Research Article

A new spectrofluorimetric method for the determination of ascorbic acid with bromocresol purple in pharmaceutical samples

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Abstract
Based on the interaction between ascorbic acid and bromocresol purple, a new simple, straightforward, and quick method for the quantification of ascorbic acid is proposed. The procedure is based on the determined quenching effect of ascorbic acid on the natural fluorescence signal of bromocresol purple in the reaction between ascorbic acid and bromocresol purple in phosphate buffer solution (pH 6). The reduction of bromocresol purple fluorescence intensity is detected at 641 nm, while excitation occurs at 318 nm. The linear relationship between the reduced fluorescence intensity of bromocresol purple and the concentration of ascorbic acid is in the range 4.65×10^{-5} to 4.65×10^{-6} mol L^{-1} ($R^2 = 0.9964$), with the detection limit of 8.77×10^{-7} mol L^{-1} and quantification limit of 2.35×10^{-5} mol L^{-1}. The findings in this study further show that the new method provides good precision and repeatability, as well as satisfactory recovery values in terms of accuracy. The new method is tested on fifteen samples with different amounts of ascorbic acid and additional components. The effects of interfering components such as citrus bioflavonoids, citric acid, folic acid, paracetamol, calcium, and magnesium carbonate on the intensity of fluorescence of bromocresol purple are also investigated. The effects of interfering components such as citrus bioflavonoids (routine and hesperidin), citric acid, folic acid, paracetamol, calcium, and magnesium carbonate on the intensity of fluorescence of bromocresol purple are also investigated. The results of iodometric titration point out that the new method is effective for the determination of ascorbic acid in pharmaceutical samples.

Article Highlights

- A new spectrofluorimetric method for determination of ascorbic acid in pharmaceutical samples using bromocresol purple.
- Determination of optimal parameters for ascorbic acid determination in a variety of pharmaceutical samples.
- Examination of the influence of additional substances in the pharmaceutical samples on the analysis.

Keywords Ascorbic acid · Spectrofluorimetry · Bromocresol purple

1 Introduction

Ascorbic acid (AA) called vitamin C is a six-carbon lactone that is synthesized withinside the liver of most mammalian species from glucose. Humans, non-human primates, and guinea pigs don't posess the enzyme gulonolactone oxidase that is crucial for the synthesis of 2-keto-l-gulonolactone, the instant precursor of ascorbic acid. As an effect of now no longer taking nutrition C thru diet, a deficiency state happens which manifests with a huge spectrum of

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medical manifestations [1]. It is required for the manufacture of carnitine, collagen, neurotransmitters, and peptide hormones, as well as various other activities in the human body, such as hydroxylation and the oxidative catabolism of aromatic acids [2–6]. Ascorbic acid is a reducing agent because it is an electron donor. A double bond between the second and third carbons contributes two electrons. Because it gives electrons, ascorbic acid works as an antioxidant, preventing other chemicals from oxidizing. However, ascorbic acid is oxidized due to the nature of these interactions. These electrons are lost in a logical sequence. Free radicals, semi-dehydroascorbic acid, and ascorbyl radicals are the products of the loss of one electron. Ascorbyl radical is relatively stable and unreactive when compared to other free radicals [1]. As an electron donor, it scavenges free radicals directly, limits the formation of new free radicals by suppressing the NADPH oxidase (NOX) pathway, and aids in the recycling of other antioxidants [5].

Ascorbic acid is commonly found in pharmaceutical supplements, and it has a bioavailability that is comparable to that of naturally occurring ascorbic acid in meals. Sodium ascorbate, calcium ascorbate, various mineral ascorbates, ascorbic acid with bioflavonoids, and additional ascorbic acid supplements are available[7, 8].

Ascorbic acid is analyzed using a variety of procedures, with the method chosen based on cost, product use, equipment availability, and time [9]. The development of new methods for determining ascorbic acid is increasing, according to the literature, due to the large range of samples for analysis and the influence of diverse matrices [10]. Titrimetric, spectrophotometric, electrochemical, chromatographic, kinetic, chemiluminescence, and spectrofluorimetric methods have all been reported for determining ascorbic acid in various types of samples [11–13]. The main advantage of spectrofluorimetry over spectrophotometry is its sensitivity, which allows it to analyze nanogram levels of the target analyte. This method is based on the notion that the analyte under investigation can show fluorescence or can react with another substance to produce a fluorescent product [14]. Changes in fluorescence intensity that occur when the analyte is added are in the form of increasing, decreasing or strongly enhacing fluorescence signal intensity [15–22]. The measurement of decreasing fluorescence intensity of methylene blue (MB) in a spectrofluorimetric approach that uses a reaction between MB and AA is based on the measurement of MB being reduced to colorless LMB and AA being oxidized to dehydroascorbic acid (DHAA) [15]. Xanthene dyes, such as pyronine Y, are a type of xanthene dye. In water, it emits a bright red–yellow glow. In the presence of nitrite, its molecular structure is broken, and the fluorescence of the solution drops dramatically. When there is only a trace of ascorbic acid in the system, the reaction proceeds slowly. This demonstrates that ascorbic acid can stifle the system's reactivity. As a result, this phenomenon is utilized to determine ascorbic acid in a range of materials [16].

