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ATOMIC ABSORPTION SPECTROMETRY IN WINE ANALYSIS
– A REVIEW –

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This article reviews methods for the determination and identification of trace elements in wine by using atomic absorption spectrometry (AAS). Wine is one of the most widely consumed beverages and strict analytical control of trace elements content is required during the whole process of wine production from grape to the final product. Levels of trace elements in wine are important from both points of view: organoleptic – Fe, Cu, Mn and Zn concentrations are directly related to the destabilization and oxidative evolution of wines, and toxicological – toxic elements content should be under the allowable limit, wine identification. The identification of metals in wine is subject of increasing interest since complexation may reduce their toxicity and bioavailability. AAS is one of widely used methods for routine analytical control of wine quality recommended by the International Organization of Vine and Wine. Two main approaches – preliminary sample digestion and direct instrumental measurement combined with AAS for trace element determination in wines are reviewed and discussed. Procedures for various sample pretreatments, for trace element separation and preconcentration are presented. Advances in metal identification studies in wines based on AAS are presented.

Key words: wine; trace elements; determination; speciation; AAS

АТОМСКАТА АПСОРПЦИОНА СПЕКТРОМЕТРИЈА ВО АНАЛИЗАТА НА ВИНО
– ПРЕГЛЕД –

Во трудот е направен преглед на методите за определување и специјација на елементите застапени во траги во вино со примена на атомската апсорпциона спектрометрија (ААС). Виното претставува еден од најупотребуваните пијалаци и затоа е потребна добра аналитичка контрола на застапеноста на елементите во траги за време на целиот производен процес од грозје до финалниот производ. Нивото на застапеност на елементите во траги во виното е важно, пред сè поради неколку причини: органолептички – концентрациите на Fe, Cu, Mn и Zn се директно поврзани со дестабилизацијата и оксидативниот процес на виното, токсиколошки – содржината на токсичните елементи треба да биде под дозволените граници, како и поради идентификација на виното. Определувањето на хемиските форми на елементите во виното е исто така важно поради тоа што нивното комплексирање може да ја намали нивната токсичност и биорасположливост. ААС е еден од широко применуваните методи за рутинска аналитичка контрола на квалитетот на виното препорачан и од Меѓународната организација за лозарство и винарството. Во трудот е даден преглед и дискусија за два главни пристапа при определувањето на елементите во траги во вино со ААС: прелиминарното разложување на примероците и директното определување. Дадени се и постапките за различни претретмани на примероците, за сепарирање на елементите во траги и за нивно претконцентрирање. Презентирани се и предностите на определувањето на хемиските форми на елементите во вино со примена на ААС.

Клучни зборови: вино; елементи во траги; определување; специјација; ААС

INTRODUCTION

Wine is a natural product, widely consumed in the world with thousands of years of tradition. The chemical composition of wine is very complex: besides ethanol, sugars and organic acids, wine contains tannins, aromatic and coloring substances and microelements. The information about the quantitative concentration of various components of wine at all stages of winemaking allows viticulturists to control the process of obtaining high quality wine that posses a certain taste, bouquet, color, flavor and transparency [1].

Another point of view on the importance of wine analysis is that recent data suggest that beverages can significantly contribute to the total dietary intake of some trace elements with the possibility of influencing their levels in tissues and body fluids. Wine is among the beverages which contributes to increasing the total dietary intake of trace elements to an extend greater than 10 % [2]. Numerous studies have shown that a moderate consumption of wine, especially red, improves good health and longevity when it is combined with a balanced diet [3]. Daily consumption of wine in moderate quantities contributes significantly to the requirements of the human organism for essential elements (B, Co, Mn, Ni, Mo, Se, Zn), even though with elements like As, Pb, Cd which are well known as toxic. Beverages of different kinds have been investigated for their content of Pb, Cd, Ni, Cr, As and Hg [4]. About a ten times higher Pb content was found in wine than in most other beverages, so wine is the most significant source of Pb. Evidently strict analytical control of trace elements levels in wine is important to asses the dietary intake of essential as well as toxic elements for humans. The maximum acceptable limits for trace element contents in wine have been established by the International Organization of Vine and Wine (OIV) but national legislation concerning allowable limits of these elements exists in almost all countries.

