

Determination of Cadmium, Lead, and Copper in Margarines and Butters by Galvanostatic Stripping Chronopotentiometry

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A galvanostatic stripping chronopotentiometric (GSCP) method for the simultaneous determination of cadmium, lead, and copper in commercial margarines and butters is described. Quantitative analyses of heavy metals in the mineralized fat samples, except standard addition method, were carried out automatically. The mean concentrations of Cd(II), Pb(II), and Cu(II) in different types of margarines and butters varied from 9.1 to 26.2, 9.2–14.2, 298.2–364.0 $\mu\text{g kg}^{-1}$, respectively, that is below the legal requirements. The detection limits are 0.02, 0.02, and 0.06 $\mu\text{g L}^{-1}$ for Cd(II), Pb(II) and Cu(II), while the reproducibilities are 3.3, 5.7, and 2.0%, respectively. The comparable precision (RSD = 0.76–4.5 and 0.87–4.9%) and recovery (96.7–102.2% and 96.1–103.2%) for the proposed GSCP and standard GFAAS methods, demonstrate the benefit of GSCP method in the routine analysis of heavy metal ions in fats.

KEYWORDS: Galvanostatic stripping chronopotentiometry; cadmium; lead; copper; margarines; butters

INTRODUCTION

The presence of heavy metal ions such as cadmium, lead and copper in oils and food fat products is a very important parameter of their quality. The content of heavy metals in fats depends on several factors. These metals are naturally present in the seed oils, because they may be absorbed from the soil and fertilizers or environmental pollutants. Moreover, the production processes, materials of packaging, and storage of food fats may be sources of heavy metals contamination. The above-mentioned heavy metals are very dangerous for human health; cadmium and lead, in particular, are cumulative poisons. Their toxicity is manifested by kidney disfunction, hypertension, hepatic injury, lung damage, bone effects, etc. (1). Toxicity of copper due to excessive intake may lead to liver cirrhosis, dermatitis, and neurological disorders (2). Moreover, trace levels of copper ions detract from the oxidative stability of edible fats, because they are powerful catalysts of their degradation (3). Oils and fats are natural nutrients in diets, because they have a high calorific value and contain essential fatty acids necessary for the correct development of human tissues. Because diet is the most important source of Cd(II), Pb(II), and Cu(II) human intake, in 1993, the Joint FAO/WHO Expert Committee for Additives and Contaminants (JECFA) reduced the tolerable lead consumption per week, from 0.05 mg kg^{-1} body weight to 0.025 mg kg^{-1} (4). However, recommended daily intake of cadmium and copper is 7 $\mu\text{g kg}^{-1}$ and 0.5 mg kg^{-1} body mass, respectively (5).

Therefore, the determination of heavy metal ions in margarines and animal fats requires a sample preparation step to release toxic elements from the fat matrix. The extraction is

the classical procedure for preconcentration of the trace and ultra-trace metal ions and the matrix removal. It offers some advantages, such as: (1) analysis of the undissolved samples, (2) the lack of calcination of samples, and (3) sample contaminations and losses of analyzed elements is avoided. The extraction of heavy metal ions from edible oils using hot hydrochloric acid (6) and Pb-piperazinedithiocarbamate complex and potassium cyanide solution (7) was noted. However, these techniques also have disadvantages: (1) the separation of phases is difficult, (2) the extraction procedure is time-consuming, (3) the results of recoveries for some metal ions are rather poor (e.g., 85 and 93% for Cd(II) (6) and Cu(II) (7), respectively). Therefore, another procedures of sample preparation for example, wet digestion (2), dry ashing (8, 9), microwave digestion (10), and alcoholic solubilization (11) were applied in analytical techniques for analysis of heavy metals in edible fats.

Among others, atomic absorption spectrometry (AAS) (2, 7, 12–19), inductively coupled plasma-atomic emission spectrometry (ICP-AES) (10, 20–22) and ion chromatography (IC) (23) are used for trace metal analysis in edible fats. The precisions of AAS methods ranged between 3 and 10% RSD (2, 16, 18), while the accuracies (expressed as recoveries) between 89 and 112% (7, 10, 18) were found for different types of oils and fats. However, the detection limits of this technique (0.4 $\mu\text{g kg}^{-1}$, 2.4 $\mu\text{g kg}^{-1}$, and 30 $\mu\text{g kg}^{-1}$ for Cd(II) (18), Cu(II) (7), and Pb(II) (10), respectively) were significantly lower in comparison with those obtained by IC method (100 $\mu\text{g Cd kg}^{-1}$, 10 $\mu\text{g Cu kg}^{-1}$, and 50 $\mu\text{g Pb kg}^{-1}$ (23), respectively). The mentioned methods require an expensive instrumentation and this limits their suitability for the routine analyses in the industrial laboratories. These constraints led to the suggestion that electrochemical methods for heavy metal ions analysis can be a matter of choice. They offer the proper sensitivity, coupled

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with multi-element speciation in situ and on-line analytical application. Electrochemical stripping analysis is generally recognized as a technique for measuring traces of metals in water (24–29), biological fluids (30–33), and foods (34–40), whereas only few applications to oil product analysis have been reported (6, 8, 9, 11). The precise (RSD ranged between 1.6 and 3.8%) direct inverse voltammetric (DIV) method was proposed for the determination of Pb(II), Cu(II), and Cd(II) in linseed, soybean, sunflower, and olive oils (11). La Pera et al. (6) and Lo Coco et al. (8) reported the derivative potentiometric stripping analysis (dPSA) used for heavy metal ions determination in olive oils. The cadmium content in all the oil samples was below the evaluated detection limits = 1.2 ng g^{-1} (6) and 5.1 ng g^{-1} (8), respectively. Moreover, a study on the application of differential pulse anodic (or cathodic) stripping voltammetry (DPASV, DPSCV) for the determination of Pb(II) and other transition metals in crude edible oil samples (9) and Cu(II), Cd(II), Pb(II) in the vegetable oils and margarine were described (23). They were found to be in good agreement with those obtained by AAS and IC methods (9, 23). However, a disadvantage of those voltammetric methods appeared to be a mercury used in stripping analysis.

The electroanalytical techniques allow the determination of trace metals of nutritional and toxicological interest in a wide range of concentrations (between a few $\mu\text{g kg}^{-1}$ and few thousand $\mu\text{g kg}^{-1}$; e.g., $1.70 \mu\text{g}$ of Pb kg^{-1} in cow milk and $3157.3 \mu\text{g}$ of Cu kg^{-1} in baby foods (37)) (6, 8, 9, 11, 34–40). The preconcentration of dissolved metal ions onto the mercury film of the working electrode allowed the achievement of low limits of detection. In addition to the precision and accuracy of electrochemical procedures were compared with those obtained by AAS method (35, 40).

In the present paper, a galvanostatic stripping chronopotentiometry (GSCP) was applied for simultaneous determination of the Cd(II), Pb(II), Cu(II) in margarine and butter samples and compared to the official standard GFAAS method for accuracy and precision. The advantage of the proposed GSCP (or constant current stripping chronopotentiometry CCSCP) method for simultaneous determination of cadmium, lead, and copper in fat samples after wet digestion was the modification of the stripping step (i.e., the replacement of chemical oxidant—toxic Hg(II) solution (6) by constant oxidative current).

