JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Evaluation of Dry Ashing in Conjunction with Ion Chromatographic Determination of Transition Metal Ions in Pig Feed Samples

MARLEEN R. VAN PAEMEL,* HERMAN DE RYCKE, SAM MILLET, MYRIAM HESTA, AND GEERT P. J. JANSSENS

Laboratory of Animal Nutrition, Faculty of Veterinary Science, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium

The contents of transition metal ions iron, copper, zinc, and manganese were simultaneously determined in pig feed using an ion chromatographic technique (IC) preceded by dry ashing. Employing ion exchange, the ions were separated on an IonPac CS5A column used in combination with a pyridine-2,6-dicarboxylic acid based eluent. The separation was followed by spectrophotometric detection after postcolumn reaction with 4-(2-pyridylazo)resorcinol. Dry ashing parameters were varied to assess their role in potential analyte loss. Quantitative recoveries (>95%) were obtained for all analytes with a dry ashing method that included a moderate temperature−time regime and ash leaching support in the form of sonication and heat treatment. The use of HCl as leaching acid and the presence of alkaline earths in the matrix solution did not interfere with the chromatographic separation.

KEYWORDS: Dry ashing; transition metal analysis; IC; pig feed; PCR; postcolumn reagent

1. INTRODUCTION

Ion chromatography (IC) is accepted as a very attractive technique for trace element analysis owing to the relative ease of operation and the satisfactory sensitivity coupled with the advantage of simultaneous determinations. Heavy and transition metals can be determined by IC through use of a chelating anionic agent in the mobile phase and visible spectrometry detection after postcolumn addition of a chromogenic reagent (*1*, *2*).

The use of IC for the determination of transition metals requires that the sample be solubilized prior to the measurement. The selection of an appropriate sample preparation is a crucial step, determining the accuracy of the whole analysis. Whether a sample preparation method is appropriate depends on the sample matrix and the analytes to be determined, as well as on its compatibility with the measurement technique used afterward. There are several preparation options for food samples such as dry ashing, wet ashing, microwave-assisted digestion, and UV photolysis. The latter three have already been used in conjunction with the described IC analysis (*3*−*5*). Dry ashing, however, has not. Generally speaking, it has some advantages over the other techniques especially for the analysis of plant material. Owing to the strong interfering and chelating properties of the organic matrix of these samples, it is necessary to achieve sufficient destruction of the organic matrix during decomposition (*6*, *7*). In addition, plant material is mostly quite heterogeneous, a problem that can be overcome through the use of large samples. Classical dry ashing allows the use of large sample sizes as well as complete destruction of the organic matrix. It was previously demonstrated that results obtained by dry ashing preceding ICP-MS did not differ with respect to accuracy from those obtained using microwave digestion and open vessel acid digestion (*8*), but these statements cannot be generalized, as in the end it all comes down to a careful selection of the sample preparation parameters for a particular application. However, taking all previous arguments into consideration, one can conclude that there is a possibility of obtaining accurate results by dry ashing and it is a technique worth screening.

The purpose of this work was the comparative investigation of four dry ashing methods for the determination of the total content of iron, copper, zinc, and manganese in plant-based pig feed by IC analysis. The total content of iron and copper is measured as Fe^{3+} and Cu^{2+} because all Fe^{2+} and Cu^{+} oxidize during the ashing process. The IC measurement technique and the whole analysis procedure using the most suitable dry ashing method were validated.

2. MATERIALS AND METHODS

2.1. Instrumentation. Chromatographic analyses were performed on a metal-free ion chromatograph equipped with a GP40 gradient pump, an IonPac CG5A (4 × 50 mm) guard column and an IonPac CS5A (4 × 250 mm) separator column, a postcolumn pneumatic controller (PC 10) for postcolumn reagent delivery, a packed-bead reaction coil, and a UV−Vis multiple-wavelength detector (all Dionex, Sunnyvale, CA). A Rheodyne injection valve model 9726 was used

* Corresponding author (telephone +32-9-2647822; fax +32-9-2647848; e-mail marleen.vanpaemel@ugent.be).

