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Application of Simplex Optimization in the Development of an Automated online Preconcentration System for Manganese Determination

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Um procedimento automatizado foi desenvolvido para determinação de quantidades traços de manganês provenientes de folhas de vegetais e águas de rio utilizando espectrometria de absorção atômica com chama (FAAS) após a pré-concentração em linha deste metal usando uma minicoluna de Amberlite XAD-4 modificada com 2-aminotiofenol. O sistema de pré-concentração baseia-se na extração em fase sólida do metal na minicoluna e eluição com ácido clorídrico 0,30 mol L^{-1}, seguido de sua determinação. As variáveis associadas ao desempenho do sistema de pré-concentração, tais como, pH, vazão de amostragem e concentração do eluente foram otimizadas utilizando um simplex básico como técnica multivariada. O método desenvolvido gerou um fator de enriquecimento de 14 vezes atingindo um limite de detecção (3 σ_B) de 2,0 mg L^{-1} e precisão expressa como desvio padrão relativo de 2,2% (%RSD, n = 10; 30,00 mg L^{-1}). O procedimento foi aplicado para a determinação de manganês em águas de rio coletada na área urbana de Jequié/Bahia. A exatidão do procedimento foi verificada usando-se materiais de referência certificados (folhas de macieira, NIST 1515 e folhas de espinafre, NIST 1570). O procedimento desenvolvido foi aplicado para a determinação de manganês em folhas de vegetais (cebolinha, espinafre, couve-flor e repolho).

An automated procedure was developed to determine trace amounts of manganese from vegetal leaves and river waters using flame atomic absorption spectrometry (FAAS) after online preconcentration of this metal using an Amberlite XAD-4 minicolumn modified with 2-aminothiophenol. The preconcentration system is based on the solid-phase extraction of the metal into the minicolumn and elution with 0.30 mol L^{-1} hydrochloric acid, followed by its determination. Variables associated with the preconcentration system performance, such as pH, sampling flow rate and eluent concentration were optimized using a basic simplex as multivariate technique. The developed method provides an improvement factor of 14 along with limit of detection (3σ_B) of 2.0 μg L^{-1}, and precision expressed as a 2.2% relative standard deviation (RSD%, n = 10, 30.0 μg L^{-1}). The procedure was applied to determine manganese in river waters collected from urban area of Jequié/Bahia. The accuracy of the procedure was checked by analysis of two certified reference materials (apple leaves, NIST 1515 and spinach leaves, NIST 1570). The developed procedure has been applied for manganese determination in vegetal leaves (chive, spinach, cauliflower and cabbage).

Keywords: vegetal leaves, natural waters, simplex optimization, manganese, automated online preconcentration

Introduction

Determination of trace metals in some samples such as vegetal leaves and natural waters at low concentrations is still a challenging task despite the availability of sensitive analytical intrumental techniques. Methods for separation of analytes from interferents in the complex matrices and selective preconcentration are frequently necessary.[1] Several online preconcentration techniques have been proposed to determine metals in various environmental samples, including knotted reactor,[2] cloud-point extraction,[3] coprecipitation[4] and solid phase-extraction.[5,6] Thus, automatization of preconcentration procedures can bring several advantages such as decrease of the analyst errors, also the risks of contamination and analyte losses due to the low numbers of manipulation steps and increase in analytical throughput.[7,8]

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The simplex method, was first suggested by Spendley *et al.*[9] This method is referred to as basic simplex method, and, in its present form was a regular figure whose number of point is defined by k+1, being k the number of variables that must be optimized and that correspond with dimensions of space. Thus, in case of two variables, k = 2, the simplex will be a triangle, three variables, k = 3, it will be a tetrahedron and in case of more dimensions, a hypertriangle.

The procedure begins by the choice of k+1 points (k is the number of dependent variables), and evaluation of the response at each, and continually forming new simplexes by reflecting the point giving the least favorable response in the hyperplane of the remaining points.

The basic algorithm works as follows: (1) generate the initial simplex according with specific factors; (2) run the experiments at the initial simplex coordinates; (3) decide from the responses which vertex represents the best (B), the next-to-best (N), and the worst (W) response; (4) calculate the new experimental point by R=P+(P-W), where P is the centroid of the face remaining if the worst vertex (W) has been eliminated from the full simplex.

