



HAL
open science

QUANTIFICATION OF LEAD AND CADMIUM IN POULTRY AND BIRD GAME MEAT BY SQUARE WAVE ANODIC STRIPPING VOLTAMMETRY

Marcello Trevisani, Matilde Checchini, Roberto Rosmini, Lorenzo Vercellotti,
Luca Taffetani

► **To cite this version:**

Marcello Trevisani, Matilde Checchini, Roberto Rosmini, Lorenzo Vercellotti, Luca Taffetani. QUANTIFICATION OF LEAD AND CADMIUM IN POULTRY AND BIRD GAME MEAT BY SQUARE WAVE ANODIC STRIPPING VOLTAMMETRY. *Food Additives and Contaminants*, 2011, 28 (02), pp.180-188. 10.1080/19440049.2010.545441 . hal-00668580

HAL Id: hal-00668580

<https://hal.science/hal-00668580>

Submitted on 10 Feb 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



QUANTIFICATION OF LEAD AND CADMIUM IN POULTRY AND BIRD GAME MEAT BY SQUARE WAVE ANODIC STRIPPING VOLTAMMETRY

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2010-257.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	23-Nov-2010
Complete List of Authors:	Trevisani, Marcello; University of Bologna, Veterinary Public Health and Animal Pathology Checchini, Matilde; Università di Bologna, Sanità Pubblica Veterinaria e Patologia Animale Rosmini, Roberto; Università di Bologna, Sanità Pubblica Veterinaria e Patologia Animale Vercellotti, Lorenzo; A.S.L. Vercelli, Servizio Veterinario Taffetani, Luca; Azienda ASL Forlì, Servizio Veterinario
Methods/Techniques:	Quality assurance, Metals analysis - stripping voltametry
Additives/Contaminants:	Heavy metals, Lead, Heavy metals - cadmium
Food Types:	Meat, Animal products – meat

SCHOLARONE™
Manuscripts

Quantification of lead and cadmium in poultry and bird game meat by Square Wave Anodic Stripping Voltammetry

M. Trevisani^{a*}, M. Cecchini^a, L. Taffetani^b, L. Vercellotti^b and R. Rosmini^a

^aDepartment of Veterinary Public Health and Animal Pathology, University of Bologna, I40064 Ozzano Emilia, Italy; ^bServizio Veterinario A..S.L., Forlì, Italy; ³Servizio Veterinario A.S.L., Vercelli, Italy

Summary

A Square Wave Anodic Stripping Voltammetric method for the analysis of lead and cadmium in chicken muscle and liver was developed and validated, and the results of a monitoring study relative to chicken and pigeon meat are reported. The voltammetric method allows the analysis of lead and cadmium at the same time in samples after acid digestion. The use of perchloric acid for digestion and of acetate buffer in the supporting electrolyte have been found suitable to reduce matrix interferences and obtain limits of quantification which were below 10 ng g⁻¹ for meat and liver samples. The regression between analytical signal and concentration of the target analytes in spiked samples and certified reference materials proved to be linear within the 10 – 100 ng g⁻¹ range for meat and within the 50 – 500 ng g⁻¹ range for liver. The analytical method was verified using available certified reference materials BCR-184 (cattle meat) and BCR-185R (cattle liver) as well as with spiked chicken samples. Precision (i.e repeatability and intermediate precision) and accuracy (% recovery and bias) were of the order of 0.3 -4.5 % for both lead and cadmium. The level of lead in muscle was in the range between 6.4 and 59.8 ng g⁻¹ in chickens and 7.9 – 63.6 ng g⁻¹ in farmed pigeons, whereas it was 8.0 – 84.4 ng g⁻¹ in chicken liver. The cadmium concentration was 0.4 – 10.4 ng g⁻¹ in chicken muscle, 10.4 – 90.6 ng g⁻¹ in chicken liver and 2.2 – 8.0 ng g⁻¹ in farmed pigeons.

