Determination of Lead Contamination in Spanish Wines and Other Alcoholic Beverages by Flow Injection Atomic Absorption Spectrometry

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A rapid, accurate, and precise method is described for the direct determination of lead in wine and other alcoholic beverages, using a flow injection−hydride generation−atomic absorption spectrometry system (FI-HG-AAS). Lead hydride was generated in $HNO_3-H_2O_2$ medium using $NaBH_4$ as the reducing agent. To increase the efficiency of lead hydride generation and to produce mineralized samples, a microwave oven was coupled on-line to the FI-HG-AAS system for some samples. This method was used with 70 samples of wine produced in Spain and with 64 samples of other alcoholic beverages widely consumed in that country. The concentrations of lead ranged in wine from not detectable (ND) to 1125.00 μg/L and in other alcoholic beverages from ND to 444.70 μg/L.

Keywords: *Lead contamination; wine; alcoholic beverages; atomic absorption spectrometry*

INTRODUCTION

Lead contents in wine may vary within a broad range, and sometimes a distinction is made between the primary "natural" lead content (depending on the kind of soil, variety of grape, etc.) and secondary contamination due to the use of pesticides and fertilizers or treatments during vinification (Henick-Kling and Stoewsand, 1993; Goossens et al., 1993). Moreover, when the bottle of wine is sealed with a metallic capsule, the lead in the capsule may cause additional contamination by carry-over during pouring (Goossens et al., 1993; Ough, 1993). According to the Royal Commission on Environmental Pollution (U.K.), the consumption of wine and beer raises blood lead concentrations; wines containing 150 mg og Pb/L can make a significant contribution to blood lead levels (Sherlock and Pickford, 1986). Therefore, the determination of lead in such samples requires the use of techniques of high sensitivity with low detection limits.

Methods based on atomic absorption spectrometry (AAS) and inductively coupled plasma−atomic emission spectrometry (ICP-AES) are widely used for the chemical characterization of wines and beverages, to investigate adulteration, and to study the changes that take place during vinification and processing (Gonzalez et al., 1988; Goossens et al., 1993; Ough, 1993). The generation of hydrides with atomic absorption spectrometry (HG-AAS) is an alternative to electrothermal atomization−atomic absorption spectrometry (ETA-AAS), which is widely used for this type of sample (Gonzalez et al., 1988; Madrid and Camara, 1994; Cabrera et al., 1994a). Flow injection for lead determination has a number of most important advantages: sample manipulation is kept to a minimum, and the volumes of sample and reagents needed are smaller than with other methods.

In this paper, a rapid, accurate, and precise method is described for the direct determination of Pb in wine and other alcoholic beverages, using a flow injection−hydride generation (FI-HG) AAS system. The results were compared with those obtained by ETA-AAS.

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MATERIALS AND METHODS

Apparatus. A Perkin-Elmer Model 1100B double-beam atomic absorption spectrophotometer with a deuterium background corrector (Perkin-Elmer Corp., Norwalk, CT) was used with an 11-mA hollow cathode lamp. Hydride atomization was done in a Perkin-Elmer quartz cell. A spectral bandwidth of 0.7 nm was selected to isolate the 217.0-nm lead line. Background correction was not used. A flow injection analysis system composed of a Gilson HP4 four-channel peristaltic pump and an Omnifit six-way sample injection valve, a glass liquid−gas phase separator, and Teflon tubes of 0.5- and 0.8-mm internal diameter (i.d.) was used. For some types of sample (beer and cider) we used a Moulinex Model FM-470 microwave oven on-line with a maximum potency of 600 W and an ice bath (Cabrera et al., 1994a). Lead hydride was generated directly from wine samples in an $HNO_3-H_2O_2$ medium and was carried by argon (99.9998% purity) to the quartz atomization cell, where it was heated by an acetylene−air flame.

A mineralization block with a thermostat and timer (Selecta) was used with Pyrex tubes of the appropriate size for brandy, rum, whiskey, gin, anisette, liquor, and spirits samples. The samples of cava (champagne-type wine), beer, and cider were degassed in an ultrasonic bath prior to analysis.

