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ANALYTICAL METHOD DEVELOPMENT AND HPLC METHOD VALIDATION FOR CHLORPHENIRAMINE MALEATE IS AN ACTIVE PHARMACEUTICAL INGREDIENT

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ABSTRACT

Design, synthesis of chlorphenamine maleate has one asymmetric carbon atom, exists as racemic mixture of R & S forms and does not show optical rotation. It is a histamine H1 receptor antagonist used as an antihistamine. The first method of drug which involves absorbance measurement at 235 nm and Linearity was obtained in the range of 2-30 µg/mL. Chromatographic separation was achieved using HPLC and the method was carried out on a kromasilC_{18} column has been developed with a mobile phase consisting of (10:90 v/v) methanol: 1 mL of hydrochloric acid in 2 L of water (pH 5.3 with orthophosphoric acid) at flow rate of 1.0 mL /min using UV detection at 235nm and The retention times of was about min 8 min. The described method was linear over a concentration range of 200-500 µg/mL with a correlation coefficient of 0.999 and the accuracy between 98.12-101.53%. The method was successfully applied for the estimation of drugs in formulation with high accuracy. Thus the developed method is simple, rapid, precise, selective, reproducible and accurate which is useful for the determination of of Chlorpheniramine Maleate in API.

KEYWORDS: HPLC, Chlorpheniramine, Maleate, 2-chloro-N,N-dimethylethanamine.

INTRODUCTION

Antihistamines are pharmaceutical agents which act by stimulating histamine action in the H1-receptors, thereby antagonizing most of the smooth muscles to alleviate or prevent the symptoms of hay fever and other allergies and put a stop to motion sickness, nausea,

vomiting, and dizziness. In addition, since antihistamines may cause drowsiness as a side effect, some of them may be used as an opponent to insomnia. Some antihistamines are used in the handling of nervous and emotional conditions to help control anxiety and to relax patients before surgery.[1] The less sedating behavior of new antihistamines have led to higher doses, which may contribute to asthma therapy by increasing vascular permeability.[2–6] Chlorphenamine, a histamine H1 receptor antagonist has been proven to reverse chloroquine resistance in Plasmodium falciparum[7] and is recommended for runny noses and seasonal allergies. Although cetirizine and levocetirizine are both important second generation antihistamines, their study has revealed that the antihistaminergic activity of the racemate is primarily due to levocetirizine.[8]

Chlorpheniramine maleate (CPM), (R/S)-3-(4-chlorophenyl)-N,N-dimethyl-3-(pyridin-2-yl)propan-1-amine maleate 2-chloropyridine (Fig. 1)[9] is a first-generation alkyl amine antihistamine, act by antagonizing H1-receptors. It is commonly used in pharmaceutical preparations for symptomatic relief of the common cold and allergic rhinitis with mild sedative property.[10] It is commonly formulated as tablets, injections and syrups as single component preparations and is one of the popular ingredients in other formulations such as cough remedies and creams. Numerous UV, HPLC and HPTLC based methods have been reported[11-16] and NMR spectroscopy,[17] polarographic method,[18] electrokinetic chromatography,[19] for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms. But no method had yet been reported for simultaneous estimation of these two drugs using HPLC in bulk drug and pharmaceutical dosage forms. Therefore, the present work was aimed to new developed synthesis and validate a new HPLC method for estimation of CPM in pharmaceutical dosage forms.

