used as the stock standard solution.

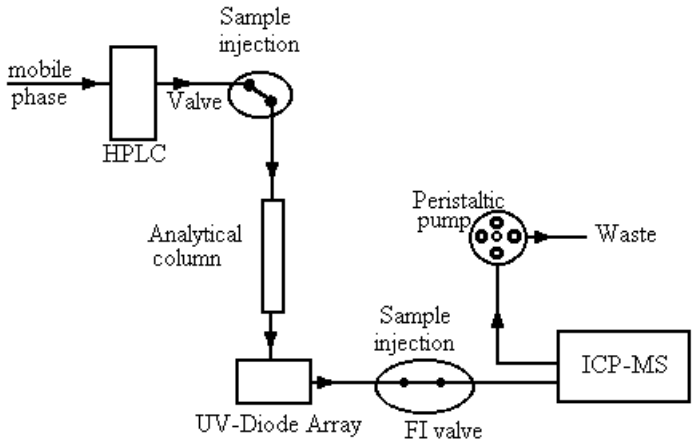

Figure 1 Schematic representation of HPLC - ICP - MS system.

Table 1. HPLC - ICP - MS instrumental parameters.

HPLC parameters	
Mobile phase	0.05 mol l^{-1} KCl in UHQ water, pH 2.5
Flow rate / ml min^{-1}	0.91
Injection volume / µl	20
Run time / s	1200
Temperature / °C	Ambient
Reversed - Phase Columns	
Hamilton C_{18}-PRP-1	Partisil C_{18}-ODS
Length / cm : 15	Length / cm : 25
Particle size / µm : 10	Particle size / µm : 5
ICP - MS parameters	
Gas flow / l min^{-1}	
Nebulizer	0.937
Coolant	14.20
Auxiliary	1.20
Forward power / W	1350
Detection mode	Time Resolved Analysis
Dwell time / µs	Variable
Spray Chamber	Water cooled 'Scott' type
Nebulizer type	V-Groove De Galan
Isotopes	^{57}Fe, ^{58}Fe, ^{63}Cu, ^{65}Cu, ^{64}Zn, ^{66}Zn
FI port	
Sample loop volume	20 µl
Teflon tube length	105 cm

Working standards were prepared by serial dilution of the stock standards on a daily basis. Citric acid stock solution was prepared by dissolving 1.094 g $H_3Cit.H_2O$ in 1000 ml of UHQ water. A 1 mg ml^{-1} ascorbic acid stock solution was prepared by AnalaR grade reagent of Janssen Chimica (Geel, Belgium) and 0.05 mol l^{-1} KCl as HPLC eluent degassed for 10 min using ultrasonic bath was used.

Enzymolysis Procedure and Trace Elemental Analysis

A synthetic digestive system (stomach and intestinal tract) was constructed to incorporate an enzymolysis procedure. The methodology adapted from the procedure developed by Crews *et al.*[22] and recommended by the Analytical Methods Committee[23] as suitable for speciation studies. Samples were homogenised with the enzyme mixture and incubated 4 h for stomach and 8 h for intestinal tract at 37 °C in a shaking water-bath. All extractions were performed in duplicate together with blanks. The first of the set was prepared in duplicate and contained only pepsin (4 h gut tract) whilst the second set after pepsin digestion had pepsin and pancreatin added (8 h intestinal tract).

Initially the samples' pH was adjusted to 2.5 after the addition of gastric juice by adding an appropriate amount of 2.5 mol l^{-1} hydrochloric acid. After 4 h the first set was removed and adjusted to pH 2.5, then centrifuged at 4500 rpm for 20 min and the supernatant filtered through a 0.45 μm Millipore filter. In order to reduce any effect from microbial activity causing changes in the composition of the polar dissolved organic compounds,[24] supernatants were stored in a fridge in the dark at 4 °C. Usually the analysis of samples was performed within hours and samples were never stored for more than 48 h.

