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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR PHENYLEPHRINE HYDROCHLORIDE ESTIMATION IN NASAL DROPS FORMULATIONS

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Simple, accurate and reproducible UV-spectrophotometric method was developed and validated for the estimation of phenylephrine hydrochloride in pharmaceutical nasal drops formulations. Phenylephrine hydrochloride was estimated at 291 nm in 1 mol·dm^{-3} sodium hydroxide (pH 13.5). Beer's law was obeyed in the concentration range of 10–100 μg·cm^{-3} (r^2 = 0.9990) in the sodium hydroxide medium. The apparent molar absorptivity was found to be 1.63×10^3 dm^3·mol^{-1}·cm^{-1}. The method was tested and validated for various parameters according to the ICH (International Conference on Harmonization) guidelines. The detection and quantitation limits were found to be 0.892 and 2.969 μg·cm^{-3}, respectively. The proposed method was successfully applied for the determination of phenylephrine hydrochloride in pharmaceutical nasal drops formulations. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 1 %), while being simple, cheap and less time consuming, and hence can be suitably applied for the estimation of phenylephrine hydrochloride in different dosage forms.

Key words: phenylephrine hydrochloride; spectrophotometry; nasal drops

РАЗВОЈ И ВАЛИДАЦИЈА НА СПЕКТРОФОТОМЕТРИСКИТЕ МЕТОДИ ЗА ОПРЕДЕЛУВАЊЕ НА ФЕНИЛЕНФРИНХИДРОХЛОРИД ВО НАЗАЛНИ ПРЕПАРАТИ

Селективен и специфичен спектрофотометриски метод е развиен и валидиран за квантитативно определување на фениленфринхидрохлорид во фармацевтски назални препарати. Фенилефринхидрохлорид е определуван на 291nm во 1 mol·dm^{-3} NaOH (pH 13.5). Во распон на концетрациите од 10 до 100 μg·cm^{-3} (r = 0.9990) важи Беровиот закон. Вредноста на моларната апсорптивност изнесува 1.63×10^3 dm^3·mol^{-1}·cm^{-1}. Методот е тестиран и валидиран за различни аналитички параметри според упатството на ICH (International Conference on Harmonisation). Вредностите на границите за докажување и квантификација изнесуваа 0,892 и 2,969 μg·cm^{-3}, соодветно. Резултатите од експериментот покажаа дека предложениот метод е прецизен, точен, репродуктивен (релативнаната стандардна девијација < 1 %), едноставен и евтин е за определување на фениленфринхидрохлоридот во фармацевтските назални препарати.

Клучни зборови: фенилефринхидрохлорид; спектрофотометрија; капки за нос

INTRODUCTION

Phenylephrine ($C_9H_{13}NO_2$, M_w 167.2 gmol^{-1}) contains not less than 99.0 % and not more than the equivalent of 100.5 % of 3-(1-hydroxy-2-methylamino-ethyl)phenol [CAS 59-42-7], calculated with reference to the dried substance. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides [1]. Converting otherwise insoluble amines into their hydrochlorides is a common way to make them water- and acid-soluble. This is particularly desirable for substances used in medications. Hydrochlorides

are salts resulting, or regarded as resulting, from the reaction of hydrochloric acid with an organic base (mostly amines). For example, reaction of phenylephrine ($C_9H_{13}NO_2$) with hydrochloric acid (HCl) yields phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$, M_w 203.7 $gmol^{-1}$). Even though this style of formulas is often used for denoting the hydrochlorides, the dot incorrectly implies that the two molecules are weakly bonded together.

Phenylephrine hydrochloride contains not less than 98.5 % and not more than the equivalent of 101.0 % of 3-(1-hydroxy-2-methylamino-ethyl)-phenol hydrochloride [CAS 61-76-7], calculated with reference to the dried substance (Fig. 1). Phenylephrine hydrochloride is white or almost white, crystalline powder, freely soluble in water and in alcohol. It melts at about 143 °C. The specific optical rotation is –43° to –47°, calculated with reference to the dried substance [2].