This paper describe new, simple, sensitive, and rapid spectrofluorimetric method for determination of ascorbic acid in pharmaceutical samples. Bromocresol purple (BCP) was chosen as the chromogenic reagent for the determination of AA in pharmaceutical samples. The effects of various reaction conditions, such as acidity, BCP concentration, reaction time, instrumental parameters, as well as the influence of possible interfearing substances were carefully investigated. To the best of our knowledge, there has been no report on the application of bromocresol purple for the determination of AA by a spectrofluorimetric method.

2 Materials and methods

2.1 Instrumentation

All fluorescence measurements were carried out on a lambda LS 55 luminescence spectrometer (Perkin-Elmer, Buckinghamshire, UK) equipped with a xenon lamp and a 1 cm quartz cell. pH adjustments were done on a digital pH meter Metler Toledo pH meter SG2 SevenGo.

3 Reagents and solutions used for spectrophotometric determination

All reagents used were analytical grade. A stock solution of ascorbic acid (Gram-mol, Croatia) 4.65×10^{-4} mol L^{-1} is always prepared fresh due to the instability of AA by dissolving 0.0041 g of AA in a 50.0 mL calibrated flask and fill up to the mark with ultra-pure water (conductivity was: 0.055 S cm^{-1}, expressed as the resistivity of 18.2 MΩ cm^{-1} and TOC was 0.02 ppb). A stock solution of bromocresol purple (BCP) (Coleman & Bell Co, Norway) 1×10^{-4} mol L^{-1} is prepared by dissolving 0.0054 g of BCP in a 100.0 mL graduated flask and filled up to the mark with ultra-pure water. All further dilutions are made with ultra-pure water. The BCP working concentration was 1×10^{-5} mol L^{-1}. Phosphate buffer, pH = 6.4 was prepared by mixing 27.0 mL of dipotassium hydrogen phosphate (Merck, Germany) in a concentration of 1 mol L^{-1} and 72.2 mL of potassium dihydrogen phosphate (Semikem, Bosnia and Herzegovina) also at a concentration of 1 mol L^{-1}. The pH is adjusted with 2.0 mol L^{-1} sodium hydroxide.

Pharmaceutical samples (tablets, powders, and effervescent tablets) used for analysis (15 samples) were commercially available. The solutions of pharmaceutical preparations were prepared by dissolving suitable amounts of

the samples in a 50.0 mL calibrated flask and diluting to the mark with ultra-pure water.

The following commercial pharmaceutical samples in the form of tablets and powders have been analyzed by the proposed method. Table 1 shows pharmaceutical samples that were analyzed with the proposed method together with the accompanying components that can be found in tablets.

For the validation experiments, a 0.01 mol L^{-1} iodine solution was prepared and standardized according to the literature. The iodine method is recommended by the European Pharmacopoeia [23] as the standard method for the determination of AA in pharmaceutical preparations.

Possible interfering substances such as rutin, hesperidin, calcium carbonate, magnesium carbonate, citric acid, paracetamol, folic acid, zinc chloride, and iron(II) sulfate were examined. The influence of possible interfering substances on the relative intensity of the fluorescence signal was examined by analyzing the standard solution of AA and three different concentrations of the interfering substances. Concentrations of interfering substances were higher, the lower and same order of magnitude as the concentration of AA.

4 General procedure for the spectrofluorimetric quantification of ascorbic acid

An aliquot of AA (2 mL) of different dilutions, BCP (2 mL), and phosphate buffer (1 mL) were mixed in a 10.0 mL calibration flask. The volumetric flasks with different AA dilutions are then incubated at 40 °C for 15 min in a water bath, then left to cool to room temperature for 5 min. The final mixture was diluted to 10.0 mL with ultra-pure water. After cooling for 10 min, the relative fluorescence intensity signal was measured at working excitation and emission wavelengths (318 nm and 641 nm) with a 10.0 mm slit width of all open filters and at 1% of transmittance (1%T). The fluorescence intensity was also measured after 30 min under the above-mentioned conditions.

5 Results

This paper informs on a new method for the determination of ascorbic acid with bromocresol purple in pharmaceutical samples that was developed. Ascorbic acid in reaction with BCP is oxidized to dehydroascorbic acid (DHAA) and BCP is reduced to his reduced (Leuko-BCP) form in phosphate buffer pH = 6.4 (Fig. 1). The effect of decreasing relative fluorescence intensity was used for the estimation of AA content in investigated samples.

6 Determination of wavelength for a maximum of excitation and emission

Excitation and emission spectra of the reagent (phosphate buffer/BCP) without AA were scanned in the wavelength range of 200–800 nm for excitation and 200–900 nm for emission spectra using 10.0 mm slit widths and scan rates of 500 nm/min for both spectra. Excitation peaks were observed at 318, 381, 509 nm, and emission peaks at 320, 361, 641 nm. Then, excitation and emission spectra of the reagent with an added analyte, AA (4.65×10^{-4} mol L^{-1}) were scanned and a decrease of intensity of fluorescence (IF) values was observed on all peaks, particularly from 895.83 to 578.75 at 318 nm excitation wavelength, and from 1000.07 to 826.60 at 641 nm emission wavelength, and these were chosen to be the working wavelengths for this method (Fig. 2).

