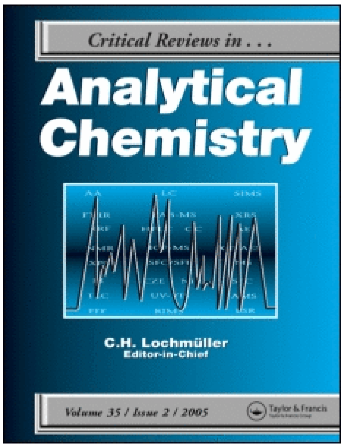

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Analytical Methods for the Determination of Trace Metals in Wine

Krystyna Pyrzyńska[a]
[a] Department of Chemistry, University of Warsaw, Warsaw, Poland

To cite this Article Pyrzyńska, Krystyna(2004) 'Analytical Methods for the Determination of Trace Metals in Wine', Critical Reviews in Analytical Chemistry, 34: 2, 69 — 83

To link to this Article: DOI: 10.1080/10408340490475858

URL: http://dx.doi.org/10.1080/10408340490475858

Critical Reviews in Analytical Chemistry, 34:69–83, 2004
Copyright © Taylor and Francis Inc.
ISSN: 1040-8347 print
DOI: 10.1080/10408340490475858

Analytical Methods for the Determination of Trace Metals in Wine

Krystyna Pyrzyńska
Department of Chemistry, University of Warsaw, Warsaw, Poland

Trace metal analyses are undertaken to determine trace contamination, their legal limits for export purposes, to monitor the content of certain salts added to some wines during processing, for authenticity purposes, and to determine metal contribution to flavor and wine quality. The most widely applied analytical methods for the determination of metal contents in wine samples are presented. Some problems with sample preparation and validation of the method are also discussed.

Keywords analytical methods, metals, sample preparation, wine

Wine has been a widely consumed beverage in the world for centuries with very obvious commercial value and social importance. Plato remarked, "No thing more excellent nor more valuable than wine was ever granted mankind by God." Numerous studies have shown that the moderate consumption of wine, especially red wines, improves good health and longevity (1, 2).

According to the Office International de la Vigne et du Vin (OIV), the total volume of wine produced and consumed in 2001 reached 25.7 and 21.9 × 10^9 L, respectively (3). The comparison between continents reveals an increase in consumption in North and South America, and to a lesser extent in Australia (Figure 1). This increase, however, is cancelled out by a steady decrease in overall consumption in most traditional producer countries in Western Europe and in South America. A list of the 10 leading wine producer and consumer countries is presented in Table 1.

Wine is a complex sample containing a variety of inorganic as well as organic substances in an ethanol-aqueous solution. The principal dissolved species are inorganic ions (such as potassium, sodium, and calcium), organic acids, polyphenols, polyhydroxy alcohols, proteins, amino acids, and polysaccharides. White wines are less abundant in dissolved substances than red wines, particularly with respect to polyphenols. Monomeric polyphenols, such as catechin, are generally considered to be more bitter, while polymeric tannins are highly astringent (4). Phenolic compounds not only contribute to the sensory characteristics of wine, such as color, flavors, and astringency, but also may act as antioxidants both free-radical scavenging and metal chelation (5). Anthocyanins contribute to the red color of grapes and wines. The major organic acid in wine is tartaric acid occurring at concentration of 0.5–5 g/L; other acids include citric, malonic, lactic, fumaric, and acetic. They contribute to tartness and mouth-feel properties of wine. Glucose and fructose are the main sugars present in grapes and they are utilized by yeast during fermentation to produce ethanol. Peptides are attributed with several different biological properties, as antioxidants, antimicrobials, or surfactant agents and they are involved in the development of sweet and bitter taste (6).

The growing concern about human exposure to different mineral components has provided the impetus for studying their content in food and beverages. In some countries alcoholic beverages, such as wine, represent over 12% of the daily intake of beverages. Thus, they could be an important source of several metal ions. Analysis of certain elements in wines is of special interest due to their toxicity in case of excessive intake as well as the effect they seem to have on the organoleptic properties of wine. A typical example is copper, which is both an essential and a potentially toxic element for humans when in excess. Several elements, including Cu, Fe, Al, and Zn, contribute to haze formation and sometimes taste effects (7). Determination of other elements, such as Pb, As, or Cd is of considerable importance due to their potential toxic effects. Moreover, the content of some metals can be used for the identification of the area from which the wine comes.

Address correspondence to Krystyna Pyrzyńska, Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland. E-mail: kryspyrz@chem.uw.edu.pl

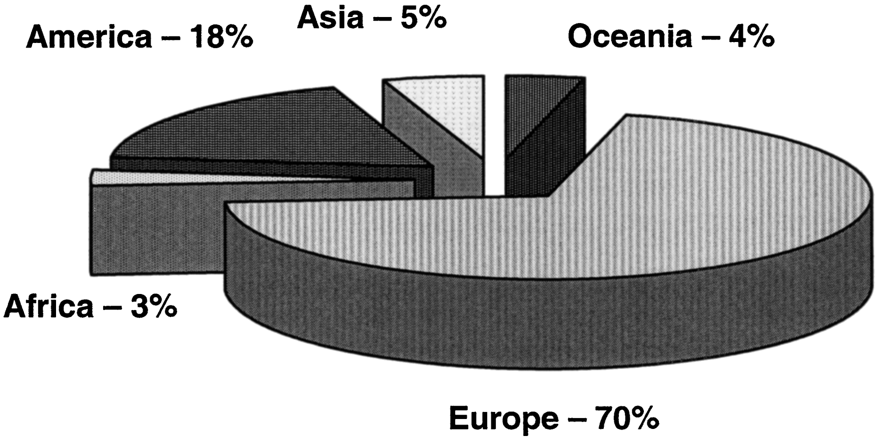

FIG. 1. Word wine production in 2001 (1).

The aim of this article is to present the main analytical methods applied for the determination of metal contents in wine samples. Some problems with sample preparation and validation of the method are also discussed.

SOURCE OF METAL IONS

The analysis of trace metals in wine is of great importance for quality and authenticity control of wine, metals bioavailability, and toxicity. The presence of these elements can influence the wine making process or can change the taste and quality of the final product. The content of metals in wine can be attributed to natural sources (the atmospheric deposition of airborne particulate matter on grapes and transfer of metals from the soil *via* the roots to the grapes and finally to wine) and to contamination during the wine-making process.

Potassium and calcium are natural components of the grape and their concentration in wine is a reflection of the grapevine status in the final stages of berry ripening. A high potassium level could result in the precipitation of potassium hydrogen tartrate. In the wine-making practice, betonite is usually applied to achieve and stabilize its clarity and to prevent the precipitation of tartaric salts in bottled wines, particularly white types. The natural content of calcium can be affected by the addition of $CaCO_3$ for deacidification and an elevated level of this metal also can lead to the onset of calcium tartrate precipitation.

TABLE 1
List of the 10 Leading Wine Producer and Consumer Countries in 2001 (3)

Production	Share (%)	Consumption	Share (%)
France	19.9	France	15.4
Italy	19.0	Italy	13.9
Spain	11.4	United States	9.7
United States	7.4	Germany	9.0
Argentina	5.9	Spain	6.4
Australia	3.8	Argentina	5.5
Germany	3.4	United Kingdom	4.6
Portugal	2.9	China	2.7
South Africa	2.4	Russia	2.5
Chile	2.1	Romania	2.2

Aluminum is also a natural component of grapes, but the concentration in both grape juice and wine is elevated by the use of betonite and, to less extent, from contact with aluminum surfaces. Increases from a juice concentration of around 0.1 mg/L to 1 mg/L in the finished wine have been observed (8). Pennington and Jones (9) reported that concentration of this metal in German wines ranged from 0.63 to 1.12 mg/L. According to their study, aluminum is mostly combined with tartaric acid and other organic acids. The mean Al concentration for red wine preserved in oak barrels it was 915.5 μg/L, whereas for those preserved in cement barrels it was only 425.6 μg/L (10). On the basis of aluminum levels found in wine samples and the annual consumption of wine in Spain, Lopez et al. (10) estimated that wine represents 82% of the total Al dietary intake from all alcoholic beverages.

Copper may be carried over into grape juice from the use of copper-based vineyard sprays, although the concentration in freshly fermented wine is generally low owing to the ability of dead yeast cells to take up this metal (11). The main source of copper in finished product is a consequence of the practice of $CuSO_4$ addition for the removal of hydrogen sulfide and other sulfidic off-odors. High residual copper can contribute to an enhanced rate of oxidative spoilage, which ultimately results in the browning of the wine. The phenomenon called "browning," particularly in white wines, constitutes one of the principal enological problems for wine producers and some metal ions (Cu, Fe, Mn) are the activators of this process. In organoleptic terms, this phenomenon translates into a process of continuous oxidation, a loss of aromatic freshness and, in the final stages, the appearance of precipitates of condensed phenolic material in the bottled wine. To minimize the incidence of these problems, it is generally recommended to maintain copper concentration below 0.3–0.5 mg/L (11). Benitez et al. (12) showed that application of ion exchange resins is extremely effective in lowering the content of copper, iron, and manganese in white wines. The treated wines presented lower polyphenolic and aromatic profiles and exhibited a reduced susceptibility to undergo browning. However, significant losses were observed in the organoleptic characteristics of the treated wines.

Iron is similar to copper in that high concentration (>5 mg/L) also will induce haze formation and oxidative spoilage. Addition of potassium hexacyanoferrate(II) eliminates part of iron content and significantly decreases concentration of other metals that also participate in the browning of white wines (13). As the use of this reagent carries with it the danger of the possible transformation of any excess into highly toxic cyanides, the ion exchange methods have been proposed for lowering the content of iron (12).