Grape variety, processing method and even the year of vinification can have a dramatic impact on the organoleptic and visual characteristics of wines. Although it is not clear that trace elements in wine can substantially affect taste, their influence on sophisticated equilibrium between different compounds in wine matrix is well known. A plethora of substances and processes can affect the elemental composition of wine during production and packing. The most important factors that determine the metal content in wines are: (i) contribution from soil on which vineyards are located and capacity of grapes to take up mineral substances; (ii) contribution from various steps of the production cycle, from grape to the finished wine (treatments prior to grape-harvest, fermentation reactions, addition of compounds with various functions); (iii) contribution from wine processing equipment, conservation and bottling. Unless exposed to significant airborne pollution grapes accumulate small amounts of toxic metals by translocation from the roots or by direct contact with vineyard sprays. Investigations carried out on the migration of toxic elements in the system soil-grapevine-grape for polluted regions showed that most of the toxic elements in grapevine are mainly due to the toxic metal containing aerosols falling from the atmosphere [5]. However Orescanin et al. [6] detected V, Cr, Mn, Fe, Ni, Cu, Zn, As and Pb in soil, grape and wine and concluded that the main source of heavy metals in grapes is absorption from the soil. Almost the same conclusion was reached by Mackenzie et al. [7]. They found that soil cation chemistry does influence the wine grape composition. Trace elements are normally absorbed onto the yeast cell and are removed from the final product during the prefermentation clarification (a process of removal of substances that produce unwanted flavors, favor the fermentation to dryness and increase the fermentation rate) [8]. The toxic elements Cd and Pb are greatly eliminated by clarification [8]. In most cases their final elevated concentrations in wine result from contamination during post-fermentation processing, and sources include contact with nonstainless steel equipment and impurities in the fining agents and filter media [9, 10]. In a model investigation, ten different bentonites have been used for wine fining and as a result statistically significant increases of most elements were observed, but in significantly lower levels of Cu, K, Rb and Zn. The addition of yeast hulls caused a statistically significant depletion of the contents of Ce, Cu, Fe, La, Sb, U, V and Y [11]. Therefore it is clear that trace element composition of grapes and wines is influenced by the type of soil, wine processing equipment and vinification, but in very specific manner for different elements [12, 13].

TRACE ELEMENTS IN WINE

Potassium is a natural component of grape and its concentrations in wine reflects the levels in grapevine in the final stages of berry ripening. High K levels affect the stability of wine with respect to the potassium hydrogen L-(+)-tartarate precipitation.

Calcium is a natural component of wine although the concentration of calcium in wine can be affected by the traditional practices of deacidification ($CaCO_3$ addition) or plastering ($CaSO_4$ addition). Elevated calcium levels can lead in some wines to calcium L-(+)-tartarate precipitation. It should be pointed that total calcium content in wine is not informative enough to predict the stability of wine and data for the free metal concentration are required [14].

Aluminum is found in grape juice, but the concentration in both juice and wine is elevated because of the use of bentonite, and to a lesser extent from contact with aluminum surfaces. It has become apparent that aluminum is strongly complexed in wine which affects its bioavailability from one side and makes haze formation unlikely from the other side.

At low concentration *iron* plays an important role in metabolism and fermentation processes as an enzyme activator, solubilizer and functional component of proteins. Above trace levels, iron has other roles: altering redox system of the wine in favor of oxidation, participating in the formation of complexes with tannins and phosphates thus resulting in instabilities.

The same can be said for copper: in trace amounts is an important inorganic catalyst for metabolic activities of microorganisms; at high levels it plays an important role in catalyzing oxidation of wine polyphenols. It should be pointed out that copper and copper complexes are more active than iron and its complexes [14]. However for both elements copper-induced and iron-induced spoilage are not related to the total metal concentration. For copper, the free active metal concentration is important and for iron the valence state determines the potential for iron-induced oxidation.

Sources of *lead* in wine were inferred from systematic assay of grapes must and wine during winemaking. It was found that Pb concentration in fermenting must vary during vinification. Lead concentration increased significantly in open-top vessels, in holding bins, and during pressing. Juice and wine stored in concrete or waxed wood have significantly higher concentration of lead compared to juice and wine stored in stainless steel. Moreover fining with bentonite or filtering with diatomaceous earth contributes further to final Pb concentration, while fermentation, both primary and secondary, removed Pb [15]. In another study measurements of 7000 wines were used to identify possible sources of Pb in wine and these showed that atmospheric–related contamination (leaded gasoline) was not responsible for elevated Pb levels in wine. It was also shown that the presence or absence of tin-lead capsules as well as the stare of tin-lead capsule corrosion had only a very minor influence on the Pb concentration in wine. It was concluded that brass is the main contamination source for elevated Pb content in wine [16].

Cadmium levels have been determined during wine making in 21 locations in France. During the alcoholic fermentation Cd elimination is almost complete with losses between 87 to 100% [17].

An interesting study for statistical evaluation of aroma and metal content in Tokay wine answered the question – how qualitative and quantitative relations of volatile organic and metal components present in traditional wines depend on the vintage, the location on which it is grown, as well as the type of wine grape, and to what extent these are characteristics of wines of given type and vintage [18]. A study revealed the correlation between trace element content, total antioxidant capacity, total phenolic content, hystamine concentrations and fruit origin of wine [19]. Wines from Jordan have been characterized for pesticides and trace metals contents and it was deremined that heavy metals showed higher values in grapes than in wines which is attributed to the removal of solids during wine preparation processes [20]. The influence of copper application on the copper content in grape and wine from Italian wine-farms was studied during the harvest of 2003. It was concluded that copper content in grape depends more strongly on the total dose applied than on the number of applications, and that the copper residue level in wine does not depend on the quantity applied in the vineyard [21].

