

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/6290987>

# Determination of lead in wine by hydride generation atomic fluorescence spectrometry in the presence of hexacyanoferrate(III)

ARTICLE *in* ANALYTICAL AND BIOANALYTICAL CHEMISTRY · JULY 2007

Impact Factor: 3.44 · DOI: 10.1007/s00216-007-1127-0 · Source: PubMed

---

CITATIONS

16

---

READS

46

5 AUTHORS, INCLUDING:



**Irina Karadjova**

Sofia University "St. Kliment Ohridski"

99 PUBLICATIONS 1,142 CITATIONS

SEE PROFILE



**Alessandro D'Ulivo**

Italian National Research Council

107 PUBLICATIONS 2,009 CITATIONS

SEE PROFILE



**Massimo Onor**

Italian National Research Council

83 PUBLICATIONS 878 CITATIONS

SEE PROFILE

# Determination of lead in wine by hydride generation atomic fluorescence spectrometry in the presence of hexacyanoferrate(III)

Irina B. Karadjova · Leonardo Lampugnani ·  
Alessandro D'Ulivo · Massimo Onor · Dimiter L. Tsalev

Received: 10 November 2006 / Revised: 8 January 2007 / Accepted: 12 January 2007 / Published online: 14 February 2007  
© Springer-Verlag 2007

**Abstract** A rapid, accurate, and precise method is described for the determination of Pb in wine using continuous-flow hydride generation atomic fluorescence spectrometry (CF-HGAFS). Sample pretreatment consists of ten-fold dilution of wine followed by direct plumbane generation in the presence of  $0.1 \text{ mol L}^{-1}$  HCl and 1% m/v  $\text{K}_3[\text{Fe}(\text{CN})_6]$  with 1% m/v  $\text{NaBH}_4$  as reducing agent. An aqueous standard calibration curve is recommended for Pb quantification in wine sample. The method provides a limit of detection and a limit of quantification of  $0.3 \text{ } \mu\text{g L}^{-1}$  and  $1 \text{ } \mu\text{g L}^{-1}$ , respectively. The relative standard deviation varies between 2–6% (within-run) and 4–11% (between-run) at  $3\text{--}30 \text{ } \mu\text{g L}^{-1}$  Pb levels in wine. Good agreement has been demonstrated between results obtained by CF-HGAFS and direct electrothermal atomic absorption spectrometry in analyses of red and white wines within the concentration range of  $9.2\text{--}25.8 \text{ } \mu\text{g L}^{-1}$  Pb.

**Keywords** Lead · Wine · Hydride generation atomic fluorescence spectrometry · Hexacyanoferrate (III)

## Introduction

Wine is one of the most widely consumed beverages and strict control of the levels of toxic elements is required according to national and international regulatory rules [1, 2]. The presence of Pb in wine could be attributed to the so-called primary sources (transfer of metal from the soil/groundwater to the grapes and finally to the wines, or atmospheric deposition of airborne particulate matter on grapes) and secondary sources including the winemaking process (clarification and fining agents), wine processing equipment (valves, pipes, pumps, bronze tanks), and bottles (cork capsules used for sealed bottles). The Organisation Internationale de la Vigne et du Vin (OIV) has set a maximum limit of  $200 \text{ } \mu\text{g L}^{-1}$  for Pb in wine.

Various methods have been developed and proposed for Pb determination in wine as reviewed recently [3–5]. Two main directions could be distinguished in sample pretreatment procedures for Pb determination in wine: sample digestion by using microwave digestion and UV-photolysis [6–9] or direct instrumental determination after wine dilution. Direct approaches are preferred for routine application. The most widely used method for direct determination of Pb in wine is electrothermal atomic absorption spectrometry (ETAAS) and is proposed as a standard method by OIV, in the presence of different modifiers:  $\text{NH}_4\text{H}_2\text{PO}_4$  [10–13],  $\text{Pd} + \text{Mg}(\text{NO}_3)_2$  [14–17], or without modifier [18]. Recent procedures based on direct inductively coupled plasma mass spectrometry (ICPMS) determination of Pb in wine [19–22] and precise Pb isotope ratios determination in wine [23–26] have been reported. A few papers dealing with speciation analysis of Pb in wine have been published, based mainly on size exclusion high-performance liquid chromatography (HPLC)-ICPMS [27] and electrochemical methods [28, 29].

I. B. Karadjova · D. L. Tsalev (✉)  
Faculty of Chemistry, University of Sofia,  
1 James Bourchier Blvd.,  
1164 Sofia, Bulgaria  
e-mail: tsalev@chem.uni-sofia.bg

L. Lampugnani · A. D'Ulivo · M. Onor  
Istituto per i Processi Chimico-Fisici (IPCF-CNR),  
Area della Ricerca di Pisa,  
Via Moruzzi 1,  
56124 Pisa, Italy

The application of direct hydride generation AAS in wine analysis is limited because of strong ethanol interference; hence, most procedures involve on line sample digestion [3, 30–33] or flow injection of relatively small sample volumes [34]. Interference from wine matrix could be significantly decreased by appropriate sample dilution. Higher dilution factors are possible with more sensitive spectrometric methods for instrumental measurements such as AFS. The aim of this study was to evaluate the possibility of applying  $K_3[Fe(CN)_6]$  as oxidizer/sensitizer for plumbane generation directly from simply diluted, untreated wine samples under relatively mild HG conditions, namely, low concentrations of hexacyanoferrate(III), tetrahydroborate(III), and NaOH. Optimal chemical conditions have been elaborated taking into account the influence of main interfering wine components on the generation of Pb hydride. To the authors' best knowledge the HGAFS technique has until now only been applied to wet digested wine [35] and biological materials [35–38].

