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A GREEN SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DROTAVERINE HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS USING FORMATION OF ION ASSOCIATION COMPLEX WITH ERYTHROSINE

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Abstract

For the determination of drotaverine hydrochloride (DRH), a new, simple and environmentally friendly non-extractive spectrophotometric method was developed, based on a direct reaction with the fluorescein dye erythrosine (ER). The color change occurs due to the formation of an ion-association complex (IA) of the protonated form of the analyte with the monoanionic form of ER. It is not necessary to use a surfactant to solubilize the IA precipitate or extract it with toxic organic solvents. A new bathochromically shifted band appears in the absorption spectrum at 560 nm. Its origin is attributed to the aggregation of dye anions. The formation of poorly soluble IA promotes the aggregation of dye anions. IA is completely formed at pH 4.4 in an acetate buffer medium after 3–5 min. The absorbance remains constant for two hours. Under optimal reaction conditions, the calibration curve was linear in the range from 0.4 to 3 μmol L^{-1} with a detection limit of 0.10 μmol L^{-1} (40 μg L^{-1}) and a correlation coefficient of 0.9996. The molar absorptivity of IA is 3.8×10^4 mol^{-1} L cm^{-1}. The stoichiometry of the complexes formed between DRH and ER, determined by Job's continuous variation method, was 1 : 1. A new simple method for calculating equilibrium constants based on visual inspection and coincidence of experimental and calculated curves describing the dependence of the mole fraction of IA and other particles on the pH of the solution was proposed. The calculated formation constant IA 2×10^6 indicates its high stability. The developed method has been successfully applied to determine DRH in pharmaceuticals.

Keywords: Drotaverine hydrochloride; Erythrosine; Spectrophotometry; Ion association complex; Pharmaceutical preparations.

ЗЕЛЕНИЙ СПЕКТРОФОТОМЕТРИЧНИЙ МЕТОД ВИЗНАЧЕННЯ ГІДРОХЛОРИДУ ДРОТАВЕРИНУ У ФАРМАЦЕВТИЧНИХ ПРЕПАРАТАХ З ВИКОРИСТАННЯМ УТВОРЕННЯ ІОННО-АСОЦІАТИВНОГО КОМПЛЕКСУ З ЕРИТРОЗИНОМ

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Анотація

Для визначення гідрохлориду дротаверину був розроблений новий, простий і екологічний безекстракційний спектрофотометричний метод, який грунтується на прямій реакції з барвником флуоресцеїнового ряду еритрозином. Зміна кольору відбувається через утворення іонно-асоціативного комплексу (ІА) протонованої форми аналіту з моноаніонною формою еритрозину. Немає необхідності використовувати поверхнево-активні речовини для солюбілізації дрібнодисперсного осаду ІА або екстракції його токсичними органічними розчинниками. В спектрі поглинання з'являється нова батохромно зміщена смуга при 560 нм. Її походження пояснюється агрегацією аніонів барвника. Утворення малорозчинних ІА сприяє агрегації аніонів барвників. ІА повністю утворюється при pH 4.4 в середовищі ацетатного буфера через 3–5 хв. Оптична густина залишається незмінною протягом двох годин. За оптимальних умов реакції градуювальна крива була лінійною в діапазоні від 0.4 до 3 мкмоль $л^{-1}$ з межею виявлення 0.10 мкмоль $л^{-1}$ (40 мкг $л^{-1}$) і коефіцієнтом кореляції 0.9996. Молярний коефіцієнт світлопоглинання ІА склав 3.8×10^4 $моль^{-1}$ л $см^{-1}$. Стехіометрія комплексів, що утворюються між дротаверином і еритрозином, отримана методом ізомолярних серій, склала 1 : 1. Запропоновано новий простий метод розрахунку констант рівноваги, заснований на візуальному огляді і співпадінні експериментальних і розрахункових кривих, що описують залежність мольної частки ІА і інших частинок від pH розчину. Розрахована константа утворення ІА 2×10^6 свідчить про його високу стійкість. Розроблений метод успішно застосований для визначення дротаверину в фармацевтичних препаратах.

Ключові слова: дротаверину гідрохлорид; еритрозин; спектрофотометрія; іонний асоціат; лікарські препарати.

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ЗЕЛЕНЫЙ СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ ДРОТАВЕРИНА ГИДРОХЛОРИДА В ФАРМАЦЕВТИЧЕСКИХ ПРЕПАРАТАХ С ИСПОЛЬЗОВАНИЕМ ОБРАЗОВАНИЯ ИОННО-АССОЦИАТИВНОГО КОМПЛЕКСА С ЭРИТРОЗИНОМ

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Аннотация

Для определения гидрохлорида дротаверина был разработан новый, простой и экологичный безэкстракционный спектрофотометрический метод, основанный на прямой реакции с красителем флуоресцеинового ряда эритрозином. Изменение цвета происходит из-за образования ионно-ассоциативного комплекса (ИА) протонированной формы аналита с моноанионной формой эритрозина. Нет необходимости использовать поверхностно-активные вещества для солюбилизации мелкодисперсного осадка ИА или экстракции его токсичными органическими растворителями. В спектре поглощения появляется новая батохромно смещенная полоса при 560 нм. Её происхождение объясняется агрегацией анионов красителя. Образование малорастворимых ИА способствует агрегации анионов красителей. ИА полностью образуется при pH 4.4 в среде ацетатного буфера через 3–5 мин. Оптическая плотность остается постоянной в течение двух часов. При оптимальных условиях реакции градуировочная кривая была линейной в диапазоне от 0.4 до 3 мкмоль $л^{-1}$ с пределом обнаружения 0.10 мкмоль $л^{-1}$ (40 мкг $л^{-1}$) и коэффициентом корреляции 0.9996. Молярный коэффициент светопоглощения ИА составил 3.8×10^4 $моль^{-1}$ л $см^{-1}$. Стехиометрия комплексов, образующихся между дротаверином и эритрозином, определенная методом изомолярных серий, составила 1 : 1. Предложен новый простой метод расчета констант равновесия, основанный на визуальном осмотре и совпадении экспериментальных и расчетных кривых, описывающих зависимость мольной доли ИА и других частиц от pH раствора. Рассчитанная константа образования ИА 2×10^6 свидетельствует о его высокой устойчивости. Разработанный метод успешно применен для определения дротаверина в фармацевтических препаратах.