Keywords: Metals analysis - stripping voltammetry; meat; lead; cadmium

* Corresponding author. Email: marcello.trevisani@unibo.it

Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional tolerable weekly intake (PTWI) for lead and cadmium (WHO, 2000; JECFA, 2005). These were set to 25 $\mu\text{g}/\text{kg}$ body weight for lead and 7 $\mu\text{g}/\text{kg}$ b.w. for cadmium. On this basis, and taking into account the food consumption patterns, maximum limits for these trace contaminants in food have been set (Berg and Licht, 2002) but many factors contribute to the uncertainty of analytical results and therefore characteristics of the analytical methods used for control and monitoring purpose have been defined (EC 2007). Although meat is not regarded as a major source of human exposure to lead and cadmium (EC, 2004a; EFSA, 2004a EFSA, 2004b) the results of monitoring programmes showed that some samples may exceed the maximum limits set and investigations are needed to trace the source to prevent contamination, and also to evaluate if they actually represent a hazard for target populations. Major concern is given indeed to offal consumption (i.e. liver) because these animal tissues accumulate toxic heavy metals, but only a few studies were addressed with respect to chicken liver, whose consumption is very popular in some regions like the Central Italy. The need for suitable methods that can produce valid data and give information on their full uncertainty has been noted in many reports especially in the context of a risk assessment. Atomic Adsorption Spectrometry (AAS) and Inductively Couple Plasma coupled with Mass Spectrometry (ICP/MS) are the most commonly used method for trace analysis and studies were already be done to evaluate their validity for analyses of many food matrices, including offal (D'Illio et al, 2008; Forte and Bocca, 2007). Benefits and drawbacks of analytical methods for trace elements analysis has been discussed taking into account difficulties due to possible interferences, detection limits, throughput and cost (Brown and Milton, 2005). Undoubtedly spectrometric techniques like Atomic Adsorption Spectrometry (AAS) and Inductively Coupled Plasma coupled with Mass Spectrometry (ICP/MS) are the preferred methods for laboratories that have to process a large number of samples for their higher throughput, but for the determination of electro-active trace elements like heavy metals stripping voltammetry has been reported to provide comparable results in terms of precision and limits of detection. In addition it has the advantage of requiring relatively inexpensive instrumentation, having low initial and running costs and also being capable of determining trace level of heavy metals like lead and cadmium at the same time (Capar and Subjoc, 1982; Locatelli, 2000; Locatelli and Torsi, 2003; Omanovic and Branica, 2003; Vos et al., 1990; Wang, 2000). With the voltammetric stripping methods, the electro active metals are concentrated at the surface of a polarized modified electrode immersed in sample solution (i.e. by application of a plating potential) and then re-dissolved (stripped) from it by means of oxido-reductants or by progressively decreasing potential ramps. The stripping potential is specific, in controlled conditions (i.e. support electrolytes and pH) for the target analyte (Bard and Faulkner,

1
2
3 1980; Wang, 1994). This study proposes a procedure for the simultaneous determination of lead and
4 cadmium in poultry (muscle and liver) samples by Square Wave Anodic Stripping Voltammetry
5 (SWASV). The method uses an optimized microwave acid digestion procedure and acetate buffer in
6 the supporting electrolyte in order to improve the recovery of target analytes and reduce the
7 interference from other electroactive compounds. One aim of this study was to identify the sources of
8 uncertainty that could affect the results. The procedure recommended for in-lab validation studies in
9 the EC report on the “relationship between analytical results, measurement uncertainty, recovery
10 factors” was followed (EC, 2004b) and the laboratory control requirements for establishing the
11 performance criteria of methods for lead and cadmium determination (EC, 2007) were tested. The
12 method was validated using two certified reference meat and liver samples (with composition closest
13 to poultry meat) as well as chicken meat and liver samples that were artificially spiked at different
14 levels in order to define the quantification limit and the overall precision and accuracy (De Silva et al.,
15 2003) in a range between 10 and 200 ng g⁻¹ of lead in muscle (or 10 - 100 ng g⁻¹ of cadmium), and
16 between 10 and 172 ng g⁻¹ of lead in liver (or 10 - 544 ng g⁻¹ cadmium). Another aim was to
17 investigate the level of these contaminants in chicken and game (pigeon) meat in regularly
18 slaughtered animals.
19
20
21
22
23
24
25
26
27
28
29
30

31 **Materials and methods**

32 ***Instruments and labware***

33
34 Voltammetric measurements were made using a potentiostat/galvanostat Autolab Pgstat12
35 (EcoChemie,NL) controlled by the software GPES 4.7 for Window. The potentiostat was linked to a
36 three electrode electrochemical cell (C3 cell stand, BAS, USA) with glassy carbon working electrode
37 (3 mm diameter), Ag/AgCl reference electrode and platinum *counter* electrode. The working electrode
38 was cleaned using aluminium oxide powder (1 µm and 0.3 µm) and an electrochemical cleaning step
39 before measurements. The samples were digested by acid oxidizing reagents in Teflon closed vessels
40 with a microwave controlled heating program (Ethos D, Milestone, USA). The labware used for dilution
41 and preparation of reagents were certified Teflon volumetric class A flasks, Teflon beakers and
42 polyethylene bottles carefully cleaned with HNO₃ 10% and washed with Ultrapure water.
43
44
45
46
47
48
49

50 ***Reagents***

51
52 Ultrapure water (grade I, std CAP and ASTM) was used to prepare all solutions and wash the labware.
53 The supporting electrolyte in the electrochemical cell was HCl 0,1M, made with Suprapur HCl 30%
54 (Merk, D). A mercury nitrate (Hg²⁺) 1000 mg·L⁻¹ solution, which is used to overlay a mercury film on
55 the graphite working electrode, was made by dissolving metallic Hg, purified by distillation (Merk, D),
56 in 5M HNO₃.and water for dilution. Reagents used for mineralization of the meat and liver samples
57
58
59
60

1
2
3 were nitric acid 65%, hydrogen peroxide 20% and perchloric acid 60-70% (Suprapur, Merck, D). An
4 acetate buffer solution (pH 4.75) was made with acetic acid in HCl 0.1 M was used to set the pH of
5 mineralized samples to pH 2 (± 0.2). Working solutions $1 \text{ mg}\cdot\text{L}^{-1}$ of lead and cadmium in 0.1M HCl
6 were made using standard $1000 \text{ mg}\cdot\text{L}^{-1}$ lead and cadmium (Titrisol, Merk, D). Certified Reference
7 Materials BCR-184 (beef) and BCR-185R cattle liver, produced by the European Community Bureau
8 of Reference, and spiked chicken meat and liver samples were used for the validation procedure.
9 These chicken meat samples at lead and cadmium concentration in a range between 10 and $100 \text{ ng}\cdot\text{g}^{-1}$
10 were prepared using samples with very low concentration of lead and cadmium.
11
12
13
14
15
16

