Compendium

UV-Vis Spectroscopic Analysis for the Beverage Industry

A compendium of application notes, SmartNotes, and case studies

thermo scientific

Introduction

Each year, U.S. beverage manufacturers sell millions upon millions of liters of sports drinks, ready-to-drink teas, beers, and other beverages. The makers of these various libations are part of a competitive beverage industry that is constantly seeking efficient testing methods to make certain that their products are safe, consistent and high quality. Product analysis using ultraviolet-visible (UV-Vis) spectroscopy has proven to be a cost-effective, non-destructive technique that can help ensure beverages are made to their manufacturers' desired specifications. This collection of application notes and other papers from Thermo Fisher Scientific highlights cases where UV-Vis analysis can provide significant advantages to beverage producers.

For many beverages, dissolved substances included in the potable solutions can be measured using UV-Visible absorption spectroscopy. Analytes measured can include flavor additives, dyes, and other components like caffeine. An application note included here shows how a Thermo Scientific™ GENESYS™ 180 UV-Visible Spectrophotometer, equipped with GENESYS Smart QC software, can be used in a sports drink manufacturing environment, to provide quality checks that incorporate Pass/Fail criteria chosen by the analyst. Another note demonstrates how the caffeine levels in a ready-to-drink tea can be easily quantified, with SmartQC software able to provide background correction.

In the craft beer segment, UV-Visible spectrophotometers can be employed in the testing process to ensure consistent quality and flavor. The instruments offer quick, simple, and affordable testing as well as the ability to utilize a variety of methods, making them more versatile than a limited instrument like a colorimeter. A case study of Pacific Northwest craft brewery pFriem Family Brewers, included here, shows how a UV-Vis spectrophotometer has become an integral part of their QC laboratory. Further specifics about how UV-Vis can be employed to analyze important beer aspects like color, taste, bitterness (IBU), or potential contaminants like diacetyl or other VDKs, are included in this compendium as a series of SmartNotes.

Lastly, a case study of midwestern craft beer yeast supplier Omega Yeast illuminates how their R&D department utilizes a GENESYS UV-Vis Spectrophotometer for OD600 measurements that correspond to various important properties including cell number, FAN, BUs, ferulic acid conversion by POF+ yeast. The yeast wranglers describe how UV-Vis analysis has helped them study topics such as hop creep, diastatic yeast, wild yeast metabolites, yeast contributions to haze, and nutritional requirements of yeast, all in the service of producing better beverages.

For industry participants ranging from small brewers to major drink producers, UV-Visible spectrophotometers from Thermo Fisher Scientific provide compact, versatile, and affordable options for beverage monitoring and quality control, as the contents of this compendium illustrate.

Table of Contents

Setting Pass/Fail Criteria with GENESYS Smart QC: Sports Drink Quality Testing 4

Caffeine Quantification in Steeped Tea: Background Correction Using GENESYSTM Smart QC 9

Let's talk Quality Control with Max Kravitz, pFriem Family Brewers 16

Smart Note: Analyze beer color using UV-Vis spectrophotometry 20

Smart Note: Measure beer vicinal diketones (VDKs) using UV-Vis spectrophotometry 22

Smart Note: Measure beer bitterness using UV-Visible spectrophotometry 25

Craft brewing case study: Omega Yeast 27

Application note

Setting Pass/Fail Criteria with GENESYS™ Smart QC: Sports Drink Quality Testing

Introduction

In manufacturing environments, testing for product quality is vital. Products must be tested not only to ensure batch-to-batch consistency, but to confirm no contaminants are present. Contaminants could have a minor effect like causing a change in the appearance of a material, or in some cases they could cause more troublesome issues like the formation of unwanted side products. These possible alternative reactions may lead to formation of an inactive substance or generate harmful byproducts. Quality checks for these possible product defects are commonplace and can be executed through a variety of different analytical techniques.

For some solution-phase products, substances included in a product can be measured using UV-Visible absorption spectroscopy, a non-destructive analytical technique. Analytes measured can include flavor additives, dyes, and other components (e.g., caffeine). In this method, ultraviolet or visible light interacts with a sample, and the sample can transmit the light or absorb it. The range of wavelengths which can be absorbed by the sample comprise the absorption spectrum, a characteristic trait of the analyte(s) of interest. As absorption arises from electronic transitions specific to the studied analyte, the resulting spectrum will be unique to the substance studied.

The absorbance of a compound at a given wavelength can be related to concentration through Beer's law (eqn. 1),

$$A = cl\varepsilon$$

Equation 1.

Where A is the measured absorbance, l is the path length of the cuvette used, ε is the molar extinction coefficient (a property unique to the analyzed compound), and c is concentration. As shown in this equation, absorbance is linearly proportional to the analyte concentration. Through this relationship, UV-Visible spectroscopy can be used to not only identify the presence of a given analyte, but to determine its concentration as well. While not all molecules absorb in the UV-Visible range, those that do can be easily measured and further quantified through this technique. As UV-Visible spectroscopy is non-destructive, the sample can be retrieved post-analysis for further testing or down-stream processing.

Herein, GENESYS™ Smart QC software in tandem with the GENESYS™ 180 UV-Visible spectrophotometer was used to analyze a set of sports drink samples as an example of product quality checks. These experiments demonstrate a scenario in which two different products are manufactured in the same facility. Using the UV-Visible spectra of the drinks as standards, equations were generated to identify if the concentration of a given sample was within an acceptable range as well as determine if one product contaminated the other. These results demonstrate the utility of the GENESYS Smart QC software for assessing product quality and providing quick pass/fail assessments.

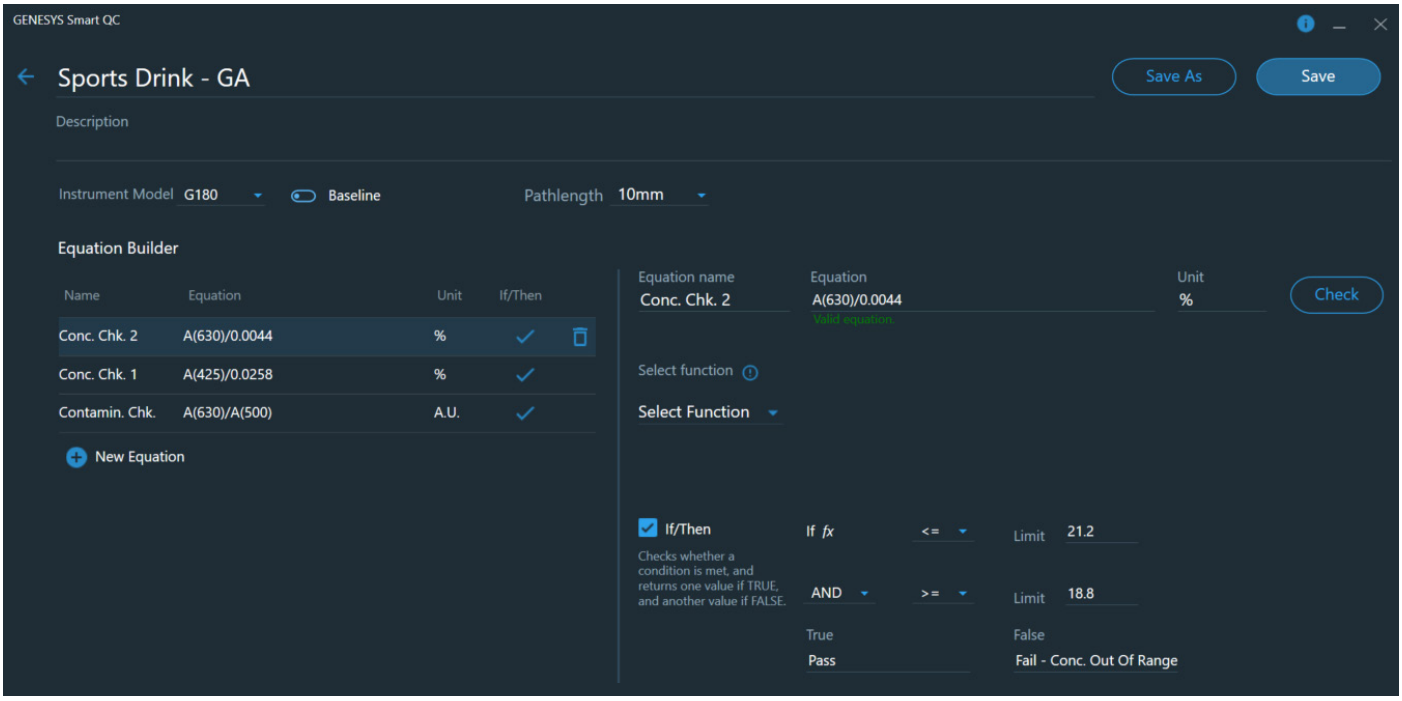

Figure 1. GENESYS Smart QC PC control software interface - Equation editor for the "Sports Drink - GA" method.

