

Method Validation for Determination of Cadmium and Lead in Offal by Means of Quadrupole Inductively Coupled Plasma Mass Spectrometry

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Offal includes viscera and internal organs that have been removed from butchered animals, that may be either directly eaten or processed for the production of other foodstuff. Such organs are able to accumulate high concentrations of potentially toxic heavy metals posing a risk for human health when ingested. Because high levels of Cd and Pb may produce damages to humans, Commission Regulation no. 1881/2006 and its amendment established maximum levels for those two elements in edible bovine, porcine, and ovine offal. In the present study, a method based on microwave acid-assisted digestion and quadrupole inductively coupled plasma mass spectrometric quantification of Cd and Pb in offal was validated according to the EU common standards. The main parameters evaluated in the validation process were: the detection and the quantification limits (LoD, LoQ), the recovery, the repeatability, the within-laboratory reproducibility, the linearity range, and the standard measurement uncertainty. The results obtained for LoD and LoQ in $\mu\text{g kg}^{-1}$ were, respectively, Cd, 1.8 and 5.4; Pb, 5.1 and 15.5; meanwhile, the mean recovery was about 98% for Cd and 103% for Pb. Repeatability was around 5% for Cd and 4% for Pb. The expanded standard measurement uncertainty, expressed as percentage and with a coverage factor of 2, was estimated as follows: Cd, 4.9%; Pb, 8.7%. For both elements, the main contribution was due to the within-laboratory reproducibility of the measurements.

KEYWORDS: method validation; offal; heavy metals; inductively coupled plasma mass spectrometry.

INTRODUCTION

Offal includes viscera and internal organs that have been removed from a butchered animals, such as beef, veal, mutton, lamb, and pork, that may be either directly eaten or processed for the production of other foodstuff. Such kinds of meat are present, in various forms, in the human diet of several countries in the world. From a nutritional point of view, a great variety of meats are richer in certain vitamins, minerals, and forms of protein compared with muscle tissue. Offal, not used for human consumption, is commonly employed for animal feed or as a fertilizer.

The concentration of elements in these meats is affected by both pollution of the rural environment where animals live and composition of the feed.

The ability of some organs in accumulating high concentrations of potentially toxic heavy metals is well-known in literature. However, high levels of these inorganic elements in offal could pose a risk for human health when ingested. Therefore, during the last years, the quantification of both

physiological and toxic elements in offal became a crucial point. To this end, different analytical methodologies were employed.

As reported in FAO/WHO, No. 46 (1), cadmium may create damages to kidneys, bones, and the nervous system, whereas lead may damage kidney and both the nervous and cardiovascular systems, as well as the heme, whose biosynthesis can be altered (2). As a consequence, at EU level, Commission Regulation no. 1881/2006 (3) and its amendment, the Commission Regulation no. 629/2008 (4), established a maximum level for the two elements in bovine, porcine, and ovine edible offal.

The exposure to cadmium, by the ingestion of offal, of a selected part of the population (smokers, vegetarians, and consumers of high-risk products) was carefully studied by authors, who recommended the routine removal of liver and kidney from older sheep, as a results of their researches (5).

The use of suitable and sensitive analytical techniques for the quantification of trace elements is strongly recommended, in fact, different analytical techniques have been employed so far in the determination of chemical elements in offal, among those, atomic absorption spectrometry and inductively coupled plasma mass spectrometry.

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There is also an increasing demand of validated methods at EU level to be employed in the official food control.

Atomic absorption spectrometry (AAS) was used in the analysis of several elements in local Jordanian and imported sheep meat and organs (6); another study, focused on quantification of arsenic, cadmium, lead, copper and zinc in liver, kidney, muscle and blood of cattle (males and females), was carried out in order to explore whether age and sex can have a role in the levels of those elements (7). Offal sampled from contaminated areas of Egypt were subjected to a washing procedure and the influence of this process on the content of some elements (Pb, Cd, Zn, Cu, Mn, and Fe) was studied by means of the same analytical technique (8).

An investigation on the relationship between the distance from the source of the emission and the level of contamination of food did not find a correlation for As, Cd, Hg, and Pb in meat samples and offal of pigs, cattle, and poultry (9). The concentrations of heavy metals in three species of owl from Korea were also studied by AAS, the values found were under their action levels (10). AAS was employed in the determination of cadmium and lead in bovine kidneys in a monitoring program, taking into account sampling, age of animals, and regional differences (11); the same analytical technique was used in the detection of Cd and Pb in meat and offal, showing exceeding tolerance levels only for Cd in some samples of porcine kidneys (12).

