

## THE VALIDATION OF THE METHOD FOR IRON DETERMINATION IN ROUMANIAN WINES USING FLAME ATOMIC ABSORPTION SPECTROMETRY

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In order to validate a method for iron determination using flame atomic absorption spectrometry an important parameter, the robustness, was analysed. With this aim there were studied the stability of iron standards during one day and then during 30 days, the effects that appeared after the modification of the slit of the monochromator and the influence of nitric acid concentration used at samples preparation on absorbances. It has been noticed that, generally, the iron standards present a good stability in time. In addition, it has been observed that the method is not robust with regard to the slit of the monochromator, meanwhile the concentration of the nitric acid in samples does not produce significant modifications of the results.

### INTRODUCTION

In certain areas, such as food analysis, the requirement for completely validated methods is requested by legislation.<sup>1-3</sup> Validation of an analytic method means a carefully examination of methods' characteristics, one of them being robustness.<sup>4,5</sup>

The method described in this paper allow the iron determination using flame atomic absorption spectrometry, using oxidant flame nitrogen oxide-acetylene, after calcinations at 550°C.

The natural iron content of wine ("physiological iron") is of C=2-6 mg/L. The iron that comes from dust deposited on grapes, treatments and from machines and metallic vessels corrosion, named "technological iron", can reach the value of 60 mg/L.

Copper, iron and manganese are responsible for changes in stability of old wines and modification of the sensory quality of wines after bottling. This phenomenon, called "browning", involves a cascade of oxidation reactions of organic components of wine, leading to loss of freshness and aroma and appearance of condensed precipitates of tannins.<sup>6</sup>

The evaluation of the robustness of the method developed in this study supposes the demonstration of confidence in analysis. The stability of solutions,

the influence of the modification of the slit of the monochromator and the influence of nitric acid used at samples preparation were monitored.

### EXPERIMENTAL PART

#### Chemical reagents

In order to obtain the calibration curve it was used a Merck iron standard solution traceable to NIST – SRM 682 Fe(NO<sub>3</sub>)<sub>3</sub> in HNO<sub>3</sub> 0.5 mol/L, 1000 mg/L Fe. Used freshly prepared standards were stored in polyethylene vessels. For the iron standards preparation and also as diluent for calcinated wine samples was used 0.1N HNO<sub>3</sub> made from Merck ultrapure concentrated HNO<sub>3</sub> 65% m/m (d=1.40g/mL). It was used ultrapure water. All used materials and vessels were kept at least 24 hours in a plastic container filled with HNO<sub>3</sub> 10% v/v and washed for several times with deionised water.

#### Instrumentation

In order to effect the measurements it was used an atomic absorption spectrometer Zeenit 700 from Analytic Jena equipped with autosampler AS52 S for dilution, 50 mm burner for nitrogen protoxide flame, monoelement lamp for iron. Also, the equipment has data processing soft, Win AAS ver:3.16.0. The Romanian Metrology National Institute calibrates the instrument. The deionised water was obtained with ELIX 3 system and the ultrapure water was obtained using Simplicity UV system, both of them provided by Millipore.

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### Procedure

The wine samples, after drying using waterbath were calcinated at 550°C. The white ashes were dissolved in a volume of 25mL 0.1 mol/L HNO<sub>3</sub> and the solutions were

transferred in polyethylene bottles. The processed samples according with this procedure were analyzed at  $\lambda=248.3\text{nm}$  using a nitrogen protoxide/acetylene flame and a calibration curve previously plotted. The optimal parameters of the instrument are described in Table 1.

Table 1

Optimum parameters FLAAS

Spectrometer		Flame	
Line, $\lambda$ (nm)	248.3	Flame	C <sub>2</sub> H <sub>2</sub> /N <sub>2</sub> O
Slit (nm)	0.2	Fuel flow (L/h)	170
Lamp type	HCL	Burner type (mm)	50
Lamp current (mA)	6.0	Burner height (mm)	5
Integration time (s)	4.0	Nebulizer rate (mL/min)	5.0
D2-HCL current (mA)	16.5		

## RESULTS AND DISCUSSION

In order to validate the method, the robustness was evaluated and the results obtained are presented in the following part.

