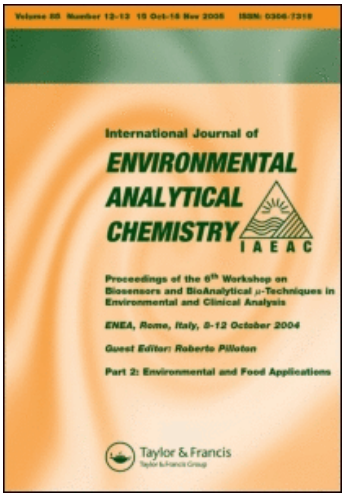

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Interface-Free Coupling Of Hplc And Atomic Spectrometry For Trace Metal Speciation By Using A High Performance Flow And Nebulization System

G. Weber[a]; H. Berndt[a]
[a] Institut für Spektrochemie und angewandte Spektroskopie, Dortmund 1, Germany

To cite this Article Weber, G. and Berndt, H.(1993) 'Interface-Free Coupling Of Hplc And Atomic Spectrometry For Trace Metal Speciation By Using A High Performance Flow And Nebulization System', International Journal of Environmental Analytical Chemistry, 52: 1, 195 — 202
To link to this Article: DOI: 10.1080/03067319308042860
URL: http://dx.doi.org/10.1080/03067319308042860

Intern. J. Environ. Anal. Chem., Vol. 52, pp. 195-202
Reprints available directly from the publisher
Photocopying permitted by license only

INTERFACE-FREE COUPLING OF HPLC AND ATOMIC SPECTROMETRY FOR TRACE METAL SPECIATION BY USING A HIGH PERFORMANCE FLOW AND NEBULIZATION SYSTEM

G. WEBER and H. BERNDT

Institut für Spektrochemie und angewandte Spektroskopie, Bunsen-Kirchhoff-Str. 11, W-4600 Dortmund 1, Germany

(Received, 20 October 1992; in final form, 4 March 1993)

The on-line coupling of HPLC with flame-AAS using a HPF/HHPN system (high performance flow/hydraulic high pressure nebulization) is described. The proposed system is used for speciation of trace metals. High molecular weight species (protein-bound metals) as well as low molecular weight ones (organic acid complexes of metals) are separated and specifically detected. Problems arising from the flow-rate and composition of the mobile phase are discussed and it is shown that these problems can be solved by optimizing the HHP-nebulizer.

KEY WORDS: HPLC, on-line AAS detection, metal speciation, hydraulic high pressure nebulization.

INTRODUCTION

The combination of HPLC with element-specific detection (in particular with atomic spectrometry) is one of the most promising concepts for trace metal speciation in environmental and biotic matrices[1]. It offers great flexibility towards separation of metal species such as oxidation states[2,3], organometals[4-6], protein-bound metals[7,8] or low-molecular-weight complexes of metals[9,10].

On-line coupling of a HPLC column to an atomic spectrometer is possible by simply connecting two capillaries (the column outlet and the nebulizer inlet capillary). However, this direct coupling drastically reduces the sensitivity because of the inefficient pneumatic nebulizer of the spectrometer. Therefore (more or less complicated) interfaces have been proposed to adapt HPLC to AAS- or ICP-instruments[3,4,11-14].

HPF/HHPN (High performance flow/hydraulic high pressure nebulization) improves the sensitivity of HPLC/AAS-coupling by optimization of the sample introduction (nebulization) step, while the simplicity of direct coupling is retained (no special interface). After

elution from the HPLC column the high pressure liquid stream is forced through a special nebulization nozzle of only 10, 15 or 20 μm. This type of nebulization[15] leads in flame-AAS to an aerosol yield of more than 50%, which corresponds to an improvement in sensitivity (as on-line detector for HPLC) of about one order of magnitude[16].

In this paper the advantages of the proposed system for separation and detection of metal species (including low- and high-molecular-weight complexes) are discussed.

EXPERIMENTAL

The experimental setup of the HPLC/flame-AAS system is shown in Figure 1. A HPF/HHPN system (KNAUER, Bad Homburg, Germany) was used consisting of a HPLC pump, solvent filter, sample injection valve (50 μL sample loop), HPLC-column and HHP-nebulizer (integrated in the spray-chamber of a Varian 1000 atomic absorption spectrometer). HHPN-adaptors for AAS instruments from Varian, Perkin-Elmer, Hitachi and Thermo-Jarrell Ash are available from KNAUER or from the respective spectrometer companies. Only equipment made of titanium or PEEK was used for chromatography.

