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A Spectrophotometric Method for the Quantitative Estimation of Trimethoprim via Oxidation and Decolonization of the Indigo Carmine

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Abstract:
The aim of this investigation was to develop a simple, rapid and sensitive spectrophotometric method for the determination of trimethoprim in bulk and pharmaceutical dosage forms. The suggested method based on the oxidation of trimethoprim with known excess of oxidizing agent (N-bromosucinimide) in acidic medium and subsequent determination of surplus oxidant by shortening of indigo carmine dye and measuring the absorbance of remaining dye at 610 nm. The absorption of residual indigo carmine is linearly and directly proportional with the concentration of trimethoprim. Beer's law was obeyed in the range of 0.2 – 5.0 µg. ml^{-1} with determination coefficient of 0.9974. The molar absorptivity was 8.07×10^4 $L.mol^{-1}.cm^{-1}$ and sandell's sensitivity index was 0.0035µg. cm^{-2}, while the limit od detection (LOD) and quantitation (LOQ) were 0.0026 and 0.0089 µg. ml^{-1} , respectively. The proposed method was satisfactory applied for the determination of trimethoprim in available dosage form and results were compared with standard addition method.
Keywords: Trimethoprim, Spectrophotometric, Estimation, Indigo carmine, N-bromosuccinimide

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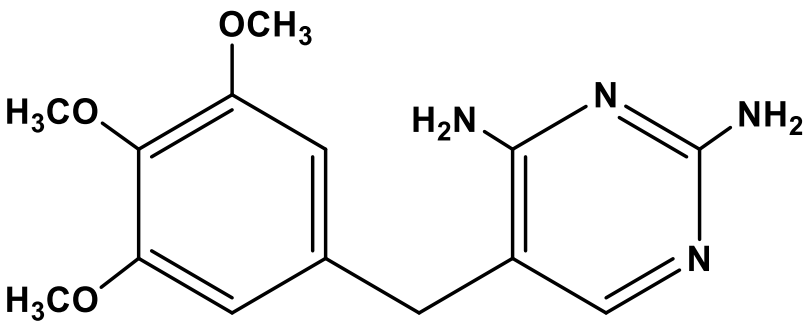
Introduction

Trimethoprim (TMP) is chemically known as (2,4-diamino-5-(3,4,5-trimethoxy benzyl) pyrimidine) [1],It's empirical formula is $C_{14}H_{18}N_4O_3$, fused between 199-203 ˚C and it's molecular weight 290.3 g/mol [2]. It's a white or yellowish white, odorless, insoluble in ether and slightly soluble in water and ethanol and soluble in methanol. It has the following formula [3]:



Chemical structure of trimethoprim

Trimethoprim is an antibiotic; inhibit the reduction of dihydrofolic acid to tetrahydrofolic acid to inhibit deoxyribonucleic acid (DNA) synthesis by binding to dihydrofolate reductase [4]. Combination of two antibiotics, trimethoprim and sulfamethoxazole (SMX) in pharmaceuticals is used to treat a wide variety of bacterial infections [5], It's an effective antimicrobial agent used to treatment urinary tract infection and as powerful bacteriostatic agent, The combination of TMP & SMX reduced the risk to develop the resistant strains of bacteria makes Co- Trimoxazole a powerful antibacterial medication [6].

Several analytical methods to determine TMP in a literature survey, have been developed they include spectrophotometry [7, 8], HPLC [9, 10], HPTLC [10, 11] , Visible and UV spectrometry [12, 13], Defferential puls polarography [14], Square wave voltammetry [15, 16], Adsorptive stripping voltammetry [17, 18] non-aqueous titrimetry and ion selective electrode (ISE) [19],Polarography and voltammetry [20],Defferential puls voltammetry and cyclic voltammetry [8], TLC [21], pH- induced spectrophotometry [13],Fluorometry [13],NMR [22].

The aim of this paper is to develop a simple and sensitive indirect spectrophotometric determination method to determine TMP. This method based on oxidation of trimethoprim by using N-bromosuccinimide, the color palace of indigo carmine dye by the residual N-bromosuccinimide.

Material and methods

All Chemicals and reagents used in this study were of analytical grade. Trimethoprim powder was purchased from the State Company of the Drug Industries and Medical Appliances Samara _ Iraq (SDI).

