

## Short communication. Use of 4-(5-Br-2 pyridylazo)-1,3-diaminobenzene (5-Br-PADAB) for the spectrometric determination of cobalt in wine

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### Abstract

The typically low content of cobalt in wines hinders its quantification by molecular absorptiometry and atomic absorption spectrometry (AAS); difficulties arise both in the preparation of samples and cobalt detection. This work proposes a highly sensitive and selective extraction-spectrometry method that uses 4-(5-Br-2 pyridylazo)-1,3-diaminobenzene (5-Br-PADAB) for the determination of cobalt in this medium. Its accuracy and reproducibility are comparable to those of flame AAS. The molar absorptivity of the complex was 116,000 L mol<sup>-1</sup> cm<sup>-1</sup>. Interference from Fe (III) and Cu (II) ions was resolved by masking them with fluoride and cupron respectively.

**Additional key words:** absorptiometry, alcoholic beverages, heavy metals.

### Resumen

#### Comunicación corta. Uso de 4-(5-Br-2 piridilazo)-1,3-diaminobenzeno (5-Br-PADAB) en la determinación espectrométrica de cobalto en vinos

El normalmente bajo contenido de cobalto en vinos trae aparejado problemas en su cuantificación, tanto en absorciometría molecular como en espectrometría de absorción atómica corriente, no solo en el tratamiento previo de las muestras sino también en el aspecto instrumental. El objetivo del trabajo fue aplicar un método altamente sensible y selectivo que usa 4-(5-Br-2 piridilazo)-1,3-diaminobenzeno (5-Br-PADAB) para la determinación de cobalto en vinos. Su exactitud y precisión fueron comparables a los obtenidos por espectrometría de absorción atómica. La absorptividad molar del complejo de Co-5-Br-PADAB fue de 116.000 L mol<sup>-1</sup> cm<sup>-1</sup>. Las interferencias debidas a Fe (III) y Cu (II) fueron resueltas por enmascaramiento con fluoruro y cuprón respectivamente.

**Palabras clave adicionales:** absorciometría, bebidas alcohólicas, metales pesados.

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Cobalt is a micronutrient required by humans and livestock (cobalt is necessary for the synthesis of vitamin B<sub>12</sub> in cattle, sheep and goats), although like most micronutrients it is toxic at high concentrations. Cobalt may enter wine during fermentation or other stages of manufacture, especially if traditional technology is used. The methods currently used to determine the low cobalt concentrations in wines suffer from selectivity problems caused by the presence (in much larger

quantities) of Fe, Cu, Zn and Mn. With the exception of atomic absorption spectrometry (AAS) (Onianwa *et al.*, 1999) in flameless atomization or plasma emission mode—which is expensive and difficult to perform—absorptiometric methods are limited in terms of their sensitivity and selectivity. Most are performed in several stages. First, the cobalt is extracted as a chelate using a suitable reagent in an organic solvent, which requires more than one contact run. This is followed by the evaporation of the organic solvent and the destruction of the organic matter in the chelate via mineralisation with an oxidising acid at high tempera-

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tures. Finally, a colour is developed with (almost exclusively) nitroso R salt, the limitations of which will be discussed later.

The aims of the present work were to use the reagent 4-(5-Br-2 pyridylazo)-1,3-diaminobenzene (5-Br-PADAB) (based on the works of Shibata, 1972; Baudino and Marone, 1980; Baudino *et al.*, 1991) to i) develop a low cost, highly sensitive and selective method for the determination of cobalt in wine, ii) to compare the results obtained with this method to those provided by flame AAS (Onianwa *et al.*, 1999), and iii) to evaluate the precision of the proposed method.

All absorptiometric measurements were made using a Shimadzu UV 150 spectrometer; pH was measured using an Orion 701 pH meter. AAS determinations were performed using an IL Model 751 AA/AE apparatus with the following operational characteristics: flame acetylene-air rate 340-127 L h<sup>-1</sup>; wavelength 240.7 nm; slit 0.08 nm; photo-multiplier voltage 800 V; aspiration rate 8 mL min<sup>-1</sup>; lamp current 8 A; scale expansion 1,000; sensitivity 0.05 µg Co/1%A.

The reagents and the calibration procedures used in cobalt extraction-spectrometry were those described by Baudino and Marone (1980). For calibration, aliquots of a 2.10<sup>-3</sup> M cobalt solution, each containing 0.6 to 25 µg Co, were pipetted into 50 mL volumetric flasks. One millilitre of 0.2 M sodium acetate-acetic acid (pH 5) and 2 mL of an ethanolic solution of 5-Br-PADAB (5.10<sup>-4</sup> M) were then added. The solutions were mixed well and allowed to rest for 10 min. Ten millilitres of concentrated HCl (dens. 1.19 g cm<sup>-3</sup>) were then added, and diluted to 50 mL with bidistilled water. Absorption was measured against a blank at 573 nm, using glass cuvettes with an optical path of 10.0 mm. The molar absorptivity of a solution of 2.5 M HCl at 573 nm was found to be 1.16 10<sup>5</sup> L mol<sup>-1</sup> cm<sup>-1</sup>, with a Sandell sensitivity of 0.51 ng cm<sup>-2</sup>. The law of Beer was adhered to over the range 0.012-0.5 µg mL<sup>-1</sup>.

