Multivariate Optimization Applied to Chromium Determination in Milk and Similar Baby Food Samples by Graphite Furnace Atomic Absorption Spectrometry

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ABSTRACT

Chromium (Cr) is an essential element, but high levels in foods can be toxic particularly for children. A fast and efficient method to determine Cr in milk and other infant foods using slurries and liquid samples is presented. Slurries were prepared in ultrapure water with 10 minutes of sonication. The liquid samples were diluted in ultrapure water when necessary. Multivariate optimization was used to establish some optimal analytical parameters through a fractional factorial design and a central composite design. Slurried and diluted samples were analyzed directly by GFAAS. The method presented a limit of detection of 1.43 µg L^{-1}, characteristic mass of 4.5 pg (recommended value of 3.0 pg), RSD from 1.5 to 11.8% (n=8), and a linear range from 4.78 to 50.0 µg L^{-1} using rhodium (500 µg) as the permanent modifier. The accuracy was evaluated by analyzing standard reference material SRM 8435 Whole Milk Powder. Good agreement was found with the obtained and certified values. The Cr concentrations of the powdered samples were between 0.042 and 1.900 µg g^{-1}.

INTRODUCTION

Food fortification is important in combating nutritional deficiencies. Moreover, processing steps, fortification, and the existence of various formulations employing different components as raw materials increase the possibility of undesirable elements in the final product. Furthermore, the interaction between constituents of foods must be evaluated because excessive addition of some nutrients may lead to the disability of others which can become extremely harmful. In this respect, infant foods deserve special attention because they are essential for developing the immune system. Thus, proper quality control of these food products is of great importance. In this context, the quantification of inorganic constituents in foods is required in order to monitor the levels of toxic and essential elements and to ensure maintenance of the quality of the food. In food analysis, sample preparation is a critical step and can consume up to 80% of the total time spent with the analysis (1). Traditional methods for preparing baby food samples includes wet or dry digestion processing (2), microwave-assisted digestion (3-5), lyophilization and ashing of the samples (6). However, these conventional methods have some disadvantages such as the high possibility of loss of volatile elements and contamination, poorer detection limits due to dilution of the samples, time-consuming and often very costly (7, 8). On the other hand, there are some alternatives for the preparation of samples that minimizes the drawbacks of decomposition such as direct introduction of the sample after solubilization, dilution or slurry preparation (9). The effectiveness of these procedures often depends on the type of food, the analytes and the employed analytical technique. There is, therefore, a need to develop faster methods with high accuracy and precision, where the losses and contamination are minimized and direct analysis or preparation is possible in a few steps.

The fact that chromium (Cr) is an essential mineral was first demonstrated by Mertz in 1969 (10) in rats and the importance of Cr in humans was demonstrated by Jeejebhoy et al. in 1977 (11). In the years following, a number of papers on the impact of Cr in human nutrition during different clinical and stress situations were published (12-17) with the main focus on the association between Cr and diabetes mellitus for type 2 diabetes (18). A number of animal trials were performed as well (19, 20). It was not until the 1990s that Cr also started to be studied intensively as an essential mineral in livestock (cattle, sheep, horses, pigs, and poultry) (21).

The association between Cr and carbohydrate metabolism has been demonstrated by trials involving livestock fed parenteral nutrition (21). The total amount of Cr in the human body ranges from 0.4 to 6.0 mg. The Cr reserve relative to body

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weight is higher in newborn children in comparison to adults (22). Numerous studies show evidence that Cr is essential for the lipid metabolism and reduces the risk of atheroenesis. It is assumed that the activity of Cr is mediated by the anabolic action of insulin, but other mechanisms cannot be ruled out. Chromium acts as a cofactor for insulin and, therefore, Cr activity in the organism is parallel to the functions of insulin. Despite its ability to enhance insulin activity, Cr cannot substitute for insulin (21). A number of studies confirm the association between Cr and the metabolism during increased physiological, pathological, and nutritional stress, e.g., fatigue, trauma, gestation, and different forms of nutritional (high carbohydrate diet), metabolic, physical, and emotional stress as well as environmental effects (23).