17 **Sample collection and preparation**

18
19 Muscle and liver samples from thirty different lots of regularly slaughtered broilers were analysed. In
20 addition muscle samples nine lots of pigeons were examined. Each sample was obtained by
21 combining aliquots (10 g each) of muscle or liver taken from 5 carcasses. Tight muscles were sampled
22 from broilers whereas breast muscle were taken from pigeons. The samples that showed the lowest
23 level of lead and cadmium were used for preparation of artificially spiked samples. The selected
24 muscle sample was spiked with lead and cadmium ($10, 50$ and $100 \text{ ng}\cdot\text{g}^{-1}$) whereas different spike
25 levels ($50, 100$ and 500 or $200 \text{ ng}\cdot\text{g}^{-1}$) was used for the liver sample. Due to the lack of certified
26 reference samples from chicken meat, the Certified Reference samples BCR 184 Bovine Muscle (Pb
27 $239 \pm 11 \text{ ng}\cdot\text{g}^{-1}$; Cd $13 \pm 2 \text{ ng}\cdot\text{g}^{-1}$) and BCR 185R Bovine Liver (Pb $172 \pm 9 \text{ ng}\cdot\text{g}^{-1}$; Cd $544 \pm 17 \text{ ng}\cdot\text{g}^{-1}$)
28 were analysed and these muscle or liver certified samples were included in every batch of analysis,
29 which also included a blank. The homogenised muscle or liver samples ($0.5 \pm 0.005 \text{ g}$) or the
30 lyophilized certified samples ($0.3 \pm 0.005 \text{ g}$) were weighted directly in a Teflon mineralizing vessel. A
31 mixture of $\text{HNO}_3:\text{H}_2\text{O}_2:\text{HClO}_4$ in the ratio 6:2:1 was used for the microwave assisted acid digestion
32 using an optimised program (250W x 2 min., 0W x 1 min, 250 W x 5 min, 450 W x 4 min, 600 W x 3
33 min, 250W x 10 min and an additional 60 min venting step). After cooling (overnight) the digested
34 samples were brought in a Teflon beaker using ultra-pure water for washing the vessels and were
35 dried (almost completely) on a hotplate set at 80°C . The dried digested samples were dissolved in
36 the supporting electrolyte (HCl 0.1 M) and transferred in Teflon volumetric flasks. After the addition of
37 small amount of acetate buffer for adjusting pH (approx. pH=2) they were diluted up to 50 mL with HCl
38 0.1M. A blank solution (reagents without sample) is also prepared for every batch of the analysis.
39 Every batch included one blank, one certified reference material, one un-spiked muscle or liver
40 sample, the same sample spiked (two repetitions) the same sample with a different spike.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

56 **Voltammetric determination**

1
2
3 Lead and cadmium concentrations were measured using a SWASV procedure and the standard
4 addition method. A deposition potential equal to -1.1 V for the deposition of lead and cadmium and the
5 anodic stripping was assessed in the range between -0.25 and -1.1 V. (Table 1). Peaks of cadmium
6 and lead can be recorded at potentials of 0.65 and 0.45 V vs Ag/AgCl (0.1 MKCl). Lead and cadmium
7 peaks were recorded in a blank solution before then in samples, in order to quantify the trace level in
8 reagents or those originating from contamination of labware and environment. An aliquot of the diluted
9 digested sample (10 mL) was put in the electrochemical cells and was purged with N₂ to remove
10 dissolved oxygen. The peaks in the voltammogram were recorded (i vs E) and their area was
11 measured. The relative concentration of lead and cadmium in the diluted samples was determined by
12 the method of Standard Addition. All instrumental measures were repeated three times, using different
13 aliquots of the diluted samples, and also the determination of the concentration in blank is done using
14 the same procedure.
15
16
17
18
19
20
21
22
23

24 **Quality controls**

25 Analytical balance and micropipettes were regularly calibrated using approved procedures and
26 checked for their precision (i.e. for the balance the precision ≤ 0.2 mg and for the micropipettes
27 systematic error $\leq 1\%$ in the range 2-10 μL or $\leq 0.4\%$ in the range 20-50 μL or $\leq 0.8\%$ at 100 μL). The
28 stability of microwave oven was checked by applying specific power/time programmes to water
29 samples (i.e. 1000 mL) and measuring the temperature increments. The measured delta value must
30 be equal to the ratio between applied potency per time and a constant (e.g. $\Delta T = 1000\text{W} \cdot 1 \text{ min}/70$).
31 The constant (70) is specified by the producers of the microwave oven and is related to leakage of
32 energy and wavelength. The allowed tolerance (CV) must be $\pm 5\%$. The stability of the potential
33 applied for the voltammetric method was also checked regularly, using a calibration cell and the
34 procedures advised in the Autolab™ user's manual. Spiking, incomplete extraction, losses through
35 evaporation, laboratory environmental contamination, standard addition calibration are covered in the
36 precision study.
37
38
39
40
41
42
43
44
45
46

