

# Experimental design optimization for the ICP-AES determination of Li, Na, K, Al, Fe, Mn and Zn in human serum

F. Bianchi<sup>a</sup>, M. Maffini<sup>a</sup>, A. Mangia<sup>a</sup>, E. Marengo<sup>b</sup>, C. Mucchino<sup>a,\*</sup>

<sup>a</sup> *Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43100 Parma, Italy*

<sup>b</sup> *Dipartimento di Scienze e Tecnologie Avanzate, Università degli Studi del Piemonte Orientale, Spalto Marengo 33, 15100 Alessandria, Italy*

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## Abstract

A chemometric approach based on experimental design and desirability functions was used to develop and validate a method for the determination of some metals of biological concern by a fast sequential ICP-AES. The elements considered are Al, Fe, Mn, Zn, Li, Na and K. The experimental design was used to investigate the effects of three instrumental most crucial parameters, such as sheath gas flow rate, pump speed and auxiliary gas flow rate. In order to improve the multielemental analysis speed, although a sequential instrument allows the use of a separate parameter set for each wavelength, regression models and desirability functions were applied to find the experimental conditions providing the highest global sensitivity. Validation was performed in terms of limits of detection (LOD), limits of quantitation (LOQ), linearity, precision and recovery. By using the 167.02 nm wavelength, aluminium LOD was  $0.5 \mu\text{g L}^{-1}$  while the highest LOD was found for K ( $65 \mu\text{g L}^{-1}$ ). A linear range of at least three orders of magnitude was statistically demonstrated for each element. Precision was evaluated by testing two concentration levels, and good results in terms of intra-day repeatability were obtained, with R.S.D. values lower than 4.1% at the lowest concentration level. Lacking a suitable certified reference material, trueness was estimated using the recovery rate on fortified samples. The validated method was then used in the quantification of the elements considered in a serum sample.

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## 1. Introduction

In the last decade an increasing interest was devoted to the neurobiologic effects of some metals, since a correlation was found between their concentration in serum and blood and some neurological diseases. A more recent and multidisciplinary approach [1,2] considers not only the metals, which are toxic in high concentrations, such as Al, Mn and Zn, but also the correlation of their presence with that of other elements, namely Na, K, Li and Fe, due to the cellular membrane exchange mechanism [3]. When the concentration levels of Al and Fe in blood are not balanced, the aluminium could penetrate through the blood–brain barrier (BBB); this fact is regarded as one of the causes starting the Alzheimer disease [4].

In addition, chronic exposure to low level of concentration of such analytes could lead to metabolic alterations since they can bind several proteins. In this latter case the simultaneous determination of others metals is of great relevance, since they could be responsible for the detoxification through a metal exchange mechanism.

For this reason, fast, reliable and multielemental methods for the determination of such analytes in biological fluids are useful. Due to the wide range of concentrations and to the matrix complexity, one of the most versatile analytical technique is the inductively coupled plasma atomic emission spectroscopy (ICP-AES); among the available types of instrumentation, the sequential spectrometer proves to be particularly flexible, since the operating parameters allow an effective optimisation of the experimental conditions. Namely, the Ultima 2 (Jobin Yvon, Longjumeau Cedex, France) spectrometer has some unique parameters, like sheath gas flow, that could be used for difficult element optimisation.

\* Corresponding author. Tel.: +39 0521 905133; fax: +39 0521 905557.  
E-mail address: [claudiom@unipr.it](mailto:claudiom@unipr.it) (C. Mucchino).

Despite several papers have been published on multielemental determinations of metals in biological fluids [5–8], an exhaustive and systematic study to investigate the effects of combined instrumental parameters on analytical results has not been yet reported. The most popular approach used is the “one variable at a time” (OVAT) one [9], that is time-consuming since it can require a high number of experiments, compared to an experimental design approach and, above all it can not provide information on the interactions of the factors investigated. Although a great number of instrumental parameters in a sequential spectrometer can be optimised, most of them, such as plasma RF power, coolant gas flow, nebuliser (primary) flow should be kept constant during a single run, since they can cause plasma perturbation or deep nebulisation efficiency changes [10]. In addition, if the number of instrumental parameters is too high, the number of measurements to be carried out could be excessive with respect to the accomplishment of the task. The aim of this work was the systematic study of the influence of some instrumental parameters on the instrumental responses for Al, Fe, Mn, Zn, Li, Na, and K, by an experimental design approach, and thus the identification of the conditions providing the highest sensitivity for the considered analytes. The experimental design made possible the study of the interactions among the variables and the modelling of multifactorial response surfaces, thus providing a great deal of information about the behaviour of the system. As the final step, when the regression models correlating the experimental responses for the seven metals were available, the predicted responses were combined into a global desirability function [11] in order to find the best compromise between the performance for all analytes and analysis time. It has to be noticed that the powerful simultaneous optimization, is not very often applied to chemical, namely analytical, problems.

