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Two step quantitation of levocetirizine dihydrochloride and phenylephrine hydrochlorideby using vierodt's method and derivative method

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Abstract
The proposed research work is described two simple, economic and fastsimultaneous quantitation of levocetirizine dihydrochloride and phenylephrine hydrochloride in solid dosage forms. Both proposed methods based on spectrophotometric analysis. First developed method is vierodt's method. It is based on solving the simultaneous equation while second method based on 1st order derivatization. First method employs formation of simple simultaneous equation using 231.0nm and 273.0nm as two analytical wavelengths for levocetirizine dihydrochloride and phenylephrine hydrochloride, respectively. Two analytical wavelengths were selected at 239.0nm & 225.5nm after derivatization of spectra for simultaneous studies of levocetirizine dihydrochloride and phenylephrine hydrochloride, respectively. The drugs obeys lambert- beer's law in the range of 2-32 µg/ml and 6-120 µg/ml& 5-90 µg/ml and 5-100 µg/ml for levocetirizine dihydrochloride and phenylephrine hydrochloride for I& II methods. Validation of the proposed methods was carried out for its accuracy, precision, specificity, ruggedness, LOD, LOQ according to ICH guidelines.

Keywords: levocetirizine dihydrochloride, phenylephrine hydrochloride, simultaneous equation method, derivative method, validation

Introduction
The levocetirizine dihydrochloride (LEVC) is chemically (R)-2-(2-(4-((4-chlorophenyl) phenyl methyl) piperazin-1-yl) ethoxy) acetic acid is a selective potent H_1- antihistamine compound indicated for the treatment of allergic rhinitis and chronic idiopathic urticaria [1, 2] and is official in Indian Pharmacopoeia 2010 and British Pharmacopoeia [3, 4].
Phenylephrine hydrochloride (PHE) is chemically (S)-1-(3-hydroxyphenyl)-2methylaminoethanol hydrochloride and is a α_1-adrenoceptor agonist which stimulates postsynaptic alpha receptor causes vasoconstriction, systolic and diastolic pressure [5]. It is indicated for nasal congestion, minor eye irritations and open angle glaucoma [6]. It is official in Indian Pharmacopoeia 2010, British Pharmacopoeia, and USP [7, 9].
Several methods have been reported for analytical determination for LEVC and PHE individually or in combination with other drugs, such as high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection or fluorescence detection and capillary electrophoresis with ultraviolet detection, Colorimetry [10, 20]. However, none of these methods were suitable for routine analysis due to some tedious steps in methodology such as solvent extraction in sample preparation [21], ultra-filtration [22], solid phase extraction and liquid–liquid extraction [23].
Literature survey reveals that there is none spectrophotometric method has been developed for simultaneous estimation of both drugs. The present developed spectrophotometric methods are having simple mathematical calculations as well as easy steps like dilutions and direct absorbance measurements from spectrophotometer. The UV spectrophotometric determination is a well-established analytical technique to determine the active pharmaceutical ingredients in their Pharmaceutical formulations. The both methods were validated according to ICH guidelines [24].

Experimental details
Instrumentation
Two UV-visible spectrophotometers, Shimadzu UV-1700 and Labindia UV 3000, with a 10 mm quartz cell were used for spectrophotometric measurements. Shimadzu – AX 200 electronic balancewas used for weighing. Freshly prepared double distilled water is used as a solvent.

Chemicals and standards
LEVC and PHE were obtained as a gift sample by Swiss Garnier Life Sciences, Mehatpur. All the chemicals and reagents used are AR grade and are purchased from Merck Pvt. Ltd., Mumbai.
Marketed formulation FC-tab (Hetero health care) was purchased from local pharmacy.

Preparation of standard solutions and calibration curve
Standard stock solutions of LEVC and PHE (1000µg/ml) were prepared separately in double distilled water. Then working standards were prepared by further appropriate dilutions of stock solution.

