Method validation for determination of heavy metals in wine and slightly alcoholic beverages by ICP-MS

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Abstract. The Organisation International de la Vigne et du Vin (OIV) fixed an uppermost level for some heavy metals in wine. Consequently, the need to determine very low concentration of elements that may be present in wine in trace and ultra trace levels occurred. Inductively coupled plasma mass spectrometry ICP-MS is considered an excellent tool for detailed characterization of the elementary composition of many samples, including samples of drinks. In this study a method of quantitative analysis for the determination of toxic metals (Cr, As, Cd, Ni, Hg, Pb) in wines and slightly alcoholic beverages by ICP-MS was validated. Several parameters have been taken into account and evaluated for the validation of method, namely: linearity, the minimum detection limit, the limit of quantification, accuracy and uncertainty.

1. Introduction

Wine is a complex matrix, which besides water, sugar and alcohol, contains a great variety of components, inorganic as well as organic. The composition of wine is influenced by many factors related to the specific production area: grape varieties, soil and climate, culture, winemaking, transport and storage [1, 2]. Moderate wine consumption contributes to the daily intake of many essential metals, including Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, Zn. Wine contains macro-elements c > 10 mg/l (Na, K, Mg, Ca), micro-elements 10 mg/l > c > 10 µg/l (Fe, Cu, Zn, Mn, Pb) and ultra-microelements c < 10 µg/l (Cr, As, Cd, Ni) [3, 4]. It should be noted that the levels of certain contaminants elements, such as Cu, Zn, As, Cd, Pb at different stages of the winemaking process are of great concern because of legal requirements [5]. The validation of the analytical method has become a basic prerequisite for those laboratories that work in an official food and beverage control [6]. The aim of this study is the validation of an analytical method for quantitative determination of toxic metals (Cr, As, Cd, Ni, Hg, Pb) in wines and slightly alcoholic beverages using the technique of inductively coupled plasma mass spectrometry ICP-MS. Thirteen wine assortments were investigated: five red wines and eight white wines.

2. Experimental

Mass spectrometry with inductively coupled plasma (ICP-MS) is a multi-element technique for analyzing liquid samples, characterized by high selectivity, sensitivity and detection limits much lower than other multi-element techniques [7, 8].
2.1. Methods
All the determinations were carried out by inductively coupled plasma quadrupole mass spectrometry (ICP-Q-MS). A Perkin Elmer ELAN DRC (e) instrument was used with a Meinhart nebulizer and silica cyclonic spray chamber and continuous nebulization. The operating conditions are: Nebulizer Gas flow rates: 0.86 l/min; Auxiliary Gas Flow: 1.2 l/min; Plasma Gas Flow: 15 l/min; Lens Voltage: 7.25 V; ICP RF Power: 1100 W; CeO/Ce = 0.031; Ba\(^{++}/Ba^{+} = 0.016.\)

2.2. Materials
Ultra-pure de-ionized water (18 M\(\Omega\) cm\(^{-1}\)) from a Milli-Q analytical reagent-grade water purification system (Millipore) and ultra-pure HNO\(_3\) 60% (Lot No B0157318 Merck) were used. All plastic ware used for the sampling were new or cleaned by soaking 24 h first in 10% HNO\(_3\) then in ultra-pure water. In order to validate the method for determining the concentration of heavy metals in wines high toxicity and alcoholic beverages weak, a synthetic slightly alcoholic sample (1% alcohol, using ethanol - reagent for analysis, minimum 95%) of 2 \(\mu\)g/l concentration As, Be, Cd, Cr, Hg, Ni, Pb, Se, Tl was prepared. To the ICP-MS analysis of wines, samples are diluted by 10 ÷ 20x volume of ultrapure water. Since wines are generally 10-12% alcohol, a synthetic sample of 1% alcohol reproduce with sufficient fidelity real matrix samples. A multielement Merck 100 mg/l As, Be, Cd, Cr, Hg, Ni, Pb, Se, Tl standard (Multi-element Standard Solution IX Certipur; Matrix 6.4% HNO\(_3\)) was used which to prepare firstly a solution of 10 \(\mu\)g/l. Using this solution, six alcoholic solutions of different concentrations (1, 2, 2.5, 5, 7, 9) \(\mu\)g/l were then prepared. The solutions of 1, 2.5, 5, 7, 9 \(\mu\)g/l were used as calibration solutions and the solution of 2 \(\mu\)g/l was employed as a synthetic sample.

3. Results and discussions
Several parameters have been taken into account and evaluated for the validation of the analytical methods for quantitative determination of toxic metals in wines and slightly alcoholic beverages (Cr, As, Cd, Ni, Hg, Pb), namely: applicability range and linearity, the minimum detection limit, the limit of quantification, repeatability, accuracy, uncertainty.

**Linearity domain:** using calibration solutions, calibration curves \(y = ax + b\) were determined (y is the signal intensity, x is the know concentration of the given analyte in the calibration solution). The linearity of the calibration curve was considered acceptable when the correlation factor R > 0.999.

