Determination of Cobalt in Animal Feces by Tungsten Coil Atomic Absorption Spectrophotometry

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An alternative analytical procedure for cobalt determination by tungsten coil electrothermal atomic absorption spectrophotometry (TCAAS) was developed to determine the liquid ruminal passage rate (turnover) of Co-EDTA in sheep feces. A matrix-matching procedure and a selective extraction of Co in 1.0 mol l^{-1} HCl were evaluated in order to correct and minimize the interference effects caused by sample matrices. As application, six sheep received at the same time one dose of the marker (Co-EDTA); thier fecal samples were collected in intervals of 6 h during 90 h. The Co amounts determined in the dry sheep feces by TCAAS were compared with those obtained by a flow injection catalytic spectrophotometric method. The characteristic mass and the detection limit, both based on peak height absorbance, were 23.1 and 19.1 pg, respectively, for 10 ml of sample volume in the samples of sheep feces. The rsd was 0.5% for 10 consecutive injections of 20.0 mg Co l^{-1}. The accuracy was assessed by employing the paired t-test at 95% confidence level; there was no significant difference for Co content determined by TCAAS and by flow injection spectrophotometry.

Keywords Tungsten coil atomizer, electrothermal atomic absorption spectrophotometry, cobalt, animal feces, animal nutrition

The determination of Co is usually required in animal nutrition studies. Because of its low concentration in plants and animals, the methodologies used for Co determination should present high sensitivity. The Co deficiency in animal nutrition can cause appetite loss, reduce growth and decrease productivity, mainly in bovines and sheeps.[1] The evaluation of the nutritional value of foods consumed by animals is frequently requested. The determination of the digestibility involving collection of total feces is difficult. One alternative is the use of the marker methodology that can supply relevant information, such as the amount of food ingested, the digesta passage rate through the gastrointestinal treat, the digestibility, the production and the fecal traffic. These studies are made by adding the marker (Co-EDTA)[2-4] to the food and by measuring the Co in feces collected at regular periods.

Once again the availability of sensitivity techniques for Co determination is essential. In the worst cases, the amount of the marker added to the food must be increased to allow accurate results in the feces. This is a limitation for studies involving expensive markers, such as the rare-earth elements.[4,5] According to these authors' proposal, the use of electrothermal atomization atomic absorption spectrophotometry is interesting owing to its high sensitivity and the capability to work with low sample volumes. It is normally carried out in a carbon atomizer, the graphite furnace, that is routinely used without major problems.[6,7]

Several tubes, tapes, strips and filaments, made of molybdenum[8,9], tantalum[9] and tungsten[9,10] have been proposed as electrothermal atomizers instead of the graphite tube. The use of metallic surfaces as atomizers in electrothermal atomization presents some favorable characteristics, such as the non-formation of carbonaceous materials, the high lifetime, the fast heating rate with a low power supply, and the smaller radiation emission in the UV-visible region.[11] Tungsten presents advantages over other metals, due to its high melting point (3410°C) and relative chemical inertness and it is available in a high degree of purity.[12] The lack of reproducibility in the physical characteristics, observed when lab-made filaments were used, was overcome by Berndt and Schaldach[13], who proposed the use of manufactured tungsten coils, which present standardized parameters in their characteristics, because they are mass produced as filaments for projector halogen lamps.[12]

The use of the simple low-cost tungsten coil electrothermal atomizer (TCA) proposed in 1988[13], could allow the implementation of this technique in nutritional laboratories, where the resources available should not be spent on high-technology and high-cost analytical techniques, such as graphite furnace atomic absorp-

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tion spectrophotometry.[11,14] Although such advantages are easily obtained with the use of the TCA, some problems could be observed such as the occurrence of interferences caused by the temperature gradient between the atomizer surface and the gaseous phase and by condensed phase reactions.