## 2. Experimental

### 2.1. Apparatus

An ULTIMA 2 instrument (Jobin Yvon, Longjumeau Cedex, France) was used for the ICP-AES analyses. The instrument was in radial configuration, with a Czerny–Turner monochromator; the optical path was continuously purged with nitrogen. Instrumental and operating parameters are listed in Table 1.

Data acquisition and processing were performed using the ICP JY v. 5.2 software (Jobin Yvon). The daily calibration of the monochromator was performed by using the carbon emission lines and each operating wavelength was individually centered before the experiment beginning. The wavelengths used and the appropriate detector voltages, set by analysing a 100 mg L<sup>-1</sup> multielement standard solution, are reported in Table 2.

Thanks to the nitrogen purging of the optic, the more sensitive 167.02 nm wavelength was selected for aluminum. A Milestone MLS-1200 MEGA microwave system (Milestone, Sorisole, BG, Italy) equipped with temperature control and high pressure PFA vessels was used for sample treatment.

Table 1  
Instrumental characteristics

Monochromator	
Model	JY 2501
Focal distance	1 m
Grating	2400 grooves mm <sup>-1</sup>
Entrance slit	10 μm, 20 μm
Exit slit	15 μm, 80 μm
Resolution	5 pm in UV range, 10 pm in vis range
Nitrogen flow rate	21 min <sup>-1</sup>
ICP-source	
Nebulizer	Meinhard
Spray chamber	Cyclonic
Argon flow rate	121 min <sup>-1</sup>
Wavelengths range	160–785 nm
Optical bench temperature	32 °C

### 2.2. Reagents

Li, Na, K, Zn, Al, Fe, Mn stock solutions (1000 mg L<sup>-1</sup>) were purchased from Merck (Darmstadt, Germany). 65% (v/v) nitric acid from J.T. Baker (Deventer, The Netherlands) was used without further purification. Standard solutions were prepared daily from the stocks, with diluted nitric acid (3%, v/v).

In order to obtain appropriate ICP-AES responses, the experiments were performed using different concentration levels as follows:

- 30 μg L<sup>-1</sup> for Al, Fe, Mn, Zn, Na, Li;
- 200 μg L<sup>-1</sup> for K.

### 2.3. Experimental design

A 2<sup>3</sup> two-level full factorial design (FFD) [12] was used to investigate the effects of the following instrumental parameters: sheath flow rate (*G*), pump speed (*Pm*) and auxiliary flow rate (*Aux*).

Low and high levels were: *G* = 0–1 L min<sup>-1</sup>, *Pm* = 7–31 rpm and *Aux* = 0–0.8 L min<sup>-1</sup>.

This experimental plan allows the evaluation of the effects of the main factors and of all the possible multi-factor interactions. All the experiments were performed in a randomised order to avoid possible memory effect of the analytical apparatus.

All the other instrumental parameters were as follows:

Table 2  
Wavelengths and voltages used for the ICP-AES determination

Element	λ (nm)	Voltage (V)	Integration	Gain	Slits
Al	167.02	580	1	10	20/15
Fe	238.204	660	1	1	20/15
Mn	257.610	550	1	1	20/15
Zn	213.856	600	1	1	20/15
Na	588.95	660	1	1	20/15
Li	670.784	690	1	1	20/15
K	766.490	620	1	100	20/80

- RF power 1000 W, nebuliser flow rate 0.6 L min<sup>-1</sup>, nebuliser pressure 3.00 bar, integration time 1 s.