47 **Detection and Quantification Limits**

48 The detection and quantification limits (D.L. and Q.L.) were calculated from the average value (mean)
49 and standard deviation (SD) of Lead and Cadmium level in the entire pool of analysed blank samples
50 (i.e. D.L. = mean + 3 SD; Q.L. = mean + 10 SD) but also calculated by extrapolating the concentration
51 of lead or cadmium in blank using the linear regression curves interpolating data from analysis of
52 artificially spiked and the certified reference samples (i.e. D.L. = $(3 \cdot Sa)/b$; Q.L. = $(10 \cdot Sa)/b$, where Sa
53 is the calculated standard deviation of intercept in the linearity study).
54
55
56
57
58
59
60

Recovery and bias

The recovery and bias were calculated on the basis of at least five replicates of the digestion procedure for each artificially spiked sample or certified reference materials, and at least three repeated measurements on separate aliquots of the digested samples. In order to define the concentration of the original samples (muscle or liver) used to prepare the spiked samples, the analytical measurements were also replicated and repeated several times (i.e. 3 replicates and 7 repetitions). The mean bias is calculated as % relative error in ANOVA (interbatch) and its uncertainty $u(\text{bias})$ with the equations:

$$u(\text{bias}) = \sqrt{[(\text{bias})^2 + (\text{SE}_{\text{bias}})^2 + (\text{RSD}_{C_{\text{ref}}})^2 + (\text{RSD}_{\text{wgt}})^2]} \quad (3); \quad \text{RSD}_{C_{\text{ref}}} = 100 - \frac{\left(\frac{CI_{C_{\text{ref}}(95\%)}}{1.96} \right)}{C_{\text{ref}}} \quad (4)$$

where C_{ref} (or C_{spike}) is the concentration of lead or cadmium in the certified reference material (or in the spiked samples) and $\text{RSD}_{C_{\text{ref}}}$ (or $\text{RSD}_{C_{\text{spike}}}$) is the Relative Standard Deviation of this value. RSD_{wgt} is the Relative Standard Deviation of the sample weight.

Repeatability, and HORRATr

Repeatability refers to degree of agreement of results in inter-batch (between-run) and intra-batch (within-run) precision within the laboratory. They were calculated through an Excel spreadsheet using formulae for an analysis of variance (ANOVA) based on the one-factor random effects model as indicated in the paper of DeSilva et al. (2003). In details, the inter-batch standard deviation (repeatability) is calculated as the square root of the ANOVA Mean Square intra-batch component MSw, if it is greater than the ANOVA Mean Square inter-batch (MSb) component (otherwise it is equal to the square root of the total ANOVA Mean Square). The inter-batch standard deviation (intermediate precision) is calculated as the square root of the total ANOVA variance components if MSb is greater than the MSw (otherwise the square root of the total ANOVA Mean Square). $\text{HORRATr} = \text{RSDr}(\text{found})/\text{RSDr}(\text{calculated})$; where calculated RSDr depends on the level of analyte (typically 15% at concentration in the order of $10 \mu\text{g kg}^{-1}$). Acceptable values for this ratio are typically 0.5-2 and for EU Regulation 333/2007 must be below 2. In this study the intermediate precision was assessed within the same laboratory, by repeating the analyses on different days and using different batch of reagents and aliquots of the same samples.

Linearity

Reference materials (spiked samples at varying concentration and certified samples) were used in linearity study in order to validate a linear dynamic range for lead and cadmium in muscle and liver.

1
2
3 Materials at four concentration level for lead and cadmium. The ISO 11095:1996 and ISO 8466-
4 2:2001 type approaches were used. The Fisher-Snedecor test was used to verify the linearity within
5 the 17.4-239.0 range for lead and within the 13.0-103.2 range for cadmium in muscle, within the 59.2-
6 509.2 range for lead and the 60.5-544.0 range for cadmium in liver.
7
8
9

10 11 ***Specificity***

12 Interference from natural constituents of samples was investigated. Occurrence and concentration of
13 electro-active compound in the chicken muscle or liver producing voltammetric peaks that can overlap
14 or drift those of lead or cadmium and their effect on measurements at different quantification levels
15 were assessed.
16
17
18
19

20 21 ***Uncertainty components quantification***

22 The up-down Eurachem/Citac method (error propagation) was used for calculating the overall
23 uncertainty (Ellison et al, 2000).
24
25
26

