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# Determination of Vitamin C in some Pharmaceutical Dosage by UV-Visible Spectrophotometer Using Bromocresol Purple as a Chromogenic Reagent

**Isam Eldin H Elgailani*, Rayan H Alghamdi**

*Department of Chemistry, Faculty of Sciences and Arts at Baljurashi, Albaha University, Albaha, Saudi Arabia*

## ABSTRACT

*A developed and validated spectrophotometric method has been suggested for the analysis of vitamin C (ascorbic acid) of some drugs namely Aspirin-C, VC500 and Centrum in their drug formulation. The proposed method used the complexation of vitamin C with Bromocresol Purple (BCP) at pH 4.0. The optimum experimental conditions were studied carefully. The $\lambda_{max}$ of the complex formed (ascorbic acid and BCP) was occurred at 430 nm with absorptivity of 0.0398 L $mol^{-1}.cm^{-1}$. The linearity was obtained in the concentration range of 0.5-100 µg/ml for iron content in hemoglobin. The Limit of Detection (LOD) and Limit Quantification (LOQ) were found to be 0.677 and 2.051 µg/ml for the iron respectively and with $R^2$ of 0.999. And the percentage recoveries ranged from 98.86-100.48%. Effects of analytical parameters on the analysis of vitamin C (Ascorbic acid) of the three drugs have been studied. This method can be applied for the estimation of vitamin C in vitamin C drugs in their pharmaceutical dosage.*

**Keywords**: Vitamin C, Ascorbic acid, Bromocresol purple, Validation, UV-Visible spectrophotometer

## INTRODUCTION

Vitamins are very important for human to keep the healthy life. They are considered to be fundamental components in metabolism processes. One of the most important vitamins is the vitamin C (also known as ascorbic acid) which helps in the body metabolic processes [1]. Vitamin C is soluble in water and considered to be antioxidant. It was found in many fruits and vegetables [2]. Vitamin C has nutritional values in many foods [3]. It's necessary in the collagen synthesis in human tissues and considered as an effective antioxidant in the living body [4-6]. Vitamin C supplementation prevents or manages the gout and other similar diseases because it minimizes uric acid levels in the blood [7]. The deficiency of vitamin C can affect health and cause scurvy disease [8]. It is necessary in the development of bone and injury recovery. Vitamin C is very fundamental in many metabolic processes and assists in fighting off infections [9]. Vitamin C helps in cholesterol and blood pressure regulation [10,11]. Several analytical methods are employed for determination of vitamin C in various samples. These methods were HPLC [12], flow injection [13], voltammetry [14], NMR spectroscopy [15], fluorometry [16] and enzymatic method [17]. Many spectrophotometric methods were used for determination of vitamin C, such as the osazone [18], silver-gelatin complex [19], oxidation with iron (III) [20], reaction with toluidine blue [21] and potassium iodate [22]. In the past, other chemicals were used e.g. fast red [23] and methyl viologen [24]. Also ascorbic acid was determined by voltammetry [25]. In this study, we validate spectrophotometric procedure for estimation of vitamin C in some drugs namely Aspirin-C, VC500 and Centrum in their pharmaceutical preparations, using Bromocresol Purple (BCP) as a chromogenic reagent.

## MATERIALS AND METHODS

All chemicals used were of analytical reagent grade, and deionized water was used to prepare all solutions. The standards of Aspirin-C, VC500 and Centrum were supplied by Bayer Bitterfield GmbH, Julphar Gulf Pharmaceutical Industries and Pfizer Canada Inc. respectively. Aspirin-C (240 mg ascorbic acid) tablets were labeled to contain 240 mg ascorbic acid per tablet, VC500 (vitamin C) tablets were labeled to contain 500 mg vitamin C per tablet and Centrum (multivitamin) tablets were labeled to contain 60 mg vitamin C per tablet.

