

Determination of Zinc in Environmental Samples by Solid Phase Spectrophotometry: Optimization and Validation Study

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A simple and specific solid-phase spectrophotometric (SPS) determination of zinc in $\mu\text{g dm}^{-3}$ level has been developed based on the reaction of Zn(II) with 4-(2-pyridylazo)resorcinol (PAR) in the presence of potassium iodide; the product was then fixed on an anionic exchanger. The absorbance of the gel, packed in a 1 mm cell, is measured directly. PAR and KI concentrations were optimized simultaneously using response surface methodology (RSM) from sequential experimental Doehlert designs. The advantages of this methodology are discussed, as opposed to univariate optimization, which is also used. The method was validated by obtaining the performance characteristics: linearity, detection and quantification limits, precision, robustness and selectivity, using suitable chemometric techniques; the trueness was confirmed on environmental samples (water and milk).

Keywords Optimization, Doehlert design, method validation, zinc, solid-phase spectrophotometry, 4-(2-pyridylazo)resorcinol, Sephadex QAE

The proposal of a new analytical method implies two important steps: the optimization of the experimental variables and the validation of the proposed method. The first one involves the selection of the values of the factors which have an influence on the analytical signal and produce the best results in the analytical process. To develop the optimization of analytical methods, two different strategies can be used. In the traditional "one-at-a-time" operation, all the values of the different factors except one are constant, and this one is the object of the survey. The alternative to this strategy is the Experimental Design; in this case the whole set of the factors comprising the system is simultaneously modified, making it possible to identify the factors which modify the analytical response. The latter strategy allows us to understand circumstances which are not explained by the traditional mode, such as those in which the analytical response is a multimodal function of the independent variables or when the effects of the variables are not additive and there is interaction among them.¹

The validation of an analytical method verifies that the characteristics of the method fit the requirements of the application domain, that is, the whole set of specifications: concentration interval of the analyte, level of uncertainty accepted, selectivity, application conditions, *etc.*² Consequently, the validation methodology involves the definition of the quantitative characteris-

tics that determine whether the different stages of the procedure are suitable for a particular analysis.

The use of chemometric techniques in the two steps makes it possible to ensure quality control in the laboratory; furthermore, less time is necessary for development of the method and improved characteristics are obtained. Thus, using experimental designs a greater quantity and higher quality of information is obtained, in relation to the variables of the analytical system and its characteristics.

Solid-phase spectrophotometry (SPS)^{3,4} is a methodology that includes the preconcentration of the analyte on a solid, with a previous or simultaneous reaction step to produce a chromogenic compound, and the subsequent measurement of the absorbance in the solid phase. This provides an increase in selectivity and sensitivity to SPS methods in relation to solution methods.

This paper describes the use of experimental design and chemometric techniques to develop and validate a SPS method for the determination of zinc in environmental samples. The optimization of variables was studied both in the traditional mode ("one-at-a-time") and using Doehlert's design⁵ in order to perform a comparative study. Robust regression^{6,7} was applied to detect the outliers and for the determination of the upper limit of the calibration graph for the 100 cm³ sample system. Saturated fractional factorial designs at two levels were used to evaluate the robustness of the proposed method. In the study of the performance characteristics, special attention was paid to the pres-

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ence of foreign ions, and to the influence of the sample matrix on the calibrating graph and on the recovery of zinc. Finally, the trueness of the proposed method was tested by using the standard addition methodology on both synthetic and real water samples as reference material and atomic absorption spectrometry as a reference method on a commercial milk sample.

Experimental

Materials and reagents

All chemicals used were of analytical grade unless stated otherwise; a Milli-Q type quality water was used for dilution of reagents and samples and all experiments were carried out at room temperature.

A Sephadex QAE A-25 anion exchanger (Sigma) was used in the chloride form as received from the supplier and without pretreatment, in order to avoid contamination.

A standard solution of zinc with 1 g dm^{-3} , prepared from ZnCl_2 and dissolved in 1 mol dm^{-3} HCl solution, was used. Working solutions were prepared daily by appropriate dilution of the standard solution in water.

