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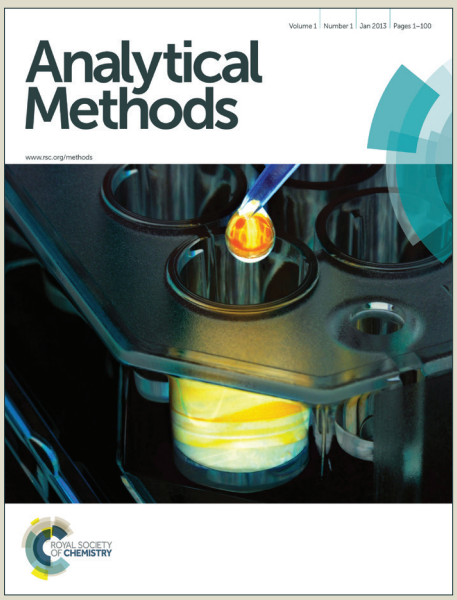

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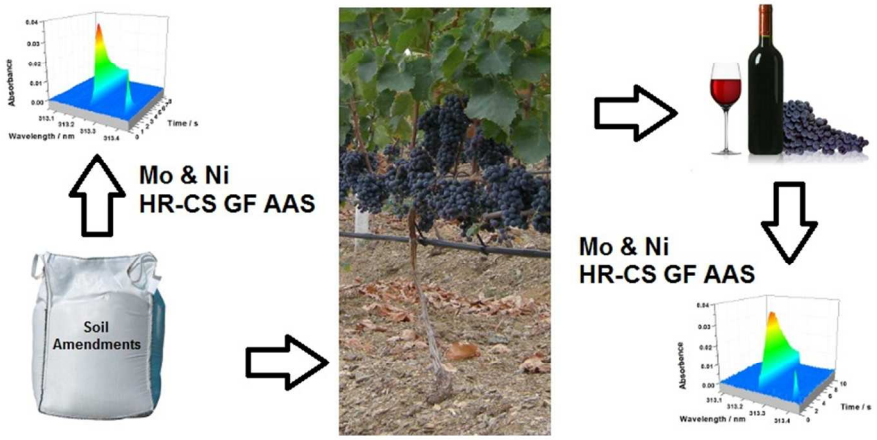

Mo and Ni have been determined simultaneously in wine and soil amendment samples
80x39mm (300 x 300 DPI)

Simultaneous determination of Mo and Ni in wine and soil amendments by HR-CS GF AAS

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Abstract

The introduction of high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GF AAS) with a linear charge coupled device (CCD) array detector, made possible the simultaneous determination of more than one element. In this work, HR-CS GF AAS has been used for the simultaneous determination of Mo (313.259 nm) and Ni (313.410 nm). Two methods have been developed: direct solid sample analysis was used for soil amendments, and direct sample injection for wine samples. For both methods a pyrolysis temperature of 1200 ºC and an atomization temperature of 2650 ºC were used. Aqueous standard solutions were used for calibration; the linear correlation coefficient was higher than 0.997 for both analytes. Detection limits of 0.05 and 0.8 μg L^{-1} for wine samples and 0.04 and 0.60 mg kg^{-1} for soil amendments were found for Mo and Ni, respectively. To investigate the accuracy of the developed method, digested and undigested wine samples were evaluated with spike recovery values between 94 and 106%. For solid samples, three CRM were evaluated, and the values found for Mo were not significantly different from the certified ones; however, the values for Ni were always too high. It has been found that this was due to a direct line overlap of the Ni with an Fe line. This effect was overcome by determining Fe using the unresolved analytical line doublet at 312.565 / 312.568 nm, and subtracting this value from the total concentration (Ni+Fe) determined at 313.410 nm. This interference was not observed in wine samples because of their low Fe concentration.

1. Introduction

Wine is known as an alcoholic beverage since the beginning of civilization, and nowadays it is widely consumed all around the world.[1] Most of this growth is due to improvements in some technical parameters, such as: grape variety, soil control, winemaking practices and storage.[2] Some countries classify their products according to the geographical production origin. In this classification, one of the main interests is using the mineral content to characterize the wines, taking into account the relationship between the mineral content in the samples and in the soil. This differentiation can be carried out using major and trace elements.[3–7]

Molybdenum (Mo) and nickel (Ni) contribute on the physiological mechanisms in plants. The most important effects of these metals are in the nitrogen metabolism and as enzymatic catalysts [8-10]. In Brazil, wines grow on a wide range of soils; the vast majority of crops are made in soils with some lack of nutrients, making necessary corrections, so that the plants are able to express their full yield potential.[11] Soil amendments, such as fertilizers and limestone, can be used to supplement the natural availability of minerals, such as Mo and Ni, compensating for their low natural concentration in soils.[12,13] However, these amendments might contain metals that represent a risk to human health if present at high concentration.[14,15]

The literature reports that the intake of high concentrations of Mo results in severe gastrointestinal irritation and death due to cardiac failure. Its excess may also cause bone deformation, teeth vulnerable to caries and disturbances in the metabolism of fat and proteins.[16,17] Molybdenum plays also an important role for the enzymatic redox reactions. It is a component of an enzyme in the human liver that oxidizes the potentially toxic sulfite ion (present in wine as a preservative compound [2,18]) to a non-toxic sulfate.[19] Nickel is considered a non-essential element for humans, but it has some potential harmful health effects. Specific responses of the human body to Ni are found in the respiratory tract and the skin. Elevated Ni concentrations can be toxic for animals as well as for humans and plants.[20]

Spectrometric techniques are widely used to determine the mineral composition of wine and soil amendments.[14,15,21] Graphite furnace atomic absorption

spectrometry (GF AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are used for trace and ultra-trace element determinations.[22–24] Flame atomic absorption spectrometry (FAAS) and inductively coupled plasma optical emission spectrometry (ICP OES) are applied for the determination of minor and major elements.[25,26] Each technique has advantages and disadvantages: ICP-MS is the most sensitive, and a multi-elemental technique, but requires sample digestion, unless electrothermal vaporization or laser ablation is used, and is relatively expensive; traditional GF AAS is a one-element-at-a-time technique, but enables the direct analysis of solid samples without significant additional effort.

