Application note

Caffeine Quantification in Steeped Tea: Background Correction Using GENESYS™ Smart QC

Introduction

UV-Visible absorption spectra can be highly useful for determining the concentration of a given analyte in solution. For QA/QC environments, UV-Visible analysis can be a vital step in ensuring the product was manufactured correctly and contains the correct amount of the substance of interest. Conversely, it can also be a useful technique for verifying the amount of a specific contaminant present is below acceptable limits. According to Beer's law (Eqn. 1),

$$A_\lambda = \varepsilon_\lambda l c \quad (1)$$

where A_λ is the absorbance measured at a given wavelength, ε_λ is the molar absorptivity of the analyte at the same wavelength, l is the path length and c is the analyte concentration, the measured absorbance is directly proportional to the analyte concentration. As UV-Visible spectrophotometers measure the absorbance spectrum of a sample through non-destructive means, these samples can be retained for further analysis.

For food products, samples are often non-ideal and can contain multiple components. In some cases, the samples studied may contain more than one absorber, which can further complicate the UV-Visible spectrum. Absorbance is additive, as shown in equation 2, where the measured absorbance ($A_{\lambda, meas}$) will be the sum of the absorbance each chromophore present ($A_{\lambda,1}$, $A_{\lambda,2}$, $A_{\lambda,n}$).

$$A_{\lambda,\,meas} = A_{\lambda,\,1} + A_{\lambda,\,2} + \ldots + A_{\lambda,\,n} \quad (2)$$

Due to the additive nature, overlapping absorption spectra can make quantification difficult. While separating the components from solution can aid in removing this obstacle, this is often a time-consuming step.

There are a couple methods that can be used when analyzing the UV-Visible data to aid in quantification. First, derivative spectroscopy has been utilized in the past to analyze more complicated spectra.[1,2] This analysis requires the first derivative of the measured spectrum be calculated and plotted as a function of wavelength. The resulting derivative spectrum can then be used for quantification through several different methods.[3,4] Each method of quantification requires some work-up of the data, typically performed outside of the instrument software. While this can be a highly useful method, in some environments it may be more useful to have the instrument software automatically perform needed calculations prior to data export.

If the interference from other absorbers is minimal, simple corrections, like single point subtraction or linear fits, can be used to estimate the contributions from these absorbers. While this does not work for all sample types, it can be used as a general, quick check before moving to the next analysis step. The Thermo Scientific™ GENESYS™ Smart QC Software can be a helpful tool to perform these calculations and automatically calculate the concentration of the analyte of interest from the corrected absorbance. In this way, a quick check can be performed without the need for further data processing outside of the instrument software.

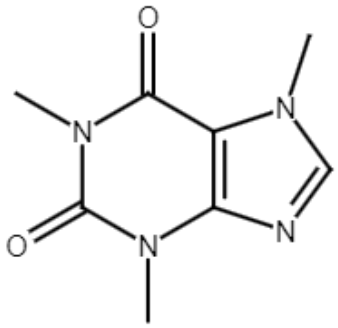


Figure 1. Caffeine chemical structure.

To demonstrate the use of the GENESYS Smart QC software for this form of correction, the analysis of caffeine content in steeped tea samples is performed. Black tea, among other tea varieties, is known to contain caffeine (Fig. 1),[5] a well-characterized UV absorber,[6] and can be extracted when steeped in heated water. However, there are a variety of other compounds, including substances which impart the characteristic brown color in tea, that can be extracted as well.[5] The presence of these absorbers can complicate the UV-Visible spectrum of a steeped tea sample, leading to difficulties when determining the concentration of caffeine.

Here in the GENESYS Smart QC PC and local control software were used to develop a method for removing the absorbance from substances other than caffeine in a set of steeped tea samples. Using UV-Visible measurements of tea samples and standard caffeine solutions collected using the Thermo Scientific™ GENESYS™ 180 Spectrophotometer, an equation to remove the background interference for additional absorbers present was devised. Once implemented in the GENESYS Smart QC method was used to measure the steeped tea samples and report the caffeine concentration for each. As a point of comparison, the absorption spectra were further analyzed through derivative spectroscopy to determine how well the developed GENESYS Smart QC method was able to correct for the overlapping absorption feature.