The influence of Fe, Cu and Mn on wine oxidation was studied and it was found that these three cations intervene ‘somehow’ the evolution of different compounds: anthocyanins, tannins, total phenol content and acetaldehyde which are sensitive to oxidation. Iron catalyzes acetaldehyde combination with phenolic compounds [22].

METHODS FOR TRACE ELEMENT DETERMINATION IN WINES BASED ON SAMPLE DIGESTION FOLLOWED BY AAS

The sample preparation step (e.g. preliminary digestion of wine sample) was included to destroy the organic matrix and/or to extract the metal ions bound in inorganic and organic complexes. In the wine industry dry ashing dates from very beginning of wine analysis: it involves the complete removal of organic matter, although volatilization losses at high temperatures are not always easy to assess and low recoveries have been observed at trace analytes levels [23]. Comparison between two mineralization methods - microwave (MW) digestion versus dry ashing for Pb determination in wines does not result in noticeable differences, but authors have been inclined to the microwave digestion due to the more reproducible results and considerable gain of time [24]. Acid wet digestion is the preferred pretreatment procedure, but reagent blanks for some elements are close to their natural contents in wine [25–33]. In some cases vanadium pentaoxide was added as a catalyst to improve completeness of sample digestion [34–36]. In order to prevent analyte losses, PTFE bombs [37] or Savillex vessel [25] have been used. As an alternative, microwave oven digestion offers advantages such as reduced losses due to volatilization, low reagents consumption, fast and complete matrix mineralization [2, 34, 38–46]. On-line MW sample digestion was used in flow injection HGAAS determination of Pb in wine [47]. Simple and very reliable sample preparation method in wine analysis is UV-photolysis which allows low blanks with minimal analyte losses [48, 49]. Wine sample digestion is unavoidable and highly recommended (OIV) procedure when HGAAS was applied in wine analysis [50, 51]. Complete digestion of wine organic matter was required in order to obtain accurate and reliable results. Flow-injection HGAAS with on line MW oxidation was used for Pb determination in wines [46, 52]; a mixture of HNO_3+$HClO_4$ has been proposed for wine digestion in thermostated vessel for Se determination by HGAAS [53]; MW digestion with HNO_3 was applied to Hg and Se determination in wines from Canary Islands [54]. An interesting approach was applied by Chuachuad et al. for Cd determination in wines by flow injection cold vapor AAS (CVAAS) [42, 55] and Pb determination by HGAAS [43] after wine MW digestion by mixture of HNO_3+H_2O_2. A volatile derivative was formed on passage of an acidified cadmium solution through a strong anion-exchange resin (Amberlite IRA-400) in the tetrahydridoborate(III) form and atomized in a quartz T-atomizer [42] or graphite furnace [55]. Strong anion-exchange resin (Amberlist A-26) in the tetrahydridoborate(III) form as reductant was used for Pb determination in wines in the presence of $K_3Fe(CN)_6$ [43]. Ozone treatment as wine pre-treatment procedure was applied for Hg determination in wine by CV AAS [56]. It is known that ethanol as main volatile component is a serious depressant in HGAAS and recently has been shown that simple ethanol evaporation is efficient for wine pre-treatment before As determination by HGAAS [28].

Although direct ETAAS is used for trace elements determination in wines, reliable results for elements like As and Sb cannot be obtained without preliminary wine digestion [26, 27, 57, 58]. Very strong matrix interferences leading to strong signal depression by 40–60 % have been observed in direct determinations, even in the presence of suitable modifier. It was suggested that wine organic matter as well as high phosphate and sulfate contents [57] are responsible for the observed interference. As far as phosphate and sulfate contents do not change after wine digestion, remarkable depression still exist and requires standard addition method to be used for calibration [27, 58]. Relatively low concentration of Pd and Ni modifiers has been recommended for efficient thermal stabilization of As, Sb [27] and Se [57] in wine digests. Complete wine decomposition in the presence of HNO_3+H_2O_2 in two different digestion systems (Tecator and Bethge) was achieved without any analyte losses before their ETAAS determination [58]. Recently Llobat-Estelles et al. [59] have shown that even for such "easy" element as Cu preliminary digestion of wine sample is preferable procedure ensuring accurate and reliable results.

Summary of the methods based on ETAAS together with detection limits (LOD) achieved are presented in Table 1. In Table 2, HG and CV methods combined with AAS and ETAAS are presented.

