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COMPARISON OF FLUORESCEIN AND POTASSIUM PERMANGANATE IN FAST LOW COST SCREENING METHODS FOR DETERMINATION OF FOLIC ACID IN PHARMACEUTICAL TABLETS

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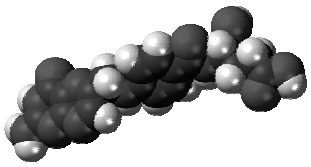
The screening methods developed, are simple, rapid and economic. Comparison showed that either of the two methods studied is suitable for the fast screening of folic acid in pharmaceutical tablets in rural areas, using as fluorescent probes either fluorescein or potassium permanganate. The fluorometric interaction between fluorescein or potassium permanganate and folic acid using fluorescence spectroscopy was studied. A decrease in fluorescein or potassium permanganate intensities was observed with the increase in the concentration of folic acid. The limit of detection was calculated with the formula C_{LOD} = 3Sb/m defined by IUPAC (where Sb is the standard deviation of the blank and m is the slope of the linear calibration curve). The percentage recoveries of folic acid were found to be 99.07 % and 97.17 %, respectively and the drug content of folic acid was found to be 4.95 mg (± 0.17%) and 4.86 mg (± 0.23%), respectively, using either fluorescein or potassium permanganate in a proposed assay as "fluorophore".



INTRODUCTION

Folic acid (FA), IUPAC name (2S)-2-[[4-[(2-amino-4-oxo-1H-pteridin-6-yl)methylamino]benzoyl]amino]pentanedioic acid[1-4] also chemically known as N-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid or pteroyl-L-glutamic acid is part of a widely distributed vitamin B family (Vitamin B_9, Vitamin B_c) often referred in the food industry as the folate (to denote the difference with folic acid) where it can be found in a variety of fruits and vegetables. In chemistry the term folate is the ionic form and folic acid the protonated ion with IUPAC clearly defining folate as the preferred synonym for pteroylglutamate and folic acid as the preferred synonym for pteroylglutamic acid. Although folic acid is sometimes in literature[5] referred to as a water soluble substance (or water-soluble B vitamin) one have to be very careful as the free acid, folic acid is only slightly soluble in water (0.01 mg/mL at 0 ºC), is insoluble in aqueous solutions below pH 5, but soluble in 1 mol/L NaOH (up to 50 mg/mL). FA is made up of the building blocks, pterin, p-amino-benzoic acid and glutamic acid, the chemistry and biochemistry of folic acid and folic acid derivatives (e.g. folates, pterins etc.), has been studied by various groups[3,4,6-13] and reviewed in various publications.[3,14,15] The various studies of folic acid (FA) revealed that depending on the pH of the aqueous solutions, FA exists in different ionic states with structural formulae of FA in a neutral, anionic, cationic and bi-cationic form[3,4,6-13] and

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Tyagi and Penzkofer[4] furthermore found that FA is only weakly fluorescent in all ionic forms. Folic acid after ingestion is transformed into its active form (tetrahydrofolate) in the small intestine, which is a coenzyme for various metabolic processes including the synthesis of purine and pyrimidine nucleotides, and hence in the synthesis of DNA.[16-18] It is also involved in some amino acid conversions and in the formation and utilization of formate. Furthermore it aids as an anti-anemia and growth factor. It is essential to life and plays an extremely important role in many biological events. Sufficient dietary intake of FA during pregnancy prevents neural tube defects of the fetus like spina bifida, while the deficiency of folic acid increases the risk of cardiovascular diseases. FA is also routinely prescribed to pregnant women and widely used in the treatment and prevention of megaloblastic anemias.[18]

Fluorescein, originally first synthesized by Adolf Baeyer in 1871 as part of the phthaleins dyes (prepared from phthalic anhydride and resorcinol),[19-21] forms part of the xanthenes derivatives as one of the most popular fluorophores with a strong fluorescent emission intensity and due to this the fluorescence of fluorescein was studied intensively.[22-26] Fluorescein in aqueous solution occurs in cationic, neutral, anionic and dianionic forms making its absorption and fluorescence properties strongly pH dependent.[22-26]