Experimental

Sample Preparation

A standard caffeine stock solution was prepared by dissolving 9.5 mg caffeine in 40 mL DI water. The stock caffeine solution was then diluted with DI water to produce 4 standard caffeine solutions (4.07 µM, 20.3 µM, 40.7 µM and 122 µM). Four stock tea samples were generated by steeping a commercially available black tea bag in 50 mL of DI water heated to 91 ˚C. The tea samples were allowed to steep in the heated DI water for 30 s, 120 s, 240 s, and 600 s, producing 4 different stock tea solutions. 300 µL of each tea sample was then filtered using a 0.22 µm hydrophilic PVDF syringe filter to remove any particulates. Each tea sample was then diluted with DI water to produce samples containing 0.5% of the original tea concentration.

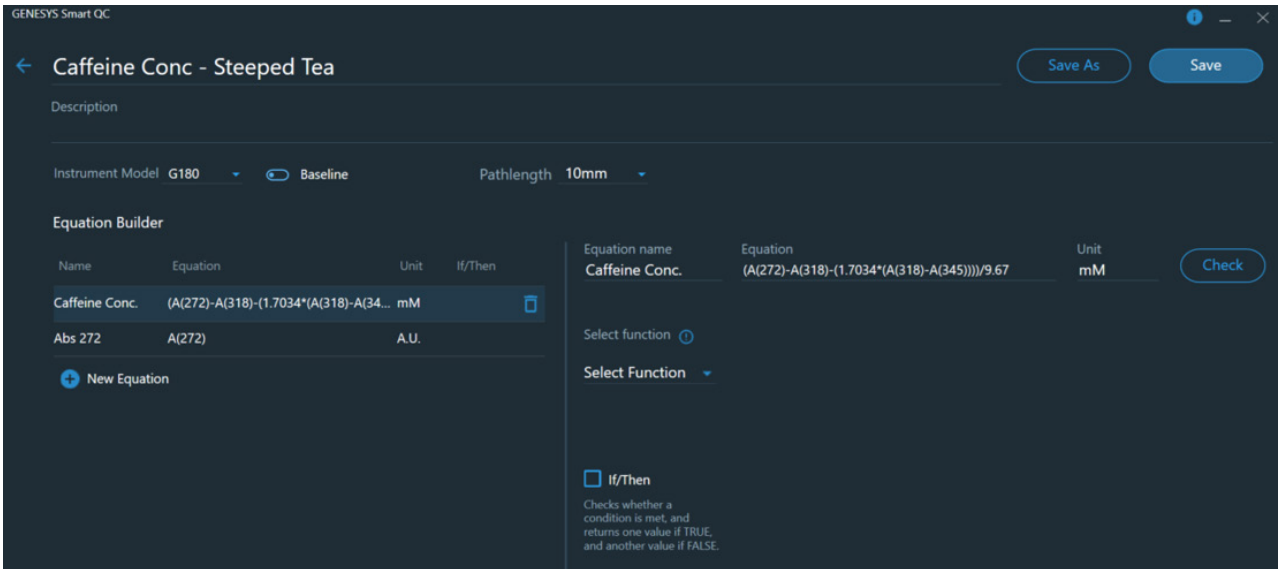


Figure 2. GENESYS Smart QC PC control software. Outlined here is the method created for analysis of caffeine in steeped tea samples.

A GENESYS Smart QC method (Caffeine Conc. – Steeped Tea) was developed using the PC control software to determine the caffeine content in each steeped tea sample. The method (Fig. 2) includes a calculation for determining the concentration of caffeine (Eqn. 3) in mM units for the dilute steeped tea samples. The "Caffeine Conc. – Steeped Tea" method was uploaded to a GENESYS 180 UV-Visible spectrophotometer (Fig. 3). The calculation for determining the caffeine concentration required derivation of a linear function from three data points to estimate and remove the background interference. More details pertaining to the development of this method are described in Equation Derivation section

(3)

$$[caffeine] = \frac{A_{272\,nm,\,raw} - A_{318\,nm} + \left(\frac{318\,nm - 272\,nm}{27\,nm}\right) * \left(A_{318\,nm} - A_{345\,nm}\right)}{(9.67\,mM^{-1}cm^{-1})\,(1.0\,cm)}$$

UV-Visible measurements were performed using a GENESYS 180 UV-Visible spectrophotometer equipped with both standard measurement applications and GENESYS Smart QC methods on the local control software. Both standards and samples outlined previously were measured using the Scan function. Spectra were collected between 200 nm and 1100 nm using a 1.0 nm data interval and slow scan rate. For the measurements collected using the "Caffeine Conc. – Steeped Tea" method, the instrument parameters are pre-defined by the GENESYS Smart QC local control software. For Scan measurements and the GENESYS Smart QC method, a 1.0 cm quartz cuvette was used to hold each standard and sample solution. DI water was used as a blank to establish the background.