10.1021/jf048653c CCC: $30.25
Published on Web 02/15/2005

Table 1. Ion Chromatographic Conditions

elution order	Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}
columns	IonPac CG5A
	IonPac CS5A
eluent	7 mM PDCA
	66 mM KOH
	5.6 mM K_2SO_4
	94 mM HCOOH
	pH 4.0
eluent flow rate (mL min^{-1})	1.2
injection volume (μL)	25
postcolumn reagent (PCR)	0.4 mM PAR
	1 M 2-dimethylaminoethanol
	0.5 M NH_3
	0.3 M $NaHCO_3$
	pH 10.4
PCR flow rate (mL min^{-1})	0.6
λ detection (nm)	530

(Rheodyne, San Francisco, CA). The chromatographic conditions are listed in **Table 1**.

2.2. Reagents and Standards. Pyridine-2,6-dicarboxylic acid (PDCA) and 4-(2-pyridylazo)resorcinol (PAR) for IC and AAS were purchased from Fluka (Buchs, Switserland). All other reagents used were of analytical grade and obtained from commercial sources. Deionized water was purified by a Milli-Q system (Millipore, Bedford, MA) to a specific resistance of 18 MΩ cm^{-1} and was used in all diluting steps. All glassware was soaked with nitric acid (10%) and rinsed with 18 MΩ water.

2.3. Calibration. Cu^{2+}, Fe^{3+}, Zn^{2+}, and Mn^{2+} 1000 mg L^{-1} stock standards (Titrisol, Merck, Darmstadt, Germany) were used to prepare working standard solutions by serial dilution before use. The calibration design was chosen in accordance with IUPAC guidelines (*9*). It contained eight equispaced concentration levels covering the 0.5−4 mg L^{-1} concentration range. Three replicated injections at each concentration level were performed with independent standard solutions, that is, separately prepared starting from the stock standards. A straight-line model was proposed for each analyte with the ordinary least squares fitting technique (OLS). To evaluate the adequacy of fit of the linear model, a lack of fit test (LOF) was performed (*10*−*12*). Following the calibration design, the number of degrees of freedom of the sum of squares residual, pure error, and lack of fit were, respectively, 22, 16 and 6.

2.4. Procedure. An experimental starter pig feed was analyzed. Four dry ashing digestion procedures were applied. Eight subsamples of 5 g for each individual method were weighed into porcelain crucibles and treated as described under methods 1−4.

2.4.1. Method 1. The samples were placed in a muffle furnace and heated consecutively at 180 °C for 3 h, at 220 °C for 1 h, at 260 °C for 1 h, at 300 °C for 1 h, and at 400 °C for 1 h and were further ashed overnight at 500 °C (16 h). The ash residue was dissolved in 5 mL of HCl (37%) and quantitatively transferred to 50 mL volumetric flasks. The flasks were sonicated for 10 min (Bransonic 52, Branson, Danbury, CT) and placed in a hot-water bath (95 °C) for 1 h. The resulting matrix solutions were filtered (DG2M, Microgon, Laguna Hills, CA) and diluted 10-fold before they were injected.

2.4.2. Method 2. The samples were placed in a muffle furnace, and the same temperature−time regime as described for method 1 was applied. The resulting ashes were treated with 2 mL of concentrated HNO_3 (69%). The samples were gently dried on a hot plate and afterward placed in muffle furnace for 30 min (500 °C). A few drops of 18 MΩ water were added to the melts, and the crucibles were sonicated (10 min). The contents of the crucibles were quantitatively transferred to 50 mL volumetric flasks, and 5 mL of HCl (37%) was added. The flasks were sonicated (10 min) and placed in a hot-water bath (95 °C) for 1 h. The flasks were made up to volume, and the resulting matrix solutions were filtered (DG2M) and diluted 10-fold before they were injected.