All absorbance measurements were made with a Split beam UV-VIS Spectrophotometer (SP-3000 Plus model, Optima, Tokyo, Japan) with 1-cm quartz cells. A pH meter (model 3305, Jenway Ltd., United Kingdom) was used. Digital water bath (Model LWB-122D, Daihan Labtech Co. Ltd., Indonesia) was also used.

An accurately weighed 1.0 g of BCP were dissolved in 20.0 ml ethyl alcohol, transferred into a 100 ml standard flask and diluted to the mark with deionized water and mixed thoroughly and then diluted to obtain concentrations ranged from 0.01-0.10% (w/v). The solution was stored in amber glass.

Buffer solutions of pH ranged from 3.0 and 11.0 were prepared and used for optimization for the reaction. An exactly weighed equivalent to 0.1 g of vitamin C in each of standard sample of the Aspirin-C, VC500 and centrum was dissolved in deionized water for each of the three drugs, transferred into a 100 ml volumetric flask and diluted to the mark with deionized water for three drugs, and mixed thoroughly to obtain 1000 µg/ml. The stock solutions of the three drugs were diluted to get an appropriate concentration for the determination. The stock solution of Aspirin-C was diluted to get concentrations ranged from 0.5-120 µg/ml.

The components of each of 3 tablets for each the three drugs (Aspirin-C, VC500 and Centrum) were powdered and well mixed. An accurately weighed amount equivalent to weight stated for each of the three drugs (Aspirin-C 240 mg, VC500 500 mg and Centrum 60 mg) was transferred into a 25 ml calibrated flask and dissolved in about 25 ml/l in distilled water for each of the three drug. The contents of the flask were stirred, stand for 5 min and then completed to volume with deionized water for the three drugs. The contents were mixed thoroughly and filtered. The prepared solution was diluted quantitatively with double distilled water for Aspirin-C, VC500 and Centrum to obtain an appropriate concentration for the determination.

The Job's method of continuous variation was employed [26]. Equi-concentration (10.0 µg/ml) aqueous solutions of Aspirin-C, VC500 and Centrum and (BCP) were prepared. Series of 10 ml portions of the master solutions of Aspirin-C, VC500 and Centrum and (BCP) were made up by forming the following ratios (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10), The solution was further treated as described under the general recommended procedure.

About 2 ml of each of the prepared solutions of Aspirin-C, VC500 and Centrum were transferred into 10 ml volumetric flask subsequently, 2 ml of pH 3.0 were added for each of the three drugs and 1 ml of 0.08% BCP was added for each of the three drugs, the solutions were heated in a thermostat at 40°C for 10.0 min for the 3 drugs, the mixtures was diluted with deionized water. The absorbance of the complex solutions (ascorbic acid and BCP) were measured at 430 nm for the three drugs against a reagent blank prepared in the same manner but no drugs.

## RESULTS AND DISCUSSION

The $\lambda_{max}$ of the complexes of Aspirin-C, VC500 and Centrum with (BCP) were shown in Figure 1. The obtained $\lambda_{max}$ were 370 nm for Aspirin-C against water and 250 nm for both VC500 and Centrum against water. The $\lambda_{max}$ of (BCP) have maximum absorption in 460 nm, and the $\lambda_{max}$ for the 3 drugs with (BCP) is 430 nm.

