

# Determination of Copper in Green Olives using ICP-OES

Intelligent Rinse function reduced analysis time by 60%, saving 191.4 L of argon



## Authors

Ryley Burgess,  
Agilent Technologies, Australia

## Introduction

The adulteration of foodstuffs is often motivated by monetary gain, but the practice can lead to serious health issues, depending on the adulteration method used. In the case of green table olives, color is often seen as an indication of freshness and market value.

Compounds containing copper have been used to enhance the color of non-fresh or low-grade produce. Copper sulfate has been used to 'coat' the olives and colorant E-141ii, which contains copper chlorophyll complexes, has been used as an additive to enhance the color. The addition of E-141ii to table olives is considered as fraudulent practice in many parts of the world. For example, US Food and Drug Administration and European Union regulations do not permit the addition of copper chlorophyll-based colorants to table olives [1, 2].

While LC/MS is widely used for the quantification of copper compounds in foods, elemental concentrations are usually determined by Flame Atomic Absorption Spectroscopy (FAAS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) or ICP-Mass Spectrometry (ICP-MS).

ICP-OES is well suited to the analysis of brines used to preserve table olives. Typically, a radially viewed plasma is used to counteract any interference effects stemming from the highly concentrated sodium matrix. However, in this study, food digests and brine samples were analyzed using the Agilent 5110 ICP-OES operating in Synchronous Vertical Dual View (SVDV) mode. SVDV with Dichroic Spectral Combiner (DSC) technology selects and combines both the axial and radial light from the vertical-oriented plasma to measure all wavelengths in a single reading. Wavelengths read axially from a vertically oriented plasma provide much lower detection limits than wavelengths read from the radial view. The vertical torch also offers reliable analysis of brine samples with up to 30% Total Dissolved Solids (TDS) meaning less cleaning, reduced downtime, and fewer replacement torches. The brine analyzed in this work contained approximately 10% TDS.

In addition to quantification of selected elements, the Agilent ICP Expert software provides rapid qualitative analysis using IntelliQuant, a semiquantitative function of the software. By carrying out a full wavelength scan, IntelliQuant can identify and calculate an approximate concentration for up to 70 elements in a sample. Elements likely to cause spectral overlaps can be quickly identified, simplifying method development. The function allows analysts in food labs to monitor additional elements in their samples, without changing their routine methods. IntelliQuant can acquire data from 167 to 785 nm, in as little as 15 additional seconds per sample.

All 5110 ICP-OES data is processed quickly using the high-speed Vista Chip II CCD detector, leading to fast analysis times. However, another function of the ICP Expert software, Intelligent Rinse, further assists with fast sample throughput. The function monitors the intensities of nominated element wavelengths during the rinse period, automatically ending the rinse when these intensities reach a user-specified threshold. This real-time optimization of rinse times improves sample throughput and reduces argon consumption, while maintaining good accuracy.

## Experimental

### Instrumentation

All measurements were performed using an Agilent 5110 SVDV ICP-OES. The sample introduction system consisted of a Seaspray glass concentric nebulizer, double-pass glass cyclonic spray chamber, and a standard single piece 1.8 mm internal diameter (id) injector torch. Instrument operating parameters used are shown in Table 1.

**Table 1.** Agilent 5110 ICP-OES instrument and method parameters.

Parameter	Setting
Read time (s)	5
Replicates	3
Sample uptake delay (s)	25
Stabilization time (s)	15
Intelligent rinse	Enabled
Intelligent rinse method	Moderate
Max rinse time (s)	30
Pump speed (rpm)	12
Fast pump during uptake and rinse (rpm)	80
RF power (kW)	1.30
Auxiliary flow (L/min)	1.00
Plasma flow (L/min)	12.0
Nebulizer flow (L/min)	0.70
Viewing height (mm)	8
Viewing mode	SVDV
Sample pump tubing	White-white
Internal standard pump tubing	Black-black
Waste pump tubing	Blue-blue
Background correction	Fitted

### Calibration standards

Working calibration standards were prepared at 0.5, 2, 5, and 10 ppm by diluting a 1000 ppm Cu single-element stock standard (Agilent Technologies). The yttrium internal standard was prepared at 2 ppm from a 1000 ppm Y single-element stock standard (Agilent).

A custom IntelliQuant calibration was created at 0.5, 5, and 50 ppm using two sets of multi-element standards, #1 and #2, from the IntelliQuant Calibration Kit (Agilent). In addition, a 1000 ppm multi-element standard consisting of Ca, K, Mg, and Na was prepared from a 5000 ppm multi-element stock solution (AccuStandard).

All blanks and standards were matched to the acidity of the brine samples using 2% HNO<sub>3</sub>. The acid was prepared from analytical grade 69% nitric acid and 18.2 MΩ Millipore water.