## Experimental

### Instrumentation

A laboratory-assembled non-dispersive AFS detector similar to those described previously [39] was employed with the following modifications. A commercially available radiation source and power supply (Pb electrodeless discharge lamp, EDL System 2, Perkin-Elmer) was used. The input power for EDL was square-wave-modulated at 500 Hz with 50% duty cycle and the operating current set at 400 mA. A bandpass optical filter model 405-B (Acton Research Corporation, MA, USA) with 40% peak transmittance at 405.8 nm and 10-nm FWHM was inserted just in front of the photocathode of the UV-VIS photomultiplier (R1527, Hamamatsu), which was operated at 750 V. Lead hydride was atomized in a miniature argon/hydrogen diffusion flame (MDF) supported on a 6-mm-i.d. quartz tube, as detailed elsewhere [40]. The miniature diffusion flame was supported with a total argon and hydrogen flow rate of 300 and 70 mL min<sup>-1</sup>, respectively. The signal output from lock-in amplifier was recorded on a computer through an analog-to-digital converter (ADC) at 20 s<sup>-1</sup> sampling frequency; post-processed raw data were filtered with a moving average of 20 points software filter for signal-to-noise improvement. Emission spectra of the lead EDL lamp and atomic fluorescence spectra of Pb in the MDF atomizer were recorded using a dispersive AFS version of the abovedescribed non-dispersive AFS spectrometer; the 405-B filter was replaced by a scanning monochromator (SPEX 270 M, Jobin Yvon, Longjumeau, France). The spectra were recorded at a scan rate of

0.5 nm s<sup>-1</sup> and spectral bandwidth of 0.45 nm. Atomic fluorescence spectra of lead in the MDF atomizer were recorded using similar CF-HG conditions which have been optimized for analytical determination.

### Hydride generation apparatus (HGAFS)

Lead hydride was generated in a continuous-flow reaction system.  $K_3[Fe(CN)_6]$  was added off-line to samples and standards. Sample/standard solutions were mixed with aqueous NaBH<sub>4</sub> in a T-junction (0.8-mm i.d., Kel-F, Ismatec) with a peristaltic pump and left to react in a 10-cm reaction coil (Teflon PFA, 0.8-mm i.d.). The plumbane formed in the reaction mixture was then stripped out in a coil (0.5 mL) by argon (150 mL min<sup>-1</sup>) added in a second T-junction. Stripping of plumbane was completed in the gas/liquid separator. Argon (150 mL min<sup>-1</sup>) and hydrogen (70 mL min<sup>-1</sup>) were added between the gas/liquid separator (GLS) and MDF atomizer. A 10-μm Teflon filter was inserted between the GLS and atomizer in order to reduce scattering signal due to aerosol carryover.

### Electrothermal AAS

The measurements were carried out with a Perkin-Elmer Model 4100 ZL atomic absorption spectrometer equipped with a transverse heated graphite atomizer (THGA), longitudinal Zeeman effect background corrector and an AS-71 autosampler. 'End-capped' THGA graphite tubes with integrated platforms (Part no. B300-0653) were used. The optimized temperature program for direct ETAAS determination of Pb was applied: (i) drying I at 80 °C (10+10 s, ramp + hold time), (ii) drying II at 130 °C (10+10 s), (iii) pyrolysis at 500 °C (20+20 s), (iv) atomization (1,300 °C (0+5 s, read), and (v) cleaning at 2,000 °C (1+2 s). Argon flow was fixed at 300 mL min<sup>-1</sup>, except for stopped-flow mode during atomization. Peak area mode of measurements (integrated absorbance,  $A_{int}$ ) was used throughout. Ten-μL aliquots of 1+1 diluted wine samples were injected.

### Reagents

Stock standard solutions used were 1,000 μg mL<sup>-1</sup> Pb, 1,000 μg mL<sup>-1</sup> Cu, and 1,000 μg mL<sup>-1</sup> Fe(III) (atomic absorption spectroscopy standard solution, Fluka, Buchs). Working standard solutions were prepared daily by appropriate dilution. Aqueous solutions of sodium tetrahydroborate(III), NaBH<sub>4</sub>, were prepared fresh daily by dissolving the solid reagent (pellets reagent for AAS, BDH, Poole, UK) in 0.01 mol L<sup>-1</sup> NaOH. Ethanol absolute (Cod. 414607, Analyticals, Erba) was used. Antifoam A emulsion from Sigma (Steinheim, Germany) was used. All other chemicals were analytical grade reagents (Carlo Erba). The

water used in all operations was prepared by means of a Milli-Q system (Millipore, Bedford, MA, USA).