To validate the method, we used a Perkin-Elmer HGA-700 graphite furnace with pyrolytically coated tubes and a L'vov platform at 283.3 nm with a spectral bandwidth of 0.7 nm. A deuterium background corrector was also used. In addition, a 45-mL-capacity microwave acid digestion bomb (Parr Instruments Co., Moline, IL) was used to mineralize samples, together with a Moulinex Model FM-460 microwave oven.

Reagents. All solutions were prepared with ultrapure water with a specific resistivity of 18 MΩ·cm, obtained by filtering double-distilled water through a Milli-Q purifier system (Millipore) immediately before use. The reagents were of analytical reagent grade or higher purity. The Pb standard solution (1.00 ± 0.002 g) was Titrisol from Merck (Darmstadt, Germany). Nitric acid (65%, Merck), hydrogen peroxide (Merck), and vanadium pentaoxide (Merck) were used. Sodium tetrahydroborate solution was preparing by dissolving sodium tetrahydroborate(III) powder (Merck) in deionized Milli-Q water and stabilizing in 1% sodium hydroxide (Merck).

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Table 1. Chemical and Physical Parameters Optimized for Lead Determination in Wine and Other Alcoholic Beverages by FI-HG-AAS and Microwave-Coupled FI-HG-AAS

	lead HG[a]			microwave oven treatment		
sample	HNO_3 v/v (%)	H_2O_2 v/v (%)	$NaBH_4$ w/v (%)	coil length (m)	i.d. of tubing (mm)	hold time (s)
wine[b]	10	2	6			
beer[b]	30	15	6	1.5	0.5	14
cider[b]	30	15	6	1.5	0.5	14
brandy[c]	10	2	6			
rum[c]	10	2	6			
whiskey[c]	10	2	6			
gin[c]	10	2	6			
anisette[c]	10	2	6			
liquor[c]	10	2	6			
spirits[c]	10	2	6			

[a] Injection volume of 500 μL. [b] No pretreatment. [c] Pretreatment with HNO_3.

Samples. We analyzed 70 commercial samples of red, white, rose, dessert, sherry, and cava wines, 23 samples of beer, and 41 samples of other alcoholic beverages including cider, brandy, rum, whiskey, gin, anisette, liquors, and spirits. All samples were obtained from products that are widely consumed in Spain. Homogeneity and reproducibility were confirmed in preliminary assays (Horwitz and Howard, 1979; Pomeranz and Meloan, 1994).

Procedure. Lead was directly determined in wine by FI-HG-AAS and in beer and cider samples by microwave FI-HG-AAS. Omitting the pretreatment step (previous mineralization of samples) had no significant effect on the quality of the data and reduced the time, amount of reagents, and number of sample manipulations required. Because they contained complex matrices, all other samples (brandy, rum, whiskey, gin, anisette, liquor, and spirits) were digested using the mineralization block before analyses by FI-HG-AAS. For sample mineralization, a 5-mL volume of alcoholic beverage (brandy, rum, whiskey, gin, anisette, liquor, spirits) was treated with 1 mL of 65% (v/v) HNO_3 and a few micrograms of V_2O_5 as a catalyst in Pyrex tubes, placed in a digestion block, and heated at 120 °C for 90 min. The solutions were left to cool to room temperature, transferred to a calibrated flask, and diluted to a final volume of 10 mL with deionized water. Lead was determined in the resulting solutions. All samples were analyzed in triplicate.

Lead Determination. FI-HG-AAS was used as the analytical technique. For all assays we injected 500 μL of sample under a nitric acid flow. An on-line microwave oven was used immediately after the samples of beer or cider were injected under nitric acid flow to enhance efficiency and decrease interferences from the matrix. An ice bath was used to cool the sample before it was placed in the oven to reduce the pressure caused by water vapor. The optimum conditions for lead hydride generation in each sample are summarized in Table 1. The same procedure was used for blanks. An aqueous calibration graph and the standard additions method were used.

