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Simultaneous Determination of Sulfamethoxazole and Trimethoprim Based on Different Reaction Rates Using H-Point Standard Addition Method

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ABSTRACT

A simple, selective, cost-effective, and reliable kinetic spectrophotometric method has been developed for the simultaneous quantification of sulfamethoxazole (SMX) and trimethoprim (TMP) in pharmaceutical formulations. The method is based on the differential oxidation reaction rates of SMX and TMP with potassium permanganate ($KMnO_4$) in an alkaline medium (NaOH), resulting in the formation of green potassium manganate (K_2MnO_4), which exhibits a maximum absorbance at 610 nm. Kinetic studies of the oxidation reaction were conducted, and simultaneous determination of SMX and TMP was achieved using the H-point standard addition method. Absorbance measurements were recorded at 610 nm after 120 and 300 seconds following the addition of TMP. The method demonstrated high accuracy and precision, with percentage errors of 1.04% to -2.24% for SMX and 3.01% to -2.33% for TMP. Repeatability, assessed over five consecutive determinations of 10 µg/mL SMX and 4 µg/mL TMP, yielded relative standard deviations of 1.39% and 0.58%, respectively. The detection limits were 0.7 µg/mL for SMX and 0.3 µg/mL for TMP. The method was successfully applied to the analysis of various tablet formulations containing both active pharmaceutical ingredients.

Highlights

- Simultaneous kinetic spectrophotometry analysis of sulfamethoxazole and trimethoprim was accomplished through this work.
- The work is based on different reaction rates using the H-point standard addition method.
- The proposed method is economical, precise and accurat

1.Introduction

Trimethoprim (TMP)-sulfamethoxazole (SMX) is used to treat respiratory tract, genitourinary and gastrointestinal infections caused by susceptible bacteria. Additionally, *Salmonella typhi,* a typhoid fever agent, is also effectively treated by this combination. The ability to treat travellers' diarrhoea caused by susceptible *E. coli* is a further benefit of using TMP-SMX (Craig and Stitzel, 2004).

Bacteria produce dihydrofolate (DHF) from p-aminobenzoic acid (PABA), then produce tetrahydrofolic acid (THF) from DHF; THF is important coenzyme for the synthesis of several major components of DNA and RNA, and these components are necessary for cell growth and replication. SMX is a sulphonamide; the chemical, pharmacological and antibacterial characteristics of SMX were varied by attaching a methylisoxazole substituent to the amido group (-SO_2-H-R). This modification has resulted in a structure similar to PABA, which inhibits the synthesis of DHF in gram-positive and gram-negative bacteria. Most bacteria are capable of synthesising DHF from precursors. Here, TMP interferes with the bacterial biosynthesis of THF, preventing the bacteria from growing or multiplying (Katzung et al., 2004, Lüllmann and Mohr, 1999).

As previously mentioned, the combination of TMP with a sulphonamide inhibits sequential processes in DHF production, leading to a significant increase in the effectiveness of both drugs. The combination often exhibits bactericidal effects, compared with the bacteriostatic activity of a sulphonamide alone (Katzung et al., 2004).

Accordingly, due to the widespread use of the TMP-SMX combination, a method for analysing this combination is important. In the literature, the determination of TMP and SMX has been described based on the combination of products and the individual ingredients, such as in analytical methods for determining TMP, including liquid chromatography (Hussein and Rasheed, 2021), chemiluminescence (He et al., 2005) and voltammetry (Metto et al., 2024). TMP was determined spectrophotometrically after reaction with persulphate in alkaline media and in a boiling water bath for 30 minutes (Qureshi et al., 1997) and reaction as a π-donor with π-acceptor reagents (bromocresol green, 2,4-dinitro-1-fluorobenzene, bromothymol blue and alizarin red S) in non-aqueous media (El-Ansary et al., 1999, Stojković et al., 2016). It was also determined using an oxidative-coupling reaction with 4-(methylamino)phenol sulphate in the presence of potassium hexacyanoferrate (III) (Raauf, 2012).

However, various coupling reagents have been used for the spectrophotometric determination of SMX following its diazotisation (Abbas et al., 2021, Azeez and Mohammed, 2022, Khalaf et al., 2017) as well as through Schiff base formation between SMX and vanillin (Mehdi, 2015). Additionally, several analytical methods have been proposed for determining of SMX, including fluorometric (Guo et al., 2024), chemiluminescence (Yang et al., 2024), HPLC (Notario et al., 2022) and electroanalytical techniques (Alberto et al., 2023, Javar et al., 2022).

