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Iodometric Determination of Milligram and Microgram Amounts of Levocetirizine in Pharmaceuticals[1]

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Abstract—Four simple, selective and sensitive methods are described for the determination of levocetirizine dihydrochloride (LCT) in bulk drug and in tablets. The methods exploit the well-known analytical reaction between iodide and iodate in the presence of acid solution. Iodide present is oxidized by iodate in an amount equivalent to the HCl present in LCT to iodine and the liberated iodine is determined by four different procedures which inturn quantify LCT at varying detection range and sensitiveness. Two direct titrimetric procedures involve titration of iodine by thiosulphate either towards starch end point (method A) or potentiometrically (method B). Both the methods have a reaction stiochiometry of 1 : 1 (LCT : liberated iodine) and have quantification ranges of 2–20 mg LCT for method A and method B. The liberated iodine is also measured spectrophotometrically at 350 nm (method C) or the iodine-starch complex measured at 570 nm (method D). In both the methods, the absorbance is found to be linearly dependent on the concentration of iodine which in turn is related to LCT concentration. The calibration curves are linear over 5–40 and 1.25–12.5 mg mL^{-1} LCT for method C and method D, respectively. The calculated molar absorptivity and Sandel sensitivity values are 1.0×10^4 L mol^{-1} cm^{-1} and 0.0435 mg cm^{-2}, respectively for method C, and their respective values for method D are 2.9×10^4 L mol^{-1} cm^{-1} and 0.0156 mg cm^{-2}. The intra-day and inter-day accuracy and precision studies were carried according to the ICH guidelines. The method was successfully applied to the analysis of two brands of tablets LCT. The accuracy was also checked by placebo blank and synthetic mixture analyses besides recovery study via standard addition procedure.

Keywords: spectrophotometry, levocetirizine, titrimetry, potentiometry, pharmaceuticals

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INTRODUCTION

Levocetirizine dihydrochloride (LCT), (2-[4-[(R)-(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride) (Fig. 1) is a third generation non sedative antihistamine[1], and is the active enantiomer of cetirizine dihydrochloride. LCT has the advantages of higher efficacy, less side effects, and longer duration over other antihistamines, and has begun to replace cetirizine in clinical therapy stepwise. It has been chemically proved that half dosage form of LCTZ (2.5 mg) has comparable antihistaminic activity to normal amount (5.0 mg) of ce-tirizine in the treatment of allergic rhinitis and chronic idiopathic urticaria [2]. In many cases, the two racemic enantiomers differ in their pharmacokinetic and pharmacodynamic properties. Replacing existing recemates with single isomers has resulted in improved safety and/or efficacy profile of various recemates [3, 4].

LCT is official in Indian Pharmacopoeia [5]. Literature survey revealed that LCT has been determined in human serum by reverse-phase high performance liquid chromatography (RP-HPLC) along with other H_1-receptor antagonists [6] and in plasma by liquid chromatography-tandem mass spectrometry [7]. Xiangping et al. have recently reported a study on the interaction of LCT with human serum albumin by molecular spectroscopy [8].

LCT in combination with a number of other drugs in tablet dosage form has been assayed by UV- spectrophotometry [9, 10], ratio derivative spectrophotometry [11], TLC-densitometry [12], RP-HPLC [13–16]. However, there are only three reports dealing with the determination of LCT when present alone in

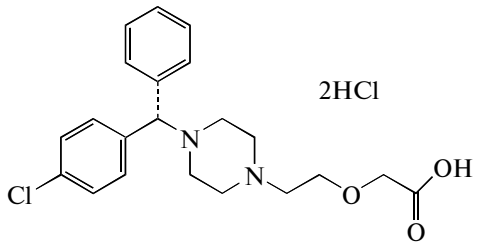

Fig. 1. Structure of LCT.

[1] The article is published in the original.

its dosage forms. Li Jing et al. have reported an UV-spectrophotometric method [17] to determine effective content of LCT in its tablets. The drug and related substance in solid oral formulation were assayed by HPLC [18]. The same technique has been applied for the stability-indicating method for the drug in bulk form and in dosage forms [19] and for the determination of LCT configuration stability in tablets using the chiral mode [20].