2.4.3. Method 3. Here, 4 mL of a 10% potassium nitrate solution was added to the samples as an ashing aid. The samples were dried overnight at 120 °C and then placed in the muffle furnace. The same temperature−time regime was applied as described for method 1. The ash residue was dissolved in 5 mL of HCl (37%) and quantitatively transferred to 50 mL volumetric flasks. The flasks were sonicated (10 min) and placed in a hot-water bath (95 °C) for 1 h. The resulting matrix solutions were filtered (DG2M) and diluted 10-fold before they were injected.

2.4.4. Method 4. The samples were placed in a muffle furnace, and the same temperature−time regime was applied as described for method 1. Ashes were treated with 2 mL of concentrated HNO_3 (69%). The samples were gently dried on a hot plate and then placed in muffle furnace for 30 min (500 °C). A few drops of 18 MΩ water were added to the melts, and the crucibles were sonicated (10 min). The contents of the crucibles were quantitatively transferred to 50 mL volumetric flasks and 5 mL HCl (37%) was added. The flasks were sonicated (10 min). The resulting matrix solutions were filtered (DG2M) and diluted 10-fold before they were injected.

2.5 Accuracy. To assess bias, a surrogate recovery experiment was done following IUPAC guidelines (*13*, *14*). Eight subsamples were spiked with 200 μL of stock standard solution (1000 mg L^{-1}) of each analyte and dried at 120 °C overnight. The samples were analyzed alongside unspiked subsamples, as described for method 2. The additional drying step was performed because liquid spikes were used. The results of these analyses allow the calculation of a recovery coefficient (R). The recovery coefficient was calculated according to the equation

$$R = (Q_{(O+S)} - Q_O) \times Q_S^{-1}$$

where $Q_{(O+S)}$ is the quantity of analyte measured for the spiked samples [peak area (p.a.)], Q_O is the quantity of analyte measured from the original samples (p.a.), and Q_S is the quantity of analyte added (i.e., spike value) (p.a.) (*14*). To verify the influence of potential contamination, a set of three blank solutions was prepared following the same procedures as described for each individual method.

3. RESULTS AND DISCUSSION

3.1. HPLC Measurement Technique. The application of IC to determine transition metals requires the use of an anionic chelating agent in the mobile phase, which has the dual purpose of reducing the effective charge on the solute cation and introducing a secondary equilibrium and thereby a further dimension of selectivity (*15*, *16*). The mixed-bed IonPac CS5A column, which was recently reported to be the most effective for the separation of transition metals, was used (*17*). The anionic chelating agents commonly used in combination with this column are PDCA and oxalic acid. Oxalic acid, however, was not an option because it is not suited for samples containing large concentrations of Ca^{2+} and Mg^{2+} as they would form insoluble oxalate complexes and precipitate as such on the column (*18*). Furthermore, $Fe(Ox)_3$ complexes are too strongly retained by the column to be eluted by the mobile phase (*17*, *19*). During preliminary tests a PDCA-based eluent composition as described by Cardellicchio et al. (*20*) did not lead to a sufficient separation of Fe^{3+} and Cu^{2+}. This coelution problem was overcome by elevating the formic acid concentration from 74 to 94 mM. This is in accordance with the fact that an acidification of the eluent increases the retention times as it leads to a smaller complexing ability of PDCA (*1*, *21*, *22*). The described IC technique has a low analytical sensitivity for alkaline earths such as Ca^{2+} and Mg^{2+}. However, these ions are reported to disturb the chromatographic separation from concentrations as low as 10 mg L^{-1}. The disturbance is due to the formation of $Mg[PDCA]_2^{2-}$ and $Ca[PDCA]_2^{2-}$ complexes that overload the anion exchange sites, affecting the efficiency of the column and the retention times (*18*). In samples containing these alkaline earths in large excess of the transition metal ions, they are often removed by an additional sample cleanup using