The average concentration of Cr in human milk is reported to be below 0.4 μg L^{-1} (24-25). However, in a study performed with Brazilian mothers from Belo Horizonte (MG-Brazil), the Cr concentration in breast milk varied from 0.69 to 17.3 μg L^{-1} (26). The recommended daily allowance as established in 1989 by the Food and Nutrition Board of the National Research Council, Canada (27) is an intake of 750 mL of milk from milk formulas and other foods. A range of Cr intake from 50 to 200 μg/day is tentatively recommended for adults. Casey et al. (25) reported that the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for infants from the age of 0 to 6 months is 10 to 40 μg Cr/day.

In this context, a method for the determination of Cr in whole milk, skim milk, milk beverage, infant formula, soy-based formula, goat's milk, and mother's milk by graphite furnace atomic absorption spectrometry (GFAAS), without sample pretreatment, was developed.

EXPERIMENTAL

Instrumentation

In this study, a PerkinElmer® model AAnalyst™ 400 atomic absorption spectrometer was used, equipped with an HGA®-800 graphite furnace, background correction with a deuterium lamp, and an AS-800 autosampler (PerkinElmer, Inc., Shelton, CT, USA). The readings were made using integrated absorbance (peak area). A PerkinElmer Lumina® hollow cathode lamp (HCL) of Cr was used, operating under the conditions as recommended by the manufacturer. A hollow cathode lamp for Cr (PerkinElmer Part No. N305-0119) was employed using a current of 30 mA, slit width of 0.2 nm, and wavelength of 357.9 nm.

Reagents, Solutions and Certified Reference Material

Deionized water (resistivity of 18.2 MΩ·cm^{-1}) was obtained with a Milli-Q™ system (Millipore Corporation, Bedford, MA, USA) and purified immediately before preparation of the solutions. Nitric acid was obtained from Merck (Darmstadt, Germany). Solutions (1000 mg L^{-1}) of iridium, ruthenium, and rhodium were obtained from Fluka (Buchs, Switzerland) and used at 1 mol L^{-1} in hydrochloric acid. Tungsten in the same solution was acquired from Merck (Titrisol®, Merck).

Plastic bottles, autosampler cups, and glassware were cleaned by soaking in 20% (v/v) HNO_3 for one day, rinsing many times with Milli-Q water, and then dried. The autosampler washing solution, containing 0.05% (v/v) Triton® X-100 (Merck) plus 0.1% (v/v) isopropanol (Sigma-Aldrich, São Paulo, Brazil), was used to avoid analyte adsorption onto the surface of the container and clogging of the capillary sampling tip, as well as to improve dispersion of the sample solution onto the platform.

Chromium stock solutions (1000 mg L^{-1}) were prepared from Titrisol® (Merck) in 5% (v/v) nitric acid.

A standard certified material NIST SRM Whole Milk Powder (National Institute of Standards and Technology, Gaithersburg, MD, USA) was used to check for accuracy of the method.

Graphite Tube Treatment

The graphite tubes were treated independently with 500 μg of each studied permanent modifier (Ir, Rh, Ru) by applying 50 μL of each metal solution (1000 mg L^{-1}) onto the wall of the tube and submitting them 10 times to a furnace temperature program as previously described (28).

Optimization Strategies

For choosing the most appropriate permanent modifier, various measures were carried out in integrated absorbance and using the background signal ratio in accordance with the manufacturer's recommended conditions. Thus, 20 μL of whole milk diluted 1 + 1 with deionized water were injected into the graphite tube treated with the permanent modifiers (W, Ru, Rh, Ir), and also in a tube without modifier. The CTAC (Cetyltrimethylammonium chloride) reagent of 0.1% (v/v) was used to prevent formation of carbonaceous residues (29-31).

In all optimization steps, 20 μL of the prepared slurry was used. A sample of liquid whole milk diluted 1+1 and spiked with 30 μg L^{-1} Cr was used since in the initial studies, using the heating program recommended by the manufacturer, the obtained analytical signal was not satisfactory. Initially, a 2^{3-1} fractional factorial design was carried out in order to investigate the influence of the main variables involved in the Cr determination process by GFAAS such as pyrolysis and atom-

ization temperatures and pyrolysis time (Table I). Based on these results, a central composite design (CCD) was applied (Table II), using the significant variables and the Statistica 6.1 software (Statsoft, Inc. / Dell, Inc., USA) (32).

Parameters of Merit

For the studies on matrix effects, the samples were divided into groups according to type. For each group, a sample was prepared by mixing equal volumes of each sample belonging to the group.

Group 1: Samples of whole milk powder, total of 5 samples (composite sample A1).

