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DIRECT DETERMINATION OF CADMIUM IN BLOOD AND URINE BY ZEEMAN EFFECT ELECTROTHERMAL AAS

by

K.R. Lum[1] and D.G. Edgar[2]

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[1] Lakes Research Branch
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, Canada L7R 4A6

[2] Nissei Sangyo Canada, Inc.
89 Galaxy Blvd.
Rexdale, Ontario, Canada M9W 6A4

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Management Perspective.

As indicated on the title page, this paper will be presented in part at the Second Nordic Symposium on Trace Elements in Human Health and Disease. Cadmium has a very long biological half-life, ca 30 years and hence is bio-accumulated. The fact that there is no metabolic pattern allowing for its elimination means that biological monitoring of physiological fluids must be carried out to determine exposure levels. Proper diagnosis and treatment depends on accurate analytical data. The work reported in this article is to our knowledge the first whereby cadmium in blood has been analysed without matrix modification or ashing agents, the addition of which can bias results because of the cadmium contributed by them. As well the method has been validated by analysing samples obtained from Dr. P. Weber of the Toxicology Centre of Laval University. The method will be of interest to fisheries biologists studying toxic metal metabolism using fish blood.

Perspective – gestion

Comme l'indique la page titre, le présent document sera présenté en partie lors du 2^{e} Colloque nordique sur les oligo-éléments en rapport avec la santé et la maladie. Le cadmium a une très longue demie-vie, environ 30 ans; il est donc bioaccumulé. Le fait qu'il n'y ait aucune voie métabolique permettant de l'éliminer signifie qu'il faut vérifier les liquides physiologiques pour connaître le degré d'exposition. Le diagnostic et le traitement adéquats dépendent de données analytiques exactes. Le travail signalé dans le présent article est à notre avis le premier selon lequel le cadmium du sang est analysé sans modification de matrice ni addition d'agents de calcination qui peuvent biaiser les résultats en raison du cadmium qu'ils contiennent. Cette méthode a également été validée par l'analyse d'échantillons fournis par le D^{r} P. Weber du Centre de toxicologie de l'Université Laval. Cette méthode intéressera les biologistes des pêches qui étudient le métabolisme des métaux toxiques à l'aide du sang des poissons.

ABSTRACT

Cadmium in blood and urine was determined using a Stabilised Temperature Platform Furnace (STPF) without matrix modification or addition of an ashing agent. Calibration was done using dilute acid standards whose concentrations had been confirmed against diluted NBS-SRM Trace Elements in Water. The procedure was validated with samples obtained from exposed workers and supplied by the Interlaboratory Comparison Program of the Toxicology Centre of Quebec, Canada.

KEY WORDS: Cadmium; blood; urine; electrothermal atomization; AAS.

RÉSUMÉ

Le dosage du cadmium dans le sang et l'urine a été fait à l'aide d'un four à plate-forme à température stabilisée (PFTS) sans modification de la matrice ni addition d'agent de calcination. L'étalonnage a été fait à l'aide d'étalons dans une solution acide dont les concentrations avaient été confirmées par rapport à des oligo-éléments NBS-SRM dilués dans l'eau. Cette méthode a été validée avec des échantillons prélevés chez des travailleurs exposés et fournis dans le cadre du Programme de comparaisons interlaboratoires du Centre de toxicologie du Québec, Canada.

MOTS CLÉS : Cadmium; sang; urine; atomisation électrothermique; SAA.

INTRODUCTION

Continuing interest in the effects of low-level exposure by cadmium and its compounds on human health has prompted the development and testing of analytical procedures for determining minute amounts of cadmium in biological tissues and physiological fluids. Progress in our understanding of the pathways of cadmium in humans depends on simple, accurate and rapid analytical methods. Electrothermal atomization AAS is an attractive instrumental technique because of its high sensitivity for cadmium and its relative simplicity and ready availability compared with electrochemical, neutron activation or emission spectrometric techniques.