**The minimum detection limit:** is the lowest concentration or quantity of analyte which can be measured with reasonable statistical certainty. To determine the limit of detection 3SD, a method developed by Perkin Elmer was used. Ultra pure water of 18.2 M\(\Omega\) cm\(^{-1}\) was aspirated and signal intensities for blank were recorded. A solution of 10 \(\mu\)g/l As, Ni, Cd, Hg, Pb, Cr was aspirated and the signal intensities for these analytes were recorded (table 1).

**Table 1. The limit of detection for As, Ni, Cd, Cr, Pb, Hg.**

| Elements | I\(_{\text{blank}}\) | RSD\(_{\text{blank}}\) | SD\(_{\text{blank}}\) | I\(_{\text{net}}\) | LOD (\(\mu\)g/L) |
|----------|-----------------|-----------------|-----------------|-----------------|-----------------|
| As       | 15.73           | 10.95           | 1.72            | 57626.89        | 0.001           |
| Ni       | 70.06           | 8.10            | 5.67            | 29042.42        | 0.006           |
| Cd       | 55.13           | 8.06            | 4.45            | 23136.62        | 0.006           |
| Hg       | 1110.16         | 4.74            | 52.66           | 63118.47        | 0.025           |
| Pb       | 421.14          | 9.94            | 41.86           | 196287.20       | 0.006           |
| Cr       | 15.78           | 8.73            | 1.37            | 22584.22        | 0.002           |

The limit of detection was calculated by the equation (1), where: SD\(_{\text{blank}}\) is the standard deviation for the signal recorded on the blank for the element studied, conc\(_{\text{sample}}\) is the concentration [\(\mu\)g/l] of the analyte in the sample, I\(_{\text{sample}}\), I\(_{\text{blank}}\) are the signal intensities recorded for the sample and blank respectively.

\[
LOD = 3 \cdot SD_{\text{blank}} \cdot \text{conc}_{\text{sample}} / \left[ I_{\text{sample}} - I_{\text{blank}} \right]
\] (1)
The limit of quantification is the lowest concentration that can be quantitatively determined with an acceptable level of repeatability accuracy. The quantification limit is generally considered to be approximately ten times the minimum detection limit. In order to verify that, three standard solution of 0.01, 0.05, 0.1 µg/l As, Cr, Ni, Hg, Pb, Cd were prepared and aspirated in the ICP-MS inlet system.

The maximum measurement limit is conditioned by the dynamics of the spectrometer detectors and limited by the requirement that the total amount of the dissolved solid must not exceed 0.2% in the sample solution (unless clogging of the nebulizer nozzels would lead to instabilities and loss of sensitivity). To test the maximum measurement limit, a slightly alcoholic sample of 2 mg/l was prepared, of which there were determinations for the elements taken into account, using calibration solutions of 0.5, 1, 2.5, 5 mg/l.

Repeatability was obtained measuring the same sample, with the same method, in the same laboratory, with the same equipment, by the same operator, in short intervals of time. To determine the repeatability, the concentration of six metals in the synthetic sample (2 µg/l) described above was determined. The obtained standard deviation was in the range 0.09-0.5 µg/l. Intermediate repeatability was obtained measuring the same sample, with the same sample, in the same laboratory, but by different operators and in different days. Standard deviation was found to lie between 0.05-0.12 µg/l.

Accuracy expresses the correlation between the arithmetic mean of the measured values and the accepted reference value. Considering the “true” concentration of 2 µg/l, the bias (%) was calculated for the elements taken into consideration (0.8-17 %).

Uncertainty estimation. All the possible sources of uncertainty have to be carefully identified and taken into account. While measuring the concentrations by ICP-MS with external standard, fluctuations in the measurement of ionic currents occurring as a result of the electrical noise in the detector, instabilities in plasma discharge, instabilities of the electrical parameters of the analyzer, lead to uncertainties in the determination of the parameters of the calibration line. Possible errors in the preparation of the calibration solutions increase these uncertainties. Let us assume that the calibration line has the form: \( y = ax + b \), where \( y \) is the signal intensity and \( x \) is the concentration of the calibration solution. Using the equations (2) and (3), the standard deviation of the parameters \( a \), \( b \) and the standard deviation unknown concentration were determined (\( n \) is the number of points that determine the calibration curve).

\[
\sigma_a = \sqrt{\frac{n \sum x^2 - (\sum x)^2}{n(n-1)}} \sigma_y
\]
\[
\sigma_b = \sqrt{\frac{\sum x^2 - (\sum x)^2}{n(n-1)}} \sigma_y
\]
\[
\sigma_y = \sqrt{\frac{\sum (y-b-ax)^2}{n}}
\]
\[
\sigma_y = x \left( \frac{\sigma_y}{y-b} \right)^2 + \frac{\sigma_a^2}{a} \right) \right)^{\frac{1}{2}} + \frac{\sigma_b^2}{b} \right)^{\frac{1}{2}}
\]