MATERIALS AND METHODS

Reagents. All reagents were of analytical grade and purchased from POCH (Gliwice, Poland). Concentrated nitric acid (60%) of Ultrapur grade (max 20 ng kg^{-1} of Cd(II), Cu(II), and Pb(II), respectively) was supplied by Merck (Darmstadt, Germany). Deionized water (DW) was used for the preparation of solutions and samples. Sodium acetate buffer containing 0.1 , 0.01 , and 0.01 mol L^{-1} of Na_2SO_4 , CH_3COOH and CH_3COONa solutions, respectively ($\text{pH} = 5.0 \pm 0.3$) was used as supporting electrolyte. Standard solution for each element ($10 \mu\text{g}$ of Cd(II) L^{-1} , $10 \mu\text{g}$ of Pb(II) L^{-1} and $20 \mu\text{g}$ of Cu(II) L^{-1}) was obtained by diluting commercial standards (1000 mg of L^{-1} , Spectrosol, United Kingdom) in the supporting electrolyte.

Apparatus. A computer-controlled instrument EcaPol consisting of the EcaSensor electrode system (Istran, Slovak Republic) was employed for all the electrochemical measurements. The compact electrode system EcaSensor was operated by the control unit fully automatically. The three-electrodes system was in the lower part of the EcaSensor and consists of carbon working electrode with a latent mercury film type D-LMF (Istran) connected to the cell body by a gold contact, the Pt auxiliary electrode and Ag/AgCl/KCl (3 M) reference electrode. A stirrer built in the EcaSensor ensures the effective mixing of the solutions. The working electrode was used only for one series of measurements (30 samples by 1 day).

Atomic Absorption Spectroscopic Analyses. Analyses were performed with an ABQ-20 graphite furnace atomic absorption spectrometer (GFAAS) (Varian) using deuterium lamp background corrector. Analyses were carried out at the most sensitive analytical spectral lines of the metals (Cu, -324.8 nm ; Cd, -228.8 nm ; Pb, -283.3 nm).

Analytical Procedure. Samples Preparation. Four commercial margarines (M-1, M-2, M-3, M-4) and four butters (B-1, B-2, B-3, B-4) were manufactured in Poland and purchased from different local markets in Toruń, Poland.

All fats in the original packing (polypropylene (PP) containers) were stored at $4 \text{ }^\circ\text{C}$ until the analysis. Fats were digested as follows: samples were accurately weighed on an analytical balance ($8.1\text{--}10.3 \text{ g}$), transferred to 250-mL conical flasks, 150 mL of concentrated nitric acid were added, and the mixture was left at room temperature overnight. The flasks were gradually heated to $150\text{--}160 \text{ }^\circ\text{C}$ on a hotplate, while hydrogen peroxide ($1\text{--}5 \text{ mL}$) was periodically added to the solutions until the digestion was completed. After cooling, the samples were transferred into volumetric flasks and made up to 100 mL with DW. Blank digestions were carried out in the same way.

GSCP Determination of Cd(II), Pb(II), and Cu(II). The analysis consisted of two stages carried out automatically: (a) In the preconcentration (deposition) step, metal ions from the sample solution were reduced and simultaneously amalgamated onto the working electrode at constant potential. (b) In the stripping step, the potentiostat was transformed into a galvanostat and the deposit re-oxidized electrochemically by application of a constant oxidative current. The change of the working electrode potential with time during the stripping of the deposited analyte was monitored and evaluated. The potential-time dependence was treated by the fast channel analyzer, which transformed the original wavelike signal into a more convenient peak signal. The inverse potential derivative with time (dt/dE) versus potential (E) curve has the form of a Gaussian curve, and the peak areas are normally proportional to the concentrations of the analytes. Since the analyzer operated under the control of the EcaPol software package, the analytical procedure can be performed completely automatically.

Hydrochloric acid (0.1 M) and acetate buffer ($\text{pH} = 5.0 \pm 0.3$) were examined as supporting electrolytes for the simultaneous determination of cadmium, lead, and copper in fat samples. However, the stripping peak for Cu(II) was poorly defined and obscured by the large background signal of the blank hydrochloric solution (0.1 M) in the potential range for copper detection. Similar results were obtained by Kadara et al. (41). The large background signal was probably due to surface reactions on the working electrode material. In the case of acetate buffer ($\text{pH} = 5.0 \pm 0.3$), when used as supporting electrolyte, a well-defined and reproducible chronopotentiometric stripping signal of Cu(II) was obtained. Therefore, acetate buffer was used as a supporting electrolyte for simultaneous determination of Cd(II), Pb(II), and Cu(II) in fat samples. In addition, the optimal conditions for simultaneous determination of Cd(II), Pb(II), and Cu(II) were partially adapted from the literature (6, 11, 25, 28). In those papers, authors used electrochemical techniques (PSA, GSCP, DIV, and dPSA, respectively) for simultaneous analysis of Cd(II), Pb(II), and Cu(II) in seawater (25), tap water, grass, sediments (28), and edible oils (6, 11).

The working electrode (D-LMF) was placed in the EcaSensor and activated with 1.0 M HCl solution at -800 mV for 2 min . When the preparation was completed, the EcaSensor was lifted and electrodes were rinsed with DW. Then, 1 mL of the sample solution and 10 mL of acetate buffer ($\text{pH} = 5.0 \pm 0.3$) were placed into the electrochemical cell. The electrodes were immersed into sample solution and measurement started. The analyses were performed in the electrochemical conditions given in **Table 1**. The quantitative analysis was done by the standard addition method, adding 0.1 mL of the standard ($10 \mu\text{g}$ of Cd(II) L^{-1} , $10 \mu\text{g}$ of Pb(II) L^{-1} and $20 \mu\text{g}$ of Cu(II) L^{-1}) to the studied samples twice. The electrochemical measurements for two standard additions were repeated in five cycles on the same fat sample upon stirring during the first (preconcentration) and second (stripping) steps. Before the start of every analysis, the EcaSensor was rinsed with DW.