The robustness of an analytic procedure represents the measurement of its capacity to remain unaffected by small, but deliberate variations in methods parameters and provides an indication of its reliability during normal usage.<sup>7,8</sup> With this purpose there were analysed the stability of standard solutions during one day and during a period of 30 days, the modification of the slit of the monochromator and of samples' acidity.

### The stability of standards during one day

Analysing the studies from literature, it is known that viability of standards with C=1000 mg/L Fe that are stored in polyethylene bottles is 2.5 years, the viability of standards with C=10-100 mg/L Fe is one month and the viability of standards with C=1-10 mg/L Fe is appreciatively one week.

The main instrument that was used in order to make the statistic control of this process is control charts. The chart presents two control limits statistically determined, one side and another of central line (LC), named superior control line (LCS) and inferior control line (LCI). The central line represents the reference value of presented characteristic and generally, is the average of considered data.<sup>9</sup> The stability of standard with a theoretical concentration of C=7.5 mg/L was verified during one day, at ½ hour intervals. All

this time, the solution was kept at room temperature, at light, in polyethylene bottle. The obtained results are presented in Table 2. The control charts for individual values,  $\bar{x}$  and mobile amplitude,  $R$ , are presented in Fig. 1. The mobile amplitude is the absolute difference between successive pairs from one series of measurements, that means the difference between the first and the second measurement, and then between the second and the third measurement and so on.<sup>9</sup>

The formulas for control charts and the values of corresponding coefficients were extracted from SR ISO 8258+C1:1999.<sup>9</sup> The control charts for individual values,  $\bar{x}$  and mobile amplitudes,  $R$ , indicate that the process is statistically controlled,<sup>9</sup> the obtained values being disposed one side and another of central line without to exceed the control limits.

It can be concluded that the standard with C=7.5 mg/L Fe is stable during one day.

### The stability of standards during 30 days

It has been conducted a study regarding the stability during 30 consecutive days for 2 iron standards of C=7.5 mg/L that were storage in different conditions in polyethylene bottles. One bottle was kept at room temperature and at light and the other was refrigerated. The obtained results (Table 3) has been analysed using control charts for individual values and mobile amplitudes (Fig. 2) and on the basis of them it has been concluded that the iron standard kept at room temperature and the refrigerated one are stable one month.

Table 2

The obtained results for stability standard iron monitoring during one day

Determination no.	Conc. Fe x (mg/L)	Mobile amplit. R (mg/L)	Determination no.	Conc. Fe x (mg/L)	Mobile amplit. R (mg/L)	Determination no.	Conc. Fe x (mg/L)	Mobile amplit. R (mg/L)
1	7.602	-	6	7.684	0.119	11	7.533	0.175
2	7.735	0.133	7	7.594	0.090	12	7.578	0.045
3	7.481	0.254	8	7.498	0.096	13	7.665	0.087
4	7.645	0.164	9	7.535	0.037	14	7.442	0.223
5	7.565	0.080	10	7.708	0.173	15	7.736	0.294
$\bar{x}=7.600$ mg/L			$s=0.0932$ mg/L			RSD%=1.227		
<b>Lines of the control chart for individual values, <math>\bar{x}</math></b>								
$LC = \bar{x} = 7.600$			$LCS = \bar{x} + E_2 \bar{R}$ $= 7.600 + 2.66 \cdot 0.141$ $= 7.975$			$LCI = \bar{x} - E_2 \bar{R}$ $= 7.600 - 2.66 \cdot 0.141$ $= 7.225$		
<b>Lines of the control chart for mobile amplitudes, <math>\bar{R}</math></b>								
$\bar{R} = LC = 0.141$ mg/L			$LCS = D_4 \bar{R}$ $= 3.267 \cdot 0.141$ $= 0.461$			$LCI = D_3 \bar{R}$ $= 0 \cdot 0.141$ (for individual values, $D_3=0$ and LCI is not represented)		

