Aspects concerning validation of a method for chromium content determination in Romanian wines by ETAAS after microwave mineralization

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Abstract

A method for the determination of chromium in Romanian wines by electrothermal atomic absorption spectrometry was proposed. The method was validated by studying quality parameters such as linearity, trueness and precision. Linearity, calculated for the linear range 5-14 µg L^{-1}, was 99.38%. Found detection and quantification limits, in order to OIV method, are 0.18 µg L^{-1} and 0.61 µg L^{-1}, respectively. Recoveries ranging between 78.53% and 115.60% were obtained from spiked samples at three concentration levels (3- 7 µg L^{-1}) and ANOVA test did not show significant bias. The obtained values for the relative standard deviation, in repeatability and intermediate precision conditions, are under the ones recommended for concentration levels present in the analyzed samples.

Keywords: *chromium, electrothermal atomic absorption spectrometry, validation method*

Introduction

Wine has been a widely consumed beverage in the world for centuries with obvious commercial value and social importance. Numerous studies have shown that the moderate consumption of wine, especially red wines, improves good health and longevity (15).

When considering vinification processes, metals determine wine quality and have a profound effect on human health and well-being. Knowledge of the metal content of wine is crucial not only for winemakers but also for consumers. Certainly, because of the nutritional value of metals in wine, analysis of the total contents of major, minor and trace metals is of a particular interest with respect to the wholesomeness and the safety of wine (14).

Metals are very good indicators of wine origin and can be used as criteria for guaranteeing authenticity, since they are not metabolized or modified during winemaking and reflect the average composition of vineyard soils (1; 4, 14). However, agricultural practices, climatic changes, environmental pollutions, and vinification processes, could change a primary pattern of metals in wine and endanger the relationship between wine and soil composition (14).

Prestige wines are among the most imitated and falsified products. Therefore, analytical methods for a suitable characterization of wines need to be developed (1).

The official methods for determination of metals in wine recommended by the OIV and the American Society of Enologists are essentially based on atomic absorption spectrometry (AAS) due to its selectivity and high sensitivity (15). Electrothermal atomic absorption spectrometry (ETAAS) has found application to determination of trace and sub-trace metals (2,6, 14, 15).

Solovyov Studies ISPU   ISSN: 2076-9210

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Trivalent chromium (Cr(III)) is required in trace amounts for sugar metabolism in humans (Glucose Tolerance Factor) and its deficiency may cause a disease called chromium deficiency. In large concentrations, chromium is harmful. Hexavalent chromium (Cr(VI)) is far greater dangerous to living organisms, due to its bigger ability to diffuse through cell tissue (7). Ingesting large amounts of chromium (VI) can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death (11). The estimated safe and required dietary intake of Cr (III) is 0.05-0.20 mg/day. Wine could contribute with an important fraction of the dietary intake of Cr (8). The origin of Cr in wine is two-fold: from natural (endogenous) sources (soil, grapes) and from exogenous sources (environmental contamination, fertilizers, pesticides, industrial processing and containers) (8, 14). It was shown that the plants growing on high-chromium soil contained higher chromium concentration than similar plants growing on low-chromium soils (8).

The validation of the analytical method is in our days an essential concern for analytical laboratories activity and illustrates in a clear manner the competence of a laboratory. In order to validate it were realized studies regarding performance of the method, tests for the evaluation of the laboratory capabilities and the intern quality control (16, 17, 18, 19, 25).

In some areas, such as food analysis, the requirement for completely validated methods it is sustained by legislation (24, 26, 27). A validation for an analytical method supposes an examination of the method's characteristics (17, 26): applicability (matrix and limit concentrations), selectivity/specificity, working range and linearity, linearity, sensibility, limit of detection, limit of determination, accuracy, precision/repeatability/ reproducibility, recovery and other criteria that can be selected as necessary conditions (robustness).

Since chromium in biotechnology is used to obtain products used as food supplements, such as vitamin and mineral complexes or enriched yeasts, knowing precisely the content of chromium (III) is essential.

The aim of this article is to present the analytical method applied for the determination of chromium content in Romanian wines and clearly some aspects, regarding the sample preparation and the validation of the method.

Material and Methods

Instrument

Measurements were carried out using a Zeenit700 Analytic Jena atomic absorption spectrometer with Zeeman background correction, graphite furnace (pyrolytically coated graphite tubes) and MPE60 auto-sampler. The atomization cell was purged with argon. A chromium hollow cathode lamp (Jena) was used as a light source. The Romanian Metrology National Institute calibrated the instrument. The method used has been previously optimized (3). Optimum instrumental conditions for ETAAS measurements were: wavelength, $\lambda=357.9$ nm; slit: 0.8 nm; lamp current: 4.0 mA; calibration mode: absorbency, peak area; Zeeman correction: 2-field. Graphite furnace temperature program is given in Table 1.