With the introduction of equipment for high-resolution continuum source atomic absorption spectrometry (HR-CS AAS) with a charge coupled device (CCD) array detector, several limitations of classical line source AAS could be overcome, particularly those associated with spectral interferences.[27] In addition, this technique makes possible the simultaneous evaluation of several absorption lines, thus permitting multi-element determinations to be carried out. However, this potential is rather limited as the currently available instrumentation only allows the simultaneous monitoring of a rather small portion of the spectrum (0.2–0.3 nm in the UV region).[28] This means that simultaneous determinations can only be performed when two analytical lines are close enough to fall within the spectral window and the elements have similar thermal behavior.

Another advantage of HR-CS AAS is that the spectral neighborhood of the analytical line becomes visible at high-resolution, so that spectral interferences can usually be detected easily and corrected using a least squares algorithm (least squares background correction, LSBC)[29] if they cannot be avoided by an appropriate temperature program. The literature reports several examples about the use of LSBC to correct for interferences due to diatomic molecules.[29-31]

The LSBC may also be used to correct for the direct overlap of atomic lines, but only if the interfering element has another atomic line within the spectral window reaching the detector. Welz et al.[32] described the well-known interference of Fe (213.859 nm) on Zn (213.856 nm) determination, and its correction using another Fe line that is close enough (213.970 nm) to be monitored simultaneously, and therefore LSBC can be applied successfully.

Based on the fact that Mo and Ni have nearby analytical lines and a similar thermal behavior, the main objective of this work was the development of two analytical methods for the simultaneous determination of Mo and Ni in wine samples and in soil amendments and investigate potential spectral interferences using high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GF AAS). For fertilizer and limestone samples, direct solid sample analysis was used, while the wine samples were analyzed directly without sample preparation using graphite tubes without platform. Digested and spiked wine samples have been analyzed for comparison.

2. Experimental

2.1 Instrumentation

A Model contrAA 700 high-resolution continuum source flame and graphite furnace atomic absorption spectrometer (Analytik Jena, Jena, Germany) equipped with a xenon short-arc lamp with a nominal power of 300 W, operating in a hot-spot mode, was used throughout. This instrument features a double monochromator with a linear CCD array detector with 588 pixels, 200 of which are used for analytical purposes, while the others are used for internal corrections. The primary resonance line at 313.259 nm has been used for the determination of Mo and the secondary line at 313.410 nm for the determination of Ni. The spectral resolution at these lines is about 1.85 pm per pixel. The measurements were made using the center pixel (CP) and two side pixels (CP±1) for both elements, corresponding to a spectral interval of 5.6 pm; however, the entire spectral range ±0.37 nm around the analytical line was displayed by the 200 pixels that were used for analytical purposes. Peak volume selected absorbance (PVSA),[33] i.e., the integrated absorbance (A_{int}) summated over three pixels around the line core (CP±1), was used for signal evaluation and quantification for both elements.

The transversely heated graphite tube atomizer, which is part of the contrAA 700, was used for all measurements with pyrolytically coated solid sampling (SS) graphite tubes (Analytik Jena Part No. 407-A81.303), SS graphite platforms (Analytik Jena Part No. 407-152.023) or pyrolytically coated standard tubes without platform, but

with a dosing hole (Analytik Jena Part No. 407-A81.011) for the analysis of liquid samples. For the analysis of soil amendment samples an M2P micro-balance (Sartorius, Göttingen, Germany) was used for weighing the samples directly onto the SS platforms. The mass was automatically transmitted to the computer of the instrument to calculate the integrated absorbance normalized to a sample mass of 0.10 mg after each measurement. This is necessary as it is impossible to always introduce exactly the same sample mass in direct SS analysis. A pre-adjusted pair of tweezers, which is part of the SSA 6 manual solid sampling accessory (Analytik Jena), was used to transfer the SS platforms to the atomizer. An MPE 60 furnace autosampler (Analytik Jena) was used for the introduction of the wine samples into the atomizer.

Argon with a purity of 99.996% (White Martins, São Paulo, Brazil) was used as purge and protective gas. A microwave oven Model Microwave 3000 (Anton Paar, Graz, Austria), equipped with an eight-vessel rotor and microwave-operated UV lamps, was used for the digestion of wine samples.

2.2 Reagents and solutions

Analytical grade reagents were used throughout. Distilled and deionized water (DDW) with a specific resistivity of 18 MΩ cm, from a Milli-Q water purification system (Millipore, Bedford, MA, USA), was used for the preparation of the standard solutions. The nitric acid (Merck, Darmstadt, Germany) used to prepare the aqueous calibration solutions was further purified by sub-boiling distillation in a quartz sub-boiling still (Kürner Analysentechnik, Rosenheim, Germany). All containers and glassware were soaked in 1.4 mol L^{-1} nitric acid for at least 24 h and rinsed three times with DDW before use.

The Mo and Ni stock standard solutions (1000 mg L^{-1} in 0.014 mol L^{-1} nitric acid) were from Specsol (Jacareí, São Paulo, Brazil). The working calibration solutions were prepared by serial dilutions of the stock solutions in 0.014 mol L^{-1} HNO_3. The following chemical modifiers have been used: $NH_4H_2PO_4$, 100 ± 2 g L^{-1} in H_2O, Pd modifier stock solution, 10.0 ± 0.2 g L^{-1} in 15% (v/v) HNO_3, Mg modifier stock solution, 10.0 ±0.2 g L^{-1} $Mg(NO_3)_2$ in 15% (v/v) HNO_3 and NH_4NO_3 extra pure; all reagents and solutions were from Merck.

2.3 Samples and sample preparation

2.3.1 Soil amendment samples

In this work, N:P:K fertilizer samples with 4:14:8 and 10:10:10 of weight percentage, a limestone sample with 23.1% K_2O, 11.3% Mg, 22.5% S and a certified reference material (CRM) NIST SRM 695, Trace Elements in Multi-Nutrient Fertilizer (National Institute of Standards and Technology, USA) were used. The fertilizer and limestone samples were acquired at local agricultural stores. Two more CRM were used to check the developed method, MESS-2 Marine Sediment - (National Research Council, Canada) and SA-A, Sandy Soil (High Purity Standards, Charleston, SC, USA).

