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Research Article

β-CORRECTION AND EXTRACTION TO OVERCOME SPECTRAL OVERLAP IN SPECTROPHOTOMETRIC DETERMINATION OF LEVOCETIRIZINE DIHYDROCHLORIDE

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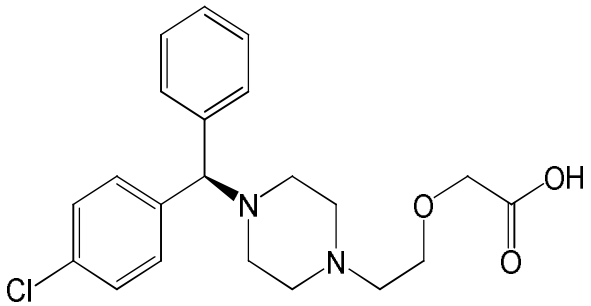
ABSTRACT

This study illustrates two methods used to overcome one of the most common problems in spectrophotometric methods of analysis which is spectral overlap resulting from the background interference. The antihistaminic drug, levocetirizine dihydrochloride, was determined spectrophotometrically using four sulphonephthaleine acid dyes, namely bromophenol blue (BPB), bromocresol green (BCG), bromothymol blue (BTB) and xylenol orange (XO). Solvent-solvent extraction was used to eliminate background interference in case of BCG and BTB, while in case of BPB the β-correction method was applied. Beer's law is obeyed in the concentration ranges 0-38.88, 4.62-41.56, 0-46.66 and 0-46.66 µg/mL for BPB, BCG, BTB and XO, respectively. Good precision is indicated from the low R.S.D.% values (0.1-1.0%). High recovery values (96.7-107.5%) were obtained. Statistical comparison of the results with the reference method using t- and F- tests shows an excellent agreement and indicates no significant difference in accuracy and precision. The methods were validated and compared with the previous spectrophotometric methods.

Keywords: β-Correction, Levocetirizine, Sulphonephthaleine, Ion-pair, Spectrophotometry.

INTRODUCTION

Levocetirizine dihydrochloride (LVC.2HCl), known chemically as 2-(2-{4-[(R)-(4-chlorophenyl) (phenyl)-methyl] piperazin-1-yl} ethoxy) acetic acid, is a third-generation antihistamine, developed from the second-generation antihistamine cetirizine. The empirical formula of LVC.2HCl is $C_{21}H_{25}ClN_2O_3.2HCl$, molecular weight of 461.8 g/mol and the chemical structure is shown in (**scheme 1**).[1]



Scheme. 1: Chemical Structure of Levocetirizine

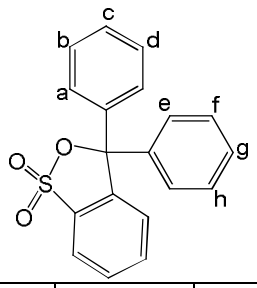
The antihistaminic activity of LVC.2HCl has been documented in a variety of animal and human models.[1-4] It is described as a long acting non-sedating antihistamine with some mast-cell stabilizing activity. It is used for the symptomatic relief of allergic conditions including allergic rhinitis and chronic urticaria.[5]

The BPB, BCG, BTB and XO acid dyes are used as pH indicators. These acid dyes dissociate in the aqueous solution liberating a proton which results in the formation of a quinoid group rather than the

lactoid ring and leaving a negative charge on the rest of the molecule. The presence of this negative charge allows the formation of ion-pair complexes with the organic compounds which contain an amino group. These reactions are used for the spectrophotometric determination of many organic compounds especially pharmaceutical compounds. The chemical structures of BPB, BCG, BTB and XO acid dyes are illustrated in (**scheme 2**). An example for the reaction between the cited acid dyes and the amino group containing organic compounds is illustrated in (**scheme 3**).