Table 1. Graphite furnace temperature program

Type	Temperature(^{0}C)	Rate (^{0}C/s)	Hold (s)	Time (s)	Air flow rate
Drying 1	90	20	10	13.5	Max
Drying 2	110	2	10	20	Max
Drying 3	130	2	10	20	Max
Pyrolysis	1300	250	10	14.7	Max
AZ (auto zero)	1300	0	5	5	Stop (read)

| Atomize | 2200 | FP | 5 | 5.1 | Stop (read) |
| Cleanout | 2550 | 500 | 4 | 4.7 | Max |

For the microwave digestion, a Millestone ETHOS PRO microwave digester was used.

Reagents

Ultrapure concentrated HNO_3 and an ultrapure $30\% H_2O_2$ solution (Merck) were used without further purification. Milli-Q water (Simplicity UV system, Millipore) was used throughout. A mono-elemental stock solution traceable to NIST – SRM 3112a $Cr(NO_3)_3$ in HNO_3 0.5 mol L^{-1}, 1000 ± 5 mg L^{-1} Cr CertiPUR® from Merck were used to prepare external standard solutions. Standard solutions were prepared daily from the stocks, in polyethylene tubes. Class A volumetric flasks and PFA-coated bottles were soaked in a 10% HNO_3 solution for at least 24 h, rinsed with Milli-Q water and dried. The pipettes regularly verified for precision and accuracy were used to sample wines and other solutions.

Wine samples

Two samples of wine were studied: two commercially available Murfatlar wines (one white: Italian Riesling, dry, DOC-CMD and one red: Merlot, medium dry, DOC-CMD) from Dobrogea Region, Romania. The wines were supplied in glass bottles with cork stoppers. The samples were chosen to represent two different types of wine matrix – the most commonly found.

Analytical procedure

Wine samples (4ml) have been added with pipette in PTF recipients and treated with 6ml HNO_3 65%. The recipients have been left in the niche for the organic matrix of the wine to stabilize, approximately 10 minutes it where added 2 ml H_2O_2 30%. Covered recipients have been introduced into the microwave furnace and mineralized according to table 2.

Table 2. The program of the microwave furnace

Stage	Time (minutes)	Temperature (^{0}C)	Microwave power (W)
1	10	150	600
2	20	150	750
3	15 – cooling	0	0

After the digestion, recipient content was quantitatively transferred into a 25 ml volumetric flask and brought down to sign with ultra pure water. From these mineralized solutions was determined Cr element. The standards used in calibration have been prepared in HNO_3 3.47 mole/l for the same acid concentration as the samples. All subsequent dilutions have been prepared with HNO_3 3.47 mole/l.

Results and Discussion

Analytical performance
Calibration

Linear range and linearity for chromium element were studied. Linear regression analysis and its statistical results were presented in table 3.

Aspects concerning validation of a method for chromium content determination in Romanian wines by ETAAS
after microwave mineralization

Table 3. Linear regression analysis and its statistics

Element	Data		
Cr	b=0.017	a=0.035	r=0.9998
	$S_b = 0.0001$	$S_a = 0.001$	$S_y = 9.5*10^{-4}$
	$LOD^{(1)}$=0.1835µg/L	$LOQ^{(1)}$=0.6117µg/L	Linear range: 5-14µg/l
	$LOD^{(2)}$= 0.0836µg/L	$LOQ^{(2)}$= 0.2788µg/L	S_{blank} = 0.0004737
	b, slope; a, intercept; r, correlation coefficient; S_a, standard deviation of the intercept; S_b, standard deviation of the slope; LOD, limit of detection; LOQ, limit of quantification; S_{blank}, standard deviation obtained from 10 measurements of the blank (HNO_3 3.47mole/L)		
	$^{(1)}$ according to OIV; $^{(2)}$ calculated by equipment		

For assignation of linear domain have been applied two statistic tests: dispersion homogeneity test and linearity test (28, 29). Linearity, calculated as $(1-S_b/b)x100$ (12), was 99.38%.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) have been set according to OIV recommended technique (20, 21, 22, 23). The two limits were based on values of the standard deviation of the intercept (S_a) and they were deduced of mathematical expressions: $DL=(3*S_a)/b$ and $DML=(10*S_a)/b$. The equipment software measures the Limit of Detection and the Limit of determination as $3S_{blank}/b$ and $10S_{blank}/b$, respectively (12). The obtained results are presented in the table 3 too.