27 **Results and Discussion**

28 29 ***Precision and accuracy***

30
31 The results of the precision and accuracy study are reported in Tables 2a and 2b. The analyses of
32 certified reference materials (BCR 184 and BCR 185R) and of artificially spiked chicken tissues show
33 that recovery of analytes (lead and cadmium) was almost complete. The mean bias for muscle was
34 indeed in the range from -0.34 to -0.70 for lead and from 0.03 to -1.03 for cadmium. The expected
35 tolerances for the mean recovery and inter-batch precision were within 5% for both lead and cadmium
36 in muscle as well as for lead in liver, but reached approximately 11% for cadmium in liver.
37 Repeatability and reproducibility of analytical determination within the lab were always good with
38 Horrat_r and Horrat_R values below 0.5 and fulfilled the EU regulation requirement (Horrat below 2).
39 Apart from uncertainty included in the precision and accuracy study other sources of uncertainty were
40 identified. Uncertainty on producing the stock solutions and the calibration standards should be
41 included in the overall uncertainty. The relative standard uncertainty related to the preparation of the
42 stock and intermediate solution is reported in Table 3. Therefore, the nominal concentration and the
43 expanded uncertainty (coverage factor k=2; 95%) of the standard solution used for calibration was
44 $100 \pm 0.24 \text{ ng}\cdot\text{mL}^{-1}$. The uncertainty values for the spiked chicken meat samples at different level of
45 lead and cadmium concentration and for the certified reference materials, which were used in the
46 precision study, is reported in Table 4. The combined uncertainties of the lead and cadmium
47 concentration in the artificially spiked were respectively 0.70% and 1.54%. The major contribution to
48 these uncertainties was given by the repeatability of determinations, weight of samples and
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 uncertainty of lead/cadmium concentration in the standard solution. It was showed that the combined
4 uncertainty of artificially spiked samples was similar to those of certified reference materials (BCR184
5 and 185R). The relative overall uncertainty for samples with 57 ng g⁻¹ of lead was 3.%, whereas for
6 samples with 53 ng g⁻¹ of cadmium was 4 % (data not shown). Therefore, the proposed analytical
7 method fulfils the requirements specified by the EU regulation 333/2007 other than relative standard
8 deviation calculated from results generated under reproducibility conditions (RSDR) which has not
9 been assessed.
10
11
12
13

14 15 16 ***Detection, Quantification Limits and Linearity***

17 The lower limit of detection and quantification (LLOD and LLOQ) calculated from the mean and
18 standard deviation of the entire pool of analysed blank samples were equal to 1. and 2 ng g⁻¹ for lead
19 (n = 73) and 0.1 ng g⁻¹ for cadmium (n= 85). The LLOQ calculated from the linearity study, i.e. ten
20 times the intercept standard deviation at lead or cadmium level equal to zero (intercept) were instead
21 1.9 and 0.9 ng g⁻¹ for lead and cadmium in muscles, 4.7 and 3.4 ng g⁻¹ for lead and cadmium in liver.
22 The linearity study showed that relations between analytes concentrations (N = 4) analysed in six
23 replicates (K = 6) and instrument responses were linear within the investigated ranges, that residual
24 standard deviations increased with the concentration of lead or cadmium in the reference materials.
25 Therefore the model with proportional residual standard deviation, as defined in ISO 11095:1996, was
26 used and the sum of squared weighted residuals (WSSE) and the sum of squared deviation due to
27 pure error (WSSP) were calculated and the ratio between variability due to lack of fit and variability
28 due to pure error proved to be not larger than the value of F (95%) calculated for N-2; N-K-N, showing
29 that there was no evidence to reject the linear model (Table 5). The present method was validated in
30 compliance with the requirements set in the EC Directive 2001/22 and it has detection and
31 quantization limit that are at least one tenth and one fifth, respectively, of the acceptable maximum
32 residual level specified in the Regulation EC 1881/2006 (i.e. in meat D.L. cadmium 5 ng g⁻¹ and lead
33 10 ng g⁻¹, in liver lead and cadmium 50 ng g⁻¹).
34
35
36
37
38
39
40
41
42
43
44
45
46

47 ***Lead and cadmium in chicken and pigeon meat***

48 Higher levels of lead in chicken muscle were approximately 60 ng g⁻¹ and most of the sample were in
49 a range from 20 and 30 ng g⁻¹. The level of cadmium was below 10.5 ng g⁻¹ in all the samples, with
50 prevalent values in the range 2 - 8 ng g⁻¹ (Table 6). Therefore, chicken muscle proved to be relatively
51 less contaminated than meat from other species commonly consumed. Level of these heavy metals
52 was relatively low also in the chicken liver samples (8.0-84.4 ng g⁻¹ for lead and 10.4 – 90.6 ng g⁻¹ for
53 cadmium) and it was observed that the concentration in liver and muscle cannot be correlated (Figure
54 1). Probably the age of animals (below 60 days) does not allow a detectable accumulation in the liver
55
56
57
58
59
60

1
2
3 and therefore consumption of chicken liver may be regarded favourably for this aspect. Level in
4 farmed games, like pigeons, also show values similar to those observed in chicken, i.e. 7.9-63.6 ng g⁻¹
5 for Pb and 2.2-8.0 ng g⁻¹ for Cd in muscles.
6
7

8
9
10 Lead and cadmium food intake assessment studies have been undertaken with the aim of establishing
11 the maximum permissible level in the different food on the basis of the Provisional Acceptable Weekly
12 Intake (PWTI) and the risk for populations and the margin of exposure with different diet in Europe
13 (EC. 2004). Meat, but also liver from chickens can only give a small contribution to consumer
14 exposure to Pb and Cd. The management choice of Food Business Operators, i.e. feed producers, in
15 controlling the sources of feedstuffs affect the level of contaminants in meat is however of great
16 importance. Regular monitoring activities, which are facilitated by use of low cost analytical methods,
17 can give insight in detecting contaminated sources of feedstuffs, and analysis of meat samples can
18 provide a valuable tool for the risk management.
19
20
21
22
23

