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Development of a Digital Imaging-Based Colorimetric Analysis Method for the Determination of Phenolphthalein in Drinking Water

İçme Sularında Fenolftalein Tayinine Yönelik Dijital Görüntüleme Temelli Kolorimetrik Analiz Yöntemi Geliştirilmesi

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Abstract

In this study, a digital imaging box-based colorimetric analysis method was developed for the determination of phenolphthalein in drinking water samples. In digital imaging, a smartphone camera was used as a sensor, and an application called "Color Detector" was used as software. Firstly, an optimization study was carried out for the variables that were considered to be effective in the analysis method. For this purpose, the distance of the sample from the phone lens, the location of the sample to be measured, the scale at the measurement location, and pH values were optimized. Then, analysis was carried out with samples with different concentrations between 0.5-5.0 mg/L. Linearity between 1-4 mg/L was observed in the analysis results. As a result of the calculations made to determine the method performance, the limit of detection (LOD) was found to be 0.14 mg/L and the percent relative standard deviation was 0.28% (n = 8). A recovery study was carried out on a drinking water sample to evaluate the performance of the method we developed in the real sample environment. As a result of the analysis, percentage recovery values were found to be in the range of 99%-106%. These values show that our method has high performance in drinking water samples.

Keywords: Phenolphthalein, Colorimetry, Digital imaging, Drinking water.

Öz

Bu çalışmada, fenolftaleinin içme suyu örneklerinde tayinine yönelik dijital görüntüleme kutusu temelli kolorimetrik analiz yöntemi geliştirilmiştir. Dijital görüntülemede algılayıcı olarak akıllı telefon kamerası kullanılmış, yazılım olarak ise "Color Detector" isimli uygulamadan yararlanılmıştır. İlk olarak analiz yöntemi üzerinde etkili olacağı değerlendirilen değişkenlere yönelik optimizasyon çalışması yapılmıştır. Bu amaçla, numunenin telefon lensine uzaklığı, numune üzerinde ölçüm alınacak konum, ölçüm konumundaki ölçek ve pH değerleri optimize edilmiştir. Daha sonra 0.5-5.0 mg/L arasında farklı derişimlere sahip örneklerle analiz yapılmıştır. Analiz sonuçlarında 1-4 mg/L arasında lineerlik gözlenmiştir. Yöntem performansının belirlenmesine yönelik yapılan hesaplamalar sonucu tayin limiti (LOD) 0.14 mg/L mg/L ve yüzde bağıl standart sapma %0,28 (n=8) olarak bulunmuştur. Geliştirdiğimiz yöntemin gerçek numune ortamındaki performansının değerlendirilmesi amacıyla içme suyu örneğinde geri kazanım çalışması gerçekleştirilmiştir. Analizler sonucunda yüzde geri kazanım değerleri %99-%106 aralığında bulunmuştur. Bu değerler yöntemimizin içme suyu örneklerinde yüksek performans gösterdiğini ortaya koymaktadır.

Anahtar Kelimeler: Fenolftalein, Kolorimetri, Dijital görüntüleme, İçme suyu.

1. Introduction

Phenolphthalein was used as a laxative in the twentieth century, but because of research, it was determined that this substance could be carcinogenic when exposed to it for a long time (Dunnick 1996). Inhalation of this substance may cause sneezing and coughing (Saeidnia and Manayi 2014). Additionally, when used in high doses and incorrectly, phenolphthalein can cause electrolyte imbalance, cardiac arrhythmia, loss of consciousness, and even death (Guo 2012, Jalilian et al. 2021). Phenolphthalein is an organic acid that's classified as an odorless and tasteless organic dye. Its chemical structure is shown in Figure 1 (Wilhelm et al. 1992, Adewuyi and Oderinde 2022, Saeidnia and Manayi 2014).

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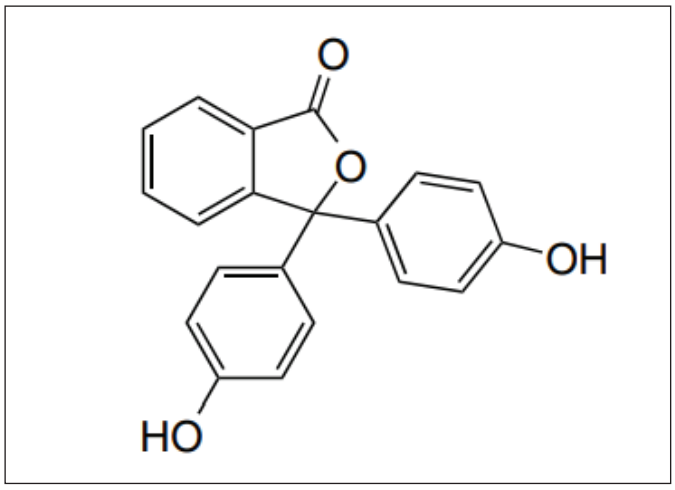

Figure 1. Chemical structure of phenolphthalein (Saeidnia and Manayi 2014).