Trimethoprim solution (100µg/ml)

This solution was prepared by weighing 0.0100 g from pure TMP (SDI) and soluble in 6 ml of methanol then completed to 100 ml of distilled water in volumetric flask.

N-Bromosuccinimide solution (1×10^{-3} M)

It was prepared by dissolving 0.0200g of NBS (FLUKA) in distilled water then completed to 100ml in volumetric flask.

Indigo carmine solution (200µg/ml)

Indigo carmine dye solution prepared by weighing 0.0200g (BDH) and dissolve in distilled water in a volumetric flask and diluting to 100ml distilled water in a volumetric flask.

Hydrochloric acid solution (2M)

It was prepared by diluting 17.16ml of concentrated HCl to 100 ml with distilled water in a volumetric flak.

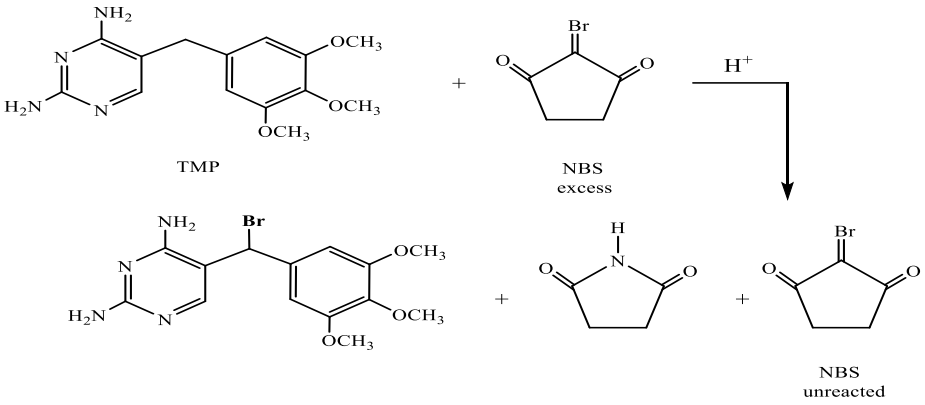
Trimethoprim tablet solution (100µg/ml)

10 tablets (Infectotrimet /Germany, each containing 200 mg TMP) were weighed and powdered. An accurately amount of the powder equivalent to 0.0100g of trimethoprim was dissolved in 6 ml of methanol, stirring and mixing well with simple heating, then filtered in conical flask and transferred to 100ml of volumetric flask completed with distilled water.

Chemical Reactions

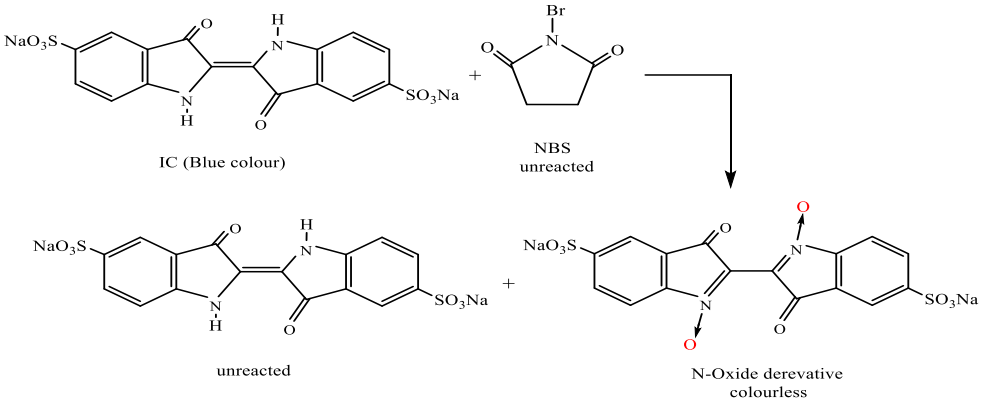
The determination process of trimethoprim based on two steps.

1- Oxidation step of trimethoprim with known excess of N-bromosccinimide in acidic medium, so suppose chemical reaction explained as follow (scheme1).



Scheme 1

2- React the unreacted of N-bromosuccinimide (NBS) to oxidized constant amount of indigo carmine to colorless product, (scheme 2), [23].