Sample pre-treatment included only milling of the samples in three cycles of 20 min each in a vibrating ball mill (Fritsch, Idar-Oberstein, Germany) with stainless steel mortar and balls. After that, the samples were passed through a 45 µm sieve and placed into an oven at 50 °C up to constant weight. The dried samples were kept in a desiccator until they were analyzed. The sample mass introduced into the graphite furnace was between 0.08 mg and 0.15 mg.

A 0.05% (v/v) Triton X-100 (Sigma, St. Louis, MO, USA) was added to the modifier solutions in order to ensure a homogeneous distribution of the chemical modifier over the solid samples. The modifier solution was added manually with a micropipette before inserting the platform into the graphite tube, followed by the heating program presented in Table 1.

2.3.2 Wine Samples

Wine samples were obtained from five different regions of Brazil: Oeste do Paraná (OPR), Vale do São Francisco (VSF), Serra Catarinense (SCa), Serra Gaúcha (SGa) and Campanha Gaúcha (CGa). All wine samples were from the same grape variety, *Cabernet sauvignon*.

The wine samples were introduced into the graphite furnace directly, i.e., without any dilution or pre-treatment. To increase the analyte mass in the graphite tube, multiple

injections were performed resulting in a final volume of 90 μL. For that, a volume of 30 μL has been injected, followed by the three drying stages (Table 2); before the pyrolysis stage the sequence has been interrupted and a new sample aliquot injected. This procedure has been repeated until the final volume was reached. The multiple injection sequence is controlled by the software of the equipment.

Due to the lack of a certified reference material for wine samples, recovery tests were used to verify the accuracy of the method. The samples, with and without the addition of the analytes, were submitted to a microwave- and UV-assisted digestion. A closed vessel system was used in the digestion procedure, with the addition of 5.0 mL of sample, 2.0 mL of 30% H_2O_2, 1.0 mL of 65% HNO_3 and 3.0 mL of DDW. The UV lamps were inserted in the quartz vessels and the following program was used: step1 — power: 900 W, ramp time: 10 min and ventilation: 127 m^3 h^{-1}; step 2 — power: 900 W, hold time: 20 min and ventilation: 127 m^3 h^{-1}; step 3 — power: 0 W and ventilation: 190 m^3 h^{-1} (cooling). After digestion and cooling, the resulting solutions were transferred to 15 mL volumetric flasks, and the volume completed with DDW. As a dilution was realized in this stage, the final volume injected into the atomizer was increased to 120 μL to obtain similar introduced analyte masses as with the direct sampling.

3. Results and discussion

3.1 Analysis of wine samples

3.1.1 Temperature program and chemical modifiers

The initial experiments were carried out using the conditions recommended by the manufacturer of the instrument, which are a pyrolysis temperature (T_p) of 1200 ºC, an atomization temperature (T_{at}) of 2600 ºC and the use of no modifier. The first results with wine samples indicated that there might be a spectral interference at the Mo and Ni lines. As it was not possible to separate that interference in time using an appropriate temperature program, the effect of different chemical modifiers has been evaluated. The 'universal modifier' Pd-Mg, Pd and Mg individually and NH_4NO_3 were investigated and the interference could not be eliminated; however, when the $NH_4H_2PO_4$ modifier was used the interference was completely removed and a more

symmetric peak was obtained, as shown in Figure 1. Based on this fact, a study of the modifier mass was performed for a wine sample and a Mo and Ni standard solution, varying the modifier mass between 0.5 and 2.0 mg. It was observed that the amount of modifier inserted into the furnace did not cause any significant increase of the integrated absorbance signal (A_{int}) and a modifier mass of 0.5 mg was sufficient to eliminate completely the spectral interference. This modifier mass was therefore employed in all further experiments.

Figure 2 shows the pyrolysis curves for standard solutions with 0.5 ng Mo and 6.0 ng Ni, respectively, and a wine sample. These curves were obtained using 0.5 mg $NH_4H_2PO_4$ and a T_{at} of 2600 ºC for Mo and Ni.

The pyrolysis curves obtained for molybdenum (Figure 2a) show a similar behavior for the standard solution and the wine sample. The A_{int} remains stable throughout the pyrolysis temperature range evaluated. The pyrolysis curves obtained for nickel (Figure 2b) exhibited a significant decrease of the A_{int} for the standard solution above 1300 ºC. This was not observed for the wine sample, however, the A_{int} for Ni decreased slowly throughout the investigated temperature range. Temperatures lower than 800 ºC and higher than 1600 ºC were not investigated as in the first case a spectral interference appeared, and in the second case the A_{int} for Ni decreased significantly. Putting together the information obtained with the pyrolysis curves for Mo and Ni it is clear that the A_{int} for Ni is more affected by temperature increases than that for Mo, which is according to expectation. For this reason, the T_p for all future measurements with the $NH_4H_2PO_4$ modifier was fixed at a compromise temperature of 1200 °C.

Figure 3 shows the atomization curves for a standard solution with 0.5 ng Mo and 6.0 ng Ni and a wine sample. Molybdenum (Figure 3a) shows a significant increase of A_{int} with temperature for both, the aqueous standard and the wine sample, which is again according to expectation. However, at temperatures higher than 2650 ºC the useful lifetime of the graphite tubes decreases significantly and it is also recommended by the instrument manufacturer that the temperature should not exceed this value. Nickel (Figure 3b), in contrast, presented a stable A_{int} throughout the temperature range investigated, for the standard solution and for the wine sample. Finally, based also on the well-defined absorbance signals for both

elements, the T_{at} was fixed at 2650 ºC for all further measurements. The final temperature program for the simultaneous determination of Mo and Ni in wine samples is shown in Table 2.

3.1.2 Figures of merit and recovery tests

The calibration curves have been established using a blank and five calibration solutions in the concentration range of 5 – 100 µg L^{-1} Mo (0.05 – 1.0 ng Mo) and of 75 – 1800 µg L^{-1} Ni (0.75 – 18 ng Ni). The figures of merit for the simultaneous determination of Mo and Ni using HR-CS GF AAS are presented in Table 3. The blank measurements were carried out according to the "zero mass response" with only the modifier solution injected into the graphite tube.[34] The instrumental limits of detection (LOD) were calculated as three times the standard deviation of 10 measurements of a blank, divided by the slope of the calibration curve. The limits of quantification (LOQ) are based on the same measurements using 10 times the standard deviation of the blank readings. LOD and LOQ were calculated considering an injection volume of 90 µL. The characteristic mass (m_0) is defined as the mass of analyte corresponding to an integrated absorbance of 0.0044 s.