While phenolphthalein can dissolve in dilute solutions of alkali hydroxides, ether, acetone, pyrene, chloroform, toluene, ethanol, and ether, it is only slightly soluble in water and can be flammable (Saeidnia and Manayi 2014). Phenolphthalein is colorless when in the benzenoid form at pH 8 or lower. However, it takes on a red-pink color in the pH range of 8-10 while in the quinonoid form. At higher pH values, it becomes colorless carbinol (Kunimoto et al. 2001).

Phenolphthalein is among the chemicals classified as toxic to the aquatic ecosystem (Silva et al. 2017). This compound can be released into the environment through various wastewater (Saeidnia and Manayi 2014). The material safety data sheet (MSDS) contains information that phenolphthalein should not be released into the environment. It is also classified as a substance of very high concern (SVHC) due to its carcinogenic, mutagenic, or reproductive toxic properties. It has also been nominated as a substance class requiring attention by the European Chemicals Agency (ECHA) (Silva et al. 2017). Some restrictions have been imposed on the use of phenolphthalein due to its toxic properties and negative effects on health (Li et al. 2022).

The detection and determination of phenolphthalein are important because it has various negative effects on living life. Many methods, including electrochemiluminescence (Guo et al. 2012), HPLC (Kim et al. 2016), LC-MS/MS (Zeng et al. 2016), capillary electrophoresis (Wang et al. 2016), and voltammetry (Geng et al. 2009), have been reported in the literature for the determination of phenolphthalein. In our study, a smartphone-based colorimetric method was used for the determination of phenolphthalein. With developing technology, we can easily access a lot of information in every aspect of our lives. Applications on smartphones are used for different purposes in daily life. These applications have made many contributions to the world of science. The digital image-based colorimetric method (DIC) is an example of a technique developed using these software applications.

This method has gained importance as a feasible method for laboratory studies due to its low cost and versatility (Barreto et al. 2020). In this developed method, color changes in images obtained with various devices are examined and the analyte can be determined using these changes (Jain et al. 2021, Fan et al. 2021, Dos Santos and Pereira-Filho 2013). Devices such as scanners, tablets, computers, smartphones, and webcams are used to acquire images, and the images are processed by software to convert the pixels of the digital image into a suitable analytical signal (Barreto et al. 2020, Resque et al. 2019). The data obtained from digital images are converted to RGB (red-green-blue) and other color scales, and the relationship between the RGB data of the images obtained and the analyte concentration is examined. In the DIC method, there are various mobile applications (Apps) specially developed to convert images into different color scales, so that the samples can be analyzed easily (Jain et al. 2021, Fan et al. 2021, Silva and Rocha 2020).

In recent years, digital imaging method has been used to determine heavy metals (El Kaoutit et al. 2013, Firdaus et al. 2019, Wongthanyakram and Masawat 2019, Kumar et al. 2020, Pessoa et al. 2017, Choodum et al. 2019), determination of peticide-herbicides (Pohanka et al. 2018, Wang et al. 2018, Sicard et al. 2015, Guo et al. 2015), determination of antibiotics (Masawat et al. 2015, Lin et al. 2018), determination of biochemical indicators (Devadhasan et al. 2015, Mahato and Chandra 2019, Ravazzi et al. 2018, Moonrungsee et al. 2018).

In our study, the digital image-based colorimetric method, which is an easy and applicable method for the determination of phenolphthalein with high accuracy and sensitivity, was used. A smartphone was used to obtain the images required to apply the method. A smartphone application called "Color Detector" was used to determine the RGB values of the images. A new box was designed to isolate the light coming from the environment while recording images and to obtain all images homogeneously. By ensuring that the positions of the cuvette and the smartphone where the standards/samples would be placed were fixed, the captured image area and focal distance were kept constant in each experiment. The smartphone was fixed to the lid of the box

and a hole was created in the box to obtain images. The box is made of a material that does not transmit light from the environment, and LED lights are placed inside to provide sufficient illumination. Figure 2 shows the designed digital imaging box.

2. Material and Methods

2.1. Instrumentation

In the sample preparation process, Shimadzu ATX224R analytical balance, Ohaus Starter 3100 pH meter, KUDOS SK 3310 HP ultrasonic water bath, and DLAB MX-S Vortex were used. An LG (Android 9.0) smartphone with a 16-megapixel camera was used for digital imaging in the colorimetric analysis. A smartphone application called "Color Detector" was used to determine RGB values during the analysis. Within the scope of the study, a digital imaging box, originally designed in our laboratory and made of lightproof white wooden material with dimensions of 24 cm x 19 cm x 17 cm (width/length/depth), was used to perform colorimetric analysis.

2.2. Chemicals

Ultrapure water obtained from the Elektro-Mag Laboratory Water Still M4 brand pure water device was used in the sample/standard preparation processes and cleaning of glassware within the scope of our study. Phenolphthalein standards used in the study were prepared by diluting 1000 mg/L standard stock solution obtained from High Purity Standards (USA). Sodium hydrogen phthalate, hydrochloric acid, sodium hydroxide, borax, sodium dihydrogen phosphate, sodium bicarbonate, and all other chemicals used were obtained from Sigma-Aldrich (Germany).

