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Speciation Analysis of Arsenic (III), Arsenic (V) and Total Arsenic by Continuous Flow HG-AAS in Thai Fruit Wines and Distilled Spirits

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Abstract: The speciation analysis of inorganic arsenic; As (III), As (V) and total arsenic in local Thai fruit wines and distilled spirits by continuous flow hydride generation-atomic absorption spectrometry (HG-AAS) was carried out. The optimized conditions were investigated in detail. It was found that the recoveries (%) of wine and distilled spirit spiked with As(III), As(V) and total As at 1.0-3.0 μg L^{-1} level were 108, 93.1 and 91.4, and 119, 91.5 and 95.8, respectively. The detection limits were found to be 1.9 μg L^{-1} and 0.63 μg L^{-1} for As(III) and As(V) in wine, 0.49 μg L^{-1} and 0.35 μg L^{-1} for As(III) and As(V) in distilled spirit, and 0.47 μg L^{-1} for total As in both beverages. From the results of both red and white wines and distilled spirits, the amount of As(III) was not detectable (n.d.) while that of As(V) ranged from n.d. -1.54 μg L^{-1} and n.d. -0.353 μg L^{-1} for wines and distilled spirits, respectively. The total As contents obtained from acid digestion method were also found to be at the ultra-trace level ranging from n.d. -1.85 mg L^{-1} and n.d. -1.64 μg L^{-1} for wines and distilled spirits, respectively. Due to differences in natural sample matrices, it is shown that As(V) can be mostly quantified in wines but As(III) can still be residual in some distilled spirits when determined with untreated samples.

Keywords: Arsenic speciation, wine, distilled spirit, hydride generation, atomic absorption spectrometry

INTRODUCTION

Wine is a widely consumed beverage throughout the world and has an obvious commercial value. Wine, in the broader sense, is a beverage resulting from the fermentation by yeast of fruit juice, although it is possible to make it from vegetable, herb and other plants, with appropriate processing and additions. A white spirit is also a beverage distilled from spirituous liquid, rice wine or locally so-called "satho" in Thailand. In the same way, other distilled spirits can also be made from many kinds of fruit and plant wines. Normally, the distilled spirits are the highest in alcohol content (45-50% v/v) (Ough *et al.*, 1982; Zoecklein *et al.*, 1995; Pena *et al.*, 2004; Esparza *et al.,* 2004). Daily consumption of wine and distilled spirit in moderate quantities contributes significantly to the requirements of the human organism for essential elements. But there are some heavy metals contaminating these beverages, particularly arsenic, cadmium, lead, mercury, iron and others are known to be potentially toxic (Bettin *et al.*, 2002; Gomez *et al.*, 2004; Fernandez-Pachon *et al.*, 2005). Analysis of certain elements in wine and distilled spirit is of special interest due to their toxicity in case of excessive intake, and the effect they may have on their organoleptic properties (Maranyi and Papp, 1998; Al Nasir *et al.*, 2001; Benitez *et al.*,

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2002; Galani-Nikolakaki *et al.*, 2002; Jos *et al.*, 2004; Gomez *et al.*, 2004; Lara *et al.*, 2005; Dugo *et al.*, 2005).

Arsenic is of great interest today due to their wide occurrence in the environment and their health impact. A regular absorption of small amounts of arsenic may cause serious effects on human health and affecting the body in several ways. People having arsenic skin manifestations and drinking contaminated beverages have high levels of arsenic in their body and organs causing various health problems (Agrawal *et al.*, 1999; Molenat *et al.*, 1999; Hsieh *et al.*, 1999; Wangkarn and Pergantis, 1999; Smichowski *et al.*, 2000; Munoz *et al.*, 2002; Shi *et al.*, 2003; Ellwood and Maher, 2003; Shi *et al.*, 2003; Kment *et al.*, 2005). In terms of limiting concentrations of arsenic normally found in wine and distilled spirit in Thailand, the standard method, the Association of Official Analytical Chemistry (AOAC), is used for the determination of arsenic in wines and distilled spirits. Arsenic present in wines and distilled spirits depends on a variety of factors such as materials used in production or using herbicides, wood preservatives, insecticides, etc. in agriculture that may be left as residues in the environment, and also on the processing, transport and storage conditions of wines and distilled spirits (Maranyi and Papp, 1998; Smichowski *et al.*, 2000; Al Nasir *et al.*, 2001; Galani-Nikolakaki *et al.*, 2002; Esparza *et al.*, 2004; Gomez *et al.*, 2004a; Jos *et al.*, 2004; Gomez *et al.*, 2004b; Dugo *et al.*, 2005; Lara *et al.*, 2005). The determination of arsenic in beverage samples can be done by various techniques including atomic absorption spectrometry (AAS) (Ough *et al.*, 1982; Galani-Nikolakaki *et al.*, 2002) and inductively coupled plasma-mass spectrometry (ICP-MS) (Perez-Jordan *et al.*, 1998; Wangkarn and Pergantis, 1999; Gomez *et al.*, 2004a). Recently, several techniques of continuous flow hydride generation-atomic absorption spectrometry (HG-AAS) (Jos *et al.*, 2004; Tasev *et al.*, 2005), microscale flow injection-ICP-MS (Wangkarn and Pergantis, 1999), continuous flow or flow injection hydride generation-atomic fluorescence spectrometry (AFS) (Segura *et al.*, 1999; Martinez *et al.*, 2001; Karadjova *et al.*, 2005) have been frequently used for the determination of arsenic species in various wine or beer samples. The use of hydride generation can selectively separate and preconcentrate the analyte from sample matrices, thereby reducing or eliminating potential chemical and/or spectral interference commonly encountered with a direct solution analysis.

