Journal of Chromatography B, 878 (2010) 34–38

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of cadmium, cobalt, copper, iron, manganese, and zinc in thyroid glands of patients with diagnosed nodular goitre using ion chromatography

Anna Błażewicz[a,*], Wojciech Dolliver[a], Suceil Sivsammye[a], Apeksha Deol[a], Rajinder Randhawa[a], Grażyna Orlicz-Szczęsna[b], Remigiusz Błażewicz[c]

[a] Department of Analytical Chemistry, Medical University of Lublin, 6 Staszica St., 20-081 Lublin, Poland
[b] Clinic of Internal Diseases, Medical University of Lublin, 16 Staszica St., 20-081 Lublin, Poland
[c] Institute of Physics, Maria Curie-Skłodowska University in Lublin, 1M. Curie-Skłodowska Square, 20-031 Lublin, Poland

ARTICLE INFO

Article history:
Received 13 August 2009
Accepted 6 November 2009
Available online 17 November 2009

Keywords:
Thyroid
Nodular goitre
Metal ions
Ion chromatography

ABSTRACT

The aim of this study was to estimate and compare the contents of selected metals in 65 pathological (diagnosed nodular goitre) and 50 healthy human thyroid tissues (taken during autopsies). Ion chromatography (IC) preceded by microwave mineralization was applied for the first time for determination of cadmium, cobalt, copper, iron, manganese, and zinc in human thyroid samples. The study proved that the concentrations of Cu^{2+}, Mn^{2+}, Fe^{3+}, and Zn^{2+} were significantly higher in the control group (healthy thyroids) in comparison with the studied group (nodular goitre) ($p < 0.05$), whereas for Co^{2+} the difference between two means of concentration (healthy *vs* pathological thyroids) was not significant statistically at 0.05 significance level. Measurement accuracy was verified by measurements of NIST standard reference material (1566a Oyster Tissue). Very good precision (RSD below 5%) and recoveries (above 90%) were evaluated.

1. Introduction

Many metals are essential to life, but in excess, these same chemicals can be poisonous. The basic tenet of toxicology—"the dose makes the poison" is still actual. Too little can lead to a deficiency, too much can result in adverse health effects. Much of the pertinent work on that subject has been summarized by World Health Organization [1]. Heavy metals are dangerous because they tend to bioaccumulate. Similarly, chronic low exposures to heavy metals can have serious health effects in the long run [2]. What is more, the toxicity of one metal can be dramatically modulated by the interaction with other toxic or essential metal [3]. Deficiency, excess and imbalance of trace elements can be the reason for pathologies.

It was reported that thyroid tissue could accumulate both highly toxic and essential elements [4]. Some literature data support the hypothesis that direct toxic heavy metal influence on thyrocytes plays a major role in thyroid cancer aetiology [5,6].

A nodular goitre is the most common pathology of the thyroid gland [7,8]. In order to study the role of particular element in thyroid pathology development it is necessary to undergo the comparative examinations in healthy subjects and well-diagnosed patients.

Traditionally, atomic absorption spectrophotometric (AAS) techniques, both flame and electrothermal have been used by most clinical chemistry laboratories to determine transition metals, e.g. in physiological fluids [9,10]. However, these techniques have their limitations, e.g. flame AAS has limited sensitivity for copper, and graphite atomic absorption spectrophotometry is susceptible to solute vaporization interferences such as depression of element signal, especially in biological samples. The protein content of the mentioned samples can cause absorption abnormalities. High sodium chloride content can hamper sensitivity, linearity, and cause burner clogging [11]. Recently high-resolution continuum source AAS (HR-CS AAS) has opened up new possibilities in determining metals in biological samples [12]. Among the other available analytical techniques used for the determination of metals ions, inductively coupled plasma (ICP)-mass spectrometry (MS) seems to be a powerful alternative to AAS [13–15]. Despite multi elemental capability and high sensitivity, which make ICP-MS an attractive technique for the analysis of biological samples, several types of non-spectral and spectral interferences can complicate the analysis of samples with complex matrices. Engström et al. described the potential of inductively coupled plasma-sector field mass spectrometry (ICP-SFMS) in the determination of 68 elements in various animal tissues [16]. Inductively coupled plasma atomic emission spectrometry (ICP-AES) [17], neutron activation analysis (NAA) [18], X-ray fluorescence spectrometry (XRF) [19], total-reflection X-ray fluorescence (TRXRF) [20], particle-induced X-ray emission (PIXE) [21] are the other most commonly

* Corresponding author. Fax: +48 081 5357350.
E-mail address: anna.blazewicz@am.lublin.pl (A. Błażewicz).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.11.014

used techniques in trace analysis of human tissues and body fluids.