Group 2: Samples of skimmed milk powder, infant formulas and enriched, total of 5 samples (composite sample A2).

Group 3: Dairy drink powder samples, total of 2 samples (composite sample A3).

Group 4: Liquid skimmed milk samples, total of 7 samples (composite sample A4).

Group 5: Powdered soybean food sample total of 1 sample (composite sample A5).

Group 6: Samples of liquid soybean meal, total of 2 samples (composite sample A6).

Then, aqueous calibration curves were constructed (0 to 50 μg L^{-1}) and the standard addition curves (0 to 50 ug L^{-1}) in each composite sample were made to verify the performance of the optimized method and also to study the matrix effects. Linearity was assessed by linear correlation coefficients. The characteristic mass was obtained through the aqueous calibration curve. The limits of detection and quantification were calculated according to IUPAC (n = 10 and parameters of the aqueous curve). The precision (repeatability) was evaluated with 8 replicate solutions in 3 different concentrations: 5, 10, and 20 μg L^{-1}. The accuracy was checked by analysis of the certified reference material NIST SRM 8435 Whole Milk Powder. Its preparation consisted of dissolution in deionized water and analysis against the aqueous curve.

RESULTS AND DISCUSSION

Optimization of the Experimental Conditions

Three permanent modifiers showing satisfactory results in the study were: W, Rh, and Ru. Since Rh showed higher sensitivity and low background absorption, this modifier was chosen for further studies.

The results of the fractional factorial design can be seen Table III. Analyzing the Pareto chart (Figure 1), it can be seen that all variables were significant at the 95% confidence level. The results obtained in the fractional factorial design were employed to optimize the method for the determination of Cr in milk and similar products by GFAAS.

The individual effect of these variables was positive for time and atomization. This suggests an increase in the values of these variables to obtain maximum response (integrated absorbance), unlike the pyrolysis temperature (PT), which showed significant negative effects, suggesting a decrease of PT to achieve the maximum response.

TABLE I
Concentration Levels Studied Using Fractional Factorial Design (2^{3-1}) in Optimizing the Method for the GFAAS Determination of Cr in Milk and Similar Baby Foods

Variable	Low Level (-1)	High Level (+1)
Pyrolysis temperature (PT) (ºC)	1300	1900
Atomization temperature (AT) (ºC)	2000	2600
Pyrolysis time (*t*) (s)	30	50

TABLE II
Concentration Levels Studied in CCD Using the Optimization Method for GFAAS Determination of Cr in Milk and Similar Baby Foods

Factors	Evaluated Levels
TP	1095 ºC (-√3), 1300 (-1), 1600 (0), 1900 (+1), 2105 (√3)
TA	1795 ºC (-√3), 2000 (-1), 2300 (0), 2600 (+1), 2805 (√3)
t	6 (-√3), 20 (-1), 40 (0), 60 (+1), 74 (√3)

TABLE III
Factorial Planning for GFAAS Determination of Cr in Milk and Similar Baby Foods Using Ru as Permanent Modifier

Test	PT (ºC)	AT (ºC)	T (s)	Integrated Absorbance (A s^{-1}) Average ± s (n=3)
1	1300 (-1)	2000 (-1)	50 (+1)	0.260 ± 0.026
2	1300 (-1)	2600 (+1)	30 (-1)	0.283 ±0.010
3	1900 (+1)	2000 (-1)	30 (-1)	0.018 ±0.002
4	1900 (+1)	2600 (+1)	50 (+1)	0.080 ±0.006

The values in parentheses correspond to the encoded value.

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From the results obtained in the fractional factorial design, a central composite design (CCD) was constructed to verify the optimum conditions for the determination of Cr in different milks and similar baby foods. The results of the CCD can be viewed in Table IV. Analyzing the Pareto chart obtained by the CCD, one can see that all variables were significant at a confidence level of 95% as well as the interactions between them (Figure 2). The PT and time of pyrolysis (*t*) variables had significant negative effects, suggesting a higher response when evaluating the PT and *t* at lower levels, unlike the atomization temperature (AT), which showed significant and positive effects, suggesting an increase of AT leads to a better response. The interaction between AT and PT had a significantly positive effect, suggesting an increase in response when working simultaneously at the lower level of PT and the higher level of AT. There is a significant negative effect on the interactions between *t* and PT and between *t* and AT, while the quadratic interaction of *t* had a significant positive effect. There is a significant negative effect on the interaction between *t* and PT and between *t* and AT, while the quadratic interaction of *t* had a significant positive effect.