Table 1

The application of ETAAS in wine analysis

Element(s)	Sample pretreatment	Modifier(s)	LOD	Ref.
Al	Direct after dilution	No modifier	40 µg l^{-1}	60
Al	Direct (dilution)	$Na_2Cr_2O_7$	2.8 µg l^{-1}	61
Al	Direct after dilution	No modifier	1 µg l^{-1}	62
Al	Digestion with HNO_3+V_2O_5	$Mg(NO_3)_2$	0.4 µg l^{-1}	35
Al	Direct	No modifier	1.5 µg l^{-1}	63, 64
Al, Cd, Pb	Sample dilution with HNO3 (add surfactant, Triton X-100)	$NH_4H_2PO_4$	1.5 µg l^{-1}	65
Al	MW digestion/solid-phase extraction	No modifier	0.021 µg l^{-1}	66
Al	Dilution	No modifier	0.125 µg l^{-1}	67
Ag, Co, Si, Zn	Direct		0.20 µg l^{-1} Ag 1.6 µg l^{-1} Co 7.9 µg l^{-1} Si 21 µg l^{-1} Zn	68
As	Digestion with HNO_3+H_2O_2	Pd	5 µg l^{-1}	27
As	Digestion with HNO_3	Pd	6.6 µg l^{-1}	26
As, Sb	Direct/better after digestion	$Pd(NO_3)_2$	–	58
Cd	Digestion/HNO_3/V_2O_5		0.5 pg	37
Cd	Digestion with $HClO_4$ and HNO_3	–	0.008 µg l^{-1}	29
Cd	Direct	$Pd(NO_3)_2$+HNO_3	0.03 µg l^{-1}	69
Cd	Direct	Pd	0.08 µg l^{-1}	70
Cd, Pb	Dilution with HNO_3	$Pd(NO_3)_2$+$Mg(NO_3)_2$	0.03 µg l^{-1} Cd 0.8 µg l^{-1} Pb	36
Cd, Pb	Microwave digestion with HNO_3	$NH_4H_2PO_4$+$Mg(NO_3)_2$	0.1 µg l^{-1} Cd 1.0 µg l^{-1} Pb	40
Cd, Cr, Pb	Direct		0.5 µg l^{-1} Cd 1 µg l^{-1} Cr 3 µg l^{-1} Pb	71–73
Cd, Cr, Pb	MW digestion	$Pd(NO_3)_2$ for Cd and Pb $Mg(NO_3)_2$ for Cr		74
Cd, Co, Cr, Mn, Pb	MW digestion	Cd: $Pd(NO_3)_2$;; Co, Mn, and Cr: $Mg(NO_3)_2$; Pb: $Pd(NO_3)_2$+$NH_4H_2PO_4$		75
Cr	Direct	No modifier or Pd	0.1–1 µg l^{-1}	76–78
Cu	Direct	No modifier	5.75 µg l^{-1}	79
Cu, Fe, Mn	Direct	No modifier		80
Cu, Pb	Direct	No modifier		81, 82
Cu, Fe	Direct/dilution (1+9) with Milli-Q water	No modifier		83
Cu	Direct	$Pd(NO_3)_2$+ $Mg(NO_3)_2$	5.0 µg l^{-1}	84
Cu	Digestion with HNO_3/$HClO_4$	No modifier	30 µg l^{-1}	21
Cu	Microwave digestion with HNO_3/H_2O_2	No modifier		59
Hg	MW digestion and extraction with APDC into MIBK	Pd	0.2 µg l^{-1}	85
Ni	Direct	Pd	1.0 µg l^{-1}	86, 87
Pb	Dilution with HNO_3	Pd+Mg	15.5 µg l^{-1}	26
Pb	Direct after dilution	$Pd(NO_3)_2$+$Mg(NO_3)_2$	19 µg l^{-1} Pb	60
Pb	Interlaboratory study by using ETAAS			88
Pb	Direct	Pd+$Mg(NO_3)_2$	0.9 µg l^{-1}	89
Pb	Direct/dilution (1+4 v/v) Triton X-100	No modifier	Not present	90
Pb	Dilution with HNO_3	$NH_4H_2PO_4$+$Mg(NO_3)_2$	6.2 µg l^{-1}	91
Pb	Dilution with HNO_3	$NH_4H_2PO_4$	LOD 4 µg l^{-1} LOQ 14 µg l^{-1}	92
Pb	Digestion with HNO_3+UV photolysis	$NH_4H_2PO_4$+$Mg(NO_3)_2$	0.12 µg l^{-1}	93
Pb	Direct	$NH_4H_2PO_4$		94, 95
Pt	Direct and mineralization	–	10 µg L^{-1}	96
Se	Digestion with HNO_3+H_2O_2	$Ni(NO_3)_2$+$Sr(NO_3)_2$	1 µg l^{-1}	57
Se	Extraction with APDC into MIBK	Ag or $Ni(NO_3)_2$+$Sr(NO_3)_2$	0.2 µg l^{-1}	57, 97
Se	Direct	Pd + hydroxylamine hydrochloride	9 µg l^{-1}	98
Tl	Extraction from 0.5 mol l^{-1} KI solution into MIBK	Pd+ascorbic acid; Ag	0.05 µg l^{-1}	99
V	Direct		4.2 µg l^{-1}	100, 52

