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Research Article

# Facile, sensitive and reagent-saving smartphone-based digital image colorimetric assay of captopril tablets enabled by long-pathlength RGB acquisition

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## Abstract

A new assay of captopril (CTP) tablets was developed based on digital image colorimetry using Ellman's reagent. For the first time, a facile technique of increasing the analytical path was applied in this work to enhance the sensitivity. For this purpose, the reaction solutions were photographed using a smartphone while they were contained in two 1-cm pathlength cuvettes which were placed side by side. The Red-Green-Blue (RGB) in term of [B/(R+G+B)] was used to plot a standard curve. Compared to using a single cuvette, double cuvettes resulted in more precise analytical signals and better linearity ($r^2$ of 0.9992). Additionally, CTP could be analyzed at low concentrations (2.5–25 μM) with LOD of 0.70 μM and LOQ of 2.13 μM, thus lowering the reagent consumption. The assay was proven to be valid, and it was greener, faster, and more affordable than the pharmacopeial chromatographic method, thereby suitable for pharmaceutical quality control.

## Keywords

smartphone, digital image colorimetry, long pathlength, captopril

## Introduction

Captopril (CTP), chemically known as 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline, is an inhibitor of the angiotensin-converting enzyme. It is used as an antihypertensive drug and for the management of congestive heart failure. In the United States Pharmacopeia and British Pharmacopoeia, the content of CTP in tablets is assayed by high performance liquid chromatography (HPLC) (USP43-NF38 2020; British PC 2022). Additionally, a literature survey revealed that several methods have been reported for quantitative determination of CTP in pharmaceutical formulations. These procedures include HPLC (Mansour and Danielson 2012; Logoyda et al. 2021; Pebriana et al. 2021), capillary electrophoresis(Hillaert and Van den Bossche 1999), colorimetry (Askal 1991; Sastry et al. 1991; El-Ashry and Ibrahim 1992; Jovanović et al. 1995), flow injection methods (Guerrero et al. 1991), and circular dichroism spectroscopy(Rahman and Khan 2019). Some of these procedures are somewhat complicated for

routine analysis, time-consuming, use unsafe chemicals for derivatization, or employ expensive or sophisticated instruments. Therefore, the development of an analytical method which overcomes these drawbacks allows pharmaceutical quality control in a more practical way.

In the digital era, smartphones have become a part of everyday life. In addition to communication, smartphones have provided a wide range of applications that can make life easier, including in chemistry. In chemical analysis, smartphone-based digital image colorimetry (SDIC) is a technique that uses a smartphone camera instead of a spectrophotometer to capture an image of a colored analyte. After delineating the colors into red-green-blue (RGB) channels, the relationship of RGB pixels and the analyte concentration is established and then used for determining the concentration of the unknown, sometimes with the aid of the mobile device's app. Because of the advantages in terms of affordability, portability, ease of use, and rapidity, SDIC is used for determining analytes in various fields, as supported by many review articles (Ong and Poljak 2020; Chen et al. 2021; Xing et al. 2022). In addition, SDIC assays can be applied for analytes in different forms e.g., homogeneous solutions (Phadungcharoen et al. 2019; Khongwichit et al. 2023), layers of immiscible liquids (Phadungcharoen et al. 2020; Caleb et al. 2023; Thanayutsiri et al. 2023), liquid droplets (Lima et al. 2017; Jain et al. 2021) and samples on solid substrates such as paper-based devices (Celikbas et al. 2020; Zhang et al. 2022). For solution samples, color measurement is usually performed while they are contained in cuvettes (Abughrin et al. 2022; Firdaus et al. 2022; Mermer et al. 2022; Minh-Huy et al. 2023) or on a microplate (Wang et al. 2020; Jing et al. 2023; Song et al. 2023). However, due to the commonly used size or dimension of these containers, the liquid pathlength, that is, the distance that the light travels through a sample, is often limited to 1 cm or less.

In UV-vis spectrophotometry, the signal measurement is governed by the Beer-Lambert Law, where the absorbance is directly proportional to the analyte concentration, the compound's specific molar absorptivity and the light's pathlength. Therefore, the absorbance measurement in a long pathlength helps in more accurate signal acquisition, especially for a dilute analyte (Melchert and Rocha 2010). In practice, however, such a technique may not be applicable to a typical UV-visible spectrometer because it is equipped with a cuvette holder designed for commonly used 1 cm cuvettes. On the other hand, the limitation of this instrument will not pose a problem for SDIC because increasing the analytical signal, i.e., color intensity of photographed solutions, may be achieved effortlessly by capturing the image of samples contained in a pair of cuvettes which are placed next to each other to double the pathlength. Since the literature review revealed no reports of SDIC assays using double cuvettes for imaging and color measurement, this study, for the first time, aimed to demonstrate its feasibility and advantages over the use of a single cuvette. For this purpure, the pathlength enhancement technique was applied to develop a new SDIC assay of captopril (CTP) tablets based on Ellman's reaction. It was demonstrated that the image capture and color measurement using double cuvettes improved several analytical performances of the assay. In addition, enhancing the sensitivity allowed the analysis of CTP at even a low concentration, thus reducing the consumption of the colorimetric reagent, promoting green and cost-saving features.