However, many of the reported methods for LCT in single-dosage form, particularly, the chromatographic methods [18–20] are complex, requiring expensive instrumental set up and skilled operator which are not always found in laboratories of developing and under developed countries. Thus, the need for a simple, selective and low-cost method is obvious, especially for routine quality control analysis of pharmaceuticals containing LCT.

There is only one report, an official method [5], on the use of titrimetric method for the determination of LCT. The method consists of the titration with 0.1 M NaOH in acetone:water medium to the potentiometric end point, but requires large amounts of acetone and large quantities of LCT. To the best of our knowledge, no visible spectrophotometric method is available for the quantification of LCT in pharmaceuticals. The present manuscript describes two fairly sensitive titrimetric and two sensitive spectrophotometric methods for the determination of LCT in both pure form and in tablet form. The methods make use of the acidity of levocetirizine dihydrochloride, iodide present is oxidized by iodate, in amounts equivalent to the acid present, to iodine which is titrated with thiosulphate to the starch end point in method A or to the potentiometric end point in method B. The absorbance of the liberated iodine is measured at 355 nm in method C and starch-iodine complex at 570 nm in method D. This reaction has earlier been exploited for the assay of a number of organic acids [21–24]. The developed methods are simpler and cost effective, than many existing methods besides being applicable to smaller amounts compared to the lone titrimetric method available [5].

EXPERIMENTAL

Apparatus. A Systronics model 106 digital spectrophotometer provided with 1-cm matched quartz cells was used for absorbance measurements.

Reagents and standards. All chemicals were of analytical reagent grade and distilled water was used to prepare solutions.

Potassium iodate. A high purity grade of the chemical (Merck, Mumbai, India) was used. A saturated solution of potassium iodate was prepared by stirring approximately 20 g of the chemical in a beaker containing 100 mL water with the help of magnetic stirrer for 60 minutes. The solution was decanted and filtered using quantitative filter paper.

Potassium iodide. A saturated solution of potassium iodide (Merck, Mumbai, India) was prepared just before use in order to prevent atmospheric oxidation to iodine.

Sodium thiosulphate. A 0.01 N sodium thiosulphate was prepared in water and standardized against 0.01 N potassium dichromate.

Saturated Borax. Approximately 30 g of borax (S.D. Fine Chem., Mumbai, India) was dissolved in 100 mL water and stirred with the help of magnetic stirrer for 15 min. The solution was decanted and filtered. The pH of the solution was between 8 and 9.

1% starch. A paste of 1 g of the chemical (potato starch, loba chemie, Mumbai, India) in cold water was dissolved in 100 mL of boiling water. Cooled before use and prepared afresh every day.

Standard Drug Solution

Pharmaceutical grade levocetirizine dihydrochloride (LCT) was received from Jubilant Life Sciences Ltd., Mysore, India, as a gift and used as received. Allercet-5 (from Microlabs Ltd. India), Xyzal-5 (from UCB India Pvt Ltd, India) and Lezyncet syrup (Mepro pharmaceuticals Pvt. Ltd.) were purchased from commercial sources in the local market. A stock standard solution equivalent to 2 mg mL^{-1} for method A and method B was prepared by dissolving 200 mg of pure drug in water and diluting to 100 mL in calibrated flask with water. The stock solution was diluted appropriately to get working concentration of 100 and 25 mg mL^{-1} with water for method C and method D, respectively.

Procedures

Method A. A 10 mL aliquot of pure LCT solution containing 2–20 mg of LCT was taken in an Erlenmeyer flask. Five mL each of saturated solution of KIO_3 and of KI were added and the flask was stopperred and let stand for 5 min with occasional swirling. Finally, 1 mL of 1% starch indicator was added and liberated iodine was titrated against standardized solution of 0.01 N $Na_2S_2O_3$ until the decoloration of blue color.