The content of other metals in wines reflects differences in grape variety, environmental factors in the vineyard (soil, climate), and the wine-processing technique. A possible source of metals can be attributed to residues of agrochemical products used as insecticides and fungicides as well as fertilizers, which contain salts of metals. A significant amount of metals is due to contact with the apparatus used in the wine production and packaging processes. Long maceration at excessively high temperatures probably causes a through extraction of metals from must. Technological features (pressing), chemical factors (alcohol content, must acidity), and physical parameters such as temperature also could affect metal extraction. On the basis of the analysis of chromium concentrations for different vintage wines of the same vineyard and winery, Cabrera-Vique et al. (14) found that Cr content significantly increased with the age of the wine. This could be the result of contamination during storage by stainless steel or after bottling by chromium oxides used for pigmentation of the bottle during the aging process.

Lithium is present in wine due to plant root uptake or to storage in glass bottles. Considering its very low natural concentration (5–50 μg/L), this metal ion is used in Italy as a denaturing agent, to mark wines not allowed for consumption (15).

Lead contamination of wine is a problem dating back to ancient times. The Romans were known to use lead vessels for storage purposes and lead acetate as a sweetener to improve acid-tasting wines (16). Lead alloys have been widely used in wine cellars due to the ease with which this metal can be mechanically formed. It has been estimated that 70% of the Pb intake by humans comes from food and drinks, and wine is the alcoholic beverage that shows the highest level of lead. An excess of ingested lead may present a real health hazard affecting both the nervous system and the biosynthesis of hemoglobin (17). In response to this health concern, the threshold limit value of Pb in wine has been reduced progressively by the OIV, and is at present 200 μg/L. The content of lead in wine may be explained by natural sources (atmospheric deposition on grapes and/or intake from groundwaters and soil) as well as those related to the production processes. Elevated Pb levels have been attributed to several possible contamination sources—leaded gasoline, tin-lead capsules used to cover the bottle neck, and brass alloys (16, 18, 19). Analysis of lead in several thousand French wines from 1951 to 1991 showed that atmospheric pollution associated with automobile emission was not a significant source of lead (16, 20). It also has been shown that the presence or absence of tin-leaded capsules as well as the state of their corrosion had only a very minor influence. There has been some evidence to suggest that brass is the main contamination source. Moreover, it is clear that the average lead concentration in wine has decreased considerably within recent years where measurements have been made, probably due to replacement of brass faucets by stainless steel.

Platinum due to its inertness was considered harmless for a long time. However, the recent widespread application of catalytic converters for automobile exhaust (which contain Pt along with Pd and Rh) is the most important source of the environmental impact of platinum. While the benefits from their use are obvious, recent studies have shown increasing Pt content in different environmental samples (21–23). According to its chemical relationship with nickel and palladium, Pt possesses a relatively high allergic potential. This metal can enter the food chain either through deposition of Pt-containing particles or by uptake from groundwater and soil. The evaluation of platinum content in different plants grown on contaminated soils (collected from areas adjacent to a German highway) showed a measurable transfer of metal from soil to plants (24). Wine samples served as an example for following the path growth, continuing with the fermentation process of grape juice, and resulting in the final product. Vineyard locations near roads with high traffic showed noticeably higher mean values for platinum content (Figure 2) (25).

SAMPLE PREPARATION

The more sensitive and accurate that analytical techniques have become, the more attention has been paid to sample preparation. It is well known that this step is very important in the whole analytical protocol. The detection limit of advanced analytical techniques is practically established by the attainable blank values which are dependent on the sample preparation stages. Within the past years the limits of detection have been improved by reducing the analytical blank (application of very pure reagents and advanced vessel materials), by improving the signal-to-noise ration (with more efficient sample oxidation), and by diluting the decomposed residue as little as possible. A possible source of error during the preparation step is sample contamination and an analyte loss, which can be related to the material used for the vessels involved in the decomposition process as well as storage. Buldini et al. (26) showed the average content of current elements in the materials most commonly applied for decomposition and storage vessels.

Aceto et al. (27) suggested the addition of nitric acid to lower pH (up to 1.5) of wine samples before their direct analysis

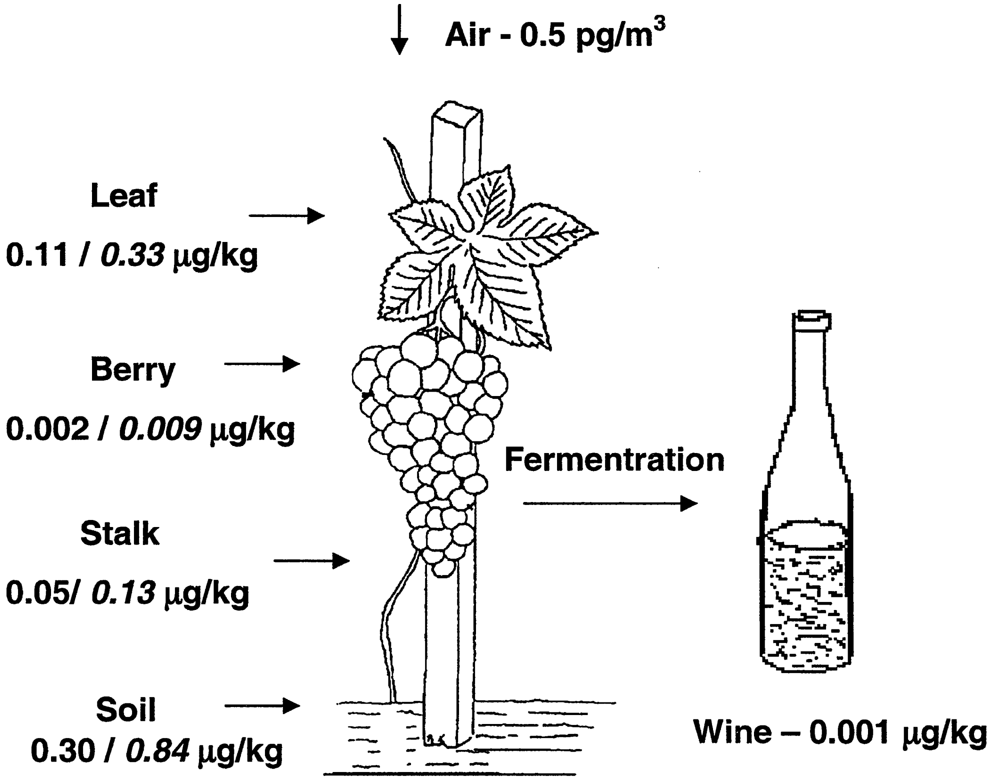

FIG. 2. Content of platinum in different parts of grape growing on traffic-free field path/*near high-traffic roads* (year of vegetation, 1991) (24).

by atomic spectroscopy techniques. Sample acidification could prevent the fermentation process and sorption of metals onto the walls of containers. Under such conditions, metal ions are released from ligands present in wine allowing atomization of analytes from free ions.

The matrix components can cause additional interferences which change the signal intensity during measurements. Wines contain ethanol in addition to a variety of inorganic and organic substances, sometimes at concentration level up to 1%. For this reason sample decomposition prior to analysis is often necessary, particularly when metal contents are close to the method's detection limit. Moreover, for the anodic stripping voltammetry method, which has been applied for determination of total metals content and their "labile" fractions, sample digestion is often required for removing the interfering substances prior to the measurement step. Wet digestion in the closed systems with the addition of reagents to oxide organic matrix is often applied for decomposition of wine samples (28–38). Usually the samples were treated with nitric acid and hydrogen peroxide. In some cases, vanadium(V) oxide as catalyst was employed (10, 30, 33).

The long time required to perform a total sample decomposition has been shortened with the use of microwave energy (29, 30, 40–44) or UV photolysis (44–46). The UV-irradiation procedure provided more precise results for determination of lead in Port wines from Portugal than those obtained by high-pressure microwave digestion (44). To overcome matrix-induced effects, some authors have simply diluted the wine samples (26, 46–50) or removed the alcohol by evaporation to dryness, following the dissolution of the residue in nitric acid (51, 52). A preliminary clean-up procedure, involving the application of C_{18} solid phase extraction (SPE) cartridges, was necessary in order to retain organic components responsible for long after run stabilization times in ion chromatography for lithium determination (15).

Preconcentration and separation are needed when the concentrations of analyte elements in wine samples are too low to be determined directly. Lead was enriched on a packed polyurethane foam column, modified by addition of 2-(2-benzothiazolylazo)-*p*-cresol (32) while cadmium was retained as the complex with 2-(5-bromo-2-pyrizylazo)-5-diethylamino phenol on the inner walls of a poli(tetrafluoro)ethylen (PTFE) knotted reactor associated with a flow injection system (38). Extraction procedures have been proposed by Cvetković et al. for selenium (34) and thallium (53) preconcentration from wine samples.

ANALYTICAL METHODS

The analyses for metal ions in wine are undertaken to monitor trace contaminations, to study the changes in a concentration of the elements during vinification, to determine metal legal limits for export purposes as well as their contribution to flavor and wine quality, and to confirm the authenticity of the final product. Several countries have rules restricting metal content in wines, which must be fulfilled by producers to gain right to export to these markets. To study the sources of metals during the wine-making process, analyses were also carried out at different stages of this procedure in grapes, grape skins, seeds, and must (39, 54). The results indicated the influence of the materials (stainless steel, brass, and betonite) used in wine-making equipment of wine cellars with respect to the heavy metals content in the final product.

