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Research Article

Simple and Selective Titrimetric and Spectrophotometric Methods for the Determination of Loratadine Using Bromate-Bromide, Methyl Orange and Methylene Blue

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ABSTRACT

One indirect titrimetric and two indirect visible spectrophotometric methods were described for the determination of loratadine in bulk drug and in its formulations. The methods used bromate-bromide, methyl orange and methylene blue as reagents. In titrimetry (method A), loratadine was treated with a known excess of bromate-bromide mixture in acidic medium and the residual bromine was back titrated iodometrically after the reaction between loratadine and *in situ* bromine was ensured to be complete. In spectrophotometric methods, the excess of bromine was estimated by treating with a fixed amount of either methyl orange (method B) and measuring the absorbance at 520 nm or methylene blue (method C) and measuring the absorbance at 680 nm. In all the methods, the amount of reacted bromine corresponded to the loratadine content. Titrimetric method was applicable over 1-8 mg range and the calculations were based on a 1:0.666 (loratadine:bromate) stoichiometric ratio. In spectrophotometry, the calibration graphs were found to be linear over 150-350 and 1.75-3.5 μg mL^{-1} for method B and method C, respectively, with corresponding molar absorptivity values of 9.15×10^2 and 1.10×10^5 L mol^{-1} cm^{-1}. Accuracy and precision of the assays were determined by computing the intra-day and inter-day variations at three different levels of loratadine. The methods were successfully applied to the assay of loratadine in tablet preparations and the results were compared with those of a reference method by applying Student's t and F-tests. No interference was observed from common pharmaceutical excipients. The reliability of the methods was further ascertained by performing recovery tests by standard addition method.

Keywords loratadine; bromate-bromide; dyes; titrimetry; spectrophotometry; pharmaceuticals.

INTRODUCTION

Now-a-days, one of the major problems is allergy due to increased global pollution and different synthetic chemicals as preservatives, artificial colorings, acidifications, taste correctors, etc., used in food industry at present.

Loratadine (figure 1), ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidine carboxylate[1] is tricyclic, long acting, non-sedative, second generation H1-antihistamine drug. It is more selective for peripheral H1-receptors as opposed to the central nervous system H1-receptors and cholinergic receptors. This selectivity significantly reduces the occurrence of adverse drug reactions, such as sedation, while still providing effective relief of allergic conditions. The reason for their peripheral selectivity is that most of these compounds are zwitterionic at physiological pH (pH ~7.4). If they are very polar, they do not cross the blood-brain barrier and act mainly outside the central nervous system, that is why they produce very little or no sedation. It is a potent and orally active that was developed as a therapeutic agent for the treatment of seasonal and perennial allergic rhinitis, allergic dermatitis and urticaria and ocular allergy[2].

Loratadine is given orally, well absorbed from the gastrointestinal tract and has rapid first-pass hepatic metabolism; it is metabolized by isoenzymes of the cytochrome P-450 system, including CYP3A4, CYP2D6 and to a lesser extent, several others. Loratadine is almost totally (97–99%) bound to plasma proteins. Its metabolite, desloratadine is largely responsible for the antihistaminergic effects. It binds to plasma proteins by 73–76%. It is official in USP1, BP2, IP3.

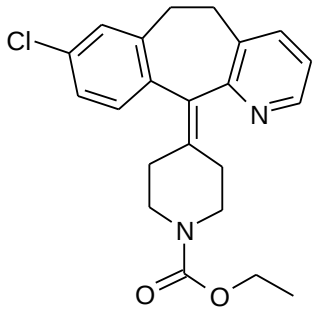

Figure 1: Molecular structure of loratadine.

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Table 1: Comparison of the performance characteristics of the proposed methods with the existing visible spectrophotometric methods.

S.No	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (µg/ml) (ε=L/mol/cm)	Remarks	Ref
1	a) molybdenum thiocyanate	DCM extractable orange red ion-pair complex formed	469.5	2.5-22.5	less sensitive, involves extraction step	23
	b) 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ)	charge transfer complex measured	588	10-80	less sensitive	
2	a) bromocresol purple	chloroform extractable ion pair complexation	409	20-55 ($\varepsilon = 0.64\times10^4$)	extraction step involved, pH adjustment required	24
	b) eosin	ion pair complex	539	3-10 ($\varepsilon = 5.05\times10^4$)	methylcellulose solution added	
3	chloranilic acid (CAA)	charge transfer complex measured in chloroform along with other drug astemizole	520	15-210 (0.96×10^3)	fairly sensitive	25
4	cobalt nitrate and ammonium thiocyanate	chloroform extractable ion pair complexes by the drugs with thiocyanate ions	618	1-12 ($\varepsilon = 2.68$ x 10^4)	extraction step involved	26
5	erythrosine B	ion-pair complex formation	550	1-6 (ε = NR)	less sensitive, pH adjustment required	27
6	3-methyl-2-benzothialinone hydrazone hydrochloride (MBTH) in presence of a) ferric chloride	oxidative coupling reaction	630	2-10 ($\varepsilon = 8.153$ x10^3)	multi-step reaction	28
	b) sodium periodate ($NaIO_4$)		624	5-25 ($\varepsilon = 9.397$ x 10^3)		
7	a) $KBrO_3$-KBr /HCl and methyl orange	Bromination of LOR and determination of unreacted Br_2 with methyl orange	520	150-350 ($\varepsilon = 9.15 \times 10^2$)	Simple method, Highly sensitive, non-stringent optimum conditions used, simple instrument employed.	Present work
	b) $KBrO_3$-KBr /HCl and methylene blue	Bromination of LOR and determination of unreacted Br_2 with methylene blue	680	1.75-3.5 ($\varepsilon = 1.01 \times 10^5$)		

