SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NAPHAZOLINE NITRATE IN BULK AND PHARMACEUTICAL PREPARATIONS BY USING 1,2-NAPHTHOQUINONE-4-SULFONATE**

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A simple, accurate, and cost-efficient UV-Visible spectrophotometric method has been developed for the determination of naphazoline nitrate (NPZ) in pure and pharmaceutical formulations. The suggested method was based on the nucleophilic substitution reaction of NPZ with 1,2-naphthoquinone-4-sulfonate sodium salt in alkaline medium at 80ºC to form an orange/red-colored product of maximum absorption (λ_{max}) at 483 nm. The stoichiometry of the reaction was determined via Job's method and limiting logarithmic method, and the mechanism of the reaction was postulated. Under the optimal conditions of the reaction, Beer's law was obeyed within the concentration range 0.5–50 μg/mL, the molar absorptivity value (ε) was 5766.5 L ·mol^{-1} ·cm^{-1}, Sandell's sensitivity value was 0.0474 μg/cm^2, the limits of detection and quantification were found to be 0.2154 and 0.6529 μg/mL, respectively. The formation constant of the complex was calculated by using the Benesi–Hildebrand equation. The suggested method was successfully applied for the quantification of NPZ in pharmaceutical formulations with good accuracy and precision. Therefore, this method can be applied for routine analysis of naphazoline.

Keywords: *determination, spectrophotometric, naphazoline, 1,2-naphthoquinone-4-sulfonate, pharmaceutical preparation.*

СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ НИТРАТА НАФАЗОЛИНА В НЕРАСФАСОВАННОМ ВИДЕ И ФАРМАЦЕВТИЧЕСКИХ ПРЕПАРАТАХ С ИСПОЛЬЗОВАНИЕМ 1,2-НАФТОХИНОН-4-СУЛЬФОНАТА

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Разработан простой, точный и экономичный спектрофотометрический метод определения нитрата нафазолина (NPZ) в чистом виде и фармацевтических препаратах в УФ-видимом диапазоне, основанный на реакции нуклеофильного замещения NPZ натриевой солью 1,2-нафтохинон-4-сульфоната в щелочной среде при 80ºC с образованием продукта оранжевого/красного цвета с максимумом поглощения λ_{max} = 483 нм. С помощью метода Джобса и предельного логарифмического метода определена стехиометрия реакции, описан механизм реакции. При оптимальных условиях реакции в диапазоне концентраций 0.5—50 мкг/мл соблюдается закон Бера, молярная абсорбционная способность ε = 5766.5 л ·моль$^{-1}$ ·см$^{-1}$, чувствительность Санделла 0.0474 мкг/см^2, пределы обнаружения и количественного определения 0.2154 и 0.6529 мкг/мл. Константа образования комплекса рассчитана с помощью уравнения Бенези–Хильдебранда. Метод успешно применен для количествен-

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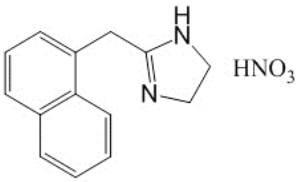
ного определения NPZ в фармацевтических препаратах с хорошей точностью и может быть использован для рутинного анализа нафазолина.

Ключевые слова: *спектрофотометрическое определение, нафазолин, 1,2-нафтохинон-4-сульфонат, фармацевтический препарат.*

Introduction. Naphazoline nitrate (NPZ): 2-(Naphthalene-1-ylmethyl)-4,5-dihydro-1H-imidazole nitrate) [1]



is a sympathomimetic drug, its effect on stimulating alpha adrenergic receptors, which results in vasoconstriction mainly on arterioles of the conjunctival and mydriasis, according to its action, this ophthalmic drug reduces the conjunctival congestion by its course of mechanism [2]. Various studies were reported for the determination of naphazoline (in the form of nitrate or hydrochloride) in each of the pure and pharmaceutical preparations, such as spectrophotometric [3–10], high-performance liquid chromatography [8, 11–13], ultra-high performance liquid chromatography [14], capillary electrophoresis [15], luminescence [16], and the voltametric method [17–19]. In the present study, we investigate a simple, sensitive, accurate, economic, environmentally friendly, spectrophotometric method that is free from organic solvents for assaying NPZ in pure and pharmaceutical formulations. The suggested procedure includes the formation of a complex between NPZ and 1,2-naphthoquinone-4-sulfonate (Folin's reagent) as a chromogenic reagent.