The amount of LCT was computed from the following formula:

$$\text{Amount, mg} = \frac{V \times M_r \times S}{n},$$

where V = mL of iodine reacted; M_r = relative molecular mass of drug; S = strength of titrant, moles/L; n = number of moles of titrant reacting with per mole of LCT.

Method B. An aliquot of the standard drug solution equivalent to 2.0–20.0 mg of LCT was measured accurately and transferred into a clean 100 mL beaker. Five ml each of saturated solution of KI and KIO_3 was added. The content was stirred magnetically and the

titrant (0.01 N $Na_2S_2O_3$) was added from a microburette. Near the equivalence point, titrant was added in 0.1 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady emf was noted. The addition of titrant was continued until there was no significant change in emf on further addition of titrant. The equivalence point was determined by applying the graphical method. The amount of the drug in the measured aliquot was calculated as described under visual titration.

Method C. A 0.5–4.0 mL of 100 μg mL^{-1} LCT was added in to a series of 10 mL calibrated flasks by means of microburette. To each flask, 1 mL each of saturated KIO_3 and KI were added flasks stoppered, content mixed and let stand for 15 min. Then 2 mL of saturated borax was added solution and made up to the mark with water. Absorbance of each solution was measured at 350 nm against reagent blank.

Method D. Different volumes (0.5–5.0 mL) of 25 μg mL^{-1} LCT were taken in a series of 10 mL calibrated flasks. One ml each of saturated KIO_3 and KI solutions were added, flasks were stoppered and content mixed. The flasks were let stand for 15 min before adding 2 mL of saturated borax and 1 mL of 1% starch to each flask, and made up to 10 mL with water. Absorbance of each solution was measured at 570 nm against reagent blank.

Standard graph was prepared by plotting the absorbance *versus* drug concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation.

Procedure for Tablets

Twenty tablets were weighed accurately and ground into a fine powder. An accurately weighed amount of the powdered tablet equivalent to 200 mg of LCT was transferred into a 100 mL calibrated flask. Sixty mL water was added and the content was shaken thoroughly for 15–20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with the water, mixed well and filtered using a Whatman No. 42 filter paper. An aliquot of the filtrate (2 mg mL^{-1} in LCT) was used for method A and method B, and diluted to required concentrations and used for the assay in method C and method D as described above.

Procedure for the Analysis of Placebo Blank and Synthetic Mixture

A placebo blank containing starch (35 mg), acacia (45 mg), sodium citrate (45 mg), hydroxyl cellulose (40 mg), magnesium stearate (50 mg), talc (40 mg) and sodium alginate (35 mg) was prepared by mixing all the components into a homogeneous mixture. A 100 mg of the placebo blank was accurately weighed and its solution was prepared as described under 'tablets,' and then subjected to analysis by following the general procedures.

To the placebo blank of the composition described above, 100 mg of LCT was added and homogenized, transferred to a 50 mL calibrated flask and the solution was prepared as described under "Procedure for tablets", and then subjected to analysis by the procedure described above. This analysis was performed to study the interference by excipients normally present in tablet preparation.

RESULTS AND DISCUSSION

Preliminary experiments showed that LCT is sufficiently acidic to release iodine from iodate-iodide mixture allowing the titrimetric and spectrophotometric determination of drug. In titrimetry, the liberated iodine was titrated with thiosulphate and the end point being located visually to the starch end point in method A, and poten-tiometric end point in method B, while in spectrophotometry, it was determined by two different color reactions.

Method Development

Method A and Method B. The quantitative nature of the reaction between LCT and iodate-iodide reagent was checked by treating 2.0–20.0 mg of drug with an excess of reagent and determining the iodine released. For the range studied (2.0–20.0 mg), 5.0 mL each of saturated solution of iodate and iodide and reaction time of 5 min was found adequate. The end point is being located visually to the starch end point in method A and end point is located potentiometrically in method B. The reaction stoichiometry is 1 : 1 (drug : liberated iodine), the COOH group in the drug moiety is not acidic enough to liberate iodine and presence of electron releasing group in the side chain attached to piperazine suppresses the acidic property of COOH group and hence the liberated iodine is due the presence of two HCl.