## Sample preparation and internal standards

Three different varieties of green olives preserved in brine were purchased from a local store in Melbourne, Australia. These samples are referred to as J1, J2, and J3. Samples of the brine from J1 and J3 were also analyzed. These samples are referred to as B1 and B3.

Both brine solutions contained salt, water, lactic acid, citric acid, and ascorbic acid. To compensate for any matrix interferences arising from the complex mixture, yttrium, monitored at 371.029 nm, was used as an internal standard. It was added online via a tee piece.

The olive samples were prepared for analysis by microwave digestion using a CEM MARS 6 Microwave Digestion System (Buckingham, UK). An acid mixture consisting of 2 mL of concentrated hydrogen peroxide and 8 mL of concentrated nitric acid were added to 0.5 g of olive sample. The microwave digestion settings are given in Table 2. The digests (green, clear solutions) were then diluted to 50 mL using Milli-Q de-ionized water (DIW).

**Table 2.** Method settings for the digestion of the green olive samples.

Setting	Value
Power (W)	1030 to 1800
Ramp time (min)	20:00 to 25:00
Hold time (min)	15:00
Temperature (°C)	210

The brine samples were filtered through filter paper to remove any olive pieces. The filtered brine samples were then diluted 1:5 using 2% (v/v) nitric acid.

## Results and Discussion

### Method detection limits

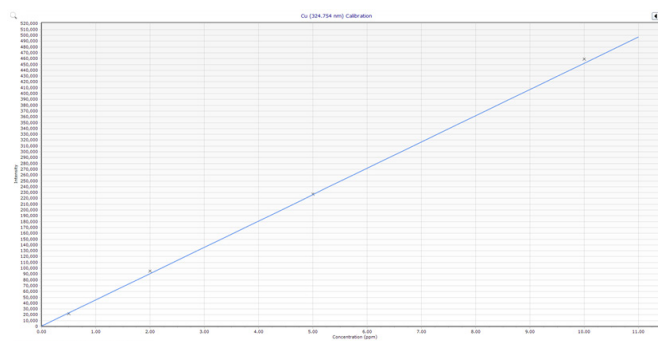
The Method Detection Limit (MDL) for Cu was based on three sigma of 10 replicate measurements of the method blank spiked with 10 ppb Cu. The result is an average of six determinations, performed on two different instruments.

**Table 3.** Element wavelength and MDL in solution.

Element and wavelength (nm)	MDL (ppb)
Cu 324.754 nm	1.04

### Calibration linearity

A linear calibration was obtained for Cu 324.754 nm up to 10 ppm, as shown in Figure 1. The calibration coefficient was greater than 0.9999 (Table 4) over the concentration range with less than 6% calibration error for each point.



**Figure 1.** Calibration curve for Cu 324.754 nm.

**Table 4.** Wavelength and working calibration range.

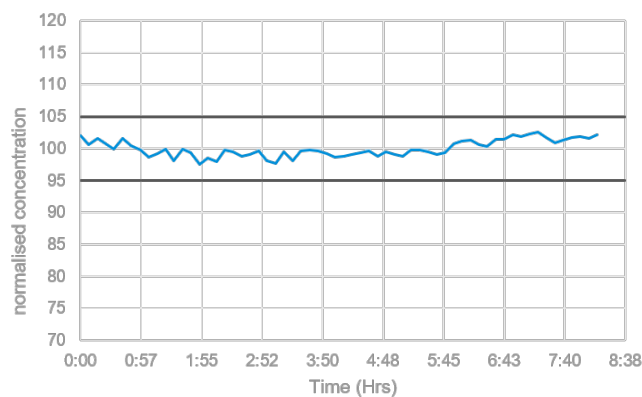
Element and wavelength (nm)	Background correction/interference correction	Concentration range (ppm)
Cu 324.754	Fitted	0.0–10.0

### Stability and precision

To check stability and precision of the 5110 ICP-OES, a brine sample was spiked with 50 ppb of Cu and then measured approximately 370 times over an 8-hour period. The plasma was stable over the duration of the run due to the vertically oriented plasma and a solid-state RF system.

There was less than 5% variation in the recovery from the expected concentration, as shown in Figure 2.

Excellent precision (less than 1.5% RSD) was obtained over the duration of the run.



**Figure 2.** Long-term stability plot for Cu 324.754 nm in 10% brine sample B3 spiked with 50 ppb Cu. The solid black lines indicate  $\pm 5\%$  variation.