The centroid is calculated using the equation 1:

$$P = \frac{1}{n}\sum_{j \neq i}^{n} y_j \qquad (1)$$

where n is the number of variables, i the index of the worst vertex to be eliminated and j is the index of the considered vertex.[10,11,12]

Simplex design methodology has been applied to optimize some analytical methods, such as solid-phase microextraction coupled to CG-MS for polycyclic aromatic hydrocarbons determination in water samples,[13] determination of amphetamine, amphetamine analogues, cocaine, and heroin in illicit tablets using capillary zone electrophoresis,[14] amoxicillin and clavulanic acid determination using sequential injection analysis with a diode-array spectrophotometer,[15] separation and determination of vitamins A and E using micellar liquid chromatography,[16] kinetic determination of nabumetone, a non-steroidal anti-inflammatory drug, by means of micellar-stabilized room temperature phosphorescence,[17] flow injection analysis system for tartaric acid determination in wines[18] and ion-pair reversed phase high performance liquid chromatographic analysis of 4-(2-pyridylazo) resorcinol chelates of Co(II), Ni(II) and Cr(III).[19]

Our research group has developed automated preconcentration system for metals determination using, as examples, cloud-point extraction where metal ions are complexed in a mixture of the reagent 2-[2′-(6-methyl-benzothiazolylazo)]-4-bromophenol (Me-BTABr) and Triton X-114. After separation, the micellar phase is trapped in a minicolumn with a filtering material.[20] Another example is the manganese preconcentration using two mini-columns packed with 4-(5′-bromo-2′-thiazolylazo)orcinol (Br-TAO) loaded polyurethane foam.[21]

This work shows the first application of a solid-phase functionalized by our research group (Amberlite XAD-4 modified with 2-aminothiophenol, AT/XAD-4) for manganese preconcentration and also presents the use of a basic algorithm simplex design to optimize an automated system for determination of this metal by FAAS.

Advantage of AT/XAD-4 resin as solid-phase is in the low backpressure generated by solid-phase used avoiding disruption of connections. Furthermore, the functionalized polymer has the advantage of reducing the leaching of the complexing reagent from solid support, increasing the column lifetime when compared to loaded sorbents. Simplex optimization was applied in this study because its advantages in comparison to other experimental design such as the simplicity and good responses for automated systems.

Experimental

Absorbance measurements were taken using a FAAS Perkin-Elmer Instruments (Norwalk, CT, USA) model AAnalyst 200. It was equipped with a manganese hollow cathode lamp and an air-acetylene flame. The instrumental parameters were as follows: 279.5 nm wavelength, 15.0 mA lamp current and 0.5 nm spectral resolution. The flow rates of acetylene, air and nebulizer were 2.0, 13.5 and 5.0 mL min^{-1}, respectively. Deuterium lamp background correction was used.

A Digimed DM 20 (Santo Amaro, SP, Brazil) pH meter was used to monitor the pH of the solutions. Sample digestion was carried out by using a Parr Instrument model 4749 (Moline, IL, USA). Acid digestion bombs enclosing a chemically inert Teflon sample cup of 23 mL were used for sample treatment.

The online preconcentration system is illustrated in Figure 1. The system consisted of a multichannel peristaltic pump model 200/4 from Millan (Colombo, Paraná, Brazil) furnished with silicone tubes to propel the liquids and four three-way direct lift solenoid valves (Vernon Hills, IL, USA) to select the preconcentration and elution steps. Solenoid valves were controlled by a microcomputer running software written in Turbo Pascal 4.0. A laboratory-made mini-column packed with DHB-XAD-4 was inserted into the online system.

Reagents and solutions

Deionized water was used to prepare all solutions. Glassware was kept overnight in a 10% v/v nitric acid solution.

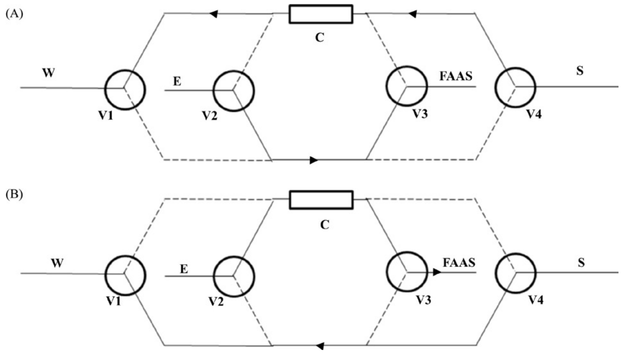

Figure 1. Schematic diagram of the automated flow system used to preconcentration and determination of manganese by FAAS. S. sample; E. eluent; P. peristaltic pump; C. AT-XAD-4 minicolumn; V1, V2, V3 and V4. solenoid valves; FAAS. flame atomic absorption spectrometer. W. waste. (A) System in the preconcentration step. solenoid valves on and (B) system in the elution position. Solenoid valves off.

