Cadmium and lead determination by ICPMS: Method optimization and application in carabao milk samples

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ABSTRACT

A method utilizing inductively coupled plasma mass spectrometry (ICPMS) as the element-selective detector with microwave-assisted nitric acid digestion as the sample pre-treatment technique was developed for the simultaneous determination of cadmium (Cd) and lead (Pb) in milk samples. The estimated detection limits were 0.09 μg kg^{-1} and 0.33 μg kg^{-1} for Cd and Pb, respectively. The method was linear in the concentration range 0.01 to 500 μg $kg^{-1}$with correlation coefficients of 0.999 for both analytes.The method was validated using certified reference material BCR 150 and the determined values for Cd and Pb were 18.24 ± 0.18 μg kg^{-1} and 807.57 ± 7.07 μg kg^{-1}, respectively. Further validation using another certified reference material, NIST 1643e, resulted in determined concentrations of 6.48 ± 0.10 μg L^{-1} for Cd and 21.96 ± 0.87 μg L^{-1} for Pb. These determined values agree well with the certified values in the reference materials.The method was applied to processed and raw carabao milk samples collected in Nueva Ecija, Philippines.The Cd levels determined in the samples were in the range 0.11 ± 0.07 to 5.17 ± 0.13 μg kg^{-1} for the processed milk samples, and 0.11 ± 0.07 to 0.45 ± 0.09 μg kg^{-1} for the raw milk samples. The concentrations of Pb were in the range 0.49 ± 0.21 to 5.82 ± 0.17 μg kg^{-1} for the processed milk samples, and 0.72 ± 0.18 to 6.79 ± 0.20 μg kg^{-1} for the raw milk samples.

Keywords: Trace metal, carabao milk, ICPMS, microwave digestion, food contaminant

INTRODUCTION

The occurrence of heavy metals like cadmium (Cd) and lead (Pb) in the environment has been the subject of a wide range of published work from analytical methods for their determination to understanding the mechanism of action leading to their toxicities (Lidsky & Schneider, 2003; Bertin & Averdeck, 2006; Patrick, 2006; Patra et al., 2011). Both Cd and Pb are ubiquitous in nature and the concentrations are highly dependent on anthropological activities. Pb was popularly used during the early days in large scale manufacture of tableware and pipes for water supply (Garcia-Lestün et al., 2010). The use of Pb in pipes, which was prevalent until the 19th century, eventually declined due to incidence of lead poisoning (Tong, 2000; Järup, 2003). But the use of Pb as anti-knock agent and as pigment in paints has persisted until recently (Garcia-Leston et al., 2010). Cd is commonly used in industries such as electroplating, pigments, synthetic chemicals, ceramics, metallurgical and photographic products, electronic and other industries (Sadegh Safarzadeh et al., 2007). These widespread uses are the most common sources of these contaminants in the environment.

Cd and Pb are of interest due to health concerns associated with these metals. The effects of Cd have been linked to higher risks of cardiovascular disease mortality in males, increased bone fragility, children's nephrotoxicity, and decreased visual ability. Pb is considered to affect the brain heavily, especially during children's developmental stage, which can result in a change in cognitive function, social behavior and slow learning ability. In adults, exposure to Pb may cause kidney problems and neurodegeneration (Genuis et al., 2010). These effects have pushed authorities to set limits for these contaminants. The Joint FAO/WHO food standard programme Codex Committee on Contaminants in Foods (CCCF) released a new list of Maximum Levels (MLs) for contaminants and toxins in food last March 2011. For Cd, the committee established a Provisional Tolerable Monthly Intake (PTMI) of 25 μg kg^{-1} body weight. However, no ML was established for Cd in milk. In the case of Pb, the Committee estimated that the previously established Provisional Tolerable Weekly Intake (PTWI) of 25 μg kg^{-1} body weight was associated with a decrease of at least 3 IQ points in children and an increase in systolic blood pressure of approximately 3 mmHg (0.4 kPa) in adults. These consequences were considered important effects when viewed on a population level and the committee therefore withdrew the PTWI as it could no longer be considered health protective. Nevertheless, they retained the ML of 0.020 mg kg^{-1}Pb in milk.

