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GREEN METHOD FOR THE SPECTROPHOTOMETRIC DETERMINATION OF PHENYLEPHRINE AND TERBUTALINE PHARMACEUTICAL FORMULATIONS

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Abstract: *Determination and evaluation active substances in chemical settings is extremely difficult, linked to chemical waste in water sewage, and may eventually have an influence on public health. As a result, efforts are being made globally to find other, perhaps less hazardous ways. Hereby, we aimed to determine two pharmaceutical products for the detection of phenylephrine hydrochloride and terbutaline sulphate using green chemistry. The procedure involves oxidizing phenylephrine hydrochloride and terbutaline sulphate using potassium permanganate as an oxidant and decolorizing the surplus of potassium permanganate in the reaction with Nile blue dye. The results confirmed that this method has provided easy and precise evaluation of phenylephrine hydrochloride and terbutaline sulphate at concentrations ranging from (0.4-4.5) µg /ml, with molar absorption capacity of ($6.9x10^4$) $l{\cdot}mol^{-1}{\cdot}cm^{-1}$ for phenylephrine hydrochloride and ($1.44{\times}10^5$) $l{\cdot}mol^{-1}{\cdot}cm^{-1}$ for terbutaline sulphate. The methodologies have precisely determined the pharmaceutical dosage forms of the tested drugs, agreeing with previously tested and stated values and standard compounds.*

Keywords: *Phenylephrine, Nile blue dye, Potassium permanganate, Terbutaline.*
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1. Introduction

The science of "green analytical chemistry" employs chemical processes and strategies to minimize the usage of hazardous substances, toxic byproducts, and raw resources during the manufacturing process. It attempts to conserve the environment by developing new chemical processes that don't pollute the earth [1].

Phenylephrine hydrochloride (PPH.HCl), $C_9H_{14}ClNO_2$, [(1R)-1-(3-hydroxyphenyl)-2-(methylamino)ethanol hydrochloride], and have the following chemical structure: $C_9H_{13}NO_2.HCl$, M. wt (203.705) $g{\cdot}mol^{-1}$. PPH.HCl is used for relieving sinus congestion and pressure. It is also used to treat pharyngitis nasal, allergic conjunctivitis, and nonspecific conjunctivitis [2, 3], by directly acting on α-adrenergic receptor agonists [4]. Terbutaline sulphate (TEBS), bis [(1RS)-1-(3,5–dihydroxyphenyl)–2-[(1,1–dimethylethyl)amino] ethanol], sulfate [2]. TEBS, a β2-adrenergic receptor agonist, is widely used to treat pulmonary hypertrophy, asthma, and bronchobronchitis. It is an orally administered bronchodilator [5].

A variety of analytical approaches existed to determine phenylephrine hydrochloride and terbutaline sulphate, including spectrophotometric [6-14], high-performance liquid chromatography [15-21], fluorescence [22], voltammetry [23-24], electrochemical [25-27].

Oxidation of PPH.HCl by NBS in low pH environment, followed by bleaching color (e.g. methyl orange dye) with N-bromosuccinamide residue, whose intensity reciprocally proportional with PPH.HCl quantities [6]. The dualistic method based on oxidative coupling reaction of PPH.HCl with PABP catalyzed by KIO_4 as an oxidant [7], with reaction of

PPH.HCl with NQS reagent in alkali milieu [8] The dualistic method based on oxidative coupling reaction PPH.HCl with DMPD.2HCl catalyzed by $FeCl_3$ in alkaline media to form soluble green-blue coloring product [9], resulting in azo dye formation from Sodium sulfacetamide diazo reacts with sodium nitrite in the presence of chlorohydric acid to form diazonium salt, which is coupled with the PPH.HCl in alkaline medium [10]. TEBS reacts with antipyrine reagent in buffer medium catalyzed by ferric cyanide solution [11], generating TEBS and eosin binary complexes in aqueous acetate buffered medium[12]. Producing nucleophilic compensation by interacting of TEBS with the reagent 9-chloroacridine in basic medium [13]. This method is on the oxidation of TEBS with Fe(Ⅲ)in the medium of nittic acid and resulting Fe(Ⅲ) subsequent chelation with 1,10-phenanthrolinein method and 2,2′-bipyridyl method B to create colored products [14].