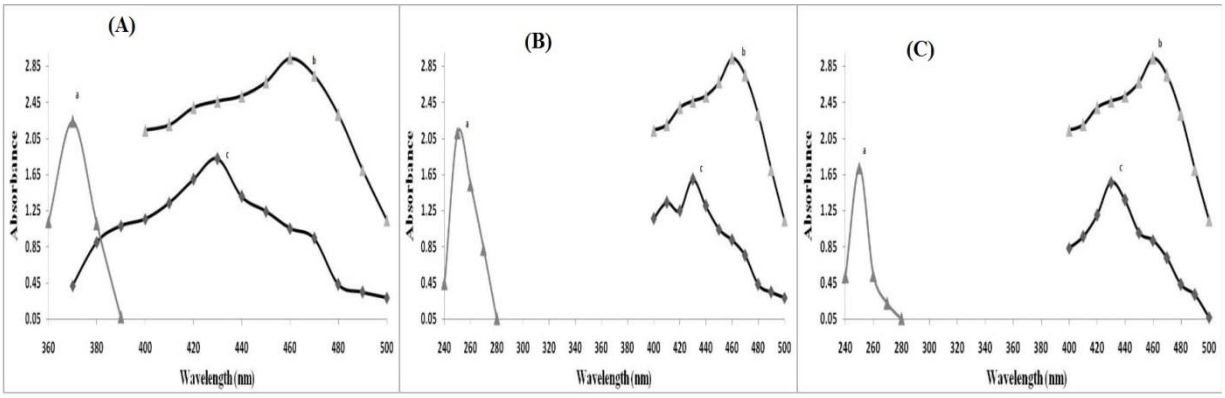


**Figure 1: Absorption spectra of (A) Aspirin-C, (B) VC500 and (C) Centrum (all of the three drugs were of 10 µg/ml): a-absorption spectra of the drug versus water, b-absorption spectra of BCP (0.08%) versus water, c-absorption spectra of reaction of the drug (10 µg/ml) with BCP (0.08%)**

The optimal analytical parameters for this method validation were determined. The ideal reaction parameters were found to be 4.0, 40°C, 15 min and 0.08% for the pH, temperature, reaction time and BCP concentration on the reaction of Aspirin-C with (BCP) respectively as revealed in Figure 2.

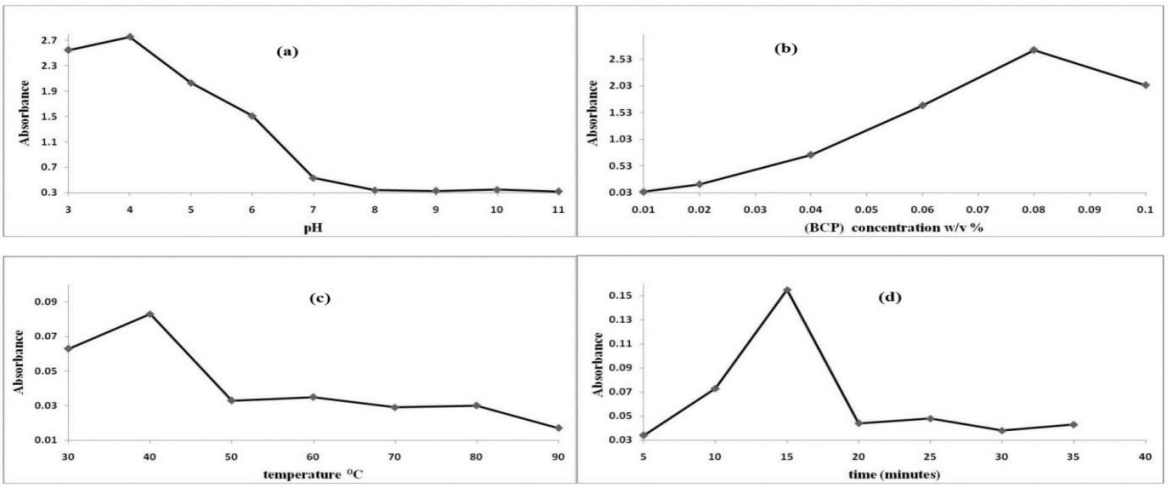


**Figure 2: Variations studies of analytical parameters on the method: (a) pH, (b) BCP concentrations, (c) Temperature and (d) Standing time**

An account for the complexation reaction between ascorbic acid and BCP was employed by Job's method, in which the amount of complex solution can be evaluated spectrophotometrically for various ratios of [ascorbic acid] to [BCP]; while the total concentration (mole numbers) of ascorbic acid and BCP is kept constant [26]. Approximately equal concentration of aqueous solutions of each of prepared drug samples (Aspirin-C, VC500 and Centrum) and (BCP) were provided. Series of drug samples solutions and BCP were made up by this ratios (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10). The solutions were used for the account of stoichiometric ratio of the reaction. The graph obtained shows the complex reaction of (BCP) with the ascorbic acid of the studied drugs samples as shown in Figure 3.