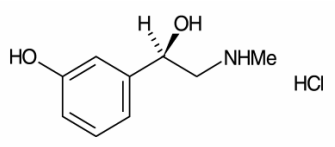

Fig. 1. Chemical structure of phenylephrine hydrochloride

Phenylephrine hydrochloride (alpha-adrenergic, sympathomimetic agent) is a useful vasoconstrictor of sustained action with little effect on the myocardium or the central nervous system. It is available in the following dosage forms: nasal drops, nasal spray, eye drops and phenylephrine injection [3]. The use of the decongestant promotes nasal and sinus drainage. Phenylephrine is available as oral tablets, chewable tablets, oral disintegrating tablet, capsules and sachets formulations. Some popular cold remedies containing phenylephrine are: Canada's hot lemon Neocitran, Serbian nasal drops Adrianol, the United Kingdom's Lemsip, and the United States' Alka-Seltzer Cold Effervescent formula, Sudafed PE Non-Drowsy Nasal Decongestant, Robitussin CF, Tylenol Sinus, and DayQuil Capsules. It is available in many combination products (with an antihistamine), such as Bromfed, Nalex-A, and AlleRX. The content of phenylephrine hydrochloride is 90.0 – 110.0 % of the stated amount.

Nasal preparations are liquid, semi-solid or solid preparations intended for administration to nasal cavities to obtain a systemic or local effect. They contain one or more active substances. Nasal preparations are non-irritating as possible, and do not adversely affect the functions of the nasal mucosa and its cilia. Aqueous nasal preparations are usually isotonic and may contain excipients, for example, to adjust the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance, or to stabilise the preparation. Nasal preparations are supplied in multidose or single-dose containers, provided, if necessary, with a suitable administration device which may be designed to avoid the introduction of contaminants. Unless otherwise justified and authorised, aqueous nasal preparations supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration, except where the preparation itself has adequate antimicrobial properties.

Various methods have been reported in the literature for the analysis of phenylephrine hydrochloride including spectrophotometry [4–8], spectrophotometry with chromogenic reagent [9], fluorometry [10], and chromatography [11, 12]. High-performance liquid chromatography [13–16], micellar liquid chromatography [17], micellar electrokinetic chromatography [18], capillary zone electrophoresis [19, 20], spectro-fluorimetric and derivative spectrophotometric methods [21], have also been reported for the determination of phenylephrine hydrochloride.

For routine analysis of phenylephrine hydrochloride, a simple and rapid analytical method is preferred. A survey of the literature has not revealed any simple validated UV spectrophotometric method for estimation of phenylephrine hydrochloride in alkaline media of nasal drops formulations and dissolution alkaline media of nasal formulations. The objective of the present study was to develop simple, precise, accurate and validated, economic analytical methods for the estimation of phenylephrine hydrochloride in pure form and in pharmaceutical formulations. The developed analytical method was validated as per the ICH (International Conference on Harmonisation) guidelines [22], and Serbian requirements [23]. Statistical tests were performed on validation data [24,25].

EXPERIMENTAL

Material and reagents

A standard of phenylephrine hydrochloride (99.97 %) was obtained as a gift from the Pharmaceutical and chemical industry Zdravlje-Actavis (Leskovac, Serbia) and used without further puri-

fication. Formulations containing phenylephrine hydrochloride were also kindly donated by Zdravlje-Actavis. Also obtained was a commercial pharmaceutical preparation for children, Adrianol-T nasal drops, labelled to contain 0.5 $mgcm^{-3}$ of phenylephrine hydrochloride. Adrianol-T contains excipients like disodium hydrophosphate dihydrate, citric acid monohydrate, methyl cellulose M.H.B. 10 000, glycerol, phenyl-mercury(II) borate, ammonium hydroxide, ethanol 96 % and pure water. A commercial pharmaceutical preparation for adulthood, Adrianol nasal drops, labelled to contain 1.0 $mgcm^{-3}$ of phenylephrine hydrochloride, was obtained. Adrianol contain excipients like disodium hydrophosphate dihydrate, citric acid monohydrate, methyl cellulose M.H.B. 10 000, glycerol, phenyl-mercury(II) borate, ammonium hydroxide, ethanol 96 % and pure water. All other chemicals and reagents used were of analytical grade (Merck Chem. Ind.).

