Application note | AN1217

UV-Vis spectroscopy

UV-Vis spectroscopy for drinking water quality

Chlorine and iron analysis

Introduction

Ensuring drinking water is free of undesired contaminants is highly important. Not only can these impurities lead to changes in the taste and appearance, but they can also lead to public health issues. As such, governing agencies, like the United States Environmental Protection Agency (EPA), have put forth requirements for safe levels of common contaminants in drinking water sources.[1] These materials can range from inorganic materials, like lead or iron, to organic compounds (e.g., carbon tetrachloride, glyphosphate, etc.), and include a very large number of substances which require identification.

While there are many different materials which need to be monitored, there is also a myriad of analytical techniques that can be used for this analysis, one of which is UV-Visible absorption spectroscopy. This method is based on the ability of UV-Visible light to initiate transitions between electronic ground state and excited state within a compound. As these electronic transitions are specific to the compound analyzed, this method can provide valuable qualitative and quantitative information unique to the measured substance. Because this method is strictly based on light interactions with a sample, it provides a fast and non-destructive method of analysis.

For water samples, a substantial number of procedures involve the use of colorimetric indicators as a detection method for analytes which do not readily absorb in the UV-Visible region. In these methods, the interaction between the analyte of interest and the colorimetric reagent produces a color change in the solution, which can then be observed through UV-Visible absorption spectroscopy. According to Beers law (Eqn. 1),

$$A_\lambda = cl\varepsilon_\lambda \quad (1)$$

where A_λ is the measured absorbance, l is the path length of the cuvette, ε_λ is the extinction coefficient and c is the concentration of the analyte, concentration is linearly proportional to absorbance. Consequently, absorption analyses provide a way for quantification of analyte content in a sample.

Herein, two colorimetric analyses were used to analyze the total chlorine and iron content in tap water samples using the Thermo Scientific™ GENESYS™ 50 UV-Visible Spectrophotometer. These analyses were carried out using pre-built methods included in the GENESYS Water Analysis software. Furthermore, using standard solutions, the pre-built total chlorine and total iron methods were tested to ensure the reported concentrations were accurate.

Experimental

Water analysis kits

For the analysis of total chlorine and total iron (Fe^{2+} and Fe^{3+}) in water samples using the pre-built UV-Visible water analysis software, the Thermo Scientific™ Orion™ AC4P72 Total Chlorine water analysis kit and the Thermo Scientific™ Orion™ AC4P78 Iron (II and III) water analysis kits were obtained and used as received. The total chlorine kit is based on the colorimetric reaction between chlorine and *N,N'*-diethyl-p-phenylenediamine sulfate (DPD) which results in a color change from colorless to pink. The iron kit uses the reaction between 1,10-phenanthroline and Fe^{2+} ions to change the color of the solution from colorless to red/orange. The latter kit also includes a reducing agent to convert residual Fe^{3+} to Fe^{2+}, allowing for a method to determine total iron content.

Sample preparation—iron standards

For the total iron analysis test, standard Fe^{2+} solutions were prepared to confirm the accuracy of the method. Briefly, a stock solution was prepared by dissolving 32.6 mg of ammonium iron(II) sulfate in 47.0 mL of DI water. The solution was then diluted to yield a 10 mg/L Fe^{2+} solution. Fe^{3+} standards were also prepared to confirm the kit can be used to accurately assess the iron content for both oxidation states.

The stock Fe^{3+} standard solution was prepared by dissolving 42.7 mg iron(III) chloride in 10.0 mL DI water. The solution was then diluted to produce a 30 mg/L Fe^{3+} solution.

Standard solutions of known Fe^{2+} and Fe^{3+} concentrations were prepared by diluting the respective stock solutions with DI water. For both iron oxidation states, three standard solutions were generated (0.35 mg/L, 0.75 mg/L and 1.50 mg/L). One packet of iron reagent powder (AC4P78 kit) was added to 10 mL of each standard solution to prepare the sample for UV-Visible analysis. The powder was allowed to dissolve completely prior to analysis. These samples were prepared in triplicate.

For the standard curve analysis, performed without using the pre-built iron analysis method, three separate stock solutions were generated. First, 32.5 mg of 1,10-phenanthroline was dissolved in 10 mL of DI water, resulting in an 18 mM 1,10-phenanthroline stock solution. Additionally, a 1.0 M sodium acetate solution was made by dissolving 820.6 mg sodium acetate in 10 mL DI water. Finally, 205.8 mg of hydroxylamine hydrochloride was dissolved in 10 mL DI water, forming a 0.30 M stock solution. Standards and samples were prepared as described in Table 1.