The aim of this study was to investigate a simple and accurate method that is suitable for routine application to arsenic determination in local Thai fruit wine and distilled spirit samples by continuous flow HG-AAS (Molenat *et al.*, 1999; Coelho *et al.*, 2002). It is well known that arsenic toxicity depends on the arsenic species present in such sample matrices and that inorganic arsenic is considered to be more toxic. As(V) was determined after reduction to As(III) by potassium iodide plus ascorbic acid in suitable buffering media, and then total inorganic arsenic and total arsenic subsequently determined (Martinez *et al.*, 2001; Trung *et al.*, 2001; Quinaia and Rollemberg, 2001). Therefore, optimal conditions were studied in detail for sample treatments of different matrices, selective measurement conditions and analytical method validation.

MATERIALS AND METHODS

Materials

All reagents used were analytical reagent grade. The AAS standard solution (1000 mg L^{-1}) of As(III) was obtained from Spectrosol (England). As(V), traceable standard (1000 mg L^{-1}), was purchased from Merck (Germany). Citric acid, hydrochloric acid, ethanol absolute, sodium acetate, sodium citrate dihydrate and sodium hydroxide were from Carlo Erba (Italy). Nitric acid (65 % w/v) and Potassium iodide were obtained from Merck (Germany). Di-sodium hydrogen phosphate anhydrous was obtained from Fluka

(Switzerland). Hydrogen peroxide, sodium borohydride and tartaric acid were from Ajex Chemical (Australia). Ascorbic acid was also from Spectrosol (England) and acetic acid from Analar (England). All aqueous solutions were prepared with deionized water (18.2 $M\Omega cm^{-1}$ resistivity) by simplicity water purification system, Model Simplicity 185, Millipore Corporation (USA). All glassware were cleaned by soaking in dilute 10% v/v HNO_3 overnight and rinsed three times with deionized water prior to use.

Instruments

For arsenic determination, the Perkin-Elmer AAnalyst 100 was equipped with a flow injection analysis system (FIAS) from Perkin-Elmer (Model FIAS-100, USA), used for continuous flow hydride generation. The FIAS-100 flow injection system consists of one peristaltic pump, five-port valve and a regulated gas control. Argon gas was used as carrier gas for the transposition of arsenic hydride from the gas-liquid separator to the electrically heated quartz tube. The electrodeless discharge lamp was used for this determination. The atomic absorption signal was measured as a peak height mode providing an analytical curve. The pH measurements were performed on pH meter from Denver Instrument, Model 251 (USA).

Wine and Distilled Spirit Samples

Wines and distilled spirits to be analyzed are widely and locally produced in various parts of Thailand. They are usually called "OTOP" products (*one "tambol" one product*), a Thai wine and distilled spirit or home-made beverages managed by local producers and allowed for sales under a quality control and assurance of legislation of Thai Industrial Standards Institute (TISI, 2003). Ten kinds of wine samples are almost made from fruits and classified as red wine (RW) and white wine (WW) according to their raw materials. Red wine samples are represented as RW1 (black galingale, Khon Kaen), RW2 (Indian mulberry, Udon Thanee), RW3 (mangosteen, Udon Thanee), RW4 ("maow" or wild compiled fruits, Sakon Nakorn) and RW5 (bel fruit, Khon Kaen). White wine samples are assigned as WW1 (longan, Loei), WW2 & WW3 (Indian gooseberry, Loei & Chaiyaphum), WW4 (black glutinous rice, Khon Kaen) and WW5 (pineapple, Khon Kaen). Five distilled spirits (DS) are all made from rice but originated from different production sites including DS1 and DS2 (Khon Kaen), DS3 (Yasothon), DS4 (Rayong) and DS5 (Patum Thanee). All samples were purchased from liquor retail markets and kept cool in refrigerator before analysis.

Analytical Methods for As(III), As(V) and Total As Determination

All of reagents were filtered through Whatman No.42 filter paper prior to use for measurement. Each standard solution (10 $\mu g\ L^{-1}$) of As(III) and As(V) was prepared by a stepwise dilution with deionized water from their stock solution (1000 mg L^{-1}). This working solution was prepared daily.

Preparation of Calibration Curve

The calibration standard for As(III) determination was established using the standard solution prepared in citrate buffer pH 5 plus ascorbic acid condition by dilution from the working solution of As(III) (Quinaia and Rollemberg, 2001; Karadjova *et al.*, 2005). The concentration of As in the standard solution was used as follows: 0.5, 1.0, 1.5, 2.0 and 2.5 $\mu g\ L^{-1}$, respectively.

The calibration standard for As(V) and total As determination was prepared by various volumes of the working solution of As(V) which was pipetted into 10 mL volumetric flask. After that, 1 mL of 10 % w/v KI plus 0.3% w/v ascorbic acid and 2 mL of concentrated HCl were added and then diluted to 10 mL with deionized water (Trung *et al.*, 2001; Frank *et al.*, 2004). The standard solution of As(V) was also prepared in the same concentration range as done for As(III).

Preparation of Carrier Solution and Reducing Reagent

A 1 mol L^{-1} HCl was prepared by an appropriate dilution of concentrated HCl with deionized water (Yang *et al.*, 2003). A 0.4% w/v $NaBH_4$ was prepared fresh daily by dissolving an accurate amount of $NaBH_4$ in 0.03 mol L^{-1} NaOH (Jos *et al.*, 2004).

Hydride Generation Method

The continuous flow HG-AAS system was controlled through a computer program (AA WinLab). Both inorganic arsenic and total arsenic in wine and distilled spirit were determined using the experimental conditions shown in Table 1.

Table 1: Instrumental parameters for as determination by continuous flow HG-AAS

Parameter	Condition
Measurement mode	Absorbance
Slit width	0.70 nm
Wavelength	193.7 nm
Quartz tube temperature	900°C
Lamp current	380 mA
Energy	53
$NaBH_4$ concentration, flow rate	0.4%w/v in 0.03 mol L^{-1} NaOH, 5.5 mL min^{-1}
HCl concentration, flow rate	1 mol L^{-1}, 10 mL min^{-1}
Argon flow rate (purge gas)	70 mL min^{-1}
Sample loop	500 μL

Selective Determination of As(III)

Preparation of Different Media Solutions

The HNO_3 and HCl solutions (1-10% v/v each) and acetic acid solution (0.05-0.40% v/v) were prepared by diluting from each concentrated HNO_3, HCl and acetic acid with deionized water, respectively (Molenat *et al.*, 1999). For citric acid and tartaric acid solutions, they were prepared in the range of 0.05-0.40 mol L^{-1} by dissolving a required amount of citric acid and tartaric acid in deionized water, respectively.