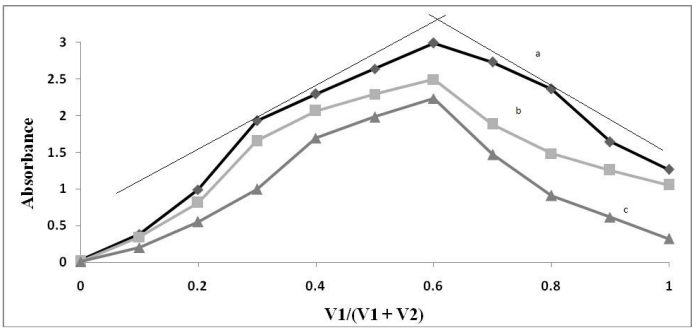


**Figure 3: Method of continuous variation for the complexation of BCP and drugs samples (V1: BCP and V2: vitamin C): a-Aspirin-C, b-VC500 and c-Centrum**

Calibration curves for the determination of prepared drug samples Aspirin-C and BCP were, established by graphing the absorbance versus concentrations (Figure 4). The linear regression line ranged in 0.5-60 µg/ml.

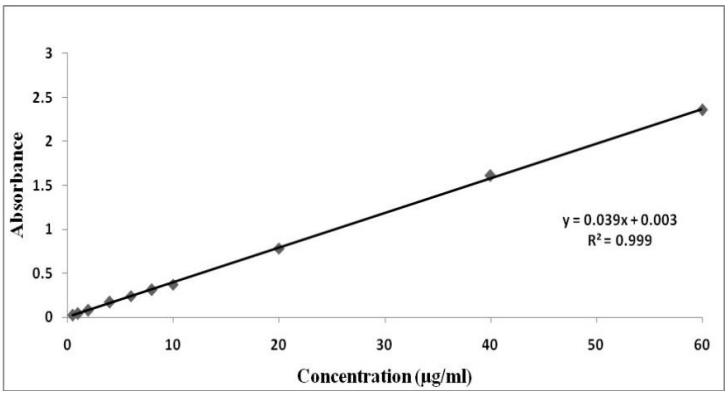


**Figure 4: Calibration curve of Ascorbic acid (vitamin C) (absorbance measurements were carried out at wavelength of 430 nm)**

The LOD and LOQ were evaluated according to International Conference on Harmonisation (ICH) [27]. The LOQ or LOD were calculated from the blank readings (n=10, SD=0.008) and the calibration graph slope. The analytical parameters for the studied methods are shown in Table 1.

**Table 1: Method analytical parameters**

| Parameter | Aspirin-C with (BCP) |
|---|---|
| $\lambda_{max}$ (nm) | 430 nm |
| Linearity limits (µg/ml) | 0.5-100 |
| Absorptivity (L $mol^{-1}$. $cm^{-1}$) | 0.0398 |
| Correlation coefficient ($R^2$-value) | 0.999 |
| Regression equation (Y) | Y = 0.039 X + 0.003 |
| Slope | 0.039 |
| Intercept | 0.003 |
| LOD (µg/ml) | 0.677 |
| LOQ (µg/ml) | 2.051 |

Robustness is the capability of the analytical procedure to be stable by small variations in working parameters. In this work the recovery percentages were estimated. Changing with small variations in the analytical parameters didn't influence procedure of the studied method as seen in Table 2.

Estimation of accuracy and precision of this work were carried out. The percentages of relative error were in the range of 0.06 - 1.60. The findings seen in Tables 3 and 4 indicate the importance of this method which can be applied for the analysis of vitamin C in the mentioned drugs in different laboratories.