A study involving a wide variety of foods available on the Greek market was carried out by means of AAS (13). High levels of Cd were determined by GF-AAS in muscle, bone, liver, and kidney of bovine living in a polluted area of Morocco (14).

A method for the quantification of Cd and Pb in calf liver by means of sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) was developed and further applied to offal of different animals, using a certified reference material (CRM) to assess the trueness (15). Limits of acceptance for As, Cd, Cr, Cu, Mn, Pb, and Zn in edible cow viscera, collected in different markets in Seoul, were also investigated by ICP-MS (16).

Cadmium and Pb were measured in blubber, liver, kidney and muscle of male and female harp seals (*Phoca groenlandica*) by ICP-MS. The results of this study confirmed the importance of quantifying these two elements in liver and kidney (17).

The levels of toxic and essential metals in liver, kidney and muscle of pigs at slaughter in Galicia (northwest Spain) were determined and compared to those obtained in other countries. An attempt to validate the method was carried out by assessing the trueness by a CRM, measuring the limit of detections and calculating the precision. This paper also highlighted the significance of determining Cd and Pb in liver and kidney (18).

In the present study, a method based on microwave acid-assisted digestion and quadrupole inductively coupled plasma mass spectrometric quantification of Cd and Pb in offal was validated according to the following EU standards: Commission Decision 657/2002 (19), UNI CEI EN ISO/IEC 17025/2005 (20), Commission Regulation (EC) No. 333/2007 (21), and Commission Regulation No.1881/2006 and its amendments (3).

The analytical technique of choice offers several advantages for its capabilities, including time-saving, high sensitivity, simultaneous multiple element determination with a wide dynamic range, and high sample throughput. For these reasons, ICP-MS is particularly suitable for demanding samples in routine analysis of trace elements in matrices or products of animal origin.

EXPERIMENTAL PROCEDURES

Materials and Methods. The present method was in-house validated on veal liver (incurred material) and was also applied on the following

Table 1. Digestion Procedure

| step | | time (min) | temperature (° C) | power (Watt) |
|------|---------|------------|-------------------|--------------|
| 1 | ramp | 5 | 100 | 1000 |
| 2 | hold | 2 | 100 | 1000 |
| 3 | hold | 5 | 180 | 1000 |
| 4 | hold | 15 | 180 | 1000 |
| 5 | cooling | 20 | | 0 |

kind of offal: veal kidney, lung and spleen, pig liver, and lamb lung and liver. An acid-assisted digestion of the samples was applied.

During the development of the method, the Certified Reference Material (CRM) Bovine Liver BCR 185R (Institute for Reference Materials and Measurements, Geel, Belgium) was used in order to assess the quality of data produced.

The liver was purchased from the market and the edible parts were homogenized in a mixer equipped with stainless steel blades (International PBI, Milan, Italy). Subsequently, this homogeneous material was divided into several portions, transferred in suitable polypropylene containers and kept in a refrigerator at -20°C .

Approximately, 2 g of liver sample and 0.5 g of CRM were weighed into previously decontaminated Teflon vessels on an analytical balance (Mod. BP 210 D, Sartorius Stedim Italy, Antella, Florence, Italy), and added with 5 mL of Suprapur concentrated nitric acid 65% (v/v) (Merck, Darmstadt, Germany) and 1 mL of Suprapur hydrogen peroxide 40% (v/v) (Merck, Darmstadt, Germany). The liver was then digested by means of a Microwave oven (Ethos plus, Milestone, FKV, Sorisole, Bergamo, Italy) equipped with a probe for the internal temperature monitoring control of samples (reference vessel). A harsh digestion program was requested for the presence of fat according to **Table 1**.

After the cooling step, the digestion solutions were quantitatively transferred into Falcon tubes of 50 mL by adding high purity deionized water with a specific resistance $\leq 18\text{ M}\Omega\text{ cm}$ (Easy Pure, PBI International, Milan, Italy) up to a weight of 20 g. The densities of these solutions were measured by weighing 1 mL of solution, in order to calculate the final volumes. The digested samples were stored in a refrigerator at $+4^{\circ}\text{C}$ up to the moment of the analysis, when they were properly diluted 1:15 and analyzed.

Quantification of cadmium and lead was performed by using the standard addition calibration approach. In particular, four standard additions were made and rhodium was used as the internal standard at the concentration of $1\text{ }\mu\text{g/L}$ in calibrants and samples. The internal standard in ICP-MS is commonly used to compensate any random fluctuations of the signals.