Experiment. A double-beam UV-Vis 1800 spectrophotometer with a 1-cm match quartz cell (Shimadzu, Japan) was used for all absorbance measurements. A thermostatically controlled water bath (type Memmert W-200, RING-Germany, a pH meter model (WTW GmbH), an inolab pH 7110 (Germany), and an analytical sensitive electronic balance ACS 120-4 (Kern & Sohn GmbH, Germany) were also used.

Naphazoline nitrate was received as a powder in pure form from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). A solution of 0.05% (w/v) of sodium 1,2-naphthoquinone-4-sulfonate (NQS) from British Drug Houses (BDH) was freshly prepared by dissolving 0.0125 g in 25-mL dark volumetric flask to protect the solution from light during use. Buffer solution of pH 10 was prepared by mixing 100 mL of 0.025 M $Na_2B_4O_7 \cdot 10H_2O$ (borax) with 24 mL of 0.1 M NaOH and complete the volume to the mark in a 200-mL volumetric flask with distilled water. Other different pH solutions were also prepared and adjusted by the pH meter.

0.1 g of NPZ was accurately weighed and dissolved in distilled water, transferred into a 100-mL volumetric flask, and diluted to the mark with the same solvent to get a stock solution of 1000 μg/mL and then kept in the refrigerator. The other working solutions were further diluted with distilled water from the stock solution within the range 2.5–250 μg/mL.

Two commercial pharmaceutical samples were purchased from local drug stores:

Ophtazolin, eye drops labeled to contain 5 mg/mL antazoline sulphate and 0.25 mg/mL NPZ is from Dar Al Dawa, Na'ur-Jordan.

Septabore, eye drops solution labeled to contain 1 mg/mL of NPZ and 10 mg/mL of boric acid is from Cooper S. A., Athens, Greece.

A suitable volume of the sample solution was diluted to get the working concentrations of 10, 20, and 30 μg/mL for analysis.

One milliliter of 2.5–250 μg/mL NPZ was added to 1.2 mL of pH 10, and 0.5 mL of 0.05% NQS was added. The volume was made up to 3 mL with distilled water. The solution was heated in a bath water thermostat at 80°C for 2 min, then the solution stood for 2 min at room temperature. The mixture was diluted to the mark (5 mL) with distilled water. The absorbance of the solution was measured at 483 nm against the reagent blank prepared in the same manner but without the drug.

Job's [20] and limiting logarithmic methods [21] were used to determine the composition of the reaction product between NPZ and NQS.

Equimolar (1.9×10^{-3} M) aqueous solutions of NPZ and NQS were prepared. Series of 1.5-mL portions of master solutions of NQS and NPZ were made up comprising different complementary proportions (0.1:1.4, 0.3:1.2, 0.5:1, 0.7:0.8, 0.9:0.6, 1.1:0.4, 1.3:0.2, and 1.4:0.1) in a 5-mL calibrated flask containing

1.2 mL of the buffer solution. The solutions were further treated as described by the general recommended procedure. The mole fraction of the drug [drug]/[drug]+[reagent] was calculated and plotted against the absorbance of each solution.