2.3. Procedure

Before starting the analysis, an optimization study was carried out for some variables that were considered to be effective on the analysis results. 1 mg/L phenolphthalein standard was used in optimization studies. Distance, measuring point, measuring radius, and pH optimization were performed. Buffers with the required pH value were prepared using Sodium hydrogen phthalate, hydrochloric acid, sodium hydroxide, borax, sodium dihydrogen phosphate, and sodium bicarbonate. Phenolphthalein standard solution was placed in a quartz cuvette with a volume of 700 µL and measurements were performed. The variables that are optimized are the distance from the smartphone camera lens,

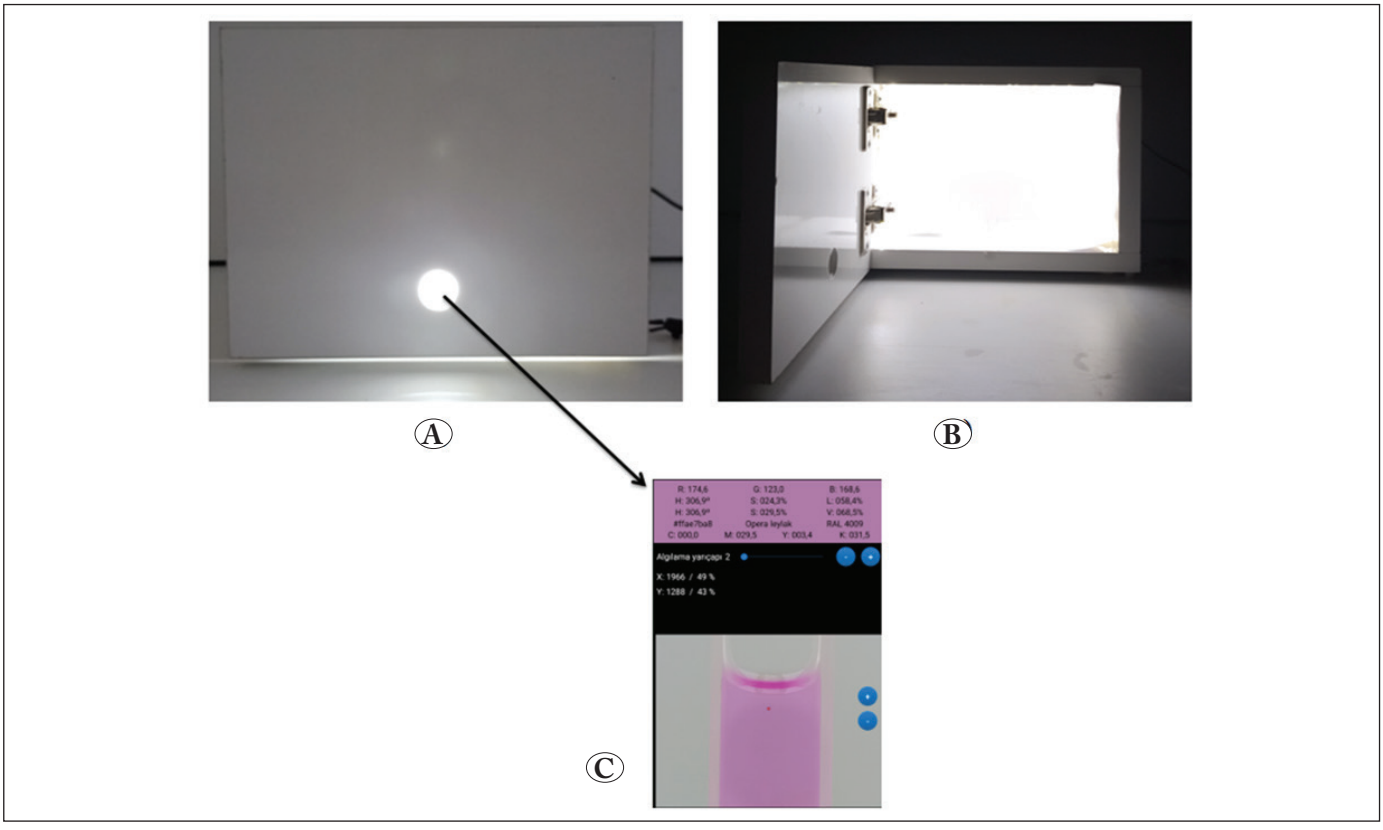

Figure 2. Digital display box **(A)** External view; **(B)** Interior view; **(C)** Sample image.

the location of the point where the measurement is made on the quartz bathtub surface, the radius of the measurement point, and the pH value. After determining the optimum conditions, phenolphthalein samples with concentrations between 0.5-5 mg/L were analyzed. Analysis results were transferred to the calibration chart and LOD, LOQ, linear region, and %RSD values were calculated. After determining the analytical performance of our method, a recovery study was carried out on a drinking water sample.