# Experimental

## Chemicals and instrumentation

CTP (purity ≥ 99.5%), CTP disulfide (purity ≥ 99.5%), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent) (purity ≥ 98.5%), were purchased from Sigma (MO, USA). CTP tablets (25 mg per tablet) were obtained from a drugstore in Thailand. Other ingredients in the tablets included microcrystalline cellulose, corn starch, anhydrous lactose, colloidal silicon dioxide and talc. An iPhone 11 Pro and white LED lamps were used as the camera and a light source, respectively. UV-vis spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Germany) was used for the optimization study of the colorimetric reaction and the comparison of the assay results with the proposed method. The Agilent 1220 Infinity LC System (Agilent Technologies, Germany) was used to perform the standard assay of CTP tablets in comparison with the proposed SDIC method.

## Preparation of the CTP solutions and the DTNB solution

Standard solutions of CTP were prepared in the range of 2.5–25 μM using 3 mM (1 g/L) edetate disodium solution as a diluent. Sample solution was prepared by dissolving the equivalent to 25 mg of CTP from a portion of powdered tablets in 20 mL of 3 mM edetate disodium solution in a 25 mL volumetric flask and sonicating for 15 min. The solution was then made up to volume with 3 mM edetate disodium solution and filtered through a 0.45 μm membrane filter. The resulting solution was further diluted with 3 mM edetate disodium solution to obtain the solution with the CTP concentration of about 20 μM. DTNB solution was prepared by dissolving appropriate amounts of the chemicals in 0.1 M sodium phosphate buffer, pH 8.0.

## Procedure for the proposed SDIC method

The colorimetric reaction was conducted by adding 350 μL of 50 μM DTNB solution to 350 μL of the standard solution (0, 2.5, 5, 10, 15, 20, 25 μM CTP) or sample solution (about 20 μM CTP) in 1.5 mL microcentrifuge tubes. The mixture was vortexed for 10 s and incubated at room temperature for 10 min.

Subsequently, the intensities of the yellow color of the solutions were measured as RGB values using transmission

mode, i.e., samples were place between a light source and camera. For this purpose, two 350-µL aliquots of the solution in each tube were pipetted and transferred into two polystyrene cuvettes with a 1 cm pathlength. To double the pathlength, both cuvettes were placed side by side in front of the light source, consisting of a light-emitting diode (LED) array illuminating white light and a white acrylic sheet acting as a light-diffusing background (Fig. 1a, b). Under an ambient lighting condition, a set of cuvettes containing the reaction solutions of standards and samples aligned in two rows were photographed in the same shot using an iPhone 11 Pro camera set on the autofocus mode with a 12 MP picture quality, f/1.8 aperture, and flash off. The distance between the smartphone camera and the cuvettes was 25 cm.

From the digital image, the RGB values of the yellow color of the solutions were measured using a free mobile application, namely the RGB Color Detector, downloaded from the App Store (iOS) or Play Store (Android) (Fig. 1c). A standard curve between the concentration of CTP and the analytical signal, i.e., $[B/(R+G+B)]_{blank} - [B/(R+G+B)]$ was plotted. The concentrations of analytes were calculated using a linear regression equation.

## Method validation

To assess the analytical performance, the method was validated according to the <1225> Validation of Compendial Procedures, described in the United States Pharmacopeia (USP) 43. The linearity was studied by constructing a standard curve of CTP over the concentration range of 2.5 to 25 µM and determining a linear regression equation and a coefficient of determination ($r^2$). From the equation, the limit of detection (LOD) and limit of quantitation (LOQ), defined as 3.3 and 10 times of standard deviation of the Y-intercept divided by the slope, respectively were calculated. The accuracy was evaluated by spiking known amounts of standard CTP at three concentration levels into the tablet placebo (n = 3 for each level). The spiked drugs were then assayed, and the results were reported as the percentage of recovery. To determine the intra-day and inter-day precision, the assays of commercial tablets were performed within a day and on three consecutive days (n = 6), respectively. The results were reported as % relative standard deviation (% RSD) of the % labeled amount. The specificity was confirmed by the assay of the known amount of CTP in the presence of excipients used in tablet formulation and an impurity, namely, CTP disulfide.

