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Design of experiments model for optimization of spectrophotometric determination of phenylephrine hydrochloride in pure and pharmaceutical formulations using *p*-Bromanil

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Abstract

A simple, fast, inexpensive and sensitive method has been proposed to screen and optimize experimental factors that effecting the determination of phenylephrine hydrochloride (PHE.HCl) in pure and pharmaceutical formulations. The method is based on the development of brown-colored charge transfer (CT) complex with *p*-Bromanil (*p*-Br) in an alkaline medium (pH=9) with 1.07 min after heating at 80 °C. 'Design of Experiments' (DOE) employing 'Central Composite Face Centered Design' (CCF) and 'Response Surface Methodology' (RSM) were applied as an improvement to traditional 'One Variable at Time' (OVAT) approach to evaluate the effects of variations in selected factors (volume of 5×10^{-3} M *p*-Br, heating time, and temperature) on the formation of the colored complex Y (absorbance) as graphical interpretation for robustness. The product was spectrophotometrically quantified at 395 nm. Beer's law is obeyed in the concentration range of 5-20 µg. mL^{-1} with detection limit of 0.4191 µg. mL^{-1}. The molar absorptivity and Sandell's sensitivity were found to be 6.07×10^{3} $L.mol^{-1}.cm^{-1}$ and 0.03356 $\mu g.cm^{-2}$ respectively and the resulting color was stable for more than 1h. Applications of the recommended method to (PHE.HCl) pharmaceutical formulations was achieved with regard to accuracy and precision.

Keywords: phenylephrine hydrochloride, *p*-Bromanil, charge transfer complexes, design of experiment, central composite design.

INTRODUCTION

Chemically, phenylephrine hydrochloride $C_9H_{14}ClNO_2$ (PHE.HCl) is (1*R*)-1-(3-Hydroxyphenyl)-2-(methylamino) ethanol hydrochloride [*61-76-7*] (Fig1) [1]. It is a selective α-adrenoceptor agonist of the phenethylamine class used locally as a decongestant for allergic conjunctivitis, sinusitis, nasopharyngitis, relieving cough and preventing or treating symptoms such as sneezing, itching of the nose, watery eyes due to colds, flu, or hay fever and throat [2-4]. Phenylephrine hydrochloride and its medicines are presented in monographs of the EP7 [5] (substance), in the USP [6] and BP [1] (substance, solution for injections, eye and nasal drops, etc.).

In any experimental procedure, several experimental variables or factors may influence the result. A screening experiment is performed in order to determine the experimental variables and interactions that have significant influence on the result, measured in one or several responses [7]. The use of 'Quality by Design' (QBD) or 'Design of Experiments' (DOE) strategy is recommended to achieve the statistical quality control monitoring, study of the factors that negatively affects the quality in pharmaceutical analysis, processes such as transfer of analytical method protocol from donor site to acceptor site, strict regulations demanded by regulatory authorities and as per recent suggestions by FDA [8].

Literature revealed some design methodologies to assess robustness of method such as; full factorial design, Asymmetrical Factorial Designs (AFD), fractional factorial designs, Central Composite Design (CCD) either as Circumscribed or Face-centered, Plackett–Burman Design (PBD), Doehlert Designs, Box–Behnken Design (BBD), Star Designs [9,10]. If the method of analysis is fast and requires testing of few factors (three or less) a good choice for robustness testing may be CCD which is widely employed because of its high efficiency with respect to the number of runs required [11].

Several methods have been reported for the determination of PHE.HCl in bulk form and performing assays of pharmaceutical dosage forms. These methods include the use of titrimetry [12,13], spectrophotometry [14-18], high performance liquid chromatography (HPLC) [19-22], flow injection analysis [23,24] and high performance thin layer chromatography (HPTLC) [25,26] methods.

This work was aimed to utilize the CCF model for screening and optimizing the more than one statistical outcome variable at a time according to the univariate optimization and preliminary experiments for developing a spectrophotometric method for estimation of PHE.HCl in bulk and pharmaceutical dosage forms using *p*-Br as π-acceptor.

EXPERIMENTAL

Equipment

A Shimadzu UV-Visible spectrophotometer 1800, Kyoto – Japan (UV probe 2.42 software) with 10 mm matched quartz cells was used for all absorbance measurements. A Professional Bench-top pH meter BP3001, Trans, Sangapor was used for pH measurements.