3. Results and Discussion

The distance of the quartz cuvette from the smartphone camera lens, the location of the measurement point on the quartz cuvette surface, the radius of the measurement point, and the pH value were optimized before starting the analyses.

3.1. Distance Optimization

The distance between the quartz cuvette placed in the digital imaging box and the smartphone camera lens was optimized before starting the analyses. For this purpose, analyses were performed for samples placed at a distance of 5, 8, and 10 cm. The distance values were determined based on the focusing feature of the smartphone's camera. Samples containing 1 mg/L phenolphthalein were used for the optimization study. RGB (red, green, blue) values obtained as a result of digital imaging-based colorimetric analysis were examined. Red intensity, which has the highest numerical value among the RGB values, was used as the measurement result in the rest of the study. When the red color intensity values of analyses at different distances were examined, it was seen that the highest value was obtained at a distance of 5 cm. In this context, it was decided to apply the distance of 5 cm in the continuation of the study. The measurement results obtained as a result of analyses are presented in Figure 3, and the graphical representation of the measurement results is presented in Figure 4.

3.2. Measuring Point Optimization

Before starting our digital imaging-based colorimetric analysis studies, an optimization study was carried out to determine the point at which the measurement would be taken on the quartz cuvette surface where the sample to be analyzed was placed. For this purpose, separate measurements were taken from the upper middle part, middle part, and lower middle part of the quartz bathtub. The sample containing 1 mg/L phenolphthalein was used during optimization. When the red color intensity of analyses taken from different points was examined, it was seen that the highest value was obtained in the middle part. In the continuation of the study, it was decided to use the middle part of the quartz bathtub as the measurement point. The measurement results obtained as a result of analyses are presented in Figure 5, and the graphical representation of the measurement results is presented in Figure 6.

3.3. Measuring Radius Optimization

The radius value of the measured point on the sample can be adjusted in the smartphone application used in digital

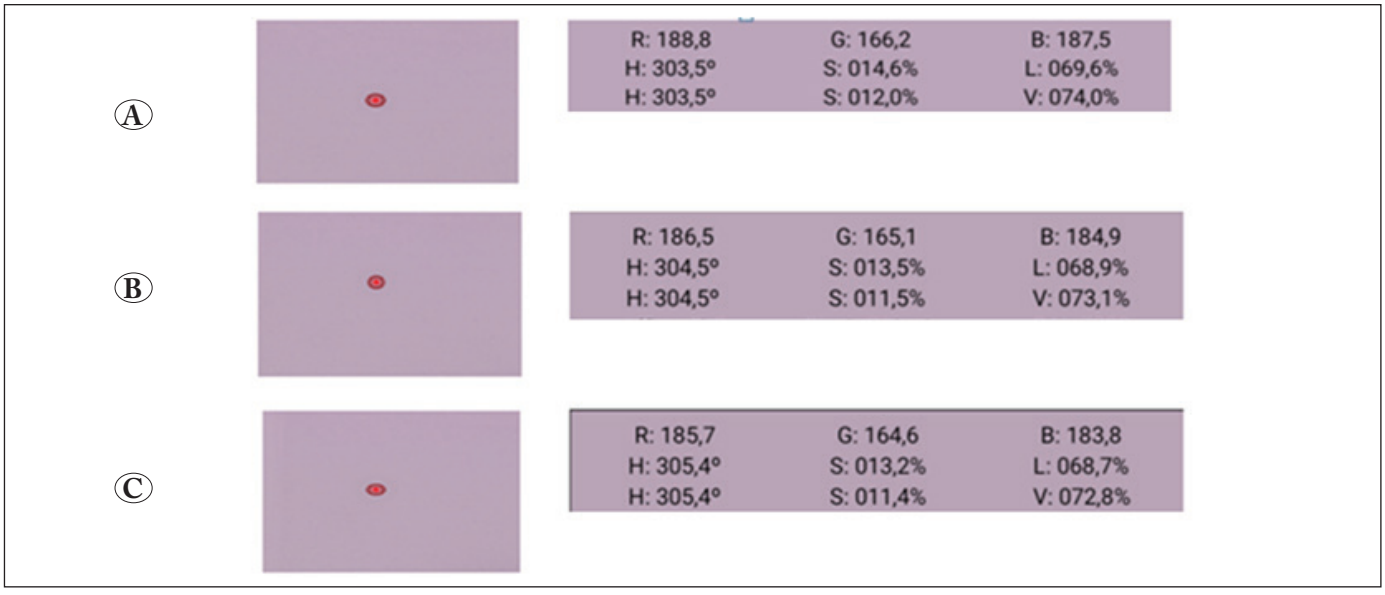

Figure 3. Distance optimization measurement results **(A)** 5 cm; **(B)** 8 cm; **(C)** 10 cm.

