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Spectrophotometric Determination of Theophylline Via Oxidation and Decomposition of the Iron (II)-bathophenanthroline Complex

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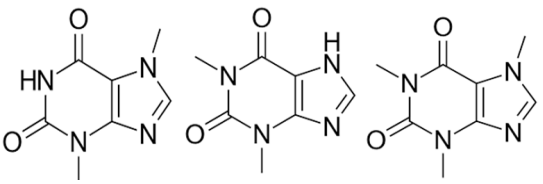
ABSTRACT

A rapid, sensitive, accurate, and selective spectrophotometric method was developed for determining theophylline (TP) in pure form, pharmaceutical tablets, and human blood serum. Theophylline is structurally related to caffeine and is widely studied as a bronchodilator, with known toxicity at high doses. The method involves oxidation of theophylline with a known excess of cerium sulfate in acidic medium at room temperature. The remaining oxidant reacts with a color complex formed between 4,7-diphenyl-1,10-phenanthroline and ferrous sulfate, producing a red color measured at 534 nm. A linear relationship was observed in the 2–23 µg/mL range. Molar absorptivity was 14,809.5 L/mol. cm, Sandell sensitivity was 0.01184 µg/cm², with LOD and LOQ of 0.05175 µg/mL and 0.17252 µg/mL, respectively. The method was successfully applied to tablets and human serum without interference from excipients, confirming its suitability for pharmaceutical and biological analysis.

Keywords: Theophylline, 4,7-diphenyl-1,10-phenanthroline, Cerium sulphate, Oxidation-reduction process.

INTRODUCTION

Theophylline is type of methyl xanthine family that hares structural similarities with theobromine and caffeine, two prevalent dietary xanthine's. Although several substituted derivatives have been created, none of them are superior to theophylline. it is also known as 1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione or theocon and it has a molecular formula $C_7H_8N_4O_2$ with molecular weight 180.164 g/mol. Its exist in both monohydrate and anhydrous forms is a white, odorless, bitter-tasting crystal powder (Fig. 1) show the chemical structure of three compound theobromine, theophylline and caffeine respectively.[1,2,3]



Theobromine Theophylline Caffeine

(3,7dimethyl 2,3,6,7(1,3dimethyl 3,7dihydro-(1,3,7 trimethyl-1H-Tetrahdro-1H-purine1H-purine2,6-dione)purine2,6(3H,7H)-dione) 2,6-dione

Fig. 1. Chemical structures of three compound Theobromine, Theophylline and Caffeine

In general, theophylline has been used for more than seventy years to treat chronic respiratory

diseases. Currently, theophylline is used as an adjunct to asthma patients who do not respond well to inhaled corticosteroids and those with severe COPD whose condition cannot be controlled with bronchodilators. PDES is an enzyme that degrades intracellular nucleotides, and theophylline is widely believed to be a weak and non-selective inhibitor of this enzyme. According to some laboratory tests, theophylline relaxes bronchial smooth muscle, although this effect only occurs when theophylline concentrations are high[4,5]. One of the side effects of theophylline is that when taken in high doses of more than 20 micrograms/mL, it may cause fever, rapid heartbeat, heartburn, and loss of appetite[6]. Aminophylline is the most common, an ethylenediamine salt that makes the drug more soluble at neutral pH for intravenous administration[7]. Several analytical techniques, such as the ones listed below, have been employed to determine theophylline such as UV-Vis spectrophotometric methods[8-12], UV-Spectroscopic method[13], High performance liquid chromatography[14-20], ion mobility spectrometry[21], Luminescence spectroscopy[22-25], solid phase extraction[26], molecularly imprinted solid-phase extraction[27,28], electrochemical sensor[29,30] and colorimetric[31]. Despite their high accuracy, these techniques are typically expensive, complex, time-consuming and typically call for professional operators who have been trained to handle certain steps in the process. Among the several analytical methods available, spectrophotometry has drawn a lot of interest due to its affordability, ease of use, and sensitivity. By measuring a sample's absorption or transmission of light at particular wavelengths. from the previous literature survey, it was noticed that this reagent was not utilized to estimate the drug being studied, theophylline in its pharmaceutical forms was determined using the reagent 4.7-diphenyl-1,10 phenanthroline. The method based on the oxidation of theophylline using a known excess of cerium sulfate tetrahydrate, by reacting with the iron(II)-bathophenothroline (red color complex), where the complex decomposes as a result of the oxidation of iron(II) to iron(III), and measuring the intensity of the absorption of the remaining color at a wavelength of 534, which is directly proportional to the concentration of theophylline.