Standards					
Standard (mg/L Fe^{2+})	Volume of 10 mg/L Fe^{32} (mL)	Volume of 18 mM 1,10-phenanthroline (µL)	Volume of 0.3 M hydroxylamine hydrochloride (µL)	Volume of 1.0 M sodium acetate (µL)	Volume of DI water (mL)
0.00	0.000	900	180	900	7.020
0.02	0.018				7.002
0.05	0.045				6.975
0.10	0.090				6.930
0.25	0.225				6.795
0.50	0.450				6.570
1.00	0.900				6.120
2.00	1.800				5.220
3.00	2.700				4.320

Samples						
Sample	Volume tap water (mL)	Volume 10 mg/L Fe^{2+} (µL)	Volume of 18 mM 1,10-phenanthroline (µL)	Volume of 0.3 M hydroxylamine hydrochloride (µL)	Volume of 1.0 M sodium acetate (µL)	Volume of DI water (mL)
0.35 mg/L Fe^{2+}	0.00	53.0	150	30	150	1.117
0.75 mg/L Fe^{2+}	0.00	113	150	30	150	1.057
1.5 mg/L Fe^{2+}	0.00	225	150	30	150	0.954
Tap water 1	7.02	0.00	900	180	900	0.00
Tap water 2	7.02	0.00	900	180	900	0.00

Table 1: Preparation of Fe^{2+} standards and samples for standard curve analysis.

Sample preparation—chlorine standards

Standard chlorine solutions were generated to confirm the validity of the pre-built method used for determining total chlorine content. According to the kit procedure, DPD is also known to react with $KMnO_4$,[2,3] producing a color change similar to the reaction with chlorine. The procedure for the AC4P72 kit outlines the response observed for a 0.891 mg/L $KMnO_4$ solution is equivalent to the response for a 1.00 mg/L chlorine. Consequently, $KMnO_4$ was used to confirm the accuracy of the test method.

A 1890 mg/L $KMnO_4$ stock solution was prepared by dissolving 18.9 mg $KMnO_4$ in 10 mL of DI water. The solution was further diluted to produce a 10 mg/L $KMnO_4$ solution. Four $KMnO_4$ solutions (0.089 mg/L, 0.22 mg/L, 0.67 mg/L, and 1.34 mg/L) were prepared by diluting the 10 mg/L $KMnO_4$ stock solution with the appropriate amount of DI water. Table 2 outlines what $KMnO_4$ concentration corresponds to a perceived chlorine concentration based on the response in the DPD method. To 10 mL of each $KMnO_4$ sample, one packet of total chlorine reagent powder from the AC4P72 kit was added and allowed to dissolve completely. This was repeated to yield three separate samples which were then analyzed using UV-Visible analysis, discussed later. To determine the LOD and LOQ for the pre-built total chlorine analysis, seven blank samples were prepared by dissolving the kit reagent packet in 10 mL of DI water for each replicate.

$KMnO_4$ Concentration (mg/L)	Accepted total chlorine concentration (mg/L)
0.089	0.10
0.22	0.25
0.67	0.75
1.34	1.50

Table 2: $KMnO_4$ concentrations and correlated chlorine concentrations.

Sample preparation—tap water

To demonstrate the analysis of drinking water, tap water samples from two different sources were collected and used for chlorine and iron analyses using the pre-built water analysis methods. As with the standards described previously, one packet of either chlorine or iron reagent powder was added to 10 mL of sample. Solutions were made and measured in triplicate.

Instrumentation

All samples were measured using a Thermo Scientific GENESYS 50 UV-Visible spectrophotometer. The GENESYS Water Analysis software was used to measure total chlorine and iron content using pre-built standard curve methods designed for the total chlorine kit (AC4P72) and the iron (II and III) kit (AC4P78). For these methods, the instrument was equipped with a test tube holder, and a 24 mm vial was used as per the method protocol.

GENESYS UV-Visible spectrophotometer test-tube holder.

According to the settings outlined for both pre-built methods, the absorbance at 510 nm was monitored and the built-in standard curve equation was used to convert absorbance to analyte concentration. The contents of one reagent packet were dissolved in 10 mL of DI water to form the blank solution, which was used to establish the background for the measurement. All standards and samples were measured in triplicate.

