ELSEVIER

Journal of Chromatography A, 835 (1999) 105–112

JOURNAL OF CHROMATOGRAPHY A

# Packed capillary high-temperature liquid chromatography coupled to inductively coupled plasma mass spectrometry

Roger Trones*, Anders Tangen, Walter Lund, Tyge Greibrokk

*Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, 0315 Oslo, Norway*

Received 15 September 1998; received in revised form 7 December 1998; accepted 7 December 1998

**Abstract**

The applicability of inductively coupled plasma mass spectrometry (ICP-MS) as a detector for high-temperature liquid chromatography with packed capillary columns has been studied. A laboratory-made micro concentric nebulizer was utilized as a interface for the coupling of the two instruments. The repeatability of peak height and area at the investigated column temperatures (75–150°C) was good, with relative standard deviation less than 1.5% ($n=3$). The limit of detection (LOD) for tetraethyl- and tetramethyllead was found to be 5 pg as Pb ($S/N=3$), and for tetraethyltin 9 pg as Sn ($S/N=3$). The effect of temperature ramping on the baseline was investigated, and no limitations regarding range or rate of temperature increase upon the ICP-MS response were found for the mobile phases used. 

*Keywords:* Interfaces, LC–MS; Liquid chromatography–mass spectrometry; High-temperature liquid chromatography; Organolead compounds; Organotin compounds

## 1. Introduction

High-temperature liquid chromatography (HTLC) with the use of relatively long packed capillary columns and non-aqueous mobile phases, is an analytical separation technique originally developed for the purpose of analyzing polymers, polymer additives, resins in crude oil and other high-molecular-mass compounds [1]. Combining this technique's high resolving power with inductively coupled plasma mass spectrometry (ICP-MS) element specific detection, is a promising combination for the determination of organometallic compounds at low concentrations.

Gas chromatography (GC) [2–5] and supercritical fluid chromatography (SFC) [6,7] have been coupled with ICP-MS detection for the above mentioned purpose by using laboratory-made interfaces. However, GC has major limitations regarding non-volatile compounds, while SFC's drawback is the rather expensive and complicated instrumental design necessary for this technique.

High-performance liquid chromatography (HPLC) coupled to ICP-MS has been used as a powerful tool for the separation and speciation of organotin [8] and metalloporphyrins [9] compounds. However, many HPLC applications for organometallic compounds demand the use of gradient elution, and a high flow-rate combined with gradient elution has been reported to result in unstable plasma conditions [10]. Biggs et al. therefore investigated ''thermal gradient liquid chromatography'' coupled with ICP atomic emission spectrometry (AES) [11], and concluded that a temperature gradient had a potential for

*Corresponding author.

0021-9673/99/$ – see front matter 
PII: S0021-9673(98)01054-1

replacing a mobile phase gradient, which was observed to have a negative influence on plasma stability. Their experiments were conducted with 2 mm or 4.6 mm I.D. columns coupled to a standard spray chamber as nebulizer.

A few commercial nebulizers are available for the coupling of conventional HPLC to ICP-MS, but they are mainly constructed for dealing with the relatively high mobile phase flow from conventional HPLC columns. Unfortunately, their rather large internal volume gives a significant contribution to band broadening [12], which makes them unsuited for capillary columns. Packed capillaries have dimensions more favorable regarding temperature programming, because of their faster response to temperature changes than conventional columns. Packed capillaries reduces temperature gradients within the column, which is known to contribute to band broadening. The micro flow-rates of packed capillary columns may be favorable because of the lowered consumption of mobile phase, improved signal to noise ratios and improved compatibility with some detectors [13].

A micro concentric nebulizer has a potential for diminishing band broadening effects, but little or no documentation exists for the use of commercially available micro concentric nebulizers at the low flow-rates ($<10$ μl/min) that are needed for packed capillary columns. We have therefore used a laboratory-made micro concentric nebulizer designed for micro-flow-rates [14], for the coupling of μ-HTLC to ICP-MS. The ICP-MS response to elevated isothermal column temperatures and temperature programming has been investigated.

The μ-HTLC–ICP-MS instrumental coupling has been studied with organotin and organolead reference compounds.