Scheme 2

Results and discussion

Optimum Reaction Condition

Amount of Indigo Carmine (IC)

To find the best amount of IC dye that can be used in the estimation of TMP which that followed the Bear's law, increasing volumes of 200 µg/ml IC dye solution were added to volumetric flask 10ml contained 0.5 ml of (2 M) HCl solution. The volume was completed with distilled water and measured the absorbance at 610 nm. The standard curve in (fig.1) offers that 1.8 ml of IC dye is the best volume that gives high absorbance within the linear relationship.

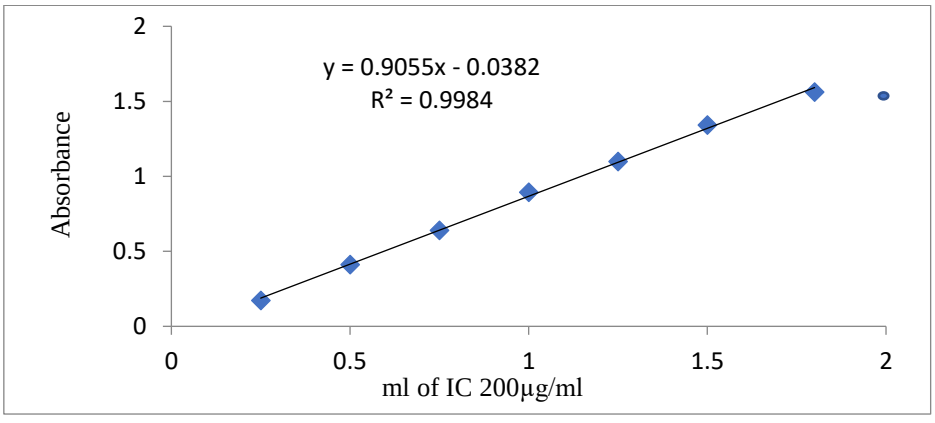


Figure 1: Standard curve of IC dye

Selection of the Oxidizing Agent

This condition was studied by adding 1.5 ml of obtainable oxidizing agents that bleaching IC dye, the oxidizing agents are (N-bromosuccinimide, N-chlorosuccinimide, Potassium periodate and ChloramineT) with concentration (200 ppm) of each oxidizing agent into 10 ml volumetric flask which contain 1.8 ml of 200µg/ml IC dye and 0.5 ml of (2 M) hydrochloric acid, then volume was completed with distilled water. The solution was stand for 10 min. and the absorbance was measured at 610 nm against reagent blank, the result in (fig. 2) showed that N-bromosuccinimide is better oxidizing agent, so it was chosen in the subsequent experiments.

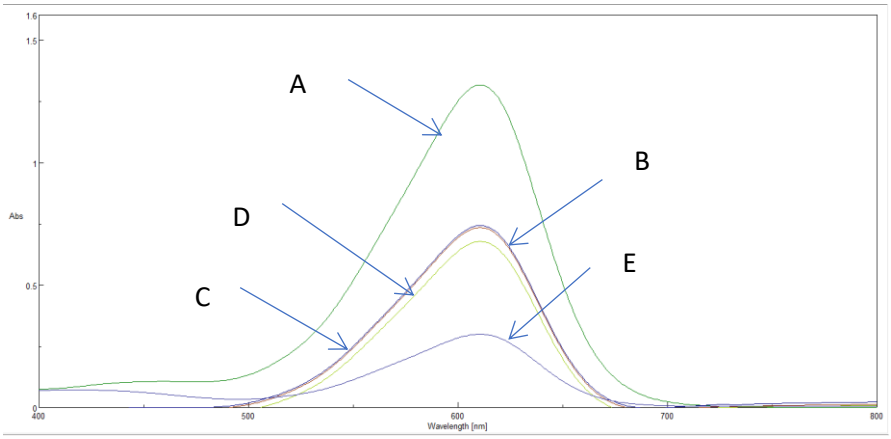


Figure 2*: (A): IC dye without oxidant, (B): IC dye with N-chlorosuccinimide, (C): IC dye with chloramine T, (D) IC dye potassium periodate, (E): IC dye with N-bromosuccinimide*

Amount of Oxidizing Agent

Effect of adding increase amounts of N-bromosuccinimide oxidizing agent to a series of 10ml volumetric flask. While other factors were constant. It found that 2.0 ml of NBS is sufficient for decolorization of IC dye (fig.3).

