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Short communication

Spectrophotometric determination of phenylephrine HCl and orphenadrine citrate in pure and in dosage forms

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Abstract

A simple and rapid spectrophotometric methods have been estimated for the microdetermination of phenylephrine HCl (I) and orphenadrine citrate (II). The proposed methods are based on the formation of ion-pair complexes between the examined drugs with alizarine (Aliz), alizarine red S (ARS), alizarine yellow G (AYG) or quinalizarine (Qaliz), which can be measured at the optimum λ_{max}. The optimization of the reaction conditions is investigated. Beer's law is obeyed in the concentration ranges 2–36 μg ml^{-1}, whereas optimum concentration as adopted from Ringbom plots was 3.5–33 μg ml^{-1}. The molar absorptivity, Sandell sensitivity, and detection limit are also calculated. The correlation coefficient was ≥ 0.9988 ($n = 6$) with a relative standard deviation of ≤ 1.7, for six determinations of 20 μg ml^{-1}. The proposed methods are successfully applied to the determination of drugs I and II in their dosage forms using the standard addition technique.

Keywords: Alizarine derivatives; Ion-pair complexes; Spectrophotometry; Dosage forms

1. Introduction

Orphenadrine citrate (RS)-(dimethyl-2-(2-methylbenzhydroxy)ethyl) amine citrate is most widely employed skeletal muscle relaxant [1]. It is centrally acting muscle relaxants drug act by depressing the appropriate neurons within the nervous system so that somatic molar nerve impulses fail to be generated. Several methods have been applied in the literature for the determination of orphenadrine citrate in dosage forms and in biological fluids. The techniques used in this connection include potentiometry [2], spectrophotometry [3], colorimetry [4,5], and high performance liquid chromatography [6].

Phenylephrine hydrochloride (Nec-synephrine) is (*R*)-1-(3-hydroxyphenyl)-2-methyl-aminoethanol hydrochloride [61-76-7]. It is closely related chemically to epinephrine. It is a useful vasconstrictor of sustained action with little effect on the myocardium or the central nervous system. It is used by topical application in nose drops. Subcutaneous injection has been employed extensively to prevent hypotension during spinal anaesthesia

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and for the treatment of orthostatic hypotension [7].

Many procedures are known for the qualitative detection and for quantitative determination of phenylephrine hydrochloride. Among the several analytical methods are titrimetric [8], colorimetric [9], spectrophotometric [10–15], fluorometry and chromatographic methods [16–19].

This study describes an accurate, sensitive, more convenient and less time-consuming spectrophotometric method for the determination of phenylephrine and orphenadrine in bulk drug and in its dosage forms. The method based on ion-pair complex formation between the studied drugs and alizarine derivatives. The coloured product is then quantified spectrophotometrically at λ_{max} for each system. The results obtained by applying the proposed method are compared with those found using the official method. The influence of interferents is also studied.

2. Experimental

2.1. Apparatus

A JASCO 530V spectrophotmeter with a 10 mm quartz cell was used for all spectral measurements and an Orion research model 601 A/digital ionalyzer pH meter was used for checking the pH of buffer solutions of pH values from 2.04 to 12.56, prepared by the recommended method [20].

2.2. Reagents

Alizarine, alizarine red S, alizarine yellow G and quinalizarine were Aldrich products and used without further purification. A stock solution (2×10^{-3} M) was prepared by dissolving the appropriate weight of ARS and AYG in doubly distilled water, while that for Aliz and Qaliz were dissolved in slightly alkaline media (0.001 M NaOH).

Orphenadrine citrate and phenylephrine HCl were supplied by Egyptian International Pharmaceutical Industrial Company (EIPICO), Egypt. Stock solution, 200 μg ml^{-1}, was prepared by dissolving 50 mg of the drug in 250 ml water. 2×10^{-3} M was also prepared by dissolving an appropriate weight in 100 ml water.