Various analytical methods for the simultaneous determination of TMP and SMX have been reported in the literature, including multivariate calibration models based on partial least squares and principal component regression (Rohman et al., 2015), as well as spectrofluorimetry (Alsayegh and Alshirifi, 2024). Although these methods may be accurate and potentially useful, they may include complex data processing, and high-cost equipment, or the extremely sensitive conditions of the experiment may prevent their routine application. Conversely, the H-point standard addition method (HPSAM) used in the current research provides an easier, less expensive and more powerful alternative. It reduces matrix interference, and does not require complex statistical models or advanced instruments, making it more feasible to apply in routine analysis in laboratories with limited resources. Shamsa and Amani proposed a method for the direct determination of SMX after diazotisation and coupling in the visible region and indirect determination of TMP in the UV

region (Shamsa and Amani, 2006). Another method was proposed for prior determination of TMP based on complexation with chloranilic acid in organic solvent followed by SMX determination after hydrolysis in H_2SO_4 and complexation (Adegoke et al., 2017). However, the different methods entail sdditional chemical modification cycles, which complicate the process and make it more time-consuming. Conversely, the proposed method enables the direct and simultaneous determination of both analytes. Additionally, several analytical methods such as HPLC (Aslam et al., 2023, Nong et al., 2025), capillary zone electrophoresis (da Silva et al., 2013, Liu et al., 2017) and voltammetry (Cesarino et al., 2013) have been reported for determining TMP and SMX. Although these techniques provide high sensitivity and selectivity, they typically require expensive instrumentation, complex optimisation, or electrode modification procedures, which can restrict their broad applicability in routine laboratories. In comparison, the HPSAM developed in this work is simpler, more economical and does not require advanced equipment, making it a practical alternative for routine analysis. A method employing HPSAM has been proposed for the simultaneous determination of both drugs; however, its application to real sample analysis is limited due to its reliance on measurements in the UV region (Givianrad et al., 2011).

The development of novel methods for the simultaneous determination of TMP and SMX is critical, given the increasing production and widespread use of these drugs in combination. In this work, a simple, low-cost, sensitive, selective, accurate and precise HPSAM was proposed for the simultaneous determination of this combination (TMP and SMX) in tablets, based on the different rates of oxidation of SMX and TMP with the common reagent $KMnO_4$ in a basic medium.

2. Materials and methods

2.1. Apparatus

A (SHIMADZU UV-1900i-Japan) UV/Visible spectrophotometer (190 - 1100nm) with a 1.0 cm path length quartz cell was used in the present work. A water bath (Lab. Companion shaking BS-11, Korea) was used since the reaction requires stirring and heat control.

2.2. Reagents

All reagents were of analytical grade, and deionised water (DW) was used for the preparation and dilution of their working solutions. The stock solution of standardised NaOH (Scharlau-Spain) and $KMnO_4$ (THORNTON & ROSS-England) was prepared daily.

2.3. Standard and sample solutions

A stock solution of 1000 µg/mL standard SMX and TMP (Awamedica Swedish expertise & quality, Erbil, Iraq) was prepared daily by dissolving 0.1 g of each analyte in 50 mL HCl (0.2 M), and then diluting to 100 mL with DW in a volumetric flask. The working solutions (100 µg/mL) were prepared by serial dilution of appropriate volume of standard solutions of both drugs with DW.

Sample solutions of different manufactured tablets, purchased from local drug stores (Table 1), were prepared by weighing ten tablets of the commercial sample then crushing and mixing them. A stock solution for HPSAM was prepared accurately by dissolving the average weight of each tablet in 50 mL HCl (0.2 M) in a beaker, shaking mechanically for 15 minutes and filtering. The residue was washed thrice with DW before diluting the filtrate to the mark with DW in a volumetric flask.

A sample solution of oral suspension was prepared after separately extracting SMX and TMP from the pharmaceutical formulation. SMX was extracted by initially diluting a 6.25 mL aliquot of oral suspension to 1 L, then transferring 10 mL from the diluted solution into a conical-bottom test tube, and adjusting the pH to 5. Subsequently, 200 µL of chloroform and 600 µL of acetonitrile were added to the aqueous phase using a glass syringe. The tube was then subjected to ultrasonic agitation for 7.5 minutes. Following sonication, the mixture was centrifuged at 5500 rpm for 5minutes. After centrifugation, solvent droplets containing SMX visibly collected at the bottom of the tube, and the resulting precipitated phase was completely separated. The solvent was evaporated and the residue

dissolved in 10mL HCl (0.2 M), followed by dilution to 25 mL with DW in a volumetric flask (Amin et al., 2023).