## 2. Experimental

### 2.1. HTLC–ICP-MS

The experimental set-up consisted of a Merck LaChrom L-7100 pump (Merck, Darmstadt, Germany), a Valco (Houston, TX, USA) Model CI4W manually operated injection valve with a 60-nl internal loop volume, a Hewlett-Packard 5700A gas chromatograph as column oven (Hewlett-Packard, Cupertino, CA, USA), and a Perkin-Elmer Sciex Elan 5000 ICP-MS (Norwalk, CT, USA). The ICP-MS instrument operating conditions and data acquisition parameters are given in Table 1. A mixture of air (18 ml/min) and argon (800 ml/min) was used as

Table 1
ICP-MS operating parameters

| | |
|---|---|
| Plasma gas flow-rate: | 15 l/min |
| Auxiliary: | 1.0 l/min |
| Nebulizer: | 0.8 l/min Ar and 18 ml/min air |
| Forward power: | 1000 W |
| Sampler (1.15 mm I.D.) | Pt |
| Skimmer (0.89 mm I.D.) | Pt |
| Injector liner (2.0 mm I.D.) | Alumina |
| Resolution: | Normal |
| Transfer frequency: | Measurement |
| Baseline time (ms): | 0 |
| Polarity: | + |
| *Data acquisition parameters (HTLC–ICP-MS)* | |
| Replicate time (ms): | 100 |
| Dwell time (ms): | 1000 |
| Scanning mode: | Peak hop |
| Sweeps/reading: | 1 |
| Readings/replicate: | 1 |
| Number of replicates: | 25 000 |
| Points/spectral peak: | 1 |

nebulizer gas. The two gases were mixed in a 1/4 in. Swagelok tee (1 in.=2.54 cm). The flow-rate of air was controlled with a Bronkhorst HI-TEC (Ruurlo, The Netherlands) mass-flow meter. The capillary column was connected to the injector by a fused-silica capillary (approximately 25 cm×30 μm I.D.× 375 μm O.D.). The fused-silica capillary transfer line (65 cm×15 μm I.D.×375 μm O.D.) from the column, to the laboratory-made micro concentric nebulizer also acted as a restrictor to prevent the mobile phase from boiling [1]. A schematic drawing of the nebulizer coupled as a interface between the chromatographic system and the detector is shown in Fig. 1.

### 2.2. Columns and mobile phases

The fused-silica capillary columns were packed with 5 μm porous Hypersil ODS (Hypersil, Shandon, UK) or Kromasil $C_{18}$ particles (Shandon Southern Products, Cheshire, UK), the I.D. was 0.32 mm, and they were 43.5 cm or 23 cm long. They were packed according to the procedure described in Ref. [14]. Mobile phases where either pure acetonitrile, or a mixture of 85% methanol, 4% acetic acid, 1% pyridine and 10% water.

### 2.3. Materials

The fused-silica capillaries were purchased from Composite Metal Services (UK), unions and ferrules from Valco (Schenkon, Switzerland). Carbon dioxide (99.998%), oxygen (99.7%) and argon (99.999%) were purchased from AGA (Oslo, Norway). HPLC grade acetonitrile, water and methanol came from Rathburn (Watherburn, UK). Acetic acid and pyridine (both analytical-reagent grade) came from Merck. Tetramethyllead (TML) and tetraethyllead (TEL) were obtained from Associated Octel (Milton Keynes, UK). Tetraethyltin (purity not available) came from Alfa-Johnson Matthey (Germany), tributyltin chloride (97%) from Fluka (Switzerland) and triphenyltin chloride (95%) from Strem (Germany).

## 3. Results and discussion

### 3.1. Elevated column temperature effect upon the ICP-MS signal response

Imposing elevated temperature on the packed capillary column, and thereby heating of the mobile phase before the nebulization process, was assumed to have a positive effect on the production of ions in the plasma, because less energy would be consumed from the plasma for evaporation of the mobile phase. However, this was not the case for the experiment performed with tetraethyl tin in the temperature interval 75–150°C using acetonitrile as mobile phase.