An *F*-test was used to compare the experimental and calculated responses at the centre of the experimental domain, to evaluate the existence of relevant quadratic effects [13]:

$$F_{\text{calc}} = \frac{(y_f - y_0)^2}{s_{\text{pe}}^2 (1/N_f + 1/N_0)} \quad (1)$$

where  $y_f$  is the mean of the estimated values obtained for the experiments of the FFD,  $y_0$  the mean of the replicated measurements at the centre of the experimental domain,  $N_f$  the number of factorial experiments,  $N_0$  the number of experiments at the centre of the experimental domain and  $s_{\text{pe}}^2$  is the experimental error estimated through the replicated measurements in the FFD, calculated as follows:

$$s_{\text{pe}} = \sqrt{\frac{v_1 s_1^2 + v_2 s_2^2 + \dots}{v_1 + v_2 + \dots}} \quad (2)$$

where

$$s_i^2 = \frac{\sum_{j=1, n_i} (y_i - \bar{y}_i)^2}{n_i - 1} \quad (3)$$

$v_i = n_i - 1$  = degree of freedom of the experimental error estimated in the conditions of the  $i$ th experiment;  $n_i$  = replicated analysis for the experimental condition of the  $i$ -th experiment.

A 95% confidence level was chosen.

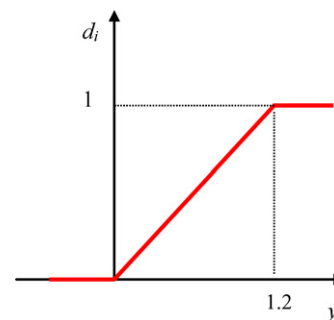
A star design [13] was added to the factorial design experiments when the *F*-test showed evidence for quadratic effects, providing thus a central composite design. Each experiment was replicated six times. The final regression models were then calculated by using the results obtained both from the full factorial design and the star design. The best regression models were then obtained by a step-wise variable selection algorithm at the confidence level of 95%.

The optimal conditions were determined by the global desirability function [11] *D*:

$$D = \left[ \prod_{i=1, q} d_i \right]^{1/q} \quad (4)$$

where  $q$  is the number of responses included in the multicriteria optimisation and  $d_i$  is the desirability of the  $i$ th response defined as follows:

$$\begin{aligned} \text{if } y_i < 0 & \quad \text{then } d_i = 0 \\ \text{if } 0 < y_i < y_{i \text{ max}} \times 1.2 & \quad \text{then } d_i = \frac{y_i}{\max y_i + 15\% \max y_i} \\ \text{if } d_i > y_{i \text{ max}} \times 1.2 & \quad \text{then } d_i = 1 \end{aligned}$$



where  $y_i$  is the value of the  $i$ th response estimated by the corresponding regression model.

Each desirability function  $d_i$  is maximum in the optimal conditions for the corresponding analyte. The maximum of *D* corresponds to the best compromise providing at the same time optimal values for each response.

The maximum of *D* was determined by a grid search algorithm [14], estimating the responses by means of the regression models.

#### 2.4. Sample treatment

A sample of human serum (30 ml) belonging to a patient with a neurological disease was provided by the Division of Neurology of the Hospital of Vaio (Parma, Italy). The sample was maintained at  $-20^\circ\text{C}$  until analysis.

Prior to the ICP-AES analysis an aliquote of the sample (2 g) was submitted to a microwave treatment with 6 ml HNO<sub>3</sub> (65%, v/v) and 1 ml H<sub>2</sub>O<sub>2</sub> (30%, v/v). The details of the microwave treatment are reported in Table 3.

#### 2.5. Validation procedure

The validation process of the method based on the ICP-AES technique was performed according to EURACHEM guidelines [15].

Detection ( $y_D$ ) and quantitation ( $y_Q$ ) limits were calculated as signals based on the mean blank ( $\bar{x}_b$ ) and the standard deviation of blank responses ( $s_b$ ):

$$y_D = \bar{x}_b + 2t s_b, \quad y_Q = \bar{x}_b + 10s_b \quad (5)$$

where  $t$  is the  $t$ -Student value (one-tailed distribution) at the confidence level chosen (95%) and degrees of freedom ( $d_f$ ).  $\bar{x}_b$  and  $s_b$  were determined by performing ten blank measurements.