Method development
Overlain UV spectra of 50 µg/ml solution of LEVC and PHE in double distilled water were recorded between 400-200 nm with medium scan speed against double distilled water blank. LEVC and PHE show maximum absorbance at 231 nm and 273 nm for method I and after derivatization of spectra LEVC and PHE shows absorbance minima at 239 nm and 225.5 nm. The absorptivity coefficients were calculated at all wavelengths.

Vierodt's method
This is one of the simplest UV-Vis spectrophotometric

methods for combined dosage form. Requirement for this method is to prepare and solve simultaneous equation for multicomponent dosage forms. The better results of analysis depend upon the well difference between absorbance maxima.

The two wavelengths 231nm and 273nm were selected for the simultaneous estimation of LEVC and PHE, respectively. The absorptivity of both drugs was calculated as average of five standard dilutions on their respective wavelengths. The concentrations of both drugs were calculated by solving the simultaneous equation as follows:

$$C_x = (A_2ay_1\text{-}A_1ay_2) / (ax_2ay_1\text{-}ax_1ay_2) \quad (1)$$

$$C_y = (A_1ax_2\text{-}A_2ax_1) / (ax_2ay_1\text{-}ax_1ay_2) \quad (2)$$

Where, ax_1 and ax_2 absorptivity values of LEVC at 231 nm and 273 nm, respectively. ay_1 and ay_2absorptivity values of PHE at 231 nm and 273 nm, respectively. C_x and C_y are the concentrations of LEVC and PHE, respectively in sample solution. A_1 and A_2 are the absorbances of mixture of LEVC and PHE at selected wavelengths 231 nm and 273 nm, respectively.

Derivative method

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second, and higherorder of derivative spectrum. The first order derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λmax of the absorbance band. A derivative spectrum shows better resolution of overlapping band and it may permits the accurate determination of absorbance maximum of the individual bands [25].

The two wavelengths were selected after first order derivatization of spectrum for measure the absorbance, at absorbance minima at 239nm for LEVC and 225.5nm for PHE. The respective concentrations of both analytes were calculated by solving the simultaneous equations (1) & (2).

Preparation of sample solutions

Accurately weighed twenty tablets of FC-tab, each of which containing 5 mg LEVC, 10 mg PHE. An accurately weighed quantity of powder equivalent to 5 mg LEVC, 10 mg PHE and transferred to standard 100 ml volumetric flask. The content of analyst was extracted by 10 min sonication with 50ml double distilled water and volume was made upto mark. Aliquot portion of this solution was further diluted to achieve final concentration. The solution was filtered with a Whattman filter paper no.1 and the absorbance was measured at their respective wavelengths.

Results and Discussions

Optimization of method

The important criterion for the selection and development of analytical methods is the Chromophore. These are the chemically unsaturated covalently bonded groups, which are responsible for electronic transitions. Chemical structure of analytes shows the richness of Chromophore, which confirms the good absorptivity of analytes for UV spectrophotometric methods. A series of solvents used for the selection of common solvents, such as water, acetic acid, acetone, methanol, ethanol, chloroform, 0.1N NaOH and 0.1N HCl etc. Double distilled water was selected as a common and cheapest solvent.

Vierodt's Method

Spectra of LEVC (figure 1) show two peaks at 231nm and 259.5nm. Linearity was observed for LEVCat both wavelength maxima. Correlation coefficients (r^2) were found 0.9995 at 231nm and 0.936 at 259. 5nm. This shows analyte has good linearity for the analysis at 231nm. So that 231nm selected as suitable wavelength for estimation of LEVC.