All chemicals used were of analytical grade. Reference manganese solutions at the µg L^{-1} level were prepared by diluting a 1000 µg mL^{-1} stock solution (Merck, Darmstadt, Germany). Hydrochloric acid solutions were prepared by direct dilution of the concentrated solution (Merck) with deionized water. Acetate (pH 3.8-5.8), phosphate (5.8-7.5), borate (pH 7.5-9.0) and ammonia (pH 9.0-10.0) buffer solutions were used to adjust the pH of the manganese solutions. Hydrochloric acid solutions were prepared by diluting a stock solution of Suprapur quality (Merck).

The functionalized resin AT-XAD-4 was synthesized by using the following reagents: 2-aminothiophenol (Merck), aluminum chloride (Vetec, Rio de Janeiro, RJ, Brazil), nitrobenzene (Merck), octanol (Merck) and methylene chloride (Vetec). AmberliteXAD-4 (specific surface area 330 m^2 g^{-1} and bead size 20-60 mesh), a PS-DVB copolymer, was purchased from Aldrich (Milwaukee, WI, USA).

Synthesis of AT-XAD-4 resin and minicolumn preparation

The sorbent AT-XAD-4 was prepared by a procedure described previously for similar resins.[22] A 3 g amount of Amberlite XAD-4 was chloromethylated by mixing the copolymer beads with dry 20 mL methylene chloride and 10 mL octanol. Anhydrous, pulverized aluminium chloride (7 g) was added in small increments to the mixture. The system was refluxed at 90 °C. The reaction was stopped after 24 h and filtered. The resin beads were cleaned with water, acetone, 1:1 v/v aqueous hydrochloric acid and again with water. The chloromethylated beads were filtered off and dried for storage. The dried chloromethylated copolymer was suspended in a mixture of 30 mL nitrobenzene and 2.0 g 2-aminotiophenol. Then the system was refluxed for 72 h at 70 °C under continuous stirring. The mixture was filtered, and the resin was washed twice with 1:1 v/v aqueous hydrochloric acid, and water, respectively. The final product was heated in ethanol for 2 h, filtered and dried in air.

A cylindrically shaped polyvinyl chloride minicolumn (3.50 cm × 4.0 mm i.d.) was packed with 100 mg of the AT-XAD-4 resin. Plastic foams were fixed at both ends of the minicolumn to prevent material losses. The minicolumn was washed with 2.0 mol L^{-1} nitric acid solution and deionized water until the effluent was acid free.

Sample preparation

Samples of river waters were collected directly from the urban area of Jequié/Bahia by the bottle immersion in the water stream. Polyethylene flasks used were previously decontaminated with nitric acid solution (10% v/v) and rinsed with ultrapure water. Samples were filtered using a membrane (0.45 µm pore diameter) through a vacuum system after sampling to remove suspended particulate material. Later, these samples were acidified to pH 2 with nitric acid and stored at 6 °C.

Plant samples (0.1 g) were digested by adding 4.0 mL of 1:1 v/v nitric acid solution to the sample. A Teflon cup was placed in a Parr 4781 digestion bomb and heated in a microwave system for 1 min at 265 W. Blank samples were prepared analogously. Sub-samples were spiked with a known amount of the analyte. After cooling at room temperature, the bomb was opened carefully in a fume cupboard. Sodium hydroxide and an appropriate buffer solution were used to adjust the pH of the final digests. The mixture was finally diluted to 25 mL with double deionized water. These solutions were analyzed immediately after preparation.

Multivariate optimization

The procedure was optimized by the simplex design methodology using the basic algorithm. Three variables were regarded as experimental variables (pH, sampling flow rate and eluent (HCl) concentration) due their great importance in the automated online preconcentration system. Initial experimental values that consist of the tetrahedron vertices of the first simplex figure were chosen with the aim of attaining the optimum values quickly. In all optimization process a 50 mg L^{-1} manganese solution and a preconcentration time of 1 min were used.

