

## **AUTOMATIC ON-LINE SORBENT EXTRACTION PRECONCENTRATION SYSTEM FOR FLAME ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION OF COPPER AND LEAD IN TABLE AND FORTIFIED WINES**

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### **ABSTRACT**

This paper reports the development of an automated FIA system for the direct determination of copper and lead in Table and Fortified Wines by flame atomic absorption spectrometry after flow injection on-line sorbent extraction preconcentration.

The studied metals were complexed using diethylammonium-N,N-diethyldithiocarbamate as chelating agent and then collected in a column packed with bonded silica reversed-phase sorbent with octadecyl functional groups (RP-C18), positioned at the loop of the FIA system injection valve. The metal complexes were afterwards eluted with ethanol and straight directed to the atomic absorption spectrometer nebulizer.

The present FIA manifold allows the attainment of a sampling rate of about 65 samples/hour for lead determinations and from 150 to 300 samples/hour for copper determinations considering its concentration level in the samples. The detection limit for both species was about 4 µg/L.

**Keywords** FIA, preconcentration, copper, lead, wines, flame atomic absorption spectrometry

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### **INTRODUCTION**

The determination of copper and lead concentrations is commonly carried out in wine control laboratories, because these metals are

highly toxic and they greatly influence the organoleptic properties of the product.

The copper content of wine is mainly exogenous, from the copper sulphate sprays used to control mildew; the endogenous fraction derives from the soil.

The methods generally used for the determination of copper in wine include voltammetry [1], ion chromatography [2] and inductively coupled plasma atomic emission

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spectrometry (ICP-AES) [3]. Nevertheless, flame atomic absorption spectrometry (FAAS) [4-6] is the most common method used. Its use is recommended by Office International de la Vigne et du Vin [5] and it is the official Portuguese method [6]. The official procedure recommends the standard additions method to reduce the effects of wine matrix complexity and overcome the FAAS sensitivity.

When copper concentration in wine samples is very low, FAAS determinations are unsatisfactory and other procedures such as electrothermal atomisation atomic absorption spectrometry (ETA-AAS) [7, 8], also called graphite furnace atomic absorption spectrometry (GF-AAS), or sample preconcentration prior to FAAS or ICP-AES determination [9,10] have been employed.

Lead contamination in wine is mainly due to environmental pollution, e.g. car exhausts and to certain oenological practices, for example the contact between wine and tubing made of bronze, brass or rubber or the use of additives or coadjuvants [9, 11-13].

Lead determination in wines has been commonly performed by colorimetry [14, 15], inductively coupled plasma mass spectrometry (ICP-MS) [16, 17], voltammetry [18, 19], FAAS [20, 21], FAAS with hydride generation [22, 23] and ETA-AAS [24-26] used as reference methodology [27-29].

Other procedures are referred to in the literature, based on semi-automated flow systems with sample preconcentration which requires pretreatment of samples and resort to colorimetry [30] or FAAS [31] as the detection

system.

The present work describes the development of a flow injection analysis (FIA) manifold that comprises a column for the sorbent extraction and FAAS detection for copper and lead determination in Table and Fortified Wines. This automated system allows the determination of both species without requiring significant changes to the manifold configuration and facilitates the direct insertion of samples into the system without prior pretreatment, despite matrices complexity of Port Wine and Madeira Wine samples. The sorbent and chelating agent used are similar to those reported by Fang et al. for the determination of the same species in water samples [32].

## EXPERIMENTAL

### Reagents and solutions

All solutions were prepared with double-deionized water with a specific conductivity less than 0.1  $\mu\text{S}/\text{cm}$ . Analytical grade chemicals were used throughout.

Diethylammonium-N,N-diethyldithiocarbamate (DDDC) 0.1% (w/v) solution was prepared daily by dissolving the solid (Aldrich) in an aqueous solution of 0.01 M acetic acid and 0.02 M ammonia (pH 9.2) [33].

Standard solutions were prepared by dilution of copper nitrate and lead nitrate solutions at concentrations of 1000 mg/L (BDH Spectrosol) in  $\text{HNO}_3$  0.2% (w/v). These

standards presented an ethyl alcohol content similar to that of the samples.