## High performance liquid chromatographic (HPLC) analysis

The USP assay of CTP tablets based on HPLC (USP43-NF38 2020) was used to compare the analysis results with those obtained from the SDIC method. Briefly, the standard solution and sample solution were prepared at 1 mg/mL (4.6 mM) of CTP. The analysis was performed on a reversed-phase column (Cosmosil 5C18-MS 4.6 mm ID × 25 cm; 5 µm, Nacalai Tesque, Inc., Nakagyo-ku, Kyoto, Japan), and an isocratic elution using a mobile phase composed of methanol, water, and phosphoric acid (45:55:0.05

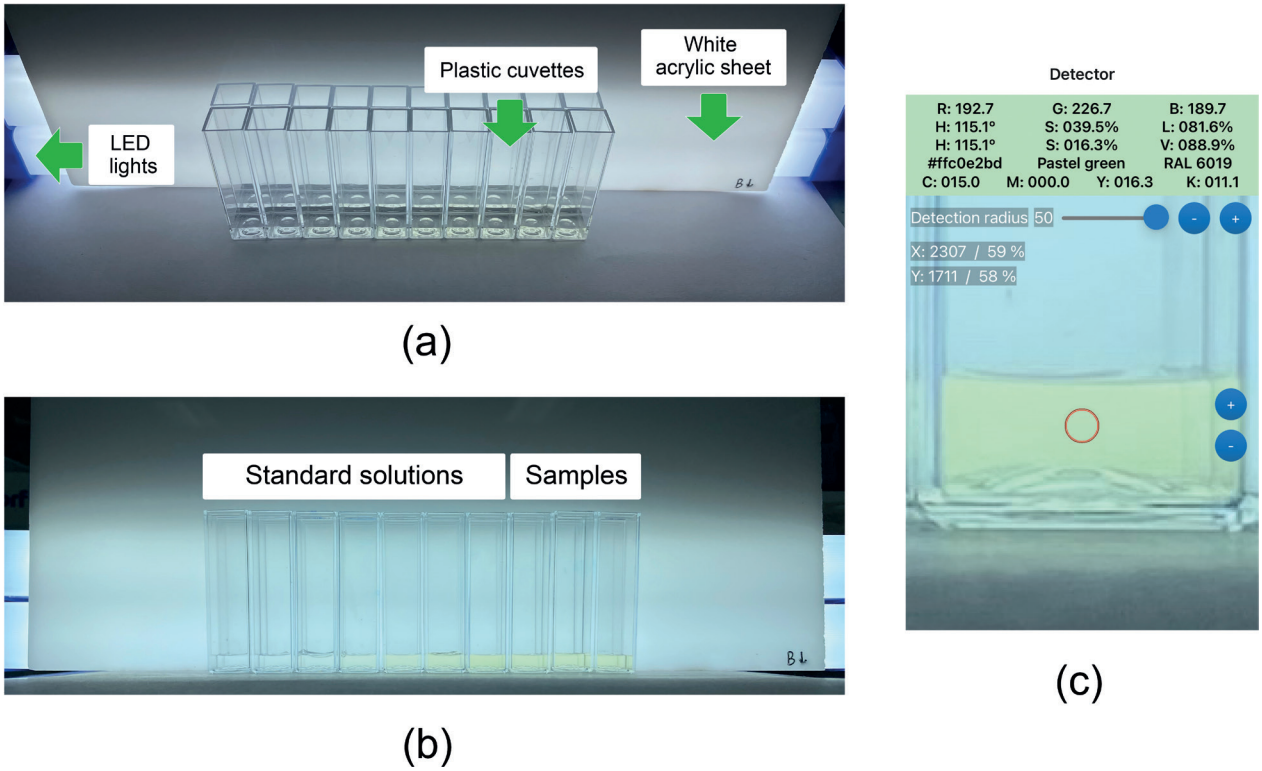


**Figure 1.** Alignment of cuvettes for photography from top view (**a**) and front view (**b**), and measurement of RGB colors on the digital image using the RGB Color Detector application (**c**).

by volume) at a flow rate of 1.0 mL/min. The UV detector was set at 220 nm. The percentage of the labeled amount of CTP in the portion of tablet was calculated using the peak areas of the sample solution and the standard solution. The chromatograms are shown in Fig. 1 of the Supplementary Data.