For the iron standard curve method, the Quant application on the GENESYS 50 UV-Visible spectrophotometer local control software was used. The absorbance of the standards outlined in Table 1 were measured at 510 nm to develop the standard curve. Subsequent samples (Table 1) were also measured at 510 nm. Each standard and sample was held in a 10 mm quartz cuvette and measured in triplicate. DI Water was used as a blank.

Scan measurements were collected using the Scan application on the GENESYS 50 UV-Visible spectrophotometer. Absorption spectra were measured between 325 and 1100 nm with a slow scan rate and 1.0 nm step size. Samples were held in a 24 mm diameter vial. Again, DI water was used as a blank.

Results/discussion

Iron analysis

The presence of iron in drinking water can lead to a change in flavor as well as discoloration of water. While the presence of iron does not lead to a significant health concern, it can be helpful to test for both aesthetic purposes (i.e., taste and smell) and to check for corrosion in facility machinery. As such, the US EPA has outlined secondary drinking water standards for materials like iron which do not pose significant health concerns, but which can still be useful to monitor for aesthetic purposes.[4]

There are multiple methods for iron quantification in water, including using the reaction between 1,10-phenanthroline and Fe^{2+} as a colorimetric indicator. In this method, the colorless water sample will turn orange in the presence of Fe^{2+} ions through the formation of ferroin, a coordination complex comprised of three 1,10-phenanthroline molecules and one Fe^{2+} ion (Figure 1a).[5] Often, a reducing agent like hydroxylamine

hydrochloride[6,7] is also used to convert residual Fe^{3+} ions to Fe^{2+}, allowing for the detection of both oxidation states of iron. While this reaction is useful as a visual inspection for iron detection, the resulting solution can be further analyzed through UV-Visible absorption spectroscopy for quantification of iron content.

Figure 1b includes the UV-Visible absorption spectra for the water analysis reagents (AC4P78 kit) with and without ammonium iron(II) sulfate. Without Fe^{2+} ions present, the absorption spectrum only includes an absorption onset at ~400 nm with a tail extending to ~550 nm. However, when Fe^{2+} is present, the resulting ferroin complex produces an absorption feature with maxima at ~480 nm and ~510 nm. For the experiments described herein, the absorbance measured at 510 nm will be used to determine the iron content.

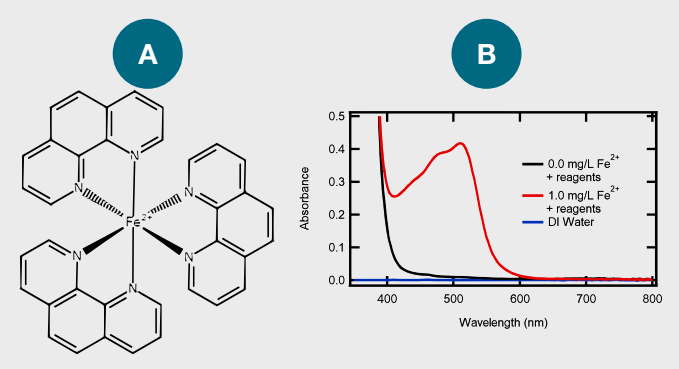

Figure 1: (a) Structure of the complex between Fe^{2+} and 1,10-phenanthroline (b) UV-Visible spectra of the iron reagents dissolved in DI water (black) and a 1.0 mg/L Fe^{2+} aqueous solution (red). Spectra were collected in a 24 mm diameter vial.

Traditionally, samples with varying concentrations of iron would be prepared and mixed with the same amount of the colorimetric reagent to construct a calibration curve (also referred to as a standard curve). The absorbance of each sample would be measured at a specified wavelength, 510 nm for this iron analysis, and plotted as a function of iron concentration. Beer's law (Eqn. 1) demonstrates that the relationship between the measured absorbance and analyte concentration is linear. As such, the standard curve is fit to a linear function to represent this relationship and can then be used to calculate the concentration of the analyte in an "unknown" sample.

However, the GENESYS Water Analysis software already includes the linear functions for methods which require a standard curve be developed. While constructing a standard curve at the beginning of the experiment is best lab practice, the inclusion of these pre-built standard curves in the software lessens the amount of time needed to analyze a sample.

