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Development of New Analytical Method and Its Validation for the Determination of Loratadine in Bulk and Marketed Formulation

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ABSTRACT

In the present work, simple, sensitive, rapid and accurate analytical methods have been developed for the estimation of Loratadine in bulk and pharmaceutical dosage form. Colorimetric method for Loratadine was based on reaction involving the formation of dark blue color complex between Loratadine and 0.02% crystal violet in the presence of 0.01M chloramine-T and 2M H_2SO_4, which obeyed Beer's law in the concentration range of 3-15 µg/ml at λmax of 601nm. The molar absorptivity and sandell's sensitivity was found to be 7.166×10^2 and 0.03846. The method was validated according to ICH guidelines. The regression equation was 0.027x + 0.001. The correlation coefficient was found to be 0.999.

Keywords: Loratadine, Chloramine-T, Crystal violet, H_2SO_4

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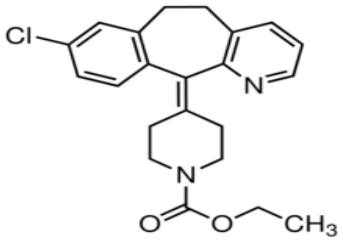
INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample [1].

Loratadine is a second-generation H1 histamine antagonist drug used to treat allergies. Structurally, it is closely related to tricyclic antidepressants, such as imipramine, and is distantly related to the atypical antipsychotic quetiapine. Loratadine is indicated for the symptomatic relief of allergy such as hay fever (allergic rhinitis), urticaria (hives), and other skin allergies. For allergic rhinitis (hay fever), loratadine is effective for both nasal and eye symptoms: sneezing, runny nose, itchy or burning eyes. Loratadine could be also used to treat mild to moderate pain from headaches [2].



IUPAC name of loratadine is ethyl 4-(8-chloro-5,6-dihydro-11 H benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate.Molecular formula is $C_{22}H_{23}ClN_2O_2$ and molecular weight is 382.88. It is insoluble in water, soluble in acetone, alcohol and chloroform [3,4].

MATERIALS AND METHODS

Loratadine was determined spectrophotometrically in bulk and marketed formulation by using crystal violet dye and chloramine-T as a strong oxidizing agent in presence of H_2SO_4.

Experimental [5-11]

Instrumentation

All experiments were performed in JASCO V-630 series UV spectrophotometer and Shimadzu 1700 with 1 cm path length matched glass cuvettes.

Preparation of standard stock solution of Loratadine

Standard stock solution was prepared by accurately weighing 100 mg of Loratadine in 100 ml calibrated volumetric flask and made up the volume with ethanol up to 100 ml to get conc. of 1000µg/ml.

Preparation of working standard solution of Loratadine

Working standard was prepared by transferring 10 ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with ethanol to get Conc. of 100µg/ml.

Preparation of Reagents

Preparation of 0.01M Chloramine-T solution

Weighed accurately 280 mg of Chloramine-T and transferred into 100 ml volumetric flask and made up the volume with distilled water.

Preparation of 2M H_2SO_4

Transferred 10.8 ml of concentrated H_2SO_4 into 100 ml volumetric flask and made up the volume with distilled water.

Preparation of Crystal violet (0.02%)

Weighed accurately 20 mg of crystal violet and added in 100 ml volumetric flask then diluted upto 100 ml with distilled water.

Determination of Absorption Maximum

An absorption maximum (or) max are the Wavelength at which maximum absorption takes place. It is important to know the absorption maximum of the substance under study, since it helps to avoid any interfering impurities.

Procedure

0.5 ml of 0.01M chloramine-T solution, 1 ml of 2M H_2SO_4, 0.9 ml of the Loratadine working standard stock solution were added to the 10 ml volumetric flask. It was kept aside for 10 minutes. 0.2 ml of 0.02% crystal violet was added in each volumetric flask and kept aside for 10 minutes for completion of reaction and made up the volume with ethanol. Absorbance against reagent blank was recorded. These solutions were scanned in UV spectrophotometer between 400-800 nm. Graph was recorded in figure no.1.

