ELSEVIER

Journal of Chromatography A, 746 (1996) 239–245

JOURNAL OF CHROMATOGRAPHY A

Liquid chromatographic determination of gallium and indium with fluorimetric detection

M.D. Prat*, R. Compañó, M. Granados, E. Miralles

Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, E-08028 Barcelona, Spain

Received 1 March 1996; accepted 9 April 1996

Abstract

A method for the determination of gallium and indium, using ion-exchange chromatography with fluorimetric detection is reported. Quinolin-8-ol-5-sulfonic acid (HQS) in an aqueous micellar medium of CTAB was used as post-column derivatizing reagent. Optimization of the separation and detection conditions were studied. Linear calibration graphs were obtained in the range 5–100 $\mu g\ l^{-1}$ for both metal ions. The detection limits, for 100 μl of sample injected, are 0.15 and 0.10 ng for Ga and In respectively. The proposed method was applied to the analysis of atmospheric aerosol samples.

Keywords: Derivatization, LC; Post-column reactors; Gallium; Indium; Quinolin-8-ol-5-sulfonic acid

1. Introduction

Gallium and indium are naturally occurring elements found at trace levels in the earth's crust. These elements are obtained industrially from bauxite and as by-products in the processing of zinc blende. Gallium and indium are used primarily in medicine for organ scanning or as antitumoral agents, and in the semiconductor industry. Due to the latter application, the world production is increasing and levels of gallium and indium in the environment are beginning to rise, mainly around industrial areas [1,2]. Although the number of studies on the risk assessment of gallium and indium compounds are limited, there is an increasing concern about their potential toxicity and impact on the environment, and some regulations have been introduced. For instance a TLV–TWA (threshold limit value–time weighted average) level for indium of 0.1 mg In/m^3 of air has been established by the American Conference of Governmental Industrial Hygienists [3].

In order to monitor these elements simple yet sensitive analytical methods are required. Several methods involving graphite furnace atomic absorption spectroscopy [4], neutron activation analysis [5] or inductively coupled plasma mass spectrometry [6] have been described for Ga and In determination, but they require expensive instrumentation which is not available to most laboratories. Sensitive determination of these elements can also be carried out by using fluorimetric methods [7,8], but extraction steps are required to separate these metal ions from each other and from others that interfere in the analysis. In this sense, the combination of fluorimetric detection with a separation technique, such as liquid chromatography, seems to be promising in order to avoid problems of selectivity.

Quinolin-8-ol (HQ) and related compounds, which are well known fluorogenic reagents for metal ions, have also been used for derivatization in liquid

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00343-3

chromatography. Depending on the separation mode, reversed-phase liquid chromatography of neutral chelates, or ion chromatography, the fluorescent complexes are formed by pre- or post-column reaction. Although some separations refer to gallium and indium [9–12], none of the applications reported takes place in the field of environmental analysis, which requires very sensitive detection.

This paper describes the use of ion-exchange chromatography followed by post-column derivatization using quinolin-8-ol-5-sulfonic acid (HQS) and fluorimetric detection to determine Ga and In at the low ppb level. The method takes advantage of the ability of cationic micellar media to enhance the fluorescence of metal–HQS complexes, which leads to an improvement in sensitivity. The method has been applied to the analysis of aerosol samples from an urban area.

2. Experimental

2.1. Instrumentation

The HPLC equipment consisted of a Gynkotek Model 480 double piston pump and a Gynkotek MSV 6 injection valve with a variable sample loop. Whatman Partisil 10 SCX (10 μm particle size, 25 cm×4.6 mm I.D.) and Hamilton PRP X-200 (10 μm particle size, 25 cm×4.1 mm I.D.) analytical columns with their respective guard columns were used. The mobile phase was run through the column for at least 2 h to equilibrate it before a chromatographic run.

Post-column reaction was achieved using a Gilson Minipuls peristaltic pump. The derivatization reagent merged with the chromatographic effluent in a T-mixer piece before its introduction into the fluorimetric detector, an Aminco-Bowman AB2 spectrofluorimeter equipped with a 25-μl flow-cell (Hellma 176.752).