Instruments

A double-beam Varian Cary-100 Conc UV–VIS spectrophotometer, connected to computer and loaded with Cary WinUV software was used. For an intermediate precision study, a different Perkin Elmer Lambda-16 UV–VIS spectrophotometer connected to computer with UV-PC software was used. Both instruments have an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm (1.0 cm) cell path length. The absorbance of phenylephrine in the selected medium at respective wavelength was determined and the apparent molar absorptivity was calculated according to the standard formulae (Table 1).

Procedure for calibration curve

One stock solution of 500 μgcm^{-3} of phenylephrine hydrochloride was prepared in sodium hydroxide (pH 13.5) by dissolving 25 mg of phenylephrine hydrochloride in 50 cm^3 of 1 $moldm^{-3}$ sodium hydroxide. For preparation of different concentrations, aliquots of stock solution were transferred into a series of 10 cm^3 standard volumetric flasks and volumes were made with the respective media. Ten different concentrations were prepared in the range of 10–100 μgcm^{-3} of phenylephrine hydrochloride in NaOH for the standard curve. In a similar way, five different concentrations were prepared in the range of 100–500 μgcm^{-3} of phenylephrine hydrochloride considering the declared value. Phenylephrine hydrochloride was estimated at 291 nm in NaOH medium.

Sample preparation

The Adrianol-T and Adrianol nasal drops preparations (Zdravlje-Actavis, Leskovac) were previously filtered. Aliquots (1 cm^3) of nasal drop solutions equivalent to 0.5 mg and 1.0 mg respectively, of phenylephrine hydrochloride were taken and suitably diluted with NaOH (1 $moldm^{-3}$) media to get a 50 μgcm^{-3} concentration and the samples were analyzed using the proposed analytical methods.

Analytical method validation

Specificity and selectivity

Phenylephrine hydrochloride solutions (50 μgcm^{-3}) were prepared in the selected media with and without common excipients (disodium hydrophosphate dihydrate, citric acid monohydrate, methyl cellulose M.H.B. 10 000, glycerol, phenyl-mercury(II) borate, ammonium hydroxide, ethanol 96 % and pure water), separately. All solutions were scanned from 400 to 200 nm at a speed of 200 nm min^{-1} and checked for change in the absorbance at respective wavelengths. In a separate study, drug concentration of 50 μgcm^{-3} was prepared independently from pure drug stock solution in selected media and analysed (n = 10). Paired t-test at 95 % level of significance was performed to compare the means of absorbance (Table 1).

Linearity

To establish linearity of the proposed methods, ten separate series of solutions of phenylephrine hydrochloride (10–100 μgcm^{-3} in 1 $moldm^{-3}$ sodium hydroxide) were prepared from the stock solutions and analyzed. Least square regression analysis was performed on the obtained data.

Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations – lower concentration (LC, 80%), intermediate con-

centration (IC, 100%) and higher concentration (HC, 120%) were prepared from independent stock solutions and analyzed (n = 10). Accuracy was assessed as the percentage relative error and mean % recovery (Table 2). To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved the addition of different concentrations of pure drug (10, 20 and 30 μgcm^{-3}) to a known preanalyzed formulation sample and the total concentration was determined using the proposed methods (n = 10). The % recovery of the added pure drug was calculated as % recovery = $[(C_t–C_s)/C_a]$ x 100, where C_t is the total drug concentration measured after standard addition; C_s, drug concentration in the formulation sample; C_a, drug concentration added to formulation (Table 3).