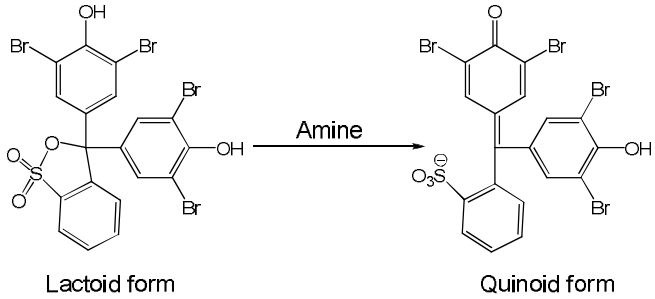
Few methods in the literature have been used for the determination of LVC.2HCl including high performance liquid chromatography (HPLC)[6-14], direct UV-determination[10], ratio derivative spectroscopy[15,16], glassy carbon electrode modified with multi-walled carbon nanotubes[17], spectrophotometry[10,16,18,19], titrimetry through the determination of the chloride of its hydrochloride[18] and voltammetry.[20]

The present study aims to propose validated simple, rapid and sensitive methods for the determination of LVC.2HCl in pure form and in pharmaceutical formulation that can be used for the quality control and assurance of this drug in industry. The methods are based on the formation of ion-pair or ion-associate complexes between the cited drug and BPB, BCG, BTB or XO. The complex of LVC.2HCl with BPB was determined in aqueous solution using the β-correction method. The ion-pair complex of XO was determined in aqueous solution by direct calibration. The ion-pair complexes of BCG and BTB were determined after extraction in chloroform. These methods are very simple in applications and less expensive in comparison to the other techniques.



	BPB	BCG	BTB	XO
A	H	CH_3	CH_3	H
B	Br	Br	Br	$CH_2N(CH_2)_2(COOH)_2$
C	OH	OH	OH	OH
D	Br	Br	$(CH_3)_2CH$-	CH_3
E	H	CH_3	CH_3	H
F	Br	Br	$(CH_3)_2CH$-	$CH_2N(CH_2)_2(COOH)_2$
G	OH	OH	OH	OH
H	Br	Br	Br	CH_3

Scheme. 2: Chemical structure of BPB, BCG, BTB and XO



Scheme. 3: The reaction between an amine and BPB

MATERIALS AND METHODS

MATERIALS

All chemicals were of analytical reagent grade purity and bidistilled water was used throughout the study. Standard LVC.2HCl was obtained from Borg Pharmaceutical Industries, Borg El Arab, Alexandria, Egypt. The acid dye reagents BPB, BCG, BTB and XO were obtained from Loba Chemie (Mumbai, India), Merck (Germany), Chemapol (Czechsolvakia) and BDH Limited Poole (England),

respectively. The commercial tablets, Allear (5 mg/tablet), were obtained from Western Pharmaceutical Industries, Cairo, Egypt.
Solutions: 1×10^{-3} mol/L standard stock solution of LVC.2HCl was prepared by dissolving an accurately weighed 0.115 g of pure drug in bidistilled water. The solution was transferred into a 250 mL measuring flask and made up to the mark by bidistilled water.
1×10^{-3} mol/L stock solutions of BPB, BCG and BTB were prepared by dissolving accurately weighed 0.167, 0.175 and 1.56 g, respectively, in the least amount (10-15 mL) of ethanol then it was completed to the mark of a 250 mL volumetric flask by bidistilled water. 1×10^{-3} mol/L XO was prepared by dissolving an accurately weighed 0.168 g in 250 mL bidistilled water. Dilute solutions were prepared by accurate dilution.

Equipments
All the spectral measurements were carried out using a Jenway 6105 UV/Vis single beam spectrophotometer equipped with 1 cm glass or quartz cell. A Scientech SA 210 digital balance was used for weighing throughout the study.

Procedures
Calibration curve method: BPB procedure
Into a series of 25 mL calibrated flasks, different volumes (0.1-1.4 mL) of 1×10^{-3} mol/L LVC.2HCl solution and 3 mL of 1×10^{-3} mol/L BPB solution were added. The solutions were completed to the mark by bidistilled water. A blank solution of BPB was prepared simultaneously without the addition of the drug. The absorbance of the blank and the complex was measured at λ_1=435 and λ_2=590 nm against bidistilled water. The following equations[21] were used to calculate the corrected absorbance values from the obtained data:

$$\alpha = \frac{A'_\alpha}{A_\alpha} = \frac{\varepsilon^{\lambda_1}_{ML}}{\varepsilon^{\lambda_2}_{ML}} \quad (1)$$