The extraction column was packed with 40-63  $\mu\text{m}$  (Sigma) bonded silica reversed-phase sorbent with octadecyl functional groups (RP-C18), that have been used as the solid sorbent.

### Instrumentation

Gilson P100, P1000 and P5000 variable volume semi-automatic pipettes were used for the accurate measurement of solutions.

Lead determination by the reference procedure was carried out in a Perkin-Elmer

4100 ZL atomic absorption spectrometer with longitudinal Zeeman background correction and equipped with a Perkin-Elmer THGA and an AS 70 autosampler. Perkin-Elmer software installed in a personal computer controlled the equipment used and an Epson LX-800 Printer displayed the results.

### Flow injection manifold

The proposed FIA manifold comprised a Rheodyne 5020 injection valve actuated by a microcomputer [34] for the accurate control of opening and closing times of the valve. The

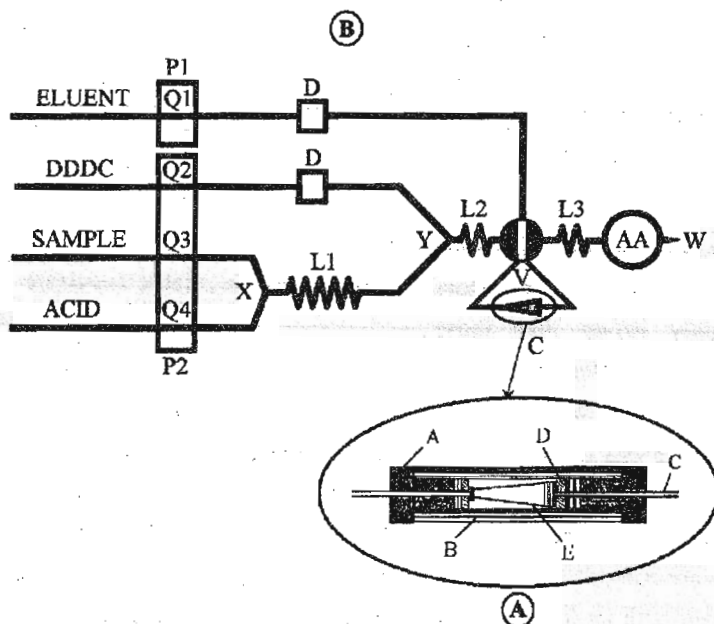


Fig. 1 (A) Detailed view of the column: A, threaded fittings; B, coating; C, PTFE tubing with an internal diameter identical to that of the manifold; D, rubber fixing ring, E, conical column with sorbent packing. (B) Flow injection manifold used for copper and lead determination: P, peristaltic pump; Q<sub>j</sub>, flow rates; D, dampers; V, injection valve; C, column; X and Y, confluences; L<sub>j</sub>, tube lengths (L<sub>1</sub> = 30 cm; L<sub>2</sub> = 3 cm; L<sub>3</sub> = 6 cm); AA, atomic absorption spectrometer; W, waste.

loop of the injection valve consisted of a home-made conic-shaped column (Fig. 1A) packed with the sorbent. To minimize sample dispersion, the column was positioned in the system so that the sample and chelant could flow from the narrowest to the largest part of the column and the eluent in the opposite direction [35]. Two Gilson Minipuls 2 peristaltic pumps were used to propel the different solutions. Tygon pump tubes and a solvent resistant silicone rubber pump tube (Isoversinic) were used for the eluent. Omnifit PTFE tubing (0.8-mm i.d.) connected by endfittings and joints of the same brand were used for the reaction coils. Home-made Perspex dampers and confluences were used [36].

A Perkin-Elmer 5000 atomic absorption spectrometer was operated according to the manufacturer's instructions and the wavelengths used were 324.8 and 283.3 nm for copper and lead respectively. The flame conditions were slightly leaner than those recommended in order to compensate for the effect of the eluent used. In these conditions the optimal aspiration flow rate was 2.5 mL/min.

The opening/closing steps of the valve, as well as the direction of rotation and speed of the peristaltic pumps were controlled by a Mtek microcomputer equipped with an Intel 486 DX data processor which also controlled elution and preconcentration times and its sequence. The analytical signals were acquired either by this control system or a Kipp & Zonen BD 111 recorder.