Precision

Repeatability was determined by using different levels of drug concentrations (same concentration levels taken in accuracy study), prepared from independent stock solutions and analyzed (n=10) (Table 2). Inter-day, intra-day and inter-instrument variation were studied to determine intermediate precision of the proposed analytical methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. The same procedure was followed for three different days to study inter-day variation (n = 10). One set of different levels of the concentrations was reanalyzed using the Lambda-16 Perkin Elmer UV–VIS spectrophotometer connected to computer with UV-PC software, to study inter-instrument variation (n = 10). The percent relative standard deviation (% R.S.D.) of the predicted concentrations from the regression equation was taken as precision (Table 3). Precision studies were also carried out using the real samples of phenylephrine nasal drops (0.5 mg cm^{-3}) in a similar way to standard solution to prove the usefulness of the method.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for phenylephrine hydrochloride by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n = 10) (Table 1).

RESULTS AND DISCUSSION

The UV spectra of phenylephrine hydrochloride in 1 moldm^{-3} NaOH medium and Adrianol excipients are shown in Fig. 2. The λ_{max} of phenylephrine hydrochloride in 1 moldm^{-3} NaOH was found to be 291 nm. The apparent molar absorptivity of the drug was found to be 1.63×10^3 dm^3·mol^{-1}·cm^{-1}.

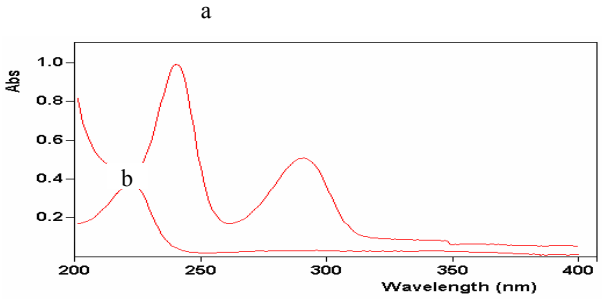

Fig. 2. UV-absorption spectra of 50 μg cm^{-3} concentration of phenylephrine hydrochloride in 1 moldm^{-3} NaOH medium (a) and Adrianol excipients (b)

The UV spectrum of Adrianol-T nasal drops preparation (phenylephrine hydrochloride with excipients) in 1 moldm^{-3} NaOH medium is shown in Fig. 3.

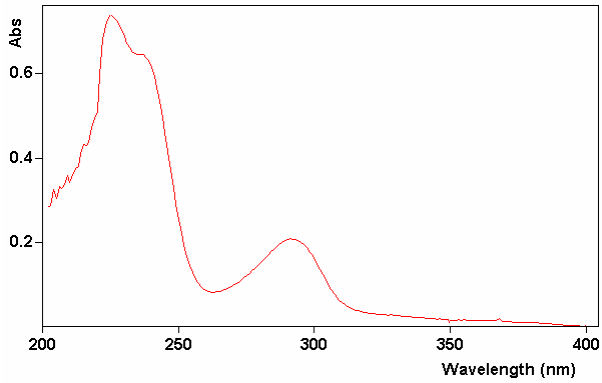

Fig. 3. UV-absorption spectar of Adrianol-T nasal drops preparation in 1 moldm^{-3} NaOH medium.

Calibration curve

In 1 moldm^{-3} NaOH, the linear regression equation obtained with a regression coefficient (r) of 0.9995 and standard deviation (SD) of 0.0016 was: A_{291} = [6.736 × C (mg cm^{-3})] + 0.0586.

Beer's law was obeyed in the concentration range of 10–100 μg·cm^{-3} (r^2 = 0.9990) in sodium hydroxide medium. The calibration curve of phenylephrine hydrochloride was in the range of 100–500 μgcm^{-3}, considering the declared value of the Adrianol-T preparation. Beer's law was not obeyed in the investigated concentration range.