The strategy to overcome interferences in the TCA is not well-established and TCA itself is poorly studied. The effect of chemical modifiers is practically unknown, except for a recent investigation presented by Bruhn et al.[15] related to the determination of Pb in blood and hair. These authors showed that alkaline and alkaline-earth interferences occurred mainly in the condensed phase and that the use of Pd can minimize them. These same interferences in Pb determination in blood were observed by Krug et al.[16] and Parsons et al.[17] and solved by a matrix matching procedure and with a phosphate modifier addition, respectively.

In this work we studied the electrothermal atomization of Co in the TCA. The Co determination in feces samples as part of a nutritional study is a challenge for the TCA due to the complexity of the sample matrix. Two procedures were investigated to overcome the interferences: the first one was based on selective extraction in acid medium to discriminate analyte and interferents[18] and the other one was based on a matrix-matching procedure.[16] For validation of the proposed method, a flow-injection catalytic spectrophotometric system (FI) was adopted.[19,21]

Experimental

Tungsten coil atomizer

A Varian SpectrAA-640 atomic absorption spectrophotometer coupled to a 150 W programmable power supply with a voltage feedback circuit power supply (Anacom Equipments and Systems, Brazil) was operated by a computer for system control and data acquisition. It allows the variation of the applied voltages between 0.02 and 15.00 V in 0.02 V increments.
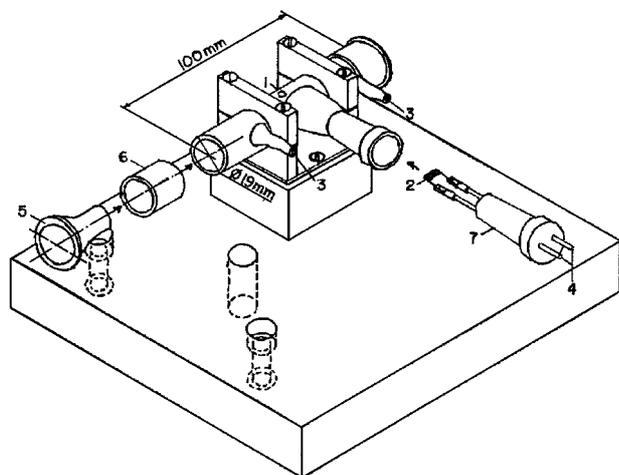
A Co hollow cathode lamp and a D_2 lamp were used for measuring atomic and background signals, respectively. The measurements were performed at the 240.7 nm Co resonance line with a spectral bandpass of 0.2 nm. The absorbance measurements were based on peak height.

The tungsten coil atomizer is depicted in Fig. 1. Details can be found in the paper published by Silva et al.[20] Osram coils (150 W, Part Number 764 3420 02, Germany) were adapted to the copper electrodes supported by a PTFE fitting (see ref. 20). A gaseous mixture containing 90%(v/v) Ar+10%(v/v) H_2 flowing at 1.0 l min^{-1} was used as purge gas. The samples were manually introduced into the tungsten coil with an Eppendorf (Germany) 10 μl micropipet.

Flow-injection system

The flow injection system[19,21] is described in Fig. 2. For the propulsion of fluids, an eight-channel peristaltic pump (Ismatec, Model ISM 761A, Switzerland) equipped with Tygon tubes with different internal diameters was used. The manifold was set up with polyethylene tubes of 0.8 mm i.d. A spectrophotometer (Femto, Model 432, Brazil) equipped with a flow cell with an optical path of 0.8 mm and coupled to a potentiometer recorder (Kipp & Zonnen, Model BD 111, Switzerland) was used to register the signals.

Reagents and reference solutions

All solutions were prepared from analytical grade reagents and distilled-deionized water was used throughout.

A 1000 mg Co l^{-1} stock solution was prepared from $CoSO_4·7H_2O$ (Merck). This stock solution was used for preparing reference solutions containing 10.0 μg Co l^{-1} in different acid media to evaluate possible effects on Co atomization: $1.4×10^{-2}$, $1.4×10^{-1}$, and 1.4 mol l^{-1} HNO_3 and $1.2×10^{-2}$; $1.2×10^{-1}$; and 1.2 mol l^{-1} HCl.