### Reference procedure

Comparison of the results obtained using the FIA methodology and those of the batch procedures, allowed evaluation of the quality of the results provided by the present automated system.

The conventional determination of metal content in Table Wines was carried out according to the recommendations of Office International de la Vigne et du Vin and therefore FAAS was used with the standard addition method for copper determinations [5], and ETA-AAS for lead [28].

Due to the lack of a reference procedure for the analysis of Fortified Wines (Port Wine and Madeira Wine), the results of the proposed methodology were compared with those obtained from previously developed methodology [7, 26].

## **RESULTS AND DISCUSSION**

The FIA system was developed from a simple configuration (Fig. 1B) that allowed adjustment of sample acidity inside the system (merging of the flows at confluence X) in order to achieve an effective complexing reaction with the chelating solution (added through channel Q2) at confluence Y. The metal complexes were preconcentrated in the column (C) placed at the injection valve (V) loop. Rotating the valve to the reverse position allowed the eluent (channel Q1) to flow through the column and the subsequent entrainment of the metal complexes to the

atomic absorption spectrometer in which the measurement took place.

The manifold parameters were optimised using the univariant method with the intention of (i) estimating copper and lead concentrations without pretreatment of samples; (ii) allowing both species to be determined without changing the configuration; (iii) analysing both Table and Fortified Wines.

The system was optimised, particularly in respect of reproducibility and sampling rate. The quality of the results obtained by the proposed methodology was assessed by comparing them with those obtained from the reference procedures using the same samples.

#### Optimisation of the flow injection manifold

*Reactors Length.* Different lengths were tried for reactor L1 positioned between X and Y joints. This reactor mixes the sample from channel Q3 with nitric acid from channel Q4.

Reactor lengths less than 30 cm provided insufficient reproducibility of the analytical signals due to inadequate mixing between the sample and the nitric acid which would cause poor retention of the species inside the preconcentration column. Longer reactors showed that there was excessive dilution of the sample that reduced the effectiveness of the preconcentration step and consequently required longer preconcentration periods, which decreased the sampling rate; selecting a 30-cm length for reactor L1 achieved a satisfactory compromise between both requirements.

The length for reactor L2 (3 cm) that connects confluence Y (in which the complexing reaction takes place) to the injection valve as well as for reactor L3 (6 cm) (through which this valve is connected to the atomic absorption spectrometer) was basically determined by the physical configuration of the manifold. It was set to the minimum possible value because the complexed metal species flowing through reactor L2 tended to be adsorbed by the reactor walls [37], whereas a higher dispersion of the species in the eluent flow would reduce the analytical signal if a longer L3 reactor were used.

*Sample/Nitric Acid Flow Rates and Acid Concentration.* The ratio of the flow rates in channels Q3 and Q4 (confluence X) conditions the sample before the metal complexes are formed after confluence Y as well as its subsequent retention in the column. The optimal conditions required for this procedure vary according to the metal studied due to their different concentration and to the differences between Table and Fortified Wines matrices.

Samples dilution and acidification at confluence X are both required because the high levels of copper in wines would otherwise cause column saturation. Alternatively, a shorter preconcentration step might be attempted if the reproducibility of the results is not affected. A four times dilution of the sample at confluence X was found to be appropriate for Table and Fortified Wines and thus Q4=1.0 mL/min and Q3=4.2 mL/min flow rates were selected.

The experimental conditions for lead

determination are much more restrictive than those required for copper determination due to the low concentrations of the former in wines. Hence the flow rates at confluence X were reduced to the lowest possible otherwise a high sample volume (time) would have to be used in the preconcentration procedure and consequently the detection limits and sampling rates would be compromised.

The flow rates selected for Fortified Wines, Q3=2.5 mL/min and Q4=1.0 mL/min, were also suitable for Table Wines. Nevertheless the less complex matrix of Table Wines allows higher sample and acid flow rates (Q3=4.4 mL/min and Q4=1.3 mL/min) and thus higher sampling rates.

The optimisation of Q3/Q4 flow rates for the estimation of copper and lead was performed considering the optimization of HNO<sub>3</sub> concentration for standards and samples in channel Q4.