A Radiometer pHM64 pH meter with an Orion combined electrode was used for pH measurements.

Aerosol samples were collected with a high volume air sampler Sierra Misco Inc. (Model 650) fitted with an indicating flow meter. The flow-rate used was 1000 m^3/24 h.

2.2. Reagents

AA-Standard solutions of gallium and indium nitrates (1 g l^{-1} as metal ion) (Alfa) in 5% nitric acid were used. Solutions of 50 mg l^{-1} were prepared by diluting the stock solution with 10^{-2} *M* sulphuric acid. Working standards were prepared daily by appropriate dilution of the intermediate solutions.

Quinolin-8-ol-5-sulfonic acid (Aldrich) was purified as the monohydrate by recrystallization from aqueous solutions.

Cetyltrimethylamonium bromide (CTAB) (Merck) was used as received.

Oxalic (Fluka), 90% lactic (Merck), dl-malic (Carlo Erba), citric (Carlo Erba) and glycolic (Fluka) acids, disodium L(+)-tartrate (Fluka), sodium 2-hydroxybutyrate (Fluka) and ethylenediamine (Carlo Erba) analytical reagent grade were used.

2.2.1. Mobile phase

The final choice for the mobile phase consisted of an aqueous solution containing 0.1 *M* lactic acid and $1.2 \cdot 10^{-3}$ *M* ethylenediamine, adjusted to pH 3.4–3.5 with sodium hydroxide. The solution was filtered through a 0.2-μm nylon membrane filter (m.s.i.) and degassed for 10 min in an ultrasonic bath.

2.2.2. Post-column reagent solution (PCR)

The PCR consisted of an aqueous solution of 10^{-3} *M* HQS and $1.2 \cdot 10^{-3}$ *M* CTAB . Both mobile phase and PCR were freshly prepared daily.

Doubly-deionized water (Culligan Ultrapure GS) of 18.3 MΩ.cm resistivity was used to prepare solutions.

All glassware used for experiments was previously soaked in 10% nitric acid for 24 h and rinsed with doubly-deionized water.

2.3. Procedure

2.3.1. Chromatographic analysis

Prepare acidic sample solutions containing Ga and In with concentrations up to 100 μg l^{-1}. After filtration through 0.45-μm nylon membranes, inject 100 μl of the sample in the chromatographic system equipped with a Partisil 10 SCX, Whatman (10 μm, 25 cm×4.6 mm I.D.) column and its respective guard column. Set both mobile phase and post-column

reagent flow-rates at 1.5 ml min^{-1}. Measure the fluorescence intensity at 529 nm using an excitation wavelength of 389 nm. Calibrate by injecting standard solutions of Ga and In in 10^{-2} *M* sulphuric acid.

2.3.2. Aerosol samples

Collect aerosol samples using cellulose filters. Digest the filter with a mixture of concentrated nitric (3 ml), sulphuric (4 ml) and hydrofluoric (3 ml) acids in a pressure digestion bomb, at 150°C for about 14 h. After digestion, evaporate to dryness in a teflon vessel. During the evaporation procedure add more sulphuric and hydrofluoric acids as well as perchloric acid if necessary. Dissolve the residue with 3 ml of $5 \cdot 10^{-3}$ *M* H_2SO_4, transfer into a 10-ml volumetric flask and dilute to 10 ml with water. Filter the solution through a 0.46-μm nylon membrane and inject 100 μl of sample into the chromatographic system. Run a blank through the entire procedure.

3. Results and discussion

3.1. Fluorescence studies

Although it has been reported that the presence of a cationic surfactant, such as cetyltrimethylammonium bromide (CTAB), can enhance the fluorescence intensity of the complexes of HQS [13,14], there are no data available on the influence of CTAB on the fluorescence of Ga– and In–HQS complexes. Therefore, in order to test the effect of the surfactant, the excitation and emission spectra of Ga(III)– and In(III)–HQS complexes were obtained with and without the presence of CTAB. Spectra obtained in the micellar medium showed an increase in relative fluorescence intensity, with an enhancement factor of 17 for Ga(III) and 12 for In(III). Moreover, a slight shift in the maxima wavelength of about 5 nm towards longer values was observed, which agrees with the behaviour described for other metal–HQS complexes [15]. The excitation and emission wavelengths maxima in this micellar medium are 384 and 525 nm for Ga(III), and 389 and 529 nm for In(III).