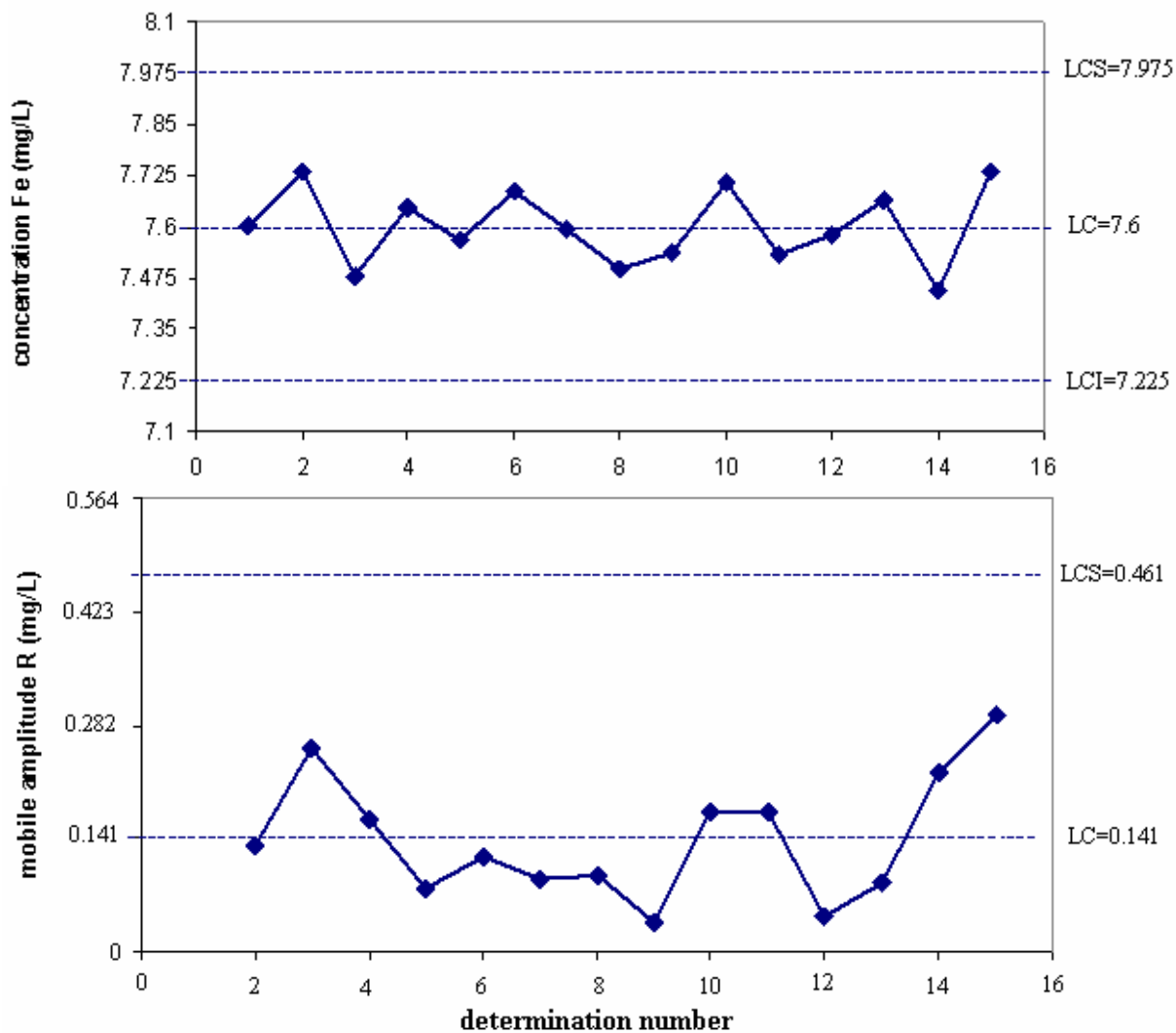


Fig. 1 – Control charts for individual values, x and mobile amplitude, R regarding the stability of iron standard during one day.