Fig. 2 is a reconstructed chromatogram of four

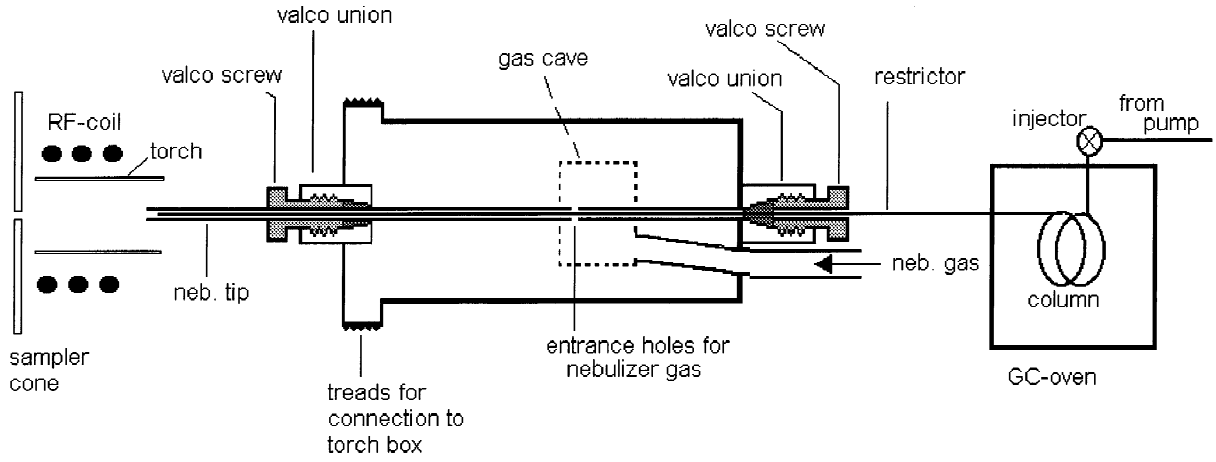


Fig. 1. Not to scale drawing of the HTLC–ICP-MS instrumental set up, showing enlarged details of the laboratory-made micro-nebulizer.

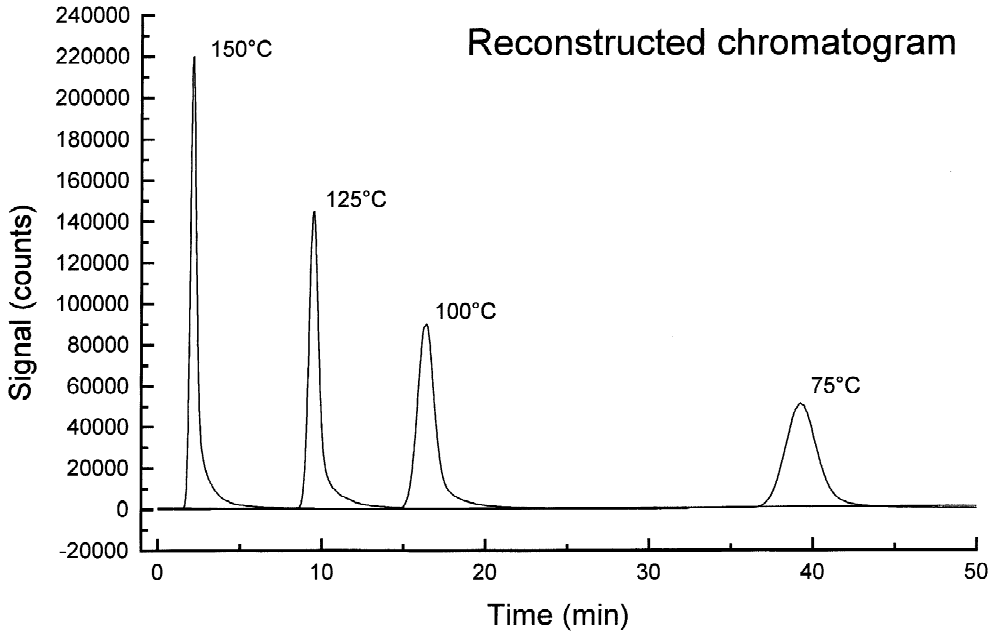


Fig. 2. Reconstructed chromatogram of four different runs using 60 nl 120 ppb tetraethyltin (Sn, mu=120) at different temperatures. The peak at 150°C is actually overlapping with the peak at 125°C. Column: 5 μm Hypersil ODS in 43.5 cm×0.32 mm I.D. Mobile phase: acetonitrile, flow=5 μl/min.

separate isothermal runs using tetraethyltin as a test substance at 75°C, 100°C, 125°C and 150°C. The three latest peaks are correctly placed according to their original retention times, but the peak at 150°C is actually overlapping with the peak at 125°C (its original retention time is approximately 8 min and 30 s). The peak height obtained at 150°C is approximately five-times as high as the signal obtained at 75°C. However, this observation can be attributed to the reduction of retention time at the higher temperature which sharpened the peaks.

