

# Development of Inductively Coupled Plasma Atomic Emission Spectrometry for Arsenic Determination in Wine

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## Abstract

This paper describes a direct method for arsenic determination and speciation in wine samples by hydride atomic emission spectrometry with inductively coupled plasma (ICP-AES). Optimization of the method included investigation of several parameters that impact the intensity of spectral lines: concentration of reaction media (hydrochloric acid), reduction reagent concentration (sodium borohydride), power generator and the carrier flow rate. The effect of ethanol on spectral lines was especially investigated because it enabled arsenic determination without prior preparation. Optimal conditions for As(III) were 8 mol/L HCl and 0.1% NaBH<sub>4</sub>, for dimethylarsinite (DMA) determination 0.005 mol/L HCl and 0.02% NaBH<sub>4</sub>, and for As(V) 4 mol/L HCl and 2% NaBH<sub>4</sub>. Total arsenic was determined in samples after their treatment in a microwave digestion unit. Results were compared to the FIA method, arsenic was determined with the standard addition method, and the results showed agreement. Detection limits (μg/L) for determination of As(V), DMA, and As(III) were found to be 0.4, 0.6, and 0.10, respectively. Good recoveries (89.9-102.0%) of added spikes were obtained for all samples. The proposed method has been validated by means of reference materials and the results obtained were in agreement with certified values.

**Keywords:** arsenic, dimethylarsinite, wine, hydride generation, inductively coupled plasma

## Introduction

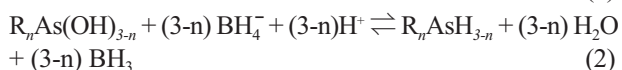
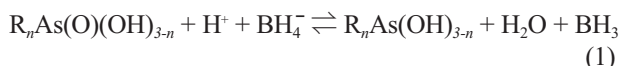
Arsenic exists in several chemical forms in nature, both organic and inorganic. The valence state of arsenic plays an important role for its behavior and toxicity in aqueous systems [1]. It is well known that As(III) is more toxic than As(V), which is more toxic than organic arsenic compounds [2, 3]. Four arsenic species are of the utmost toxic significance and they are numbered according to their toxicity: inorganic As(III), arsenite, inorganic As(V), arsenate, organic monomethylarsonate, MMA (CH<sub>3</sub>As(OH)<sub>2</sub>O<sup>-</sup>), and DMA ((CH<sub>3</sub>)<sub>2</sub>As(OH)O<sup>-</sup>) [4]. The levels of arsenic in the environment are continuously increasing due to natural

(volcanic activity) and anthropogenic sources (e.g. agriculture, industry) [5]. The continuous release of arsenic into the environment leads to accumulation in the food chain, within which marine food is a major source of arsenic in the diet [6]. Arsenic (III) and arsenic (V) are the most often determined species in natural waters, soils, and sediments, while organic arsenic species are common constituents of biological tissues and fluids [7]. Hence there are many methods described for arsenic determination.

Hydride generation (HG) is a well-known technique for determination of arsenic at trace levels. It consists of the reaction of some arsenic compounds with sodium tetrahydroborate in acidic medium to produce arsines. The mechanism of hydride formation is [8]:

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The type and concentration of the used reagents have critical effects on the hydride generation response of arsenite, arsenate, MMA, and DMA [9]. Therefore, it is difficult (if not impossible) to find the right acid concentration to obtain the same response for all arsenic species [10].

Arsenic concentration in wines depends on various factors, including the effect of aerosoles on grapes, grape sorts, climate conditions, soil, pesticide usage, and storage conditions [11]. It is difficult to foresee oxidation states of arsenic in wines, both organic and inorganic, and some results can be found in literature: As(III) [12], As(III) + As(V) [13], As(III) + As(V) + MMA + DMA [5]. Legislation concerning arsenic contents exists in some countries. In the particular case of Serbia, the legislation specifies limits of 1 mg/L. Traditionally, the official method for determining arsenic in wines is hydride generation atomic absorption spectrometry [12]. The hydride technique normally requires a previous decomposition of the wine sample, which is time consuming and may lead to high values or even loss of analyte [14]. Inductively coupled plasma with optical emission spectrometry (ICP-OES) is a multi-element method with very good detection power and offers the right conditions for reliable and rapid determination of the analytes [15]. In a mentioned paper fifteen wine samples from six German wine-growing regions were tested for fifteen elements. In the last decade, the appearance of very sensitive multi techniques, such as inductively coupled plasma mass spectroscopy (ICP-MS), opened new prospects in this area [16]. But the results obtained for As and Ga were not comparable, due to methodological influence, so ICP-OES is still the best choice.