Materials

Pharmaceutical grade PHE.HCl drug was provided by the state company for drug industries and medical appliances SDI (Samarra-Iraq). Analytical grade chemicals (*p*-Bromanil, sodium tetraborate, potassium hydrogen phthalate, boric acid, potassium dihydrogen phosphate, ammonium chloride) were used throughout. HPLC grade acetonitrile and acetone were used in the experiments. Nasophrin nasal drops (product of SDI, Iraq) labeled to

contain 0.25% of PHE.HCl, Vibrocil nasal drops (product of Novartis, Switzerland) labeled to contain 2.5 mg of PHE.HCl per 1 mL were purchased from commercial source.

Standard and reagents

A stock solution of PHE.HCl (500 μg. mL^{-1}) was prepared by dissolving 0.05 gm in 100 mL acetonitrile and working solutions of lower concentrations were freshly prepared by serial dilution.

p-Bromanil solution (5×10^{-3} M) was daily prepared by dissolving 0.0529 gm of the compound in 25 ml of acetonitrile and more dilute solutions were prepared by suitable dilution with acetonitrile.

Alkaline tetraborate buffer solution [1] (Borax) (pH=9) was prepared by dissolving 1.006 gm of sodium tetraborate in 100 ml of distilled water. Different pH of sodium borate buffer ranged from 3 to 12 were prepared by adjustment with (~ 0.1 M) of NaOH or HCl.

An accurate volume (from the two nasal drops) equivalent to contain 5 mg of the studied drug was dissolved in acetonitrile in a 50 mL calibrated volumetric flask and this solution was further diluted stepwise to the requisite concentrations with the same solvent.

Preliminary investigations for the determination of PHE.HCl

In a calibrated 5 mL-volumetric flask, 1.0 mL of PHE.HCl solution (containing 20μg) was added successively to 1 mL of 5×10^{-3} M of *p*-Br solution with shaking, the solution was warmed at 50 °C for 5 minutes on water bath. 1.0 ml of pH 9 borax buffer solution was added to the mixture which kept in dark for 3 min. The solution is made up to final volume with distilled water and the absorption spectrum of the resulted CT product was measured against the reagent blank to determine the λ_{max} (Fig 2).

RESULTS AND DISCUSSION

p-Br has been used as chromogenic reagent for the determination of several drugs [27-31] via CT-complex formation. These methods are based on the interaction between electron donors (drugs) and *p*-Br that acts as an electron acceptor producing intensely colored CT-complexes. A probable mechanism was suggested for the formation of PHE.HCl–*p*-Br complex and is given in Fig 3. Optimization of this reaction have been made with two approaches:

One Variable at Time (OVAT) approach

In this approach series of experiments were conducted to screen the most appropriate factors affecting the formation of the colored CT product; reagent concentration, heating time, temperature, volume of buffer, pH, reaction time, type of buffer, order of addition, kind of diluting solvent and stability.

Effect of reagent concentration

To fix the optimum reagent concentration for complete color development in a total volume of 5 ml, the concentration of *p*-Bromanil was varied. The optimum amount of 5×10^{-3} M of *p*-Bromanil solution was found to be 0.7 ml (Fig 4). Furthermore 1.0 ml 35×10^{-4} M reagent was used in all absorption measurement to ensure complete color development.

Effect of time and temperature

The optimum reaction time was determined by following the color development at different temperatures. Complete color intensity was attained after heating at 60 °C (Fig 5) in a water bath for 3 min (Fig 6).

Effect of volume of buffer solution

To fix the optimum buffer volume for complete color development in a total volume of 5 ml, the volume of borate buffer was varied. The optimum amount of borate buffer was found to be 1 ml (Fig 7) that will be used in all absorption measurement to ensure complete color development.

Effect of pH and kind of the buffer solution

A detailed study of the reaction in various buffer media (phthalate buffer, borate buffer, phosphate dipotassium buffer, phosphate dihydrogen buffer, ammonium chloride buffer of different pH values), showed that borate buffer solution increased the sensitivity of the complex (Table 1). Moreover, borate buffer solution of pH 9 was necessary for complete color development and highest absorbance value (to obtain high acute and precise results) as shown in Fig 8.

Effect of time on reaction before dilution

The optimum reaction time on reaction before dilution was determined by following the color development. Complete color intensity was attained after 5 min in the dark (Fig 9).

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: phenylephrine hydrochloride- p-Bromanil- borate buffer (Fig 10).