2.3. General procedures

An aliquot containing 20–360 μg of drug I or II were transferred into a 10 ml calibrated flask. 1.0 ml of 2×10^{-3} M of ARS and AYG for both drugs I and II, 2.0 ml of 2×10^{-3} M of Aliz-II and 1.5 ml for Aliz-I and Qaliz-II were used. Whereas 2.0 ml of 2×10^{-3} M was used for Qaliz-I complex Table 1. 4.0 ml of the optimum buffer media of the optimum pH value for each system as recorded in Table 1 was added and then completed to the mark with bidistilled water. The absorbance was measured for each system at the optimum wavelength (Table 1) against a reagent blank prepared in the same way without addition of the examined drug.

2.4. Application to various dosage forms

The contents of 20 tablets (orphamole) were weighed and mixed. An amount of the tablet powder equivalent to 20 mg of II was weighed, dissolved in bidistilled water and any remaining residue was removed by filtration. The clear solution was diluted to 100 ml with water in a 100 ml calibrated flask. The drug content of this solution was obtained by applying the standard addition method to aliquot containing 10 μg ml^{-1} of the drug as described above. For eye drops (I prefrin), the same procedures were done.

2.5. Stoichiometric relationship

Job's method of continuous variations was employed; a 2×10^{-3} M solution of drug I or II and 2×10^{-3} M solution of reagents under consideration were used. A series of solutions were prepared in which the total volume of drug and reagent was kept at 2.0 ml. The reagents were mixed in various proportions and diluted to volume in a 10 ml calibrated flask following the above-mentioned procedure.

Table 1
Quantitative parameters for the ion-pair complexation of drugs I and II with Aliz, ARS, AYG and Qaliz

Parameter	I				II			
	Aliz	ARS	AYG	Qaliz	Aliz	ARS	AYG	Qaliz
Buffer	Borate	Borate	Phosphate	Borate	Phosphate	Acetate	Phosphate	Borate
pH	5.2	6.6	4.2	5.2	4.88	5.5	3.4	8.0
λ_{max}	480	495	385	520	533	512	440	550
Reagent (2×10^{-3})	1.5	1.0	1.0	2.0	2.0	1.0	1.0	1.5
Stability (h)	8.0	6.0	9.0	6.0	6.0	8.0	8.0	9.0
Stability constant	4.95	6.80	4.40	5.35	4.75	5.65	3.90	7.40
Beer's law limit (μg ml^{-1})	2–23	2–25	2–30	2–27	2–28	2–27	2–24	2–36
Ringbom concentration/μg ml^{-1}	3–21	3.5–22	3–27	2–25	3.5–25.5	3–25	3–22	3.5–33
Molar absorptivity/1 mol^{-1} cm^{-1} ($\times 10^3$)	4.2	4.6	5.2	2.6	3.39	4.99	3.19	7.990
Sandell sensitivity/μg cm^{-2}	0.072	0.063	0.095	0.08	0.090	0.061	0.096	0.038
Detection limits/μg ml^{-1}	0.4	0.5	0.35	0.3	0.3	0.4	0.2	0.2
Regression equation[a]								
Slope	0.09	0.12	0.09	0.018	0.011	0.016	0.0102	0.026
Intercept	−0.011	0.001	0.007	−0.008	0.010	−0.007	0.013	0.005
Correlation coefficient (r)	0.9988	0.9988	0.9990	0.9992	0.9988	0.9994	0.9990	0.9996
RSD[b] (%)	0.8	1.2	1.4	1.7	1.5	1.7	1.6	1.0
Range of errors	1.10	1.30	1.6	2.0	1.8	2.0	1.7	1.0

[a] $A = a + bc$ (where c is the concentration of drug in μg ml^{-1}).
[b] Average of six determinations.