Among several methods for metals determination, techniques of atomic spectrometry as well as electrochemical methods have been commonly used. Most of the elements present in wine can be determined with these techniques at concentrations ranging from mg/L^{-1} to the μg/L^{-1} level.

Atomic Absorption Spectrometry

The official methods for the determination of metals in wine recommended by the OIV and the American Society of Enologists are essentially based on atomic absorption spectrometry (AAS) due to its selectivity, high sensitivity, and capability for direct measurements.

Alkali metals and alkali earth metals as well as many of the transition metals like Mn, Cu, Fe, or Zn are all atomized with good efficiency using a common air/acetylene flame with typical detection limits in the sub-ppm range (55). For flame atomic absorption spectrometry (FAAS), short-term precision is in the range of 0.1% to 1%. Long-term precision depends on the spectrometer optics; double-beam types are capable of reaching precision of 1–2%, while the long precision for single-beam types is typically in the 10% range. The high concentration of potassium in wines (generally 300–1500 mg/L^{-1}) acts as a natural ionization buffer. This is a critical factor for alkaline metals determination because it causes a decrease in their ionization and a corresponding increase in the signal. Moreover, in the presence of high concentration of phosphate (300–800 mg/L^{-1} as PO_4^{2-}) Ba, Ca, Mg, Sr, and Al may be converted to their refractory phosphates in the flame environment; absorbance is expected to decrease in this case (27). The usual procedure suggests the addition of a releasing agent (normally LiCl) to samples.

Electrothermal atomic absorption spectrometry (ETAAS) is mainly used for trace metals determination in wine samples (10, 14, 29, 30, 33–37, 40, 45, 47). However, during the direct analysis of complex matrixes, the nonspecific background absorption from interfering chemical species often occurs and could cause serious problems. To minimize the unspecific absorption, the application of a continuum deuterium source or Zeeman effect as the background correction system is recommended. The interferences depend mainly on the details of the applied analytical conditions for measurement, and a careful optimization of the temperature programs for the particular elements is needed. Freschi et al. (30) observed that large amount of fumes developed during the pyrolysis step and the accumulation of carbonaceous residue after several firings which strongly affected performance. These drawbacks were minimized by using an additional pyrolysis stage at 400°C.

The considerable volatility of some metals requires their thermal stabilization in the graphite furnace and it could be realized by the application of suitable chemical modifiers. The general aim of chemical modification in ETAAS is the improvement of analytical performance by in situ changing (modifying) the thermochemical behavior of both the analyte and the matrix. Chemical modification is recommended in order to maximum permissible pyrolysis temperature and to minimize interferences as well as to alter carbon-analyte interactions. According to Tsalev et al. (56), different chemical modifiers are effective for the determination of different metals. Presently, compounds of the platinum group of metals appear to be the most effective and universal chemical modifiers, sometimes in combination with $Mg(NO_3)_2$ (57, 58). $Pd(NO_3)_2$ thermally stabilized Cd and Pb in wine samples and enhanced the sensitivity of their determination (30, 40, 47). Mena et al. (33) obtained the best results for cadmium using $NH_4H_2PO_4$ or $Mg(NO_3)_2$ as a chemical modifier. However, Kristl et al. (40) reported that $NH_4H_2PO_4$ increased nonspecific background absorption, which can be ascribed to this modifier also stabilizing the sample matrix, and its application cannot be recommended. The addition of $Mg(NO_3)_2$ or $NaVO_3$ did not influence the thermal stability of chromium in wine samples but both modifiers improved the sensitivity of Cr signals.

The applicability of ETAAS has recently been extended with the development of simultaneous multielement instruments (59). The direct and simultaneous determination of several trace elements in wine samples is particularly advantageous for routine laboratories because of the saving of time and the minimum sample preparation that is required (30, 47). The availability of commercial simultaneous multielement electrothermal spectrometers was utilized for direct lead determination in wine, and bismuth was selected as internal standard taking into account similar physicochemical properties and electrothermal behavior between Pb and Bi (47). Internal standardization become a simple and efficient method to compensate for random and systematic error.

Hydride generation-atomic absorption spectrometry (HG-AAS) is also applied for the determination of some elements (e.g., As, Sb, Se, Pb, Sn, and Bi) in wine samples (32, 60). The important advantage of the HG-AAS technique is the possibility of separation of analytes from nonvolatile matrix components, which should eliminate a number of interferences. The volatile hydrides are usually generated using sodium tetrahydroborate as the reducing agent in an acidic medium. Prior to analysis, the organic matrix must be completely decomposed and all species of a given element oxidized into inorganic ions. This step is performed mainly by a closed-vessel microwave technique because of its efficient sample throughput and the ability to control the reaction parameters such as temperature and pressure (61). To increase the efficiency, a microwave oven is coupled on-line to a flow-injection system (40, 62). High temperatures and long digestion times are essential to assure the quantitative oxidation in samples with a high protein content (63).

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-OES can be used to perform multielement determination in wine, but the detection limit for most metals is in the range of 1 to 10 μg/L^{-1}. This method has considerably wide dynamic range, up to 10^5, making it a more suitable technique for highly concentrated samples or samples with wide concentrations of the analyte. The sensitivity of the ICP-OES method can be improved applying preconcentration of the analyte prior to detection. Cadmium from wine samples was enriched as the complex with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol using a knotted reactor (38). A total enhancement factor of 216 was obtained with pneumatic nebulization. The axial viewing ICP-OES results in lower limits of detection than the radial viewing mode and the careful selection of the operating conditions (e.g., power, carrier gas flow rate) enables the use of a larger linear working range (64). ICP-OES accuracy proved to depend on wine composition, since it showed severe positive interferences from inorganic cations, especially calcium and potassium, and negative interferences from glucose and ethanol, the major organic components of wine (15). Although these interferences are opposite in sign, there is little probability that they can compensate each other due to the large variability of wine composition.

Two of the strengths of the ICP-MS technique are its multielement capability and the low detection limits obtained for most elements. One limitation is the polyatomic interferences produced in the plasma. The polyatomics are formed from elements frequently present in complex matrices and also from argon. Various systems have been described to overcome those isobaric interferences, from modified sample introduction to generation of cold plasma (65). An alternative is to search for an increased resolution of the mass analyzer or to use the collision cell (66).

The introduction of large amounts of volatile solvents can cause plasma instability due to energy withdrawal, formation of carbon-containing molecular species, and carbon deposition on the torch and sample cones (42, 67, 68). To correct for signal variations, a sample either must be pretreated to reduce its carbon content or analyzed along with matrix-matched standards or with appropriate internal standards. An internal standard should be selected with consideration to the type of sample and the ionization efficiency of the element. Among investigated different standards (Se, Y, In, and Sb) for arsenic determination, indium compensated adequately for matrix effects caused by the wine sample (46). Employing matrix-matched standards is extremely difficult, particularly for samples containing unspecified or variable amounts of carbon (e.g., alcoholic beverages). A microscale flow-injection system employing a microconcentric nebulizer, which incorporates a desolvatation membrane to remove the organic solvent from the aerosol, was used to reduce signal enhancement effects caused by alcohol (46). However, this nebulization system did not overcome matrix effects from other organic and inorganic components of the wine samples (28). Similar problems were observed in determination of lead in port wines from Portugal (68). Port wine as an aged fortified wine is very rich in alcohol, particles in suspension, and polymeric organic compounds, particularly sugars. Alcohol caused suppression of the signal and its instability, while the polymeric matter caused blockage of the injector tube and cones of the ICP, preventing the flux of the analyte ions to the MS detector. The maximum dilution allowed on account of sensitivity (10 times) was not sufficient to reduce either alcohol or sugar to acceptable levels. Thus, decomposition of the sample matrix was necessary and it was found that the UV-irradiation procedure provided more precise results than those obtained after high-pressure microwave digestion.

For rapid analysis and a high sample throughput the procedure of a semiquantitative mode is advantageous (28, 43, 50, 69). This type of analysis is based on a precalibrated internal response for all elements, which can be updated with a single-point calibration and can lead to rough estimates of true concentration values with deviations up to a factor of 2. Comparison of concentrations measured for Mn, Fe, Ni, Cu, Zn, Rb, Sr, and Pb by ICP-MS with In as internal standard and by total reflection X-ray fluorescence spectrometry (TXRF) as an independent method indicated that the semiquantitative approach was sufficiently accurate (28). Thus, this mode of analysis can be an important tool for comparison of elemental compositions of different wines, for instance, for provenance testing.