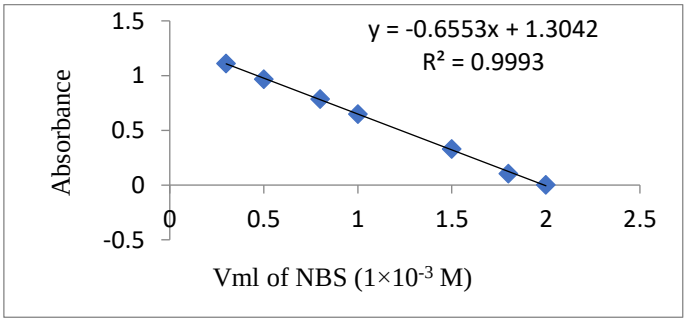


Figure 3: Amount of oxidant agent for decolorizat ion IC

Selection of the Best Acid

Different kinds of strong and weak acids were studied, the results shown in (table 1) that hydrochloric acid is an ideal medium for the reaction. In addition, 0.5 ml of 2M HCl was chosen as optimum amount (table 2).

Table 1: Selection of the best acid

Acid used (2M)	Absorbance	Final pH
HCl	0.712	1.8
H_2SO_4	0.519	2.14
HNO_3	0.419	1.7
H_3PO_4	0.457	2.3
CH_3COOH	0.657	3.4

Table2: Selection the best volume of acid

ml of (2M) HCl	Absorbance / µg of TMP in 10 ml					R^2
	10	20	30	40	50	
0.2	0.342	0.606	0.904	1.253	1.300	0.9645
0.3	0.342	0.656	0.901	1.118	1.321	0.9919
0.4	0.363	0.694	0.903	1.197	1.389	0.9825
0.5	**0.368**	**0.701**	**0.937**	**1.191**	**1.432**	**0.9972**
0.6	0.362	0.687	0.989	1.150	1.388	0.9849

Effect of Oxidation Time

This effect has been determined by adding reaction components before adding IC dye. After shaking the flasks and waiting for suitable time, IC dye was added. Again shaking the flasks for several minutes to complete oxidation reaction. (Table 3) shows that 10 min. is the best time for oxidation of trimethoprim, and 5 min. was suitable time for oxidation of IC dye.

Table 3: Effect of Oxidation Time

Standing time before addition IC . min.	Absorbance/ Standing time after addition IC and before dilution ,min.						
	5	10	15	20	30	40	60
After addition	0.693	0.697	0.699	0.701	0.700	0.702	0.701
5	0.709	0.711	0.708	0.709	0.711	0.710	0.709
10	0.710	0.712	0.711	0.711	0.708	0.708	0.705
15	0.735	0.735	0.732	0.737	0.735	0.730	0.727
20	0.723	0.726	0.720	0.720	0.721	0.719	0.719

Effect of Temperature and Stability

The effect of temp. was studied with different degrees (0-30 ˚C) on the intensity color and stability of the dye .The results in the table 4 shows that the absorbance is stable for one hour at room temperature (25˚C ±2).

Table 4: Effect of Temperature on Absorbance

Temp. ˚C	Absorbance of 20µg TMP / min. standing						
	5	10	20	30	40	50	60
0	0.596	0.546	0.590	0.592	0.580	0.570	0.563
10	0.682	0.686	0.687	0.689	0.686	0.688	0.684
25±2	0.707	0.711	0.710	0.708	0.705	0.717	0.705
30	0.629	0.624	0.626	0.628	0.627	0.618	0.613

Effect of Surfactants

The effect of different type of surfactants (neutral, negative and positive) on dye absorption were studied, the results in (table 5) show the addition of surfactants lead to decrease the absorption, therefor , they excluded in subsequent experiments.

Table 5: Effect of Surfactants

Surfactants	Absorbance/ ml of surfactants			
	0.0	0.5	1.0	2.0
Triton X-100(2%)	0.709	0.484	0.451	0.448
SDS(1×10^{-3}M)		0.583	0.554	0.550
CBC (1×10^{-3}M)		0.327	0.338	0.330
CTAB (1×10^{-3} M)		0.372	0.337	0.306

Final Absorption Spectrum

When trimethoprim was treated according to the optimized conditions established in suggested method , the final absorption spectrum in (fig.4) illustrated the spectrum for only dye versus blank and for 20µg trimethoprim versus the blank and spectrum of blank versus distilled water.