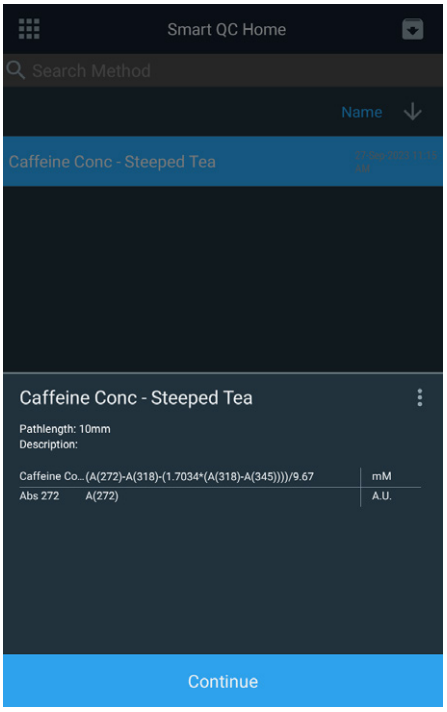


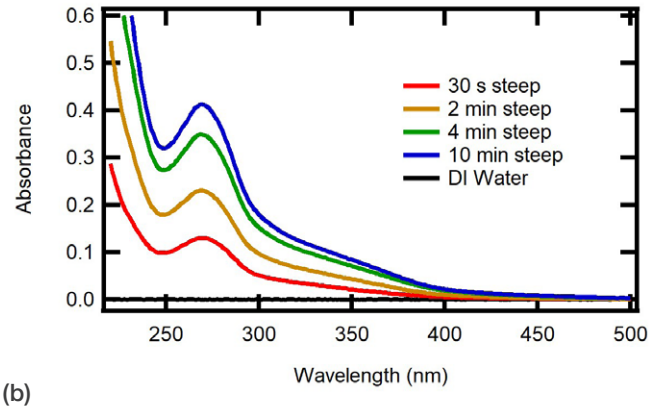
Figure 3, GENESYS Smart QC local control method "Caffeine Conc. – Steeped Tea".

Results/Discussion

UV-Visible Spectra of Caffeine Standards and Steeped Tea

The absorption spectra for four different tea samples were collected as shown in Figure 4a. These spectra include a peak at ~ 272 nm as well as a broad absorption feature starting at ~425 nm and extending into the deep UV (λ < 200 nm). With increasing steep time, the absorbance of the samples increases as well. This result matches expectations, as the longer the tea leaves are able to steep, the more components can be extracted. Additionally, as these samples were filtered, minimal scatter is expected. This is further suggested by the lack of an absorption feature at wavelengths longer than 450 nm. There is the possibility that some particulates smaller than the pore size for the filters were present, however the extent to which these particles have affected the absorption spectra is minimal

(a)



(b)

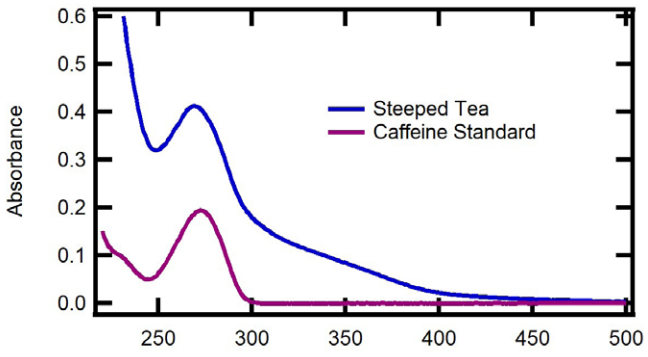


Figure 4. (a) Absorption spectra of tea samples steeped for 30 s (red), 2 min (orange), 4 min (green) and 10 min (blue) measured in a 1.0 cm cuvette. (b) Comparison of the absorption spectra for 10 min steeped tea sample (blue) and 20.3 µM caffeine standard solution

For comparison against a caffeine standard, the absorbance of 20.3 µM caffeine was measured. Figure 4b includes the absorption spectra of the caffeine standard and a tea sample steeped for 10 min. Both spectra include an absorption feature centered at 272 nm, while the caffeine standard does not absorb at wavelengths longer than 300 nm, unlike the tea samples. The UV-Visible spectrum of the caffeine standard agrees with literature.[1,6] As expected, the match between the absorption spectrum of caffeine and the steeped tea sample confirms the presence of caffeine in the steeped tea. However, as absorption is additive (Eqn. 2) a correction method is needed to eliminate background interference from additional chromophores extracted from the tea. The exact identity of these chromophores is out of scope for this analysis.

