

## UV/Visible Spectroscopy

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## Determination of Wine Color and Total Phenol Content Using the LAMBDA PDA UV/Vis Spectrophotometers

### Introduction

Historically, the earliest evidence of viniculture is approximately 8,000 years ago and worldwide it has become increasingly more prevalent in recent years. The

expansion of markets and producers has resulted in an escalation in methods used to guarantee product safety and quality of wine.

Wine contains over 600 nutritional substances including vitamins, organic acids and more importantly polyphenols. The seeds and skin of the grape provide a valuable source of polyphenols, and with increasing interest in their health-enhancing properties as antioxidants, research has gathered pace over the last 15 years. The key benefits found have been aiding age prevention and cardiovascular disease by preventing the oxidation of Low Density Lipoprotein (LDL).<sup>1</sup>

The versatility of PerkinElmer's LAMBDA™ 265 and LAMBDA 465 PDA UV/Visible Spectrophotometers allows quantification of the total phenol content in the wines, and also wine color to be measured to determine quality and any potential contamination.

## Measuring Wine Color Intensity and Hue

Wine quality and possible contamination can be observed by quantifying 'Wine Color Intensity' and 'Wine Hue'. A straightforward method of determining 'Wine Color Intensity' using the LAMBDA 265 instrument is to set up an equation to calculate the summation of the absorbance of three wavelengths occurring in the visible region, typically 420 nm, 520 nm and 620 nm. An additional parameter to calculate is the 'Wine Hue', executed by changing the equation calculation to obtain the ratio of the absorbance at 420 nm and 520 nm ( $A_{420}/A_{520}$ ).

### Reagents and Apparatus

1. Red wine samples
2. Deionised (DI) water
3. LAMBDA 265 (or LAMBDA 465) PDA UV/Visible Spectrophotometer
4. UV Lab™ software
5. Cuvettes (1 cm pathlength)

## Experimental

Using the UV Lab software, the LAMBDA 265 instrument parameters were set as shown in Figure 1. The initial method was established to record the 'Wine Color Intensity', as shown in Equation 1. An additional calculation was setup to calculate 'Wine Hue' as shown in Equation 2.

Red wine samples were diluted by a factor of 5 and measured on a LAMBDA 265 with a standard 1 cm pathlength quartz cuvette. Using DI water as a blank, the instrument was zeroed and each red wine sample analysed in turn using the 'Wine Color Intensity' and 'Wine Hue' methods.

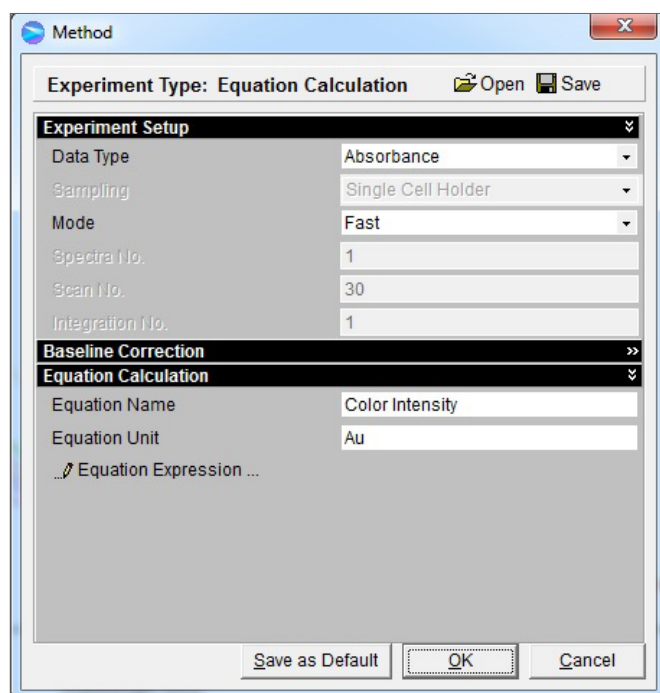


Figure 1. Instrument parameters and method setup.

### Equation 1.

$$\text{Wine Color Intensity} = A_{420} + A_{520} + A_{620}$$

### Equation 2.

$$\text{Wine Hue} = A_{420} / A_{520}$$

## Results

Figure 2, shows the spectra of seven red wine samples, and the corresponding absorbance values to calculate 'Wine Color Intensity' and 'Wine Hue' are presented in Table 1. As shown in Figure 2, the absorption spectra all have the same characteristic spectral features, albeit varying absorbance values. Furthermore, variation in 'Wine Color Intensity' and 'Wine Hue' for all wines is observed, as shown in Table 1.