Experimental

Two different sports drinks, green apple flavored ("GA") and strawberry lemonade flavored ("SL"), were acquired from a local supermarket and used as received for all samples and standards made herein. Standard GA and SL solutions were made in triplicate. Each standard solution was prepared by adding 0.5 mL of the respective stock solution to 2.0 mL of DI water, resulting in a final concentration of 20% v/v. The absorption spectrum of all six solutions were measured using the GENESYS 180 UV-Visible spectrophotometer from 350 nm to 700 nm. The scans were collected using a slow scan speed and a 1.0 nm data interval.

A standard curve was generated using solutions of 4%, 10%, 20%, 30% and 40% v/v GA in water in order to determine the extinction coefficient (ε, units: $\%^{-1}\ cm^{-1}$). The spectra were collected using the scan application on the GENESYS 180 UV-Visible spectrophotometer. Specifically, the absorbances at 425 nm and 630 nm were monitored and used for the standard curve analysis. The absorption spectra were collected between 350 nm and 700 nm using a slow scan speed and 1.0 nm data interval, similar to previous measurements. Each standard was analyzed in triplicate.

Four samples were made using the appropriate stock sports drink and DI water to be analyzed using the GENESYS Smart QC software. The amount of each component included in each sample is described in Table 1.

Sample	Volume GA Stock (mL)	Volume water (mL)	Volume SL Stock (µL)
GA-1	0.50	2.00	---
GA-2	0.25	2.25	---
GA-3	0.50	2.00	50
SL-1	---	2.00	500

Table 1. Solution volumes used to prepare each sample analyzed using the GENESYS Smart QC software.

The Smart QC method, labeled Sports Drink – GA, was generated using the GENESYS Smart QC PC control software (Figure 1). Three equations with pass/fail criteria were established – one (Contamin. Chk.) to identify if a contaminant was present in the sample and two (Conc. Chk. 1, Conc. Chk. 2) to check for sample concentration (see Table 2). The results from the standard curve measurements were used to estimate ε at 425 nm and 630 nm, which were included in the Conc. Chk. 1 and Conc. Chk. 2 equations, respectively. The method was exported, then loaded onto a GENESYS 180 UV-Visible spectrophotometer local control platform. The Sports Drink – GA method was then used to analyze all samples described previously. Each sample was analyzed using a 1.0 cm glass cuvette.

Equation Name	Equation	Pass/Fail Criteria
Contamin. Chk.	$\frac{A(630)}{A(425)}$	$1.4 \leq \frac{A(630)}{A(425)} \leq 1.8$
Conc. Chk. 1	A(425)	$18.8\% \leq \frac{A(425)}{0.0258} \leq 21.2\%$
Conc. Chk. 2	A(630)	$18.8\% \leq \frac{A(630)}{0.0044} \leq 21.2\%$

Table 2. Equations generated using the GENESYS Smart QC PC control software.

Results/Discussion

Setting Acceptance Criteria

To demonstrate a possible quality check, a method was developed to check the concentration of GA, a sports drink, and determine if a contaminant, SL sports drink, is present. This scenario mimics a possible manufacturing environment in which more than one product is handled using either the same equipment or in close proximity to one another. It is important to note that there are a variety of substances which can be analyzed using UV-Visible techniques, such as caffeine or flavorings, which are not related to the color of the sample. For the purposes of this experiment, only absorption features related to the dyes used in the studied beverages are analyzed.

In order to develop this method, the spectra of 20% v/v GA, referred to as the GA standard, were collected in triplicate. From the collected spectrum the absorption maxima were found at 630 nm and 425 nm. According to Beer's law (eqn. 1), the absorbance is directly proportional to the concentration of the absorptive component in solution. Consequently, the absorbance at both of these absorption maxima (0.087 and 0.50 at 630 nm and 425 nm, respectively) was used as a standard value. However, more information is needed to create a pass/fail value in terms of GA concentration (units: %).

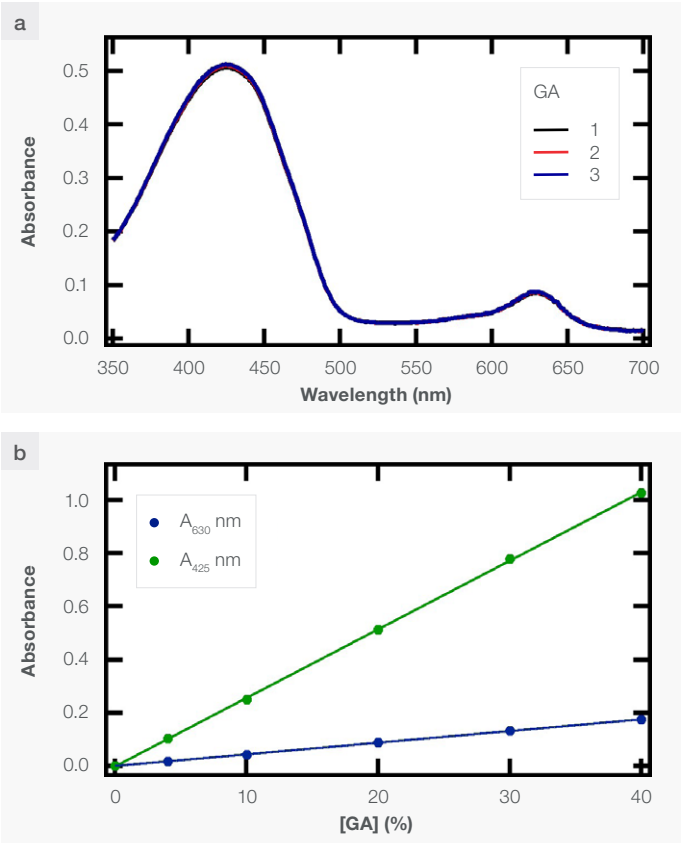

Figure 2. (a) UV-Visible spectra of 20% v/v GA. (b) Standard curve of GA samples of varying concentration, including error bars. Samples were measured in triplicate.

In order to convert the recorded absorbance to concentration, the extinction coefficient is required, as outlined through Beer's law (eqn. 1). To determine the extinction coefficient, standard curves were generated using absorption measurements of GA samples with varying concentration as described in the Experimental section (Figure 2b). The extinction coefficient is then estimated using the slope of the line best fit to the data. From the standard curves using data collected at 425 nm and 630 nm, the extinction coefficients were found to be 0.0258 ± 0.0001 $\%^{-1}$ cm^{-1} and 0.00438 ± 0.00003 $\%^{-1}$ cm^{-1}, respectively. Using these results, equations were created to convert the measured absorbance at 425 nm and 630 nm to concentrations values. The pass/fail criteria were then created to determine if the concentration was within acceptable limits. For the purposes of this experiment, the passing concentration range was selected to be 18.8% - 21.2%. A failed test will report "Fail – Conc. Out Of Range".

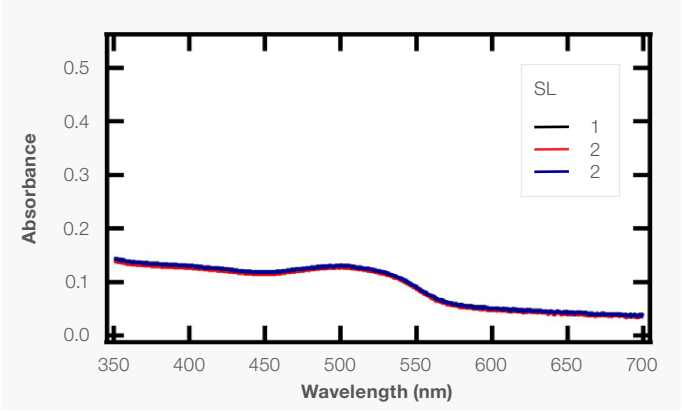

Figure 3. UV-Visible absorption spectra of 20% v/v SL collected in triplicate.