Various techniques such as non-aqueous titrations[3], UV method[4], UV area under curve method[5], derivative spectrophotometry[6,7], stability indicating methods[8,9], spectrofluorimetric method[10,11] and cyclic voltammetry[12] have been described for the determination of LOR.

The chromatographic techniques[13-22] were the most widely used for the determination of LOR. Although, the procedures were specific, most of the described methods were time consuming and required multistage extraction procedures. On the other hand, the reported spectrophotometric methods suffer from one or the other disadvantage such as poor sensitivity, use of organic solvent, scrupulous control of experimental variables and special equipments (Table 1). Titrimetry and spectrophotometry are well established techniques and owing to their speed, selectivity, reduced costs and versatility of application, they can be considered to be advantageous alternatives to sophisticated and expensive techniques normally used in pharmaceutical analysis.

By considering these drawbacks, the present work is aimed for developing titrimetric and spectrophotometric methods that would overcome many of the problems encountered in the reported methods. This work describes one titrimetric and two spectrophotometric methods for the determination of LOR in pharmaceuticals based on bromination reaction using bromate-bromide mixture and by employing two dyes, methyl orange and methylene blue. The methods were successfully applied to the determination of LOR in two different brands of tablets with good accuracy and precision and without detectable interference by

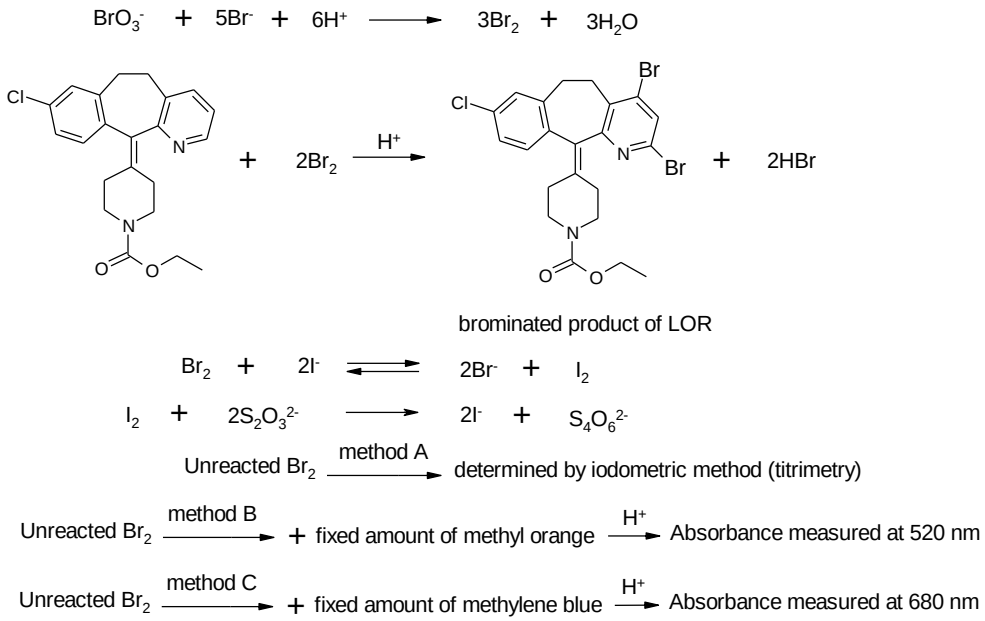

Figure 2: Probable reaction scheme showing bromination of LOR and determination of *in situ* generated bromine by titrimetry and spectrophotometric methods.