To assess the accuracy of the pre-built iron analysis method, three Fe^{2+} standards of varying concentration (0.35, 0.75 and 1.5 mg/L) were analyzed using the GENESYS Water Analysis software method "AC4P78, Iron, II & III." Table 3 includes the measured absorbance and calculated concentrations based on the pre-built iron analysis method. The percent difference from the anticipated concentration was calculated using Equation 2 to demonstrate how close the values are to the accepted concentration.

$$\% \, Diff = \frac{2|C_t - C_o|}{(C_t + C_o)} \qquad (2)$$

In this equation, C_t is the true analyte concentration and C_o is the observed concentration. These results imply the GENESYS Water Analysis iron method was able to produce an accurate calculated concentration with percent difference from the true concentration below 5%.

As a point of comparison, a standard curve was constructed using a separate set of standards prepared without the kit reagents (see Experimental for details). Samples with the same Fe^{2+} concentrations (0.35, 075 and 1.5 mg/L) were analyzed using this manually constructed standard curve (Figure 2) and the Fe^{2+} was reported (Table 3). As outlined previously, this standard curve does not use the colorimetric reagents from the AC4P78 iron analysis kit, however this curve was constructed using the same colorimetric reaction between Fe^{2+} and 1,10-phenanthroline.

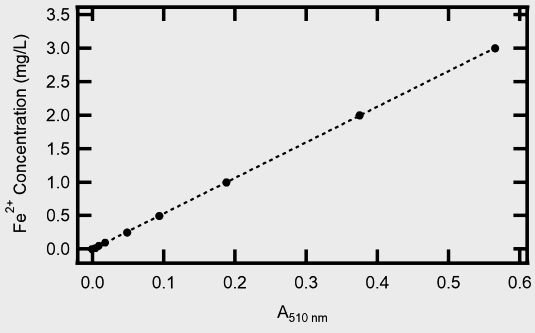

Figure 2: Manually constructed standard curve for the analysis of iron content in the presence of 1,10-phenanthroline.

Accepted Fe^{2+} concentration (mg/L)	Water analysis method			Standard curve method		
	$A_{510\ nm}$	Calculated Fe^{2+} concentration (mg/L)	Percent difference (%)	$A_{510\ nm}$	Calculated Fe^{2+} concentration (mg/L)	Percent difference (%)
0.35	0.158 ± 0.004	0.335 ± 0.009	4.48	0.067 ± 0.005	0.34 ± 0.03	2.02
0.75	0.331 ± 0.001	0.743 ± 0.003	0.94	0.147 ± 0.001	0.750 ± 0.007	0.04
1.50	0.66 ± 0.02	1.52 ± 0.04	1.15	0.286 ± 0.008	1.45 ± 0.04	3.30

Table 3: Absorbance, calculated Fe concentration, and associated percent differences for standard Fe^{2+} solutions using both the GENESYS Water Analysis and standard curve methods for Fe^{2+} standard solutions.

As shown in Table 3, the standard curve method was also able to accurately determine the concentration of Fe^{2+} in each sample with percent difference values also under 5%. Note that the absorbances for each sample concentration do not match between analysis methods. This difference is readily explained by the use of different size sample containers: As the kit requires the use of a 24 mm vial and the standard curve was constructed using a 10 mm quartz cuvette, a lower absorbance is expected for the latter method according to Beer's law (Eqn. 1). The results included in Table 3 indicate that even though it is best practice to manually construct a standard curve, this pre-built iron method is accurate within the concentration range outlined and can be readily used in place of a manually constructed standard curve.

In the 1,10-phenanthroline method, a reductant is often required to convert free Fe^{3+} ions to Fe^{2+} as the former ion is unable to form ferroin, the complex monitored for this colorimetric assay. To ensure the reagents in the AC4P78 kit were able to appropriately reduce Fe^{3+} to Fe^{2+} three samples with known Fe^{3+} concentrations (0.35, 0.75 and 1.5 mg/L) were prepared and analyzed according to the appropriate kit instructions. Absorbance measurements were collected using the "AC4P78, Iron, II & III" method in the GENESYS Water Analysis software. Table 4 includes the absorbance measured and calculated concentration for each Fe^{3+} sample. Much like the Fe^{2+} results, the percent difference is <5%, suggesting the pre-built method and associated kit works well for Fe^{3+} samples within the concentration range specified.

Accepted Fe^{3+} concentration (mg/L)	$A_{510\ nm}$	Calculated Fe^{3+} concentration (mg/L)	Percent difference (%)
0.35	0.164 ± 0.008	0.35 ± 0.02	0.19
0.75	0.337 ± 0.009	0.76 ± 0.02	1.19
1.50	0.661 ± 0.002	1.524 ± 0.006	1.59

Table 4: Absorbance, calculated Fe concentrations and associated percent differences for standard Fe^{3+} solutions analyzed using both the GENESYS Water Analysis software and standard curve methods.