7 Optimization of experimental condition for the proposed method

To optimize the proposed method, the effects of various reaction conditions, such as pH, BCP concentration, reaction time, instrumental parameters (slits, open filters, and 1%T) were carefully investigated. Variables were changed in the experiments one by one, while others were kept constant. Furthermore, various validation parameters such as accuracy, precision, repeatability, etc. were also investigated and calculated to prove the validity of results provided by this method.

7.1 Influence of pH

The spectrophotometric measurement of AA with bromocresol purple in acidic conditions at pH = 3,0 was reported by Elgailani and Alghamdi [24]. However, this was not shown to be applicable for the spectrofluorimetric determination of AA. As a result, the acidity/basicity range in which the reaction occurs was tested between 3 and 8. Between pH 5 and 7, the best results were obtained. So the working pH of the phosphate buffer was further examined by 0.2 pH units in the range of 5 and 7. According to the literature data for electroanalysis of ascorbic acid using poly(bromocresol purple) [25] pH, 6.4 was the best. Our result also showed that the best reproducibly was achieved at pH 6.4 and this pH was taken for further experiments.

7.2 Influence of the concentrations of BCP

The effect of the concentration of BCP on the relative fluorescence intensity was examined in the concentration range of BCP from 1×10^{-7} to 1×10^{-4} mol L^{-1}. The

Table 1 Commercially available pharmaceutical samples that were analyzed

Sample	Pharmaceutical samples of AA	Additional components	Manufacturer
1	Zink + C	Acidifier citric acid, acidity regulator sodium hydrogen carbonate, L—ascorbic acid, filler sorbitol, flavors, sweetener sodium cyclamate and sodium saccharin, zinc citrate (3.3 mg zinc), dye riboflavin	DM, Karlsruhe, Germany
2	Vit C + Zn	Filler sorbitol, L-ascorbic acid (vitamin C), palm fat (completely hardened), filler xylitol, acidifier citric acid, acid regulator monosodium citrate, thickener sodium carboxymethyl cellulose, flavor, zinc oxide (5 mg), medium-chain triglycerides, release agent magnesium salts of fatty acids, sweeteners	Doppel Herz, Flensburg, Germany
3	Eisen + C	Acidifier citric acid, acidity regulator sodium hydrogen carbonate, filler sorbitol, flavors, maize starch, beetroot powder, L—ascorbic acid, sweetener sodium cyclamate, and sodium saccharin, iron—II—lactate, dye riboflavin	DM, Karlsruhe, Germany
4	Fabricet	Paracetamol (300 mg), L-ascorbic acid, sodium hydrogen carbonate, sodium carbonate, citric acid, lactose monohydrate, docusate sodium, sodium saccharin, sodium benzoate, povidone	Hemofarm, Vrsac, Bosnia and Herzegovina
5	Multivita plus	Acidifier citric acid, acidity regulator sodium hydrogen carbonate, L—ascorbic acid, filler sorbitol, inulin, sweetener sodium cyclamate, maize starch, tricalcium phosphate, beetroot juice powder, the aroma of orange and mango, sodium saccharin, calcium-D-pantothenate, DL-alpha-tocopheryl acetate, pyridoxine hydrochloride, thiamine hydrochloride, riboflavin, petroyl-mnoglutamic acid, cyanocobalamine	Nutrilo GmbH Germany
6	Vitamin C protect	L-ascorbic acid, bulking agent cellulose, anticoagulant silicon dioxide, talc, magnesium salts of fatty acids	Esensa, Belgrade, Serbia
7	Vit C Cek	L-ascorbic acid (with a protective layer of ethylcellulose of tartaric acid), sorbitol, magnesium stearate, silicon dioxide, sodium cyclamate, the aroma of orange	Farmalabor, Canosa di Puglia, Italy
8	Hansal Vit C 20	Acidifier citric acid, acidity regulator sodium hydrogen carbonate, L—ascorbic acid, filler sorbitol, flavors, sweetener sodium cyclamate and sodium saccharin, dye riboflavin	Sanotact, GmbH Germany
9	Hansal multivit	Acidifier citric acid, acidity regulator sodium hydrogen carbonate, filler sorbitol, flavors, maize starch, L-ascorbic acid, maltodextrin, sweetener sodium cyclamate and sodium saccharin, DL-alpha-tocopheryl acetate, beetroot juice powder, nicotinamide, calcium-D-pantothenate, dye riboflavin pyridoxine hydrochloride, thiamine mononitrate, petroyl-mnoglutamic acid, D-biotin, cyanocobalamine	Sanotact, GmbH Germany
10	Vit C (multivita)	Acidifier citric acid, acidity regulator sodium hydrogen carbonate, L—ascorbic acid, filler sorbitol, inulin, starch, tricalcium phosphate, beetroot juice powder, sweetener sodium cyclamate, dye riboflavin	Nutrilo GmBH Germany
11	Plivit C	L-ascorbic acid, maize starch, talc, povidone, magnesium stearate	Pliva, Zagreb, Croatia
12	Vitamin C 1000	L-ascorbic acid, magnesium stearate, polyethylene glycol, silicon dioxide, hydroxypropyl methylcellulose, cellulose gel, citrus bioflavonoid complex (*Citrus spp*.) (20 mg, 10%, 10 mg hesperidin and rutin, rosehip 20 mg)	Optimum, Alkmaar, The Netherlands
13	Bio-C	L-ascorbic acid, thickener hydroxypropyl methylcellulose, maize starch, silicon dioxide, magnesium salts of fatty acids, color titanium dioxide, talk, olive oil, a complex of bioflavonoids from the peel of citrus fruit (*Citrus spp*.) (40 mg, 30% flavonoids calculated as hesperidin)	Dietpharm, Bestovje, Rakitje, Croatia
14	Natural wealth	L-ascorbic acid, dicalcium phosphate, hypromellose, cellulose (plant origin), magnesium stearate, bioflavonoids from the peel of citrus fruit (*Citrus spp*.) (25 mg), wild Rosehips	Wealth, Zagreb, Croatia);
15	Vit C alkaloid	L-ascorbic acid, microcrystal cellulose, sodium-croscarmellose, magnesium stearate, lactose, starch	Alkaloid, Skopje, North Macedonia