Ion chromatography (IC) has been widely applied in the analysis of ionic species in many samples of various origins. What is more, due to its sensitivity (down to the ng/g concentration range) and the possibility of simultaneous multi element determination IC method requires small amounts of biological samples. The stability of the separation system, good selectivity and speed of analysis allow the range of IC applications to continuously broaden [22].

Recently, the application of ion chromatography to samples of complex matrices has received much attention [23]. Unfortunately most of the applications of IC in clinical samples analysis focus mainly on biological fluids (blood, serum, urine, saliva, teardrops) because they are easy to obtain and generally do not require complicated digestion procedures. However, it has to be emphasized that the most appropriate information on the content of trace metals comes from the tissues. It is well known, that, e.g. infectious diseases or strenuous physical activity affects trace element content in blood as a result of short-term intensified biochemical and metabolic processes [24]. Our investigations concerned pathological human thyroid glands removed during a surgical procedure (diagnosed nodular goitre) and healthy thyroids taken from individuals killed in accidental events (control group).

It is obvious, that chemical digestion in appropriate conditions is required before IC analysis of tissues. Microwave mineralization in a closed system—where the contamination problems are significantly reduced can be recommended for such samples.

The aim of the present study was to evaluate the concentrations of selected metals in human thyroids. To the best of our knowledge it is the first time the ion chromatography method has been used to carry out metal determination in healthy and pathological thyroid tissues. The present paper also presents the basic validation parameters of determining the transition metal ions with the use of IC method together with the digestion conditions of the studied samples.

2. Experimental

2.1. Instrumentation and reagents

The measurements were carried out on a Dionex model DX-500 ion chromatograph (Sunnyvale, CA, USA), which was composed of a IP25 isocratic pump, a pre-column CG5A, a CS5A column, and a UV–Vis detector AD20. A Chromeleon (Dionex) chromatography workstation was used for instrument control and data acquisition. The detection was accomplished by visible absorbance after post column reagent. 1 L of the post column reagent (PCR) was prepared with 0.5 mmol of 4-(2-pyridylazo)resorcinol (PAR), 1.0 mol of 2-dimethylamino-ethanol, 0.3 mol of sodium bicarbonate, and 0.50 mol of ammonium hydroxide dissolved in deionized water (18 MΩ cm).

All mobile phases, analyte solutions and derivatizing reagent were made up in water purified with an Easypure system (Barnstead, USA). All reagents were of analytical grade. Eluent was filtered and degassed before use. The pyridine-2,6 dicarboxylic acid (PDCA) eluent was used for iron, copper, nickel, zinc, cobalt, cadmium, and manganese. The composition of 1 L of PDCA eluent concentrate (this concentrate simplifies eluent preparation and improves reproducibility), which was 5 times diluted with deionized water and used as mobile phase during chromatographic analysis, was as follows: 7.0 mmol of PDCA, 66 mmol of potassium hydroxide, 5.6 mmol of potassium sulfate, and 74 mmol of formic acid.

All mobile phase components were obtained from Sigma–Aldrich, Germany. Aqueous solutions of metal salts were prepared by dilution of Titrisol standard metal salt solutions (Merck). Working solutions were freshly prepared from standard metal salt solutions by dilution with doubly distilled water. PAR was obtained from Dionex, USA.

All analyses were carried out using pre-washed polypropylene flasks and vials.