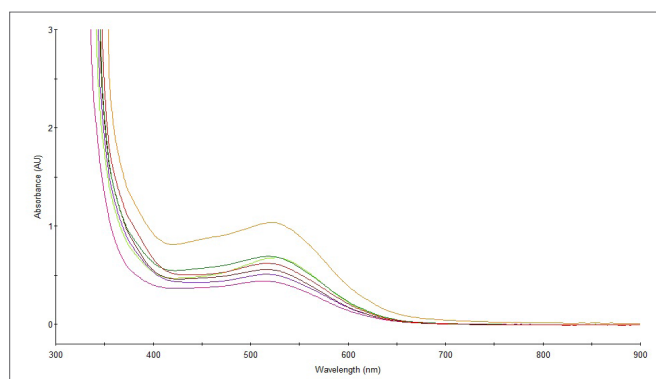


Figure 2. UV/Vis spectra of red wine samples.

Table 1. Wine Color Intensity' and 'Wine Hue' of red wine samples using LAMBDA 265.

	Wine Color Intensity (Au)	Wine Hue	Absorbance at 420 nm	Absorbance at 520 nm	Absorbance at 620 nm
Red Wine 1	2.095	0.787	0.814	1.034	0.247
Red Wine 2	0.889	0.840	0.367	0.437	0.084
Red Wine 3	1.274	0.694	0.469	0.676	0.130
Red Wine 4	1.048	0.862	0.438	0.509	0.101
Red Wine 5	1.122	0.831	0.463	0.557	0.102
Red Wine 6	1.376	0.792	0.548	0.692	0.136
Red Wine 7	1.259	0.834	0.517	0.620	0.122

## Total Phenol Content of Wine

Gallic acid, a type of phenolic compound, is a common standard used for determining the phenol concentration of various analytes, such as phenolics found in wine. The Folin-Ciocalteu (FC) assay uses a redox reagent (a mixture of phosphomolybdate and phosphotungstate) for the colorimetric assay. Upon reduction of the FC reagent, a blue colored complex (chromogen) develops which absorbs strongly at 740 nm and thus can be detected spectrophotometrically.<sup>2</sup> As a result, total phenol concentration of wine samples can be quantified by the LAMBDA PDA UV/Vis spectrophotometer.

## Reagents and Apparatus

1. 5 red wine samples and 2 white wine samples
2. DI water
3. LAMBDA 465 (or LAMBDA 265) PDA  
UV/Visible Spectrophotometer
4. UV Lab software
5. Cuvette (1 mm pathlength)
6. Solutions of 0 - 500 mg/L gallic acid
7. 0.2 N Folin-Ciocalteu reagent
8. Saturated solution of sodium carbonate

## Experimental

Using the UV Lab software in Quantification mode, the LAMBDA 465 instrument parameters were set as shown in Figure 3.

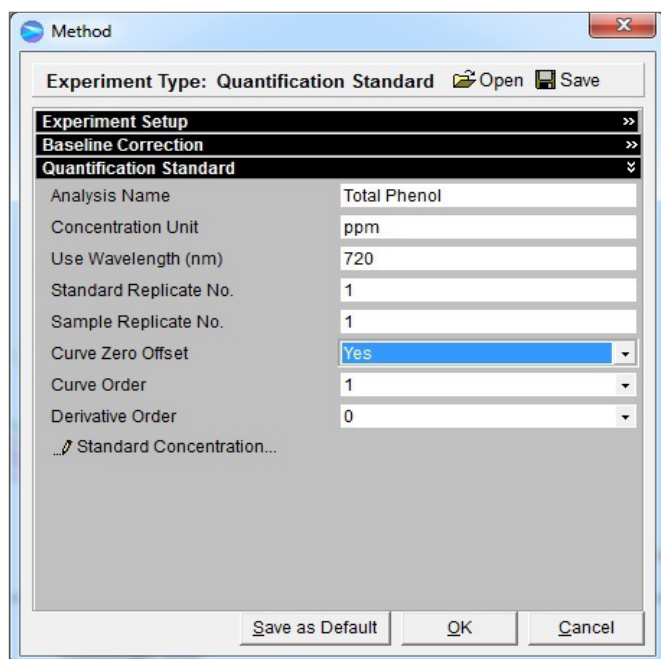


Figure 3. Instrument parameters for Total Phenol measurement.

Red wine samples were diluted by a factor of 6, while no dilution was required for white wine samples. Each of the standards of known concentration (0 – 500 mg/L gallic acid) and unknown diluted wine samples were treated in the same way prior to measurement. Initially, 5 ml of FC reagent was added to 1 ml of each of the standards and samples and a waiting time of 3 - 5 minutes was allowed. Following this, 4 ml of  $\text{Na}_2\text{NO}_3$  was added and the solutions left for two hours. A calibration curve was constructed by taking the absorbance of each of the standards at 740 nm. Finally the absorbance values of each of the wine samples were measured, thus enabling the determination of the total phenol concentration.

## Results

Figures 4 and 5 show the spectra and calibration curve of the gallic acid standards respectively. The linear regression coefficient ( $R^2$ ) obtained from the calibration curve, displayed in Figure 5, is greater than 0.999 indicating a high level of data correlation.