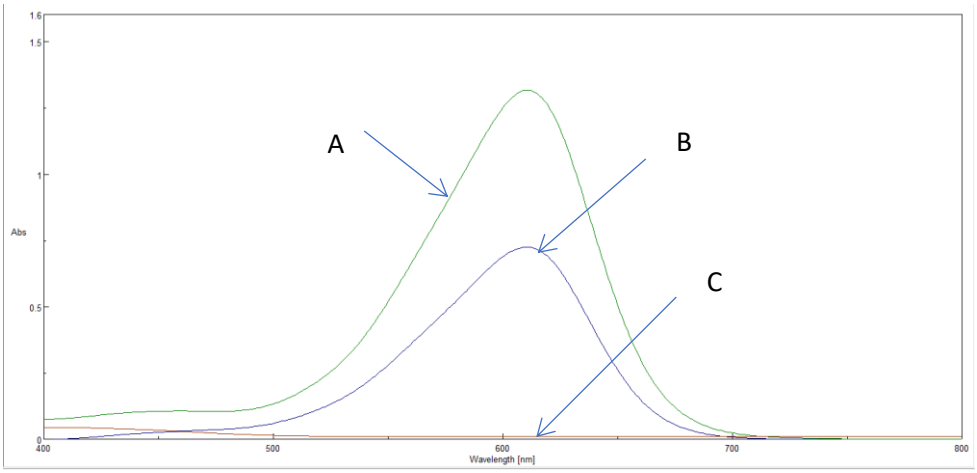


Figure 4: Final Absorption Spectrum for

(A) dye versus blank, (B) 20µg TMP versus blank, (C) blank versus distilled water

Recommended Procedure and Calibration Curve

According to the optimum condition of the suggested method, different amounts of trimethoprim solution transferred in to a series of 10 ml volumetric flask, to cover the range of concentration between 0.2-5.0 µg/ml in final volume. To each flask 0.5 ml of 2M HCl was added and followed by 2ml of 1×10^{-3} M N-bromosuccinimide solution with mixing and left for 10 minutes to complete oxidation process of TMP, then 1.8 ml of 200ppm of indigo carmine dye(IC) was added. The flasks were kept at room temperature (25˚C±2) for 5 minutes to complete oxidation of the dye and complete with distilled water to the mark, then the absorbance of the residual dye versus blank was measured at 610 nm. A linear calibration curve (fig.5) was obtained over the range (0.2-5.0)µg/ml of trimethoprim. The value of the molar absorptivity was found to be 8.07×104 L. $mol^{-1}.cm^{-1}$ and sandell's sensitivity value was 0.0035µg. cm^{-2} . While the value of detection limit (LOD) and quantitative limit (LOQ) were 0.0026 and 0.0089µg. ml^{-1} respectively.

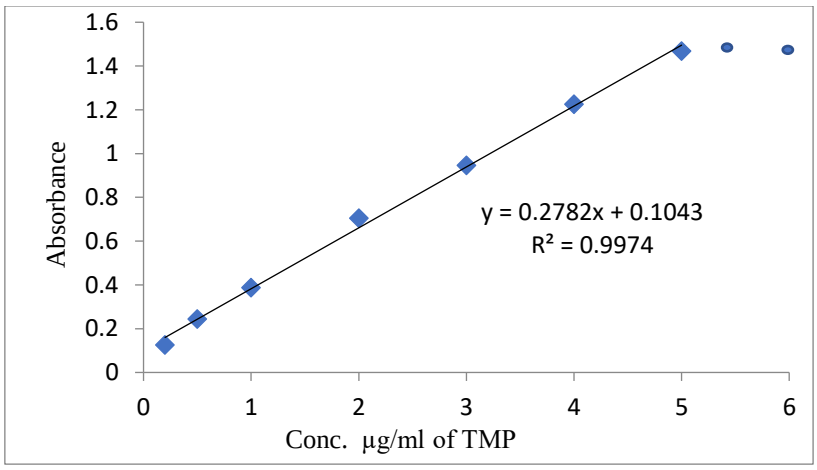


Figure 5: Calibration Curve of TMP Determination

Accuracy and Precision

The accuracy and precision of the suggested method were examined by calculating the by analysis two different indicate that the method is satisfactory concentrations (10, 20) µg/ml of TMP and the result in the table 6.