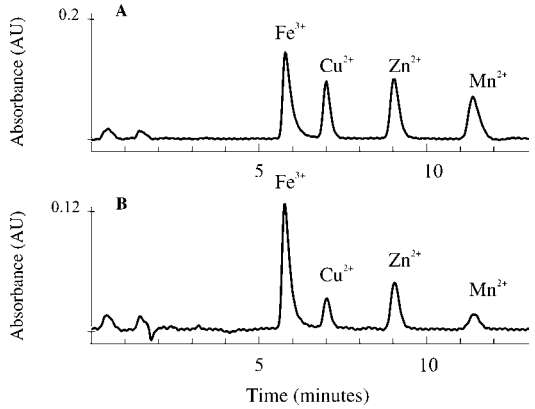

Figure 1. Chromatograms of (**A**) a mixed 4 mg L^{-1} standard and (**B**) a pig feed sample. The sample preparation was as described for method 2. For both chromatograms the chromatographic conditions are listed in **Table 1**.

a precolumn. The Ca^{2+} and Mg^{2+} content of the feed was determined using an IC technique that included a separation on a Metrosep Cation 1-2 column (Metrohm, Herisau, Switserland) with an eluent containing tartaric acid and PDCA in combination with a conductivity detector (*23*). The feed contained 0.888% Ca^{2+} and 0.223% Mg^{2+}, which led, for all applied sample preparation methods, to concentrations of 88.8 mg L^{-1} Ca^{2+} and 22.3 mg L^{-1} Mg^{2+} in the resulting matrix solutions. As these alkaline earth concentrations potentially hinder the transition metal determination, the injection volume was kept small. There were no differences observed in retention times and column efficiency between the analyses of pig feed matrix samples and the analyses of aqueous standard solutions. Chromatograms of a mixed aqueous standard and a feed sample are shown in **Figure 1**.

3.2. Validation. To verify whether a calibration with aqueous standards is suited to determine the analytes in the feed matrix solutions, a preliminary general matrix test was performed (*9*). For the four analytes two sets of three calibration curves were constructed, namely, one set of curves with aqueous standards and one set with the stock standards serially diluted with a matrix solution prepared as described for method 2. For each curve three equispaced standards were used ranging from 0.5 to 4 mg L^{-1}. A *T* test was applied to compare the average of the slopes of the two sets of curves. The obtained *P* values for Fe^{3+}, Cu^{2+}, Zn^{2+}, and Mn^{2+} were, respectively, 0.36, 0.06, 0.10, and 0.78. Therefore, a significant difference in analytical sensitivity due to matrix effects could not be demonstrated, and aqueous standards were used to perform the calibrations. The data from the calibration procedure are shown in **Table 2**. The LOF test results indicate no lack of fit, and a straight line with OLS fitting is an appropriate model for the four calibrations. The assumptions of normality and homoscedasticity of the data were verified by the residual plots, which did not reveal any patterns that questioned these assumptions.

All measured signals were well above 10-fold the baseline detector noise, and therefore all measurements were within the region of quantitation (*24*, *25*).

3.3. Sample Preparation. The accuracy of the analysis is determined not only by the measurement technique but also by the preceding decomposition procedure. The latter should be given great attention because it often affects the results to a greater extent (*26*, *27*).