Table 2

HG and CV methods with AAS, ETAAS and AFS detection in wine analysis

Element(s)	Technique	Sample pretreatment	Reaction media	Reductant	LOD	Ref.
As(III), As(V) total As	HGAAS	Direct (ethanol evaporation) MW digestion	8 mol l^{-1} HCl	$NaBH_4$ (0.2% or 0.6% m/v)	0.1 µg l^{-1} As(III), As(V), total As	31
Cd	FI-CVAAS	Digestion	0.2 mol l^{-1} HNO_3; 1% *m/V* thiourea, 1 mg l^{-1} Co	Amberlite IRA-400/ tetrahydroborate(III) form	0.032 µg l^{-1}	43
Cd	FI-CV ETAAS	Digestion	0.2 mol l^{-1} HNO_3; 1% *m/V* thiourea, 1 mg l^{-1} Co	Amberlite IRA-400/ tetrahydroborate(III) form	0.09 µg l^{-1}	58
Hg	CVAAS	Digestion	1 mol l^{-1} HCl	$SnCl_2$ (20% m/v)	1.0 µg l^{-1}	42
Hg (white wines)	FI-CVAAS	Ozonation	1 mol l^{-1} HCl	$SnCl_2$ (20% m/v)	0.5 µg l^{-1}	59
Pb	FI-HGAAS	Direct	0.1 mol l^{-1} HNO_3 3% *m/V* $K[Fe(CN)_6]_3$	Amberlite IRA-400/ tetrahydrido-borate(III) form	3.1–5.2 µg l^{-1}	46
Pb	FI-HGAAS	Digestion	HNO_3+H_2O_2	$NaBH_4$ (6 % *m/V*)	10 µg l^{-1}	50
Pb	FI-HGAAS	Direct	HNO_3	H_2O_2	10 µg l^{-1}	55
Pb	HGAAS	Direct (dilution with HCl)	HCl H_2O_2 (7.5%)	$NaBH_4$ (21% *m/V*)	24 µg l^{-1}	101
Sb	HG-ETAAS	Direct (Pd modifier)	HCl+thiourea	$NaBH_4$ (1 % *m/V*)	0.13 µg l^{-1} Sb	102
Se	HGAAS	Digestion with HNO_3	7 mol l^{-1} HCl	$NaBH_4$ (0.6% *m/V*)	0.1 µg l^{-1} Se	42

DIRECT METHODS FOR TRACE ELEMENTS DETERMINATION IN WINE

Atomic Absorption Spectrometry in Flame, Electrothermal and Hydride generation modes is particularly suitable for direct determination of trace elements in wine. However wine is a complex matrix containing ethanol and other organic compounds which influence the transport properties of the sample toward atomization device due to the changes in viscosity and surface tension in comparison with aqueous standard solutions. Wine contains high concentrations of K, which acts as natural ionization buffer and should be taken into account in calibration procedures. Inorganic components in wine like sulphates and phosphates could interfere with the atomization of elements (FAAS) or cause strong background absorption due to radicals formed in ETAAS. FAAS is most widely used and easily accessible technique for the determination of Ag, Ca, Fe, K, Mn, Mg, Na and Zn in wines [31, 65, 103–105]. Conventional ionization buffers (CsCl) and ethanol are added to the calibration solution in order to obtain matrix-matched standard solutions and La(III) chloride is used as releasing agent to overcome phosphate atomization interferences in the determination of Ba, Ca, Mg and Sr. Sample dilution with HNO_3 is recommended for FAAS determination of transition metals Cu, Fe, Mn and Zn. In order to increase sample throughput, an automatic flow injection system based on zone sampling technique has been developed for the determination of Ca, K, Mg and Na in wines [106] as well as a flow injection system based on stream splitting for Cu determination in wines [107]. Direct application of HGAAS with quartz tube or quartz burner with Ar/H_2 flame as atomizers in wine analysis is limited because of drastic ethanol interference [28, 101, 108, 109]. It was shown recently that ethanol probably enters as an aerosol from gas/liquid separator into the atomizer, thus interfering with the atomization of hydrides [28, 108]. The magnitude of this interference strongly depends on the type of the atomizer used – it is not observed if hydride trapping in graphite furnace or inductively coupled plasma are employed as atomizers. This fact is well documented as successful direct determination of Sb in wine using HGAAS with hydride trapping into the graphite furnace was reported [102]. Sample dilution [101, 108] or flow injection mode [109] are proposed to overcome ethanol interference and to achieve accurate determination of As in wine samples. Recently sample matrix-assisted photo-induced chemical vapor generation has been proposed for ultrasensitive detection of Hg in wines [110]. Ethanol e.g. wine matrix component under UV-irradiation reduces mercury compounds or ions to atomic mercury thus playing a role of reductant for CVAAS determination of Hg. The application of direct hydride generation with different detectors is summarized in Table 2.