TMP was extracted with four 50 mL portions of chloroform after adding 30 mL of NaOH (0.1 M) to 5 mL of the oral suspension and shaking, each extract being washed with the same two 10 mL portions of 0.1 M NaOH. The combined chloroform extracts were reserved and extracted with four 50 mL portions of 1 M acetic acid. The combined acetic acid extracts were washed with 5 mL of chloroform and diluted to 250 mL with 1 M acetic acid solution; 12.5 mL from this solution was diluted to the mark with DW in a 100mL volumetric flask (Office and Commission, 2008).

Table 1. Samples analysed by the present method

Sample No.	Name	Manufacture-Country	SMX (mg/tablet,5mL)	TMP (mg/tablet,5mL)
1	Trimazol Awa (Tablet)	Awamedica-Iraq	400	80
2	METHEPRIM (Tablet)	SDI- Iraq	400	80
3	Trimoks (Tablet)	ATABAY-Turkey	400	80
4	TMS forte (Tablet)	T&D Pharma-Germany	800	160
5	Trimoks (Oral suspension)	ATABAY-Turkey	200	40

2.4. Procedure

The mixtures of SMX and TMP were transferred to a volumetric flask (10 mL) in a water bath at 40^{o} C with shaking, followed by standard addition of different concentrations of TMP in the range of 2.0 - 8.0 µg/mL; 2mL of NaOH (0.5 M) and 1 mL of $KMnO_4$ (0.005 M) were then added. The volume was diluted to the mark with DW after 120 s (t_1), and the absorbance was measured immediately against a blank at the λ_{max} (610 nm Figure 1). The second absorbance was then read after shaking for 300 s (t_2) in the water bath. SMX had the same absorbance in both measurements; therefore, it was considered an interferent. Conversely, TMP was considered an analyte because it has different absorbances at the selected measurement times (Figure 1). Two straight lines were obtained by plotting the absorbance against the corresponding added TMP concentrations so that they had a common point with the H coordinates ($-C_H$, A_H), where ($-C_H$) is the unknown analyte concentration (TMP) and A_H is the analytical signal due to the interferent (SMX). Therefore, SMX was determined by plotting A_H in the standard SMX constructed calibration graph at t_2 and 610 nm with a regression equation of y_{t2} = 0.0174x - 0.0016 and a correlation coefficient of 0.9999.

3. Results and discussion

The proposed method is a beneficial analytical technique for analysing binary and multicomponent mixtures of drugs when the rate constants of the two components are time-dependent (Bosch-Reig et al., 1991). The well-known oxidising agent, $KMnO_4$ has been proposed for kinetic spectrophotometric HPSAM for simultaneous determination (Issa et al., 2013). Validation of the current method was performed as a simple analytical method for the simultaneous determination of the two drugs.

The comparison revealed that spectrofluorimetry (Alsayegh and Alshirifi, 2024) provided the best sensitivity, with limit of detections (LODs) as low as 0.015–0.016 µg/mL; however it requires advanced instrumentation and strict control of experimental conditions so may not be accessible in many routine laboratories. HPLC (Nong et al., 2025) also offers good accuracy, although in this case, its reported precision (relative standard deviation (RSD) up to 2.83%) is lower than that of several other methods, and the method remains expensive and solvent-consuming.

Voltammetry (Cesarino et al., 2013) and the conventional HPSAM (Givianrad et al., 2011) exhibit good recovery and sensitivity but have high variability (RSD values of 2.0%) and practical concerns such as electrode preparation or long analysis times.

In comparison to these, the suggested HPSAM achieves detection limits of 0.3 µg/mL (TMP) and 0.7 µg/mL (SMX), which, while not as low as for spectrofluorimetry, remain competitive with voltammetry and conventional HPSAM. More significantly, this method provides high recovery (≈99%–102%) and excellent precision (RSD <1.5%), making it comparable to or better than several advanced methods. Its key advantage lies in its simplicity, low cost, and ability to simultaneously determine TMP and SMX without the need for expensive instruments, derivatisation or complex statistical modelling. Table 2 shows the results.

In the preliminary investigation, 0.5 mL of 200 µg/mL SMX and TMP (10 µg/mL) were separately added into two different 10 mL volumetric flasks, then 1 mL of 0.5 M NaOH and 1 mL 0.005 M $KMnO_4$ were added to each volumetric flask and both solutions were diluted to the mark with DW. The green colour formed faster with SMX than with TMP due to the different rates of their oxidation reactions with $KMnO_4$.