Table 3  
Microwave operating conditions

Step	Time (min)	Power (W)
1	2:00	250
2	2:00	0
3	6:00	250
4	5:00	400
5	5:00	600

Vent: 5:00

The concentration values corresponding to the detection limit (LOD) and quantitation limit (LOQ) were obtained from the corresponding signals  $y_D$  and  $y_Q$  through a calibration plot  $y = f(c)$ . For this purpose an appropriate calibration curve satisfying the  $0.5x_l \leq \text{LOD} < x_l$  condition was constructed (where  $x_l$  is the lowest concentration value of the calibration samples).

Linearity was established over three orders of magnitude for all the analytes in the following ranges: 2–2000  $\mu\text{g L}^{-1}$  for Al, Fe and Mn, 7–7000  $\mu\text{g L}^{-1}$  for Zn and Li, and 16–16,000  $\mu\text{g L}^{-1}$  for Na and 125–125,000  $\mu\text{g L}^{-1}$  for K. Ten concentration levels were analysed, performing six measurements at each level. Homoscedasticity was verified by applying the Bartlett test, lack-of-fit and Mandel's fitting test were also performed to check the goodness of fit and linearity [16]. The significance of the intercept (significance level 5%) was established by a *t*-test.

Intra-day repeatability [17] was calculated in terms of R.S.D. (%) on two concentration levels (20 and 1400  $\mu\text{g L}^{-1}$  for Al, Fe, Mn, Zn, Na and Li; 140  $\mu\text{g L}^{-1}$  and 12,500  $\mu\text{g L}^{-1}$  for K), performing six replicates at each level.

Trueness was estimated in terms of the presence of matrix effect and of recovery rate from fortified samples of human serum (2 g) at the final concentrations of 0.05  $\text{mg kg}^{-1}$  for Mn, 0.25  $\text{mg kg}^{-1}$  for Al, 7  $\text{mg kg}^{-1}$  for Zn, 130  $\text{mg kg}^{-1}$  for Fe, 0.40  $\text{mg kg}^{-1}$  for Li, 5300  $\text{mg kg}^{-1}$  for Na and 500  $\text{mg kg}^{-1}$  for K. The spike was added prior to the microwave treatment described in sample treatment section.

Appropriate dilutions in 3% (v/v) nitric acid were always performed before ICP-AES analysis.

Recovery rate (RR%) [15] was calculated as follows:

$$\text{RR} = \frac{c_1 - c_2}{c_3} \times 100\% \quad (6)$$

where  $c_1$  is the concentration of the fortified sample,  $c_2$  the concentration of the sample before fortification and  $c_3$  is the concentration of fortification.

The presence of matrix effect was evaluated by comparing the slopes of the regression models obtained by using the external standard and the standard addition method, respectively.

## 2.6. Statistical analysis

All statistical analyses were performed using the statistical package SPSS 10.0 for Windows (SPSS, Chicago, IL, USA).

## 3. Results and discussion

With the aim to find the optimized instrumental conditions for the subsequent determination of the investigated analytes in human serum, an experimental design approach was carried out by using standard solutions.

The parameter choice was in a first stage restricted to the ones which have not a monotonic trend in an optimization experiment: as an example, the optimization of RF power normally ends at the highest possible value of the experimental domain. In addition, since from our experience with this instrument, the optical bench behaviour was never an issue, the typical parameters of the monochromator were not considered.

Subsequently, a choice was made among parameters that have a similar effect on the signal intensity, i.e. nebulisation flow and pump rate.

This sequential spectrometer has some unique features, e.g. the sheath gas flow that is an auxiliary gas flow introduced around the aerosol main stream. The effect is a local temperature decrease which relatively enhances the intensities of spectral lines of easily ionized elements, such the alkali ones. The plasma perturbation due to the sheath gas is negligible so it can be used for the optimisation of the experimental conditions for the determination of alkali metals in the same run, by modulating its flow value for each element or spectral line taking a further advantage from the sequential configuration. Due to the instrumental hardware, the sheath gas flow values must be pre-set to three different levels and each spectral line could be associated to one of the pre-set values. On the contrary, the auxiliary gas must be kept constant during a single run since it influences the plasma shape and the height of the normal analytical zone whereas the optical alignment is usually carried out at the beginning of the run only.