ETAAS permits determination of toxic trace elements in wine samples much below their permissible limits (OIV, national legislation) and therefore is widely used for wine quality control. The choice of efficient modifier for trace element thermal stabilization, optimal temperature program for the graphite furnace and suitable calibration method are the most popular topics of investigation. An advantage of ETAAS is the possibility to develop accurate direct methods for trace element determination in wine without any sample pretreatment. Expected matrix interferences are associated with wine organic matter which may cause high values of nonspecific absorption and ethanol content in wine sample which impairs sample delivery into the graphite furnace. Problems connected with reproducible sample injection are most frequently solved by injection into a preheated platform or graphite tube ('hot injection'), while sample sputtering is avoided by applying two-stage drying step [60]. The use of Zeeman background correction is preferable to overcome high nonspecific absorbance, thus greatly improving the accuracy of measurements. Stabilized temperature platform furnace (STPF) conditions should be fulfilled in order to obtain accurate and reliable results [79]. Aluminum levels in wine are high enough to permit high dilution factors to minimize matrix effects and allow for external calibration in assays. [63, 65, 67]. For port wine, however, a product with the most complex matrix which composition differs considerably from traditional table wines, potassium dichromate was proposed as modifier for Al determination together with end-capped Transverse Heated Graphite Atomizers (THGA$^{®}$) [61]. Trace elements (Ag, Co, Si, and Zn) were determined in port wine by ETAAS, and FAAS [68]. Cadmium and Pb are elements predominantly determined in wine samples by ETAAS moreover that ETAAS is an official method of analysis for Cd and Pd in wine by European regulations [71, 72, 111]. Typically sample dilution with HNO_3 is the only sample pretreatment and the chemical modifiers used for thermal stabilization of both elements in wine samples are $Pd(NO_3)_2$ [34, 69, 74, 89], $Pd(NO_3)_2$+$Mg(NO_3)_2$ [35, 77], $NH_4H_2PO_4$ [92, 94, 95], and $NH_4H_2PO_4$+$Mg(NO_3)_2$ [91]. Method of standard addition is frequently recommended as calibration procedure for Cd and Pb quantification in wines. An alternative approach is presented by Jorhem and Sundstrom [90]: Pb is determined in wine without any modifier by utilizing relatively low atomization temperature. It should be mentioned that the wine matrix contains by itself enough phosphate and Mg to act as a thermal stabilizer ("internal modifier"). Successful simultaneous determination of Cd and Pb in wines was reported in the presence of $Pd(NO_3)_2$ as modifier and by using two stage ashing to avoid formation of carbonaceous residue inside the atomizer [35]. Although it is not very typical for ETAAS, Bi as an internal standard has been proposed for Pb determination in wine [89]. The employment of internal standard could minimize absorbance variations due to changes in experimental conditions such as atomizer temperature, integration time, graphite tube surface, sample composition etc. Chromium levels in French wine and grapes and in Spanish wines were determined by direct ETAAS after careful optimization of temperature programs [76, 78]. Fast temperature programs with high sample throughput were developed for Cu determination in wines [84]. Useful models which permit the detection of possible sources of bias errors were applied to the determination of Cu in wine [59]. Manganese, Ni and V levels were defined in French wines and grapes from different regions and in Californian wines by using ETAAS [86, 100, 112]. Vanadium determination by ETAAS from the view point of matrix interferences and calibration procedures was discussed [49]. Selenium is an essential element, unfortunately present at very low levels in wine. Direct determinations are hampered by strong matrix interferences [57] and even by different behavior of both oxidation states [98].

Comparison of results obtained for trace elements content by ETAAS and ICP-AES with ultrasonic nebulization shows very good agreement [29]. Methods for direct trace element determination in wines by ETAAS are complied in Table 1.

TRACE ELEMENTS SEPARATION AND PRECONCENTRATION PRIOR TO WINE ANALYSIS

Separation and preconcentration procedures have been recommended for trace analytes determination in wines in cases when the concentration of elements are below the detection limits of instrumental method available in laboratory or strong matrix interferences restricted direct application of instrumental method. Liquid/liquid extraction is proposed for the determination of Se [57, 97], Tl [99] and Hg [33] due to their extremely low content in wine samples – typically less than 0.1 μg l^{-1}. Liquid/liquid extraction is usually combined with FAAS and ETAAS, most extraction systems are based on chelate extraction of dithiocarbamate or ion associate complex of the analyte. Solid phase extraction is more frequently used in wine analysis due to the possibility to achieve fast automatic analysis of trace elements and to combine with less expensive and easily available FAAS or spectrophotometry [113–115].