To study the effects caused by concomitants, solutions were prepared containing 10 mg Co l^{-1} plus 0;

Fig. 1 Tungsten coil atomizer: (1) sample introduction, (2) tungsten coil, (3) gaseous symmetric entrances, (4) copper electrodes, (5) quartz window, (6) rubber connection, (7) Teflon cone.
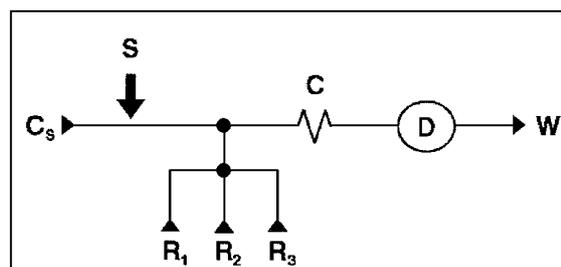
Fig. 2 Flow injection system. Cs, carrier; R_1 masking solution; R_2 reagent; R_3, oxidant; S, sample/standard loop; C, reaction coil; D, detector and W, waste.

1.0; 10; 50; 100; 200 and 500 mg l^{-1} of each element: Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{3+}, PO_4^{3-}, Al^{3+}, Zn^{2+}, SO_4^{2-}, Cu^{2+}, Cr^{3+}, Yb^{3+}, SiO_3^{2-} and Mn^{2+}. All solutions were prepared in 1.2×10^{-2} mol l^{-1} HCl medium. Later on, to obtain a better understanding of the interference effects, a sample of sheep feces, without Co marker, was extracted by the hydrochloric procedure and their metal contents were determined by ICP-AES[22], to know actually the amounts of concomitants present in this kind of material. Then, a Co solution was prepared in a medium containing all elements to simulate the feces sample matrix: 20.0 mg Co l^{-1} plus 1.5 mg l^{-1} SO_4^{2-}, 2.0 mg l^{-1} Mn^{2+}, 3.0 mg l^{-1} Fe^{3+}, 2.0 mg l^{-1} Al^{3+}, 20.0 mg l^{-1} Mg^{2+}, 50.0 mg l^{-1} Ca^{2+}, 25.0 mg l^{-1} Na^+, 35.0 mg l^{-1} K^+, and 50.0 mg l^{-1} PO_4^{3-} in 2.5×10^{-1} mol l^{-1} HCl.

For obtaining the TCAAS analytical curve the matrix matching procedure was adopted. Fecal samples without Co were weighed and known amounts of Co stock solution were added. These spiked samples were acid extracted following the same procedure as used for samples. The Co concentration in the final solutions ranged from 20.0 to 100.0 μg Co l^{-1}.

Sample preparation (Co-EDTA complex)

Amounts of about 45 g of Co-EDTA were prepared as described elsewhere:[23] to a 300 ml of water were added 37.5 g Co(II) acetate, 43.8 g EDTA disodium and 6.0 g NaOH. The solutions were heated to dissolve the salts and, after being cooled, received 30.0 ml of 30% H_2O_2. After 3 h, 450 ml ethanol (95%(v/v)) were added; the Co-EDTA solution was stored overnight under refrigeration and it was filtrated through Whatman #4 filter paper with a vacuum, washed with 80%(v/v) ethanol and dried overnight at 100°C.

The dosing amount for determining liquid turnover is about 50 – 60 mg for sheep, and the easiest way to get the animals to accept the dose of Co-EDTA is to mix it thoroughly in the concentrate feed. Three sheep received a single dose of 330 mg Co-EDTA as marker (one dose is equivalent to 56 mg Co[23]). The feces were collected at the end of the digestive tract (rectum) to avoid contamination in intervals of 6 h during 90 h. The samples were dried in a forced-air oven at 65°C and passed through 1 mm sieve.