Results and Discussion

Optimization procedure

The variables expected to have the greatest effect on the automated manganese preconcentration system were sampling flow rate, pH and eluent (HCl) concentration and they were used for the optimization step. The initial simplex design was consisted of four vertices (one more than the number of variables) and it was established by the initial values (represented by the letter I in Table 1). The experiments described by the initial simplex design were performed and their analytical signal (absorbance) values are also presented in Table 1. The simplex figures were moved in the direction given by the rules of the basic algorithm.[11] Thus, the worst vertex was rejected and the new simplex was established by calculating the next reflection vertex.

Figures 2(a), (b) and (c) show the values of the three parameters as a function of the vertex number. The sampling flow rate and pH profiles present trends to higher values when the vertices are dislocated to the optimal region showing the great influence of these variables in the preconcentration process. However, HCl concentration showed considerable variation but no obvious trend.

The experiments were then performed using the established vertices until no further improvement in the analytical signal was observed, as shown in Figure 3. As it can be seen, the highest absorbance value was obtained at vertex 17. Thus, the optimum conditions of experiment 17 (7.2 mL min^{-1} for sampling flow rate, 8.9 for pH and 0.30 mol L^{-1} for HCl concentration) were selected to establish the analytical characteristics for the automated system.

Analytical characteristics

Under optimum working conditions and using 1 min as preconcentration time, the analytical figures of merit obtained for the automated online solid-phase extraction system for Mn are summarized in Table 2. The regression curve obtained from the preconcentration process was $A = 0.00223\ C_{Mn} + 0.0093$, where A is the absorbance and C_{Mn} is the manganese concentration in solution, in µg L^{-1}. It was observed that lack of fit was not significant (p-value = 0.8516 > 0.05 for a 95% confidence level) for this regression. Conventional analytical curve (without preconcentration) resulted in the following equation: $A = 0.000159\ C_{Mn} + 0.0048$. This equation was obtained under optimum conditions of the spectrometer. Again, lack of fit was not significant for this regression (p-value = 0.4695 > 0.05).

Table 1. Values obtained from simplex optimization

Experiment	Vertex	Sampling flow rate (mL min^{-1})	pH	HCl (mol L^{-1})	Analytical signal (A)
01	I	2.4	5.0	0.20	0.013
02	I	3.6	5.0	0.20	0.022
03	I	4.2	5.9	0.20	0.034
04	I	4.2	5.3	0.36	0.025
05	R	5.6	5.8	0.31	0.030
06	R	5.7	6.3	0.38	0.061
07	R	6.1	6.7	0.23	0.068
08	R	5.0	6.8	0.23	0.074
09	R	7.0	7.3	0.36	0.071
10	R	6.3	7.6	0.17	0.081
11	R	6.1	7.8	0.28	0.093
12	R	4.6	7.5	0.09	0.064
13	RN	7.0	7.3	0.36	0.069
14	R	6.3	8.5	0.13	0.102
15	R	7.8	8.4	0.29	0.066
16	RN	4.7	7.5	0.10	0.064
17	R	7.2	8.9	0.30	0.120
18	R	5.3	8.4	0.18	0.062
19	R	7.7	8.4	0.29	0.112
20	R	8.0	9.4	0.20	0.086

I = initial simplex design, R = reflection of the vertex with the worst response, RN = reflection of the vertex with the second worst response.