The second digestion set was analytically prepared as above and then incubated for a further 4 h at 37 °C after the pH had been adjusted with saturated $NaHCO_3$ to pH 7.4 and intestinal juice added. After 4 h (total 8 h), the second digestion set (intestinal tract) was adjusted to neutral pH, and then centrifuged at 4500 rpm for 20 min and filtered through a 0.45 μm Millipore filter. After filtration, aliquots were injected through a FI port and HPLC columns and analysed by ICP-MS. Trace elements and dietary component were added to the enzymolysis procedure as follows. 0.1 mg l^{-1}

Cu(II) and Zn(II), 5 mg l^{-1} Fe(III) of elemental spikes, 5g of *Kellogg's All-Bran* fibre and 1.5g of *Boots multivitamin* tablets.

Results and Discussion

In this work two of the more popular reversed-phase columns have been used in speciation studies one polymer based and the other silica based in conjunction with a diode-array and ICP-MS detectors to investigate the effect of the column stationary phases and solid support may have on speciation using a series of soluble fractions obtained for the benefit of this study from the standard acid solutions and a synthetic digestive system.[5]

The substrate of the column packing material can be an important consideration in elemental speciation studies and needs therefore to be investigated for the influence trace elements and low molecular weight organic species may have on it. This paper therefore will initially focus on the comparison between the separation characteristics of reversed-phase C_{18}-ODS (silica based) and C_{18}-PRP-1 (styrene based) columns for two organic acids (ascorbic acid and citric acid) and three trace elements (Cu(II), Zn(II), Fe(III)). Figure 2 shows the UV/diode array chromatograms and ICP-MS data for four selected examples as follows. (2a) C_{18}-ODS: H_2Asc with three elements, Cu(II), Zn(II) and Fe(III), (2b) C_{18}-PRP-1: H_2Asc and the same three elements as for (2a), (2c) C_{18}-ODS: H_3Cit and the same three elements as for (2a) and (2d) C_{18}-PRP-1: H_3Cit and the same three elements as for (2a). The overlapped chromatogram represents the ICP-MS data. The chromatogram under the ICP-MS chromatogram corresponds to the HPLC-UV/diode array set at the wavelength of 230 nm. Although four wavelengths, 210, 230, 254 and 280nm, were selected on the diode-array to help characterise the UV peaks to which the metal peaks of three elements, Cu, Zn, Fe, may be associated, however only the UV chromatogram at 230nm is shown for simplicity.

The C_{18}-ODS column seems to give a complete separation of the citric and ascorbic acid although both are identified in the two systems at all wavelengths for ascorbic acid, however only 210 nm and 230 nm gave response signals for the citric acid system. This result suggests that the C_{18}-ODS column offers a better separation of

the organic species present but this has correspondingly poorer resolution of the elemental distribution due to the uncapped polar silanol groups, dispersion or salt deposition at the orifice of the ICP-MS. A gradual decrease in total MS counts were also noticed in each run when samples were injected which was thought to be due to the salt deposition inside of the injector tube of ICP-MS.[16] The separation characteristics observed for the C_{18}-ODS column may simply be attributed to the particle size (5μm) of the packing material and the column length (25 cm) compared to the C_{18}-PRP-1 column which has a 10μm particle size of the packing material and 15 cm length. However, longer columns increase the retention volume and potentially decrease the peak resolution of the retained species and impairing the detection limit. Thus, the column packing material substrate appears to be an important aspect of the separations observed indicating that the column may have properties associated with manufacture that may influence separations of the type described.

In figure 2a, UV chromatograms of ascorbic acid for the C_{18}-ODS column show responses which are attributed to the OH bonded chromophores (210 nm), C=C double bonds in which OH bonds conjugated cause bathochromic shift and a hyperchromic effect, increasing the intensity of absorption, C=O double bonds (230 and 254 nm), and the five membered carbon ring (280 nm). A large solvent front peak is also observed at 230 nm associated with unretained, OH bonded, and polar species in the solvent. Peak associated with ascorbic acid retained by C_{18}-ODS column was displayed a shoulder which may indicate an additional organic species, possibly a complex with iron.