Sample solubilization (nitric–perchloric digestion)

A mass of 0.500 g of each sample was weighed, in triplicate, and transferred to digestion tubes. A volume of 6 ml of a nitric–perchloric acid mixture (2:1(v/v)) was added to each sample and left overnight. The tubes were placed in a heating block at an initial temperature of 70°C. The temperature was increased at a rate of 1°C min^{-1} to a final temperature of 240°C. After that, the tubes stayed under heating until the reduction of the volume of the digested solution to 1 ml in each tube. The resulting clear solutions were transferred to volumetric flasks and the volumes were made up to 50 ml with water.

Table 1   Co heating program in the TCAAS

Step	Time/s	Voltage applied/V
Drying	5	0.70
	5	0.65
	5	0.60
	5	0.50
	5	0.40
	10	0.30
Pyrolysis	5	2.30
Atomization	1	13.00

Hydrochloric acid extraction

A mass of 250.0 mg of fecal samples was weighed and transferred to glass flasks. A volume of 12.5 ml of a 1.0 mol l^{-1} HCl solution was added to each flask. The samples were heated in a water bath at 65°C during 15 min and shaken for 30 min. Then the volume of all flasks was made up to 50.0 ml with distilled-deionized water. The samples were filtrated with Whatman filter paper. The Co content of the resulting solutions was measured by a flow injection catalytic spectrophotometric method and by tungsten coil electrothermal atomic absorption spectrophotometry (TCAAS). The heating program adopted is shown in Table 1. In both methods, samples were diluted ten-fold before measurements. Each extraction was carried out in duplicate.

Flow injection system

Reference Co solutions in the range 0.5 – 40.00 μg Co l^{-1} in 2.5×10^{-1} mol HCl l^{-1} were prepared by proper dilution of the Co stock solution for obtaining the FI analytical curve (Fig. 2).

Both reference solutions and sample carrier stream were prepared in 2.5×10^{-1} mol l^{-1} HCl. According to the chemistry involved and the conditions previously established[19,21], the reagents were: R_1, masking solution, 1.0×10^{-1} mol l^{-1} $Na_2P_4O_7$ plus 2.5×10^{-1} mol l^{-1} NaOH; R_2, color reagent, 1.0×10^{-3} mol l^{-1} Tiron, and R_3, oxidant, 0.1%(v/v) H_2O_2. This latter solution was prepared immediately before use. All other conditions were identical to those recommended by Nogueira *et al.*[21]

Methodology

Observation height and sensitivity.   The sensitivity and the repeatability are critically dependent on the correct choice of the observation height. The observation height was varied from a position tangential to the coil to positions upwards. After, the position for measurements was fixed at the maximum sensitivity point. All measurements were made by manually introducing 10.0 ml of Co reference solution onto the coil and were based on peak height.