Preparation of Buffer Solutions

Acetate buffer was prepared by adding an appropriate volume of 0.1 mol L^{-1} acetic acid to 0.1 mol L^{-1} sodium acetate solution. Citrate buffer (0.1 mol L^{-1}) was prepared by adding an appropriate volume of 10% w/v citric acid solution to 100 mL volumetric flask. Then, 10% w/v sodium citrate solution was added and diluted to 100 mL with deionized water. Citric phosphate buffer was also prepared in the same manner as described above. All buffer solutions were prepared at pH 3.5, 4.0, 5.0 and 6.0, respectively.

Preparation of Standard As Solution in Different Media and Buffer Solutions

For optimized conditions, the selective determination of As(III) was studied by preparing 1 μg L^{-1} standard solution of both As(III) and As(V) in each concentration of media and for each pH of the buffer solutions. Then, their solutions were determined according to the instrumental parameters shown in Table 1.

Prereduction of As(V)

Preparation of KI Solution and KI Plus Ascorbic Acid Solution

The KI solutions between 0.5 and 15.0% w/v were prepared by dissolving an appropriated amount of KI to 100 mL with deionized water. The KI solution was prepared daily. A 10% w/v KI plus each concentration of ascorbic acid, including 0.1, 0.3, 0.5, 0.7, 1.0 and 3.0% w/v was prepared by dissolving 10 g of KI with 20 mL deionized water. Then, a required amount of ascorbic acid was dissolved with 20 mL deionized water, respectively. After that, the two solutions were mixed in 100 mL volumetric flask and diluted up to the mark with deionized water.

Effect of KI and Ascorbic Acid Concentrations
A 1.00 mL of the working solution of As(V) (1 μg L^{-1}) was pipetted into 10 mL volumetric flask. After that, 1 mL of each KI concentration and 2 mL of concentrated HCl were added and then diluted to 10 mL with deionized water. This solution was left to stand for 1 h prior to measurement. This parameter was studied in the same manner as mentioned above, but using various concentrations of reducing mixture solution.

Effect of Matrix Ions
Each interference ion, including 100 mg L^{-1} Na^{+} and Mg^{2+}, 10 μg L^{-1} Cu^{2+}, Zn^{2+}, Mn^{3+}, Fe^{3+} and Al^{3+}, and 5 μg L^{-1} Ni^{2+}, Pb^{2+} and Cr^{3+} was added to 1.0 μg L^{-1} arsenic model solution. These solutions were introduced to arsenic determination.

Preparation of Wine and Distilled Spirit Samples
Wine or distilled spirit samples (20 mL) were evaporated in an oven at 80°C until dryness (Tasev *et al.*, 2005; Karadjova *et al.*, 2005). Evaporated wine or distilled spirit samples were dissolved with 0.1% v/v HCl and filtered through Whatman No.42 filter paper into 20 mL volumetric flask until the volume reached 20 mL.

As(III) Determination in Wine and Distilled Spirit
A 1 mL solution of the wine sample was pipetted into 10 mL volumetric flask. Then, 1 mL of 0.3% w/v ascorbic acid solution was added and diluted to the mark with citrate buffer pH 5, and then determined by the instrument. In case of distilled spirit, 5 mL of the sample solution was used following the same procedure for the preparation of wine samples.

As(V) Determination in Wine and Distilled Spirit
A 2 mL of the solution obtained from wine sample was pipetted into 10 mL volumetric flask. After that, 1 mL of 10% w/v KI plus 0.3% w/v ascorbic acid and 2 mL of concentrated HCl were added and then diluted to the mark with deionized water. This solution was left to stand for 1 h prior to analysis. The concentration of As that was obtained from the above step of the procedure was the concentration of a sum of inorganic arsenic, As(III) + As(V) (Tasev *et al.*, 2005). Therefore, the As(V) concentration in the sample can be calculated by the difference in concentration between As(III) and sum of the inorganic arsenic. To determine As(V) in a distilled spirit sample, the same procedure for the preparation of wine sample was followed but using 5 mL of the sample solution.

Total As Determination in Wine and Distilled Spirit
Evaporated sample (wine or distilled spirit) was treated by acid digestion method using only concentrated HNO_3 as oxidizing agent in the ratio of 10:4 or 50:1 by volume, respectively (Tasev *et al.*, 2005). Then, the digested sample was dissolved with deionized water and filtered through Whatman No.42 filter paper into 20 mL volumetric flask until the volume was up to the mark. To determine total As, 2.5 mL of the solution was pipetted into 10 mL volumetric flask. Then prereduction was done following the same procedure as described earlier.

RESULTS AND DISCUSSION

Firstly, experiments were carried out to optimize hydride generation conditions for the speciation of arsenic species. Such hydride generation parameters were optimized as concentrations of HCl, $NaBH_4$ and NaOH for both As(III) and As(V), KI and ascorbic acid concentrations and the reaction time necessary to complete the reduction of As(V) to As(III), and selectively for As(III) in different media.

