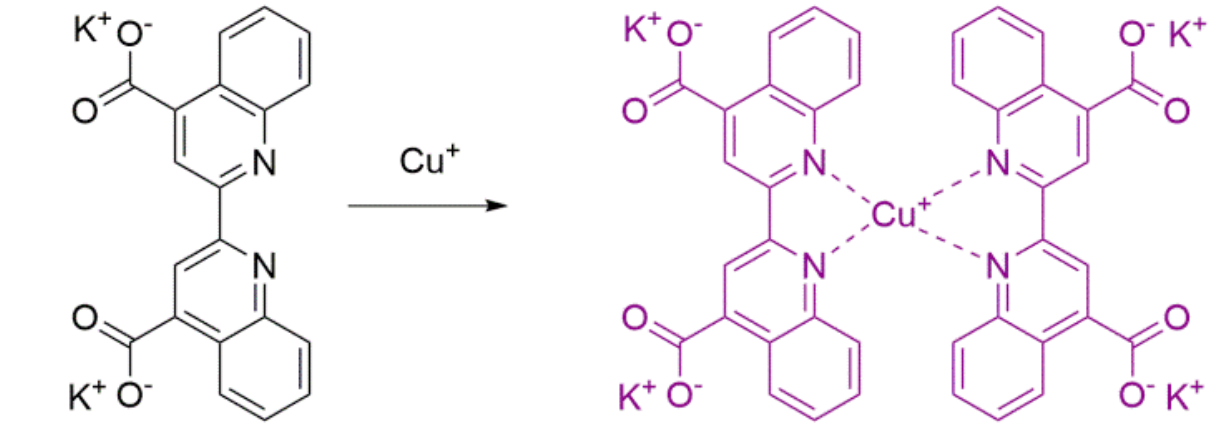
Method & Procedure

The Determination of Total Cu in White Wine by BCA Colorimetric Analysis

Version 1. Feb 2018



Bicinchoninic acid (BCA) potassium salt

BCA-copper complex

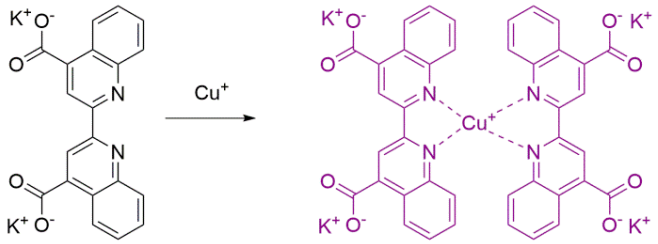
Nikolaos Kontoudakis[a,b], Mark Smith[c], Paul A. Smith[c], Eric N. Wilkes[c], Andrew C Clark [a,b]

a National Wine and Grape Industry Centre, Mambarra Drive, Wagga Wagga, NSW 2678, Australia
b School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
c Australian Wine and Research Institute, Glen Osmond, SA 5064, Australia

Wine Australia for Australian Wine

Method Overview

This method allows the determination of total copper concentration in white wine. It utilises the colorimetric reagent 2,2'-bicinchoninic acid dipotassium salt (BCA) to react with copper(I) and form a purpled coloured complex, which absorbs at 563 nm. During the analysis, ascorbic acid is added to aid the conversion of copper(II) to copper(I). Also, silver(I) nitrate is added in order to induce dissociation of suspended copper(I) sulfide in wine, and thereby to provide non-sulfide bound copper ions and silver(I) sulfide.



Bicinchoninic acid (BCA) potassium salt

BCA-copper complex

The reaction for the formation of the purple BCA-copper complex

Equipment and Reagents Required

- Preferably de-ionised water, and if possible ultra-pure water (18.2 μΩ), for the preparation of Cu standards and reagents. This will ensure as little Cu contamination within the water as possible.
- Analysis of metals at concentrations below 1 mg/L often involves soaking glassware with 10 %(v/v) nitric acid, and rinsing with copious amounts of ultra-pure water prior to use. However, in a winery setting, washing glassware with 1 %(w/v) citric acid dissolved in de-ionised water, followed by 5-6 rinses with deionised water should suffice.
- General glassware: 50 mL volumetric flask, 10 mL volumetric flask, 6 x 25 mL beakers, 1 L volumetric flask, 100 mL volumetric flask.
- Spectrophotometer: containing a standard visible lamp and the capability to insert a 40 mm cuvette. The spectrophotometer should be turned on 20 minutes before the analysis. The spectrophotometer should be zeroed on water in the 40 mm cuvette prior to analysis.
- Analytical balance.
- Micropipettes: 1 x 1-5 mL range, 1 x 0.1-1.0 mL range, and 1 x 0.02-0.20 mL range (or 0.01-0.10 mL range). The accuracy and precision of this technique will depend on the accuracy and precision of the micropipettes utilised. Pipettes must be calibrated for accuracy every 6 months.
- 40 mm glass cuvette: can be purchased for around $ 30-40. Quartz cuvettes (40 mm) can also be used but will be 10-times more expensive.
- 0.20 or 0.45 μm syringe filter. Regenerated cellulose is preferred but any should be fine.
- 10 mL syringes.