Standards

Ferritin (from horse spleen) was obtained from Pharmacia. Cytochrom C (95%, from horse heart), Metallothionein (from rabbit liver) and Cyanocobalamin (99%) were obtained from Sigma. All other chemicals were of analytical grade (Merck p.a.). Tartrate and citrate species were prepared by mixing copper(II)- and iron(III)-standards with a ten fold molar excess of the respective acid.

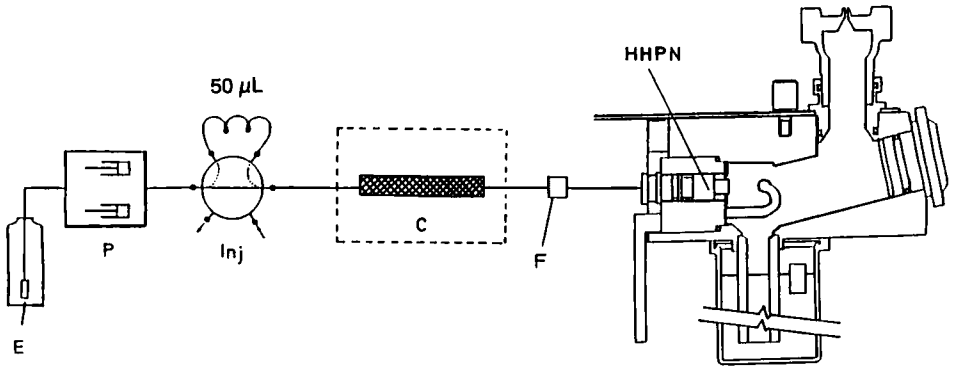

Figure 1 HPLC/Flame-AAS system
E: eluent reservoir with filter, P: HPLC pump, Inj: Sample injection, c: HPLC column, F: filter (5 μm), HHPN: hydraulic high pressure nebulizer (integrated in the atomic absorption spectrometer).

HPLC separation conditions

a) Low-molecular-weight species:
Spherisorb (5 μm) ODS 2 column (250 × 4 mm),
ammoniumsulfate/sulfuric acid pH 2.5 (ionic strength: 0.1 M),
0.5 mL/min

b) High-molecular-weight species:
Spherisorb (10 μm) Diol (250 × 4.6 mm),
sodium-acetate(0.1 M)/sodium-sulfate(0.1 M) pH 5.0,
0.5 mL/min

RESULTS AND DISCUSSION

Chromatographic conditions for the separation of metal species depend on the kind of species present (organometals, oxidation states, complexes etc.) and on the matrix (environmental, biotic). These conditions, namely the composition and flow-rate of the mobile phase, can have a considerable influence on the sensitivity of atomic spectrometric detection. Often the parameters, which are necessary for optimal separation of compounds, are far from being ideal for atomic spectrometric detection. A typical example is the determination of protein-bound metals by using size-exclusion or gel-permeation chromatography. In Figure 2 the separation of two iron-proteins (ferritin and cytochrom c) and cyanocobalamin (vitamin B12) is shown. The Diol-column can be used for size separation of proteins[17] and for most proteins a linearity of elution volume versus logarithm of molecular weight is valid. One problem with this kind of separation is the low flow-rate (0.5 mL/min), another is the relatively high concentration of inorganic salts. If a conventional system is used (HPLC coupled to flame-AAS with pneumatic nebulizer) the quantitation of these species is possible only in the μg-range (with respect to the injected amount of metal). In contrast to the conventional system the hydraulic high pressure nebulizer (HHPN) not only enhances the sensitivity by one order of magnitude[16], but also allows an optimal adaptation to the low flow-rate by choosing an optimal nebulization nozzle (10 μm diameter). In Figure 3 the dependence of signal height on the flow-rate and on the nebulization nozzle diameter is shown. At 0.5 mL/min the 10 μm nozzle offers a signal enhancement factor of 2.5 compared to the 20 μm nozzle. Such an adaptation is impossible for conventional nebulizers, so the overall gain in sensitivity with respect to a conventional system is about a factor of 25. Additionally, the HHPN system is not affected by high salt contents, which do affect the pneumatic nebulization.