In this study, single element 1000 mg/L stock standard solutions of Ba, Cd, Ce, Mg, In, Pb, Rh, and U in 2% (v/v) HNO_3 were employed (Spex Industries Inc., Edison, NJ).

Instrumentation. Determination of the elements of interest was performed by means of quadrupole inductively coupled plasma mass spectrometry, ICP-QMS (Elan DRC II, Perkin-Elmer SCIEX, Norwalk, CT). The instrumental setting and data acquisition parameters for ICP-QMS are reported in **Table 2**.

Method Validation. As regards the methods of analysis, the CR No.333/2007 (21) states that "Methods of analysis used for food control purposes shall comply with the provisions of points 1 and 2 of Annex III to Regulation (EC) No. 882/2004" (22) furthermore, as provided by the last Regulation, wherever possible, methods of analysis shall be characterized by the appropriate criteria set out in Annex III. According to what is listed in Annex III, the following parameters have been taken into account for the in-house validation of the proposed method in offal: selectivity, trueness by the recovery at three levels of concentration, repeatability, and within-laboratory reproducibility at three levels of concentration, instrumental/method detection limits (LoDs) and quantification limits (LoQs), range of linearity, standard measurement uncertainty.

The stability studies were not carried out, being the concentrations of the elements of interest in the digested solutions rather high (about 0.05 mg/L). Moreover, a previous study on low level trace elements determination in milk demonstrated the stability of the solutions for four weeks (23).

Table 2. Instrumental Parameters for ICP-QMS

| | |
|---|--|
| spectrometer | Elan DRC II (Perkin-Elmer SCIEX, Norwalk, CT, USA) |
| sample uptake rate (mL/min) | 1 |
| sample introduction | Meinhard nebulizer with cyclonic spray chamber |
| RF power (W) | 1450 |
| gas flow rates (L/min) | plasma, 15; auxiliary, 1.0; nebulizer, 0.85 |
| dwelt time (ms) | 50 |
| no. of replicate | 5 |
| interface | Pt cones |
| extraction lens voltage | optimized for maximum I (⁵⁶ Fe) |
| acceptance limits for optimization solution | Mg > 6000 cps, In > 30 000 cps, U > 20 000 cps, mass 220 (background) < 2 cps, CeO/Ce < 3%, Ba ²⁺ /Ba ⁺ < 3% |
| scanning mode | peak hopping |
| analytical masses (amu) | standard mode ¹¹⁴ Cd, ²⁰⁸ + ²⁰⁷ + ²⁰⁶ Pb, ¹⁰³ Rh (internal standard), ¹¹⁸ Sn |

Table 3. Levels of Addition for Cd and Pb (mg/kg)

| | concentration in mg/kg | |
|---------|------------------------|-------|
| | Cd | Pb |
| level 1 | 0.250 | 0.250 |
| level 2 | 0.500 | 0.500 |
| level 3 | 0.750 | 0.750 |

Table 4. LoD, LoQ, and Linearity Range

| params (units) | Cd | Pb |
|--|--------------|------------|
| instrumental LoD and LoQ ($\mu\text{g/L}$) | 0.035; 0.106 | 0.475; 1.4 |
| LoD and LoQ of the method ($\mu\text{g/kg}$) | 1.8; 5.4 | 5.1; 15.5 |
| range of linearity ($\mu\text{g/L}$) | 2–20 | 2–20 |

The quantification of Cd and Pb in reagent blanks and control of the instrument drift for readings were always carried out as internal quality control (IQC) procedure. In particular, reagent blanks were checked before digesting the liver and a reference standard sample was measured after the calibration curve and each ten samples to evaluate the instrumental drift (maximum acceptable drift set $\pm 10\%$). The definitions applied during this study are those reported in EU standards for foodstuff (19, 21).

Selectivity. Selectivity is the capability of a method to discriminate the elements of interest in presence of potential interferent ions in the matrix. Investigation of selectivity is always necessary. In ICP-MS, the interference studies are one of the most significant parts; for this reason, in this paper, selectivity is discussed in the Interference Study section.

Recovery Study. Although a number of CRMs on freeze-dried liver are offered by different suppliers, the trueness of the method was assessed by using the recovery tests. In fact, a freeze-dried material cannot be representative when an incurred material has been chosen for the validation.