EXPERIMENTAL

Instruments

All absorbance measurements were conducted using a SHIMADZU spectrophotometer, model UV-1900i spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The pH measurements were carried out using a pH Meter, model BP3001 (Boeco, Germany), The weighing was performed using an electronic sensitive balance, model BEL (BEL Engineering, Italy).

Chemical Reagents and Standard Solution

All chemicals and reagents used in this research were highly purified and supplied by reputable companies such as Fluka, Searle, or BHD. All materials were used without any prior purification.

Standard solution of theophylline (TP) (200 µg/mL): It was prepared by dissolving 0.0200 g in 100 mL volumetric flask and completed to the mark with ethanol.

Solution of dilute hydrochloric acid 1 M (approximately): It was prepared with transfer 8.4 mL of concentrated hydrochloric acid into 91.6 mL distilled water in a 100 mL of volumetric flask.

Solution of dilute sulfuric acid 0.5 M (approximately):It was prepared by transferred of 2.7 mL of concentrated sulfuric acid into 97.3 mL of distilled water in 100 mL of volumetric flask.

Cerium sulfate tetrahydrate $Ce(SO_4)_2.4H_2O$ (3×10^{-3} M): The solution was prepared by dissolving 0.1213 g of cerium sulphate in 100 mL of (0.5 M) sulfuric acid in a volumetric flask.

4,7-diphenyl-1,10-phenanthroline (3×10^{-3} M): In a 100 mL volumetric flask, dissolve 0.0997 g of the pure reagent in 100 mL of ethanol and finish to the mark.

Ferrous sulphate solution $FeSO_4.7H_2O$ (3×10^{-3} M): This solution was prepared by dissolving 0.0834 g of ferrous sulfate (provide by Fluka Company) in the distilled water, then completing the volume to 100 mL with distilled water by a volumetric flask.

Iron(II)-bathophenanthroline complex solution: was prepared by mix of 75 mL of 4,7-diphenyl-1,10-phenanthroline at 3×10^{-3} M with 25 mL of Ferrous sulfate solution $FeSO_4.7H_2O$ at 3×10^{-3} M In a 100 mL volumetric flask (The reaction of Ferron complex is (1:3).

Pharmaceutical Preparation

Theophylline dosage forms solutions (200 µg/mL): ten tablets of each of two dosage forms of theophylline (ASMASAM, Samara/Iraq, which contain120 mg/tablet) and (PHP Theophylline, Kyiv/ Ukraine, which contain 300 mg/tablet) were weighed, crushed and mixed thoroughly 0.0268 g of PHP Theophylline and 0.0373 g of ASMASAM, equivalent to 0.0200 g of pure TP, were weighed and dissolved and made up to the mark with ethanol using a 100 ml volumetric flask.

Initial procedure

Add 0.5 mL of 200 µg/mL TP solution to a 10 mL volumetric flask, add 1 mL of 3×10^{-3} M oxidizing agent cerium sulfate, and let the solution stand for a 5 min with shaking. Then add 1 mL of reagent Iron (II)-bathophenanthroline complex (IBA-C) and left the solution stand for 5 minutes. The volume was then filled to the mark with distilled water, and the absorbance was measured at 534 nm.