$$\beta = \frac{A_o}{A'_o} = \frac{\varepsilon^{\lambda_2}_{L}}{\varepsilon^{\lambda_1}_{L}} \quad (2)$$

$$A_c = \frac{(\Delta A' - \beta \Delta A)}{(1-\alpha\beta)} \quad (3)$$

Where A'_o and A_o are the absorbance of the blank solution (L) at λ_1=435 nm and λ_2=590 nm, respectively. $A`_\alpha$ and $A_\alpha$ are the absorbance of the complex (ML) at the same wavelengths $\lambda_1$ and $\lambda_2$, respectively. $A_c$ is the absorbance of the complex after correction. $\Delta A`$ and ΔA are the absorbance of the complex against the blank solution at λ_1 and λ_2, respectively.

BCG procedure
Varying volumes of 5×10^{-4} mol/L LVC.2HCl solution (0.2-1.4 mL) were added to a series of 60 mL separating funnels. 2 mL of 5×10^{-4} mol/L BCG solution were added to each funnel and mixed well. The formed yellow colored complexes were extracted into 5 mL chloroform by shaking for 2 minutes then the organic layer was separated into a 10 mL volumetric flask and completed to the mark by chloroform. The absorbance of the extract was measured at 425 nm against a blank solution prepared simultaneously by the same way without the addition of LVC.2HCl. The reading of the device was taken after 5 minutes to allow the instrument reading to stabilize. The corresponding blank solutions have no absorbance at the wavelength of measurement.

BTB procedure
Into a series of 60 mL separating funnels, different aliquots of 1×10^{-3} mol/L LVC.2HCl solution (0.2-1.2 mL) were added. Then after, 2 mL of 1×10^{-3} mol/L BTB solution were added to each separating funnel and the resulting yellow colored complex was extracted into 5 mL chloroform. The organic layer was separated and completed to 10 mL by chloroform in a 10 mL calibrated flask. The absorbance was measured at 413 nm against a blank solution prepared simultaneously by extracting 2 mL of 1×10^{-3} mol/L BTB without the addition of the drug solution.

XO procedure
Into a series of 10 mL measuring flasks, different volumes (0.2-1.4) of 5×10^{-4} mol/L LVC.2HCl solution and 2 mL of 5×10^{-4} mol/L XO solution were added. The volume of the solutions was completed to the mark by bidistilled water. The absorbance of a blank solution prepared simultaneously from XO without the addition of LVC.2HCl was measured at 590 nm against the formed ion-pair complexes.

The decrease in the blank color was measured at 590 nm because the blank reading is higher than that of the formed complex at this wavelength.

Procedure for tablets

Ten tablets of the drug Allear (5 mg LVC/tablet), Western Pharmaceutical Industries, Cairo, Egypt, were accurately weighed and the average tablet weight was calculated. An accurately weighed amount of the intimately ground tablets powder equivalent to 5 mg LVC.2HCl was dissolved in 10-15 mL bidistilled water. The resulting solution was filtered and the remained excipients were washed several times by bidistilled water to make sure that the entire active ingredient in the powder was dissolved. This step is very hard and requires a lot of time because the LVC.2HCl is adsorbed on the starch surface; so the use of ultrasonic water bath for 30 minutes is preferred in this step. The filtrate was transferred quantitatively into a 100 mL calibrated flask and completed to the mark by bidistilled water. Different volumes of this solution were taken and treated by the same procedures described above.

The association constant (K_c)

The association constants (K_c) of the formed complexes were estimated using Benesi-Hildebrand method[22] which depends on the experimental condition that one of the two component species should be present in a large excess, so that its concentration is virtually unaltered on formation of the complex. Different volumes of BPB (1×10^{-3} mol/L), BCG (1×10^{-4} mol/L), BTB (1×10^{-4} mol/L) or XO (1×10^{-4} mol/L) were transferred to 60 mL separating funnels. 1 mL of 5×10^{-2} mol/L, 1 mL of 1×10^{-3} mol/L, 1 mL of 1×10^{-3} mol/L or 2 mL of 1×10^{-3} mol/L LVC.2HCl solution were added to BPB, BCG and BTB or XO solutions, respectively. In case of BCG and BTB the contents were mixed gently and the complexes formed were extracted into 5 mL chloroform then the organic layer was separated and completed to 10 mL by chloroform. In case of BPB and XO, the solutions were completed to 10 mL by bidistilled water. Relations between $1/[\text{reagent}]_o$ on the x-axis and $[\text{LVC}]_o/[\text{A}]$ on the y-axis were plotted.