## Spike recoveries

To check the accuracy of the method, all samples were spiked with Cu, and the recoveries were calculated. Table 6 shows the measured concentrations and recovery results for the five different samples. The spike recoveries are the average of the results obtained from three analytical runs on two instruments (n=6). The recovery results for each sample type were within  $\pm 10\%$  of the spike concentration values. These excellent recoveries demonstrate the ability of the 5110 ICP-OES to accurately determine Cu at both ppb and ppm levels, in both simple and complex matrices.

**Table 6.** Spike recovery results for the digested olive samples (J1, J2, and J3) and the brine samples (B1 and B3).

Sample	Measured conc of diluted sample (ppm)	Spiked conc in diluted sample (ppm)	Measured conc of spiked diluted sample (ppm)	Recovery (%)
J1	0.684	2	2.567	94.1
J2	0.0197	0.05	0.0668	94.2
J3	0.0198	0.05	0.0663	93.0
B1	1.694	2	3.791	104.9
B3	0.0353	0.05	0.0879	105.2

## Optimizing rinse times

The concentration of Cu present in the olive and brine samples was expected to vary, so Agilent's Intelligent Rinse software function was used to optimize the rinse times. For a sample load of 35 samples, an average of 11 minutes was saved over the duration of the run, using a 'moderate' Intelligent Rinse setting (Table 7). Sample throughput can be further increased using a 'quick' Intelligent Rinse setting—an ideal setting for low concentration samples.

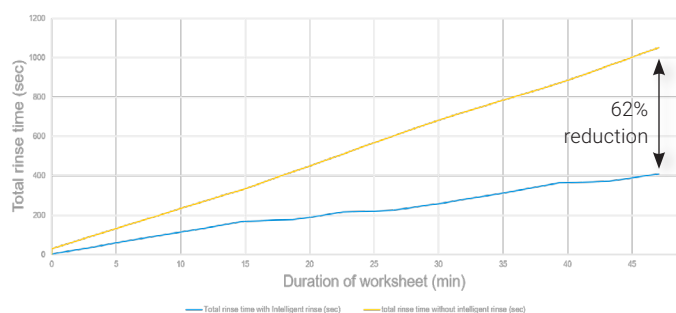
During the spike recovery test, the total rinse times were noted for each sample run (n=6) and averaged to calculate the total time saved by the Intelligent Rinse function. If the default rinse setting had been used for all measurements, all samples, including those with only trace amounts of Cu would have required 30 seconds rinse time.

With Intelligent Rinse, samples containing Cu at the low end of the calibration range required a rinse time of around 3 seconds. As expected, the higher concentration samples required a longer rinse time (up to 20 seconds) to reach the user-specified intensity threshold. Total rinse times for the 35 samples with and without Intelligent Rinse are shown in Figure 3. Almost 60% less time was needed for the analysis using Intelligent Rinse.

Shorter rinse times between samples lead to an increase in sample throughput and a reduction in argon consumption per sample. In this study, a total of 191.4 L of argon was saved over the duration of the 35 sample run using Intelligent Rinse (Table 7).

**Table 7.** Comparison of Intelligent Rinse and normal rinse settings (n=6) for a 35 sample run.

Total rinse time without Intelligent Rinse (min)	Average total rinse time with Intelligent Rinse (min)	Average time saved (min)	Average argon gas saved (L)	Flow rate of argon* (L/min)
17.5	6.5	11	191.4	17.4



\* The argon flow rate includes polypurge

**Figure 3.** Total rinse time over the duration of the sample run, using Intelligent Rinse (blue line) compared to the default rinse time of 30 seconds (orange line).

## Automatic full spectrum scan

Qualitative data from a full scan can be invaluable when monitoring samples for evidence of adulteration or contamination. Agilent's IntelliQuant function enables rapid identification and an estimation of the concentration of up to 70 elements per sample in less than 15 seconds.

A full spectrum scan of brine sample (B1) and B1 spiked with Cu were obtained using IntelliQuant. The software generates a periodic table heat map to visually represent the relative concentrations of elements present in a sample.

Heat maps for the unspiked and spiked B1 samples are shown in Figures 4a and 4b, respectively. Elements present at low concentrations are shown in yellow, medium concentrations are shown in orange, and high concentrations are shown in red. The concentration ranges are user-adjustable. The heat maps for the unspiked and spiked brine samples clearly show an increase in concentration for Cu.

The slight variation in concentration for Ca and K in the two heat maps is because separate aliquots of brine were used as samples.