The C_{18}-PRP-1 column shows similar peaks to the C_{18}-ODS column, for ascorbic acid the only difference being the absence of the shoulder, which interestingly may be present in the Fe in ICP-MS data (figure 2b). From ICP-MS chromatogram, zinc shows no retention on both C_{18}-ODS (figure 2a) and C_{18}-PRP-1 columns (figure 2b). Iron shows two apparent peaks on the C_{18}-ODS column (figure 2a) splitting into an unretained and retained form, however, on C_{18}-PRP-1 column most of the iron is retained and only a small shoulder appears after the ascorbic acid peak (figure 2b). Poor resolution was obtained on both columns for copper, which eluted later than the zinc and iron.

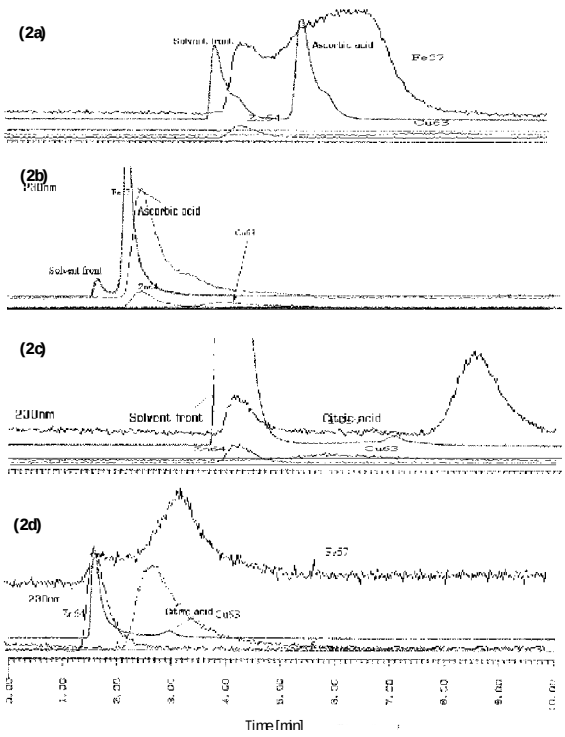

Figure 2. Comparisons of reverse phase C_{18}-ODS and C_{18}-PRP-1 columns responses: HPLC/UV diode array chromatograms at 230 nm overlapped with ICP-MS chromatograms of standard solutions (2a) C_{18}-ODS: H_2Asc and trace elements, (2b) C_{18}-PRP-1: H_2Asc and trace elements, (2c) C_{18}-ODS: H_3Cit and trace elements, (2d) C_{18}-PRP-1: H3Cit and trace elements.

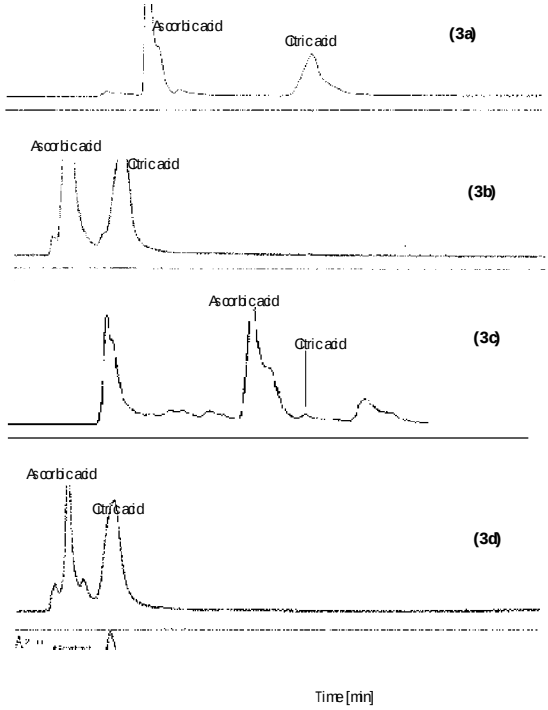

Figure 3. Comparisons of reverse phase C18-ODS and C_{18}-PRP-1 columns responses: HPLC/UV diode array chromatograms at 230 nm (3a) C_{18}-ODS: pH 2.5, H_3Cit and H_2Asc in gut system, (3b) C_{18}-PRP-1: pH 2.5, H_3Cit and H_2Asc in gut system, (3c) C_{18}-ODS: pH 7.4, H_3Cit and H_2Asc in intestinal system, (3d) C18-PRP-1: pH 7.4, H_3Cit and H_2Asc in intestinal system.