Table 3

The obtained results after monitoring the stability of iron standard during 30 days in different conditions

Determination no.	Room temp., light		Refrigerated		Determination no.	Room temp., light		Refrigerated	
	Conc. Fe, x (mg/L)	Mobile amplit. R (mg/L)	Conc. Fe, x (mg/L)	Mobile amplit. R (mg/L)		Conc. Fe, x (mg/L)	Mobile amplit. R (mg/L)	Conc. Fe, x (mg/L)	Mobile amplit. R (mg/L)
1	7.34	-	7.34	-	13	7.09	0.654	7.41	0.283
2	7.739	0.399	6.928	0.412	14	7.207	0.117	7.291	0.119
3	7.536	0.203	7.374	0.446	15	7.395	0.188	7.211	0.08
4	7.8	0.264	7.647	0.273	16	7.696	0.301	7.676	0.465
5	7.136	0.664	7.536	0.111	17	7.1	0.596	7.429	0.247
6	7.819	0.683	6.964	0.572	18	7.214	0.114	7.778	0.349
7	7.119	0.7	7.508	0.544	19	7.793	0.579	7.129	0.649
8	7.87	0.751	6.909	0.599	20	7.255	0.538	7.09	0.039
9	7.843	0.027	7.297	0.391	21	7.572	0.317	7.756	0.666
10	7.013	0.83	7.446	0.149	22	7.177	0.395	7.231	0.525
11	7.812	0.799	7.02	0.426	23	7.027	0.15	7.561	0.33
12	7.744	0.068	7.693	0.673	-	-	-	-	-

Room temp., light	$\bar{x} = 7.448 \text{ mg/L}$	Refrigerated	$\bar{X} = 7.358 \text{ mg/L}$
	$s = 0.3136 \text{ mg/L}$		$s = 0.2671 \text{ mg/L}$
	$\text{RSD}\% = 4.211$		$\text{RSD}\% = 3.630$
	<b>The lines of the control chart for individual values, <math>x_i^9</math></b>		<b>The lines of the control chart for individual values, <math>x_i^9</math></b>
	$\text{LC} = \bar{x} = 7.448$		$\text{LC} = \bar{X} = 7.358$
	$\text{LCS} = \bar{x} + E_2 \bar{R} = 7.448 + 2.66 \cdot 0.424 = 8.576$		$\text{LCS} = \bar{X} + E_2 \bar{R} = 7.358 + 2.66 \cdot 0.379 = 8.366$
	$\text{LCI} = \bar{x} - E_2 \bar{R} = 7.448 - 2.66 \cdot 0.424 = 6.32$		$\text{LCI} = \bar{X} - E_2 \bar{R} = 7.358 - 2.66 \cdot 0.379 = 6.35$
	<b>The lines of the control chart for mobile amplitudes, <math>R_i^9</math></b>		<b>The lines of the control chart for mobile amplitudes, <math>R_i^9</math></b>
	$\text{LC} = \bar{R} = 0.424$		$\text{LC} = \bar{R} = 0.379$
	$\text{LCS} = D_4 \bar{R} = 3.267 \cdot 0.424 = 1.385$		$\text{LCS} = D_4 \bar{R} = 3.267 \cdot 0.379 = 1.238$
$\text{LCI} = D_3 \bar{R} = 0 \cdot 0.424$ (for individual values LCI is not represented)	$\text{LCI} = D_3 \bar{R} = 0 \cdot 0.379$ (for individual values LCI is not represented)		