When analysing standard copper solutions with nitric acid concentrations ranging from 0 to 3M (Fig. 2) the signal amplitude was constant, whereas for samples of both wines the best response was obtained at HNO<sub>3</sub> concentration of 1M and above (Fig. 2). The concentration of 1M was therefore selected.

For lead determination, it was found that the analytical signal peak height attained for standards did not significantly vary up to about 9M acid concentration. The samples, however, provided increasing peaks up to 6M nitric acid concentration (Fig. 2), which was therefore selected as optimal HNO<sub>3</sub> concentration.

#### *Chelating Agent Concentration and Flow*

*Rate.* The chelating agent concentration in channel Q2 solution was assessed within a concentration range of 0.02% to 0.2% (w/v), and using differently concentrated standards and samples. No significant differences in the analytical signal amplitude attained for both were found. Therefore the mean concentration (0.1%) of the chelating agent was selected for complexing copper and lead in both Table and Fortified Wines, although the lowest concentration would be sufficient to prevent its consumption by other metals that may be present in higher concentrations in other samples, whose composition may vary in relation to those used in our study.

After establishing the chelating solution composition, its flow rate at confluence Y in which the acidified sample is mixed with this solution was also evaluated. This mixing step is greatly affected by the limited length of reactor L2 discussed previously. The propulsion of fluids by peristaltic pumps inevitably produces pulses in the flow, even when pulse dampers are used. The mixing of two flows at a confluence is never fully accomplished before it reaches the reactor placed next to the confluence. Optimal conditions for perfect mixing at confluence Y were sought without changing the reactor length due to the effects mentioned above and it was found that this occurs at flow rates higher than 1.0 mL/min.

The selection of the flow rate for the chelating solution (Q2), 1.0 mL/min, was determined by the fact that higher flow rates at Q2 in addition to those set for Q3 and Q4 produced an overpressure on the preconcentra-

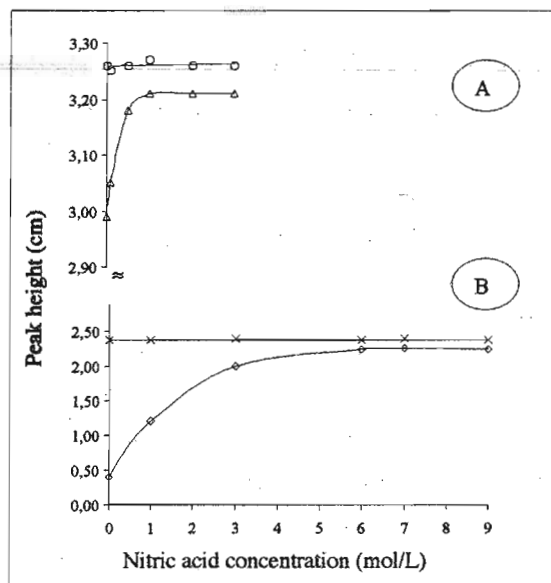


Fig. 2 Effect of nitric acid concentration on the peak heights of (A) copper standards and samples (o and Δ) and (B) lead standards and samples (x and ◇).

tion column positioned at the loop of the injection valve and, consequently, reduced reproducibility.

*Eluent Flow.* As methanol and ethanol are widely used for the elution of hydrophobic sorbents [33], these solvents were tested in the proposed system (channel Q1) and no significant effects on the analytical signal amplitude and precision were found. Therefore, ethanol was used as the eluent due to its lower toxicity.

When optimising this parameter, a slight overpressure on the nebulizer was imposed compared to the optimum recommended for the atomic absorption spectrometer when operating under conventional conditions with this solvent (2.5 mL/min).

The use of overpressure in atomic absorp-

tion spectrometer coupled to FIA systems produces better reproducibility of results, as already stressed by some of us in prior works [38, 39].

Flow rates ranging from 2.3 to 3.7 mL/min were tested and it was found that lower flows produced lower sampling rates and that the highest flows gave rise to high consumption of the eluent as well as excessive overpressure inside the manifold. This determined the selection of a 2.8 mL/min flow rate for the eluent determined by the peristaltic pump.