Carabaos (water buffalos) in the Philippines were mainly raised to augment manpower in farming until early 1990s when the use was diverted towards meat and milk production. Buffalos contribute 72 million tons of milk and 3 million tons of meat annually to world food, much of it in areas that are prone to nutritional imbalances (Singh & Barwal, 2010). In 2010, the total carabao milk production in the Philippines reached about 5.8 million liters (BAS, 2011). The nutritional content of milk makes it important as a food source in the Filipino diet either as raw milk or processed as cheese, yogurt, butter, cream and native Filipino sweets. The increasing utilization of carabao milk for food production in the Philippines intensifies the need for a method suitable for the determination of relevant heavy metals. In this work, we present the use of microwave-assisted acid digestion for the complete mineralization of milk samples prior to the simultaneous detection of Cd and Pb using ICPMS as the element-selective detector. This simple and reliable method was aimed for use in routine monitoring of these environmental pollutants in milk samples. In addition, we report for the first time the analysis of these elements in carabao milk, both raw (obtained directly from the farm) and processed (commercially available) milk samples in the Philippines.

METHODOLOGY

Chemicals and reagents

All chemicals and reagents used in this work were of analytical reagent grade unless otherwise specified. Nitric acid was obtained from Merck (Darmstadt, Germany), hydrochloric acid was obtained from Mallinckrodt Chemicals (Thailand), and hydrogen peroxide was from Sigma-Aldrich (Steinheim, Germany). Single-element standards of Cd, Pb and rhenium (Re) with concentrations equivalent to 10,000

± 30 μg mL^{-1} and indium (In) with concentration equal to 1,000 ± 3 μg mL^{-1} were purchased from CPI International (Santa Rosa, CA, USA). A certified reference material, BCR 150 (spiked skim milk powder), was obtained from the European Commission, Joint Research Center, Institute for Reference Materials and Measurements (EC-JRC-IRMM, Geel, Belgium). Another reference material, NIST 1643e (Trace elements in water) was obtained from the National Institute of Science and Technology (Gaithersburg, MD, USA) and was used for validation. All dilutions and solution preparations were done using ultrapure water prepared using a Barnstead system (18.2 MΩcm resistivity, Thermo Fisher Scientific, Selangor Darul Ehsan, Malaysia).

Instrumentation

A Multiwave 3000 microwave digestion system (Anton Paar, Graz, Austria) fitted with a 16-position rotor for high digestion performance was used for the complete mineralization of the samples. An Agilent 7500cx ICPMS (Agilent Technologies, Germany) equipped with a MicroMist glass concentric nebulizer and an integrated autosampler (I-AS with type A vials, 89 x 6 mL capacity) was used as the element-selective detector. The monitored masses were *m/z* 111 for Cd and *m/z* 208 for Pb. The analysis was performed using standard mode and the optimum conditions typically used for the analyses, based on a previous study (Torres et al., 2010), are as follows: Rf power = 1550 W, carrier gas flow rate = 0.85 $Lmin^{-1}$, make-up gas flow rate = 0.25 L min^{-1}, nebulizer pump operated at 0.01 rps, sampling depth at 8.0 mm and the spray chamber was kept at 2.0°C.

Samples and sample preparation

Raw milk samples and processed milk samples were obtained from different towns and cities in Nueva Ecija, Philippines. Nueva Ecija is the largest province in Central Luzon covering an area of 5, 284 km^2 where majority of its land is utilized for agricultural purposes. As of January 1, 2011, the total carabao population in the province is 51,442 (BAS, 2011). Nueva Ecija gathers more milk from carabao than any other place in the Philippines and is the major supplier of carabao milk in Metro Manila (PCC, 2011). The location, age, breed and type of diet of the animal sources for the raw milk samples are summarized in Table 1, along with the details about the processed (commercially available) milk samples. All samples were stored in polyethylene containers and kept at 4.0°C during transport to the laboratory. Triplicate aliquots (0.500 mL each) of the milk samples were subjected to the optimized mineralization procedure prior to ICPMS analysis.