The purpose of the presented work was to develop an analytical method that could enable the arsenic speciation and direct detection of (As(III), As(V), and DMA) without previous reduction. To the best of our knowledge there were no papers with direct determination of arsenic species in wine by ICP-AES. Effects of HCl and NaBH<sub>4</sub> concentration, generator power, and carrier rate effects were investigated, as well as experimental conditions for arsenic speciation. Determination of As(V) without its prereduction was important because it causes a problem when applying hydride techniques.

## Experimental Procedure

### Instrumentation and Apparatus

For all measurements an atomic emission spectrometer with inductively coupled plasma iCAP-6500 Duo (Thermo scientific, United Kingdom) was used. The system is equipped with an integrated unit for hydride generation.

The detector was a RACID86 Charge injector device (CID). All measurements were at three wavelengths: 189.0, 193.7, and 197.0 nm.

Microwave digestion was performed in a pressurized microwave (Ethos 1, Advanced Microwave Digestion System, Milestone, Italy) equipped with a rotor holding 10 cuvettes (PTFE).

### Reagents and Chemicals

Chemicals used were p.a. grade. All glassware was soaked in 4 mol/L HNO<sub>3</sub> for a minimum of 12 hours and rinsed well with distilled water. Sodium borohydride solutions (Merck, Germany) were stabilized with sodium hydroxide (Merck, Germany). NaOH concentration matched the NaBH<sub>4</sub> concentration (up to 0.5%). More concentrated NaBH<sub>4</sub> solutions were prepared in 0.5% NaOH.

Stock As(III) solution (1000 ppm) was prepared by dissolving 0.1326 g As<sub>2</sub>O<sub>3</sub> in a minimal amount of 4.0 mol/L NaOH, it was then neutralized with the same amount of 4.0 mol/L HCl and diluted with water to 100 cm<sup>3</sup>.

Stock As(V) solution (1000 ppm) was arsenic(V) standard solution for atomic absorption (Merck, Germany).

Stock DMA solution (1000 ppm) was prepared by dissolving 0.1880 g cacodylic acid in 100 ml water.

Stock solutions were prepared monthly and stored in glass volumetric bottles at 4°C. Working solutions were prepared by diluting the stock solutions and its pH value was adjusted with hydrochloric acid.

There is no wine standard reference material with arsenic and therefore for evaluations of the accuracy of the analytical method all recovery experiments were done with the reference material of ground water.

All solutions were prepared using water from a Millipore Simplicity 185 System incorporating dual UV filters (185 and 254 nm) to remove carbon contamination.

### Determination of Arsenic Species in Wine

The wine was introduced directly in the ICP-AES, and by selecting the HCl and NaBH<sub>4</sub> concentration (optimization described below) one of the arsenic species was measured, either As(III), As(V), or DMA.

### Wine Sample Preparation

Two procedures were used for preparation for wine samples. Only for direct determination, due to wine fizzing in gas liquid separator, were four drops of 10% water solution of silicone debubbler added. It was not necessary for determination of total arsenic in wine by microwave digestion.