All vessels were pre-cleaned by leaching for 24 h in 15% HNO<sub>3</sub> and 15% HCl, followed by rinsing with deionized water. Commercially available wines from Italy, Macedonia, and Bulgaria were used as wine samples.

#### Procedures for wine sample preparation

##### HGAFS determination of Pb

A 2.5-mL wine sample was placed in a 25-mL calibrated flask; 2.5 mL 1 mol L<sup>-1</sup> HCl, 2.5 mL of 10% m/v K<sub>3</sub>[Fe(CN)<sub>6</sub>], and 5 µL of 0.1% v/v antifoam were added and the sample was made up to the mark with deionized water. Blanks and standards are prepared in a similar manner with deionized water and diluted Pb(II) aqueous standard solutions.

##### ETAAS determination of Pb

Wine sample was diluted 1+1 (v/v) with 0.2 mol L<sup>-1</sup> HNO<sub>3</sub>. Sample aliquots of 10 µL were injected. Standard addition method was used for calibration. The detection limit is 2.1 µg L<sup>-1</sup> and the relative standard deviation varied in the range from 3 to 8% for a lead content in wine sample of 3–30 µg L<sup>-1</sup>.

## Results and discussion

### Optimization of instrumental parameters

The effects of the various instrumental parameters including the lamp current, wavelengths, and Ar and H<sub>2</sub> flow rates were investigated with a view to best signal-to-noise ratio. Optimized parameters are summarized in Table 1.

### Non-dispersive atomic fluorescence detection

The AF spectra of lead and emission spectra of the EDL Type II employed as the excitation source are reported in Table 2. Two AFS setups based on solar blind PMT tubes fitted with optical bandpass filters have been evaluated within spectral ranges of 160–320 nm and 185–680 nm, respectively.

In the first setup, a solar blind PMT tube (Hamamatsu R166, 160–320 nm) detects efficiently only the minor intensity AF line at 261.4 nm, while it attenuates significantly the intensity of the 283.3-nm line. At the same time the limiting noise is controlled by the OH radical emission bands (280–320 nm) of the MDF atomizer. The insertion of bandpass optical filters to reduce the radiation

**Table 1** Experimental conditions for non-dispersive HGAFS determination of lead

Parameter(s)	Value
Radiation source	Operating current 400 mA, amplitude modulation 500 Hz, square-wave, 50% duty cycle
Photomultiplier type and voltage	Hamamatsu 1527, 750 V
Wavelength selection	404.9 nm AF line, optical bandpass filter at 405.8 nm
Gas flow rates	H <sub>2</sub> 70–80 mL min <sup>-1</sup> , Ar 300–320 mL min <sup>-1</sup>
Reductant flow rate	2 mL min <sup>-1</sup>
Sample flow rate	4 mL min <sup>-1</sup>

from OH radical (e.g., 190-B or 220-B Acton Research filters tried in our work) is of little utility because they cut AF radiation and OH radical emission to similar extents.

An alternative setup of the AFS detector was evaluated and adopted in this work by the replacement of the solar blind PMT with a UV-VIS, low dark current noise PMT (Hamamatsu R1527, 185–680 nm, peak wavelength 400 nm), while the intense AF line of lead at 404.9 nm was isolated by the bandpass optical filter (404-B Acton Research, 40% transmittance at the peak wavelength of 405.8 nm, 10-nm bandpass) which is able to cut efficiently the relatively far OH radical emission bands. This setup improves sensitivity by a factor of ca. 30, as seen from data in Table 2. The limit of detection (LOD) and limit of

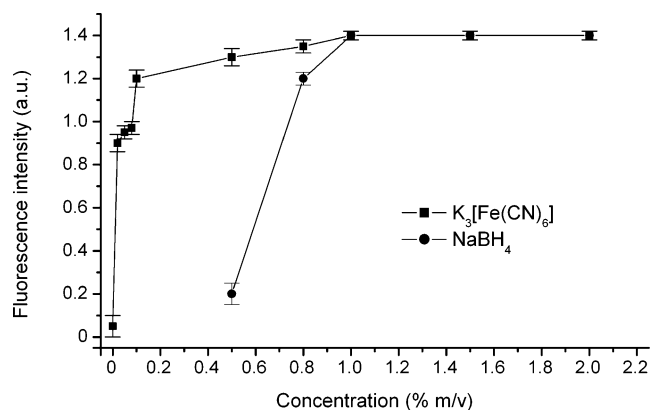
**Table 2** Observed EDL emission and atomic fluorescence spectra for lead<sup>a</sup>

Wavelength (nm)	Relative intensity	
	EDL emission	AF emission <sup>b</sup>
261.4	1.3	2.9
266.2	0.9	— <sup>c</sup>
279.9	2.7	—
283.3	6.7	60.0
287.2	1.8	—
356.8	5.3	—
363.1	36.0	40.0
367.8	44.4	17.1
373.2	11.1	—
400.9	1.3	—
404.9	100.0	100.0
415.9	0.9	—