The fluorescent properties of HQS as a fluorogenic reagent were compared with those of 5,7-dichloro-2-methyl-quinolin-8-ol (DCMHQ). This reagent was selected for a comparative study because previously it was observed that DCMHQ forms a highly fluorescent complex with Ga(III) in different micellar media [16]. The results obtained showed that both Ga–HQS, and Ga–DCMQH exhibited a similar response, whereas fluorescence of In–HQS complex was about 8 times greater than that given by the DCMHQ complex, indicating HQS is more sensitive than DCMHQ for In. Moreover, whereas Ga(III)–DCMHQ complex is more fluorescent than that of In(III), in the case of HQS both complexes showed similar fluorescence intensity, which seems more convenient from the point of view of an environmental application.

3.2. Optimization of the reaction detection system

The on-line reaction involved in the post-column derivatization and the fluorimetric detection of Ga(III) and In(III) was investigated by using a flow injection system. In order to optimize experimental variables, a three-channel system was used, which facilitated the variation of HQS and CTAB concentrations as well as pH. HQS, CTAB and buffer solutions were mixed before injection of 100 μl of 100 $\mu g\,l^{-1}$ Ga(III) or In(III) solutions in the resulting derivatizing solution. The total flow-rate was 2.7 ml min^{-1} and the individual flow-channel ratios were 4:4:1 (surfactant:buffer:reagent) (Fig. 1). Concentration values given in the following refer to the global reagent stream.

The fluorescence intensity of Ga and In complexes did not vary noticeably between pH 3 and 6 (Fig. 2),

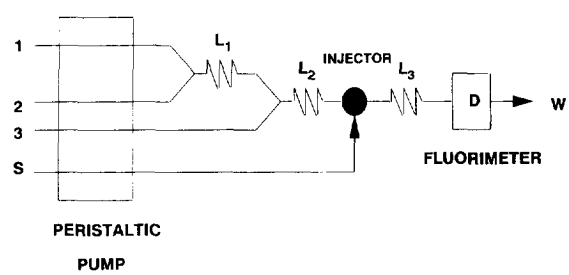

Fig. 1. Flow injection manifold used in the optimization of the derivatizing reaction. 1, buffer solution. 2, CTAB solution. 3, HQS solution. S, sample solution. L_1 and L_2, mixing coils. L_3, reaction coil. D, detector. W, waste.

but more symmetrical peaks and better signal-to-noise ratio were obtained in the lower pH range, close to 3. On the other hand, relative fluorescence intensity increased with CTAB concentration until it stabilized for $3 \cdot 10^{-3}$ *M* CTAB. The investigation of the effect of HQS concentration on peak height showed that the signal increased with increasing HQS concentration up to about 10^{-4} *M* and had no influence on the fluorescence signal over the range 10^{-4}–$6 \cdot 10^{-4}$ *M*.

The influence of the reaction time was studied by measuring the peak height obtained for several coil lengths. No significant differences were observed when reaction coils (0.5 mm I.D.) over the range 30–60 cm were used, but longer coils led to an increase in peak width, and therefore to a loss of sensitivity.

As a result of this study the composition of the derivatization solution was fixed to $5 \cdot 10^{-4}$ *M* HQS, $6 \cdot 10^{-3}$ *M* CTAB and pH 3.5, and a 30 cm×0.5 mm I.D. reaction coil was used in further experiments.