Two sets of experiments were performed using the general procedure. The first series of experiments was performed using different concentrations of NQS (1.1×10^{-4}–4.2×10^{-4} M) at a fixed concentration of NPZ (4.6×10^{-4} M). The second series of experiments was performed by using different concentrations of NPZ (1.8×10^{-5}–3.6×10^{-4} M) at a fixed NQS concentration (1.9×10^{-3} M). The logarithms of the absorbance (log*A*) for the reaction of NPZ with NQS were plotted against the logarithms of the concentrations of NQS and NPZ in the first and second series of experiments, respectively. The slopes of the suitable lines were determined for both sets of experiments.

Results and discussion. The absorption spectrum of the reaction product between NPZ and NQS was recorded against the reagent blank in Fig. 1. The orange/red-colored product exhibited λ_{max} at 483 nm, and the λ_{max} of 364 nm was observed for NQS. The λ_{max} of the reaction product was found to be red – shifted by 204 nm from the λ_{max} of NPZ 279 nm. All measurements were performed at 483 nm to remove the interference.

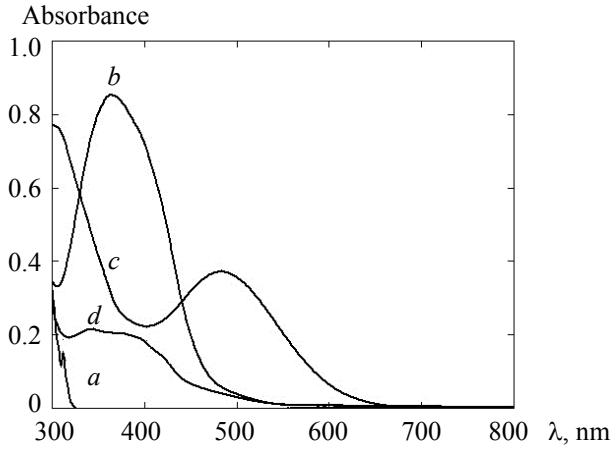


Fig. 1. Absorption spectra of (a) NPZ (5 μg/mL), (b) NQS (0.05%), (c) NPZ with NQS (20 μg/mL) after optimization and (d) blank solution.

NQS derivatization gained significant attention for the quantitative analysis of many pharmaceutically active compounds [22, 23]. To achieve maximum absorption of the colored product, the optimal conditions were investigated by changing the parameters one at a time while the other is kept constant.

The effect of pH on the absorbance of the NPZ–NQS product was investigated by changing pH from 8 to 13. As seen in Fig. 2a, the absorbance of the reaction product increased rapidly until it reached its maximum value at pH 10. This is attributed to the maximum degree of nucleophilic substitution reaction. However, at pH>10, the absorbance decreased significantly. This is probably due to an increase in the amount of OH^-. The strong nucleophilic ability of the hydroxide ion can hold back the nucleophilic substitution between NPZ and NQS. In addition, the high alkaline media may lead to NPZ hydrolysis, as well as the product of the reaction. Another reason for reducing the absorbance may be the instability of NQS in high alkaline media [24]. Therefore, all experiments were performed at pH 10 to keep the high sensitivity for NPZ determination.

The influence of NQS concentration was examined within the range 0.03–0.5% (w/v), as seen in Fig. 2b. The concentration of NQS that led to more product up to an amount of 0.05%, after which the absorbance decreased. In other word, the increase in the concentration of NQS had no enhancement of the absorbance values, which may be due to formation of new species. Therefore, 0.05% (0.5 mL) was considered to be the optimal concentration.

The influence of the amount of buffer solution was tested by keeping pH at 10 and changing the volume over the range 0.3–1.8 mL. We found that in the absence of buffer solution (alkaline medium), the chemical reaction is not complete. So, adding the buffer is necessary to get an orange/red-colored product. Also, the absorbance of the colored product increased with the increase in the volume of buffer from 0.3 to 1.2 mL,

and any extra volume of the buffer had no effect on the improvement of absorbance of the measured species (Fig. 2c). Thus, the volume of 1.2 mL was chosen to achieve the highest absorbance of the product.