### 3.1. Experimental design

The experimental domain was defined taking into account instrumental and operating limits, namely:

the sheath gas flow was varied in all the allowed instrumental range, auxiliary gas flow values higher than 0.8  $\text{L min}^{-1}$  coupled with the highest value of the sheath flow rate could produce plasma extinction, pump rate values lower than 7 rpm caused a poor nebulisation, whereas the value of 31 rpm was the highest allowed by the instrument used.

For each analyte the main and interaction effects were calculated and the presence of curvature was verified by the *F*-test, as described in the Section 2. All the analytes revealed a significant curvature, suggesting the need to include square terms in the regression models. An additional set of measurements was then performed, according to a star design scheme, leading to a central composite design.

The regression models obtained using the step-wise variable selection algorithm are reported in Table 4. They are all satisfactory as indicated by the  $r^2$  values, in fact the lowest values ( $r^2 = 0.820$  and  $0.868$ ) were obtained for Na and K, usually not determined at these low concentration levels. In Fig. 1 are depicted the response surfaces of Fe and K.

The regression models suggested that a different behaviour characterises Li, K and Na with respect to Al, Fe, Mn and Zn. In particular, for Li, K and Na the sheath flow resulted to be significant, with positive coefficients, whereas negative values were obtained for the other analytes. Taking advantage of the instrumental configuration with the possibility of using different values of the sheath flow, the regression models of the seven analytes were used to find the optimal sheath flow values for each element. Al, Fe, Mn and Zn revealed an optimal sheath flow of 0.0  $\text{L min}^{-1}$ , whereas Li, Na and K a value of 1  $\text{L min}^{-1}$ . As previously described, the optimal sheath flow for the alkali

Table 4

Regression coefficients of the polynomial functions calculated using the step-wise method (standard error in parentheses)

$$\text{Al} = 1.12 (\pm 0.02) - 0.36 (\pm 0.01) \times G + 0.06 (\pm 0.01) \times \text{Pm} - 0.06 (\pm 0.01) \times \text{Aux} - 0.08 (\pm 0.01) \times G \times \text{Pm} + 0.07 (\pm 0.01) \times G \times \text{Aux} - 0.38 (\pm 0.02) \times G \times G - 0.08 (\pm 0.02) \times \text{Pm} \times \text{Pm} - 0.09 (\pm 0.02) \times \text{Aux} \times \text{Aux}$$

$$r^2 = 0.951$$

$$\text{Fe} = 1.014 (\pm 0.003) - 0.171 (\pm 0.002) \times G + 0.015 (\pm 0.002) \times \text{Pm} - 0.015 (\pm 0.002) \times \text{Aux} - 0.038 (\pm 0.002) \times G \times \text{Pm} + 0.018 (\pm 0.002) \times G \times \text{Aux} + 0.16 (\pm 0.004) \times G \times G$$

$$r^2 = 0.991$$

$$\text{Mn} = 1.012 (\pm 0.004) - 0.2 (\pm 0.002) \times G + 0.379 (\pm 0.002) \times \text{Pm} - 0.015 (\pm 0.002) \times \text{Aux} - 0.048 (\pm 0.003) \times G \times \text{Pm} + 0.017 (\pm 0.003) \times G \times \text{Aux} + 0.192 (\pm 0.004) \times G \times G$$

$$r^2 = 0.991$$

$$\text{Zn} = 1.059 (\pm 0.006) - 0.178 (\pm 0.003) \times G + 0.02 (\pm 0.003) \times \text{Pm} - 0.025 (\pm 0.003) \times \text{Aux} - 0.022 (\pm 0.004) \times G \times \text{Pm} - 0.03 (\pm 0.004) \times G \times \text{Aux} + 0.167 (\pm 0.006) \times G \times G - 0.023 (\pm 0.006) \times \text{Pm} \times \text{Pm} - 0.026 (\pm 0.006) \times \text{Aux} \times \text{Aux}$$

$$r^2 = 0.980$$

$$\text{Na} = 1.228 (\pm 0.006) + 0.044 (\pm 0.005) \times G + 0.026 (\pm 0.005) \times \text{Pm} + 0.026 (\pm 0.005) \times G \times \text{Pm} - 0.12 (\pm 0.008) \times G \times G$$