Ключевые слова: дротаверина гидрохлорид; эритрозин; спектрофотометрия; ионный ассоциат; фармацевтические препараты.

Introduction

Spectrophotometric methods are widely used in the analysis of pharmaceuticals due to ease of use, rapidity, accessibility in most chemical analysis laboratories, sufficiently high sensitivity and selectivity. The formation of intensely colored IA complexes between active ingredients of pharmaceutical preparations and organic dyes is often used in the analysis of pharmaceutical formulations.

Since the spectra of the dye and IA are very similar, it is common to carry out the step of separating the excess dye. Extraction is mainly used for this operation, but this is not always acceptable due to the toxicity and flammability of organic solvents. The use of organic solvents does not allow this analytical technique to be classified as green analytical chemistry. In addition, the partial co-extraction of the simple salt of the dye limits a further increase in the sensitivity.

Several extraction-free spectrophotometric methods using xanthene anionic dyes were earlier proposed for the determination of drugs containing amino groups based on formation of binary [1–3] or ternary complexes [4–7].

In one of the approaches, the formation of binary IA between the protonated form of drug and anionic form of xanthene dye such as eosin or ER is used. Typically, low soluble salt of IA is solubilized using non-anionic surface-active substances.

In this study, this approach was improved. It was shown that it is not necessary to use surface-active substances to solubilize the IA precipitate. In previous works, it was not explained the nature of the color change for this type of analytical spectrophotometric reactions. We have assumed that the aggregation of dye anions is responsible for the observed phenomenon.

Drotaverine hydrochloride (DRH) is an antispasmodic drug chemically known as 1-(3, 4-diethoxybenzylidene)-6, 7-diethoxy-1, 2, 3, 4-tetrahydroisoquinoline. It is widely used to treat smooth muscle spasms and pain associated with gastrointestinal colics, renal colics, biliary colics, irritable bowel syndrome, postsurgical spasm, and uterine neck spasm [8]. As can be seen from the structure of DRH (Fig. 1), a secondary amino group is present in the drug molecule.

Several analytical methods have been reported for the determination of DRH either individually or in combination with other drugs including voltammetry [9], high-performance liquid chromatography (HPLC) [10–12], thin layer chromatography (TLC) [13], and a few spectrophotometric methods [14–17]. However, the existing spectrophotometric methods suffer from certain limitations. Some of them require heating, other methods are time-consuming, need extraction with toxic organic solvents, or the addition of surfactant. Some of these methods require complicated chemometric calculations.

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Therefore, there is a need in developing simple and green spectrophotometric methods for the determination of DRH that avoid the aforementioned disadvantages of the previous methods and at the same time can compete with sophisticated methods such as HPLC.

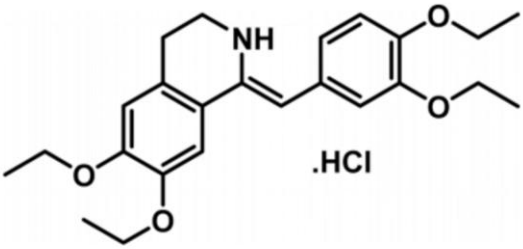

Fig. 1. Structure of DRH

In this work, a simple, sensitive, precise, inexpensive, and environmentally-friendly non-extractive spectrophotometric method was developed and validated for the determination of DRH. The applicability and accuracy of the method were evaluated by analyzing pharmaceutical preparations containing DRH.

Experimental part

Reagents and instrumentations. All chemicals were of analytical grade purity and all solutions were freshly prepared in distilled water. A standard solution (1×10^{-3} mol L^{-1}) of Drotaverine hydrochloride (M_w = 433.968) was prepared by dissolving 43.4 mg of pure drug in 100 mL bidistilled water and made up to 100 mL with bidistilled water. The working standard solution (1×10^{-5} mol L^{-1}) was prepared by further dilution of the standard solution in 100 mL of bi-distilled water. The acetate buffer solution (pH 4.4) was prepared by mixing appropriate volumes of 0.1 mol L^{-1} sodium acetate and 0.1 mol L^{-1} acetic acid.

ER was purchased from Sigma-Aldrich and used without purification. 1 mmol L^{-1} ER stock solution was prepared by dissolving a precise amount (0.880 g) of ER in 10 mL of ethanol and diluting to 100 mL with distilled water. The working solution (10^{-4} mol L^{-1}) was prepared by diluting of stock ER solution with distilled water.

The absorbance of solutions was measured with a UV-VIS spectrophotometer SF-46 (LOMO, Russia) using a glass cell with an optical path length of 5 cm. The pH measurements and adjustments were performed using a pH-150 MI pH-meter (Russia).