The various experimental factors were studied and optimised. The orders of addition of the reagents were studied. Mixing SMX or TMP with NaOH and adding $KMnO_4$ exhibited high sensitivity. The influences of NaOH and $KMnO_4$ were studied separately using different volumes of 0.5 - 3 mL. The highest result was obtained with 2 mL NaOH (0.5 M) and 1 mL $KMnO_4$ (0.005 M). For increased simplicity and better temperature control, the effect of temperature was studied by placing the reaction vessel in a water bath at different temperatures with a constant heating time of 5 minutes with shaking. The maximum absorbance was achieved at 40° C, indicating complete oxidation of drugs and formation of the green product. Above 40° C, the absorbance decreased due to a decrease in the green product due to the formation of yellowish-brown MnO_2.

Table 2. Comparison of the proposed method with previously reported methods.

Method	Analyte	LOD (µg/mL)	LOQ (µg/mL)	R (%)	RSD (%)	Reference
Spectrofluorimetry	TMP	0.016	0.036	101.29	0.64	(Alsayegh and Alshirifi, 2024)
	SMX	0.015	0.033	100.96	0.56	
HPLC	TMP	---	1.88	99.62	2.83	(Nong et al., 2025)
	SMX	---	0.19	105.35	1.25	
HPSAM	TMP	0.11	---	103.33	2.01	(Givianrad et al., 2011)
	SMX	0.15	---	102.55	1.63	
Voltametry	TMP	0.009	---	99.4	1.8	(Cesarino et al., 2013)
	SMX	0.006	---	102	2.5	
Proposed method	TMP	0.3	0.97	101.51	0.58	This work
	SMX	0.7	2.22	98.96	1.39	

3.1. Initial-rate method

A rate expression is written as follows:

$$Rate = K[KMnO_4]^m[Drug]^n \quad \text{..... (1)}$$

where *m* and *n* are the orders of the reactions. Under optimised experimental conditions (using excess $KMnO_4$), the above equation is written as follows:

$$Rate = \check{K}[Drug]^n \quad \text{..... (2)}$$

$$\log Rate = \log \check{K} + n \log C \quad \text{..... (3)}$$

In this context, $\check{K}$ refers to the pseudo-order rate constant, *n* is the reaction order, and C stands for the concentration of either SMX or TMP. The reaction rate can be determined by the change in absorbance over time ($\Delta A/\Delta t$), where *A* represents the absorbance and *t* denotes the time in seconds. Taking the logarithms of the rates and concentrations (Table 3) from the limiting logarithmic plot (Figures 2 and 3), the above equation is written as follows:

$$\log Rate = 3.802 + 1.0368 \log[SMX] \quad \text{..... (4)}$$

$$\log Rate = 3.6303 + 0.9944 \log[TMP] \quad \text{..... (5)}$$

The reactions are first order concerning both drugs. This distinct kinetic behaviour was used to

simultaneously determine SMX and TMP. Table 3 presents the calibration graph parameters for the separate determination of SMX and TMP using the initial-rate method (El-Didamony and Abo-Elsoad, 2017, Issa et al., 2013).

Table 3. Logarithms of the rates for different concentrations of SMX and TMP and calibration graph characteristics, for determination of SMX and TMP using the initial-rate method.

Log (ΔA/Δt)	Log[SMX]	Log (ΔA/Δt)	Log[TMP]
-1.82	-5.40	-1.79	-5.46
-1.46	-5.1	-1.5	-5.16
-1.16	-4.8	-1.2	-4.86
-0.86	-4.5	-0.90	-4.55
-0.68	-4.32	-0.72	-4.38
-0.55	-4.19	-0.6	-4.25
-0.45	-4.10	-0.5	-4.16
Linear range (mol/L)	3.94×10^{-6} - 7.89×10^{-5}		3.44×10^{-6} - 6.88×10^{-5}
Linear range (μg/mL)	1-20		1-20
Slope	1.0368		0.9944
Intercept	3.802		3.6303
R^2	0.9992		0.9999

3.2. Applying HPSAM

The rate of the oxidation reaction between SMX and $KMnO_4$ was faster than that of TMP and $KMnO_4$. As demonstrated in Figure 4, the reaction of SMX was almost complete after 120 s, while the reaction of TMP was not complete even at 300 s under the same conditions. In this case, the times 120 and 300s were selected for measuring the absorbance at which the produced green compound had the same signal over the range between these two times in the presence of the interferent (SMX). Conversely, the signals differed in the presence of the analyte (TMP). The analytical signal of the mixture of SMX and TMP equalled the sum of the individual signals of the two drugs. The two straight lines obtained at two selected wavelengths differed considerably with respect to their slopes. Therefore, the HPSAM can be used for the simultaneous determination of SMX and TMP (Bosch-Reig et al., 1991).