Another example for protein separation is shown in Figure 4. A metallothionein standard (Sigma, both isoforms I and II are present) was injected, containing zinc and cadmium. It can be seen, that cadmium and the main fraction of zinc are bound to different forms. It should be noted, that these metalloproteins do not fit exactly into the linear relationship between log(molecular weight) and elution volume, because the respective retention mechanisms do not simply depend on the size of the molecule. Therefore, the two forms of metallothionein can be separated by this simple system. The small zinc peak at the retention time of cadmium corresponds to 7 ng zinc, which is very close to the detection limit. It is

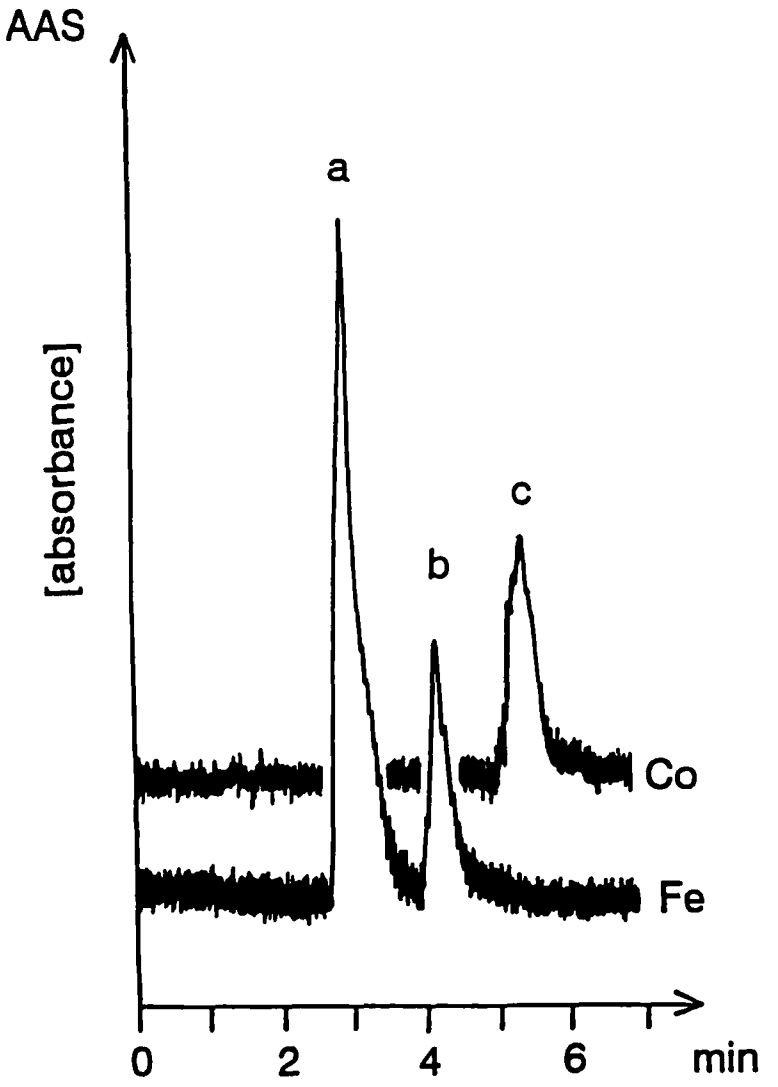

Figure 2 Separation of high molecular weight species of Fe and Co
a: Ferritin (=400 ng Fe), b: Cytochrom C (=113 ng Fe), c: Cyanocobalamin (=110 ng Co)

difficult to give exact detection limits, because they depend on the flow-rate, the composition of the mobile phase and also on the retention of the respective species (peak broadening), but generally the detection limit lies in the range of 5 to 15 ng (injected amount of metal).

The problem of low flow-rate is not only present in the separation of high molecular weight species, but sometimes also in the separation of low molecular weight species. In Figure 5 the separation of organic acid complexes of iron and copper is shown as an example. Organic acids are important for the binding of trace metals in some foods[10] and also play a