Effect of solvent

Several solvents, i.e. acetone, acetonitrile and distilled water were investigated. Among these solvents, the most intense absorption was obtained in distilled water (Table 2)

Stability

The effect of time on formed CT product was investigated by allowing standing for varying times. The results showed that the complex remains stable at least for 60 minutes (Fig 11).

Central Composite Face Centered Design (CCF) model

Results obtained from OVAT approach illustrated that *p*-Bromanil concentration, heating time, and temperature have the greatest effect on the chromogenic reaction. To study the simultaneous variations of the independent selected factors (X1, X2, X3) on the dependent response Y (absorbance), a multivariate approach DOE with CCF was employed to obtain predictive model describing the changes in the responses within the experimental domain. A second order polynomial that describes the mathematical relationship between the response and the factors was used to predict the response for any condition within the experimental space and to define the response surface. The model is specified by:

$$\mathbf{Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_1X_3 + \beta_6X_2X_3 + \beta_7X_1^{\,2} + \beta_8X_2^{\,2} + \beta_9X_3^{\,2}}$$

Where:

Y: the response.

X's: the significant effects factors.

β's: the coefficients represent the magnitude of the effect of the different factors in the model.

Each factor had three levels which were upper, central point and lower (– 1, 0, +1) and the ranges of values used in the design are shown in Table (3). After setting the boundary conditions for each variable, A total 20 different combinations (including six replicates of center point in which each had the coded value 0) is chosen in random order according to a CCF configuration for the three factors as shown in Table (4). The absorbance of these 20 experiments was fed into the modified DOE program, a measured absorption signal was fed again to the program and the process was repeated successively until optimum conditions were obtained similarly to those obtained by the univariate method.

The final optimum CCF conditions for the spectrophotometric estimation of PHE.HCl were 0.94 mL of 5×10^{-3} M *p*-Br, heating time 1.07 min, and temperature of 80 °C as shown in Fig (12).

Table 1: Type of the buffer solutions.

Type of buffer	Absorbance
KH_2PO_4	0.2863
NH_4Cl	0.0540
K_2HPO_4	0.1498
$C_8H_5KO_4$	0
Borax	0.4041

Table 2: Effect of different of diluting solvent on the absorbance of 20µg.mL^{-1} PHE.HCl.

Type of solvent	Absorbance
D.W	0.4041
Acetone	0
Acetonitrile	0.1740

Table 3: Independent factors and levels of the CCF model.

Factor	Symbol	Coded factor levels [a]		
		-1	0	+1
Volume of 5×10^{-3} M *p*-Bromanil	X1	0.1	0.55	1.0
Heating time	X2	0	5	10
Temperature	X3	10	45	80

[a] For passage from coded level to natural variable, the following equations were used: x_1 = (Vol. *p*-Br − 0.55)/0.45; x_2 = (Heating time −5 / 5; x_3 = (Temp.− 45) / 35.

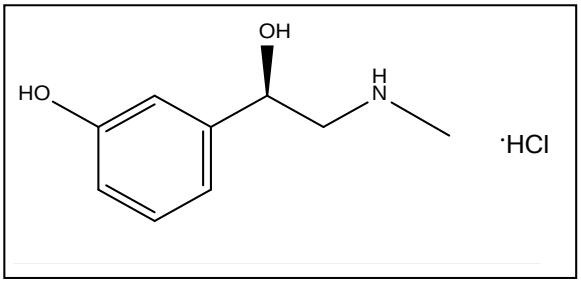

Fig 1 Chemical structure of PHE.HCl

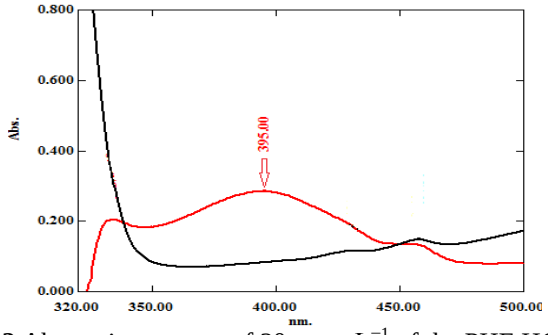

Fig 2 Absorption spectra of 20 µg. mL^{-1} of the PHE.HCL-*p*-Br complex against reagent blank which measured against acetonitrile.