Procedure for the spectrophotometric determination of DRH. An amount of sample corresponding to a final concentration of DRH in the range from 0.4 to 4 μmol L^{-1} was placed in a 25 mL calibrated volumetric flask. Then 2.5 mL of acetate buffer (pH 4) and 2.5 mL of 1×10^{-4} mol L^{-1} ER solution were added, the solution was mixed thoroughly and diluted to the mark with distilled water. The absorbance was measured at 560 nm against a blank reagent using glass cells with a path length of 50 mm.

Analysis of pharmaceutical formulations containing DRH. Five tablets of drug were weighed, ground in a mortar, and finely powdered. An amount of drug formulation equivalent to one tablet was accurately weighed and dissolved in hot double distilled water, the solution was filtered through a 0.45 μm Whatman paper filter, transferred to a 100 mL volumetric flask, and diluted to the mark with distilled water. Further dilutions were made with double distilled water to obtain a concentration corresponding to the middle part of the calibration graph. The solutions were analyzed as described in section 2.2. The concentration of DRH was calculated from the regression equation.

Results and discussion

The color reaction between DRH and ER. It was found that under appropriate conditions DRH forms low-soluble IAs with ER. The color of the solution changes from orange to pink instantly after the addition of DRH. ER has an absorption maximum at 526 nm in aqueous solution (Fig. 2). The electronic spectrum of the dye is markedly changed in the reaction with DRH. The absorbance in the maximum of the band decreases, the spectrum broadens, and a new bathochromically shifted band appears. This band is absent in spectra of all the protonated forms of ER. The wavelength of 560 nm which corresponds to the maximum in difference spectrum was chosen as an analytical wavelength.

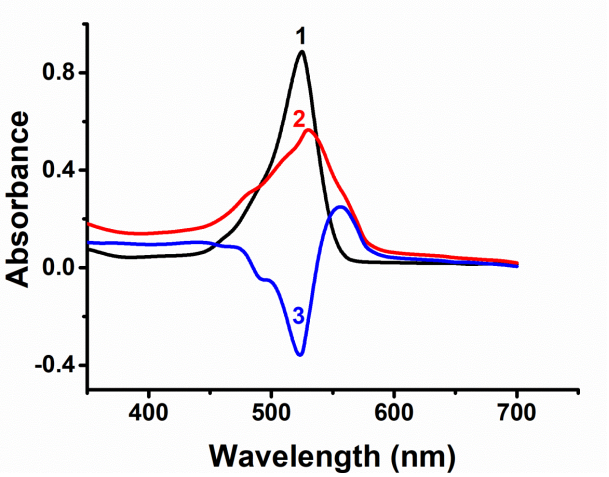

Fig. 2. Spectra of IAs formed between DRH and ER: (1) dye spectrum, (2) IA spectrum, (3) difference spectrum. Experimental conditions: C_{dye} = 10 μM, C_{DRH}= 7 μM, pH = 4, l =1cm

The nature of color changes by the formation of IA in aqueous solution is rarely discussed in the literature. It was assumed recently by investigation of the formation of IA formed between cationic polymethine dyes and anionic halide complexes of metals that appearance of new bands related with the formation of dye aggregates. It was suggested that the formation of a low soluble finely dispersed phase of IA precipitate is the prerequisite for the aggregation process. Close contact between IA molecules in low-soluble aggregates favors the non-covalent dipole-dipole bonding interaction between π-electronic systems of the dye molecules.

The appearance of one or two new bands by the formation of dye aggregates is commonly explained by the overall accepted theory of molecular exciton coupling as the formation of an excitonic state through the electronic coupling of the tightly packed dye molecules. The result of exciton splitting of excited states in a composite molecule is the appearance of strong spectral shifts (which can be of the order of 2000 cm^{-1}) of absorption bands for constituent molecules [18].

The direction and magnitude of the band shift testify in favor of the formation of a J-aggregate. Similar changes occur in the spectra of the dyes when representatives of polymethine [19–21], rhodamine [22–24], and triphenylmethane [25] basic dyes form low soluble IAs with suitable counterions.

The optimization of the reaction conditions. The optimal conditions for the formation and stability of the IA formed between DRH and ER have been studied aiming to achieve complete IA formation, maximum absorbance, and highest possible sensitivity. Several factors have a significant influence on the formation of IA, including acidity, ER concentration, and time of complex formation.

Chemistry of the reaction and effect of solution pH. The pH of the solution plays a very important role in the formation of specific IAs. In the studied system, both anionic dye and drug undergo protolytic equilibria that should be taken into account when choosing optimal pH. Therefore, the effect of pH on the formation of IA was investigated over a pH range from 2 to 5. As can be seen from Fig. 3, the dependence of the IA formation on pH has a maximum at pH 4.4-4.5. Hence, a pH 4.4 was employed for further work.

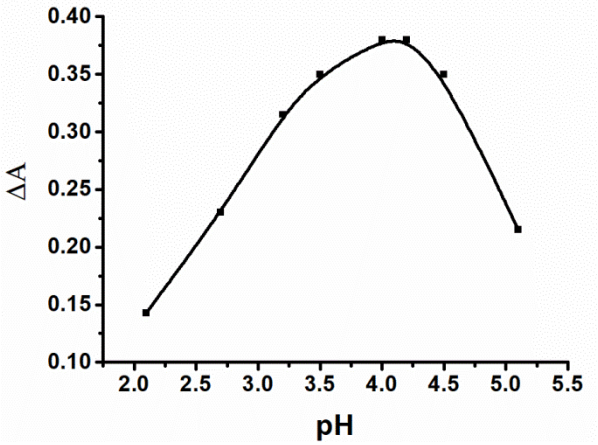

Fig. 3. The effect of the pH on the absorbance of the formed IA complex. Experimental conditions: C_{dye} = 10 μM, C_{DRH}= 2 μM

At lower pH, the decrease of the absorbance is explained by the destruction of the IA due to the protonation of the dye with the formation of a molecular form. At higher pH the absorbance decrease is caused by further deprotonation of the dye with the formation of a di-anionic form. In addition, deprotonation of drug can be responsible for IA destruction.