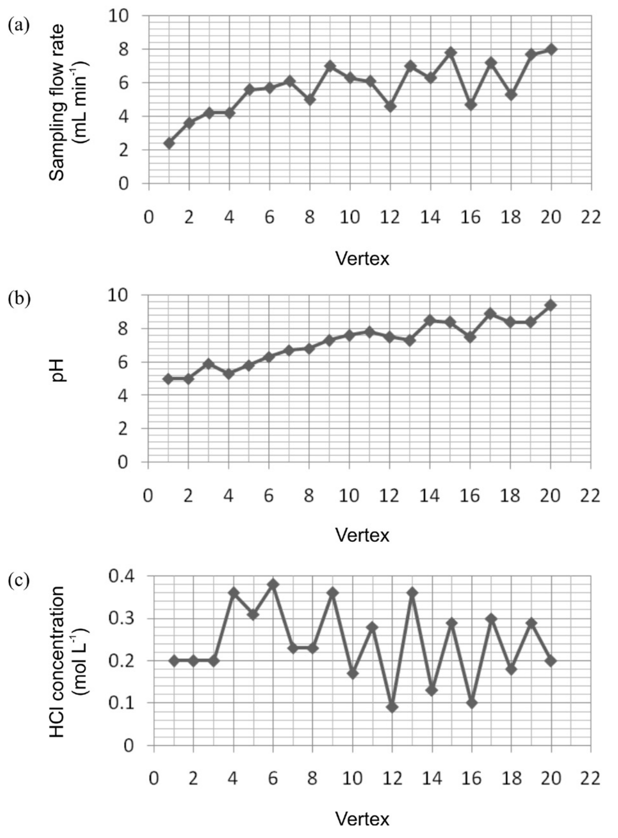

Figure 2. Evolution of the variables levels values with the trials defined by the simplex design method, (a) sampling flow rate, (b) pH and (c) eluent concentration.

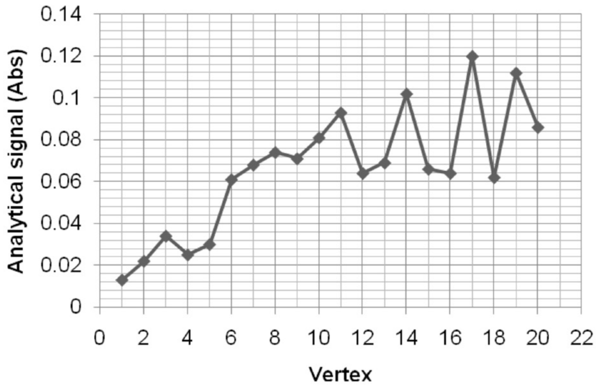

Figure 3. Evolution of analytical signal with the trials defined by the simplex design method.

The enrichment factor was calculated as the ratio of the slopes of the linear section in calibration graphs for preconcentration and direct aspiration, respectively.[25] An enrichment factor of 14 was obtained for this system.

The concentration efficiency (CEF) was defined as the product of the enrichment factor and the sample frequency, expressed in min^{-1}. The consumptive index (CI) was also calculated. This parameter was defined as the volume of the sample consumed to achieve a unit of EF.[25] It was calculated by the ratio of the sample volume, in milliliters, and EF.

Table 2. Analytical characteristics of the automated online preconcentration system for manganese determination using AT/XAD-4

Limit of detection (LOD)	2.0 µg L^{-1}
Limit of quantification (LOQ)	6.8 µg L^{-1}
Precision	6.3% (50 µg L^{-1}); 5.4% (100 µg L^{-1})
Enrichment factor	14
Concentration efficiency	12 min^{-1}
Consumptive index	0.51 mL
Sampling frequency	51.3 h^{-1}

The calibration graph for manganese was linear up to 100 µg L^{-1}. The limit of detection (LOD) was calculated from the calibration curve, as LOD = 3 s / m, where s was the standard deviation of 12 measurements of a reagent blank, and m was the slope of the calibration curve. The limit of quantification (LOQ) was calculated as LOQ = 10 s/m. From these equations, LOD and LOQ were found to be 2.0 and 6.8 µg L^{-1}, respectively. The precision, assessed as the relative standard deviation (R.S.D.) of Mn determination was obtained by measuring the analytical signal for 10 cycle times at levels of 50 and 100 µg L^{-1} Mn. The R.S.D. obtained were 6.3% (50 µg L^{-1}) and 5.4% (100 µg L^{-1}). Memory effect was not observed using the developed procedure probably because a 0.30 mol L^{-1} solution was enough to clean the inner walls of tubes and connections from preconcentration system.