24 25 26 **Conclusions**

27
28
29 Efficient sample digestion and pH adjustment was recognized as an essential pre-treatment for the
30 trace element measurement, however high qualitative standards are achievable using Square Wave
31 Anodic Stripping Voltammetry for the assessment of lead and cadmium in chicken muscle and liver.
32 Although our results have to be confirmed by full collaborative studies to assess the reproducibility and
33 robustness of this method, they showed that it can comply with the requirements set in the regulation
34 of the European Union regarding the official food control, with good performance in term of detection
35 and quantification limits, recovery, within-laboratory repeatability and measurement uncertainty for
36 both lead and cadmium. Very low values of lead and cadmium were measured in chicken and pigeon
37 meat and due to their very young age also chicken liver showed very low values..
38
39
40
41
42
43
44

45 46 **References**

- 47
48
49 Bard A.J. and Faulkner L.R. (1980). *Electrochemical Methods*. John Wiley & Sons ISBN 0-471-05542-
50 5. p 413-420.
51
52
53
54 Brown J.C.R. and Milton M.J.T. (2005). Analytical techniques for trace element analysis: an overview.
55 *Trends in Analytical Chemistry* 24(3). 266-274.
56
57
58
59
60

- 1
2
3 Capar S.G. and Subjoc G.A. (1982). Defining a lowest level of reliable measurement for lead in foods.
4 J Assoc Off Anal Chem. 65.1025-1029.
5
6
7
- 8 Danzer K. and Currie L.D. (1998). Guideline for calibration in analytical chemistry. Part 1:
9 Fundamentals and single component calibration. Pure & Appl. Chem. 70(4). 993-1014.
10
11
- 12 De Silva B.. Smith W.. Weiner R.. Kelley M.. Smolec J.. Lee B.. Khan M.. Tacey R.. Hill H.. Celniker A.
13 (2003). Recommendations for the bioanalytical method validation of ligand-binding assay to support
14 pharmacokinetic assessment of macromolecules. Pharmaceutical Research 20(11) 1885-1900.
15
16
17
- 18 D'ILio S., Petrucci F., D'Amato M., Di Gregorio M. Senofonte O. and Violante N. (2008). Method
19 Validation for determination of cadmium and lead in offal by means of quadruple inductively coupled
20 plasma mass spectrometry. J. Agric. Food Chem. 56,11584-11588.
21
22
23
24
25
- 26 EC (2001) Commission Directive 2001/22/EC of 8 March 2001 laying down sampling methods of
27 analysis for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs.
28 Official Journal of the European Union. L77 of 16.3.2001. pp. 14-21.
29
30
31
- 32 EC (2004a). European Commission SCOOP 2004. task 3.2.11. Assessment of the dietary exposure to
33 arsenic. cadmium. lead and mercury of the population of the EU Member States. European
34 Commission. Directorate-General Health and Consumer Protection. Reports on tasks for scientific
35 co-operation March 2004.
36
37
38 [http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/scoop_3-2-](http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf)
39
40
41
42
43
- 44 EC (2004b). Report on the relationship between analytical results. Measurement uncertainty. recovery
45 factors and the provisions of EU food and feed legislation. with particular reference to Community
46 legislation concerning: - contaminants in food (Council Regulation (EEC) no 315/93 of 8 February
47 1993 laying down community procedures for Contaminants in food);- undesirable substances in feed
48 (directive 2002/32/ec of the European parliament and of the Council of 7 may 2002 on Undesirable
49 substances in animal feed). Available at web address:
50 ec.europa.eu/food/food/chemicalsafety/contaminants/report-anpling_analysis_2004_en.pdf –
51
52
53
54
55 Accessed on April 2008.
56
57
58
59
60

- 1
2
3 EC (2006). Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels
4 for certain contaminants in foodstuffs. Official Journal of the European Union. L364 of 20.12.2006.
5 pp. 5-24.
6
7
8
9
10 EC (2007). Commission regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of
11 sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-
12 MCPD and benzo(a)pyrene in foodstuffs. Official Journal of the European Union. L88 of 29.3.2007.
13 pp. 29-38.
14
15
16
17
18 EFSA (2004a). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from
19 the Commission related to lead as undesirable substance in animal feed. *The EFSA Journal* (2004)
20 71. 1-20.
21
22
23
24 EFSA (2004b). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from
25 the Commission related to cadmium as undesirable substance in animal feed. *The EFSA Journal*
26 (2004) 72. 1-24.
27
28
29
30
31 Ellison S.R.M. Rosslein M., Williams A. (2000). Quantifying Uncertainty in Analytical Measurement. 2nd
32 ed. Eurachem/Citac. ISBN 0 948926 15 5.
33
34
35
36 Forte G. and Bocca B. (2007). Quantification of cadmium and lead in offal by SF-ICP-MS: method
37 development and uncertainty estimate. *Food Chemistry* 105, 1591-1598.
38
39
40
41 ISO 8466-1 1990. "Water quality – Calibration and evaluation of analytical methods and estimation of
42 performance characteristics – Part 1: Statistical evaluation of the linear calibration function."
43
44
45
46 ISO 8466-2 2001. "Water quality – Calibration and evaluation of analytical methods and estimation of
47 performance characteristics – Part 2: calibration strategy for non-linear second-order calibration
48 functions."
49
50
51
52 ISO 17025 2005. "General Requirements for the Competence of Testing and Calibration
53 Laboratories". In: Compendium of international analysis of methods – OIV. Guide for the validation –
54 quality control
55
56
57
58
59
60