As expected, most papers describing Pb determination in wines applied flow injection analytical mode [8 30, 41, 116, 117]. A specially designed for Pb^{2+} imprinted polymer Pb-Spec allows direct determination of Pb in wine without any sample pretreatment and without any significant matrix interferences [39]. Automatic on-line sorbent extraction preconcentration system (diethylammonium-N,N-diethyldithiocarbamate complexes are collected in a column packed with bonded silica reversed-phase sorbent with octadecyl functional groups) combined with FAAS allows determination of Pb with sampling rate of 65 samples/hour and for Cu sampling rate is from 150–300 samples per hour [8]. Determination of free Pb^{2+} and total Pb after sample digestion could be peformed by using sorption of Pb on packed polyurethane foam column, modified by addition of 2-(2-benzothiazolylazo)-*p*-cresol [30]. The main idea of a series papers for trace element preconcentration from wine samples is sorption of analyte complexes with different reagents e.g. bathocuproinedisulfonic acid [44], dithizone [43], KSCN [44], on inert sorbents like Chromosorb 108, diaion HP-2MG or XAD-7 respectively. Recently column solid phase extraction procedure using rubeanic acid as complexing reagent and Sepabeads SP70 (divinylbenzene copolymer) as sorbent was proposed for Pb, Fe, Cd and Mn determination in MW digested wine samples [118]. A chelating resin consists of pyrocarechol violet immobilised on an Amberlite XAD-1180 support was used for Al preconcentartion from preliminary digested wines [66]. A natural sorbent modified rice husks was characterized and successfully applied for Cd and Pb determinations in wines [119]. Rice husks have been shown to be a homogeneous and stable adsorbent in which more than 100 preconcentration/elution cycles provide a relative standard deviation of less than 6 %.

FRACTIONATION AND SPECIATION OF TRACE ELEMENTS BY USING AAS

The understanding of the physicochemical forms under which a metal is present in wines deserves interest because complexation with wine organic matter may reduce their toxicity and their bioavailability for humans. It is recognized that the extent of the toxic effects caused by trace metals (As, Cd, Pb, Hg) is not governed by their total concentration but it is regulated by the forms of the metals that can efficiently interact with biologically active ligands [86]. It also well known that wine instability and haze formation depends on the exact chemical form of trace elements like Fe, Cu, Mn and Zn [22]. Wine is a very complex matrix and the accurate determination of exact chemical species of trace metals in wine is real analytical challenge. The possible physical form of trace elements (e.g. dissolved or suspended) can be determined by using filters of different pore size [120] and these results are ecologically very important because this colloid fraction destroys the quality of wine [120]. Analytical procedures based on flame and ETAAS spectrometry in combination with solid-phase or liquid-liquid extraction have been developed for Cu, Fe and Zn fractionation in wines [121–127]. Iron is one of the most widely investigated elements in wine. The efforts are concentrated on the determination of labile species of Fe(II) and Fe(III) as well as iron bounded to wine organic matter (wine polyphenols and proteins) and wine organic acids. Sequential cloud point extraction is used to differentiate between insoluble-suspended Fe and aqueous Fe [123]. The determination of labile Fe(II) and labile Fe(III) species in accordance with the redox processes in wines influenced by the pH-value, oxygen content and matrix constituents is very difficult. Most frequently solid phase extraction or liquid/liquid extraction is used for selective determination of Fe(II) or Fe(III) and the other form is calculated by the difference from the total Fe content. HPLC with AAS and electrochemical detection is applied for Fe speciation in wines (e.g. determination of Fe(II) and Fe(III) bound with wine organic acids) and it was found that both Fe species are in complex with tartaric acid. However less than 12 % of total Fe is found in this fraction, the rest could be bound to other organic compounds of wine [128]. A scheme was presented for fractionation of wine components (polyphenols, proteins polysaccharides) and Fe, Cu and Zn determination in different fractions [121]. The resin XAD-8 is used for the separation of wine polyphenols in complex with wine proteins and polysaccharides. Around 20–30 % of Fe, 30 % of Cu and 15 % of Zn are found in this fraction. Dowex ion exchange resins were used for the separation of cationic and anionic species of Cu, Fe and Zn. As a rule the concentration of labile Fe(II) is higher than the concentration of labile Fe(III). Less than 5 % of Cu and Fe are bound to wine polysaccharides and around 50 % of Cu and 60 % of Zn are presented in wines as positively charged labile species. The ability of plant polysaccharides to bind cations is due to the presence of a high proportion of negatively charged glycolsyl-residues. Their complexation capacities increase between pH 3 and pH 7 due to the dissociation of the carboxylic acid groups. The total capacity of pectic polysaccharides to complex metal ions is directly related to their degree of polymerization and their glycosyl-residue composition [127].