To avoid column effects, we replaced the packed capillary column with a relative long open capillary tubing, and utilized temperatures from ambient to as high as 200°C using tetraethyltin. The higher temperatures did not result in a significant gain in signal counts compared to ambient temperature. From a practical point of view, we did not evaluate higher temperature than 200°C. Using temperatures above 200°C on a reversed-phase packing would probably irreversibly damage it. Also the danger of decomposition of the organometal at higher temperatures imposes limitations upon the temperature range to be used [15]. A conclusion based on practical limitations indicated that elevated column temperature did not affect the signal response.

### *3.2. Chromatographic performance at elevated temperature*

Increasing the temperatures may improve the peak symmetry of strongly retained peaks [1], but the opposite is seen in Fig. 2, due to negative contribution from extra-column dead volumes. The time the solute spend in the extra-column volumes remains fairly constant at all temperatures, whereas the time spent traveling through the column decreases dramatically at the higher temperatures. Consequently it is reasonable to assume that the contribution to band broadening from the extra-column volume is more critical at the higher temperatures, due to the low retention factor observed at these temperatures.

The temperature programs performed with the use of TML and TEL in Fig. 3a and b did not show an extra gain in signal height or area. The energy needed for evaporating acetonitrile at ambient tem-

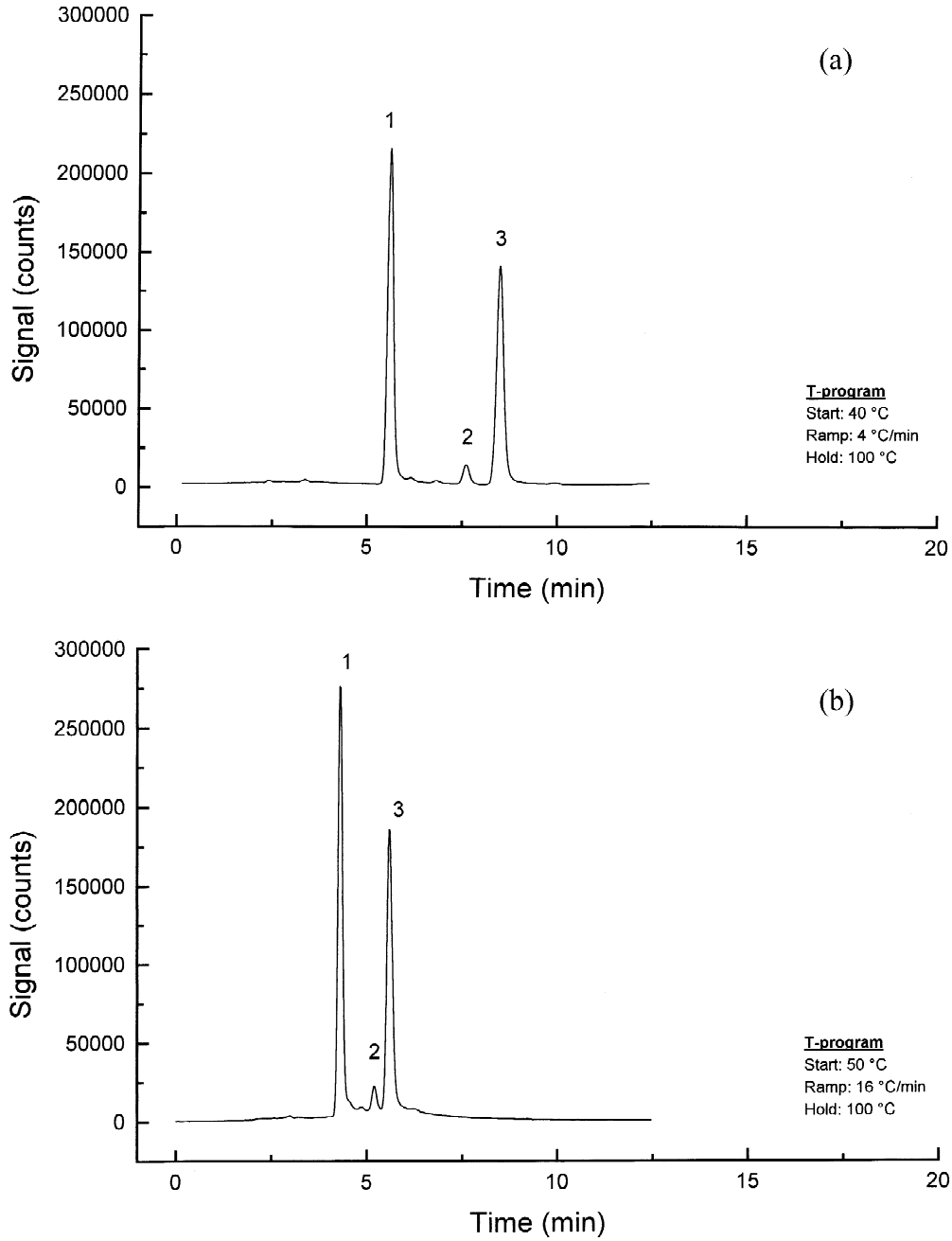


Fig. 3. (a) Temperature programmed separation of (1) tetramethyl lead (TML), (2) unknown and (3) tetraethyllead (TEL). (Pb, mu=208). Start: 40°C, then 4°C/min up to 100°C. (b) Temperature programmed separation of (1) tetramethyllead (TML), (2) unknown and (3) tetraethyllead (TEL). (Pb, mu=208). Start: 50°C, then 16°C/min up to 100°C. Injected 60 nl 10 ppm of each known compound. Column: 5 μm Kromasil RP-18 in 23 cm×0.32 mm I.D. Mobile phase: acetonitrile, flow=10 μl/min.