**Table 2: Robustness of the proposed spectrophotometric method for Aspirin-C sample**

| Condition | Aspirin-C (10.0 µg/ml) | % Recovery ± RSD* |
|---|---|---|
| pH | 3.8 | 98.80 ± 0.40 |
| | 4.2 | 101.02 ± 0.26 |
| (BCP) concentration (w/v %) | 0.07 | 98.12 ± 0.40 |
| | 0.09 | 99.49 ± 0.52 |
| Temperature (°C) | 38 | 99.06 ± 0.65 |
| | 42 | 101.38 ± 0.53 |
| Reaction time (min) | 13 | 99.06 ± 0.39 |
| | 17 | 100.03 ± 0.39 |

*RSD is the relative standard deviation; (n=3)

**Table 3: Accuracy and precision of the suggested method**

| Sample | Taken | Found | Relative error% | SD | RSD% |
|---|---|---|---|---|---|
| Aspirin-C (µg/ml) | 8.00 | 7.872 | 1.60 | 0.052 | 0.661 |
| | 10.00 | 10.120 | 1.20 | 0.039 | 0.385 |
| | 20.00 | 19.880 | 0.06 | 0.054 | 0.272 |

SD is the standard deviation; (n =3)

**Table 4: Interday and intraday accuracy determination**

| Amount added (µg/ml) | Interday (n=3) | | | | Intraday (n = 3) | | | |
|---|---|---|---|---|---|---|---|---|
| | Found | Recovery % | Mean ± SD | % RSD | Found | Recovery % | Mean ± SD | % RSD |
| 4 | 3.915 | 97.875 | 0.039 | 0.996 | 3.906 | 97.65 | 0.064 | 1.600 |
| 8 | 8.103 | 101.288 | 0.052 | 0.642 | 7.974 | 99.68 | 0.068 | 0.850 |
| 10 | 9.949 | 99.490 | 0.068 | 0.683 | 10.103 | 101.03 | 0.064 | 0.633 |

Mean value; (n=3)

The results obtained by this investigated method were obviously adequate for the analysis of vitamin C. Thus the determination of vitamin C in each of the three drugs (Aspirin-Co, VC500 and Centrum), were carried out individually as seen in Table 5.

**Table 5: Analysis of the investigated drugs in their pharmaceutical formulation**

| Drug sample | drug description | % ± SD* |
|---|---|---|
| Aspirin-C | 240 mg of vitamin C/tablet | 99.45 ± 0.83 |
| VC500 | 500 mg of vitamin C/tablet | 100.23 ± 0.65 |
| Centrum | 60 mg of vitamin C/tablet | 99.28 ± 0.32 |

*Mean of (n=3)

Three different concentrations of standard Aspirin-C were added to a constant concentration of standard drug samples and then the total was determined by this method. Each test was performed in triplicate. The range of % recoveries was found 98.86-100.48% for vitamin C in drug sample as shown in Table 6.

**Table 6: Method percentage recovery**

| Sample | Aspirin-C (µg/ml) | Standard Aspirin-C Added (µg/ml) | Found (µg/ml) | Recovery (% ± RSD)* |
|---|---|---|---|---|
| Aspirin-C (µg/ml) | 8.0 | 4.0 | 11.863 | 98.86 ± 0.45 |
| | 8.0 | 8.0 | 16.077 | 100.48 ± 0.32 |
| | 8.0 | 10.0 | 17.863 | 99.24 ± 0.22 |

*Mean of (n = 3)

## CONCLUSION

The current work showed the effectiveness determination of BCP as a chromogenic reagent in the analysis of vitamin C containing drugs namely Aspirin-C, VC500 and Centrum, each one in its pharmaceutical dosage. The investigated spectrophotometric method is superior to past reported one for determination of vitamin C (ascorbic acid in Aspirin-C, VC500 and Centrum samples, in terms of their simplicity and cheapness. The other advantages include that, the method involve the measurement of stable coloured species, and rapid formation of the complex of the ascorbic acid and BCP. Therefore, the method is applicable for analysis of each investigated drug.

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