Electrochemical Methods

The electrochemical stripping methods of anodic stripping voltammetry (ASV) and stripping potentiometry (PSA) are very useful for determination of trace levels of some heavy metals in wine samples (11, 31, 48, 70–72). The deposition step in both of these techniques is similar, but the stripping step is quite different. In the PSA technique, the reduced analytes are reoxidized chemically from the amalgam and the change of potential with time is recorded. Dissolved oxygen and mercury ions, either together or separately, are commonly used for chemical reoxidation. In ASV, stripping is achieved electrochemically; the metal at its characteristic potential is reoxidized from the amalgam state resulting in a flow of measured current. However, ASV requires sample decomposition to remove interfering substances, while stripping potentiometry is not affected by the adsorption of organic compounds at the electrode surface. This

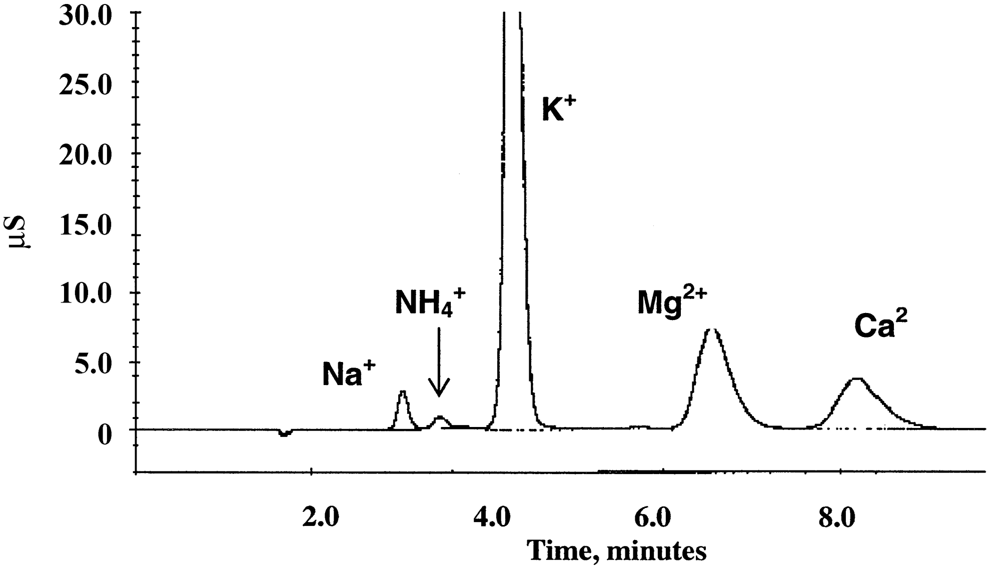

FIG. 3. Ion chromatogram of a red wine. Dionex IonPac CS12A (250 × 4.6 mm) analytical column and IonPac CG12 guard column. Eluent: 20 mM methanesulfonic acid, flow rate 1 mL/min^{-1}, 25 μL injection volume.

allows the direct measurement and makes PSA an ideal technique for speciation assessment.

It must be noted that electrochemical stripping procedures discriminate between the labile and inert fraction of the particular metal, whereas the application of ion selective electrodes in potentiometry gives only the results for free and uncomplexed metal ion concentrations. The labile metal concentration determined by electrochemical stripping methods includes contributions from free metal ion and its readily dissociated complexes. The difference between the total and labile concentration is the inert fraction.

Green et al. (70) and Wiese and Schwedt (71) noted that the sample dilution factor was a critical parameter in the determination of copper. For wines analyzed initially with a dilution factor of 8 to minimize matrix effect, the total Cu concentration was always underestimated by PSA compared to FAAS. Good agreement of both methods was obtained by decreasing the dilution factor up to 4 and keeping copper concentration in the test solution always higher than 50 μg/L^{-1}.

Chromatographic and Electrophoretic Methods

Ion chromatography (IC) with conductivity detection, which is commonly used for determining alkaline and alkaline earth metals in different types of samples, was also applied for quantification of these cations in wine (15). This method is less subject than ICP-OES to interferences from other matrix components since the analyte peaks can be isolated quite well. Aqueous methanesulfonic acid at a concentration of 20 mM and a flow rate of 1 mL/min^{-1} was used as eluent. It was found that the accuracy of IC determination conditions was less sensitive to wine composition than to instrumental conditions, which can be effectively controlled (15). However, a preliminary clean-up procedure with SPE C_{18} cartridges had to be adopted to separate matrix components responsible for long stabilization times. Figure 3 shows the chromatogram of the red wine sample with well-resolved peaks for sodium, ammonium, potassium, magnesium, and calcium ions (73).

Capillary zone electrophoresis (CZE), as an alternative technique to IC, can be readily applied to the analysis of both cations and anions. The content of some metals (Na, K, Ca, Mg, Mn, and Li) in a variety of red wines produced in different geographical zones of Galicia (northwestern Spain) was determined in a single run by CZE (74). The running buffer solution contained 6.5 mM α-hydroxyisobutiric acid and 2 mM 18-crown-6-ether at a final pH of 4.5. Samples were injected using hydrostatic injection for 30 s and indirect UV detection at 214 nm was applied. The developed method showed good precision for the migration time of the analytes (0.09–0.209% RSD) and for the peak area (2.5–3.4% RSD).

Quality Assurance

The most efficient way for the quality control of the analytical results is through the analysis of Certified Reference Materials (CRM). However, reference wines with certified concentrations of minor, trace, or ultratrace elements are not available. Wines with certified total lead concentration, such as BCR C (dry white), BCR D (sweet), and BCR E (red), from the Standards Measurement and Testing Program (Community Bureau of Reference, Brussels, Belgium) are now out of stock.

The accuracy of the published procedures for determination of metals in wine were usually checked using recovery assays by adding known amounts of selected metals to the different randomly chosen samples prior to the digestion step and processing the samples in the same way as experimental wine

TABLE 2
Content of Selected Metals in Wines Determined by Different Methods

Metal	Type of wine	Range content	Ref.
FAAS			
Na*	Australian, white	5–118	(55)
K*		265–1100	
Mg*		78–718	
Ca*		30–120	
Fe*		0.1–4.0	
Cu*		0–1.8	
ETAAS			
Al*	Spanish, white	0.07–1.25	(10)
	Spanish, red	0.19–1.68	
Cr	French, white	6.6–43.9	(14)
	French, red	7–90	
Cd	Brazilian, red	0.03–0.19	(30)
Pb	and white	10.3–55.0	
Cd	Spanish	0.1–15.5	(33)
Se	Macedonian	0.79–0.93	(34)
As	California, red	9.7	(35)
	French, white	2.7	
	German, white	7.5	
V	French, red	10.7–447	(37)
	California, red	4.6–183	
Cd	Slovenian	0.2–0.5	(40)
Cr		5.2–23.1	
Pb		18.4–31.3	
HG-AAS			
Sb	Spanish	up to 11.25	(41)
ICP-OES			
Cd	Argentinian, white	0.80–2.16	(38)
	Argentinian, red	0.37–1.29	
ICP-MS			
Mn*	German, red	0.95–1.23	(28)
Fe*		6.69–7.45	
Ni		54–60	
Cu*		2.74–3.39	
Zn*		6.01–10.9	
Sn		16–71	
Ce		0.5–17.6	
Pb		324–477	
As	French, white	9.7–10.4	(46)
	French, red	12–13.2	
	Spanish, white	7.2–7.9	
Li	Spanish, red	18.9–44	(50)
Mg*		77–98	
Al*		0.8–1.36	
Mn*		0.20–0.94	
Co		2–4	
Cd		0.21–0.64	
Hg		0.7–0.13	
Tl		0.08–0.26	
PSA			
Cd	German, red	0.003–0.98	(31)
Pb	and white	4–254	
Cu		0.05–3.94	
Cu	Australian, white	0.23–0.67	(71)
CZE			
Li	Spanish, red	19–85	(74)
Na*		1.0–67.7	
K*		83.9–1883	
Mg*		16.8–85.8	
Ca*		22.8–92.7	
Mn*		0.4–1.2	

Note. Metals with $*$ expressed in mg/L^{-1}; all other metals expressed in μg/L^{-1}.

Abbreviations: FAAS, flame atomic absorption spectrometry; ETAAS, electrothermal atomic absorption spectrometry; HG-AAS, hydride generation-atomic absorption spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; PSA, anodic stripping potentiometry; CZE, capillary zone electrophoresis.

samples (10, 29, 33, 35, 39, 40, 46, 47, 50, 53). In some cases, the results of measurement by direct analysis were compared with those obtained after acid decomposition (30, 31, 35, 37, 40, 45). Additionally, the proposed methods were validated by comparison with other analytical techniques, for example, TXRF or neutron activation analysis (28, 34, 36, 38, 43, 48, 50).

The validation of ICP-MS determination has been evaluated by intercomparison using different nebulization systems (51) or by analysis of reference materials different from wines but containing a similar level of investigated metals (75). Some authors prepared "house-hold reference material" that has the mean composition of a normal wine (10). Comparison of the external calibration with standard addition methodologies also has been applied (45, 46).

The Institute for Reference Materials and Measurements organized an interlaboratory comparison program (the International Measurement Evaluation Program, IMEP-16) focused on the evaluation of measurement performance for the determination of the Pb mass fraction in a commercial red wine (39). One hundred twenty-nine laboratories from 33 countries participated in this IMEP-16 program. Lead concentration in the average test samples was certified using the combination of a straightforward microwave sample digestion protocol and inductively coupled plasma isotope dilution quadrupole mass spectrometry as a primary method of measurement. The results were spread over a broad range of values (from 1 up to 2900 μg/L^{-1}) and nearly one-third were outside the ±50% uncertainty region. Underestimation of the uncertainty statement attached to the measurement result was clearly the problem for many participants, irrespective of the self-declared experience and the type of analytical technique applied. The results obtained using ETAAS method (recommended in EC Regulation 2676/90) were not significantly different from those obtained using other techniques. Nearly 13% of the reported results were obtained with ICP-based techniques (below 5% for ICP-MS and about 8% for ICP-OES). For comparison, the results obtained by 14 National Measurement Institutes in the frame of the pilot study organized by the Comitè Consultatif pour la Quantitè de Matière on identical samples were in very good agreement, well within ±10% of the IMEP-16 reference value (39).