The purpose of this green analytical method study is to develop a simple and accurate colourimetric assay In order to determine phenylephrine chloride terbutaline sulfate in pure and pharmaceutical products, eliminating the usage of hazardous ingredients and waste generation.

2. Experimental part

2.1. Instrumentation:

A Shimadzu (UV-1800, PC UV-visible double, spectrophotometer) was used to measure the absorbance and spectra.

2.2. Reagents and Chemicals:

The "State Company for Drug Industries and Medical Appliances" supplies terbutaline sulphate and phenylephrine hydrochloride. Every chemical used is of the standard of an analytical reagent.

PPH.HCl, and TEBS: Solutions (100)µg/mL were prepared via 0.01gram of each drug added and dissolved in (100 ml) of (D.W) in a container.

NB dye: To prepare Nile blue dye (50)µg/mL, 0.01gram was dissolved in absolute ethanol in a (200) mL container.

$KMnO_4$: prepared using (0.016)g added to (100)ml of DW.

HCl: To prepare (1) M hydrochloric acid, (20) ml of concentrated HCl (5M) was diluted with DW in a (100) ml volumetric bottle.

2.3. Pharmaceutical preparation

Safadrop (1%): One millilitre of (1%) safadrop diluted with (100)ml of DW to obtain (100)µg/mL of PPH.HCl pharmaceutical.

Pioneer drop (0.25%): To attain PPH.HCl at (100)µg/mL, (1)mL of 0.25% Pioneer drop mixed with (24)ml of DW.

Terbutaline sulphate (Bricanyl Inhalation powder, 120 doses, each dose containing (0.5) mg TERS, equivalent to (60) mg total was used, and the drug was diluted by DW to obtain (100) µg /ml solution.

Standard Operating Procedure: Two series of (10) mL containers were filled with aliquots of solutions containing (0.3) µg/mL of PPH, HCl, and TEBS separately. Followed by adding 0.8 mL (160) µg/mL of $KMnO_4$ and 1 mL of (1M) HCl solution. After giving the solutions a gentle shake, they were allowed to oxidize for ten minutes at room temperature. Next, each solution received the addition of (2) mL (50) µg/mL NB dye. After adding DW to dilute the flasks to the appropriate level, were carefully mixed, and the absorbance at room temperature (638) nm was measured in comparison to the reagent blank.

3. Results and Discussion

The suggested method for determining phenolic medicines PPH, HCl, and TEBS comprises oxidizing them with $KMnO_4$ in an acidic medium and bleaching the blue colour of NB dye [28]. Adding $KMnO_4$ to increasing amounts of the drug reduces oxidant concentration and increases NB dye absorbance (Figure 1).