Spectra of PHE also show two peaks at 273nm and 213.5nm (figure 1). Correlation coefficient was found to be 0.9997 and 0.9879, respectively. According to the correlation coefficient, 273nm was showing good linearity for detection of PHE. Therefore, 231 nm and 273 nm were selected as the analyte wavelength for Vierodt's method. Then the optical parameters were calculated for analytes such as molar absorptivity1.55x 10^4 L/mol.cm and 2.03x 10^4L/mol.cm and sandell's sensitivity 2.9 x 10^{-2} and 9.9 x 10^{-2} for LEVC and PHE, respectively. The concentration of LEVC and PHE was determined by using following equations:

$$C_{LEVC} = A_2 \times 0.00423 - A_1 \times 0.00974 / 0.0011 \times 0.00423 - 0.0339 \times 0.00974 \quad (3)$$

$$C_{PHE} = A_1 \times 0.0011 - A_2 \times 0.0334 / 0.0011 \times 0.00423 - 0.0339 \times 0.00974 \quad (4)$$

C_{LEVC} is the concentrations of LEVC in µg/ml; C_{PHE} is the concentrations of PHE in µg/ml; A_1 is the absorbance of sample at λ_{231}nm and A_2 is the absorbance of sample at λ_{273} nm.

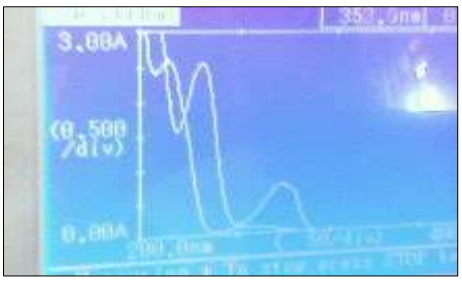

Fig 1: Overlay spectra of LEVC and PHE

Derivative method

Among the 1st,2nd, 3rd and 4th order of derivative spectra of both drugs. First order of derivative spectra shows strong peaks and valley. Therefore, first order of derivative spectra is selected for the study(fig.2). It shows absorption maximum at 224nm and absorption minimum at 239nm for LEVC. The correlation coefficient was found to be 0.874 and 0.9983 at 224nm and 239nm, respectively. Therefore, 239nm shows good linearity and selected as a wavelength for estimation of LEVC.

Figure (3) shows derivative spectra of PHE. A derivative spectrum shows a strong absorption maxima and absorption minima at 264.5nm and 225.5nm, respectively. Molar absorptivity of PHE was too low at 264.5nm, while excellent at 225.5nm. Also, the correlation coefficient was shows good linearity at 225.5nm. Therefore, 239 nm and 225.5 nm were selected as a study wavelength for derivative spectroscopic method. Then the optical parameters were calculated for both analytes such as Molar 3.50 x 10^4 L/mol.cm and 7.07 x 10^4L/mol.cm and Sandell's sensitivity 5.8 x 10^{-2} and 6.5 x 10^{-2} for LEVC and PHE, respectively. The concentration of LEVC and PHE was determined by using following equations:

C_{LEVC}= A_2 × 0.00127 – A_1 × 0.01752 / 0.03025 × 0.00127 – 0.01593 × 0.01572 (5)

C_{PHE}= A_1× 0.03025 – A_2 × 0.01593 / 0.03025 × 0.00127 – 0.01593 × 0.01572 (6)

Where C_{LEVC} is the concentrations of LEVC in µg/ml; C_{PHE} is the concentrations of PHE in µg/ml; A_1 is the absorbance of sample at λ_{239}nm and A_2 is the absorbance of sample at $\lambda_{225.5}$ nm.

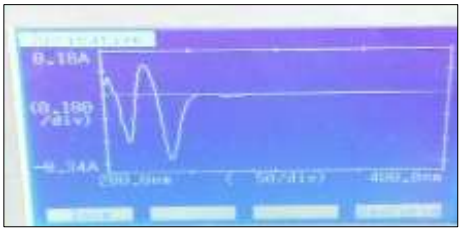

Fig 2: First order derivative spectra of LEVC

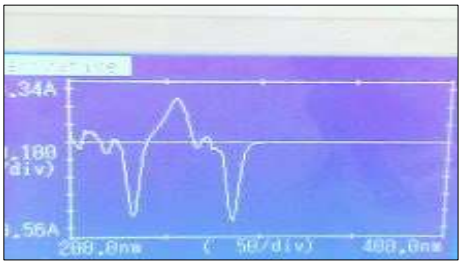

Fig 3: First order derivative spectra of PHE

Validation of Developed Methods

The methods were validated according to the ICH guidelines for validation of analytical procedures in order to determine linearity and range, precision, specificity, accuracy, robustness, LOD and LOQ for each method. The results of validation studies were statistically proved.