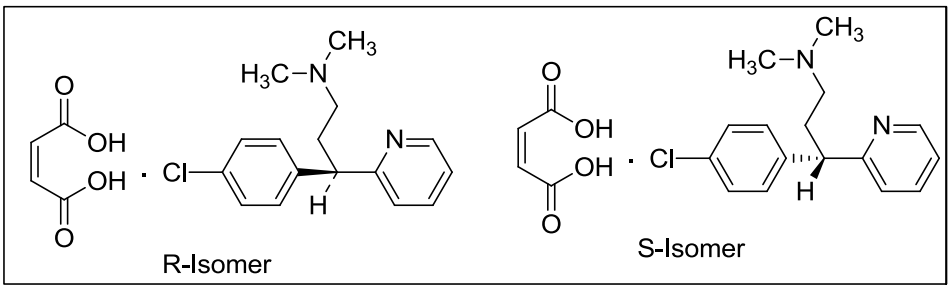
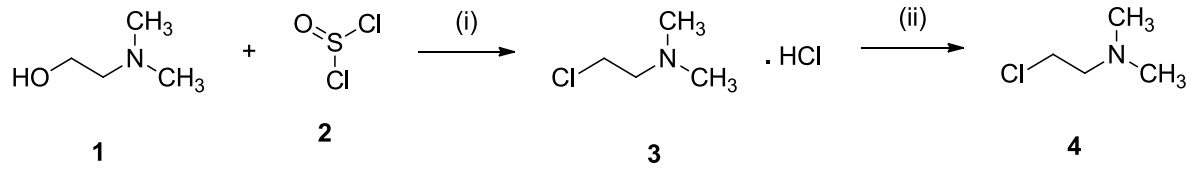
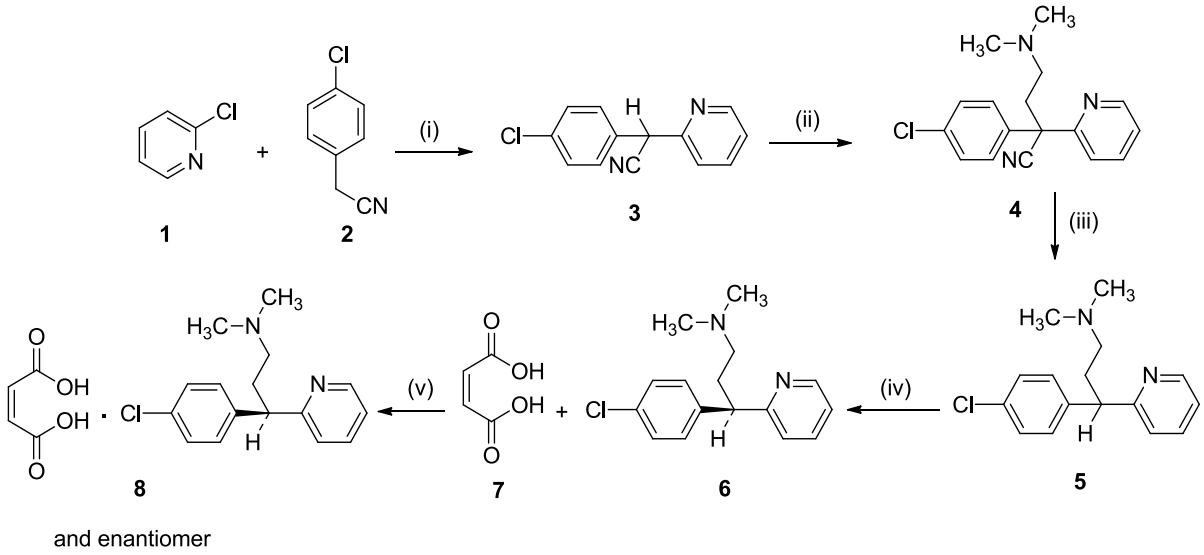


Figure 1: Chemical structures of R/S-chlorpheniramine maleate.



1 2 3 4

Scheme 1: Reaction conditions: (i) O–Xylene, MeOH, reflux, 5 h (ii) NaOH (Lye) + H_2O, O–Xylene, Na_2SO_4, reflux, 4 h.



1 2 3 4 5 6 7 8

and enantiomer

Scheme 2: Preparation of target R and S configurations (8).

Reagents and conditions: (i) $NaNH_2$, O–xylene, MeOH, reflux, 7 h (ii) MeOH-H_2O, 2-chloro-N,N-dimethylethanamine, rt, 8 h (iii) KOH, H_2O, rt, 6 h (iv) High vacuum distillation (v) Isopropanol, reflux, 4 h, yield 89%.