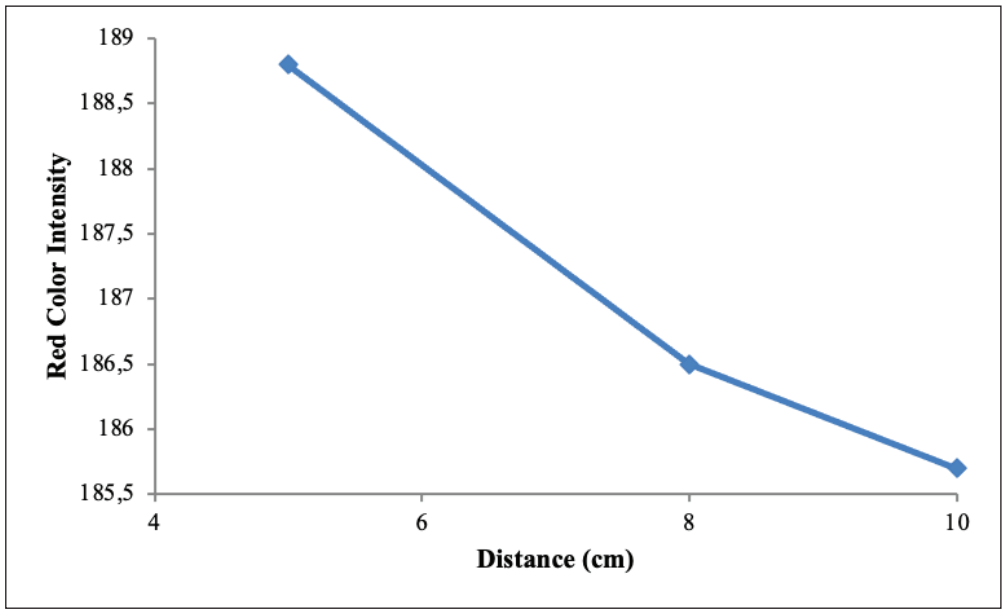

Figure 4. Distance optimization measurement results comparison graph.

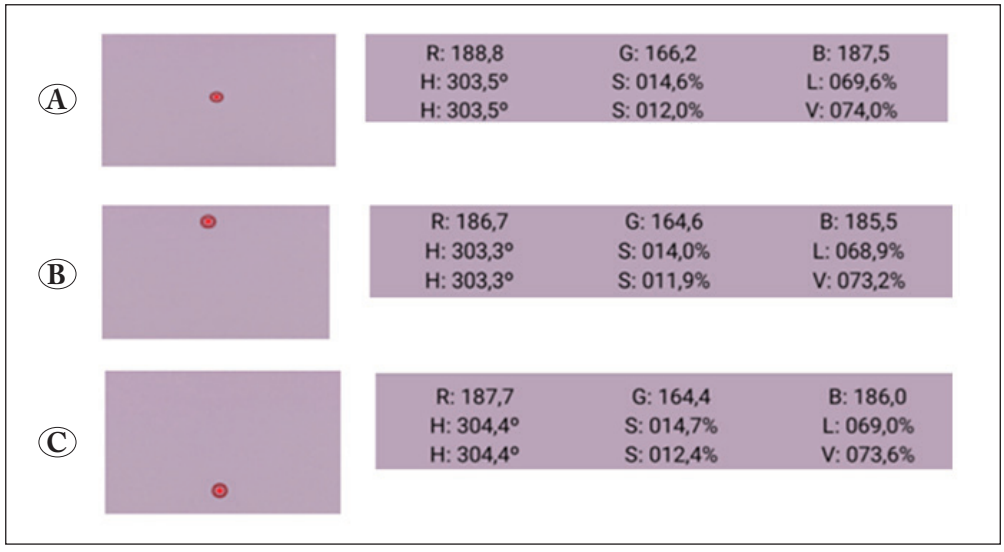

Figure 5: Measurement point optimization measurement results **(A)** Middle part; **(B)** Upper middle part; **(C)** Lower middle part.

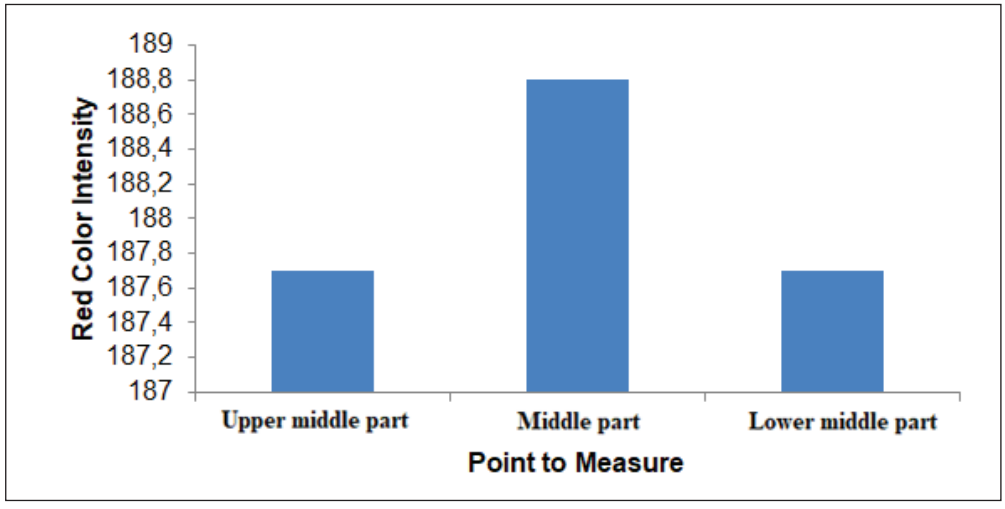

Figure 6: Measuring point optimization results comparison chart.