Linearity

The linearity is the ability of an analytical procedure which shows that obtained test results are directly proportional to the concentration of analyte in the sample. Aliquots of working standard solution were transferred into a series of 10 ml stopper volumetric flasks. The volumes were made up to mark with double distilled water. The absorbance of LEVC and PHE were measured at 231nm and 273 nm, respectively as well as at 239nm and 225.5nm against double distilled wateras the reagent blank, and the calibration curve was plotted (fig 4 & fig 5). The linearity range was followed 2-30µg/ml and 6-120µg/ml & 5-90µg/ml and 5-100µg/ml for LEVC and PHE, for Vierodt's method and for derivative method,respectively. The results were incorporated in table 1. The regression equations show the analytes are obey the Beer- Lambert's law at their respective wavelengths:

At 231.0 nm (LEVC) Y = 0.0335x + 0.0032 (n=15, r^2 = 0.9995) (7)

At 273.0 nm (PHE) Y = 0.0092x + 0.0099 (n=14, r^2 = 0.9997) (8)

At 239.0 nm (LEVC) Y = 0.0164x + 0.0175 (n=11, r^2 = 0.9983) (9)

At 225.5nm (PHE) Y = 0.0140x + 0.0276 (n=12, r^2 = 0.9998) (10)

Table 1: Optical data analysis for Vierodt's method and Absorbance ratio method

Optical parameters	Vierodt's method		Derivative method	
	LEVC 231nm	PHE 273nm	LEVC 239nm	PHE 225.5nm
Beer's law limits (µg/ ml)	02-30	06-120	05-90	05-100
Molar Absorptivity (L.mol-1.cm-1)	1.56 x10^4	2.038 x10^4	3.503 x10^4	7.065 x10^4
Sandell's sensitivity (µg.cm-2)	0.029614	0.099914	0.05814	0.065359
Regression equation (y= mx+c)	y= 0.0335x +0.0032	y=0.0092x + 0.0099	y = 0.0164x +0.0175	y = 0.014x +0.0276
Correlation coefficient (r^2)	0.9995	0.9997	0.9983	0.9998
Slope, m	0.0335	0.0092	0.0164	0.014
Intercept, c	0.0032	0.0099	0.0175	0.0276

Where, LEVC = levocetirizine dihydrochloride, PHE = Phenylephrine hydrochloride.