Specificity and selectivity

The UV-spectrum of phenylephrine hydrochloride was not changed in the presence of common excipients used in the formulation of nasal drops. The absorption spectrum of pure drug sample was matching those of the formulation samples in the selected NaOH media (Fig. 2). The calculated *t*-values were found to be less than that of the tabulated *t*-values, indicating that statistically there was no significant difference between the mean absorbance of solutions prepared from pure drug sample and the formulation samples (Table 1). Therefore the proposed analytical method is specific and selective for the drug.

Linearity

The linearity range for phenylephrine hydrochloride was found to be 10–100 μgcm^{-3} (r^2 = 0.9990) in 1 moldm^{-3} NaOH (Table 1). The low values of the standard error (S.E.) of slope and intercept (Table 1) indicated high precision of the proposed methods. Also, the mean slope and intercept values are within the 95 % confidence interval. The quality of the fit of the regression equations was supported by the high regression coefficient values (Table 1).

T a b l e 1

Optical characteristics, statistical data of the regression equations and validation parameters for phenylephrine hydrocloride (n = 10).

Parameter	1moldm^{-3} NaOH
Optical characteristics	
Apparent molar absortivity (dm^{3}mol^{-1}cm^{-1})	1.63 x 10^3
Regression analysis	
Slope (S.E.[a])	6.736 (0.0718)
95% confidence limits of slope	6.698; 6.802
Intercept (S.E.) [a]	0.0586 (0.0019)
95% confidence limits of intercept	0.0577; 0.0595
Regression coefficient (r^2)	0.9990
Validation parameters	
Specificity and selectivity – *t* [b]	0.98
Linearity (μgcm^{-3})	10–100
Limit of detection (LOD)(μgcm^{-3})	0.892
Limit of quantification (LOQ)(μgcm^{-3})	2.969

[a] Standard error of mean.
[b] Theoretical values at 95% confidence limits *t* = 2.225.

Accuracy

The accuracy ranged from 40 to 60 μgcm^{-3} in 1 moldm^{-3} NaOH (Table 2). The excellent mean % recovery values, close to 100 %, and their low standard deviation values (S.D. < 1.0) indicate high accuracy of the analytical methods. The validity and reliability of the proposed methods was assessed by the recovery studies. In 1 M NaOH, the mean % recoveries (% R.S.D.) for lower, intermediate and higher concentrations were found to be 100.025 (40 μgcm^{-3}), 100.16 (50 μgcm^{-3}) and 99.95 (60 μgcm^{-3}), respectively.

T a b l e 2

Accuracy and method precision data for the developed method (n = 10).

Level	Estimated concentration (μgcm^{-3})[a]			Mean % recovery	Accuracy[b]
	Range	Mean (±S.D)	% R.S.D.	(±S.D)	(%)
LC (40 μgcm^{-3})	39.85–40.26	40.01 ± 0.3368	0.842	100.025 ± 0.257	0.025
IC (50 μgcm^{-3})	49.81–50.35	50.08 ± 0.3696	0.738	100.16 ± 0.498	0.16
HC (60 μgcm^{-3})	59.56–60.38	59.97 ± 0.5487	0.915	99.95 ± 0.392	- 0.05

[a] Estimated concentration of phenylephrine hydrocloride was calculated by linear regression equation.
[b] Accuracy is givin in % relative error [=100 × (predicted concetration – nominal concetration)/ nominal concetration].

The validity and reliability of the proposed methods was further assessed via recovery studies by the standard addition method (Table 3). The mean % recoveries (%R.S.D.) for the intermediate concentration were found to be 99.87 (0.58), 100.50 (0.64) and 99.84 (0.53), respectively. These results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical methods.

Table 3

Standard addition of phenylephrine hydrochloride for accuracy (n = 10).