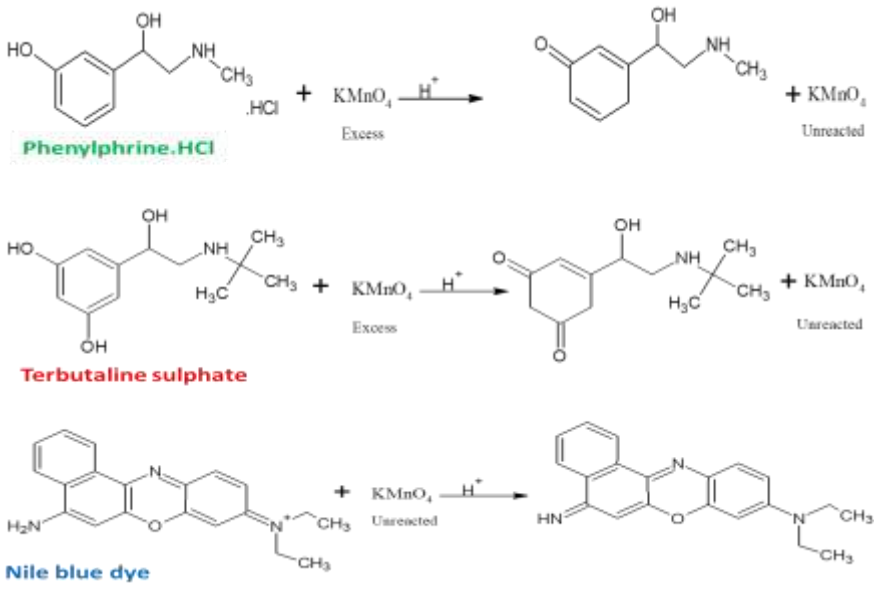

Fig. 1. Proposed chemical reactions between the tested drugs and $KMnO_4$.

3.1. Reaction condition optimization

Effect of Nile Blue Dye Concentration: Preliminary studies were conducted to establish the optimal concentration of NB dye using a spectrophotometer. The findings showed that (10) µg/mL of NB dye was an effective reaction agent (Figure 2A).

Effect of the type and concentration of the stabilizer: the reaction demonstrated that (0.8) mL of (160) µg/mL $KMnO_4$ mixture was sufficient to achieve higher decolorizing of the colour of NB dye. This was suggested in further studies (Figure 2B).

The Effect of Oxidant Reagents: it has been demonstrated that $KMnO_4$ behaves as beneficial oxidants, alongside other oxidants tested in the present study [chloramine-T, N-bromosuccinamid, and bromate-bromide], these latter have no extra-advantage over $KMnO_4$ (Figure 2C). The reaction confirmed that (0.8) ml of (160) µg /ml KMnO4 liquid was surplus to reach optimum decolorizing dependent NB dye, subsequently used in further steps.

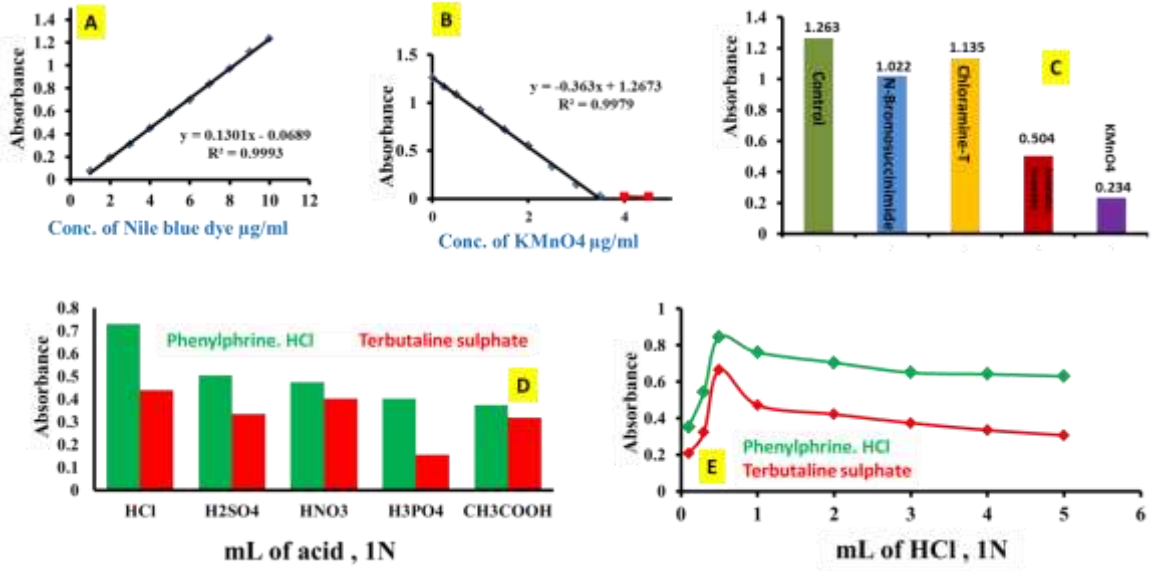

Fig. 2. Optimization conditions of reactant and reaction (A) Calibration curve of NB dye (B) Calibration curves for measuring oxidant concentration in (10) µg/mL NB dye in acidic solution. (C) Testing $KMnO_4$ compared to other types of oxidizing agents (D) Effect of acids on tested drugs (E) Effect of HCl quantity on tested drugs