The regression equations at the two selected times t_1=120 s and t_2=300 s could be described as follows:

$$A_{t_1} = S_{t_1}C_{t_1} + In_{t_1}$$
$$A_{t_2} = S_{t_2}C_{t_2} + In_{t_2}$$

Here (A) is the absorbance, (S) is the slope, (C) is the concentration and (In) is the intercept. The intersection point of the pair of calibrations is the H-point with coordinate ($-C_H$, A_H), where $-C_H$ is the concentration of analyte TMP and A_H is the analytical signal of interferent SMX. Thus, the constant analyte (TMP) concentration ($-C_H$) can be calculated as follows:

At the H-point,

$$A_{t_2} = A_{t_1}$$
$$S_{t_2}C_{t_2} + In_{t_2} = S_{t_1}C_{t_1} + In_{t_1}$$
$$S_{t_2}C_{t_2} - S_{t_1}C_{t_1} = In_{t_1} - In_{t_2}$$
$$C_{t_1} = C_{t_2} = -C_H$$
$$-C_H(S_{t_2} - S_{t_1}) = In_{t_1} - In_{t_2}$$
$$-C_H = \frac{In_{t_1} - In_{t_2}}{S_{t_2} - S_{t_1}}$$

For the interferent (SMX) determination, the analytical signal of SMX at the H-point (A_H) was substituted in the individual regression equation of the SMX and calculated as follows:

$$C_{t_2} = C_{t_1}$$
$$\frac{A_{t_2} - In_{t_2}}{S_{t_2}} = \frac{A_{t_1} - In_{t_1}}{S_{t_1}}$$
$$S_{t_2}(A_{t_1} - In_{t_1}) = S_{t_1}(A_{t_2} - In_{t_2})$$
$$S_{t_2}A_{t_1} - S_{t_2}In_{t_1} = S_{t_1}A_{t_2} - S_{t_1}In_{t_2}$$
$$S_{t_2}A_{t_1} - S_{t_1}A_{t_2} = S_{t_2}In_{t_1} - S_{t_1}In_{t_2}$$
$$A_{t_2} = A_{t_1} = A_H$$
$$A_H(S_{t_2} - S_{t_1}) = S_{t_2}In_{t_1} - S_{t_1}In_{t_2}$$
$$A_H = \frac{S_{t_2}In_{t_1} - S_{t_1}In_{t_2}}{S_{t_2} - S_{t_1}}$$

3.3. Accuracy and specificity

The accuracy and specificity of the method were assessed using the proposed HPSAM for analysing several mixtures with a constant TMP concentration (4 µg/mL) and different SMX concentrations (6, 8 and 10 µg/mL, Figure 5). Conversely, different TMP concentrations (4, 6 and 8 µg/mL) and a constant SMX concentration (10 µg/mL) were analysed (Figure 6). Table 4 provides the results. The results confirm that SMX and TMP different concentration ratios can be simultaneously and accurately determined by applying the proposed HPSAM.

Table 4. Accuracy of HPSAM

		Concentration (µg/mL)		TMP determination (µg/mL)		SMX determination (µg/mL)	
Regression equations	**R^2**	**TMP**	**SMX**	**TMP**	**%E**	**SMX[a]**	**%E**
y_{t1} = 0.0071x + 0.1308	0.9998	4	6	4.05	1.16	5.96	-0.7
y_{t2} = 0.0157x + 0.1656	0.9996						
y_{t1} = 0.007x + 0.1664	0.9994	4	8	3.91	-2.33	8.08	1.04
y_{t2} = 0.0156x + 0.2	0.9997						
y_{t1} = 0.0072x + 0.1976	0.9991	4	10	4.07	1.75	9.78	-2.24
y_{t2} = 0.0157x + 0.2324	0.9997						
y_{t1} = 0.0072x + 0.1976	0.9991	4	10	4.07	1.75	9.78	-2.24
y_{t2} = 0.0157x + 0.2324	0.9997						
y_{t1} = 0.0071x + 0.2124	0.9994	6	10	6.13	2.17	9.82	-1.85
y_{t2} = 0.0155x + 0.2642	0.9999						
y_{t1} = 0.0069x + 0.2276	0.9987	8	10	8.24	1.75	9.90	-0.96
y_{t2} = 0.0152x + 0.296	0.9999						

[a]calculated from calibration curve of SMX at 300s (t_2) with regression equation of y_{t2} = 0.0174x - 0.0016

3.4. Repeatability

Five replicate experiments with 4 µg/mL TMP and 10 µg/mL SMX solution mixtures were conducted to investigate the repeatability of the method (Table 5). The concentrations of the analyte TMP and interferent SMX were calculated in each test solution using the HPSAM. The relative standard deviations for the five replicate analyses of the TMP and SMX mixtures were 0.58% and 1.39% respectively.