<sup>a</sup> Most intensive lines observed in the spectral range of 200–480 nm

<sup>b</sup> CF-HG conditions: sample 4 mL min<sup>-1</sup>, 2 mg L<sup>-1</sup> Pb in 0.1 mol L<sup>-1</sup> HCl + 0.5% m/v K<sub>3</sub>Fe(CN)<sub>6</sub>; reductant 2 mL min<sup>-1</sup>, 1 % m/v NaBH<sub>4</sub> in 0.3% m/v NaOH; Ar 320 mL min<sup>-1</sup>, H<sub>2</sub> 320 mL min<sup>-1</sup>

<sup>c</sup> Not detected

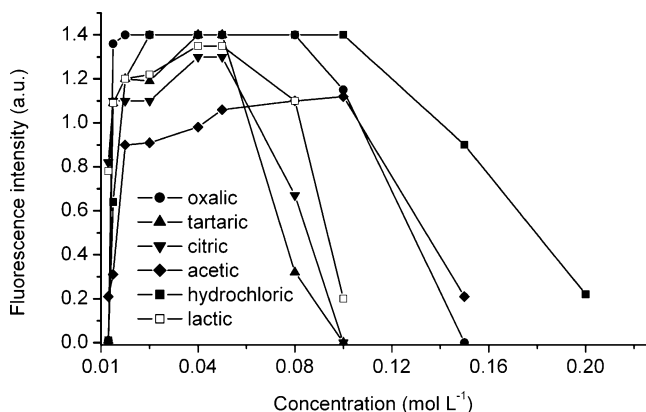


**Fig. 1** Effect of hexacyanoferrate(III) (1% m/v  $K_3[Fe(CN)_6]$ ) and tetrahydroborate(III) (1% m/v  $NaBH_4$ ) on atomic fluorescence signal of Pb ( $1 \mu g L^{-1}$ ) in the presence of  $0.1 mol L^{-1}$  HCl

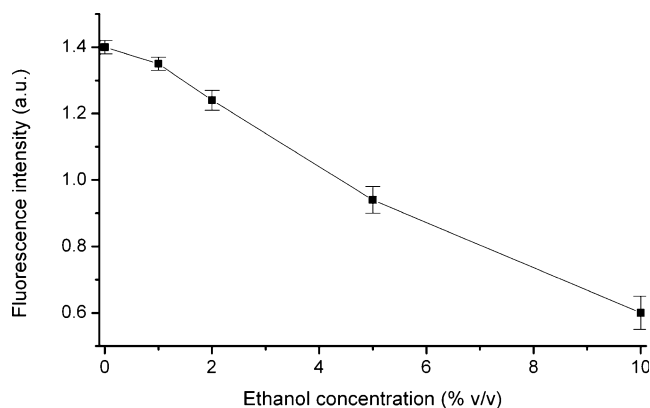
quantification (LOQ) for Pb in pure aqueous solutions with this HGAFS system are  $0.03$  and  $0.1 \mu g L^{-1}$ , respectively.

#### Optimization of chemical parameters

Plumbane generation was achieved by using  $NaBH_4$  in the presence of  $K_3[Fe(CN)_6]$ , which is one of the most used additives enhancing the generation efficiency of lead hydride [30, 41, 42]. The effect of  $NaBH_4$  and  $K_3[Fe(CN)_6]$  concentrations were studied in the presence of  $0.1 mol L^{-1}$  HCl (Fig. 1). Because of the well-known dependence of lead hydride generation on the acidity of the reaction medium we decided to prepare fresh  $NaBH_4$  solutions daily and use as low as  $0.01 mol L^{-1}$  NaOH for their stabilization. Considering the role of  $K_3[Fe(CN)_6]$  in plumbane generation [43], the oxidation of Pb(II) to Pb(IV) by  $K_3[Fe(CN)_6]$  seems unlikely with a view to electrochemical potentials involved in the redox equation ( $E^\circ [Pb(IV)/Pb(II)] = 1.69 V$ ,  $E^\circ [Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-} = 0.36 V$ ). The enhancement in plumbane generation can be the result of the interaction of  $K_3[Fe(CN)_6]$  with the tetrahydroborate–Pb(II) reaction system, forming reaction

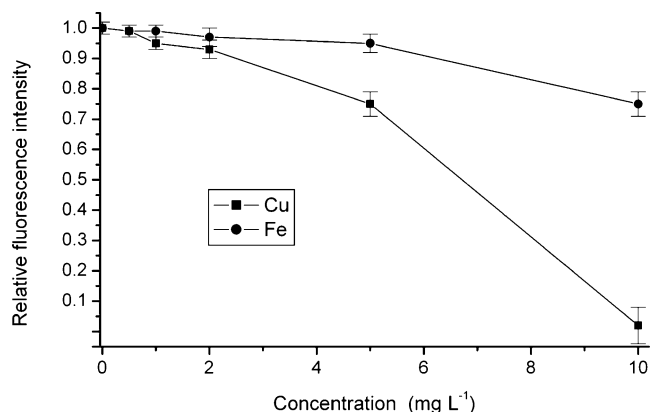


**Fig. 2** Effect of different acids on atomic fluorescence signal for Pb (II) ( $1 \mu g L^{-1}$ ) in the presence of 1% m/v  $K_3[Fe(CN)_6]$  and 1% m/v  $NaBH_4$