RESULTS AND DISCUSSION

The method is generally based on the oxidation of TP using a known excess of the oxidizing agent, cerium sulfate, in an acidic medium. After that, the unreacted cerium sulfate is reacted with the iron(II) bathophenothroline complex (red complex), where the complex decomposes as a result of the oxidation of iron(II) to iron(III). The absorbance of the remaining color of the complex is measured at a wavelength of 534 nm, which is directly proportional to the concentration of theophylline, various parameters were studied such as complex volume ,amount of cerium sulfate, type of acid and concentration of acid and also stability of complex, The effect of these parameters on the oxidation- reduction reaction was studied to choose the optimum conditions for determination TP in pharmaceutical preparations.it is worth noting that all experiments in this paper are based on the use of 0.5 mL of TP (200 µg/mL) solution in a final volume of 10 ml with measuring the absorbance of the resulting solution measured at 534 nm.

Optimum amount of complex

The required volume iron (II)-batho-phenanthroline complex (IBA-C) was determined by adding increasing amounts of it to a series of 10 mL volumetric flasks, filling the volume to the mark with distilled water, performing spectroscopic absorption measurements at 534 nm, and plotting the standard curve. Fig. 2 shows that 2 mL of complex was the optimal volume for the best estimate, R^2 = 0.9967, and this was adopted in subsequent experiments.

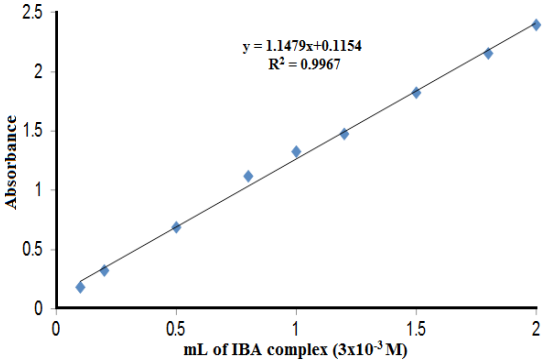


Fig. 2. Standard curve of Iron (II)-bathophenanthroline complex (IBA-C)

Selection of oxidizing agent

Several types of oxidizing agent such as, [Potassium dichromate ($K_2Cr_2O_7$), sodium periodate ($NaIO_4$), potassium periodate (KIO_4), N-Bromosuccinimide (NBS) and Cerium sulfate tetrahydrate ($Ce(SO_4)_2.4H_2O$)] were tested with the aim of choosing the best agent to oxidize 0.5 mL of TP, by the added 0.5 mL of HCl and then adding 1 mL of each of them at a concentration of 3×10^{-3} M and then added 0.5 mL of HCl and wait for 5 min and finally added 2 mL of complex and left the solutions for 5 min also before dilution with distilled water, Table 1. Show study of the effect of different oxidizing agents on the solution absorbance. The result shows the absorbance resulting from the addition of different oxidizing agents with the aim of determining the optimal agent for oxidizing (TP), noting that $Ce(SO_4)_2\bullet4H_2O$ is the only agent that showed a clear absorbance response.

Table 1: Selection of oxidizing agent

Oxidizing agent	Absorbance
$K_2Cr_2O_7$	No colour contact
$NaIO_4$	No colour contact
KIO_4	No colour contact
NBS	No colour contact
$Ce(SO_4)_2.4H_2O$	0.812

Effect amount of oxidizing agent (Cerium sulfate tetrahydrate)

The amount of oxidizing agent required to oxidize 0.5 mL of 200 µg/mL TP was studied using several volumes ranging from (0.5-1.5 mL) and also other compounds of reaction were added.

Fig. 3 shows that the volume of 1 mL gave the highest absorption.