General Reagents

- 2,2'-Bicinchoninic acid dipotassium salt (BCA) regent: 0.05 %(w/v) in water. Weigh 25 mg in a 50 mL volumetric flask, dissolve in ~45 mL water and make up to 50 mL with water. Prepare reagent fresh.
- Ascorbic acid: 80 g/L in water. Weigh 0.8 g of ascorbic acid into a small beaker and add 10 mL of water. Prepare this reagent fresh. Accuracy of preparation is not critical for this reagent.
- Silver(I) nitrate: 1 g/L silver(I) in water (which is 1.57 g/L silver(I) nitrate). Weigh 32 mg of silver(I) nitrate into a small beaker and add 20 mL of water. Prepare this reagent fresh.

Reagents that must be prepared accurately

- 500 mg/L copper(II): weigh 1.965 g copper(II) sulfate pentahydrate into a 1 L volumetric flask and dissolve in 800 mL of water and then make the volume accurate to 1L with water.
- 20.0 mg/L copper(II): accurately take 4.00 mL of 500 mg/L copper(II) and dilute it to 100 mL with water in a 100 mL volumetric flask.

Methodology

For each wine sample the four solutions indicated below require preparation. This includes: i) a wine blank, ii) wine non-blank, iii) wine + 0.1 mg/L Cu, and iv) wine + 0.3 mg/L Cu sample. A shortened version of the method may involve skipping the 'wine + 0.3 mg/L Cu' sample but this may lower the accuracy of the method. Replicate measures will provide an indication of precision of the measurement, and triplicate preparation of each solution is recommended. The spectrophotometer can be zeroed on water at 563 nm prior to the measurements below.

Solution to add	Wine blank	Wine non-blank	Wine + 0.1 mg/L Cu	Wine + 0.3 mg/L Cu
Sample	10	10	10	10
Ascorbic acid	0.05	0.05	0.05	0.05
Silver(I)	0.1	0.1	0.1	0.1
BCA	---	0.5	0.5	0.5
Copper	---	---	0.05	0.15
Water	0.65	0.15	0.10	---

All additional volumes listed are in mL.

After the additions in the table above, the samples are mixed and after 30 minutes at room temperature filtered with a syringe filter. A small portion of the sample (~0.5-1.0 mL) is used to rinse the 40 mm glass cuvette, and then the remaining sample loaded into the 40 mm glass cuvette and the absorbance measured at 563 nm. Measurement should be made as soon as possible after the filtration step.

Calculation

1. If any replicates for the samples above were recorded, the average values should be calculated for the 'Wine blank', 'Wine+0.1 mg/L Cu' and 'Wine+0.3 mg/L Cu' samples. The 'Wine non-blank' replicates can be left as individual absorbances at this stage.
2. The average blank absorbance value should be subtracted from each of the 'Wine non-blank', 'Wine+0.1 mg/L Cu' and 'Wine+0.3 mg/L Cu' absorbances to provide Awine_rep, A0.1, A0.3.
3. Within excel, prepare a chart with x values of 0, 0.1 and 0.3, and then with y values with the absorbances corresponding to Awine_rep, A0.1, A0.3.
4. Fit a trendline to the data and show the equation for the trendline. The equation for the trendline will be in the format of y = a x + b, were a and b are numbers.
5. The copper concentration within the wine can be provided by calculating: (b / a), and the answer will be in mg/L.
6. Perform steps 3-5 for additional wine replicates and then average them for the final result.