One method for correcting the absorbance includes fitting the data at longer wavelengths to a linear function. Typically, this method is used to correct for a sloped baseline by fitting to a line a section of the data where there is no absorption. The fit is then extrapolated out to the full measurement range and subtracted from the raw spectrum. For the tea samples, this method can be used as a rough correction to remove the overlapping absorption feature as the absorbance between 320 nm and 350 nm fits well to a linear function. Keep in mind, this method is not exact as it assumes a linear function fits well for all points across the spectrum, which may not be the case. Under these circumstances, this method will likely under-correct the data, leading to a higher calculated concentration than the true concentration. Herein, this correction method will be implemented through the "Caffeine Conc. – Steeped Tea" method generated using the GENESYS Smart QC PC control software.

Equation Derivation

As the GENESYS Smart QC software allows for the user to include a calculation using multiple analysis wavelengths, a simplified equation was needed to include in the "Caffeine Conc. – Steeped Tea" method. Equation 4 outlines the basic correction needed to remove background interference from additional absorbers present in the tea samples,

(4)

$$A_{272\,nm,\,corr} = A_{272\,nm,\,raw} - C_{272\,nm}$$

where $A_{272\,nm,corr}$ is the corrected absorbance, $A_{272\,nm,\,raw}$ is the measured absorbance and $C_{272\,nm}$ refers to the background absorbance. As described previously, to determine the background correction needed, a linear function would need to be fit to the data. To determine this equation, a generic linear function is used (Eqn. 5),

(5)

$$C_\lambda = m\lambda + b$$

where C_λ is the absorption at a given wavelength λ, m is the slope of the line and b is the y-intercept. The slope can be further simplified as the change in the y-axis of a graph divided by the change in the x-axis (Eqn. 6). For the data sets described herein, the slope can be determined by dividing the change in absorbance between two points by the change in wavelength for both points.

(6)

$$m = \frac{rise}{run} = \frac{A_1 - A_2}{\lambda_1 - \lambda_2}$$

(7)

$$m = \frac{A_{345\,nm} - A_{318\,nm}}{27\,nm}$$

For the tea samples, the absorbance at 318 nm and 345 nm (Eqn. 7) were chosen to define the linear fit as they were far enough away from where caffeine is expected to absorb. Additionally, these points covered the apparent linear section of the background interference well.

The y-intercept can be determined by solving the original linear equation using one of the known points. Equation 8 outlines the generic formula for determining the y-intercept and equation 9 describes the y-intercept equation derived to analyze the tea samples.

(8)

$$b = A_1 - m\lambda_1 = A_1 - \left(\frac{A_1 - A_2}{\lambda_2 - \lambda_1}\right)\lambda_1$$

(9)

$$b = A_{318\,nm} - \left(\frac{A_{318} - A_{345}}{27}\right) * 318\,nm$$

Using equations 3, 4, 5, and 7, the simplified form of the corrected absorbance can be written as shown in equation 10. Using the tea sample as an example, equation 11 can then be derived.

(10)

$$A_{\lambda,\,corr} = A_{\lambda,\,raw} - A_1 + \left(\frac{\lambda_1 - \lambda}{\lambda_2 - \lambda_1}\right) * \left(A_1 - A_2\right)$$

(11)

$$A_{\lambda,\,corr} = A_{\lambda,\,raw} - A_{318\,nm} + \left(\frac{318\,nm - \lambda}{27\,nm}\right) * \left(A_{318\,nm} - A_{345\,nm}\right)$$

Using equation 11, the linear fit was subtracted across the entirety of the measured UV-Visible spectrum for the tea sample steeped for 10 min. As is shown in Figure 5, when normalized to the absorbance at 272 nm, the corrected absorbance spectrum for the steeped tea sample is similar to the absorption spectrum of the caffeine standard. Though there is some variation in the overall shape of the corrected absorption spectrum comparatively, this observation suggests the correction method can remove the majority of the background interference for these samples.