Three levels of additions of 0.5, 1.0, and 1.5 times the maximum levels for Cd and Pb in liver were selected on the basis of the criterion indicated in CD No.657/2002 (19). A maximum level of 0.50 mg/kg is set in CR No.1881/2006 (3) for cadmium in liver and for lead in offal of bovine animals, sheep, pig, and poultry, whereas a maximum of 1.0 mg/kg is set for cadmium in kidney of the same species.

According to the scheme of **Table 3**, six independent aliquots of liver were spiked, before the digestion, with the right amount of standard solution of Cd and Pb in the three different levels of concentration (mg/kg), so as to obtain eighteen 18 spiked samples and 9 samples at their natural level.

As regards the analysis of veal kidney, lung and spleen; pig liver; and lamb lung and liver, recovery tests were also performed on these matrices at the maximum levels for Cd and Pb in order to check the performance of the method. In particular, for cadmium in kidney, an addition of 1.0 mg/kg was made.

Table 5. Method Performance

| parameters (%) | Cd | | | Pb | | |
|-----------------------------------|---------|---------|---------|---------|---------|---------|
| | level 1 | level 2 | level 3 | level 1 | level 2 | level 3 |
| recovery | 98 | 98 | 99 | 101 | 103 | 104 |
| repeatability | 6.0 | 5.3 | 3.9 | 4.0 | 3.9 | 4.0 |
| within-laboratory reproducibility | 4.2 | 2.2 | 3.0 | 4.1 | 4.1 | 5.1 |
| expanded uncertainty | 4.9 | | | 8.7 | | |

The recovery was calculated as follows

$$\text{recovery \%} = 100C/\text{spiked concentration}$$

where C is the element concentration found. An acceptance range of 90–110% of the target concentration was selected in compliance with CD No. 657/2002 (19).

Repeatability and Within-Laboratory Reproducibility. Three independent sets of samples (six spiked and three unspiked) were analyzed on three different days to calculate the repeatability and within-laboratory reproducibility as a percentage variation coefficient (CV %).

The analytical conditions were kept unchanged for the calculation of the repeatability during the three days of measurements. In fact, as stated in CR No.333/2007 (21) and CD No.657/2002 (19), repeatability is the precision under repeatability conditions, that are those conditions where independent test results are obtained with the same method on the same sample in the same laboratory by the same operator using the same equipment in a short interval of time. Data from the recovery of the set of samples at three levels of concentration were used for the first day of analysis for repeatability. On the other hand, being the within-laboratory reproducibility the precision obtained in the same laboratory under predetermined conditions over long time intervals, some changes were applied to the analytical conditions. The measurements were performed during three weeks and the following changes were made: first day, no changes; second day, operator and instrument (two instruments of the same kind); third day, spleen was analyzed instead of liver.

Instrumental/Method Detection and Quantification Limits. The calculation of the instrumental/method limit of detection (LoD) and limit of quantification (LoQ) was based on the commonly adopted 3σ and 10σ approaches, respectively. A pool of reagent blanks was used for the calculation of instrumental LoD and LoQ, whereas a pool of digested liver was used for the calculation of the method LoD and LoQ. To this end, 20 measurements of the reagent blanks/digested liver and 20 measurements of a reagent blanks/digested liver spiked with 0.5 $\mu\text{g/L}$ of Cd and 1 $\mu\text{g/L}$ of Pb were carried out.

The values obtained for method LoDs and LoQs were multiplied by the final dilution factor (300) and divided by the mean sample weight. The formula used was the following

$$\text{LoD} = 3\text{sd}C_{\text{spike}}/I_{\text{spike}} - I$$

$$\text{LoQ} = 10\text{sd}C_{\text{spike}}/I_{\text{spike}} - I$$

where C is the concentration expressed in $\mu\text{g/L}$, sd is the standard deviation, and I is the signal intensity of the element.

Requirements for LoQ in elements with a maximum level can be found in Regulation no. 333/2007 (21). For Cd and Pb, LoQ must be less than one-fifth of the maximum level of Regulation No.1881/2006 (3). In the present study, in offal, LoQ for lead should be <0.10 mg/kg, whereas in liver and kidney, LoQ for cadmium should be <0.10 and <0.20 mg/kg, respectively.

Range of Linearity and Calibration Curve. The working concentration range of a method plays an important role in method validation. In fact, the range shall be chosen in a purpose-adapted way according to the level of concentration of the elements and care shall be taken not to exceed this established range. In this paper, the addition calibration approach has been used so as to match the composition of the sample and at least 5 points (blank not included) were prepared as recommended by CD no. 657/2002 (19).