Pyrolysis and atomization curves.   The pyrolysis and atomization curves were obtained with solutions containing 10.0 mg Co l^{-1} in 1.2×10^{-2} mol l^{-1} HCl, 1.4×10^{-2} mol l^{-1} HNO_3 and 1.4×10^{-2} mol l^{-1} $HClO_4$ acid media.
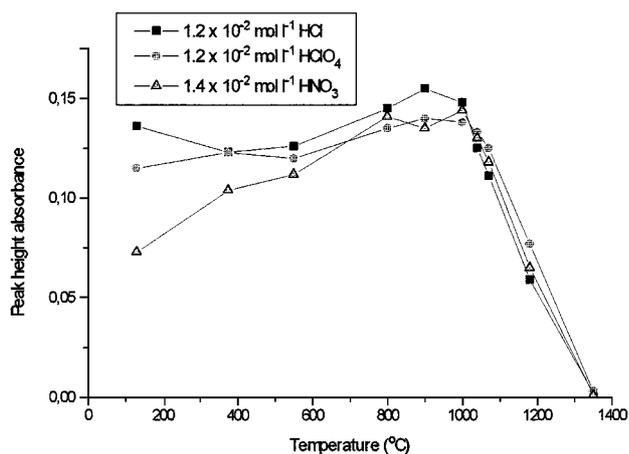
Fig. 3   Pyrolysis curves of 20.0 μg Co l^{-1} in different acid media
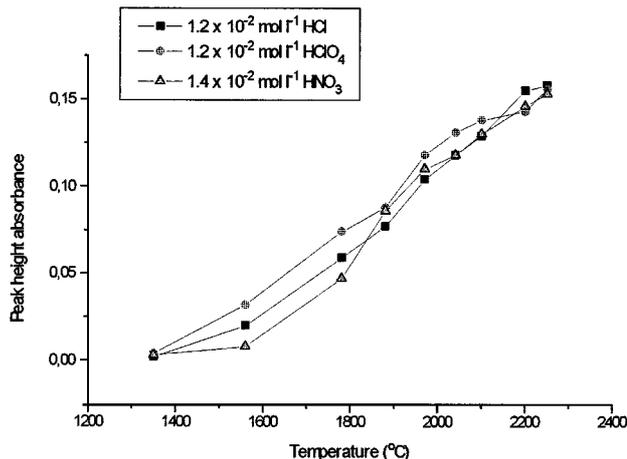
Fig. 4   Atomization curves of 20.0 μg Co l^{-1} in different acid media

These acids were chosen since they are frequently employed as extractors or decomposing reagents for preparation of animal or vegetable materials. For obtaining the temperature pyrolysis curves, the atomization temperature was kept at 2200°C (13.00 V) and the pyrolysis temperature was augmented starting from 120°C (0.30 V) until complete disappearance of the absorbance signal at 1350°C (4.50 V). In each condition, the pyrolysis step was carried out in 5 s. The temperature atomization curves were obtained by fixing the pyrolysis temperature at the point previously established and increasing the atomization temperature from 1280°C (4.00 V) to 2250°C (14.00 V). In each condition, the atomization step was carried out in 1 s.

The effect of pyrolysis time was evaluated by varying it from 0 to 30 s in a pyrolysis temperature of 900°C (2.30 V). The atomization was carried out by applying 13.00 V (2200 °C) during 1 s.

Results and Discussion

Observation height and sensitivity

The best sensitivity was attained when the Co resonance line passed through the tungsten coil. However, the noise was increased in this observation height owing to the tungsten coil expansion under heating and radiation scattering. The best compromise between sensitivity and signal noise was reached by placing the radiation beam tangentially to the upper side of the tungsten coil. This is in agreement with previous studies dealing with Pb[16] and Ba.[20] This position was kept in all further measurements.

Pyrolysis and atomization curves

The pyrolysis and atomization temperatures are important to decrease interferences and to increase sensitivity. In graphite furnace AAS the pyrolysis step is essential to remove interferents without losing the analyte and is a critical parameter towards stabilized tem-

perature platform furnace (STPF) conditions.[24] The pyrolysis and atomization curves together with physical data, such as boiling and melting points, are useful indications of the possible mechanism of atomization and could help to propose strategies to minimize interferences.

In Figs. 3 and 4, the pyrolysis and atomization curves for 20 mg l^{-1} Co in 1.2×10^{-2} mol l^{-1} HCl; 1.4×10^{-2} mol l^{-1} HNO$_3$ and 1.2×10^{-2} mol l^{-1} HClO$_4$ media are presented.

In HCl medium, when the voltage increased from 0.30 to 0.70 V there was a decrease of about 10% in the absorbance signal. From 1.10 V to 2.30 V there was a continuous increment in absorbance signals, attaining the maximum value at the highest voltage (2.30 V, 900°C). For higher applied voltages, there was a continuous and pronounced decrease in the absorbance signals due to analyte losses.