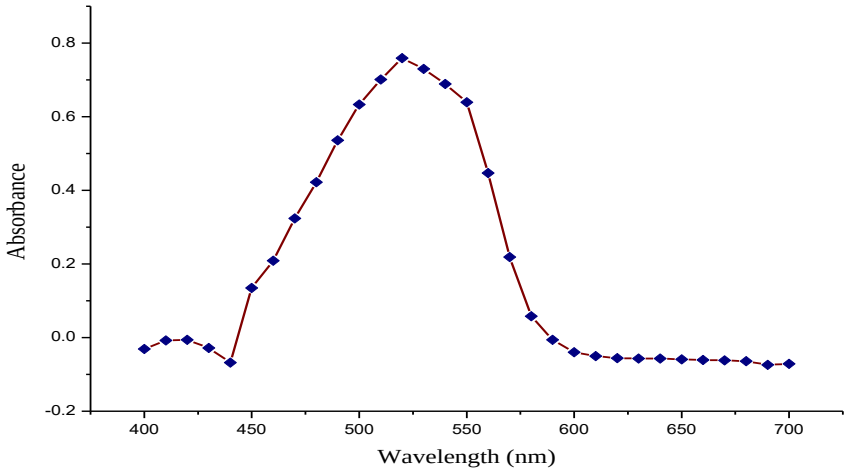

Figure 3: Absorption spectrum of method B - 325 µg/ml LOR with methyl orange.

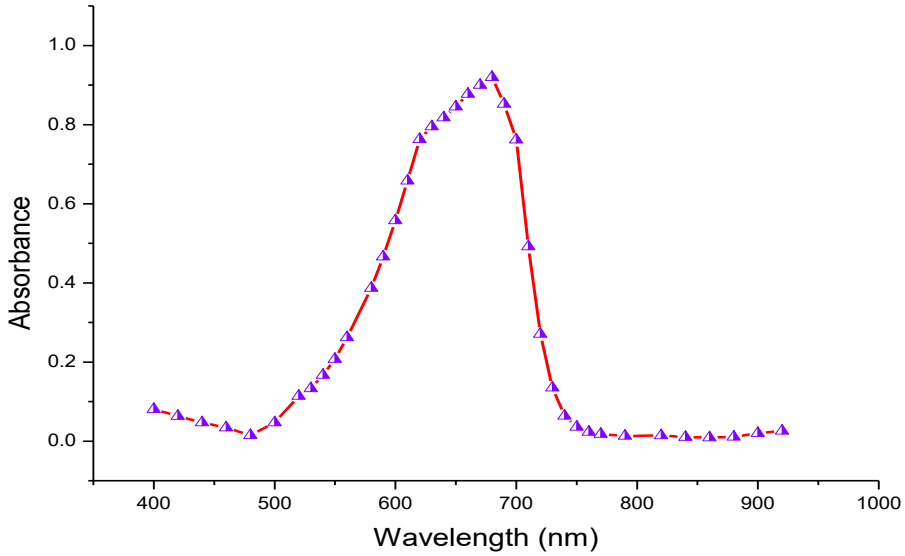

Figure 4: Absorption spectrum of method C – 3.5 µg/ml LOR with methylene blue.

excipients. The accuracy was further ascertained by placebo blank and synthetic mixture analyses and also by recovery experiments *via* standard-addition procedure and the methods were to be simple, accurate and easy to apply for the routine analysis of LOR.

Table 2: Sensitivity and regression parameters for spectrophotometric methods.

Parameter	Method B	Method C
λ_{max}, nm	520	680
Linear range, μg mL^{-1}	150-350	1.75-3.5
Molar absorptivity(ε), L mol^{-1} cm^{-1}	9.15×10^2	1.01×10^5
Sandell sensitivity*, μg cm^{-2}	4.19×10^{-7}	3.80×10^{-9}
Limit of detection (LOD), μg mL^{-1}	60.92	0.67
Limit of quantification (LOQ), μg mL^{-1}	184.62	2.03
Regression equation, Y**		
Intercept (a)	-0.0683	-0.1100
Slope (b)	0.0026	0.2949
Standard deviation of a (S_a)	0.0080	0.0057
Standard deviation of b (S_b)	3.12×10^{-5}	0.0021
Variance (S_a^2)	6.4×10^{-5}	3.2×10^{-5}
Regression coefficient (r)	0.9995	0.9998

*Limit of determination as the weight in μg/mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.
**Y=a+bX, Where Y is the absorbance, X is concentration in μg mL^{-1}, a is intercept, b is slope

EXPERIMENTAL

Apparatus

A Systronics model 105 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and Materials

All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation. Pure LOR (Pharmaceutical grade, 99.8% pure) sample was kindly provided by Orchid Pharmaceuticals, Chennai, India as a gift and used as received. Two brands of tablets, namely, Lorfast Meltab (Cadila, India) and Lorinol-10 (Microlabs Ltd, Mumbai, India) used in the investigation were purchased from local pharmacy in Chennai, Tamilnadu.