perature is 32.94 kJ/mol ($\Delta H_{vap}$), while the energy needed at 81.6°C (boiling point for acetonitrile) is 29.75 kJ/mol ($\Delta H_{vap}$) [16]. The relative small difference in $\Delta H_{vap}$ is probably too small to contribute to a higher production of ions in the plasma compared to the energy produced of the plasma. Fig. 4 shows three subsequent injections at 150°C ($t_r$ is close to $t_0$), with good peak height repeatability (RSD= 1.3%). Area repeatability at the temperatures utilized (75–150°C) ranged from 0.9 to 1.4% (RSD, $n$=3).

Efficiency measured as plate numbers was not impressive, but these measurements were clearly influenced from the extra column volumes introduced by the extra long fused-silica tubing necessary for the nebulizer coupling. Efficiency measurements performed by using UV on-column detection in our earlier work has shown far better values [17], indicating that the columns are adequately packed.

### *3.3. Response to temperature programming*

A temperature increase performed during a run decreases the viscosity which consequently gives a temporarily increasing flow-rate until the end temperature in the program is reached. This process can be observed by monitoring the pressure display on the pump. The momentary expanding mobile phase induced by the temperature ramp gives a short and sudden increased pressure, before the pressure decreases as a function of the reduced viscosity.

Our previous work with HTLC using packed capillary columns and UV detection showed that elevated column temperature had an improving effect upon asymmetric peak shapes of late eluting compounds. Besides, the analysis could be performed in one-fifth of the time by using an adequate temperature program, compared to an isothermal run at 50°C [1]. Unfortunately, UV detection showed a negative effect of temperature programming, observed as a rising baseline. Introducing a steep temperature gradient in a non-aqueous μ-LC system, that contained a mobile phase with a UV-cutoff close to the maximum absorption wavelength of the compounds to be analyzed, gave a steeply rising baseline. This phenomena is caused by a large refractive index change of the mobile phase molecules induced by the temperature gradient. However, this problem can be solved by using dual-wavelength absorbance detec-