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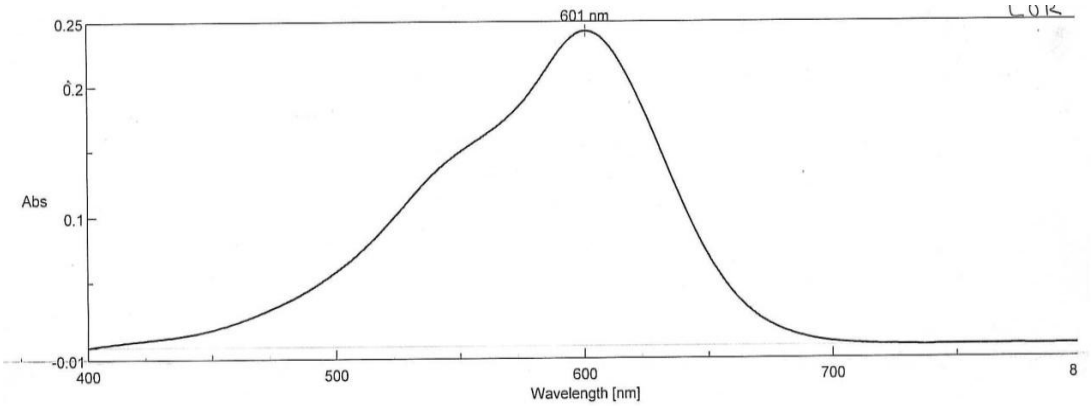

Figure no. 1: λmax graph for Loratadine with chloramine-T and crystal violet

Model: JASCO V-630
Band width: 1.5 nm
Response: Medium
Measurement: 800-400 nm
λmax: 601 nm
Absorbance: 0.246

Study of Beer-Lambert's Law

Standard curve was prepared by using pure Loratadine in the conc. range of 3-15 µg/ml by this method and selecting absorbance maximum at 601 nm.

Procedure

0.5 ml 0.01 M chloramine-T solution, 1 ml 2M H_2SO_4 and 0.3, 0.6, 0.9, 1.2 and 1.5 ml of working standard of Loratadine were taken in 5 volumetric flasks of 10 ml and kept aside for 10 minutes. 0.2 ml of 0.02 % of crystal violet solution was added and kept aside for 10 minutes for completion of reaction. Make up the volume with ethanol. Absorbance was taken against reagent blank at 601 nm. The result was recorded in table no.1 and figure no.2

Table no.1: Absorbance of different concentrations of Loratadine at 601nm

Sr. No.	Vol. Of working standard drug	conc. of drug (µg/ml)	Absorbance
1	0.3 ml	3	0.078
2	0.6 ml	6	0.165
3	0.9 ml	9	0.247
4	1.2 ml	12	0.326
5	1.5 ml	15	0.407

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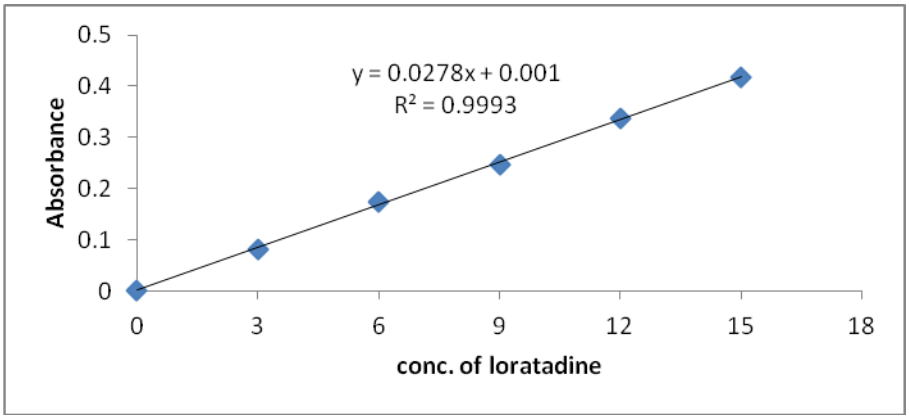

Figure no.2: Standard Curve and linearity for Loratadine

Analysis of Marketed Formulation

Loratadine is marketed as Alaspan of 10 mg tablet manufactured by Encore health care were taken for analysis.