EXPERIMENTAL SECTION

All the chemicals were purchased from commercial suppliers and were used without further purification. All reactions were performed under inert nitrogen atmosphere employing dry solvents. Precoated TLC silica gel plates (Kieselgel 60 F254, Merck) were used for monitoring reactions and the spots are visualized under UV lamp (254 nm). Purification was performed by column chromatography using silica gel (particle size 60-120 mesh, Merck). Melting points were determined in open capillary tubes on cintex melting point apparatus and are uncorrected. IR (KBr) spectra were recorded on a Perkin–Elmer 400 FTIR spectrometer (υ_{max} in cm^{-1}) or a Varian 670-IR FT-IR spectrometer (ATR) in the frequency range of 600-4000 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Bruker DRX-300 (300 MHz FT NMR) or Varian Mercury 500 MHz spectrometer. Proton chemical

shifts are presented in δ ppm with reference to TMS. *J* values are presented in Hz. Mass spectra were recorded using Jeol SX-102 spectrometer.

Chlorphenamine maleate is synthesized in four step process accompanied by the usual centri fugation, drying, pulverizing and sifting operations.

Preparation of 2-chloro-N,N-dimethylethanamine **(4)**

2-(dimethylamino)ethanol (**1**) (DMAE) (2 g, 1.3 mol) is reacted with thionyl chloride (**2**) (0.12 g, 0.05 mol) in presence of anhydrous methanol (10 mL), O-xylene (5 mL) in reflux conditions for 5 h to gave 2-chloro-N,N-dimethylethanamine hydrochloride (**3**) (DMC. HCl). Thus, the obtained compound **3** (1.8 g, 1.2 mol) is treated with caustic soda lye in presence of O-xylene (8 mL) at reflux for 4 h to obtained intermediate 2-chloro-N,N-dimethylethanamine (**4**) (DMC Base).

Synthesis of (R)-3-(4-chlorophenyl)-N,N-dimethyl-3-(pyridin-2-yl)propan-1-amine maleate

2-chloropyridine (**1**) is reacted with 2-(4-chlorophenyl)acetonitrile (**2**) in presence of sodium amide followed by condensation with 2-chloro-N,N-dimethylethanamine (DMC) base in anhydrous condition, in presence of O-xylene, at controlled temperature. The cyano base **4** thus obtained is further decyanated with caustic potash flakes to yield crude chlorphenamine base (**6**) in good yield. The crude chlorphenamine base is then purified to pure chlorphenamine base by high vacuum distillation to obtained R-isomer of chlorphenamine base, further it can be treated with different acids in presence of isopropanol is mixed with maleic acid and reflux condition for 4 h to gave different substituent acid enansiomers, dried with 89 % yield its salt.

(R)-3-(4-chlorophenyl)-N,N-dimethyl-3-(pyridin-2-yl)propan-1-amine maleate: Crystalline white solid, mp. 130-135 °C; IR (KBr, υ_{max}, cm^{-1}): 864.14, 883.43, (maleate), 1356 (C-O str), 1473.66 (C=N str), 1570.11 (C=N str), 1586.54, 1618.33 (Ar-C=C str), 1703.20 (C=O str), 2436.18-2692.72 (N^{+}-H str), 2943.47, 2960.50 (CH str), 3066.92 (Ar-CH str); ^{1}H NMR (300 MHz, D_2O): δ_{ppm} 8.37–8.39 (m, 1H, Py-H), 7.73–7.78 (m, 1H, Py-H), 7.25 (s, 4H, Ar-H), 7.20–7.36 (m, 2H, Py-H), 6.17 (s, 2H, COOH), 4.79 (t, 1H, CH), 2.41 (m, 4H, CH_2), 2.81 (s, 6H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.10, 148.22, 139.93, 138.58, 134.72, 132.26, 129.10, 128.80, 123.33, 122.83, 56.09, 49.06, 42.80, 28.60; ESI-Ms m/z 230.0 $(M+H)^{+}$.

Elemental analysis

The elemental analysis of Chlorphenamine Maleate EPCRS and Working Standard was carried out for determination of carbon, hydrogen, nitrogen and oxygen. The carbon, hydrogen, nitrogen and oxygen analysis was carried on CHNS-O Analyser Flash EA 1112 Series (please see supporting data).