Trueness

Taking into account that we had not CRM available for this kind of samples, recovery assays were carried out for trueness study purpose (9, 12, 13). A red and white wines were fortified, before mineralization, at i levels (i=3) of concentration and were analyzed in j replicates (j=3). Fortification to obtain concentrations in sample between the interval 3-7µg/l for Cr was performed. The recovery for each sample (R_{ij}) was obtained, the average recovery for each level ($\overline{R_i}$) and the global recovery for chromium ($\overline{R}$) were calculated (20, 21, 22, 23). It has been observed that the recoveries in the both cases were in range recommended by the literature of the specialty (17) (table 4-5).

Table 4. Statistical results obtained by application of ANOVA test to recovery for Cr in a white wine by ETAAS

ANOVA: single factor							
SUMMARY							
Groups (addition)	Recovery (%)			Count	Sum	Average	Variance
A (3mg/L)	104.40	87.33	78.53	3	270.26	90.09	173.01
B (5mg/L)	92.40	86.00	81.20	3	259.60	86.53	31.57
C (7mg/L)	84.16	89.30	94.87	3	268.33	89.44	28.69
					Overall mean	88.69	
ANOVA							
Source of Variation		SS	df	MS	F-calculated		F-critical
Between Groups		21.56	2	10.78	0.139		5.143

M. ARTIMON, I. GH. TĂNASE, M. PELE, GH. CAMPEANU, G. VASILE

Within Groups	466.56	6	77.76	
Total	488.12	8		

Table 5. Statistical results obtained by application of ANOVA test to recovery for Cr in a red wine by ETAAS

ANOVA: single factor						
SUMMARY						
Groups (addition)	Recovery (%)			Count	Sum	Average	Variance
A (3mg/L)	87.67	102.00	93.67	3	283.34	94.44	51.79
B (5mg/L)	99.40	88.60	115.60	3	303.60	101.20	184.68
C (7mg/L)	87.14	91.86	100.29	3	279.29	93.10	44.38
					Overall mean	96.25

ANOVA					
Source of Variation	SS	df	MS	F-calculated	F-critical
Between Groups	113.10	2	56.55	0.604	5.143
Within Groups	561.69	6	93.62		
Total	674.79	8			

One-way ANOVA was performed to find out possible bias contribution due to between levels variance. After ANOVA calculations were performed, no significant bias was detected.

Precision

Repeatability for the two studied wines (white and red wines) and intermediate precision for the both wines were evaluated. For this, each sample was analyzed six times in a short period, without changing any operational condition. The relative standard deviation in repeatability conditions was calculated. On the other hand, by analyzing the same wines in intermediate precision conditions, the relative standard deviation was evaluated.

The precision was evaluated through calculations of standard deviation of repeatability (s), relative standard deviation (RSD%), standard deviation of the average (s_m), repeatability limit (r), Horrat value (Ho_r) and also of the confidence interval for the average of obtained results (10, 20, 21, 22, 23; 25,). The confidence level considered was 95%.

In all cases, the obtained results show RSD% values under the ones recommended by the Horwitz equation (17, 20, 21, 22, 23) and by AOAC for concentration levels present in the analysed samples (17) (table 6,7,8,9).

Table 6. The obtained results for repeatability for white wine

Sample	1	2	3	4	5	6
x_i Cr (µg/L)	43.375	40.387	41.531	40.850	46.612	42.100

• Determination number (n): 6	• Horwitz value r : 17
• Average, $\bar{x}$: 42.476 µg/L	• Horrat value, Ho_r : 0.315
• s: 2.278 µg/L	• s_m : 0.9302
• RSD%: 5.364	• The confidence interval for the average:
• Repeatability limit, r: 6.316 µg/L	40.085÷44.866

Table 7. The obtained results for repeatability for red wine

Sample	1	2	3	4	5	6
x_i Cr (µg/L)	113.5	119.72	112.06	111.38	113.56	116.81

• Determination number (n): 6	• Horwitz value r : 14.63
• Average, $\bar{x}$: 114.51 µg/L	• Horrat value, Ho_r : 0.19
• s: 3.1672 µg/L	• s_m : 1.293

Aspects concerning validation of a method for chromium content determination in Romanian wines by ETAAS after microwave mineralization

- RSD%: 2.766
- Repeatability limit, r: 8.78 µg/L
- The confidence interval for the average: 111.18÷117.83