One of the advantages of using ICP-MS is to have a linear concentration range of at least 3 orders of magnitude. Nevertheless,

the linearity range was evaluated by checking the linear regression coefficient (r^2) of a calibration curve constructed with 10 standard additions on digested unspiked liver. The linearity of the calibration curve was considered acceptable when $r^2 > 0.999$.

Measurement Uncertainty. Uncertainty is defined as: “Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used” (24). Measurement uncertainty characterizes the range of values within which the true value is asserted to lie, with a specified level of confidence. Random and systematic errors in a measured result contribute to uncertainty. In the estimation of the overall uncertainty the major contributions have to be identified and quantified (25).

To the aims of this study, the contributions to combined measurement uncertainty were chosen as follows: the preparation of the standard solutions (u_f), the standard uncertainty associated to the recovery (u_{rec}) and the within-laboratory reproducibility of the measurements (u_m). Each component was calculated as follows

$$u_{rec} = \frac{rsd}{\sqrt{n}}$$

where rsd is the ratio between the standard deviation of the mean recovery and the mean recovery value and n is the number of spiked samples; the uncertainty u_f associated with the preparation of the standard solutions was considered by the sum of the following contributors: pipettes volume, scale, and stock solutions:

$$\frac{u_f}{C} = \sqrt{\left(\frac{u_v}{V}\right)^2 + \left(\frac{u_b}{M}\right)^2 + \left(\frac{u_{cal}/\sqrt{3}}{cal}\right)^2}$$

where u_v is the standard uncertainty of the pipettes volume, u_{cal} is the standard uncertainty of the stock standard solutions and u_b is the standard uncertainty of the scale.

The combined uncertainty (u_c) was calculated as the square sum of the selected contributors:

$$\frac{u_c}{C} = \sqrt{u_m^2 + u_{rec}^2 + u_f^2}$$

whereas, the expanded measurement uncertainty (U):

$$U = u_c(x)k$$

where the coverage factor k is 2, which considers a normal distribution of measurements with a 95% confidence level.

RESULTS AND DISCUSSION

The performance of the mass spectrometer was daily checked by aspirating the optimizing standard solution containing 1 $\mu\text{g/L}$ of Ce, In, Mg, U and 10 $\mu\text{g/L}$ of Ba in HNO_3 0.5%, according to the information provided by Perkin-Elmer as reported in **Table 2**. In case of spectrometer underperformance, the common corrective actions were undertaken.

During the development of the method, selectivity was investigated by studying all possible interferent ions that could hamper the quantitative determination of the elements of interest. The ICP mass spectrometer was employed in standard mode, because no interferences correction was required in the measure of cadmium and lead by means of DRC technology. The next paragraph illustrates the interferences study and a further discussion on the accomplishments to the EU requirements for each parameter validated is provided together with some applications of the method on different kind of offal.

Interferences Study. Cadmium. The potential interferences of some relevance on the analytical mass ^{114}Cd were: $^{98}\text{Mo}^{16}\text{O}$, $^{79}\text{Br}^{35}\text{Cl}$, $^{38}\text{Ar}^{76}\text{Ge}$, and ^{114}Sn . As already mentioned, a daily test on the oxides formation was performed; the above interference was negligible since the percentage of oxides on CeO/Ce masses was kept below 3% by adjusting the optimization

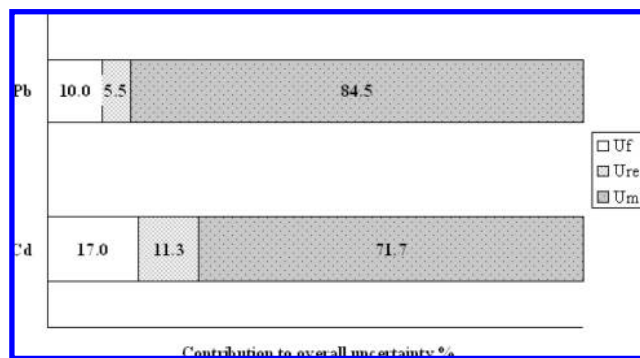


Figure 1. Contribution of each factor to the overall combined uncertainty for Cd and Pb.

parameters (i.e., nebulizer gas flow, lens voltage, RF power), furthermore the content of Mo in the matrices under study was very low. As the formation of the molecular ion BrCl did not occur; the presence of ArGe was negligible because of the limited presence of Ge. In addition, the isobaric interference of ^{114}Sn was corrected by using a mathematical equation:

$$-0.027250^{118}\text{Sn}$$

Lead. The analytical signal of lead was corrected for the sum of the most abundant isotopes: ^{206}Pb , ^{207}Pb , and ^{208}Pb . This correction is frequently used in the analysis of lead, because in the natural state, the abundance of its isotopes is not regular. The potential interferences on these isotopes are: $^{192}\text{Os}^{16}\text{O}$, $^{190}\text{Os}^{18}\text{O}$, and $^{191}\text{Ir}^{17}\text{O}$. Being Ir and Os present in these matrices at ultratrace level, this oxides formation was negligible.