As the mobile phase and stationary phase functional groups are the same on both columns one must conclude that it is the uncapped polar silanol groups on the C_{18}-ODS column are causing the more polar separations seen for the iron.

The second ligand studied, citric acid, contains carboxyl groups, which have the observed chromophores at 210 and 230 nm for both the C_{18}-PRP-1 and the C_{18}-ODS columns. The retention time were shifted to longer times due to difference in column length. C_{18}-ODS column, which offers better resolution for compounds on both ICP-MS and UV systems except for the copper citrate system on the ICP-MS chromatogram. Copper appears to be separated on both columns suggesting the copper complex may be more non-polar than the one existing for Fe and rely on the reversed-phase properties of the columns. It can be seen from the chromatograms that citric acid may be associated

with iron and copper rather than zinc which has a lower stability constant than the other two elements.[25] Iron has the highest stability constant of the organic ligands studied, thus it has the more likelihood of complexing immediately with citric acid (figure 2c & 2d). The different behaviour of copper may also be a result of its absorption onto the walls of the tubing between the column and the nebulizer.[12] For simple solutions such as those used in figures from 2a to 2d C_{18}-ODS column gives better resolution with silanol activity possibly playing an important role for iron speciation. Figure 3 shows the UV/diode-array chromatograms at 230 nm for four selected examples as follows. (3a) C_{18}-ODS: pH: 2.5; H_3Cit and H_2Asc in Gut system, (3b) C_{18}-PRP-1: pH: 2.5; H_3Cit and H_2Asc in Gut system, (3c) C_{18}-ODS: pH: 7.4; H_3Cit and H_2Asc in intestinal system, and (3d) C_{18}-PRP-1: pH: 7.4; H_3Cit and H_2Asc in intestinal system. In chromatographic experiments if the pH of the mobile phase is 2.5 and the sample pH is 7.4, the question remains on to what will happen to the sample when it undergoes separation at a different pH. There are many effects or influences on the sample pH apart from the mobile phase pH. For instance, interactions with column material, which could further modify the pH of the sample through the release of hydrolysed H^+ or OH^- ions.[26] In the synthetic digestive system, the pH of the intestinal tract is 7.4 and so the pH level of the sample when injected into the reversed-phase column could be anything between pH 7.4 and pH 2.5. Thus as the pH changes concentration of the species present will change.

In the synthetic digestive system used in this study, the pH of the intestinal tract is at 7.4 and so the pH level of the sample injected onto the reversed-phase column might be kept at that pH although the mobile phase for the chromatographic separation may be at pH 2.5. Thus samples may interact with residual silanol groups and as a result produce artefacts of the chromatography related to the retention mechanism, peak tailing, and loss of chromatographic resolution.

Four different dietary samples from the intestinal part of the gut model were injected onto the C_{18}-PRP-1 and C_{18}-ODS columns. These were; intestinal control (only pepsin-pancreatin digest), Kellogg's All-Bran fibre, multivitamins, and a mixture of Kellogg's All-Bran fibre and multivitamins added into the pepsin-pancreatin digest. For the intestinal control, pepsin-pancreatin digest, using the C_{18}-PRP-1 column were

examined and found to contain no traces of iron however some zinc and copper are present with the zinc appearing as an unretained peak. The very low levels of copper concentration observed were thought to be interference from sodium salts (as $^{40}Ar^{23}Na^{+}$) present at more than 1g L^{-1} in the samples. Following the addition of fibre, the zinc peak became split and the copper peak shifted to a longer retention time. The second zinc peak and the copper peak were found to be associated with the chromophores observed with the UV/diode-array at 254 nm suggesting the presence of C=C and C=O double bonded organic groups. Most of the iron was seen to elute with the solvent front and this might be due to the low level of iron present in the supernatant as polar inorganic species, which have limited interactions with column material. In the presence of fibre and multivitamins, most of zinc and copper and to a less extent iron appeared to be associated with the retention time of ascorbic acid. In samples containing only multivitamins, copper concentrations were negligible compared to fibre and fibre&multivitamin samples. This suggests that the fibre sample is rich in copper. Most of the zinc and copper appear to be complexed with ascorbic acid and to a lesser extent with organic species associated with fibre and multivitamins. The small amount of copper and iron which eluted with the solvent front in the presence of multivitamins are most likely to be highly polar inorganic species or complexes such as copper nitrate, copper chloride, etc. possibly associated with the reagents used to adjust conditions in the gut model system which have limited interactions with column material.