### Determination of Total Arsenic in Wine by Microwave Digestion

Ethanol removal was performed under an IR lamp at a temperature not higher than 70°C. Ethanol was removed from all the samples to avoid high pressure and to complete

mineralization of organic substances in wine. Samples (5.0 ml) were transferred into PTFE cuvette, 1 ml of concentrated  $\text{HNO}_3$  and 0.5 ml 30%  $\text{H}_2\text{O}_2$  were added. Digestion was performed under the following programme: warm up for 10 mins to  $200^\circ\text{C}$  and held for 10 mins at that temperature. After a cool-off period samples were quantitatively transferred into a volumetric flask (25 ml). One of the issues was dissolved nitrogen oxides as a result of microwave digestion, so it was important to reduce them prior to hydride generation. In paper [13] urea and hydrazine hydrochloride were tested as reduction agents, and hydrochloride was more effective than urea.

Reference material of ground water was prepared using the same procedure for microwave digestion as wine samples.

## Discussion and Results

### Effect of HCl Concentration (0.01-8 mol/L) on Arsine Signal Intensities at Various $\text{NaBH}_4$ Concentrations

In the first series of experiments standard solutions with arsenic species were used ( $20 \mu\text{g/L}$  each). By changing experimental conditions (concentration of HCl and  $\text{NaBH}_4$ , RF generator, flow of carrier gas) optimal conditions for selective determination of each arsenic species were found. Then the determination of arsenic species in the mixtures was carried out and the results were compared with expected values.

The effect of HCl concentration on arsine signal intensity was investigated using a series of experiments:  $\text{NaBH}_4$  solution concentrations ranged from 0.02 to 2%, and acid solution was 0.01 or 8 mol/L. Results are presented in Figs. 1-3. Different behaviours were observed for the different arsenic species.

As expected, As(III) and As(V) signal intensities were weak for low  $\text{NaBH}_4$  concentration (0.02%) and all acid concentrations of HCl, whereas DMA signals decreased with increases of acid concentration (Fig. 1).

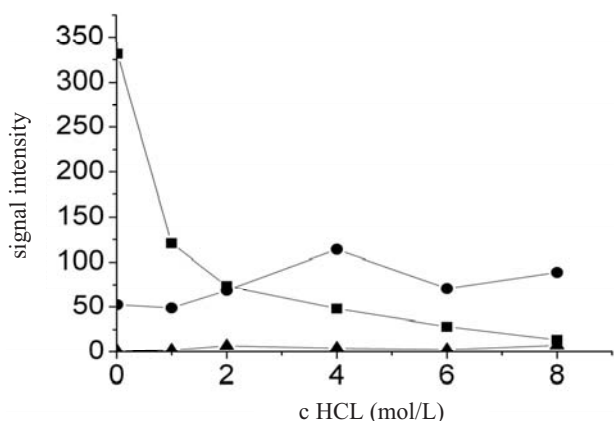


Fig. 1. Impact of different HCl solutions on signal intensities for As(III), As(V), and DMA when  $\text{NaBH}_4$  was 0.02% (■ is DMA, ▲ is As(V), and ● is As(III)).

Higher signal intensities for DMA were achieved when diluted HCl and less concentrated  $\text{NaBH}_4$  solutions were used. Therefore, the impact of the dilute HCl solution was investigated with 0.005 mol/L HCl, and  $\text{NaBH}_4$  concentration varied in the range from 0.02% to 0.10%. The highest signals for DMA determination were obtained for 0.005 mol/L HCl and 0.02%  $\text{NaBH}_4$ . This set of experiments helped us to choose the optimal conditions for DMA determination.

Results show that the As(III) signal increases with an  $\text{NaBH}_4$  concentration increase for any acid concentration, but the highest signals were obtained for As(III) when 4 mol/L or 8 mol/L were used (Fig. 2).

However, when 8 mol/L HCl solution was used with different  $\text{NaBH}_4$  solutions it was found that with 0.1%  $\text{NaBH}_4$  solution only As(III) could be determined while the signals for As(V) and DMA were considerably lower (Fig. 3). Therefore, optimal conditions for As(III) determination were: 8 mol/L HCl and 0.1 %  $\text{NaBH}_4$ .