Calibration Curves. Calibration curves were obtained for increasing concentrations of analytes standard solutions using acetate buffer as supporting electrolyte (measurements conditions, **Table 1**). Five calibration curves for each metal were plotted on the same day. The

Table 1. Electrochemical Conditions for Determination of Cd(II), Pb(II), and Cu(II) by GSCP Method

electrochemical parameters	values
deposition potential [mV]	-900
starting potential [mV]	-700
end potential [mV]	250
stripping current [μ A]	10
quiescence time [s]	10
stripping time [s]	30
regeneration potential [mV]	100
regeneration time [s]	10
deposition time – sample [s]	60
deposition time – background [s]	10
deposition time – standard [s]	60
stand-by potential [mV]	-200

Table 2. Analytical Parameters for the GSCP Determination of Cd(II), Pb(II), and Cu(II) in Fat Samples

regression parameters	metals		
	Cd(II)	Pb(II)	Cu(II)
concentration range (μ g L ⁻¹)	0.07–0.42	0.06–0.36	2.0–6.0
slope of regression line ^a (<i>b</i>) (ms L μ g ⁻¹)	3359.3	3226.4	371.8
standard deviation of slope ^a (<i>s_b</i>) (ms L μ g ⁻¹)	93.1	79.6	2.2
intercept of regression line ^a (<i>a</i>) (ms)	38.9	25.7	17.9
standard deviation of intercept ^a (<i>s_a</i>) (ms)	25.1	18.5	9.2
standard deviation of <i>y</i> -residuals (<i>s_{y/x}</i> = <i>s_B</i> – standard deviation of blank) ^a (ms)	27.0	19.9	7.3
coefficient of determination ^a (<i>R</i> ²)	0.997	0.998	1.000
relative standard deviation of slope ^a (RSD) (%)	2.8	2.5	0.58
relative standard deviation RSD ^b (%)	2.0	2.3	2.8
detection limits (μ g L ⁻¹)	0.02	0.02	0.06

^a *n* = 5 ^b Five replicate solution containing 0.28 μ g of Cd L⁻¹, 0.24 μ g of Pb L⁻¹, and 4.4 μ g of Cu L⁻¹

within-day precision (repeatability) was found by regression analysis of (d*t*/d*E* – peak area) = *f*(*c*) curves, and expressed as the relative standard deviation (RSD %, *n*=5) of the slope (Table 2) (42). The calibration plots were linear (*R*² = 0.997, 0.998, and 1.000) in the concentration ranges between 0.07 and 0.42, 0.06–0.36, and 2.0–6.0 μ g L⁻¹ for Cd(II), Pb(II) and Cu(II), respectively. The regression parameters were calculated and listed in Table 2. The sensitivity of the reported GSCP method for Cd(II), Pb(II), and Cu(II) determination is given by the slopes of the calibration plots, which were 3359.3, 3226.4, and 371.8 ms μ g⁻¹ L, respectively. The sensitivity for the cadmium assay was better than that for lead (*b_{Cd}*/*b_{Pb}* = 1.04) for the similar concentration range under the same electrochemical conditions (Table 1). However, the analytical curve (2.0–6.0 μ g L⁻¹) for copper ions presents the smallest slope (*b* = 371.8 ms μ g⁻¹ L). The relative standard deviations (*n* = 5) of the curves slopes were 2.8, 2.5, and 0.58% for cadmium, lead, and copper ions, respectively. The repeatability was 2.0, 2.3, and 2.8% at concentration levels of 0.28 μ g of Cd L⁻¹, 0.24 μ g of Pb L⁻¹ and 4.4 μ g of Cu L⁻¹. The values of RSD were less than 3%, indicating reasonable intra-day precision of GSCP method for simultaneous determination of Cd(II), Pb(II), and Cu(II).

Detection Limits. The detection limits (LOD) of the studied method were defined as *y* = *y_B* + 3*s_B*, where *y_B* (= *a*), the average concentrations of metals (*n* = 5) giving a signal equal to the blank, where *s_B* (= *s_{y/x}*), the standard deviation of the blank (42). Therefore, detection limits were calculated utilizing the expression LOD = 3*s_{y/x}*/*b*, where *s_{y/x}* and *b* are the estimated standard deviation of *y*-residuals (the peak threshold) and the slope (sensitivity of method) of the analytical calibration function of each metal (95% confidence level, Table 2), respectively. Analytical blank solutions for all metal determinations were prepared according to the general procedure at conditions listed in Table 1.

Precision and Accuracy of the Analytical Procedures. The content of Cd(II), Pb(II), Cu(II) in the mineralized samples determined (five portions of each fat were digested and each solution analyzed three times within 1 day) by the studied method were compared with the

official standard GFAAS method (12, 13). The reproducibility of the method was checked by five replicate determinations of Cu(II), Cd(II), and Pb(II) in the same fat samples (M-1, M-2, B-3) over the period of 3 days. Both methods were compared for within-day (repeatability) and between-day (reproducibility) precision using *F*-test and accuracy expressed as recovery values. The recovery experiments were performed as follows: fat samples (8.1–10.3 g) were heated to 80 °C upon stirring, standard solutions of the examined ions (15 μ g of Cd L⁻¹, 10 μ g of Pb L⁻¹ and 200 μ g of Cu L⁻¹) were added; the obtained mixtures were stirred at the same temperature for 12 h, and elaborated wet digestion procedure was executed.

RESULTS AND DISCUSSION

Determination of Heavy Metal Ions in Fats. The typical set of stripping chronopotentiograms and the results of the simultaneous determination of Cd(II), Pb(II), and Cu(II), in sample B-1 is presented in Figure 1.

This stripping curve revealed three potential peaks of Cu(II), Pb(II), and Cd(II), which were oxidized at 100, -350, and -530 mV, respectively, under the experimental conditions described in Table 1. For comparison, stripping peaks of copper, lead, and cadmium in honey samples were at 30, -410, and -590 mV, while a more negative deposition potential = -1200 mV and much longer deposition time equal to 1200 s were used (38).

Metal concentrations in B-1 sample, obtained by the standard addition method, yielded calibration lines (*y* = 3398.5*x* + 277.4, *y* = 3247.5*x* + 297.8 and *y* = 373.8 + 1226.1 with correlation coefficients *R*² = 0.999, 0.999, and 1.000 for Cd(II), Pb(II) and Cu(II), respectively) (Figure 1B). Those values of correlation coefficients suggest a good linearity of the used standard addition method in the range of examined concentrations. In comparison, Lo Coco et al. (8) found a similar value of *R* = 0.998 for analysis of cadmium in olive oils by dPSA method. Moreover, the within-day precision (expressed as RSD% of the slope for sample B-1, *n* = 5) were 0.53, 0.82, and 0.65% for Cd(II), Pb(II), and Cu(II), indicating reasonable repeatability of the studied GSCP method.

Instrument time used for simultaneous determination of Cd(II), Pb(II), and Cu(II) in fat samples by GSCP method did not exceed 180 s, whereas 480 s were necessary for dPAS analysis of Pb(II) and Cd(II) in wheat (39), which suggests the usefulness of the proposed method in fast analyses.

In our studies, the detection limits were 0.02, 0.02, and 0.06 μ g L⁻¹ for Cd(II), Pb(II), and Cu(II), respectively (electrochemical conditions, Table 1). Wahdat et al. (11) obtained an LOD of 0.5 ng g⁻¹ for Cd(II) in oil samples after 120 s of electrolysis time. In addition, LODs of Cd(II), Pb(II), and Cu(II) in olive oil samples were 1.2, 5.9, and 3.6 ng g⁻¹, respectively (plating time = 180 s and stripping time = 10 s) (6). In comparison, LODs obtained for cadmium, lead, and copper ions, in our study, were two orders lower than those for dPSA analyses of these metals in olive oils at similar electrolysis time (6). It is noteworthy that LOD in electrochemical techniques depends on the determined element, matrix composition, and electrolysis time (6, 25, 39). Therefore, the proposed method offers the possibility of improving the detection limits and reducing the analysis time.