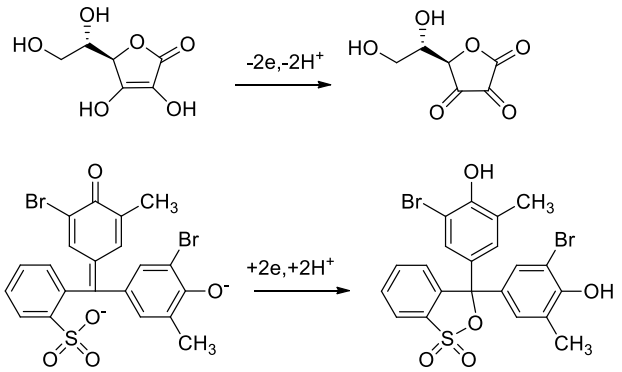

Fig. 1 Reaction between ascorbic acid and bromocresol purple

highest IF was found to be at the concentration value of 1×10^{-5} mol L^{-1}, (Fig. 3) whereas higher concentrations showed lower IFs, and with lower concentrations, it was not possible to construct the calibration curve.

7.3 Calibration Curve, LOD and LOQ

Under optimal conditions, the calibration curve was found to be linear in the concentration range of AA from 4.65×10^{-5} to 4.65×10^{-6} mol L^{-1} with a determination coefficient of $R^2 = 0.9964$, and the linear equation $y = -61.836x + 8.5902$. and recorded at 1%T.

The linearity area is narrow, which is also a limitation of the method. The limit of detection was (LOD) 8.77×10^{-7} mol L^{-1}, (calculated as 3σ) and limit of quantification (LOQ) 2.35×10^{-5} mol L^{-1}, (calculated as 10σ) were determined using nine (n = 9) standard solutions of AA of the same concentration (2.48×10^{-5} mol L^{-1}), by measuring their IFs. Measurement precision (expressed as RSD) was found to be 8.46% for this concentration.

The calibration curve was also recorded with the instrument parameter all filters opened. It was found to be linear in the concentration range from 4.77×10^{-5} to 4.77×10^{-6} mol L^{-1} with a determination coefficient of $R^2 = 0.9809$, and the linear equation $y - 6 \times 10^6 + 981.28$ under the optimal condition Table 2.

7.4 Reaction time

The stability of the IF signal of Leuko-BCP concerning time was determined by measuring the reaction progression of an AA solution (4.65×10^{-4} mol L^{-1}) with the reagent for