**Fig. 3** Effect of ethanol on atomic fluorescence signal of Pb(II) ( $1 \mu g L^{-1}$ ) in the presence of 1% m/v  $K_3[Fe(CN)_6]$  and 1% m/v  $NaBH_4$  in the presence of  $0.1 mol L^{-1}$  HCl

intermediates which evolve to plumbane. The role of  $K_3[Fe(CN)_6]$  (1% m/v, i.e., about  $0.03 mol L^{-1}$ ) on plumbane generation could be similar to that identified recently in bismuthine generation [44]. In the case of bismuthine generation from Bi(III) ( $10^{-6}$ – $10^{-4} mol L^{-1}$ ), the presence of  $K_3[Fe(CN)_6]$  at millimolar level eliminates the formation of elemental bismuth and greatly enhances the formation of the hydride. The enhancement of bismuthine generation at high Bi(III) concentration level was not due to the re-oxidation of Bi(0) to Bi(III) by  $K_3[Fe(CN)_6]$  but to the formation of reaction intermediates which evolved preferentially toward the formation of the hydride rather than to the element [44]. At least 1% m/v  $NaBH_4$  is required for the reduction of Pb bound in such intermediate species. Taking into account that wine matrix tends to foam, it was decided to apply as low as possible  $NaBH_4$  concentration: 1% m/v  $NaBH_4$  and 1% m/v  $K_3[Fe(CN)_6]$  were accepted as optimal. These optimal concentrations of reagents avoid excessive foaming and aerosol formation. The influence of the type and concentration of three widely used acids,  $HNO_3$ ,  $HClO_4$ , and HCl, on plumbane generation was studied. The highest absorbance signal for Pb is obtained at mineral



**Fig. 4** Depressive effect of Cu(II) and Fe (III) on relative atomic fluorescence signal for lead.  $0.1 mol L^{-1}$  HCl, 1% m/v  $K_3[Fe(CN)_6]$ , 1% m/v  $NaBH_4$



**Table 3** Slopes (mean  $\pm$  s) of calibration and standard addition equations for Pb in tenfold diluted wine in different reaction media ( $n=3$ )

Samples	Reaction media			
	0.02 mol L <sup>-1</sup> HCl	0.05 mol L <sup>-1</sup> HCl	0.1 mol L <sup>-1</sup> HCl	0.05 mol L <sup>-1</sup> tartaric acid
Aqueous standard	1.24 $\pm$ 0.02	1.28 $\pm$ 0.02	1.28 $\pm$ 0.02	1.25 $\pm$ 0.02
Chardonnay (white)	0.24 $\pm$ 0.03	0.75 $\pm$ 0.03	1.26 $\pm$ 0.02	0.32 $\pm$ 0.04
Cabernet (red)	0.21 $\pm$ 0.03	0.63 $\pm$ 0.03	1.29 $\pm$ 0.02	0.43 $\pm$ 0.04
Chianti 2003 (red)	0.22 $\pm$ 0.04	0.78 $\pm$ 0.03	1.30 $\pm$ 0.02	0.45 $\pm$ 0.04

acid concentrations from 0.02 to 0.15 mol L<sup>-1</sup>. Therefore the choice of the acid medium used would depend mainly on practical considerations such as reagent purity, availability, and price. In our case, the lowest blank value was obtained from analytical grade HCl.

Wine represents a complex, variable matrix and most of the carboxylic and hydroxylcarboxylic acids are present at varying and unpredictable concentration levels. Most of these acids are also well-known complexing agents for Pb (II). Tartaric acid is the strongest of the acids found in wine, the pH of which generally depends on the concentration of this acid. Therefore the next step was investigation of the influence of the concentrations of various acids on the degree of plumbane generation (Fig. 2). As seen the combined effect of all types of acids present in wine sample on the degree of plumbane generation cannot be easily predicted and evaluated. However taking into account that the maximum permissible limits for tartaric and citric acids are 4–8 g L<sup>-1</sup> and 1 g L<sup>-1</sup>, respectively [45], a tenfold dilution of wine sample should be able to eliminate the influence of wine acidity on the efficiency of lead hydride generation.

#### Influence of wine matrix

Ethanol is the main interfering component in wine sample. Literature results are ambiguous [33, 34], with prevailing opinion that ethanol interferes with Pb hydride generation. Ethanol solutions in the range 1–10% v/v were spiked with 1  $\mu$ g L<sup>-1</sup> Pb, and plumbane was generated under optimal

chemical conditions, namely, 0.1 mol L<sup>-1</sup> HCl, 1% m/v K<sub>3</sub>[Fe(CN)<sub>6</sub>], and 1% m/v NaBH<sub>4</sub>. The degree of depressive effect of ethanol is shown in Fig. 3. It is worth mentioning that attempts to remove this interference by increasing the K<sub>3</sub>[Fe(CN)<sub>6</sub>] or NaBH<sub>4</sub> concentrations failed. Ethanol does not interfere with the hydride generation but with the atomization of the hydrides in the diffusion flame [46]. A radical, yet time-consuming approach would be to remove the depressive effect of ethanol by mild evaporation of wine samples. The higher sensitivity of HGAFS vs. HGAAS does allow the application of high dilution factors, thus reducing levels of C<sub>2</sub>H<sub>5</sub>OH below 1% v/v in final solutions. Transition metals are another serious interfering component in wine samples, which is most pronounced for Cu(II) and Fe(III). It is known that their interference strongly depends on the hydride reaction conditions. Model investigations performed applying the abovementioned optimal parameters are presented in Fig. 4. Copper(II) is a more serious interferent than Fe(III), but there is still a good margin of tolerance to these potential interferents after tenfold dilution of wine. The actual concentrations of Cu and Fe in wine would hardly increase by 1 order of magnitude above their maximum permissible levels (1 and 5  $\mu$ g mL<sup>-1</sup>, respectively), still giving  $\leq 5\%$  signal depression.