Validation of the Proposed GSCP Method. The concentrations of heavy metal ions in the analyzed margarines and butters determined by GSCP method were compared with these obtained by GFAAS. (Tables 3 and 4).

The content of Cd(II) in margarines samples obtained by GSCP method (23.9, 25.5, 26.2, and 23.5 μ g kg⁻¹ for M-1, M-2, M-3, and M-4, respectively) was fairly constant and significantly

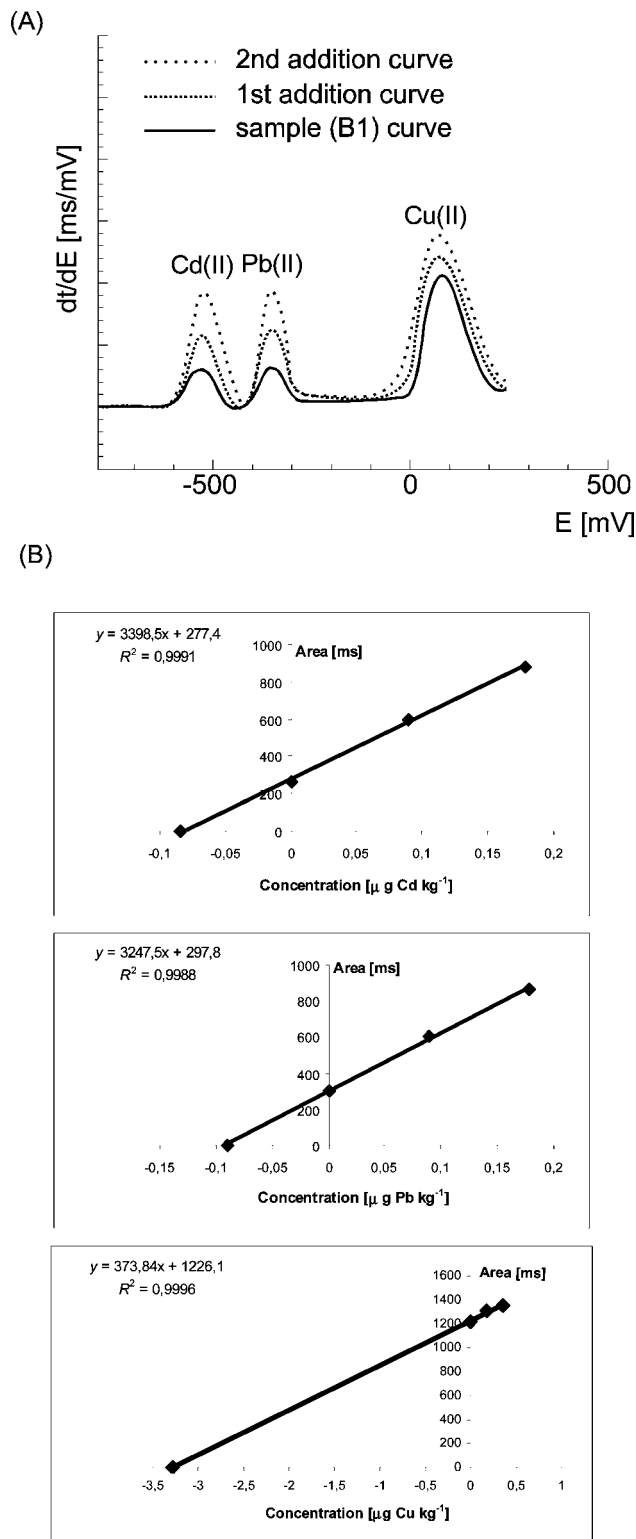


Figure 1. (A) Stripping chronopotentiograms. (B) Results of the simultaneous determination of Cd(II), Pb(II), and Cu(II) in B-1 sample.

higher when compared to studied butters (9.1, 16.4, 16.1, and 16.2 $\mu\text{g kg}^{-1}$ for B-1, B-2, B-3, and B-4, respectively). It can be noted that, Cd(II) concentration in B-1 (9.1 $\mu\text{g kg}^{-1}$) was the lowest among analyzed fats and well below the Polish standard values 20 and 30 $\mu\text{g kg}^{-1}$ (for butters and margarines, respectively) (22, 43). Moreover, the level of Cd(II) in the discussed fats was significantly lower than that in the margarine analyzed by Buldini (about 50 $\mu\text{g kg}^{-1}$) (23). On the other hand, the concentration of Cd(II) in vegetable oils reported by Wahdat

were below the detection limits of voltammetric method (0.5–1 μg of Cd kg^{-1}) (11).

The level of lead in the analyzed fats was much lower than that recommended by Polish Standard PN-EN ISO 12193:2000 (13), 100 $\mu\text{g kg}^{-1}$, and varied between 9.2 and 14.2 $\mu\text{g kg}^{-1}$. The content of Pb(II) in all the studied fats was about four times lower when compared to results obtained by Buldini et al. (23) and Wahdat et al. (11) ($c_{\text{Pb(II)}} \approx 50 \mu\text{g kg}^{-1}$ of fat). Similar concentrations of Pb(II) (10.2, 14.1, 12.9, and 13.4 $\mu\text{g kg}^{-1}$) and Cd(II) (9.1, 16.4, 16.1, and 16.2 $\mu\text{g kg}^{-1}$ for B-1, B-2, B-3, and B-4, respectively) in the studied butter samples were observed. However, the results listed in Table 3 indicate a 2-fold amount of Cd(II) in relation to Pb(II) in all margarines. For comparison, in Buldini's work, concentrations of Cd(II) and Pb(II) in edible oils and margarine were in the same range (<50 $\mu\text{g kg}^{-1}$) (23).

Among the determined heavy metal ions, the copper content was the highest in all fat samples and ranged between 298.2 and 364.0 $\mu\text{g kg}^{-1}$; however, this is below the Polish Standard PN-EN ISO 8294:2000 (12) prescribed limit of 400 $\mu\text{g kg}^{-1}$. It is noteworthy that the levels of Cu(II) in analyzed fats were lower in comparison with the reported results (2470 $\mu\text{g kg}^{-1}$ for margarines and 400–680 $\mu\text{g kg}^{-1}$ for vegetable oils (2), 601–1354 $\mu\text{g kg}^{-1}$ (11), 648–1289 $\mu\text{g kg}^{-1}$ for edible oils (44)), although surprisingly low amounts of Cu(II) in margarine (49 $\mu\text{g kg}^{-1}$ (23)) and edible oils (12.8–50.5 and 15.94–58.51 $\mu\text{g kg}^{-1}$) were determined by Buldini et al. (23) and La Pera et al. (6), respectively.