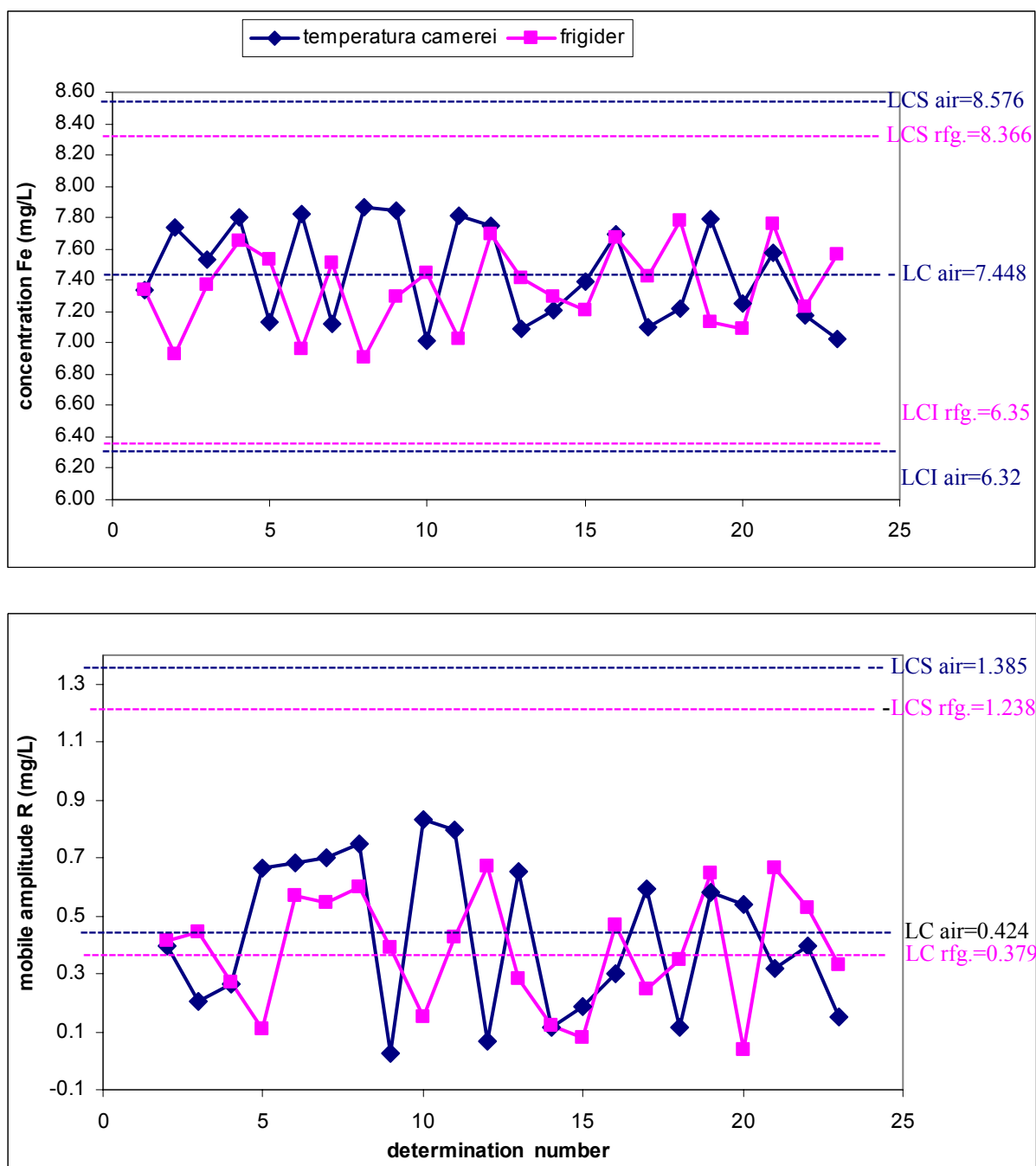


Fig. 2 – The control charts for x and R regarding the stability of the standards during 30 days.

### Modification of the slit of the monochromator

The modification of the slit of the monochromator depends on a series of factors such as: type and the usage level of the hollow cathode lamp, the wavelength and the sensibility of the photomultiplier. There have been used many values of the slit: 0.2nm, 0.5nm, 0.8nm and 1.2nm.