RESULTS AND DISCUSSION

LVC.2HCl reacts with the cited dyes to produce intense and stable yellow colored complexes. Chemically, the structure of LVC.2HCl possesses two tertiary aliphatic nitrogen atoms in the biperazine ring which features its basic nature. This structure suggests the possibility of utilizing acidic dyes as chromogenic reagents. The formation of intense yellow colored product with an absorption maximum at 590, 425, 413 and 590 nm for BPB, BCG, BTB and XO, respectively, (Fig. 1), is due to an opening of the lactoid ring and subsequent formation of a quinoid group which reacts with the drug forming ion-pair or ion-associate.

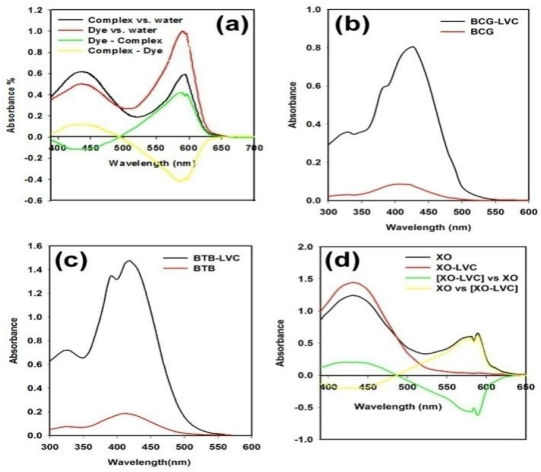


Fig. 1: Visible absorption spectra of LVC.2HCl complexes with (a) BPB, (b) BCG, (c) BTB and (d) XO

Optimization of variables and method development

Effect of reagent concentration

The influence of the concentration of BPB, BCG, BTB and XO on the intensity of the developed color was studied as shown in (Fig. 2) The obtained results indicate that 3 mL of BPB and 2 mL of BCG,

BTB or XO (1×10^{-3} mol/L) were sufficient to produce maximum and reproducible color. The presence of excess reagent does not cause any increase in the absorbance values.

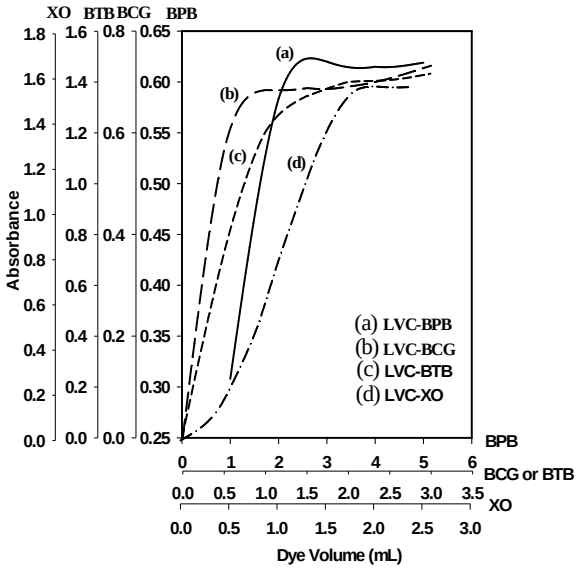


Fig. 2: Effect of reagent volume on the formation and stability of the formed complexes

Reaction time

The effect of time on the formation of the yellow colored complexes was studied at ambient temperature (25±2 ºC) and it was found that a 5 minutes standing time was sufficient for maximum color development for BPB, BCG, BTB and XO. The formed colors were stable up to 50 minutes in all cases. These results indicate the stability of the complexes and their spontaneous formation which allow their determination easily over a wide range of time. (Fig. 3) shows the effect of the reaction time on the stability of the formed complexes.