The spectra of 20% v/v SL (SL standard), a possible contaminant, were collected using the same instrument parameters to see if the absorption spectrum overlaps with the spectrum for GA. A broad absorption feature can be found between 475 nm and 550 nm as well as a baseline artifact extending throughout the spectrum, possibly from scatter (Figure 3). As GA has minimal absorption in the range containing an absorptive feature from SL, the absorbance collected at this range can be used to determine if the GA sample is contaminated with SL. For this experiment, a ratio between the absorbance at 630 nm, where GA absorbs, and 500 nm, where SL absorbs, is used to test if the GA sample is contaminated with SL (Table 2). A failed test will report "Fail – Contaminated".

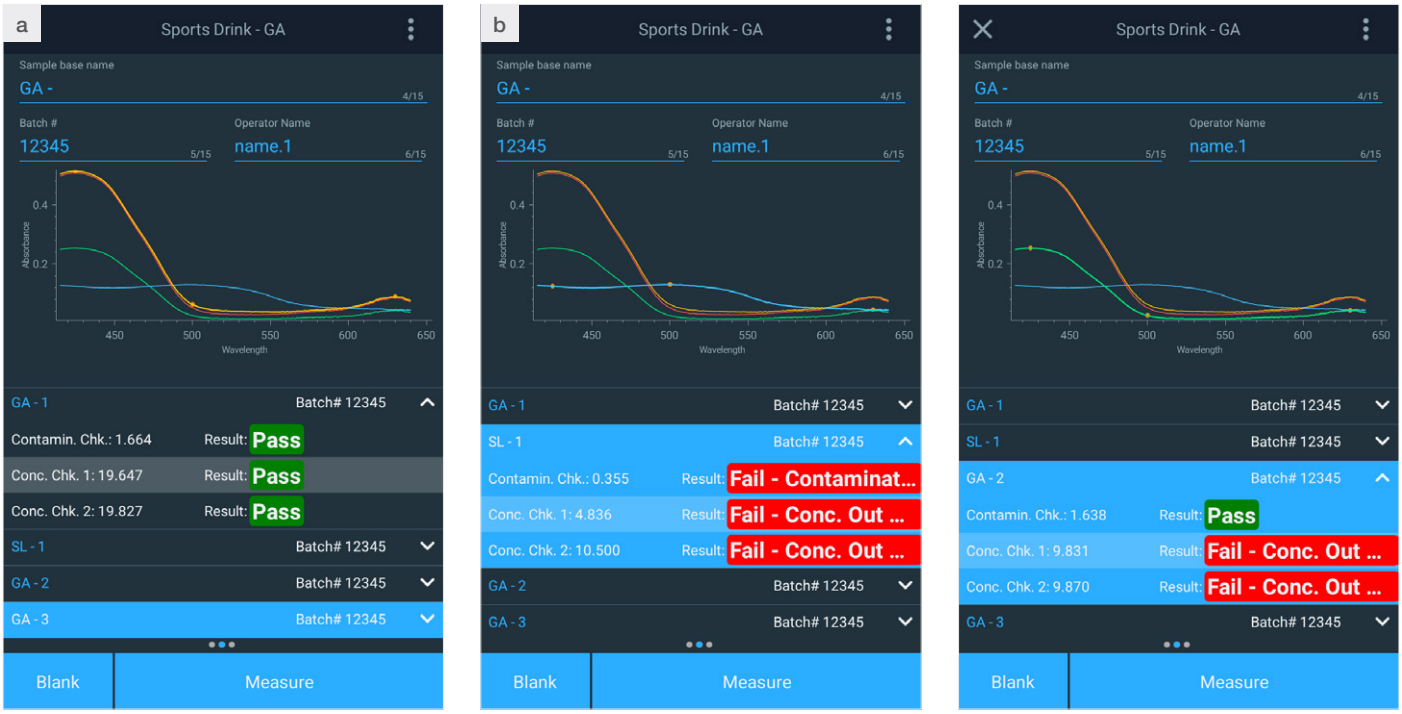

Figure 4. GENESYS Smart QC "Sports Drink – GA" method results for (a) GA-1: 20% v/v GA standard and (b) SL-1: 20% v/v SL standard.

Figure 5. GENESYS Smart QC "Sports Drink – GA" method results for GA-2: 10% v/v GA.

Analysis with GENESYS Smart QC

Four different samples were then used to test the established pass/fail criteria outlined in Table 2. First, the absorption spectrum for both the GA and SL standards were measured and checked against the generated acceptance criteria through the GENESYS Smart QC local control software. As expected, the GA standard passed all tests while the SL standard failed all tests (Figure 4). This result indicates to the user that the sample at the least contains a contaminant and at most may also not be the correct concentration. This failure can then be used as an indicator that the sample or batch the sample came from should be further checked to ensure the correct substance was analyzed.

To simulate a scenario where the product was improperly mixed or diluted, a 10% v/v GA samples was prepared. When analyzed using the GENESYS Smart QC local control software (Figure 5), both Conc. Chk. 1 and Conc. Chk. 2 failed, indicating the concentration was outside of the acceptable range as expected. However, note that though the concentration was incorrect, the ratio between the absorbance at 630 nm and 500 nm still passes, indicating no contaminant was present. As the ratio between these two numbers will remain the same regardless of the sample concentration, the user will be able to differentiate whether the error is strictly from the amount of analyte present, or if it arises from a contaminant. Note that if a contaminant does not absorb in the UV-Visible region this method cannot rule out the presence of a contaminant. Under these circumstances, an additional quality test should be administered.

Figure 6. (a) Images of GA samples with (left) and without (right) SL contamination. (b) GENESYS Smart QC results for GA-3: 20% v/v GA spiked with SL (SL concentration = 2.0 %).

Finally, a sample was generated which intentionally included a contaminant, SL, that absorbs in the UV-Visible region. In solution, the amount of SL present was 2% v/v, an order of magnitude smaller than the concentration of GA (20% v/v). As can be seen in Figure 6a, by eye the standard GA without SL contamination appears the same as the contaminated GA sample. However small variations can be observed in the UV-Visible spectrum (Figure 6b). When the equations are applied, the concentration of GA is shown to be in range, however the contaminant is detected as shown by the "Fail – Contaminated" result in Figure 6b.

Conclusion

As demonstrated in these experiments, UV-Visible absorption spectroscopy can be a useful tool for checking product quality. Through the GENESYS Smart QC software, acceptance criteria can be set, allowing for a quick pass/fail check of a product based on the measured absorption spectrum. In the example described herein, equations were included which identified the presence of a possible contaminant as well as determined if the sample concentration was within the appropriate limits. While the analysis of samples with different colorants is used as an example herein, there are a wide variety of other solution phase compounds which can be checked for quality purposes using UV-Visible techniques.

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

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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.

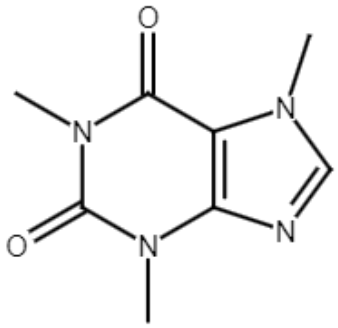


Figure 1. Caffeine chemical structure.

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.

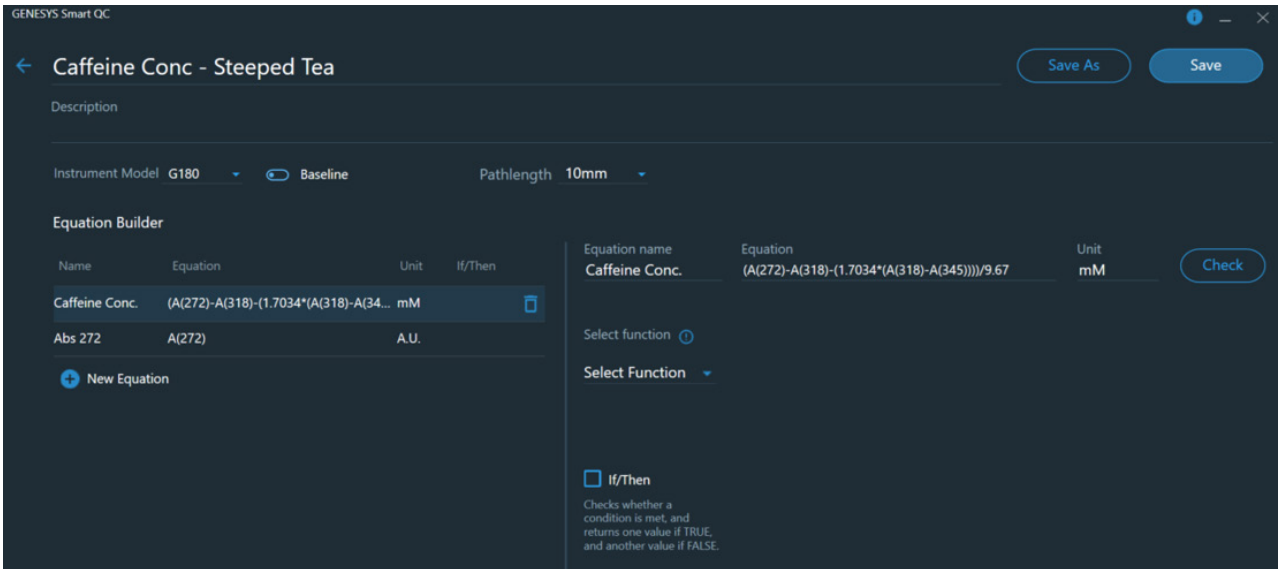


Figure 2. GENESYS Smart QC PC control software. Outlined here is the method created for analysis of caffeine in steeped tea samples.