The intra-day precision (repeatability) of proposed GSCP and standard GFAAS methods was tested by analyses of all commercial samples (four margarines and four butters) in five replicates. The values of RSD were below 1.1% for copper determination, indicating reasonable repeatability of used methods (Tables 3 and 4). For comparison, RSD values obtained for Cu(II) determination in margarine by DPASV and IC methods were insignificantly higher (1.6 and 2.1%, respectively) (23). Moreover, a satisfactory intra-day precision (expressed as $\text{RSD} < 5\%$) can also be obtained for very low cadmium and lead concentrations (<30 $\mu\text{g kg}^{-1}$) in the real samples. In addition, the RSD values and confidence limits for both methods were comparable. Also, similar content of those elements in milk powder were detected using DPASV and ETAAS methods, but obtained RSD values were larger than our results ($c_{\text{Cd(II)}} = 21.0 \mu\text{g kg}^{-1}$, $\text{RSD} = 5.7\%$; $c_{\text{Pb(II)}} = 52.0 \mu\text{g kg}^{-1}$, $\text{RSD} = 23.1\%$ for DPASV method, and $c_{\text{Cd(II)}} = 22.2 \mu\text{g kg}^{-1}$, $\text{RSD} = 5.0\%$; $c_{\text{Pb(II)}} = 52.0 \mu\text{g kg}^{-1}$, $\text{RSD} = 15.4\%$ for ETAAS method) (35).

Although, results listed in Tables 3 and 4 indicate that the average concentrations of heavy metal ions in fats and their standard deviations obtained by standard GFAAS method were somewhat higher than those obtained by GSCP procedure, F-test revealed no significant difference between the variances (squares of the standard deviations) of the applied methods at $P = 0.05$. The calculated F_1 , F_2 , and F_3 values (the variance ratio of standard GFAAS and proposed GSCP methods for Cd(II), Pb(II), and Cu(II) determinations, respectively), ranging from 1.06 to 4.57, are below $F_{\text{theoretical}} = 6.39$. Therefore, the proposed GSCP and standard GFAAS methods do not significantly differ in their precision. However, the experimental t -values for the studied metal ions in most fat samples were higher than the critical value $t_4 = 2.78$ ($P = 0.05$). The results collected in Tables 3 and 4 (t -values and confidence intervals) indicate that there are significant differences between the mean concentrations of Cd(II) in M-1, M-2, M-3, and B-1; of Pb(II) in M-1, M-2,

Table 3. Determination of Cd(II), Pb(II) and Cu(II) in Margarines ($\mu\text{g kg}^{-1}$ Wet Weight)

statistical parameters	samples/methods							
	M-1		M-2		M-3		M-4	
	GSCP	GFAAS	GSCP	GFAAS	GSCP	GFAAS	GSCP	GFAAS
$C_{\text{Cd(II)}}^a$	23.9	27.8	25.5	27.1	26.2	27.1	23.5	24.1
SD ^a	0.25	0.40	0.36	0.38	0.49	0.52	0.53	0.70
RSD ^a (%)	1.1	1.4	1.4	1.4	1.9	1.9	2.3	2.9
confidence limit ^b	23.9 ± 0.3		27.8 ± 0.5		25.5 ± 0.5		27.1 ± 0.5	
$F_1^a = s_2^2/s_1^2$	2.55		1.09		1.11		1.74	
t_{calcd}	26.34		7.44		3.77		1.47	
$C_{\text{Pb(II)}}^a$	11.3	12.4	14.2	15.3	10.9	13.4	9.2	9.4
SD ^a	0.4	0.4	0.2	0.4	0.2	0.4	0.2	0.4
RSD ^a (%)	3.2	3.3	1.6	2.5	1.6	2.8	2.6	4.3
confidence limit ^b	11.3 ± 0.4		12.4 ± 0.5		14.2 ± 0.3		15.3 ± 0.5	
$F_2^a = s_4^2/s_3^2$	1.30		2.79		4.57		2.76	
t_{calcd}	6.63		4.93		10.94		0.90	
$C_{\text{Cu(II)}}^a$	298.2	315.0	356.4	377.8	316.4	342.2	320.0	326.6
SD ^a	2.6	3.2	2.7	3.3	2.3	4.4	3.0	3.5
RSD ^a (%)	0.87	1.0	0.76	0.87	0.94	1.3	0.94	1.1
confidence limit ^b	298.2 ± 3.2		315.0 ± 3.9		356.4 ± 3.4		377.8 ± 4.1	
$F_3^a = s_6^2/s_5^2$	1.49		1.47		2.24		1.06	
t_{calcd}	6.67		11.04		9.46		3.23	

^a $n = 5$; $C_{\text{Cd(II)}}$, $C_{\text{Pb(II)}}$, $C_{\text{Cu(II)}}$, concentrations of Cd(II), Pb(II), and Cu(II), respectively ($\mu\text{g kg}^{-1}$). ^b Probability level, $P = 0.05$; SD, standard deviation; RSD, relative standard deviation, s_1^2 , s_2^2 , s_3^2 , s_4^2 , s_5^2 , s_6^2 , variance of results of Cd(II), Pb(II), and Cu(II) determinations for GSCP and GFAAS methods, respectively; $F_{\text{theoretical}}^b = 6.39$; $t_{\text{theoretical}}^b = 2.78$; $t_{\text{theoretical}}^b = 8.61$ ($P = 0.001$).

Table 4. Determination of Cd(II), Pb(II) and Cu(II) in Butters ($\mu\text{g kg}^{-1}$ Wet Weight)

statistical parameters	sample/method							
	B-1		B-2		B-3		B-4	
	GSCP	GFAAS	GSCP	GFAAS	GSCP	GFAAS	GSCP	GFAAS
$C_{\text{Cd(II)}}^a$	9.1	10.5	16.4	17.1	16.1	17.0	16.2	17.4
SD ^a	0.3	0.4	0.7	0.8	0.5	0.8	0.5	0.9
RSD ^a (%)	3.5	4.1	4.5	4.8	3.0	4.6	3.3	4.9
confidence limit ^b	9.1 ± 0.4		10.5 ± 0.5		16.4 ± 0.9		17.1 ± 1.0	
$F_1^a = s_2^2/s_1^2$	1.82		1.25		2.71		2.53	
t_{calcd}	6.84		1.17		1.75		2.12	
$C_{\text{Pb(II)}}^a$	10.2	10.8	14.1	16.6	12.9	15.1	13.4	14.2
SD ^a	0.5	0.5	0.3	0.5	0.4	0.6	0.4	0.5
RSD ^a (%)	4.5	4.6	1.8	3.0	3.2	4.1	3.1	3.7
confidence limit ^b	10.2 ± 0.6		10.8 ± 0.6		14.1 ± 0.3		16.6 ± 0.6	
$F_2^a = s_4^2/s_3^2$	1.17		4.03		2.20		1.65	
t_{calcd}	2.19		8.61		4.90		4.33	
$C_{\text{Cu(II)}}^a$	364.0	382.8	343.8	357.4	300.0	324.2	305.8	329.4
SD ^a	3.8	4.2	3.7	4.2	2.4	3.4	3.1	3.9
RSD ^a (%)	1.1	1.1	1.1	1.2	0.78	1.1	1.0	1.2
confidence limit ^b	364.0 ± 4.7		382.8 ± 5.2		343.8 ± 4.6		357.4 ± 5.2	
$F_3^a = s_6^2/s_5^2$	1.19		1.30		2.13		1.53	
t_{calcd}	10.29		9.71		10.01		10.93	

^a $n = 5$; $C_{\text{Cd(II)}}$, $C_{\text{Pb(II)}}$, $C_{\text{Cu(II)}}$, concentrations of Cd(II), Pb(II), and Cu(II), respectively ($\mu\text{g kg}^{-1}$). ^b Probability level, $P = 0.05$; SD, standard deviation; RSD, relative standard deviation, s_1^2 , s_2^2 , s_3^2 , s_4^2 , s_5^2 , s_6^2 , variance of results of Cd(II), Pb(II), and Cu(II) determinations for GSCP and GFAAS methods, respectively; $F_{\text{theoretical}}^b = 6.39$; $t_{\text{theoretical}}^b = 2.78$; $t_{\text{theoretical}}^b = 8.61$ ($P = 0.001$).