Stoichiometry of the complexes

The reaction stoichiometry in case of BCG, BTB and XO was found to be 1:1 (LVC:Dye) as shown from Job's method of continuous variation[23], (Fig. 4), and the molar ratio method[24], (Fig. 5), while in case of BPB it was found to be 1:1 and 1:2 (LVC:Dye). The Benesi-Hildebrand method, (Fig. 6), was used to determine the association constants (K_c), (Table 1), of the complexes.

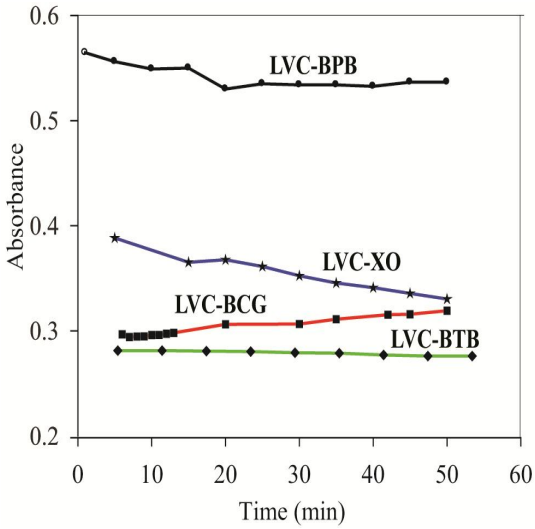


Fig. 3: Effect of time on the stability of the complexes

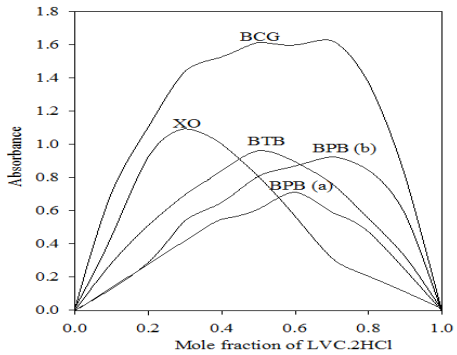


Fig. 4: Job's method of continuous variation for the determination of reaction stoichiometry (a) [BPB]=1×10^{-3} mol/L, [LVC]=1×10^{-3} mol/L, Total volume=1 mL, (b) [BPB]=5×10^{-4} mol/L, [LVC]=5×10^{-4} mol/L, Total volume=3 mL, [BCG]=[BTB]=1×10^{-3} mol/L, [LVC]=1×10^{-3} mol/L, Total volume=2 mL, [XO]=1×10^{-3} mol/L, [LVC]=1×10^{-3} mol/L, Total volume=1 mL.

The formation constants were calculated from Job's method of continuous variation and the *log* K_f values were found to be 4.18, 4.19, 3.67 and 3.75 for BPB, BCG, BTB and XO methods, respectively. The values of K_f and K_c, (Table 1), indicate the stability of the formed complexes. Eq. (4) was used to calculate the formation constants from the data of Job's method of continuous variation[24]:

$K_f=[A/A_m]/[1-A/A_m]^{n+1}\ C_M\ n^2$ (4)

Table 1: Analytical parameters for the determination of LVC.2HCl with BPB, BCG, BTB and XO

Parameters	Reagents			
	BPB	BCG	BTB	XO
λ_{max}, (nm)	435, 590	425	413	590
Beer's law range, (µg/mL)	7.78-38.88	4.62-41.56	19.44-46.66	7.78-46.66
Ringbom range (µg/mL)	7.78-38.88	4.62-32.33	7.78-38.88	7.78-23.33
Molar absorpitivity, (L/mol cm)	7.30×10^{3}	16.99×10^{3}	11.68×10^{3}	17.32×10^{3}
Sandell sensitivity, (µg/cm)	62.9×10^{-3}	27.2×10^{-3}	39.5×10^{-3}	26.7×10^{-3}
LOD, (µg/mL)	2.08	0.49	1.07	1.28
LOQ, (µg/mL)	6.92	1.63	3.56	4.27
Slope (a), (mL/µg cm)	15.9×10^{-3}	36.8×10^{-3}	25.3×10^{-3}	37.5×10^{-3}
Intercept (b)	0.001	0.080	0.05	0.023
Correlation coefficient (r^2)	0.990	0.985	0.995	0.996
R.S.D.%	1.1	0.6	0.9	1.6
K_c^{AD}, (L/mol)×10^3	1.3	26.8	5.2	1.3
ΔG^o, (kcal/mol)	-5.700	-5.714	-5.005	-5.114
K_f, (L/mol)	15.14×10^{3}	15.49×10^{3}	4.68×10^{3}	5.62×10^{3}