TABLE IV
Results of Central Composite Design Optimization in the GFAAS Determination of Cr in Milk and Similar Baby Foods

Test	PT (ºC)	AT (ºC)	*t* (s)	Integrated Abs. (A s^{-1}) Average ± s (n=2)
1	1095 (-√3)	2300 (0),	40 (0)	0.860 ± 0.023
2	1300 (-1)	2000 (-1)	20 (-1)	0.760 ± 0.007
3	1300 (-1),	2000 (-1)	60 (+1)	0.731 ± 0.028
4	1300 (-1),	2600 (+1)	20 (-1)	0.929 ± 0.006
5	1300 (-1),	2600 (+1)	60 (+1)	0.923 ± 0.012
6	1600 (0)	1795 (-√3)	40 (0)	0.062 ± 0.083
7	1600 (0)	2300 (0)	6 (-√3)	1.146 ± 0.198
8	1600 (0)	2300 (0),	40 (0)	0.776 ± 0.017[a]
9	1600 (0)	2300 (0),	74 (√3)	0.657 ± 0.000
10	1600 (0)	2805 (√3)	40 (0)	0.997 ± 0.030
11	1900 (+1)	2000 (-1)	20 (-1)	0.287 ± 0.001
12	1900 (+1)	2000 (-1)	60 (+1)	0.071 ± 0.009
13	1900 (+1)	2600 (+1)	20 (-1)	0.762 ± 0.055
14	1900 (+1)	2600 (+1)	60 (+1)	0.256 ± 0.054
15	2105 (√3)	2300 (0),	40 (0)	-0.001 ± 0.004

[a] Five replicates were performed at the midpoint. The figures in brackets refer to the coded values.

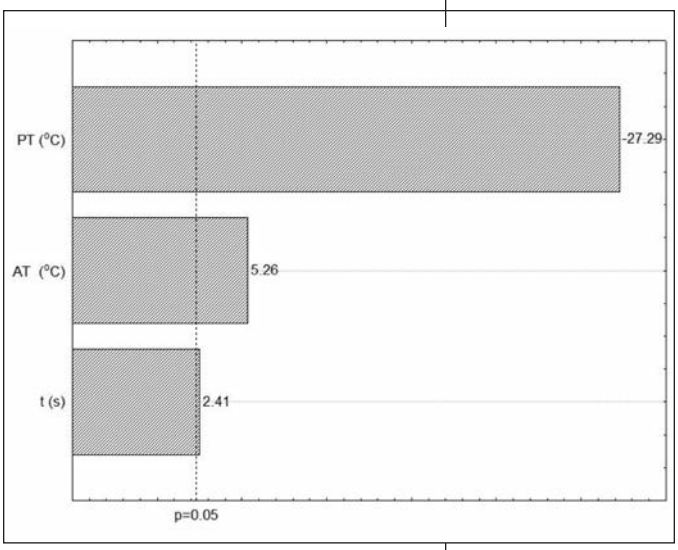

Fig. 1. Pareto chart obtained with the fractional factorial design.

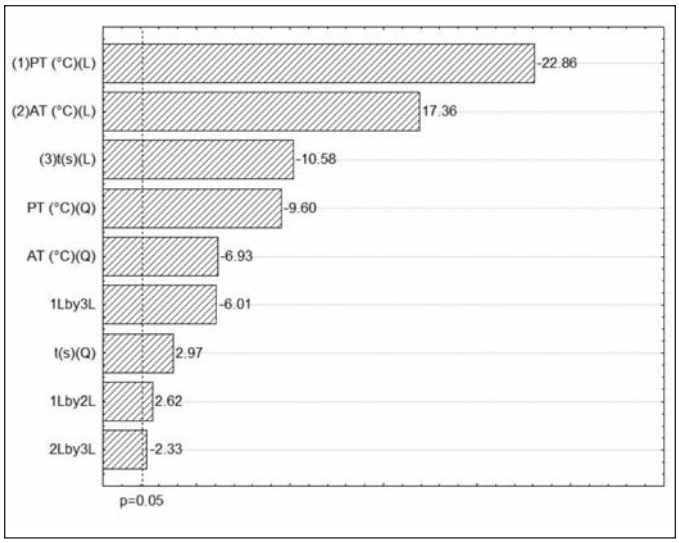

Fig. 2. Pareto chart obtained with the CCD, showing the estimated effect of the variables: PT, pyrolysis temperature; AT, atomization; t, pyrolysis time, Q, quadratic interaction, L-linear interaction.