However, in the C_{18}-ODS case the elemental association is not so obviously with ascorbic or citric acid. What was apparent with both columns is that both zinc and copper species seem to be highly polar in nature, an effect not attributed to an artefact of the column stationary phase. In addition the apparent speciation of zinc and copper is associated with fibre rather than vitamin additions, which suggests the possible presence of metallothionein type complexes.[27] Iron seems seriously effected by the C_{18}-ODS column stationary phase compared to the C_{18}-PRP-1 column and little information can be deduced for it other than to assume that the highly polar nature of the iron species present are reacting with uncapped silanol groups on the C_{18}-ODS column. In general for the systems described the C_{18}-ODS column was found to give a better separation of

the organic species present compared to the C_{18}-PRP-1 column. This is thought to be due to the attraction of highly polar species to uncapped silanol groups present in the C_{18}-ODS column. The C_{18}-PRP-1 column on the other hand is a styrene-based column producing an effective passive substrate surface and which will lead exclusively to active C_{18} phase separations. Such separations are more commonly associated with larger organic non-polar compounds rather than smaller compounds of the type studied. Thus the column packing material substrate appears to be an important aspect of the separations observed indicating that the column may have properties associated with manufacture that may influence separations of the type described.

Conclusions

Analysis of elemental species generated using a standard solutions and a synthetic digestive system with HPLC coupled ICP-MS methodology identified possible limitations with the chromatographic columns studied. Using the synthetic digestive system, dietary components such as multivitamins and fibre were added to alter the potential speciation, so changing the chromatographic separations. Whilst no specific complex forms were identified from the method it clearly indicated that sample preparation and factors such as pH and ligand concentration were important parameters to control. It was evident that all the species observed had a low molecular weight and were structurally similar to compounds such as citrate and ascorbate. The chromatographic method used whilst capable of separating low molecular weight organic elemental complexes did appear subject to experimental artefacts associated with methodology used. In general for the systems described the C_{18}-ODS column was found to give a better separation of the organic species present compared to the C_{18}-PRP-1 column. This is thought to be due to the attraction of highly polar species to uncapped silanol groups present in the C_{18}-ODS column. The C_{18}-PRP-1 column on the other hand is a styrene-based column producing an effective passive substrate surface and which will lead exclusively to active C_{18} phase separations. Such separations are more commonly associated with larger organic non-polar compounds rather than smaller compounds of the type studied. Thus the column packing material substrate

appears to be an important aspect of the separations observed indicating that the column may have properties associated with manufacture that may influence separations of the type described. The modelling of speciation reactions may be used to gain insight into processes such as the toxicity and bioavailability of metals in biological systems. In addition the study illustrated the importance for care in selection of column materials of metal speciation studies when they are to be based on column separations. Further the use of computer programs may prove helpful in selecting suitable columns.