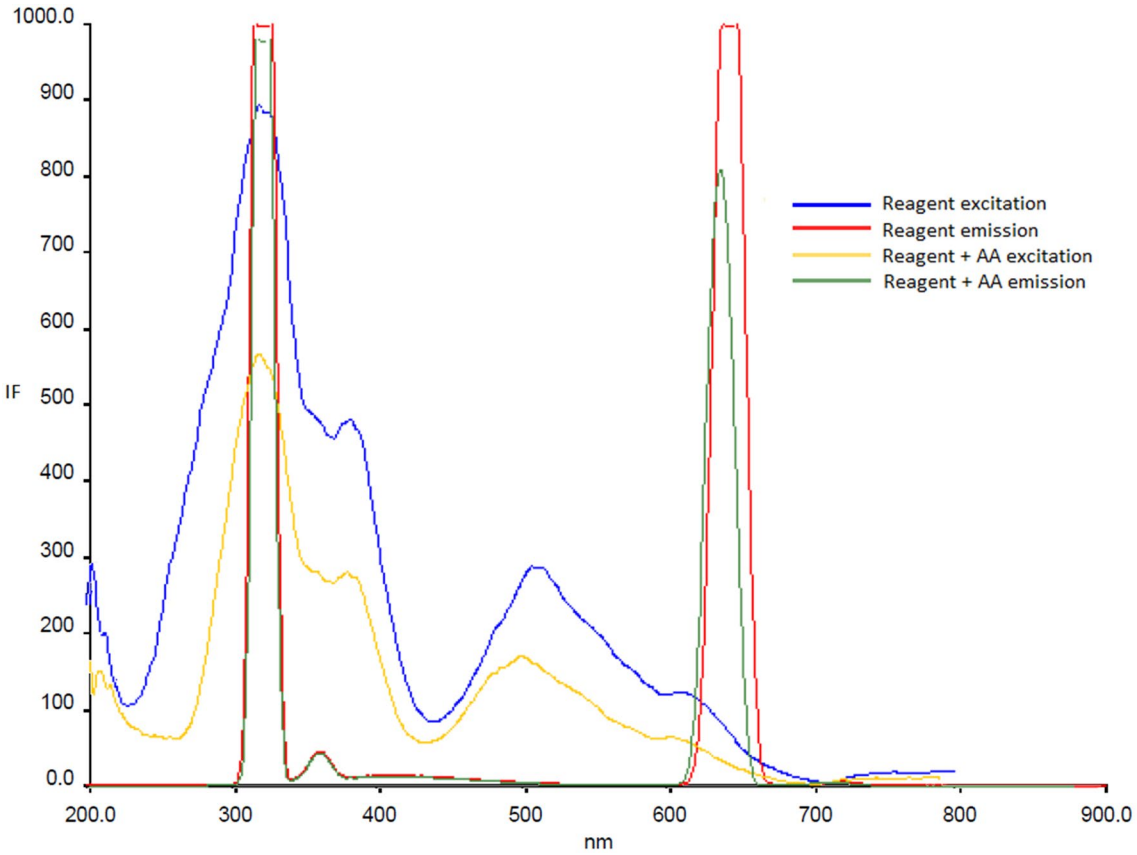

Fig. 2 Excitation and emission spectra of the reagent (phosphate buffer/BCP) without AA and with added AA

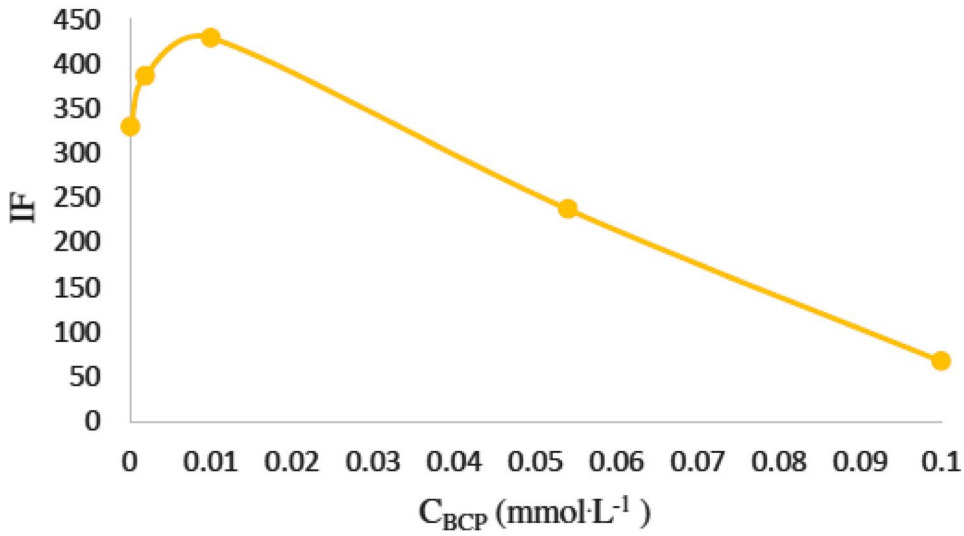

Fig. 3 Effect of BCP concentration on the relative IF of the reaction system

Table 2 Method analytical parameters

Parameters	Optimal condition
Excitation wavelength	318 nm
Emission wavelength	641 nm
Concentration BCP	1×10^{-5} mol L^{-1}
Slit	10 mm
Stability of signal	After 9 min
Linearity (1%T)	4.65×10^{-5} to 4.65×10^{-6} mol L^{-1}
Linearity (all filters open)	4.77×10^{-5} to 4.77×10^{-6} mol L^{-1}
Linear Eq. (1%T)	y = -61.836x + 8.5902
Linear equation (all filters open)	$y=-6\times10^{6}+981.28$
LOD	8.77×10^{-7} mol L^{-1}
LOQ	2.35×10^{-5} mol L^{-1}
SD	2.097×10^{-5} mol L^{-1}
RSD	8.46%

13 min, and it was shown that the signal is stable 9 min after the reaction was initiated.

8 Accuracy, precision, and repeatability

The accuracy of this spectrofluorimetric method was assessed by analyzing AA solutions in the concentration range from 0.0480 mmol L^{-1} to 0.0058 mmol L^{-1} (n = 10 for each concentration) and by calculating SD, %RSD, and Recovery values (Table 3), which showed satisfactory accuracy.

To estimate precision and repeatability, the IF of one newly-prepared AA solution 0.477 mmol L^{-1} was measured periodically, in four replicates with n = 10 (Table 4). Obtained results were taken for calculating SD and %RSD.