The major drawback of ETA-AAS for the analysis of blood and urine arises from the large amounts of organic matter and salt which if not removed generates molecular absorption and light scatter, which greatly increase the background absorbance and impair the precision and accuracy of the determination. Thus, matrix modification procedures and pre-treatment of whole blood and urine samples are usually required (e.g. ref 1-10) . In this paper we describe the validation of a direct injection procedure for analysing cadmium in these matrices without matrix modification or addition of an ashing agent. The advantage of such a procedure would be the virtual elimination of the reagent blank.

ANALYTICAL METHODOLOGY

Analyses were performed on an Hitachi Model Z-8000 Polarized Zeeman Effect AAS equipped with an autosampler, and using pyrolytically-coated graphite tubes and platforms supplied by Ringsdorf Gmbh. An optical-temperature sensor was used for work with platforms to ensure maximum heating rate. The time-resolved absorbance signals were displayed on an integral CRT and printed out on the thermal printer of the instrument's computer. A Hamamatsu hollow cathode lamp was operated at 5 ma.

Cadmium standards were prepared by serial dilution of 1000 mg/l Spex standard (Spex Industries, Inc., Metuchen, N.J.) in 1% nitric acid (sub-boiling distilled from Seastar Chemicals, Sidney, B.C) and subsequently stored in acid-cleaned polyethylene bottles. Distilled water was passed through a Milli-Q system (Millipore Ltd., Mississauga, Ontario) before use.

Blood and urine samples were kindly provided by Dr. Jean-Philippe Weber of the Centre de Toxicologie du Quebec (Universite Laval, 2705 boul. Laurier, Quebec G1V 4G2), as part of an Interlaboratory Comparison Program. Whole blood was diluted 5 to 11 times with deionized-distilled water prior to injection into the graphite furnace. Urine was injected without dilution.

New pyrolytically-coated tubes and platforms were conditioned as described previously (ref 11), except that atomization temperature was kept constant at the pre-selected value.

RESULTS

Optimum temperature program.

The optimum temperature program for whole blood analysis is shown in Table 1. The drying program was selected by visually monitoring the sample drying process using a mirror supplied by the instrument's manufacturer, and a pen-light while observing the temperature on the video display to ensure that excessive boiling, bumping or splattering of the sample did not occur. Because we do not add Triton X-100 or other chemicals to prevent splattering, this was a critical step in the procedure.

Ashing temperatures between 300 and 1100 C were investigated at an atomization temperature of 1800 C. Peak area absorbance increased up to 900 C beyond which loss of analyte was observed (Table 2).

Atomization efficiency was studied in the temperature range 1600 to 2300 C and ashing temperature set at 700 C. The results (Table 3) show that analyte absorbances were constant in the range 1800 to 2300 C; relative standard deviation of the averaged atomic absorption signals in this temperature range was 2.6%. The average background absorbance was 0.1143 with a relative standard deviation of 5.2%.

Time-resolved absorbance profiles.

Atom formation processes and vapour phase interferences can be investigated with the aid of time-resolved absorbance profiles. As one would expect the atomic absorption signals for a 1 ug/l cadmium standard in 1% nitric acid is clean and well-resolved from the practically non-existent background (Fig 1a). Using the temperature program listed in Table 1, a spiked seawater sample was studied (Fig 1b). The analyte signal is partially resolved from the very high background absorbance signal which peaks at ca 3 absorbance units. The overlap in the two signals occurs in an area where the background is less than

1.5 absorbance units and hence Zeeman correction can be expected to give an accurate result. Note that the appearance time of the analyte is increased relative to the cadmium standard suggesting that seawater contains substance(s) which decrease the rate at which atomization occurs. In the case of blood serum, the background absorbance signal shows the presence of two types of potentially interfering substances (Fig 1c). Partial overlap occurs only with the first of the two peaks and the background absorbance is well-within the range where efficient correction can be achieved. The appearance time of cadmium in serum is identical to that for the cadmium standard. The profile for undiluted urine shows complete resolution of analyte and background absorbance signals (Fig. 1d), although efficient correction of any direct overlap could be expected as neither of the two background peaks are over 1 absorbance unit. Cadmium in urine also has a similar appearance time as cadmium in dilute nitric acid matrix. The last profile is for whole blood diluted 11 times with deionized distilled water (Fig. 1e). The time scale shows the ashing stage and one can clearly see the very large (>1.5 AU) background signal during this step and the dramatic effect this has on the analyte signal. During atomization, however, the background doublet is not large especially in the region of overlap with the analyte signal.