For more realistic samples, two separate tap water specimens were collected and tested using both the pre-built water analysis method and the standard curve methods outlined previously. It is expected these samples will not include a large concentration of iron, though they will reflect a sample matrix containing more than one analyte. The absorbance measured at 510 nm for both samples (Table 5) was well below the noise level for the GENESYS 50 spectrophotometer and therefore below the limit of detection. Therefore, if iron is present in these samples, it is in such low quantities that it cannot be detected via either of these UV-Visible techniques. According to the EPA's guidelines on secondary standards, the acceptable iron content should be < 0.3 mg/L.[4] As expected, the tap water tested herein adhere to this requirement.

Samples	Water analysis method		Standard curve method	
	$A_{510\ nm}$	Calculated Fe concentration (mg/L)	$A_{510\ nm}$	Calculated Fe concentration (mg/L)
Tap water 1	-0.006 ± 0.003	Out of range	0.002 ± 0.001	0.016 ± 0.008
Tap water 2	-0.0044 ± 0.0007	Out of range	0.001 ± 0.003	0.01 ± 0.01

Table 5: Measured absorbance and calculated iron concentration for tap water samples.

Chlorine analysis

While iron content is related more to the appearance and taste of drinking water, there are other contaminants which can pose a risk to public health. For example, the growth of bacteria can be a concern for drinking water. To avoid this, disinfectants are often used to prevent the growth of bacteria or remove the colonies already grown. These disinfectants often include chlorine and/or chloramine.[2] While this removes the risk of bacterial growth, too much of these disinfectants can also be harmful. Consequently, it is important that the amount of chlorine present is known to ensure it does not exceed safe levels.

A commonly used method for determining the chlorine content uses UV-Visible absorption spectroscopy. This method, which the Orion water analysis kit AC4P72 is based on, uses the reaction between *N*, *N*-diethyl-p-phenylenediamine sulfate (DPD) and free chlorine.[2] The resulting product changes the color of the solution from colorless to pink, producing absorption maxima at 475 nm, 511 nm and 553 nm, as shown in Figure 3. For samples which also contain chloramine, potassium iodide is often used to react with chloramine. This in turn produces a compound which can further react with residual DPD.[3,8] By monitoring the absorbance at 510 nm as a function of chlorine concentration a standard curve can be developed, providing a method for quantifying the free chlorine content present. The GENESYS Water Analysis Software includes pre-built methods for the DPD colorimetric analysis of free chlorine, such as the kit used in the experiments described herein (AC4P72).

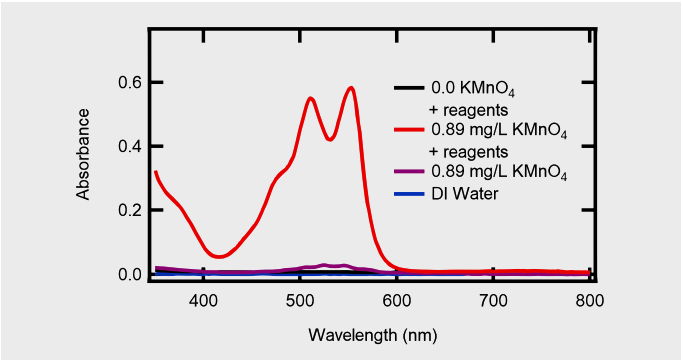

Figure 3: UV-Visible absorption spectra of the chlorine analysis reagents dissolved in water (black), 0.89 mg/L $KMnO_4$ (purple) and 0.89 mg/L $KMnO_4$ in the presence of dissolved chlorine analysis reagents (red). All samples were held in a 24 mm diameter vial.

To assess the accuracy of the pre-built free chlorine method, a set of four standards with varying concentration were prepared and analyzed using the "AC4P72" method. As chlorine solutions are often unstable and can degrade over time, a secondary standard must be used to assess the pre-built method. As described previously, $KMnO_4$ can also react with DPD in a similar fashion as chlorine and therefore is often used as a standard for this procedure. Herein, a set of $KMnO_4$ standards (0.089, 0.22, 0.67 and 1.34 mg/L) were used to check the accuracy of the GENESYS Water Analysis software.

