

ETAAS DETERMINATION OF SOME TRACE ELEMENTS IN WINE**T. STAFILOV^A, J. CVETKOVIĆ^B, S. ARPADJAN^C, I. KARADJOVA^C**^a Institute of Chemistry, Faculty of Science, Sts. Cyril and Methodius University, POB 162, 1000 Skopje, Macedonia; e-mail: trajcest@iunona.pmf.ukim.edu.mk^b Institute of Agriculture, Bull. A. Makedonski bb., 1000 Skopje, Macedonia^c Faculty of Chemistry, University of Sofia, 1126 Sofia, Bulgaria**Abstracts**

Methods for trace elements determination in wine by electrothermal atomic absorption spectrometry (ETAAS) directly in untreated wines and in previously decomposed wines are given. The investigations on the atomization behaviour of the microelements in wine, on the reasons for strong wine matrix interferences, on the accuracy of the calibration procedures are presented. Appropriate procedures for preliminary separation and preconcentration of ultra trace elements from wine matrix are compared and discussed. Optimal analytical methods for direct ETAAS determination of As, Cd, Cr, Ni, Pb and extraction ETAAS determination of Se, Tl are developed and validated. Results for the content of trace elements in Macedonian and Bulgarian wines are presented.

Key words: trace elements, wine, determination, electrothermal atomic absorption spectrometry

1. Introduction

The analysis of trace metals in wines is of great importance for quality control of wine, authenticity control of wine, wine metals toxicity, bioavailability etc. These elements might be essential or toxic in the human body, they can influence the wine making process or they can change the wine taste and quality. The presence of trace elements in wine is the consequence of the atmospheric deposition of airborne particulate matter on grapes and/or of the intake of these elements by the grapevine from ground water and soil, pesticides and fertilizers [1]. Trace elements in wine samples can be measured by different techniques: stripping voltammetry [2-9], instrumental neutron activation analysis [10], ICP-AES, ICP-MS [11-16] or UV-Vis spectrophotometry [17-20].

However official methods for the determination of heavy metals in wine produced by the Office International de la Vigne et du Vin and the American Society of Enologists are essentially based on atomic absorption spectrometry (AAS). Some elements with relatively high concentration in wine could be analyzed by flame atomic absorption spectrometry (FAAS) [21-37]. But for many elements present in wine with low concentration, hydride generation techniques with FAAS (HG-AAS) [38-44] or mostly electrothermal atomic absorption spectrometry (ETAAS) [45-77] is used. For some elements direct determination (or using simple dilution) of trace elements in wine by ETAAS was applied [50,53,58-64,73]. However, very often some additional operations in the preparation of wine samples are necessary because of matrix interferences [51-53,59,64,66,72,73]. In order to avoid these interferences and because of the very low concentration of trace elements in the samples investigated, it is necessary to use matrix modifier [45,48,55,56,65-69] or to separate and concentrate these elements [45,46,77]. To reach this goal, different methods could be employed: extraction, ion exchange, precipitation, etc.

In this work, the possibilities of electrothermal atomic absorption spectrometry for the determination of As, Cd, Cr, Ni, Pb, Se and Tl in different wines (white, red and rose) are evaluated. Optimal instrumental parameters (temperature programs, modifiers, atomizers) for direct ETAAS determination of As, Cd, Cr, Ni, Pb and extraction ETAAS determination of Se and Tl are presented. Suitable calibration procedures are proposed. The accuracy, precision

and selectivity of the developed methods were evaluated. Comparative analysis by using different analytical procedures and atomic absorption instruments were used as validation procedure. Results for the content of trace elements in Macedonian and Bulgarian wines are presented.

2. Experimental

2.1. Instrumentation

The electrothermal atomic absorption measurements were carried out on a Perkin-Elmer Zeeman 3030 spectrometer coupled with an HGA-600 atomizer and on a Varian SpectraAA 880 spectrometer coupled with GTA-100. The light sources were hollow cathode lamps for As, Cd, Cr, Pb, Ni, Se and Tl (Varian) and hollow cathode lamps for Cr, Ni, Pb and Tl (Perkin-Elmer) and electrodeless lamps for Cd and Se (Perkin-Elmer), the spectral bandpass and the wavelengths used were as recommended by Perkin-Elmer and Varian. Uncoated graphite tubes and uncoated graphite tubes with pyrolytic platforms (Perkin-Elmer) and with centre fixed platforms (Varian) were used as atomizers. Solutions (10 μ l) were introduced manually into the graphite furnace by using Eppendorff pipettes for the Perkin-Elmer instrument and by using autosampler for SpectraAA 880. The graphite furnace operating parameters are presented in Table 1. Only peak areas were used for quantification.