Since the detection system was based on the formation of fluorescent complexes of the analytes, it was important to study the behaviour of the fluorescent signals in the presence of some complexing agents such as carboxylic acids, commonly used in ion-exchange chromatography of polyvalent metal ions. In order to reproduce the chromatographic detection, a two-channel manifold was used: the sample was injected in the carrier stream containing the carboxylic acid, which simulated the mobile phase, and was mixed in a T-piece with the post-column reagent solution, which contained HQS, CTAB and buffer. Both channels were delivered at 1.5 ml min^{-1}. The concentration of the complexing agents ranged between 0.001 and 0.1 *M*. Lactic, glycolic, 2-hydroxybutyric, malic and tartaric acids were shown to have no effect on the fluorescence response, indicating that their Ga and In complexes were displaced by HQS, whereas citrate and oxalate strongly reduced the fluorescence response of the gallium complex, which suggests that their Ga complexes were stable or sufficiently inert so as not to experience substitution by HQS. The presence of ethylenediamonium in the eluent, which is used as a competing cation in ion-exchange chromatography, did not have any influence on the signal.

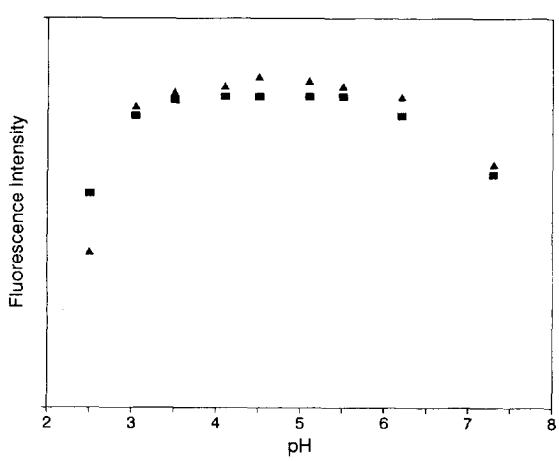

Fig. 2. Influence of pH on the fluorescence intensity of (■) Ga(III) complex, (▲) In(III) complex in the presence of CTAB. $c_{Ga}=100$ $\mu g\,l^{-1}$; $c_{In}=100$ $\mu g\,l^{-1}$; $c_{HQS}=5 \cdot 10^{-4}$ *M*; $c_{CTAB}=10^{-2}$ *M*.

3.3. Chromatographic studies

The chromatographic separation of Ga(III) and In(III) was attempted by ion-exchange chromatography using a strong cation exchanger. Since gallium is usually found in natural samples associated with aluminium, and this element provides signal in the conditions used for Ga and In detection, the preliminary tests for chromatographic separation also took Al behaviour into consideration.

When cations, such as trivalent ions, show high interaction with the sulphonate group, eluents usually contain a complexing agent and a competing cation [17]. The role of the complexing agent is to reduce the effective charge of the cation, and hence its affinity for the exchange sites, and to increase the selectivity factor between cations, which can be very poor when ion-exchange is the only retention mechanism in operation. Moreover, the competing cation leads to a reduction in retention times.

Mobile phases assayed in this study consisted of aqueous solutions of polyfunctional carboxylic acids, which act as complexing reagents of metal ions. The pH of the mobile phases was fixed between 3.4 and 3.5, which avoids the presence of hydroxocomplexes that would cause broadening of the Ga(III) and In(III) peaks, and on the other hand, the carboxylic acid acts itself as a buffer system. Moreover it is not necessary to modify the effluent pH before detection

as gallium and indium are well detected. Tests were made with a Partisil 10 SCX column, a cation-exchange silica-based column of high capacity (500 μeq/g).

This study showed that separation between Ga(III) and In(III) was not achieved with eluents containing 2-hydroxybutyric acid in the concentration range 0.05–0.1 *M*. With regard to glycolic acid, Ga and In peaks were resolved, but, as retention times were relatively high, broad peaks and hence poor sensitivity were obtained. On the other hand, mobile phases containing malic or tartaric acids provided good separation between both metal ions, but Ga was eluted with the dead volume and overlapping between In and Al peaks was observed. The best results were achieved with lactic acid as eluent. Moreover, aluminium was effectively separated from Ga and In with a mobile phase containing 0.1 *M* lactic acid.

The peak obtained for aluminium was very broad, regardless of the composition of the eluent. This fact has also been reported for similar systems to those studied here [18], and it has been proposed that strong aluminium–silica interaction and relatively slow complexation kinetics between the carboxylic acid and aluminium ions were probably responsible for this behaviour.