Performance of the method.

The procedure was validated by analysing six samples each of urine and blood (diluted 11x) obtained from exposed workers using the temperature program listed in Table 1. Each sample was loaded onto the autosampler carousel and interspersed with aqueous standards containing 1 ug/l of cadmium in 1% nitric acid. This standard had been checked for accuracy against diluted NBS-SRM 1643b Trace Elements in Water. Five replicate injections of samples and standards were run and the results for analyses on two separate weeks are shown in Table 4. One of our main objectives here was to assess whether samples run in this fashion, without operator attention and monitoring tube performance with number of injections, would yield accurate results.

The results are generally well within 15% of the target concentrations. The latter are set by elementary statistical analysis of the results reported by the laboratories (not including ours) participating in the Interlaboratory Comparison Program (ref 12). Usually the number of laboratories was greater than 16. The significant deviation of our result for C-81 from the assigned target concentration was likely caused by unusual behaviour of this sample during the drying stage. In the development of the procedure we encountered one sample which did not dry completely following the general drying program and a

modified program was established for this sample. This is of course not possible using an autosampler.

The detection limit of the method was calculated from the average absorbance values of the 1 ug/l standard througout the development and testing of the procedure and the average blank absorbance value. These were 0.102 (s.d. = 0.015) and 0.0015 (s.d. = 0.0003) respectively. Taking twice the blank value as the practical limit of detection gives a concentration of 0.03 ug/l.

Conclusions.

The procedure described in this article offers a simple means of determining cadmium in whole blood and urine by direct injection without the need for adding a matrix modifier or ashing agent. Calibration of the analysis is done using a dilute acid standard which had previously checked against diluted NBS-SRM Trace Elements in Water. The analysis of samples from exposed workers shows that the method yields accurate results. Variations in the constituents of blood samples can result in poor accuracy during unattended operation with an autosampler, because of loss of sample analyte during the drying stage. This problem can easily be rectified by modifying the drying program accordingly.

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Table 1. Temperature Program for the determination of cadmium in whole blood and urine.

Program Step	Temperature, C	Time
Dry	90 - 120	15 s
	120 - 150	30 s
	150 - 600	10 s
Ash	600 - 600	30 s
Atomization	2000- 2000	7 s
Clean	2500- 2500	3 s

Table 2. Effect of ashing temperature on absorbance signals at an atomization temperature of 1800 C, n = 5.

Ashing Temperature	Peak Area Absorbance (standard deviation)	
300 C	0.0524	(0.0027)
400 C	0.0368	(0.0024)
500 C	0.0451	(0.0019)
600 C	0.0531	(0.0009)
700 C	0.0558	(0.0055)
800 C	0.0669	(0.0081)
900 C	0.0689	(0.0055)
1000C	0.0532	(0.0046)
1100C	0.0129	(0.0041)

Table 3. Effect of atomization temperature on peak absorbance of cadmium in blood, n = 5.

Atomization Temperature	Peak Area Absorbance (standard deviation)	
1600 C	0.05683	(0.0071)
1700 C	0.05863	(0.0057)
1800 C	0.06137	(0.0071)
1900 C	0.06023	(0.0030)
2000 C	0.05830	(0.0031)
2100 C	0.05807	(0.0019)
2200 C	0.06150	(0.0023)
2300 C	0.06130	(0.0051)

Table 4. Cadmium in blood and urine provided by Centre de Toxicologie du Quebec. Concentrations in ug/l.

SAMPLE	TARGET CONCENTRATION	THIS STUDY
	URINE	
D-56	3.0	2.70
D-57	0.6	0.62
D-58	4.1	4.21
D-62	5.5	4.98
D-63	2.2	2.19
D-64	4.0	3.29
	BLOOD	
C-73	1.5	1.50
C-74	4.5	3.50
C-75	6.0	6.20
C-79	5.0	4.70
C-80	9.0	8.08
C-81	7.0	5.40