Aqueous solutions of 4-(2-pyridylazo)resorcinol (PAR) (Merck) of an appropriate concentration were prepared weekly. The appropriate amounts of KI (Panreac PRS) were dissolved in reverse osmosis water. Solutions of the required pH were prepared from 0.2 mol dm^{-3} or 0.5 mol dm^{-3} sodium hydroxide (Panreac PA) and 0.2 mol dm^{-3} or 0.5 mol dm^{-3} boric acid (Merck).

Instruments and apparatus

A Perkin Elmer Lambda 2 UV/Vis spectrophotometer connected to an IE 486 computer fitted with a Perkin Elmer software package (PECSS) was used for the measurements and data processing.

Furthermore, an Agitaser Model 2000 rotating-bottle agitator, a URA 2610 desk centrifuge and a Crison Digit-501 pH-meter with a combined glass-calomel electrode were used.

Software

Data analysis was performed by QUIMIO⁸ for the regression model, STATGRAPHICS⁹ for the experimental design, and PROGRESS¹⁰ for the robust regression.

Absorbance measurements

The absorbance of the Zn(II)-PAR in presence of KI fixed on the exchanger was measured in a 1 mm cell at 500 nm (absorption maximum) and 750 nm (within the range where the solid phase only caused attenuation of light) against a 1 mm cell well-packed with exchanger, equilibrated with water. In the same way the absorbance of the blank (a 1 mm cell packed with exchanger equilibrated with blank solution) was measured at the same wavelengths. The blank absorbance

is due to the PAR fixed on the exchanger.

The net absorbance, A_{RP} , for the reaction product was obtained by: $A_{\text{RP}} = A_{\text{S}} - A_{\text{B}}$; here: $A_{\text{S}} = A_{500} - A_{750}$ (for the sample) and $A_{\text{B}} = A_{500} - A_{750}$ (for the blank).

Analytical procedures

Sample system of 100 cm³. An aliquot of the sample (volume depending on analyte concentration) containing up to $70 \mu\text{g dm}^{-3}$ of Zn(II) was added to a 100 cm^3 calibrated flask with 2 cm^3 of $3.92 \times 10^{-4} \text{ mol dm}^{-3}$ PAR solution, 2 cm^3 of 0.10 mol dm^{-3} potassium iodide solution, 10 cm^3 of 0.2 mol dm^{-3} buffer solution (pH 8.7); the volume was completed by adding water. The solution was transferred to a 1000 cm^3 polyethylene bottle and 0.080 g of the Sephadex QAE A-25 exchanger was added. The mixture was mechanically shaken for 10 min, after which the colored solid phase beads were collected by suction filtration and, with the aid of a pipette, packed into a 1 mm cell together with a small portion of the filtrate. The cell was centrifuged for 30 s at 25g. Absorbances were measured at 500 and 750 nm for the sample against a 1 mm cell reference, similarly packed with exchanger and equilibrated with water. A blank solution containing all the reagents was prepared and treated as described for the sample. Net absorbance, A_{RP} , was measured for the reaction product as described under "Absorbance measurements".

Sample system of 500 cm³. An aliquot of the sample (volume depending on analyte concentration) containing up to $7.85 \mu\text{g dm}^{-3}$ of Zn(II) was placed in a 500 cm^3 calibrated flask and the volume was completed with water. The solution was then transferred to a 1000 cm^3 polyethylene bottle and the following, in this order, were added: 2 cm^3 of $1.28 \times 10^{-3} \text{ mol dm}^{-3}$ PAR solution, 2 cm^3 of 0.77 mol dm^{-3} potassium iodide solution, 5 cm^3 of 0.5 mol dm^{-3} buffer solution (pH 8.7) and 0.080 g of the Sephadex QAE A-25 exchanger. The mixture was mechanically shaken for 40 min. The absorbance of the colored beads was measured by the above procedure.