1
2
3 Locatelli C. (2000). Proposal of new analytical procedures for heavy metal determinations in mussels.
4 clams and fishes. Food Additives and Contaminants 17. 769-774.
5
6

7
8 Locatelli C. Torsi G. (2003). Analytical procedures for the simultaneous voltammetric determination of
9 heavy metals in meals. Microchemical Journal 75 233–240.
10
11

12
13 OIV (2005). Compendium of International Analysis of Methods – OIV. Guide for the validation – quality
14 control. Practical guide for the validation. quality control. and uncertainty assessment of an
15 alternative oenological analysis method (Resolution 10/2005). MA-E-AS1-12-GUIVAL
16
17

18
19 Omanovic D., Branica. M. (2003). Pseudopolarography of trace metals Part I. The automatic ASV
20 measurements of reversible electrode reactions. Journal of Electroanalytical Chemistry 543. 83-92.
21
22

23
24 Vos G. Lammers H. Kan CA. (1990). Cadmium and lead in muscle tissue and organs of broilers.
25 turkeys and spent hens and in mechanically deboned poultry meat. Food Additives and
26 Contaminants 7. 83-91.
27
28

29
30 Wang J. (2000). Analytical Electrochemistry. VCH Verlagsgesellschaft. New York.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1 – Parameters used for Lead and Cadmium Anodic Stripping Voltammetry

<i>Pre-treatment</i>	<i>Measurement</i>
Conditioning potential = -0,2 V x 1 min Deposition potential = -1,1 V x 2 min ^a Equilibration time = 30 sec	Frequency 15Hz Scan rate = 0.045V/s Initial potential = -1.1V End potential = -0.25V Step potential = 0.003V

Notes:

^a Deposition time may be increased up to 5 minutes if the peaks are too little to be measured

Table 2a - Results of precision and accuracy study relative to chicken muscle

Lead concentrations (ng·g ⁻¹)		Spiked chicken muscle			BCR-184
		10+7,41	50+7,41	100+7,41	239
Results	n	19	19	18	26
Accuracy	Mean bias (%RE)	-0.34	-0.70	-0.55	-0.50
	LCL	-0.34	-0.72	-0.58	-0.57
	UCL	-0.33	-0.68	-0.53	-0.43
Precision	Intra-batch(%CV)	1.11	2.22	0.44	3.62
	Inter-batch(%CV)	0.94	1.89	0.85	3.07
Accuracy + Precision	lmeanl + Inter-batch	1.28	2.59	1.40	3.57
90% expected tolerance level	Lower Limit (%RE)	-0.36	-0.86	-0.68	-1.49
	Upper Limit (%RE)	-0.31	-0.54	-0.42	0.49
	Horrat r	0.05	0.12	0.03	0.26
	Horrat R	0.04	0.11	0.05	0.22
Cadmium concentrations (ng·g ⁻¹)		Spiked chicken muscle			BCR-184
		10+3.2	50+3.2	100+3.2	13
Results	n	19	19	18	28
Accuracy	Mean bias (%RE)	-1.03	-0.97	-0.88	0.03
	LCL	-1.04	-0.99	-0.89	0.02
	UCL	-1.03	-0.96	-0.86	0.03
Precision	Intra-batch(%CV)	0.95	0.55	0.64	2.03
	Inter-batch(%CV)	1.71	1.10	0.86	1.75
Accuracy + Precision	lmedial + Inter-batch	2.74	2.07	1.74	1.77
90% expected tolerance level	Lower Limit (%RE)	-1.06	0.55	-1.00	0.00
	Upper Limit (%RE)	-1.00	1.10	-0.75	9.90
	Horrat r	0.06	0.03	0.04	0.14
	Horrat R ^a	0.11	0.07	0.05	0.12

Notes:

^a repeatability within the same laboratory

Table 2b - Results of precision and accuracy study relative to chicken liver

Lead concentrations (ng·g ⁻¹)		Spiked chicken liver			BCR-185R
		50+9.2	100+9.2	500+9.2	172
Results	n	18	18	18	18
Accuracy	Mean bias (%RE)	-0.29	-0.75	-0.41	0.46
	LCL	-0.31	-0.77	-0.43	0.37
	UCL	-0.27	-0.73	-0.39	0.56
Precision	Intra-batch(%CV)	0.26	0.58	1.53	4.35
	Inter-batch(%CV)	0.64	1.93	3.16	3.81
Accuracy + Precision	lmeanl + Inter-batch	0.93	2.68	3.57	4.27
90% expected tolerance level	Lower Limit (%RE)	-0.38	-1.02	-0.85	-0.49
	Upper Limit (%RE)	-0.19	-0.48	0.02	1.41
	Horrat r	0.02	0.04	0.10	0.28
	Horrat R	0.04	0.12	0.20	0.24
Cadmium concentrations (ng·g ⁻¹)		Spiked chicken liver			BCR-185R
		50+10.4	100+10.4	200+10.4	544
Results	n	18	18	18	21
Accuracy	Mean bias (%RE)	-0.33	-0.81	-0.32	-3.84
	LCL	-0.35	-0.83	-0.34	-4.68
	UCL	-0.31	-0.79	-0.30	-3.01
Precision	Intra-batch(%CV)	0.19	0.51	0.52	4.35
	Inter-batch(%CV)	0.63	1.91	1.18	6.30
Accuracy + Precision	lmedial + Inter-batch	0.96	2.72	1.50	10.14
90% expected tolerance level	Lower Limit (%RE)	-0.43	-1.08	-0.49	-8.81
	Upper Limit (%RE)	-0.24	-0.55	-0.16	1.13
	Horrat r	0.01	0.03	0.03	0.28
	Horrat R ^a	0.04	0.12	0.07	0.40