It is known that around 90–95% of Pb in wine is bound to dimers of the pectic polysaccharide ramnagalacturonan-II [27]; therefore, a final conclusion about optimal reaction media was made after spike recovery experiments with different type of wines. Three brands of typical wines, viz., Chardonnay, Cabernet, and Chianti 2003, were diluted tenfold and spiked with known amounts of Pb(II). Different concentrations of HCl in the range 0.02–0.1 mol L<sup>-1</sup> and tartaric acid 0.5 mol L<sup>-1</sup> using optimal 1% m/v NaBH<sub>4</sub> and 1% m/v K<sub>3</sub>[Fe(CN)<sub>6</sub>] were tested as reaction media for direct plumbane generation from diluted wine. The slopes of calibration graphs ( $\Delta I_{AF}/\Delta C$ ) obtained for reagent-matched aqueous standards and spiked wine samples are presented in Table 3. Lower slopes of addition equations in more diluted HCl or tartaric acid could be explained by the stability of Pb complex with the dimers of ramnagalacturonan-II. Equal slopes of aqueous standard calibration graph and spiked wine samples were achieved only in the

**Table 4** Comparative results for Pb contents in wine by HGAFS and ETAAS ( $\mu$ g L<sup>-1</sup>, mean  $\pm$  s,  $n=6$ )

Wine brand	HGAFS	ETAAS
Chardonnay (Bulgaria; white)	15.3 $\pm$ 0.5	15.8 $\pm$ 0.6
Pinot Chardonnay (Italy; white)	9.2 $\pm$ 0.4	9.6 $\pm$ 0.5
Sauvignon Blanc (Macedonia; white)	21.4 $\pm$ 0.4	21.7 $\pm$ 0.6
Chianti 2003 (Italy; red)	10.7 $\pm$ 0.3	10.9 $\pm$ 0.5
Cabernet Sauvignon (Bulgaria; red)	25.8 $\pm$ 0.5	26.2 $\pm$ 0.6
T'ga za Jug (Macedonia; red)	22.6 $\pm$ 0.4	22.9 $\pm$ 0.5
Rosso di Montalcino (Italy)	12.1 $\pm$ 0.4	12.5 $\pm$ 0.5

presence of  $0.1 \text{ mol L}^{-1}$  HCl. In order to prove that naturally present lead in wine sample behaves in the same way as does the added ionic lead(II), the same wine samples were further diluted (up to 15- and 20-fold total dilution), resulting in unified slopes for all studied wines and three spiked samples. Apparently, as observed previously [27], the ionic Pb(II) spike could be bound to excess 'free' dimers of ramnagalacturonan-II. As far as the slopes of calibration graphs obtained for spiked wine samples and aqueous standards are statistically equal, calibration against aqueous standard calibration curve could be recommended for Pb quantification in wines. A 5- $\mu\text{L}$  aliquot of 0.1% (v/v) antifoam agent solution should be employed to suppress foaming in the gas/liquid separator, even after tenfold dilution of wine sample. Antifoam agent is by itself slightly depressive, hence its levels should be matched in aqueous calibration standards as well. It could be expected that some information on the content of lead in 'free' and 'complexed' Pb in wine could be obtained from the examination of the degree of plumbane generation in the presence of two HCl concentrations: more diluted and more concentrated.

#### Figures of merit

The limit of detection (LOD) and limit of quantification (LOQ) of the proposed procedure were 0.3 and  $1 \mu\text{g L}^{-1}$ , calculated from 3 and 10 standard deviations of ten runs of wine samples with low Pb contents in the range 2–5  $\mu\text{g L}^{-1}$  Pb (red or white wine, tenfold diluted). The relative standard deviation varied between 2–6% (within-run) and 4–11% (between-run) at a 3–30  $\mu\text{g L}^{-1}$  lead level in wine. The accuracy of this procedure was demonstrated by comparative analysis using direct ETAAS. Very good agreement was obtained between the results (by *t*-test, one-way ANOVA, 95%) in all cases (Table 4). The slope of the regression line of the bivariate plot of HGAFS results (*X*) and ETAAS determinations (*Y*) for 13 wine samples was  $1.03 \pm 0.02$ , with a regression coefficient of 0.991.