Wine samples with and without BCA

Example Calculation for Sample Preparation in Triplicate

As an example the following data was obtained for samples prepared in triplicate:

Wine blank: 0.017, 0.023, 0.020
Wine non-blank: 0.121, 0.120, 0.120
Wine + 0.1 mg/L Cu: 0.165, 0.167, 0.167
Wine + 0.3 mg/L Cu: 0.260, 0.257, 0.257

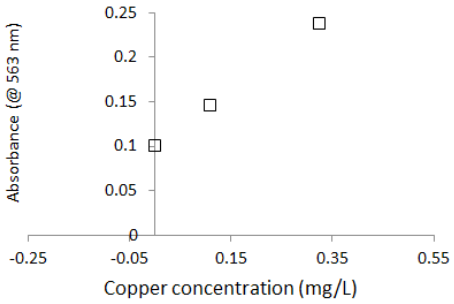
Step 1 The average is calculated for the Wine blank, and the samples with added Cu:

Average(Wine blank) = (0.017 + 0.023 + 0.020) / 3 = 0.020
Average(Wine + 0.1 mg/L Cu) = (0.165 + 0.167 + 0.167) / 3 = 0.166
Average(Wine + 0.3 mg/L Cu) = (0.260 + 0.257 + 0.257) / 3 = 0.258

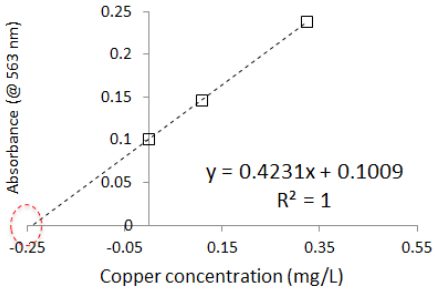
Step 2 Subtract the average blank from each of the replicates of the 'Wine non-blank' and from the averages of the samples with added Cu.

Awine_rep1 = 'Wine non-blank'(replicate 1) – 'Wine blank' = 0.121 – 0.020 = 0.101
Awine_rep2 = 'Wine non-blank'(replicate 2) – 'Wine blank' = 0.120 – 0.020 = 0.100
Awine_rep3 = 'Wine non-blank'(replicate 3) – 'Wine blank' = 0.121 – 0.020 = 0.100
A0.1 = 'Wine 0.1 mg/L Cu'(average) – 'Wine blank' = 0.166 – 0.020 = 0.146
A0.3 = 'Wine 0.3 mg/L Cu'(average) – 'Wine blank' = 0.258 – 0.020 = 0.248

Step 3 In excel, prepare a graph for the quantification of each wine replicate. For Wine replicate1, the x values are the Cu concentrations of 0, 0.1 and 0.3, while the y values would be the absorbances of Awine_rep1, A0.1, A0.3. For replicate1, the y values are 0.101, 0.146 and 0.248, providing the following graph:



Step 4 Fit a trendline to the graph and add the equation (as shown below). In this case, the equation for wine replicate1 is y = 0.4569 x + 0.1009 (make sure to show four decimal places in the equation). If the general equation format is written as y = a x + b, in this case a = 0.4231 and b = 0.1009.



Step 5 The intercept of the trendline on the x-axis will indicate the copper concentration in wine replicate1. In this case it can be given by b / a = 0.1009 / 0.4231 = 0.238 mg/L copper.

Step 6 The identical approach is taken for wine replicate2 and wine replicate3 is performed, utilising their Awine_rep2 and Awine_rep3 values (see step 2) of 0.100 and 0.100, respectively. This should provide copper concentrations in wine replicates 2 and 3 of 0.235 and 0.235 mg/L copper. Therefore, the average of the three replicates would provide a concentration of (0.238 + 0.235 + 0.235) / 3 = 0.236 mg/L. The standard deviation of the replicates can also be calculated within excel (=STDEV(0.238, 0.235, 0.235) = 0.002 mg/L). Therefore, the answer can be quoted as 0.236 ± 0.002 mg/L (n=3).

Photos of Equipment

Figure 2. Typical spectrophotometer used for the analysis (top left), 40mm cuvette within the spectrophotometer (top right), BCA reagent, sample solutions, including the clear/yellow coloured blank sample, and syringe filter (bottom left), filtration of the samples after the 30 minute reaction time (bottom right).

Validation Parameters

The validation parameters of the technique are as follows:

- Recovery. The average recovery was 104 ± 9 % for 12 white wines with addition of 0.1 mg/L copper(II).
- Accuracy. The accuracy of the technique for determination of total copper in white wines was equivalent to that of ICPOES.
- Repeatability. The repeatability was assessed in terms of the average relative standard deviation of total copper concentrations, and was 3 ± 2 % for 12 white wines.
- Specificity. The BCA reagent is specific for Cu in wine, with no influence from Mg, Mn, Fe, Zn and Al.
- Linearity. The technique is linear within the range tested from 0.04 to 1.0 mg/L copper.
- Stability. Samples should be filtered 30 minutes after addition BCA to wines, and then measured immediately afterwards.
- Matrix effects. Eliminated with silver(I) additions to wine and with the use of quantification via standard additions.