Once the absorbance for the tea samples is corrected, a relationship between the corrected absorbance and the concentration of caffeine needs to be established. To accomplish this, the UV-Visible spectra of four caffeine standard solutions of varying concentration were measured (Fig. 6a). The absorbance collected at 272 nm ($A_{272\ nm}$) was plotted as a function of caffeine concentration and fit to a linear function (Fig. 6b).

In this way, the molar absorptivity could be determined by using the fit parameters and knowledge of Beer's law (Eqn. 1). Based on the measurements outlined herein, the molar absorptivity was estimated to be 9.67 x 10^{-3} μM^{-1} cm^{-1}, similar to the molar absorptivity reported in literature.[6] Using Beer's law and the calculated molar absorptivity, an equation can be made using the corrected absorbance at 272 nm (Eqn. 3). As mentioned in the experimental section, Equation 3 was included in the "Caffeine Conc. – Steeped Tea" method, as shown in Figure 2, which was then deployed to the GENESYS 180 UV-Visible spectrophotometer.

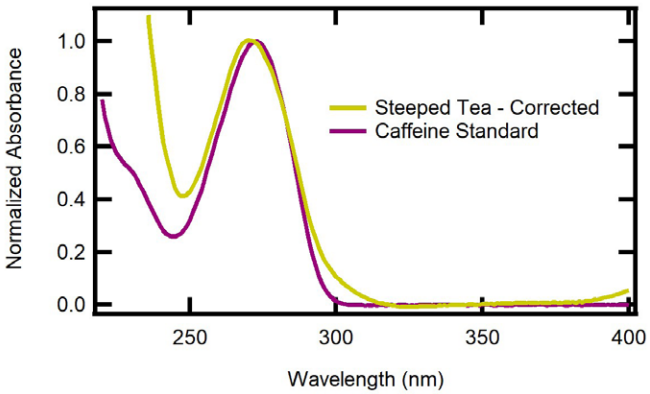


Figure 5. Absorption spectra of the 20.3 µM standard caffeine solution (purple) and the corrected 10 min steeped tea solution (orange) normalized to the absorbance at 272 nm.

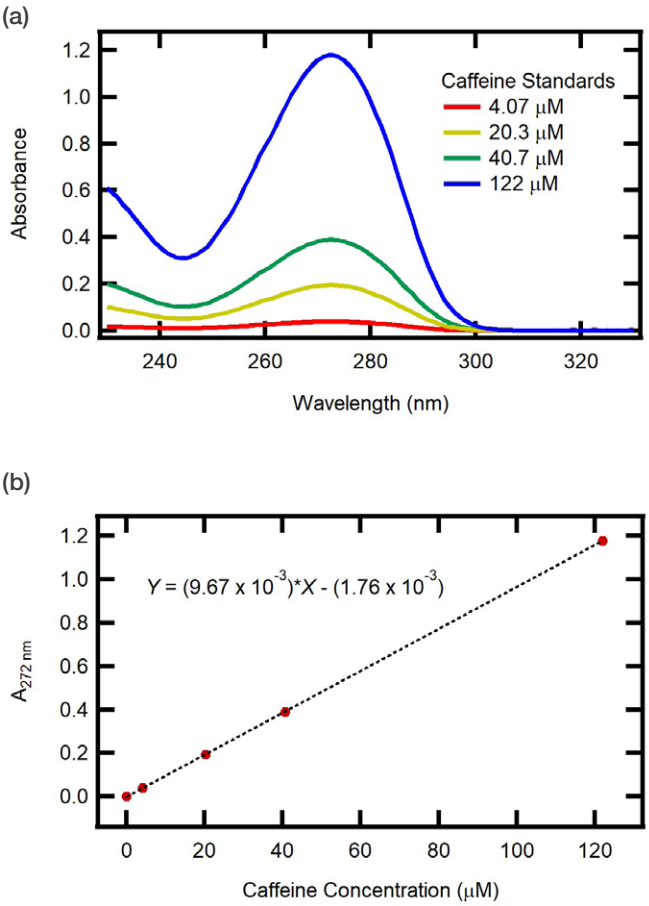


Figure 6. (a) Absorption spectra of caffeine standards measured in a 1.0 cm quartz cuvette. (b) Absorbance at 272 nm as a function of caffeine concentration.