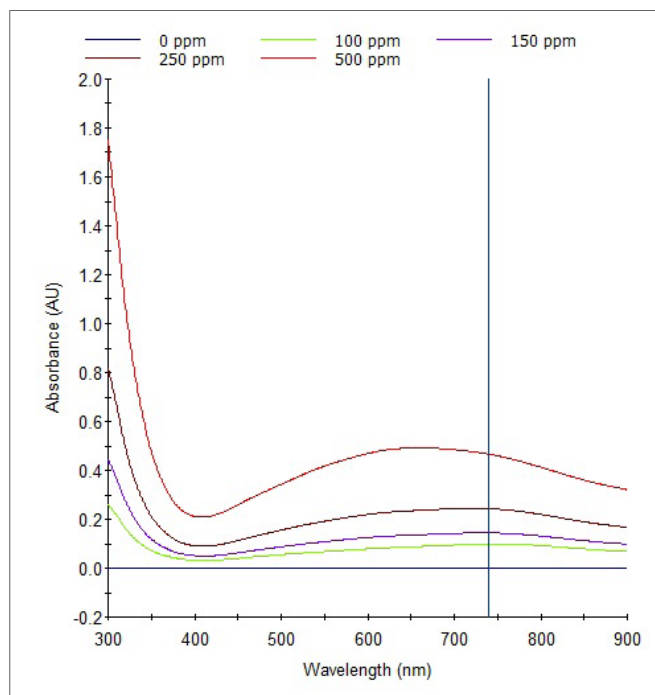


Figure 4. UV/Vis spectra of calibration standards of gallic acid.

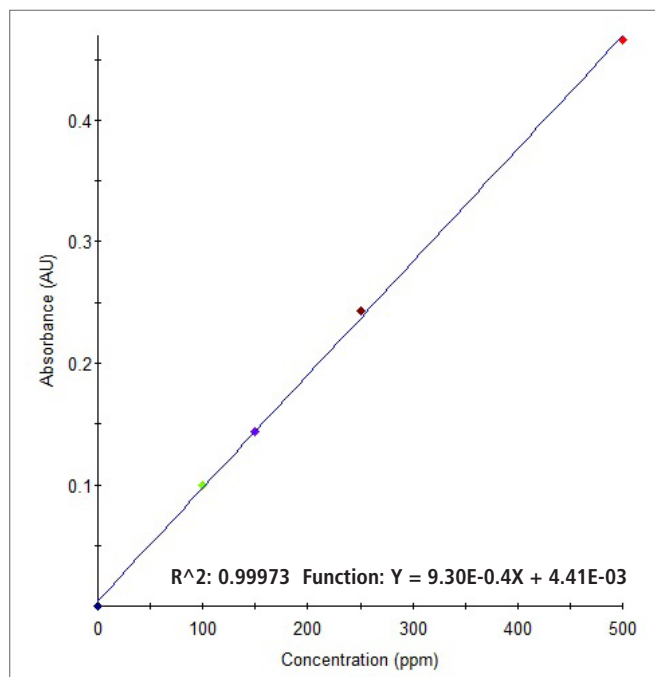


Figure 5. Calibration curve of gallic acid standards.

### Calculation of Total Phenol Concentration in Wine

Using the calibration curve in Figure 5, the total phenol concentration of each wine sample was obtained as shown in Table 2. In the case of the red wine samples, the dilution factor of 6 needed to be taken into account and thus the concentration of phenol determined from the calibration curve was multiplied by the dilution factor. It was observed that red wine samples had a greater total phenol concentration, ranging between 1000 - 3000 mg/L, while the white wines ranged between 200 - 300 mg/L.

Table 2. Total phenol concentration of red and white wine samples.

	Concentration (mg/L)	Absorbance at 740 nm	Dilution Factor	Original Concentration (mg/L)
Red Wine 1	453.69	0.427	6	2722.14
Red Wine 2	332.99	0.314	6	1997.94
Red Wine 3	198.84	0.189	6	1193.04
Red Wine 4	397.80	0.375	6	2386.80
Red Wine 5	169.72	0.162	6	1018.32
White Wine 6	277.63	0.263	1	277.63
White Wine 7	210.02	0.200	1	210.02

### Conclusion

Versatility of instruments is becoming increasingly more important to help optimise capital expenditure and the low cost LAMBDA 265 PDA UV/Vis Spectrophotometer has been shown to be a particularly versatile instrument for multiple applications, all while using minimal bench space. Operation of the instrument is simple with the UV Lab software and in the example above, spectra of wines were obtained rapidly and Wine Color Intensity and Wine Hue determined by simple equation calculations. The quantification of Total Phenol content in wines could also be determined using the LAMBDA 265. The LAMBDA 465 offers higher sensitivity for these measurements and is the ideal instrument for a range of more demanding applications in research and routine testing environments.

### References

1. Scalbert, A; Johnson, I; Saltmarsh, M. (2005) Polyphenols: antioxidants and beyond. *Molecules*. 81 (1).
2. Blainski, A; Lopes, GC; Palazzo de Mello, JC. (2013) Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. *Molecules*. 18 (6).