To study the displacement effect of a competing cation, ethylenediamonium (EDA) was added to the lactic acid eluent. As shown in Table 1 the retention of gallium was not affected but indium eluted earlier showing an increase in efficacy and peak height. Aluminium also elutes earlier, but there is good resolution between In(III) and Al(III) peaks if EDA concentration is kept below $3.6 \cdot 10^{-3}$ *M*. Optimum separation was obtained with an eluent containing 0.1 *M* lactic acid, $1.2 \cdot 10^{-3}$ *M* EDA at pH 3.4–3.5 adjusted with NaOH (Fig. 3). It appears that in these conditions the elution mechanism is a combination of the pushing effect of the ethylenediamonium cation and the complexing effect of lactic acid.

Some tests were carried out using a cation-exchange polymeric column (Hamilton PRP X-200, 25 cm×4.1 mm I.D., 10 μm particle size) of low capacity (35 μeq/g). The eluent used was 0.1 *M* lactic acid adjusted to pH 3.45 with NaOH. A change in the elution order was observed in the polymeric column, since aluminium eluted before indium. In this polymeric column In(III) was strongly retained. As a consequence, a broad peak was obtained, which led to a loss of sensitivity in the detection of indium. Addition of EDA to the mobile phase (Table 1) resulted in a slight decrease of In(III) retention time,

Table 1
Effect of EDA concentration on Ga(III), In(III) and Al(III) retention times

EDA (m*M*)	Retention time (min)		
1. Partisil 10 SCX			
	Ga	In	Al
–	3.1	5.6	9.4
0.6	3.0	5.1	8.2
1.2	3.0	4.7	7.9
1.8	3.1	4.5	7.7
3.6	3.1	4.1	7.0
7.5	3.2	3.5	6.5
2. Hamilton PRP X-200			
	Ga	Al	In
–	2.7	10.9	13.2
0.3	2.6	5.8	12.4
0.75	2.6	4.2	11.9
1.5	2.5	3.3	10.6

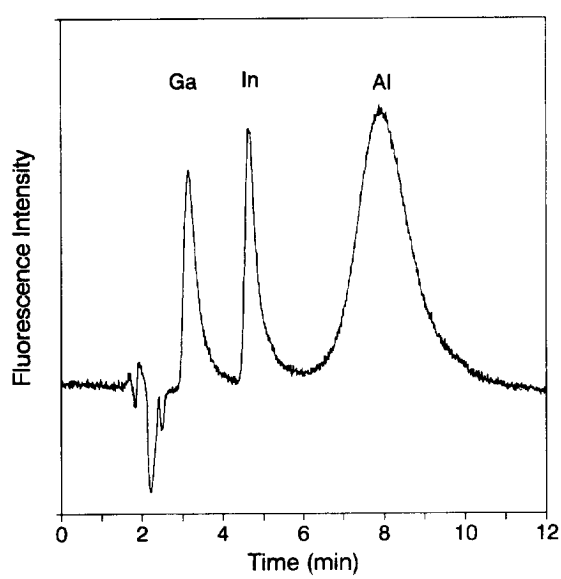

Fig. 3. Chromatogram of a standard mixture of 50 $\mu g\,l^{-1}$ of Ga, 50 μg^{-1} of In and 1 $mg\,l^{-1}$ of Al. Column: Whatman, Partisil 10 SCX, 25 cm×4.6 mm I.D. Mobile phase: 0.1 *M* lactic acid, $1.2 \cdot 10^{-3}$ *M* ethylenediamine, pH adjusted to 3.45 with sodium hydroxide; flow-rate 1.5 $ml\,min^{-1}$; injection volume 100 μl.

but in a clear diminution of that of aluminium which approaches to the gallium peak. This could be a major drawback in the analysis of samples with high content of aluminium, because its peak might overlap with that of gallium. As a result of these findings it was decided to perform further experiments with a Partisil 10 SCX silica-based cation-exchange column and an eluent containing 0.1 *M* lactic acid, $1.2 \cdot 10^{-3}$ *M* EDA at pH 3.4–3.5 adjusted with NaOH, and a flow-rate of 1.5 ml min^{-1}.