Distribution measurements

PAR, KI, buffer solution and 0.080 g of Sephadex QAE A-25 exchanger in this order were added to an aqueous solution, 100 cm^3 or 500 cm^3 respectively, containing 4.74×10^{-5} or $3.87 \times 10^{-5} \text{ mmol}$ of Zn(II); the solution was stirred for 15 min for the 100 cm^3 sample and for 50 min for the 500 cm^3 sample. The concentration of Zn(II) in the solution was measured as described under "Analytical procedures". Subsequently, the filtrated solution was treated in the same way with a further batch of exchanger (0.080 g), and the Zn(II) left in that was determined by the above procedure. In these latter determinations the concentration of Zn(II) was under the detection limit of the procedures, and therefore it is possible to say that the zinc had been fixed on the exchanger in the previous equilibration. The distribution ratio D (mmol of zinc fixed per g of exchanger/mmol of Zn(II) per cm^3 of solution) was calculated

from the initial concentration in the solution and the concentrations corresponding to the detection limits. The average values of $D=(1.4\pm 0.4)\times 10^4$ and $(10\pm 2)\times 10^4$ cm^3/g were obtained for the 100 cm^3 and 500 cm^3 sample system, respectively, from three replicate determinations. The fixation process may be termed quantitative, as values of D higher than 10^4 are obtained in both procedures.¹¹

Results and Discussion

Absorption spectra

The product of the reaction between Zn(II) and PAR in the presence of potassium iodide shows a red-orange color with an absorption maximum at 493 nm (pH 8.7) in solution. This compound was fixed on different anionic solid phases (Dowex resin and Sephadex) and exhibited an absorption maximum centered at 494–500 nm. The maximum absorbance was found for the Sephadex QAE A-25 phase. The net absorption spectrum in this exchanger showed a band centred at about 500 nm, the position of which did not change with pH over the range 4.5–9.5.

Influence of pH

The influence of pH on the net absorbance of the complex is studied. The optimum pH range is 7.8–9.5. Therefore, we chose a pH value of 8.7 as a suitable one.

Boric acid/sodium borate was chosen as the buffer solution. The increase in the ionic strength due to the buffer solution produced a slight decrease both of the blank and the complex, and therefore the net absorbance presents stability over the range studied from $2\times 10^{-2}\text{ mol dm}^{-3}$ to $4\times 10^{-2}\text{ mol dm}^{-3}$. A buffer concentration of 0.02 mol dm^{-3} was considered the most appropriate for later experiments.

Effect of PAR and KI on net absorbance (100 cm^3 sample system)

The influence of the concentration of these reagents has been studied jointly, using the response surface methodology (RSM) from sequential experimental Doehlert designs.¹² The optimization was also carried out in the traditional mode ("one-at-a-time") with the intention of comparing the two methodologies (multivariate and univariate).

In the *Multivariate Methodology*, two Doehlert designs were used to reach the maximum net absorbance region¹², but as the calculated maximum ($[\text{PAR}]=6.28\times 10^{-6}\text{ mol dm}^{-3}$ and $[\text{KI}]=11.75\times 10^{-3}\text{ mol dm}^{-3}$) from the experimental data of the second design is close to the limits of the experimental domain, a third design was employed to confirm the position of the obtained optimum.

The analysis of the results obtained in the third design by means of ANOVA showed that neither PAR nor KI were statistically significant. The quadratic effects of PAR and KI and the interaction term between the two

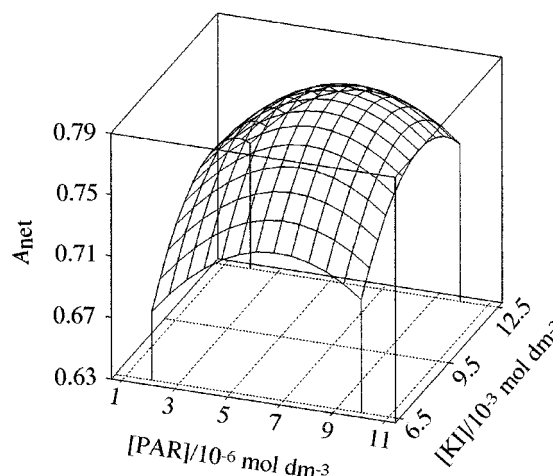


Fig. 1 Net absorption response of the PAR-Zn(II) complex fixed on Sephadex QAE with respect to PAR and I⁻. Response surface corresponding to Design III (100 cm^3 sample system).

variables were not significant either.

Figure 1 shows the response surface function developed by the model from the experimental data of the third design. The region explored approximates a plane of equal response (the PAR concentration values are between 5×10^{-6} and $8\times 10^{-6}\text{ mol dm}^{-3}$ and KI concentrations lie between 9×10^{-3} and $11\times 10^{-3}\text{ mol dm}^{-3}$); the calculated maximum ($[\text{PAR}]=6.67\times 10^{-6}\text{ mol dm}^{-3}$ and $[\text{KI}]=10.05\times 10^{-3}\text{ mol dm}^{-3}$) is included in the experimental domain, which confirms the results obtained from the second design.