$KMnO_4$ concentration (mg/L)	Accepted "chlorine" concentration (mg/L)	$A_{510\ nm}$	Accepted "chlorine" concentration (mg/L)	Percent difference (%)
0.089	0.10	0.057 ± 0.002	0.082 ± 0.004	20.2
0.22	0.25	0.152 ± 0.007	0.24 ± 0.01	2.6
0.67	0.75	0.419 ± 0.009	0.72 ± 0.02	4.7
1.34	1.50	0.86 ± 0.01	1.53 ± 0.02	2.1

Table 6: Calculated Cl_2 concentration of standard solutions. Percent difference also included.

Table 6 includes the calculated chlorine concentration for each $KMnO_4$ sample measured as well as the expected chlorine concentration. The percent difference for each sample, with the exception of the lowest concentration sample, are below 5%, suggesting these values are accurate. The 20% difference calculated for the 0.089 mg/L $KMnO_4$ sample implies either the sample was not prepared properly, or this concentration may be below the limit of quantification (LOQ) and/or limit of detection (LOD).

The LOD and LOQ are two separate values to assess the reliability of measurements for samples with low analyte concentration. The LOD outlines the lowest measurable quantity that can be distinguished from the blank (i.e., lowest absorbance).[10,11] Here, the blank refers to the sample with no analyte, but which contains the colorimetric reagents. This value can be calculated using the standard deviation of the response (i.e., absorbance) measured for replicate blank solutions as well as the average of the blank measurements. Equation 3 includes the calculation of LOD through this method,

$$LOD = \overline{x_b} + 3.3 * s_b \quad (3)$$

where $\overline{x_b}$ is the average absorbance of the blank samples, s_b is the standard deviation of the blank. Alternatively, if the data is fit to a linear function, then the detection limit can be calculated using the slope of the line and the standard error of the estimate[11] as shown in Equation 4,

$$LOD = \frac{3.3 s_y}{m} \quad (4)$$

where s_y is the standard error of the estimate, and m is the slope of the linear curve. However, as the pre-built methods do not provide this data, Eqn. 3 can be used instead.

For the total chlorine analysis used previously, seven blank solutions were measured using the pre-built total chlorine method. Using the standard deviation and average of the collected absorbance for these blank samples, the LOD was found to be 0.018 A, corresponding to a concentration of 0.014 mg/L. It should be noted that the LOD can be used to define the point at which a method and/or instrument is capable of determining the presence of an analyte, however the LOQ defines the lowest response which can be used for quantification and can be calculated as shown in in Equation 5.[10]

$$LOQ = \overline{x_b} + 10 * s_b \quad (5)$$

For the total chlorine method, the LOQ was found to be 0.041 A, which correlates to a concentration of 0.053 mg/L. For the lowest concentration sample studied here (0.089 mg/L $KMnO_4$, corresponding to a chlorine concentration of 0.1 mg/L) the measured absorbance is above the calculated LOD and LOQ, suggesting this sample may have been improperly prepared.

Sample	$A_{510\ nm}$	Calculated Chlorine concentration (mg/L)
Tap water 1	0.012 ± 0.003	0.005 ± 0.004
Tap water 2	0.025 ± 0.003	0.027 ± 0.005

Table 7: Calculated Cl_2 concentration of tap water samples.

Much like the iron analysis described previously, two tap water samples were tested to determine total chlorine content using the total chlorine method in the GENESYS Water Analysis software (Table 7). Tap water sample 1 resulted in a reported absorbance of 0.012 ± 0.003 A. As described earlier, the LOD for this analysis is 0.018 A, indicating this method was unable to detect chlorine present in the sample. Given this information, it can be concluded that the chlorine content present in this sample is below the acceptable limit of 4.0 mg/L.[9] The second tap water sample had a markedly higher absorbance measured through this method (0.025 ± 0.003 A). As this concentration is above the LOD, it can be determined that chlorine was detected in the sample, however this absorbance is below the LOQ. Consequently, the total chlorine concentration should be reported as <0.053 mg/L. Much like tap water sample 1, tap water sample 2 is also below the acceptable limits for total chlorine content in drinking water,[9] as expected.

Conclusions

While colorimetric assays provide an indirect method for analyzing iron and chlorine content in water samples, the experiments detailed herein outline the accuracy of UV-Visible absorption techniques, even at low analyte concentrations. These results also detail the reliability of the pre-built GENESYS Water Analysis methods for quantitative determination of common substances found in drinking water. Additionally, the analysis of two water samples demonstrated the water was below the EPA's designated levels for chlorine and iron, as expected, further demonstrating the ability to use this fast and non-destructive technique for analysis of drinking water.

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