3. Results and discussion

3.1. Absorption spectra

The absorption spectra of drug I or II and their complexes with alizarine derivatives under the optimum conditions are shown in Fig. 1. The absorption band of reagents showed λ_{max} at 590, 550, 362 and 490 nm for Aliz, ARS, AYG and Qaliz, respectively, whereas that of I-complexes are located at 480, 495, 385 and 520 nm, using Aliz, ARS, AYG and Qaliz, respectively. For II complexes, the maximum absorbance was appeared at 533, 512, 440 and 550 nm, respectively. However, in all instances, the absorbance was measured at those λ_{max} against a reagent blank prepared under identical conditions.

Investigations were carried out to establish the most favourable conditions to give a highly intense colour and to achieve maximum colour development in the quantitative determination of the examined drugs I and II. The influence of each of the following variables on the reaction was tested.

3.2. Effect of pH

The effect of pH on the drug–reagent complex was investigated over the pH range 2.04–12.56 using different types of buffer solutions (acetate, borate, phosphate, thiel and universal buffers [20]). Phosphate buffer solution is used to maintain the optimum one for I-AYG, II-Aliz and II AYG, whereas borate buffer solution is the optimum for drug I with Aliz, ARS and Qaliz and II-Qaliz. Acetate buffer solution is the best for II-ARS complex formation to obtain highest absorbance value in addition to the stability of the colour without affecting the absorbance. For drug I-complexes, the pHs 5.2, 6.6, 4.2 and 5.2 were used for Aliz, ARS, AYG and Qaliz, respectively, whereas for drug II-complexes, the optimum pH values were 4.9, 5.5, 3.4 and 8.0, respectively (Fig. 2). Moreover, the optimum volume of buffer solution added to 10 ml to give

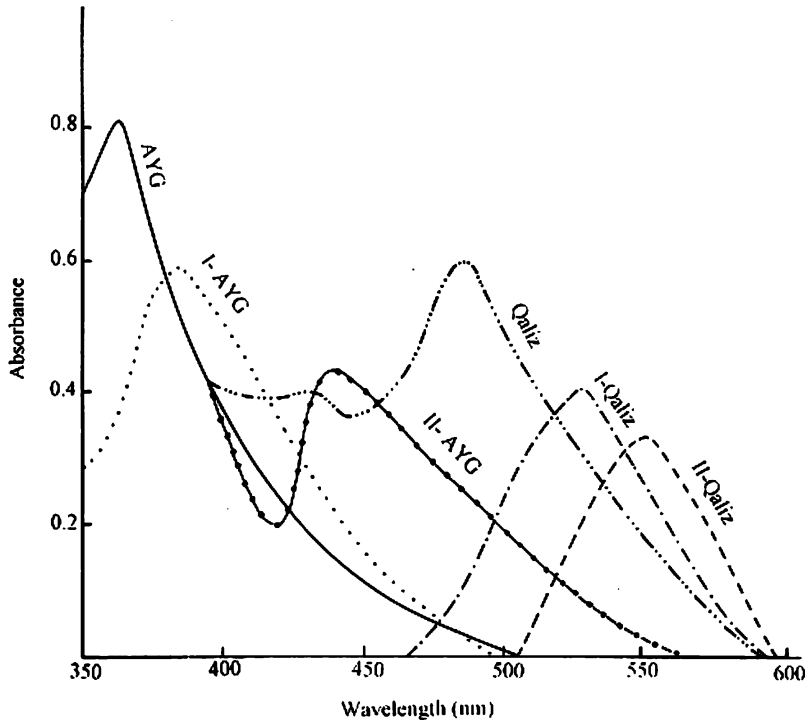


Fig. 1. Maximum absorbance spectra of AYG and Qaliz and their complexes with phenylephrine HCl (I) and orphenadrine citrate (II).

constant absorbance value was also studied and found to be 4.0 ml.