The influence of temperature on the color development was studied by changing the temperature from 25 to 90°C. As shown in Fig. 2d, there were significant differences between the absorbance at room temperature and those obtained at high temperatures up to 80°C after which absorbance reduced. Thus, 80°C is optimal.

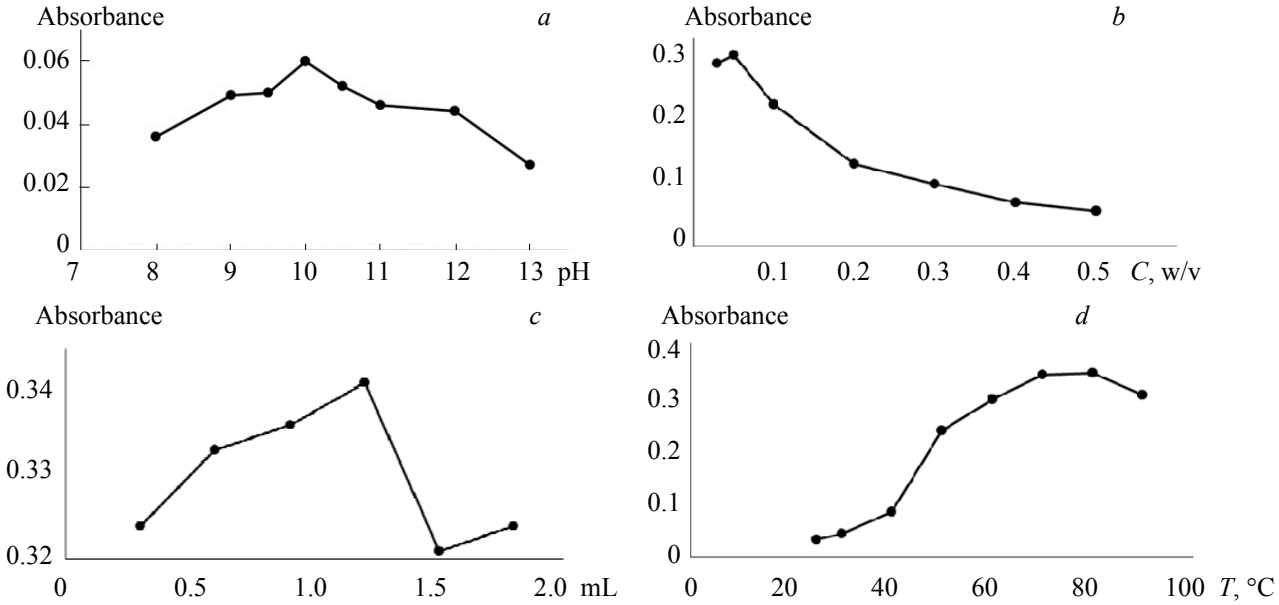


Fig. 2. Effect of (a) pH, (b) NQS concentration, (c) buffer solution, (d) temperature on the reaction of NPZ-NQS (20 μg/mL).

By changing the order of addition of reagents, it is possible to increase the absorbance value and hence increase the yield of the reaction product. Thus, several studies have been carried out to assess the effect of the sequence of addition for the reactants on color improvement. The order of addition should be followed as in sequence (i), otherwise a decrease in color intensity was observed. The data are listed in Table 1.

TABLE 1. Effect of the Sequence of Addition on Color Improvement for Naphazoline Nitrate

S. No.	Sequence of addition	Absorbance	Recommended sequence of addition
i	B+D+R	0.360	
ii	R+D+B	0.357	
iii	B+R+D	0.357	i
iiii	D+R+B	0.347	
iiiii	D+B+R	0.345	

N o t e. B: buffer D; R: reagent.

The reaction between NPZ and NQS in the presence of buffer solution at pH 10 was studied at different heating times from 1 to 5 min. As shown in Fig. 3, 2 min was sufficient to complete the reaction and get the maximum absorbance. Increasing the heating time neither accelerated the reaction nor presented reproducible results; thus, 2 min is the optimal heating time.