Acid Types and Concentrations: Experiments have shown that $KMnO_4$ oxidizes NB dye and drugs in an acidic solution. To obtain high sensitivity, (3) µg/mL of each drug was tested against different acid types at concentrations of (1M) HCl was found to be the most proper acid for the reaction (Figure 2D). Furthermore, (0.5) mL of (1M) HCl was chosen as the optimal concentration for the two drugs (Figure 2E).

Time impacts on Oxidation: To test the impact of the reaction of oxidation duration on PPH, HCl, and TEBS drugs, (0.8) mL of (160) µg/mL $KMnO_4$ was mixed with (3) µg/mL of each drug in 0.5 mL of (1M HCl). The mixtures were concussed and leave it to stand at 25°C for various amounts of time. Next, add (2) ml of (50) µg/ml NB dye to each drug solution. The mixture was stirred and topped up to (10) mL. After five minutes of standing, the absorbance of leftover NB dye was detected at 638 nm against a blank solution. Table 1 indicates that drug oxidation takes 15 minutes and maintains a consistent absorbance for two hours.

Table1. Oxidation behaviour of the tested drugs versus NB dye regarding the time frame

Standing time before adding NB & Dilution (min)	Absorbance/standing time after adding NBD & dilution(min)									
	5	10	15	20	25	30	40	50	60	120
Phenylephrine										
After addition	0.540	0.536	0.532	0.530	0.527	0.526	0.524	0.521	0.520	0.515
5min	0.791	0.780	0.772	0.770	0.796	0.765	0.764	0.762	0.760	0.759
10min	0.835	0.830	0.828	0.828	0.827	0.826	0.824	0.824	0.823	0.822
15min	0.934	0.934	0.933	0.931	0.930	0.929	0.929	0.926	0.925	0.925
20min	0.906	0.909	0.906	0.904	0.903	0.901	0.909	0.908	0.907	0.906
Terbutaline										
After addition	0.440	0.438	0.436	0.433	0.432	0.431	0.429	0.428	0.427	0.427
5min	0.544	0.545	0.547	0.548	0.549	0.549	0.550	0.551	0.553	0.555
10min	0.646	0.648	0.649	0.649	0.650	0.652	0.654	0.655	0.656	0.658
15min	0.683	0.685	0.688	0.690	0.692	0.693	0.695	0.697	0.697	0.699
20min	0.634	0.632	0.635	0.637	0.638	0.638	0.638	0.637	0.636	0.636

3.2. Calibration Curves

Standard calibration curves were created by graphing absorbance against concentration for phenylephrine hydrochloride and terbutaline sulphate pharmaceuticals using NB dye under the experimental conditions stated (Figure 3).