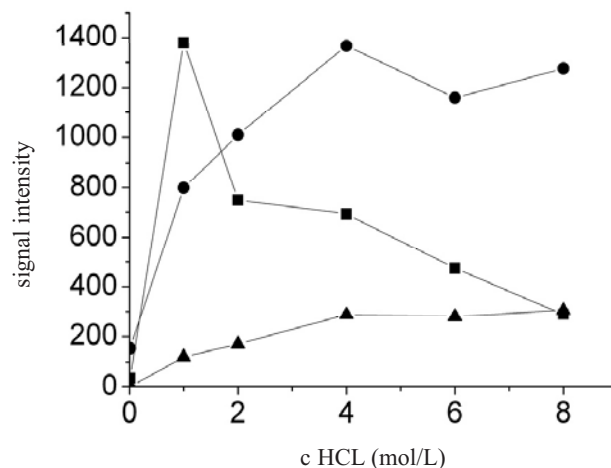


Fig. 2. Impact of different HCl solutions on signal intensities for As(III), As(V), and DMA when  $\text{NaBH}_4$  0.5% are used. (■ is DMA, ▲ is As(V) and ● is As(III)).

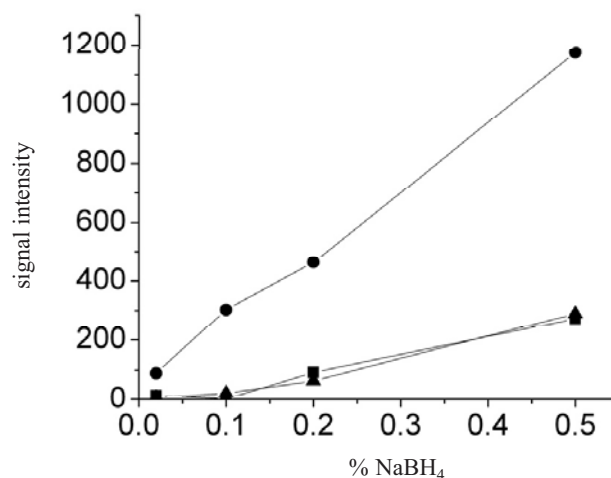


Fig. 3. Effect of  $\text{NaBH}_4$  concentration on As(III), As(V), and DMA ( $20 \mu\text{g/L}$  each) signal intensities, at 189.0 nm, for 8 mol/L HCl. (■ is DMA, ▲ is As(V) and ● is As(III)).

Table 1. Experimental conditions for determination of arsenic in the mixtures.

Arsenic	HCl (mol/L)	NaBH <sub>4</sub> %	Range (µg/L)	R	Calibration run	DL*
DMA	0.005	0.02	0-100	0.9871	Y=(21.06±3.42) C	0.60
As(III)	8	0.1	0-100	0.9957	Y=(0.78±0.07) C	0.10
As(V)	4	2	0-20	0.9985	Y=(42.07±2.30) C	0.30

\*Detection limit (µg/L) calculated for three times the S.D. of replicated blanks.

The highest intensity of As(V) signal was obtained for high concentrations of HCl acid and the highest concentration of NaBH<sub>4</sub> (2%). However, there was a problem with plasma stability in high concentrations of acid. When 4 mol/L HCl and 2% NaBH<sub>4</sub> solutions were used, obtained signals corresponded to As(V). Optimal conditions are presented in Table 1.

Further optimization of the method included adjusting other parameters at preselected concentrations of HCl and NaBH<sub>4</sub>. These parameters were: effect of RF value (generator power on signal intensities at all three wavelengths) and effect of carrier gas flow rate. Fig. 4 shows linear dependence between signal intensity and the power value for As(III) and DMA, while it was not the case for As(V). Therefore, 1250 W was chosen for As(V) and 1150 W for As(III) and DMA.

Dependence of spectral line intensities on carrier flow rates at the fixed power value was presented in Fig. 5. The intensity of signals reached maximum at 0.4 L/min (for As(III) and DMA) and 0.5 L/min (for As(V)). Further investigations were performed at the chosen flow rates.

### The Ethanol Effect

The next parameter to be optimized was the ethanol effect. Calibration runs for As(III) and As(V) were recorded in the range from 0 to 100 µg/L in aqueous solution and in solutions containing 5% and 10% ethanol.