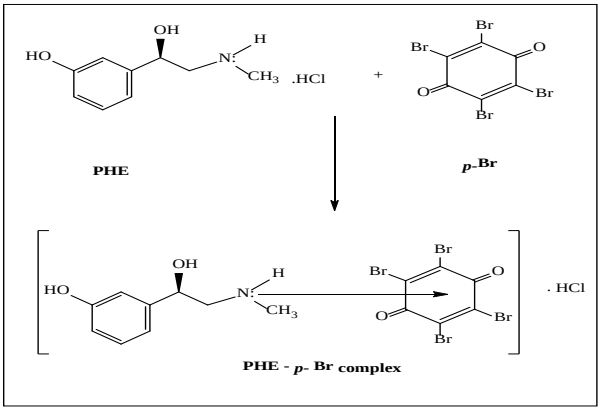

Fig 3 Steps of the main reactions between PHE.HCl and *p*-Br.

Table 4: Arrangement of the CCF (uncoded values) for the three independent factors used in the present study.

Exp. No	Factors		
	(X1) Vol. of *p*-Br (mL)	(X2) Heating time (min.)	(X3) Temperature (°C)
1	0.2	0.0	10.0
2	0.2	0.0	80.0
3	0.2	10.0	10.0
4	0.2	10.0	80.0
5	1.0	0.0	10.0
6	1.0	0.0	80.0
7	1.0	10.0	10.0
8	1.0	10.0	80.0
9	0.2	5.0	45.0
10	1.0	5.0	45.0
11	0.6	0.0	45.0
12	0.6	10.0	45.0
13	0.6	5.0	10.0
14	0.6	5.0	80.0
15	0.6	5.0	45.0
16	0.6	5.0	45.0
17	0.6	5.0	45.0
18	0.6	5.0	45.0
19	0.6	5.0	45.0
20	0.6	5.0	45.0

Table 5: Optical characteristics, statistical data of the regression equations and validation parameters of PHE.HCl by OVAT and CCF methods.

Parameter	Value	
	OVAT	CCF
Optical characteristics		
1. λ_{max} (nm) **2.** Apparent molar absorptivity ($L.mol^{-1}.cm^{-1}$) **3.** Sandell's sensitivity ($\mu g.cm^{-2}$)	395 4114.05 0.04951	395 6069.25 0.03356
Regression analysis		
1. Slope (mL. μg^{-1}) **2.** Intercept **3.** Regression coefficient (r)	0.0202 0.0315 0.9978	0.0298 -0.0616 0.9993
Validation parameters		
1. Beer's Law Limit (Linearity, µg. mL^{-1}) **2.** Limit of detection (µg. mL^{-1}) **3.** Limit of quantitation (µg. mL^{-1})	2-20 0.65346 1.98020	5-20 0.46100 1.39700

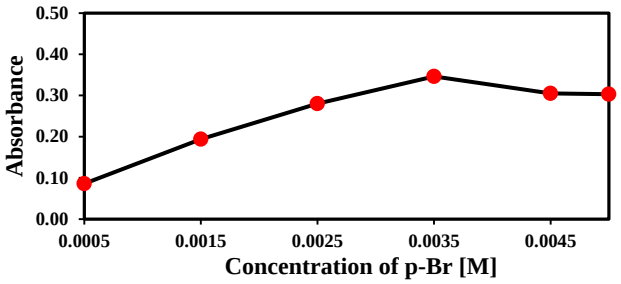

Fig 4 Effect of concentration of *p*-Br on the absorbance of 20 µg. mL^{-1} of PHE.HCl.

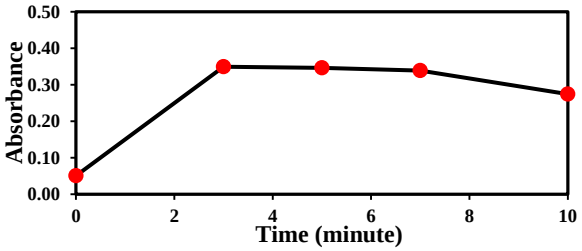

Fig 5 Effect of time reaction on the absorbance of 20µg. mL^{-1} of PHE.HCl- *p*-Br complex.

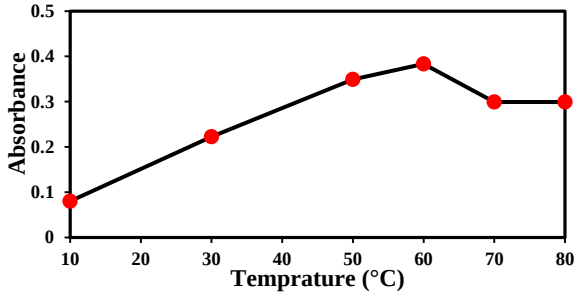

Fig 6 Effect of temperature on the absorbance of 20 µg. mL^{-1} of PHE.HCl - *p*-Br complex.