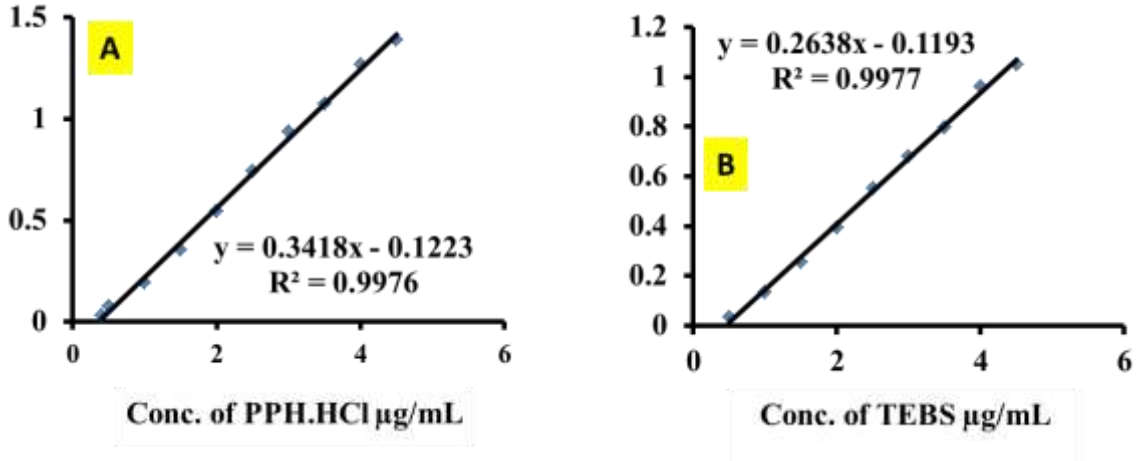

Fig. 3. Calibration graphs of phenylephrine hydrochloride (A) and terbutaline sulphate (B)

The limits of "Beer's law" (BL), "molar absorptivity" (MA) values, and "Sandell sensitivity" (SS) [29] were assessed and listed in table 2, and the limits of detection (LOD) and

quantitation (LOQ) [30] were computed using the subsequent formulas: LOQ = $10\sigma C_{low}/\bar{X}$ and LOD = $3\sigma C_{low}/\bar{X}$, where $\bar{X}$=mean absorbance of the lowest amount
σ= standard deviation of absorbance of minimum amount.

Table 2. Determination parameters of tested drugs (analytical and statistical)

Parameter	Phenylephrine	Terbutaline
Linearity range (µg/ml)	0.4 -4.5	0.5 -4.5
Slope	0.3418	0.2638
Intercept	0.1223	0.1193
R^2	0.9976	0.9977
MA ($l.mol^{-1}.cm^{-1}$)	6.9×10^4	1.44×10^5
SS ($\mu g/cm^2$)	0.002925	0.0038
LOD* (µg/ml)	0.0203	0.0263
LOQ* (µg/ml)	0.0678	0.0879
*Average of six determinations of blank, R^2=Determination coefficient, MA=molar absorptivity values, SS=Sandell sensitivity, (LOD=limits of detection), (LOQ=limits of quantitation).		

"Accuracy and Precision": By detecting the rate of the recovery and the reciprocal SD "(standard deviation)" of three distinct levels of each tested drug ingredient, the accuracy and precision were assessed. The findings presented in Table 3, show that the proposed approach is very accurate and precise.

Table 3. Determination parameters of tested drugs (accuracy and precision)

Durg	Conc. of drug(µg/ml)		Recovery* (%)	Mean Recovery (%)	RSD (%)
	Conc. used	Conc. detected			
Phenylephrine	1	0.9546	95.46	98.57	1.99
	2	1.952	97.60		1.154
	3	3.08	102.66		0.983
Terbutaline	1	0.9715	97.15	98.61	2.35
	2	1.949	97.45		1.07
	3	3.037	101.23		0.989
***** Average of three determinations.					

Analytical Applications of the method: The proposed approach was tested to determine phenylephrine hydrochloride and terbutaline sulphate in pharmaceutical dosage forms. Drug concentrations were evaluated using a direct calibration curve (Table 4 and Table 5), and a standard addition approach (Figure 4). Both methods produced equivalent results, suggesting that the recommended approach is free of interference.

Table 4. Determination of study drugs in pharmaceutical dosage.