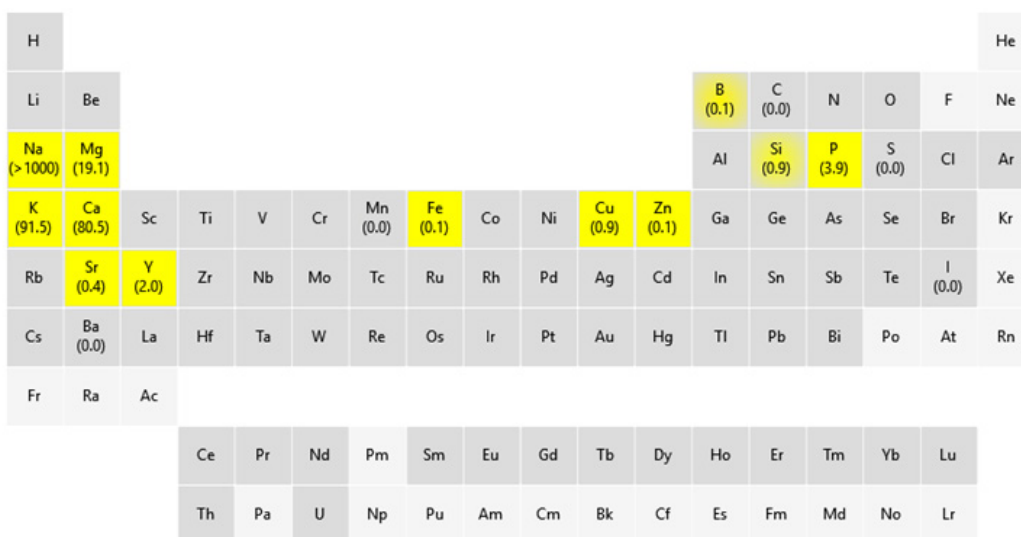


Figure 4a. IntelliQuant heat map of unspiked B1.

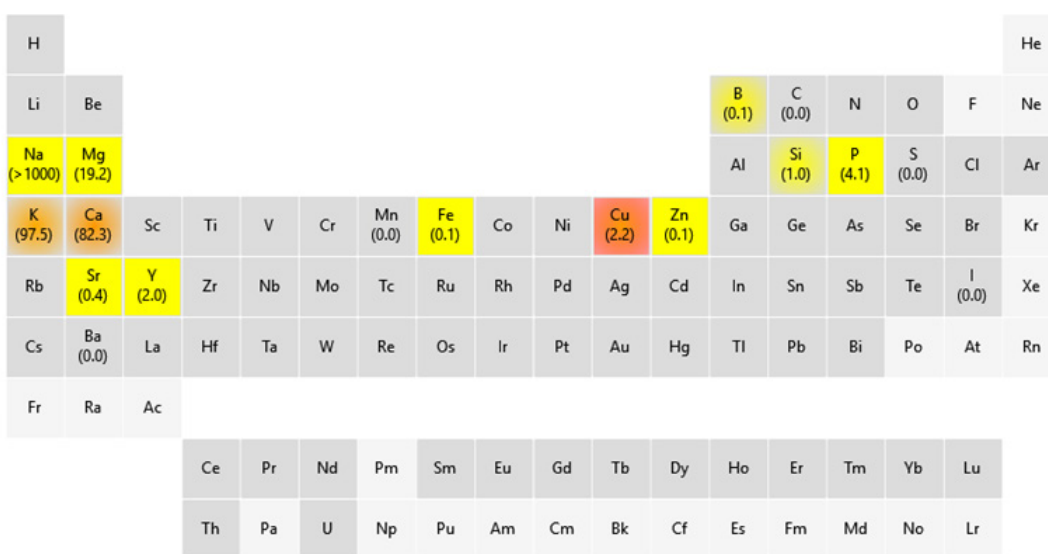


Figure 4b. IntelliQuant heat map of spiked B1.

## Conclusions

The Agilent 5110 SVDV ICP-OES is suitable for the accurate quantification of copper in green olives and the brine used to preserve the produce.

A quick semiquantitative analysis of the brine sample was also carried out using IntelliQuant. By collecting data from the entire wavelength range from 167 to 785 nm in <15 seconds, IntelliQuant is a valuable tool for monitoring samples for contaminants and metal-containing compounds used in the adulteration of samples.

All 5110 ICP-OES data is processed quickly using the high-speed Vista Chip II CCD detector leading to fast analysis times and reduced argon consumption per sample.

The productivity and cost-effectiveness of the method were further improved using the Intelligent Rinse function of ICP Expert software. Intelligent Rinse shortened the rinse duration of this study by almost 60%, leading to a saving in argon consumption of over 190 L.

## References

1. US FDA Color Additive Status List For Industry, 2017-11-15. Accessed February 2018, [www.fda.gov/forindustry/coloradditives/coloradditiveinventories/ucm106626.htm](http://www.fda.gov/forindustry/coloradditives/coloradditiveinventories/ucm106626.htm)
2. Scientific Opinion on re evaluation of copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)) as food additives, *European Food Safety Authority (EFSA) Journal*, Volume 13, Issue 6, **2015**

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