2.2. Patient population and preparation of samples

The studied material consisted of 115 human thyroids. Investigations were performed in 65 people who constituted the studied group (40 female and 25 male, the average age was 35) and had fragments of their thyroid glands (diagnosed nodular goitre) removed during a surgical procedure. The patients underwent thyroidectomy in 3 hospitals in Lublin (a town of 380,000 population in the south-east region of Poland which suffered as a result of the accident at the Chernobyl nuclear power plant in 1986). The diagnoses were confirmed by histopathological examination. All the patients diagnosed with a nodular goitre were in euthyreosis and did not suffer from hyperthyroidism. The protocol was accepted by the local Ethics Committee. The samples obtained were fixed in formalin (according to the standard procedure).

Healthy human thyroids were obtained from individuals mainly from the south-east region of Poland and killed in accidents. The control group included 50 people (9 female and 41 male, the average age was 25 years). No morphological changes were detected in any of the removed tissues. Each sample of tissues was split with a quartz knife. All samples were transported and stored in polypropylene containers. Both kinds of thyroids (healthy and pathological) were treated the same way. Such uniform sample preparation allowed to perform comparative analysis of healthy and thyroid tissues.

All the samples were washed three times with deionized water and placed in pre-cleaned laboratory glass containers in a hot-air laboratory oven at 120 °C until being completely dry. After cooling the containers were placed in desiccators over silica gel and later weighted accurately. After weighing they were transferred to pre-cleaned polypropylene containers fitted with screw caps.

Before IC analysis thyroid samples (each time 0.5 g of dry tissue) were mineralized using a microwave-assisted high pressure digestion system (UniClever BM-1, Plazmotronika, Poznań, Poland). Each time the acidic digestion with 99% HNO_3 (Sigma–Aldrich, Germany) water solution was applied (2 mL of HNO_3:8 mL H_2O). A program of microwave digestion consisted of the following four stages: 1) 60% of power of microwave generator, heating for 3 min; 2) 80% of power of microwave generator, heating for 5 min; 3) 100% of power of microwave generator, heating for 8 min; and 4) cooling for 10 min. The conditions of mineralization procedure had been previously optimized in terms of mass of the sample and time of matrix destruction (i.e. the effects of mass of the sample and mineralization time on the measured concentrations of ions were checked). Each time the completeness of the digestion was monitored with the ion chromatography. It turned out that the optimal time of mineralization should last 26 min since longer time did not affect the assay values significantly. Shorter times of the described procedure caused some disturbances to the base line on chromatograms. Therefore in this study 26-min procedure (including a cooling step) was applied. The mass of the sample taken for the mineralization should be compatible with the used digestion procedure as well. It turned out that the determined concentration values of investigated ions were not significantly affected by the mass of the sample (which varied from 0.5 to 1.0 g) [25]. After digestion, the Teflon vessels (with screw caps) were cleaned thoroughly twice with dilute

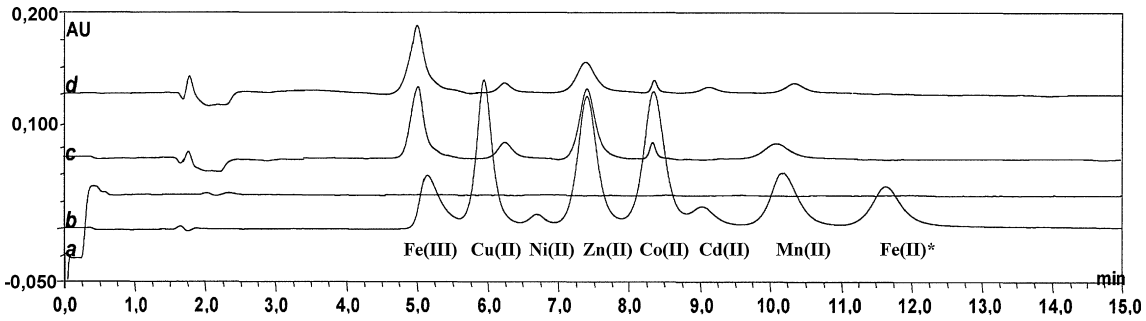

Fig. 1. Chromatograms of blank sample (a), standard mixture (b), sample of thyroid from the control group (c), and sample of thyroid of a patient with diagnosed nodular goitre (d). Each sample was injected at least in triplicate; all operating conditions are described in the text.
Because of the drastically oxidative environment applied during described digestion procedure it was not possible to detect the presence of iron (II) in the studied samples, however, IonPac CS5A enables separation of Fe(III) and Fe(II) what can be seen in b.

digestion mixture in order to avoid memory effects (adsorption by the walls of containers).