RESULTS AND DISCUSSION

Optimization and validation of method

The certified reference material BCR 150 was used for the method optimization and validation studies. This material is available in powder form but it is the only available certified reference material which is closely matched in terms of matrix to the target samples for the application of the method. In the method optimization, parameters were varied such as amount of sample, maximum power, and different acid and acid mixtures used in digestion.The use of microwave digestion for sample pre-treatment has been reported by Dobrzański et al. (2005) and Ataro et al. (2008). These previous studies used different microwave digestion systems compared to the one available to us, thus we found it necessary to optimize the conditions. Initially, different mineralization agents were added: nitric acid only, acid mixtures (nitric acid and hydrochloric acid), as well as mixtures of acid and oxidizing agent (nitric acid: hydrogen peroxide) at different proportions (2:1 and 3:1, v:v) while other parameters(amount of sample: 0.2 g, digestion period: 45 min, and maximum power of digestion: 600 W) were held constant. Figure 1a shows the percent recovery of Cd and Pb achieved with the different acid and acid mixtures used in the mineralization process. After subjecting to ANOVA, it was apparent that the results obtained do not have significant differences (at $\alpha < 0.05$), thus the subsequent optimization step was focused on the use of lowest amount of acid (2 mL nitric acid) added to 6.0 ml of water. This choice of mineralization agent will ensure that possible contamination during the sample preparation is minimized due to addition of only one

Table 1. Milk samples obtained from the different cities and municipalities of Nueva Ecija with product description (for the processed milk samples) and information about the animal source (for the raw milk samples)

Processed milk samples (commercially available)

Sample	Place of Origin	Product Description
CMCA1	Talavera, Nueva Ecija	Pasteurized milk with Coffee Flavor
CMCA2	Talavera, Nueva Ecija	Pasteurized milk with Buko-pandanFlavor
CMCA3	Talavera, Nueva Ecija	Pasturized milk with Chocolate Flavor
CMCA4	Talavera, Nueva Ecija	Pasteurized milk

Raw milk samples

Sample	Place of Origin	Age (years)	Diet	Breed
CMC1	Palayan City, Nueva Ecija	5	grass only	Philippine Carabao
CMC2	Cabanatuan City, Nueva Ecija	7	grass only	Philippine Carabao
CMC3	Cabanatuan City, Nueva Ecija	10	grass only	Philippine Carabao
CMC4	Palayan City, Nueva Ecija	7	grass only	Philippine Carabao
CMQ1	Quezon, Nueva Ecija	4	grass and rice hay	Bulgarian Murrah Buffalo
CMQ2	Quezon, Nueva Ecija	7	grass and rice hay	Bulgarian Murrah Buffalo
CMQ3	Quezon, Nueva Ecija	5	grassand rice hay	Philippine Carabao
CMQ4	Quezon, Nueva Ecija	7	grassand rice hay	Bulgarian Murrah Buffalo
CMQ5	Quezon, Nueva Ecija	6	grass and rice hay	Bulgarian Murrah Buffalo
CMQ6	Quezon, Nueva Ecija	6	grass and rice hay	Mestiza (Hybrid Carabao)
CMQ7	Quezon, Nueva Ecija	6	grass and rice hay	Mestiza (Hybrid Carabao)
CMQ8	Quezon, Nueva Ecija	7	grass and rice hay	Mestiza (Hybrid Carabao)
CMSD1	Sto. Domingo, Nueva Ecija	12	grass and rice hay	Bulgarian Murrah Buffalo
CMSD2	Sto. Domingo, Nueva Ecija	11	grass and rice hay	Bulgarian Murrah Buffalo
CMSD3	Sto. Domingo, Nueva Ecija	4.5	concentrate	Bulgarian Murrah Buffalo
CMSD4	Sto. Domingo, Nueva Ecija	5	concentrate	Bulgarian Murrah Buffalo
CMSD5	Sto. Domingo, Nueva Ecija	11	concentrate	Bulgarian Murrah Buffalo
CMSD6	Sto. Domingo, Nueva Ecija	10	concentrate	Bulgarian Murrah Buffalo
CMSD7	Sto. Domingo, Nueva Ecija	12	concentrate	Bulgarian Murrah Buffalo
CMSL1	San Leonardo, Nueva Ecija	15	grass only	Philippine Carabao
CMSL2	San Leonardo, Nueva Ecija	10	grass only	Philippine Carabao
CMSL3	San Leonardo, Nueva Ecija	8	grass only	Philippine Carabao
CMSL4	San Leonardo, Nueva Ecija	5	grass and rice hay	Bulgarian Murrah Buffalo
CMSL5	San Leonardo, Nueva Ecija	10	grass only	Philippine Carabao
CMSL6	San Leonardo, Nueva Ecija	12	grass only	Philippine Carabao