Table 1. Optimal experimental conditions for ETAAS determination of As, Cd, Cr, Ni, Pb, Se and Tl in wine

| Element | Pretreatment temperature (°C) | | Atomization temperature (°C) | | Atomizer | Modifier | Sample pretreatment | Calibration |
|------------------------------|-------------------------------|--------|------------------------------|--------|----------------------|---|--------------------------|----------------------------------|
| | D ₂ | Zeeman | D ₂ | Zeeman | | | | |
| As white wine red wine | 1400 1400 | - - | 2600 2600 | - - | Platform Platform | 5 μ g Pd 5 μ g Pd | direct digestion | aqueous st.sol. standard add. |
| Cd | 300 | 400 | 1600 | 1000 | Platform | no modifier | direct | standard add. |
| Cr | 1300 | 1500 | 2600 | 2500 | Wall | no modifier | direct | aqueous st.sol |
| Ni | 800 | 1300 | 2400 | 2300 | Wall | no modifier | direct | aqueous st.sol |
| Pb | 400 | 700 | 2100 | 1500 | Platform | no modifier | direct | standard add. |
| Se | | 1400 | | 2400 | Platform | Ag ^{***} , Ni+Mg(NO ₃) ₂ | digestion, extraction | standard add. |
| Tl | 350 | 900 | 2400 | 1500 | Platform | Ag,* tartaric acid | digestion, extraction | standard add. |

* Varian SpectraAA 880; ** Perkin-Elmer Zeeman 3030; *** modifier for measurements by Perkin-Elmer

2.2. Reagents

All reagents and solvents were of analytical-reagent grade (E. Merck). Aqueous standard solutions were prepared by appropriate dilution of 1000 μ g ml⁻¹ stock solutions, which were made from Titrisol concentrates (Merck, Darmstadt, Germany). The matrix modifiers used were 10 % aqueous solution of tartaric acid, 10 g l⁻¹ Ag as silver nitrate, Ni+Mg(NO₃)₂ and 0.3 g l⁻¹ Pd as tetrachloroammonium palladate(II) (Fluka AG). Ammonium pyrrolidin-1-yl dithioformate [ammonium pyrrolidinedithiocarbamate (APDC)] as 2% aqueous solution was used. The organic solvent isobutylmethyl ketone (IBMK) was applied without additional purification. Doubly distilled water was used throughout.

3. Results and Discussion

3.1. Direct ETAAS determination of As, Cd, Pb, Cr and Ni in wine

As a first step the possibilities for the direct ETAAS determination of the studied elements is investigated. Analytical methods for the determination of As, Cd, Pb, Cr and Ni could be developed only after careful optimization of the instrumental parameters (temperature programs, modifiers, calibration procedures) since natural content of these elements in wines is above their characteristic masses. In order to obtain optimal temperature programs pretreatment and atomization curves (wall and platform atomization) were constructed for As, Cd, Pb, Cr and Ni in the presence of different wine samples - filtered and untreated wines, wines obtained after different treatment procedures and bottled wines obtained from the market. The optimal instrumental parameters obtained for both studied instruments are summarized in Table 1. Wine matrix thermally stabilizes Cd and Pb and no modifier is proposed for their determination. The thermal behavior of As was studied for spiked wines (50 ng/ml As as As(III)) only with SpectraAA 880. The most effective modifier ensuring highest loss-free pretreatment temperature in presence of wine is Pd (5 µg Pd).

The wine matrix interference on the atomization of As, Cd, Pb, Cr and Ni was evaluated through the ratio of the slopes of the calibration curves obtained in presence of wine (b_m) and for aqueous standard solutions (b_o). For Cr and Ni this ratio is 0.98 ± 0.03 . The strongest matrix interference was observed for As determination in red wines, the slope ratio was lower than 0.5 under optimal instrumental parameters for the both instruments. For white wine this ratio for As is 0.92 ± 0.05 , for Cd and Pb - 0.93 ± 0.06 . As can be expected platform atomization removes in some extent matrix interference in the analysis of As, Cd and Pb for all types of the investigated wines. But even in this case standard addition calibration should be used as calibration procedure. It should be mention that the behaviour of the standard addition is significantly different when it is previously prepared in the wine sample and when the addition is performed directly in the graphite furnace by using the possibilities of the autosampler. This behaviour is also significantly different for different types of wine and the depression is more pronounced for treated and bottled wines. Hence, the standard addition should be performed to each wine sample prior to injection into the graphite furnace.