Bromate–bromide mixture

A bromate-bromide solution equivalent to 5mM $KBrO_3$-50 mM KBr was prepared by dissolving accurately weighed 209 mg of $KBrO_3$ and 1.5 g of KBr (Merck, India) in distilled water and diluting to the mark in a 250 mL calibrated flask and this solution was used in titrimetric work. For use in spectrophotometric study, a 1000 μg mL^{-1} $KBrO_3$ solution containing a large excess of KBr was prepared by dissolving 100 mg of $KBrO_3$ and 1 g of KBr in distilled water and diluting to the mark in a 100 mL calibrated flask. This was diluted stepwise to get 25 μg mL^{-1} and 75 μg mL^{-1} bromate solutions for use in method B and method C respectively.

Methyl Orange

A 500 μg ml^{-1} stock solution was prepared by dissolving 58.82 mg of the dye (S.d. Fine Chem. Mumbai, India, 85 % dye content) in water and diluting to the mark in a 100 ml volumetric flask. This was appropriately diluted to obtain a 100 μg ml^{-1} solution for method B.

Methylene blue

The solution was prepared by dissolving 0.025 mg of dye (S.d. Fine Chem, India, dye content 93%) in 100ml of distilled water (250 μg mL^{-1}) and then diluted to 120 μg mL^{-1} in a 100ml calibrated flask.

Hydrochloric acid solution

2M and 5M HCl solutions prepared by diluting the appropriate volume of concentrated acid (Fisher Scientific, India, Sp. grade 1.18) with distilled water.

Sodium thiosulphate solution

0.03M sodium thiosulphate solution was prepared by dissolving 7 g of the chemical (S.d. Fine Chemicals, India) in 1 litre of distilled water. This solution was standardized[29].

Potassium iodide

10% KI solution prepared by dissolving 10 g of chemical (Merck, India) with distilled water.

Starch indicator

1 % starch paste containing 1 g of starch (S.d. Fine Chemicals, India) was poured slowly into 100 ml boiling water and cooled.

Loratadine standard solution

A 1 mg mL^{-1} standard drug solution was prepared by dissolving 250 mg of pharmaceutical grade LOR in methanol. The volume was made upto 250 mL in a calibrated flask with methanol and was used in titrimetry. This solution (1000 μg mL^{-1}) was then diluted with methanol to get 500 μg mL^{-1} and 5 μg mL^{-1} solutions for use in method B and method C respectively.

General analytical procedures

Titrimetry (method A)

An aliquot of pure LOR solution 1-8 mL containing 1-8 mg of LOR (1 mL/mg) was transferred accurately into a 100 mL Erlenmeyer flask and the total volume was made upto 10 mL with distilled water. The solution was acidified by adding 5 mL of 2M HCl. 10 mL of bromate-bromide solution (5 mM w.r.t $KBrO_3$) was transferred to the flask by means of a pipette. The flask was stoppered, the content mixed well and kept aside for 15 min with occasional swirling. The stopper was then washed with 5 mL of water and 5 mL of 10% potassium iodide solution was added to the flask. The liberated iodine was titrated with 0.03M sodium thiosulphate to a starch end point. A blank titration was run under identical conditions by omitting the drug solution. The amount of LOR in the measured aliquot was calculated from the following formula:

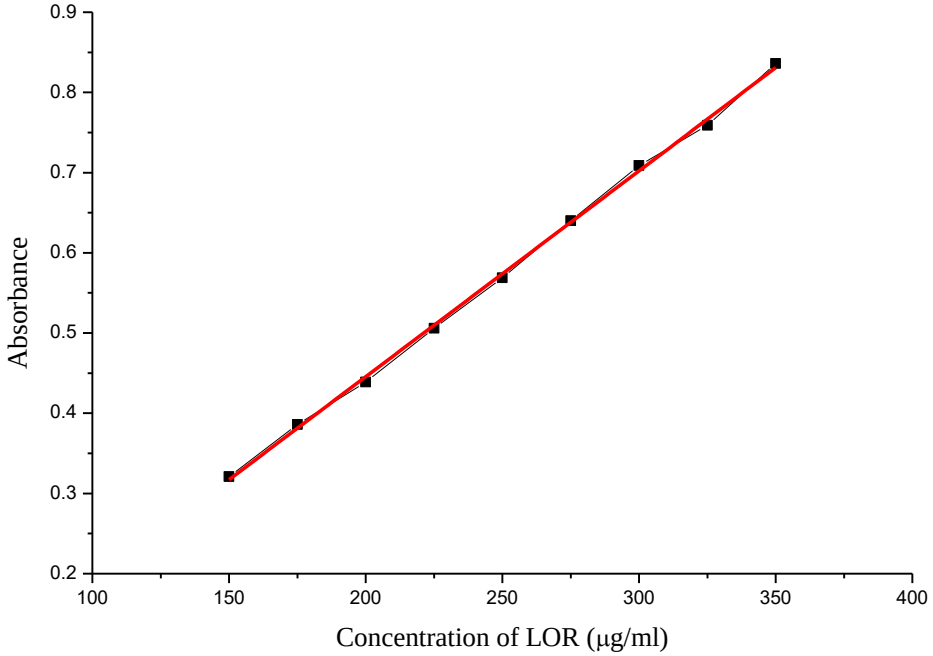

Figure 5: Beer's law limit for method B.

