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Spectrofluorimetric Determination of the H1 blocker Drug Levocetirizine dihydrochloride Based on its Oxidation with Cerium (IV) In Bulk and Pharmaceutical formulations

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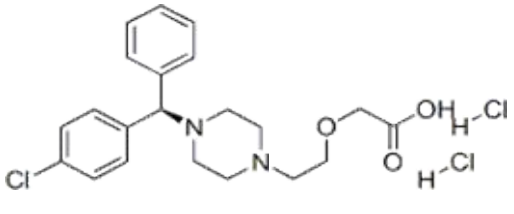
Abstract: A simple, accurate and sensitive spectrofluorimetric method has been developed and validated for determination of Levocetirizine dihydrochloride (LCTZ). The method was based on the oxidation by cerium (IV) in the presence of sulphuric acid and monitoring the fluorescence of cerium (III) formed at λ_{ex} 249 nm and λ_{em} 354 nm. All variables affecting the reaction conditions as cerium (IV), sulphuric acid concentrations, heating time, temperature and dilution solvents were carefully studied. Linear calibration graphs were obtained in the range of 50-5000 ng/ml. The limit of detection and limit of quantification were 10 ng/ml and 50 ng/ml respectively. The effect of potential interference due to common ingredients as glucose, sucrose, lactose, citric acid, and propylene glycol was investigated. Applying standard addition method shows a recovery of 99.36-100.27 from their corresponding dosage forms. The method was applied successfully for the assay of the studied drug in pure and pharmaceutical dosage forms as tablets.

Key words: Oxidation reduction; Levocetirizine dihydrochloride; Spectrofluorimetry; Interference study; Pharmaceutical analysis.

INTRODUCTION

Levocetirizine dihydrochloride, (R)-2-[2-[4-(4-chlorophenyl)-phenylmethyl] phenylmethyl-1]-piperazinyl-] ethoxylacetic acid dihydrochloride (figure1) is an anti-histamine; its principal effects are mediated via selective inhibition of H_1receptors[1,2].Levocetirizine dihydrochlorideis official in IP 2007[3].Different spectrophotometric[4],HPLC[5-8] and LCMS[9,10] methods have been reported for the determination of cetirizine in pharmaceutical formulations and biological fluids. The dosage forms of LCTZ are available in the market for the prophylaxis and treatment of allergic rhinitis.

Figure 1 Structure of LCTZ



One spectrofluorimetric method[11] have been reported for cetirizine by using eosin dye. An advantage of developed method by using cerric ammonium sulphate was high sensitivity and not requires any extraction procedure. The objective of this work was to develop and validate an accurate, specific, sensitive, precise, repeatable spectrofluorimetric method for determination of LCTZ in pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments and Apparatus

A Shimadzu RF 1501 Spectrofluorophotometer with 1 cm quartz cells was used for recording the spectra and carrying out fluorescence measurements. The calibration and linearity of the instrument were checked at frequent intervals with standard quinine sulphate (0.01 µg ml^{-1}). Wavelength calibration was performed by measuring λexcitation and λemission of the same standard of quinine sulphate at λ_{ex} 275 nm and λ_{em} 430 nm, although no variation in the wavelength was observed. All fluorescence measurements were recorded at the lower set sensitivity. In addition, a thermostatically controlled water-bath was used.

Analytical grade chemicals were used as received. Doubly distilled water was used throughout. Sulphuric acid 0.25 M was prepared by mixing 13.6 ml of concentrated sulphuric acid in 1000 ml double distilled water.

Cerric ammonium sulphate (Sigma Chemie GmbH, West Germany) 0.005 M was prepared by dissolving 3.175 g in 1000 ml 0.25 M sulphuric acid. The prepared solution was kept in the refrigerator and used for one week during which it is checked at frequently intervals by measuring its fluorescence intensity.

Standard sample Levocetirizine dihydrochloride was obtained as gift from Cadilla Healthcare, Ahmadabad, and Gujarat, India. Tablet formulation containing Levocetirizine dihydrochloride 5 mg was procured from local pharmacy.