Reagent and chemicals

- Working standard stock solution (100µg/ml)
- 0.01M Chloramine-T solution
- 2M H_2SO_4
- 0.02% Crystal violet

Preparation of sample solution

10 tablets were weighed and crushed properly using a mortar and pestle. Then powder weight equivalent to 100mg was weighed and transferred to 100ml of volumetric flask and dissolved in ethanol and filtered through whatmann filter paper in to another 100ml volumetric flask and made up to mark with same diluent which give the solution of 1000µg/ml conc. Again 10 ml of solution was taken in 100 ml volumetric flask to get conc.,of 100µg/ml. Further dilution was performed to get a concentration of 10µg/ml. The result was recorded in table no.2

Table no.2: Assay result of marketed formulation of Loratadine

Sample	Labelled amount	Amount found	% Recovery
Loratadine	10 mg	9.876 mg	98.76 %

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METHOD VALIDATION

Linearity

A linear relationship should be evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance (by dilution of a standard stock solution) and using the proposed procedure. This method obeys the Beer- Lambert's law in the concentration range of 3-15 µg/ml.

Accuracy of recovery studies

The accuracy of the methods was determined by calculating % recovery of Loratadine by standard addition method. Known volumes of standard solutions of Loratadine were taken for recovery studies in 3 different levels 50%, 100%, 150% and recovery study was carried out. The result was recorded in **table no.3**

Table no.3: Accuracy study of Loratadine

Drug	Amount Present In Formulation (µg/ml)	Amount Added (%)	Amount Recovered µg/ml	% Recovery
Loratadine	10	-	9.876	-
		50	4.96	99.20%
		100	9.902	99.02%
		150	14.83	98.87%

Method precision (% Repeatability)

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 6 µg/ml without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD). Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable**.** The result was recorded in table no.4

Table no.4: Method Precision (% Repeatability) of Loratadine

Conc. in µg/ml	Absorbance
9	0.246
9	0.247
9	0.249
9	0.244
9	0.245
9	0.247
Mean	0.246
SD	0.001788
%RSD	0.7271 ± 0.001465

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Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:

$LOD = 3.3\ \sigma/S$
$LOQ = 10\ \sigma/S$

Where, σ = the standard deviation of the response,
S = slope of the calibration curve.

The result was recorded in table no.5

Table no.5: LOD and LOQ for Loratadine

Drug	LOD (µg/ml)	LOQ (µg/ml)
Loratadine	0.2185	0.6622

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed methods were performed by analysing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Loratadine(9,12,15 µg/ml). The results were reported in terms of relative standard deviation (RSD). The result was recorded in table no.6 and table no.7

Table no.6: Intermediate Precision (Interday) Of Loratadine

Conc. (µg/ml)	Day	Absorbance	Mean (X)	%RSD = 100 SD/X	Acceptance criteria
9	1	0.247	0.247	1.032 ±0.00294	% RSD < 2
	4	0.249			
	7	0.244			
12	1	0.328	0.326	0.6135 ± 0.00230	
	4	0.326			
	7	0.324			
15	1	0.406	0.403	0.6563 ± 0.00305	
	4	0.402			
	7	0.401			

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Table no.7: Intermediate Precision (Intraday) Of Loratadine

Conc. (µg/ml)	Time	Absorbance	Mean (X)	%RSD = 100 SD/X	Acceptance criteria
9	2	0.247	0.244	1.1948 ± 0.00336	% RSD < 2
	4	0.244			
	6	0.240			
12	2	0.328	0.326	0.5313 ± 0.0020	
	4	0.327			
	6	0.325			
15	2	0.406	0.405	0.3904 ± 0.00182	
	4	0.405			
	6	0.403			

Table no.8: Statistical data for Loratadine by colorimetric method

Parameter	Result
λmax (nm)	601 nm
Beer's law limits (µg/ml)	3-15 µg/ml
Sandell's sensitivity (µg.cm^2/ 0.001 Au)	0.03846
Molar absorptivity (1/mol.cm)	7.166×10^2
Regression equation (y=a+bc) Slope (b) Intercept (a)	 0.027 0.001
Correlation coefficient(R^2)	0.999
% Recovery	98.76%
%RSD	0.7271
LOD	0.2185
LOQ	0.6622

RESULTS AND DISCUSSION

The objective of the proposed work was to develop new analytical methods for the determination of Loratadine and validate the methods according to ICH guidelines and applying the same for its estimation in marketed formulation.