Commercial product	Available dose	Conc. used (µg.ml-1)	Conc. detected (µg.ml-1	Recovery (%)
Phenylephrine hydrochloride				
Nazafrin Nasal drops (Safa Co, Diala, Iraq)	1%	1	0.978	97.80
		2	1.937	96.85
		3	3.14	103.66
		3.5	3.51	102.31
Nasofen Nasal drops (Pioneer Co, Sulaimani, Iraq)	0.25%	1	0.980	98
		2	1.987	99.35

		3	3.102	103.4
		3.5	3.564	101.82
Terbutaline sulphate				
Bricanyl Turbohaler Inhalation Powder (AstraZeneca, Switzerland)	0.5mg/dose 120 dose	1	0.967	96.70
		2	1.972	98.61
		3	3.01	100.3
		3.5	3.393	96.96

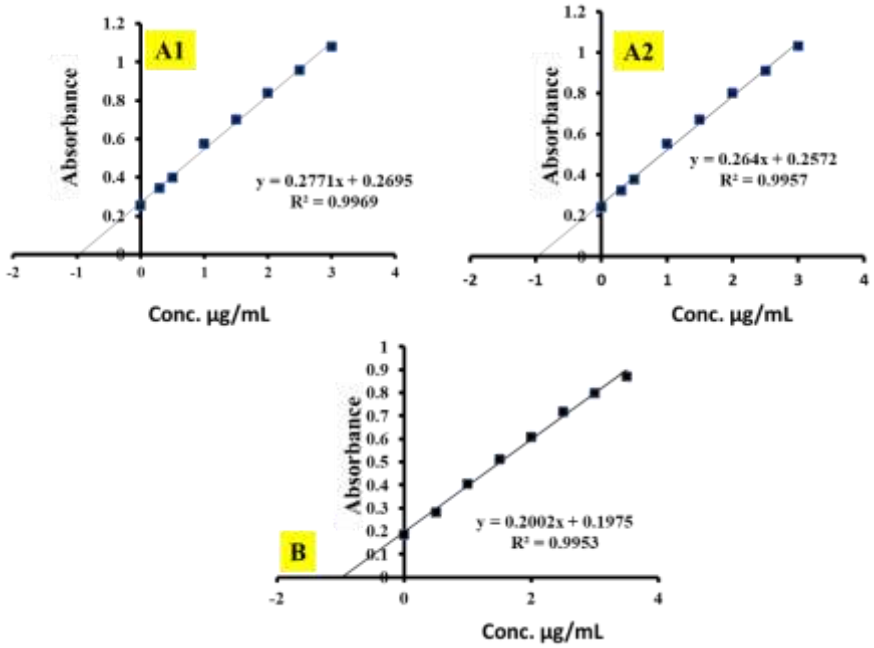

Fig. 4. Determination of phenylephrine hydrochloride (A1, Á2) and terbutaline sulphate(B) using standard addition technique.

Table 5. Detection of the studied drugs in the commercial products using present versus standard methods.

Commercial product	Available dose	Recovery (%)	Drug content found (mg)	
			Present method	Standard addition
Phenylephrine hydrochloride				
Nazafrin Nasal drops (Safa Co, Diala, Iraq)	1%	97.04	0.9780	0.9704
Nasofen Nasal drops (Pioneer Co, Sulaimani, Iraq)	0.25%	97.42	0.9809	0.9742
Terbutaline sulphate				
Bricanyl Turbohaler Inhalation Powder (Astra Zeneca, Switzerland)	0.5mg/dose 120 dose	98.65	0.9677	0.9865

3.3. Comparison of the proposed method with reported methods

The suggested reaction showed positive outcome compared to other available spectrophotometric methods (Table 6), being easier, no buffer or surfactant or heating steps, with the color stays put for over an hour. Using magnesium permanganate as an oxidizing agent

provide ecofriendly method for spectrophotometric evaluation (Figure 5 and Table 6).

Table 6. Comparison of the suggested versus available spectrophotometric methods.