Preparation of Standard Solution

An accurately weighed quantity of about 10 mg LCTZ was transferred into 100 mL volumetric flask. About 50 ml of double distilled water was added and sonicated to dissolve. The solution was cooled at room temperature and made up to volume with double distilled water. Aliquot 1ml of above solution in 10 ml volumetric flask& diluted up to mark with double distilled water to get final concentration of 10µg/ml. From this solution a series of dilution was prepared quantitatively in double distilled water to obtain standard solutions having concentration range of 0.05-5µg/ml.

Preparation of sample (tablet dosage form) Solution

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 5 mg of LCTZ was transferred to a 100 ml volumetric flask. The content was mixed with 50 ml double distilled water, sonicated for 15 min. to dissolve the drug as completely as possible. The solution was filtered through a whatman filter paper no. 41. The residue on the filter paper was washed with double distilled water and washing were collected in filtrate. A measured volume of the filtrate was diluted quantitatively with double distilled water to yield a sample solution having a concentration assumed to be 1µg/ml of LCTZ.

Optimization of reaction condition

A series of experiments were conducted to establish the optimum analytical conditions for the oxidation of LCTZ by Ce (IV). The parameters optimized were performed on all the studied LCTZ by altering each variable in turn while keeping the others constant.

Effect of cerric ammonium sulphate concentration

The effect of Ce (IV) concentrations was investigated using 1 ml of different concentrations of the reagent in the range of 0.001–0.01 M employing concentrations of the studied 2 µg/ml for LCTZ. Maximum relative fluorescence intensity was obtained with a Ce (IV) concentration of 0.005 M, above which it slightly decreased.

By applying different volumes (0.1-1.2 ml) of the same concentration, a volume of 1 ml in a total volume of 10 ml was found to be quite enough for maximum fluorescence intensity.

Effect of acid concentration

Different acids as HNO_3, HCl and H_2SO_4 were tested to determine the most suitable for optimum reaction development. Sulphuric acid could be used as the fluorescence of Ce (III) is high. Nitric acid could not be used due to the inhibitory effect of nitrate ions on the fluorescence of Ce (III). The effect of sulphuric acid concentration on the sensitivity of the method was studied by using 0.1-0.5 M sulphuric acid; relative fluorescence intensity increases up to 0.25 M ammonium cerric sulphate, and then decreased up on using higher concentrations. Therefore, 0.25 M sulphuric acid was adopted for this method.

Effect of Heating Time and Temperature

The influence of different heating temperature and incubation time were studied using a thermostatic water bath. The effect of different heating temperature on the sensitivity of the method was studied by using

40-100°C heating temperature. The optimum temperature found to be 80 °C and complete oxidation was attained in a period of 80 min.

Effect of diluting solvents

Dilution effect with different solvents on the relative fluorescence intensity revealed that best solvents were water, methanol, and ethanol as they were all more or less comparable to each other. However water was the solvent of choice for economic and environmental safety purposes. The induced fluorescence intensity was found stable for more than 3 h.

General Procedure

One milliliter of sample or standard solution was transferred by a pipette into a 10-ml calibrated flask. A volume of 1 ml of cerric ammonium sulphate was added. The solution was mixed well and heated in a thermostatic water bath at 80 °C for 80 min. The solution was then cooled, diluted to volume with double distilled water and measured spectrofluorimetrically at λ_{ex} 249 and λ_{em} 354 nm against a blank experiment treated similarly.

Procedure for Calibration Curve

For preparation of different drug concentrations, aliquots of stock solution were transferred into a series of 10 ml standard flasks and follow general procedure. A total of 8 different concentrations (0.05, 0.1, 0.3, 0.5, 1, 3, 5µg/ml)) of LCTZ were prepared and the fluorescence intensity were measured at an excitation wavelength of 249 nm and an emission wavelength of 354 nm and was plotted vs. concentration to give calibration curve, and regression equation was calculated. The emission spectra and calibration curve of LCTZ was shown in figure 2 and 3.