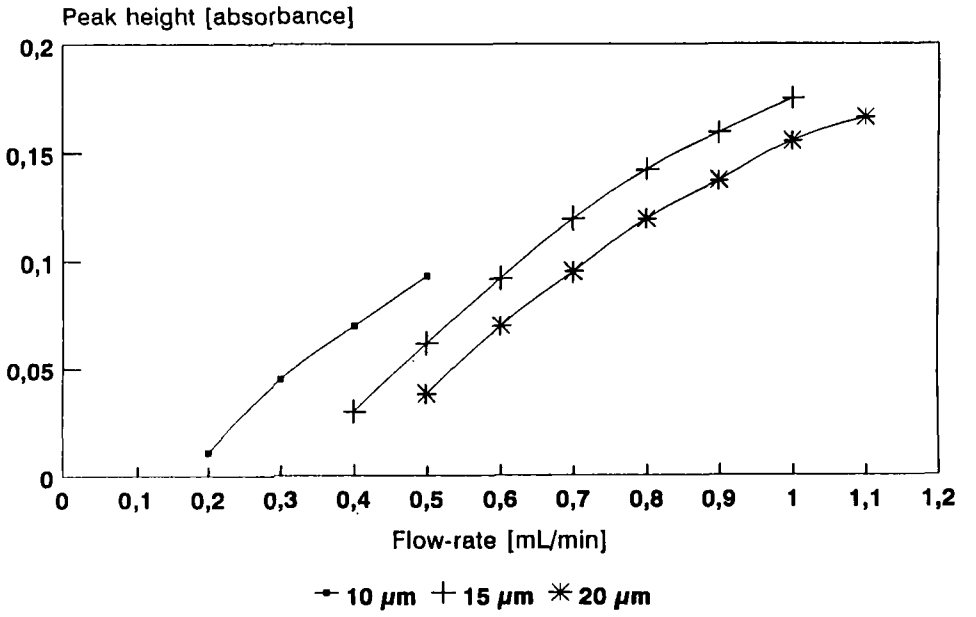

Figure 3 Flow-characteristics of different nebulization nozzles.

role in trace metal absorption and metabolism[18]. For the separation of organic acids flow rates similar to those of protein separations (0.5 mL/min) are preferred[19,20]. Using these optimized conditions and the HPF/HHPN system, different metal complexes can be separated: copper- and iron-tartrate elute at 5.4 minutes and the corresponding citrates at 8.3 minutes (copper) and 9.5 minutes (iron). Note that the two metal citrates elute at slightly different retention times (which also differ from that of free citric acid). This is typical for low molecular weight complexes of metals, which often do NOT coelute with the corresponding ligand. In most cases the complexes are more polar than the ligand and therefore elute at shorter retention times in reversed-phase systems.

One problem with the separation of metal complexes (esp. at low pH) is the risk of dissociation of the species. In the case of the copper and iron complexes investigated here a considerable degree of dissociation was not observed (recovery of injected species > 90%, no additional peaks for free metals). However, no attempts were undertaken to investigate the exact structure of the complexes before and after chromatography (e.g. stoichiometry, charge). Therefore, changes of the species with respect to metal/ligand ratio (from 1:2 to 1:1) or similar effects cannot be excluded.