When the applied temperature−time regime is composed, several factors need to be taken into consideration. In the literature, consensus exists of the fact that a moderate charring phase is of critical importance to avoid losses of analyte from the sample through local overheating and subsequent loss of solid particles of smoke (*28*, *29*). Mader et al. (*29*) reported that the removal of organic components from plant material proceeds much more quickly than in the case of soft animal tissues and that an initial charring temperature below 200 °C is opportune. The final ashing temperature should, as a rule, be kept as low as possible because of the danger of losing volatile inorganic compounds, but it must at the same time be high enough to ensure complete combustion of all the organic matter (*30*). Mader et al. (*28*), who reviewed the literature published on classical dry ashing of biological material from 1978 on, noted that no researchers ashed above 550 °C and that the most frequently used ashing temperatures were 450 and 500 °C. In addition, Mader et al. (*29*) communicated that a decrease of ashing temperature from 500 to 450 °C led to an incomplete decomposition of readily combustible plant material such as potato tubers. Based on this information the following temperature−time regime was composed and applied. The samples were consecutively heated at 180 °C for 3 h, at 220 °C for 1 h, at 260 for 1 h, at 300 °C for 1 h, and at 400°C for 1 h and were finally ashed at 500 °C overnight (16 h). This moderate temperature regime was chosen to prevent spray, dust, and volatilization losses. Verifying to what extent the precautions are necessary to avoid losses was not an objective. Therefore, the temperature−time regime was not varied among the sample preparation methods.

When method 1 was applied, the resulting ash still contained charred particles. If ashing does not lead to a complete decomposition of the organic matrix and thus does not generate a carbon-free ash, an ashing aid that increases the oxidation power is required (*28*, *31*). To solve this problem, the two most frequently used oxidation aids, namely, the addition of nitric acid in combination with an additional ashing step and the addition of nitrates before ashing, were incorporated in, respectively, methods 2 and 3. The addition of KNO_3 before ashing turned out to be not effective. In addition to an incomplete ashing, crust formation was observed when samples were dried after addition of the KNO_3 solution. Crust formation hinders the ashing process as it reduces the permeability for oxygen (*29*). Method 2 was effective in completely decomposing the feed matrix, and a white siliceous residue was obtained after the leaching procedure.

Table 2. Lack of Fit Test Results for the Calibration Data of Fe^{3+}, Cu^{2+}, Zn^{2+}, and Mn^{2+} Including the Test Statistic *F*[a] and the Corresponding *P* Value

		sum of squares	mean squares	*F*	*P* value
Fe^{3+}	residuals	7.15×10^8	3.25×10^7		
	pure error	6.66×10^8	4.16×10^7		
	lack of fit	4.89×10^7	8.16×10^6	0.196	0.973
Cu^{2+}	residuals	6.22×10^8	2.80×10^7		
	pure error	5.05×10^8	3.16×10^7		
	lack of fit	1.17×10^8	1.95×10^7	0.618	0.713
Zn^{2+}	residuals	3.20×10^8	1.50×10^7		
	pure error	2.42×10^8	1.51×10^7		
	lack of fit	8.77×10^7	1.46×10^7	0.968	0.477
Mn^{2+}	residuals	6.14×10^8	2.79×10^7		
	pure error	4.21×10^8	2.63×10^7		
	lack of fit	1.94×10^8	3.22×10^7	1.228	0.343

[a] *F*, the test statistic, follows an *F* distribution with 6, 16 degrees of freedom and is calculated as the ratio of the lack of fit mean of squares to the pure error mean of squares.

Table 3. Results of the Pig Feed Analyses (Mean ± SD)[a] Using Four Different Dry Ashing Sample Preparation Methods

method	Fe^{3+} (mg kg^{-1})	Cu^{2+} (mg kg^{-1})	Zn^{2+} (mg kg^{-1})	Mn^{2+} (mg kg^{-1})
1	245.7 ± 29.4	146.8 ± 7.9	182.3 ± 5.0	79.3 ± 8.5
2	253.6 ± 31.1	133.6 ± 19.4	180.3 ± 9.1	80.1 ± 11.7
3	230.6 ± 40.9	137.0 ± 8.5	180.2 ± 12.7	79.6 ± 9.1
4	214.5 ± 29.6	132.5 ± 5.5	187.9 ± 3.5	80.1 ± 7.0

[a] Eight replicates.