Table 1: Elemental analysis of Chlorphenamine Maleate.

Elements	% Carbon	% Hydrogen	% Nitrogen	% Oxygen
Theoretical Values	61.45	5.93	7.17	16.39
EPCRS	61.40	5.70	7.31	16.23
Chlorphenamine Maleate	60.42	5.74	7.16	16.33

Reagents and Chemicals

DMC used the standard as such and use % potency on as is basis for calculations and obtained from reputed companies, formulation tablets were purchased from local pharmacy. Hydrochloric Acid (AR grade), Purified water (Milli Q or Equivalent), Methanol (HPLC Grade), water and Acetonitrile were purchased from Merk specialties pvt limited, orthophosphoric acid (Merck), HCl (Merck), NaOH (Merck), H2O2 (S.D.Fine), Sodium bisulphate (S.D.Fine) Mumbai. 0.45µm nylon membrane filter papers were obtained from Pall Life Sciences, Mumbai. A combined dosage tablet MIGNAR MF was purchased from local market.

Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system (Hamilton PRP X 100, 250 x 4.1 mm, 10µ) equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Determination of maximum absorbance

The standard solution of Chlorphenamine Maleate was scanned in the range of 200-400 nm against mobile phase as blank. CPM shows maximum absorbance at 235 nm. Thus the wave length selected for the determination of CPM is 235 nm (Fig. 7).

Preparation of standard stock solution

Weigh and transfer accurately about 50 mg of DMC working standard into 25 mL volumetric flask. Add 15 mL of Mobile Phase and sonicate to dissolve and dilute to volume with Mobile Phase. Dilute 1 mL of this solution to 100 mL with Mobile Phase (Concn. 20ppm). Weigh and transfer accurately about 500 mg of Chlorpheniramine Maleate sample into a 50 mL volumetric flask. Add 35 mL of Mobile Phase and sonicate to dissolve. Dilute to volume with Mobile Phase. (Concn. 10000 ppm). Inject equal volumes of Mobile Phase in sample and standard solution in six replicate injections. The Retention time of DMC is about 1.6 min. The test is not valid unless the Relative standard deviation of area counts of DMC for replicate injections of standard solution should not be more than 5.0%.

Preparation of Mobile phase; Mobile Phase A: Mix 1 mL of hydrochloric acid in 2 L of water, Mobile Phase: Mix Mobile Phase A and Methanol in the ratio (90:10). Filter and degas. **Chromatographic Condition**: Column: Hamilton PRP X 100, 250 x 4.1 mm, 10µ; Detection: Refractive Index; Sensistivity at 256; Internal Temp. 30 °C; Injection Volume: 20 µL; Flow Rate : 1.0 mL / minute; Run Time : 8 min; Column Oven Temp 60 °C;

RESULTS AND DISCUSSION

Validation of the method

Validation of the optimized HPLC method was carried out with respect to the following parameters.

System suitability

A system suitability test of the chromatography system was performed before each validation run. The system suitability test was applied to this drug Chlorpheniramine Maleate to check the various parameters such as tailing factors, resolution, theoretical plates, and repeatability against the specifications set for the method. The tailing factor was within ≤ 2, theoretical plate number of ≥ 2000, which satisfied the acceptance criteria.

Linearity and range

Linearity of Chlorpheniramine Maleate detector response of assay method was found by injecting seven standard solutions with concentration ranging from 2 µg/mL to 30 µg/mL for Metformin of the test concentration and a graph was plotted for concentration versus peak area. Good linear relation was observed within the concentrations and the study. Regression equation was found to be y=1062x-23325 (r^2=0.999) for Chlorpheniramine Maleate. Y=slope, m=intercept, c=correlation coefficient. The results were shown in **Table 2, Fig 2.**

