Research Article

Development and Validation of First-Order Derivative Spectrophotometry for Simultaneous Determination of Levocetirizine Dihydrochloride and Phenylephrine Hydrochloride in Pharmaceutical Dosage Form

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A simple, precise, accurate, and economical spectrophotometric method has been developed for simultaneous estimation of levocetirizine dihydrochloride (LCT) and phenylephrine hydrochloride (PHE) by employing first-order derivative spectrophotometric method. The first-order derivative absorption at 240 nm (zero crossing point of PHE) was used for quantification of LCT and 283.2 nm (zero crossing point of LCT) for quantification of PHE. The linearity was established over the concentration range of 4–24 $\mu\text{g/mL}$ and 8–48 $\mu\text{g/mL}$ for LCT and PHE with correlation coefficients (r^2) 0.9964 and 0.9972, respectively. The mean % recoveries were found to be in the range of 99.14%–100.43% for LCT and 98.73%–100.83% for PHE. The proposed method has been validated as per ICH guideline and successfully applied for the simultaneous estimation of LCT and PHE in combined tablet dosage form.

1. Introduction

The chemical name of levocetirizine dihydrochloride (LCT) is [2-[4-[(R)-(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride [1]. The structure of LCT is given in Figure 1. LCT is a third generation nonsedative antihistamine, developed from the second generation antihistamine cetirizine. Chemically, LCT is the active L-enantiomer of cetirizine racemate. It works by blocking H_1 histamine receptors. It is used in the treatment of the allergic rhinitis and conjunctivitis, hay fever, pollinosis—control sneezing, runny but not blocked nose, and red, watering, and itchy eyes [2].

The chemical name of phenylephrine hydrochloride (PHE) is (R)-3-Hydroxy- α [(methylamino)methyl] benzenemethanol hydrochloride [3]. The structure of PHE is

given in Figure 2. PHE is a direct acting sympathomimetic agent. It is a selective α_1 adrenoceptor agonist and has negligible β action. It is also a vasoconstrictor, because it has little cardiac action. It is mainly used as a nasal decongestant and for producing mydriasis when cycloplegia is not required. It tends to reduce intraocular tension by constricting ciliary body blood vessels [2].

LCT and PHE in combined dosage form are used as a nasal decongestant.

The review of the literature revealed that various analytical methods involving spectrophotometry, HPLC, HPTLC, and LC-MS have been reported for LCT alone and in combination with other drugs [4–14]. Several analytical methods have been reported for PHE alone and in combination with other drugs including spectrophotometry, HPLC, HPTLC,

FIGURE 1: Chemical structure of LCT.

FIGURE 2: Chemical structure of PHE.

LC-MS/MS, and electrophoresis [15–23]. However, to the best of our knowledge, no spectrophotometric method is published for the simultaneous determination of LCT and PHE in tablet dosage form. The present work describes the development of a simple, precise, accurate, and reproducible spectrophotometric method for the simultaneous estimation of LCT and PHE in combined dosage forms. The developed method was validated in accordance with ICH guideline and successfully employed for the assay of LCT and PHE in combined tablet dosage form.

2. Materials and Methods

2.1. Chemicals and Reagents. Analytically, pure LCT and PHE were provided as gratis samples by Baroque Pharmaceuticals, Khambhat, Gujarat, India. Tablet of LCT and PHE, levocet-D⁺ manufactured by Hetero Healthcare Ltd., Hyderabad, India, and rinostat-L manufactured by RPG Life Sciences Ltd., Maharashtra, India, were purchased from a local pharmacy.

2.2. Instruments. A Shimadzu UV-Visible 1700 PharmaSpec double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells, and UV probe 2.34 software was used. Calibrated analytical balance Shimadzu BP211D (Sartorius Gottingen AG, Germany) was used for weighing purpose. All statistical calculations were carried out using Microsoft Excel 2007 analytical tool.

2.3. Preparation of Standard Stock Solutions. Accurately weighed 25 mg of LCT and PHE standards was transferred to a separate 25 mL volumetric flask and dissolved in 10 mL distilled water. The flasks were shaken and volume was made

up to the mark with distilled water. These solutions are 1000 $\mu\text{g/mL}$ LCT and 1000 $\mu\text{g/mL}$ PHE respectively.