The absorption of the colored product was found to be stable for 1 h, as seen in Fig. 4. After 24 h, the absorbance decreases by 0.05, this allowed the handling of many samples with comfortable measurements. It provides the developed procedure with high efficiency that can be applied to the analysis of many samples in the quality control laboratories.

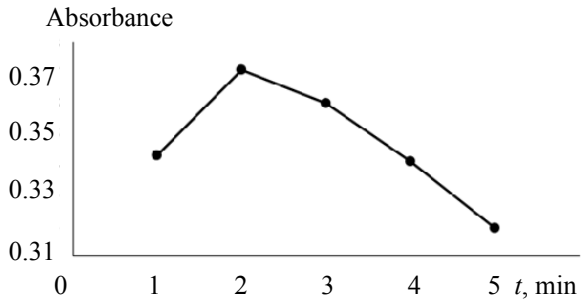


Fig. 3. Effect of heating time on the reaction of NPZ-NQS (20 μg/mL).

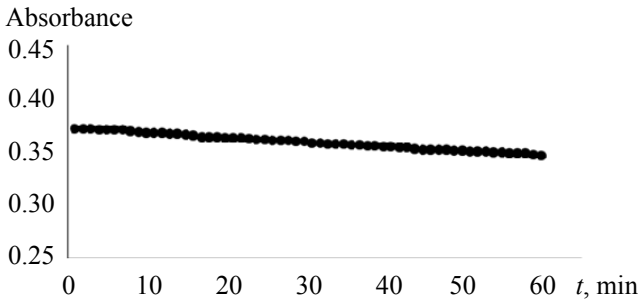


Fig. 4. Stability of the colored product.

According to Job's method, stoichiometry for NPZ–NQS was found to be 1:1, as shown in Fig. 5. Two straight lines were obtained in the limiting logarithmic method (Fig. 6). The slope values of the two lines were 0.9233 and 1.2925, supporting the ratio of 1:1.

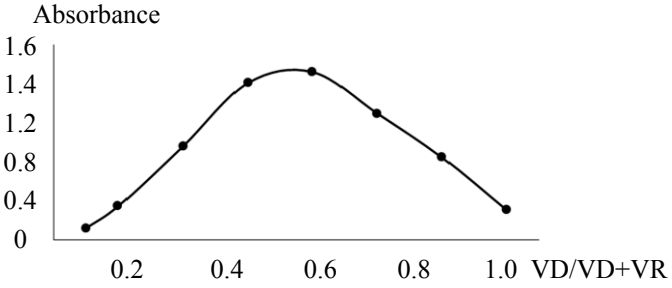


Fig. 5. Job's method.

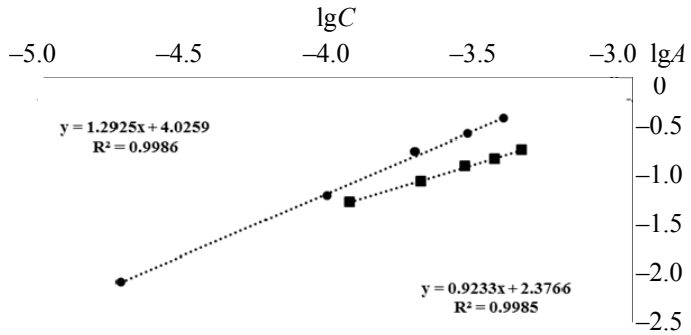
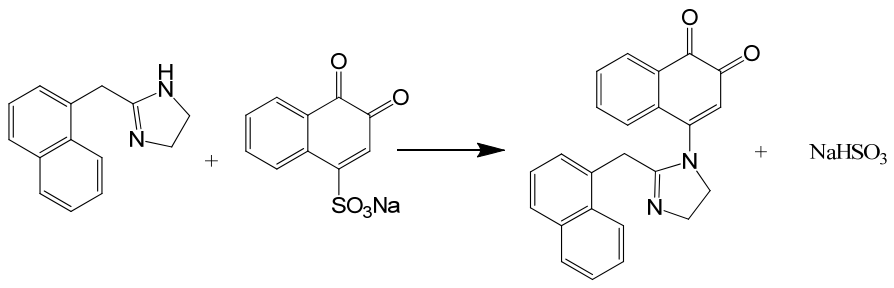