The extent of oxidation of an organic substrate by potassium permanganate depends on the pH of the medium. The heptavalent manganese changes to Mn(VI) in alkaline medium while in neutral and acidic medium the permanganate is further reduced forming ultimately Mn(II).[27]

Several methods have been reported in the literature for the determination of folic acid. These include spectrofluorimetric,[28-31] electrochemical,[32-35] chromatographic,[36-39] and chemiluminescence methods.[40-43] Both Waibadur *et al.*[40] and Zhao *et al.*[43] used complex materials as reagents for the chemiluminescence (CL) determination of folic acid; Waibadur *et al.*[40] used tris(2,2'-bipyridyl)ruthenium(II) and Zhao *et al.*[43] $KAuBr_4$ in a Au(III)-luminol CL system. Furthermore both groups used flow injection analysis assemblies in their proposed systems that are expensive and more complex to be used by normal operators in in-field on-site areas.

The main purpose of this work was to find a fast low cost optical screening method for the determination of folic acid in pharmaceutical tablets comparing the fluorescence of fluorescein and potassium permanganate as possible fluorophore reagents. Fluorescein for example cost far less than tris(2,2'-bipyridyl)ruthenium(II) (with a factor of more than 100 times expensive) and $KAuBr_4$ (with a factor more than 150 times more expensive) and in the case of potassium permanganate the price factor of tris(2,2'-bipyridyl)ruthenium(II) and $KAuBr_4$ is even by far larger and more expensive.

EXPERIMENTAL

1. Reagents and materials

Folic acid (FA), potassium permanganate ($KMnO_4$), dimethylsulfoxide (DMSO), sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$) and sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) were obtained from Sigma-Aldrich. Fluorescein was purchased from Fluka.

Phosphate buffer solutions (PBS, 0.1 mol/L) with pH = 4-8 were obtained by using different ratios between NaH_2PO_4 and Na_2HPO_4. The pH was adjusted with 0.1 mol/L HCl or NaOH solutions to the required pH needed in the measurements. All solutions were prepared using deionized water obtained from a Direct-Q 3 Water Purification system (Millipore Corporation, France). The analyzed pharmaceutical products were Acifol tablets produced by Zentiva S.A., Roumania, 1 tablet containing 5 mg of folic acid; they were collected from a local pharmacy.

The standard stock solution of folic acid (10^{-3} mol/L) was prepared by dissolving 0.044 g in 0.1 mol/L NaOH and diluting the volume to 100 mL with deionized water. The standard solutions of FA (10^{-4} – 10^{-8} mol/L) were prepared from the stock solution by serial dilution and buffered with phosphate buffer of different pHs. The prepared stock solutions were stored in a refrigerator and were protected from light.

A stock fluorescein solution (10^{-2} mol/L) was prepared by dissolving 0.332 g in DMSO and then was diluted up to mark using PBS (pH = 8.0) in a 100 mL volumetric flask. The standard solution of fluorescein (10^{-3} mol/L) was prepared from the stock solution by serial dilution and buffered with phosphate buffer of different pHs.

The standard stock solution of potassium permanganate (10^{-3} mol/L) was prepared in deionized water and 0.1 mol/L HCl. Working solutions were prepared daily before use by preparing required dilutions of the stock solutions.

2. Apparatus

Fluorescence measurements were conducted with a spectrometer QE65000 from Ocean Optics (Dunedin, Florida) equipped with a xenon lamp (HPX 2000). The pH measurements were performed using a CyberScan PCD 6500 Multiparameter.

3. Procedure

The proposed screening method is very simple, quick and efficient and was developed for the determination of folic acid based on the quenching effect on the fluorescence intensity of fluorescein or potassium permanganate used as fluorescent probes. Fluorometric interaction between fluorescein or potassium permanganate and folic acid was studied using fluorescence spectroscopy.

4. Sample preparations

Ten tablets of Acifol (1 pharmaceutical tablet of Acifol contains 5 mg of folic acid) were used. Each tablet was placed in a 100 mL volumetric flask and buffered with phosphate buffer (pH = 8.0). The dissolved solution of this drug was filtered using a filter (CA 0.2 μm) to isolate the insoluble excipients. Finally, the samples were diluted with PBS pH = 8.0 to meet the analyte concentration within the range of the calibration curve. The solutions were kept in the fridge.