The values of LOD and m_0 for Mo were compared with those reported in the literature. Calvo et al.[35] determined Mo in human urine by GF AAS and direct sampling, evaluating different modifiers; the LOD and m_0 values were 0.2 µg L^{-1} and 14.1 pg, respectively. Baralkiewicz et al.[36] determined Mo in water samples using multiple injections without any sample pre-treatment and modifier and the LOD obtained was 0.07 µg L^{-1}. These values are comparable with those obtained in this work. The use of the Ni line at 313.410 nm is not reported in the literature. Although the LOD and m_0 values obtained for Ni using this line are relatively high, they are adequate for the determination of Ni in wine samples.

To check the trueness of the developed method for the determination of Mo and Ni in wine, samples were spiked by adding appropriate aliquots of 1000 mg L^{-1} stock standard solution to obtain spikes with 7.5 µg L^{-1} Mo and 100 µg L^{-1} Ni, respectively. The spiked samples were analyzed directly, without any pre-treatment, and also after microwave-assisted acid digestion. Recoveries of the analytes added to wine

samples varied between 94% and 106% for direct analysis and for digested samples, with a relative standard deviation of less than 5% for direct sampling and less than 10% for digested samples, which might be considered appropriate for the purpose of this work. The values obtained with the two methods were not significantly different according to a t-test at a 95% confidence level.

3.1.3 Determination of Mo and Ni in wine samples

Based on the results of the recovery test and the figures of merit, the method was considered adequate for the determination of Mo and Ni in red wine samples. Five different samples were analyzed and the results are shown in Table 4. According to Brazilian legislation,[37] the maximum allowed concentration of Ni in wine is 0.1 mg L^{-1}; there is no value specified for Mo; however, the European Food Safety Authority (EFSA)[38] proposed that the adequate intake of Mo for adults is 65 µg per day. The results obtained for Ni in wine samples are in agreement with the Brazilian regulations, and for Mo, as suggested by EFSA, wine can contribute for the daily consumption.

The mineral content in wine is of importance for its characterization and classification according to the geographical origin. The developed method can be applied with precision and accuracy for this purpose. However, the values for some elements might be affected by the use of soil amendments, such as fertilizer and limestone, so that the analysis of these materials has been included in the present work.

3.2. Analysis of soil amendments

3.2.1. Temperature program and chemical modifiers

Initially, three different soil amendments, two fertilizers and one limestone sample, were evaluated using the conditions recommended by the manufacturer for the direct analysis of solid samples, i.e., a T_p of 1200 ºC, a T_{at} of 2600 ºC and no modifier . The time-resolved absorbance spectrum of the fertilizer N:P:K -4:14:8, which is shown in Figure 4a, exhibited a spectral interference, which had the same pattern as that found in the wine samples. Therefore, the effect of the chemical modifier $NH_4H_2PO_4$

in Triton medium was evaluated and, as shown in Figure 4b, the spectral interference was eliminated.

A study was conducted to optimize the mass of the chemical modifier. The A_{int} values for Mo and Ni in the standard solution and in the fertilizer sample were not significantly affected by different masses of $NH_4H_2PO_4$, except for a slight decrease in sensitivity for masses higher than 0.15 mg. This is significantly lower than the value found for the wine samples, which might be due to the high natural phosphate content of the fertilizer. More important than the influence of the chemical modifier on the sensitivity is its contribution to remove the spectral interference. A mass of 0.15 mg $NH_4H_2PO_4$ was chosen and its influence on the absorbance signal at the three analytical pixels of each element is shown in Figures 5 and 6. The interference has only a small contribution on the A_{int} values for Mo, but the A_{int} values for Ni are significantly affected by the interference, mainly at the CP-1 pixel. The action of the modifier in removing the spectral interference provided more symmetric and better defined peaks for both analytes.

The influence of the sample mass introduced into the furnace on the integrated absorbance of Mo and Ni has also been investigated and is shown in Figure 7. For masses up to 0.2 mg the A_{int} values for Mo increase linearly, but for higher masses the value of A_{int} starts to deviate from linearity. For Ni the ratio between sample mass and A_{int} remains linear through the entire mass range evaluated; this difference is most likely due to the much lower A_{int} values obtained for Ni compared to Mo. So, for further experiments a sample mass around 0.1 mg was used.

As fertilizers have a matrix that is significantly different from that of wine samples, pyrolysis curves were established for the N:P:K - 4:14:8 fertilizer and Mo and Ni standard solution. This was also considered necessary as a different type of graphite tube and platform, and also a different modifier mass has been used. Figures 8a and 8b show that the standard solution for Mo and Ni present a small declination in the A_{int} values with increasing temperature for both analytes. Figure 8a also shows high values for the A_{int} of Mo at T_p lower than 1200 ºC, because the spectral interference is not completely eliminated, even using the $NH_4H_2PO_4$ modifier. Above 1200 ºC the interference is eliminated and the Mo signal is not affected anymore. The A_{int} values for Ni in aqueous standard solutions were declining more rapidly for T_p > 1200 º C, whereas the A_{int} for the fertilizer sample did not decrease up to 1600 ºC. It is obvious

that this additional stabilizing effect is due to a matrix compound that has not been removed yet. It is actually wrong to assume that the complete removal of the matrix in the pyrolysis stage is a precondition for an interference-free determination of the analyte. There are many examples in the literature that, particularly in direct solid sample analysis, the matrix can have a stabilizing effect for the analyte; and there are even more examples that show that aqueous standards can be used for the determination of trace elements using direct solid sample analysis in a graphite furnace.[39] For all further measurements 1200 ºC was chosen as the best T_p as no interference was observed above this temperature.

The atomization curves are shown in Figure 9; they exhibit a behavior very similar to that for the wine samples for both analytes. Molybdenum reached the maximum A_{int} values at a T_{at} of 2650 ºC, whereas the A_{int} values for nickel remain essentially stable over the temperature range evaluated. For further experiments the T_{at} has been fixed at 2650 ºC. The final temperature program for determination of Mo and Ni in soil amendments is shown in Table 1.