The *Univariate Methodology* was used to explore the influence of PAR and KI concentrations on net absorbance, in the ranges of 3.92×10^{-6} – $1.57\times 10^{-5}\text{ mol dm}^{-3}$ and 0.0 – $1.9\times 10^{-2}\text{ mol dm}^{-3}$, respectively.

There is a constant influence of the PAR concentration on the net absorbance in the range of values of 5.88×10^{-6} – $1.57\times 10^{-5}\text{ mol dm}^{-3}$. It is evident that the value calculated with multivariate methodology lies within this range.

The net absorbance rises with the increase in KI concentration to $9.50\times 10^{-3}\text{ mol dm}^{-3}$; it then remains constant between $9.50\times 10^{-3}\text{ mol dm}^{-3}$ and $1.9\times 10^{-2}\text{ mol dm}^{-3}$. Again, the value calculated with multivariate methodology is in the zone of constant maximum net absorbance.

The comparison of the two optimization methodologies enables the following conclusions to be drawn:

The efficiency of the maximum estimation was better with multivariate than with univariate methodology, *i.e.* fewer experiments are required, while it is possible, at the same time, to identify the existence of interactions between the variables. The agreement between the maxima found by the two procedures might be due to the fact that the interaction term is not statistically significant in any case.

Moreover, using multivariate methodology, better

information on the examined system, *i.e.* response function and response surface, can be obtained. In addition, the influence on the analytical signal of one variable when the value of the other variables is constant can be estimated using the response function.

In accordance with the results obtained, an optimum PAR concentration of 7.84×10^{-6} mol dm⁻³ was selected for the analytical procedure.

The use of 10^{-2} mol dm⁻³ KI (maximum obtained with the two methodologies) produces good sensitivity in the determination of Zn²⁺; however, it produces a high variability in the experimental results. It was found that using a lower concentration of KI, 2×10^{-3} mol dm⁻³, better repeatability is obtained with no significant loss in sensitivity. Therefore, the latter concentration was used in the analytical procedure.

Effect of PAR and KI on net absorbance (500 cm³ sample system)

Optimization of PAR and KI concentrations was carried out using two Doehlert designs.¹² From processing of the obtained data, it can be concluded that the maximum absorbance corresponds to PAR and KI concentrations of 6.48×10^{-6} mol dm⁻³ and 3.00×10^{-3} mol dm⁻³, respectively. These values are used in the analytical procedure.

Other experimental conditions

The order of addition used here was: Zn²⁺+PAR+KI+buffer+exchanger. Other orders tested present higher data variability. For all measurements, 0.080 g of exchanger was used as a compromise between maximum sensitivity and operativity.

Optimum stirring time, at 77 rpm, was found to be 10 min for the 100 cm³ sample system and 40 min for the 500 cm³ sample system. The centrifugation time used here was 30 s at 2500 rpm (approximately 25g) in all cases.

Both the blank absorbance and the complex absorbance fixed on the solid phase were stable for at least 1 h.

Validation of the method

In order to check the analytical methods, the follow-

ing performance characteristics were obtained: calibration range, linearity, quantification and detection limits, precision, sensitivity, robustness, selectivity and trueness.

Some of the performance characteristics of the proposed methods are summarized in Table 1.

Calibration

Calibration graphs, by applying univariate linear regression, were established in the ranges 8.3 – 70 µg dm⁻³ (100 cm³ sample system) and 1.1 – 7.9 µg dm⁻³, lack-of-fit P-value¹³ is 3.5% (500 cm³ sample system).

The linearity of a calibration function is a characteristic of great importance^{13,14}, although it is necessary to distinguish between "in line" linearity (goodness-of-fit) and "on line" linearity (degree of dispersion of the data around the calibration line).¹⁵ "In line" linearity is usually checked by the lack-of-fit test¹³, although there are other procedures for its evaluation.¹⁵ "On line" linearity, however, is checked by means of: $100[1-RSD(b)]$, where RSD(b) is the relative standard deviation of the slope¹⁵ (Table 1).