RESULTS AND DISCUSSION

1. Effect of pH

For the determination of the optimum pH, accurate volumes of standard 10^{-4} mol/L folic acid and 10^{-3} mol/L fluorescein solutions with the pH values between 4 and 8 were added in different volumetric flasks of 10 mL. The fluorescence spectra of the solutions in the mixture (FA with Fluorescein at different pH values) (Fig. 1) were measured in a 1 cm quartz cell at a wavelength of 531.55 nm. The integration time was 180 ms, scans to average of 10 and boxcar width of 8.

The fluorescence intensities were plotted against pH (Fig. 2). As shown in Fig. 2 the maximum intensity of fluorescence was achieved at a pH 8. Therefore, pH = 8 was chosen as the optimum pH.

2. Influence of folic acid concentration on fluorescein

According to Tyagi and Penzkofer[4] FA is only weakly fluorescent in all ionic forms and this was confirmed by the overlay fluorescence spectra of different concentrations of FA alone at pH = 8 in Fig. 3 where the fluorescence intensity (counts) varies between 2460 and 2580. If the overlay fluorescence spectra solutions of different concentrations of FA (10^{-3} – 10^{-8} mol/L FA) in 10^{-3} mol/L fluorescein (Fig. 4) and of a solution of 10^{-3} fluorescein alone (Fig. 4) are compared, the following results were revealed.

(i) The fluorescence intensity (counts) increases tremendously to an increased value between 2460 and 8000 due to the fluorophores interaction from fluorescein.

(ii) Folic acid has a quenching effect on the dipole-dipole interaction in fluorescein that became so strong that the fluorescence intensity of the solution of a mixture of 10^{-3} mol/L FA and 10^{-3} mol/L fluorescein is less that the fluorescence intensity of 10^{-3} mol/L fluorescein alone.

(iii) Furthermore the relative quenching effect of different concentrations of FA is indirect proportional to the increase in fluorescence intensity values of 10^{-3} mol/L fluorescein in the mixtures with decreasing concentrations from 10^{-4} mol/L FA to 10^{-8} mol/L FA.

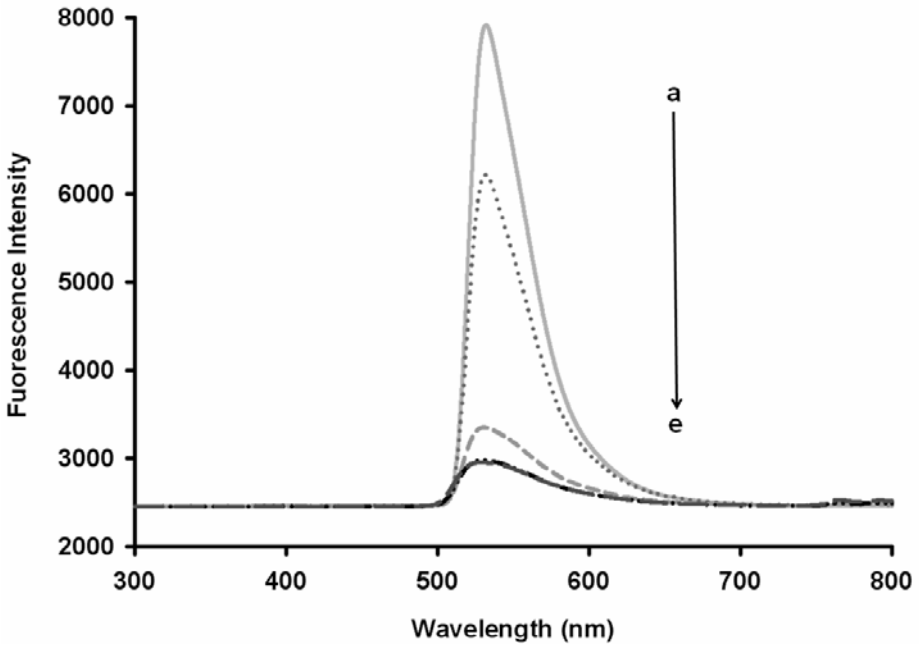

Fig. 1 – Overlay fluorescence spectra of mixed solutions (FA, 10^{-4} mol/L; Fluorescein, 10^{-3} mol/L, PBS); (a) pH = 8, (b) pH = 7, (c) pH = 6, (d) pH = 5 and (e) pH = 4.