Method Validation. The proposed method was validated by comparison with the ETA-AAS technique, and lead was determined in mineralized samples using a microwave digestion bomb. A volume of 2.0 mL of samples (wine, beer, cider, and other alcoholic beverages) was treated with 2.5 mL of 65% (v/v) HNO_3 and a few micrograms of V_2O_5 at the maximum setting for 90 s and brought to a final volume of 25 mL with deionized water. Lead was determined in the resulting solutions by ETA-AAS. An aliquot of 10 μL was injected into a graphite tube and run under optimized conditions (Cabrera et al., 1994b, 1995). CAUTION: Start with small samples, small amounts of acid, and short digestion times and then increase only if necessary. Cooling the digestion bomb as described in the operating instructions is a particularly important safety consideration. It is also recommended that the bomb should remain in the microwave oven after the heating cycle for a period equal to the heating time (National Bureau of Standards, 1994). The results obtained for the two methods (Table 2) were compared with the F test, and no significant differences at the 95% confidence level were observed. We therefore concluded that the method can be used as an alternative to ETA-AAS.

Table 2. Lead Concentrations in Alcoholic Beverages Determined by FI-HG-AAS and ETA-AAS

	lead[a] (μg/L, $x \pm$ SD)	
sample	FI-HG-AAS	ETA-AAS
wine	97.7 ± 0.7	98.0 ± 0.4
beer	40.5 ± 0.4	35.5 ± 1.0
cider	111.9 ± 0.6	115.0 ± 0.7
brandy	14.3 ± 0.5	15.0 ± 1.0
rum	ND[b]	ND
whiskey	71.4 ± 0.8	72.5 ± 0.3
gin	35.7 ± 0.6	34.9 ± 0.2
anisette	216.7 ± 0.4	215.5 ± 0.5
liquor	ND	ND
spirits	358.0 ± 1.0	356.0 ± 2.0

[a] Mean value ± standard deviation at the 95% confidence level (Student's t), $n = 10$. [b] Not detectable.

Table 3. Analytical Characteristics for Lead Determination in Wine and Other Alcoholic Beverages by FI-HG-AAS and Microwave-Coupled FI-HG-AAS

sample	detection limit[a] (μg/L)	characteristic mass[b] (ng)	precision[c] (RSD, %)	accuracy[d] (%)	slope ratio blank/addition
wine	10	0.8	5−6	98.7 ± 0.5	1.10
beer	12	1.0	6−7	99.0 ± 1.0	1.05
cider	12	1.0	5−6	98.0 ± 0.7	1.10
brandy	10	1.2	4−5	99.5 ± 0.5	1.05
rum	10	1.2	5−6	99.5 ± 0.7	1.05
whiskey	10	1.2	5−6	99.0 ± 1.0	1.10
gin	10	1.2	4−5	98.7 ± 1.0	1.20
anisette	10	1.2	6−8	98.5 ± 1.5	1.15
liquor	10	1.2	4−5	98.0 ± 1.0	1.15
spirits	10	1.2	7−8	99.5 ± 0.5	1.10

[a] Calculated according to IUPAC rules. [b] Characteristic mass in ng/0.0044A·s. [c] Ten replicate determinations on each of three different samples. [d] Recovery assays in five different samples.

RESULTS AND DISCUSSION

The proposed method (FI-HG-AAS) was highly efficient and required small amounts of samples and reagents and a minimum of sample manipulation. The method was versatile (more than 120 samples/h), free from interferences, and relatively cheap. FI-HG-AAS can be used as an alternative to conventional methods to determine Pb in wine and other beverages. Modifying the chemical and physical conditions of Pb hydride generation and sample pretreatment would allow the proposed method to be used to determine Pb in other similar samples such as grape juice and juices in general and in most soft drinks. To evaluate the analytical characteristics of the method, the characteristic mass and precision were evaluated, and the detection and quantification limits were calculated according to IUPAC rules (Long and Winefordner, 1983). Accuracy was tested with recovery assays by adding Pb at concentrations of 25, 50, 100, and 150 ng/mL to different randomly chosen samples and processing the mixtures as described above in triplicate. The results are shown in Table 3 as the mean ± standard deviation at the 95% confidence level. The technique was accurate and reproducible. The data for 10 determinations in three different samples were analyzed statistically, and these results are also shown in Table 3.

To determine the selectivity of the method for each type of sample, standard additions graphs were pre-

Table 4. Lead Contamination Levels in Spanish Wines

sample	*n*	lead (μg/L) mean	lead (μg/L) range
red wine	36	222.31	57.50−1125.00
white wine	11	150.40	ND[a]−350.00
rose wine	6	135.30	99.20−183.30
sherry	5	127.66	50.00−283.30
oloroso sherry	7	278.00	ND−769.30
cava	5	278.63	139.50−379.50

[a] Not detectable.