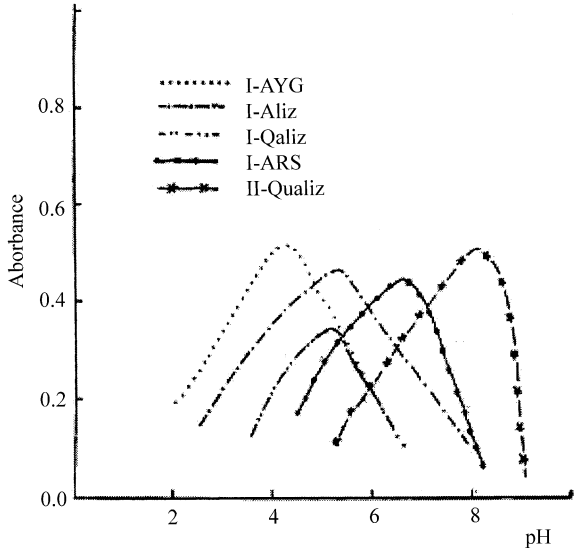


Fig. 2. Effect of pH on the absorbance of drugs (I) with Aliz, ARS, AYG and Qaliz and (II) with Qaliz.

3.3. Effect of reagent concentrations

When the general procedure was followed with varied amounts of 2×10^{-3} M reagent concentration, maximum and constant absorbance was obtained with 1.0 ml for I-complex with Aliz, ARS and AYG and for II-complex with ARS and AYG. For I-Qaliz and II-Aliz, 1.5 ml of 2×10^{-3} M was sufficient for high absorbance values, whereas 2.0 ml was the best for II-Qaliz complex.

3.4. Effect of solvent

The solvents studied (methanol, ethanol, propanol, acetone, dioxane and dimethylformamide) have not affected the intensity of the formed complex.

3.5. Effect of time and temperature

The optimum reaction time was investigated by following the colour development at ambient temperature (25 ± 2 °C). Complete colour inten-

sity was attained after 2.0 min of mixing for all complexes. Raising the temperature up to 50 °C has no effect on the absorbance of the formed complexes, whereas raising above 50 °C, the absorbance start to decay. The absorbance remains stable for at least 6 h (Table 1).

3.6. Sequence of additions

The most favourable sequence is "drug–reagent buffer" for the highest absorbance for all complexes. Other sequences needed longer time in addition to lower stability. The complexes with this sequence remain stable for at least 6 h.

3.7. Stoichiometric ratio

The molar ratio of the drug to reagent in the complex formed was investigated by the Job's method of continuous variations which found to be 1:1 Fig. 3. The stability constants calculated using Harvey and Manning equation [17] applying the data obtained from the continuous variation method was calculated and recorded in Table 1.

3.8. Effect of interferences

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany drugs I and II in their dosage forms (lactose, glucose, galactose, glycerol, saccarose, magnesium streate and starch) was studied. The results indicated that up to 100-fold molar excess of them not interfere (absorbance change by ± 3.0% is non-interferent). Also there is no interference from the degradate product resulted from hydrolytic degradation, indicating a high selectivity for determining the studied drugs I and II in their dosage forms.

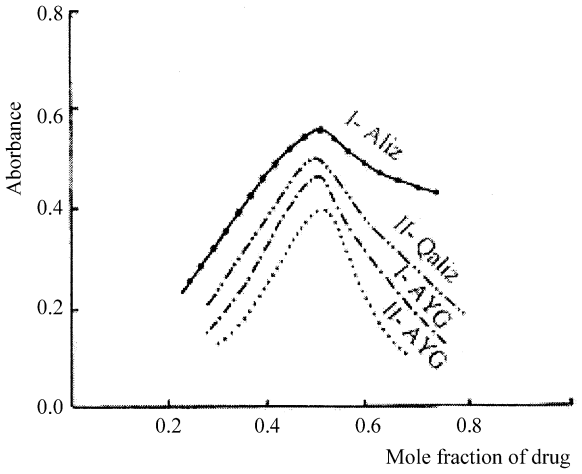


Fig. 3. Continuous variation method for the complexes of drugs (I) and (II).

3.9. Analytical data

Under the optimum experimental conditions, there was a linear relationship between absorbance and drug concentration in the range 2.0–36 μg ml^{-1} with a correlation coefficient ≤ 0.9988 (Table 1). For more accurate analysis, Ringbom optimum concentration range were calculated and recorded in Table 1. The regression analysis, the apparent molar absorptivity and Sandell sensitivity were also calculated from the calibration graph applying least-square method (Table 1).