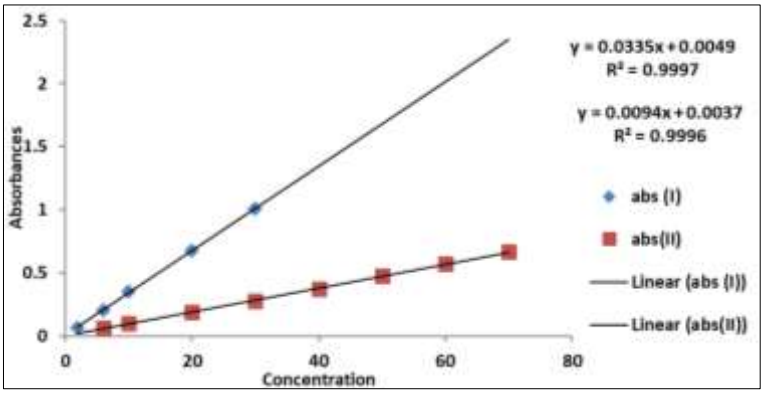

Fig 4: Calibration curve of LEVC and PHE for vierodt's method

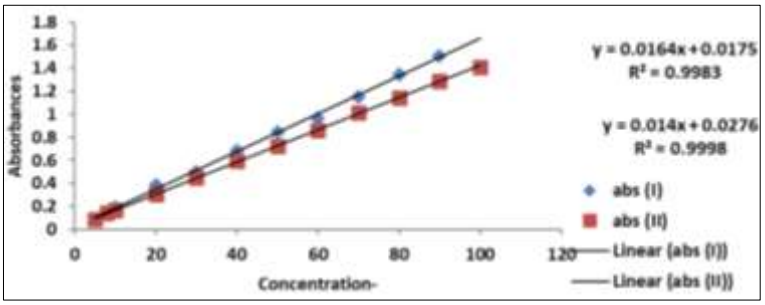

Fig 5: Calibration curve of LEVC and PHE for derivative method

Precision

The precision of an analytical method was determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation, relative standard deviation and standard error of mean. Precision was determined as intraday and interday precision with three concentrations and three replicates. The obtained data were statistically analyzedand the relative standard deviation for Vierodt's method found to be 0.622 and 0.278 for LEVC and PHE, respectively and for derivative method found to be 0.273 and 0.767 for LEVC and PHE, respectively. The validation results show that the developed method is highly précised. The results are shown in table 2.

Table 2: Precision of LEVC and PHE (Interday precision and intraday precision)

Method	No. of aliquots	Interday precision		Intraday precision	
		LEVC	PHE	LEVC	PHE
Vierodt's method (method I)	9	$\bar{X}$ = 99.72 SD= 0.11 RSD= 0.62% SEM= 0.0635	$\bar{X}$ = 100.55 SD= 0.74 RSD= 0.736% SEM= 0.427	$\bar{X}$ = 100.30 SD= 0.98 RSD = 0.977% SEM= 0.566	$\bar{X}$= 100.10 SD= 1.11 RSD= 1.108% SEM= 0.641
Derivative Method (method II)	9	$\bar{X}$ = 98.95 SD= 0.278 RSD= 0.281% SEM= 0.161	$\bar{X}$ = 98.78 SD= 1.402 RSD= 1.42% SEM= 0.809	$\bar{X}$ = 99.39 SD= 0.875 RSD = 0.880% SEM= 0.505	$\bar{X}$= 98.74 SD= 0.314 RSD= 0.318% SEM= 0.181

X = mean; SD=Standard deviation; RSD=Relative standard deviation; SEM=standard mean error

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the observed value.Accuracy of the methods was confirmed by recovery studies. Accuracy was assayed by using nine determinations over three concentration levels, covering the specified range. The percentage recovery found to be 101.26, 100.86 and99.91, 100.01 for LEVC and PHE, respectively for both methods. The recovery of analyte indicates that the proposed methods are accurate. The results are shown in table 3.

Table 3: Recovery study of LEVC and PHE

Method	Recovery level (added amount)	% Recovery of LEVC	% Recovery of PHE
Vierodt's method (method I)	80%	$\bar{X}$ (mean ± SD) X = 100.94±0.301 RSD = 0.298% SEM = 0.1003	$\bar{X}$ (mean ± SD) X = 100.01±0.725 RSD = 0.725% SEM = 0.242
	100%	$\bar{X}$ = 101.21±0.704 RSD = 0.696% SEM = 0.235	$\bar{X}$ = 101.13±0.672 RSD = 0.665% SEM = 0.224
	120%	$\bar{X}$ = 101.63±0.173 RSD = 0.170% SEM = 0.0576	$\bar{X}$= 101.44±0.376 RSD = 0.371% SEM = 0.125
Derivative Method (method II)	80%	$\bar{X}$ (mean ± SD) X = 99.65±0.21 RSD = 0.211% SEM = 0.07	$\bar{X}$ (mean ± SD) X = 99.53±0.160 RSD = 0.161% SEM = 0.0534
	100%	$\bar{X}$ = 99.75±0.88 RSD = 0.883% SEM = 0.294	$\bar{X}$ = 100.23±0.320 RSD = 0.319% SEM = 0.107
	120%	$\bar{X}$ = 100.33±0.763	$\bar{X}$= 100.27±0.568