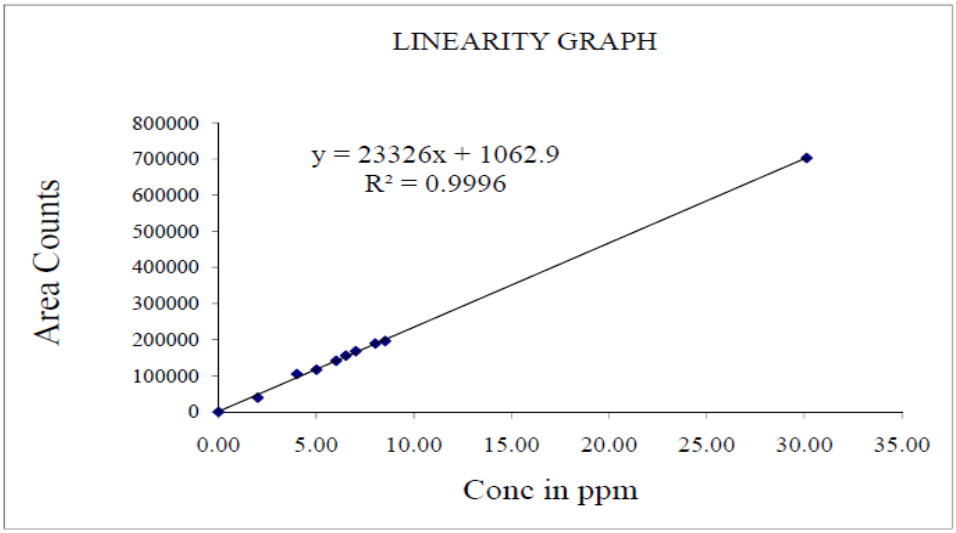


Figure 2: Linearity of sample (CPM).

Table 2: Linearity of chlorpheniramine maleate

		Chlorpheniramine Maleate	
Sr. No	**Level**	**Concn (µg/mL)**	**Response (Area Counts)**
	Linearity-1	2.01	
	Linearity-2	4.02	
1	Linearity-3	5.02	39368
2	Linearity-4	6.02	104856
3	Linearity-5	6.53	116647
4	Linearity-6	7.03	141238
5	Linearity-7	8.03	155772
6	Linearity-8	8.53	167717
7	Linearity-9	30.12	189268
8			196118
9	Slope	23325.66	702905
	Intercept	1062.91	
	Correlation Coefficient	0.99969	

Acceptance Criteria: Correlation Coefficient should not be less than 0.99.

Precision

The method was checked by injecting six replicate injections of the solution 350 µg/mL and 35 µg/mL of chlorpheniramine maleate and the RSD was found to be 1.68% and 6.73% values respectively. Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter-day precision). The results obtained for intra-day precision (RSDs) were 1. 68% and 6.73%. The results were shown in **Table 3.**

Table 3: System Precision.

	CPM (%w/w) As such sample	CPM (Standard Area Counts)
# Injection		
1	0.008	175621
2	0.008	170692
3	0.008	170472
4	0.007	171488
5	0.007	177657
6	0.008	173571
Mean	0.008	173250
SD	0.0005	2915.87
% RSD	6.735	1.683

Acceptance Criteria: RSD should not be more than 10.0 % and 5.0% respectively.

Accuracy

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% levels of standard concentration for CPM. The solutions were analyzed in triplicate at each level as per the proposed method. % recovery for each case was calculated and was found to be within the acceptance criteria of 98-101% for both drugs. This showed that the recoveries of chlorpheniramine maleate by the proposed methods are satisfactory. RSD accepted within the limit of 2. The results are shown in **Table 4.**

Table 4: Results for accuracy of chlorpheniramine maleate.

S.NO	% Recovery	Concentration in µg/mL			Amount Found	% recovery	RSD
		Target	Spiked	Total			
1	50%	200	100	300	294.55	98.19	0.65
2		200	100	300	298.42	99.47	
3		200	100	300	296.65	98.88	
4	100%	200	200	400	399.83	99.95	0.78
5		200	200	400	394.96	98.74	
6		200	200	400	394.08	98.52	
7	150%	200	300	500	507.67	101.53	1.71
8		200	300	500	490.63	98.12	
9		200	300	500	497.87	99.57	

Ruggedness

Ruggedness was performed by the analyst to analyst variability, the percent relative standard deviation (% RSD) was calculated. The value of percentage RSD was below 2.0%. Acceptable RSD was obtained. The results are shown in **Table 5.**

Table 5: Result for ruggedness of chlorpheniramine maleate.