Fig. 6. Limiting logarithmic method, (■) first set of experiments, NQS concentrations (1.1×10^{-4}–4.2×10^{-4} M) at NPZ concentration (4.6×10^{-4} M), (●) second set of experiments, NPZ concentrations (1.8×10^{-5}–3.6×10^{-4} M) at NQS concentration (1.9×10^{-3} M).

Depending on the molar ratio observation, it was presumed that the reaction pathway would proceed as



The formation constant of the 1:1 complex was calculated by using the Benesi–Hildebrand equation [25], as shown below:

$$[NQS]/A_c = 1/\varepsilon_c + (1/\varepsilon_c K_c)\times(1/[Drug]),$$

where [NQS] and [Drug] are the total concentration of the reagent and drug respectively, A_c is the absorbance of the complex, ε_c is the molar absorptivity of the complex, and K_c is the formation constant of the (drug–NQS) complex. The K_c value can be found by plotting $[NQS]/A_c$ versus 1/[Drug]. In this plot, the 1:1 complex could yield a straight line, in which K_c = (intercept/slope) and ε_c = (1/intercept), as shown in Fig. 7, and the results are listed in Table 2.

TABLE 2. Complex Formation Constant between the Drug and NQS

Parameter	Observation
Intercept	0.0006
Slope	7×10^{-8}
Correlation coefficient (r)	0.9999
ε_c, L · mol^{-1} · cm^{-1}	1666.7
K_c, L/mol	8571.4
ΔG, KJ/mol	−26.5833

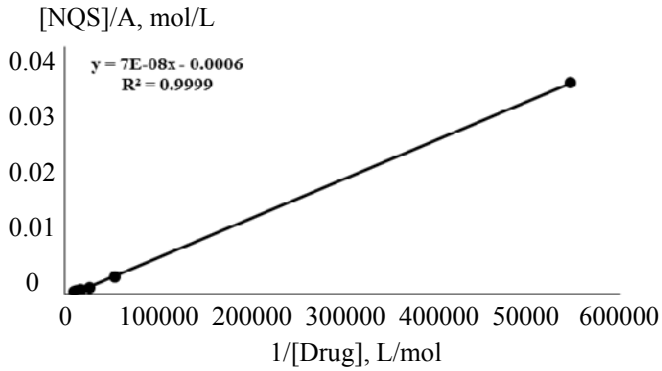


Fig. 7. Benesi–Hildebrand plot for the NPZ–NQS complex.

Under the optimal conditions, there is a linear relationship between the absorbance and the concentrations of the drug at λ_{max} = 483 nm, as shown in Fig. 8. The limit of detection (LOD) and quantification (LOQ) of the analytical method are estimated to be 3.3(σ/s) and 10(σ/s), respectively, where σ is the standard deviation of the blank absorbance and s is the slope in the equation of the calibration curve.

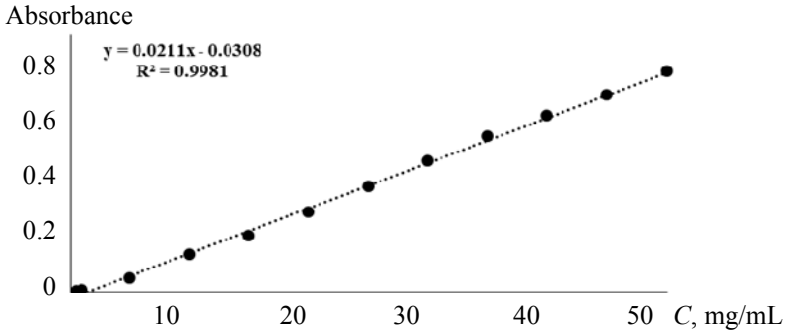


Fig. 8. Calibration curve for NPZ at 483 nm.

