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Spectrophotometric microdetermination of phenylephrine hydrochloride in pure and in pharmaceutical formulations using haematoxylin

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Abstract

A simple, rapid, selective and sensitive method for the micro amount determination of phenylephrine hydrochloride either in pure form (raw material) or in pharmaceutical formulations is described. The method is based on the development of violet colour charge transfer complex with haematoxylin in alkaline medium with 10 min after heating at 65 °C. The wavelength and maximum absorption range was found in the range from 640 to 620 nm. Molar absorptivity and Sandell sensitivity were found to be 2.38×10^4 L mol^{-1} cm^{-1} and 8.6 ng cm^{-2} respectively. A linear correlation was found between absorbance (at the λ_{max}) and concentration. The resulting colour is stable for more than 10 h. Results of the analyses of pure drug and dosage forms by the proposed method are in good agreement with those of the official BP 1998 procedure.

Keywords: Phenylephrine HCl analysis; Haematoxylin; Spectrophotometry; Pharmaceutical formulations

1. Introduction

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 vasoconstrictor of sustained action with little effect on the myocardium or the central nervous system. It is used by topical application in nose drops. Sub-cutaneous injection has been employed extensively to prevent hypotension during spinal anaesthesia and for the treatment of orthostatic hypotension [1].

Many procedures are known for the qualitative detection and for the quantitative determination of phenylephrine hydrochloride. Among the several analytical methods are titrimetric, [2] colorimetric, [3–5] spectrophotometric, [6–11] fluorometry [12] and chromatographic [13–16] methods.

Haematoxylin [7,11b-dihydrobenz-(b)-indeno-[1,2-d]-pyran3,4,6a,9,10-6(h)-pentol] (517-28-2) is a catechol derivative from heart wood or logwood (Haematoxylin campeochi-anum) and is an important stain.[17] Haematoxylin or its oxidized form (hematein) has been widely used for the detection and determination of several metal ions as aluminum,[18] arsenic, [19] tin [20] and molybdenum. [21] Oxidized haematoxylin has been used for the spectro-photometric determination of penicillins and cephalosporins [22] and some antihistaminesb [23].

The proposed method in this work involves the use of haematoxylin as a chromogenic reagent for the spectrophotometric determination of phenylephrine hydrochloride. This work was undertaken to apply the above-mentioned reaction to the spectrophotometric microdetermination of this drug in pure form (raw material) and in pharmaceutical formulations. The reaction conditions were thoroughly studied and the molar ratio of the reaction was calculated.

2. Experimental

2.1. Equipment and reagents

A Perkin-Elmer Lambda 3B spectrophotometer with 10 mm quartz cells was used for all absorbance measurements. An Orion research model 601 A/digital ionalyzer pH meter was used for pH measurements.

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Analytical grade chemicals were used throughout. 6.6×10^{-3} M haematoxylin (Aldrich) was prepared by dissolving 200 mg of the compound in 100 ml of absolute ethanol.

Misr Company for Pharmaceutical Industrial Company, Cairo, Egypt, supplied phenylephrine hydrochloride sub-standard, 10^{-3} M was prepared by dissolving 20.4 mg in 100 ml bidistilled water. Working solutions of lower concentrations were freshly prepared by dilution.

The phosphate buffer solutions of different pH-values (5.6–11.1) were prepared as recommended [24].

2.2. General Procedure

To a series of 10-ml calibrated flasks, 1.0 ml of haematoxylin, 2.0 ml of absolute ethanol and 5.0 ml of pH 7.5 buffer solution were added successively. Aliquots of phenylephrine hydrochloride solution containing 5–300 μg was added and the mixture was kept in a water bath at 65±1 °C for 10 min. The resulting violet colour solution was removed from the bath, cooled and diluted to 10 ml with bidistilled water and the absorbance measured against a reagent blank prepared similarly, at 638 and 620 nm. The amount of phenylephrine hydrochloride was computed from its calibration graph.