2.4. Selection of Analytical Wavelength. Working standard solutions of 4–24 $\mu\text{g/mL}$ of LCT and 8–48 $\mu\text{g/mL}$ of PHE were prepared in distilled water by appropriate dilution, and the spectrum was recorded between 200 and 400 nm, and all zero-order spectrums (D^0) were converted to first derivative spectrums (D^1) using delta lambda 2 and scaling factor 7. The overlain first derivative spectrums of LCT and PHE at different concentrations were recorded. The zero crossing point (ZCP) of LCT was found to be 283.2 nm, and ZCP of PHE was found to be 240 nm.

2.5. Method Validation [24]. The proposed method was validated in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (% relative standard deviation—% RSD) was expressed with respect to the repeatability, intraday, and interday variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

2.6. Linearity. Appropriate volume of aliquot from LCT and PHE standard stock solution was transferred to volumetric flask of 10 mL capacity. The volume was adjusted to the mark with distilled water to give working standard solutions containing 4–24 $\mu\text{g/mL}$ for LCT and 8–48 $\mu\text{g/mL}$ for PHE respectively. All D^1 spectra were recorded using the above spectrophotometric condition. D^1 absorbance at 240 nm and 283.2 nm was recorded for LCT and PHE, respectively, ($n = 5$). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

2.7. Accuracy. Accuracy was assessed by the determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at 3 different concentration levels 80, 100, and 120%, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times, and average recoveries were measured.

2.8. Precision. The repeatability was evaluated by assaying 6 times the sample solution prepared for assay determination. The intraday and interday precision study of LCT and PHE was carried out by estimating different concentrations of LCT (12, 16, and 20 $\mu\text{g/mL}$) and PHE (24, 32, and 40 $\mu\text{g/mL}$), 3 times on the same day and on 3 different days (first, second, third) and the results are reported in terms of % RSD.

2.9. Detection Limit and Quantitation Limit. ICH guideline describes several approaches to determine the detection and

FIGURE 3: Overlain D^1 spectra of LCT in distilled water. FIGURE 4: Overlain D^1 spectra of PHE in distilled water.

quantitation limits. These include visual evaluation, signal-to-noise ratio, and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10\sigma/S$ criteria, respectively, where σ is the standard deviation of the y -intercepts of the regression lines and S is the slope of the calibration curve.

2.10. Reproducibility. The absorbance readings were measured at a different laboratory for sample solution using another spectrophotometer by another analyst, and the values obtained were evaluated using t -test to verify their reproducibility.

TABLE 1: Summary of validation parameters.

Parameters	LCT	PHE
% Recovery	99.14%–100.43%	98.73%–100.83%
Precision (% RSD)		
Repeatability ($n = 6$)	1.06	0.96
Intraday ($n = 3$)	0.53–0.66	0.79–1.11
Interday ($n = 3$)	0.87–1.10	1.01–1.60
Limit of detection ($\mu\text{g/mL}$)	0.19	0.64
Limit of quantification ($\mu\text{g/mL}$)	0.57	1.94
Solution stability	Stable for 24 hrs.	Stable for 24 hrs.

FIGURE 5: Overlain D^1 spectra of LCT and PHE in distilled water.

2.11. Determination of LCT and PHE in Their Combined Tablet Dosage Form. Twenty tablets were weighed accurately. A powder quantity equivalent to 10 mg PHE and 5 mg LCT was accurately weighed and transferred to volumetric flask of 100 mL capacity. 50 mL of distilled water was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken, and the solution was filtered through Whatman filter paper (0.45μ). Then, the volume was made up to the mark with distilled water. From this solution, the 2.4 mL was transfer to 10 mL volumetric flask. The volume was adjusted to the mark with the distilled water to give a solution containing $12\mu\text{g/mL}$ of LCT and $24\mu\text{g/mL}$ of PHE. The resulting solution was analyzed by the proposed method. The quantitation was carried out by keeping these values to the straight line equation of the calibration curve.

3. Results and Discussion

First-order derivative spectrophotometric method was developed for determination of LCT and PHE.

The overlain first-order derivative spectrum of LCT and PHE at different concentrations revealed that at

TABLE 2: Statistical data of LCT and PHE.

Parameters	LCT at 240 nm	PHE at 283.2 nm
Linear range	4–24 $\mu\text{g/mL}$	8–48 $\mu\text{g/mL}$
Slope	0.0152	0.0457
Intercept	0.0045	0.0443
Standard deviation of intercept	0.0008	0.0008
Regression coefficient (r^2)	0.9964	0.9972

TABLE 3: Accuracy data.