HGAAS is very suitable technique for speciation purposes due to different response obtained from different analyte chemical species. Selective hydride generation of different arsenic species (As(III), As(V), DMA, MMA) is achieved by using different reaction media, hence arsenic speciation in wine could be performed. Applying this approach it was shown that As(III) is major arsenic species in wines [28, 108]. Wifladt et al. [102] showed by using HGAAS that Sb(III) as well Sb(V) are present in wine samples.

Most important procedures recommended for trace element speciation are presented in Table 3.

Table 3

Speciation analysis of trace elements in wine

Element	Species	Separation procedure	Detection method	Ref.
As	As(III), As(V), MMA, DMA	Ion exchange, cation exchange resin AG 50 W-X8; anion exchange resin AG1-X8	HGAAS, 1.4% m/V $NaBH_4$	129
As	Total, As(III), As(V)	As(III), As(V): selective reaction media Total: wine MW digestion	HGAAS	28
Al, Ca, Cu, Fe, K, Na, Pb	Metals in real solutions, colloids or suspensions	Ultrafiltration through 0.2 and 0.45 μm membrane filters	FAAS, ETAAS	130
Cu, Pb	Total Cu and Pb;bioavailable Cu and Pb, complexed Cu and Pb	RP-HPLC, C_{18}218TP54 column, gradient elution 0–30% ethanol in 20 mmol L^{-1} KH_2PO_4, off line. Bioavailable fractions: gastrointestinal digestion	Total Pb: ETAAS; Total Cu: FAAS; Pb and Cu in dialysates: ETAAS Complexed Pb: SWCV Complexed Cu: potentiometry, ISE	81, 82
Cu, Fe, Zn	Fractionation	Fractions of Cu, Fe and Zn bound to polyphelons, proteins and polysaccharides. Labile species of Cu, Fe(II), Fe(III) and Zn.	FAAS ETAAS	121
Fe	Fe species	IC	FAAS	131
Fe	Total and Fe(III)	Fe(III):extraction of thiocyanate complex into MIBK, total Fe: FAAS	Sequential injection analysis by FAAS	122
Fe	Free and bounded Fe	Sequential cloud point extraction	FAAS	123
Fe	Fe(II), Fe(III), Organically bounded Fe	Liquid/liquid extractuion (thiocyanate, o-phenantroline) Column solid phase extraction	FAAS	124
Fe	Labile Fe(II) and Fe(III)	Solid phase extraction by using 1,10-phenantroline and 8-hydroxy-7-iodoquinoline-5-sulphonic acid	FAAS	125
Fe	Fe(III), total Fe	HPLC, Spherisorb S5 ODS 2 column, mobile phases: 50 mM CH_3COONH_4+CH_3OH (70+30 v/v) pH 4; CH_3COOSO_4/H_2SO_4 pH 2.5	Electrochemical Fe(II) FAAS	128

QUALITY ASSURANCE

Validation of developed analytical procedures including quality control of analytical results obtained is important characteristic presented or discussed in most of the papers dealing with wine analysis. It is well known that analysis of certified reference materials is the best way to confirm accuracy and reliability of analytical methods; however, reference wines with certified concentrations of minor, trace or ultratrace elements are not available [132]. That is way in common case added/found method has been used to establish the accuracy and precision of the analytical method developed. Another alternative widely used when direct method of analysis is tested is parallel determination of trace analytes by using previous wine sample digestion [28, 30, 36, 49, 57, 58, 71, 86, 109]. The compatibility of two methods (AAS and TXRF) was validated by parallel analysis of five samples for Fe and Cu and an agreement within the statistical uncertainty involved in both techniques was found [38]. Arsenic content determined by HG AAS or HG AFS is typically confirmed by ETAAS after wine sample digestion [28, 108]. In the frame of Comparison 16 of the International Measurement Evaluation Programme (IMEP) focused on the evaluation of measurement performance for the determination of the Pb mass fraction in a commercial red wine most widely used instrumental method was ETAAS, around 5% of results were obtained with ICP-MS and about 8% with ICP-AES) [133]. It was concluded that the results obtained using electrothermal atomic absorption spectrometry (ETAAS, recommended in EC Regulation 2676/90) were not significantly different from those obtained using other techniques.

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
APDC	Ammonium pyrolidinedithiocarbamate
CVAAS	Cold vapour atomic absorption spectrometry
DI	Direct injection
DMA	dimethylarsinate
ETAAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FI	Flow injection
ICP-AES	Inductively coupled plasma – atomic emission spectrometry
ISE	Ion selective electrode
HGAAS	Hydride generation atomic absorption spectrometry
HPLC	High-performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MIBK	Methylisobutyl ketone
MMA	Monomethylarsonate
MW	Microwave
OIV	International Organization of Vine and Wine
PTFE	Polytetrafluoroethylene
SPE	Solid phase extraction
STPF	Stabilized temperature platform furnace
SWCV	Square-wave cathodic stripping voltammetry
TXRF	Total reflextion X-ray fluorescence spectrometry
UV	Ultraviolet

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