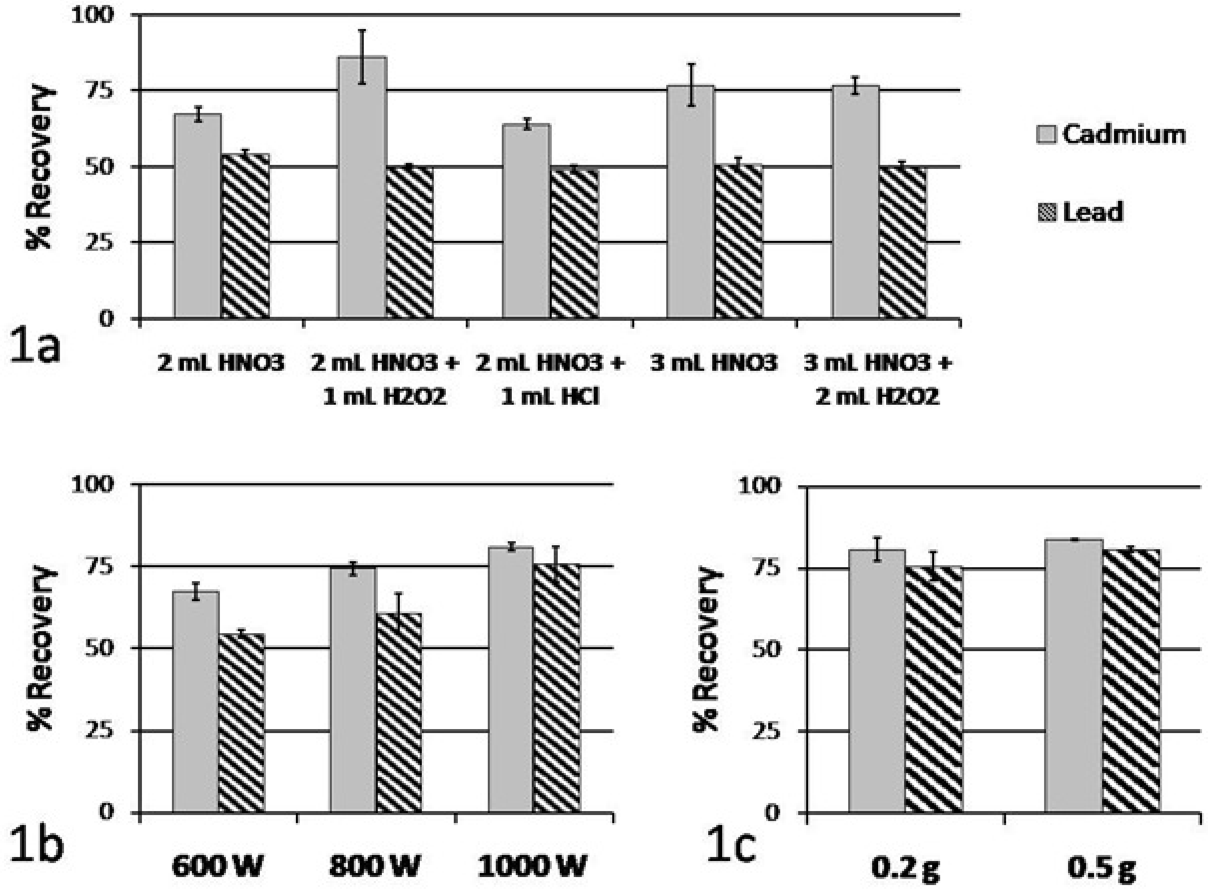

Figure 1. Percent recoveries of Cd and Pb as a function of different digestion parameters (acid used in the digestion, maximum power and amount of sample): (1a) effect of the use of different acid, acid mixtures, and mixture of acid and oxidizing agent on the percent recovery of the analytes in the reference material (amount of sample: 0.2 g; power: 600 W; digestion period: 45 min); (1b) effect of the maximum power used in digestion and (1c) effect of the amount of sample digested

reagent. Also, the use of only one mineralization agent is more economical if the method is intended for use in routine monitoring.