In this paper, "in line" linearity in the 100 cm³ sample system was checked using Least Median Squares Regression (LMS) by means of PROGRESS software. It was thus possible to detect outliers, to check the fit of the experimental points and to establish the upper limit of the linear calibration.⁷ In the 500 cm³ sample system, the lack-of-fit test was carried out according to the recommendations of the Analytical Methods Committee.¹³

Detection and quantification limits

There are different ways to estimate the detection limit;^{14,16,17} however, we chose the IUPAC recommendations¹⁶, as they are the most widely used for instrumental methods and with the aim of providing a parameter comparable to those in other studies. The fluctuations in the background absorbance measured for the blank, calculated as the average of 10 within-day independent determinations¹⁸ and expressed as SD units, for the 100 cm³ and 500 cm³ sample systems were 0.019 and 0.015A, respectively. The lower limits are shown in Table 1.

Table 1 Performance characteristics of the analytical methods

Feature (unit)	Sample system	
	100 cm ³	500 cm ³
Intercept	0.0123	0.0045
Slope (dm ³ µg ⁻¹) ^a	0.0227±0.0003	0.1427±0.0017
<i>s</i> ^b	0.0204	0.0182
"On line" linearity (%)	98.7	98.8
Detection limit (µg dm ⁻³)	2.5	0.3
Quantification limit (µg dm ⁻³)	8.3	1.1
Repeatability (RSD%)	4.3	2.1
Reproducibility (RSD%)	5.0	4.4

a. Mean±standard deviation (*n*=8, for 100 cm³; *n*=5 (in triplicate), for 500 cm³). b. Standard deviation of regression.

Precision

The precision of the proposed methods, expressed as repeatability and reproducibility, is shown in Table 1.

The data were obtained with $25 \mu\text{g dm}^{-3}$ Zn(II) for the 100 cm^3 sample and $4.9 \mu\text{g dm}^{-3}$ Zn(II) for the 500 cm^3 sample system; $n=10$ in all cases, except for repeatability for the 100 cm^3 sample system ($n=30$).

Robustness

The robustness of the 500 cm^3 sample system was checked according to Cuadros Rodríguez *et al.*¹⁹ To carry out the experiments, two two-level fractional saturated factorial designs (2^{7-4} and 2^{3-1}), centered on the experimental optimum, were used. In order not to modify the structure of the first design, a "dummy" variable, confounded with the most probable interaction (exchanger quantity-stirring time), was added. The conclusion reached is that the analytical procedure proposed for the 500 cm^3 sample system is robust for variations of $\pm 10\%$ of PAR or KI concentrations, and ± 0.1 units of pH value, and $\pm 5\%$ on exchanger quantity. However, a strict control of the stirring time and final volume is required.

Sensitivity

In SPS methodology, the sensitivity can be enhanced in proportion to the volume of the sample to be analyzed. The ratio between the slopes of the calibration graphs obtained of the different sample volumes is a measure of the increase in sensitivity from the use of a larger sample volume.⁴ In this case, the slope ratio for the analytical procedures proposed is: $S_{500}/S_{100}=6.3$, where the subscripts represent the sample volume (cm^3) and the improvement of sensitivity in the 500 cm^3 sample system is evident.

The sensitivities of the proposed methods, expressed as apparent molar absorptivity, are compared in Table 2 with those of spectrophotometric procedures of published methods. The higher sensitivity of our methods is apparent.

The other method for determination of zinc using

SPS, which uses Zincon as a reagent²⁰, requires the prior separation of the analyte as a ZnCl_4^{2-} complex which is fixed on an anionic exchanger; the reaction between the zinc-chloride complex (which is not stripped from the exchanger) and Zincon is then carried out on the solid phase. However, the use of the procedures proposed in this paper makes it possible to determine the zinc concentration without any prior separation of the analyte from the matrix. Therefore, fewer operations are required and, consequently, the possibility of human error is diminished. Moreover, the sensitivity of the method has been improved (Table 2).

Selectivity

We studied the interference by foreign ions which are commonly found in water and/or which cause a similar reaction of the analyte in the determination of zinc at the $25 \mu\text{g dm}^{-3}$ level. Tolerance is defined as the concentration of foreign ions which produces an error of $\pm 5\%$ in the determination of the analyte. The results are summarized in Table 3.