The standard deviation of the absorbance measurements was 0.0033. The limits of detection (3σ) was calculated and recorded in Table 1. The relative standard deviations were obtained from a series of six standard each containing 20.0 μg ml^{-1} of drug I or II and the results are recorded in Table 1.

3.10. Analytical applications

The proposed methods was successfully applied to various dosage forms, viz. tablets (orphamole, II) and eye drops (prefrin, I). The results are recorded in Table 2, and compared statistically with the official method. For further confirmation, the standard addition method was applied to test the reliability and recovery of the proposed methods. The recovery studies were carried out after adding known quantities of pure drug to the preanalyzed formulations. The percentage recoveries were found to be close to 100% (Table 2). The high percentage recoveries indicates no interferences from ingredients and excipients that might be found in different dosage forms. Consequently,

Table 2
Analysis of phenylephrine HCl and orphenadrine citrate using Aliz, ARS, AYG and Qaliz and official methods

Reagent drug taken ($\mu g\ ml^{-1}$)	Aliz found ($\mu g\ ml^{-1}$) (R%) ($\pm$S.D.%)		ARS found ($\mu g\ ml^{-1}$) (R%) ($\pm$S.D.%)		AYG found ($\mu g\ ml^{-1}$) (R%) ($\pm$SD%)		Qaliz found ($\mu g\ ml^{-1}$) (R%) ($\pm$S.D.%)		Official methods found[a] ($\mu g\ ml^{-1}$) (R%) ($\pm$S.D.%)
	I	II	I	II	I	II	I	II	
4	4.01 (100.25) ($\pm$0.62)	4.03 (100.75) ($\pm$0.35)	3.95 (98.75) ($\pm$0.29)	3.99 (99.75) ($\pm$0.41)	3.99 (99.75) ($\pm$0.25)	4.03 (100.25) ($\pm$0.45)	4.02 (100.5) ($\pm$0.23)	3.96 (99.00) ($\pm$0.36)	3.91 (97.75) ($\pm$0.51)
8	7.99 (99.86) ($\pm$0.25)	7.95 (99.40) ($\pm$0.55)	7.92 (99.00) ($\pm$0.49)	7.97 (99.63) ($\pm$0.35)	7.92 (99.00) ($\pm$0.66)	7.93 (99.13) ($\pm$0.46)	8.03 (100.37) ($\pm$0.84)	7.94 (99.25) ($\pm$0.42)	7.84 (98.00) ($\pm$0.46)
10	10.04 (100.40) ($\pm$0.70)	10.02 (100.20) ($\pm$0.44)	9.89 (48.90) ($\pm$0.55)	9.99 (99.90) ($\pm$0.65)	9.91 (99.10) ($\pm$0.43)	9.94 (99.40) ($\pm$0.49)	10.04 (100.40) ($\pm$0.31)	9.87 (98.70) ($\pm$0.65)	10.1 (101.00) ($\pm$0.96)
16	16.09 (100.56) ($\pm$0.39)	16.08 (100.5) ($\pm$0.70)	15.93 (99.56) ($\pm$0.62)	16.01 (100.06) ($\pm$0.71)	15.89 (99.31) ($\pm$0.69)	15.94 (99.65) ($\pm$0.39)	16.03 (100.18) ($\pm$0.63)	15.92 (99.50) ($\pm$0.62)	15.83 (98.93) ($\pm$0.99)
18	17.96 (94.78) ($\pm$0.85)	17.95 (99.72) ($\pm$0.43)	17.98 (99.50) ($\pm$0.62)	18.03 (100.16) ($\pm$0.66)	17.85 (99.72) ($\pm$0.32)	17.98 (99.89) ($\pm$0.21)	18.06 (100.33) ($\pm$0.36)	17.92 (99.56) ($\pm$0.49)	17.92 (99.56) ($\pm$1.01)
20	20.05 (100.25) ($\pm$0.65)	20.03 (100.15) ($\pm$0.55)	19.8 (99.00) ($\pm$0.59)	20.01 (100.05) ($\pm$0.49)	19.91 (99.55) ($\pm$90.33)	20.03 (100.15) ($\pm$0.29)	20.1 (100.5) ($\pm$0.52)	19.93 (99.65) ($\pm$0.71)	19.3 (96.50) ($\pm$1.01)
24	–	–	–	–	–	24.11 (100.45) ($\pm$0.43)	24.03 (100.13) ($\pm$0.62)	23.96 (99.83) ($\pm$0.66)	24.3 (101.25) ($\pm$0.62)
28	–	–	–	–	–	–	–	27.81 (99.32)	27.75 (99.11)