The comparison of the performance of the methodologies used in two distinct laboratories for multielement analysis in three different wine samples was published (44). These laboratories have been using ICP-MS apparatus (quadrupole mass analyzers) of different brands as well as different methodologies of wine preparation for analysis. In the first laboratory a pretreatment by UV-irradiation was performed, while in the second a microconcentric nebulizer was used for direct analysis of the wines. The two methodologies provided similar values for detection limits and similar precisions with RSDs of 0.5–5% for most of the elements. Comparable results were found at both laboratories for Li, V, Co, Ni, Cu, Zn, Rb, Sr, Ba, and Pb; the differences were lower than 10%. For the rare earth elements (REEs) arsenic and gallium, some discrepancies were observed. However, the differences for REEs were still in the acceptable range due to the sub-ppb levels involved.

SPECIATION/FRACTIONATION ANALYSIS

Until now, the research has been focused mainly on the determination of the total amount of metals present in wine or any food or beverage regardless of what species were present. There is considerable evidence that metal bioavailability and toxicity are not governed by its total concentration. Instead, it is regulated by the chemical forms (whose identification and characterization is called speciation) in which it is present at the site of absorption to overcome the intestinal barrier. Moreover undesirable turbidities which occur in wines, caused by heavy metals such as iron and copper, seem to be more related to the type of species present (11). Metals may exist in wines as free ions, as complexes with organic acids as well as species with large molecules of pectic polysaccharides, peptides, or proteins and polyphenols, namely, the condensed tannins. The speciation of metals with biological ligands is a subject of increasing interest since complexation may reduce their toxicity and bioavailability.

Most of the reported studies in wines are related to Pb species (11, 18, 19, 32, 49, 72, 76–78). Wine is belived to be a significant source of this metal in the human diet even through the lead content of wines has decreased considerably over the past 30 years. The speciation analysis of ionic organolead compounds in wines was proposed by Łobinski et al. (18, 76). These compounds may arise in wine due to the natural biomethylation processes from inorganic Pb present during the fermentation. The analytes were extracted as diethyldithiocarbamate complexes into hexane and propylated with a Grignard reagent. The derivatized extract was analyzed by capillary gas chromatography/ microwave-induced plasma optical emission spectrometry. Some wines from southern France showed elevated concentrations of Me_3Pb^+ (8.1–112 ng/L^{-1}). Ethyllead compounds generally were not found in wines produced in rural areas away from the highway. Wines made from grapes grown close to industrialized centers showed an elevated content of triethyllead (Figure 4).

Attempts to determine "electrochemically labile" lead, which is supposed to be bioavailable and thus potentially toxic, by ASV and PSA methods, showed little or no labile fraction at its natural pH of 3.2–3.4 (11, 70, 72). Fractionation patterns using ultrafiltration demonstrated that the behavior of Pb is significantly different from the other metals (79). In red wine there was a sudden decrease between 100,000 and 30,000 nominal molecular weight cutoff (NMWCO), while in white wine a gradual decrease in the lead concentration was observed by 100,000 NMWCO ultrafilter. According to McKinnon and Scollary (79), lead is bounded in red wine by a polymeric species, most probably, tannin.

More recent work has attempted to identify the ligands primarily responsible for lead complexation in wines (77). Application of size-exclusion high-performance liquid chromatography with on-line ICP-MS detection evidenced that this metal is associated with a dimer of a pectic polysaccharide, rhoamnogalacturonan II (RG-II), with molecular mass on the order 10 kDa. Other minor species with apparent molecular masses in the range of 500 to 3000 Da were not identified. The fact that lead can be found in the naturally occurring RG-II isolated from plant cell walls indicates the potentially vital role of this polysaccharide in the Pb uptake by plants. The excess of unbounded RG-II in wine (its complexation capacity equals *ca.* 2 mg/L^{-1}) apparently can be used to complex lead ions taken with other foodstuffs. However, in order to claim a detoxification capability of this compound, the stability of the Pb-RG-II complex in the gastrointestinal system needs to be investigated.

The combination of potentiometry, ASV, and kinetic photometry permitted an "operationally defined" speciation of copper in wine (71). It was found that the content of free copper ions and its labile species is in the range of 3.3–31% and 33–86%, respectively. However, the distribution of metal species in investigated wine samples cannot be explained by the total copper and protein concentrations. Other matrix components, like tannins, must have an influence.

Evaluation of iron species in wines is of major importance either to the changes in stability it may cause and its effect on

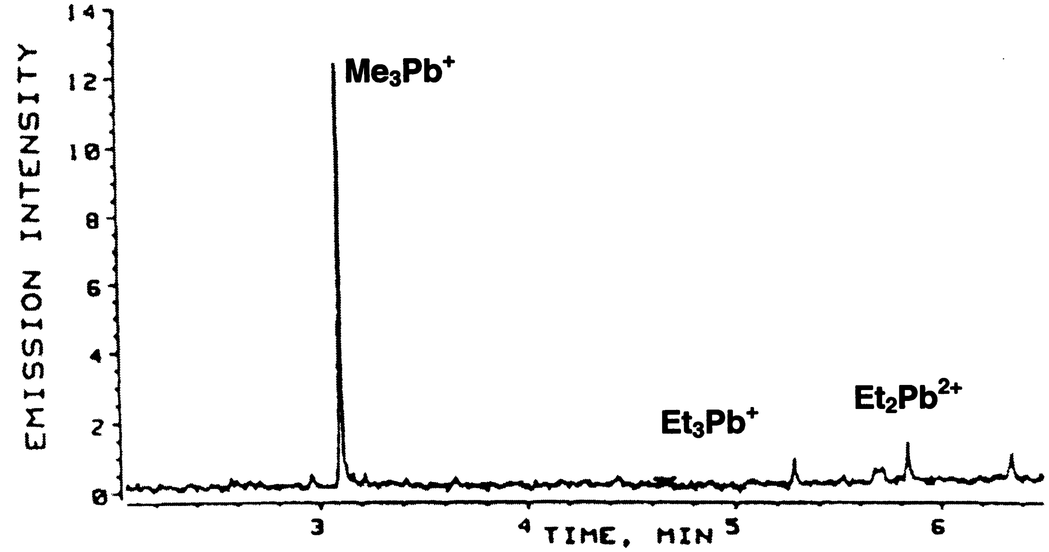

FIG. 4. Chromatogram of the organolead compounds in a white wine sample. Peaks: Me_3Pb^+ (48.0 ng/L^{-1}), Et_3Pb^+ (3.6 ng/L^{-1}), Et_2Pb^{2+} (6.3 ng/L^{-1}) (76).

the oxidation and wine aging. When protected from air, wine exhibits reducing characteristics and iron is present mostly as Fe(II). Fe(III) is present in much lower concentrations and usually complexed with citric and oxalic acids, although it is capable of promoting precipitation phenomena with phosphate and tannin depending on its content. Ajlec and Štupar applied different solid sorbents for separation of iron compounds in wine (80). In 0.1 M HNO_3 medium ionic species of Fe(II) and Fe(III) were sorbed on cation-exchange resin Dowex 50 W × 8, while uncharged organic iron was passed through the column. The sorbed species were then eluted with 2.25 M hydrochloric acid and determined by AAS. For separation of negatively charged citrate complexes of Fe(III), anion-exchange resin Dowex 1 × 8 was used and uncharged organic iron was enriched on an Amberlite XAD-2 column. It was found that in bottled wines the content of Fe(III) was higher (75.8–94.2%) than in local wines from small growers (32.0–82.6%). For determination of Fe(III) and total iron in Portuguese table wines, sequential injection analysis with AAS detection was applied (81). Fe(III) was extracted as its thiocyanate complex with methylisobutylketone. Sixty percent to 87% of iron in Hungarian wines was found in colloid solution as this fraction was removed by filtration through a 0.2 μm pore membrane filter (82). Enologically the colloidal fraction is very important, because it destroys the quality of wines.

A survey of butyltin compounds in Chinese alcoholic beverages purchased from retail markets was carried out by the headspace solid-phase microextraction followed by gas chromatography coupled with flame photometric detection (83). Organotin compounds have been used extensively as polyvinyl chloride stabilizers, wood preservatives, and agrochemicals. The levels of monobutyltin (MBT) and dibutyltin (DBT) ranged from <0.016 to 5.87 and from <0.0022 to 33.26 μg/L^{-1} as Sn, respectively. The results indicated that dry wines generally contained more DBT than sweet wines. The level of dibutyltin decreased with time; after 110 days of storage the concentration of DBT was 27% of the original content. It was presumed that butyltin compounds degradate during the storage period.

A scheme was proposed for fractionation of wine components and determination of Fe, Cu, and Zn in different fractions (84). The charge of metal species was established using cation and anion exchangers. Amberlite XAD-8 sorbent was applied for separation of wine polyphenols in complexes with wine proteins and polysaccharides. Labile species of Fe(II) was separated by sorption of its complex with 1,10-phenantroline. The analytical procedure used for wine component fractionation is presented in Figure 5. The results indicated that almost 30% of Fe was complexed with polyphenols and proteins in untreated wines, while the same fraction equals *ca.* 20% in finished and bottled wines. Less than 5% of iron was found in the polysaccharide fraction. Approximately 50% of Cu was present as labile ion and organically bound copper was found mainly in the polyphenol/protein fraction while less than 4–5% in the polysaccharide fraction. More than 60% of zinc in investigated wines was present as a positively charged labile ion and less than 15% was retained on the XAD-8 sorbent as a complex with polyphenols.