Table 3 Accuracy assessment results for standard AA solutions

Prepared C_{AA} (mmol/L)	IF ± SD	Calculated C_{AA} (mmol/L)	%RSD	Recovery (%)
0.0480	5.514 ± 0.129	0.0490	2.39	102.08
0.0230	7.115 ± 0.158	0.0240	2.22	104.35
0.0058	8.238 ± 0.074	0.0057	0.89	98.28

Table 4 Precision and repeatability estimation results for one standard AA solution

#	C_{AA} (mmol/L)	IF ± SD	%RSD	Average IF ± SD	Average %RSD
1	0.477	7.08 ± 0.15	2.10	6.85 ± 0.15	2.19
2	0.477	6.75 ± 0.15	2.22		
3	0.477	6.79 ± 0.14	2.06		
4	0.477	6.79 ± 0.16	2.36		

9 Sample preparation

The samples used for analysis were in different forms, with a different ascorbic acid content, and commercially available. Pharmaceutical samples (tablets, effervescent tablets, and one powdery form) were first weighed, then powdered in a mortar (except the powdery form of AA, sample). A suitable amount of powdered sample was dissolved in ultra-pure water in a 50.0 mL calibrated flask, which was the stock solution. Then further dilutions (1:10, 1:20, 1:100) were prepared with ultra-pure water in 10.0 mL calibrated flasks, which were the working sample solutions used for analysis. Simultaneously, the stock solution aliquots of

2.0 mL were centrifuged at 5000 rpm for 10 min at 20 °C. After the samples were centrifuged, identical dilutions were made with ultra-pure water as with the non-centrifuged stock solution to prepare working solutions. The samples were further treated according to the procedure and optimized conditions pH, the concentration of BCP reagent, time reaction, and instrumental parameters. Samples were measured in different concentrations (1:10, 1:20, 1:100), centrifugated and non-centrifugated, immediately after cooling and 30 min after, and with all filters open and 1%T. The results were calculated from a two-calibration curve using AA as a standard. Results are presented in Table 5.

Samples containing zinc (samples 1 and 2) must be centrifuged and analyzed 30 min after the reaction. Sample 1 contains fluorescent riboflavin and it requires analysis with 1% T. The dilution corresponding to this type of sample is 1:10. Samples 3 (containing iron(II) lactate), sample 4 (containing paracetamol), and sample 5 (also containing other vitamins—multivitamin) require to be centrifuged, diluted 1:10, and analyzed after 30 min with 1% T. Sample 6 requires to be centrifuged because it contains cellulose and talc which can interfere with the analysis if not precipitated. It requires a 1:20 dilution and is analyzed with 1% T.

Samples 8 and 9 can be analyzed without centrifugation immediately after the completing of the reaction. Sample 4 was diluted 1:10 and analyzed with all filters opened.

Samples 5 and 9 are multivitamins, but sample 5 requires centrifugation because it contains inulin which is not well soluble in water, i.e., its solubility changes with temperature [26, 27]. The difference in the analysis is that sample 5, which is multivitamin and contains a small amount of ascorbic acid (40 mg/tablet) should be diluted in a ratio of 1:20 and analyzed with 1% T due to the influence of other vitamins present in the sample. Samples 10 and 11 must be centrifuged before analysis because they contain inulin and talc which may limit the analysis especially in sample 11 where the AA content is lower. Also, both samples are diluted at 1:10. Sample 6 was also diluted at 1:20, centrifuged, and 30 min after the reaction was complete with all filters open. A sample of vitamin C containing 20 mg or less of bioflavonoids and 40 times more ascorbic acid was analyzed at a 1:10 dilution and in all filters open. Samples containing more than 20 mg of bioflavonoids must be diluted at 1:20 and worked with 1% T. The ascorbic acid content is 20 times higher than the bioflavonoid content.

Sample 15 could not be analyzed by the proposed method, even after centrifugation and dilution. It is believed that in addition to containing microcrystalline cellulose and magnesium stearate, it also contains croscarmellose sodium, which is cross-linked carboxymethyl cellulose. The crosslinking of carboxymethyl cellulose reduces the solubility in water, and at the same time allows the material to swell and absorb multiple weights in water. As a result, it provides superior drug dissolution and disintegration characteristics, thus improving the bioavailability of the formulas by bringing the active ingredients into better contact with body fluids. Its purpose in most pills, including dietary supplements, is to help the pill break down quickly in the gastrointestinal tract. If the tablet disintegrant is not included, the tablet may break down too slowly, in the wrong part of the gut, thus reducing the effectiveness and bioavailability of the active ingredients.

Table 5 Results of analysis of pharmaceutical samples determined by the developed method and by the iodine titration

Sample	Proposed method (mg AA/tablet)	Labeled value (mg AA/tablet)	Titration with Iodine (mg)	Recovery (%)	P test	T test
1	390.51 ± 0.06	400	576.50 ± 0.07	97.62	0.0005	4.85
2	470.49 ± 0.25	500	486.80 ± 0.07	94.09	0.4790	0.78
3	134.35 ± 0.08	134	81.50 ± 0.001	100.26	0.5671	0.59
4	201.62 ± 0.08	200	198.40 ± 0.07	100.81	0.1191	1.88
5	48.76 ± 0.29	60	66.10 ± 0.07	81.26	0.0001	1.84
6	462.07 ± 0.12	500	442.20 ± 0.07	92.41	0.2454	1.36
7	1210.34 ± 0.18	1000	1175.38 ± 0.07	121.03	0.0007	0.53
8	257.62 ± 12.60	240	331.30 ± 0.21	107.34	0.0001	9.37
9	44.63 ± 0.92	40	116.00 ± 0.35	111.58	0.0003	5.25
10	224.58 ± 0.08	250	268.00 ± 0.14	89.83	0.0001	21.24
11	47.15 ± 0.18	50	44.18 ± 0.07	94.30	0.7650	0.53
12	906.94 ± 6.57	1000	1351.20 ± 0.07	90.67	0.0297	3.31
13	631.88 ± 0.13	500	537.18 ± 0.07	126.38	0.0013	5.80
14	646.04 ± 0.13	500	437.86 ± 0.07	129.21	0.0029	6.48
15	1082.84 ± 1.56	500	533.47 ± 0.07	216.57	0.0001	36.21