Final insight on sensitivity impact for arsenic determination was obtained with ethanol addition. A steeper slope was obtained for ethanol solutions instead of aquatic ones, which means that increased sensitivity and improved detectability were achieved. The strongest impact of ethanol was observed at 189.0 nm. After these experiments 189.0 nm was chosen as optimal wavelength for arsenic determination in wine samples. A further increase of ethanol concentration (over 10 %) did not improve sensitivity.

### Microwave Digestion Optimization

Hydrazine hydrochloride was added after microwave digestion and prior to total arsenic determination in order to remove nitrogen oxides that decreased the yield of arsine up to 25%. The impact of reduction time was also investigated and it was found that the yield of hydride increased with time (Fig. 6). After microwave digestion, 1 g of hydrazine hydrochloride was added to samples and they were left for 6 hours before the measurement.

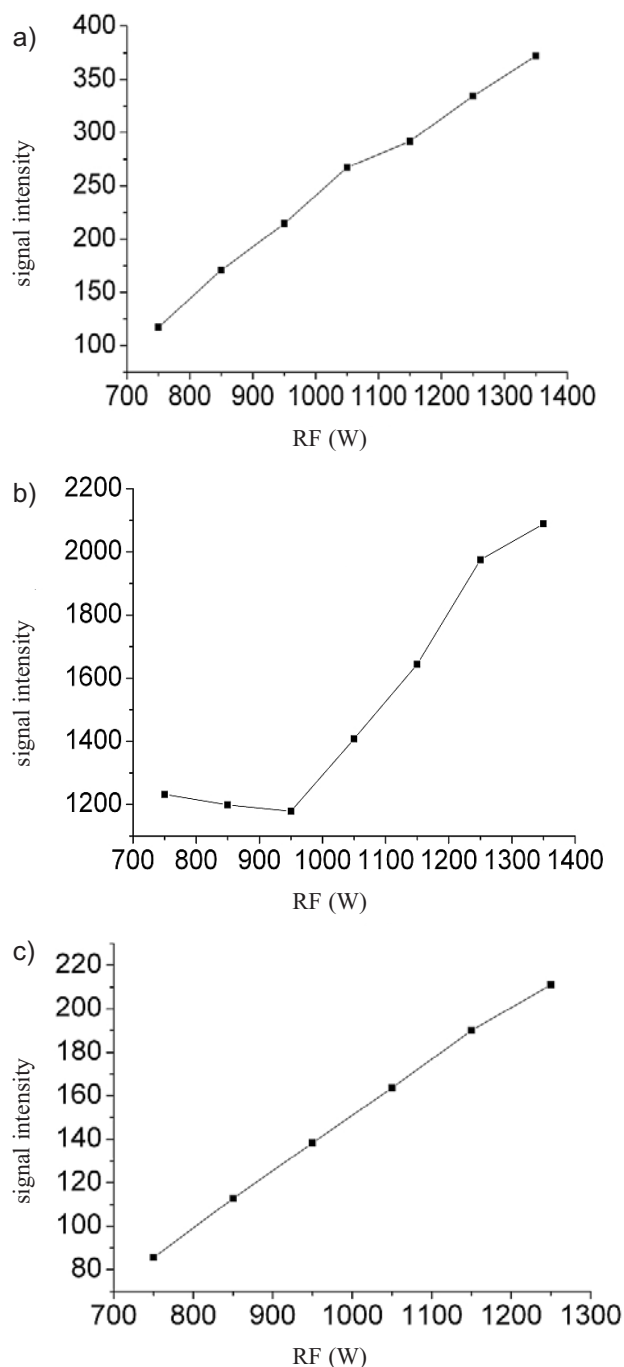


Fig. 4. RF value dependence on signal intensity a) As (III) at optimal conditions: 8 mol/L HCl, 0.1 % NaBH<sub>4</sub> b) As (V) at optimal conditions: 4 mol/L HCl, 2 % NaBH<sub>4</sub> c) DMA at optimal conditions: 0.005 mol/L HCl, 0.02 % NaBH<sub>4</sub>. Wavelength 189.0 nm, 20 µg/L each.