CLASSIFICATION OF WINES USING METALLIC CONTENTS

It is of considerable interest to be able to determine the geographic origin and varieties of grapes. Wine is one of the most widely consumed beverages in the world and the evaluation of its quality is important for manufacturers, merchants, and consumers. On the one hand, customers are interested in

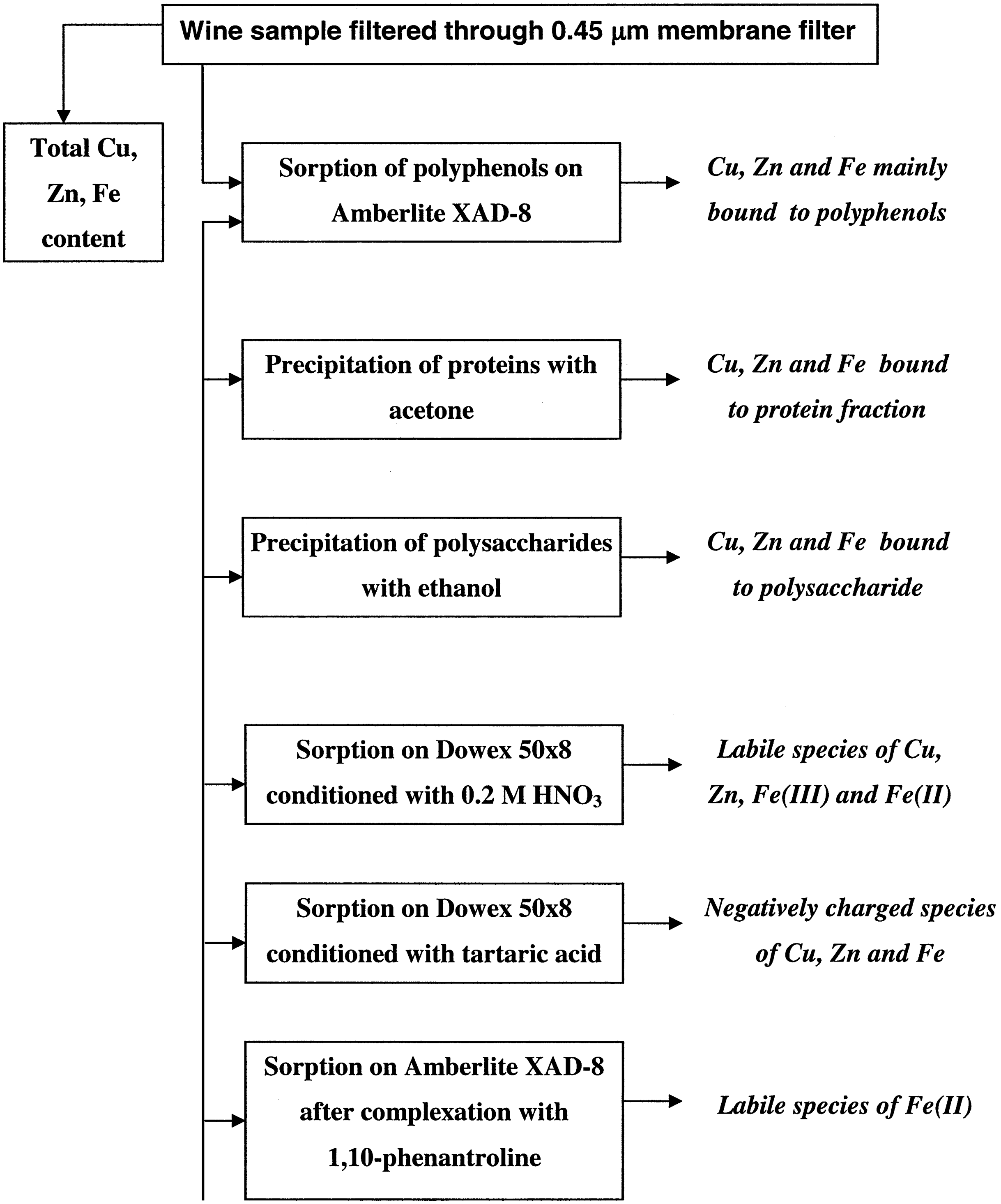

FIG. 5. Analytical procedure for wine component fractionation and determination of Cu, Fe, and Zn in different fractions (84).

obtaining reliable information on wine-growing regions and grape varieties, because the connections always exist between these attributes and the quality of wines. On the other hand, the identity of wines has to be verified by testing laboratories and offices to prevent fraud. Historically, the quality or geographical origin of wine was determined only through tasting by wine experts. Recently, more advanced instrumental techniques have become available.

In order to achieve effective fraud control different physicochemical parameters have been used, the values of which allow establishment of a link between the wine samples and their geographical place of origin. Some studies combine different kinds of physicochemical parameters. Content of volatile compounds, phenols, metals, anthocyans, and organic acids was applied to characterize red wines from different parts of France (85) and to distinguish German wines from the Rhein-Pfaz and Mosel region (86).

A simple approach is to measure traces of strontium in wine to track its origin. Among stable and naturally occurring strontium isotopes, only ^{87}Sr is radiogenic and its concentration gradually increases in minerals due to the radioactive decay of the ^{87}Rb isotope. Thus, ^{87}Sr/^{86}Sr isotope ratio of wine reflects that of the soil on which the grapes were grown and it is a promising fingerprint of wine origin (87, 88). The advantage of such an approach is that the processes of fermentation and aging do not alter the strontium-isotope ratios in grapes and wine. However, some fertilizers may add strontium to the soil and change the isotope ratio. The results obtained so far are very promising but the analysis is not simple and fast, and it is just too expensive to be routine.

The content of metals may provide good differentiation about wine geographical origin due to the direct relationship with soil composition. Although it does require a necessary prerequisite that the concentrations within a group of elements or species depend only or at least mainly on the composition of the soil in the cultivation region but will not be significantly influenced from wine production, transport, or storage. The differentiation was carried out using major, trace, and ultratrace elements (51, 69, 87–95). The group of the REEs gained interest because, owing to their chemical similarity, they offer a chance to avoid problems of selective changes of their concentration distribution as can serve a database for the application of chemometrical methods (51, 69, 87). It was shown that the REEs distribution in the soil is reproduced in the roots, leaves, grapes, and finally in grape juice with only minor modification (96).

German researchers studied 165 wines from six grape-growing regions (91). It was shown that testing just the three elements—barium, silicon, and vanadium—and three organic compounds, it was possible to correctly identify the geographic background of as many as 90% of the wines tested. According to the authors, the tested organic compounds reflect wine-making procedure: the content of 2-furancarboxaldehyde is determined by aging, 4-methyl-1-pentanol reflects the amount of fermentation, and 1-octanol reflects the wine's maturity.

Eleven elements were used to characterize sweet and dry wines from the islands of El Hierro, La Palma, and Lazarote (Canary Islands, Spain) (94). Among the three islands significant differences in mean content were found with the exception of iron and copper, metals whose content is influenced by the technology of wineries. Cluster analysis and principal component analysis showed differences in wines according to the island of origin and ripening state of grapes. Linear discriminant analysis using Rb, Na, Mn, and Sr, the most discriminant elements, gave 100% recognition ability and 95.6% prediction ability.

REFERENCES

1. D. M. Goldberg and I. L. Bromberg, Health effects of moderate alcohol consumption: a paradigmatic risk factor, *Clinica Chimica Acta* 246 (1996):14–16.
2. M. Gronbaek, M. Deis, T. I. Sorensen, U. Becker, P. Schnohr, and P. Jensen, Mortality associated with moderate intakes of wine, beer or spirits, *British Medical Journal* 10 (1995):1165–1169.
3. G. Dutuc-Rosset, Report on the state of vitiviniculture in the word, presented at XXVIII Congress, Bratislava, Slovakia, June 24, 2002.
4. S. E. Ebeler, Analytical chemistry: unlocking the secrets of wine flavor, *Food Reviews International* 17 (2001):45–64.
5. A. Peña-Neira, T. Hernandez, C. Garcia-Vallejo, I. Estrella, and J. A. Suarez, A survey of phenolic compounds in Spanish wines of different geographical origin, *European Food Research and Technology* 210 (2000):445–448.
6. M. V. Moreno-Arribas, E. Pucyo, and M. C. Polo, Analytical methods for the characterization of proteins and peptides in wines, *Analytica Chimica Acta* 458 (2002):63–75.
7. K. A. Riganakos and P. G. Veltsistas, Comparative spectrophotometric determination of the total iron content in various white and red Greek wines, *Food Chemistry* 82 (2003):637–643.
8. M. Laroque, J. C. Cabanis, and L. Vian, Determination of aluminium in wines by direct graphite furnace atomic absorption spectrometry, *Journal of AOAC International* 77 (1994): 463–466.
9. J. A. T. Pennington and J. W. Jones, Aluminum in health: a critical review, ed. H. Fitelman (Marcel Dekker, New York, 1989).
10. F. F. Lopez, C. Cabrera, M. L. Lorenzo, and M. C. Lopez, Aluminium levels in wine, beer and other alcoholic beverages consumed in Spain, *The Science of the Total Environment* 220 (1998):1–9.
11. G. R. Scolary, Metals in wine: contamination, spoilage and toxicity, *Analusis* 25 (1997): M26–M30.
12. P. Benitez, R. Castro, and C. G. Barroso, Removal of iron, copper and manganese from white wines through ion exchange techniques: effects on their organoleptic characteristics and susceptibility to browning, *Analytica Chimica Acta* 458 (2002): 197–202.
13. J. Oszmianski, V. Cheynier, and M. Mountounet, Iron-catalyzed oxidation of (+) catechin in model system, *Journal of Agriculture and Food Chemistry* 44 (1994):712–1715.
14. C. Cabrera-Vique, P. L. Tessedre, M. T. Cabanis, and J. C. Cabanis, Determination and levels of chromium in French wines and grapes by graphite furnace atomic absorption spectrometry, *Journal Agriculture and Food Chemistry* 45 (1997):1808–1811.
15. O. Zerbinati, F. Balduzzi, and V. Dell'Oro, Determination of lithium in wines by ion chromatography, *Journal of Chromatography A* 881 (2000):645–650.
16. A. Kaufmann, Lead in wine, *Food Additives and Contaminants* 15 (1998):437–445.
17. T. Nielsen, K. A. Jensen, and P. Grandjean, Organic lead in normal human brains, *Nature* 274 (1978):602–603.
18. R. Łobiński, J. Szpunar-Łobińska, F. C. Adams, P. L. Teissedre, and J. C. Cabanis, Speciation analysis of organolead compounds

in wine by capillary gas chromatography/microwave-induced-plasma atomic emission spectrometry, *Journal of AOAC International* 76 (1993):1262–1267.