# Results and discussion

## Method development

### *Optimization of DTNB concentration and reaction time*

In the assay, the CTP containing a thiol group reacted with DTNB, to form a yellow-colored product, namely, 2-nitro-5-thiobenzoic acid ($TNB^-$) (Fig. 2). To achieve the reaction condition in which the intense yellow color was rapidly produced, the optimal concentration of DTNB and the reaction time were investigated. At pH 8, which is used in a standard protocol for Ellman's reaction, the optimal condition was investigated by fixing the concentration of CTP at 25 μM (i.e., the maximum drug concentration of the standard curve), and varying the concentration of the DTNB reagent (30, 37.5, 50 μM). For each reaction, the absorbance values at 412 nm were monitored over a period of 20 min. As shown in Fig. 3, the reaction in which 50 μM of DTNB solution was used, produced the highest absorbance when compared to the others. In addition, at this DTNB concentration, the yellow-colored product formed the fastest, giving the maximum absorbance in 10 min. Therefore, 50 μM of the DTNB with a 10-min reaction time was used in the assay procedure.

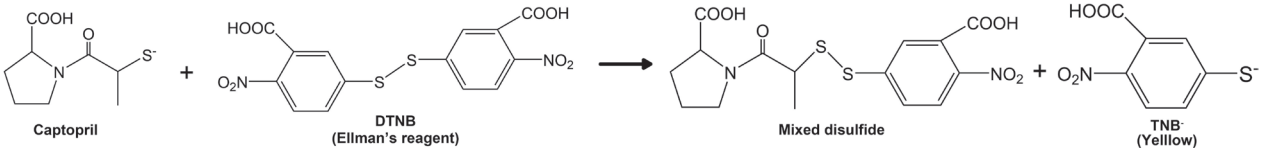


**Figure 2.** Reaction of CTP with DTNB.

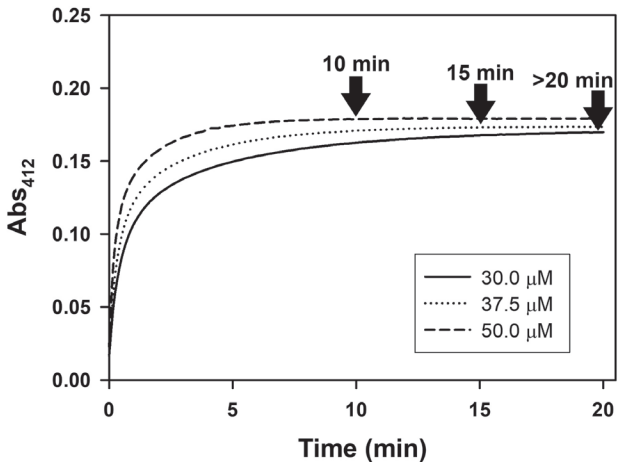


**Figure 3.** Effect of the DTNB concentration on the reaction time course.

### *Selection of effective analytical signal for the SDIC*

After reacting with the DTNB under the optimal condition, a series of the CTP standard solutions (0–25 μM) were transferred into 1 cm pathlength cuvettes aligned in a single row. The cuvettes were photographed, and the RGB intensities of the yellow solutions were read out. As shown in Table 1, B channel ($B_0 - B$, where $B_0$ was of a blank) gave a linear regression equation with a higher slope as well as a better linear relationship between the analytical signal (Y) and the drug concentration (X) ($r^2$ of 0.9313) than did R and G. With the additional transformation of B into B/(R+G+B), the linearity was further improved as indicted by a higher $r^2$ of 0.9881. For the following experiments, $[B/(R+G+B)]_0 - [B/(R+G+B)]$ was thus used as the effective analytical signal for the assay.

**Table 1.** Regression equations and $r^2$ values determined using different Y functions.

| Y | Regression equation | $r^2$ |
|---|---|---|
| $R - R_0$ | y = 0.5499x + 2.2689 | 0.9263 |
| $G - G_0$ | y = 0.2915x + 3.5850 | 0.7684 |
| $B_0 - B$ | y = 0.8232x – 4.1061 | 0.9313 |
| $\log (B_0/B)$ | y = 0.0017x – 0.0081 | 0.9319 |
| $[B/(R+G+B)]_0$- $[B/(R+G+B)]$ | y = 0.0013x – 0.0021 | 0.9881 |

### *Enhancement of pathlength by using dual cuvettes*

In UV-vis spectrophotometry, increasing the cuvette pathlength would increase the absorbance, which could aid in more accurate quantification, especially for dilute samples. However, long-pathlength (>1 cm) measurement is not practical in many laboratories since typical UV-vis spectrophotometers are equipped with a cell holder designed for the most commonly used 1 cm cuvette. While this issue leads to additional requirements of special cuvettes and instrumentation, it is not a problem or limitation for SDIC. This is because measuring color intensity of a solution in double pathlength can be done effortlessly by photographing a pair of cuvettes which are filled with the same sample and then placed next to each other. In this work, experiments were therefore conducted to demonstrate the feasibility and advantages of this technique by comparing the use of double cuvettes (two 1 cm pathlength cuvettes) with a single cuvette (one 1 cm pathlength cuvette).