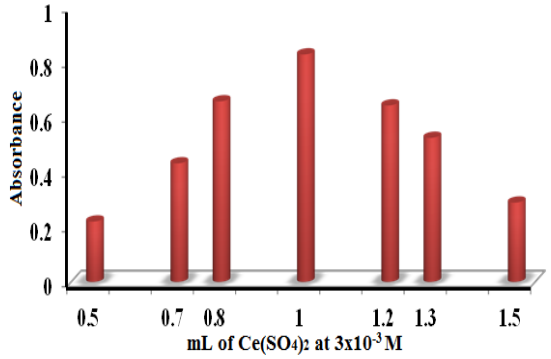


Fig. 3. The optimum amount of cerium sulfate

Effect of acidic medium on oxidation–reduction process

A study was conducted on the effect of various available acids on the oxidation process of (TP) solution such as (hydrochloric acid, sulfuric acid, nitric acid and acetic acid)by adding 0.5 mL of each one at a concentration of 1 M. The results shown in Table 2 HCl was chosen because it gave the highest absorption, which indicates that the largest amount of the compound (TP) undergo oxidation.

Table 2: Effect of acidic type on oxidation–reduction process

Absorbance	0.5 mL of 1M acid
0.839	HCl
0.627	H_2SO_4
Turbid	HNO_3
0.429	CH_3COOH

Effect of hydrochloric acid volume

A study was conducted on the effects of different volumes (0.25–1.250 mL) of 1M hydrochloric acid solution versus different amount of standard TP solution (2-20 µg/mL) Referring to the results in Table 3, show demonstrates the influence of varying volumes of 1 M hydrochloric acid on the absorbance of TP solution . The optimal volume of 0.7 mL was selected based on the highest determination coefficient (0.9939).

Table 3 : Effect of amount of acid on absorbance

mL of 1M HCl	Absorbance/µg of TP					
	4	6	10	16	20	R^2
0.25	0.045	0.183	0.305	0.437	0.49	0.9434
0.5	0.589	0.932	1.065	1.293	1.436	0.9729
0.7	0.675	0.838	1.215	1.634	1.876	0.9939
1	0.607	0.819	1.145	1.474	1.718	0.9879
1.25	0.657	0.798	1.323	1.567	1.825	0.9597

Effect of time on oxidation-reduction process

The time required to complete the oxidation of theophylline to 0.5 mL of (TP) was also studied using the previously calculated amount of oxidizing agent (Cerium sulfate tetrahydrate), in an acidic medium, and with an oxidation time for the complex of 5 min before the dilution. The results in the Table 4, showed that the best time for the oxidation of theophylline is 10 minutes.

Table 4 : The effect of time on oxidation of theophylline

Standing time for oxide. (TP)	Immediately	5	10	15	20	25
Absorbance	0.917	1.073	1.216	1.183	1.136	1.123

After selecting the theophylline oxidation time, the time required for the complex to oxidize was studied by waiting for various periods of time before diluting with distilled water. According to the results shown in the Table 5, the optimal oxidation time for the complex was found to be 15 minutes.

Table 5 : The effect of time on oxidation of complex

Standing time for oxid. the complex	Immediately	5	10	15	20	25
Absorbance	1.183	1.213	1.225	1.234	1.192	1.158

Effect of order of addition

To study how the addition order affects the intensity of the colored product, several samples were prepared with different addition orders, and the results, as shown in Table 6, The effect of changing the order of adding reaction components on the absorption value shows that the first order (I) is optimal for achieving the highest absorption of the colored compound.

Table 6 : The sequence of additions

Reaction component	Order number	Absorbance
$S+H^++OX+R$	I	1.243
$OX+S+H^++R$	II	1.218
$OX+H^++S+R$	III	1.137
$OX+R+H^++S$	IIII	0.083

S (Theophylline), H^+ (Hydrochloric acid), OX (Ceric sulphate), R (IBA complex).

The effect of different solvents on the absorption spectrum

Several organic solvents were used in addition to distilled water in the dilution process. The results indicate that acetone gave slightly

higher absorption than distilled water. In subsequent experiments, distilled water was continued because it is available, safe and cheap. The results are shown in the Figure 4.

