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Spectrophotometric Determination of Mesalazine via Oxidative Coupling Reaction

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ABSTRACT
A simple and sensitive spectrophotometric method for the determination of a mesalazine in the aqueous solution. The method is based on the oxidative coupling reaction between mesalazine and Phenothiazine in the presence of potassium dichromate as oxidizing agent to forming a green color water soluble dye showing maximum absorbance at 610nm. Beer's law is obeyed in the concentration range of (0.1-11) µg/ml, with Correlation confection (R^2) value more than 0.999.Molar absorptivity ($2.055x10^4$) $L.mole^{-1}.cm^{-1}$ and Sandall's sensitivity (0.007451) $µg/cm^2$. This method has been successfully applied for the determination of mesalazine in various pharmaceutical preparations.

Keywords: Oxidative Coupling- Mesalazine- Phenothiazine

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INTRODUCTION

Mesalazine or 5-amino salicylic acid (5-ASA) which is also known as mesalamine[1][17]. Mesalazine is crystal white or pink powder with melting point of 280 °C and can dissolve in hot water, and some solutions acids and bases diluted [2]. It has molecular formula ($C_7H_7O_3$) and The chemical structure in the following figure-1- [3]

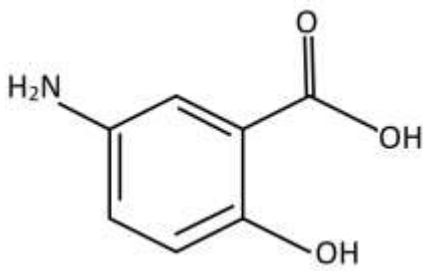

Figure 1: 5-Amino-2-hydroxybenzoic acid M.wt= 153.14 $g.mole^{-1}$

It is prepared chemically out of reducing the chemical compound and meta nitrobenzoic acid by using zinc powder and hydrochloric acid [4].

Mesalazine is used as an effective treatment for Chronic bowel inflammation which is a group of autoimmune diseases that cause inflammation of the small and large intestine. This inflammation includes two forms, one of which is moderate to severe Crohn's disease and the other ulcerative colitis [5] and one of the symptom of include rectal and anal inflammation medicines are taken to treat these infections in several forms including mouth ,injections and suppositories [6]. Among the drugs used for this purpose is 5- amino sassilic acid as well as drugs for immunomodulators such as Azathioprine .Studies have shown that most pharmaceutical compounds have side effects such as headache, diarrhea, nausea and lower abdominal pain [7].

Many and various analytical methods have been used to determinate Mesalazine of which are diazotization and coupling with various Reagents [8, 9] which depend on reaction conditions. There are several Spectrophotometer methods that have been described using oxidative coupling reactions [10]. There are other methods which depend on the reaction of Schiff bases forming [11], and also reactions of forming charge transfer complexes [12], also determind by electrochemical methods [13], and chromatographic methods such as (UPLC), has been used [14].

EXPERIMENTAL

Apparatus: Absorption measurements have been recorded and all spectral measurements by using Shimadzu UV-1800 double –beam spectrophotometer, supplied with 1-cm matched quartz cells.

Chemical and Reagents

All reagents and chemical substances used are of the high purity.

Standard mesalazine solution (100 µg/ml)

The solution has been prepared by dissolving (0.01 g) of pure mesalazine in 5 ml ethanol and dilute the solution with distilled water up to the mark in a 100 ml volumetric flask. The solution is stable for many days so, it has been preserved in a dark color flask.

Phenothiazine ($5x10^{-3}$ M)

This solution was prepared by dissolving (0.0996 g) of Phenothiazine in(10)ml Absolute ethanol and completed to 100 ml in a volumetric flask by same solvent.

Potassium Dichromate Solution ($5x10^{-3}$ M)

The oxidizing agent solution has been prepared by dissolving (0.147 gram) of Potassium dichromate in an amount of distilled water and diluted to the mark in 100ml volumetric flask with same solvent.

Recommended procedures

1ml of phenothiazine(0.005 M) was added in to a series of 25ml volumetric flask and 1ml potassium dichromate (0.005 M) followed by the addition of increasing concentrations (0.1-11) µg/ml of mesalazine then the mixtures were shaken

and diluted to the mark with distilled water. The absorbance was measured after 45min at 610nm against corresponding reagent blank.