As shown in Fig. 4, the standard curve of the double cuvette-based measurement had twice the slope and the higher $r^2$ than that of a single cuvette, reflecting the

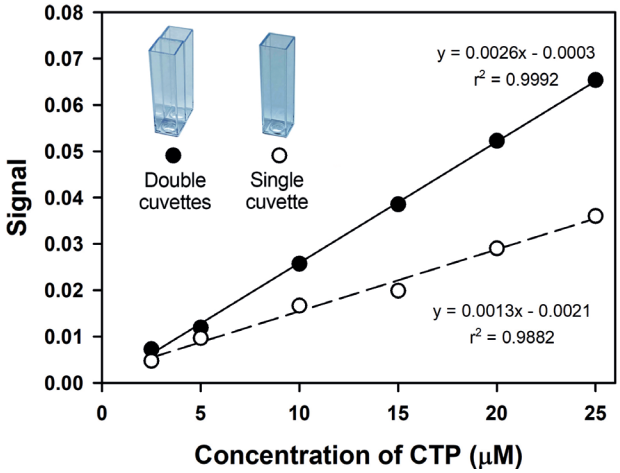


**Figure 4.** Standard curves obtained from signal acquisition using a single cuvette and double cuvettes.

increased sensitivity and linearity. It was likely that when the pathlength increased, the color captured in the image was more intense. This resulted in a more accurate RGB measurement, and finally, the lowered scattering of data points around a regression line. In another experiment, the SD values which represented the variation of the analytical signals were examined by analyzing the replicate samples having three different CTP concentrations (n = 10). The results depicted in Fig. 5 revealed that the color measurement of dilute samples with a pale color encountered a problem related to the high variation of the signals obtained. For example, the greatest SD value was found when 2.5 μM of the CTP solutions were measured, especially in a single cuvette. When the CTP concentration increased, the effect was less pronounced. Interestingly, the color measurements done by using double cuvettes could reduce the variation in signals, due to the acquisition of more intense color in the image. This finding suggested that the use of double cuvettes offered the advantage in giving more precise analytical signals.

In addition to being proven by the higher slope of the standard curve, the increment of sensitivity due to using

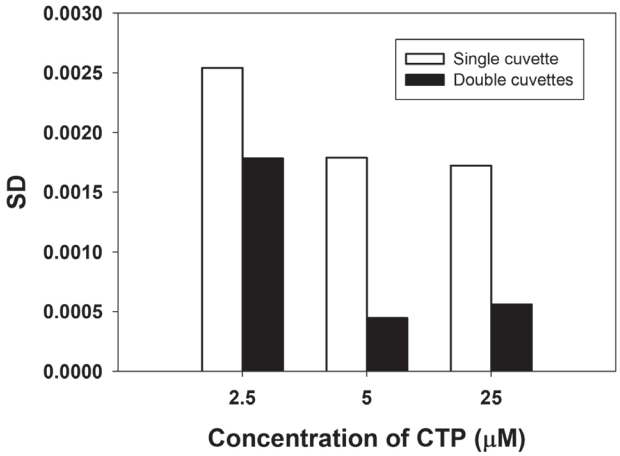


**Figure 5.** Standard deviations (SD) of B/(R+G+B) signals measured using single cuvette versus double cuvettes at different CTP concentrations (n = 10).

double cuvettes was shown by the analysis's LOD and LOQ. Compared to that of a single cuvette, the standard curve based on double cuvettes had a higher slope and lower SD of the Y-intercept. Consequently, their LOD and LOQ as calculated by the ratio of the SD of the Y-intercept to the slope, were lower. For double cuvettes, the LOD and LOQ were 0.70 and 2.13 μM, respectively. For the single cuvette, the LOD and LOQ were 2.74 and 8.30 μM, respectively. The lower values of LOD and LOQ confirmed that using double cuvettes enhanced sensitivity. From these results, it can be concluded that increasing the pathlength by using double cuvettes in the measurement of color intensity helped improve sensitivity, linearity, and precision for the SDIC, especially in the assay where the color intensity of the sample is low or the sample solution is dilute. To the best of our knowledge, this simple but effective technique to improve the analytical performance of the SDIC has never been reported in the literature.

While a longer pathlength can be helpful in obtaining more accurate analytical signals of dilute sample, there are also limitations. Too many stacks of cuvettes and/or exceedingly long rows might produce an image with an overly wide angle of the relevant objects. In other words, the cuvettes positioned far away from the center might be obscured by the walls of the others. This, in turn, rendered the RGB measurement difficult and unreliable. Therefore, two rows with a maximum of 10 cuvettes aligned in each row were used in this study.