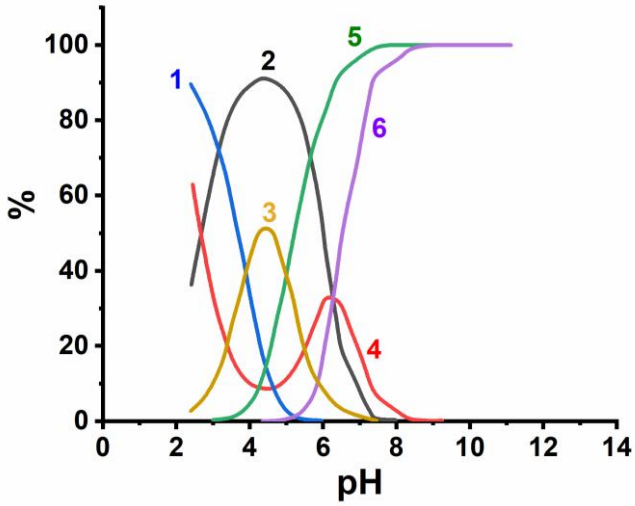

Fig. 4. Influence of pH on the composition of the solution by the formation of ion association complex between Drotaverine and Erythrosine. The curves in the figure refer to the following forms of substances: Erythrosine: molecular H_2ER (1), monoanionic HER^- (3), dianionic ER^{2-} (5), drotaverine: protonated DRH^+ (4), molecular DR (6) and ion associate DRH+ HER^- (2)

In correspondence with the general theory of the IAs, both counterions have to be univalent to form stable compound. In other words, in the optimal conditions from one hand the drug should be completely converted to univalent positively charged protonated form and on the other hand, the anionic dye should be in the mono-anionic form. The pK_a value for DRH is 6.3 [26]. Hence the protonated cationic form of DRH is dominant at a pH lower than 6.3. ER is a diprotic weak acid (H_2L) with $pKa_1 = 3.9$ and $pKa_2 = 5.0$ in aqueous solution [27].

In order to confirm the hypothesis on formation of ion associate between single charged ions of DRH and ER, equilibrium concentrations and percentage of different protonated forms of DRH and ER as well as IA were calculated. The calculations were made by program developed by us in the medium of Embarcadero Delphi 2021. Calculations of equilibrium concentrations are based on the projection algorithm proposed by Leon Every [28]. This method allows to calculate equilibrium concentrations in very complex systems where equilibrium constants differ in many orders of magnitude by using very rough initial approximations of calculated equilibrium concentrations.

In the calculations, we used the above-mentioned dissociation constants of protonated forms, the dissociation constant of water, and the assumed formation constant of IA (K_4). In the studied system, there is a total 5 equilibria listed below.

$$H_2ER \leftrightarrows H^+ + HER^- \qquad K_1 = 1.26\times10^{-4} \quad (1)$$

$$HER^- \leftrightarrows H^+ + ER^{2-} \qquad K_2 = 1\times10^{-5} \quad (2)$$

$$DRH^+ \leftrightarrows H^+ + DR \qquad K_3 = 5.01\times10^{-7} \quad (3)$$

$$DRH^+HER^- \leftrightarrows DRH^+ + HER^- \qquad K_4 = 5\times10^{-7} \quad (4)$$

$$HOH \leftrightarrows H^+ + OH^- \qquad K_5 = 1.8\times10^{-16} \quad (5)$$

To solve the problem, it was necessary to compile a matrix of coefficients for all substances. An example is given below (Table 1).

Table 1

Matrix of coefficients for the equations of reactions (1) – (5) used in the calculation of equilibrium concentrations

Equation number	Specie								
	H^+	OH^-	HOH	H_2ER	HER^-	ER^{2-}	DRH^+	DR	IA
1	1	0	0	–1	1	0	0	0	0
2	1	0	0	0	–1	1	0	0	0
3	1	0	0	0	0	0	–1	1	0
4	0	0	0	0	1	0	1	0	–1
5	1	1	–1	0	0	0	0	0	0

The formation constant for IA formed between ER and DRH was unknown. It was evaluated using the method of curve coincidence. We achieved the coincidence of the experimental curve of the dependence of the yield of IA on acidity and the one calculated using the program so that these curves visually coincide. Of course, this is a less accurate method than using programs that allow you to minimize the sum of deviations of the residuals using the nonlinear least squares method [29], but these programs are very difficult to use and require experienced users. On the contrary, the approach we have proposed is very simple, requires only drawing up the simplest scheme of equilibria and allows you to quickly estimate the necessary constants and schemes of equilibria. In our work, we used it to prove the proposed above equilibrium scheme.