Kc^{AD} is the association constant calculated from Benesi-Hildebrand method, ΔG^o is the free energy change and K*f* is the formation constant calculated from Job's method of continuous variation

Where A and A_m are the observed maximum absorbance and the absorbance values when the entire drug present is associated, respectively. C_M is the molar concentration of the drug at maximum absorbance and n is the stoichiometry.

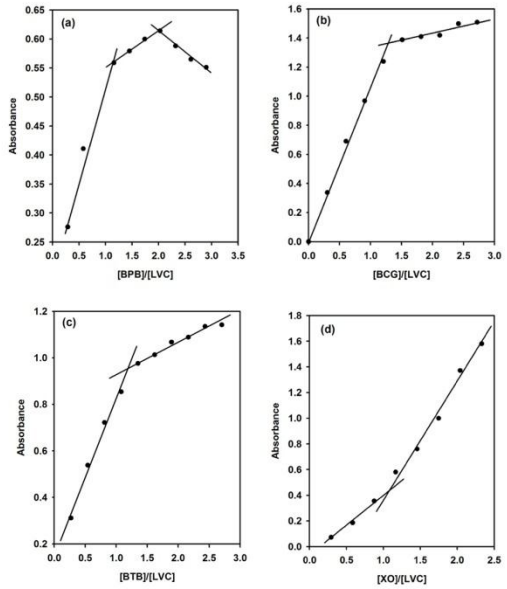


Fig. 5: Molar ratio method for the determination of reaction stoichiometry between the cited drug and (a) BPB, (b) BCG, (c) BTB and (d) XO

The association constant and the free energy change

The association constants of 1:1 complexes were determined using Benesi–Hildebrand method applying Eq. (5):

$$\frac{[A_o]}{A_\lambda^{AD}} = \frac{1}{\varepsilon_\lambda^{AD}} + \frac{1}{K_c \varepsilon_\lambda^{AD}} \frac{1}{[D_o]} \qquad (5)$$

Where $[A_o]$ and $[D_o]$ are the total concentrations of LVC.2HCl and BPB, BCG, BTB or XO, respectively. A_λ^{AD} and ε_λ^{AD} are the absorbance and the molar absorpitivity of the complex at 435 and 590, 425, 413 and 590 nm for BPB, BCG, BTB and XO, respectively. K_c is the association constant of the complexes. A straight line was obtained, (Fig. 6). However, it should be noted that ε^{AD} which is the molar absorpitivity of the complex itself should not be confused with any stoichiometric values calculated with reference to the amount of any analyte being determined. The latter is best described as Beer's value while the former is Benesi–Hildebrand's value. The association constants K_c and the molar absorbitivities ε^{AD} are listed in (Table 1).

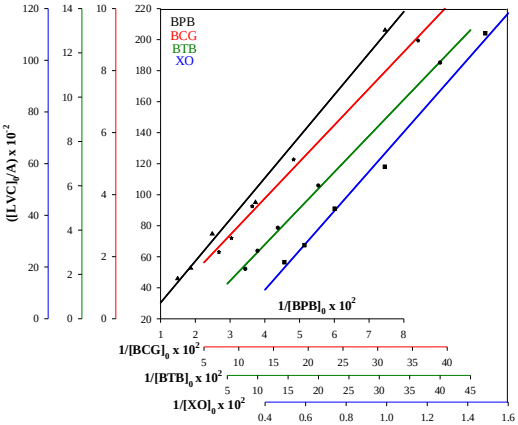


Fig. 6: Benesi-Hildebrand method for the determination of the association constants of the formed complexes

The standard free energy change of the reaction, ΔG^o, is related to the association or the formation constant by:

$$\Delta G^o = -2.303\ RT \log K \qquad (6)$$

Where ΔG^{o} is the free energy change of the complex, R is the gas constant 1.987 cal/mol.degree, T is the temperature in Kelvin and K is the association or formation constant of the drug-reagent complex (L/mol). The negative values of the calculated free energy change (ranging from -5.714 to -5.005 Kcal/mol) indicate the stability of these complexes and their spontaneous formation as indicated in (Table 1).