S.NO	CPM
1	1271782
2	1274409
3	1271736
4	1253883
5	1271272
6	1258271
RSD	0.67

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, wavelength, pH. The changes are tested ±5. The results are obtained small variations which do not show influence in the results indicates that the proposed method is robustness. The results are shown on the **Table 6.**

Table 6: Results for robustness of chlorpheniramine maleate.

S.No	Condition	Change	CPM	
			Area	% Change
1	Standard	……..	1266430	……..
2	MP 1	45:55(v/v)	1281012	1.15
3	MP 2	35:65(v/v)	1250273	1.28
4	WL 1	240nm	1282037	1.23
5	WL 2	236nm	1280197	1.08
6	pH 1	5.2	1274709	0.65
7	pH 2	5.4	1255694	0.84

Limit of Detection and Quantification Limit

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. Based on determination of Prediction linearity, six replicate injections were made for LOD and LOQ. The results are shown on the **Table 7.**

Table 7: Limit of Detection and Limit of Quantification.

Chlorpheniramine Maleate		
	LOD	**LOQ**
1	16020	29273
2	15437	31399
3	17740	29955
4	17750	33674
5	17591	31750
6	19360	30472
Mean	17316	31087
SD	1402.28	1560.75
% RSD	8.098	5.021
% w/w	0.02	0.03

Formulation

The prepared concentration of the tablet solution was injected into the HPLC. The resulting peak areas were compared with the standard peak areas and the assay was calculated for the method. % assay was found to be 99.51% for chlorpheniramine maleate. High % assay of more than 99.5% was found for the both drugs. Hence the method can successfully apply for the simultaneous estimation of chlorpheniramine maleate in pharmaceutical formulation. Results of the formulation analysis were shown in **Table 8.**

Table 8: Result for formulation of Chlorpheniramine Maleate.

S.NO	Drug	Brand	Dosage	Amount Prepared	Amount Found	%Assay
1	Chlorpheniramine Maleate	MIGNAR MF	500mg	350	348.317	99.51

System stability

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommendation of storage conditions, and retests periods to be established. The standard samples of Chlorpheniramine Maleate does not change their concentration up to 24 h. After the 24 h concentration will be changed for both the drugs. The results were shown in **Table 9.**

Table 9: Results for stability.

Time in hrs	Area % Assay	
	For CPM	for CPM
0	400718	100
1	398745	99.50
2	399548	99.70
4	398547	99.45
6	395325	98.65
12	397145	99.10
18	396231	98.90
24	394988	98.56
26	389547	97.21
28	385478	96.20

Table 10: X-Ray Powder Diffraction Pattern of Chlorphenamine Maleate.

2θ	d (Å)	I/I°
13.057	6.7751	57.7
18.821	4.7111	21.2
19.341	4.5855	100.0
20.297	4.3716	99.7
21.921	4.0514	28.9
24.163	3.6802	39.5
24.719	3.5988	20.5
25.040	3.5534	25.5
25.725	3.4602	28.4
26.319	3.3835	35.0