Table 5. Results for five replicate analyses of TMP and SMX by HPSAM

		Concentration (µg/mL)		Found (µg/mL)	
Regression equations	**R^2**	**TMP**	**SMX**	**TMP**	**SMX[a]**
y_{t1} = 0.0072x + 0.1976	0.9991	4	10	4.07	9.78
y_{t2} = 0.0157x + 0.2324	0.9997				
y_{t1} = 0.0069x + 0.1996	0.9984	4	10	4.09	9.95
y_{t2} = 0.0155x + 0.2348	0.999				
y_{t1} = 0.007x + 0.197	0.9969	4	10	4.05	9.79
y_{t2} = 0.0155x + 0.2314	0.999				
y_{t1} = 0.0071x + 0.2032	0.9984	4	10	4.02	10.14
y_{t2} = 0.0157x + 0.2378	0.9996				
y_{t1} = 0.0066x + 0.1964	0.9971	4	10	4.07	9.82
y_{t2} = 0.0154x + 0.2318	0.9998				
Mean				4.06	9.90
SD				0.24	0.14
$RSD_{(n=5)}$				0.58	1.39
%R				101.51	98.96

[a]calculated from calibration curve of SMX at 300s (t_2) with regression equation of y_{t2} = 0.0174x - 0.0016

3.5. Limit of detection and limit of quantification

LOD and LOQ were calculated based on the blank determination method, applied when the

blank measurements yielded a non-zero standard deviation. Moreover, in the case of the HPSAM, the analysis involved two calibration curves with distinct slopes (Shrivastava and Gupta, 2011) as follows:

$$LOD = X_b + 3S_b$$
$$LOQ = X_b + 10S_b$$

Here, X_b is the mean concentration of several (n = 5) replicated measurements of the blank, resembling TMP or SMX and S_b is the standard deviation of the blank. The corresponding values obtained were a LOD of 0.3 µg/mL and a LOQ of 0.97 µg/mL for TMP, and a LOD of 0.7 µg/mL and a LOQ of 2.22 µg/mL for SMX.

3.6. Interferents

The potential interference from common pharmaceutical excipients (starch, povidone, cellulose, and magnesium stearate) was evaluated by adding each excipient to a solution containing known concentrations of SMX and TMP. Each excipient was introduced at a concentration equivalent to a maximum excess of 0.3 g per tablet, relative to the active pharmaceutical ingredients, into one litre of a solution comprising 400 µg/mL SMX and 160µg/mL TMP. This mixture was then diluted to obtain a working solution containing 100 µg/mL SMX and 40 µg/mL TMP. Finally, analytical determinations were conducted at concentrations of 10 µg/mL for SMX and 4 µg/mL for TMP in the presence of the added excipients. The results indicated that the tested excipients did not cause significant interference with the analysis of either compound.

3.7. Application

Some pharmaceuticals have been analysed to confirm the applicability of the proposed HPSAM in the simultaneous determination of TMP and SMX. The concentrations were obtained via the triplicate analysis of each pharmaceutical sample (Table 1). The precision was investigated and found to be less than 2.36% (RSD) for TMP and 1.10% for SMX. The quality of the results obtained with the HPSAM was assessed by determining TMP and SMX using the present and a standard method of the British Pharmacopoeia (Office and Commission, 2008) as mentioned in Table 6. These results show good agreement between the two methods. The results of the present method were compared statistically with those obtained by the standard method employing a *t*-test and *F*-test. The results indicated no significant difference between the accuracy and precision of the two methods at a 95% confidence level.