$$r^2 = 0.820$$

$$\text{Li} = 1.161 (\pm 0.003) + 0.044 (\pm 0.002) \times G + 0.024 (\pm 0.002) \times \text{Pm} + 0.0140 (\pm 0.002) \times G \times \text{Pm} - 0.04 (\pm 0.004) \times G \times G - 0.11 (\pm 0.004) \times \text{Pm} \times \text{Pm}$$

$$r^2 = 0.927$$

$$\text{K} = 1.026 (\pm 0.002) + 0.026 (\pm 0.001) \times G + 0.009 (\pm 0.001) \times \text{Pm} + 0.009 (\pm 0.002) \times G \times \text{Pm} + 0.004 (\pm 0.002) \times G \times \text{Aux} + 0.019 (\pm 0.003) \times G \times G - 0.071 (\pm 0.003) \times \text{Pm} \times \text{Pm}$$

$$r^2 = 0.868$$

metals was, as expected, an high value: in fact, the sheath flow decreases the plasma temperature, thus reducing the available energy and allowing a relative better ionization of the alkali metals only. On the contrary, for Al, Fe, Mn and Zn, with high ionization energies, the optimal sheath flow was found in correspondence of  $0.0 \text{ L min}^{-1}$ , thus meaning that this additional gas is not required for these elements.

Data obtained from the regression models showed also different optimal values both for the auxiliary gas flow and the pump speed, thus requiring the use of the multicriteria method of the desirability functions in order to find the optimal ICP-AES conditions (in terms of auxiliary gas flow and pump speed) for the simultaneous determination of the metals. The calculations were done taking into account the responses predicted by the regression models of all seven analytes and by introducing, for each element, the optimal values of the sheath flow as constant term (0 for Al, Fe, Mn, Zn and 1.0 for Li, Na and K).

A global desirability value  $D = 0.86$  was calculated for a pump rate of 31 rpm, an auxiliary gas flow rate of  $0.3 \text{ L min}^{-1}$  and sheath flows (previously calculated) of 1 and  $0.0 \text{ L min}^{-1}$  for each alkaline and the group of Al, Fe, Mn and Zn, respectively. In these conditions all the single desirabilities were greater than 0.83. For all the elements the main effect of the variable pump was found to be significant with the highest value, thus obtaining an higher amount of analytes inside the plasma. Finally, regarding the role of the auxiliary gas, it was found that the value of  $0.3 \text{ L min}^{-1}$  optimised the sampling zone within the plasma.

The method was then validated, operating in the optimised conditions.

### 3.2. Method validation and applications

The method was validated according to the EURACHEM guidelines, in terms of detection limits, quantitation limits, lin-

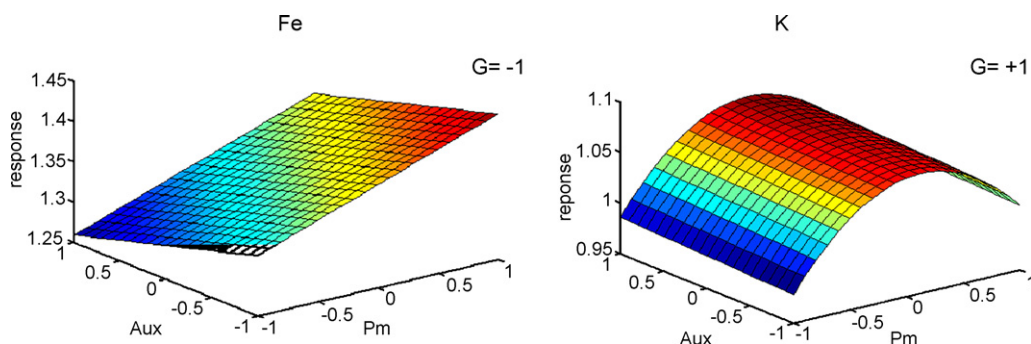


Fig. 1. Response surfaces depicted from the regression models calculated by step-wise regression analysis.