Analytical parameters	Present method using NB dye	Literature method						
	PPH.HCl	TEBS	PPH.HCl [6]	PPH.HCl [7]	PPH.HCl [8]	TEBS [11]	TEBS [12]	TEBS [13]
λ_{max} (nm)	638	638	518	512	484	550	545	589
Beer's law (µg/ml)	0.4-4.5	0.5-4.5	0.1-1.25	2-20	0-20	4-20	0.5-10	0.1-6
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	6.9×10^4	$1.44\times10_5$	107050	0.552×10^4	4521-4074	1.1905×10^4	3.169×10^3	1.075410^5
Medium of method	Acidic	Acidic	Acidic	-	Basic	Acidic	Acetate buffer	Basic
Reagent	NB dye	NB dye	Methyl orange	p-aminobenzophenone	NQS	Amino antipyrine	EosinY	9-Chloroacridine
Type of reaction	Oxidation-reduction	Oxidation-reduction	Oxidation-reduction	Oxidative coupling	Sheff's base	Oxidation-reduction	Ion-pair	neucleophilic substitution
RSD%	0.983-1.99	0.989-2.35	-	0.0212-0.0715	≤3.3211	0.937	≤0.72	1.57
Sandell's sensitivity (S) μ/cm^2	0.002925	0.0038	-	0.0368	0.0451	0.0368844	-	-
Correlation Coefficient R^2	0.9976	0.9977	0.9999	0.9986	0.9952	0.9993	0.9984	0.9994
Limit of detection LOD (mg/l)	0.0203	0.0263	-	0.0094	3.2260	0.0811	0.030	0.0983
Limit of quantifcation LOQ (mg/l)	0.0678	0.0879	-	0.0313	9.7758	0.2460	0.103	0.2978
Application	Nasal drops	Inhalation Powder	Nasal drops	Drops -injection	Nasal drops-tablets	tablets	tablets	tablets

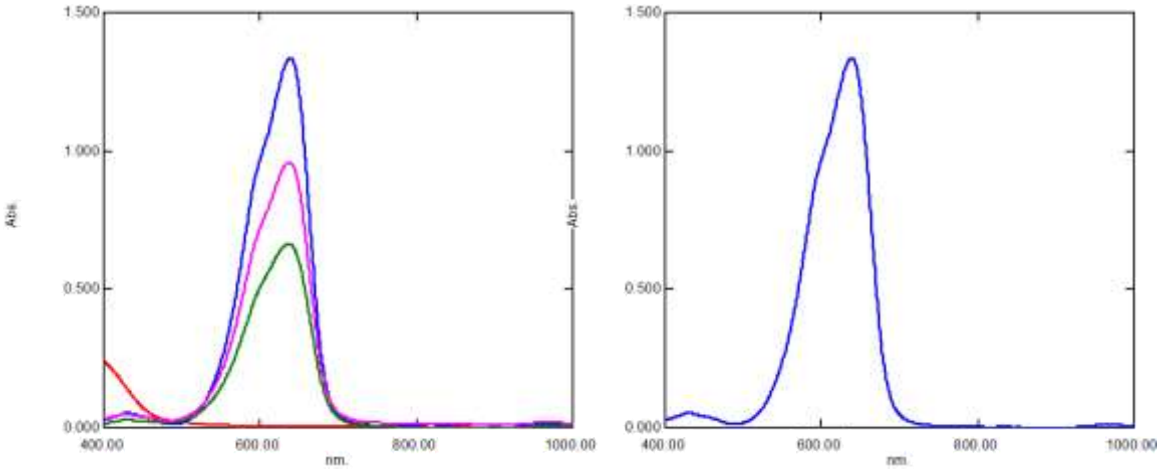

Fig. 5. Spectrophotometric evaluation of the proposed methods.

4. Conclusion

The present study has provided a new platform for the discovery of an easy, precise, and sensitive spectrophotometric technique for the evaluation and determination of phenylephrine hydrochloride and terbutaline sulphate in pharmaceutical products harnessing potassium permanganate as oxidant agents of the two commercial drugs. The surplus of potassium permanganate was then quantified by reacting with Nile blue dye in a bleaching reaction, this method provide a good determination step of phenylephrine hydrochloride and terbutaline sulfate in pharmaceutical products.