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O,$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6.$$

Method C and Method D. Absorbance of the liberated iodine or starch-iodine complex was measured at 350 or 570 nm as deduced from the absorption spectra of the colored species (Fig. 2). In both the methods, the reaction was relatively fast in the beginning and iodine continued to be liberated even after 15 min. Since most of the iodine was liberated within 15 min, the reaction was stopped by adding borax to the reaction mixture after a standing time of 15 min. The absorbance remained constant for 45 and 30 min in method C and method D, respectively. Attempts to hasten the reaction by heating were unsuccessful

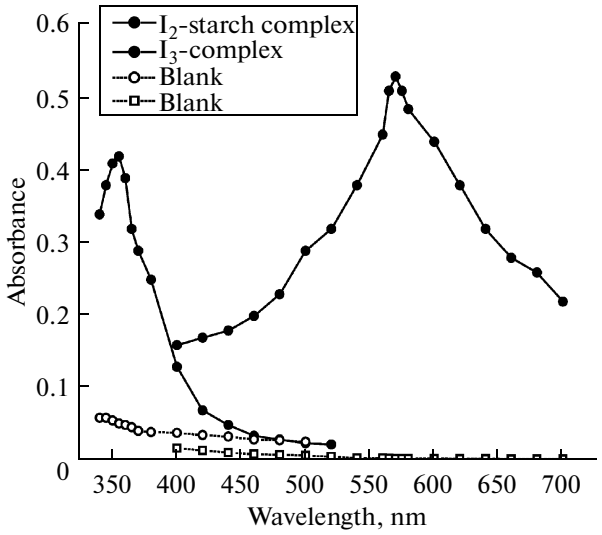

Fig. 2. Absorption spectra.

owing to the volatility of iodine and dissociation of iodine-starch complex at elevated temperature.

The possible reaction schemes responsible for change in absorbance as a function of LCT concentration are represented below:

$$IO_3^- + 5I^- + 6H^+ (LCT) \rightarrow 3I_2 + 3H_2O$$

(Method C: measured at 350 nm),

$$I_2 + Starch \rightarrow I_2—Starch\ complex$$

(Method D: measured at 570 nm).

METHOD VALIDATION

Linearity, Detection and Quantification Limits

Under the optimum conditions a linear relation was obtained between absorbance and concentration of LCT in the ranges given in Table 1. The calibration graph in each instance is described by the equation:

$$Y = a + bX,$$

(Where Y = absorbance, a = intercept, b = slope and X = concentration in μg mL^{-1}). The correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity and sandell sensitivity values, the limits of detection (LOD) and quantification (LOQ) are calculated as per the current ICH guidelines [26] and compiled in Table 1. LOD and LOQ were calculated according to the same guidelines using the following formulae:

$$LOD = \frac{3.3 \times \sigma}{S} \ \&\ LOQ = \frac{10 \times \sigma}{S},$$

where σ is the standard deviation of six reagent blank determinations and S is the slope of the calibration curve.

Selectivity. The results obtained from placebo blank and synthetic mixture analyses revealed that the inactive ingredients used in the tablet preparation did not interfere in the assay of active ingredient. The absorbance values obtained from the placebo blank solution were almost equal to the absorbance of the blank which revealed no interference from the adjuvants. To study the role of additives added to the syn-

Table 1. Sensitivity and regression parameters

Parameter	Method C	Method D
λ_{max}, nm	350	570
Color stability, min	45	30
Linear range, μg mL^{-1}	5–40	1.25–12.5
Molar absorptivity(ε), L mol^{-1} cm^{-1}	1.0×10^4	2.9×10^4
Sandell sensitivity*, μg cm^{-2}	0.0435	0.0156
Limit of detection (LOD), μg mL^{-1}	0.34	0.17
Limit of quantifcation (LOQ), μg mL^{-1}	1.05	0.51
Regression equation, Y**		
Intercept (a)	0.00243	0.0192
Slope (b)	0.0228	0.0677
Regression coeffcient (r)	0.9982	0.9970
Standard deviation of intercept (Sa)	0.0223	0.0862
Standard deviation of slope (Sb)	0.0007	0.0128

Notes: * Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and $\underline{1} = 1$ cm;

** $Y = a + bX$, Where Y is the absorbance, X is concentration in μg/mL, a is intercept, b is slope, $\pm t\bar{S}_a / \sqrt{n}$ = confidence limit for intercept, $\pm t\bar{S}_b / \sqrt{n}$ = confidence limit for slope.