### Determination of Arsenic Content in Wine

The determination of arsenic species in wine samples was carried out under the optimal conditions found in experiments with standard solutions.

Arsenic(III) was determined directly in wine samples from calibration curve based on 5% solutions of ethanol in water. Experimental conditions were chosen on the basis of preliminary experiments: 8 mol/L HCl, 0.1 % NaBH<sub>4</sub>, flow rate 0.4 L/min and 1150 W. During microwave digestion

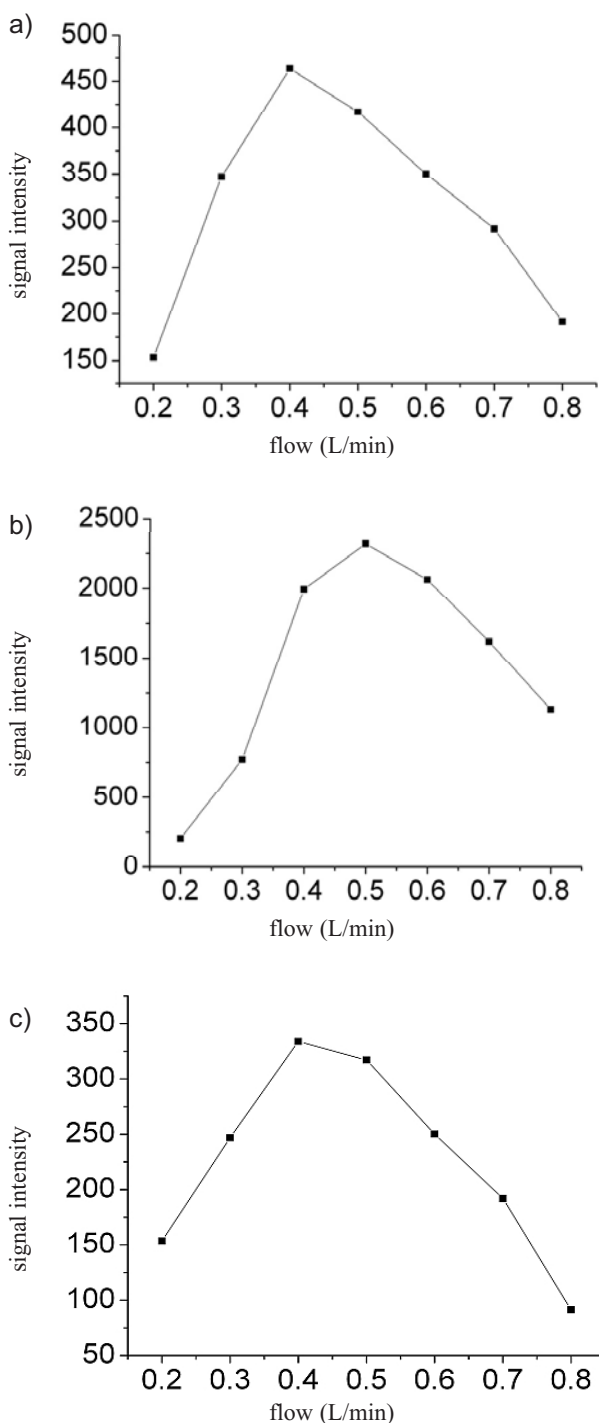


Fig. 5. Effect of carrier flow rate on signal intensities at optimal conditions at 1150 W for As(III) (a), 1250 W for As(V) (b), and 1150 W for DMA (c).

with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, all arsenic species were converted to As(V). It was determined from the calibration run with aqueous standards. The conditions were: 4 mol/L HCl, 2 % NaBH<sub>4</sub>, flow rate 0.5 L/min, 1250 W.

Results were compared to those obtained with the standard addition of all arsenic species and they showed good agreement, which indicated that the wine base is insignificant.