M-3, B-2, B-3, and B-4; and of Cu(II) in all fat samples, assayed by both analytical methods. Comparison of two experimental means of metals contents ($n = 5$) in studied fats indicated that the proposed method in some cases is affected by systematic errors, although the results of Cd(II) and Pb(II) determinations in all fat samples (with the exception of M-1 and M-3, respectively) obtained by the two analytical methods do not differ significantly at $P = 0.001$ because $t_{\text{calculated}}$ are below $t_{\text{theoretical}} = 8.61$ (Tables 3 and 4). However, at high copper concentration, the proposed GSCP method does not give accurate results ($t_{\text{calculated}} > t_{\text{theoretical}}$, at $P = 0.001$).

On the other hand, the regression lines for comparing the two analytical methods were used.

The correlation plots between the obtained results of Cd(II), Pb(II), and Cu(II) determinations in different fat samples using

proposed GSCP and standard GFAAS methods were presented in Figure 2. A relatively high correlation coefficients ($R^2 = 0.973$ and 0.939 , $n = 8$ for Cd(II) and Cu(II) in all fat samples, respectively) indicate a good agreement between both methods. A somewhat lower correlation coefficient ($R^2 = 0.899$) was noted for lead determinations in the concentration range between 9.2 and $16.6 \mu\text{g kg}^{-1}$. Moreover, the slopes (b) of the regression lines (1.04 ± 0.18 , 1.22 ± 0.41 , 0.96 ± 0.25 for Cd(II), Pb(II), and Cu(II), respectively) were close to the model value of 1. In addition, the confidence limits of the intercepts ($a = 0.66 \pm 3.56$, -1.28 ± 4.98 and 32.04 ± 79.99 for Cd(II), Pb(II) and Cu(II), respectively) include the ideal value of 0. Therefore, the comparison between the results obtained by standard GFAAS and proposed GSCP methods indicates that the two

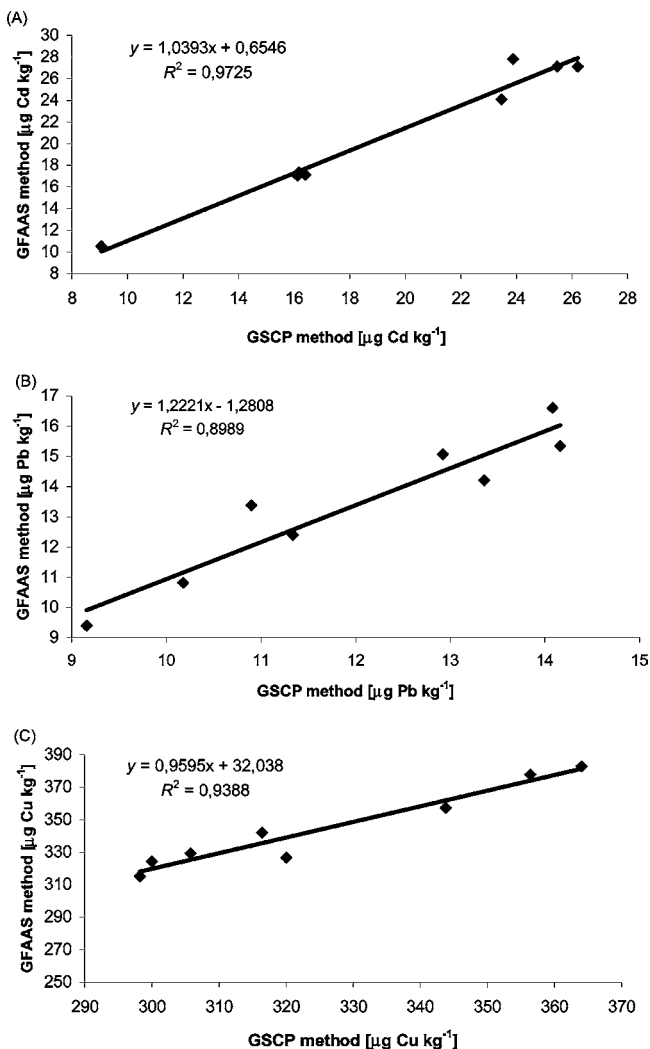


Figure 2. Correlation between GFAAS and GSCP methods for the determinations of (A) Cd(II), (B) Pb(II), and (C) Cu(II) in fat samples.

procedures give statistically comparable values of metal concentrations in fat samples.

The accuracy of the proposed and standard methods were expressed also as a recovery study, and the results are presented in Table 5.

The recoveries of cadmium, lead, and copper added to real samples solutions ranged between 96.7 and 101.0%, 99.1 and

Table 6. Reproducibility Test

sample	statistical parameters			
	C_{mean}^a ($\mu\text{g kg}^{-1}$)	SD ^a ($\mu\text{g kg}^{-1}$)	RSD ^a (%)	confidence limit ^b ($\mu\text{g kg}^{-1}$)
M-1	298.9	6.1	Cu(II)	298.9 ± 3.4
M-2			Cd(II)	
B-3	13.2	0.75	Pb(II)	13.2 ± 0.4

^a $n = 15$, each value is the mean of five determinations; each determination was repeated three times; C_{mean}^a , mean concentration; SD, standard deviation; RSD, relative standard deviation. ^b Probability level, $P = 0.05$

102.2%, and 98.9 and 101.0% for the studied method and 96.1 and 101.2%, 96.2 and 103.2%, and 98.2 and 99.6% for GFAAS method, respectively (Table 5). Moreover, repeatability (calculated using RSD, $n = 5$) for Cd(II), Pb(II), and Cu(II) determinations by both methods did not exceed 5%. La Pera et al. reported lower recovery values for cadmium ($84.52 \pm 9.86\%$), lead ($100.68 \pm 0.67\%$), and copper ($97.34 \pm 2.72\%$) determinations in olive oils by dPSA (6). As it can be expected, no statistically significant differences between the two techniques were obtained for all fat samples examined.

Reproducibility (inter-day precision) of the proposed method was evaluated by performing the determination within 3 days on M-1, M-2, and B-3 samples ($n = 5$), and results are reported in Table 6.