As can be seen from Fig. 4, under the found optimal conditions, the percentage of IA yield reaches 90%, and its maximum yield is reached at pH 4.5 and coincides with the experimentally found one. Such a good agreement between the calculated and experimentally found dependences confirms that the IA contains singly charged ions. In addition, it can be seen that the curves for the monoanionic form of erythrosine and for the protonated form of drotaverine have extrema at pH 4.5, which means that these substances are spent on the formation of the IA. The found constant for equilibrium (4) corresponds to stability constant for the formation of IA of 2×10^6 that proves high stability of the IA formed between DRH and ER.

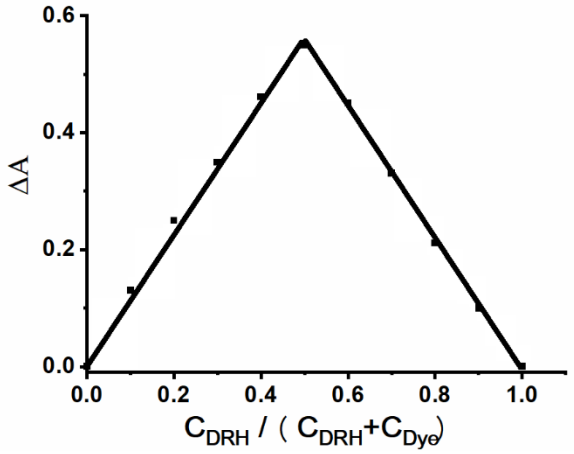

Fig. 5. Determination of IA composition using Job's method of continuous variation

The reaction stoichiometry and composition of the IA formed between DRH and ER were determined by Job's method of continuous variation (Fig. 5). The maximum on curve corresponds to the mole fraction of 0.5 for DRH or dye which proves the formation of a 1 : 1 drug–dye IA complex, confirming that one cation of the DRH associates with one anion of ER and each of participating compounds is in the single charged form. The form of the curve of the continuous variation method indicates that obtained IA has high stability.

It is shown in [30] that by dissociation of ER molecule, the first proton splits out from the phenolic hydroxyl group but not from carboxylic since the presence of iodine atoms makes more acidic phenolic protons. DRH is protonated on the secondary amino group. Based on these statements we can propose the following reaction mechanism for the formation of IA (Fig. 6).

Effect of ER concentration on the absorbance of IA. The effect of ER concentration was investigated in the range of 2–14 μmol L^{-1}. As can be seen from Fig. 7, both the absorbance of the IA and the blank solution are strongly increased with increasing ER concentration. However, the curve of deviation from additivity flattened out starting from ER concentration of 6 μmol L^{-1}. 10^{-5} mol L^{-1} ER concentration was chosen as optimum to ensure the complete formation of IA. In addition, this concentration of dye is sufficient for the determination of higher than 2 μM concentrations of DRH.

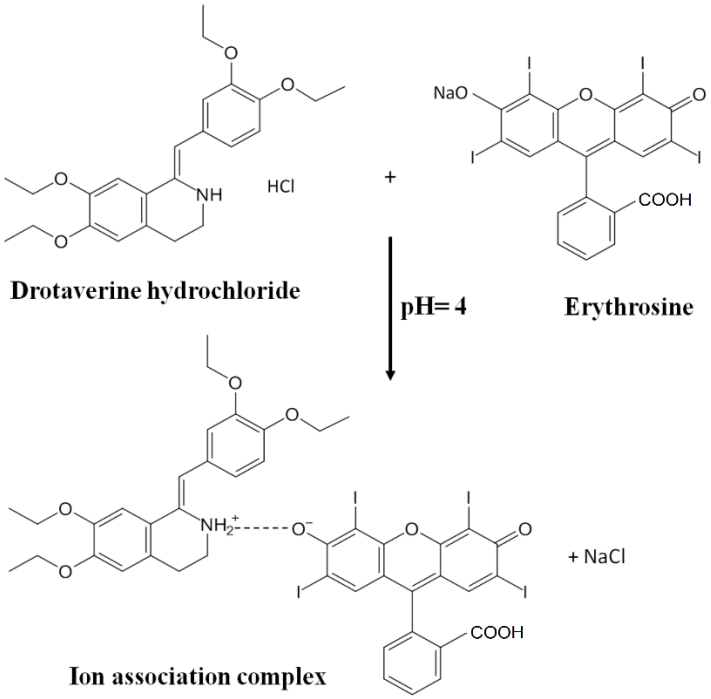

Fig. 6. The suggested scheme of chemical reaction for the formation of the IA complex between DRH and ER

This dependence was also calculated using the above program. We systematically varied the total concentration of the dye and calculated the equilibrium concentrations of all forms, including the concentration of the associate. In this case, we used the found value of the stability constant of the IA, the concentration of drotaverine was taken as 2 μM. It is obvious that both the shape of the curve and all the found values of the yield of the complex are in excellent agreement with the experimentally obtained values. The saturation area also starts at about 6 μM.

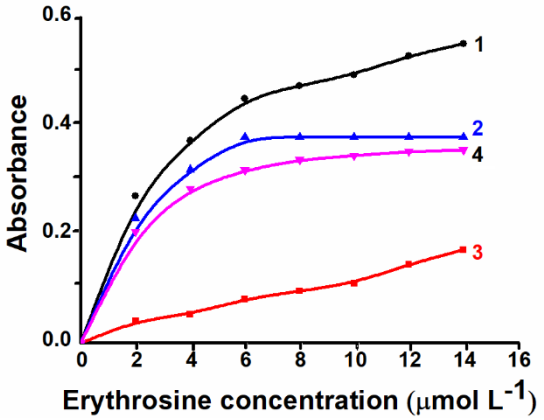

Fig. 7. Effect of ER concentration on the IA complex formation: (1) IA, (2) ΔA, (3) blank. (4) – calculated curve. Experimental conditions: C_{DRH}= 2 μM and pH = 4.