3.2. ETAAS determination of As, Cd, Pb, Cr and Ni after wine digestion

Wine matrix digestion followed by ETAAS measurement was performed in order to check the accuracy of the results obtained for Cd, Cr, Pb and Ni and the possibilities to decrease the interferences in the analysis of As.

The experiments provided showed that a mixture of HNO₃ and H₂O₂ ensures complete and relatively fast wine matrix decomposition. Digestion procedure was performed in open vessel (bakers covered with watch glasses), closed system under reflux and microwave digestion system (Milestone). The mixture of HNO₃ and H₂O₂ plays a role of modifier for Cd and Pb and these elements are determined in absence of modifier. Palladium was used as modifier for As. The matrix interference was evaluated again through the ratio of the slopes of the calibration curves. As expected the digestion procedure equalizes the behavior of all types of the investigated wines. Still the matrix interferences in As determination in red wine are relatively high and the standard addition method should be used for calibration. For Cd, Cr Ni and Pb calibration against aqueous standards is possible. Parallel analysis performed for all studied elements in different wines showed very good agreement between direct ETAAS determination and ETAAS determination after digestion. The comparison of the results obtained with different digestion procedures confirmed the suggestion that there are no losses of volatile elements during the digestion in open vessel.

3.3. Extraction ETAAS determination of Se and Tl

The natural content of Se and Tl in wines is below the detection limits of these elements achieved by ETAAS, therefore a preconcentration step should be used for their determination. Liquid/liquid extraction was chosen as simple, fast and suitable for routine application method for separation and preconcentration of Se and Tl from wine samples. It is well known that only Se(IV) forms extractable dithiocarbamate complex in presence of 1 mol l⁻¹ HCl and both Tl(I) and Tl(III) form extractable dithiocarbamate complexes in wide pH range (pH 1 to pH 6). The degree of extraction of Se (IV) was studied directly from wine samples acidified to 1 mol l⁻¹ HCl and from digested wine samples evaporated to dryness and acidified to 1 mol l⁻¹ HCl. The degree of extraction directly from wine samples was lower than 30 %, but from digested wine samples it was in the range 95%-99% which can be used for Se determination. For thallium determination relatively higher volumes of wine samples should be used for preconcentration and the degree of extraction of the dithiocarbamate complexes of Tl(I) and Tl(III) is in the range of 65-68 % when extracted directly from wine and 75-80 % from digested wines. Therefore the extraction system KI-IBMK was investigated as another possibility for preconcentration of Tl. It was found that in presence of 0.5 mol l⁻¹ KI the extraction of both Tl(I) and Tl(III) into IBMK is quantitative. Unfortunately the degree of extraction directly from wines is only around 70 %, therefore extraction from digested wine samples (degree of extraction 95-97%) is proposed for routine determination of Tl in wines. It should be mentioned that thermal behaviour of organic species of Se and Tl extracted into IBMK is significantly different for both instruments studied. Silver is proposed as modifier for thermal stabilization of both Se extracted as dithiocarbamate complex and Tl extracted as iodide complex for Zeeman 3030. However for Varian the most appropriate modifier for Se stabilization in organic phase is Ni+Sr(NO₃)₂ and for Tl - tartaric acid which ensures higher loss-free pretreatment temperature with highest sensitivity of measurement. The optimal instrumental parameters are depicted also in Table 1.

3.4. Wine analysis

Different Macedonian and Bulgarian wines were analyzed for As, Cd, Cr, Ni, Pb, Se and Tl according to the analytical procedures developed. The results obtained as a mean range are presented in Table 2.

Table 2. Concentration range of As, Cd, Cr, Ni, Se and Tl in Macedonian and Bulgarian wines (in µg ml⁻¹)

| Element | Macedonian wines | | Bulgarian wines | | Other wines |
|---------|------------------|------------|-----------------|-------------|--------------|
| | White, n= 10 | Red, n= 16 | Red, n= 16 | White, n= 9 | |
| As | 6-10 | 8 - 12 | 8-12 | 5-9 | 3-20 |
| Cd | 0.1-6 | 0.1-4 | 0.1-0.4 | 0.07-0.3 | 0.25-0.7 |
| Cr | 9-31 | 10-30 | 10-30 | 12-20 | 30-60 |
| Ni | 9-18 | 10-40 | 10-40 | 8-15 | 30-50 |
| Pb | 50-250 | 50-250 | 30-90 | 20-70 | 30-100 |
| Se | 0.5-0.8 | 0.5-1.3 | 0.5-1.3 | 0.4-0.7 | 0.6-13 |
| Tl | 0.1-0.5 | 0.3-0.7 | 0.3-0.7 | 0.1-0.5 | not recorded |

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