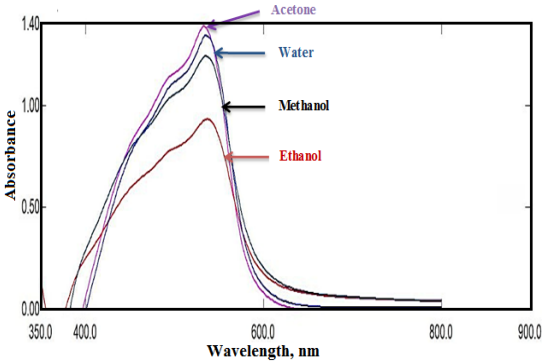


Fig. 4. The effect of different solvents

Effect Stability of remaining complex

The effect of time on the absorption of the remaining color of the compound was studied by taking two different amounts (10 and 16 µg/mL) of theophylline solution at a concentration of 200 µg/mL. The results of the experiment shown in Table 7, The absorbance value of the remaining color of (IBA-C) product remained stable for 60 min, a period sufficient to complete the spectrophotometric measurements.

Table 7 : Effect Stability remaining of dye

	Absorbance/time, (minute)							
MBZ µg/mL	Immediately	5	10	20	30	40	50	60
10	1.219	1.217	1.215	1.211	1.211	1.212	1.205	1.209
16	1.637	1.635	1.633	1.631	1.633	1.629	1.625	1.625

The procedure and final absorption spectrum:

Under optimized reaction conditions, 0.5 mL of (200 µg/mL) TP solutions were transferred in 10 mL volumetric flask with 1 mL of cerium sulfate at 3×10^{-3} M for each one in acidic medium by adding 0.7 mL of hydrochloric acid. After leaving for10 min, followed by adding 2.0 mL of IBA complex was added and left at room temperature for 15 min before filling the volumetric flask to the mark with distilled water. The absorbance of this solution was measured against the blank sample at 534 nm. Fig. 5 shows the final absorption spectrum of this process.

Preparation the calibration curve

The proposed method was applied according to the procedure mentioned in the preparation of the final absorption, using increasing concentrations covering the range of 2-26 µg/mL of theophylline solution at a concentration of 200 µg/mL. The standard curve was drawn, which gave a linear relationship in the concentration range of 2-23 µg/mL (Fig. 6), while the molar absorption coefficient value was 14809.5 L/mol.cm, and the Sandell index value was 0.012165 µg/cm².

The limit of detection (LOD) and limit of quantification (LOQ) values were calculated by preparing ten blank samples and measuring the absorbance at a wavelength of 534 nm using the following mathematical relationships[32].