## Analytical performance

As summarized in Table 2, the proposed SDIC assay performed by the sample measurement in double cuvettes showed good linearity and sensitivity over a low concentrations range of CTP. Compared with the USP assay in which the sample solution for HPLC analysis was prepared at 4.6 mM, in the SDIC method the target concentration for sample preparation in the SDIC method was as low as 20 μM. The proposed method was accurate (% re-

**Table 2.** Linearity, accuracy and precision of the proposed SDIC assay.

| Parameters | Results |
|---|---|
| Regression equation | y = 0.0026x – 0.0003 when $Y = (\frac{B}{R+G+B})_0 - (\frac{B}{R+G+B})$ and X is concentration of CTP (μM) |
| $r^2$ | 0.9992 |
| Range | 2.5–25 μM |
| LOD | 0.70 μM |
| LOQ | 2.13 μM |
| Accuracy | |
| % Recovery (n=3 for each level) | 101.02 ± 1.17 % (low; spiked with 8 uM)<br>100.45 ± 0.46 % (medium; spiked with 12 uM)<br>100.46 ± 1.07 % (high; spiked with 16 uM) |
| Precision | |
| Inter-day precision (n=18) | 1.15 % RSD |
| Intra-day precision (n=6) | 0.57 % RSD |

coveries within 98–102% at all three concentrations tested) and precise (% RSD of less than 2% for both repeatability and intermediate precision). In terms of specificity, the SDIC method was free from the interference by excipients in tablets as evidenced by the nearly 100% of recoveries in the accuracy study. In addition, the method was unaffected by an impurity, namely, CTP disulfide which is possibly found in CTP. Without a free thiol group, the CTP disulfide did not produce the yellow color with the DTNB, thereby giving an analytical signal which did not differ from that of the blank (Table 3). In addition, when this impurity co-existed with the drug, CTP's analytical signal did not change.

**Table 3.** Effect of CTP disulfide on the proposed SDIC assay (n=5).

| Test | B/(R+G+B) | |
|---|---|---|
| | Average ± SD | *t*-test results |
| Blank versus CTP disulfide | | |
| Blank | 0.3673 ± 0.0016 | no difference |
| CTP disulfide | 0.3668 ± 0.0019 | ($t_{cal}$ = 0.437, $t_{crit}$=1.859) |
| CTP versus CTP + CTP disulfide | | |
| CTP | 0.3111 ± 0.0009 | no difference |
| CTP + CTP disulfide | 0.3108 ± 0.0009 | ($t_{cal}$ = 0.496, $t_{crit}$=1.859) |

For the evaluation of robustness, the influence of two parameters, i.e., room lighting levels and LED brightness, which might affect the image capture were studied by the assay of the CTP solutions (≈20 μM) under different conditions. The results revealed that while the image acquisition in this work was normally carried out in the area in which the room ceiling lights were on, turning off the lights, resulting in a dimmer room, did not affect the assay results (Table 4). It was probable that the LED lights placed behind the cuvettes dominated the ambient light in the photography. In addition, lowering the brightness of the LED from a full to a half level did not cause a statistically significant difference between the obtained assay results. This can be explained by the fact that different LED brightness might produce images with different RGB intensities. However, if all the standard solutions and sample solutions were photographed in the same shot and the calculations were made using their own standard curve, there was no difference between the different conditions in the results of the final analysis. From these findings, the proposed assay was considered robust, and additional equipment such as a dark box used to control ambient light was not required.

**Table 4.** Robustness of proposed SDIC assay (n = 6).

| Parameter | Concentration of CTP calculated (μM) | % RSD | *t*-test results |
|---|---|---|---|
| Room lighting condition | | | |
| Light on (217 lux) | 20.84 ± 0.20 | 1.07 | no difference |
| Light off (31 lux) | 20.91 ± 0.26 | | ($t_{cal}$ = -0.469, $t_{crit}$ = 1.812) |
| Brightness level of LED | | | |
| Full brightness (2,487 lux) | 20.84 ± 0.20 | 1.83 | no difference |
| Half brightness (1,754 lux) | 20.98 ± 0.52 | | ($t_{cal}$ = -0.633, $t_{crit}$ = 1.943) |

## Economical, green and time-saving features

The economical and green features of the proposed method were demonstrated by comparison with previously reported methods. Compared with the smartphone-based assay relying on the formation of complex with expensive palladium (II) chloride (Ravazzi et al. 2018), the present method is cheaper, safer, and eco-friendlier since palladium ion can be harmful by interfering with enzymatic metabolisms and organ functions (Tang et al. 2020). In addition, palladium is very toxic to aquatic organisms, thereby causing environmental hazards and pollution (Zimmermann et al. 2017; Merck 2023). Compared with the spectrophotometric assay of CTP using DTNB and 1-cm cuvette for the absorbance measurement (Hosseinimehr et al. 2004), the SCIC method proposed in this work was capable of analyzing CTP in the linear range of about 8 times lower concentrations than the spectrophotometric method, and significantly saved >10 times the amount of DTNB used (Table 5). In addition, the SDIC assay in the present work was compared with the SDIC assay of another thiol drug, namely D-penicillamine, which was also based on Ellman's reaction (Phadungcharoen et al. 2019). In our experiment, it showed that D-penicillamine reacted with DTNB to form a yellow product in the same way as CTP, as evidenced by their standard curves with negligible difference (Fig. 2 in Supplementary data). This was because both drugs have one thiol group in a molecule, and the colorimetric reaction produces the same $TNB^-$ product. As shown in Table 5, the CTP