Effect of time. In the studied range of IA concentrations, it exists in the form of a finely dispersed low soluble phase. It turned out that formed nano-particles of precipitate are very stable for a long time even without using a solubilizer.

The effect of time on the IA complex formation and stability was studied within the range from 1 to 120 min. Fig. 8 shows that most of the IA color appears immediately, after that the absorbance develops more slowly and reaches a maximum within about 3-5 min. The color of IA remains constant for at least 2 hours. Thus, 5.0 min was taken as optimum time in the developed procedure.

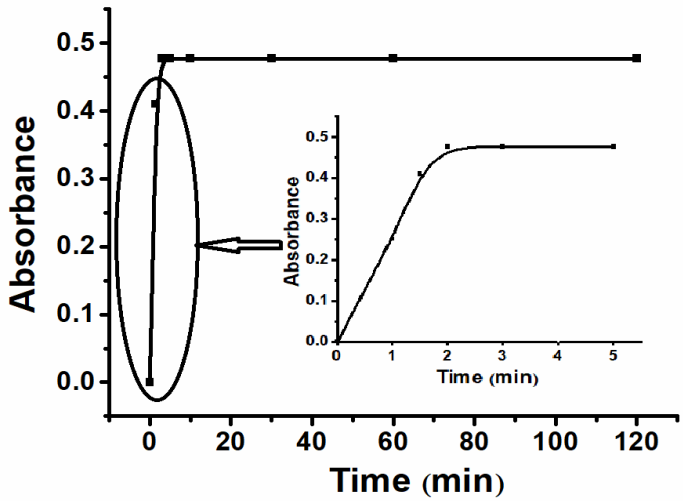

Fig. 8. Effect of reaction time on the IA complex formation. Experimental conditions: C_{dye} = 10 μM, C_{DRH}= 2 μM and pH = 4

Calibration Graph. Under the optimum experimental conditions, the calibration curve was linear in the range from 0.4 to 3 μmol L^{-1} of DRH (Fig. 9). Determination of higher DRH concentrations is complicated due to the rapid formation of a precipitate which makes the measurements badly reproducible. The linear regression equation was ΔA = 0.193 × C(DRH) (where ΔA is the absorbance and C(DRH) is the concentration of drug expressed in μmol L^{-1}) with an acceptable correlation coefficient of R^2 = 0.9996. The limit of detection was found to be 0.10 μmol L^{-1} (40 μg L^{-1}) of DRH, the molar absorptivity of the IA was 3.8×10^4 mol^{-1} L cm^{-1} at 560 nm. The precision of the developed method was evaluated at three concentration levels where DRH concentration was equal to 1, 2, and 3 μmol L^{-1}. Relative standard deviation was 2.7, 1.5, and 1.8, respectively.

Analysis of pharmaceutical formulations. In order to evaluate the precision and accuracy of the developed method in the analysis of real objects, the proposed spectrophotometric method was applied for the determination of DRH in commercial pharmaceutical tablets. Five different samples of pharmaceutical formulations have been analyzed as described above in sections 2.2 and 2.3.

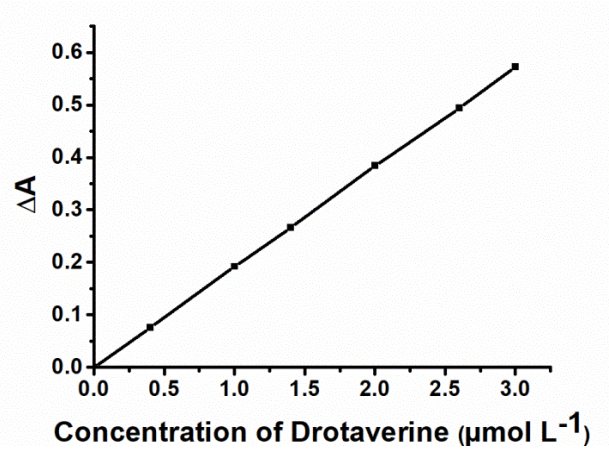

Fig. 9. Calibration graph for the spectrophotometric determination of DRH.

The analyzed tablets (ARTERIUM (Kyivmedpreparat), drotaverine (Darnitsa) and, No-spa (Sanofi)) contain only one active substance. The obtained results are presented in Table 2 and show a good agreement between the content of DRH determined by the proposed method and the DRH content guaranteed by producer that confirms the accuracy of the suggested method. Precision of the developed method varies in the range 0.01-0.02 units of S_r.

Table 2

Results for the determination of DRH in pharmaceutical preparations using the proposed methods (n = 5, P= 0.95)

Drug (producer)	Weight of DRH per one tablet, mg	S	S_r	X±Δ
Arterium (Kyivmedpreparat)	40 mg	0.79	0.02	39.7±1.0
Drotaverine (Darnitsa)	40 mg	0.37	0,009	40.0 ± 0.4
No-spa (Sanofi)	40 mg	0.68	0.017	40.2 ± 0.8

Conclusions

In this study, a new, rapid, simple, sensitive, precise, inexpensive, and environmentally friendly non-extractive spectrophotometric method has been developed for the determination of DRH in pharmaceutical preparations. From the scope of characteristics, the developed method outperforms previously proposed spectrophotometric procedures. The proposed method can compete even with HPLC and could be considered as a successful alternative method.