Experiments carried out with various reducing or complexing compounds show that the absorbance difference between standard and blank did not undergo any variation. Quantities of 250 mg dm^{-3} of thiourea, 100 mg dm^{-3} of ascorbic acid and oxalate ions, 50 mg dm^{-3} of phosphate ions and 10 mg dm^{-3} of thiosulfate ions do not affect the sensitivity of the reaction, although they do not decrease the interference of the metal ions. It has been demonstrated that a concentration of 100 mg dm^{-3} of tartrate ions is effective at reducing interference from Al(III), Mn(II), Pb(II), Co(II) and Cd(II) (Table 3).

Applications of the method

To check the trueness of the proposed SPS method, the analytical procedures were applied to the determination of zinc content in different samples: synthetic, distilled and mineral water and milk samples.

Synthetic samples. Different synthetic samples were prepared containing $24.7 \mu\text{g dm}^{-3}$ of zinc and different

Table 2 Comparison of sensitivity of some zinc methods

Method	Molar absorptivity/ $\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$	Reference
Hydrazidazol/Triton X-100	2.7×10^4	22
PAN/CTMAB	5.0×10^4	23
PPRT	5.5×10^4	24
PPST	6.1×10^4	25
PAR/(CTMAB-PO)	8.1×10^4	26
Zincon ^a (1000 cm^3 sample) ^b	3.1×10^6	20
PAR ^a (100 cm^3 sample) ^b	1.5×10^7	This paper
PAR ^a (500 cm^3 sample) ^b	9.4×10^7	This paper

CTMAB, cetyltrimethylammonium bromide; PPRT, 1-(phenyl-2-pyridyl)carbilidene-5-resorcilidenethiocarbohydrazone; PPST, 1-(phenyl-2-pyridyl)carbilidene-5-salicylidene thiocarbohydrazone; PO, Pregel O. a. Solid phase spectrophotometry. b. Apparent molar absorptivity (absorbance value of the complex fixed on the exchanger from a 1 mol dm^{-3} aqueous solution of zinc and measured in a 10 mm optical path cell).

Table 3 Effect of foreign ions on the determination of 25 $\mu\text{g dm}^{-3}$ of zinc

Foreign ion	Tolerance level/ $\mu\text{g dm}^{-3}$
NO_3^-	300000
Br^-	250000
Ca(II) , SO_4^{2-} , F^-	100000
IO_3^-	50000
Cl^- , Mg(II)	25000
CO_3^{2-} , CH_3CO_2^-	10000
Al(III)^a	50 (250)
Bi(III)	25
Fe(III)	20
Cr(III)	10
Pb(II)^a	5 (100)
Mn(II)^a , Cu(II)	2 (5)
V(V) , Cd(II)^a , Ni(II)	1 (5)
Co(II)^a	1 (2)

a. In brackets, tolerance levels in presence of 100 mg dm^{-3} tartrate.

amounts of the usual ions in natural water. For all measurements, the 100 cm^3 sample system was used. Aliquots of 85 cm^3 of the samples were analyzed as described under *Analytical procedures*. The average value (3 determinations) and standard deviation for zinc concentrations calculated from the calibration graphs are shown in Table 4. Recoveries of 96–103% indicate the trueness of the method even in the presence of foreign ions when there are 100 mg dm^{-3} of the tartrate ion as a masking agent.

Distilled and mineral water. The distilled water samples were collected in glass flasks from a single distillation system with metallic electrodes. The mineral water samples (a commercially available mineral water) were collected directly from the commercial bottles. Both samples were analyzed without prior treatment.