Pharmaceutical Solutions

Tablets: Ten tablets of measalizen (400 mg) have been taken and weighed on form finely powdered, then an amount equivalent to one tablet was taken and dissolved in (5 ml) of ethanol and (75ml) distilled water, then filtered through a Whatmann41 filter paper and the filtrate was diluted with distilled water to 100 ml in a calibrated flask.

The Pharmaceutical Solution- (Asacol) Suppository

Asacol suppositories: Weight and mix the contents of Five suppositories of [each one containe 500mg measalizen pure] and an accurately weighed of powder equivalent to 0.01g measalizen was dissolved in (5 ml) absolute ethanol and amount of distilled water with heating in a water-bath to complete the dissolving process .After that filteration and washing paper filtration with distilled water.Then the volume of filterat was completed to100ml distilled water.

Pharmaceutical Solutions- Enema

The enema contains one gram of mesalazine dissolved in 100 ml of water. Emptied contents of the enema in a volumetric flask of (1000 ml) and then shaking for about five minutes to obtain the homogeneity of the solution and complete the volumetric flask to the mark with distilled water, After that the solution is filtered and the filtrate is a of the pharmaceutical preparation solution with a concentration of (1000 µg/ml). Different quantities equivalent to concentrations (2, 4, 6 µg/ml) were prepare according to the proposed method to find a concentrations mesalazine in pharmaceutical preparation.

RESULTS AND DISCUSSION

One ml of solutions prepared from the reagent and the oxidative agent has been used and added to one ml of mesalazine solution of (100 µg/ml) concentration in a volumetric flask of (25 ml). Then the measurement of absorption of the coloured product at wavelength (610 nm) against the blank solution.

Principle of the Method

The study of the reaction was under the optimized conditions using Job's method and a molar ratio method. The results obtained Fig. (2) Shows that a (1:1) mesalazine to Phenothiazine reagent was formed.

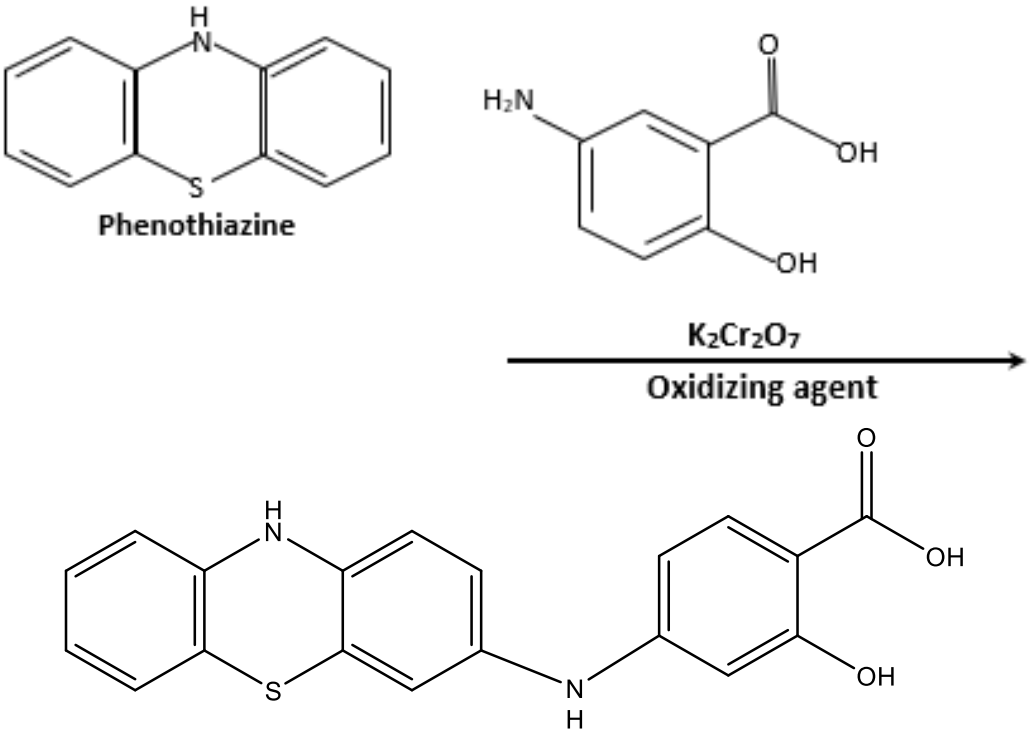

Figure 2: oxidation and coupling

Studying Reaction Conditions

Various factors influencing the absorption of colored dye as a result of the oxidative coupling reaction have been studied.