Estimation of LCTZ in pharmaceutical dosage forms

Sample solution was monitored at λ_{ex} 249 nm and λ_{em} 354 nm. There was no interference from excipients commonly present in the tablets. The LCTZ content was found to be 98.81 ±0.47%, of the label claim. The low value of % RSD indicated the method was suitable for routine analysis of the LCTZ in pharmaceutical dosage forms.

Method Validation

The developed method was validated for its accuracy, precision, reproducibility and selectivity. The validation parameters were described in table 1.The accuracy of the method was determined by performing recovery studies on tablet formulation and for prepared solutions containing known amount of drug by standard addition method in which preanalyzed samples were taken and standard drug was added at three different levels. The result was shown in table 2. Also, the experiment was repeated three times in a day to determine intra-day precision and on three different days to determine interday precision. The percent relative standard deviation (%RSD) was calculated at each concentration level and the result was given in table 3. The reproducibility was confirmed by repeating the method by three different analysts and the % RSD was calculated. The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by visual detection. The selectivity of the method was checked by monitoring a standard solution of LCTZ in presence of excipients (starch, methylcellulose, hydroxypropylmethylcellulose) at the same concentration levels as used in tablets using the method described in the procedure for calibration curve in pharmaceutical tablet drug which could be potentially employed for its estimation in pharmaceutical formulations.

Figure 2 Emission spectra of LCTZ

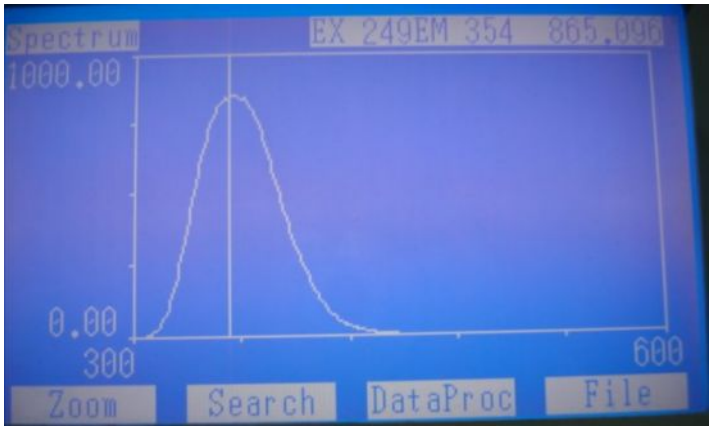


Figure 3 Calibration curve of LCTZ

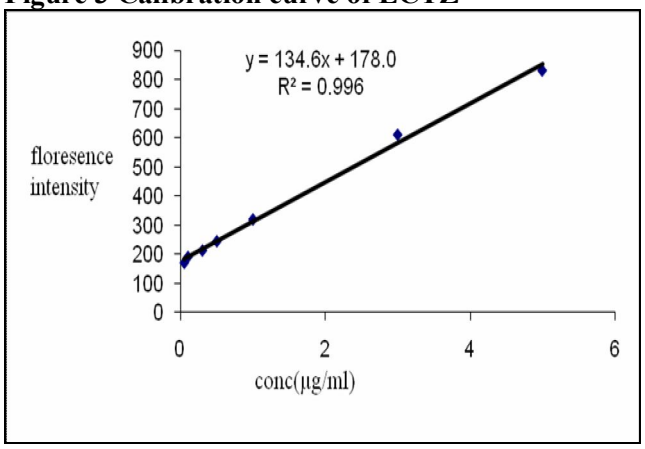


Table 1 Validation parameters of LCTZ

Parameters		Result
Linearity(µg/ml)		0.1-5
Regression equation y=mx+c		y = 134.6x + 178
%Recovery ± SD,(n=3)	Level 1	99.74 ±1.08
	Level 2	100.27 ± 0.67
	Level 3	99.36 ±0.70
Repeatability (%RSD, n=6),		0.242
Interday precision (%RSD) (n = 3) at 3 range		0.564 -0.957
Intraday precision (%RSD) (n = 3) at 3 range		0.201-0.438
LOD (µg/ml)		0.03
LOQ(µg/ml)		0.1