The slope and intercept of the linear graph, LOD, LOQ, and other analytical parameters are presented in Table 3. The accuracy and precision, repeatability (intra-assay), and intermediate precision (inter-assay) were assessed by using three different concentrations of NPZ (10, 20, and 30 μg/mL) replicated three times for every concentration during the same day under optimal conditions and replicated three times over three

days, respectively (Table 4). The accuracy was evaluated as percentage relative error (RE%), repeatability and intermediate precision as relative standard deviation (RSD%). The low values of RSD and RE reflect the high accuracy and precision of the proposed method.

The analytical method for the determination of NPZ in pharmaceutical formulations (eye drops) was successfully applied by analyzing three different concentrations (10, 20, and 30 μg/mL) of pharmaceutical preparations using the proposed procedure directly. No interference was observed from the sample matrix. As shown in Table 5, the results were satisfactory (accurate and precise), as indicated by the good recovery percentage, which is consistent with the labeled claim.

TABLE 3. Analytical Parameters for the Proposed Method

Parameter	Value
Wavelength (λ_{max})	483
Beer's law range, μg/mL	0.5–50
Regression equation	$y = 0.0211x - 0.0308$
Slope	0.0211
Intercept	0.0308
Correlation of linearity (r^2)	0.9981
Limit of detection, LOD, μg/mL	0.2154
Limit of quantification, LOQ, μg/mL	0.6529
Molar absorptivity, $L \cdot mol^{-1} \cdot cm^{-1}$	5766.5
Sandell's sensitivity, $\mu g/cm^2$	0.0474

TABLE 4. Intra-assay and Inter-assay Assessment of Accuracy and Precision

Taken, μg/mL	Intra-day (n = 3)			Inter-day (n = 3)		
	Found*, μg/mL	RSD%	RE%	Found*, μg/mL	RSD%	RE%
10	9.6904	0.5647	−3.0964	9.7536	1.7520	−2.4645
20	19.7694	0.7324	−1.1532	20.0537	0.4920	0.2686
30	29.5956	1.8970	−1.3481	30.8278	1.3075	2.7593

*Mean value of three determinations.

TABLE 5. Spectrophotometric Determination of NPZ in Eye Drops by the Proposed Method

Eye drops brand name	Weight* found, mg	Concentration, μg/mL		Recovery, %	RSD, %
		taken	found*		
Ophtazolin 0.25 mg	0.2671	10.000	10.6856	106.8	1.1162
	0.2390	20.000	19.1216	95.6	0.1431
	0.2422	30.000	29.0585	96.9	0.1883
Septobore 1 mg	0.9972	10.000	9.9724	99.72	1.9583
	1.0810	20.000	21.6209	108.1	0.1201
	1.1098	30.000	33.2932	111.0	0.8616

Conclusions. The present research explained the successful assessment of Folin's reagent (Naphazoline nitrate) in the implementation of the visible spectrophotometric method for the accurate quantitative determination of naphazoline in pure drug and eye drops. The suggested method has many advantages of speed, simplicity, accuracy, and precision, and it is highly sensitive, as can be seen from the molar absorptivity value. In addition, it does not involve a critical reaction or tedious preparation of samples. Furthermore, it does not require sophisticated and expensive equipment and all analytical reagents are cheap and accessible in every analytical laboratory. Therefore, the proposed method is valuable for the quality control and the routine analysis of Naphazoline nitrate in pharmaceuticals as there is no interference from the different excipients commonly found in commercial eye drops.

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