Studying the Optimal Amount of the Reagent ($5x10^{-3}$ M)

In a series of volumetric flasks of (25 ml), then adding increased volumes of Reagent (0.5-3 ml) and (2 ml) of oxidizing agent both have a concentration of ($5x10^{-3}$ M), then (1 ml) of Mesalazine were added (100 µg/ml) at room

temperature. Then all flasks have been filled up to 25 ml with distilled water to the mark. Absorption measurements at wavelength of (610 nm) against its blank solutions. The results obtained in Table (1) indicate that the use of 1 ml of (5×10^{-3}M) Phenothiazine reagent gave the maximum colour intensity, and this volume was used in later studies.

Table 1: Effect the volume of Reagent quantity on absorption

Volume of (ml) reagent $5x10^{-3}$M	0.5	1	1.5	2	2.5	3
Absorbance	0.537	0.544	0.542	0.541	0.540	0.536

Effect of the Oxidizing Agent

A study of different types of oxidative agent such as potassium dichromate, iron nitrates, and Cerium (IV) sulfate were investigated. The results summarized in table (2) indicated that the best oxidizing agent has been potassium dichromate whose concentration was ($5x10^{-3}$M). Additional increased volumes of the oxidizing agent of potassium

dichromate have been added to a series of volumetric flasks of (25 ml) with fixed amount of Reagent (1 ml), then (1 ml) of mesalazine of (100 µg/ml) concentration, has been added, then diluted by distilled water and the solutions have been left for five minutes; afterwards, solution absorbance was measured against their blank solution, It has been found that the optimal amount of the volume of the oxidizing agent was (1 ml). The results obtained in table (3) indicate that the use of (1) ml of

(5×10^{-3}M) oxidizing agent gave the maximum colour intensity, and this volume was used in subsequent experiments.

Table 2: the effect of the type of the oxidizing agent on absorbance

Oxidizing agent (0.005)M	$K_2Cr_2O_7$	$Ce(SO_4)_2.4H2O$	$Fe(NO_3)_3.9H2O$
Absorbance	0.544	0.475	0.482

Table 3: the effect of potassium dichromate oxidizing agent

Volume of (ml) 0.005M $K_2Cr_2O_7$	0.5	1.0	1.5	2.0	2.5	3
Absorbance	0.531	0.547	0.545	0.543	0.539	0.530

Studying Oxidizing Time

1 ml of reagent and 1 ml of oxidizing agent were added to a series of 25 ml calibrated flasks, solutions were left to study oxidation time at different times, then 1ml of mesalazine was added, then complete the volume to the mark of the distilled water. The absorbance was measured at wavelength 610 nm is among the results obtained, as in Table No. (4). The best time is 5 minutes, which was relied upon in the subsequent study.

Table 4: Studying oxidizing time

(min) Time	3	5	10	15	20	25	30
Absorbance	0.547	0.553	0.550	0.548	0.543	0.542	0.540

Studying the effect of acid and base (0.1 M)

Use hydrochloric acid and sodium hydroxide, volume: (1 ml), concentration (0.1 M) independently, have been added to the constituents of the reaction which showed decrease a few in absorption, so in the following tests any adding of the acid and base has been excluded.

Studying effect of the Order of Addition

Various series of experiments have been carried out to determine the best additional order of the reacting substances. Table (5) shows the sequence of additions, according to which number (1) was the strongest to demonstrate the maximum absorption.

Table 5: the effect of addition order

Order number	Sequence of addition	Absorbance
I	R+O+D	0.553
II	D+O+R	0.534
III	D+R+O	0.523

Studying the effect of the surfactants

Study effect of various anionic, cationic and neutral surfactants tested for the investigation of the sensitivity of method. The results reveal that the presence of the surfactants has no remarkable effect on the intensity of the colour. Therefore, surfactants were excluded from this study. Therefore, the method has been carried out without using surfactants from the shown results in table (6).