*Preconcentration and Elution Time Intervals.* Adequate preconcentration and elution times depend upon copper and lead concentration of the samples; these times can be changed whenever necessary by the computer which controls all the system. The

optimisation of these parameters was carried out in the current study considering the usual concentration ranges of these metals even under unfavourable conditions. Therefore, the length of the preconcentration step was optimised using solutions with concentrations near the lowest concentration levels whereas for the determination of the best elution time, solutions with concentrations near to the highest values were used.

The optimal times for copper preconcentration and elution were assessed within a copper concentration range of 25 to 200 µg/L and 200 to 1250 µg/L. Times of 16/9s and 4/8s were accomplished for the lowest and highest concentrations, respectively.

For lead determinations, a concentration range of 20 to 100 µg/L was used in the optimisation of the same parameters, with a preconcentration step of 50s and a 10s elution.

#### Working characteristics of the flow systems

After optimisation of the manifold parameters, the system working characteristics were evaluated.

The developed system allowed the attainment of sampling rates for copper determinations of about 150 samples/hour with concentrations ranging from 25 to 200 µg/L (Fig. 3), and about 300 samples/hour at higher concentrations. In both conditions, the reproducibility of the system was good, providing results with a relative standard deviation (RSD) of 1.6% for 11 consecutive injections of samples with an intermediate

concentration taken from the considered range of concentrations. The detection limit evaluated as the concentration corresponding to three-times the standard deviation of the background signal [40], corresponded to 4 µg/L for the low concentration levels.

The determination of lead at concentrations of 20 to 100 µg/L was accomplished at a sampling rate of about 65 samples/hour. A detection limit of 4 µg/L [40] and a RSD of 1.9% for 11 consecutive injections were obtained.

#### Application to wine analysis

Precision of the results obtained from the automated system was assessed by comparison with those from the reference methods, namely FAAS standard addition method [5] for copper determinations (Table 1) and ETA-AAS [28] for lead determinations (Table 2). As there are no reference methods for comparison of the results obtained with Fortified Wine samples, these were compared with those given by ETA-AAS, the procedure used for the determination of both species [7, 26].

After comparison of the results obtained for a set of 19 Table and Fortified Wines, the relationship  $CF = C_0 + S \times CR$ , where CF corresponds to the concentration obtained by the FIA methodology and CR to that given by the reference method, was established.

A  $C_0$  of -3.2 µg/L and an S value of 1.01 were obtained for copper determinations. A correlation coefficient of 0.9996 between the current and the reference methods was found



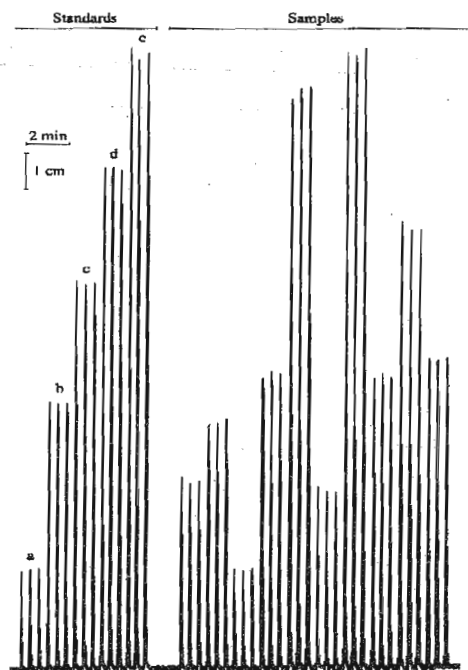


Fig. 3 FIA register obtained for the determination of copper in wines corresponding to injections of standards [(a) 25, (b) 50, (c) 100, (d) 150 and (e) 200  $\mu\text{g/L}$ ] and samples.

for the 19 samples analysed, showing that there was an excellent agreement between both methodologies. The mean relative deviation of the automated procedure was less than 2%, the highest corresponding to 5%, for the different wine samples in copper concentrations ranging from 29.0 to 1230.0  $\mu\text{g/L}$ . The comparison with the results provided by ETA-AAS methodology [7] showed that there was also a good agreement between both methodologies (Table 1).