Table 4. Results of an ANOVA Procedure To Verify Whether Complete Decomposition, Addition of an Ashing Aid, and the Use of Heat Treatment Significantly Influence Trace Element Analysis

	complete decomposition			use of ashing aid			heat treatment		
	signif diff			signif diff			signif diff		
	no	yes	*P* value	no	yes	*P* value	no	yes	*P* value
Fe^{3+}	∨		0.175	∨		0.370		∨	0.025
Cu^{2+}	∨		0.568	∨		0.103	∨		0.846
Zn^{2+}	∨		0.976	∨		0.616	∨		0.080
Mn^{2+}	∨		0.914	∨		0.959	∨		0.996

Hydrochloric acid and nitric acid are the most commonly used leaching acids. The choice is often determined by the determination technique used afterward due to possible interferences of these acids with the latter (*6*). Preliminary stock standards were serially diluted with HCl and HNO_3 solutions of the same concentration as those obtained in the matrix solution after sample preparation. Neither of them interfered with the chromatographic separation.

The ash of plant-based material contains a siliceous fraction, which remains undissolved by HCl and HNO_3 leaching. Analytes can be quite strongly retained in the siliceous residue with a consequent risk of them not being solubilized during the leaching procedure (*32*−*34*). Mader et al. (*34*), who investigated analyte retention in insoluble residues, reported that ultrasonic mixing results in a powerful increase in the efficiency of the analyte release. Hence, sonication was applied in all methods. Heat treatment was also suggested as a leaching support and was applied in methods 1−3. Remarkably, after method 2, a white residue remained in the flasks, whereas after method 4, a reddish precipitate was formed.

The results of the iron, copper, zinc, and manganese determinations of the pig feed are given in **Table 3**. ANOVA (SPSS 11.0.1 for Windows) was used to verify whether statistically significant differences could be observed due to the following sample preparation characteristics: attainment of a complete decomposition leading to a carbon-free ash (methods 2 and 4), the use of an ashing aid (methods 2−4), and the implementation of heat treatment to support the ash leaching (method 4). The results are listed in **Table 4**. For the analytes Cu^{2+}, Zn^{2+}, and Mn^{2+} it could not be demonstrated that any of the three characteristics significantly influenced the sample preparation efficiency. For Fe^{3+} the measurements were significantly lower when a hot-water bath treatment was omitted (method 4). Mader et al. (*34*) proposed heat treatment as a possible leaching support but also stated that when sonication was already applied, efficiency was not further improved. However, Fe^{3+} measurements were not performed in their experiments. It can be concluded that the present results do not contradict these statements but add to the conclusion that heat treatment is required when Fe^{3+} is to be dissolved.

3.4. Accuracy. The following *R* values were obtained: 0.98 for Fe^{3+}, 0.99 for Cu^{2+}, 1.04 for Zn^{2+}, and 1.07 for Mn^{2+}. As all *R* values were >95%, there is no indication of an incomplete transfer of the analytes from the feed matrix to the matrix solution, that is, measurement phase. For each method three blanks were prepared and analyzed to verify whether contamination influenced the accuracy of the analyses and therefore also the recovery results. On none of the chromatograms were peaks detected. To avoid contamination, all recipients and syringe filters were acid-washed and thoroughly rinsed with 18 MΩ water. It can therefore be concluded that with measures such as the implementation of a moderate temperature−time regime and ash leaching support, it was possible to accurately determine iron, copper, zinc, and manganese in a pig feed matrix.

ABBREVIATIONS USED

AU, absorption units; IC, ion chromatography; PDCA, pyridine-2,6-dicarboxylic acid; PAR, 4-(2-pyridylazo)resorcinol; OLS, ordinary least squares; LOF, lack of fit; *R*, recovery coefficient.

LITERATURE CITED

(1) Haddad, P. R.; Jackson, P. E. Eluents for Ion-Exchange Separations. In *Ion Chromatography*, *Principles and Applications. Journal of Chromatography Library*; Elsevier: Amsterdam, The Netherlands, 1990; Vol. 46, pp 101−105.