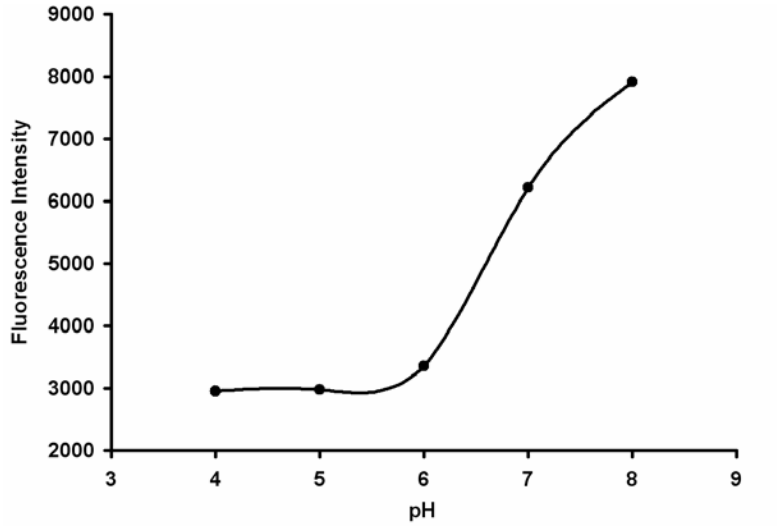

Fig. 2 – Influence of pH on fluorescence intensity. Conditions: [FA], 10^{-4} mol/L; [Fluorescein], 10^{-3} mol/L; PBS pH = 4-8.

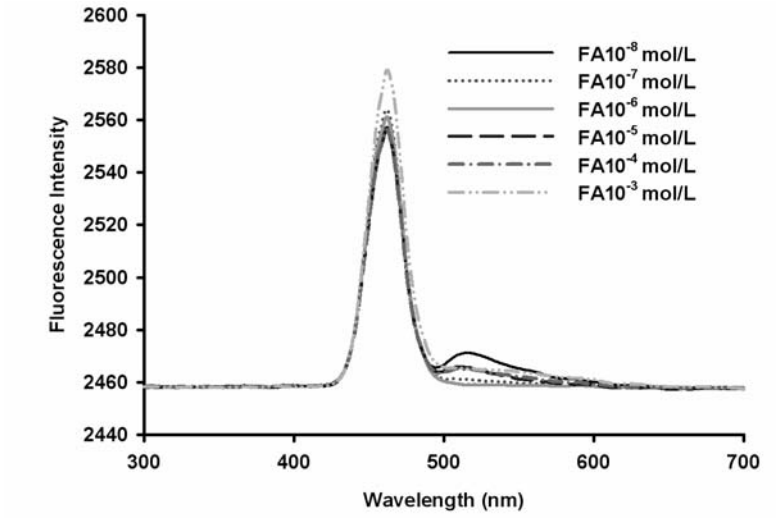

Fig. 3 – Fluorescence spectrum of 10^{-4} mol/L FA solution. Conditions: PBS pH = 8.