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.

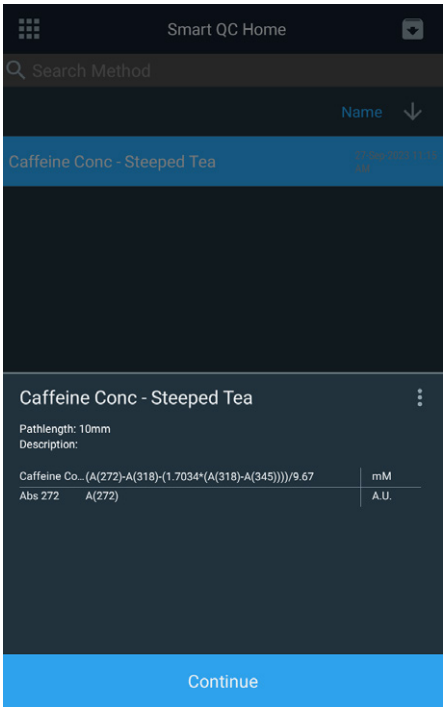


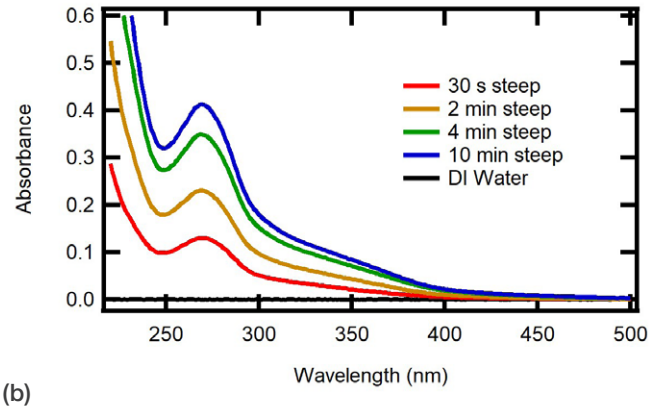
Figure 3, GENESYS Smart QC local control method "Caffeine Conc. – Steeped Tea".

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)

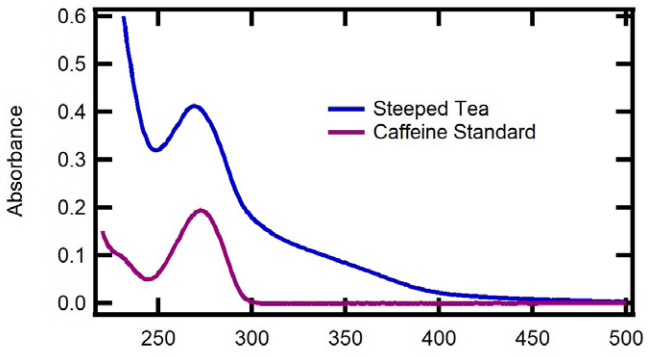


Figure 4. (a) Absorption spectra of tea samples steeped for 30 s (red), 2 min (orange), 4 min (green) and 10 min (blue) measured in a 1.0 cm cuvette. (b) Comparison of the absorption spectra for 10 min steeped tea sample (blue) and 20.3 µM caffeine standard solution

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.

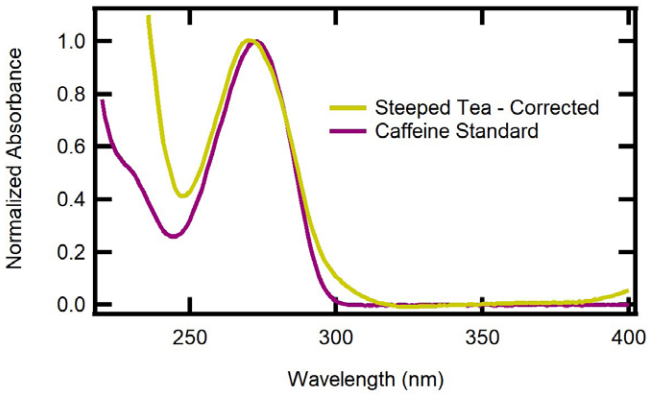


Figure 5. Absorption spectra of the 20.3 µM standard caffeine solution (purple) and the corrected 10 min steeped tea solution (orange) normalized to the absorbance at 272 nm.

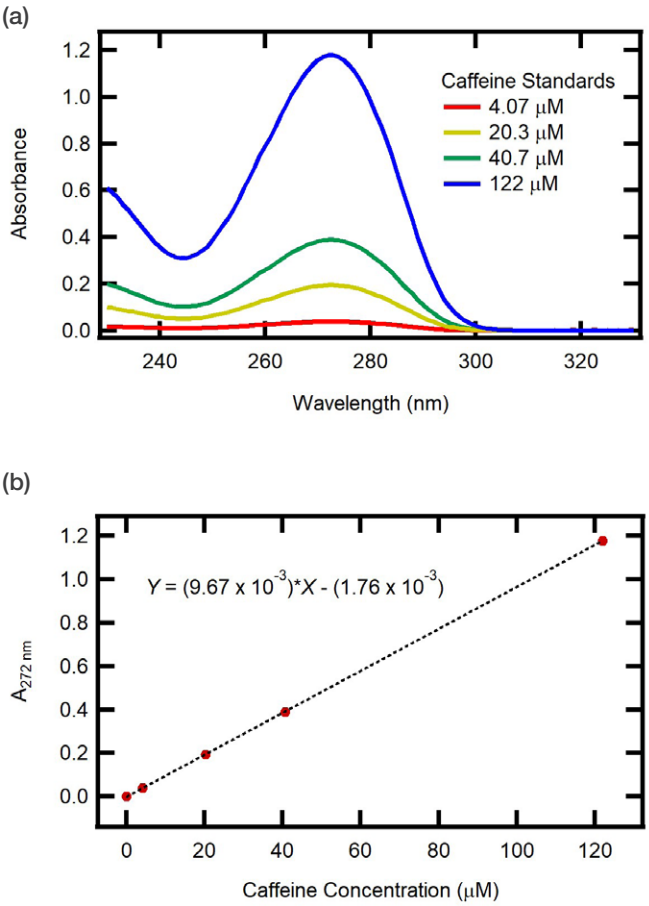


Figure 6. (a) Absorption spectra of caffeine standards measured in a 1.0 cm quartz cuvette. (b) Absorbance at 272 nm as a function of caffeine concentration.

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).

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

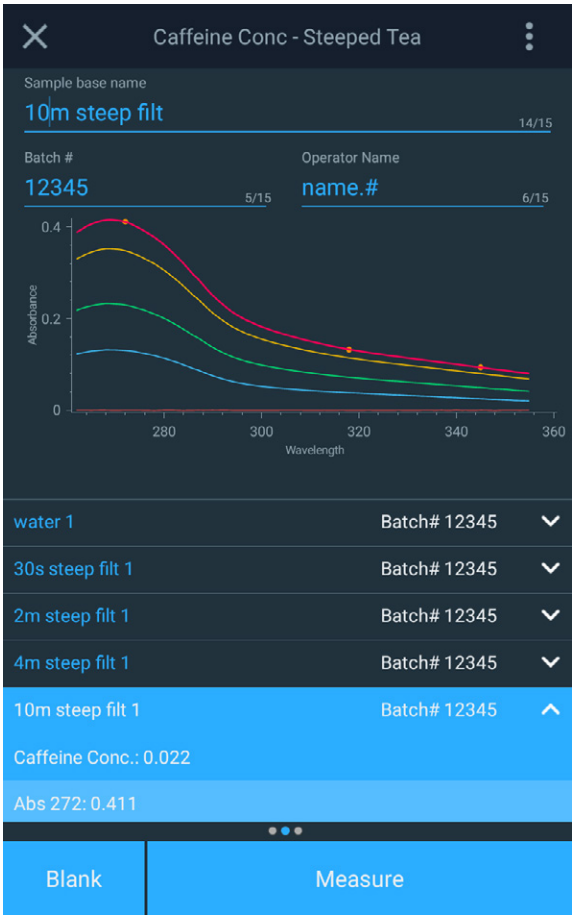


Figure 7. GENESYS Smart QC results for steeped tea samples analyzed using the “Caffeine Conc. – Steeped Tea” method.