The average contents were $298.9 \mu\text{g}$ of Cu kg^{-1} in margarine M-1, with RSD = 2.0%; $25.8 \mu\text{g}$ of Cd kg^{-1} in margarine M-2, with RSD = 3.3%; and $13.2 \mu\text{g}$ of Pb kg^{-1} in butter B-3, with RSD = 5.7%. The confidence intervals of the mean values were 298.9 ± 3.4 , 25.8 ± 0.5 , and $13.2 \pm 0.4 \mu\text{g kg}^{-1}$ for Cu(II), Cd(II), and Pb(II), respectively. Comparison of the RSD values for repeatability (0.87, 1.4, and 3.2% for Cu(II), Cd(II), and Pb(II), respectively) with the reproducibility data (2.0, 3.3, and 5.7%) revealed that the inter-day precision was approximately two times higher than the intra-day precision of the proposed method. The same relation between intra-day and inter-day precisions (3 and 6%, respectively) was observed in the case of Cd(II) determination in edible oils and fats by ETAAS method (18).

The proposed GSCP method is relatively simpler and shorter (c.a. 180 s) than standard GFAAS method; therefore, it can be

Table 5. Recovery Tests

metal concn ($\mu\text{g kg}^{-1}$)	method	sample							
		M-1	M-2	M-3	M-4	B-1	B-2	B-3	B-4
		found ^a concentration							
Cd(II)	GSCP	37.6 (1.7)	40.8 (2.1)	40.9 (2.1)	38.1 (2.2)	23.8 (1.6)	30.6 (3.1)	31.4 (2.1)	31.2 (2.5)
	GFAAS	41.9 (2.3)	42.6 (3.6)	41.6 (2.8)	38.6 (3.9)	24.5 (3.9)	31.6 (4.2)	31.5 (2.8)	31.9 (3.3)
Pb(II)	GSCP	21.1 (3.5)	24.0 (2.9)	21.3 (1.9)	19.5 (3.6)	20.6 (3.2)	23.9 (2.6)	23.4 (4.2)	23.6 (1.3)
	GFAAS	21.9 (3.7)	25.4 (3.0)	23.0 (3.0)	19.8 (4.5)	21.0 (3.4)	25.6 (2.9)	25.9 (4.9)	24.1 (3.1)
Cu(II)	GSCP	492.7 (0.54)	550.7 (0.47)	515.2 (1.1)	517.4 (0.88)	566.8 (0.34)	549.1 (0.73)	494.4 (0.36)	508.2 (0.41)
	GFAAS	511.0 (0.26)	575.4 (0.58)	538.6 (0.94)	521.7 (0.72)	579.3 (0.59)	550.6 (0.81)	514.7 (0.95)	525.2 (0.66)
		recovery (%)							
Cd(II)	GSCP	96.7	100.7	99.3	99.0	98.8	97.5	101.0	100.0
	GFAAS	97.9	101.2	98.8	98.7	96.1	98.4	98.4	98.5
Pb(II)	GSCP	99.1	99.2	101.9	101.6	102.0	99.2	102.2	100.9
	GFAAS	97.8	100.4	98.3	102.1	101.0	96.2	103.2	99.6
Cu(II)	GSCP	98.9	99.0	99.8	99.5	100.5	101.0	98.9	100.5
	GFAAS	99.2	99.6	99.3	99.1	99.4	98.8	98.2	99.2

^a $n = 5$; Found concentrations of Cd(II), Pb(II) and Cu(II) in studied fats after addition standard solutions of Cd(NO₃)₂ ($15 \mu\text{g L}^{-1}$), Pb(NO₃)₂ ($10 \mu\text{g L}^{-1}$), and Cu(NO₃)₂ ($200 \mu\text{g L}^{-1}$) to the samples; RSD (%) values are given in parentheses

useful for the simultaneous determination of Cd(II), Pb(II), and Cu(II) in vegetable and animal fats after wet digestion. Moreover, the cost of the instrumentation is considerably lower than that in the case of standard GFAAS method. It is noteworthy that our method does not require a toxic mercury solution or mercury drop electrode and technical gases for sample deaeration.