Method validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are accuracy, precision, detection limit, quantitation limit, linearity and range.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed “trueness”. For more accurate results in photometric analysis, it is very important to select a suitable concentration range and to evaluate the analysis of accuracy than to prove conformity to Beer’s law. In order to select a suitable concentration range and evaluate the accuracy in photometric analysis, Ringbom plots for optimum concentration ranges were obtained by plotting the photometric data of percent transmittance (%T) as ordinates against the normal logarithm of concentration as abscissas. The optimum Ringbom ranges have been investigated and found to be 7.78-38.88, 4.62-32.33, 7.78-38.88 and 7.78-23.33 µg/mL for BPB, BCG, BTB and XO methods, respectively.

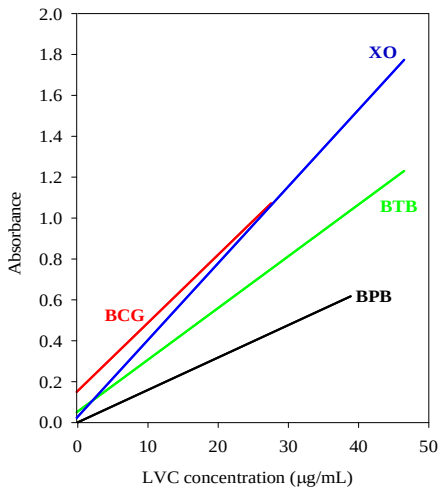


Fig. 7: Calibration Curves for the determination of LVC using BPB, BCG, BTB and XO

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The proposed methods are proved to be precise as indicated from the low values of %R.S.D. as shown in (Table 2). The %R.S.D. values for the proposed methods are 1.1, 0.6, 0.9 and 1.6 for BPB, BCG, BTB and XO methods, respectively.

Table 2: A comparison between the proposed methods and the previous spectrophotometric methods for the determination of LVC.2HCl

Method	Solvent	Λmax (nm)	Concentration range (µg /mL)	ε (L/ mol cm)	References
-Derivative spectrophotometry	Water	239 (^{1}D) 233-243 (^{2}D)	1.2-10.0 (^{1}D) 0.8-10.0 (^{2}D)	-----	10
-Chloranil	DMF	556	120-250	1.55×10^3	10
-Ratio derivative spectroscopy	Methanol	238.4	2-6	4.16×10^5	15
-Derivative ratio spectrum method	0.1 N HCl	225.4	8.5-25.5	2.73×10^3	16
-Hg (II)-Diphenylcarbazone complex	Water	540	0-40	5.87×10^3	18
-UV spectrophotometry	Water	231	8-24	1.44×10^4	19
-Bromocresol purple	Chloroform	407	5-15	2.85×10^4	26
-Eosin	Methanol	539	8-24	1.72×10^4	26
-Base catalyzed condensation of mixed anhydrides of organic acids (Citric/Acetic)	Methanol	543	3-7	8.48×10^4	26
-Bromocresol purple	Chloroform	409	1-16	1.89×10^4	27
-Bromophenol blue	Chloroform	414	1.5-21	1.21×10^4	27
Proposed methods					
-β-correction with BPB	Water	435, 590	7.78-38.88	7.30×10^3	-
-Bromocresol green (BCG)	Chloroform	425	4.62-41.56	15.37×10^3	-
-Bromothymol blue (BTB)	Chloroform	413	19.44-46.66	11.68×10^3	-
-Xylenol orange (XO)	Water	590	7.78-46.66	17.64×10^3	-

Limit of Detection (LOD) and Limit of Quantification (LOQ)

According to ICH recommendation[25] the approach based on the S.D. of the response and slope (b) of the calibration curve was used to determine the LOD and LOQ. The following equations [Eq. (7) and (8)] were used to calculate the LOD and LOQ

LOD=K×S.D./b, where K=3 (7)

LOQ=K×S.D./b, where K=10 (8)

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. From the results of the calibration curves, it is obvious that the proposed analytical methods are linear over very wide concentration ranges (0-38.88, 4.62-41.56, 0-46.66 and 0-46.66 µg/mL for BPB, BCG, BTB and XO methods, respectively). The correlation coefficients of the calibration curves are 0.990, 0.985, 0.995 and 0.996 for BPB, BCG, BTB and XO methods, respectively which indicates good linearity of the calibration curves.