19. P. L. Teissedre, R. Łobiński, M. T. Cabanis, J. Szpunar-Łobińska, J. C. Cabanis, and F. C. Adams, On the origin of organolead compounds in wine, *The Science of the Total Environment* 153 (1994):247–252.
20. K. Rosman, W. Chisholm, S. Jimi, J. P. Candelone, C. Boutron, P. L. Teissedre, and F. C. Adams, Lead concentrations and isotopic signatures in vintages of French wine between 1950 and 1991, *Environmental Research* 78 (1998):161–167.
21. J. Schäfer, J. W. Eckhardt, Z. A. Berner, and D. Stüben, Time-dependent increase of traffic-emitted platinum-group elements (PGE) in different environmental compartments, *Environmental Science & Technology* 33 (1999):3166–3170.
22. K. Pyrzyńska, Monitoring of platinum in the environment, *Journal of Environmental Monitoring* 2 (2000):99N–103N.
23. T. Hees, B. Wenclawiak, S. Lusting, P. Schramel, M. Schwarzer, M. Schuster, R. Dams, D. Verstrache, and E. Helmers, Sources of anthropogenic platinum-group elements (PGE): automotive catalysts versus PGE-processing industries, *Environment Science and Pollution Research* 5 (1998):223–230.
24. E. Helmers, Elements accompanying platinum emitted from automobile catalysts, *Chemosphere* 33 (1996):405–419.
25. F. Alt, H. R. Eschnauer, B. Mergel, J. Messerschmidt, and G. Tölg, A contribution to the ecology and enology of platinum, *Fresenius Journal of Analytical Chemistry* 357 (1997):1013–1019.
26. P. L. Buldini, L. Ricci, and J. L. Sharma, Recent applications of sample preparation techniques in food analysis, *Journal of Chromatography A* 975 (2002):47–70.
27. M. Aceto, O. Abollino, M. C. Buzzoniti, E. Mentasti, C. Sarzanini, and M. Malandrio, Determination of metals in wine with atomic spectroscopy: flame-AAS, GF-AAS and ICP-AES, *Food Additives and Contaminants* 19 (2002):126–133.
28. M. M. Castiňeira, R. Brandt, A. van Bohlen, and N. Jakubowski, Development of a procedure for the multi-element determination of trace elements in wine by ICP-MS, *Fresenius Journal of Analytical Chemistry* 370 (2001):553–558.
29. C. Cabrera-Vique, P. L. Teissedre, M. T. Cabanis, and J. C. Cabanis, Determination of platinum in wine by graphite furnace atomic absorption spectrometry, *Journal of AOAC International* 80 (1997):57–62.
30. G. P. G. Freschi, C. S. Dakuzaku, M. de Moraes, J. A. Nórega, and J. A. G. Neto, Simultaneous determination of cadmium and lead in wine by electrothermal atomic absorption spectrometry, *Spectrochimica Acta Part B* 56 (2001):1987–1993.
31. P. Ostapczuk, H. R. Eschnauer, and G. R. Schollary, Determination of cadmium, lead and copper in wine by potentiometric stripping analysis, *Fresenius Journal of Analytical Chemistry* 358 (1997):723–727.
32. V. A. Lemos, M. de la Guardia, and S. L. Ferreira, An on-line system for preconcentration and determination of lead in wine samples by FAAS, *Talanta* 58 (2002):475–480.
33. C. Mena, C. Cabrera, M. L. Lorenzo, and M. C. Lopez, Cadmium levels in wine, beer and other alcoholic beverages: possible sources of contamination, *The Science of the Total Environment* 181 (1996):201–208.
34. J. Cvetković, T. Stafilov, and D. Mihajlocić, Nickel and strontium nitrates as modifiers for the determination of selenium in wine by Zeeman electrothermal atomic absorption spectrometry, *Fresenius Journal of Analytical Chemistry* 370 (2001):1077–1981.
35. B. T. Kildahi and W. Lund, Determination of arsenic and antimony in wine by electrothermal atomic absorption spectrometry, *Fresenius Journal of Analytical Chemistry* 354 (1996):93–96.
36. S. Galani-Nikdakaki, N. Kallithrakas-Konos, and A. A. Katsanos, Trace element analysis of Cretan wines and wine products, *The Science of the Total Environment* 285 (2002):155–163.
37. P. L. Teissedre, M. Krosniak, K. Portet, J. Gase, A. L. Waterhouse, J. Serrano, J. C. Cabanis, and G. Cross, Vanadium levels in French and Californian wines: influence on vanadium dietary intake, *Food Additives and Contamaminants* 15 (1998):585–591.
38. R. F. Lara, R. G. Wuilloud, J. A. Salonia, R. A. Olsina, and L. D. Martinez, Determination of low cadmium concentrations in wine by on-line preconcentration in a knotted reactor coupled to an inductively coupled plasma optical emission spectrometer with ultrasonic nebulization, *Fresenius Journal of Analytical Chemistry* 371 (2001):989–993.
39. C. R. Quetel, S. M. Nelms, L. van Nevel, I. Papadakis, and P. D. P. Taylor, Certification of the lead fraction in wine for comparison 16 of the International Measurement Evaluation Programme, *Journal of Analytical Atomic Spectrometry* 16 (2001):1091–1100.
40. J. Kristl, M. Veber, and M. Slekovec, The application of ETAAS to the determination of Cr, Pb and Cd in samples taken during different stages of the winemaking process, *Analytical and Bioanalytical Chemistry* 374 (2001):314–204.
41. C. M. Mena, C. Cabrera, M. L. Lorenzo, and M. C. Lopez, Determination of lead contamination in Spanish wines and other alcoholic beverages by flow injection atomic absorption spectrometry, *Journal of Agriculture and Food Chemistry* 45 (1997): 1812–1815.
42. C. M. Almeida and M. T. Vasconcelos, UV-irradiation and MW-digestion pretreatment of Port wine suitable for the determination of lead isotope ratios by inductively coupled plasma mass spectrometry, *Journal of Analytical Atomic Spectrometry* 14 (1999):1815–1821.
43. C. M. Almeida and M. T. Vasconcelos, Advantages and limitations of the semi-quantitative operation mode of an inductively coupled plasma-mass spectrometer for multi-element analysis of wines, *Analytica Chimica Acta* 463 (2002):165–175.
44. C. M. Almeida, M. T. Vasconcelos, M. Barbaste, and B. Medina, ICP-MS multi-element analysis of wine samples—a comparative study of the methodologies used in two laboratories, *Analytical and Bioanalytical Chemistry* 374 (2002):314–322.
45. T. Wierzbicki and K. Pyrzyńska, Determination of vanadium content in wine by GFAAS, *Chemia Analityczna (Warsaw)* 47 (2002):449–454.
46. S. Wangkarn and S. A. Pergantis, Determination of arsenic in organic solvents and wines using nicroscale flow injection inductively coupled plasma mass spectrometry, *Journal of Analytical Spectrometry* 14 (1999):657–662.
47. K. G. Fernandes, M. de Moraes, J. A. G. Neto, J. A. Nóbrega, and P. V. Oliveira, Evaluation and application of bismuth as an internal standard for the determination of lead in wines by simultaneous electrothermal atomic absorption spectrometry, *Analyst* 127 (2002):157–162.
48. Ø. Mikkelsen and K. H. Schrøder, Voltammetry using dental amalgam electrode for heavy metal monitoring of wines and spirits, *Analytica Chimica Acta* 458 (2002):249–256.