Samples containing talc, inulin, cellulose, magnesium salts of fatty acids (in the form of magnesium stearate) require centrifugation before analysis. Samples containing hydroxypropylmethylcellulose do not require centrifugation because this form of cellulose is soluble in water. Multivitamin samples must be analyzed at 1% T. Samples containing zinc, iron, paracetamol should be diluted 1:10, centrifuged, and analyzed at 1%T.

Examination of the influence of individual interferences that can be found in pharmaceutical samples confirmed that additional components that are in addition to ascorbic acid must be taken into account because the preparation of the sample and the analysis itself depend on it.

Results obtained with the proposed method show good agreement with those obtained by the titrimetric method and the amount of AA stated on the declarations provide by the manufacturers, of simpler composition.

The content of AA for the sample that contains bioflavonoids calculated as hesperidin (Bio-C), samples 13 and 14, was higher than the result obtained by the titrimetric method. For sample 4 that contained paracetamol and some additional components, the content of AA measured with the proposed method is in agreement with the content of AA according to the labeled value. The presence of Zn in the investigated sample showed an impact on the relative fluorescence intensity. The AA content results in samples (samples 1 and 2) were higher than that obtained by titration.

Based on this data, the influence of bioflavonoids, metals such as zinc, iron, calcium, and magnesium in form of their salts, paracetamol, and folic acid on the relative fluorescence intensity were examined (Table 6).

Table 6 Influence of interferences on the relative intensity of fluorescence for the determination of ascorbic acid.

Interfering substance	Concentration (mol L^{-1})	Change in signal (%)
Rutin	1×10^{-2}	−99.06
	1×10^{-3}	−94.35
	1×10^{-4}	−21.07
Hesperidine	1×10^{-3}	+66.33
	1×10^{-4}	+0.55
	1×10^{-5}	+35.89
Citric acid	1×10^{-2}	+14.95
	1×10^{-3}	+30.55
	1×10^{-4}	+6.73
Folic acid	1×10^{-2}	−99.49
	1×10^{-3}	−93.24
	1×10^{-4}	−32.78
Zinc chloride	1×10^{-3}	+112.99
	1×10^{-4}	−0.88
Iron (II) sulphate	1×10^{-6}	−23.28
	1×10^{-7}	−17.78
Paracetamol	1×10^{-2}	+200.40
	1×10^{-3}	+8.26
	1×10^{-4}	+36.46
Calcium carbonate	1×10^{-3}	−2.99
	1×10^{-4}	+35.48
Magnesium carbonate	1×10^{-2}	−5.27
	1×10^{-3}	+7.73
	1×10^{-4}	−18.49

10 Interferences

Rutin and hesperidin as citrus bioflavonoides, calcium carbonate, magnesium carbonate, citric acid, paracetamol, zinc chloride, folic acid, and iron(II) sulfate were investigated as possible interfering agents. By evaluating standard solutions of AA at one concentration of three different concentrations of the interfering substances, the effect of probable interfering substances on the relative intensity of the fluorescence signal of bromocresol purple was investigated. The concentrations of possible interfering substances were the same, higher, or lower than AA concentrations (Table 6). All of these chemicals can be detected as ingredients or other components in pharmaceutical samples alongside AA. The concentrations of most possible interfering substances were greater than those present in pharmaceutical samples.

From the results presented in Table 6, it is clear that interfering substances like bioflavonoids or metals could interfere with the fluorescence intensity and give the wrong results on the quantity of AA. Paracetamol, folic acid, bioflavonoids show to have a major impact on signal intensity. Other investigated substances were investigated in a higher concentration than those which are actually present in the sample of AA. The interfering activity of magnesium carbonate, or some other type of magnesium salts like magnesium salts of fatty acids, calcium carbonate could be prevented by centrifugation or by diluting the sample unlike bioflavonoids, Zn, Fe, etc., which required some other different treatments.

11 Discussion

The purpose of this paper is to develop a new spectrofluorimetric method for the determination of ascorbic acid in pharmaceutical samples because spectrofluorimetry is a simple, low-cost, sensitive method that enables the measurement of low concentrations of the test analyte. After

optimizing the method, which required testing different conditions under which the reaction between AA and BCP takes place, the goal was to test the method on as many pharmaceutical preparations containing various additional components besides AA.