2.3. IC analysis

Appropriate concentrations of standards were prepared from 1 g/L stock standards solutions (Merck, Darmstadt, Germany). All standards, samples and reagents were stored in polyethylene bottles. The detailed procedure of the standard solutions preparation has already been described [26]. However, for the purpose of clarity of the present paper, some main concepts have to be mentioned here as well. The solutions used to determine the calibration curves were prepared by weight dilution (i.e. both solution being diluted and water were weighed on an analytical balance) using standard solutions of 1000 ppm concentration (Merck, Darmstadt, Germany). For each cation a series of 20 standard solutions in the range of 0.001–100 ppm were obtained. In the next stage the peak areas were determined within individual series by interpolation (for extreme concentrations, if necessary, by extrapolation) for exactly identical assumed concentrations. It was found that the data are of the heteroscedastic type, therefore, the weighted regression method was applied. The weights for the individual points of the curve were calculated by the standard method [27]. The parameters of the correlation equations were determined using the TableCurve 2D v5.0 (ASIN Software Inc.) program. Solutions obtained after mineralizations were 10-fold diluted with deionized water and analyzed using the ion chromatograph. In the present study the total number of injections on the chromatographic column amounted to 1035 (each sample from 115 subjects was cut into three parts before launching the digestion procedure and then injected at least in triplicate).

Before injection, the column was equilibrated applying PDCA eluent. IonPac CS 5A analytical column (250 mm × 4.6 mm I.D., 9 μm bead diameter ethylvinylbenzene functionalized with both quaternary ammonium and sulfonate functional groups which is compatible with pH 0–14) allowed the analysis of acidic solutions of digested tissues. The detector wavelength was 530 nm, the sample loop volume was 25 μL, the eluent flow rate was set at 0.3 mL/min, while the post column reagent flow rate was fixed at 0.15 mL/min. The column backpressure was approximately 1900 psi (13.1 MPa). All measurements were made at 25 ± 1 °C.

After equilibration of the analytical column, three calibration blank standards were analyzed to establish a representative blank level (Fig. 1a). Then, the prepared calibration standards were analyzed (Fig. 1b). When the blank level was acceptable (concentration value lower than method detection limit), the samples were analyzed. Since the concentration range should encompass values expected in samples to be measured, all the samples were previously 10-fold diluted to avoid the exceeding of the concentration range (0.001–100 ppm). Samples having concentrations higher than the established concentration range were diluted and reanalyzed.

The quality control standard, i.e. NIST Standard Reference Material oyster tissue (SRM 1566a), an independent standard made from certified reference solutions was used for the initial verification of calibration (accuracy). 50 mg of SRM samples were digested in a mixture of HNO_3 and H_2O (volume ratio 1:4) which was also used for digestion of thyroid samples.

3. Results and discussion

3.1. Validation parameters

Ion chromatography as a highly sensitive method enables the analysis of small amounts of sample materials. This is very important since in many cases only a small volume of thyroid sample is taken from a patient during surgical operation. Table 1 presents the IC results obtained for 3 independent measurements, including 3 sample digestions, and the comparison with certified values. Limits of detection and quantitation are included as well. Good agreement with the certified values was found for all the metals studied.

Method precision was determined in terms of repeatability (intra-assay precision) and quantified by the relative standard deviations (RSD) of the replicate measurements, and was assessed at

Table 1
Comparison of metal ions concentrations measured by IC and certified values.