Optimization of Experimental Conditions
The influence of HCl concentration in carrier solution was investigated in the range of 1-7 mol L^{-1}. Higher concentrations of HCl did not improve the analytical signal and it would cause severe effervescence and splashing of solution droplets on the gas-liquid separator inner walls due to fast reaction. Thus, a 1 mol

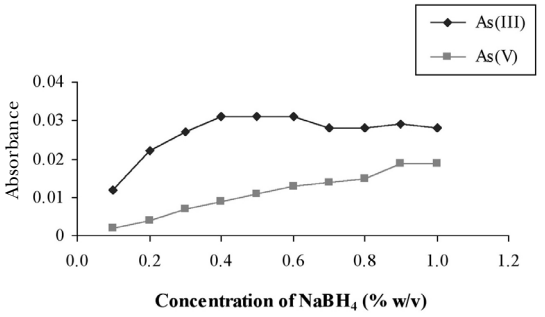


Figure 1: Effect of $NaBH_4$ concentration on absorbance of each 1 μg L^{-1}As(III) and As(V) in 10 % v/v HCl; 1 mol L^{-1} HCl and 0.05 mol L^{-1} NaOH (n = 5)

L^{-1} HCl solution was enough to use in this work. $NaBH_4$ concentration is an important parameter for arsine generation, since it is formed in the presence of hydrogen generated by $NaBH_4$ in acidic medium. Figure 1 shows that the hydride generation from As(V) is gradual and arsine generation is slower compared with As(III), making it necessary to increase the $NaBH_4$ concentration to obtain a good comparability between the results found for As(V).

At the same time, higher concentrations of $NaBH_4$ (> 0.6% w/v) would also cause serious effervescence and splashing of solution droplets on the gas-liquid separator inner walls. Then, water vapor and a mist of reagent might condense on the transfer line and consequently trap the volatile arsenic hydride, resulting in the significant decrease in the signal intensity. A low $NaBH_4$ concentration (< 0.4% w/v) could not perhaps afford complete reduction of the analyte leading to low signal intensity. Therefore, the $NaBH_4$ concentration of 0.4% w/v was selected for the determination of As(III). The NaOH was used to stabilize $NaBH_4$ for a given high analyte signal. It was found that absorbance signal was stable at NaOH concentration higher than 0.04 mol L^{-1} for both As species. Therefore, 0.04 mol L^{-1} NaOH was also used in this study. Since the transformation of As(V) to As(III) and the ensuring generation of arsine by hydride generation is considerably slower at lower acidity, the selective determination of As(III) can be achieved by controlling the reaction pH of the hydride generation. Therefore, different media including citric, tartaric, acetic, hydrochloric and nitric acids were also studied (Quinaia *et al.*, 2001; Shi *et al.*, 2003). As the results shown in Figure 2(a)-(e), the absorbance signals of both As(III) and As(V) were slightly increased with the organic acid concentration. When citric acid and tartaric acid were used as a medium, higher sensitivity to As(III) was obtained. Higher concentration of citric acid (> 0.15 mol L^{-1}) and acetic acid (> 0.6% v/v) caused strong bubble formation in the gas-liquid separator resulting in arsine transport problems. However, the selective determination of only As(III) could be achieved in the presence of acetate, citrate and citric phosphate buffers (Figure 3). The absorbance signals of both As(III) and As(V) decreased as the pH of each buffer solution was increased, with the absorbance of As(V) was decreasing to zero.

Taking into account the recoveries of 1 μg L^{-1} As(III) solution which had been spiked with 1-5 μg L^{-1} As(V) in 0.1 mol L^{-1} tartaric acid, citrate buffer pH 4, citrate buffer pH 5 and citric phosphate buffer pH 4 were comparatively studied. As shown in Figure 4, citrate buffer pH 5 showed preferred recovery, despite small potential interference from As(V). Therefore, citrate buffer pH 5 was used as a medium for the selective determination of As(III) in this study.

It is known that at room temperature and at low pH (pH <1), the oxidation state of As(V) is reduced relatively slowly by $NaBH_4$ whereas As(III) is instantaneously converted to arsine upon reaction with $NaBH_4$. The arsine atomic absorption peak heights are usually decreased by one-fourth to one-third for As(V) when compared to As(III). In order to speed up the reaction and to increase the sensitivity of the method, all As(V) should be converted to the As(III) form before reacting with $NaBH_4$. The reducing agent investigated in this study was KI plus ascorbic acid because of wide