**Table 5.** Comparison of the assay of D-penicillamine and CTP.

| Characteristic | CTP assay proposed in this work | CTP assay (Hosseinimehr et al. 2004) | D-penicillamine assay (Phadungcharoen et al. 2019) |
|---|---|---|---|
| Thiol in the reaction (μM) | 1.25–12.5 | 10–100 | 17–134 |
| LOD (μM) | 0.70 | 0.32 | 6.61 |
| Reaction volume (μL) | 700 | 1,820 | 300 |
| Signal measurement | RGB | Absorbance | RGB |
| Container used for signal measurement | Double cuvettes | Single cuvette | 96-well microplate |
| Pathlength (cm) | 2 | 1 | 0.88 |
| DTNB consumed per reaction (nmole) | 17.5 | 200 | 75 |
| Light source | LED and a white acrylic sheet | UV-vis spectrophotometer | Illuminating iPad screen |

assay in which the color measurement was performed by using double cuvettes (total pathlength of 2 cm) was capable of determining the analyte in a linear range with lower concentrations, and having a lower LOD and LOQ than that of D-penicillamine in which the color analysis was done on the samples contained in a 96-well microplate (pathlength of about 0.88 cm). Consequently, despite more reaction volume, the CTP assay required 4.3 times less DTNB reagent per reaction (on a molar basis) than the D-penicillamine assay. This feature helped reduce the cost of chemicals. In addition, minimization of the colorimetric reagent consumption aligned with the 12 principles of Green Analytical Chemistry (Gałuszka et al. 2013) and White Analytical Chemistry (Nowak et al. 2021) in which the environmental impacts and human safety are carefully concerned during the development and implementation of analytical procedures. In addition to reagent saving benefits, the equipment that was used in this assay was less expensive. For example, instead of a tablet gadget, LED lamps and a white acrylic sheet were used as the light source for photography.

Compared with the USP method, the proposed assay was greener since it did not involve the use of an organic solvent and did not require expensive HPLC equipment and column. Since the SDIC method was facile, rapid, and capable of analyzing multiple samples simultaneously, the assay could be achieved in a significantly shorter time. Excluding the time required for column equilibration and washing, the USP method analyzed samples individually with a run time of 10 min per sample. In contrast, the SDIC method could analyze a set of 6 standards and 6 samples within 15–20 min, indicating a higher sample throughput. Using the Analytical Greenness Metric (AGREE), greenness had a high score of 0.77 with 0.7 being considered green. (Fig. 6a). In addition, the Green Analytical Procedure Index (GAPI) illustrated that the SDIC method possessed 8, 2, and 4 green, yellow and red shaded sections, respectively (Fig. 6b). These findings confirmed that the method proposed in the present work is greener, faster, and more affordable.

## Applicability of the proposed method

### *Comparison with other methods*

The applicability of the SDIC method was demonstrated by quantifying the CTP in commercial tablets. For this purpose, the assay results, reported as the % labeled amounts, were compared with that obtained from the USP chromatographic method as well as the spectrophotometric method in which the samples prepared in the SDIC method were subjected to the measurement of absorbance instead of RGB values. The results showed that % labeled amounts as determined by SDIC, spectrophotometry and the USP method were 95.7±1.5, 95.1±1.1 and 95.2±0.7%, respectively, which all met the acceptance criteria for the CTP tablets (90.0–110.0%). According to the one-way ANOVA at a 95% confidence level, these assay results were not significantly different (calculated and critical F-values were 0.4228 and 3.6823, respectively). Therefore, the SDIC assay could be applied for the analysis of the CTP content in tablet formulation, giving results in accordance with that determined from the USP method.

### *Feasibility of using a cuvette with a 2 cm pathlength*

In this work, double 1 cm cuvettes were placed side by side to obtain a 2 cm pathlength, as cuvettes of this size are the most commonly available. Nevertheless, a cuvette with a pathlength of 2 cm is also commercially available. Therefore, the feasibility of using this special type of cuvette was studied. As seen in Fig. 7, the standard curve constructed using the color intensities of standard solutions contained in cuvettes possessing a 2 cm pathlength resembled that in double cuvettes with a 1 cm pathlength. Furthermore, the assay results determined by using these two types of cuvettes, calculated from their own standard curve, showed no statistically significant difference (calculated and critical t-values were -0.267 and 2.306, respectively). This finding confirmed that both types of cuvettes could be used for the analysis, depending on the availability in a laboratory.