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FENİLEFİRİN VƏ TERBUTALİNİN FARMASEFTİK FORMALARININ SPEKTROFOTOMETRİK TƏYİNİ ÜÇÜN YAŞIL ÜSUL

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Xülasə: Kimyəvi aktiv maddələrin su hövzələrində müəyyən edilməsi və qiymətləndirilməsi çox çətin məsələdir, cünki bu proses çirkab sulara atılan kimyəvi tullantılarla əlaqədardır və nəticədə əhalinin sağlamlığına təsir göstərə bilər. Bu səbəbdən də potensial olaraq başqa, daha az təhlükəli alternativ metodların axtarışı üçün dünya miqyasında səylər göstərilir. İşin məqsədi fenilefirin hidroxlorid və terbutalin sulfatın aşkarlanması üçün yaşıl kimyadan istifadə etməklə iki əczaçılıq məhsulunun müəyyən edilməsi olmuşdur. Bu prosedurda oksidləşdirici kimi kalium permanqanatdan istifadə etməklə, fenilefirin hidroxlorid və terbutalin sulfat oksidləşdirilir, kalium permanqanatın artığı isə Nil mavisi boyası ilə reaksiya nəticəsində rəngsizləşdirilir. Nəticələr göstərir ki, bu metod fenilefirin hidroxlorid və terbutalin sulfatın (0.4-4.5) µg/ml qatılıq intervalında asan və dəqiq qiymətləndirilməsini təmin etmişdir (fenilefirin hidroxloridin molyar absorbsiyası ($6.9x10^4$) $l{\cdot}mol^{-1}{\cdot}cm^{-1}$, terbutalin sulfat üçün isə ($1,44\times10^5$) $l{\cdot}mol^{-1}{\cdot}cm^{-1}$). Sınaqdan keçirilmiş dərmanların farmasevtik formaları bu metod ilə dəqiq müəyyən edilmişdir. Alınmış nəticələr əvvəllər sınaqdan keçirilmiş standart birləşmələr və standart kimi qəbul olunmuş qiymətlərlə uygunluq təşkil edir.

Açar sözlər: Fenilefirin, Nil mavisi boyası, Kalium permanqanat, Terbutalin.

ЗЕЛЕНЫЙ МЕТОД СПЕКТРОФОТОМЕТРИЧЕСКОГО ОПРЕДЕЛЕНИЯ ФАРМАЦЕВТИЧЕСКИХ ФОРМ ФЕНИЛЭФРИНА И ТЕРБУТАЛИНА

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Резюме: Определение и оценка активных веществ в химических средах чрезвычайно сложны, поскольку они связаны с химическими отходами в сточных водах и в конечном итоге могут оказать влияние на здоровье населения. По этой причине, по всему миру предпринимаются усилия по поиску других альтернативных, возможно, менее опасных путей. В данной работе мы стремились определить два фармацевтических продукта для обнаружения гидрохлорида фенилэфрина и сульфата тербуталина с использованием зеленой химии. Процедура включает окисление гидрохлорида фенилэфрина и сульфата тербуталина с использованием перманганата калия в качестве окислителя и обесцвечивание избытка перманганата калия в реакции с красителем Нильским-синим. Результаты подтвердили, что этот метод обеспечивает легкую и точную оценку гидрохлорида фенилэфрина и сульфата тербуталина в концентрациях в диапазоне (0.4-4.5) мкг/мл, с молярной абсорбционной способностью ($6.9x10^4$) л·моль$^{-1}$·см$^{-1}$ для гидрохлорида фенилэфрина и (1.44×10^5) л·моль$^{-1}$·см$^{-1}$ для сульфата тербуталина. Методиками точно были определены фармацевтические лекарственные формы испытуемых препаратов, согласующиеся с ранее протестированными и заявленными значениями и стандартными соединениями.

Ключевые слова: фенилэфрин, краситель нильский синий, перманганат калия, тербуталин.