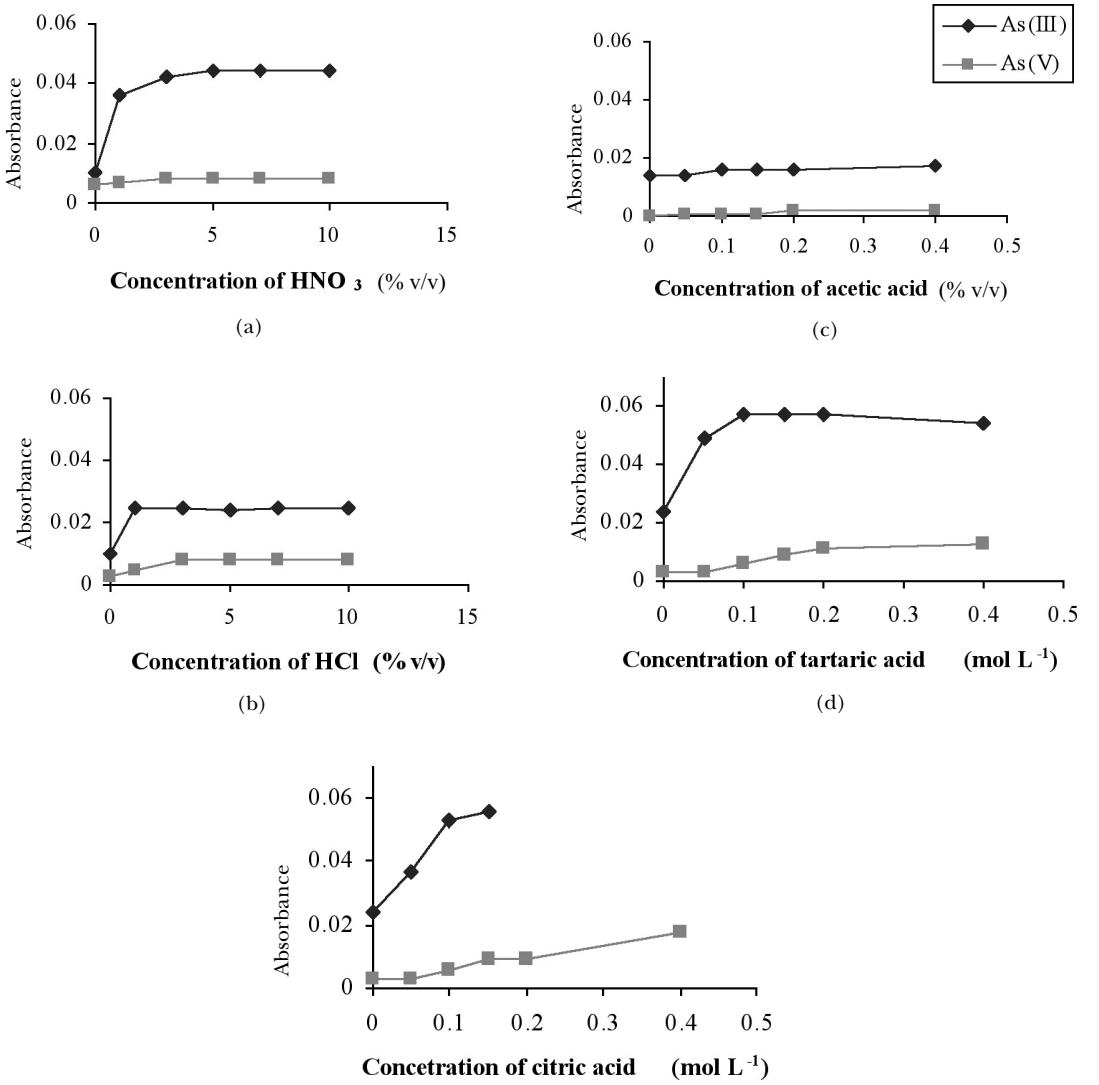
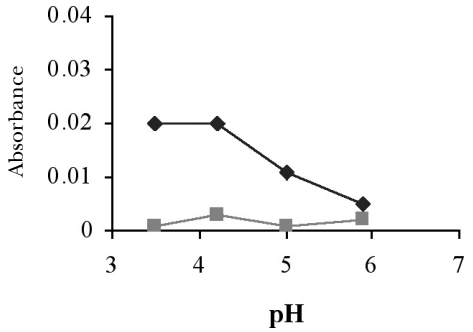
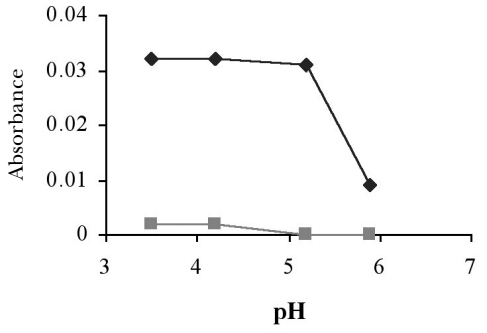


Figure 2: Selective determination of As(III) in different media; each 1 μg L^{-1} As(III) and As(V) (n = 5) (a) HNO_3, (b) HCl, (c) acetic acid, (d) tartaric acid, and (e) citric acid.

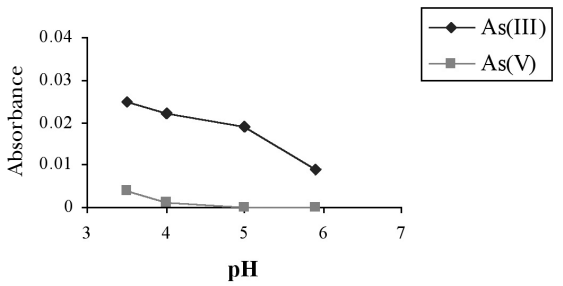
acceptance as the most suitable for this propose. Ascorbic acid used in association with KI can prevent the oxidation of iodide to triiodide by air or with an oxidant like Fe^{3+} (Segura *et al.*, 1999; Trung *et al.*, 2001). Then HCl, KI plus ascorbic acid concentration and reducing time were also important parameters to be considered at the reducing step. Thus, 1 mL of various concentrations of the prereduction solution was added into the model solution, followed by 2 mL concentrated HCl and filled up to 10 mL with deionized water. As shown in Figure 5, the concentration of 10% w/v KI was sufficient for the quantitative prereduction of As(V). The concentration of ascorbic acid for stabilization of 10% w/v KI was also studied and the optimized condition was attained in the range of 0.1-1.0% w/v. Higher than 1.0% w/v of ascorbic acid caused a decrease in absorbance



(a) acetate buffer



(b) citrate buffer



(c) citric phosphate buffer

Figure 3: Selective determination of As(III) in each pH of different buffer solutions; 1 μg L^{-1}As(III) and As(V) (n=5) (a) acetate buffer, (b) citrate buffer, and (c) citric phosphate buffer

signal. Therefore, 10% w/v of KI plus 0.3% w/v of ascorbic acid was used.

Signal intensities increased with increasing contact times of a reduction of As(V) both in the presence and in the absence of 0.3% w/v ascorbic acid, and reached a maximum plateau at about 60 min. Consequently, 10% w/v KI plus 0.3% w/v ascorbic acid, 2 mL of concentrated HCl and 1 hour contact time was found to be suitable for the quantitative prereduction of As(V) to As(III) in this study. Good recoveries for the determination of As(III) (101.2%), As(V) (105.7%) and total inorganic arsenic, As(III) + As(V), (103.9%) with 1 μg L^{-1} As spiked concentration were also obtained when using the proposed conditions.