Table 8. The obtained results regarding intermediate precision for white wine

Sample		1	2	3	4	5	6
x_i Cr, (µg/L)	Analyst 1	41.186	44.182	44.562	42.787	40.131	46.725
	Analyst 2	44.550	43.787	45.397	44.450	39.983	42.286
	Analyst 3	41.191	43.732	46.356	42.247	42.994	40.412
Parameter	Analyst 1	Analyst 2	Analyst 3	Analyst 1+2	Analyst 1+3	Analyst 2+3	Analyst 1+2+3
n	6	6	6	12	12	12	18
$\bar{x}$, (µg/L)	43.262	43.409	42.822	43.335	43.042	43.115	43.164
s, (µg/L)	2.403	1.974	2.105	2.098	2.166	1.969	2.052
RSD_r%	5.554	4.547	4.915	4.841	5.032	4.567	4.755
r, (µg/L)	6.660	5.471	5.834	5.815	6.003	5.458	5.689
Horwitz value r	17	17	17	17	17	17	17
Horrat value Ho_r	0.327	0.267	0.289	0.285	0.296	0.269	0.280
s_m, (µg/L)	0.981	0.806	0.859	0.606	0.625	0.568	0.484
Interval confidence, (µg/L)	40.741÷ 45.783	41.338÷ 45.480	40.614÷ 45.030	42.003÷ 44.668	41.667÷ 44.417	41.865÷ 44.366	42.144÷ 44.185

Table 9. The obtained results regarding intermediate precision for red wine

Sample		1	2	3	4	5	6
x_i Cr, (µg/L)	Analyst 1	113.50	112.06	111.38	118.19	121.25	116.78
	Analyst 2	113.94	122.88	116.56	119.08	120.43	117.26
	Analyst 3	121.22	114.75	116.74	119.12	112.48	116.78
Parameter	Analyst 1	Analyst 2	Analyst 3	Analyst 1+2	Analyst 1+3	Analyst 2+3	Analyst 1+2+3
n	6	6	6	12	12	12	18
$\bar{x}$, (µg/L)	115.53	118.36	116.85	116.94	116.19	117.60	116.91
s, (µg/L)	3.87	3.14	3.09	3.67	3.41	3.07	3.39
RSD_r%	3.35	2.65	2.64	3.14	2.93	2.61	2.90
r, (µg/L)	10.72	8.69	8.56	10.17	9.45	8.51	9.41
Horwitz value r	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Horrat value Ho_r	0.23	0.18	0.18	0.22	0.20	0.18	0.20
s_m, (µg/L)	1.58	1.28	1.26	1.06	0.984	0.887	0.800
Interval confidence, (µg/L)	111.47÷ 119.58	115.07÷ 121.65	113.61÷ 120.09	114.61÷ 119.27	114.02÷ 118.35	115.65÷ 119.55	115.22÷ 118.60

Conclusions

Since Romania was accepted as a part of European Union in January 2007, incontestably, the national economy has to be adjusted, including agro-alimentary field, to the standards and norms of the Common Market. The assurance of the safety food it is a major desideratum for each country, which has to be found, again in every decision took at micro and macroeconomic level.

The penetration and the maintaining of the Romanian wines on the Europe market are linked directly to the production of wines proving accentuate characteristics. Romanian wine remains a true ambassador of its country. Each type of wine is defined by a set of unique

features, given by the soil where the vineyard grows, the variety of vineyard, the region, the essence of wood from which casks are made and nevertheless, by the skill and passion of producers. This combination gives the wine a specific bouquet which differentiates it from other several tens of thousands alternatives. Due to an elevated commercial value, it is often doctored, either with the purpose of hiding some flaws of its preparation or storage or to sell an inferior wine under the label of a superior product. In this case, the setting of some methods for the physical and chemical analysis of wines, able to identify the fakes, is fully justified.

The aim of this study was to prove the analytical performances of a quantitative method for chromium determination in wine samples (white and red wines) by ETAAS method after their microwave mineralization. The method, previously optimized, was validated.

The performance parameters obtained such as linearity, detection and determination limits, trueness, followed as recovery and, precision, evaluated as repeatability and intermediate precision of the two wine samples investigated (white and red wines), respect the theoretical values provided by specialty literature permitting, in this way, validation of the method.

This study confirms the suitability of the proposed methods for determination chromium content in wine for routine and quality control laboratories.

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Aspects concerning validation of a method for chromium content determination in Romanian wines by ETAAS
after microwave mineralization

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