imaging-based colorimetric analysis. In the analysis to be carried out, measurement radius optimization was made to determine the radius that would yield the highest measurement result. For this purpose, a separate analysis was carried out on the radius scale at 2, 5, and 10 radius values. When the red color intensity of analyses performed at different radius values was examined, it was seen that the highest value was obtained when the radius was 2. In the rest of the study, the radius value was applied as 2. The measurement results obtained as a result of the analysis are presented in Figure 7, and the graphical representation of the measurement results is presented in Figure 8.

3.4. pH Optimization

Within the scope of our work to develop a digital imaging-based colorimetric analysis method in water samples, pH optimization was carried out to determine the most suitable environmental pH. Potassium hydrogen phthalate was used in the preparation of buffers between pH 4-6, tris was used for pH 7, and borax was used for pH 8-10. Figure 9 shows the measurement results at pH values ranging from 4.0 to 10.0. When the measurement results were examined, it was seen that the highest value was obtained at pH 9.0. The color transformation of phenolphthalein occurs between pH 8-10. The highest red color value was obtained at pH 9. At pH 9, the red color intensity in the sample medium was higher than at pH 10. At pH 10, the red-green-blue color intensity of the sample medium increased less in favor of red. In the continuation of the study, the pH of the environment was determined to be 9.0.

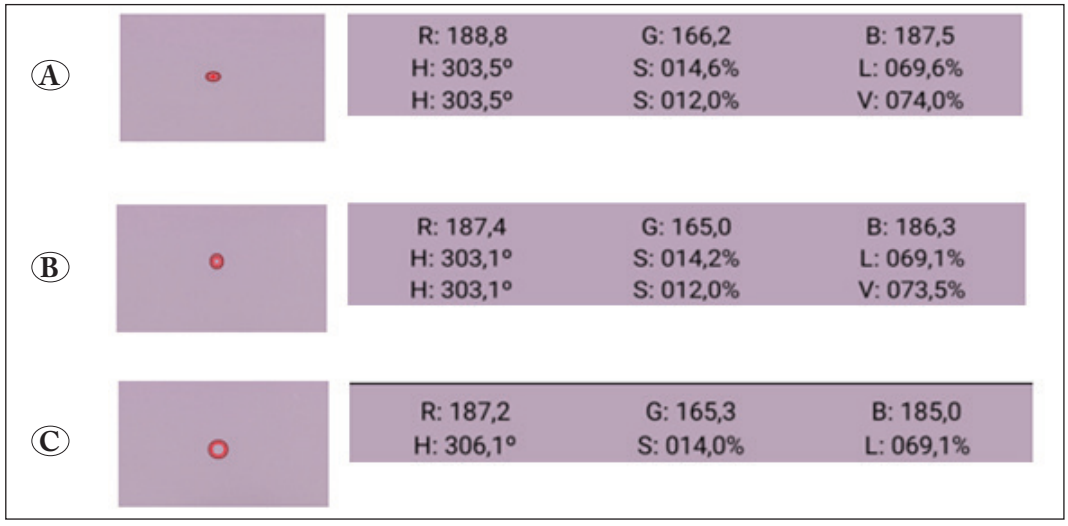

Figure 7: Radius optimization measurement results **(A)** 2; **(B)** 5; **(C)** 10.