The potential interference metal ions in citrate buffer pH 5 for As(III) determination and in 10% w/v KI plus 0.3% w/v ascorbic acid for total arsenic determination were investigated. The results in Table 2 show that 100 mg L^{-1} Na^{+} and Mg^{2+}, 10 mg L^{-1} Zn^{2+}, Mn^{2+} and Al^{3+}, and 5 mg L^{-1} Pb^{2+}, Ni^{2+} and Cr^{3+} did not interfere in the analysis of both As(III) and total arsenic determination. However, the poor As(III) recoveries as a result of citrate buffer pH 5 could be the effect of hindered arsine production by Cu^{2+} and oxidation of As(III) by Fe^{3+}. The extents of their effect on the determination of 1 μg L^{-1} As(III) were examined by spiking with increasing levels of copper and iron interferences (Figure 6). It was found that the tolerable concentration ratio of Cu^{2+} and Fe^{3+} to As(III) were 7000 and 2000, respectively. Addition of ascorbic acid was used to avoid the effect of Fe^{3+} on the absorption intensity of As(III). It was noted that good recovery of 1 μg L^{-1} As(III) in 7 mg L^{-1} Fe^{3+} associated with various concentrations of ascorbic acid (0.1-0.5% w/v) was also not effected. Thus 0.3% w/v ascorbic acid was confirmed for use in this study.

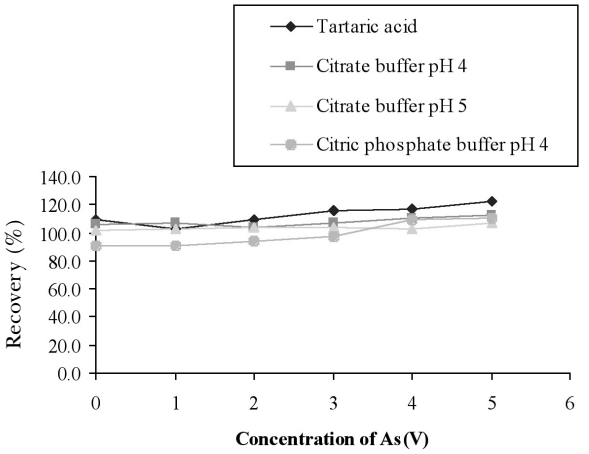


Figure 4: Recoveries of 1 μg L^{-1}As(III) spiked with various amounts of As(V) (mg L^{-1}) in different media (n = 5)

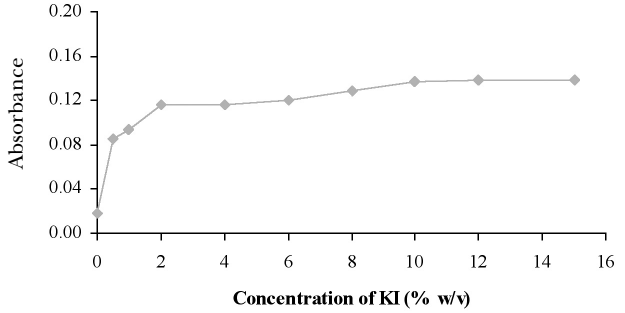


Figure 5: Effect of KI concentration on absorbance of As(V) that was reduced to As(III); 1 μg L^{-1} As(V) (n = 5)

Table 2: Effect of some matrix ions on the recovery of each 1 μg L^{-1} As(III) and As(V) in different media (n = 4)

Ion	Added as	Ion concentration (mg L^{-1})	Recovery (%) ± S.D.		
			Citrate buffer	KI + ascorbic acid	
			As(III)	As(III)	As(V)
Na^{+}	NaCl	100	102.1 ± 0.011	99.60 ± 0.008	97.50 ± 0.005
Mg^{2+}	$Mg(NO_3)_{21}$	100	101.5 ± 0.005	98.80 ± 0.004	95.60 ± 0.005
Cu^{2+}	$CuCl_2$	10	86.90 ± 0.013	174.5 ± 0.023	167.9 ± 0.015
Zn^{2+}	$ZnCl_2$	10	103.5 ± 0.007	103.8 ± 0.003	105.2 ± 0.004
Mn^{3+}	$MnCl_3$	10	105.8 ± 0.034	101.5 ± 0.007	101.8 ± 0.006
Al^{3+}	$AlCl_3$	10	102.6 ± 0.005	97.30 ± 0.009	94.60 ± 0.007
Fe^{3+}	$FeCl_3$	10	53.20 ± 0.014	99.40 ± 0.006	98.80 ± 0.005
Pb^{2+}	$Pb(NO_3)_2$	5	95.00 ± 0.015	99.00 ± 0.006	96.20 ± 0.004
Ni^{2+}	$Ni(NO_3)_2$	5	102.5 ± 0.006	102.2 ± 0.013	98.30 ± 0.003
Cr^{3+}	$Cr(NO_3)_3$	5	101.4 ± 0.004	109.3 ± 0.007	100.7 ± 0.002

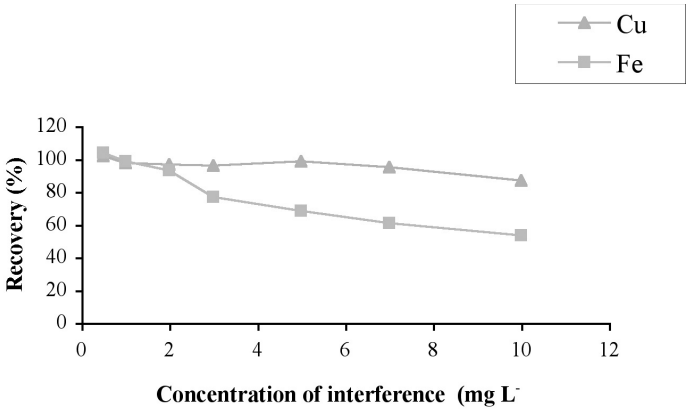


Figure 6: Effects of Cu^{2+} and Fe^{3+} ions interfering in the determination of As(III); 1 μg L^{-1} As(III) (n = 5)