In HClO$_4$ medium an initial increase of the absorbance value was observed and the maximum signal was again obtained at 900°C. Similar behavior was observed in HNO$_3$ medium, indicating that at relatively low pyrolysis temperatures an oxidant medium improves Co atomization. The highest absorbance value was attained at 1000°C (2.50 V).

It can be concluded that the atomization of Co was not markedly affected by the acid media of the samples; thus the ideal conditions for sample preparation can be more easily reached. This same comment is true for the atomization curve; in all media the best sensitivity was attained at 2200°C (13.00 V). According to the procedure previously described, the effect of pyrolysis time on Co atomization in different acid media was evaluated; the results obtained are presented in Fig. 5.

In HCl medium, the best sensitivity was attained with a 3 s pyrolysis time. A 20% loss in signal was observed when the pyrolysis was performed in 30 s. It should be mentioned that for the tungsten coil atomizer the applied voltage was kept constant in each step, but this does not mean that the temperature was also kept
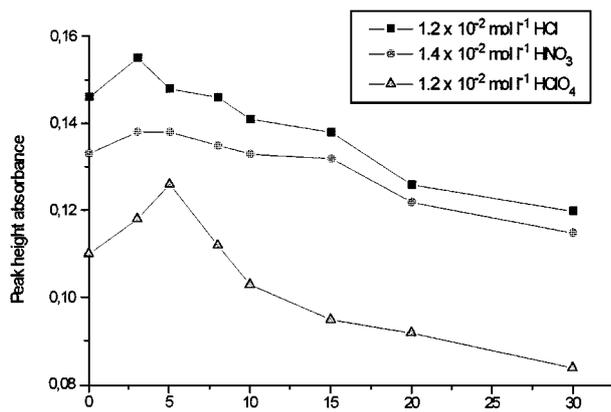
Fig. 5   Pyrolysis time in 1.2×10^{-2} mol l^{-1} HCl; 1.2×10^{-2} mol l^{-1} HClO$_4$ and 1.4×10^{-2} mol l^{-1} HNO$_3$.

Fig. 6   Interference study. Concomitant concentrations up to 500.0 μg l^{-1} in 10.0 μg Co l^{-1}. Heating program according to Table 1.

constant. The coil atomizer temperature is not completely controlled, and the temperature gradually increases during a long step at a constant applied voltage. Changes in the resistance coils could induce unexpected losses of refractor compounds. In HNO$_3$ medium only 13% of signal reduction were observed after a 30 s pyrolysis time.

The effect of pyrolysis time was more intense in HClO$_4$, showing a higher increment at short pyrolysis time and a 35% of signal reduction after 30 s. These effects are not clearly understood, but they should be related to the thermal behavior of the compounds formed in condensed phase in each media.

Effects caused by concomitants

The effect caused by concomitants was studied by employing the heating program shown in Table 1. The elements used in this investigation were chosen considering their presence in vegetable and animal samples and also their behavior as previously observed in the tungsten coil atomizer.[16,20] The element Cr was investigated because it is commonly used as a marker in nutritional studies and thus it could be present in high concentrations.

The cations Na$^+$, K$^+$ and Zn^{2+} up to a 500 mg l^{-1} concentration did not cause interference. Solutions containing Al^{3+} and Yb^{3+} up to 100 mg l^{-1} concentration did not cause interference. A 20% and 30% negative interferences were observed when 200 mg l^{-1} of Al^{3+} or Yb^{3+} were added, respectively.