#### Conclusion

Direct continuous-flow HGAFS determination of lead in wine with reagent-matched calibration is feasible and provides limits of detection as low as  $0.3 \mu\text{g L}^{-1}$  owing to (1) straightforward atomization in a miniature Ar–H<sub>2</sub> diffusion flame, (2) high dilution factors (> tenfold) possible with this highly sensitive technique, (3) bringing levels of interference from ethanol, Cu(II), and Fe(III) below their tolerance limits upon dilution, (4) hydride generation under relatively mild conditions ( $0.1 \text{ mol L}^{-1}$  HCl and 1% m/v NaBH<sub>4</sub>) in the presence of hexacyanoferrate(III), and (5) efficient foam control.

**Acknowledgments** The authors gratefully acknowledge funding by NATO Outreach Fellowship 219.34 to IBK and partial financial support from Bulgarian Ministry of Education, NSFB Project VYX-12/05.

#### References

- Pedersen GA, Mortensen GK, Larsen EH (1994) Beverages as a source of toxic trace-element intake. *Food Addit Contam* 11:351–363
- European Economic Community (1990) Official methods of analysis of wine: method 2676/90. *Official Journal L272*:1–192
- Baluja-Santos C, Gonzalez-Portal A (1992) Application of hydride generation to atomic-absorption spectrometric analysis of wines and beverages: a review. *Talanta* 39:329–339
- Pyrzynska K (2004) Analytical methods for the determination of trace metals in wine. *Crit Rev Anal Chem* 34:69–83
- Stafilov T, Karadjova I (2006) Methods for determination and speciation of trace elements in wine. *Int J Pure Appl Chem* 1:273–305
- Kim M (2004) Determination of lead and cadmium in wines by graphite furnace atomic absorption spectrometry. *Food Addit Contam* 21:154–157
- Kristl J, Veber M, Slekovec M (2002) The application of ETAAS to the determination of Cr, Pb and Cd in samples taken during different stages of the winemaking process. *Anal Bioanal Chem* 373:200–204
- Almeida CM, Vasconcelos MTSD (2002) Advantages and limitations of the semi-quantitative operation mode of an inductively coupled plasma-mass spectrometer for multi-element analysis of wines. *Anal Chim Acta* 463:165–175
- Myors RB, Hearn R, Mackay LG (2005) The high-accuracy analysis of lead in wine by exact-matching isotope dilution mass spectrometry using ICP-MS. *J Anal At Spectrom* 20:216–219
- Matthews MR, Parsons PJ (1993) A simple method for the determination of lead in wine using Zeeman electrothermal atomization atomic-absorption spectrometry. *At Spectrosc* 14:41–46
- Mindak WR (1994) Determination of lead in table wines by graphite furnace atomic absorption spectrometry. *J AOAC Int* 77:1023–1030
- Zuo ZY, Zhang M, Sun ZA, Wang DS (2002) Determination of lead in grape wine by graphite furnace atomic absorption spectrometry with ammonium dihydric phosphate as modifier. *Spectrosc Spectr Anal* 22:859–861
- Tripković M, Todorović M, Holclajtner-Antunović I, Raižić S, Kandić A, Marković D (2000) Spectrochemical determination of lead in wines. *J Serb Chem Soc* 65:323–329
- Bruno SNF, Campos RC, Curtius AJ (1994) Determination of lead and arsenic in wines by electrothermal atomic-absorption spectrometry. *J Anal At Spectrom* 9:341–344
- Freschi GPG, Dakuzaku CS, de Moraes M, Nóbrega JA, Gomes Neto JA (2001) Simultaneous determination of cadmium and lead in wine by electrothermal atomic absorption spectrometry. *Spectrochim Acta Part B* 1987–1993
- Almeida AA, Bastos ML, Cardoso MI, Ferreira MA, Lima JLFC, Soares ME (1992) Determination of lead and aluminium in port wine by electrothermal atomic-absorption spectrometry. *J Anal At Spectrom* 7:1281–1285
- Fernandes KG, de Moraes M, Neto JAG, Nobrega JA, Oliveira PV (2002) Evaluation and application of bismuth as an internal standard for the determination of lead in wines by simultaneous electrothermal atomic absorption spectrometry. *Analyst* 127:157–162