2.3. Procedure for eye drops

The contents of 5.0 bottles of eye drops were mixed and the average volume of one bottle was determined. An accurate volume equivalent to contain 5.0 mg of the studied drug was dissolved in 50 ml water in a 50 ml calibrated flask and completed to the mark with water. This solution was further diluted stepwise to the requisite concentration with water and analysed as described under the general procedure described above.

3. Results and discussion

3.1. Absorption spectra

The spectral characteristics of the systems (phenylephrine-haematoxylin-buffer under optimum conditions) are shown in Fig. 1. Under the same conditions the reagent blank shows very small absorbance quantity ($A=0.0042$) in the region of interest, so all the absorbance measurements were carried out against a reagent blank.

The following factors were found to affect the formation of the coloured species; reagent concentration, solvents, pH values, order of addition, time and temperature.

3.2. Effect of reagent concentration

To fix the optimum reagent concentration for complete colour development in a total volume of 10 ml, the concentration of haematoxylin was varied. The optimum amount of 6.6×10^{-3} M of haematoxylin solution was found to be 0.8 ml. 1.0 ml 6.6×10^{-3} M reagent was used in all absorption measurement to ensure complete colour development.

3.3. Effect of solvent

Several organic solvents, i.e methanol, ethanol, propanol, acetone and dioxane were investigated. Most of these solvents suffer from precipitation with low absorbance values. Ethanol was found to be the best solvent. The ratio of solvent was also investigated and it was found that 30% (v/v) ethanol gave a clear coloured solution and the highest absorbance value. At <20% (v/v) precipitation occurs and at >50% (v/v) absorbance decrease.

3.4. Effect of pH

A detailed study of the reaction in various buffer media (acetate, borate, phosphate, and universal buffer solutions of different ph values), showed that phosphate buffer solution was the optimal one. Moreover phosphate buffer solution of pH 7.5 was necessary for complete colour development and highest absorbance value, in addition to the stability of colour for at least 10 h (to obtain high acute and precise results).

3.5. Effect of order of addition

From the experiments in which the reagent was added in all possible sequences, it was concluded that the maximum absorbance is attained only with the following order: haematoxylin-solvent-buffer-phenylephrine hydrochloride.

3.6. Effect of time and temperature

The optimum reaction time was determined by following the colour development at different temperatures. Complete colour intensity was attained after heating at 65±1 °C in a water bath for 10 min. The colour of the complex remained stable for 10 h

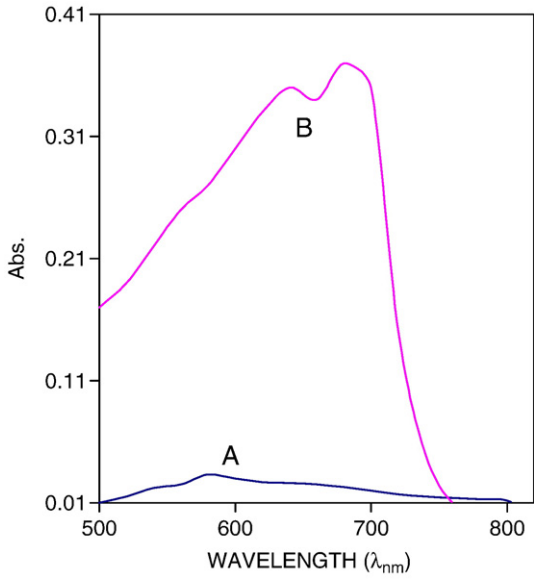

Fig. 1. Absorption spectra of [A]−6.6×10^{-4} M reagent, and [B]− Reagent−1.8 μg/ml drug complex.

after which the absorbance gradually decreased at the rate of 10% for 1 h (Fig. 2).