Arsenic content was determined in the following wine samples: "Shardonnay selekcija", "Shardonnay", "Rajnski rizling", "Graševina", "Rose" (vinarija "Radovanovic" from Serbia), and "Kratošija" (wine originating from FRY Macedonia). Table 2 sums up determination results. Results were compared to the FIA method [1]. The results obtained by the two methods are not statistically different [17].

Results for As(III) and As(V) after digestion in all study wines were essentially the same (Table 2). Therefore, the presence of As(V) and DMA in original wine were negligible, since during oxidative digestion As(III) and DMA were converted to As(V).

### Validation of the Proposed Method

The accuracy of As(V) determination was tested by the analysis of certified reference material of groundwater (high content) CRM 610. The sample was treated as wine samples (microwave digestion) and arsenic was determined as As(V). Very good agreement with declared value was obtained (Table 3).

Recovery values for spiked wine samples obtained by direct determination were presented in Table 4. Detection limits (μg/L) for determination of As(V), DMA and As(III) were found to be 0.4, 0.6, and 0.10, respectively.

### Conclusions

Simple and rapid method for As(III), As(V), and DMA determination in wine samples by ICP-AES was developed. This method can be used for routine analysis of arsenic species in wine samples. Using various concentra-

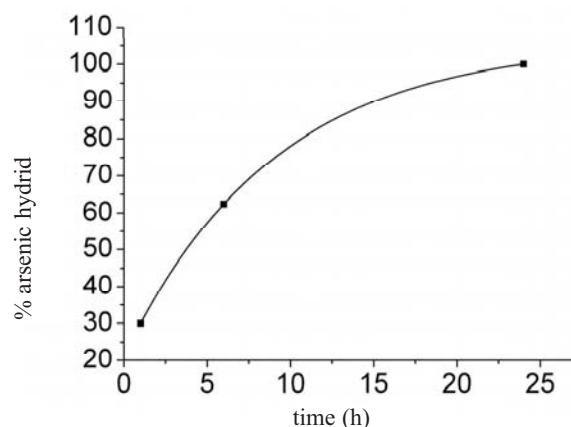


Fig. 6. Effect of time on hydrazine hydrochloride on the yield of arsenic hydride.

Table 2. Results for arsenic determination in wine samples.

Sample	Developed method		FIA method [1]	
	As(III) µg/L <sup>a</sup>	As(V) after wine sample digestion (µg/L)	As(III) µg/L	As(III) + As(V) µg/L
Kratošija	18.9±0.1	19.2±0.2	19.2±0.4	19.2±0.4
Shardonnay selekcija	4.65±0.2	4.74±0.2	4.8±0.5	4.9±0.5
Shardonnay	4.52±0.2	4.50±0.2	4.7±0.5	4.8±0.5
Rajnski rizling	5.07±0.2	5.05±0.2	5.2±0.4	5.3±0.4
Graševina	15.07±0.1	15.06±0.1	15.3±0.6	15.3±0.6
Rose	8.30±0.2	8.45±0.2	8.5±0.4	8.6±0.4

<sup>a</sup> mean±SDTable 3. Results<sup>a</sup> for certified material CRM 610 obtained by the developed method.

Sample	Developed method [µg As /kg]	Certified [µg As /kg]
CRM 610 high content	10.6±0.3	10.8±0.4

<sup>a</sup> mean±standard deviation (n=3).

Table 4. Recovery of As(III), As(V), and DMA for spiked wine samples.

Initial concentration (µg/L)		Spiked concentration (µg/L)	Found (µg/L)	Recovery (%)
As (III)	5.07 ± 0.2	5	10.8	101.0
		10	15.4	102.0
As(V)	N.D.	5	4.7	94.6
		10	9.6	96.0
DMA	N.D.	5	4.5	89.9
		10	9.2	92.3

tions of HCl and NaBH<sub>4</sub> (as well as apparatus adjustment) it is possible to differentiate and determine arsenic species. Use of ethanol/water mixtures increased the sensitivity of the method. The advantage was that there was no need for As(V) reduction. Optimal conditions were found for direct determination of arsenic species in wine samples. In addition, total arsenic as As(V) was determined after oxidative microwave digestion.

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