GENESYS Smart QC and Derivative Analysis Comparison

Figure 7 includes the GENESYS Smart QC results for the “Caffeine Conc. – Steeped Tea” method. The tea sample steeped for 10 min is highlighted here, reporting a concentration of 0.022 mM caffeine in the diluted sample. The caffeine concentration calculated using the Smart QC method for the diluted tea samples steeped for 30 s, 2 min and 4 min are included in Table 1. Based on the concentration of the diluted samples, the caffeine concentration for the stock steeped tea samples were calculated and reported in Table 1 as well. As anticipated, with increasing steep time, a higher concentration of caffeine is observed.

As a point of comparison, the tea samples were analyzed through derivative spectroscopy. This method is commonly used for complex matrices containing overlapping absorption features, as discussed previously. For these purposes, the first derivative of the absorption spectrum for each caffeine standard solution was calculated (Fig. 8a). Using the “peak-to-peak” method,3 a standard curve was constructed relating the sum of the positive and negative peak amplitudes, $|A_{260\ nm}|$ and $|A_{285\ nm}|$ (Eqn. 12), to caffeine concentration (Fig. 8b).

$$\left|A_{260\ nm}\right| + \left|A_{285\ nm}\right| \quad (12)$$

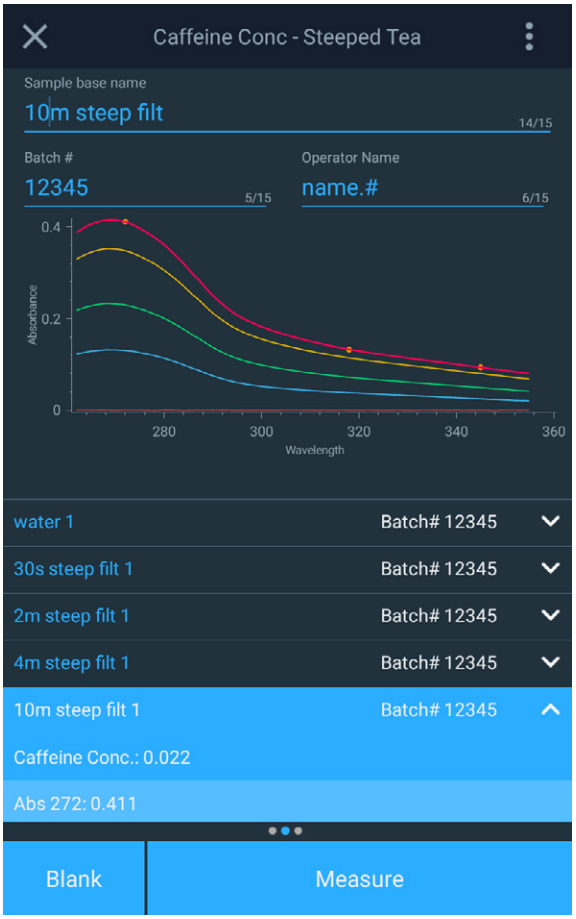


Figure 7. GENESYS Smart QC results for steeped tea samples analyzed using the “Caffeine Conc. – Steeped Tea” method.