Robustness study[24,25] was carried out using Placket Burman design 2^{3-1} (Table 5). For this manganese preconcentration system, modifications in the optimum value for sampling flow rate above 2.8% have significant effects on the signal (see Pareto Graph in Figure 4). On the other hand, modifications in the optimum values for pH

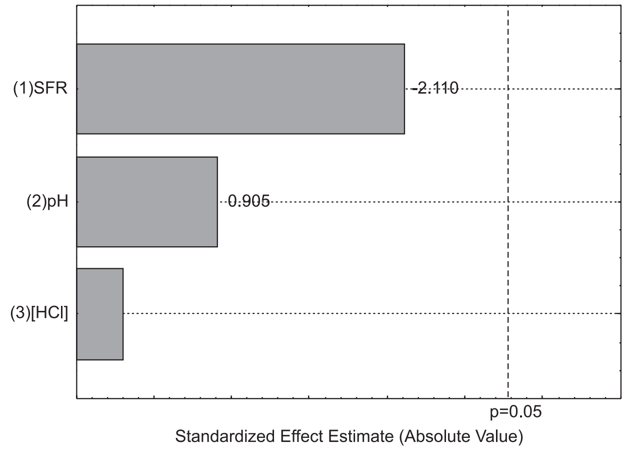

Figure 4. Pareto graph for robustness study. SFR: sampling flow rate; [HCl]: acid concentration in mol L^{-1}.

and eluant concentration below 3.4% have none significant effects on the system performance.

Accuracy and application

Manganese was determined in two standard reference materials (spinach leaves, NIST 1570 and apple leaves, NIST 1515) in order to evaluate the accuracy of the developed procedure. These samples were digested using the procedure described previously. Table 3 presents the results of this experiment. The manganese contents established by the present procedure agreed well with the certified values. The results indicate the effectiveness and accuracy of the procedure.

Table 3. Determination of manganese in (a) standard reference material and (b) vegetal samples (n = 3)

Sample	Certified value	Found value	P-value
	(a) (µg g^{-1})		
Apple leaves (NIST 1515)	54 ± 3	57 ± 3	0.353
Spinach leaves (NIST 1570)	75.9 ± 1.9	74 ± 4	0.834
	(b) (µg g^{-1})		
Chive	-	46 ±6	-
Spinach	-	236 ± 7	-
Cauliflower	-	22 ± 2	-
Cabbage	-	17.1 ± 0.5	-

The proposed procedure was applied to determine manganese in vegetal leaves (chive, spinach, cauliflower and cabbage) and also river waters collected from Jequié city urban area. Results are presented in Table 3 and 4. Analysis of variance (ANOVA) was applied to these two groups of results. For a confidence level of 95%, p-values have demonstrated that there are none significant differences between these groups (p-value > 0.05).

Results demonstrate the applicability of the proposed online system to determine manganese in these matrices. Considering the mass of the food, the limit of detection of the procedure is 1.0 µg g^{-1}.

Table 4. Determination of manganese in river waters and spike tests (µg L^{-1}**,** n = 3)

Sample	Added	Found	Recovery (%)
River water 1	0	19.2 ± 0.2	-
	20.0	40.2 ± 0.8	105
River water 2	0	13.8 ± 0.1	-
	20.0	32.5 ± 0.4	94
River water 3	0	67.6 ± 0.5	-
	20.0	88.1 ± 0.2	103

Table 5. (a) Modification levels from optimum value and (b) Plackett-Burman 2^{3-1} design for robustness study of the preconcentration system for manganese determination

(a)			
Variable	+1	0	−1
SFR (mL min^{-1})	7.0 (−2.8%)	7.2	7.4 (+2.8%)
pH	8.6 (−3.4%)	8.9	9.2 (+3.4%)
[HCl] (mol L^{-1})	0.29 (−3.4%)	0.30	0.31 (−3.4%)
(b)			
SFR (mL min^{-1})	pH	[HCl] (mol L^{-1})	Absorbances
−1	−1	−1	0.119/0.122
1	−1	1	0.118/0.119
-1	1	1	0.121/0.121
1	1	−1	0.119/0.120

SFR: Sampling flow rate.

Conclusions

The use of a basic simplex design method allowed the easy and fast optimization of an automated online preconcentration system for manganese determination in river water and vegetal leaves. The automation of the procedure diminished the errors associated with the operation of manual valves normally used in these kinds of systems and increased the FAAS performance, presenting analytical characteristics that allow manganese determination at low concentration levels. The developed system is simple, rapid, easy to use, selective, and with adequate sensitivity. The manganese contents found in the analyzed matrices are at an acceptable level, suggesting that these foods are adequate for human consumption. The developed method is presented as a good alternative for the routine determination of manganese in vegetal leaves and river waters samples.

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