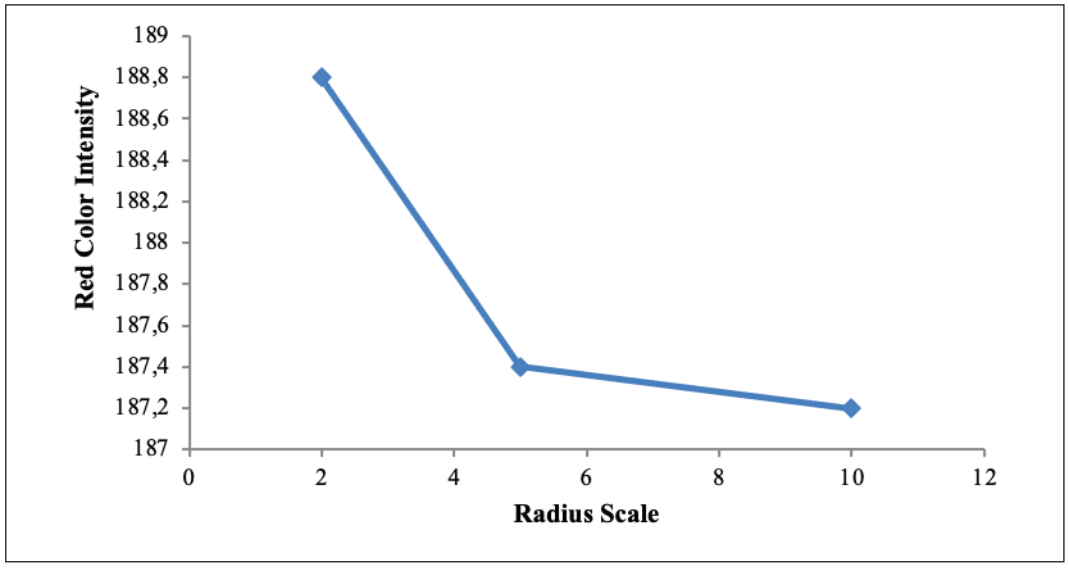

Figure 8: Radius optimization measurement results comparison graph.

3.5. Analytical Performance of the Method

Analyzes were carried out to determine the analytical performance of the digital imaging-based colorimetric analysis method we developed for the determination of phenolphthalein. For this purpose, colorimetric analysis of phenolphthalein solutions with different concentrations was carried out under the determined optimum conditions to obtain a calibration chart. The calibration graph obtained by plotting the measurement results obtained as a result of the analysis is shown in Figure 10.

When Figure 10 is examined; The signal change was almost linear against the concentration varying between 1-4 mg/L. Deviation from linearity occurs at concentrations below 1 mg/L and above 4 mg/L. Deviation occurs when the concentration is below 1 ppm and when it is above 4 ppm. The method we developed in these regions does not provide linear results. In the calibration graph drawn for our linear region of 1-4 mg/L, the correlation coefficient (R^2) was calculated as 0.9955. The closeness of the correlation coefficient to 1 reveals the linearity of our measurement results. LOD (Limit of detection) and LOQ (Limit of quantification)

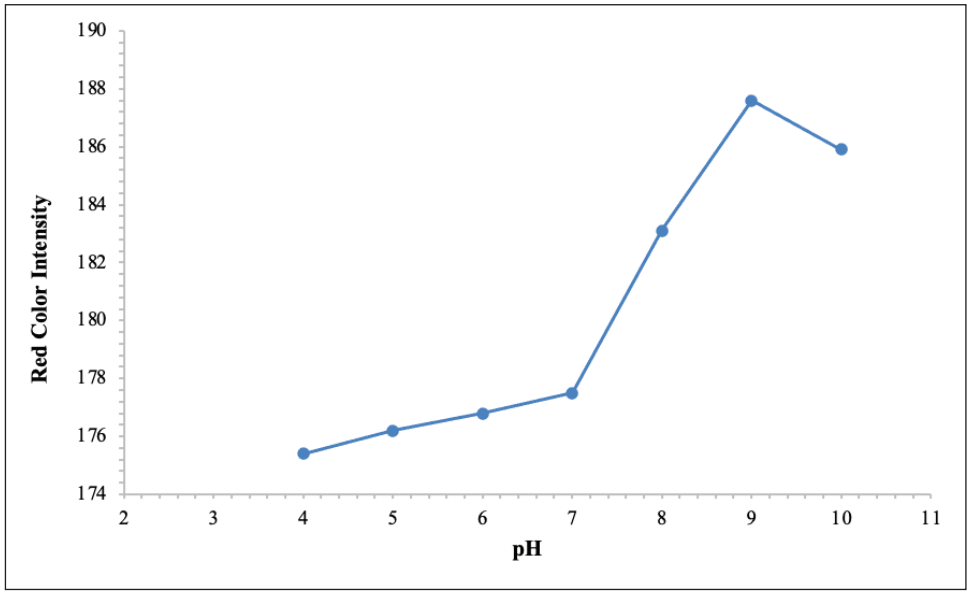

Figure 9. pH optimization measurement results.