Metal ion	LOD (LOQ)* (μg/mL)	Measured value (μg/g) ± SD	Certified value (μg/g) ± SD
Cd	0.022 (0.073)	3.75 ± 0.45	4.15 ± 0.38
Co	0.026 (0.087)	0.66 ± 0.15	0.57 ± 0.11
Cu	0.048 (0.16)	65.1 ± 5.9	66.3 ± 4.3
Fe	0.009 (0.03)	586 ± 17.2	539 ± 15
Mn	0.006 (0.020)	11.9 ± 2.1	12.3 ± 1.5
Ni	0.006 (0.020)	2.14 ± 0.59	2.25 ± 0.44
Zn	0.056 (0.19)	857.2 ± 45	830 ± 57

* LOD—limit of detection; LOQ—limit of quantitation; LOQ = 10/3·LOD (values of LOD and LOQ are taken from the previously published work [26].

Table 2
Recoveries of Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} from spiked thyroid samples of 3 different subjects.

Ion	Sample number	Recovery (%) Concentration of ion spike (ppm)		
		0.25	0.50	1.0
Cd^{2+}	1	97.0	90.0	96.5
	2	100.0	98.5	99.1
	3	95.6	94.9	102.7
Co^{2+}	1	108.0	92.0	111.0
	2	102.0	99.5	99.8
	3	97.6	99.9	109.7
Cu^{2+}	1	98.5	99.2	100.5
	2	94.9	98.9	92.0
	3	96.6	100.0	100.0
Fe^{3+}	1	95.5	99.0	100.0
	2	97.3	101.5	100.0
	3	99.4	108.0	103.0
Mn^{2+}	1	92.2	99.7	101.0
	2	101.0	96.5	97.8
	3	96.1	90.2	90.7
Zn^{2+}	1	100.0	100.0	100.0
	2	98.0	99.9	102.0
	3	99.6	101.0	101.0

four unique concentrations in replicates of six, on four separate occasions (i.e. $4 \times 6 \times 4$). RSD was determined to be 5.0% for Cd^{2+}, 1.6% for Co^{2+}, 4.18% for Cu^{2+}, 2.21% for Fe^{3+}, 4.25% for Mn^{2+}, and 1.9% for Zn^{2+}.

Recoveries ranging from 94.9 to 102.7% for Cd^{2+}, from 92.0 to 109.7% for Co^{2+}, from 92.0 to 100.5% for Cu^{2+}, from 95.5 to 108% for Fe^{3+}, from 90.2 to 101% for Mn^{2+}, and from 98 to 101% for Zn^{2+}were determined by examining samples spiked with various amounts of all the studied ions (Table 2).

3.2. Comparison of metals' contents in healthy and pathological tissues

The results of IC determinations of the studied ions are presented in Table 3.

It is very difficult to compare our results with previously published studies involving the ion chromatography technique because according to the authors' best knowledge, there are no such data available. Despite of the lack of such data, our results for copper and zinc are similar to the literature data reportedly obtained by a different method (involving X-ray fluorescence) [28] where authors determined 2.82 ± 0.62 μg/g of copper and 38.3 ± 2.2 μg/g of zinc in nodular goitre tissue. However, one can find in literature a significantly higher value of zinc concentration in human thyroid samples obtained during operations on people living in regions of Belarus, i.e. 341 ± 16 μg/g which was determined by the use of ICP-MS [29].

Generally iron, copper, and zinc concentrations are in good agreement with literature data (despite different sample pretreatment procedures and different analysis methods, e.g. NAA [30]), except, as above described ICP-MS measurement of zinc [29]. Values for cobalt in the healthy thyroids were higher by a factor of 3.1–4.7, and for manganese lower by factor of 1.9, respectively, compared with the literature values (Table 3). In the present study cadmium was present only in NG group, whereas nickel was not detectable for either the studied group or the control group. Interestingly, significant discrepancies in the results found in literature concern selectively some metals, whereas for others the good agreement can be found regardless of the method applied. Addi-

Table 3
Values of mean concentrations and concentrations ranges (μg/g of dry tissue) of studied metals in thyroid tissues determined using IC method.