The maximum power used in the digestion was then varied (from 600 W to 800 W, and then to 1000 W) while keeping all other parameters constant. The power used in the microwave system linearly affects the temperature inside the digestion vessels. Higher digestion power (1000 W) used ensures that the temperature inside the vessels reaches the range between 180°C to 200°C, which favors mineralization of the organic constituents in the samples. The digestion program employed was as follows: the system was ramped to 600 W for 5 min, kept at this condition for 5 min, ramped to 1000 W for 5 min and held at this condition for another 20 min, before allowing the system to cool. This digestion program made certain that the temperature in the system reached only a maximum value of 200°C. This maximum power was chosen to provide a safe distance from the set value of 210°C, which is the maximum tolerable temperature of the microwave digestion unit. When the set value is reached, the instrument will force the microwave system to instantaneously abort the program. The apparent increase in the percent recoveries of both Cd and Pb with increasing maximum power used can be clearly seen in Figure 1b. It was then decided to use 1000 W for the succeeding optimization steps.

To further optimize the method, the amount of sample was increased from 0.2 g to 0.5 g, while maintaining other parameters at their respective values (period of digestion: 45 min; maximum power: 1000 W; and acid used in digestion: 2 mL nitric acid). The suggested minimum amount of the reference material that should be used for validation studies is 0.1 g. The data shown in Figure 1c reflect that the recovery of both Cd and Pb slightly improved when higher amount of sample was used. This was expected due to the use of more of the homogenized sample. Although there may be insufficient mineralization with larger amount of sample used, the results suggest that the digestion parameters were still suitable with the higher amount of sample. With this, it was deemed better to use higher amount of sample for better results.

For subsequent analyses, the optimized parameters were as follows: 0.5 g of sample was digested with 2 mL nitric acid and 6.0 ml of water at 1000 W for a period of 45 min. After digestion, the digests were transferred to 15 ml polyethylene containers and the volume was adjusted to 10 ml using ultrapure water. These pretreatment procedures take about 2 hr (for triplicate runs of 5 samples/CRMs) from the weighing step until the digests are ready for ICPMS analysis. The digests were then subjected to ICPMS analysis using the optimized parameters in the Agilent 7500 ICPMS system (Torres et al., 2010). Internal standards composed of In (*m/z*= 115) and Re (*m/z*= 185) were incorporated to the sample via peristaltic pump. Internal standards were added to compensate for any instrument drift and possible signal suppression or enhancement from matrix effects (Vanhaecke et al., 1992). The choice of internal standards is based on several requirements: that it should be near the *m/z* ratio of the target analytes, it should have comparable ionization energy and it should not be widely available in nature (Park & Song, 2005). For our purpose, we selected the internal standards In for Cd and Re for Pb.