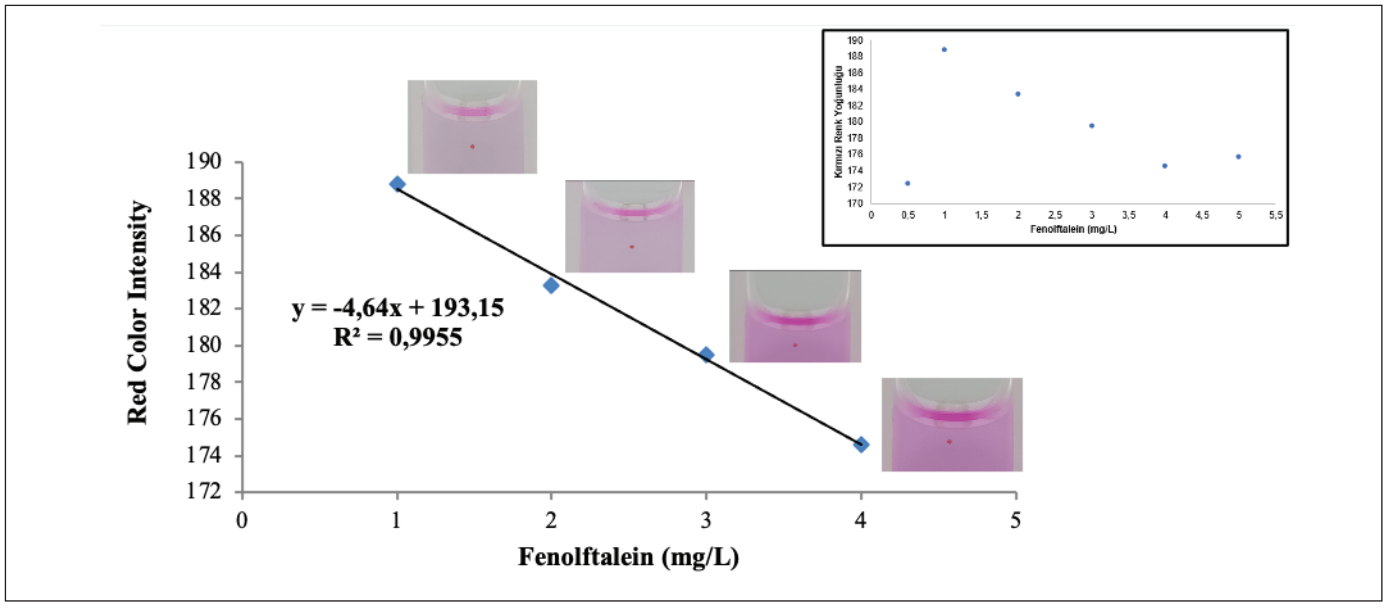

Figure 10. Calibration graph.

Table 1. Comparison of the detection limits achieved using different methods for the determination of phenolphthalein.

Analytical method	LOD (mg/L)	Reference
Digital Imaging-Based Colorimetry	0.14	Our study
Capillary electrophores	0.18	(Wang et al. 2016)
Thin layer chromatography	10	(Fang et al. 2016)
Liquid Chromatography	0.47	(Nasr et al. 2017)
High-performance liquid chromatography	0.5	(Wilhelm et al. 1992)

values were calculated to determine the accuracy and sensitivity of the analysis method we developed. Eight repeated measurements were taken for the lowest concentration in the calibration chart we drew to calculate LOD and LOQ values. The standard deviation (SD) value was calculated using our measurement results obtained as a result of the analysis. In our calculations, the formula 3SS/m (m=slope of the calibration graph) was used for the LOD value and the formula 10SD/m was used for the LOQ value. As a result of the aforementioned procedures, the LOD value was found to be 0.14 mg/L and the LOQ value was 0.48 mg/L. The percentage relative standard deviation value (%RSD) of the developed analytical method was calculated as 0.28%. This value shows that the method has high reproducibility. A comparison of detection limits obtained using different methods for the determination of phenolphthalein is given in Table 1. When Table 1 is examined, it can be seen that the LOD value of the method we developed is much lower than other methods.

3.6. Recovery Study on Drinking Water Sample

To demonstrate the performance of our method in a real sample environment, a recovery study was conducted in drinking water. Within the scope of recovery experiments, analyses were made by adding 1, 2, and 3 mg/L phenolphthalein standards to the drinking water sample, respectively. As a result of the analysis, percentage recovery values were found to be in the range of 99%-106%. These values show that our method has high performance in drinking water samples.

4. Conclusion and Suggestions

Our study aimed to develop a digital imaging-based analysis method for the determination of phenolphthalein in drinking water samples. First of all, an imaging box was designed to perform digital imaging. In the design of this box, care was taken to prevent light from outside and to use reflective material inside. An application called Color Detector, which can be downloaded to smartphones free of charge was used in the analysis processes. Before starting the analyses, variables that had an impact on the measurements were optimized. For this purpose, distance, radius, measurement point, and pH optimization were carried out. After the optimum values were determined, the analysis of phenolphthalein samples with increasing concentrations was started. As a result of the analysis of samples with concentrations varying between 0.5 mg/L and 5 mg/L, a linear region between 1 and 4 mg/L was detected. As a result of the calculations, the LOD value was found to be 0.14 mg/L and the LOQ value was 0.48 mg/L. The percentage relative standard deviation value (%RSD) of the developed analytical method was calculated as 0.28%. This value shows that the method has high reproducibility. A recovery study was carried out on a drinking water sample to evaluate the performance of the method we developed in the real sample environment. As a result of the analysis, percentage recovery values were found to be in the range of 99%-106%. These values show that our method has high performance in drinking water samples. When the studies in the literature developed using different analysis methods for the determination of phenolphthalein are examined, it is seen that the analytical performance of our method is quite satisfactory.

5. Acknowledgment

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Author contribution

Author Yağmuroğlu and Author Yücel: Planned and designed the study, gathered and analyzed data about the study, and wrote the article by analyzing the study.

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