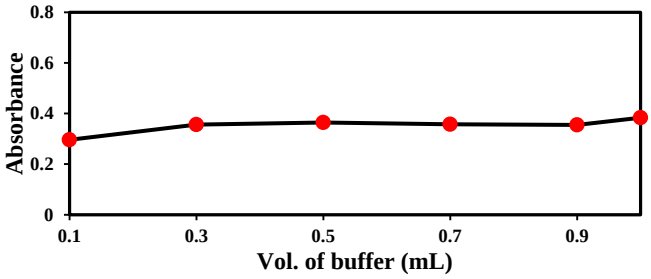

Fig 7 Effect of volume of buffer on the CT- complex formation.

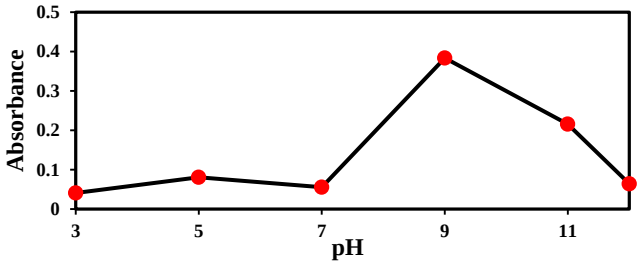

Fig 8 The influence of pH on the formation of CT-complex (PHE.HCl - *p*-Br).

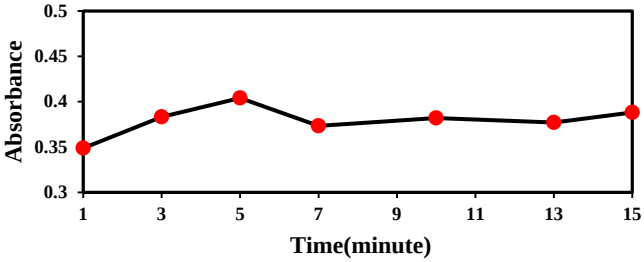

Fig 9 The influence of time on the development of the CT-complex (PHE.HCl - *p*-Br).

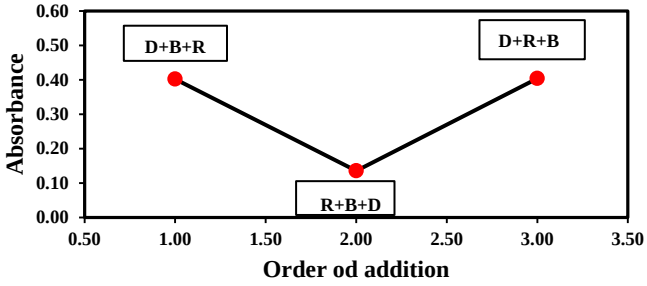

Fig 10 Effect of order of addition (***D**: Drug, **R**: Reagent, **B**: Buffer*).

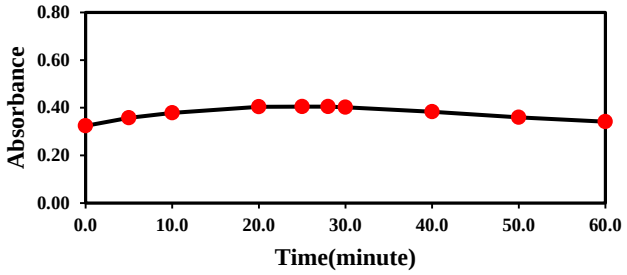

Fig 11 Effect of time on the stability of PHE.HCl- *p*-Br complex.

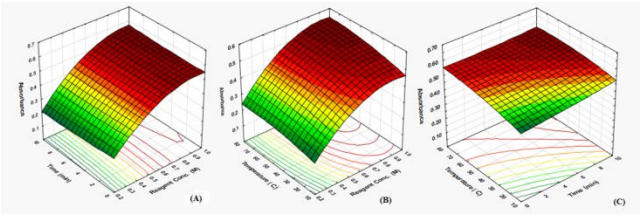

Fig 12: The response surface plots for the absorbance of PHE.HCl- *p*-Br complex as a function of any pair of the studied variable while keeping the other variable constant.