3.4. Characteristics of the analytical method

Calibration graphs obtained from both peak areas and peak heights were linear over the range 5–100 $\mu g\, l^{-1}$. Higher metal concentrations were not tested. Duplicate injections of standard solutions were made. Regression data for calibration lines ($n=7$) are:

$h_{Ga} = -0.1(0.7) + 1.11(0.01)\ C$ $\quad r=0.9996$
$A_{Ga} = -2.5(2.8) + 2.85(0.04)\ C$ $\quad r=0.9991$
$h_{In} = 0.1(1.0) + 1.51(0.01)\ C$ $\quad r=0.9995$
$A_{In} = -2.8(1.8) + 3.70(0.02)\ C$ $\quad r=0.9998$

where h is peak height, A peak area, C concentration in $\mu g\, l^{-1}$ and values in parenthesis refer to standard deviations. The detection limits, defined as the concentration of metal ions that produces an analytical signal three times the standard deviation of the background signal, are 1.5 $\mu g\, l^{-1}$ for gallium and 1.0 $\mu g\, l^{-1}$ for indium, which correspond to 0.15 ng and 0.10 ng respectively for 100 μl of injected sample.

Short-term precision was evaluated from a series of 10 injections of a standard solution containing 25 $\mu g\, l^{-1}$ of Ga and In. Relative standard deviations obtained in peak-height mode were in the range 2.7–3.0, and in the range 5.2–6.2 in the peak-area mode. Since the greatest precision was found when using peak-height mode, this was chosen to quantify.

Interference studies were carried out by analyzing solutions containing 50 $\mu g\, l^{-1}$ of Ga and In and 1 $mg\, l^{-1}$ of the foreign ion. Two kinds of potential interferences were taken into account: metal ions, such as Zn(II), Cd(II), Al(III), Be(II) and Mg(II), which form strong fluorescent complexes with HQS, and Fe(III), Ni(II), Cu(II), As(III) and As(V) ions, which have a quenching effect on the fluorescence of Ga and In complexes. It was found that Zn(II), Cd(II) and Al(III) appeared at higher retention times and were well separated from the Ga(III) and In(III) signals, whereas Mg(II) and Be(II) were not detected at the working conditions. Finally, the potential quenchers did not change the chromatograms of the analytes significantly.

3.5. Determination of gallium and indium in environmental samples

The proposed method was applied to the determination of Ga and In in atmospheric aerosols collected in an urban area. The accuracy of the proposed method was assessed by determining the recoveries of gallium and indium from spikes. The samples were spiked with amounts of Ga and In which correspond to levels reported for an industrial area (about 1.5 ng metal ion/m^3) [1,2].

Table 2
Determination of gallium and indium in atmospheric aerosol samples

Sample	Found[a]		Found[b]		Recovery (%)	
	Ga (ng)	In (ng)	Ga (ng)	In (ng)	Ga	In
S1	54	n.d.	311	242	103	97
S2	76	n.d.	328	240	101	96
S3	60	n.d.	295	218	94	87
S4	41	n.d.	308	236	107	94
S5	68	n.d.	324	223	102	89
Mean value					101	93
Standard deviation					5	4

[a] Natural sample.
[b] Sample spiked with 250 ng of Ga and 250 ng of In.
n.d.=not detected.