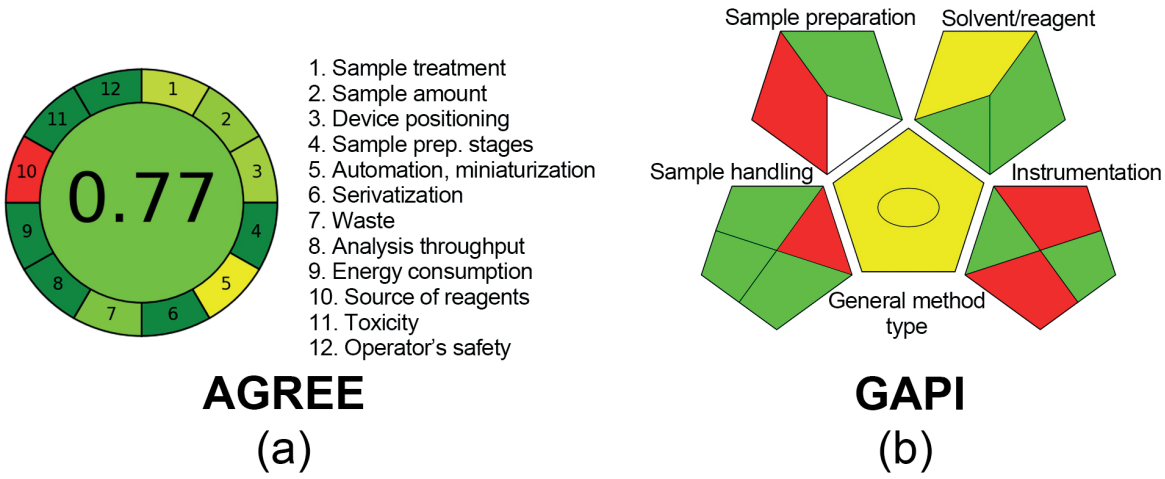


**Figure 6.** Greenness of the proposed SDIC assay assessed by (**a**) Analytical GREEnness (AGREE) and (**b**) Green Analytical Procedure Index (GAPI).

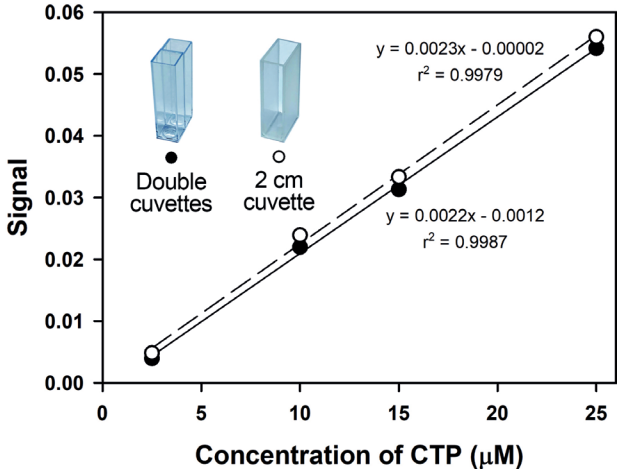


**Figure 7.** Standard curves obtained from signal acquisition using double 1-cm pathlength cuvettes and a 2-cm pathlength cuvette.

## Conclusion

A smartphone-based colorimetric method relying on Ellman's reaction was developed for the assay of CTP tablets. By aligning two 1 cm cuvettes containing the same sample solution side by side, the pathlength was doubled, and more intense color of the photographed samples was effortlessly attained. This allowed the quantification of the CTP at low concentrations in which the reaction might produce pale colored solutions, thereby not only increasing sensitivity, linearity and precision of the analytical signal acquisition, but also reducing the consumption of the colorimetric reagent. The method was proven accurate, insensitive to different light conditions, and unaffected by interference from excipients or the CTP disulfide in tablets. Unlike the HPLC method of the USP, the proposed SDIC was greener, faster, and more affordable. These features encouraged its applicability and suitability in quality control laboratories. Furthermore, the simple but powerful means of increasing the pathlength using double cuvettes, first reported in this work, may be used to develop SDIC assays with enhanced sensitivity for other substances.

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## Supplementary material 1

### Supplementary data

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Data type: docx

Explanation note: **fig. S1.** HPLC chromatograms of (a) standard mixture of CTP and CTP disulfide, and (b) tablets. **fig. S2.** Relationship of the yellow color intensities as determined by absorbance measurement and concentrations of D-penicillamine and CTP.



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