R%: recovery.
[a] Average of six determinations.

the methods are simple, rapid, accurate and stability indicating assay.

The results obtained from the proposed methods were compared with those obtained using the official method [21]. The accuracy via t-value and the assessment of precision via F-value for five degrees of freedom and a 95% confidence level were calculated and the results indicated that there is no significance difference between them (Table 2).

3.11. Stoichiometry of the complex

The stoichiometry of the complex formed between alizarine derivatives (Aliz, ARS, AYG and Qaliz) with phenylephrine HCl (I) and orphenadrine cetrate (II) was investigated at pH 5.2, 6.6, 4.2 and 5.2 for I-alizarine derivatives and 4.88, 5.5, 3.4 and 8.0 for II-alizarine derivatives complexes, respectively, by the continuous variation method [22].

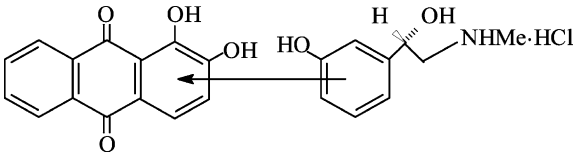
The result indicates the existence of 1:1 charge transfer complexes. The complexes formation between the two drugs under investigation with reagents under consideration may be confirmed by charge transfer (CT) complex, the alkaloids (I and II) behave as a donor and the reagents behave as acceptor [23]. Moreover, the two drugs molecules containing more than donating groups (ion-pair of electrons on the nitrogen of NHMe and oxygen of OH group). Furthermore, low ionization potential of alkaloids was determined according to the equation

$$I_p = a + bv_{CT},$$

where the coefficients a and b reported by Aloisi and Pignataro [24] and Mourad [25].

The stability constant of the complex formed are calculated applying Harvey and Manning equation [26] and are found to be 8.0, 6.0, 9.0 and 6.0 for drug I with Aliz, ARS, AYG and Qaliz, respectively, and 6.0, 8.0, 8.0 and 9.0 for drug II with the same reagent, respectively.

To confirm the structure, the solid samples were prepared and analysed using, elemental analysis and IR spectra. The fundamental IR spectra of the samples is the sum of reagents (Aliz, ARS AYB and Qaliz) and alkaloids with that of reagents predominating in the region of 550–1750 cm^{-1}. A significant difference in the region 2300–3000 cm^{-1} is observed. In this concern, the wide band of the alkaloids (phenylephrine HCl and orphenadrine citrate) in this region is shifted to higher frequencies with increasing intensity (weak band) due to the overlap of N–H of the alkaloids and C=O of the reagents during complexcation. The following structure of I-alizarine complex is suggested:



4. Conclusion

It is clear that alizarine derivatives (Aliz, ARS, AYG and Qaliz) are highly sensitive reagents for the determination of drugs under investigation (I and II). The present procedures uses a relatively small amount of drug and is simple, direct, fast, accurate and applicable over a convenient range of concentrations of two drugs under investigatigation. Therefore, these procedures can be recommended for rotine quality control analysis of phenylephrine HCl and orphenadrine citrate (I and II, respectively). The procedures are considered as a stability indicating assay since there are no interferences from other ingredients.

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