(2) Haddad, P. R.; Jackson, P. E. Detection by Post-column Reaction. In *Ion Chromatography*, *Principles and Applications. Journal of Chromatography Library*; Elsevier: Amsterdam, The Netherlands, 1990; Vol. 46, pp 400−402.

(3) Buldini, P. L.; Cavalli, S.; Mevoli, A.; Sharma, J. L. Ion chromatographic and voltammetric determination of heavy and transition metals in honey. *Food Chem.* **2001**, *73*, 487−495.

(4) Fredrikson, M.; Carlsson, N.; Almgren, A.; Sandberg, A. Simultaneous and sensitive analysis of Cu, Ni, Zn, Co, Mn, and Fe in food and biological samples by ion chromatography. *J. Agric. Food Chem.* **2002**, *50*, 59−65.

(5) Lu, H.; Mou, S.; Riviello, J. M. Use of ion chromatography for the determination of heavy and transition metals in biochemical samples. *J. Chromatogr. A* **1999**, *857*, 343−349.

(6) Koplik, R.; Curdova, E.; Suchanek, M. Trace element analysis in CRM of plant origin by inductively coupled plasma mass spectrometry. *Fresenius' J. Anal. Chem.* **1998**, *360*, 449−451.

(7) Buldini, P. L.; Cavalli, S.; Mevoli, A. Sample pretreatment by UV photolysis for the ion chromatographic analysis of plant material. *J. Chromatogr. A* **1996**, *739*, 167−173.

(8) Ming, Y.; Bing, L. Determination of rare earth elements in human hair and wheat flour reference materials by inductively coupled plasma mass spectrometry with dry ashing and microwave digestion. *Spectrochim. Acta B* **1998**, *53*, 1447−1454.

(9) Thompson, M.; Ellison, S. L. R.; Wood, R. Harmonized guidelines for single-laboratory validation of methods of analysis. *Pure Appl. Chem.* **2002**, *74*, 835−855.

(10) Neter, J.; Wasserman, W.; Kutner, M. H. Diagnostics and remedial measures. In *Applied Linear Statistical Models*, 3rd ed.; Irwin: Flossmoor, IL, 1990; pp 131−140.

(11) Van Loco J.; Elskens, M.; Croux, C.; Beernaert, H. Linearity of calibration curves: use and misuse of the correlation coefficient. *Accredit. Qual. Assur.* **2002**, *7*, 281−285.

(12) Vial, J.; Jardy, A. Taking into account both preparation and injection in high-performance liquid chromatography linearity studies. *J. Chromatogr. Sci.* **2000**, *38*, 189−194.

(13) Thompson, M.; Ellison, S. L. R.; Fajgelj, A.; Willetts, P.; Wood, R. Harmonised guidelines for the use of recovery information in analytical measurement. *Pure Appl. Chem.* **1999**, *71*, 337−348.