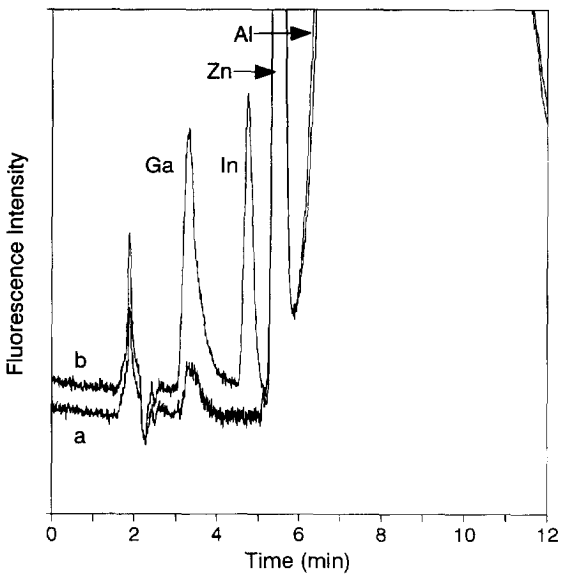

Fig. 4. Chromatograms of an atmospheric aerosol sample from urban area. (a) Non-spiked sample; (b) sample spiked with 250 ng of Ga and 250 ng of In. Conditions as in Fig. 3.

A volume of 650 m^3 of air was sucked in through a cellulose acetate filter (Millipore, 20×25 cm^2). After collection, each filter was divided in four equivalent parts. Two of them were spiked with 250 ng of Ga and In, while the others were kept unchanged. The four parts were analyzed as described above (see procedure).

The results (Table 2) showed that good recoveries and precision were achieved. Chromatograms of a natural and a spiked sample are shown in Fig. 4. The peaks which appear after that of In correspond to Zn and Al present in the sample. The amount of detected gallium corresponds to an air concentration of 0.4 ng m^{-3}, while indium content is below the detection limit of the method.

Acknowledgments

The authors thank the Comissionat per Universitats i Recerca de la Generalitat de Catalunya (1995 SGR 00458) for supporting this study. E. Miralles also thanks CIRIT for an FPI grant.

References

[1] E. Merian, Metals and their Compounds in the Environment: Occurrence, Analysis and Biological Relevance, Weinheim, New York, 1991.
[2] J.E. Fergusson, The Heavy Elememts: Chemistry, Environmental Impact and Health Effects, Pergamon Press, Oxford, 1991.
[3] R.L. Hayes, in H.G. Seiler, H. Sigel and A. Sigel (Editors), Handbook on Toxicity of Inorganic Compounds, Marcel Dekker, New York. 1988, Ch. 27
[4] Y. Hayashibe, M. Kurosaki, F. Takekawa and R. Kuroda, Mikrochim. Acta, II (1989) 163.
[5] E.L. Lakomaa, P. Manninen, R.J. Rosenberg and R. Zilliacus, J. Radional. Nucl. Chem., 168 (1993) 357.
[6] K.L. Orians and E.A. Boyle, Anal. Chim. Acta, 282 (1993) 63.
[7] A. Fernández Gutiérrez and A. Muñoz de la Peña, in S.G. Schulman (Editor), Molecular Luminescence Spectroscopy, John Wiley, New York, 1985, Part 1, Ch. 4.
[8] G.G. Guilbault, in G.G. Guilbault (Editor), Practical Fluorescence, Marcel Dekker, New York, 1990, Ch. 5.
[9] B.D. Karcher and I.S. Krull, J. Chromatogr. Sci., 25 (1987) 472.
[10] B.D. Karcher and I.S. Krull, Chromatographia, 4 (1987) 705.
[11] P. Jones, K. Barron and L. Ebdon, Anal. Proc., 22 (1985) 373.
[12] T. Williams and N.W. Barnett, Anal. Chim. Acta, 264 (1992) 297.
[13] K. Kina, K. Tamura and N. Ishibashi Bunseki Kagaku, 23 (1974) 1404 (C.A. 82: 164 470).
[14] D.A. Phillips, K. Soroka, R.S. Vithanage and P.K. Dasgupta, Mikrochim. Acta, (1986) 207.
[15] K. Soroka, R.S. Vithanage, D.A. Phillips, B. Walker and P.K. Dasgupta, Anal. Chem., 59 (1987) 629.
[16] M.D. Prat, R. Compañó, J.L. Beltrán, R. Codony, J. Fluorescence, 4 (1994) 279.
[17] P.R. Haddad and P.E. Jackson, Ion Chromatography. Principles and Applications (Journal of Chromatography Library, Vol. 46), Elsevier, Amsterdam, 1990.
[18] P. Jones, L. Ebdon and T. Williams, Analyst, 113 (1988) 641.