3.2.2. Figures of merit

The calibration curves have been established using a blank and five calibration solutions in the concentration range of 5 – 100 µg L^{-1} Mo (0.05 – 1.0 ng Mo) and of 75 – 1800 µg L^{-1} Ni (0.75 – 18 ng Ni).The figures of merit for the simultaneous determination of Mo and Ni using HR-CS GF AAS are presented in Table 5. The limits of detection (LOD) and quantification (LOQ) have been calculated for a sample mass of 0.10 mg as three and ten times, respectively, the standard deviation of ten measurements of the blank, divided by the slope of the calibration curve. The characteristic mass (m_0) is defined as the mass of analyte corresponding to an A_{int} of 0.0044 s.

The values found in this work for Mo are comparable or better than published values. Shaltout et al.[40] determined Mo in dust samples by HR-CS GF AAS without a modifier using direct solid sample analysis; they reported LOD and m_0 values of 50 µg kg^{-1} and 28 pg, respectively. Rello et al.[41] determined Mo in dried urine by HR-CS GF AAS using Pt as modifier and direct solid sample analysis; they found a LOD of 1.5 µg L^{-1}. No report was found for the determination of Ni at 313.410 nm.

3.2.3. Sample and CRM analysis

To check the trueness of the developed method for the determination of Mo and Ni in soil amendment samples, first the NIST SRM 695 Trace Elements in Multi-Nutrient Fertilizer was analyzed. As shown in Table 6, the result for Mo was in agreement with the certified value with 95% of confidence according to a *Student* t-test; however, the value found for Ni was significantly higher than that reported for the CRM. Therefore two more CRM, a sediment and a soil, were analyzed; the results are also presented in Table 6. Again, the values found for Mo were in agreement with the certified or informed value, but the values for Ni were higher than the certified ones.

The most probable explanation for these too high results is the presence of some spectral interference at the analytical line of Ni. We found in the literature[42] that iron has an absorption line at 313.411 nm, which is practically overlapping with the analytical line of Ni used in the proposed method. Hence, the contribution of Fe to the absorbance at the Ni signal was investigated. In all the analyzed CRM the Fe concentration was relatively high; in the NIST SRM 695 the certified Fe concentration is 40 g kg^{-1}, in MESS-2 sediment it is 69.9 g kg^{-1}, and in SA-A soil it is 9.27 g kg^{-1}. A standard solution with 100 mg L^{-1} Fe (1000 ng) was evaluated at the 313.410 nm line, and it was possible to observe that there was a contribution of Fe on this wavelength. No reference about this iron interference on Ni was found in the literature for this wavelength.

In order to correct for this interference we were looking for another iron line of similar sensitivity, and the unresolved doublet at 312.565 / 312.568 nm was found to be most appropriate. The A_{int} values for both iron lines were very similar, with a ratio ($A_{int(313.410\ nm)}/A_{int(312.565/312.568\ nm)}$) of 1.13 between their integrated absorbance values. Using the sensitivity ratio as a correction factor and the A_{int} at 312.565/312.568 nm, the iron contribution at 313.410 nm could be subtracted. After that correction the NIST SRM 695 was re-evaluated and the result of 134±8 mg kg^{-1} was now in agreement with the certified value (Table 6) with 95% of confidence using a *Student* t-test.

It was found that only Fe concentrations above 50 mg L^{-1} (500 ng) contributed to a significant increase of the A_{int} value of Ni at 313.410 nm. As the wine samples

showed no iron concentration that could have any contribution to the A_{int} values at 313.410 nm, no correction for this interference was necessary.

As the above results prove that the Fe interference on Ni could be efficiently corrected, the developed method could be applied to determine Mo and Ni in soil amendment samples. All samples were evaluated at both Fe lines and the corrected results for two fertilizers and one limestone sample are shown in Table 7. Considering that all three soil amendment samples analyzed in this work presented a significant content of Mo and Ni, they might have an influence on the soil composition, i.e., more Mo and Ni can be bioavailable for the plant metabolism. Consequently, for a more precise information about the geographical production origin, the soil of some fertilized regions should be investigated, in which could be the topic of some future work.

4. Conclusion

A method has been developed for the simultaneous determination of Mo and Ni in two distinctly different kind of samples, wine using direct sampling without any sample preparation, and soil amendments using direct solid sample analysis. The fact that the same temperature program and calibration against aqueous standard solutions could be used for both types of samples demonstrates one more time the robustness of HR-CS GF AAS. The spectral interference of Fe at the 313.410 nm Ni line was not described in the literature up to now, but it could be corrected measuring the Fe absorption at the 312.565 / 312.568 nm doublet and subtracting the corresponding contribution of Fe at the 313.410 nm nm Ni line. Although the number of applications for the simultaneous determination of two elements using HR-CS GF AAS is limited due to the relatively narrow spectral window that reaches the detector, the present work offers one more example, and there might be more, which can increase the sample throughput and economize measurement time and consumption of graphite parts.

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Figure Captions

Figure 1: Time-resolved absorbance spectra for Mo and Ni in 90 µL of a wine sample; a) without modifier; b) with 0.5 mg $NH_4H_2PO_4$; T_p = 1200 °C; T_{at} = 2600 °C.

Figure 2: Pyrolysis curves for a) ● 0.5 ng Mo in aqueous solution and ■ a wine sample; b) ● 6.0 ng Ni in aqueous solution and ■ a wine sample, using a mass of 0.5 mg of $NH_4H_2PO_4$; T_{at} = 2600 °C.

Figure 3: Atomization curves for a) ● standard solution with 0.5 ng Mo and ■ Mo in a wine sample; b) ● standard solution with 6.0 ng Ni and ■ Ni in a wine sample using a mass of 0.5 mg $NH_4H_2PO_4$; T_p = 1200 °C.

Figure 4: Time-resolved absorbance spectra for Mo and Ni for a N:P:K - 4:14:8 fertilizer; a) without modifier; b) with 0.15 mg $NH_4H_2PO_4$ and 0.05% Triton-X; T_p = 1200 °C, T_{at} = 2600 °C.