It can be noticed that adding surfactants does not increase absorbance sensitivity [15].

Table 6: the effect of the surfactants

Surfactant added used	Absorbance / (mL) of Surfactant added				
	0.5	1.0	1.5	2.0	2.5
SDS($1x10^{-3}$) M	0.525	0.518	0.520	0.519	0.512
CTAB($1x10^{-3}$) M	0.533	0.529	0.517	0.522	0.516
Triton(X-100) 2%	0.535	0.524	0.528	0.527	0.514

The surfactants have been excluded in the following experiments for they have led to less absorbance.

Temperature and Reaction Time

The time effect on the stability of the absorbance of the coloured substance produced at different temperatures has been studied. The results obtained are given in Table (7), which shows that the room temperature was the best where it showed the highest absorbance for the dye produced after 45 minutes and was stable for 30 minutes.

Table 7: The effect of temperature on stability

After dilution	R.T	50	(0-5)
0	0.553	-	-
5	0.554	0.490	0.304
10	0.555	0.510	0.315
15	0.559	0.516	0.325
20	0.563	0.528	0.337
25	0.567	0.531	0.349
30	0.570	0.529	0.353
35	0.575	0.525	0.360
40	0.579	0.520	0.365
45	0.582	0.519	0.422
50	0.582	0.515	0.422
55	0.582	0.512	0.421
60	0.582	0.505	0.421
65	0.582	0.501	0.420
70	0.582	0.498	0.420
75	0.582	0.492	0.420
80	0.580	0.487	0.417
90	0.575	0.482	0.410
110	0.558	0.478.	0.406
120 (2hr.)	0.543	0.47	0.402

Final Absorption spectrum

After determining the optimum conditions described above, the final absorption spectrum was recorded for the coloured product wavelength of maximum absorption was appear at 610nm in contrast to the reagent blank which shows small absorption at λmax. Therefore, the 610nm wavelength of maximum absorption has been selected for subsequent experiments.

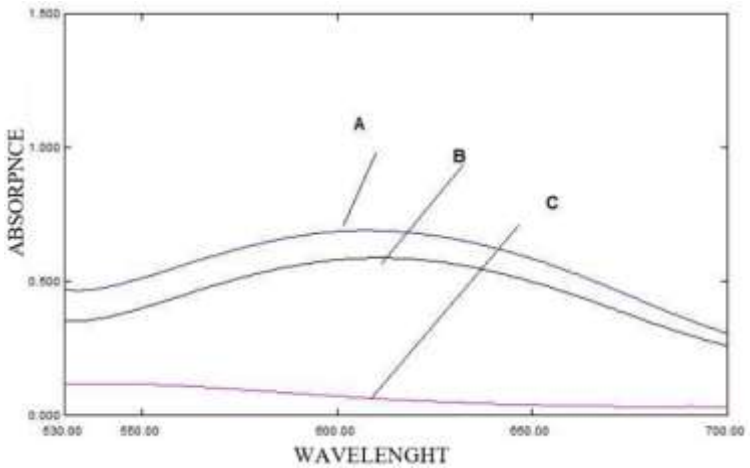

Figure 3: drawing of final absorption spectrum of 4µg/ml of the mesalazine (A) against distilled water (B) against blank solution (C) the blank solution against distilled water

General Method and Calibration graph

By using the optimal reaction conditions, the standard curve for mesalazine was prepared by adding increasing quantities of mesalazine (100 µg/ml) concentration in volumetric flasks of (25 ml) to cover the limits of Beer's Law for a range of concentrations from (0.1-11 µg/ml) to a fixed each one of volume of Reagent and oxidizing agent each (1 ml), with (0.005 M) concentration, and diluted with distilled water to the mark, The solutions were left at room temperature and the absorption of solutions was measured against their blank solutions at wavelength of (610 nm) as shown in figure (4), the molarity absorption value ($2.055x10^4$ $L.mole^{-1}.cm^{-1}$) and Sandall's sensitivity value (0.007451 g/cm^2) and the limit of detection LOD (0.043577322 µg/ml) and the limit of quantity LOQ(0.1452577422 µg/ml).