3.7. Nature of the complex

The stoichiometry of the complex formed between haematoxylin and phenylephrine hydrochloride was investigated at pH 7.5 by using the molar ratio and continuous variation methods. The results obtained indicated the formation of 1 : 1 and 1:2 (R:D) complexes. The reaction of phenylephrine HCl with haematoxylin occurs through the formation of a charge transfer complex.

Sastry et al [22,23] used the oxidized haematoxylin as a reagent for the spectrophotometric determination of penicillins, cephalosporins and antihistamines in pure and in pharmaceutical formulations. The method is based on acid hydrolysis of drugs with 5.0 M HCl and subsequent treatment with oxidized haematoxylin to form a charge transfer complex. The advantage of the present method over Sastry et al [22,23], seems from consumed time of complex formation in addition to canceling the chloramine T used (to oxidize haematoxylin) more sensitive, in addition to selectivity of the present work.

3.8. Interferences

Several pharmaceutical formulations of phenylephrine HCl are associated with flavouring agents, diluents, excipients such as liquifilm (polyvinyl alcohol), antipyrine, benzalkonium chloride, prednisolone acetate, phemylmercuric nitrate, polysorbate, edetate disodium, sodium phosphate dibasic, sodium phosphate monobasic and sodium thiosulphate. In preliminary experiments, these compounds were tested with haematoxylin under the same conditions and found not to interfere up to 200-fold excess in the assay of phenylephrine HCl. Moreover all β-lactam antibiotics and antihistamines do not interfere up to 250-fold excess.

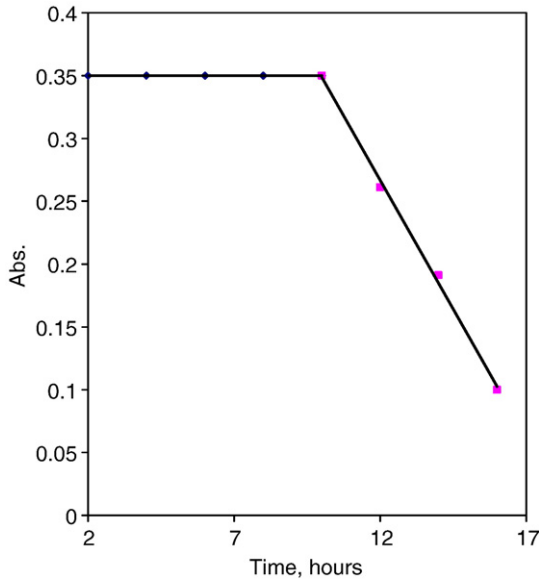

Fig. 2. Rate of colour stability.

Table 1
Evaluation of accuracy and precision of the proposed method

Taken µg/ml	Found* µg/ml		SD %	RSD %	Standard Error	Confidence limit
	P	O				
4.0	3.98	4.10	0.03	0.68	0.013	3.98±0.036
8.0	8.04	8.15	0.02	0.76	0.008	8.04±0.025
12.0	12.12	11.80	0.05	0.51	0.021	12.12±0.062
16.0	15.90	16.25	0.07	0.39	0.030	15.90±0.083
20.0	19.85	20.25	0.04	0.87	0.017	19.85±0.051
24.0	24.20	24.40	0.08	0.96	0.034	24.20±0.098
28.0	27.80	28.50	0.09	0.79	0.038	27.80±0.115

P : Proposed method O : Official method [25].
* Average of six determinations.

3.9. Quantification

A linear correlation was found between absorbance and concentration in the range of 0.5–30 µg/ml. For more accurate analysis Ringbom optimum concentration ranges were determined by plotting log[*C*] in µg/ml against percent transmittance and the linear part of the S-shape, curve gave the accurate range of analysis. The molar absorptivity and Sandell sensitivity (calcutated as the F.Wt of drug by the molar absorptivity) were found to be 2.38×10^4 L mol^{-1} cm^{-1} and 8.6 ng cm^{-2}. Regression analysis using the method of least squares was made and found to be $A=0.0042+0.012$ °C (where C in µg/ml).