CONCLUSIONS

The on-line coupling of HPLC with flame-AAS using the HHP nebulizer improves the

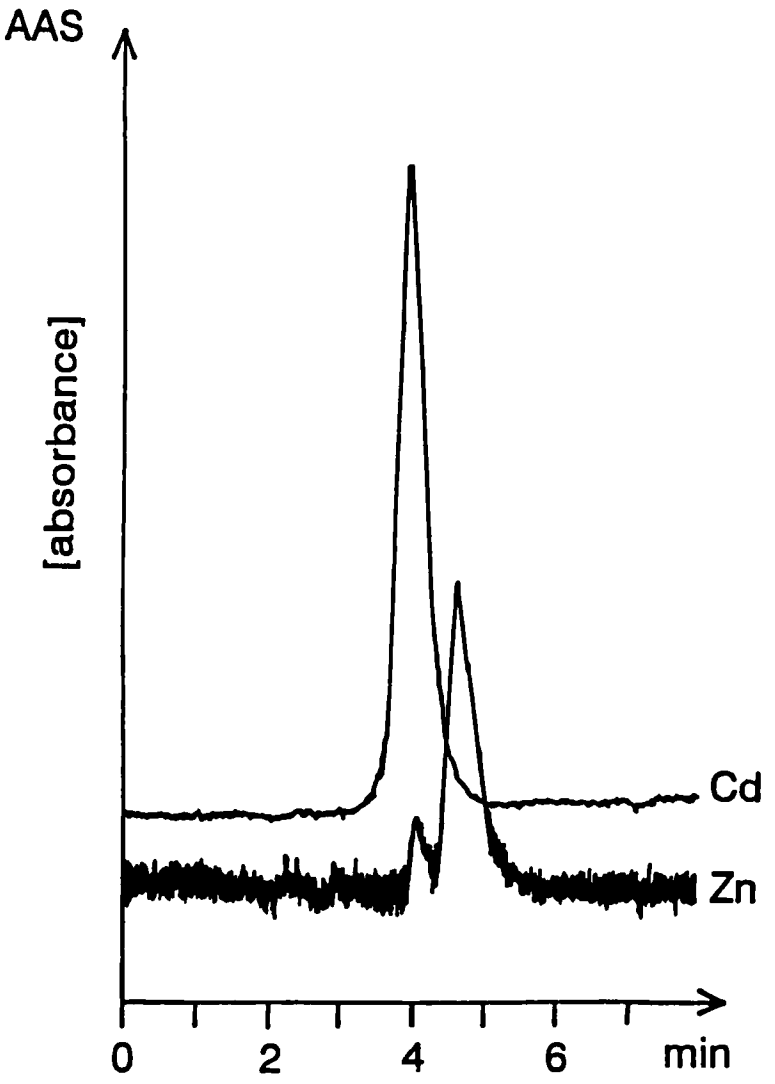

Figure 4 Separation of Zn- and Cd-Metallothioneins
The Cd peak corresponds to 470 ng Cd, the Zn peaks to 7 and 71 ng Zn.

detection limit (compared to conventional nebulization) by at least one order of magnitude. For low flow-rates even higher enhancements are possible due to the optimization of nebulization nozzle diameter. Element-specific detection of metals is possible for low- and high-molecular weight species at separation conditions, that would be problematic for conventional AAS detectors.