Developed colorimetric method was found to be rapid, simple, precise, accurate and economic for routine estimation of Loratadine in commercial dosage forms.

Estimation of Loratadine is based on oxidation reaction. In this method chloramine- T is used as oxidizing agent in presence of H_2SO_4. After completion of reaction known quantity of crystal violet is added. Part of crystal violet is oxidized by reacting with excess of chloramine-T and remaining part gives dark blue color. Color of the solution indicates the amount of drug present. The solution was analysed at λmax 601 nm.

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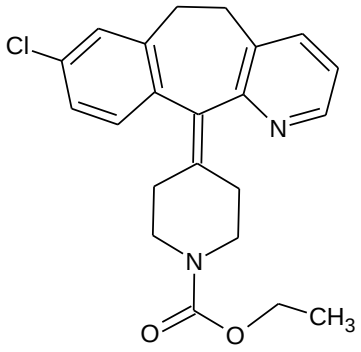
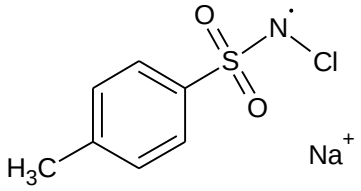
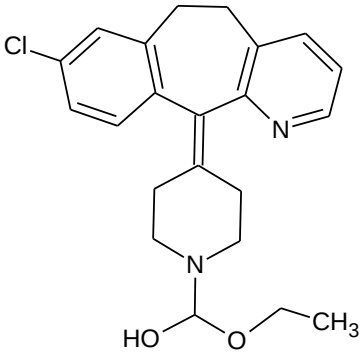


Loratadine

+

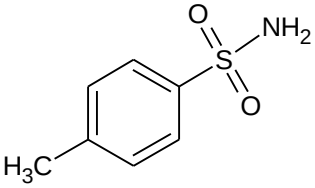


Chloramine T

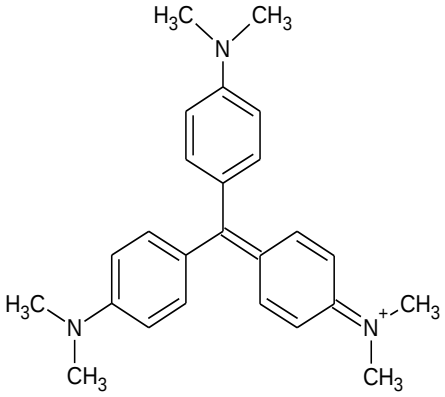


p- toluene sulphonamide

+

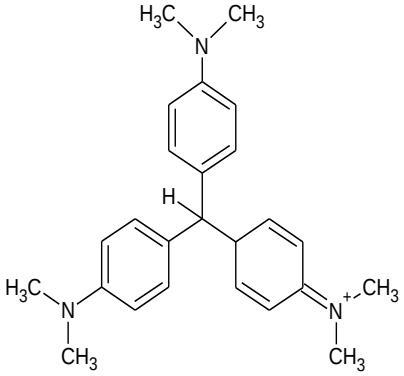


+ NaCl



Crystal violet
(colored)

Unreacted
chloramine T



Leuco form (CV)

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analyzing large number of samples in a short time period with due accuracy and precision.

A very few analytical methods appeared in the literature for the determination of Loratadine includes HPLC, HPTLC, and UV-Visible spectrophotometric methods. In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical.

In the present investigation, colorimetric method for the quantitative estimation of Loratadine in bulk drug and pharmaceutical formulations has been developed.

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