Table 6. Application of the proposed HPSAM for determination of TMP (2µg/mL) and SMX (10µg/mL) in different pharmaceutical samples

	TMP determination						SMX determination					
Sample	HPSAM (µg/ml±SD)	RSD *(n=3)*	BP (µg/ml±SD)	%E	*t*-test	*F*-test	HPSAM[a] (µg/ml±SD)	RSD *(n=3)*	BP (µg/ml±SD)	%E	*t*-test	*F*-test
1	2.06±0.04	1.94	2.01±0.02	2.48	1.93	0.25	9.86±0.06	0.6	9.72±0.09	1.44	2.24	2.25
2	1.99±0.04	2.01	1.97±0.03	1.01	0.69	0.56	9.98±0.11	1.10	9.7±0.16	2.88	2.49	0.47
3	2.06±0.03	1.46	2.003±0.03	2.84	2.32	1	9.95±0.07	0.70	9.69±0.18	2.68	2.33	6.61
4	2.04±0.01	0.49	2.02±0.02	0.99	1.54	4	10.01±0.04	0.30	9.9±0.04	1.11	3.81	1.77
5	1.99±.04	2.36	2.01±0.01	-0.99	0.08	0.01	9.88±0.07	0.79	9.98±0.06	-1.00	1.87	0.73

[a]calculated from calibration curve of SMX at 300s (t_2) with regression equation of (y_{t2} = 0.0174x - 0.0016) Tabulated values: $t_{95\%\ (3+3-2)}$ = 2.776 , $F_{(2,2)}$= 19

4. Conclusion

The study of the proposed method confirms that the kinetic spectrophotometric HPSAM can be beneficial in solving the difficulty when TMP and SMX react simultaneously with $KMnO_4$ under the same reaction conditions, while simultaneously determining both with satisfactory simplicity. The proposed method requires no expensive resources, unlike chromatographic and spectrofluorimetric methods, which require expensive tools, solvents or complicated calibration models, and can still provide satisfactory sensitivity, accuracy and precision. Furthermore, a good recovery percentage was attained for the spiked sample analysis. It was also concluded that the proposed method is

selective; the matrix effect can be eliminated during the analysis of TMP and SMX in pharmaceutical formulations. Most importantly, the proposed methods are sensitive (low LODs) and can be conducted with good precision and accuracy. Notably, the approach provides a good balance between usability and performance, and its sensitivity is moderate compared to other methods (Table 2); however it provides excellent compromise between accessibility and analytical reliability. These strengths make the method appropriate and valid for use in laboratories lacking liquid chromatographic instruments for quality control of the drugs.

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Statements and declarations
The author declares no conflict of interest, financial or otherwise.

Conflict of interest
The author declares no competing interests.

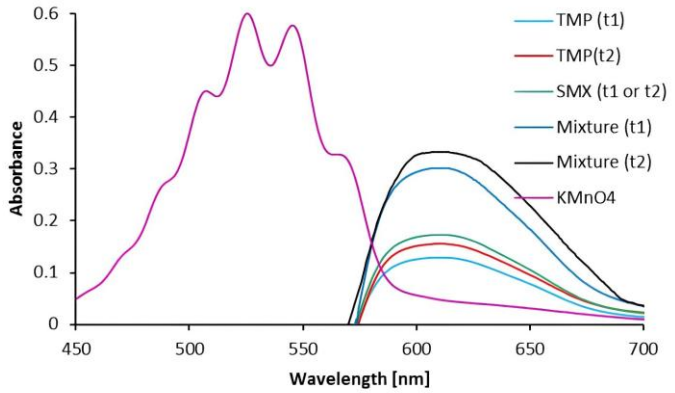

Figure 1. Absorption spectra showing the selected wavelength and time for HPSAM. (—) 10µg/mL TMP at 120s, (—) 10µg/mL TMP at 300s, (—)10µg/mL SMX at 120s or 300s, (—)mixture of 10µg/mL TMP and 10µg/mL SMX at 120s, (—) mixture of 10µg/mL TMP and 10µg/mL SMX at 300s in the presence 2mL NaOH (0.5M) and 1mL $KMnO_4$ (0.005M) and (—) $KMnO_4$ ($2.5x10^{-4}$M.

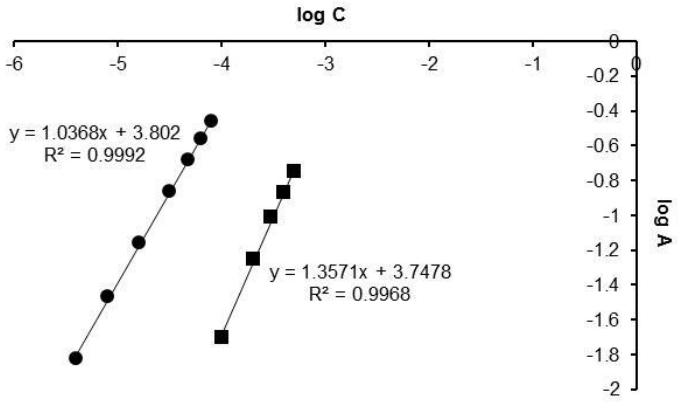

Figure 2. Limiting logarithmic plot for the molar ratio (■) LogA vs. Log [$KMnO_4$] and (•) LogA vs. Log [SMX].