The method is based on the formation of a specific IA complex between the cationic form of DRH and monoanionic form of ER as a color reagent in acetic buffer medium (pH 4.4). We for the first time proposed to interpret the change occurring in the absorption spectrum by formation of such type of IA (low-soluble ion association complexes formed between fluorescein xanthene dyes and monoprotonated nitrogen containing drugs) as result of dye aggregation (formation of weak π-π bond between aromatic systems of the dye). Formation of the IA crystals facilitates approximation of dye ions. Formation of ion association bond is confirmed by the method of continuous variations. The composition of IA complex is 1:1.

The mechanism of IA formation was proposed. The complete formation of IA occurs when simultaneously two conditions are fulfilled, namely both dye and drug should be in monoprotonated form. These conditions are met to the maximum extent at the optimum pH.

The proposed method is suitable for routine quality control applications. The reagents used are inexpensive and available in any analytical laboratory. The approach avoids the use of extraction separation of excess dye. In this way, toxic organic solvents are excluded from the procedure making the developed method belonging to green chemistry. Earlier solubilisation with surface active substance was applied to stabilize low soluble phase of IA in the solution. In this work it was shown that the obtained solution can be stable for a long time, even without solubilizer. Moreover, use of surfactant can be controversial since IA can be partly dissolved in the micellar medium leading to decreased sensitivity.

The results of the analysis of drugs by the proposed spectrophotometric method showed a satisfactory agreement with the drug contents labeled on pharmaceutical preparations.

References

[1] Derayea, S. M. S. (2014). An application of eosin y for the selective spectrophotometric and spectrofluorimetric determination of mebeverine hydrochloride. *Anal. Methods, 6* 2270–2275. https://doi.org/10.1039/c3ay41371c

[2] Abd El-Hay, S. S., El-Mammli, M. Y., Shalaby, A.A. (2016). Determination of clemastine hydrogen fumarate, desloratadine, losartan potassium and moxepril HCl through binary complex formation with eosin. *Arab. J. Chem., 9*, S541–S547. https://doi.org/10.1016/j.arabjc.2011.06.021

[3] El-Brashy, A. M., Metwally, M. E., El-Sepai, F. A. (2004). Spectrophotometric determination of some fluoroquinolone antibacterials by binary complex formation with xanthene dyes. *Il Farmaco, 59*, 809–817. https://doi.org/10.1016/j.farmac.2004.07.001

[4] Yamaguchi, T., Nakao, M., Nakahara, R., Nishioka, Y., Ikeda, C., Fujita, Y. (2009). Spectrophotometric Determination of Quinolone Antibiotics by an Association Complex Formation with Aluminum(III) and Erythrosin. *Anal. Sci., 25*, 125–128. https://doi.org/10.2116/analsci.25.125

[5] Kelani, K., Bebawy, L.I., Abdel-Fattah, L. (1999). Determination of astemizole, terfenadine and flunarizine hydrochloride by ternary complex formation with eosin and lead(II). *J. Pharm. Biomed. Anal., 18*, 985–992. https://doi.org/10.1016/S0731-7085(98)00107-1

[6] Abdellatef, H. E., Ayad, M. M., Taha, E. A. (1999). Spectrophotometric and atomic absorption spectrometric determination of ramipril and perindopril through ternary complex formation with eosin and Cu(II). *J. Pharm. Biomed. Anal., 18*, 1021–1027. https://doi.org/10.1016/S0731-7085(98)00113-7

[7] El Walily, A. F. M., Belal, S. F., Bakry, R. S. (1996). Spectrophotometric and spectrofluorimetric estimation of ciprofloxacin and norfloxacin by ternary complex formation with eosin and palladium(II). *J. Pharm. Biomed. Anal., 14*, 561–569. https://doi.org/10.1016/0731-7085(95)01662-7

[8] Kuralla, H., Saripilli, R., Kolapalli, V. R. M. (2018). Preparation and evaluation of drotaverine hydrochloride orally disintegrating tablets using melt granulation. *J. Appl. Pharm. Sci., 8*, 39–46.

https://doi.org/10.7324/JAPS.2018.81006

[9] Ziyatdinova, G. K., Samigullin, A. I., Budnikov, G. K. (2007). Voltammetric determination of papaverine and drotaverine. *J. Anal. Chem., 62*, 773–776. https://doi.org/10.1134/S1061934807080138

[10] Maher, H. M., Belal, T. S. (2012). HPLC-dad stability indicating determination of the fixed-dose combination of nifuroxazide and drotaverine hydrochloride in capsules. *J. Liq. Chromatogr. Relat. Technol., 35*, 2001–2020. https://doi.org/10.1080/10826076.2011.627617

[11] Sastry, B. S., Gananadhamu, S., Rao, G. D. (2008). Development of RP-HPLC method for estimation of drotaverine hydrochloride in pharmaceutical formulations. *Int. J. Chem. Sci., 6*, 2055–2061.