Chromatograms

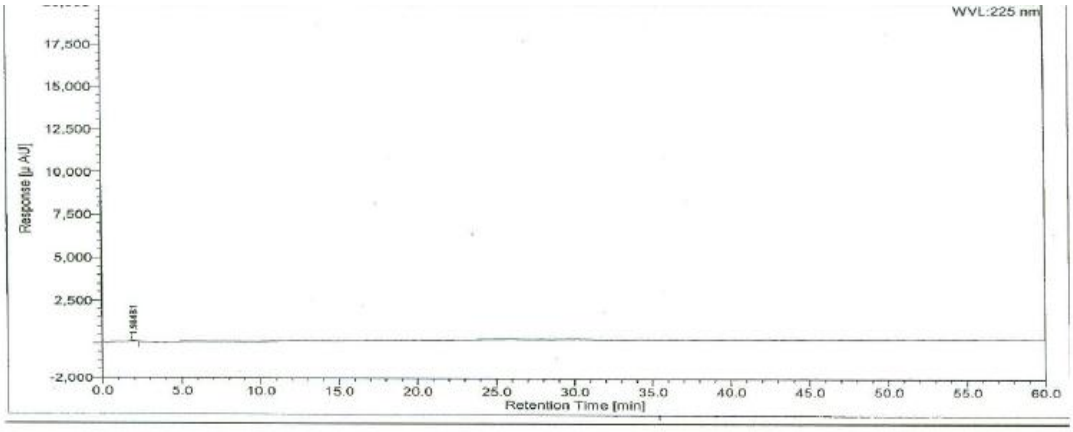


Figure 3: Representative Blank Chromatogram of sample Chlorpheniramine Maleate.

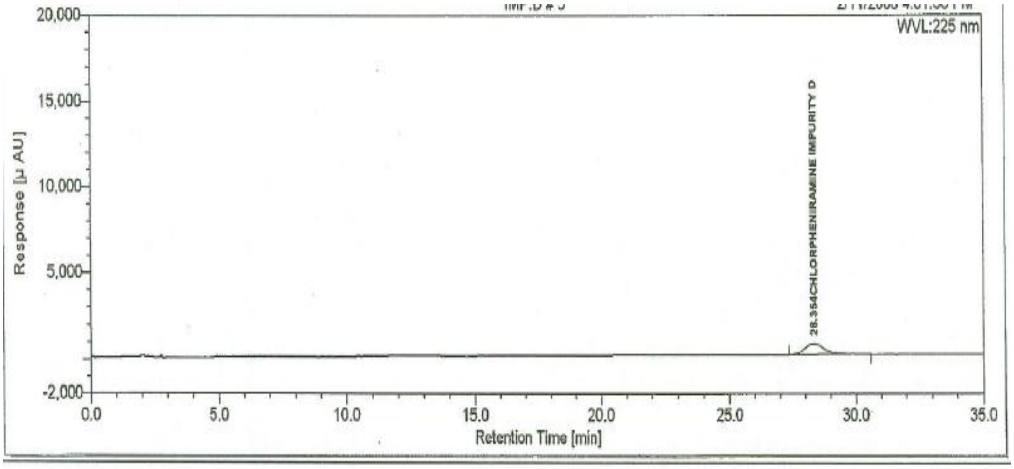


Figure 4: Representative Blank Chromatogram of sample Chlorpheniramine Maleate

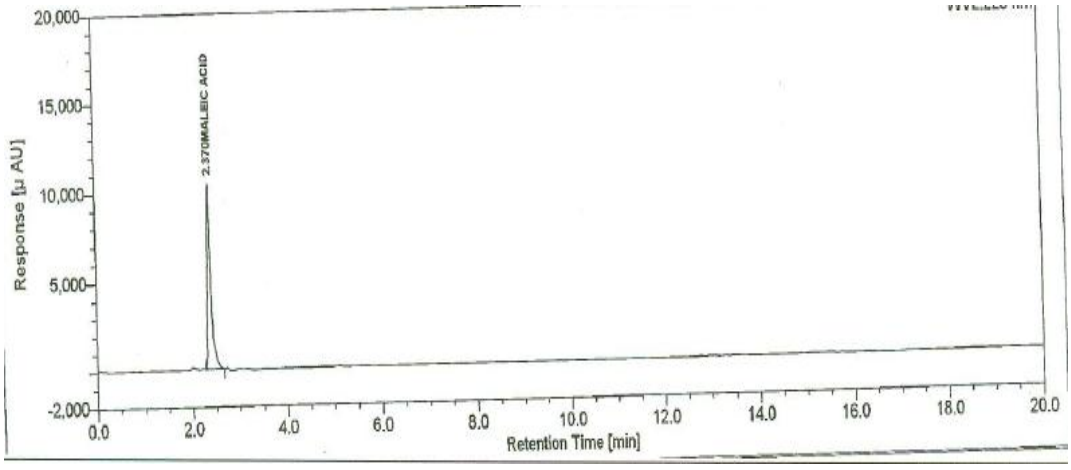


Figure 5: Representative Blank Chromatogram of sample Maleic acid.