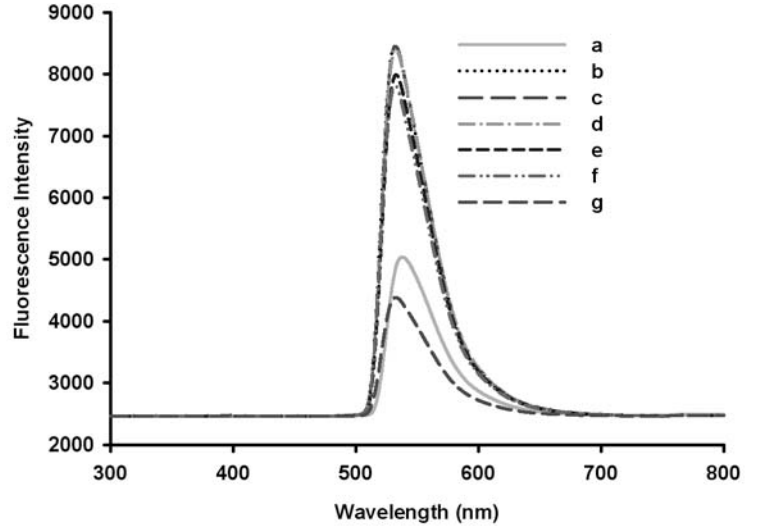

Fig. 4 – Overlay fluorescence spectra of mixed solutions (Conditions: PBS pH = 8):
(a) Fluorescein alone, 10^{-3} mol/L, and also Fluorescein, 10^{-3} mol/L with: (b) FA, 10^{-8} mol/L, (c) FA, 10^{-7} mol/L, (d) FA, 10^{-6} mol/L, (e) FA, 10^{-5} mol/L, (f) FA, 10^{-4} mol/L and (g) FA, 10^{-3} mol/L.

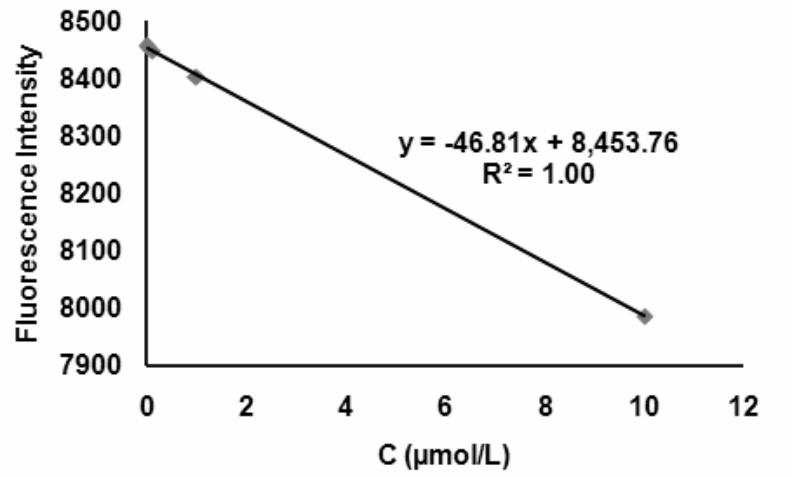

Fig. 5 – Influence of concentration of FA on fluorescence intensity. Conditions: [Fluorescein], 10^{-3} mol/L; [FA], 10^{-8} mol/L to 10^{-5} mol/L; PBS pH = 8.

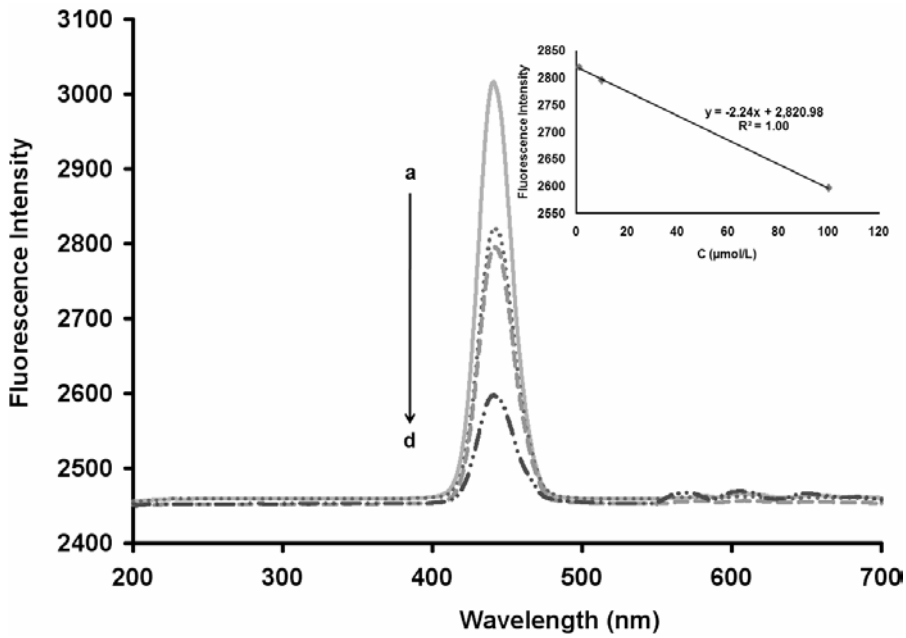

Fig. 6 – Fluorescence quenching of $KMnO_4$ upon addition of increasing amount of FA; (a) 0 µmol/L, (b) 1 µmol/L, (c) 10 µmol/L, (d) 100 µmol/L. Inset: Calibration curve of concentration of FA against fluorescence intensity. Conditions: [$KMnO_4$], 10^{-3} mol/L; PBS pH = 3.