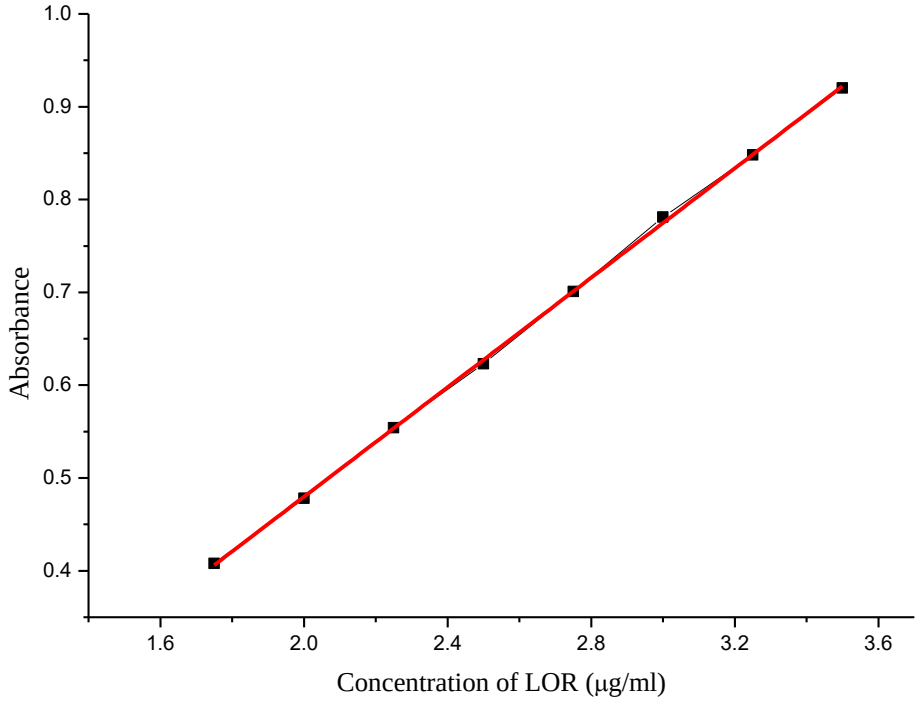

Figure 6: Beer's law limit for method C.

$$Amount\ of\ LOR(mg) = \frac{(B - S)MwR}{n}$$

where B = volume of thiosulphate consumed in the blank titration without drug, ml; S = volume of thiosulphate consumed in the sample titration, ml; M_w = relative molecular mass of LOR; R = molar concentration of bromate; n = number of moles of bromate reacting with each mole of LOR.

Spectrophotometric procedure using Methyl Orange (Method B)

Different aliquots (3.0-7.0mL) of 500 µg mL^{-1} LOR solution were accurately measured into a series of 10 mL calibrated flasks and the total volume was adjusted to 7.0 mL with distilled water. To each flask, added 1 mL of each 5 M hydrochloric acid and bromate-bromide solution (25 µg mL^{-1} w.r.t. $KBrO_3$). The content was mixed well and let stand for 10 min with occasional shaking. Then 1 mL of 100 µg mL^{-1} methyl orange solution was added to each flask and diluted to the mark with water. The absorbance of each solution was measured at 520 nm against a reagent blank after 5 min.

Spectrophotometric procedure using Methylene blue (Method C)

Table 3: Intra-day and inter-day precision and accuracy studies.

		Intra-day (n=7)			Inter-day(n=5)		
Method	LOR taken[a]	LOR found[a]	%RSD[b]	%RE[c]	LOR found[a]	%RSD[b]	%RE[c]
	2.0	2.06	1.26	3.00	1.98	1.59	1.00
A	4.0	3.92	2.05	2.00	3.89	1.84	2.75
	6.0	6.11	1.94	1.83	6.08	1.37	1.33
	200.0	200.18	1.62	0.09	199.91	1.46	0.05
B	250.0	249.89	1.58	0.04	249.93	1.98	0.03
	300.0	300.11	1.39	0.04	299.88	1.44	0.04
	2.0	2.03	2.11	1.50	1.96	1.87	2.00
C	2.5	2.47	1.35	1.20	2.53	1.56	1.20
	3.0	3.08	1.67	2.67	2.96	1.60	1.33

[a]Mean value of n determinations; The values were in mg for method A and µg mL^{-1} for method B and method C.
[b]Relative standard deviation (%)
[c]Relative error (%)

Table 4: Results of robustness and ruggedness.