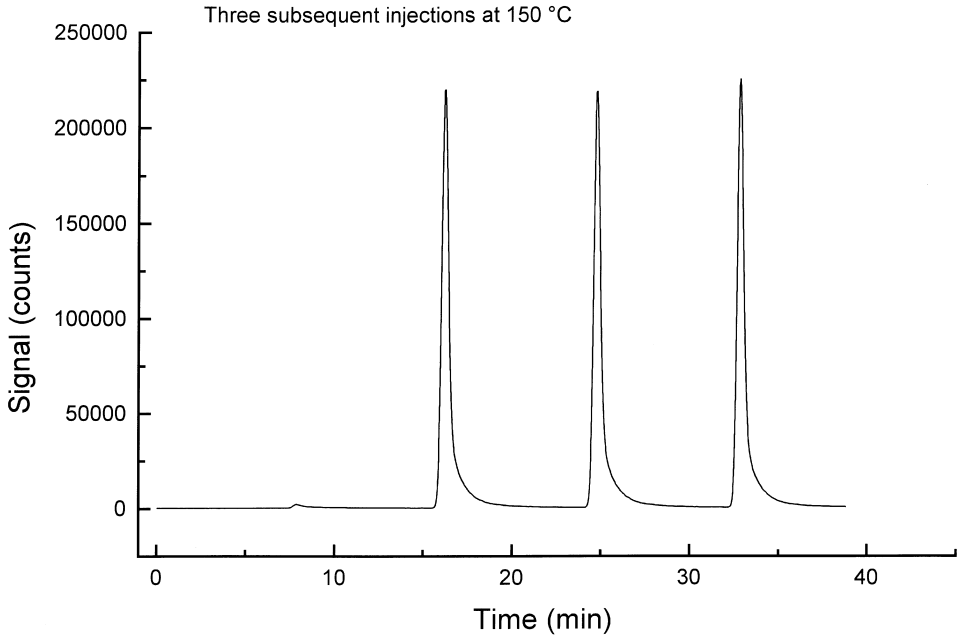


Fig. 4. Three subsequent injections of 60 nl 120 ppb tetraethyltin (Sn, mu=120) at 150°C. Column: 5 μm Hypersil ODS in 43.5 cm×0.32 mm I.D. Mobile phase: acetonitrile, flow=5 μl/min.

tion [18], or by using a wavelength further away from the mobile phase UV-cutoff.

When HTLC was coupled with evaporative light scattering detection (ELSD) [19], temperature ramps steeper than 6°C/min in a wide temperature interval resulted in a rising baseline, believed to be due to momentary expansion of the mobile phase. Analysis could be performed by the use of steeper temperature ramps than 6°C/min, but then the range from start to the end temperature had to be minimized [19].

Fig. 3a and b show two different temperature programmed separations of TML and TEL performed by HTLC–ICP-MS, (a) with a temperature ramp of 4°C/min, while b is performed with 16°C/min. Steeper ramps did not reveal any negative influence upon the baseline. Fig. 5 shows a temperature programmed separation of three organotin compounds by the use of a 32°C/min temperature ramp. Peak number 3 (tributyltin chloride) shows strong tailing, probably due to methanolysis or hydrolysis on the column. The same analysis performed with a 16°C/min temperature ramp did not show any difference regarding tailing, neither did a run at ambient temperature with the same mobile phase and column. These organotin separations were performed with a 10% aqueous mobile phase (+85% methanol, 4% acetic acid, 1% pyridine). Additionally, the danger of degradation of the silica based reversed-phase packings at elevated temperature by the use of aqueous mobile phases may restrict the use of such systems.

With the present mobile and stationary phases, ICP-MS did not show any dependency or sensitivity towards steep temperature programs, in contrast to the UV detector and ELSD.

### *3.4. Limit of detection*

The HTLC–ICP-MS instrument coupling was capable of detecting tetraethyltin (Sn, mu (mass units)=120) at a concentration level of approximately 150 ppb, using an injection volume of 60 nl, which gives a total mass of 9 pg detected (referring to the metal). Others have detected 51 pg with an injection volume of 100 μl using conventional HPLC coupled to ICP-MS [8]. TML was detected (as Pb,