References

1. Batley, G. E., (Ed.), *Analytical Methods and Problems*, CRC Press, Florida, 1989, pp. 319.
2. Bernhard, M., Brinckman, F. E., Sadler, F, (Eds.), *The Importance of Chemical Speciation in Environmental Processes*, Springer-Verlag, Berlin, 1987, pp. 7.
3. Underwood, E. J., *Trace Elements in Human and Animal Nutrition*, Academic press, New York, 1977, pp. 146.
4. Branch, S., Ebdon, L., O'Neill, P., *J. Anal. At. spectrom.* **1994**, *9*, 33.
5. Crews, H. M., Burrell, J. A., McWeeny, D. J., *Lebensm. Unters. Forsch.* **1985**, *180*, 221.
6. Crews, H. M., Massey, R., McWeeny, D. J., *J. Res. Natl. Bur. Stand.* **1988**, *93*, 349.
7. Crews, H. M., Dean, J. B., Ebdon, L., Massey, R. C., *Analyst*, **1989,** *114*, 895.
8. McWeeny, D. J., Crews, H. M., Massey, R. C., in Subramanian, Ivengar, K. G., Okamoto, G. V., (Eds.), *Biol. Trace Element Res.*, ACS Sym. Series 445, Washington, 1991, pp. 248.
9. Durrant, S. F., *Trends in Anal. Chem.* **1992,** *11*, 68.
10. Dean, J. R., Ebdon, L., Crews, H. M., Massey, R. C., *J. Anal. At. Spect.* **1988,** *3,* 349.
11. Thompson, J. J., Houk, R. S., *Anal. Chem.* **1986,** *58,* 2541.
12. Beauchemin, D., Micklethwaite, R. K., Van Loon, G. W., Hay, G. W., *Chemical Geology* **1992,** *95,* 187.
13. Rivaro, P., Zaratin, L., Frache, R., Mazzucotelly, A., *Analyst* **1995,** *120*, 1937.
14. Branch, S., Ebdon, L., O'Neill, P., *J. Anal. At. Spectrom.* **1994,** *9,* 33.
15. Ding, H., Wang, J. S., Dorsey, J. G., Caruso, J. A., *J. Chromatography* **1995,** *694*, 425.
16. Dundar, M. S., Haswell, S.J., *Analyst* **1995,** *120,* 2085.
17. Claessens, H. A., Vermeer, E. A., Cramers, C.A., *LC-GC Int.* **1993,** *6,* 692.
18. Jiang, S., Palmieri, J. S., Fritz, J. S., Houk, R. S., *Anal. Chim. Acta* **1987,** *200,* 559.
19. Thompson, J. J., Houk, R. S., *Anal. Chem.* **1986,** *58,* 2541.
20. Suyani, H., Heitkemper, D., Creed, J., Caruso, J., *Appl. Spectrosc.* **1989,** *43,* 962.
21. Ahuja, S., (Ed.), *Chromatography and Separation Chemistry*, American Chemical Society, ACS Symposium series 297, Washington, 1986, pp. 83.
22. Crews, H. M., Burrell, J. A., McWeeny, D. J., *Lebensm. Unters. Forsch.* **1985,** *180,* 221.
23. Analytical Methods Committee **1976,** *92*, 320.
24. Brown, L., Haswell, S. J., Rhead, M. M., O'Neill, P., Bancroft, K. C. C., *Analyst* **1983,** *108,* 1511.
25. Martell, A. E., Smith, R. M., *Critical Stability Constants*, vol.3, Plenum press, London, 1977, pp. 163.
26. Neue, U. D., Philips, D. J., Walter, T. H., Capparella, M., Alden, B., Fisk, R. P., *LC-GC Int.* **1995,** *8,* 30.
27. Stillman, M. J., Shaw, C. F., Suzuki, K. T., (Eds.), *Synthesis, Structure and Properties of Metallothioneins, Phytochelatins and Metal-Thiolate Complexes*, VCH, New York, 1992, pp. 14.

Povzetek

V prispevku so prikazani vplivi kolone (C_{18}-ODS oziroma C_{18}-PRP-1) in kromatografskih pogojev (mobilna faza s pH 2,5; standardna raztopina s pH 2,5 oziroma 7,4) na speciacijo kovinskih zvrsti (bakra, cinka in železa) v prisotnosti askorbinske in citronske kisline. Po kromatografski separaciji so bile organske komponente detektirane spektrofotometrično, kovine pa z ICP-MS. Izkazalo se je, da se pri izbranih pogojih samo bakrove in železove zvrsti delno zadržijo na obeh kromatografskih kolonah, vendar je bila ločljivost med obema kovinama slaba. Cink se pri teh pogojih ni zadržal na kolonah.