Drug in formulation (μgcm^{-3})	Pure drug added (μgcm^{-3})	Total drug found (μgcm^{-3}) (±S.D)	% Recovery (±R.S.D)
50	0	50.21 ± 0.34	100.42 ± 0.67
50	10	59.92 ± 0.32	99.87 ± 0.58
50	20	70.35 ± 0.45	100.50 ± 0.64
50	30	79.87 ± 0.42	99.84 ± 0.53

Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability (% R.S.D.) ranged from 40 to 60 μgcm^{-3} in 1 $moldm^{-3}$ NaOH, at all three levels of phenylephrine hydrochloride concentrations (Table 4). The repeatability results indicated the precision under the same operating conditions over a short interval of time and inter-assay precision. Intermediate precision expresses within-laboratory variations in different days and in different instruments. In intermediate precision study, % R.S.D. values were not more than 1.0 % in all the cases (Table 4).

Table 4

System precision study (n = 10)

Concentration (μgcm^{-3})	Estimated concentration / μgcm^{-3} (Intra-day repeatability % R.S.D., n = 10) Day 1	Day 2	Day 3	(Intra-instrument repeatability % R.S.D., n = 10) *
40	40.01 (0.842)	39.84 (0.675)	40.12 (0.984)	39.96 (0.986)
50	50.08 (0.738)	49.88 (0.698)	49.93 (0.651)	50.09 (0.616)
60	59.97 (0.915)	60.15 (0.784)	60.07 (0.811)	59.83 (0.694)

*Calculation: $RSD(\%)=\frac{S}{<x>}\cdot 100\%$ $S=\sqrt{\frac{\sum(x-<x>)^2}{n-1}}$

R.S.D. values for the proposed analytical method were well within the acceptable range, indicating that the method have excellent repeatability and intermediate precision. The % R.S.D. values for the precision studies with real samples of phenylephrine nasal drops were found to be less than 1.

LOQ and LOD

In 1 $moldm^{-3}$ NaOH, LOD and LOQ were found to be 0.892 and 2.969 $\mu g \cdot cm^{-3}$ for phenylephrine hydrochloride (Table 1).

Estimation of formulations

In 1 $moldm^{-3}$ NaOH, the assay values of phenylephrine hydrochloride for nasal drops formulations ranged from 99.40 % to 101.20 %, with standard deviation of not more than 0.77 %. The assay values for the formulations were same as mentioned in the label claim, indicating that the interference of excipient matrix is insignificant in the estimation of phenylephrine hydrochloride by the proposed analytical method. The estimated drug content with low values of standard deviation established the precision of the proposed method. The calculated Student's t-values did not exceed the tabulated values (Table 5).

Table 5

Application of spectrophotometric method to the determination of phenylephrine hydrochloride from pharmaceutical dosage forms (n = 10).

Sample (preparation)	Adrianol-T
Total phenylephrine hydrochloride found ($mgcm^{-3}$) (±S.D)	0.506 ± 0.0091
% Recovery	101.20
Acuracy [a] (%)	1.20
t [b]	1.98
Sample	**Adrianol**
Total phenylephrine hydrochloride found ($mgcm^{-3}$) (±S.D)	0.994 ± 0.0134
% Recovery	99.40
Acuracy [a] (%)	– 0.60
t [b]	2.09

[a] Accuraci is givin in % relative error [=100 × (predicted concetration – nominal concetration)/ nominal concetration].

[b] Theoretical values at 95% confidence limits t =2.225

CONCLUSION

A UV-spectrophotometric method was developed for phenylephrine hydrochloride determination. The analytical method is simple, sensitive, rapid and specific and it can be conveniently employed for the routine analysis and the quality control of phenylephrine hydrochloride in pharmaceutical dosage forms. The method was suitable to determine concentrations in the range 0.01 to 0.1 mgcm^{-3}, precisely and accurately. The limits of detection and quantitation for phenylephrine hydrochloride with a lower concentration were 0.892 and 2.969 μg·cm^{-3}, respectively, values which are under the lowest expected concentrations in the sample. The sample recovery from the formulation was in good agreement with its respective label claim.

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