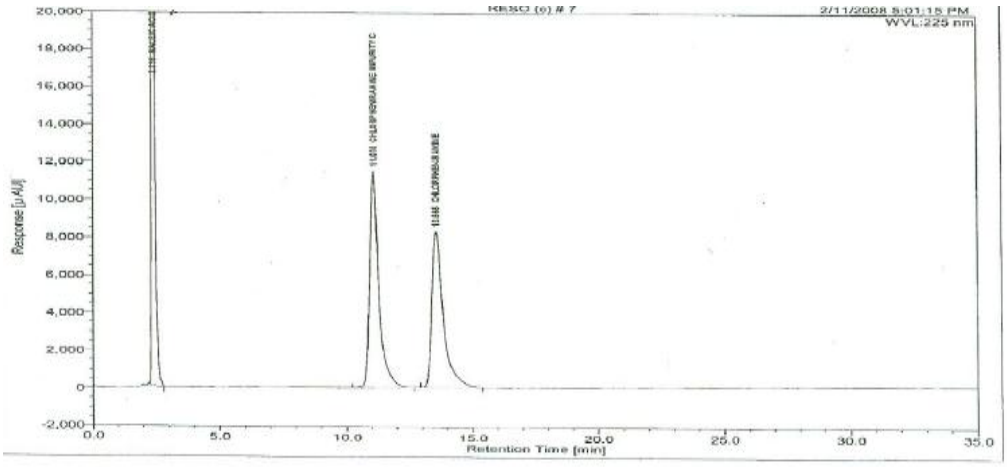


Figure 6: Representative chromatogram of sample with reference solution chlorpheniramine.

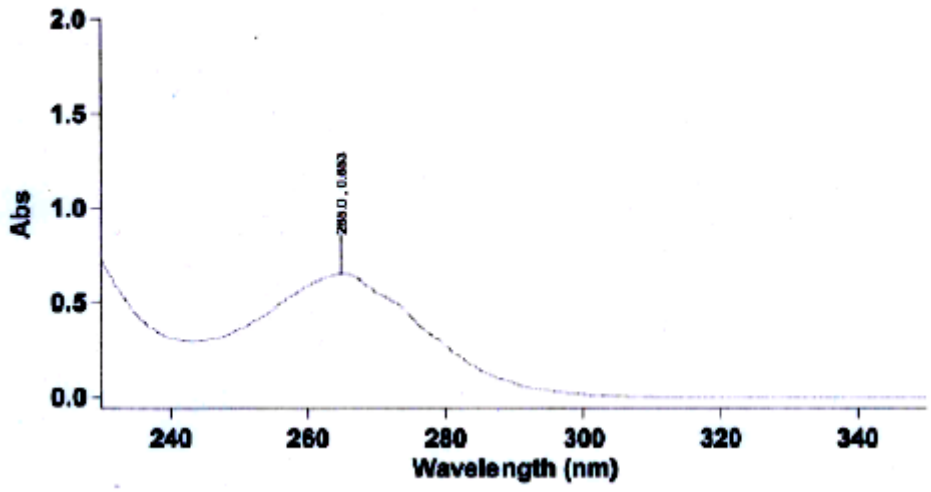


Figure 7: UV spectrum of Chlorphenamine Maleate.

CONCLUSION

The synthesis of new method developed CPM preparation is a simple, rapid, accurate, and reliable procedure for the HPLC analysis of R/S-chlorphenamine maleate in histamine action in the H1-receptors, meeting all requirements for the validation of an analytical methodology. The rapid, single step, histamine preparation coupled with the simple HPLC–UV isocratic chromatographic apparatus makes the method cost-effective and suitable for analysis of a large number of samples. The method was fully validated to meet the requirements for absorbance, sensitivity, accuracy, linearity, stabilty and precision which is useful for the determination of chlorpheniramine maleate in active pharmaceutical ingredient.

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