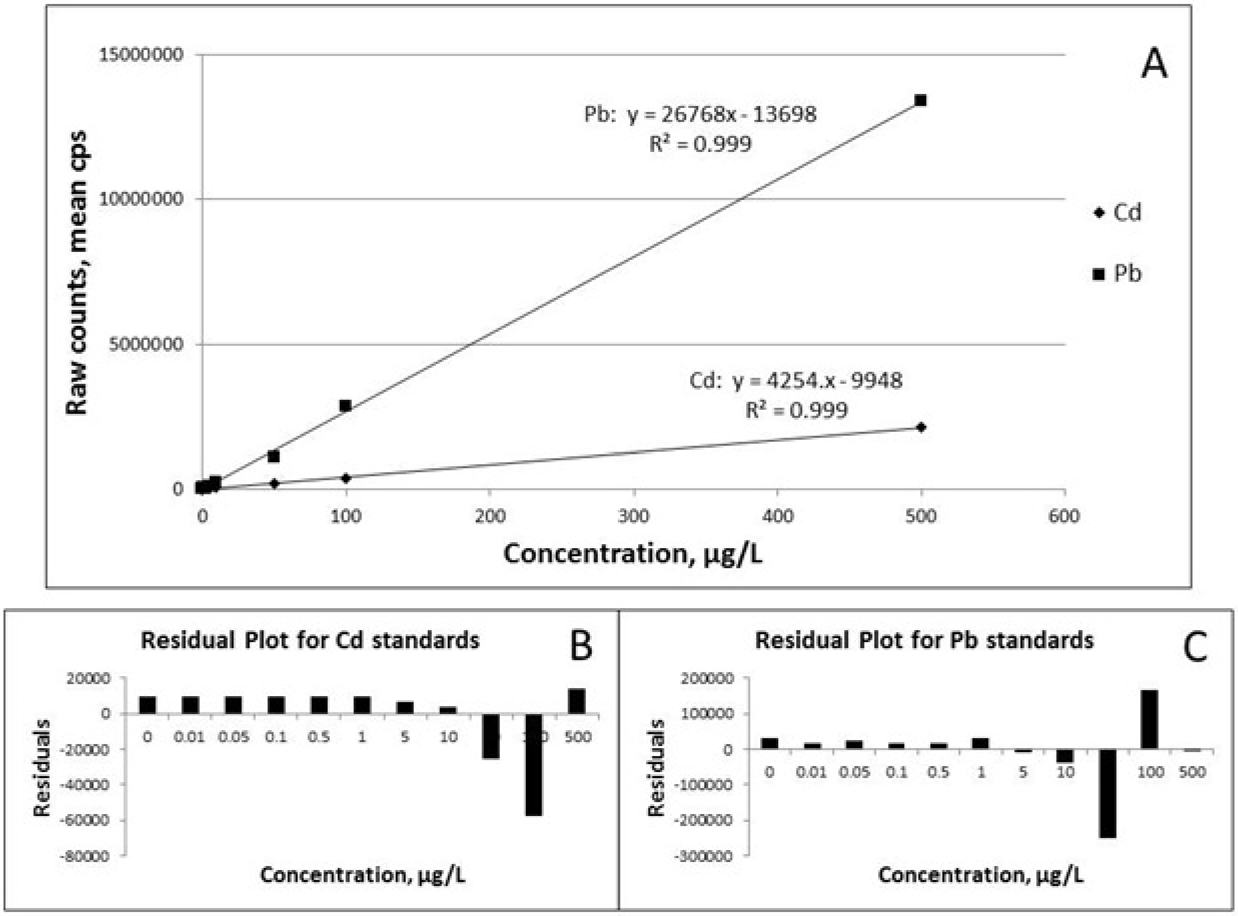

Figure 2. Evaluation of the linearity of the calibration standard solutions. (2A) shows the linearity of the calibrations standard solutions; (2B) and (2C) show results of the residual analyses for Cd and Pb standard solutions, respectively.

Table 2. Determined concentration of Cd and Pb in the milk samples (expressed as mean concentration, n = 3)

Sample	Cadmium		Lead	
	Concentration (ì gkg^{-1})	SD	Concentration (ì gkg^{-1})	SD
Processed milk samples (commercially available)				
CMCA1	< MDL		5.82	0.17
CMCA2	< MDL		3.48	0.12
CMCA3	5.17	0.13	2.74	0.17
CMCA4	0.11	0.07	0.49	0.21
Raw milk samples				
CMC1	< MDL		< MDL	
CMC2	0.20	0.05	< MDL	
CMC3	< MDL		1.17	0.12
CMC4	0.31	0.07	1.09	0.05
CMQ1	0.12	0.08	2.12	0.31
CMQ2	0.22	0.08	2.98	0.21
CMQ3	0.18	0.08	0.78	0.17
CMQ4	0.11	0.08	2.77	0.20
CMQ5	0.12	0.07	0.72	0.18
CMQ6	0.17	0.07	1.08	0.08
CMQ7	0.11	0.09	3.99	0.15
CMQ8	0.45	0.09	< MDL	
CMSD1	0.20	0.06	2.75	0.11
CMSD2	< MDL		< MDL	
CMSD3	0.14	0.06	< MDL	
CMSD4	0.16	0.05	< MDL	
CMSD5	0.20	0.07	< MDL	
CMSD6	0.12	0.07	1.29	0.07
CMSD7	0.11	0.07	6.79	0.20
CMSL1	0.23	0.08	6.10	0.23
CMSL2	0.29	0.08	2.89	0.16
CMSL3	0.26	0.08	2.63	0.16
CMSL4	0.33	0.07	4.93	0.16
CMSL5	0.25	0.08	2.97	0.14
CMSL6	0.15	0.07	1.89	0.14

*MDL – method detection limit

Table 3. Comparison of reported concentrations of Cd and Pb concentrations in milk samples by other researchers