The elements included in Fig. 6 exerted a more pronounced interferent effect. Their effects started up to 1.0 mg l^{-1}, causing losses in the Co absorbance. The worst effect was caused by the ion Fe^{3+}. Amounts of 1.0 mg Fe^{3+} l^{-1} decreased the Co signal by 25%. This is a critical problem due to the elevated Fe concentrations in samples of plants and animal feces. Another element that caused a marked negative interference and is a major component of these samples is P (as PO$_4^{3-}$). Other major elements, such as Ca^{2+} and Mg^{2+}, only presented significant interference for concentrations higher
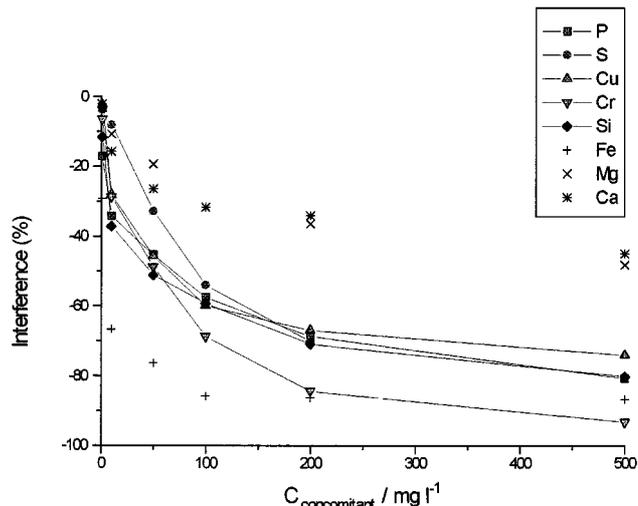
than 50 mg l^{-1}.

An alternative to solve the Fe^{3+} and PO$_4^{3-}$ interferences would be the adoption of a selective extraction procedure with hydrochloric acid in which Co is quantitatively extracted, but Fe^{3+} and PO$_4^{3-}$ are not.[18,21] In this extraction procedure, organic matter is extracted and the extraction solution containing the analyte is introduced onto the coil. It can be supposed that the effects caused by organic matter on tungsten coil surface could be solved by an efficient pyrolysis step.

When Co was atomized from these extracts, was observed a 25% reduction in the negative interferences when compared to signals generated from the acid digested solutions. This is an appreciable reduction of interference effects, but not suitable to attain the necessary accuracy. In spite of the Co selective extraction, the extracted fraction of undesirable concomitants is still high enough to provoke negative interferences.

Taking into account that the main objective of this work is the proposal of a simple, sensitive and low cost method for Co determination in samples originating from nutritional studies, it was decided to employ a matrix matching procedure to correct interferences, mainly by the homogenous presented in the studied samples, and because the modifiers were not yet well established.[16,20] In this sense, the loss in sensitivity caused by interferents was not critical because the procedure presented suitable sensitivity for the Co levels measured in this work.

When the tungsten coil is critically appraised, it is possible to observe that the main obstacle to it is dissemination as a low cost atomizer is the occurrence of interferences; this is needs special strategies to be corrected and can cause deterioration of analytical characteristics. The use of a matrix-matching procedure in this study is straigtforward, because the sample matrix is essentially constant and thus it can be easily simulat-

Table 2 Results with the proposed TCAAS method and by FI, in mg Co kg⁻¹ of dry matter