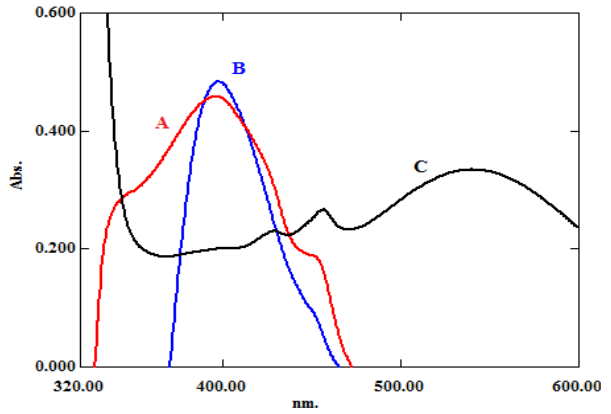

Fig 13: Absorption spectra: (A) 20 µg. mL^{-1} of PHE.HCl- *p*-Br complex against reagent blank under OVAT conditions, (B) 20 µg. mL^{-1} of PHE.HCl- *p*-Br complex under CCF conditions, (C) the reagent blank measured against distilled water.

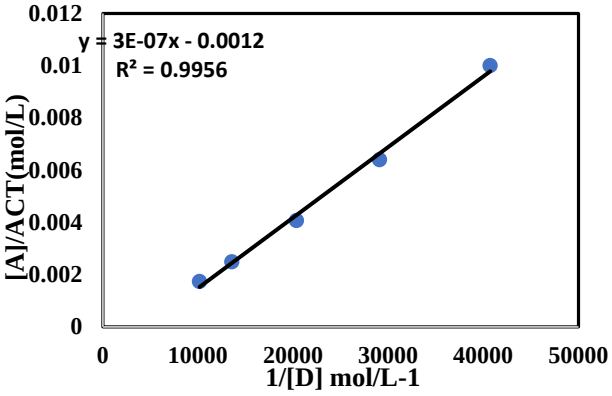

Fig 14: Benesi–Hildebrand plot for PHE.HCl –*p*-Br complex.

Table 6: Accuracy and precision of the method.

Method	Taken µg. mL^{-1}	*Found* µg. mL^{-1}*	*RE%*	*RSD%*
OVAT	5.00	5.2228	-4.4554	0.05097
	10.00	9.7277	2.7228	0.1028
	20.00	19.6266	1.8672	0.15734
CCF	7.00	7.0895	1.284	0.0355
	15.00	14.6846	-2.1029	0.0136
	20.00	20.0873	-0.4363	0.0249

Average of three measurements.

Table 7: Parameter from Benesi–Hildebrand plots for the complex.

Parameter	Observation
Intercept	-0.0012
slope	3E-07
Correlation coefficient (r)	0.997798
[a] ε^{CT} (L.mol^{-1}.cm^{-1})	833.3333
[b] K^{CT}	4000
Log K^{CT}	3.60206
[c] ΔG^o, J/mol	-24346.11
ΔG^o, KJ/mol	-24.3461

a. ε^{CT} = 1/Intercept
b. K^{CT} = Intercept/Slope
c. (ΔG^o=-2.303RT Log K^{CT})

Final Absorption Spectra

Figure (13) shows the final spectra of CT product under OVAT and CCF conditions respectively.

Validation of Beer's law (Linearity, accuracy and precision)

Applying the conditions established by OVAT and CCF methods for spectrophotometric determination of PHE.HCL, calibration range was obtained and Table (5) summarize the optical and statistical characteristics of the two optimization methods.

The precision and accuracy of the OVAT and CCF approaches were evaluated by performing three replicate analyses on pure drug solutions at three different concentration levels within the Beer's law limits. The percent error (RE%) and relative standard deviation (RSD) values presented in Table (6) reveal the high accuracy and precision of the methods.

Table 8: CCF method for determination of PHE.HCl in pharmaceutical sample.

Sample	Amount of drug (mg)	Taken (µg.ml^{-1})	Found* (µg.ml^{-1})	RE%	RSD%
Nasophrin (PHE.HCl 0.25%) S.D.I/ Iraq	2.5	5	5.2769	5.5383	0.0315
		10	10.2232	-2.2315	0.2614
		20	19.6939	-1.5304	0.0882
Vibrocil (PHE.HCl 2.5mg/mL) Novartis/ Switzerland	2.5	5	4.7667	6.666	0.2743
		10	9.6999	-3.0013	0.0366
		15	16.0493	-6.9953	0.0567

Average of three measurements.

Association constant (Benesi–Hildebrand equation)

The association constants of CLNZ- *p*-Br complex has been calculated via Benesi-Hildebrand Equation [32]. On plotting the values of $[A^o]/A^{CT}$ versus $1/[D^o]$, straight line was obtained (Fig 14) and the result is presented in Table (7). The negative value of ΔG^o refers to the spontaneously of the reaction.