LITERATURE CITED

- Cabrera, C.; Lloris, F.; Gimenez, R.; Olalla, M.; Lopez, C. Mineral content in legumes and nuts: contribution to the Spanish dietary intake. *Sci. Total Environ.* **2003**, *308*, 1–14.
- Onianwa, P. C.; Adeyemo, A. O.; Idowu, O. E.; Ogabiela, E. E. Copper and zinc contents of Nigerian foods and estimates of the adult dietary intakes. *Food Chem.* **2001**, *72*, 89–95.
- Paz, I.; Molero, M. Catalytic effect of solid metals on thermal stability of olive oils. *JAOCs* **2000**, *77*, 127–130.
- Joint FAO/WHO (World Health Organization) Expert Committee for Additives and Contaminants (JECFA): 33. Report: *Evaluation of Certain Food Additives and Contaminants*. WHO Techn. Report Series 837: Geneva, 1993.
- Joint FAO/WHO (World Health Organization) Expert Committee for Additives and Contaminants (JECFA): 33. Report: *Evaluation of Certain Food Additives and Contaminants*. WHO Techn. Report Series 776: Geneva, 1989.
- La Pera, L.; Lo Curto, S.; Visco, A.; La Torre, L.; Dugo G. Derivative potentiometric stripping analysis (dPSA) used for the determination of cadmium, copper, lead, and zinc in sicilian olive oils. *J. Agric. Food Chem.* **2002**, *50*, 3090–3093.
- Bati, B.; Cesur, H. Determination of copper in edible oils by atomic absorption spectrometry after lead piperazinedithiocarbamate solid-phase extraction and potassium cyanide back-extraction. *Anal. Sci.* **2002**, *18*, 1273–1274.
- Lo Coco, F.; Ceccon, L.; Ciruolo, L.; Novelli, V. Determination of cadmium(II) and zinc(II) in olive oils by derivative potentiometric stripping analysis. *Food Control* **2003**, *14*, 55–59.
- Tamrakar, P. K.; Pitre, K. S. Analysis of crude oil for its trace metals content. *Indian J. Chem. Sect. A.: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.* **2000**, *39*, 779–783.
- Allen, L. B.; Siitonen, P. H.; Thompson, H. C. Determination of copper, lead, and nickel in edible oils by plasma and furnace atomic spectroscopies. *JAOCs* **1998**, *75*, 477–481.
- Wahdat, F.; Hinkel, S.; Neeb, R. Direct inverse voltammetric determination of Pb, Cu, and Cd in some edible oils after solubilization. *Fresenius J. Anal. Chem.* **1995**, *352*, 393–394.
- Standard Method for the Analysis of Animal and Vegetable Fats and Oils. Determination of copper, iron, and nickel contents. Graphite furnace atomic absorption method. PN-EN ISO 8294: 2000, 2000.
- Standard Method for the Analysis of Animal and Vegetable Fats and Oils. Determination of lead content. Graphite furnace atomic absorption method. PN-EN ISO 12193: 2000, 2000.
- Hendrikse, P. W.; Slikkerveer, F. J.; Zaalberg, J.; Hautfenne, A. Determination of copper, iron, and nickel in oils and fats by direct graphite furnace atomic absorption spectrometry. Results of a collaborative study and the standardized method. *Pure. Appl. Chem.* **1988**, *60*, 893–900.
- Capar, S. G. Determination of copper, iron, and nickel in oils and fats by direct graphite furnace atomic absorption spectrometry: summary of collaborative study. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 320–321.
- Lynch, S.; Littlejohn, D. Development of a slurry atomization method for the determination of cadmium in food samples by electrothermal atomization atomic absorption spectrometry. *Talanta* **1990**, *37*, 825–830.
- Firestone, D. Direct graphite furnace-atomic absorption method for determination of lead in edible oils and fats: summary of collaborative study. *J. Assoc. Off. Anal. Chem.* **1994**, *77*, 951–954.
- van Dalen, G. Determination of cadmium in edible oils and fats by direct electrothermal atomic absorption spectrometry. *J. Anal. At. Spectrom.* **1996**, *11*, 1087–1092.
- Volynsky, A. B. Graphite atomizers modified with high-melting carbides for electrothermal atomic absorption spectrometry. II. Practical aspects. *Spectrochim. Acta, Part B* **1998**, *53*, 1607–1645.
- Standard Method for the Analysis of Animal and Vegetable Fats and Oils. Determination of heavy metals contents by atomic emission spectrometry method. Determination of copper contents. PN-A-86939-3:1998, 1998.
- Standard Method for the Analysis of Animal and Vegetable Fats and Oils. Determination of heavy metals contents by atomic emission spectrometry method. Determination of lead contents. PN-A-86939-5:1998, 1998.
- Standard Method for the Analysis of Animal and Vegetable Fats and Oils. Determination of heavy metals contents by atomic emission spectrometry method. Determination of cadmium contents PN-A-86939-7:1999, 1999.
- Buldini, P. L.; Ferri, D.; Sharma, J. L. Determination of some inorganic species in edible vegetable oils and fats by ion chromatography. *J. Chromatogr. A* **1997**, *789*, 549–555.
- van Staden, J. F.; Matoetoe, M. C. Simultaneous determination of copper, lead, cadmium, and zinc using differential pulse anodic stripping voltammetry in a flow system. *Anal. Chim. Acta* **2000**, *411*, 201–207.
- Riso, R. D.; Le Corre, P.; Chaumery, Ch. J. Rapid and simultaneous analysis of trace metals (Cu, Pb, and Cd) in seawater by potentiometric stripping analysis. *Anal. Chim. Acta* **1997**, *351*, 83–89.
- Chow, Ch. W. K.; Kolev, S. D.; Davey, D. E.; Mulcahy, D. E. Determination of copper in natural waters by batch and oscillating flow injection stripping potentiometry. *Anal. Chim. Acta* **1996**, *330*, 79–87.
- Omanović, D.; Peharec, Ž.; Pižeta, I.; Brug, G.; Branica, M. A new mercury drop electrode for trace metal analysis. *Anal. Chim. Acta* **1997**, *339*, 147–153.
- Beinrohr, E.; Csémi, P.; Manová, A.; Dzurov, J. Absolute analysis of trace metals through galvanostatic stripping chronopotentiometry with signal accumulation. *Fresenius J. Anal. Chem.* **1994**, *349*, 625–632.
- Muñoz, E.; Palmero, S.; García-García, Ma A. A continuous flow system design for simultaneous determination of heavy metals in river water samples. *Talanta* **2002**, *57*, 985–992.
- Moreno, M. A.; Marin, C.; Vinagre, F.; Ostapczuk, P. Trace element levels in whole blood samples from residents of the city Badajoz, Spain. *Sci. Total Environ.* **1999**, *229*, 209–215.
- Giroussi, S. T.; Voulgaropoulos, A. N.; Ayiannidis, A. Simultaneous voltammetric determination of molybdenum and copper in biological samples. *Fresenius J. Anal. Chem.* **1997**, *357*, 429–432.
- Gozzo, M. L.; Colacicco, L.; Callà, C.; Barbaresi, G.; Parroni, R.; Giardina, B.; Lippa, S. Determination of copper, zinc, and selenium in human plasma and urine samples by potentiometric stripping analysis and constant current stripping analysis. *Clin. Chem. Acta* **1999**, *285*, 53–68.
- Locatelli, C. Analytical procedure for the simultaneous voltammetric determination of toxic metals in dialysis fluids. *Anal. Bioanal. Chem.* **2003**, *376*, 518–523.
- Gillain, G.; Rutagengwa, J. Determination of zinc, cadmium, lead, copper, antimony, and bismuth in milk by differential pulse anodic stripping voltammetry with a hanging mercury drop electrode following two independent mineralization methods. *Anal. Chim. Acta* **1985**, *13*, 471–473.
- Karadjova, I.; Girousi, S.; Iliadou, E.; Stratis, I. Determination of Cd, Co, Cr, Cu, Fe, Ni, and Pb in milk, cheese, and chocolate. *Mikrochim. Acta* **2000**, *134*, 185–191.
- Queirolo, F.; Stegen, S.; Restovic, M.; Paz, M.; Ostapczuk, P.; Schwuger, M. J.; Muñoz, L. Total arsenic, lead, and cadmium levels in vegetables cultivated at the Andean villages of northern Chile. *Sci. Total Environ.* **2000**, *255*, 75–84.

- (37) Tripathi, R. M.; Raghunath, R.; Sastry, V. N.; Krishnamoorthy, T. M. Daily intake of heavy metals by infants through milk and milk products. *Sci. Total Environ.* **1999**, *227*, 229–235.
- (38) Sanna, G.; Pilo, M. I.; Piu, P. C.; Tapparo, A.; Seeber, R. Determination of heavy metals in honey by anodic stripping voltammetry at microelectrodes. *Anal. Chim. Acta* **2000**, *415*, 165–173.
- (39) Lo Coco, F.; Monotti, P.; Fiecchi, V.; Ceccon, L. Determination of lead(II) and cadmium(II) in hard and soft wheat by derivative potentiometric stripping analysis. *Anal. Chim. Acta* **2000**, *409*, 93–98.
- (40) Sancho, D.; Vega, M.; Debán, L.; Pardo, R.; González G. Determination of copper and arsenic in refined beet sugar by stripping voltammetry without sample pretreatment. *Analyst* **1998**, *123*, 743–747.
- (41) Kadara, R. O.; Newman, J. D.; Tothill, I. E. Stripping chronopotentiometric detection of copper using screen-printed three-electrode system – application to acetic-acid bioavailable fraction from soil samples. *Anal. Chim. Acta* **2003**, *493*, 95–104.
- (42) Miller, J. N.; Miller, J. C. In *Statistic and Chemometrics for Analytical Chemistry*, 4th ed.; Pearson Education: Harlow, England, 2000.
- (43) Official Journal Republic of Poland, no. 9, item 72, Warsaw, Poland, 2001.
- (44) Reddy, K. H.; Prasad, N. B. L.; Reddy, T. S. Analytical properties of 1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone: simultaneous spectrophotometric determination of copper(II) and nickel(II) in edible oils and seeds. *Talanta* **2003**, *59*, 425–433.

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