Figure 5: Absorbance profile of Mo in the N:P:K – 4:14:8 fertilizer at the three analytical pixels; black line - without modifier, sample mass 0.117 mg, A_{int} = 0.3674 s; red line - with 0.15 mg $NH_4H_2PO_4$ and 0.05% Triton-X; sample mass 0.100 mg, A_{int} = 0.3182 s. T_p = 1200 °C; T_{at} = 2600 °C.

Figure 6: Absorbance profile of Ni in the N:P:K – 4:14:8 fertilizer at the three analytical pixels; black line - without modifier, sample mass 0.117 mg, A_{int} = 0.0761 s; red line - with 0.15 mg $NH_4H_2PO_4$ and 0.05% Triton-X; sample mass 0.100 mg, A_{int} = 0.0429 s; T_p = 1200 °C; T_{at} = 2600 °C.

Figure 7: Dependence of the integrated absorbance signal from different N:P:K – 4:14:8 fertilizer masses introduced into the graphite furnace with 0.15 mg $NH_4H_2PO_4$ and 0.05% Triton-X. a) Mo; b) Ni. T_p = 1200°C; T_{at} = 2600 °C.

Figure 8: Pyrolysis curves for a) ● 0.5 ng Mo in aqueous solution and ■ N:P:K - 4:14:8 fertilizer; b) ● 6.0 ng Ni in aqueous solution and ■ N:P:K - 4:14:8 fertilizer; the integrated absorbance values for the solid samples are normalized for a sample mass of 0.1 mg; 0.15 mg $NH_4H_2PO_4$ and 0.05% Triton-X was used as the modifier; T_{at} = 2600 °C.

Figure 9: Atomization curves for a) ● 0.5 ng Mo in aqueous solution and ■ N:P:K - 4:14:8 fertilizer; b) ● 6.0 ng Ni in aqueous solution and ■ N:P:K - 4:14:8 fertilizer; the integrated absorbance values for the solid samples are normalized for a sample

mass of 0.1 mg; 0.15 mg $NH_4H_2PO_4$ and 0.05% Triton-X was used as modifier; T_p = 1200 °C.

Table 1: Graphite furnace heating program for the simultaneous determination of molybdenum and nickel in soil amendment samples, using HR-CS GF AAS.

Stage	Temperature °C	Ramp °C s^{-1}	Hold time s	Gas flow rate L min^{-1}
Drying 1	90	10	20	2
Drying 2	110	15	20	2
Pyrolysis	1200	300	20	2
Atomization	2650	3000	10	0
Cleaning	2650	0	5	2

Table 2: Graphite furnace heating program for the simultaneous determination of molybdenum and nickel in wine samples, using HR-CS GF AAS.

Stage	Temperature °C	Ramp °C s^{-1}	Hold time s	Gas flow rate L min^{-1}
Drying 1	90	10	30	2
Drying 2	100	5	20	2
Drying 3	120	5	10	2
Pyrolysis	1200	300	20	2
Atomization	2650	3000	8	0
Cleaning	2650	0	5	2

Table 3: Figures of merit for the simultaneous determination of Mo and Ni in wine using HR-CS GF AAS, T_p = 1200ºC, T_{at} = 2650ºC.

Parameters	Mo	Ni
m_o(pg)	8.3	189
LOD (pg / µg L^{-1})*	4.2 / 0.05	73 / 0.81
LOQ (pg /µg L^{-1})*	14 / 0.16	240 / 2.7
Linear regression equation	y=0.0034+0.515m (ng)	y=0.0024+0.022m (ng)
R	0.9995	0.9984

*Total injected volume: 90 µL.

Table 4: Values of Mo and Ni determined in five different red wine samples by HR-CS GF AAS; n = 3 measurements ± standard deviation.

Wine samples	Mo (μg L^{-1})	Ni (μg L^{-1})
Oeste do Paraná (OPR)	9.26 ± 0.12	8.8 ± 0.1
Vale do São Francisco (VSF)	2.10 ± 0.07	26.1 ± 0.8
Serra Catarinense (SCa)	2.36 ± 0.03	13.2 ± 0.3
Serra Gaúcha (SGa)	0.87 ± 0.02	12.8 ± 0.3
Campanha Gaúcha (CGa)	2.35 ± 0.08	7.7 ± 0.4

Table 5: Figures of merit for the simultaneous determination of Mo and Ni in soil amendment samples using HR-CS GF AAS, T_p: 1200 ºC, T_{at}: 2650 ºC.

Parameters	Mo	Ni
m_o(pg)	7.0	136
LOD (pg / µg kg^{-1})*	3.8 / 38	60 / 600
LOQ (pg / µg kg^{-1})*	11 / 110	200 / 2000
Linear regression equation	y=0.0027+0.5639m (ng)	y=0.0013+0.03145m (ng)
R	0.9969	0.9993

*LOD and LOQ calculated for 0.10 mg of sample for both analytes

Table 6: Results obtained for Mo and Ni in certified reference materials using the developed method.

CRM		Mo (mg kg^{-1})	Ni (mg kg^{-1})
NIST SRM 695	Certified	20.0±0.3	135±2
	Found	19.7±1.8	190±20
MESS-2	Certified	2.85±0.12	49±2
	Found	2.88±0.09	90±7
SA-A	Certified	1.0±0.1	3.3±0.3
	Found	0.92±0.03	20±2

Table 7: Determination of Mo and Ni in three mineral fertilizers by HR-CS GF AAS using direct solid sample analysis and calibration against aqueous standard solutions.