Drug	Level	Amount taken (total) ($\mu\text{g/mL}$)	Amount added ($\mu\text{g/mL}$)	Amount recovered ($\mu\text{g/mL}$)	% Recovery $\pm$ SD
LCT	80%	14.4	6.4	14.35	99.69 $\pm$ 0.450
	100%	16	8	16.003	100.01 $\pm$ 0.405
	120%	17.6	9.6	17.51	99.52 $\pm$ 0.370
PHE	80%	28.8	12.8	28.82	100.06 $\pm$ 0.765
	100%	32	16	31.85	99.53 $\pm$ 1.066
	120%	35.2	19.2	35.04	99.54 $\pm$ 0.625

TABLE 4: Precision data at 240 nm for LCT.

Conc. ($\mu\text{g/mL}$)	Intraday Mean $\pm$ S.D. ($n = 3$)	% RSD	Interday Mean $\pm$ S.D. ($n = 3$)	% RSD
12	0.229 $\pm$ 0.001	0.66	0.228 $\pm$ 0.002	1.10
16	0.287 $\pm$ 0.001	0.53	0.287 $\pm$ 0.002	0.87
20	0.353 $\pm$ 0.002	0.56	0.355 $\pm$ 0.003	0.98

TABLE 5: Precision data at 283.2 nm for PHE.

Conc. ($\mu\text{g/mL}$)	Intraday Mean $\pm$ S.D. ($n = 3$)	% RSD	Interday Mean $\pm$ S.D. ($n = 3$)	% RSD
24	0.153 $\pm$ 0.001	0.99	0.156 $\pm$ 0.002	1.60
32	0.192 $\pm$ 0.001	0.79	0.197 $\pm$ 0.002	1.01
40	0.225 $\pm$ 0.002	1.11	0.226 $\pm$ 0.003	1.55

TABLE 6: Assay results of marketed formulations.

Formulation	Drug	Labeled claim (mg)	Amount found per tablet (mg)	% Label Claim $\pm$ S.D.
Levocet-D ⁺	LCT	5	4.98	99.66 $\pm$ 0.808
	PHE	10	9.96	99.66 $\pm$ 0.950
Rinostat-L	LCT	5	4.98	99.63 $\pm$ 1.266
	PHE	10	10.03	100.3 $\pm$ 0.9

283.2 nm a different concentration of LCT possesses zero D^1 absorbance, whereas PHE possesses significant D^1 absorbance (Figure 3). In a similar manner, at 240 nm different concentrations of PHE possess zero D^1 absorbance, whereas LCT possesses significant D^1 absorbance (Figure 4). Considering the above facts, wavelengths 283.2 nm and 240 nm were selected for the estimation of PHE and LCT,

FIGURE 6: D^1 spectrum of marketed formulation 1.

respectively. Figure 5 shows overlain D^1 spectra of LCT and PHE at different concentrations.

The proposed method has been extensively validated as per ICH guidelines. Summary of validation parameters for the proposed method is given in Table 1.

Linearity was assessed for LCT and PHE by plotting calibration curves of the D^1 absorbance versus the concentration over the concentration range 4–24 $\mu\text{g/mL}$ and 8–48 $\mu\text{g/mL}$, respectively. The correlation coefficients (r^2) for LCT and PHE were found to be 0.9964 and 0.9972, respectively (Table 2). The following equations for straight line were obtained for LCT and PHE:

$$\begin{aligned} \text{linear equation for LCT, } y &= 0.0152x + 0.0457, \\ \text{linear equation for PHE, } y &= 0.0045x + 0.0443. \end{aligned} \quad (1)$$

The % recoveries were found to be 99.14%–100.43% for LCT and 98.73%–100.83% for PHE (Table 3). Precision was determined by repeatability, intraday, and interday variation for both drugs and was expressed as % RSD (Tables 4 and 5).

The proposed spectrophotometric method was successfully applied to LCT and PHE in combined tablet dosage form as shown in Figures 6 and 7 (Table 6).

4. Conclusion

The proposed first-order derivative method provides simple, specific, precise, and accurate quantitative analysis for simultaneous determination of LCT and PHE in combined tablet dosage form. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), and reproducibility. The proposed method can be used for routine analysis and quality control assay of LCT and PHE in combined dosage form.

FIGURE 7: D¹ spectrum of marketed formulation 2.

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