The quadratic model obtained from the CCD is expressed according to the following equation:

$A = 7.8894 + (3.3163 \times 10^{-3})(PT) - (1.4166 \times 10^{-6})(PT)^2 + (4{,}8945 \times 10^{-3})(AT) - 1.02285 \times 10^{-6})(AT)^2 + (4.1597 \times 10^{-7})(PT)(AT) - (1.432291 \times 10^{-3})(PT)(t) - (5.552083 \times 10^{-6})(AT)(t)$

where A is the absorbance integrated response, PT is the pyrolysis temperature, AT is the atomization temperature, and *t* is time of pyrolysis.

The critical point was calculated from the partial derivatives $[\partial A/\partial(PT)] = 1277$ °C, $[\partial A/\partial(AT)] = 2512$ °C and $[\partial A/\partial(t)] = 52$ s, corresponding to the region with greater sensitivity. The maximum, minimum, and critical points are presented in Table V. For visualization of the response surface, it was necessary to set a variable obtained in two graphs (see Figures 3 and 4). Figure 5 shows that under optimum conditions, the background absorption was properly corrected.

Thus, the optimal experimental conditions for determining Cr in different milks and similar baby foods have been established: Rh as permanent modifier, pyrolysis temperature of 1280 °C, atomization temperature of 2510 °C, and pyrolysis time of 52 seconds.

TABLE V
Points Observed by CCD in the Optimization Method for the GFAAS Determination of Cr in Milk and Similar Baby Foods

Parameters	Minimum Observed	Critical Point	Maximum Observed
Pyrolysis Temp. (°C)	1095	1277	2105
Atomization Temp. (°C)	1795	2512	2805
Pyrolysis Time (s)	6	52	74

Figures of Merit

Using the above optimized method, the performance characteristics were determined.

The results obtained in the study of matrix effects are listed in Table VI. The samples were grouped according to similarity and composite samples were prepared. Comparing the slopes of the curves

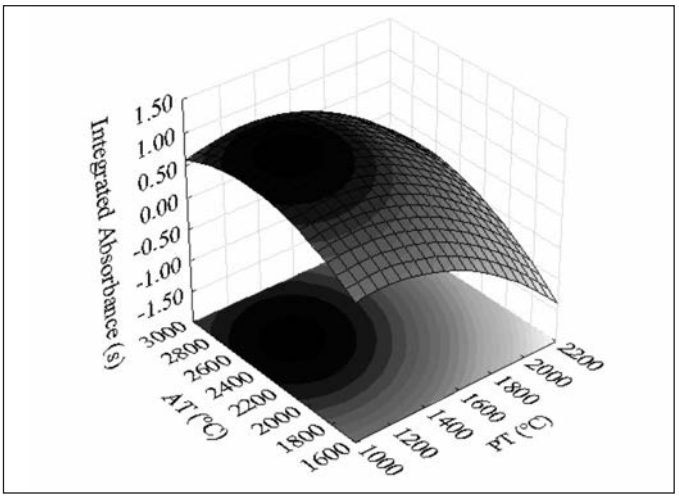

Fig. 3. Response surface and the contours obtained by the CCD setting pyrolysis time of 52 s.

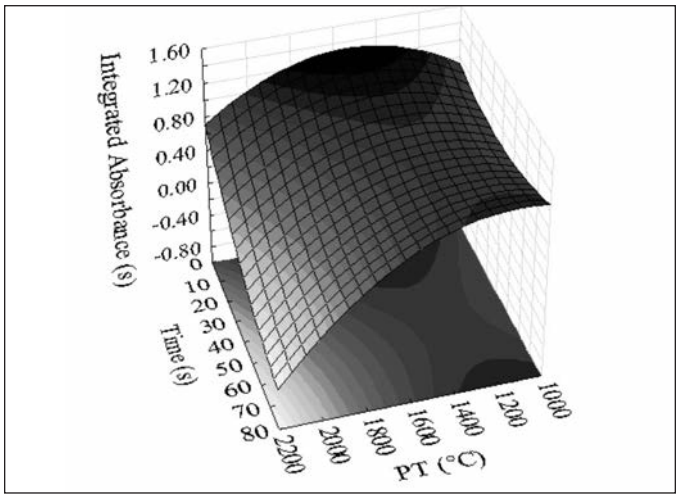

Fig. 4. Response surface and the contours obtained by the CCD fixing the atomization temperature at 2512 °C.