The reproducibility of the procedure was determined by running eight replicate samples, each containing 18 µg/ml of phenylephrine HCl in the final assay solution. At this concentration, the relative standard deviation was 0.64%.

The performance of the method was assessed by calculation of the *t*- and *F*-values compared with the official method [25] (Based on potentiometric titration using 0.1 M ethanolic sodium hydroxide). Mean values were obtained in a student's *t*- and *F*-test and 95% confidence limits for five degrees of freedom.[26] The results showed that the calculated *t*- (1.09) and *F*-values (2.24) did not exceed the theoretical ones (2.57 and 5.05, respectively).

3.10. Accuracy and precision

In order to determine the accuracy and precision of the method, solutions containing seven different concentrations of phenylephrine HCl were prepared and analysed in quintuplicate. Although the official method has higher detection limits compared to the proposed method, a high concentration of drug was used for the official ones and diluted for the proposed method. The measured standard deviation (S.D.), relative standard deviations. (R.S.D.), the standard analytical errors and confidence limits (Table 1) can be considered satisfactory at least for the levels of concentrations examined.

Comparison of the recovery obtained with the proposed method with the purity of phenylephrine HCl as determined using British Pharmacopoeia [25] showed a high accuracy of the proposed method. The proposed method is simple, less time consuming, more sensitive and highly selective than the official

Table 2
Determination of phenylephrine HCl in different formulations using the proposed and official method [25]

Sample	Manifested by	Label to content	Taken μg/ml	Found*		Recovery %	S.D. %
				Official	Proposed		
Eye drops							
PREFRIN	EIPICO	1.2 mg/ml	7.0	7.15	7.05	100.71	0.62
			14.0	14.35	13.85	98.93	0.98
			21.0	21.50	21.25	101.19	1.13
			28.0	27.70	28.20	100.71	0.57
BLEPHAMIDE	EIPICO	1.2 mg/ml	5.0	4.9	4.94	98.80	1.06
			15.0	15.30	15.10	100.67	0.51
			25.0	25.50	24.80	99.20	0.73
OPTO FRIN ZINC	Nile	1.2 mg/ml	8.0	7.88	7.95	99.38	0.47
			16.0	15.60	15.80	98.75	0.99
			24.0	23.50	23.70	98.75	0.87
			30.0	29.30	29.60	98.67	1.11
PHENYLEPHRINE	Kahira	1.2 mg/ml	6.0	5.95	6.00	100.00	0.36
			12.0	11.85	11.90	99.58	0.43
			18.0	18.20	18.15	100.83	0.75
			24.0	23.75	24.25	101.04	0.97
			30.0	30.40	29.75	99.17	0.82

*Average of six determinations.
EIPICO: Egyptian International Pharmaceutical Industries Company, Egypt (Under license from Allergan U. S. A.).
Nile: Nile Company for Pharmaceutical and Chemical Industries, Cairo, Egypt.
Kahira: Kahira Pharmaceutical and Chemical Industries Company, Cairo, Egypt.

method. Moreover, the proposed method could be used for the routine determination of phenylephrine HCl in pure form or in pharmaceutical formulations.

3.11. Analytical Applications

The proposed method was applied to some pharmaceutical formulations containing phenylephrine HCl. The results in Table 2 indicate high accuracy and selectivity. The proposed method is suitable for the determination of phenylephrine in drug formulations without interferences from excipients such as glucose, lactose, and starch or from degradation products.

4. Conclusions

The proposed method is simpler, less time consuming, sensitive and more selective than the official method. Although the colour development of the CT the complex at room temperature requires 75 min for completion, this can be shortened to 10 min by raising the temperature to 65±1 °C. Comparing with the Sastry et al [22,23] method using the oxidized haematoxylin as a reagent for the spectrophotometric determination of some drugs, the proposed method is more sensitive, less time consuming without addition of chloramine T, in addition to selectivity. Although polyvinyl alcohol, benzalkonium chloride and polysorbate enhanced the color of hematoxylene and its complexes in presence of dispersing agents and organized assemblies of micelles, these compounds have neither effect nor reaction under the optimum experimental conditions of the proposed method. Regarding the interference of excipience and additives, in addition to the degradate products, there are no interferences in the microdetermination of phenylephrine HCl in pure or in pharmaceutical formulations, suggesting applications in bulk analysis.