Table 5  
LOD and LOQ values, and linearity for the ICP-AES technique, calibration fitting:  $y = a + bx$

Element	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )	Range ( $\mu\text{g L}^{-1}$ ), Mandel's fitting test, $F_{\text{calc}}^a$	$a (\pm\sigma_a)$	$b (\pm\sigma_b)$	$r^2 (n=66)$
Al	0.5	0.97	2–2,000 7.0	2.7 ( $\pm 0.4$ )	0.8 ( $\pm 0.2$ )	0.984
Fe	0.91	2.0	2–2,000 6.8	3.88 ( $\pm 0.05$ )	0.68 ( $\pm 0.02$ )	0.965
Mn	0.54	1.3	2–2,000 4.9	3.76 ( $\pm 0.05$ )	0.72 ( $\pm 0.02$ )	0.971
Zn	3.9	6.5	7–7,000 1.7	3.34 ( $\pm 0.04$ )	0.89 ( $\pm 0.01$ )	0.990
Na	13	16	7–7,000 3.9	2.53 ( $\pm 0.05$ )	0.95 ( $\pm 0.02$ )	0.989
Li	3.3	5.9	16–16,000 2.0	2.5 ( $\pm 0.7$ )	1.02 ( $\pm 0.02$ )	0.982
K	65	124	125–125,000 0.20	0.23 ( $\pm 0.04$ )	1.04 ( $\pm 0.01$ )	0.996

<sup>a</sup>  $F_{\text{tab}}(1, 63) = 7.05$ , confidence interval = 99%.

earity, precision and accuracy by using the experimental settings providing the optimised conditions.

Limits of detection ranging from 0.5 to 65  $\mu\text{g L}^{-1}$  were obtained. As expected the highest LOD found was that for K: 65  $\mu\text{g L}^{-1}$ . All the obtained values are reported in Table 5 with the corresponding LOQ values.

A good linearity was established over three orders of magnitude for all the analytes (Mandel's fitting test).

The method precision was evaluated at two concentration levels. Good results were obtained in terms of intra-day repeatability: R.S.D. values lower than 3.4% at the highest concentration, and lower than 4.1% at the lowest one, were obtained.

Due to the analytical technique chosen and the mineralization step in the sample treatment a simplified procedure, not involving certified samples, was adopted for trueness: matrix effect was evaluated by comparison of the two regression models obtained by using the external standard and the standard addition method, respectively. The absence of proportional systematic errors was verified, since no significant difference was observed between the slopes of the regression models ( $t_{\text{calc}} < t_{\text{tab}}(\alpha=0.05, 24)$ ), so that it can be concluded that the investigated analytes can be determined using the external standard approach.

Table 6  
Extraction recoveries from fortified human samples

Element	Concentration ( $\text{mg kg}^{-1}$ ) <sup>a</sup>	Recovery (%) <sup>b</sup>
Al	0.17 ( $\pm 0.02$ )	88.9 $\pm$ 2.1
Fe	52.8 ( $\pm 0.6$ )	91.5 $\pm$ 1.8
Mn	0.017 ( $\pm 0.001$ )	88.6 $\pm$ 1.3
Zn	2.7 ( $\pm 0.6$ )	90.7 $\pm$ 2.4
Na	2211 ( $\pm 19$ )	89.1 $\pm$ 1.1
Li	0.13 ( $\pm 0.01$ )	90.2 $\pm$ 1.7
K	371 ( $\pm 68$ )	89.3 $\pm$ 1.6

<sup>a</sup> Before spiking.

<sup>b</sup>  $n = 3$ .

Extraction recoveries greater than 88.6  $\pm$  1.3% ( $n = 3$ ) were obtained from fortified samples prepared by addition of the analytes to a human serum sample. Data obtained, are reported in Table 6. In the same table are also reported the values obtained by analysing the human serum sample before the spiking procedure, thus corresponding to the concentration levels of the element under investigation in the real matrix.

#### 4. Conclusion

An efficient and fast sequential ICP-AES method for the determination of Al, Fe, Mn, Zn, Na, Li and K was developed and validated.

By applying a chemometric approach based on the use of experimental design methods, the optimal conditions corresponding to the highest sensitivity for the considered analytes were calculated. The application of the multicriteria decision method of the desirability functions was found to be crucial.

Since different optimized conditions were found for single group of elements, the method shows good performance in terms of detectability, linearity and precision for the analysis of the metals under investigation: K and Na can be determined at lower concentration levels not common with the ICP-AES techniques. The method proved also to be adequate to the purpose of simultaneously determining seven analytes in human serum.

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