Application of the method to pharmaceutical preparation

CCF method was applied successfully to determine PHE.HCl in the commercial dosage form as nasal drops (2.5 mg) and the obtained results are given in Table (8). The obtained values of recovery percentage revealed the reliability of the recommended method for the determination of the cited drug in their pharmaceutical formulations.

CONCLUSION

A simple and inexpensive spectrophotometric method was developed and validated successfully for the analysis of and sensitive A charge-transfer complexation between phenylephrine hydrochloride with *p*-Br reagent occurred with a 1:1 stoichiometry and maximum wavelength of absorption at 395 nm. The proposed method is beneficial over univariate method due to its sensitivity, accuracy, low relative standard deviation and high percentage of recovery and therefore it can be used in rapid quantitative determination of phenylephrine hydrochloride in both pure and dosage form.

REFERENCES

1) "British Pharmacopoeia" Her Majesty's Stationary Office, London 2013: 1223.
2) A.C. Moffat, J. V. Jackson and MSD. Moss, "Clarke's isolation and identification of drugs", The pharmaceutical press, London, (1986) 893.
3) A. Goodman, T. Rall, A. Nier, and P. Taylor. "The Pharmacology Bases of Therapeutics", McGraw-Hill, New York. 1996.
4) A. Goth, "Medical Pharmacology Principle and Concepts",10th ed., The Mosby C.V. Company, 1981.
5) European Pharmacopoeia, 6th Ed. and suppl. – Strasbourg: Council of Europe – 1 Electron. Optic. Disc (CD-ROM) (2007).
6) United States Pharmacopoeia, 1 Electron. Optic. Disc (CD-ROM) (2007).
7) T. Lundstedt, E. Seifert, L. Abramo, B. Thelin, A. Nystrom, J. Pettersen, R. Bergman, "Experimental design and optimization", Chemometrics and Intelligent Laboratory Systems, (1998) 42, 3–40.
8) P.K. Walter, "Top 10 changes in FDA's process validation guidance". 2011. BioProcess. Int. 9, 72.
9) P. K. Sahu, R. N. Rao, T. Cecchi, S. Swain, C. S. Patro, J. Panda, "An Overview of Experimental Designs in HPLC Method Development and Validation", Journal of Pharmaceutical and Biomedical Analysis, (2018) 147, 590-611
10) J. Goupy, "What kind of experimental design for finding and checking robustness of analytical methods?", Anal. Chim. Acta, (2005) 544,184–190.
11) S. B. Ganorkar, D. M. Dhumal, A. A. Shirkhedkar, "Development and validation of simple RP-HPLC-PDA analytical protocol for zileuton assisted with Design of Experiments for robustness determination", Arabian Journal of Chemistry (2017) 10, 273–282
12) Indian Pharmacopoeia, Vol III, Government of India, Ministry of Health and Family Welfare, Published by the Controller of Publications: Delhi, 2010; 1899-1900.
13) United States Pharmacopoeia, 32th edition, The United States Pharmacopoeial convention Inc: Rockville, 2009; 3283-3286.
14) HM. Elfatatry, MM. Mabrouk, SF. Hammad, FR. Mansour, AH. Kamal, S. Alahmad,"Development and Validation of Chemometric-Assisted Spectrophotometric Methods for Simultaneous Determination of Phenylephrine Hydrochloride and Ketorolac Tromethamine in Binary Combinations", J AOAC Int. (2016) ; 99(5):1247-51.
15) NM. Mostafa , GM. Elsayed , NY. Hassan , DA. El Mously, "Development and Validation of Eco-Friendly Liquid Chromatographic and Spectrophotometric Methods for Simultaneous Determination of Coformulated Drugs: Phenylephrine Hydrochloride and Prednisolone Acetate", J AOAC Int. (2017) ;100(6):1761-1770.
16) S.Wankhede, K.Lad and S. Chitlange, "Development and validation of UV spectrophotometric methods for simultaneous estimation of Cetirizine Hydrochloride and Phenylephrine Hydrochloride in tablet dosage form", Int. J. Pharm. Sci. Drug Res., (2012); 4(3): 222-226.
17) L. Soni, T. Narsinghani and C. Saxena. "UV-spectrophotometric estimation of Ebastine and Phenylephrine Hydrochloride in tablet formulation using absorbance ratio method", Der Pharm. Sinica, 2011; 2 (6):11-16.
18) TS. Belal , DS. El-Kafrawy , MS. Mahrous , MM. Abdel-Khalek , AH. Abo-Gharam, "Validated spectrophotometric and chromatographic methods for simultaneous determination of ketorolac tromethamine and phenylephrine hydrochloride", Ann Pharm Fr. (2016);74(4):267-82.
19) British Pharmacopoeia, Vol II, The Department of Health, Social Services and Public Safety: London, 2010; 1666-1668.
20) MR. Rezk , AS. Fayed , HM. Marzouk , SS. Abbas, "Chromatographic Determination of Cyclopentolate Hydrochloride and Phenylephrine Hydrochloride in the Presence of Their Potential Degradation Products", J AOAC Int. (2017);100(2):434-444.
21) P. Kotaiah, S. Kamarapu, "Method development and validation of RP-HPLC method for simultaneous estimation of Dextromethorphan Hydrobromide, Phenylephrine Hydrochloride and Triprolidine Hydrochloride in bulk and combined tablets dosage forms", Int. J. Pharm. Biosci., 2013; 3(3): 172-179.
22) T. Tonde, H. Ramesh, S. Bhandarkar, T. S. Bobade and S. M. Hiradeve, "Simultaneous estimation of Cetrizine hydrochloride and Phenylephrine hydrochloride in tablet dosage form by RP-HPLC", International Journal of Biomedical and Advance Research, 2017; 8(12): 465-472.
23) Y. Fustermestre, L. Zamora, "Determination of Phenylephrine Hydrochloride by flow injection analysis with chemiluminescence detection", J. Assoc. Offi. Agri. Chem. Int., 2001; 84(1): 13-18.
24) R. Rocha, C. Galhardo, M. Auxiliadora and J. Masini, "Spectrophotometric determination of Phenylephrine Hydrochloride in pharmaceuticals by flow injection analysis exploiting the reaction with Potassium Ferricyanide and 4-amino antipyrine", J. Assoc. Offi. Agri. Chem. Int., 2000; 85: 875-878.
25) FA. El Yazbi , EM. Hassan , EF. Khamis , MA. Ragab , MM. Hamdy, "Development and Validation of a High-Performance Thin-Layer Chromatographic Method for the Simultaneous Determination of Two Binary Mixtures