Table 2 Recovery Data of LCTZ

Level of Standard Addition (%)	Florescence intensity	% Recovery	Mean % Recovery ± SD*
50 %	380.914	100.23	99.74 ±1.08
	374.339	98.5	
	381.94	100.5	
100 %	451.272	100.87	100.27 ± 0.67
	445.322	99.54	
	449.259	100.42	
150 %	508.029	98.7	99.36 ± 0.70
	511.117	99.3	
	515.235	100.1	

*__SD__- standard deviation

Table 3 Inter-day and Intra-day precision

Concentration of LCTZ (µg/mL)	Intraday precision	Interday precision
	Florescence intensity± %RSD (n=3)	
0.3	211.977±0.232	210.625±0.564
1	318.698±0.438	317.844±0.957
3	608.029±0.201	608.029±0.823

*__RSD__- Relative standard deviation

Table 4 Assay Result of LCTZ tablet

Sample No.	Label Claim	Fluorescence intensity	Amount Found	% Label Claim
	Levocetirizine (mg/tab)		Levocetirizine (mg/tab)	Levocetirizine (mg/tab)
1	5	310.667	4.928195	98.56
2	5	311.749	4.968388	99.37
3	5	310.964	4.939227	98.78
4	5	309.959	4.901895	98.03
5	5	311.458	4.957578	99.15
6	5	311.235	4.949294	98.99
Mean				98.81
Standard Deviation				0.47

RESULT AND DISCUSSION

Cerium (IV) has been used as an oxidizing agent for the determination of LCTZ by monitoring the fluorescence of their Ce (III) formed. However, Ce (III) is more fluorescent therefore; measurement of its fluorescence can be used as a very sensitive method for the determination of LCTZ. In the present work LCTZ was oxidized by Ce (IV) and fluorescence intensity of the induced Ce (III) was monitored at λ_{ex} 249 nm and λ_{em} 354 nm. To avoid interference due to the presence of Ce (III), the fluorescence intensities of Ce (IV) were measured to obtain appropriate blank correction. Result of all validation parameters are shown in Table 1.The proposed flourimetric method was found to be linear in the range of 0.05 to 5 µg/ml with correlation coefficient (r^2) 0.996, slope 134.6 and intercept 178.0. The method was validated in terms of accuracy, precision, reproducibility and the results are recorded in Tables 2 and 3. The accuracy of the method was determined by performing recovery studies by standard addition method in which preanalyzed samples were taken and standard drug was added at three different levels. Values of recovery±SD greater than 99.0% indicate that the proposed method is accurate for the analysis of drug. The precision of the proposed method was estimated in terms of inter-day precision and intraday precision wherein the method was repeated on three different days and repeated for three different time periods in the same day respectively. The results are shown in table 3 indicating % RSD of less than 2% at each level clearly indicate that the proposed method is precise enough for the analysis of drug. The reproducibility of the method was confirmed by performing the proposed method by three different analysts. The values of % RSD less than 2% indicate that the proposed method is reproducible for the analysis of LCTZ. The selectivity of the method was checked by monitoring a standard solution of LCTZ in presence of other compounds of tablet. The excipients too did not show any effect on the estimation of LCTZ. Hence, the determination of LCTZ in the tablet is considered to be free from interference due to other excipients. Rigorous analysis of the results indicates that the presence of excipients in tablet formulation did not interfere with the final determination of the active component. This reveals the potential utility of this developed method for the routine analysis of LCTZ in pharmaceutical preparations. The assay result is described in table 4. The LCTZ content was found to be 98.81 ±0.47%, of the label claim. The low value of % RSD indicated the method was suitable for routine analysis of the LCTZ in pharmaceutical dosage forms.

CONCLUSION

The present work is the first to describe a fully validated spectrofluorimetric procedure for the assay of LCTZ without interference from common excepients. Hence, it can be recommended for the routine quality control of LCTZ in its pharmaceutical dosage forms. Another advantage, is that, compared to the existing spectrofluorimetric method of cetirizine it is more sensitive. From the economical point of view, the proposed method is simple, rapid and inexpensive and not requires any extraction procedure so it is a good alternative to reported other methods and to high cost HPLC with an equivalent sensitivity.

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