$$C_{LOD}=\frac{3\sigma_B}{Slope}\ ,\ C_{LOQ}=\frac{10\sigma_B}{Slope}$$

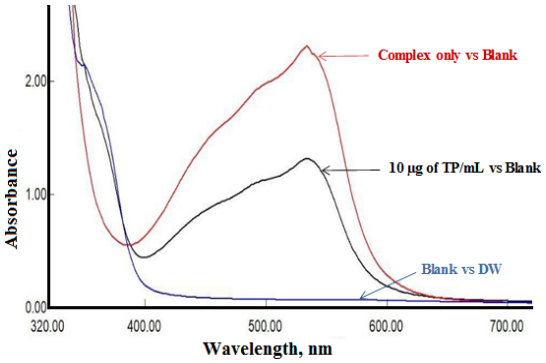


Fig. 5. Final absorption spectrum

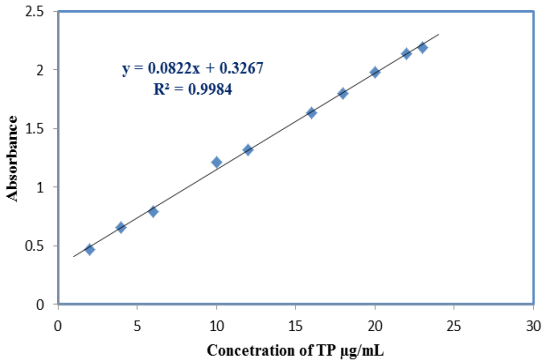


Fig. 6. The calibration curve for TP

Stoichiometric section

The continuous change method was applied (Job's method)[33] to determine the molar reaction ratios between theophylline and the cerium sulfate. Equal concentrations of theophylline and the cerium ion were prepared at a concentration of 1.11×10^{-3} M. The results shown in Fig. 7(a, b) shows that the reaction ratio between theophylline and the cerium ion is 1:1.

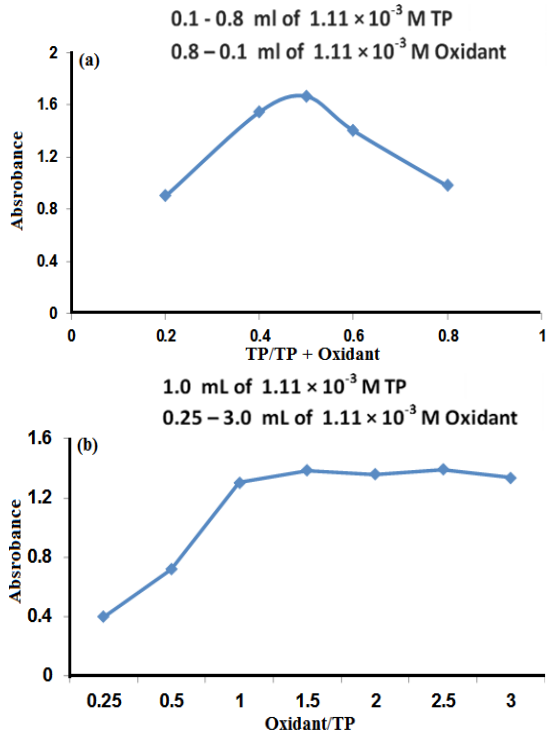


Fig. 7. Plot of (a) Job's method and (b) mole ratio for theophylline and cerium ion

Accuracy and precision

We conducted a study of the accuracy and precision of the method by calculating the recovery percentage, relative error, and relative standard deviation for three different concentrations (6, 10 and 16 µg/mL) of standard theophylline. The results in Table 8 indicate that the method has good accuracy and precision and error percentage is within the acceptable range.

Table 8 : Accuracy and precision

Taken of amount TP µg/mL	Found amount of TP µg/mL	Recovery *%	Relative error*, %	Relative standard deviation*, % of TP µg/mL
6	5.99	99.83	-0.17	0.97
10	10.15	101.5	-1.5	1.29
16	16.29	101.81	-0.898	2.34

*Average for five determinations

Application of the proposed method

The proposed method was applied to pharmaceutical preparation solutions of the drug compound in tablet form from two different companies, at different concentrations (6, 10, and 16 µg/mL) for each solution. The results shown in Table 9 demonstrate the success of the method in estimating theophylline concentration in pharmaceutical preparations with acceptable results.

Table 9 : Application of the proposed method

Pharmaceutical preparation	Present amount of TP (µg/mL)	Found amount of TP (µg/mL)	Recovery* %	Relative* error, %	Relative standard deviation*%
ASMASAM 120 mg TP/tablet Samaraa/Iraq	6	6.14	102.33	2.33	1.71
	10	10.32	103.2	3.2	2.94
	16	15.89	99.31	-0.69	1.42
PHP Theophylline 100 mg TP/tablet Kyiv/Ukraine	6	6.1	101.67	1.67	3.1
	10	10.18	101.8	1.8	2.46
	16	16.37	102.32	2.31	1.65

*Average for five determinations.