Notes:

^a repeatability within the same laboratory

Table 3 - Relative standard uncertainty related to the preparation of stock and intermediate solutions

Source	Tolerance	Uncertainty	Relative uncertainty
Standard weight and purity 1000 mg	± 2	1.02000	0.1020%
Volumetric flask. 1000 mL	± 0.2	0.08165	0.0082%
Pipette. 500 μ L	$\pm 1.6\%$	0.00656	0.0131%
Volumetric flask. 50 mL	± 0.06	0.02450	0.0490%
Pipette. 1000 μ L	$\pm 1\%$	0.04127	0.0041%
Volumetric flask. 100 mL	± 0.1	0.04083	0.0408%
Combined uncertainty			0.1206%

Table 4 - Relative standard uncertainty related to the preparation of the Certified Reference materials and the artificially spiked samples used for precision analysis

Source	Tolerance	Uncertainty	Relative uncertainty
BCR184 purity:			
Lead 239 ng·g ⁻¹	± 11	5.61	2.35%
Cadmium 13 ng·g ⁻¹	± 2	1.02	7.85%
weight 0.3 g	0.002 g linearity; 0.001 g readability	± 0.0014	0.47%
combined uncertainty			
lead			2.39%
cadmium			7.86%
BCR185R purity			
Lead 172 ng·g ⁻¹	± 9	4.59	2.67%
Cadmium 544 ng·g ⁻¹	± 17	8.67	1.59%
weight 0.3 g	0.002 g linearity; 0.001 g readability	± 0.0014	0.47%
Combined uncertainty			
lead			2.71%
cadmium			1.66%
Chicken muscle:			
Lead 7.41 ng·g ⁻¹	series of measures	± 0.044 ^a	0.59%
Cadmium 3.21 ng·g ⁻¹	series of measures	± 0.048 ^a	1.49%
weight 0.5 g	0.002 g linearity; 0.001 readability	± 0.0018	0.36%
Standard solution 100 ng·g ⁻¹ (lead and cadmium)	0.103% ^b	0.01206	0.12%
Pipette. 50 µL (10 ng·g ⁻¹)	± 1%	0.006532	0.0131%
Pipette. 250 µL (50 ng·g ⁻¹)	± 2%	0.008187	0.0033%
Pipette. 500 µL (100 ng·g ⁻¹)	± 1%	0.006560	0.0013%
Combined uncertainty			
Lead ^c			0.70%
Cadmium ^c			1.54%

Notes:

^a St.Dev. result of 7 independently repeated determinations;

^b calculation reported in Table 3;

^c for standards at 10, 50 and 100 ng g⁻¹

Table 5 – Results of the linearity study

		Muscle		Liver	
		Pb	Cd	Pb	Cd
Linearity range		17-239 ng g ⁻¹	13-103 ng g ⁻¹	59-509 ng g ⁻¹	60-544 ng g ⁻¹
LLOQ		1.9 ng g ⁻¹	0.9 ng g ⁻¹	4.7 ng g ⁻¹	3.4 ng g ⁻¹
Lack of fit	WSEE-WSSP	1.92·10 ⁻⁵	4.11·10 ⁻⁵	3.04·10 ⁻⁴	1.36·10 ⁻⁴
	$\hat{\tau}_l^a$	9.59·10 ⁻⁶	2.06·10 ⁻⁵	1.52·10 ⁻⁴	6.81·10 ⁻⁵
Pure error	WSSP	1.60·10 ⁻³	2.90·10 ⁻³	3.66·10 ⁻³	2.31·10 ⁻³
	$\hat{\tau}_p^b$	7.98·10 ⁻⁵	1.45·10 ⁻⁴	1.83·10 ⁻⁴	1.16·10 ⁻⁴
F ratio ^c	$\hat{\tau}_l / \hat{\tau}_p$	0.12 *	0.14 *	0.83 *	0.59 ^a

Notes:

^a $\hat{\tau}_l$ = variability due to lack of fit; ^b $\hat{\tau}_p$ = variability due to pure error;

^c $F = \hat{\tau}_l / \hat{\tau}_p < F_{1-0.05(N-2;NK-N)} = 3.49$

Table 6 – Concentration (in ng g⁻¹) of lead and cadmium in broilers and pigeons

	broilers' muscle		broilers' liver		pigeons' muscle	
	Lead	Cadmium	Lead	Cadmium	Lead	Cadmium
mean	21.6 ± 11.5	4.7 ± 2.7	35.1 ± 20.7	41.9 ± 22.5	23.6 ± 16.9	4.5 ± 1.8
Range (min-max)	6.4 – 59.8	0.4 – 10.4	8.0 – 84.4	10.4 – 90.6	7.9 – 63.6	2.2 – 8.0
Lots (n) ^a	26	29	26	27	9	9

Notes:

^a each lot sample was made by pooling aliquots from five different animals

For Peer Review Only

Figure 1 – Correlation between lead or cadmium concentration in broilers’ muscle and liver