Table 1

Determination of folic acid in pharmaceutical tablets

Sample	**Amount labeled of acid folic (mg)**	**Amount found of folic acid (mg) ± RSD (%)***	**Recovery (%)**
Acifol	5	4.95 ± 0.17(i)	99.07
		4.86 ± 0.23(ii)	97.17

* Relative standard deviation for ten measurements (n = 10)
(i) With fluorescein; (ii)With potassium permanganate

A series of 6 standard solutions containing folic acid (FA) solutions with concentrations ranging from 10^{-8} to 10^{-3} mol/L and a solution concentration of 10^{-3} mol/L fluorescein in each were prepared in PBS pH = 8 and these solutions were used to measure the fluorescence intensity. We used an integration time of 200 ms, scans to average of 10 and width boxcar of 8. The reaction of folic acid with fluorescein had a maximum around 532 nm. The influence of the concentration of folic acid in the different standard FA solutions at maximum fluorescence intensity in the concentration range 10^{-8}-10^{-3} mol/L were measured revealing a linear concentration range only between 10^{-8} and 10^{-5} mol/L (Fig. 5) and the correlation coefficient was found to be 0.9999. (We could not use the fluorescence intensities for 10^{-4} and 10^{-3} mol/L FA due to the "enhanced" quenching effects of FA on fluorescein at these concentrations). The limit of detection was 1.42 x 10^{-8} mol/L, calculated with the formula C_{LOD} = 3Sb /m defined by IUPAC (where Sb is the standard deviation of the blank signals and m is the slope of the calibration curve). Fig. 5 shows that the higher the concentration of folic acid the lower the fluorescence intensity of fluorescein.

3. Influence of folic acid concentration on potassium permanganate

A series of 4 standard solutions containing folic acid (FA) solutions with concentrations ranging from 10^{-6} to 10^{-3} mol/L and a solution of 10^{-3} mol/L potassium permanganate in each were prepared at a pH = 3 and these solutions were used to measure the fluorescence intensity. The influence of the concentration of folic acid in the mixture on maximum fluorescence intensity in the concentration range 10^{-6}-10^{-3} mol/L; linear range of concentration was between 10^{-6} and 10^{-4} mol/L (Fig. 6). The correlation coefficient and detection limit were 0.9998 and 2.95x10^{-6} mol/L, respectively.

A maximum around λ = 441 nm was obtained after the reaction of folic acid with potassium permanganate (Fig. 6). A decrease in the intensity of potassium permanganate with an increase in the concentration of folic acid is observed in Fig. 6.

4. Analytical application

The proposed method was applied for the determination of folic acid in pharmaceutical tablets (Acifol tablets). The obtained results for the determination of FA in the tablets using the linear calibration curve, were compared with those declared and are presented in Table 1. There were no significant differences between the amount declared and that obtained by the method that was presented.

CONCLUSIONS

The fluorometric interaction between fluorescein or potassium permanganate and folic acid using fluorescence spectroscopy was studied. A decrease in fluorescein or potassium permanganate intensities was observed with the increase in the concentration of folic acid.

The method was successfully applied for the determination of folic acid in pharmaceutical tablets. The screening methods developed, are simple, rapid and economic. Comparison showed that either of the two methods studied is suitable for the fast screening of folic acid in pharmaceutical tablets in rural areas, using as fluorescent probes either fluorescein or potassium permanganate. The limit of detection was calculated with the formula C_{LOD} = 3Sb/m defined by IUPAC (where Sb is the standard deviation of the blank and m is the slope of the linear calibration curve). The percentage recoveries of folic acid were found to be 99.07 % and 97.17 %, respectively and the drug content of folic acid was found to be 4.95 mg (± 0.17%) and 4.86 mg (± 0.23%), respectively, using either fluorescein or potassium permanganate in a proposed assay as "fluorophore".

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