Samples	Mo(mg kg^{-1})	Ni(mg kg^{-1})
N:P:K - 04:14:08	6.9±0.4	14.7±0.7
N:P:K - 10:10:10	5.6±0.1	7.0±0.1
Limestone	1.00±0.05	6.5±0.1

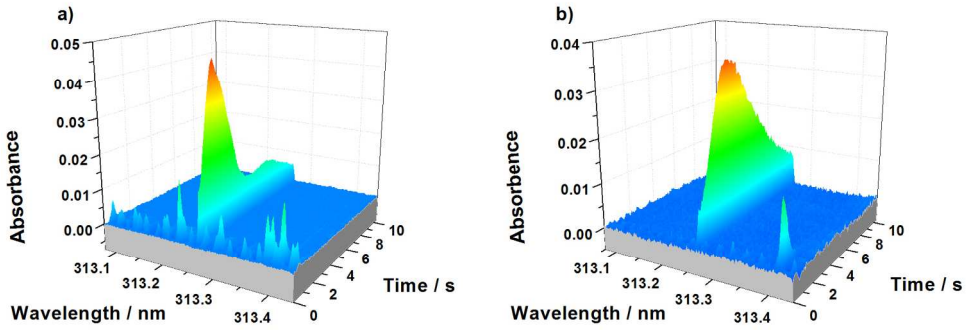


Figure 1: Time-resolved absorbance spectra for Mo and Ni in 90 µL of a wine sample; a) without modifier; b) with 0.5 mg NH4H2PO4; Tp = 1200 °C; Tat = 2600 °C.
170x60mm (300 x 300 DPI)

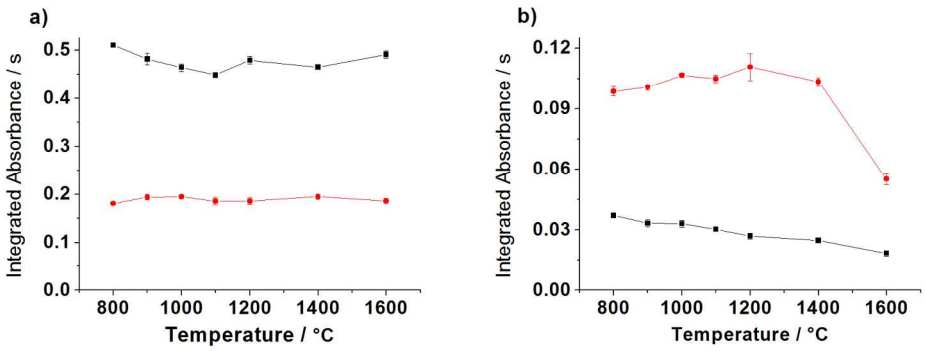


Figure 2: Pyrolysis curves for a) □ 0.5 ng Mo in aqueous solution and ■ a wine sample; b) □ 6.0 ng Ni in aqueous solution and ■ a wine sample, using a mass of 0.5 mg of NH4H2PO4; Tat = 2600 °C.
170x60mm (300 x 300 DPI)

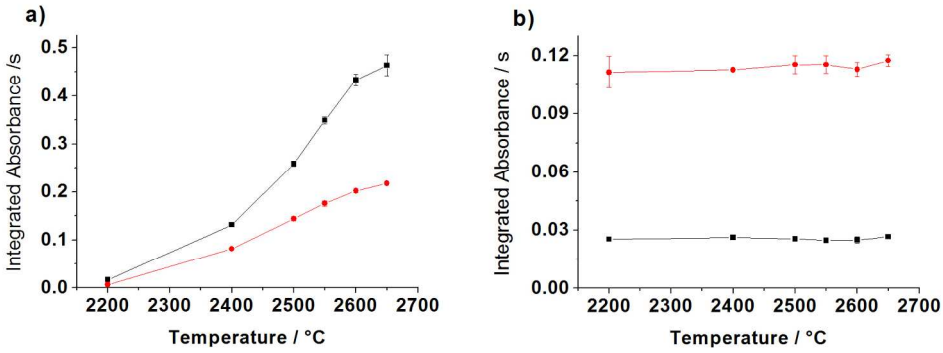


Figure 3: Atomization curves for a) □ standard solution with 0.5 ng Mo and ■ Mo in a wine sample; b) □ standard solution with 6.0 ng Ni and ■ Ni in a wine sample using a mass of 0.5 mg NH4H2PO4; Tp = 1200 °C.
170x60mm (300 x 300 DPI)

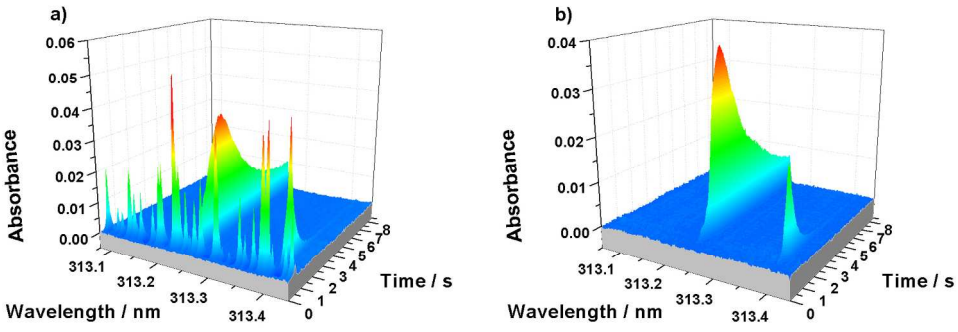
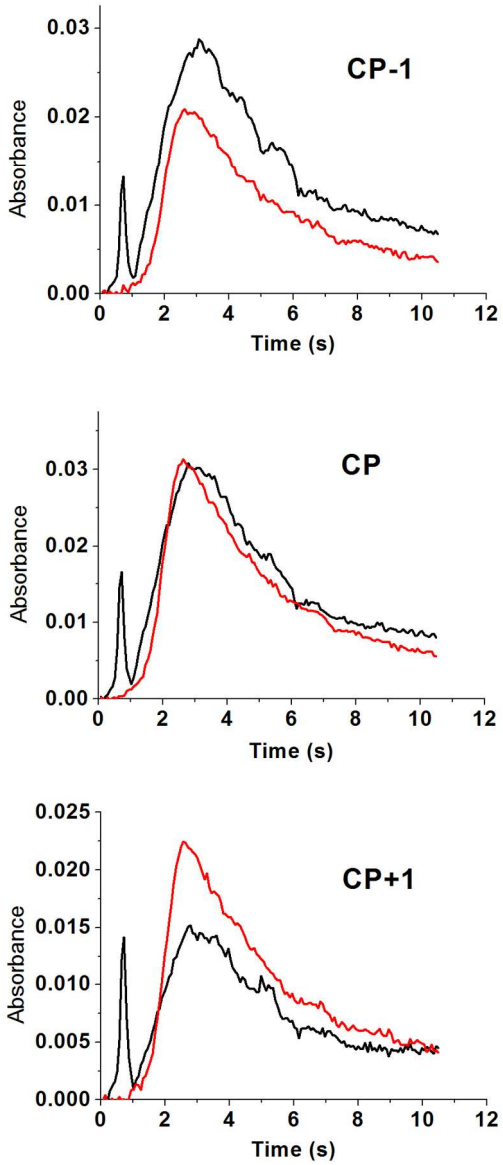