References

[1] A. Goth, Medical Pharmacology Principle and Concepts, 10th Ed., The Mosby C.V. Company, 1981.
[2] M.A. El-Sayed, A.C. Obiakara, S.U. Uzodinma, J. Assoc. Off. Anal. Chem. 64 (1981) 860.
[3] E.T. Koshy, H. Mitchner, J. Pharm. Sci. 52 (1963) 802.
[4] N.M. Sanghavi, J.J. Vyas, Indian Drugs 34 (1997) 463.
[5] G.S. Bhuee, J. Singh, S.N. Rostogi, J. Inst. Chem. (India) 55 (1983) 229.
[6] M. Knochen, J. Giglio, Talanta 64 (2004) 1226.
[7] S.A. Shama, J. Pharm. Biomed. Anal. 30 (2002) 1385.
[8] I. Shoukrallah, Anal. Lett. 24 (1991) 2043.
[9] H. Mahgoub, Drug. Dev. Ind. Pharm. 16 (1990) 2135.
[10] M.M. Amer, A.M. Taha, S.R. El-Shabouri, P. Khashaba, J. Assoc. Off. Anal. Chem. 65 (1982) 894.
[11] P. Solich, C.K. Polydorou, M.A. Koupparis, C.E. Efstathiou, J. Pharm. Biomed. Anal. 22 (2000) 781.
[12] M.A. Martin, D. Delcastillo, P. Prados, Talanta 40 (1993) 1719.
[13] J.O. De Beer, C.V. Vandenbroucke, D.L. Massart, J. Pharm. Biomed. Anal. 12 (1994) 1379.
[14] M.J. Smelo, R. Stromberg, J. Assoc. Off. 74 (1991) 289; Anal. Abstr. 54 (1991) 28.
[15] V.E. Ruchkin, N.A. Tyuka Vkina, I.A. Rulenko, N.K. Akhtonova, A. Kolesnik, V.I. Litvinenko, Farm. Zh. (hiev) 662 (1991); Anal. Abstr. 55 (1993) 2G4.
[16] N. Okamura, H. Miki, T. Harada, S. Yamoshita, Y. Masaoka, Y. Nakamoto, Y. Nakamoto, M. Tsuguma, A. Yoshitomi, A. Yagi, J. Pharm. Biomed. Anal. 20 (1999) 363.
[17] D.A. Johnson, Plant Microtechnique, Tata McGraw–Hill, Bombay, 1946.
[18] I. Sarghie, E. Sirghie, Rev. Chem., Bucharest 35 (1984) 535.
[19] V. Croitoru, E. Pirlog, Ser. Chim-Metal. 39 (1977) 17.
[20] C.L. Leong, Analyst 102 (1977) 837.
[21] Z. Zhang, J. Oiu, C. Lin, Gaodeng Xuexiao Huaxue Xuebao 5 (1984) 39.
[22] C.S.P. Sastry, P.V. Satyanarayana, A.R. Rao, N.R.P. Singh, Mikrochim. Acta 1 (1989) 17.
[23] C.S.P. Sastry, A.S.R.P. Tipirmeni, M.V. Suryanarayana, Indian Drugs 26 (1989) 351.
[24] H.T.S. Britton, Hydrogen Ions, 4th Ed., Chapman and Hall, 1952.
[25] British Pharmacopoeia, Her Majesty's Stationary Office, London, 1998, p. 516.
[26] J.C. Miller, J.N. Miller, Statistics in Analytical Chemistry, 2nd Ed., Ellis Horwood, Chichester, 1988.