t^a	TCAAS (mg Co kg⁻¹)			FI (mg Co kg⁻¹)		
	Sheep 1	Sheep 2	Sheep 3	Sheep 1	Sheep 2	Sheep 3
0	1.62±0.09	1.56±0.8	1.46±0.08	1.94±0.07	1.98±0.07	2.03±0.09
6	38.0 ±0.1	37.9 ±0.1	37.4 ±0.1	40.9 ±0.1	40.9 ±0.1	42.0 ±0.1
12	73.7 ±0.1	73.9 ±0.1	74.1 ±0.1	79.2 ±0.1	75.9 ±0.1	81.1 ±0.1
18	84.0 ±0.1	82.6 ±0.1	80.6 ±0.1	88.6 ±0.1	87.2 ±0.1	90.2 ±0.1
24	65.4 ±0.1	64.9 ±0.1	63.8 ±0.1	70.2 ±0.1	69.2 ±0.1	72.1 ±0.1
30	60.0 ±0.1	61.5 ±0.1	60.7 ±0.1	62.3 ±0.1	61.4 ±0.1	60.1 ±0.1
36	45.2 ±0.1	46.6 ±0.1	45.6 ±0.1	43.2 ±0.1	45.2 ±0.1	40.2 ±0.1
42	36.4 ±0.1	38.8 ±0.1	38.1 ±0.1	32.5 ±0.1	36.2 ±0.1	29.8 ±0.1
48	32.0 ±0.1	31.5 ±0.1	30.5 ±0.1	28.6 ±0.1	29.4 ±0.1	20.1 ±0.1
54	24.6 ±0.1	24.8 ±0.1	23.4 ±0.1	20.6 ±0.1	22.1 ±0.1	16.2 ±0.1
60	15.2 ±0.1	14.6 ±0.1	13.3 ±0.1	12.8 ±0.1	13.6 ±0.1	10.1 ±0.1
66	9.05±0.09	8.69±0.10	8.11±0.09	8.56±0.09	8.33±0.08	7.30±0.09
72	6.01±0.07	5.90±0.09	5.80±0.06	5.20±0.07	5.64±0.06	4.23±0.09
78	3.68±0.09	3.62±0.08	3.55±0.09	3.54±0.07	3.21±0.08	3.31±0.07
84	2.67±0.07	2.50±0.09	2.45±0.08	2.48±0.08	2.10±0.07	2.51±0.06
90	1.82±0.06	1.80±0.06	1.61±0.06	1.74±0.06	1.82±0.08	1.70±0.06

a. Collected time, in hours. Mean±relative standard deviation (n=4).

ed in the reference solutions.

The flow injection catalytic spectrophotometric method adopted for comparison also is affected by Fe^{3+} interferences, but in this case the problem was solved by proper dilution of the samples before sample introduction. This is completely acceptable for marked fecal animal samples, but could not be used for samples in which the Co concentration is lower.

Analytical application

The Co amounts in sheep feces, collected each 6 h during 90 h (t(h), in Table 2), were determined with the developed TCA method and with a flow injection catalytic spectrophotometric method adopted for comparison (Table 2). A paired t-test showed a good agreement at these results at a 95% confidence level. The TCA method presented a detection limit of 19.1 pg and a characteristic mass of 23.1 pg. This detection limit is close to that obtained by FI method[21] and higher than that obtained by GFAAS.[6,25] It should be pointed out that the TCA limit of detection was established using matrix-matched solutions which deteriorated its value. However, the matrix-matching approach is essential to correct interferences. The typical coil lifetime was around 200 firings.

The passage rate of the marker is shown in Fig. 7. The profile of the curve reflects the kinetics of the liquid digestion expected for ruminants. According to a recommended procedure[4,26], the natural log of fecal marker concentration was plotted against time, and the ruminal passage rate was calculated as the slope of the linear descending portion of the line. This mathematical treatment of the curves allowed the estimation of the passage rate. For the 3 sheep investigated, the passage rate was 5.54±0.05% in the dry matter. The passage rate calculated by the FI method was 5.68±0.04%,
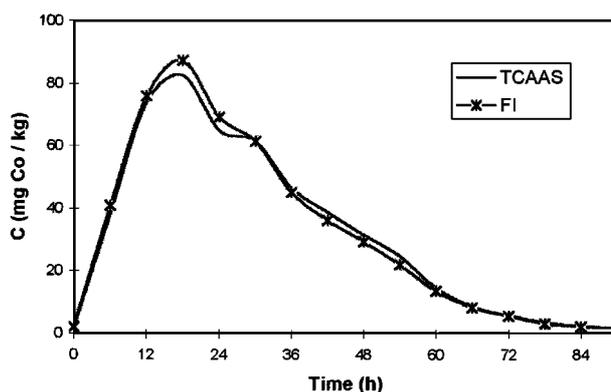
Fig. 7 Passage rate of marked Co-EDTA in the sheep 1, in mg Co kg⁻¹ of dry matter, by the proposed TCAAS (heating programme described on Table 1) and by FI.

showing again the good agreement between the two methods.

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