Table 6: Accuracy and Precision for the method

Amount of TMP µg/ 10ml	Recovery,*%	Relative error,* %	Relative standard deviation, * %
10	98.98	-1.01	0.973
20	99.28	-0.71	1.128

* Average of five determination

Effect of excipients

The effect of the presence of some substances that are often added to pharmaceutical preparations were studied under optimum conditions by adding varying amounts of these additives from (50,100,150) µg to 20µg TMP /10ml , and it was found that the additives don't interfere with the suggested method and results in table 7.

Table 7: Effect of Excipients

Excipients	Recovery% of 20 µg TMP		
	µg of excipients added		
	50	100	150
Starch	97.41	97.63	101.30
Glucose	98.21	99.38	101.44
Sucrose	100.27	98.65	98.21
Arabic gum	98.9	96.2	97.73

Application of the Method

The proposed method was applied to the estimation of trimethoprim in it's available sample od dosage form (INFECTOTRIMET , tablet(200mg), Germany,Hoppenheim) with two different concentration The results in (table 8) prove the success of the proposed method for estimation of TMP.

Table 8: Application of the Method

Pharmaceutical preparation	Amount of TMP taken (µg)	Recovery,* %	Relative error,*%	Relative standard deviation,*%
INFECTOTRIMET (200mg) tab., Germany	10	100.20	0.207	0.725
	20	99.10	-0.891	1.377

*Average of five determinations

Evaluation of the Suggested Method by Standard Addition

Standard addition method was applied to prove the efficiency of the proposed method and it's success in estimation of trimethoprim in it's pharmaceutical preparations, and no interferences with additives exit in pharmaceutical preparations. The results in the figure 6 and table 9 indicate that the standard addition method is in good agreement with the proposed method.

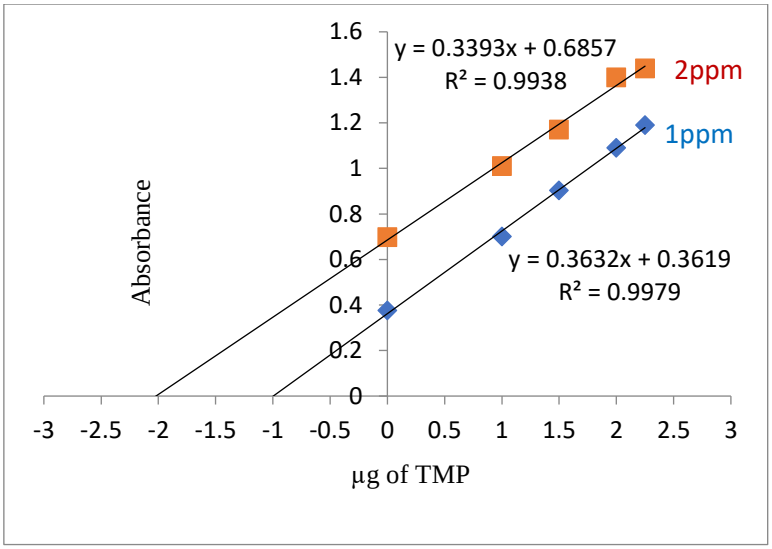


Figure 6: Plot of using standard addition method in estimation of TMP in tablet.

Table 9: Estimation of TMP in pharmaceuticals by standard addition method

Pharmaceutical preparation	Amount of TMP taken µg/ml	Amount of TMP measured µg/ml	Recovery, %
INFECTOTRIMET (200mg) tablet, Germany	1.0	0.996	99.64
	2.0	2.02	101.04

Conclusion

An indirect Spectrophotometric method has been proposed for the determination of trimethoprim. The proposed method required only N-bromosuccinamid and indigo carmine dye in acidic medium. The method was an accurate, quick and sensitive, and also was characterized by Simplicity as it did not need to be organized temperature, pH adjustment and extraction steps. The method has been successfully applied for analysis of trimetheprim in its pharmaceutical preparation (tablets), also it was of good accuracy and precision.

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