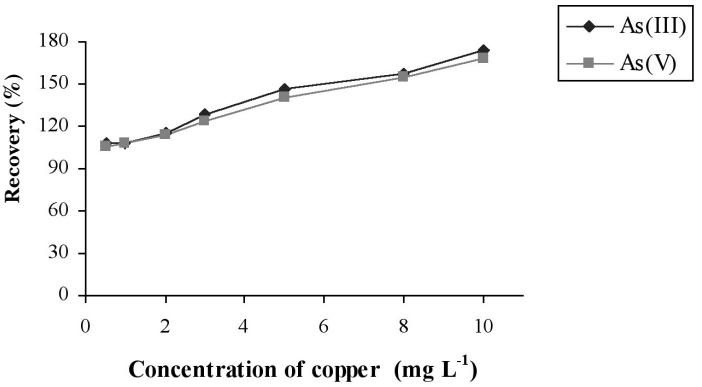


Figure 7: Effect of Cu^{2+} ion interfering in the determination of As(V) and total As;1 μg L^{-1} As(V) (n = 5)

It is known that the concentration of copper in wine and distilled spirit samples is almost less than 0.2 mg L^{-1} (TISI, 2003). Moreover, wine and distilled spirit samples were diluted before using the proposed procedure. Thus, there was no interferance from Cu when using this method. For total inorganic As and total As determination, the tolerable concentration ratio of Cu to As(III) and As(V) in 10% w/v KI plus 0.3% w/v ascorbic acid was 2000 (Figure 7). In order to eliminate the influence of Cu on total As determination, EDTA and thiourea were added to a model solution and the concentration of ascorbic acid solution increased (Yang *et al.*, 2003; Yin *et al.*, 2003). It was found that they did not decrease interference from Cu. Normally, Cu content is frequently found to be lower than 0.2 mg L^{-1} in wine and distilled spirit samples. Thus, it does not interfere with the total As determination under this condition. Some samples have high Cu content so the sample may be diluted prior to analysis to a level that does not interfere with the total inorganic As and total As determinations.

In all cases preliminary evaporation of ethanol is performed in order to avoid over-pressure and to ensure better conditions for complete mineralization of wine organic matter in the sample. The interference effect

of ethanol as one of the main volatile compounds in wine (9-12% v/v) and distilled spirit (38-45% v/v) on the absorbance signal of As was studied. A model solution in citrate buffer pH 5 mixed with ethanol to 0.5% v/v concentration of ethanol was also preliminary directed to analysis by HG-AAS. It was found that a strong bubble formation and fine aerosol formed in the gas-liquid separator which entered into the transfer line together with arsine are responsible for the decreased degree of arsine atomization. Therefore, the samples were evaporated in an oven at 80°C for elimination of ethanol.

For determination of As(III) and As(V) in wine samples, evaporated samples were diluted 10 folds and 5 folds, respectively, as using lower dilution levels causes bubble formation in the gas-liquid separator. For distilled spirit sample, evaporated samples were diluted 2 folds for both determination of As(III) and As(V). For total As determination, evaporated samples (both wine and distilled spirit) were treated by acid digestion method. Then, the digested samples were diluted 4 folds. Lower than 4 folds dilution can be given for background high absorbance signal.

Analytical Performance Characteristics

After system optimization, the validation of methods including limits of detection (LOD) and of quantitation (LOQ), precision and accuracy, and recovery of the proposed procedure for As determination were investigated. The precision in terms of relative standard deviation (RSD) was expressed by analyzing each standard solution containing 1 μg L^{-1} As(III), As(V) and total As (n=10). The percentages of RSD were 3.1, 2.2 and 2.7 for the determination of As(III), As(V) and total As, respectively. The regression equation associated with correlation coefficient (r^2) for As(III) and total As was obtained as $y = 0.0348x - 0.0006$ ($r^2 = 0.9999$), and $y = 0.0592x + 0.0018$ ($r^2 = 0.9998$), respectively.

The LOD was calculated according to $3s_o/s$ or 3σ, where s_o was obtained from the standard deviation for 10 replicate measurements of a blank solution and s is the slope of the calibration graph, and LOQ was 10s (Wineforder and Long, 1983). The LODs were 1.9 μg L^{-1} and 0.49 μg L^{-1} for As(III) and As(V) in wine; 0.63 μg L^{-1} and 0.35 μg L^{-1} for As(III) and As(V) in distilled spirit, respectively and 0.47 μg L^{-1} for total As in both wine and distilled spirit. Also, the LOQs were 6.3 μg L^{-1} and 1.6 μg L^{-1} for As(III) and As(V) in wine, respectively; 2.1 μg L^{-1} and 1.2 μg L^{-1} for As(III) and As(V) in distilled spirit, respectively and 1.6 μg L^{-1} for total As in both wine and distilled spirit.

The accuracy of the proposed procedure for As determination was investigated and good recoveries of spiked samples were found as shown in Table 3. Under the optimized conditions, standard calibration for inorganic As and total As determination in wine and distilled spirit samples were proved by comparative analysis using standard addition method, and the results are presented in Table 4. It was found that a very good agreement between the results obtained from standard calibration curve and standard addition method, expressed by *t*-test value for a confidence level of 95.0%, resulted. Therefore, the standard calibration curve could be used for quantitative determination of all As in wine and distilled spirit samples analyzed in this study without any effect on the sample matrix interference.