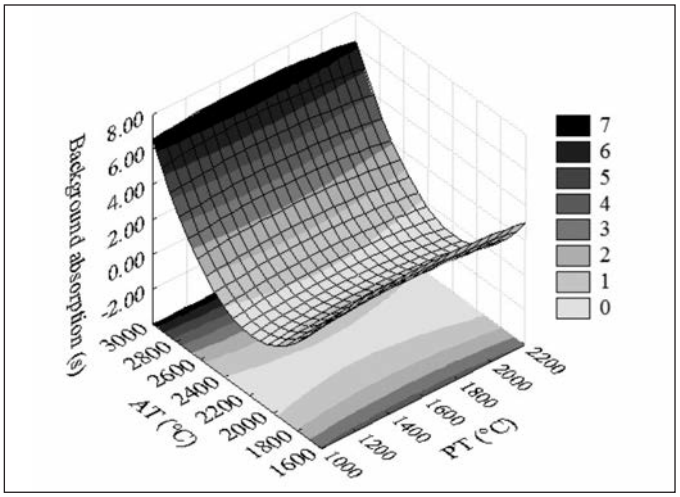

Fig. 5. Contour graph of response surface for the background absorption setting pyrolysis time of 52 s.

performed in conditions of repeatability, it can be seen that the slopes of the standard addition curves A2, A4, A5, and A6 are statistically similar to the slope of the aqueous curves (95% confidence level). This suggests the use of aqueous calibration for the determination of Cr in whole milk samples, skimmed milk, infant formulas and enriched powder, liquid skimmed milk and the powder, and liquid soybean meal samples since there is no matrix effect on the analytical signal. For other types of matrices, standard addition calibration should be used to obtain more accurate results. The other analytical parameters of merit were obtained based on aqueous calibration (Table VII).

The linear working range was established between the limit of quantification and the last point of the calibration curve. The limit of detection (1.43 ± 0:09 μg L^{-1}) and the limit of quantification (4.78 ± 0.29 ug L^{-1}) were calculated by the IUPAC criterion, according to LOD = 3 sblank, n = 10 / s and LOQ = 10 sblank, n=10 / s.

Sensitivity was evaluated by calculating the characteristic mass, which was (4.5 ± 0.4 pg) and when compared with the recommended value (3 pg) is considered appropriate. This mass value is slightly higher and allowed for a wider working range up to 50.0. By comparison, Quinaia and Nobrega (33) determined Cr in milk by GFAAS obtaining a characteristic mass of 7.8 pg. The relative standard deviations obtained by the authors of this study for three different concentrations were within acceptable values of precision, not exceeding 11.8% and 7.8 pg. The concentration of Cr in the certified reference material SRM 8435 Whole Milk Powder was only informed value; however, the value obtained was very close to the value reported, indicating that the accuracy of the present method is appropriate.

TABLE VI
Calibration Results Through the Standard Addition Calibration Curves for the Composite Samples and the Aqueous Curve for Evaluating the Matrix Effect in Determining Cr in Milk and Similar Foods by the Optimized GFAAS Method

	Standard Addition Calibration Curves		Aqueous Calibration Curves[a]	
Sample	Slope and Deviation Obtained by Regression	r	Slope and Deviation Obtained by Regression	r
A1	0.0210 ± 0.0013	0.9964	0.0182 ± 0.0002	0.9993
A2	0.0175 ± 0.0001	1.0000	0.0182 ± 0.0002	0.9993
A3	0.0215 ± 0.0024	0.9993	0.0182 ± 0.0002	0.9993
A4	0.0177 ± 0.0012	0.9951	0.0182 ± 0.0002	0.9993
A6	0.0166 ± 0.0006	0.9985	0.0158 ± 0.0005	0.9987
A7	0.0169 ± 0.0004	0.9995	0.0156 ± 0.0002	0.9993

[a] Aqueous curves obtained from repeatability conditions relative to the correspond ing standard addition curves.