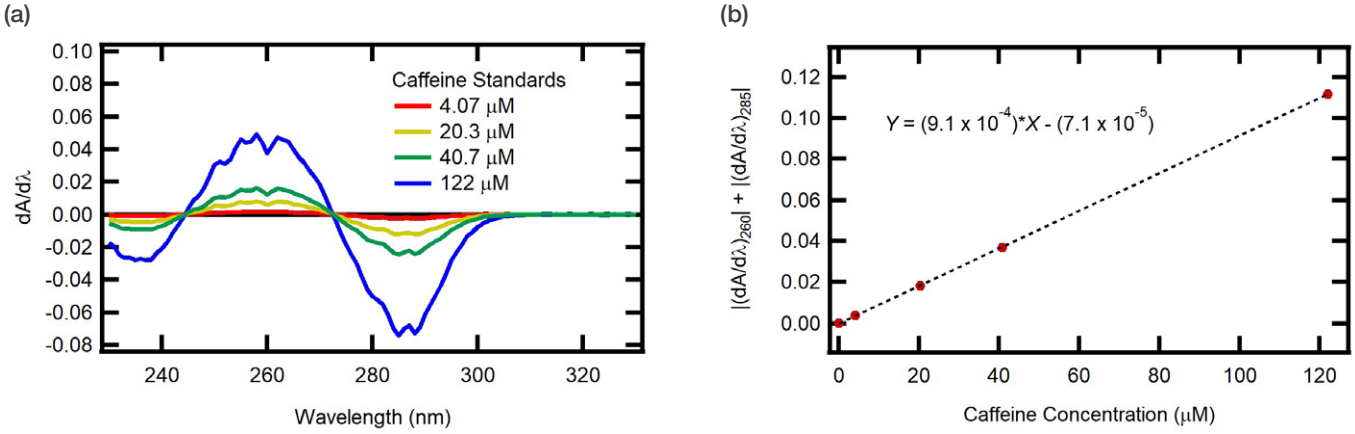


Figure 8. (a) First derivative spectra of caffeine standards measured in a 1.0 m quartz cuvette. (b) Standard curve and linear fit generated through the peak-to-peak method.

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.

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2. Karpińska, J., Derivative Spectrophotometry – Recent Applications and Directions of Developments, Talanta, 2004, 64, 801 – 822.
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Learn more at **thermofisher.com/smartqc**

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CASE STUDY 53172

Let's talk Quality Control with Max Kravitz, pFriem Family Brewers

Pfriem brewery (top of page) and beer on tap (above). Photos courtesy of pFriem Family Brewers.

To create award-winning artisanal beer with a unique Pacific Northwest hometown flair, pFriem Family Brewers took inspiration from great Belgian and German brewers. Their Quality Laboratory Manager, Max Kravitz, shares how their testing processes ensure consistent quality and flavor and the added benefits provided by integrating UV-Vis spectrophotometry methods.

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Pfriem laboratory. Photo courtesy of pFriem Family Brewers.

"...we've found the spectrophotometer to be an invaluable tool for routine beer testing on numerous levels. It is so amenable to many types of testing that it has quickly become an integral part of our quality laboratory. After having had a spectrophotometer, I could not imagine running a beer quality program without one."

Max Kravitz, Quality Laboratory Manager

1. **Could you provide a brief introduction to pFriem Family Brewers?**
   pFriem is a 15 barrel (bbl) brewery located in beautiful Hood River, Oregon. We make over 120 unique styles of beer a year with emphasis on classic German lagers, hop-forward American ales, and traditional Belgian-inspired beers.

2. **What is your brewery's yearly capacity?**
   We are on track to brew ~30,000 bbls of beer in 2019.

3. **What kind of quality testing procedures do you use in your brewery? What tests do you perform, what equipment do you use, how often do you test, etc.?**
   We have dozens of quality checkpoints for every single batch of beer, which extend from the brewhouse all the way to the final package and beyond. Our on-site quality laboratory focuses on yeast quality, fermentation consistency/performance, microbiological testing, packaging quality, refermentation monitoring, and sensory panels. We use a variety of different instrumentation from optical dissolved oxygen meters, carbon dioxide meters, densitometers, pH meters, turbidimeters, spectrophotometers, amongst many others.

4. **Do you have any examples, from your own experience, that demonstrate the value of UV-Visible (UV-Vis) spectrophotometry for QA/QC testing?**
   Dry hopping beers is a critical component to all of our hop-forward beers. However, the process of dry hopping has proven to disrupt the maintenance of otherwise terminal vicinal diketone (VDK) values*. This phenomenon has led to cellaring nightmares with highly extended VDK stands that had numerous negative quality impacts on the beer. With VDK quantification by [UV-Vis] spectrophotometry, we were able to identify key variables in our dry hopping process that not only allowed us to avoid this issue, but greatly improve the hop flavor, aroma, and consistency of all of our dry hopped beers.

5. **What is the value of in-house testing versus testing at outside analytical labs?**
   In a brewery production setting, we need answers right away. Unfortunately, we don't have the luxury for the overwhelming majority of our production beers to send samples out for analysis and wait for results. Many of the analytes we measure in our laboratory are very sensitive and must be processed right away as their true values can change based on sample collection and handling. Having an on-site quality laboratory was one of the first additions we made to our brewing program when pFriem first began in 2012.

6. In your experience, what are some of the advantages to having a UV-Vis spectrophotometer for analytical testing in a brewery?

I must admit, originally I was considerably hesitant as to whether we really needed a spectrophotometer in our laboratory. However, since incorporating it into our quality program, we've found the spectrophotometer to be an invaluable tool for routine beer testing on numerous levels. It is so amenable to many types of testing that it has quickly become an integral part of our quality laboratory. After having had a spectrophotometer, I could not imagine running a beer quality program without one.

7. Why use UV-Vis technology rather than other analytical instrumentation techniques? Or do you see other techniques as complementary?

UV-Vis technology is generally much less expensive than more advanced analytical testing like HPLC or GC-MS. We've done a considerable amount of validation for our numerous photometric-based assays by comparing our results to those measured by third-party laboratories for the same samples. Overall, we've found great correlation of our spectrophotometric results with those that were measured by more advanced instruments. We do still send samples out occasionally for third-party testing, but typically only for validation of our current methods or for measurement of challenging analytes.

8. If a brewery is already using sensory testing for their beer, what advantage does a spectrophotometer add to improving brewing quality?

We feel that sensory is ultimately the single most important quality test we have here at pFriem. However, measuring samples by spectrophotometry and generating quantification values for important beer analytes has been critical for us staying constantly in control of our process, quickly identifying drifting, and ensuring consistent quality for all of our beer.

9. For what experiments do you currently use a spectrophotometer and what future experiments do you hope to adopt?

We use our spectrophotometer to run the following right now: Color, Bitterness, VDKs, Acetaldehyde and Sulfur Dioxide. We are hoping to soon incorporate Free Amino Nitrogen and Wort Thiobarbituric Index.

10. What was your experience using the Thermo Scientific™ BeerCraft™ Software on the touchscreen of the Thermo Scientific™ GENESYS™ 50 UV-Vis spectrophotometer?

We found the BeerCraft software to be pretty slick all around. It was easy to navigate and was a simple platform both for measuring and presenting results.

11. What was your experience using the Beer Color and Beer Bitterness methods on the BeerCraft Software?

Beer color and bitterness were both very straightforward and easy to use.

12. Discuss your experience with working with Thermo Fisher Scientific. Would you recommend the company to others?

Our overall experience with Thermo Fisher laboratory equipment has been very positive. We enjoy the high quality of their products and their technical support. We would greatly recommend them for any beer quality laboratory.

With 20 ASBC methods, Beer Craft Software and GENESYS UV-Vis Spectrophotometers make it easy to select and test various critical parameters. Store and organize data by batch names for easy retrieval later.