| Metal | a (µg/L) | b (µg/L) | σa | σb | Y (µg/L) | X (µg/L) | σX (µg/L) | 2σX (µg/L) |
|-------|---------|---------|----|----|----------|---------|-----------|-----------|
| As    | 6255.48 | 26.85   | 86.07 | 387.95 | 12368.91 | 121.69 | 0.070 | 0.070 | 0.141 |
| Cd    | 2706.56 | -160.46 | 50.06 | 225.63 | 4987.42 | 82.85 | 1.902 | 0.055 | 0.191 |
| Hg    | 13950.70 | 946.40 | 274.47 | 1652.52 | 33925.86 | 213.74 | 2.364 | 0.128 | 0.256 |
| Pb    | 24703.50 | 595.31 | -16336.6 | 2683.05 | 54523.05 | 1275.57 | 2.183 | 1.449 | 2.897 |
| Ni    | 3476.13 | -290.32 | 61.00 | 273.07 | 6325.46 | 88.78 | 1.903 | 0.089 | 0.178 |
| Cr    | 8038.78 | 0.96 | 39.00 | 10.06 | 7890.32 | 81.43 | 1.998 | 0.023 | 0.046 |

* Expanded uncertainty (k = 2) represents interval that there is a probability of 95% to be the true value.
It is important to observe that the uncertainty estimation calculated in this manner should be associated with the concentration domain taken into account and with the analyst skill. Table 2 presents the uncertainty for As, Cd, Hg, Pb, Ni and Cr in the 2 µg/l concentration range by ICP-MS. The extended uncertainty is between 0.04-0.25 µg/l (except for lead).

4. Application of the method to wine samples
In an attempt to characterize Romanian wines from the point of view of their heavy metal content, 13 samples representing different wine assortments were studied. Thirty metals were identified with concentrations ranging from 0.02 µg/l to 6 mg/l. Light metals: Na, Mg, K, Ca were the largest share, showing concentrations of the order of mg/l (ppm). Some metals considered to be micronutrients (Cu, Zn) were found in concentration of the order of several µg/l. Heavy metals with high toxicity like Pb, Cd, As showed concentrations below the limits imposed by OIV (table 3).

Table 3. Metals content of some Romanian wines.

| Vineyard       | Assortment of wine       | Type of wine     | As  | Cd  | Hg  | Pb  | Ni  | Cr  |
|----------------|--------------------------|------------------|-----|-----|-----|-----|-----|-----|
| Cotnari, Moldova | Feteasca Alba 2005       | semi-sweet white | 3.26| 1.42| 81.14| 21.74| 31.32| 73.68|
|                | Selected wine 2005       | semi-sweet white | 7.66| 1.22| 200  | 1.98 | 7.66 | 400.50|
|                | Tamăioasa romaneasca 2008| sweet white      | 9.44| 0.44| <LOD | 28.30| 17.92| 203.04|
|                | Tamăioasa romaneasca 2005| sweet white      | 0.29| 0.40| <LOD | 16.80| 16.80| 99.74 |
|                | Bussiuaca de Moldova 2003| sweet white      | 2.08| 0.38| <LOD | 13.44| 34.46| 70.90 |
|                | Muscat Ottonel 2004      | semi-sweet white | 12.2| 0.84| 53.94| 24.16| 78.02| 144.42|
| Recas          | Burgund Mare, Frankokva  | semi-sweet red   | 5.26| 0.28| 10.4 | 9.82 | 93.94| 46.38 |
|                | Rosu Dulce               | sweet red        | 6.30| 0.28| <LOD | 14.76| 14.76| 66.02 |
| Prahova        | Vinoi Casei Riesling 2007| dry white        | 4.80| 0.28| <LOD | 38.58| 35.36| 244.64|
|                | Cabernet Sauvignon 2007  | dry red          | 0.96| 0.08| <LOD | 10.48| 37.32| 81.64 |
|                | Cabernet Sauvignon 2007  | dry red          | 10.64| 0.38| <LOD | 22.74| 69.94| 210.64|
| Murgatlar      | Dry Muscat 2006          | semi-dry white   | 5.90| 0.64| <LOD | 47.38| 68.58| 102.04|
|                | Pinot Noir 2007          | semi-sweet red   | 3.42| 0.72| 0.643| 15.82| 15.82| 128.88|

5. Conclusion
In this study a method of quantitative analysis for the determination of toxic metals (Cr, As, Cd, Ni, Hg, Pb) in wines and slightly alcoholic beverages by ICP-MS was validated and applied. Several statistical parameters have been taken into account and evaluated for the validation of method: the limit of detection ranged between 0.002-0.03 for the six metals studied ensures the limit of quantification required for quantitative determinations of the concentrations of these elements in slightly alcoholic beverages; good linearity (correlation factor 0.9998 ≥ R ≥ 0.9994) for most elements recommend the method described for determination at trace and ultra-trace level; maximum limit of measurement is limited by the requirement that the total amount of solid dissolved in the sample solution must not exceed 0.2%. The uncertainty estimation takes into account the uncertainty influence in determining the parameters of the calibration curve; values determined lies between 0.04-0.25 mg/l. The results obtained for some Romanian wines are in agreement with the values reported in literature by other authors [9, 10]. Wide variability in the concentration of metals at trace level suggests the possibility to correlate it with the origin of wine.

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