[12] Bolaji, O. O., Onyeji, C. O., Ogungbamila, F. O., Ogunbona, F. A., Ogunlana, E. O. (1993). High-performance liquid chromatographic method for the determination of drotaverine in human plasma and urine. *J. Chromatogr. B Biomed. Sci. Appl., 622*, 93–97. https://doi.org/10.1016/0378-4347(93)80254-2

[13] Ayad, M. M., Youssef, N. F., Abdellatif, H. E., Soliman, S. M. (2006). A comparative study on various spectrometries with thin layer chromatography for simultaneous analysis of drotaverine and nifuroxazide in capsules. *Chem. Pharm. Bull., 54*, 807–813. https://doi.org/10.1248/cpb.54.807

[14] Abdellatef, H. E., Ayad, M. M., Soliman, S. M., Youssef, N. F. (2007). Spectrophotometric and spectrodensitometric determination of paracetamol and drotaverine HCl in combination. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc., 66*, 1147–1151. https://doi.org/10.1016/j.saa.2006.05.028

[15] Amin, A. S., El-Sheikh, R., Zahran, F., Fetouh Gouda, A. A. E. (2007). Spectrophotometric determination of pipazethate HCl, dextromethorphan HBr and drotaverine HCl in their pharmaceutical preparations. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc., 67*, 1088–1093. https://doi.org/10.1016/j.saa.2006.09.027

[16] Metwally, F. H. (2008). Simultaneous determination of Nifuroxazide and Drotaverine hydrochloride in pharmaceutical preparations by bivariate and multivariate spectral analysis. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc., 69*, 343–349. https://doi.org/10.1016/j.saa.2007.04.004

[17] Makhani, A. A., Devhare, L. D. (2017). Development and validation of Vierordt's spectrophotometric method for simultaneous estimation of Drotaverine and Nimesulide combination, Research Chronicle in Health Sciences, *3*, 22–28. https://healthscires.com/index.php/rchs/article/view/56

[18] Vishnikin, A.B. (2005). Novel indirect spectrophotometric methods for determination of phosphate and arsenate using polyoxometalates and micellar medium. *J. Molec. Liquids, 118* (1-3), 51–55. https://doi.org/10.1016/j.molliq.2004.07.012

[19] Vishnikin, A. B., Al-Shwaiyat, M. K. E. A., Bazel, Y. R., Andruch, V. (2007). Rapid, sensitive and selective spectrophotometric determination of phosphate as an ion associate of 12-molybdophosphate with Astra Phloxine. *Microchim. Acta, 159*, 371–378. https://doi.org/10.1007/s00604-007-0754-7

[20] Khlyntseva S., V., Vishnikin, A. B., Al-Shwaiyat, M. K. E. A., Sklenářová, H., Solich, P., Bazel, Y. R., Andruch, V. (2011). Sequential injection determination of orthophosphate as ion associate of 12-molybdophosphate with Astra Phloxine. *Talanta, 84*, 1355–1360. https://doi.org/10.1016/j.talanta.2011.02.046

[21] Hedjazi, M., Vishnikin, A. B., Khudyakova, S. N. (2019). Rapid, highly sensitive and selective spectrophotometric determination of cadmium(II) as an ion associate of tetraiodocadmiate(II) with Astra Phloxine. *J. Chem. Technol., 27*, 293–303. https://doi.org/10.15421/081930

[22] Ramakrishna, T. V., Aravamudan, G., Vijayakumar, M. (1976). Spectrophotometric determination of mercury(II) as the ternary complex with Rhodamine 6G and iodide. *Anal. Chim. Acta, 84*, 369–375. https://doi.org/10.1016/S0003-2670(01)82236-4

[23] Filik, H., Tütem, E., Apak, R. (2004). Use of the molybdenum-thiocyanate-rhodamine 6G ternary complex for spectrophotometric molybdenum determination without extraction. *Anal. Chim. Acta, 505*, 77–82. https://doi.org/10.1016/S0003-2670(03)00624-X

[24] Hernandez Córdoba, M., López Garcia, I., Sánchez-Pedreño, C. (1984). Spectrophotometric determination of mercury with thiocyanate and Rhodamine B, *Mikrochim. Acta, 84*, 467–475. https://doi.org/10.1007/BF01197161.

[25] Motomizu, S., Wakimoto, T., Tôei, K. (1983). Spectrophotometric determination of phosphate in river waters with molybdate and Malachite Green. *Analyst, 108*, 361–367. https://doi.org/10.1039/AN9830800361

[26] Dahivelkar, P. P., Bhoir, S. I., Bari, S. B., Surana, S. J., Bhagwat, A. M. (2012). Simultaneous determination of diclofenac potassium and drotaverine hydrochloride in human plasma using reversed-phase high-performance liquid chromatography. *J. Chromatogr. Sci., 50*(8), 694–701. https://doi.org/10.1093/chromsci/bms026

[27] Derayea, S. M., Hamad, A. A., Ali, R., Ali, H. R. H. (2019). Investigating erythrosine B as a fluorimetric probe for the determination of benzimidazole drugs via facile complexation reaction. *Microchem. J., 149*, 104024. https://doi.org/10.1016/j.microc.2019.104024

[28] Every, L. (1982). A numerical method for finding the concentrations of chemicals in equilibrium. *J. Chem. Phys., 76* (6), 3242–3248. https://doi.org/10.1063/1.443317

[29] Vishnikin, A., Miekh, Yu., Denisenko, T., Bazel, Ya., Andruch, V. (2018). Use of sequential injection analysis with lab-at-valve and an optical probe for simultaneous spectrophotometric determination of ascorbic acid and cysteine by mean centering of ratio kinetic profiles. *Talanta, 188*, 99–106. https://doi.org/10.1016/j.talanta.2018.05.056

[30] Wang, J., Liu, Z., Liu, J., Liu, S., Shen, W. (2008). Study on the interaction between fluoroquinolones and erythrosine by absorption, fluorescence and resonance Rayleigh scattering spectra and their application. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc., 69*, 956–963. https://doi.org/10.1016/j.saa.2007.05.057