The results obtained from the determination of lead concentration in the samples (varying from 22.8 to 92.0  $\mu\text{g/L}$ ) were also compared

and presented a CO of 0.006  $\mu\text{g/L}$ , an S value of 0.996 and a correlation coefficient of 0.998. The mean relative deviation of the proposed methodology was less than 1% (the maximum being about 4%).

## CONCLUSIONS

On-line sorbent extraction preconcentration by FIA coupled to flame AAS is an advantageous alternative to conventional procedures for copper and lead determination in Table and Fortified Wines.

The automated procedure described in this

Table 1 Results in  $\mu\text{g/L}$  obtained from copper determination in Table Wines (W- White; R- Red) and Fortified Wines (PW- Port Wine; MW- Madeira Wine) by on-line sorbent extraction preconcentration FIA methodology (FIA), by the reference method (OIV) and by electrothermal atomisation (ETA-AAS)<sup>(1)</sup>

Sample	Method			RD (%) <sup>(2)</sup>	
	FIA	OIV	ETA-AAS	a	b
PW1	378.0	382.6	379.0	-1.2	-0.9
PW2	494.3	500.5	505.0	-1.2	0.9
PW3	181.4	187.9	180.0	-3.4	-4.2
PW4	588.4	603.3	599.0	-2.5	-0.7
PW5	1148.5	1140.4	1130.0	0.7	-0.9
MW1	323.7	340.0	334.0	-4.8	-1.8
MW2	1243.3	1230.0	1250.0	1.1	1.6
MW3	585.9	592.0	598.0	-1.0	1.0
R1	52.5	51.0	53.0	2.9	3.9
R2	129.2	123.0	121.0	5.0	-1.6
R3	35.6	36.0	35.9	-1.1	-0.3
R4	28.9	29.0	29.2	-0.3	0.7
R5	35.9	36.0	34.9	-0.3	-3.1
R6	62.9	60.0	56.0	4.8	-6.6
W1	866.8	860.0	864.0	0.8	0.5
W2	80.9	80.0	82.0	1.1	2.5
W3	103.3	103.0	104.0	0.3	1.0
W4	596.8	587.6	586.0	1.6	-0.3
W5	161.7	169.0	170.2	-4.3	0.7

(1) Please see text.

(2) Relative deviation of the developed methodology to the reference method (a) and ETA-AAS (b).

study presents great advantages in relation to the methods previously described in the literature for lead determination [30, 31] because samples are inserted into the system without prior treatment. This allows automation of the whole analytical procedure as well as the attainment of good sampling rates that are not affected by the need for pretreatment of samples.

When comparing the present methodology

to electrothermal atomisation AAS it is found that column preconcentration and subsequent flame AAS determination provides high sampling rates in addition to results of similar precision. The manifold presents other advantageous features such as easy handling and less expensive maintenance and working conditions.

It should also be stressed that this FIA manifold only requires the instrumentation usually

Table 2 Results in  $\mu\text{g/L}$ , obtained from lead determination in Table Wines (W-White; R- Red) and Fortified Wines (PW- Port Wine; MW- Madeira Wine) by on-line sorbent extraction preconcentration FIA methodology and by the reference method

Sample	FIA preconcentration	Reference method	RD (%) <sup>(1)</sup>
PW1	53.8	55.0	-2.2
PW2	60.8	61.8	-1.6
PW3	39.6	40.0	-1.0
PW4	74.9	74.0	1.2
PW5	91.3	92.0	-0.8
MW1	47.2	48.0	-1.7
MW2	51.9	52.0	-0.2
MW3	49.1	49.0	0.2
W1	34.9	34.0	2.6
W2	31.6	32.4	-2.5
W3	62.2	61.9	0.5
W4	63.1	63.4	-0.5
W5	53.7	53.4	0.6
R1	34.3	34.4	-0.3
R2	27.5	26.6	3.3
R3	29.0	29.4	-1.4
R4	25.7	25.4	1.2
R5	21.9	22.8	-3.9

(1) Relative deviation of the developed methodology to the reference method.

available in control laboratories and that is the same detection system as that of conventional procedures and avoids the use of ETA-AAS for lead determinations in wines.

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