Evaluation of the suggested method

The standard addition method was applied to test the selectivity of the proposed method and its absence of any drug interactions, by taking two concentrations (6 and 10 µg/mL) of each pharmaceutical preparation from the two companies. The results shown in Fig. 8 and Table 10.

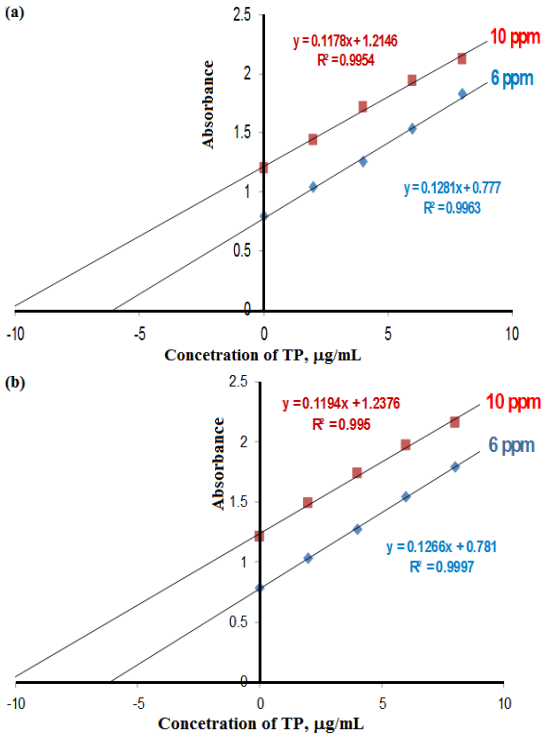


Fig. 8. Plots of standard addition method for determination of TP in pharmaceutical dosage forms

Table 10 : The results of standard addition method

Pharmaceutical preparation	Presence amount of TP µg/mL	Measured amount of TP µg/mL	Recovery%
PHP Theophylline 100 mg TP/tablet Kyiv/Ukraine	6	6.1	101.66
	10	10.32	103.2
ASMASAM 120 mg TP/tablet Samara/Iraq	6	6.07	101.12
	10	10.28	102.8

Based on the results shown in Fig. 8 and Table 10, the proposed method is considered to have excellent selectivity with no interferences.

Application the method on human blood serum

Blood samples were taken from healthy individuals who had not previously taken theophylline. The serum was then separated by centrifugation at 4,000 rpm for 15 minutes[34]. Theophylline was determined in the serum sample using 0.1 mL of serum, adding two different volumes of theophylline at a concentration of 200 µg/mL, adding the remaining components according to the approved method, and completing the volume to the mark with distilled water. Absorbance values were measured at 534 nm, and the necessary calculations were performed. The Table 11 show the absorbance values measured at 534 nm after adding theophylline to the serum according to the approved procedure. The results show no interference between the serum components and the theophylline.

Table 11: Results of application the method on human blood serum

Concentration of TP, µg/mL Present amount	Found amount	Recovery %*	RE, %*	RSD%*
6	5.87	97.83	-2.17	1.78
10	10.14	101.14	1.14	2.31

*Average for three determinations

CONCLUSION

A simple and sensitive spectrophotometric method was suggested for the determination of theophylline in pure form and in dosage forms and application on blood serum by oxidation–reduction process. The method was based upon formation of red complex between iron(II) and 4,7-diphenyl-1,10-phenanthroline, it's a complex with high stability under normal condition the absorbance of the complex was measured at 534 nm. The method is characterized by ease of implementation and accurate results without the need for separation, purification or heating processes, thus being an not cost and economical method. This method showed good sensitivity, as the molar absorption value reached 14809.5 L/mol.cm with a good analytical range 2-23 µg/mL and acceptable recovery percentage approximately close to 100%. Thus, the method succeeded in achieving reliable analytical results, which enhances the possibility of its use in the routine analysis of theophylline in pharmaceutical preparations and biological analysis, without the influence of interferences and additives.

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Conflict of interest

The author declare that we have no conflict of interest.

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