Method	LOR taken[a]	Robustness (%RSD) Parameters interchanged		Ruggedness (%RSD)	
		Volume of 5M HCl[b] (ml)	Reaction time[c] (min)	Inter analysts (n=3)	Inter instruments (n=3)
A	5.0	1.53	1.38	1.53	2.08
B	250.0	1.79	1.67	1.41	1.23
C	2.5	1.06	1.54	1.38	1.96

[a]The values were in mg for method A and µg mL^{-1} for method B and method C.
[b]In method A, volumes of 5M HCl were 4.0, 5.0 and 6.0 ml and in method B and C, volumes of acid added were 0.8, 1.0 and 1.2 ml.
[c]In method A, the reaction times were 12, 15 and 18 min maintained during contact time while in method B and C, they were 9, 10 and 11 min.

Varying aliquots of standard LOR solution (3.5-7.0 mL; 5 µg mL^{-1}) were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 7 mL by adding distilled water. To each flask, 1 mL of 5 M HCl and 1 mL of bromate-bromide solution (75 µg mL^{-1} w.r.t. $KBrO_3$) were added. After mixing the content, the flasks were allowed to stand for 10 min with occasional shaking. Then, 1 mL of 120 µg mL^{-1} methylene blue solution was added to each flask and diluted to the mark with water. The absorbance was measured at 680 nm against a reagent blank after 10 min.
In method B and method C, a calibration graph was prepared by plotting absorbance versus concentration of LOR and the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Analysis of dosage forms

Twenty tablets each containing 10 mg of LOR were weighed accurately and pulverized. An amount of tablet powder equivalent to 100 mg was transferred into a 100 mL volumetric flask. The content was shaken well with about 25 mL of methanol for 15 min. The mixture was diluted to the mark with methanol. It was filtered using Whatmann No. 42 filter paper. First 10 mL portion of the filtrate was discarded and a 5 mL aliquot was subjected to analysis following the procedure described in method A. For method B and method C, the tablet solution (1000 µg mL^{-1} in LOR) was diluted appropriately with methanol to get 500 and 5 µg mL^{-1} LOR and suitable portions were used in the analysis by following the general spectrophotometric procedures described for pure drug.

Analysis of placebo blank and synthetic mixture

A placebo blank containing talc (250 mg), starch (300 mg), lactose (30 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (70 mg) and magnesium stearate (100 mg) was extracted with methanol and solution made as described under "analysis of dosage forms". A convenient aliquot of solution was subjected to analysis by titrimetry (method A) and spectrophotometry (method B and method C) according to the recommended procedures. A synthetic mixture was prepared by adding 100 mg of LOR to the placebo blank prepared above, homogenized and the solution was prepared as done under "analysis of dosage forms". The filtrate was collected in a 100-mL flask and a 5 mL aliquot was assayed by method A. The synthetic mixture solution (1000 µg mL^{-1} in LOR) was appropriately diluted to get 500 and 5 µg mL^{-1} solutions, and appropriate aliquots were subjected to analysis by method B and method C, separately.

RESULTS AND DISCUSSION

Reaction mechanism

The determination of LOR was based on bromination reaction by bromine generated *in situ* by the action of acid on bromate-bromide mixture. In titrimetry, the reaction was followed by back titration of the residual bromine iodometrically. The stoichiometry was expressed as the number of moles of bromate reacting with each mole of the

Table 5: Comparison of assay results of the reference and the developed methods.

Tablet brand name	Nominal amount, mg	Found % (of nominal amount ± SD) *		
		Reference Method	Proposed methods	
			Method B	Method C
Lorinol[a]	10	98.06 ± 0.29	100.09 ± 0.84 t = 1.52 F = 2.43	99.86 ± 1.37 t = 1.93 F = 2.25
Lorfast Meltab[b]	10	99.14 ± 1.35	100.13 ± 0.33 t = 1.21 F = 1.34	99.14 ± 1.63 t = 1.39 F = 2.42

Mean value of five determinations; [a]Manufactured by Micro labs Ltd., South Sikkim, India.; [b]Manufactured by Cadila Pharmaceuticals Ltd., India.; Tabulated t-value at the 95% confidence level is 2.78.; Tabulated F-value at the 95% confidence level is 6.39.

drug. Titrimetry was found to be applicable over the range 1-8 mg. Outside these limits, deviant inconsistent was obtained. The relationship between the titration end point and the amount of LOR was evaluated by calculating correlation coefficient, *r*, *via* the linear least square method and was found to be -0.9959, suggesting that the reaction between loratadine and bromate proceeds stoichiometrically in the ratio 1:0.666. Since 2/3 mole of bromate were consumed in the reaction, two moles of bromine were believed to have been used up for the bromination of the pyridine ring at 2nd and 4th position. The probable reaction scheme was shown in figure 2.