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About Max Kravitz

Max Kravitz has been the Quality Laboratory Manager at pFriem Family Brewers for 4 years. He is a graduate of the University of Vermont with degrees in Molecular Biology and French. Previous to working at pFriem, Max worked in neurobiology research studying nicotinic acetylcholine receptors in embryonic chick retinas, dopaminergic nuclei in knockout mice and zebrafish husbandry, and neuroelectrophysiology. All the meanwhile at the lab bench, Max was completely and utterly obsessed with beer making. Max joined the pFriem team in 2015 to begin their Quality Laboratory program and hasn't looked back since. Making world-class beer is a never-ending and continually challenging pursuit that always presents something new to learn.

About pFriem Family Brewers

Josh Pfriem (pronounced "freem") had been on this career path his entire adult life—soaking up brewing knowledge, devouring literature, concocting experimental varietals in his shed, biking across Belgium, professionally brewing, and knocking on the doors and picking the brains of some of the greatest brewers in the world. Introduced at a child's birthday party, Josh Pfriem, Ken Whiteman, and Rudy Kellner formed the brain power that would eventually lead to the launch and rapid growth of pFriem Family Brewers. They shared a deep love of family, the Columbia River Gorge, and of course, great beer—but more importantly, each brought his own unique skill set to the table, creating the means and vision to not only make great beer, but to operate a great business. A year-and-a-half and thousands of hours of hard work later, in August 2012, pFriem Family Brewers opened its doors for the first time, and the three founders realized the beginning of their unique dream—championing family, community, and creating one of the world's best Premium Craft Beers.

Pfriem Tasting Room, Hood River, Oregon. Photo courtesy of pFriem Family Brewers.

* Vicinal diketones (VDKs) occur in beer as a by-product of the fermentation process. Increased VDKs can negatively alter the flavor of beer giving a buttery flavor.

Yeast serves to reabsorb VDKs over multiple days, so the ability to measure the concentration of VDKs in beer can increase brewing productivity saving time and money.

For more information on VDKs, see Smart Note SN53085 - Vicinal Diketones (VDKS)

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

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SmartNotes

As breweries continue to grow, the importance of beer quality becomes increasingly more significant, and basic microbiological testing is no longer enough to guarantee adequate consistency. Larger breweries who already test for beer quality are always looking for ways to simplify their testing efforts by utilizing different technology to limit the time and expense of quality testing. UV-Visible spectrophotometers are a perfect solution to help breweries meet their quality standards by offering quick, simple and affordable testing as well as the versatility to utilize a large variety of methods compared to more limited instrumentation like a colorimeter.

Beer Color

Color is one of the most important attributes of a beer. It foreshadows taste and governs consumer preference. Assuring consistency in color, aroma and taste is critical to a brand's success. Whether the color ranges from a light yellow Pilsner to a rich dark Porter, it is essential that each brew meets customer expectations.

Analyzing beer color with spectrophotometry is a simple option for assuring your beer's color and quality is consistent from bottle to bottle, batch to batch.

Figure 1: EBC and SRM color chart

Question: How do I analyze beer color using UV-Vis spectrophotometry?

Answer:

Beer color can be measured by a spectrophotometer in multiple ways. A single wavelength measurement of the absorbance at 430 nm on a turbidity-free sample can be used to obtain a color measurement in Standard Reference Method (SRM) units or European Brewing Convention (EBC) units (Figure 1). A measurement at 700 nm is used to confirm the absence of turbidity. There are limitations to this method as certain beer samples having the same color as determined by single-wavelength measurements can differ significantly in appearance.

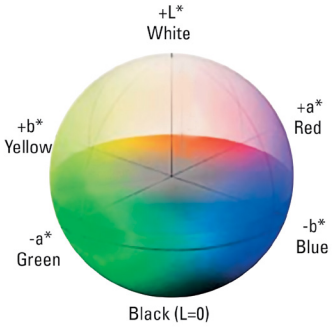

Figure 2: L*a*b* color space

Tristimulus analysis can be used as an alternative to the single wavelength method and provides a better representation of beer color in three-dimensional color space by mathematically transforming %T values measured between 380 nm and 780 nm into the color coordinates L*, a*, and b* (Figure 2).

Materials needed

Thermo Scientific™ GENESYS™ UV-Visible Spectrophotometer with BeerCraft™ Software, beer, reagent water (18Ω resistivity or greater), and 10 mm cuvettes.

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Procedure

Beer color

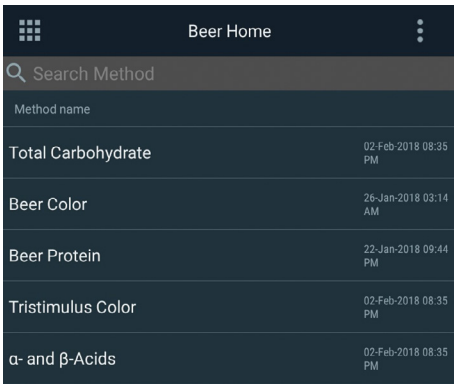
1. Select the Beer Color method on your GENESYS UV-Vis Spectrophotometer. Choose the pathlength conversion factor of 1.27 if using 10 mm cuvettes. Enter the dilution factor of your sample (DF = 1 for undiluted samples, DF =2 for 1:1 dilution, etc.).
2. Fill a cuvette with reagent water and use it to blank the instrument.
3. Fill a cuvette with beer ensuring no air bubbles are present and select Measure to determine the absorbance (A) at 430 and 700 nm.
4. If the beer is not free of turbidity, repeat the measurement after centrifuging or filtering your beer sample.

Tristimulus color

1. Decarbonate beer in an Erlenmeyer flask by shaking until gas no longer escapes.
2. Select the Tristimulus Color method on your GENESYS Spectrophotometer.
3. Fill a cuvette with reagent water and use it to blank the instrument.
4. Fill a cuvette with degassed beer and select Measure to determine L*, a*, b* values.

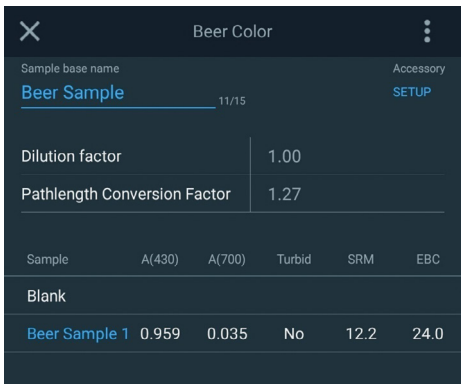
Thermo Scientific™ GENESYS™ 150 UV-Vis Spectrophotometer

Easily measure beer color:



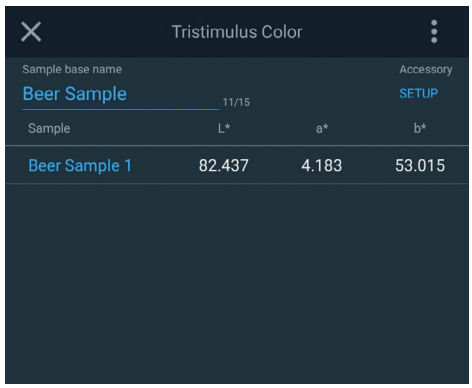
1

In the GENESYS instrument, open the BeerCraft software methods



2

Use the Beer Color method to obtain color measurements in SRM or EBC units



3

Use the Tristimulus Color method to obtain L*, a* and b* color values.

Results

The absorbance of the beer sample at 430 nm and 700 nm measured 0.959 and 0.035 respectively. The sample was turbidity free. The calculations determined that the beer color was 12.2 SRM or 24.0 EBC. The Tristimulus Color method resulted in color coordinates of L*=82.437, a* =4.183, and b*=53.015.

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SmartNotes

As breweries continue to grow, the importance of the quality of their beer becomes increasingly more significant, and basic microbiological testing is no longer enough to guarantee adequate consistency. Larger breweries that already test for beer quality are always looking for ways to simplify their testing efforts by utilizing different technology to limit the time and expense of quality testing. UV-Visible spectrophotometers are a perfect solution to help breweries meet their quality standards by offering quick, simple, and affordable testing as well as the versatility to utilize a large variety of methods compared to more limited instrumentation like a colorimeter.