(14) Burns, D. T.; Danzer, K.; Townshend, A. Use of the terms recovery and apparent recovery in analytical procedures. *Pure Appl. Chem.* **2002**, *74*, 2201−2205.
(15) Janvion, P.; Motellier, S.; Pitsch, H. Ion-exchange mechanisms of some transition metals on a mixed-bed resin with a complexing eluent. *J. Chromatogr. A* **1995**, *715*, 105−115.
(16) Timerbaev, A. R.; Bonn, G. K. Complexation ion chromatography—an overview of developments and trends in trace metal analysis. *J. Chromatogr. A* **1993**, *640*, 195−206.
(17) Ding, X.; Mou, S. Retention behavior of transition metals on a bifunctional ion-exchange column with oxalic acid as eluent. *J. Chromatogr. A* **2001**, *920*, 101−107.
(18) Ding, X.; Mou, S.; Liu, K.; Siriraks, A.; Riviello, J. Ion chromatography of heavy and transition metals by on- and post-column derivatizations. *Anal. Chim. Acta* **2000**, *407*, 319−326.
(19) Lu, H.; Mou, S.; Yan, Y.; Tong, S.; Riviello, J. M. On-line pretreatment and determination of Pb, Cu and Cd at the μg L^{-1} level in drinking water by chelation ion chromatography. *J. Chromatogr. A* **1998**, *800*, 247−255.
(20) Cardellicchio, N.; Cavalli, S.; Ragone, P.; Riviello, J. M. New strategies for determination of transition metals by complexation ion-exchange chromatography and post-column reaction. *J. Chromatogr. A* **1999**, *847*, 251−259.
(21) Bin Abas, M. R.; Takruni, I. A.; Abdullah, Z.; Tahir, N. M. On-line preconcentration and determination of trace metals using a flow injection system coupled to ion chromatography. *Talanta* **2002**, *58*, 883−890.
(22) Weiss, J. Ion-Exchange Chromatography (HPIC). In *Ion Chromatography*, 2nd ed.; VCH Publishers: New York, 1995; pp 190−194.
(23) Läubli, M. W.; Kampus, B. Cation analysis on a new poly-(butadiene-maleic acid)-based column. *J. Chromatogr. A* **1995**, *706*, 99−102.
(24) Jenke, D. R. Chromatographic method validation: a review of current practices and procedures. II. Guidelines for primary validation parameters. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19*, 737−757.
(25) Long, G. L.; Winefordner, J. D. Limit of detection. A closer look at the IUPAC definition. *Anal. Chem.* **1983**, *55*, 712a−724a.
(26) Curdova, E.; Szakova, J.; Miholova, D.; Mestek, O.; Suchanek, M. Evaluation of various mineralization methods and measurement techniques for trace element analysis of plant materials. *Analusis* **1998**, *26*, 116−121.
(27) Poykio, R.; Torvela, H.; Peramaki, P.; Kuokkanen, T.; Ronkkomaki, H. Comparison of dissolution methods for multi-element analysis of some plant materials used as bioindicator of sulphur and heavy metal deposition determined by ICP-AES and ICP-MS. *Analusis* **2000**, *28*, 850−854.
(28) Mader, P.; Szakova, J.; Curdova, E. Combination of classical dry ashing with stripping voltammetry in trace element analysis of biological materials: review of literature published after 1978. *Talanta* **1996**, *43*, 521−534.
(29) Mader, P.; Haber, V.; Zelinka, J. Classical dry ashing of biological and agricultural materials. Part I. Following the course of removal of organic matrix. *Analusis* **1997**, *25*, 175−183.
(30) Bock, R. Oxidizing procedures. In *A Handbook of Decomposition Methods in Analytical Chemistry*; International Textbook: Glasgow, U.K., 1979; pp 123−153.
(31) Pomeranz, Y.; Meloan, C. E. Ash and Mineral Components. In *Food Analysis: Theory and Practice*; Avi Publishing; Westport, CT, 1971; pp 515−522.
(32) Hoenig, H.; Baeten, H.; Vanhentenrijk, S.; Vassileva, E.; Quevauviller, Ph. Critical discussion on the need for an efficient mineralization procedure for the analysis of plant material by atomic spectrometric methods. *Anal. Chim. Acta* **1998**, *358*, 85−94.
(33) Ledent, G.; de Borger, R.; Vanhentenrijk, S. Etude de deux minéralisations appliquées aux dosages des éléments minéraux dans les végétaux. *Analusis* **1984**, *12*, 393−395.
(34) Mader, P.; Szakova, J.; Miholova, D. Classical dry ashing of biological and agricultural materials. Part II. Losses of analytes due to their retention in an insoluble residue. *Analusis* **1998**, *26*, 121−129.

Received for review August 10, 2004. Revised manuscript received December 22, 2004. Accepted December 23, 2004. This research was funded by BOF (Ghent University).

JF048653C