Table 2. Evaluation of intra-day and inter-day accuracy and precision

Method	LCT taken*	Intra-day accuracy and precision			Inter-day accuracy and precision		
		LCT found**	RE, %	RSD, %	LCT found**	RE, %	RSD, %
A	6.0	5.83	1.25	1.96	5.75	2.15	2.85
	12.0	11.80	2.29	1.22	11.71	1.78	3.46
	18.0	17.77	1.25	0.43	17.69	2.06	3.58
B	6.0	5.89	1.76	1.73	5.81	2.58	3.26
	12.0	11.84	1.25	1.02	11.69	3.04	2.48
	18.0	17.84	1.15	0.90	17.75	2.36	2.46
C	10.0	9.83	1.69	1.73	9.78	2.46	2.76
	20.0	19.71	1.44	0.95	19.65	1.74	3.54
	30.0	29.43	1.88	1.54	29.14	2.85	3.26
D	5.0	4.91	1.70	0.70	4.88	2.64	3.15
	7.5	7.38	1.47	1.91	7.35	1.92	2.64
	10.0	9.77	2.23	1.94	9.76	2.37	3.07

* The amount of LCT is in mg for method A and method B; and μg mL^{-1} for method C and method D;
** Mean value of three determinations.

Table 3. Results of analysis of tablets by the proposed methods and statistical comparison of the results with the reference method

Tablets analysed**	Label claim, mg/tablet	Found* (Percent label claim ±SD)				
		Reference method	Method A	Method B	Method C	Method D
[a]Allercet	5.0	96.48 ± 1.12	97.13 ± 1.14 $t = 1.39$ $F = 2.14$	98.56 ± 1.42 $t = 2.13$ $F = 1.60$	98.84 ± 1.74 $t = 1.68$ $F = 2.41$	97.26 ± 1.85 $t = 1.86$ $F = 2.72$
[b]Xyzal	5.0	98.56 ± 1.36	97.47 ± 0.96 $t = 2.71$ $F = 2.00$	98.04 ± 1.26 $t = 2.44$ $F = 1.16$	99.38 ± 1.84 $t = 2.11$ $F = 1.87$	97.68 ± 1.95 $t = 2.70$ $F = 1.47$

Notes: * Mean value of 5 determinations. Tabulated t-value at the 95 % confidence level and for four degrees of freedom is 2.77. Tabulated F-value at the 95 % confidence level and for four degrees of freedom is 6.39;
** Marketed by: [a]Micro labs Ltd, [b]UCB India Pvt. Ltd.

thetic sample, the analysis of synthetic mixture solution prepared as described earlier yielded percent recoveries of 98.3 ± 2.13, 99.1 ± 1.76, 97.86 ± 1.94 and 101.34 ± 1.91 (n = 5) for method A, method B, method C and method D, respectively, demonstrated the accuracy as well as the precision of the proposed method and complement the findings of the placebo blank analysis with respect to selectivity.

Precision. The precision of the method was calculated in terms of intermediate precision (intra-day and inter-day) [26]. Three different concentration of LCT were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good (Table 2).

Accuracy. The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for LCT (Bias %). The results obtained are compiled in Table 2 and show that the accuracy is good for the method.