Diacetyl

Diacetyl (2,3-butanedione) is naturally present in beer as a by-product of the fermentation process. A similar compound 2,3-pentanedione is also produced, and they are collectively known as vicinal diketones (VDKs). Increased 2,3-butanedione content can negatively alter the flavor of beer giving a butter or butterscotch flavor while 2,3-pentanedione tends to impart a honey flavor to the beer. Monitoring the production and consumption of VDKs is important as they are easily detectable in beer with generally reported flavor thresholds of 0.1 mg/L for 2,3-butanedione and 0.9 mg/L for 2,3-pentanedione. As VDKs are a natural part of fermentation, they cannot be completely eliminated from beer, however healthy yeast also serves to reabsorb VDKs over time. The reabsorption of VDKs occurs over multiple days, so the ability to measure the concentration of VDKs in beer is advantageous as it can increase brewing productivity saving time and money. An organoleptic test is often done to detect VDKs consisting of a panel of testers tasting and smelling the sample. While this test is useful to report the presence or absence of VDKs, it is limited by the specific sensory perception of each person and is not sufficient to monitor yeast performance over time to determine if it is finished reducing the VDKs in the beer.

Question: How do I measure beer Vicinal Diketones using UV-Vis spectrophotometry?

Answer:

The concentration of vicinal diketones in beer can be determined by distillation of a beer sample followed by color development of the distillate. The absorbance of the sample is then measured at 530 nm and the result is compared to a previously generated standard curve to obtain the concentration of VDKs.

Materials needed

Thermo Scientific™ GENESYS™ UV-Visible Spectrophotometer with BeerCraft™ Software, beer, α-naphthol, isopropanol, vegetable carbon, creatine, potassium hydroxide, diacetyl, distillation apparatus, volumetric flasks, pipettes, graduated cylinders, 10 mm cuvettes.

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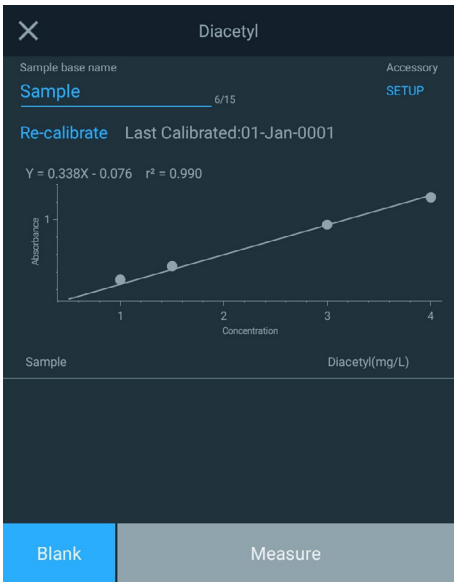
Procedure

1. Prepare a α-naphthol solution by dissolving 4.0 g of α-naphthol in 100 mL isopropanol. Then add 0.5 g of vegetable carbon. The solution is stirred for 0.5 h and filtered.
2. A KOH-creatine solution is made by dissolving 0.3 g creatine in 80 mL of 40% KOH and filtering.
3. The diacetyl standard curve is made by pipetting 0.5, 1.0, 1.5, 3.0, and 4.0 mL of a 20 mg/L diacetyl solution into 10 mL volumetric flasks followed by adding water to bring the volume to 5 mL. Since the upcoming beer distillation step concentrates the VDKs in the sample by 4, the standard solutions are prepared at 4 times the normal concentration to eliminate the need for additional calculations. These 5 mL solutions represent concentrations of 0.5, 1.0, 1.5, 3.0, and 4.0 mg/L.
4. Distill 100 mL of decarbonated beer into a graduated cylinder containing 5 mL water. Collect ~15 mL of distillate and fill to 25 mL with water. Pipette 5 mL of this solution into a 10 mL volumetric flask.
5. Pipet 5 mL of water into a 10 mL volumetric flask to serve as a blank.
6. To each standard, sample, and blank volumetric flask add 1 mL of the α-naphthol solution and 0.5 mL of the KOH-creatine solution and fill with water to the mark. Then shake for 1 min.
7. Select the Diacetyl method on your GENESYS UV-Vis spectrophotometer.
8. Select Re-calibrate and then select Calibrate to enter the concentration values of your prepared standards.
9. Blank the instrument and measure the standard solutions.
10. Return to the method home screen and measure the sample to obtain the concentration of diacetyl in beer in mg/L.

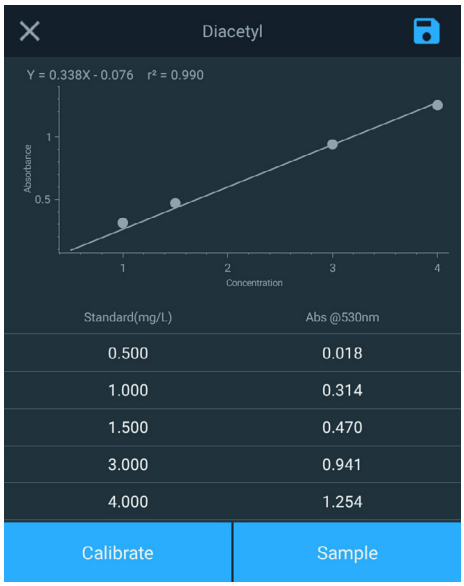
Thermo Scientific™ GENESYS™ 150 UV-Vis Spectrophotometer

Easily measure beer diacetyl:



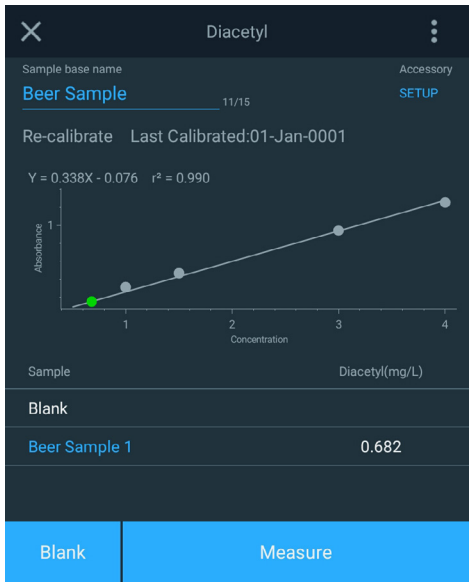
1

Open the GENESYS BeerCraft Software and select Diacetyl. Select Re-calibrate



2

Select Calibrate to enter the concentration of the standards and measure the absorbance



3

Blank the instrument and measure the sample to determine the diacetyl concentration

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Results

The calculations determined that the VDK concentration in this beer sample was 0.682 mg/L. This high VDK concentration could indicate the need for additional time during fermentation for the yeast to properly break down the VDKs. The spectroscopic test for VDKs is advantageous over the organoleptic test in that it can save the brewer days of production with a certainty they can move to the next step by giving quantifiable numbers in a shorter time. At a relatively low cost, the GENESYS UV-Vis Spectrophotometer is a worthwhile investment that will provide significant payback over time. A GC method is often used for measuring VDKs, but this method has a number of limitations compared to the spectroscopic method including a large cost investment on equipment, the need for a skilled operator, various testing and safety factors, the need to purchase gas tanks, and extended run times for each analysis. The spectroscopic method of VDK analysis offers brewers the ability to obtain analytical data on the quality of their beer sample at an affordable price.

References

1. *ASBC Methods of Analysis, online. Beer 25. Broad Spectrum Method for VDK Approved 1964. American Society of Brewing Chemists, St. Paul, MN, U.S.A: 10.1094/ASBCMOA-Beer-25*

Related chemicals ordering information

Description	CAS number
α-naphthol	90-15-3
isopropanol	67-63-0
vegetable carbon black	7440-44-0
creatine	57-00-1
potassium hydroxide	1310-58-3
diacetyl	431-03-8

SmartNotes

QA

As breweries continue to grow, the importance of the quality of their beer becomes increasingly more significant, and basic microbiological testing is no longer enough to guarantee adequate consistency. Larger breweries that already test for beer quality are always looking for ways to simplify their testing efforts by utilizing different technology to limit the time and expense of quality testing. UV-Visible spectrophotometers are a perfect solution to help breweries meet their quality standards by offering quick, simple, and affordable testing as well as the versatility to utilize a large variety of methods compared to more limited instrumentation like a colorimeter.

Beer bitterness units

Bitterness is one of the most fundamental characteristics of beer and a chief component in its flavor quality. The bitterness of beer is imparted from the use of hops primarily through the iso-α-acids extracted out of the hop cones during brewing. The overall bitterness of beer is measured in International Bitterness Units (IBU or BU). The final BU of beer is a result of both the amount and type of hops used during the brewing process as well as when the hops are added. In order to maintain consistency in quality, bitterness needs to be tightly monitored and controlled.