Element	Nodular goitre (NG) (studied group) $n = 65$	Concentration range	Healthy tissues (control group, CG) $n = 50$	Concentration range	Literature values of mean concentration
Cd	0.125 ± 0.006	0.080–0.180	n.d.***		healthy: 0.0161 (NAA) [6]
Co*	0.110 ± 0.003	0.090–0.109	0.124 ± 0.01	0.105–0.142	healthy: 0.0404 ± 0.0028 (NAA) [5]; healthy: 0.026 ± 0.031 (NAA) [30]
Cu**	3.35 ± 0.93	1.890–4.921	5.24 ± 0.51	4.543–6.210	goitre: 2.82 ± 0.62 (TRXRF) [28]
Fe**	201.59 ± 23.45	162.4–239.5	241.44 ± 21.96	204.120–272.00	pathological: 182 ± 12 (ICP-MS) [29]; healthy: 237 ± 15 (NAA) [5]
Mn**	0.40 ± 0.22	0.079–0.712	0.68 ± 0.10	0.520–0.891	healthy: 1.34 ± 0.68 (NAA) [30]
Ni	n.d.		n.d.		no data
Zn**	41.83 ± 7.19	29.721–53.450	101.30 ± 10.90	89.310–125.3	pathological: 341 ± 16 (ICP-MS) [29]; 38.3 ± 2.2 (TRXRF) [28]; healthy: 87 ± 4 (NAA) [5]; healthy: 129 ± 67 (NAA) [30]

*The difference between two means (NG *vs* CG) was not significant statistically ($p < 0.05$); **significantly lower concentration in the studied group ($p < 0.05$); ***n.d.—not detected under the applied conditions.

tional difficulty is related to the limited information about sample pre-treatment procedures. Some important differences may be attributed to the sample storage as well. Therefore, additionally, our next study will be undertaken in order to compare fresh-frozen and formalin-fixed thyroid samples in context of metals' determination using ion chromatography method.

Statistical analysis revealed over 2-fold reduction of zinc concentration in the pathological tissues (the mean value was 41.83 ± 7.19 μg/g) in comparison with the control group (101.30 ± 10.90 μg/g).

The concentrations of the studied metals in healthy tissues are slightly higher (except 2-fold increase of zinc concentration) than those in NG tissues. The statistical significance between the NG and CG (Table 3) for variables with normal distribution was evaluated with *t*-Student test for variables not related at 0.05—significance level. The statistically significant differences between mean concentrations (NG *vs* CG) concerned copper, iron, manganese, and zinc for which level of concentrations were higher in CG ($p < 0.05$).

Fig. 1 presents the chromatograms obtained for blank sample (a), standard mixture of metal ions (b), and also in thyroid sample from the control group (c), and sample of thyroid of a patient with diagnosed nodular goitre (d). Chromatograms for NG samples differed remarkably from those obtained for the healthy ones. The main difference to the healthy thyroid samples is the lower concentration of zinc, iron, copper, manganese, and cobalt, and additionally the presence of small amounts of cadmium. This effect was observed for all thyroid samples available in the present study. Explanation of this phenomenon will be the aim of the next investigations.

Our results were difficult to compare with published data due to differing techniques used in tissue digestion and different methods of analysis. An additional problem in comparing our results from healthy subjects with the results from the control group arose due to the fact that most literature data is related to the analysis of animal tissues. To the best of authors' knowledge, this is the first time IC method has been applied for metal determination in healthy and pathological human thyroid tissues.

4. Conclusions

Ion chromatography preceded by microwave-assisted acidic digestion of samples allowed determining Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} in nodular goitre and healthy human thyroids. Cd^{2+} was detected only in nodular goitre samples. An evaluation of the obtained data indicated that the mean values found for iron, copper, and zinc are within the values presented in literature. Values for cobalt in thyroids were higher by a factor of 3.1–4.7, and for manganese lower by factor of 1.9, respectively, compared with the literature values. Nickel was not detected either in healthy or in pathological thyroids.

There were no significant differences between Co^{2+} content in healthy and nodular goitre thyroid tissues. For Cu^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} significantly lower ($p < 0.05$) concentrations in the studied group were observed.

The main assets of the presented method lie in its simplicity and the practicality of determining analytes from samples of various origins. Suitability of the developed IC method was supported by validation results. Generally, very good results of precision (RSD below 5%) and recoveries (above 90%) were evaluated.

This study was supported by grant from the Medical University of Lublin, Poland.