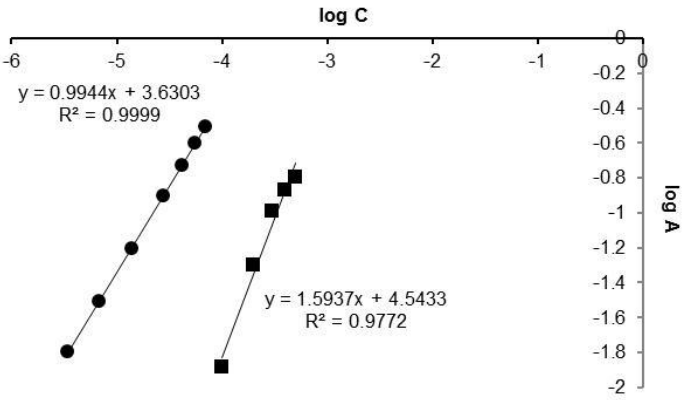

Figure 3. Limiting logarithmic plot for the molar ratio (■) LogA vs. Log [$KMnO_4$] and (•) LogA vs. Log [TMP].

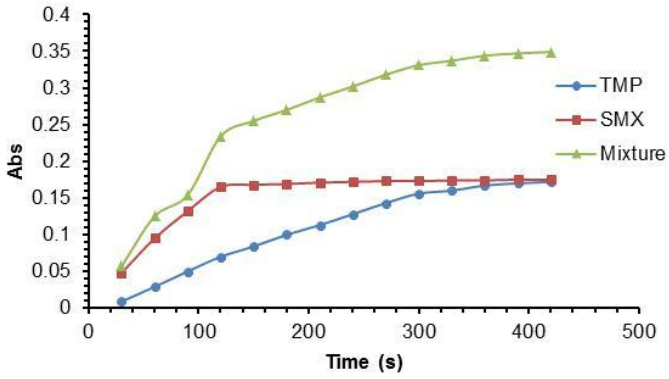

Figure 4. Absorbance-time plots for (•) 10µg/mL TMP, (■) 10µg/mL SMX and (▲) mixture of SMX and TMP in the presence 2mL NaOH (0.5M) and 1mL $KMnO_4$ (0.005M).

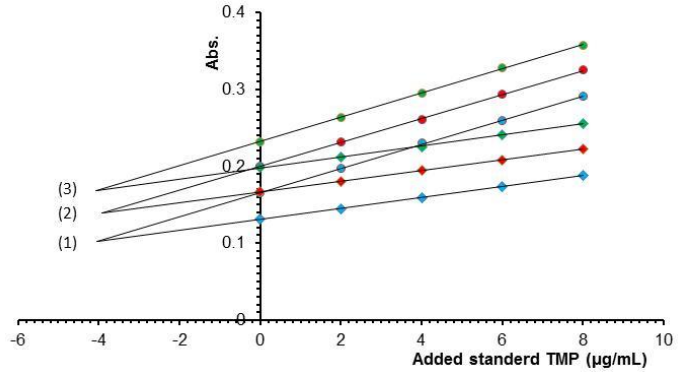

Figure 5. Plots of HPSAM between the absorbance at t_1 (■) and t_2 (•) and the added concentration of TMP to the mixture of fixed TMP (4µg/mL) and SMX (1) 6µg/mL (2) 8µg/mL (3) 10µg/mL.

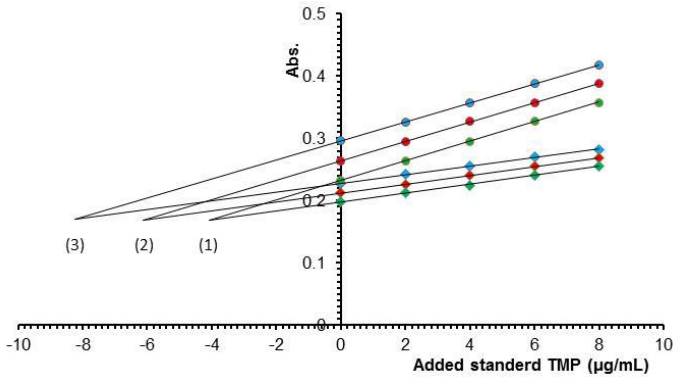

Figure 6. Plots of HPSAM between the absorbance at t_1 (■) and t_2 (•) and the added concentration of TMP to the mixture of fixed SMX (10µg/mL) and TMP (1) 4µg/mL (2) 6µg/mL (3) (8µg/mL).

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