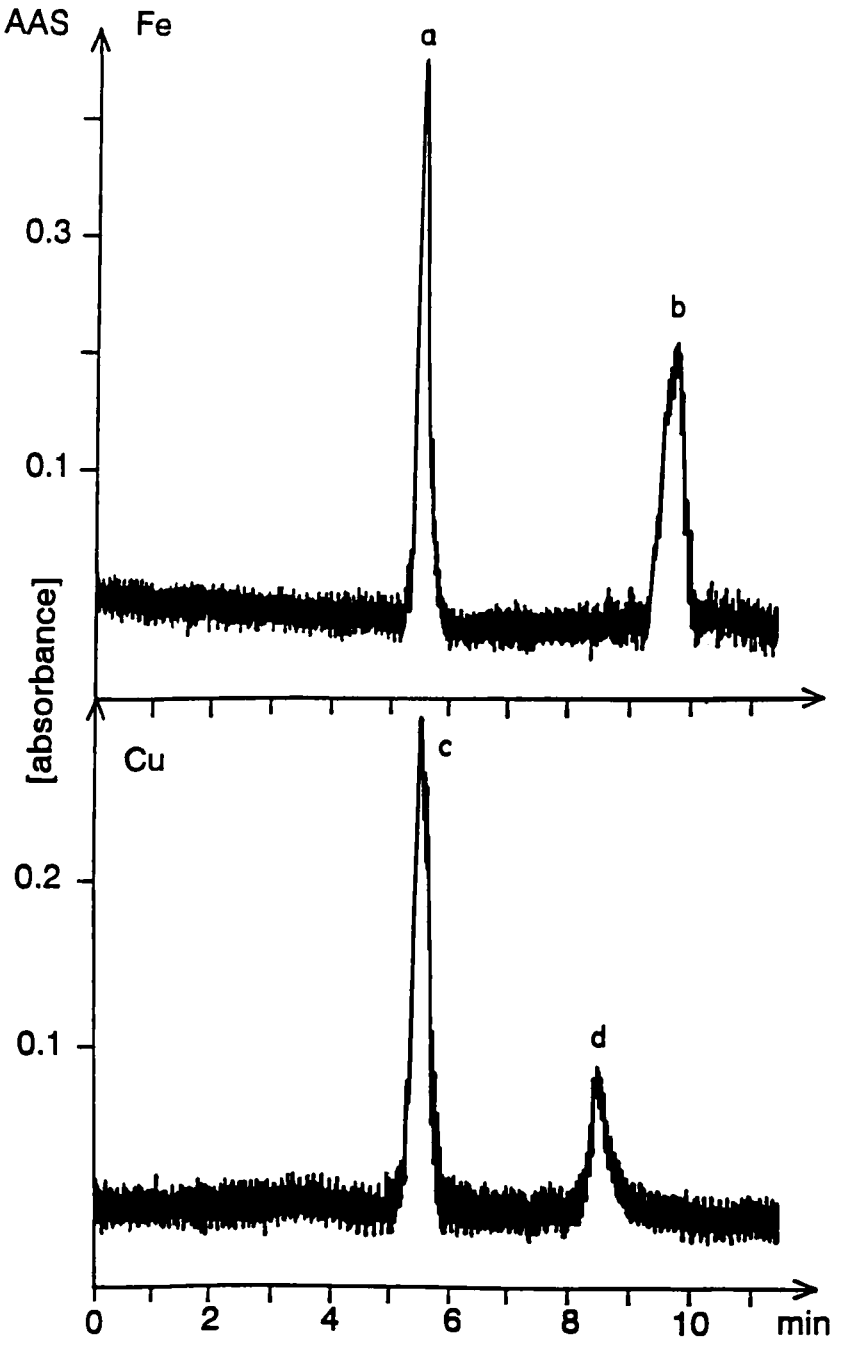

Figure 5 Separation of low molecular weight species of Cu and Fe
a: Fe-tartrate (=160 ng Fe), b: Fe-citrate (=150 ng Fe), c: Cu-tartrate (=190 ng Cu), d: Cu-citrate (=47 ng Cu)
Injected samples contained a 10-fold excess of the ligands.

Acknowledgement

This work was supported by the "Ministerium für Forschung und Technologie der Bundesrepublik Deutschland" and by the "Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen".

References

1. L. Ebdon, S. Hill and R. W. Ward, *Analyst*, **112,** 1–16 (1987).
2. S. Ahmad, R. C. Murthy and S. V. Chandra, *Analyst*, **115,** 287–289 (1990).
3. S. B. Roychowdhury and J. A. Koropchak, *Anal. Chem.*, **62,** 484–489 (1990).
4. O. Nygren, C. A. Nilsson and A. Gustavsson, *Spectrochim. Acta*, **44B,** 589–595 (1989).
5. D. Beauchemin, M. E. Bednas, D. D. Berman, J. W. McLaren, K. W. M. Siu and R. E. Sturgeon, *Anal. Chem.*, **60,** 2209–2212 (1988).
6. H. Suyani, J. Creed, T. Davidson and J. Caruso, *J. Chromatogr. Sci.*, **27,** 139–143 (1989).
7. H. M. Crews, J. R. Dean, L. Ebdon and R. C. Massey, *Analyst*, **114,** 895–899 (1989).
8. H. vanBeek and A. J. Baars, *At. Spectrosc.*, **2,** 70–74 (1990).
9. G. Weber, *J. Trace Elem. Electrolytes Health Dis.*, **2,** 61–65 (1988).
10. G. Weber, *Fresenius J. Anal. Chem.*, **340,** 161–165 (1991).
11. A. Gustavsson and O. Nygren, *Spectrochim. Acta*, **42B,** 883–888 (1987).
12. L. Ebdon, S. Hill and P. Jones, *Analyst*, **112,** 437–440 (1987).
13. S. J. Haswell, R. A. Stockton, K. C. C. Bancroft, P. O'Neill, A. Rahman and K. J. Irgolic, *J. Autom. Chemistry*, **9,** 6–14 (1987).
14. O. Nygren, C. A. Nilsson and W. Frech, *Anal. Chem.*, **60,** 2204–2208 (1988).
15. H. Berndt, *Fresenius J. Anal. Chem.*, **331,** 321–323 (1988).
16. G. Weber and H. Berndt, *Chromatographia*, **29,** 254–258 (1990).
17. D. E. Schmidt, R. W. Giese, D. Conron and B. L. Karger, *Anal. Chem.*, **52,** 177–182 (1980).
18. R. B. Martin, *J. Inorg. Biochem.*, **28,** 181–187 (1986).
19. R. M. Marce, M. Calull, R. M. Manchobas, F. Borrull and F. X. Rius, *Chromatographia*, **29,** 54–58 (1990).
20. S. H. Ashoor and M. J. Knox, *J. Chromatogr.*, **299,** 288–292 (1984).