The synthesis of 5-Br-PADAB was undertaken as follows:

— *Preparation of 5-Br-2-aminopyridine*: A solution was prepared with 18.8 g of 2-aminopyridine and 200 mL of 95% ethanol in a round-bottomed flask provided with refluxed coolant. Forty grams of bromine were then added drop by drop, while keeping the temperature under 20°C. The solid thus formed was collected on filter paper, washed through a funnel three times with 3-4 mL of methanol, and finally dissolved in distilled water. This aqueous solution was then weakly alkalised with a 0.5 N NaOH solution, trans-

ferred to a decanting flask, and stirred for 5 min with an equal volume of ethyl ether. This last operation was repeated twice. The ether extracts were then evaporated to dryness by warming in a water bath. The residue containing the 5-Br-aminopyridine was dissolved by successively adding two 10 mL aliquots of petroleum ether. The remaining solid was dissolved in hot benzene and re-precipitated by cooling. Purification was performed by repeating this last operation, which resulted in the formation of a yellow crystalline powder with a melting point of 137°-138°C.

— *Diazotation*: A solution of 6 g of 5-Br-2-aminopyridine in 80 mL of absolute ethanol was prepared and placed in a 250 mL round bottomed flask provided with refluxed coolant. Some 5.46 g of recently prepared isoamyl nitrite were then added, along with 2.4 g of sodium amide. The mixture was heated for 2 h under reflux conditions in a hot water bath, and then cooled, thus producing the final diazotate solution for use in copulation.

— *Copulation*: First, 8.4 g of m-phenylenediamine hydrochloride was dissolved in 50 mL of distilled water in a precipitation vessel. The solution obtained was then poured into the diazotate solution. The compound formed was left to rest overnight at room temperature; it was then filtered through a filter composed of a funnel with a sinterized glass bed. The dark reddish crystals formed during the reaction were washed three times over the filter using 5 mL aliquots of cold distilled water. The solid obtained was dissolved in hot ethanol and re-precipitated by adding cold distilled water as necessary.

Following the same procedure, the dark-reddish solid obtained was re-crystallized twice. The portion of the reagent destined for spectral studies was purified by sublimation at low pressure (T° > 200°C; 30 mmHg).

All the glass materials in contact with the samples were pre-treated by removing all traces of grease using an alcoholic solution of 5% KOH. They were then subjected to contact with HNO<sub>3</sub> (1:1 v:v) for 2 to 3 h at room temperature before washing repeatedly with running tap water and bidistilled water. The HNO<sub>3</sub> and HCl solutions used should be of great purity (if necessary, purification should be performed by distillation in Pyrex equipment). The remaining precautions taken to prevent the contamination of the reagents and samples are described in Thiers (1957).

Samples of white wine and red wines (grape varieties indicated in Table 1) were obtained from wineries (harvest 2004), four from the Province of Mendoza,

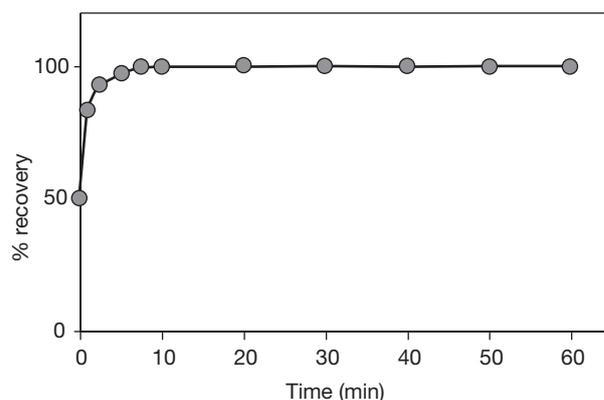
**Table 1.** Cobalt content in wines as determined by the proposed and flame AAS (Onianwa *et al.*, 1999) methods. The results are the means of five determinations

Samples	Type	Origin	Cultivar	Cobalt content ( $\mu\text{g L}^{-1}$ )			
				Proposed method	SD	AAS	SD
M1	Table wine	Mendoza	Malbec	1.72	0.041	1.69	0.040
M2	Table wine	Mendoza	Cabernet Sauvignon	1.70	0.040	1.72	0.040
M3	Table wine	San Juan	Syrah	1.82	0.046	1.85	0.039
M4	Varietal wine	San Juan	Malbec	1.70	0.046	1.68	0.043
M5	Fine varietal wine	San Juan	Torrontes	1.88	0.044	1.93	0.047
M6	Fine varietal wine	San Juan	Sauvignon Blanc	1.80	0.043	1.80	0.041
M7	Fine varietal wine	San Juan	Chardonnay	1.60	0.040	1.65	0.042
M8	Varietal	Mendoza	Bonarda	2.01	0.046	1.96	0.041
M9	Varietal	Mendoza	Tannat	1.63	0.048	1.70	0.043
M10	Varietal	San Juan	Syrah	1.67	0.051	1.68	0.048