49. S. Razic, M. Todorovic, I. Holclajtner-Antunovic, and M. Stoilkovic, Determination of metal traces in wine by argon stabilized d.c. arc, *Fresenius Journal of Analytical Chemistry* 365 (1999):367–370.
50. M. Y. Perez-Jordan, J. Soldevila, A. Salvador, A. Pastor, and M. de la Guardia, Inductively coupled plasma mass spectrometry analysis of wines, *Journal of Analytical Atomic Spectrometry* 13 (1998):33–39.
51. S. Augagneur, B. Medina, J. Szpunar, and R. Łobiński, Determination of rare earth elements in wine by inductively coupled plasma mass spectrometry using microconcentric nebulizer, *Journal of Analytical Atomic Spectrometry* 11 (1996):713–721.
52. J. D. Greenough, H. P. Longerick, and S. E. Jackson, Trace element concentrations in wines by ICP-MS: evidence for the role of solubility in determining uptake by plants, *Canadian Journal of Applied Spectroscopy* 41 (1996):76–80.
53. J. Cvetković, S. Arpadjan, I. Karadjova, and T. Stafilov, Determination of thallium in wine by electrothermal atomic absorption spectrometry after extraction preconcentration, *Spectrochimica Acta Part B* 57 (2002):1101–1106.
54. V. R. Angelova, A. S. Ivanov, and D. M. Braikov, Heavy metals (Pb, Cu, Zn and Cd) in the system soil-grapevine-grape, *Journal of the Science of Food and Agriculture* 79 (1999):713–721.
55. L. Sauvage, D. Frank, J. Starne, and M. B. Millikan, Trace metal studies of selected white wines: an alternative approach, *Analytica Chimica Acta* 458 (2002):223–230.
56. D. L. Tsalev, V. I. Slaveykova, L. Lampugnani, A. D'Ulivo, and R. Georgieva, Permanent modification in electrothermal atomic absorption spectrometry—advances, anticipations and reality, *Spectrochimica Acta Part B* 55 (2000):473–490.
57. A. B. Volynsky, Mechanisms of action of platinum group modifiers in electrothermal atomic absorption spectrometry, *Spectrochimica Acta Part B* 55 (2000):103–150.
58. A. B. Volynsky and R. Wennrich, Mechansms of the action of platinum metal modifiers in electrothermal atomic absorption spectrometry: aims and existing approaches, *Spectrochimica Acta Part B* 57 (2002):1301–1316.
59. M. Hoening and A. Cilissen, Performance and practical applications of simultaneous multi-element electrothermal atomic absorption spectrometry the case of the SIMAA 6000, *Spectrochimica Acta Part B* 52 (1997):1443–1449.
60. C. Baluja-Santos and A. Gonzalez-Portal, Application of hydride generation to atomic-absorption spectrometric analysis of wines and beverages: a review, *Talanta* 39 (1992):329–339.
61. A. Taylor, S. Branch, A. Fisher, D. Halls, and M. White, Atomic Spectrometry Update. Clinical and biological materials, foods and beverages, *Journal of Analytical Atomic Spectrometry* 16 (2001):421–446.
62. L. Schloske, H. Waldner, and F. Marx, Optimisation of sample pre-treatment in the HG-AAS selenium analysis, *Analytical and Bioanalytical Chemistry* 372 (2002):700–704.
63. M. J. Moya, R. Torralba, M. A. Palacios, and M. M. Bonilla, Comparison of three digestion methods for the FI-HG-AAS determination after preconcentration, *Quimica Analitica* 20 (2001):125–130.
64. M. Chausseau, E. Poussel, and J. M. Mermet, Self-absorption effects in radially and axially viewed inductively coupled plasma-atomic emission spectrometry—the key role of the operation conditions, *Fresenius Journal of Analytical Chemistry* 370 (2001):341–347.
65. N. Jakubowski, L. Moems, and F. Vanhaecke, Sector field mass spectrometers in ICP-MS, *Spectrochimica Acta Part B* 53 (1998):1739–1763.
66. M. Iglesias, N. Gilon, E. Poussel, and J. M. Mermet, Evaluation of an ICP-collision/reaction cell-MS system for the sensitive determination of spectrally interfered and non-interfered elements using the same gas conditions, *Journal of Analytical Atomic Spectrometry* 17 (2002):1240–1247.
67. S. A. Lehn, K. A. Warner, Mg, and G. M. Heieftje, Effect of sample matrix on the fundamental properties of the inductively coupled plasma, *Spectrochimica Acta Part B* 58 (2003):1785–1806.
68. I. I. Stewart and J. W. Olesik, Steady state acid effects in ICP-MS, *Journal of Analytical Atomic Spectrometry* 13 (1998):1313–1320.
69. N. Jakubowski, R. Brandt, D. Stuewer, H. Eschnauer, and S. Gortges, Analysis of wines by ICP-MS: is the pattern of the rare earth elements a reliable fingerprint for the provenance? *Fresenius Journal of Analytical Chemistry* 364 (1999):424–428.
70. A. M. Green, A. C. Clark, and G. R. Scollary, Determination of free and total copper and lead in wine by stripping potentiometry, *Fresenius Journal of Analytical Chemistry* 358 (1997):711–717.
71. C. Wiese and G. Schwedt, Strategy for copper speciation in white wine by differential pulse anodic stripping voltammetry, potentiometry with an ion-selective electrode and kinetic photometric determination, *Fresenius Journal of Analytical Chemistry* 358 (1997):718–722.
72. M. T. Vasconcelos, M. Azenha, and V. de Freitas, Electrochemical studies of complexation of Pb in red wines, *Analyst* 125 (2000):743–748.
73. K. Pyrzyńska and M. Biesaga, unpublished results.
74. M. Nùñez, R. M. Peña, C. Herrero, and S. Garcia-Martin, Analysis of some metals in wine by means of capillary electrophoresis. Application to the differentiation of *Ribeira Sacra* Spanish red wines, *Analusis* 28 (2000):432–437.
75. L. Halicz, Y. Erel, and A. Veron, Pb isotope measurement by ICP-MS; accuracy, precision and long time drift, *Atomic Spectroscopy* 175 (1996):186–189.
76. R. Łobiński, W. M. R. Dirkx, J. Szpunar-Łobińska, and F. Adams, Speciation analysis of organolead coumpounds by gas chromatography with atomic spectrometric detection, *Analytica Chimica Acta* 286 (1994):381–390.
77. J. Szpunar, P. Pellerin, A. Makarov, T. Doco, P. Williams, B. Medina, and R. Łobiński, Speciation analysis for biomolecular complexes of lead in wine by size-exclusion high-performance liquid chromatography-inductively coupled plasma mass spectrometry, *Journal of Analytical Atomic Spectrometry* 13 (1998):749–754.
78. C. Marin and P. Ostapczuk, Lead determination in wine by potentiometric stripping analysis, *Fresenius Journal of Analytical Chemistry* 334 (1992):881–886.
79. A. J. McKinnon and G. R. Scollary, Size fractionation of metals in wine using ultrafiltration, *Talanta* 44 (1997):1649–1658.
80. R. Ajlec and J. Štupar, Determination of iron species in wine by ion-exchange chromatography—flame atomic absorption spectrometry, *Analyst* 114 (1989):137–142.
81. R. C. Campos Costa and A. N. Araújo, Determination of Fe(II) and total Fe in wines by sequential injection analysis and flame atomic absorption spectrometry, *Anal. Chim. Acta* 438 (2001):227–233.

82. Z. Murányi and L. Papp, Enological metal speciation analysis, *Microchimical Journal* 60 (1998):134–142.
83. J.-Y. Liu and G.-B. Jiang, Survey on the presence of butyltin compounds in Chinese alcoholic beverages, determined by using headspace solid-phase microextraction coupled with gas chromatography-flame photometric detection, *Journal of Agricultural and Food Chemistry* 50 (2002):6683–6687.
84. I. Karadjova, B. Izgi, and S. Gucer, Fractionation and speciation of Cu, Zn and Fe in wine samples by atomic absorption spectrometry, *Spectrochimica Acta Part B* 57 (2002):581–590.
85. E. M. Diaz-Plaza, J. R. reyero, F. Pardo, and M. R. Salinas, Comparison of wine aromas with different tannic content aged in French oak barrels, *Analytica Chimica Acta* 458 (2002):139–145.
86. M. Ortega-Heras, M. L. Gonzalez-SanJose, and S. Beltran, Aroma composition of wine studied by different extraction methods, *Analytica Chimica Acta* 458 (2002):85–93.
87. C. M. Almeida and M. T. Vasconcelos, ICP-MS determination of strontium isotope ratio in wine in order to be used as a fingerprint of its regional origin, *Journal of Analytical Atomic Spectrometry* 16 (2001):607–611.
88. M. Barbaste, K. Robinson, S. Guilfoyle, B. Medina, and R. Łobiński, Precise determination of the strontium isotope ratios in wine by inductively coupled plasma sector field multicollector mass spectrometry, *Journal of Analytical Atomic Spectrometry* 17 (2002):135–137.
89. M. J. Baxter, H. M. Crews, M. J. Dennis, I. Goodall, and D. Anderson, The determination of the authenticity of wine from its trace element composition, *Food Chemistry* 60 (1997):443–450.
90. L.-X. Sun, K. Danzer, and G. Thiel, Classification of wine samples by means of artificial neural networks and discrimination analytical methods, *Fresenius Journal of Analytical Chemistry* 359 (1997):143–149.
91. K. Danzer, D. De la Calle Garcia, G. Thiel, and M. Reichenbäch, Classification of wine samples according to origin and grape varieties on the basis of inorganic and organic trace analysis, *American Laboratory* (1999):26–34.
92. E. Hernandez-Caraballo, R. M. Avila-Gomez, T. Capote, F. Francklin Rivas, and A. G. Perez, Classification of Venezuelan spirituous beverages by means of discrimination analysis and artificial neural networks based on their Zn, Cu and Fe concentrations, *Talanta* 60 (2003):1259–1267.
93. M. Barbaste, B. Medina, L. Sarabia, M. C. Ortiz, and J. P. Perez-Trujillo, Analysis and comparison of SIMCA models for denominations of origin of wines from de Canary Islands (Spain) builds by means of their trace and ultratrace content, *Analytica Chimica Acta* 472 (2002):161–174.
94. S. Frias, J. E. Conde, J. J. Rodrigez-Bencomo, F. Garcia-Montelongo, and J. P. Perez-Truijllo, Classification of commercial wines from Canary Islands (Spain) by chemometric techniques using metallic content, *Talanta* 59 (2003):335–344.
95. S. Perez-Magariñ, M. Ortega-Heras, and M. L. Gonzalez-San Jose, Multivariate classification of rosé wines from different Spanish protected designations of origin, *Analytica Chimica Acta* 458 (2002):187–190.