18. Jorhem L, Sundstrom B (1995) Direct determination of lead in wine using graphite furnace. *At Spectrosc* 16:265
19. Goossens J, Desmaele T, Moens L, Dams R (1993) Accurate determination of lead in wines by inductively coupled plasma-mass spectrometry. *Fresenius J Anal Chem* 347:119–125
20. Goossens J, Moens L, Dams R (1994) Determination of lead by flow-injection inductively-coupled plasma-mass spectrometry comparing several calibration techniques. *Anal Chim Acta* 293:171–181
21. Medina B, Augagneur S, Barbaste M, Grouset FE, Buat-Meard P (2000) Influence of atmospheric pollution on the lead content of wines. *Food Addit Contam* 17:435–445
22. Quétel CR, Nelms SM, Van Nevel L, Papadakis I, Taylor PDP (2001) Certification of the lead mass fraction in wine for comparison 16 of the International Measurement Evaluation Programme. *J Anal At Spectrom* 16:1091–1100
23. Almeida CMR, Vasconcelos MTSD (1999) Determination of lead isotope ratios in port wine by inductively coupled plasma mass spectrometry after pre-treatment by UV-irradiation. *Anal Chim Acta* 396:145–153
24. Almeida CMR, Vasconcelos MTSD (1999) UV-irradiation and MW-digestion pre-treatment of Port wine suitable for the determination of lead isotope ratios by inductively coupled plasma mass spectrometry. *J Anal At Spectrom* 14:1815–1821
25. Augagneur S, Medina B, Grouset F (1997) Measurement of lead isotope ratios in wine by ICP-MS and its applications to the determination of lead concentration by isotope dilution. *Fresenius J Anal Chem* 357:1149–1152
26. Barbaste M, Halicz L, Galy A, Medina B, Emteborg H, Adams FC, Lobinski R (2001) Evaluation of the accuracy of the determination of lead isotope ratios in wine by ICP MS using quadrupole, multicollector magnetic sector and time-of-flight analyzers. *Talanta* 54:307–317
27. Szpunar J, Pellerin P, Makarov A, Doco T, Williams P, Medina B, Łobiński R (1998) Speciation analysis for biomolecular complexes of lead in wine by size-exclusion high-performance liquid chromatography inductively coupled plasma mass spectrometry. *J Anal At Spectrom* 13:749–754
28. Azenha MAGO, Vasconcelos MTSD (2000) Pb and Cu speciation and bioavailability in port wine. *J Agric Food Chem* 48:5740–5749
29. Vasconcelos MT, Azenha M, de Freitas V (2000) Electrochemical studies of complexation of Pb in red wines. *Analyst* 125:743–748
30. Dědina J, Tsalev DL (1995) Hydride generation atomic absorption spectrometry. Wiley, Chichester, pp 288–307
31. Chuachud W, Tyson JF (2005) Determination of lead by flow injection hydride generation atomic absorption spectrometry with tetrahydroborate immobilized on an anion-exchange resin. *J Anal At Spectrom* 20:282–288
32. Madrid Y, Meseguer J, Bonilla M, Cámara C (1990) Lead hydride generation in a lactic acid-potassium dichromate medium and its application to the determination of lead in fish, vegetable and drink samples. *Anal Chim Acta* 237:181–187
33. Cacho J, Ferreira V, Nerin (1992) Determination of lead in wines by hydride generation atomic absorption spectrometry. *Analyst* 117:31–33
34. Cabrera C, Madrid Y, Cámara C (1994) Determination of lead in wine, other beverages and fruit slurries by flow-injection hydride generation atomic-absorption spectrometry with online microwave digestion. *J Anal At Spectrom* 9:1423–1426
35. Wan Z, Xu ZR, Wang JH (2006) Flow injection on-line solid phase extraction for ultra-trace lead screening with hydride generation atomic fluorescence spectrometry. *Analyst* 131:141–147
36. Wang JH, Yu YL, Du Z, Fang ZL (2004) A low cost and sensitive procedure for lead screening in human whole blood with sequential injection-hydride generation-atomic fluorescence spectrometry. *J Anal At Spectrom* 19:1559–1563
37. Shi J, Gong XY, Zhu YQ (2004) Determination of trace lead in the traditional Chinese medicine using hydride generation atomic fluorescence spectrometry. *Guang Pu Xue Yu Guang Pu Fen Xi, Spectroscopy Spectral Anal* 24:1451–1453
38. Lu XH, Lu H, Ma LD, Cui YJ (2006) The determination of lead by MD-HG-AFS in APMP-QM-P3 comparison. *Accred Qual Assur* 11:208–211
39. D'Ulivo A, Dedina J (1996) Interferences in hydride atomization studied by atomic absorption and atomic fluorescence spectrometry. *Spectrochim Acta Part B* 59:725–793
40. Dědina J, D'Ulivo A (1997) Argon shielded highly fuel-rich, hydrogen-oxygen diffusion microflame-a new hydride atomizer. *Spectrochim Acta Part B* 52:1737–1746
41. Vereda Alonso E, García de Torres A, Rivero Molina A, Cano Pavon JM (1998) *Quim Anal* 17:167–175
42. Chen S, Zhang Z, Yu H, Liu W, Su M (2002) Determination of trace lead by hydride generation-ICP-MS. *Anal Chim Acta* 463:177–188
43. D'Ulivo A (2004) Chemical vapor generation by tetrahydroborate (III) and other borane complexes in aqueous media. A critical discussion of fundamental processes and mechanisms involved in reagent decomposition and hydride formation. *Spectrochim Acta Part B* 59:725–793
44. D'Ulivo A, Battistini SST, Pitzalis E, Zamboni R, Zoltan M, Sturgeon RE (2007) Effect of additives on chemical vapour generation of bismuthane by derivatization by tetrahydroborate (III). *Anal Bioanal Chem* (in press)
45. Vereda Alonso E, García de Torres A, Rivero Molina A, Cano Pavón JM (1998) Determination of organic acids in wines. A review. *Quím Anal* 17:167–175
46. Karadjova IB, Lampugnani L, Dědina J, D'Ulivo A, Onor M, Tsalev DL (2006) Organic solvents as interferents in arsenic determination by hydride generation atomic absorption spectrometry with flame atomization. *Spectrochim Acta Part B* 61:525–531