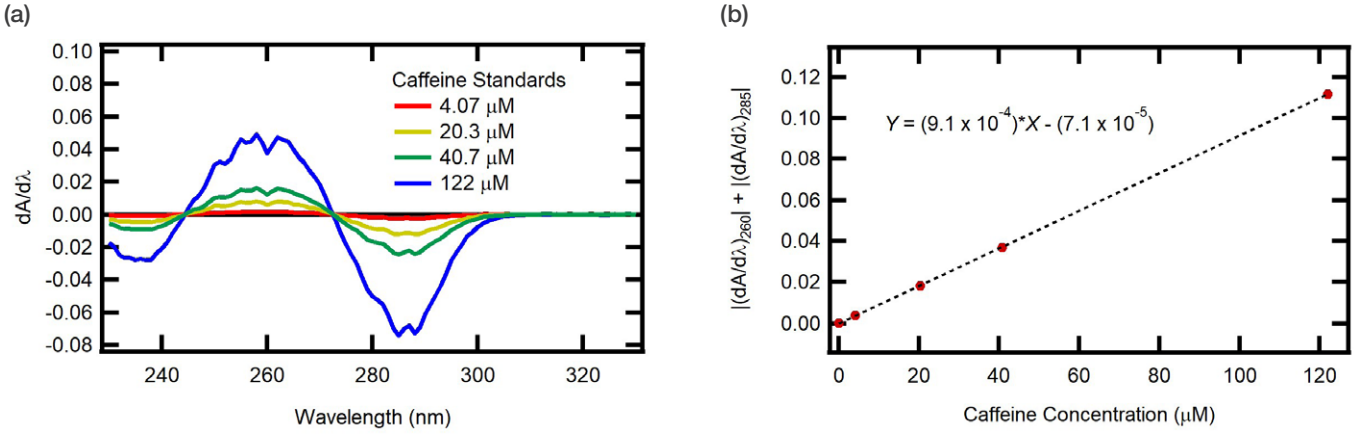


Figure 8. (a) First derivative spectra of caffeine standards measured in a 1.0 m quartz cuvette. (b) Standard curve and linear fit generated through the peak-to-peak method.

Includes the caffeine concentrations for both the 0.5% tea sample and the tea stock solution using the first derivative and Smart QC methods. The resulting concentrations calculated from both methods are similar to one another. The Smart QC method does overestimate the concentration, as expected, compared with the results calculated using derivative spectroscopy. This appears to be more significant for the samples exhibiting a higher raw absorption spectrum. While there is some variation, the similarity in the results demonstrates the "Caffeine Conc. – Steeped Tea" method could be used to provide a quick check for the caffeine concentration in the samples studied. Note that though this correction has worked well for this sample set, this correction may not be appropriate for all data sets. The method should be tested and thoroughly vetted beforehand to ensure the correction is acceptable and will not grossly over or under estimate the concentration.

Tea Sample	Smart QC Method		Derivative Method	
	[Caffeine] $_{Sample}$ (mM)	Caffeine] $_{Stock}$ (mM)	[Caffeine] $_{Sample}$ (mM)	[Caffeine] $_{Stock}$ (mM)
30 s Steep	7.0×10^{-3}	1.4	6.8×10^{-3}	1.4
2 min Steep	1.2×10^{-2}	2.4	1.1×10^{-2}	2.2
4 min Steep	1.8×10^{-2}	3.6	1.6×10^{-2}	3.2
10 min Steep	2.2×10^{-2}	4.3	1.9×10^{-2}	3.8

Table 1. Caffeine concentrations calculated for each sample and steeped tea stock solution. Concentrations were calculated using the GENESYS Smart QC and derivative methods.

Conclusions

As shown through the data described here in, the developed GENESYS Smart QC method was able to correct the absorbance at 272 nm to remove the contributions from interfering substances. From this corrected absorbance, the concentration of caffeine for each sample was reported. In comparison to the concentration of caffeine determined through derivative spectroscopy, this analysis did overestimate the concentration of caffeine present. However, the variation is minimal between both calculation methods, suggesting this method can provide a quick concentration check on the instrument software without the need to further process the data manually.

References

1. Habtamu, D.; Belay, A., First Order Derivative Spectra to Determine Caffeine and Chlorogenic Acids in Defective and Nondefective Coffee Beans, Food Sci. Nutr., 2020, 8, 4757 – 4762.
2. Karpińska, J., Derivative Spectrophotometry – Recent Applications and Directions of Developments, Talanta, 2004, 64, 801 – 822.
3. Talsky, G.; Mayring, L.; Kreuzer, H., High-Resolution, Higher-Order UV/VIS Derivative Spectrophotometry, Angew. Chem., Int. Ed. Engl., 1978, 17, 785 – 799.
4. Thermo Fisher Scientific, Analysis of Vitamin A Within Cod Liver Oil: Use for Derivatives in UV-Visible Absorption Techniques, https://assets.thermofisher.com/TFS-Assets/CAD/Application-Notes/vitamin-a-%20quantification-evo-app-note-AN54651.pdf
5. Scharbert, S; Hofmann, T., Molecular Definition of Black Tea Taste by Means of Quantitative Studies, Taste Reconstitution and Omission Experiments, J. Agric. Food Chem., 2005, 53, 5377 – 5384.
6. Belay, A.; Ture, K.; Redi, M.; Asfaw, A., Measurement of Caffeine in Coffee Beans with UV/vis Spectrometer, Food Chem., 2008, 108, 310 – 315.

Learn more at **thermofisher.com/smartqc**