Table 5. Lead Contamination Levels in Beer and Other Alcoholic Beverages Widely Consumed in Spain

sample	*n*	lead (μg/L) mean	lead (μg/L) range
beer	23	48.23	ND[a]−245.00
cider	9	123.42	63.00−176.20
brandy	4	9.28	ND−25.00
rum	5	18.00	ND−70.00
whiskey	5	85.33	20.00−195.40
gin	4	8.92	ND−35.70
anisette	6	225.95	85.70−444.70
liquor	2	ND	ND
spirits	6	276.83	164.80−389.70

[a] Not detectable.

pared for blanks and for each sample. The slopes of the calibration lines for spiked samples were similar to the slope of the calibration line for the standard in acid medium (Table 3); thus, matrix effects were considered to be negligible.

The levels of Pb contamination in wine and other alcoholic beverages are given in Tables 4 and 5, respectively. In both cases there was considerable variability in the results, which reflect the presence of lead contamination in the raw materials used during production or secondary contamination from different technological processes. Lead was detected in all samples of wine analyzed, although the levels were similar to those reported by other authors (Gonzalez et al., 1988; Baluja-Santos and Gonzalez-Portal, 1992; Henick-Kling and Stoewsand, 1993; Cabrera et al., 1994a). We observed considerable variability in the results, not only between the different types of wine but also between samples from the same region or the same vintage year. This emphasizes the large number of unknown factors that can increase the presence of this toxic element in wine.

The Pb content in wine depends on both natural and exogenous factors. Natural factors include soil type and composition, grape variety, and climate. Exogenous factors can include the fermentation process (Marin and Ostapczuk, 1992), the vinification system, contamination from vehicle exhaust fumes, industrial emissions, the use of organochloride-containing pesticides and Pb arsenates (now prohibited), the proximity of a vineyard to ceramic factories, and the use of lead-containing capsules to seal the bottle (Smart et al., 1990). Although the presence of Pb in grapes is affected by the factors mentioned above, normal must contains little or no lead because most of this element tends to precipitate out as lead tartrate during fermentation. The normal Pb content in wine varies from 0.4 to 0.5 mg/L (Gonzalez et al., 1988; Teissedre et al., 1994). Lead is not a natural substance in plant nutrition; this element can be present in soil, but plants accumulate only small amounts. Long maceration at excessively high temperatures may cause large amounts of Pb to be extracted into wine, but other factors such as alcohol content and must acidity can also increase lead levels.

In this study the highest Pb concentrations in wine were found in cava and oloroso sherry (Table 4). A high Pb content in oloroso sherry was also observed in a previous study (Lopez-Artiguez et al., 1990). The elevated levels of Pb in cava may be due to the acidity of this type of wine and to the presence of CO_2. High concentrations of Pb were found in red wines, probably because the lees remain in prolonged contact with must. In addition, dark grapes usually contain higher concentrations of Pb than white grapes (Marin and Ostapczuk, 1992). The high concentrations of sulfur dioxide in these wine samples may also affect Pb content. The differences between Pb levels between white and rose wines were not statistically significant, although Pb levels were higher in some of the white wines. During white wine-making, Pb is probably removed when grape skins and lead-attracting yeasts on the skins are eliminated prior to fermentation; this is not done in red wine vinification.

In the samples of beer analyzed, the mean lead concentration was 48.23 μg/L. Canned beers had the highest levels, probably because low-quality cans were used; lower concentrations were found in draft beers. However, another study reported that Pb content increased in draft beer because of the dispensation equipment (Newton et al., 1992). Among the alcoholic beverages we analyzed, the highest levels of Pb were found in spirits, anisette, and cider samples.

Periodic determinations are advisable in view of the potential medium- and long-term risks associated with lead contamination. A few clinical reports have suggested that heavy alcohol intake may augment the absorption and toxicity of lead. More likely explanations for the apparent synergism are increased lead exposure and nutritional deficiencies in some alcoholics (Falcone, 1991). In addition to continuing efforts to reduce the level of Pb contamination, current efforts to reach an international agreement to stop the use of metallic capsules together with continuous surveillance of all processes during which contaminants can appear are the most effective approaches to the prevention of Pb contamination.

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