Location	Concentration		Milk source	Method	Reference
	Cd	Pb			
Croatia	0.037 ± 0.007 mg kg^{-1}	0.27 ± 0.06 mg kg^{-1}	Cow (raw)	EAAS	Pavlovic et al. (2004)
Egypt	0.007 mg kg^{-1}	0.327 mg kg^{-1}	Cow (raw)	ICP-AES	Elsayed et al. (2011)
Turkey	0.257 ìg L^{-1}	6.83 ìg L^{-1}	Cow (raw)	GFAAS	Ay and Karayunlu (2008)
Italy	0.162 ìg g^{-1}	0.109 ìg g^{-1}	Sheep (raw)	ICP-AES	Coni et al. (1996)
	0.128 ìg g^{-1}	0.059 ìg g^{-1}			
	0.183 ìg g^{-1}	0.078 ìg g^{-1}			
	0.181 ìg g^{-1}	0.094 ìg g^{-1}			
	0.161 ìg g^{-1}	0.050 ìg g^{-1}	Goat (raw)		
	0.130 ìg g^{-1}	0.049 ìg g^{-1}			
Pakistan	0.076 ± 0.014mg L^{-1}	18.870 ± 0.062mg L^{-1}	Cattle (raw)	AAS	Javed et al. (2009)
	0.084 ± 0.003 mg L^{-1}	42.687 ± 0.051 mg L^{-1}	Goat (raw)		
India	0.23 ± 0.02 ìg ml^{-1}	0.85 ± 0.11 ìg ml^{-1}	Cow (raw)	AAS	Patra et al. (2008)
Spain	4.88 mgL^{-1}	14.82 mg L^{-1}	Cow (raw)	EAAS	Rodríguez et al. (1999)
	4.30 mgL^{-1}	10.25 mg L^{-1}	Cow (Processed)	EAAS	
	7.81 mg L^{-1}	11.86 mg L^{-1}	Goat (raw)	EAAS	
Lithuania	0.37 ìg kg^{-1}	0.47 ìg kg^{-1}	Cow (raw)	ICP-SFMS	Valiukenaite et al. (2006)
	0.18 ìg kg^{-1}	0.54 ìg kg^{-1}			
Nueva Ecija,	0.11 ± 0.07 to 5.17 ± 0.13 ìg kg^{-1}	0.49 ± 0.21 to 5.82 ± 0.17 ìg kg^{-1}	Carabao (processed)	ICP-MS	This study
Philippines	0.11 ± 0.07 to 0.45 ± 0.09 ìg kg^{-1}	0.72 ± 0.18 to 6.79 ± 0.20 ìg kg^{-1}	Carabao (raw)	ICP-MS	This study

For the method parameters, we assessed linearity, reproducibility and the method detection limit. The correlation coefficients calculated were 0.999 for both Cd and Pb within the range of 0.01 to 500 μg kg^{-1}of the calibration solutions as shown in Figure 2A. Further analysis of residuals reflected no visible trend with the determined concentration against the known concentration of the calibration standard solutions indicating that the change in counts monitored is linear with the change in concentration (shown in Figures 2B and 2C). The instrument drift was monitored by analyzing repeatedly the calibration standard containing 1.0 μgkg^{-1} of both Cd and Pb throughout the ICPMS analysis sequence. The repeated determination of one standard will give an indication on the instrument performance and day-to-day variability of the measurements. The calculated RSD for the drift standards, on different ICPMS analysis days, were less than 2% for both Cd and Pb (n = 7) indicating that the instrument performance was reliable throughout the measurements. The estimated method detection limits were 0.09 μgkg^{-1} and 0.33 μgkg^{-1} for

Cd and Pb, respectively. These were calculated by getting the standard deviation of the measurements of seven replicates of a solution containing 1 μg kg^{-1}of both Cd and Pb, then subsequently multiplying it by the student's t-value (n = 7; Student's t-value = 3.143; Ripp, 1996).

After assessment of the method performance, the optimized conditions were applied to triplicate aliquots (0.5 g of BCR 150 weighed to the nearest 0.1 mg) of the reference material and the determined values were 18.24 ±0.18 μgkg^{-1} and 807.57 ± 7.07 μg kg^{-1} for Cd and Pb, respectively. These determined values correlate well with the certified values (Cd: 21.8 ± 1.4 μg kg^{-1} and Pb: 1000 ± 40.0 μg kg^{-1}) in the reference material. These recovery values account for 83.7% and 80.7% in the reference material of Cd and Pb, respectively. The values fall within the limits of acceptable recovery percentages (80-110%) as a function of the analyte concentration, as suggested by Taverniers et al. (2004). Since the reference material available to us is in the solid form, we also analyzed another certified reference material NIST 1643e (triplicate samples of 0.500 mL) and the determined concentrations were 6.48 ± 0.10 μg L^{-1} for Cd and 21.96 ± 0.87 μg L^{-1} for Pb, which were in good agreement with the certified values in the material 6.568 ± 0.073 μg L^{-1} and 19.63 ± 0.21 μg L^{-1}, for Cd and Pb, respectively. After these validation experiments, we subjected actual samples to the pretreatment procedures prior to the actual analysis by ICPMS.