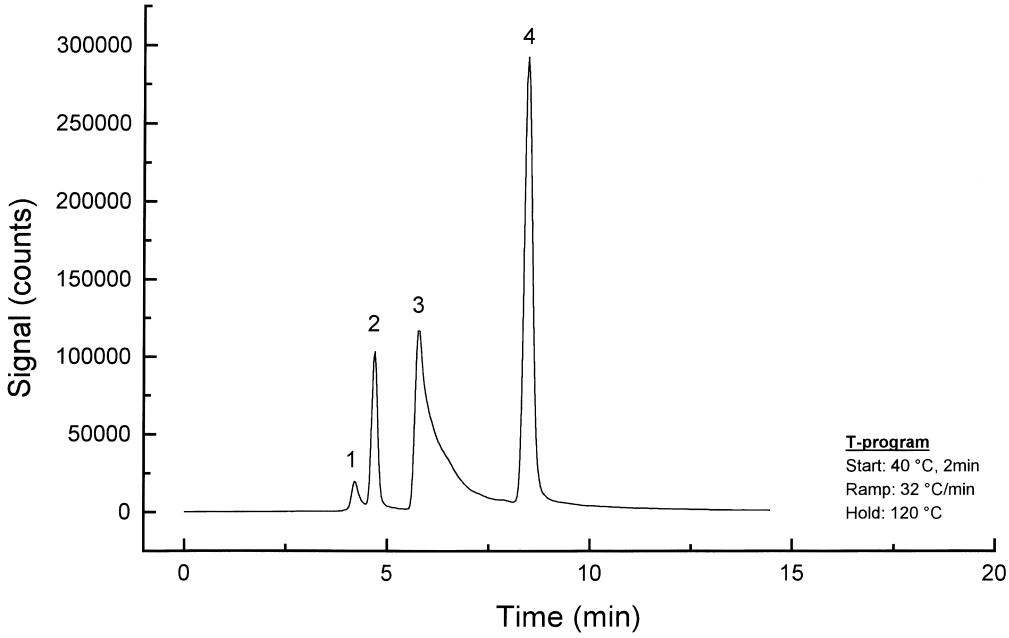


Fig. 5. Temperature programmed separation of (1) unknown, (2) triphenyltin chloride (37 ppm), (3) tributyltin chloride (29 ppm), (4) tetraethyltin (40 ppm). Start: 40°C (2 min), then 32°C/min up to 120°C. Column: 5 μm Kromasil RP-18 in 23 cm×0.32 mm I.D. Mobile phase: 85% methanol, 4% acetic acid, 1% pyridine and 10% water, flow=5 μl/min, injection volume=60 nl.

mu=208) at a concentration level of 100 ppb, equal to a total mass of 5 pg ($S/N=3$). With large volume injection and on-column focusing [20], much lower concentrations are available with the packed capillary columns.

## Acknowledgements

R.T. was supported by the Norwegian Research Council (Science and Technology). Einar Brevik and Norunn Foelsvik at NIVA supplied us with organotin compounds. The authors want to thank Rune Bjornstol and Bjorn Dalbakk at the engineering workshop at the Department of Chemistry, for valuable technical assistance.

## References

[1] R. Trones, A. Iveland, T. Greibrokk, J. Microcol. Sep. 7 (1995) 505.
[2] T. De Smaele, L. Moens, R. Dams, P. Sandra, LC·GC Int., March (1996).
[3] W.G. Pretorius, L. Ebdon, S.J. Rowland, J. Chromatogr. 646 (1993) 369.
[4] B. Lalère, J. Szpunar, H. Budzinski, P. Garrigues, O.F.X. Donard, Analyst 120 (1995) 2665.
[5] E.M. Krupp, J. Feldmann, A.V. Hirner, W.R. Cullen, Poster at the 1997 European Winter Conference on Plasma Spectrochemistry, Ghent, 1997.
[6] E. Blake, M.W. Raynor, D. Cornell, J. Chromatogr. A 683 (1994) 223.
[7] E. Blake, M.W. Raynor, D. Cornell, J. High Resolut. Chromatogr. 18 (1995) 33.
[8] C.F. Harrington, G.K. Eigendorf, W.R. Cullen, Appl. Organomet. Chem. 10 (1996) 339.
[9] L. Ebdon, E.H. Evans, W.G. Pretorius, S.J. Rowland, J. Anal. Atom. Spectrom. 9 (1994) 939.
[10] W.R. Biggs, J.C. Fetzer, Anal. Chem. 61 (1989) 236.
[11] W.R. Biggs, J.C. Fetzer, R.J. Brown, Anal. Chem. 59 (1987) 2798.
[12] C. Rivas, L. Ebdon, S.J. Hill, J. Anal. Atom. Spectrom. 11 (1996) 1147.
[13] D. Ishii, Introduction to Microscale High-Performance Liquid Chromatography, VHC, New York, 1988.
[14] A. Tangen, R. Trones, T. Greibrokk, W. Lund, J. Anal. Atom. Spectrom. 12 (1997) 667.
[15] P.S.C. Craig, Organometallic Compounds in the Environment – Principles and Reactions, Longman, New York, 1986.
[16] D.R. Lide, CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 72nd ed., 1991–1992.
[17] R. Trones, P. Molander, T. Greibrokk, manuscript in preparation.
[18] C.N. Renn, R.E. Synovec, Anal. Chem. 63 (1991) 568.
[19] R. Trones, T. Andersen, I. Hunnes, T. Greibrokk, J. Chromatogr. A 814 (1998) 55.
[20] P. Molander, T.E. Gundersen, C. Haas, T. Greibrokk, R. Blomhoff, E. Lundanes, J. Chromatogr A, in press.