TABLE VII
Performance Characteristics of the Optimized Method for GFAAS Determination of Cr in Milk and Similar Baby Foods

Parameters	Results
Linear working range	4.78 – 50.00 μg L^{-1}
Limit of detection, LOD	1.43 ± 0.09 μg L^{-1}
Limit of detection, LOD	28.6 ± 1.8 μg kg^{-1}
Limit of quantification, LOQ	4.78 ± 0.29 μg L^{-1}
Limit of quantification, LOQ	95.6 ± 5.8 μg kg^{-1}
Precision, RSD[a]	1.5 to 11.8%
Accuracy (SRM 8435)	0.461 ± 0.035[b] μg g^{-1}
Recovery[c]	82.6 – 96.8%
Characteristic mass[c]	4.5 ± 0.4[d] pg

[a] 8 samples, concentration levels 5, 10, and 20 μg L^{-1}.
[b] Informed value: 0.5 μg g^{-1}.
[c] 10, 20, and 30 μg L^{-1}.
[d] Recommended characteristic mass: 3 pg.

Analytical Application

An optimized method was applied to the determination of Cr in 17 samples obtained at a local market. The analysis results of the milk and similar baby food samples are listed in Table VIII. For the analyzed samples, the Cr concentrations were in the range of 0.042 to 1.900 μg g^{-1}, and 0.092 to 0.449 μg g^{-1} (n = 3) for whole milk, 0.098 μg $^{-1}$ for milk drink (n = 1), 0.042 to 0.191 μg $^{-1}$ for skimmed milk, 0.047 μg $^{-1}$ to 0.131 μg g^{-1} for infant formula (n = 7), 0.140 and 0.154 μg g^{-1} for soy food and soy infant formula (n = 2), 1.15 to 1.90 μg g^{-1} for goat's milk (n = 2). The Cr content was higher in the goat milk sample powder and lower in a skim milk sample. Comparative studies, such as by Güler (34), reported a Cr concentration of 0.77 ± 0.08 μg g^{-1} (dry basis) in a raw goat milk sample by ICP-OES after microwave digestion (34). In the work of these authors, the Cr content in goat milk samples are much lower than those

observed in the present study (1.15 and 1.90 μg g^{-1}). Saracoglu et al. (35) found Cr concentrations in the range 2.02 to 68.8 μg g^{-1} in 19 infant formula samples analyzed by GFAAS after microwave digestion (35). By comparison, the values for infant formula obtained in the present work were very low, between 0.047 to 0.151 μg g^{-1}.

CONCLUSION

In this study, a fast method is proposed to determine Cr in milk and similar infant food samples using slurries and liquid samples. The slurries preparation process is very simple and consists in a sonication of the powdered samples diluted in ultrapure water for 10 minutes. Chemical modification was very important to stabilize the analyte and the use of Rh (permanent modifier) permitted the direct determination of chromium in undigested milk and similar food samples. The multivariate optimization approach was an adequate tool to obtain optimal conditions. The figures of merit were satisfactory, and there was good agreement with the NIST SRM 8435 Whole Milk Powder certified sample. It was found that the Cr concentration in some goat milk samples was much higher than in whole milk, soy-based drink samples, and in an infant formula milk-based drink.

The results of this study show that the method proposed is unique and can be used for chromium (and probably other chemical elements) concentrations in various kinds of milk and similar samples by GFAAS with a simple sonication in ultrapure water.

TABLE VIII
Chromium Concentrations in Milk and Similar Baby Foods

Samples	Concentrations (μg g^{-1}), n=3 Samples
Milk drink M3	0.098 ± 0.010
Whole milk IE1	0.449 ± 0.076
Whole milk I5	0.105 ± 0.013
Whole milk I6	0.092 ± 0.004
Skim milk D2	0.191 ± 0.015
Skim milk D1	0.042 ± 0.006
Infant formula F1	0.047 ± 0.021
Infant formula F2	0.064 ± 0.004
Infant formula DE1	0.076 ± 0.017
Infant formula DE2	0.131 ± 0.054
Infant formula F5	0.114 ± 0.006
Infant formula F6	0.101 ± 0.008
Infant formula F8	0.110 ± 0.010
Infant soy formula FS2	0.140 ± 0.007
Soy food S1	0.154 ± 0.018
Goat milk C1	1.900 ± 0.600
Goat milk C2	1.150 ± 0.120

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