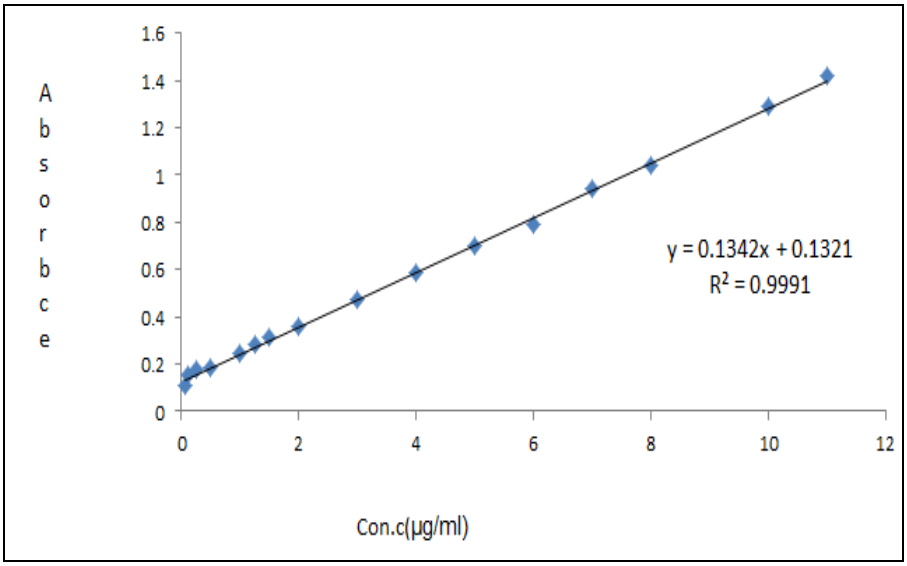

Figure 4: Calibration graph to the proposed method of estimating mesalazine

Accuracy and Precision

Three different concentrations of the mesalazine solution (100 µg/ml) studied and these three concentrations limits to Beer's Law, the average recovery and relative standard deviation have been calculated as illustrated in table (8). The results have been highly precise and accuracy. The results illustrated in (Table 8) indicate that the method was satisfactory.

Table 8: Accuracy and Precision

Mesalazine concentration of (µg)	Mesalazine concentration found	Recovery* (%)	RE* (%)	RSD* (%)
2	1.977	98. 85	-1. 15	0.714
4	3.975	99. 37	- 0.625	0.1402
6	5. 921	98. 68	-1. 316	0.208

* Average of five determination

Nature of the Complex

Both Job's method (the continuous variations method) and Mole- ratio method have been used to study the colored complex of the resulting from the oxidative coupling process between mesalazine and phenothiazine [16].

The results obtained in figure (5) indicate that the product of oxidative coupling was formed in the ratio of (1:1) for reagent and drug (mesalazine).

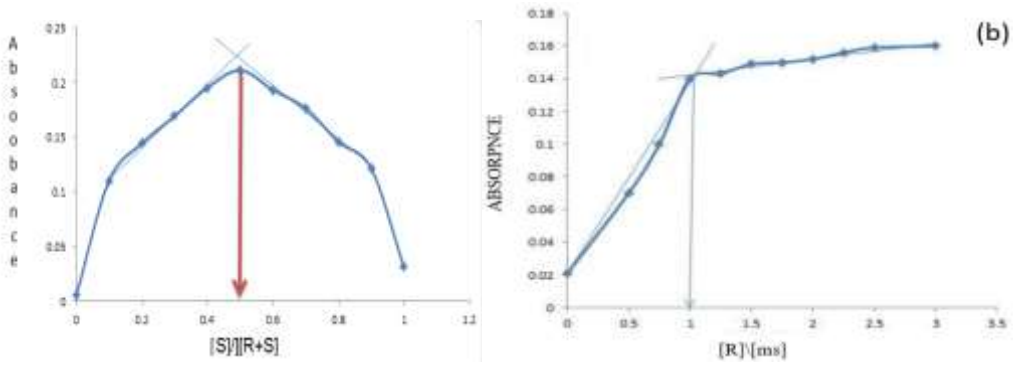

Figure 5: (a) Job's method and (b) Mol ratio method

INTERFERENCES

The effect of a variety of excipients that have been added to pharmaceutical preparations, such as starch, sucrose, lactose, glucose, has been shown by the findings that there has been no substantial intervention of excipients or additives up to (500 µg) in the proposed method as shown in Table (9).