Validation Parameters. The overall performance of the method proposed is summarized in **Tables 4** and **5**. A maximum value for LoQ of 0.1 mg/kg in cadmium and lead is fixed by the CR No.333/2007 (21), whereas a value of 0.005 mg/kg for Cd and 0.015 mg/kg for Pb is reached by this method. The values obtained are well below the limit imposed by the regulation.

The results for the recovery tests perfectly fall in the acceptance range. Probably, the high levels of concentration of Cd and Pb set for the validation justify this high-quality findings.

The within-laboratory reproducibility, expressed in terms of CV %, does not exceed the limit of 15% for a concentration range of 100 $\mu\text{g/kg}$ to 1000 $\mu\text{g/kg}$, as suggested by the CD No. 657/2002 (19).

The expanded standard measurement uncertainty, expressed as percentage and with a coverage factor of 2, was estimated according to the mentioned procedure as follows: Cd, 4.9%; Pb, 8.7%. The contribution of each component to the overall uncertainty is plotted in **Figure 1** for Cd and Pb as a percentage. For both elements, the main contribution is due to u_m , the within-laboratory reproducibility of the measurements. The estimated standard measurement uncertainty for Cd is fairly low; instead, for Pb seems to be rather high. In particular, for lead, the high value can be ascribable to major fluctuations of the signal during the 3 day measurements.

The “fitness-for-purpose” approach was applied to the uncertainty data in order to assess the suitability of this method for official food control. In fact, methods can be checked if they are able to produce results with standard measurement uncertainties (u_c) less than the maximum standard measurement uncertainty (u_m) calculated using the formula reported in point C.3.3.2 (21)

Table 6. Measurement Uncertainty Check by the "Fitness-for-Purpose Approach"

| Element | u_M (mg/kg) | u_c (mg/kg) | $u_M > u_c$ |
|---------|----------------------|----------------------|-------------|
| Cd | 9.0×10^{-2} | 1.4×10^{-2} | passed |
| Pb | 9.0×10^{-2} | 2.2×10^{-2} | |

Table 7. Results for Offal Samples Analyzed

| offal | concentration \pm sd ($\mu\text{g}/\text{kg}$) | | results for the recovery (%) | |
|-------------|--|-----------------|------------------------------|-----|
| | Cd | Pb | Cd | Pb |
| veal kidney | 103.4 \pm 3.6 | 23.3 \pm 0.7 | 114 | 119 |
| veal lung | <5.4 | <15.5 | 104 | 106 |
| veal spleen | <5.4 | <15.5 | 103 | 106 |
| pig liver | 105.1 \pm 10.7 | <15.5 | 109 | 110 |
| lamb lung | <5.4 | 63.6 \pm 14.0 | 104 | 106 |
| lamb liver | <5.4 | 289.3 \pm 7.3 | 104 | 106 |

$$u_M = \sqrt{\left(\frac{\text{LoD}}{2}\right)^2 + (\alpha C)^2}$$

where u_M is the maximum standard measurement uncertainty; LoD is the limit of detection of the method; C is the concentration of interest; α is a numeric factor to be used depending on the value of C .

Table 6 shows that this method well meets the performance criteria set out by the regulation.

Application of the method to offal samples. Cadmium and lead were quantified in six different kinds of offal and the recovery test was performed to check the trueness. The recoveries for both elements were rather satisfactory (always above 100%). Three independent samples were analyzed for each kind of offal. Data are reported in **Table 7**.

The present method was validated in compliance with the requirements set in the regulations of the European Union regarding the official food control. Furthermore, the method demonstrated to meet the performance criteria with satisfactory results in terms of detection and quantification limits, recovery, within-laboratory reproducibility and measurement uncertainty for both elements.

Quadrupole inductively coupled plasma mass spectrometry well matched the requisites for an analytical technique in method validation and it turned out to be a useful means of quantification for cadmium and lead in routine and research analysis.

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