General IBU Guide	
Double or imperial IPA	80-100 IBU
Barleywine	70-100 IBU
India pale ale (IPA)	60-80 IBU
Stout	30-50 IBU
English bitter	30-40 IBU
Porter	20-40 IBU
Scottish ale	10-20 IBU
American light lager	8-12 IBU

Question:
How do I measure beer bitterness using UV-Visible spectrophotometry?

Answer:

The overall bitterness of beer can be determined by extracting an acidified beer solution into isooctane followed by measuring the absorbance at 275 nm. The absorbance is then multiplied by 50 to obtain the bitterness in BU.

Materials needed

Thermo Scientific™ GENESYS™ UV-Visible Spectrophotometer with BeerCraft™ Software, chilled beer, isooctane, hydrochloric acid, octanol, mechanical shaker, centrifuge, centrifuge tubes, pipettes, and 10 mm cuvettes.

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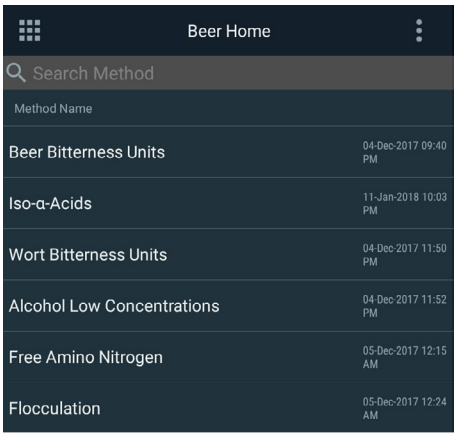
Procedure

1. Add 10 mL of beer to a 50 mL centrifuge tube followed by 1 mL of 3M HCl, 20 mL of isooctane and 50 µL octanol.
2. Cap the centrifuge tube and shake the sample vigorously with a mechanical shaker for 15 minutes to mix the layers.
3. Allow 5–10 minutes for the organic and aqueous layers to separate. Centrifuge the sample if needed to achieve better separation.
4. Select the Beer Bitterness Units method on your GENESYS UV-Vis Spectrophotometer.
5. Prepare a solution of 20 mL isooctane with one drop of octanol and transfer a portion of this mixture into a cuvette to blank the instrument.
6. A portion of the upper organic layer was transferred from the centrifuge tube to a cuvette and measured to determine its bitterness in BU.

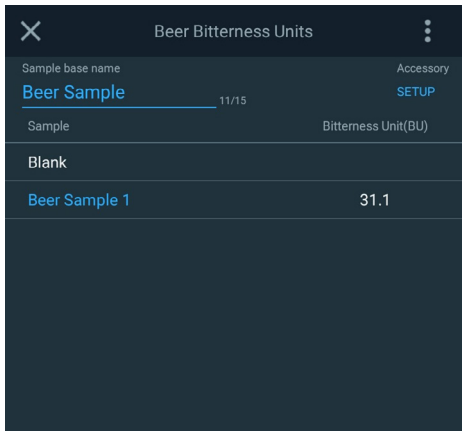
Thermo Scientific™ GENESYS™ 50 UV-Vis Spectrophotometer

Easily measure beer bitterness:



1

Open the GENESYS BeerCraft Software and select Beer Bitterness Units



2

Blank the instrument and measure the beer sample to determine its bitterness in BU

Results

The calculations determined the bitterness of this beer sample was 31.1 BU. This is a typical bitterness measurement for a number of beer styles including pilsners, porters, and stouts.

Related chemicals ordering information

Description	CAS number
Isooctane[1]	540-84-1
Hydrochloric Acid	7647-01-0
Octanol[2]	111-87-5

1. Spectrophotometric grade or equivalent.
2. Reagent grade or redistilled equivalent.

References

1. *ASBC Methods of Analysis, online. Beer 23. Bitterness Units Approved 1968, rev. 2018. American Society of Brewing Chemists, St. Paul, MN, U.S.A: 10.1094/ASBCMOA-Beer-23*

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CASE STUDY CS53348

Craft brewing case study: Omega Yeast

Omega Yeast was founded in 2013 with the goal of providing reliable, made to order yeast and bacterial pitches for professional and home brewers all over the world. They work with breweries large and small to offer advice for strain selection and troubleshooting.

What is your current procedure regarding quality testing of your yeast?

Quality analysis of our cultures comprises a range of testing including microbiological growth/plating, cell counting with hemocytometer, cell mass by percent solids, PCR for potential spoilage organisms, pH and apparent extract. We also measure pH, apparent extract, FAN and BUs in our batches of propagation medium (i.e., wort!).

The pieces of equipment we use most often in the quality lab are our density meter, pH meter, microscope, centrifuge, PCR thermocycler and of course, the GENESYS UV-Vis Spectrophotometer.

Our R&D department utilizes the GENESYS UV-Vis Spectrophotometer for OD600 measurements corresponding to cell number, FAN, BUs, ferulic acid conversion by POF+ yeast. We have designed a few in house assays. For example, measurements of dextrin metabolism with iodine as a starch indicator and measurements of cell growth/survival by the acidification of medium using bromocresol blue pH indicator. We have also used our GENESYS UV-Vis Spectrophotometer for the analysis of water/minerals that would traditionally use a colorimeter.

What is the value of in-house testing versus outside analytical testing lab testing?

With in-house testing we can employ rigorous monitoring on every brew and every culture we produce. With bulk throughput and continuous sampling we can identify outliers and reject anything that is out of our specifications. Third party analysis is often more expensive and results are not relayed fast enough to integrate into our quality program. We do resort to third party analysis for methods that are more specialized and outside of the scope of our quality lab.

In your experience, what are some of the advantages to having a spectrophotometer for analytical testing in the brewing industry?

Easy, cost-effective, fast, versatile! We have really found more uses for the spec than we initially anticipated.

Why use UV-Vis technology vs. other analytical instrumentation techniques? Or do you see other techniques as complementary?

Other techniques are often complementary. The UV-Vis has been versatile and allowed us to get the most bang for our buck from our quality lab to our R&D projects.

ThermoFisher SCIENTIFIC

How did you first hear about the Thermo Scientific™ BeerCraft™ Software on the Thermo Scientific™ GENESYS™ Spectrophotometer?

A couple of us are classically trained yeast biologists and had experience using spectrophotometers in University labs. We knew that we needed a UV-Vis spec to be a workhorse in our lab. The GENESYS Spectrophotometer and the BeerCraft Software are both high end. We were also happy that we wouldn't be limited to kits that tied us to specific reagents or protocols.

What has your experience been with using the BeerCraft Software on the GENESYS 50 onboard software and touch screen?

The software and touch screen are very user friendly. It's no different then using an app on your phone. Exporting the data is very user friendly as well.

You have an active R&D program focused on yeast research. How have the findings from this research been implemented in your business?

A big collaboration between our quality and R&D teams has been in the development of better assays to detect diastatic yeast. We have improved upon some of our traditional screening methods that we use to QC our yeast cultures, and have also developed novel functional screening tools to measure starch degrading properties of suspect contaminants. The GENESYS UV-Vis was a critical tool for the development of these functional assays. Our diastatic yeast study was recently accepted and will be published by the American Society of Brewing Chemists (ASBC).*

How has the GENESYS Spectrophotometer with BeerCraft Software helped with your yeast research? What methods are you utilizing the spectrophotometer for in your work?

There is a lot of screening involved in the development of new yeast strains. The GENESYS has been integral here. Also, we find that we rely heavily on colorimetric assays as affordable screening tools for research. The spec has helped us study topics such as hop creep, diastatic yeast, wild yeast metabolites, yeast contributions to haze, nutritional requirements of yeast.

We have had great success with Thermo Fisher Scientific. There are several people that have helped us along the way and they have always been there for us! We look forward to growing this relationship.

* Improved Functional Assays and Risk Assessment for STA1+ Strains of Saccharomyces cerevisiae.

Omega Yeast Representative

Laura Burns is the Director of Research and Development at Omega Yeast. Laura earned her Ph.D. in Cell and Developmental Biology from Vanderbilt University, where she studied cellular responses to stress in Saccharomyces cerevisiae. With this vast knowledge of yeast under her belt, she decided to apply it to her passion in beer and fermentation. She worked in production brewing for 5 years as Head Brewer and Director of Quality Assurance and gained the experience and knowledge of what it means to be a craft brewer. Now back at the bench at Omega Yeast, Laura has the opportunity to develop new yeast, new tools and better solutions for craft brewers, which keeps her continuously inspired. She is incredibly grateful for the support of her colleagues and the great minds at Omega Yeast.

Find out more at **thermofisher.com/beercraft**

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