The values of the absorbencies are presented in Table 4.

The graphical representation of the absorbance versus concentration for different slit is presented in Fig. 3.

The characteristics of the calibration function calculated for the four different values of slit are presented in Table 5.

Table 4

The values of the obtained absorbance at different slit

Concentration (mmol/L)	Absorbance at different slit			
	0.2nm	0.5nm	0.8nm	1.2nm
0.026786	0.0335	0.0381	0.0312	0.0256
0.053571	0.0654	0.0647	0.0582	0.0449
0.080357	0.0988	0.0930	0.0765	0.0635
0.107143	0.1283	0.1214	0.0994	0.0846
0.133929	0.1602	0.1442	0.1264	0.1035
0.160714	0.1874	0.1806	0.1456	0.1235
0.187500	0.2203	0.2121	0.1682	0.1437
0.214286	0.2492	0.237	0.187	0.1606
0.241071	0.2842	0.2584	0.2087	0.1776
0.267857	0.3100	0.2852	0.234	0.2023

Table 5

Resume of characteristics of calibration function for different slit: 0.2nm, 0.5nm, 0.8nm and 1.2nm

Parameter	Series 1 (0.2nm)	Series 2 (0.5nm)	Series 3 (0.8nm)	Series 4 (1.2nm)
$y = a+bx$	$0.0046+1.1484x$	$0.0099+1.0427x$	$0.0116+0.8274x$	$0.0062+0.7247x$
Slope, b	1.1484	1.0427	0.8274	0.7247
y-intercept, a	0.0046	0.0099	0.0116	0.0062
Standard deviation about the linear regression, $s_y$	$1.91 \cdot 10^{-3}$	$3.91 \cdot 10^{-3}$	$2.34 \cdot 10^{-3}$	$1.60 \cdot 10^{-3}$
Standard deviation of the slope, $s_b$	$5.93 \cdot 10^{-3}$	$1.21 \cdot 10^{-2}$	$7.26 \cdot 10^{-3}$	$4.96 \cdot 10^{-3}$
Standard deviation of the intercept, $s_a$	$9.85 \cdot 10^{-4}$	$2.02 \cdot 10^{-3}$	$1.21 \cdot 10^{-3}$	$8.25 \cdot 10^{-4}$
Correlation coefficient, R	0.9998	0.9990	0.9994	0.9996
Determination coefficient, $R^2$	0.9996	0.9981	0.9989	0.9993
Degrees of freedom	8	8	8	8

