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

O. M. Baudino¹*, M. M. de Pedernera², C. E. Guardia Calderon² and E. A. Suero¹

¹ Instituto de Biotecnología. Facultad de Ingeniería. Universidad Nacional de San Juan. 
Avda. Libertador, 1109 oeste. 5400 San Juan. Argentina
² Área de Bromatología, Ensayo y Valoración de Medicamentos. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. Ejército de los Andes, 950. 5700 San Luis. Argentina

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⁻¹ cm⁻¹. 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 pyridylazo)-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 pyridylazo)-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⁻¹ cm⁻¹. 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.
Use of 5-Br-PADAB in the spectrometric determination of cobalt in wine

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5-Br-PADAB was then added, and diluted to 50 mL with bidistilled water. Absorption was measured against a blank at 573 nm, 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 synerthesized 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 HNO₃ (1:1 v:v) for 2 to 3 h at room temperature before washing repeatedly with running tap water and bidistilled water. The HNO₃ 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,
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 50ºC, from 20ºC up to 500ºC, allowing time enough at each temperature for fume production to cease. Finally, the muffle furnace was closed and the temperature increased to 620ºC. 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.

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Type</th>
<th>Origin</th>
<th>Cultivar</th>
<th>Cobalt content (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proposed method</td>
</tr>
<tr>
<td>M1</td>
<td>Table wine</td>
<td>Mendoza</td>
<td>Malbec</td>
<td>1.72</td>
</tr>
<tr>
<td>M2</td>
<td>Table wine</td>
<td>Mendoza</td>
<td>Cabernet Sauvignon</td>
<td>1.70</td>
</tr>
<tr>
<td>M3</td>
<td>Table wine</td>
<td>San Juan</td>
<td>Syrah</td>
<td>1.82</td>
</tr>
<tr>
<td>M4</td>
<td>Varietal wine</td>
<td>San Juan</td>
<td>Malbec</td>
<td>1.70</td>
</tr>
<tr>
<td>M5</td>
<td>Fine varietal wine</td>
<td>San Juan</td>
<td>Torrontes</td>
<td>1.88</td>
</tr>
<tr>
<td>M6</td>
<td>Fine varietal wine</td>
<td>San Juan</td>
<td>Sauvignon Blanc</td>
<td>1.80</td>
</tr>
<tr>
<td>M7</td>
<td>Fine varietal wine</td>
<td>San Juan</td>
<td>Chardonnay</td>
<td>1.60</td>
</tr>
<tr>
<td>M8</td>
<td>Varietal</td>
<td>Mendoza</td>
<td>Bonarda</td>
<td>2.01</td>
</tr>
<tr>
<td>M9</td>
<td>Varietal</td>
<td>Mendoza</td>
<td>Tannat</td>
<td>1.63</td>
</tr>
<tr>
<td>M10</td>
<td>Varietal</td>
<td>San Juan</td>
<td>Syrah</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Figure 1. Recovery of cobalt: time elapsed between the addition of 5-Br-PADAB (time zero) and the extraction of cobalt with chloroform.
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 (µg 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-P ADAB, 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-P ADAB complex. This low sensitivity means larger sample volumes are needed. The distribution coefficient of the ion-pair Co-5-Br-P ADAB-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-P ADAB need not be removed since, at the cobalt complex absorption peak, it shows negligible absorbance. This is not the case in methods that employ nitroso R salt.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cobalt added (µg)</th>
<th>Cobalt detected (µg)</th>
<th>Recovery percentage</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.56</td>
<td>0.52</td>
<td>94.6</td>
<td>0.033</td>
</tr>
<tr>
<td>M2</td>
<td>1.12</td>
<td>1.15</td>
<td>102.7</td>
<td>0.034</td>
</tr>
<tr>
<td>M3</td>
<td>1.68</td>
<td>1.66</td>
<td>98.9</td>
<td>0.045</td>
</tr>
<tr>
<td>M4</td>
<td>2.24</td>
<td>2.30</td>
<td>102.6</td>
<td>0.037</td>
</tr>
</tbody>
</table>

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### References


BAUDINO O.M., GUARDIA CALDERÓN C.E., DE PEDERNERA M., 1991. Uso de 4-(5-Br-2 pyridylazo)-1,3-diaminobenzene (5-Br-PADAB) en la determinación espectrométrica de cobalto en agua. Anales de Química 87(1), 99-104. [In Spanish].