		RSD = 0.760 SEM = 0.254	RSD = 0.566 SEM = 0.189

Where, X= mean; RSD = relative standard deviation; SEM= standard error of mean

Robustness

The robustness is the ability of an analytical procedure to remain unaffected by small deliberate change in method. It is performed by making deliberate change in wavelength. Original method and changed method results deviates 0.56%, 0.72% and 0.35%, 0.15% for LEVC and PHE respective at increment of 2nm while deduction of two nm percentage deviation obtained 0.40%, 0.66% and 0.30%, 0.45% for LEVC and PHE, respectively in both methods. This indicates that the developed method is robust and unaffected by minor change in the wavelength. The results are given in table 4.

Table 4: Percent deviation in robustness (increase/decrease inλmax)

Method	wavelength		Active drug in %		Percentage deviation	
	LEVC	PHE	LEVC	PHE	LEVC	PHE
Vierodt's method (method I)	231 229 233	273 271 275	99.48 99.08 98.92	100.82 100.16 100.10	0.40% 0.56%	0.66% 0.72%
Derivative method (method II)	239 237 241	225.5 223.5 227.5	98.70 99.00 98.35	98.75 99.20 98.60	0.30% 0.35%	0.15% 0.15%

Specificity

Specificity predicts the interference of excipients or impurities in analysis of pharmaceutical dosage form. Specificity was done by spiking the drug substance with appropriate levels of excipients and demonstrating the assay results that were unaffected by the presence of these extraneous materials. Placebo was prepared in the same way as the sample under the conditions prescribed in the proposed methods. Duplicate dilution was taken and observed any significant absorbanceat the analyte wavelength. The percentage interference of significant absorbance was found to be 0.536 and 0.611 for LEVC and PHE, respectively for Vierodt's method and 0.613 and 0.614 for LEVC and PHE, respectively for derivative method which implies that the developed methods are specific for the analytes in the tablet dosage form. The results are given in table 5.

Table 5: Summary of analytical method validation

Validation parameters	Vierodt's method		Derivative method	
	LEVC	PHE	LEVC	PHE
Linearity Corr. Coefficient (R^2)	2-30µg/ml 0.9995	6-120µg/ml 0.9997	5-90µg/ml 0.9983	5-100µg/ml 0.9998
Interday precision (%RSD)	0.622%	0..278%	0.273%	0.767%
Intraday precision (%RSD)	0.457%	0.499%	0.477%	0.570%
Robustness (incr.) (%deviation) (decr.)	0.56% 0.40%	0.72% 0.66%	0.35% 0.30%	0.15% 0.45%
Accuracy (%recovery)	101.26	100.86	99.91	100.01
Specificity	0.5362	0.6112	0.6132	0.6139
LOQ	0.299	2.174	0.610	1.429
LOD	0.099	0.717	0.201	0.472

Where LOD= Detection Limit; LOQ= Quantitation Limit; RSD = relative standard deviation

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

This shows the sensitivity of analyte for the particular analytical methods. They were calculated from the standard deviation (σ) of the absorbance (n=5) of the sample and the slope of the corresponding calibration curve (S) in accordance to the following equations:

$$LOD = 3.3\times\sigma/S \quad (13)$$

$$LOQ = 10\times\sigma/S \quad (14)$$

Conclusions

The developed Simultaneous equations and Derivative spectrophotometric methods have been successfully applied for simultaneous determination of LEVC and PHE in combined dosage form. This shows the proposed method is enable to save time and tedious methodology for prior separation treatment of sample. Standard deviation, relative standard deviation and standard mean of error were satisfactorily low for the accuracy and precision. Thus the developed methods in present investigation were rapid, simple, specific, sensitive, accurate and precised. Once the equations were constructed, analysis required only measuring the absorbance values of the sample solution at the selected wavelengths followed by few simple calculations. The suggested methods were validated and results are statistically proved. Recovery studies and specificity indicated that practically there was no interference from tablet additives, so these methods can be easily and conveniently adopted for routine quality control simultaneous analysis of LEVC and PHE.

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