Containing Ketorolac Tromethamine with Phenylephrine Hydrochloride and with Febuxostat", J Chromatogr Sci. 2016; 54(5): 819-28.

26) G.Joshi, "Development and validation of HPTLC method for an anticold formulation", J. Anal. Bioanal. Tech., 2012; 3(7): 12-16.
27) S. A. Alkahtania, A. M. Mahmoudb, and S. S. Abu Al-Rubb, "Novel analytical study for the charge-transfer reactions of omeprazole with 2,3-dichloro-naphthoquinone and 2,3,5,6-tetrabromo- 1,4-benzoquinone: application for the development of microwell assay of omeprazole" Journal of advances in chemistry, (2018); 15 (1), 6099-6115.
28) N. Z. Alzoman, M. A. Sultan, H. M. Maher, M. M. Alshehri, T. A. Wani and I. A. Darwish, "Analytical Study for the Charge-Transfer Complexes of Rosuvastatin Calcium with π-Acceptors", Molecules 2013; 18, 7711-7725.
29) UM. Rabie, MH. Abou-El-Wafa, H. Nassar, "Interaction of thiazolidine-2-thione with 2,3,5,6-tetrabromo-1,4-benzoquinone: a set of sequential interactions involving redox and substitution reactions after an initial charge transfer complexation", Spectrochim Acta A Mol Biomol Spectrosc., 2012; 86: 252-255.
30) J. Anichina, Y. Zhao, SE. Hrudey, A. Schreiber, XF. Li, "Electrospray ionization tandem mass spectrometry analysis of the reactivity of structurally related bromo-methyl-benzoquinones toward oligonucleotides", Anal Chem. 2011; 83(21): 8145-8151.
31) BC. Ghosh, N. Deb, AK. Mukherjee, "Determination of individual proton affinities of ofloxacin from its UV-Vis absorption, fluorescence and charge-transfer spectra: effect of inclusion in beta-cyclodextrin on the proton affinities", J Phys Chem B. 2010;114(30): 9862-9871.
32) H. A. Benesi and J. H. Hildebrand, "A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons", Journal of the American Chemical Society 71.8 (1949): 2703-2707.