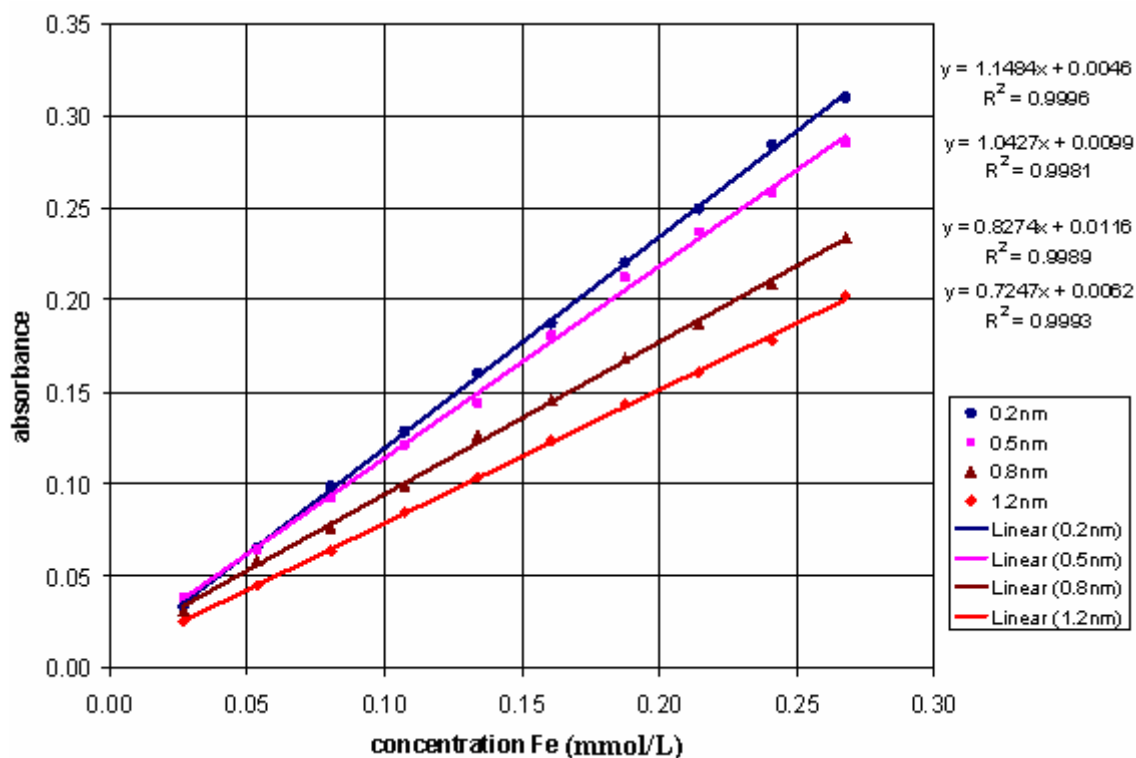


Fig. 3 – The graphical representation of the absorbance versus concentration for different slit: 0.2nm, 0.5nm, 0.8nm and 1.2nm.

The experimental data show that the slope of the calibration curve increases with the slit of the monochromator from 0.2nm to 1.2nm. The best results regarding the correlation coefficient, R, background, the slope (the method's sensitivity) and the y-intercept, has been obtained for a slit of 0.2nm. Regarding the standard deviation about the regression, the standard deviation of the slope and intercept, the best results are obtained for a slit of 1.2nm but the differences are not significant in comparison with those obtained with a slit of 0.2nm. So, as consequence, the iron determination it will be conducted using a slit of 0.2nm. The significant modification of the slope with the slit,

will determine a significant modification of the results and shows that the method is not robust one as regards the slit of the monochromator.

### The modification of the acidity of the analysed solution

It has been studied the influence of the medium acidity on the absorbance determinations using a standard solution of 7 mg/L Fe. The obtained values for the solution that has been prepared using nitric acid with variable concentrations 0.1 mol/L to 3.0 mol/L are given in Table 6, Fig. 4.

Table 6

The absorbance values obtained for a standard solution of 7 mg/L Fe prepared with nitric acid (different molarities)

Nr.crt.	Conc. HNO <sub>3</sub> (mol/L)	Absorbance	Nr.crt.	Conc. HNO <sub>3</sub> (mol/L)	Absorbance
1	0.10	0.2038	7	1.84	0.2029
2	0.39	0.2040	8	2.13	0.2022
3	0.68	0.2044	9	2.42	0.2022
4	0.97	0.2042	10	2.71	0.2008
5	1.26	0.2049	11	3.00	0.2011
6	1.55	0.2047	-	-	-
$\bar{x} = 0.2032$		$s = 1.444 \cdot 10^{-3}$	RSD%=0.7104		$\bar{x} \pm ts/\sqrt{n} :$ 0.2032±0.0010