the remainder from the Province of San Juan. In all cases, the sample wines were manufactured using stainless steel fermenting equipment. One hundred milliliters aliquots were placed in platinum capsules and slowly heated using a blanket heater until dry. The capsules were then placed in a cold open muffle furnace, raising the temperature by steps of  $50^{\circ}\text{C}$ , from  $20^{\circ}\text{C}$  up to  $500^{\circ}\text{C}$ , allowing time enough at each temperature for fume production to cease. Finally, the muffle furnace was closed and the temperature increased to  $620^{\circ}\text{C}$ . These conditions were maintained until a residue free of organic matter was obtained. The residue from mineralisation was collected with 4 mL of hydrochloric acid (1:1 v:v), vaporized in a hot water bath until almost dry, and then completely transferred along with 2 mL of bidistilled water into a 50 mL separating funnel with a Teflon stop cock containing 1 mL of 0.5 M sodium acetate-acetic acid, 1 mL of 0.8 M sodium fluoride and 0.5 mL of 0.1 M cupron ethanol solution to mask the iron and copper respectively [Fe (III) and Cu (II) ions could cause serious interference; the sodium fluoride and cupron prevent this by selectively complexing with these metals], 1 mL of  $10^{-3}$  M 5-Br-PADAB, and 0.5 mL of 96% ethanol. This mixture was left to rest for 10 min, stirring sporadically, before adding 1 mL of 1% (w:v) anthraquinone sulphonate solution and shaking with 10 mL of chloroform (without washing) for 10 min. It was then left to rest in order to separate the phases. The chloroform phase was decanted into another flask or extraction tube, washing the aqueous phase twice with 2 mL chloroform each time. If the organic phase took the form of an emulsion it was centrifuged or dehydrated by adding a small quantity of sodium

sulphate. The organic extract was then shaken with 5 mL of 2.4 M HCl for approximately 30-40 s. The absorbance of the acidic solution at 573 nm was measured against a blank of reagents prepared in a similar fashion. The cobalt concentration was calculated using the calibration curve. In the presence of the masking substances used to reduce cation interference, the time required for the cobalt to be quantitatively complexed by the 5-Br-PADAB is longer than in their absence. The results of tests showed that the minimum resting time required before proceeding to extraction was 10 min (Fig. 1).

The accuracy of the method was checked against the standard flame AAS technique proposed by Onianwa *et al.* (1999). Table 1 shows that results obtained with both methods are concordant.

**Figure 1.** Recovery of cobalt: time elapsed between the addition of 5-Br-PADAB (time zero) and the extraction of cobalt with chloroform.

**Table 2.** Over-aggregate analysis: results are the means of three determinations

Sample	Cobalt added ( $\mu\text{g}$ )	Cobalt detected ( $\mu\text{g}$ )	Recovery percentage	SD
M1	0.56	0.52	94.6	0.033
M2	1.12	1.15	102.7	0.034
M3	1.68	1.66	98.9	0.045
M4	2.24	2.30	102.6	0.037

In addition, the recovery of the aggregated cobalt was performed before proceeding to the mineralisation stage (over-aggregate method) in order to detect possible operational or methodological errors. As shown in Table 2, the results for the recovery of the aggregated cobalt were satisfactory.

The precision of the proposed method was also analysed. The mean value of the cobalt for 15 determinations was  $1.83 \mu\text{g L}^{-1}$ , the standard deviation (SD) was 0.0477, and the coefficient of variation (CV) was 2.6.

Among the extraction-spectrometry methods used to determine cobalt in trace amounts, those that employ nitroso R salt as a colouring reagent are the most common. However, compared to the proposed method they have a number of disadvantages. They all first require the use of some reagent to make a complex (Dewey and Marston, 1971; AOAC, 1990), the extraction of the cobalt, the evaporation of the solvent, the destruction of the extracting agent with acidic-oxidising mixtures, and the generation of a colour with nitroso R salt. When using 5-Br-PADAB, complexing, extraction and colour generation are achieved with just one reagent, which reduces the time required and operational costs. In addition, fewer chances arise for contamination and there are fewer losses due to sample projection. Moreover, the coloured complex obtained with the nitroso R salt has a lower order of magnitude extinction coefficient than the Co-5-Br-PADAB complex. This low sensitivity means larger sample volumes are needed. The distribution coefficient of the ion-pair Co-5-Br-PADAB-anthraquinone sulphonate is large enough to allow the quantitative extraction of cobalt in just one contact run, whereas the nitroso R salt methods need at least three consecutive extractions. With respect to interference from other ions, the proposed method is highly selective and, using fluoride and cupron as iron and copper masks respectively, it is practically specific. Finally, it should be noted that excess 5-Br-PADAB need not be removed since, at the cobalt complex ab-

sorption peak, it shows negligible absorbance. This is not the case in methods that employ nitroso R salt.

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