References

[1] Environmental Health Criteria Monographs, No. 17 (1981), 108 (1991), 134 (1992), 200 (1998), 221 (2001), World Health Organization, Geneva.
[2] R. Masters, in: J. Rose (Ed.), Environmental Toxicology, Gordon and Breach, London, 1998.
[3] M.L. Alonso, F.P. Montana, M. Miranda, C. Castillo, J. Hernandez, J.L. Benedito, BioMetals 17 (2000) 389.
[4] B.E. Saltzman, S.B. Gross, D.W. Yeager, B.G. Meiners, P.S. Gartside, Environ. Res. 52 (1990) 126.
[5] V.Ye. Zaichick, A.F. Tsyb, B.M. Vtyurin, Analyst 120 (1995) 817.
[6] M. Yaman, Curr. Med. Chem. 13 (2006) 2513.
[7] J.D. Mortensen, L.B. Woolner, W.A. Bennet, J. Clin. Endocrinol. Metab. 15 (1995) 1270.
[8] H. Voltzke, J. Ludemann, D.M. Robinson, K.W. Spieker, C.S. Schwahn, A. Kramer, U. John, W. Meng, Thyroid 13 (2003) 803.
[9] F.W. Sunderman Jr., Hum. Patrol 4 (1973) 549.
[10] F.W. Sunderman Jr., Ann. Clin. Lab. Sci. 5 (1975) 421.
[11] Application Note 108, Determination of Transition Metals in Serum and Whole Blood by Ion Chromatography, Dionex Corporation (USA), 1998.
[12] D.L. Gallindo Borges, A. Furtado da Silva, B. Welz, A.J. Curtius, U. Heitmann, J. Anal. Atom Spectrom. 21 (2006) 763.
[13] P. Schramel, I. Wendler, Fresenius J. Anal. Chem. 361 (1998) 487.
[14] I. Rodushkin, E. Engstrom, A. Stenberg, D.C. Baxter, Anal. Bioanal. Chem. 380 (2004) 247.
[15] I. Rodushkin, M.D. Axelsson, Sci. Total Environ. 305 (2003) 23.
[16] E. Engstrom, A. Stenberg, S. Senioukh, R. Edelbro, D.C. Baxter, I. Rodushkin, Anal. Chim. Acta 521 (2004) 123.
[17] R. Rahil-Kazen, B.J. Bolann, A. Myking, R.J. Ulvik, J. Trace Elem. Med. Biol. 16 (1) (2002) 15.
[18] R. Zeisler, R.R. Greenberg, Biol. Trace Elem. Res. 71–72 (1999) 283.
[19] M.L. Carvalho, A.F. Marques, X-Ray Spectrom. 30 (2000) 397.
[20] N. Szoboszlai, Z. Polgari, V.G. Mihucz, G. zaray, Anal. Chim. Acta 633 (2009) 1.
[21] M.L. Carvalho, T. Magalhaes, M. Becker, A. Von Bohlen, Spectrochim. Acta Part B 62 (2007) 1004.
[22] J. Weiss, Handbook of Ion Chromatography, vol. 1, WILEY-VCH Verlag GmbH&Co. KGaA, Weinheim, 2004.
[23] P.N. Nesterenko, P. Jones, J. Chromatogr. A 770 (1997) 129.
[24] M. Speich, A. Pineau, F. Ballereau, Clin. Chim. Acta 312 (2001) 1.
[25] A. Błażewicz, unpublished data.
[26] A. Błażewicz, T. Baj, R. Świeboda, Ł. Świątek, Polish J. Environ. Stud. 16 (2007) 191.
[27] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, John Wiley & Sons, New York, 1984.
[28] M. Kucharzewski, J. Braziewicz, U. Majewska, S. Góźdź, Biol. Trace Elem. Res. 93 (2003) 9.
[29] S.F. Boulyga, J.S. Becker, A.F. Malenchenko, H.-J. Dietze, Microchim. Acta 134 (2000) 215.
[30] Y. Katoh, T. Sato, Y. Yamamoto, Biol. Trace Elem. Res. 90 (2002) 57.