Table 9: the effect of added excipients on the estimation of (MS)

Excipients	Recovery (%) of 100 µg Mesalazine / µg of amount the foreign compound added		
	100(µg)	250(µg)	500(µg)
Strach	98.62	96.74	96.56
Glucose	103.94	100.68	98.97
Lactose	97.08	97.94	97.25
Sucrose	97.94	98.97	97.77

Application of the Method

The present method has been applied to pharmaceuticals such as tablets, suppositories, and enemas under optimal reaction conditions of oxidative coupling between the Reagent Phenothiazine, the oxidizing agent and the mesalazine as is shown in table (10) the method successful in estimating mesalazine, whereby recovery the relative error, relative standard deviation less from %5 have been found that shows that the proposed method is good accuracy and Precision.

Table 10: Analytical application of the Method

Pharmaceutical Preparations	µg Taken	µg Found	Recovery (%)	Relative error (%)	Relative Standard deviation (%)	Average Recovery (%)
Awasalazine 400mg /tablet,(Iraq)	2	1.941	97.063	-2.934	0.315	97.296
	4	3.892	97.324	-2.675	0.294	
	6	5.850	97.501	-2.498	0.250	
Asacol Suppository 500mg Switzerland	2	1.946	97.340	-2.659	0.324	97.818
	4	3.913	97.83	-2.161	0.199	
	6	5.897	98.284	-1.715	0.146	
Pentasa enema 1g/100ml Czech	2	1.981	99.038	- 0.941	0.428	97.987
	4	3.910	97.975	-2.024	0.700	
	6	5.816	96.948	-3.051	0.192	

Evaluation of the proposed methods

For the purpose of proving the efficiency and success of the method in estimating mesalazine in pharmaceutical preparations in tablet form. The standard addition method was applied, and from the results reached in Figure (5) represented by the standard addition curve to estimate the mesalazine at a concentration of (2،4) µg / ml and table (11) shows the recovery rate. The standard addition method is in good agreement with the proposed method within the acceptable error range of ± 5% and the method is free of additive interference indicating satisfactory method selectivity. It is successfully applied to the pharmaceutical preparations tablets.

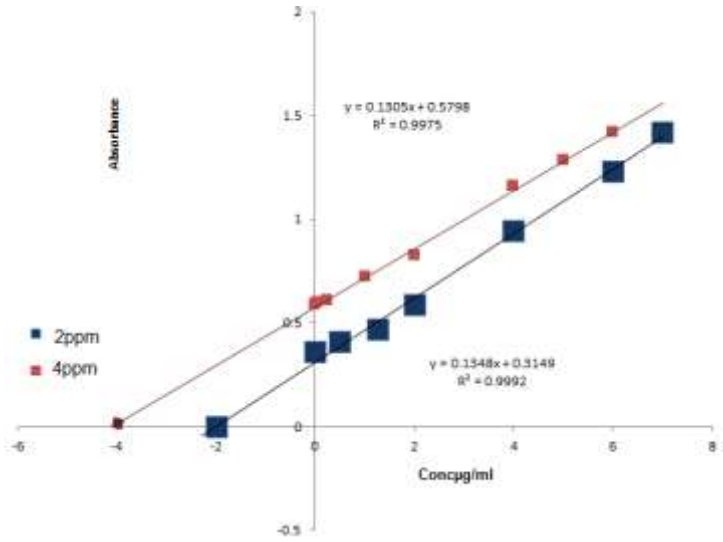

Figure 6: standard addition method

Table 11: standard addition method the of estimating mesalazine

Pharmaceutical Preparation	Amount Present µg/ml	Amount Fount µg/ml	% Recovery
Mesalazine	2	1.970	98.503
	4	3.989	99.74

* Average of five determination

CONCLUSIONS

A simple and sensitive spectrophotometric method for estimating Mesalazine has been developed through forming a fixed and coloured dye due to oxidative coupling between Mesalazine and phenothiazine in aqueous solution in the presence of potassium dichromate which followed the principle of Beer limits Law between (0.1-11 µg/ml) and the developed method is of high accuracy and compatibility.

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