Real Sample Analysis

Several samples of local Thai fruit wines and distilled spirits were analyzed under the optimum conditions of inorganic As and total As described above. Selective reduction at different acid media allowed the speciation study of As in wines and distilled spirits. The fact that As(III) and As(V) have different sensitivities of absorbance measurements in the absence of KI prompted the selectivity of As(III) determination, prereduction of As(V) and hydride generation conditions to be investigated. Optimized hydride generation conditions were then created using 1 mol L^{-1} HCl as carrier and 0.4% w/v $NaBH_4$ in 0.03

Table 3: Recovery of As(III), As(V) and total As determination in spiked wine and distilled spirit samples (n = 5)

Sample/ As species	Concentration (μg L^{-1}) ± S.D.			Recovery[3] %
	Added	Standard curve[1]	Standard addition[2]	
Wine				
As(III)	3.0	n.d.	3.231 ± 0.122	108
As(V)	2.0	1.227 ± 0.042	3.089 ± 0.134	93.1
Total As	2.0	1.059 ± 0.072	2.887 ± 0.039	91.4
Distilled spirit				
As(III)	1.0	n.d.	1.186 ± 0.091	119
As(V)	1.0	0.3530 ± 0.004	1.268 ± 0.036	91.5
Total As	1.0	0.5110 ± 0.043	1.469 ± 0.004	95.8

n.d. = not detectable
1 contents found in wine and distilled spirit samples
2 contents found in spiked wine and distilled spirit samples
3 calculated from differences in concentration found between standard addition and standard curve methods

Table 4: Concentration of As in samples comparing between calibration curve and standard addition methods (n = 4)

Sample/ As species	Concentration (μg L^{-1}) ± S.D.		test[3]
	Calibration curve	Standard addition	
Wine[1]			
As(III)	1.925 ± 0.062	2.081 ± 0.045	3.527
As(V)	2.440 ± 0.103	2.285 ± 0.056	2.290
Total As	2.155 ± 0.061	2.296 ± 0.112	1.915
Distilled spirit[2]			
As(III)	1.010 ± 0.043	1.045 ± 0.024	1.231
As(V)	1.492 ± 0.048	1.434 ± 0.036	1.674
Total As	1.273 ± 0.033	1.353 ± 0.021	3.542

1 spiked with 2.0 μg L-1 standard solution of As(III)
2 spiked with 1.0 μg L-1 standard solution of As(III)
3 for a confidence level of 95.0%

mol L^{-1} NaOH as the reducing agent. Selective determination of As(III) was presented in citrate buffer pH 5. Prereduction of As(V) by using 10% w/v KI plus 0.3% w/v ascorbic acid, and concentrated HCl added to standard solution and allowing for 1 h prior to measurement resulted in improved sensitivity. The inorganic As species were determined directly in wine and distilled spirit samples after diluted and ethanol evaporated samples. The total As content was determined after acid digestion, using appropriate ratio of the samples and concentrated HNO_3, and then the digested samples were diluted 4 folds.

The results obtained are shown in Table 5. The concentration of As(V) was in the range of 0.687-1.54 and 0.0920-0.353 μg L^{-1}, while that of the total As was 0.622-1.85 and 0.511-1.64

Table 5: As(III), As(V) and total As contents found in wine and distilled spirit samples (n = 3)

Sample	Concentration (μg L^{-1}) ± S.D.			
	Inorganic species			Total As after sample digestion
	As(III)	As(V) [Inorg. As minus As(III)]	Inorg. As	
RW 1	n.d.	1.17	1.17 ± 0.008	1.18 ± 0.100
RW 2	n.d.	0.687	0.687 ± 0.008	0.741 ± 0.020
RW 3	n.d.	n.d.	n.d.	n.d.
RW 4	n.d.	1.54	1.54 ± 0.024	1.41 ± 0.078
RW 5	n.d.	0.748	0.748 ± 0.009	0.622 ± 0.006
WW 1	n.d.	n.d.	1.13 ± 0.096	1.13 ± 0.048
WW 2	n.d.	n.d	n.d.	0.691 ± 0.068
WW 3	n.d.	1.05	1.05 ± 0.023	1.85 ± 0.120
WW 4	n.d.	0.879	0.879 ± 0.085	0.960 ± 0.040
WW 5	n.d.	n.d	n.d.	n.d.
DS 1	n.d.	0.353	0.353 ± 0.004	0.511 ± 0.043
DS 2	n.d.	n.d.	n.d.	n.d.
DS 3	0.915 ± 0.044	0.0920	1.01 ± 0.003	0.953 ± 0.039
DS 4	1.48 ± 0.18	n.d.	1.30 ± 0.026	1.64 ± 0.025
DS 5	n.d.	n.d.	n.d.	n.d.

n.d. = not detectable
RW = red wine; WW = white wine; DS = distilled spirit

μg L^{-1} for wine and distilled spirit, respectively. The As(III) content found in two distilled spirit samples was in the range of 0.915-1.48 μg L^{-1}, while the As(III) content in all wine samples was not detectable. It is shown that the contents of an inorganic As species, especially As(III), in some representative samples of wine and distilled spirit to be tested this time are not detectable because the As(III) existing in their samples is lower than the detection limit of the proposed method. It is noted that there is no fruit wine and distilled spirit sample with As concentration above 2 μg L^{-1}. However, As(III) was detected, ranging from 1.3-7.6 μg L^{-1} in some wine samples produced in European countries (Karadjova *et al.*, 2005). While the content of As(V) in those wines was not detectable due to lower than LOD (0.4 μg L^{-1}), there is no report of arsenic speciation in distilled spirit yet. Therefore, this data would be useful for quality control analysis of such beverages. These results indicate that both wine and distilled spirit production conform to the national legislation that allows for a maximum allowance limit (0.1 mgL^{-1} As) of Thai Industrial Standards Institute. It can be concluded that almost all As in the wine and distilled spirit sample are of the inorganic As species.

No interference effect was observed in the studied wine and distilled spirit samples, owing to the fact that the interfering levels of metallic ions were below the interference threshold and also because the samples were diluted prior to analysis. Quantification was carried out by both standard addition and simple calibration curve methods. The paired t-test analysis showed no significant difference. Thus, the As(III) aqueous analytical curves in each media could be used for the calibration of As determination in wines and distilled spirits.

CONCLUSION

The results obtained demonstrate that the contents of inorganic arsenic, As(III) and As(V), and total arsenic were slightly different. Most arsenic species in the wine and distilled spirit sample are of the inorganic arsenic form. Consequently, the direct determination of sum inorganic arsenic in the distilled spirit sample is an adequate representative for the distilled spirit quality control. For wines, it was found that the limit of detection of sum inorganic arsenic determination was higher than that of the total arsenic determination.

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