Loratadine has pKa=5. Due to its pKa value (weak acid) and weak basic pyridine ring, LOR reacted with both acidic and basic dyes. So loratadine is mainly in unionized form. In spectrophotometry, it was followed by change in absorbance of red colour of methyl orange at 520 nm (figure 3) or blue colour of methylene blue at 680 nm (figure 4), the change being caused by the bleaching action of bromine on the dyes. The discoloration was caused by the oxidative destruction of the dyes. Loratadine when added in increasing concentrations to a fixed concentration of insitu generated bromine, consumes the latter proportionately and there occurs fall in the concentaration of bromine. When a fixed concentration of dye was added to the decreasing concentration of bromine, an increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ_{max} was observed with increasing concentration of LOR. Thus, in situ generation of bromine is carried out using a mixture of potassium bromide and potassium bromate in the presence of HCl according to the following equation:

Absorption spectra

The resulting absorption spectra are due to the red colour of residual unoxidized methyl orange at 520 nm (Figure 3) or blue colour of residual unoxidized methylene blue at 680 nm (Figure 4).

Method development Titrimetry - Optimizations of critical response parameters

The reaction stoichiometry was found to be unaffected in the presence of 3-8 mL of 2 M HCl in a total volume of 23-25 mL and 5 mL was chosen as the optimum volume and better results and consistent stoichiometry were obtained in the preferred HCl medium than the other acid media studied (H_2SO_4, H_3PO_4 and CH_3COOH). The bromination reaction was found to be complete in 15 min and contact time up to 30 min had no effect on the stoichiometry or the results. A 10 mL volume of 5 mM bromate solution in the presence of a large amount of bromide was found adequate for quantitative bromination of LOR in the range investigated.

Spectrophotometry

Preliminary experiments were performed to fix the upper limits of the dye concentrations that could be measured spectrophotometrically and these were found to be 100 µg mL^{-1} and 120 µg mL^{-1} for methyl orange and methylene blue, respectively. A bromate concentration of 2.5 µg mL^{-1} was found to irreversibly destroy the red colour of 10 µg mL^{-1} methyl orange whereas 7.5 µg mL^{-1} bromate was required to bleach the blue colour due to 12 µg mL^{-1} methylene blue in acid medium. Hence, different concentrations of LOR were reacted with 1.0 mL of 25 µg mL^{-1} bromate in method B and 1.0 mL of 75 µg mL^{-1} bromate in method C in the presence large excess of bromide and in acid medium followed by the determination of the residual bromine as described under the respective procedures.

None of the acids (H_2SO_4, H_3PO_4 and CH_3COOH) showed precise and accurate results than HCl. Therefore hydrochloric acid was the medium of choice for the bromination of LOR. The absorbance of the dyes was not affected in 0.25-1.5 M hydrochloric acid concentration for method B and method C, respectively. However, since 1 mL of 5 M acid in a total volume of about 5.0 and 8.0 mL for method B and method C, respectively, was found sufficient to cause bromination of drug in a reasonable time of 10 min, respectively, the same concentration (0.5 M overall) was maintained for the determination of unreacted bromine with the dyes. The specified acid concentration for bromination reaction was not critical. The bromination reaction was found to be complete in 10 min for both methods B and method C, respectively and contact times up to 60 min had no effect on the absorbance of the dyes. A contact time of 5 min (method B) and 10 min (method C) was necessary for the bleaching of the dye colour by the residual bromine. The absorbance of either dye solution even in the presence of the brominated drug product was found to be stable for more than 12 hours under these optimized conditions.

Method Validation

Analytical parameters of spectrophotometric methods

Table 6 Results of recovery study by standard addition method

Tablet studied	Method A				Method B				Method C			
	LOR in tablet taken (mg)	Pure LOR added (mg)	LOR total found (mg)	Pure LOR recovered[a] (percent ± SD)	LOR in tablet taken (µg/ml)	Pure LOR added (µg/ml)	LOR total found (µg/ml)	Pure LOR recovered[a] (percent ± SD)	LOR in tablet taken (µg/ml)	Pure LOR added (µg/ml)	LOR total found (µg/ml)	Pure LOR recovered[a] (percent ± SD)
Lorinol (10 mg)	3.0	1.0	4.02	102.00 ± 0.29	200.0	25.0	225.13	100.52 ± 1.38	2.0	1.0	3.03	103.00 ± 0.59
	3.0	1.5	4.46	97.33 ± 2.83	200.0	50.0	249.89	99.78 ± 1.69	2.0	1.5	3.48	98.67 ± 2.13
	3.0	2.0	5.05	102.50 ± 0.56	200.0	75.0	275.12	100.16 ± 0.84	2.0	2.0	3.96	98.00 ± 2.84
Lorfast Meltab (10 mg)	3.0	1.0	3.98	98.00 ± 1.72	200.0	25.0	225.09	100.36 ± 2.06	2.0	1.0	2.97	97.00 ± 1.92
	3.0	1.5	4.53	102.00 ± 1.05	200.0	50.0	249.91	99.82 ± 2.34	2.0	1.5	3.52	101.33 ± 0.79
	3.0	2.0	5.07	103.50 ± 0.37	200.0	75.0	275.05	100.07 ± 1.81	2.0	2.0	4.04	102.00 ± 1.26