Analytical parameters

A linear relation was found between the absorbance of the formed complexes and the concentration of the cited drug in the ranges given in (Table 1). These linear relations indicate the wide ranges of determination (0-38.88, 4.62-41.56, 0-46.66 and 0-46.66 µg/mL for BPB, BCG, BTB and XO, respectively.) of the cited drug. The calibration graph in each case is described by:

A=a+b C (9)

Where A=absorbance, a=intercept, b=slope and C=concentration in µg/mL. Correlation coefficient (r), intercept and slope for the calibration data are also summarized in (Table 1). Correlation coefficient values ranging from 0.985 to 0.996 indicate the direction and strength of the linear relationship. Sensitivity parameters such as molar absorpitivity (ε), Sandell's sensitivity and the limits of detection (LOD) and quantification (LOQ) are also given in the (Table 1).

Application to the analysis of pharmaceutical tablets

In order to evaluate the applicability of the proposed methods to the determination of LVC.2HCl in tablets, the obtained results from the study were compared with those of the reference method[10] by applying the student's t-test for accuracy and F-test for precision. The results show that the student's t- and F-values at 95% confidence level are less than the theoretical values, indicating that there is a good agreement between the obtained results by the proposed methods and the reference method with respect to accuracy and precision. (Table 3) summarizes the statistical treatment of the obtained results.

Table 3: Statistical treatment of results

Method	Taken (µg)	Found (µg)	%Recovery±S.D.[a]	t- value	F- value
Official method	46.18	46.27	100.2±0.01	-------	-------
BPB	18.47	18.19	98.5±0.02	2.12	0.25
BCG	46.18	45.67	98.9±0.01	2.27	1
BTB	46.18	45.86	99.3±0.01	1.14	1
XO	46.18	45.72	99.0±0.03	1.34	0.1

[a] Mean±standard deviation of three replicate analyses

Recovery and interference studies

To further ascertain the accuracy and validity of the proposed methods, recovery studies were performed. Different concentrations of the commercial tablet solution were analyzed by the proposed methods and the results showed that the recovery is good and the excipients of the tablets don't cause any significant interference except in case of XO. This interference from the tablet excipients can be eliminated by applying the standard addition method. (Table 4) shows the recovery studies of the proposed methods and (Fig. 7) illustrates the calibration graphs.

Table 4: Recovery studies for determination of LVC.2HCl in tablets, Allear (5 mg/tablet)

Reagent	Taken (µg)	Found (µg)	%Recovery±S.D.
BPB	20	20.24	101.2±0.1
	30	30.72	102.4±0.2
BCG	20	21.5	107.5±1.0
	25	25.2	100.7±0.8
BTB	18.5	17.9	96.9±0.1
	36.9	35.7	96.7±0.3
XO[a]	18.74	19.02	103±0.2

[a] The recovery studies in case of XO were carried out using the standard addition method to eliminate the matrix interference

CONCLUSION

The paper describes new methods for the determination of LVC.2HCl in pure form and in pharmaceutical formulation. The methods are based on the formation of ion-pair or ion-associate complexes between the drug and BPB, BCG, BTB and XO acid dyes. Compared with the existing UV-visible spectrophotometric methods (Table 2), the proposed methods are simple, accurate, sensitive and cost-effective and can be used in routine analysis. Moreover, the proposed methods are free from tedious experimental steps. The most attractive feature of these methods is there relative freedom from interference by the usual tablet diluents and excipients. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. Hence, the recommended methods are well suited for the routine analysis of LVC.2HCl in quality control laboratories.

A comparison between the proposed methods and the previous spectrophotometric methods which were used for the determination of LVC.2HCl is illustrated in (Table 2) which indicates that the proposed methods are selective (high values of ε) and have wider ranges of determination with respect to all of the previous spectrophotometric methods.

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