Figure 4: Time-resolved absorbance spectra for Mo and Ni for a N:P:K - 4:14:8 fertilizer; a) without modifier; b) with 0.15 mg $NH_4H_2PO_4$ and 0.05% Triton-X; Tp = 1200 °C, Tat = 2600 °C.
170x60mm (300 x 300 DPI)



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80x180mm (300 x 300 DPI)

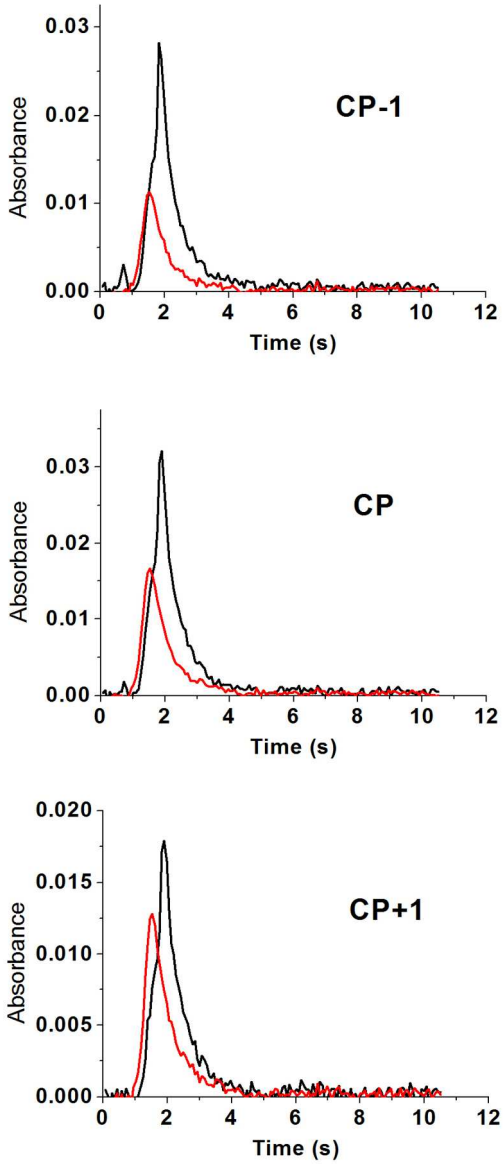
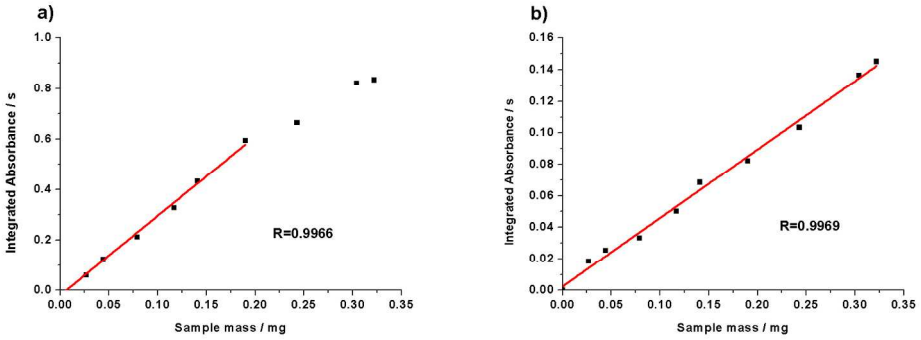


Figure 6: Absorbance profile of Ni in the N:P:K – 4:14:8 fertilizer at the three analytical pixels; without modifier, sample mass 0.117 mg, Aint = 0.0761 s; with 0.15 mg NH4H2PO4 and 0.05% Triton-X; sample mass 0.100 mg, Aint = 0.0429 s; Tp = 1200 °C; Tat = 2600 °C.
80x180mm (300 x 300 DPI)



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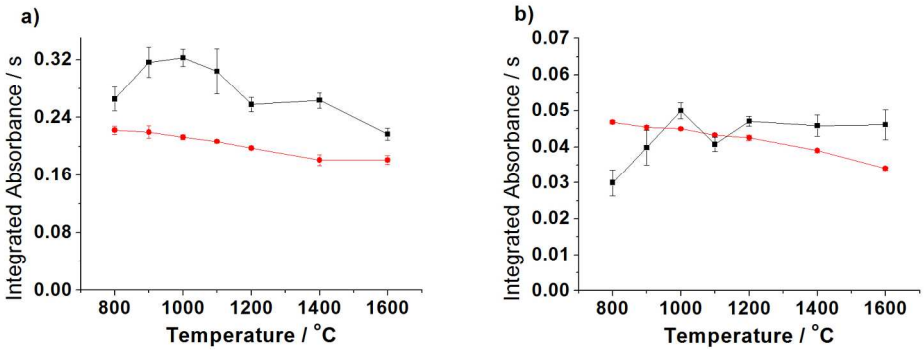

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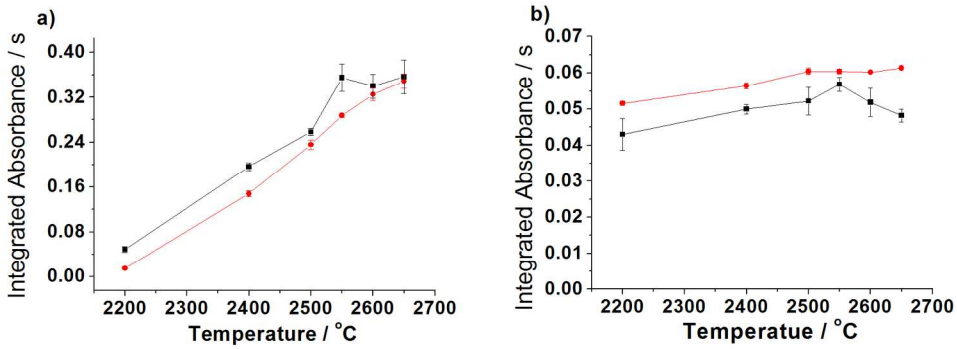

170x60mm (300 x 300 DPI)