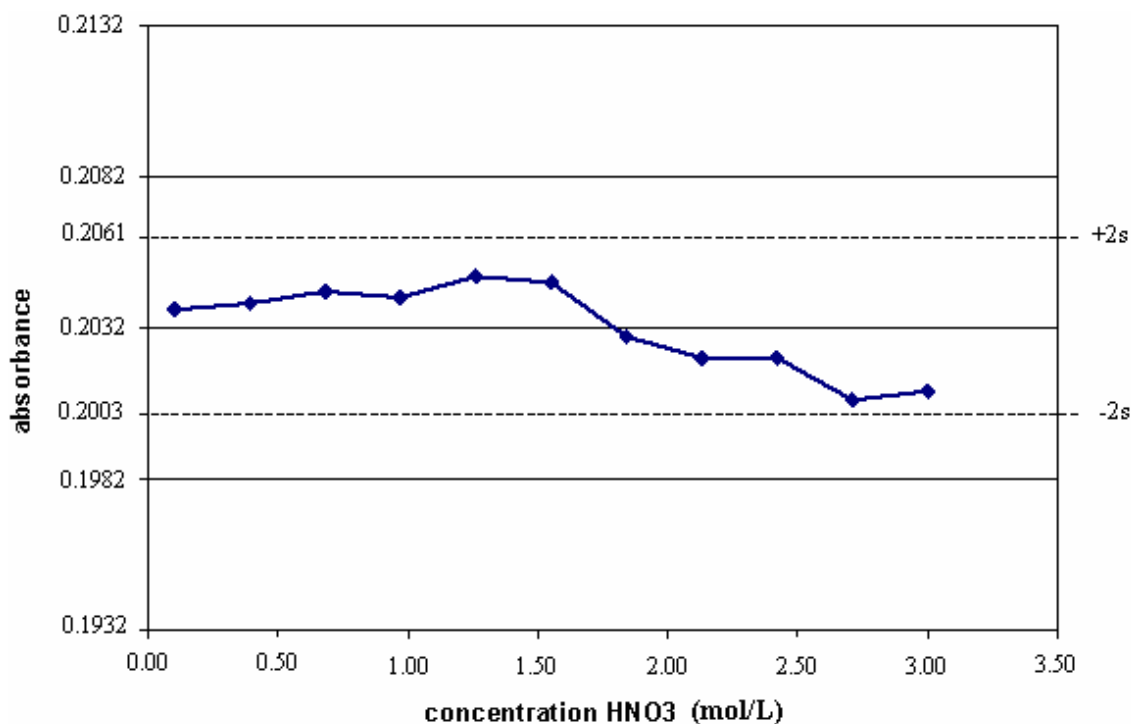


Fig. 4 – The graphical representation of the absorbance for an iron standard (7 mg/L) as a function of nitric acid molarities.

Initially, the absorbance increases slightly with the increase of the acidity, nitric acid molarities

respectively, up to 1.5 mol/L and after this value the absorbance decreases. The medium value of the

absorbance obtained after determination using iron standards prepared in nitric acid of different concentrations (0.1 mol/L up to 3.0 mol/L) is 0.2032. The standard deviation is  $1.444 \cdot 10^{-3}$ ,  $RSD\% = 0.7104$  and a confidence interval of the average is  $\pm 0.0010$  ( $\pm ts/\sqrt{n}$ , where  $t=2.23$  for  $n-1=10$  degrees of freedom,  $A=0.2022-0.2042$ ).

It can be noticed that the obtained values are in the interval  $\pm 2s$  beside the average. These results show that a variation of the nitric acid concentration from  $C=0.1$  mol/L up to  $C=3.0$  mol/L does not produce significant modifications of the results.

### CONCLUSION

In order to validate a determination method for iron using flame atomic absorption spectrometry using nitrogen protoxide/acetylene flame after calcinations has been evaluated the robustness of the method. In order to achieve this purpose, it has been monitored the stability of the standards, the influence on determinations of the modification of the slit of the monochromator and the effect produced by the modification of the samples' acidity. It has been observed using Shewhart charts, a stability of the standards ( $C=7$  mg/L) during one day and then during 30 days.

Also, it has been observed that the modification of the slit of the monochromator produces a

significant modification of the slope of calibration curve, so, from this point of view the method is not a robust one. The concentration of the nitric acid ( $C=0.1-3$  mol/L) from samples did not affected very much the results, these being  $\pm 2s$  beside the medium value.

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