[a]Mean value of three determinations

A linear correlation (figure 5 and 6) was found between absorbance at λ_{max} and concentration of LOR in the ranges given in Table 2. The graphs were described by the regression equation:

$Y = a + bX$

(where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration of LOR in µg mL^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and the values were presented in Table 2. A plot of absorbance and concentration yielded straight lines with slopes equal to 0.9995 and 0.9998 for method B and method C respectively. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values[30] of both methods were also given in Table 2. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines[31] using the formulae:

LOD = 3.3 S/b and LOQ = 10 S/b

(where S is the standard deviation of blank absorbance values and b is the slope of the calibration plot) were also presented in Table 2. The high values of ε and low values of Sandell sensitivity and LOD indicate the high sensitivity of the developed methods.

Accuracy and precision

To analyse the accuracy and precision, the developed methods were repeated seven times within the day to determine the repeatability (intra-day precision) and five times on five different days to determine the intermediate precision (inter-day precision). These assays were performed for three levels of different concentrations. The results of this study were summarized in table 3. The percentage relative standard deviation (%RSD) values were ≤ 2.11% (intra-day) and ≤ 1.98% (inter-day) indicating high precision of the methods. Accuracy was evaluated as percentage relative error (%RE) between the measured mean concentrations and taken concentrations for LOR. Bias {bias % = [(Concentration found - known concentration) x 100 / known concentration]} was calculated at each concentration and these results were also presented in table 3. Percent relative error (%RE) values of ≤ 3% demonstrate the high accuracy of the proposed methods.

Selectivity

In all the three developed methods, results of placebo blank and synthetic mixture analyses revealed that the inactive ingredients used in the preparation had no role in the assay of active ingredient. To study the role of additives added to the synthetic sample, 4ml, 5 ml and 6 ml of the resulting solution was assayed (n=5) by titrimetry which yielded a % recovery of 99.24 ± 0.82, 98.69 ± 1.08 and 100.09 ± 0.59. The synthetic mixture analysis by spectrophotometric methods yielded percentage recoveries of 97.56 - 102.03 with %RSD values in the range 1.13 - 2.06. These results demonstrated the accuracy as well as the precision of the developed methods and complement the findings of the placebo blank analysis with respect to selectivity.

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of acid and contact time. The effect of these changes was studied on the absorbance of the developed methods. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (≤ 1.79%). Method ruggedness was expressed as %RSD of the same procedure applied by three different analysts as well as using three different instruments (burettes in method A and spectrophotometers in method B and C). The inter-analysts %RSD were within 1.53 whereas the inter-instruments RSD for the same LOR concentrations ≤ 2.08 suggesting that the developed methods were rugged.

Application to formulations

The proposed methods were applied to the determination of LOR in two representative tablets. The results in table 4 showed that the methods were successful for the determination of LOR and that the excipients in the dosage forms did not interfere. The results obtained were statistically compared with the reference method[11]. The results obtained by the developed methods agreed well with those of reference method by applying the Student's t-test for accuracy and F-test for precision and with the label claim. The calculated Student's t- value and F-value at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, for four degrees of freedom. Hence, no significant difference exists between the developed methods and the reference method with respect to accuracy and precision.

Recovery study

To further assess the accuracy of the developed methods, recovery experiments were performed by applying the standard-addition techniques. The recovery was done by determining the agreement between the measured standard concentration and added known concentration to the sample. The tests were done by spiking the pre-analyzed tablet powder with pure LOR at three different concentrations of the content present in the tablet powder (taken) and the total was found by the developed methods. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 97.00 and 103.50% with relative standard deviation in the range 0.29 - 2.84%. Closeness of the results to 100 % showed the good accuracy of the developed methods. The results are shown in Table 6.

CONCLUSIONS

Three useful methods for the determination of LOR using bromate-bromide mixture, titrimetry, methyl orange and methylene blue have been developed and validated according to ICH guidelines. The developed titrimetric procedure was simple since it was free from critical working conditions and does not use any expensive instrumentation. The developed spectrophotometric methods did not require any expensive equipment and specialized technicians. The developed methods were one of the most sensitive ever reported for LOR and were much simpler than the existing spectrophotometric methods with respect to optimum conditions. The developed methods depended on the use of simple and inexpensive chemicals. An additional advantage of the methods is that the measurement was made at longer wavelengths where the interferences from the co-formulated substances were far less than that at shorter wavelengths.

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