Analysis of actual samples

The samples analyzed in this study were carabao milk samples from the different cities and municipalities in the province of Nueva Ecija, which is the top producer of carabao milk in the Philippines. The determined Cd and Pb levels for all samples are summarized in Table 2. All the determined Pb levels were below the suggested maximum level of the joint FAO/WHO Food Standards programme for milk, which is 0.020 mg kg^{-1}. The concentrations of Pb were in the range 0.49 ± 0.21 to 5.82 ± 0.17 μg kg^{-1}for the processed milk samples, and 0.72 ± 0.18 to 6.79 ± 0.20 μg kg^{-1} for the raw milk samples. Although there is no maximum limit established for Cd in milk, it can be observed that the levels of Cd in the samples were detectable. The Cd levels determined were in the range 0.11 ± 0.07 to 5.17 ± 0.13 μg kg^{-1}for the processed milk samples, and 0.11 ± 0.07 to 0.45 ± 0.09 μg kg^{-1} for the raw milk samples. The detection of these metal contaminants in carabao milk being supplied for human consumption highlights the need for monitoring of these metals in this foodstuff.

The values reported in this study were generally lower compared to the values reported by other researchers from other countries. Table 3 shows the results obtained in the analysis of Cd and Pb in milk samples from different countries as well as the animal source and method used in the analysis. The differences in the reported values with what was determined in this study may be due to the animal source in the other studies, which is cow, and in this study, which is carabao. There are studies showing that the concentrations of heavy metals in cow's milk are lower as compared to buffalo's or even goat's milk (Enb et al., 2009). Other factors that may have influenced the differences in the reported values are the analytical techniques used for the determination and the actual grazing areas for the animals used as sources of the milk samples. Aside from these factors, the heavy metal concentrations may have been affected by conditions such as age, type of diet and breed of the carabao. Although we have noted the information about the carabaos used as milk sources in this work, the correlation that we got is only indicative of possible effect of these factors on the metal load of the milk samples. For further studies, a wider scope with more sampling periods may be conducted since the results show that the optimized method offers a simple way of determining these contaminants in this type of sample.

This is the first time that the analysis of Cd and Pb in carabao milk in the Philippines was performed. Thus, this information can be useful for further toxicological studies and to estimate the average intake of Cd and Pb due to consumption of carabao milk. For future work, it might be worthwhile to conduct a comparative study of the metal content of the different milk samples (cow, carabao, goat, formula and breast milk) in the Philippine setting. Processing of the milk samples might also have effects on the metal concentrations. It has been reported that the processing was related to the observed increase of metal concentrations in the processed milk and milk products (Enb et al., 2009). Also for further work, it might be sensible to carry out more detailed correlation studies which will look at the animal source, the raw milk and the processed milk. Analysis of more samples may also give more conclusive results.

CONCLUSION

A method suitable for the simultaneous determination of Cd and Pb in milk samples was optimized and validated. The method is based on the use of microwave-assisted acid digestion as the sample pre-treatment step prior to detection using ICPMS. The optimized method showed excellent linearity and low estimated detection limits, which are desirable for trace analysis of these contaminants in foodstuffs. The method was validated using certified reference materials, BCR 150 (spiked-skim milk powder) and NIST 1643e (trace elements in water), and the determined Cd and Pb values agree well with the certified values in the reference materials. The application of the method to milk samples collected from different cities and municipalities in Nueva Ecija in the Philippines showed that low concentrations of Cd and Pb were detected in these samples. The determined amounts were below the maximum limits set by the authorities but the presence of these contaminants in this important nutrient source necessitates for routine monitoring of these metals in these samples. The method presented is a simple, rapid and repeatable method which can be used for the routine monitoring of both Cd and Pb in milk samples.

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