Application to tablets analysis. The proposed methods were applied to determine LCT in two brands of tablets The results were statically compared with those obtained by the official Indian Pharmacopoeial method [5] for accuracy and precision by applying the Student's t-test and variance ratio F-test. The official method consisted of acid-base titration to the potentiometric end point in acetone: water system. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant

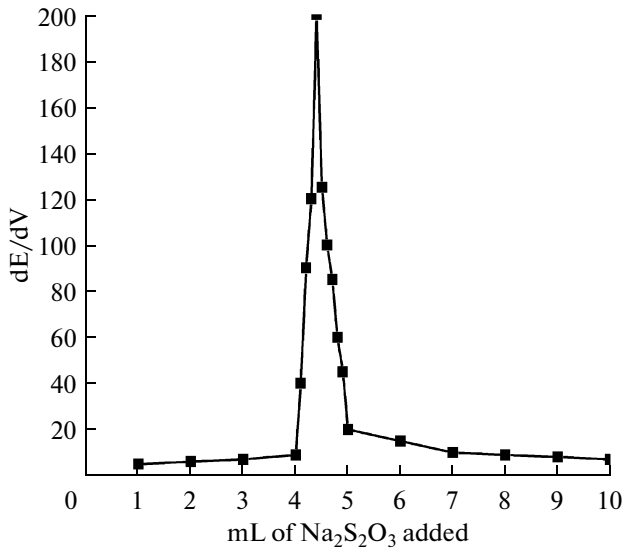

Fig. 3. Potentiometric titration curve for 10 mg LCT vs. 0.01 N $Na_2S_2O_3$.

difference between the proposed methods and the literature method at the 95% confidence level with respect to accuracy and precision (Table 3).

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure LCT at three concentration levels (50, 100 and 150% of that in tablet powder) and the total was found by the proposed methods. In all cases, the added LCT recovery percentage values ranged between 96.67 and 103.24. The results of this study given in Table 4 indicated that the recovery was good, and that the co formulated substances did not interfere in the determination.

CONCLUSIONS

Four methods have been developed for determination of levocetirizine dihydrochloride in bulk drug and in its dosage forms and validated as per the current ICH guidelines. The methods use cheap and readily available chemicals, compared to the lone titrimetric method [official method], the presented methods are rather simple and sensitive. The reported methods suffer from such draw backs as high cost, and also several clean-up steps (HPLC). They are time consuming and often poorly reproducible, some require toxic organic solvents. Any method chosen for routine analysis should be reasonably simple, used materials should be readily available in the laboratory or readily obtainable, and require a minimum amount of equipment. The methods are selective as none of the common tablet excipients contain acidic groups to interfere with the present proposed methods. The proposed spectrophotometric methods are free from rigid experimental variables such as pH control, heating or extraction step and/or use of organic solvents. They are characterized by high selectivity and comparable sensitivity with respect to the existing methods. The accuracy, reproducibility, simplicity and cost-effectiveness of the methods suggest their application in the quality control laboratories where the modern and expensive instruments are not available.

Authors thank Jubiliant Life Sciences Limited. Mysore, India. for gifting pure levocetirizine. Authors

Table 4. Accuracy assessment by recovery experiments

Tablet studied	Method	LCT in tablet powder taken*	Pure LCT added*	Total found*	Pure LCT recovered (Percent ± SD**)
Allercet (5 mg)	A	5.83	3.00	8.73	96.67 ± 1.36
		5.83	6.00	11.66	97.23 ± 1.28
		5.83	9.00	14.72	98.15 ± 1.46
	B	5.91	3.00	8.87	98.90 ± 0.94
		5.91	6.00	11.85	99.08 ± 0.82
		5.91	9.00	14.82	98.64 ± 1.24
	C	14.86	10.0	24.72	98.68 ± 1.07
		14.86	15.0	29.72	99.08 ± 2.12
		14.86	20.0	35.12	101.30 ± 1.26
	D	3.89	2.00	5.91	100.53 ± 0.78
		3.89	4.00	7.99	102.78 ± 0.32
		3.89	6.00	10.01	103.24 ± 1.28

Notes: * The amount of LCT is in mg for method A and method B; and μg mL^{-1} for method C and method D;
** Mean value of three determinations.

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