The 500 cm^3 sample system was applied to the two samples. The volume of water used was 500 cm^3 for the distilled water and 250 cm^3 for the mineral water. The appropriate amount of tartrate ions (20 or 30 mg dm^{-3} , respectively) was added as a masking agent. The

trueness of the method proposed was checked using the statistical methodology proposed by Cuadros Rodríguez *et al.*²¹, which is based on the establishment of three calibration graphs: standard calibration (SC), standard-addition calibration (AC) and Youden calibration (YC). The comparison, using the *t*-test, of the slopes of the lines obtained from SC and AC calibrations makes it possible to distinguish two circumstances: either a) the difference between the two slopes is significant, indicating there is a systematic proportional error which makes it unsuitable for use as a test of trueness, or b) the difference between the two slopes is not significant, in which case standard-addition calibration can be used to validate the method. In the latter case, it is then necessary to check whether the intercepts of the SC and YC regression graphs are significantly different, which would indicate the existence of a systematic error due to a matrix effect in the sample. In this case, the true blank of the sample (Youden's blank) is calculated as the difference between the two intercepts. Finally, the trueness of the results is verified by the comparison, using a *t*-test, of the means of the analyte concentrations obtained from the SC and AC calibration graphs. If the difference is not significant, it proves that the method is true. Table 5 summarizes the statistics obtained from the different calibration graphs for both types of samples. As there is no significant difference between the slopes of the SC and the AC graphs, the standard calibration graph can be used, in all cases, for the determination of zinc. The trueness of the results is apparent, there being no significant differences between the values obtained from the two calibration graphs.

Milk samples. An aliquot of a commercially available UHT milk was collected from the commercial packaging and analyzed without prior treatment or delay. The standard addition method was used in the SPS method and the constant error was corrected using the Youden blank. An atomic absorption spectrometry method (AAS) was used as a reference method. The results obtained from the SPS and AAS methods, expressed as mean \pm standard deviation, were 9.7 ± 0.4 and 9.4 ± 0.1 , respectively. The statistical comparison of these results by means of a Student *t*-test showed no significant dif-

Table 4 Determination of 24.7 $\mu\text{g dm}^{-3}$ zinc in synthetic samples^a

Sample	Amount found ^b $\pm s_{n-1}$ ($\mu\text{g dm}^{-3}$)	% Relative error	Recovery, %
A	23.8 \pm 0.6	-3.6	96
B	25.5 \pm 1.5	3.2	103
C	24.2 \pm 0.6	-2.0	98
D	24.0 \pm 1.9	-2.8	97

a. In the presence of 100 mg dm^{-3} of tartrate. b. Mean ($n=3$). [Sample A] Composition: Al(III) ; Cd(II) ; Co(II) ; Mn(II) ; Pb(II) ; Cl^- and NO_3^- . Concentration equal to tolerance level. [Sample B] Composition: Fe(III) ; Cu(II) ; Ni(II) ; Cl^- and NO_3^- . Concentration equal to tolerance level. [Sample C] Composition: Ca(II) ; Mg(II) ; Cl^- ; NO_3^- ; SO_4^{2-} and F^- . Concentration equal to tolerance level. [Sample D] Same composition and concentration as sample A plus sample B plus sample C.

Table 5 Statistics for the determination of zinc in water samples

Sample	Results			
Distilled water ^a	AC	SC	YC	
	Slope	0.1267	0.1331	0.2181
	<i>n</i>	12	5	3
	<i>s</i>	0.0290	0.0140	0.0011
There is no constant error bias (YB=0). There is no significant difference between the AC and SC slopes. Trueness test: $t_{cal}=0.732 < t_{crit}=2.145$ ($\alpha=0.05$, Df=14). There is no significant difference. Amount found, $1.88 \mu\text{g dm}^{-3}$ ($n=3$); RSD=4.4%. Zinc in sample, $1.9 \mu\text{g dm}^{-3}$.				
Mineral water ^b	AC	SC	YC	
	Slope	0.1248	0.1370	0.3946
	<i>n</i>	8	10	4
	<i>s</i>	0.0429	0.0565	0.0141
There is constant error bias (YB \neq 0). There is no significant difference between the AC and SC slopes. Trueness test: $t_{cal}=1.601 < t_{crit}=2.131$ ($\alpha=0.05$, Df=15). There is no significant difference. Amount found, $1.53 \mu\text{g dm}^{-3}$ ($n=3$); RSD=5.7%. Zinc in sample, $3.1 \mu\text{g dm}^{-3}$.				

a. In the presence of 20 mg dm^{-3} of tartrate. b. In the presence of 30 mg dm^{-3} of tartrate. *n*=number of measurements in a least-squares fitting set of data. *s*= standard deviation of the regression fitted line. YB=Youden blank. Df=degrees of freedom.

ference (P-value of 20%). We then have clear indications of the suitability of the proposed method for these samples.

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