To achieve the goal, the method was tested on fifteen different samples having different AA content as well as additional components including bioflavonoids, iron, zinc, cellulose, citric acid, folic acid, paracetamol, etc. All samples were tested without prior preparation as well. with prior preparation involving centrifugation. Samples were analyzed with 1% T or with all filters open, as well as immediately after incubation and 30 min after incubation. Also, samples were prepared in three different dilutions to reduce the effect of interference, which was also subsequently examined. Since there is no universal method for all types of samples, this method also has its limitations and requirements related to sample preparation and analysis. Linearity area is narrow, which represents a limitation of this method and requires that samples be prepared in such a narrow concentration range. Samples that contain iron, zinc, paracetamol, magnesium salts like magnesium salts of fatty acids, cellulose, talc, inulin demand to be centrifugated before analysis. In samples that contain hydroxypropylmethylcellulose, it is not necessary to be centrifugated because this form of cellulose is soluble in water. Samples that contain bioflavonoids and riboflavin must be analyzed with 1%T because these components tend to fluoresce. Multivitamin samples must be analyzed at 1% T. Finally, samples that have a smaller amount of AA, and a lot of additional components are required to be diluted in ratio 1:20 so the influence of additional components can be reduced.

Table 7 compares performance characteristics such as linearity range and limit of detection reported spectrofluorimetric methods. It is visible that some of the methods have a narrow linear range while others have a wider. Comparing the proposed method with other methods in the literature in terms of the field of linearity [14, 28, 29] the proposed method does not have a wide field of

Table 7 Comparison between previously reported spectrofluorimetric methods for the determination of ascorbic acid and the proposed method

Reagent(s) Used	λ_{ex} i λ_{em} (nm)	Linearity range (μmol L^{-1})	LOD (μmol L^{-1})	Samples	References
2,3-Diaminonaphthalene	400, 520	11–1700	2.27	Pharmaceutical samples	[28]
1,2-Diamino-4,5-dimethoxybenzene	370, 458	7.3–1130	0.0051	Blood serum	[17]
Cerium(IV) ion	303, 340	0.1–8.0	0.016	Pharmaceutical samples	[21]
Mono-3-[6-N(4-carboxyphenyl)]-beta-cyclodextrin	280, 352	0.28–450	0.068	Real samples	[22]
o-Phenylenediamine	360, 430	1.0–5.6	nd	Beer, wine, urine and pharmaceutical samples	[28]
o-Phenylenediamine	356, 440	11–567	7.38	Pharmaceutical samples	[29]
o-Phenylenediamine	360, 430	0.5–170 0.3–230	0.113 0.034	Pharmaceutical samples, non-alcoholic beverages and blood serum	[19, 22, 30]
Ti(III)	227, 419	1–10	0.8	Real samples (leon juice, hematinic capsule, shampoo)	[31]
Methylene blue	664, 682	0.3–6	0.25	Pharmaceutical samples	[15, 32]
Methylene blue	660, 694	0.6–230	1.3	Non-alcoholic beverages	[33]
Methylene blue	664, 686	5.68–45.42	1.124	Tablet, sweetlet, effervescent tablet, and ready to drink product	[34]
Pyronine Y	516, 554	0.13–2.0	0.068	Pharmaceutical samples, fruits, vegetables and juices	[16]
TPTZ i $FeCl_3$	393,790	5.4–540	0.77	Pharmaceutical samples	[14]
Acriflavine	265,505	11.36–56.78	0.4542	Pharmaceutical nutritional supplements	[35]
L-tyrosine	275, 300	0.03–30	0.01	Tablets, injections, effervescent tablet, chewable tablets, multivitamine formula with mineras	[36]
Thiamina and potassium ferricyanide	367, 441	0.086–1.5	0.026	Tablets and juices	[37]
Bromocresol purple	**318, 641**	4.65–46.5 4.77–47.7	**0.877**	Pharmaceutical samples	**Present work**

The bold values represent the characteristics of the proposed method

linearity Table 2, which is also its biggest limitation. The detection limit Table 7 is also not better than few other proposed methods [14–16, 21, 29, 31] for the determination of AA in pharmaceutical samples. However, this method is promising for the simple and relative rapid determination of AA. It can be applied to many pharmaceutical samples containing various additional components..

12 Conclusions

For the quantification of ascorbic acid, a variety of spectrophotometric and spectrofluorimetric methods are routinely utilized. They're utilized in the regular analysis since they're typically simple, operative, quick, cheap, and sensitive. Because there is no single method for all types of samples, each documented method, including this one, finds use for a different type of sample, which necessitates a different type of sample, which demands a new form of sample preparation.

The proposed approach is simple to use and has a high sensitivity. Except for samples that include interfering compounds that must be eliminated before analysis, sample preparation is simple. Depending on the additional components present in the sample, the sample will be processed and evaluated differently. The method has the benefit of being able to be used on a large number of pharmaceutical samples. The method showed good precision and repeatability as well as satisfactory recovery values in terms of accuracy. A limitation of the method is the narrow linearity range. To our knowledge, there are no other reports on the spectrofluorimetric method for estimating AA content with bromocresole purple.

Further research could include applying this method to the real samples or trying to add some components or metal ions that could affect on the reaction to achieve better reproducibility, reaction rate, better linearity range, and maybe reduce sample preparation.

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Availability of data and material On the request from the corresponding author.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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