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UPLC METHOD DEVELOPMENT & VALIDATION OF CHLORPHENIRAMINE & TRITHIOPARAMETHOXY PHENYLPROPENE IN ITS BULK & TABLET DOSAGE FORMS – A STABILITY STUDIES

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Abstract: Analytical Method Development and Validation for Chlorpheniramine andTrithioparamethoxy Phenylpropene in bulk and Combined Dosage Form RP- UPLC, Newmethod was established for simultaneous estimation of Chlorpheniramine and Trithioparamethoxy Phenylpropene by RP-UPLC method. The chromatographic conditions were successfully developed for the separation of Chlorpheniramine and Trithioparamethoxy Phenylpropene by using BEH C18 (2.1 × 50 mm, 1.7 µm) particle size, flow rate was 1.0 ml/min, mobile phase ratio was Methanol: TEA buffer: ACN (50:25:25 v/v) 3.8 (pH was adjusted with orthophosphoric acid), detection wavelength was 210nm. The retention times were found to be 2.246 mins and 5.461mins. The % purity of Chlorpheniramine and Trithioparamethoxy Phenylpropene was found to be 101.27% and 99.76% respectively. The system suitability parameters for Chlorpheniramine and Trithioparamethoxy Phenylpropene such as theoretical plates and tailing factor were found to be 5387, 0.97 and 5398 and 1.26, the resolution was found to be 2.97.

***Keywords*:** Chlorpheniramine and Trithioparamethoxy Phenylpropene, Method Development, Validation, Accuracy.

INTRODUCTION

The antiallergic medication chlorpheniramine maleate belongs to the antihistamine medicine class. Histamines are produced by the body in response to allergens such dust, pollen, pet dander, etc.This leads to runny or blocked nostrils, watery eyes, rashes on the skin, itching, and sneezing. Chlorpheniramine maleate reduces the activity of histamine to lessen these symptoms.[1,7]Chlorpheniramine maleate (CPM) is chemically 2-[pChloro-α-[2-(dimethylamino) ethyl]benzyl] pyridine maleate, with the molecular formula C16H19ClN2. [6] It is a white, crystalline, odorless powder with a bitter flavor that dissolves readily in ether and benzene and partially in water, alcohol, and chloroform. This alkyl amine belongs to the class of efficient first-generation H-1 receptor antagonists.[2,9]

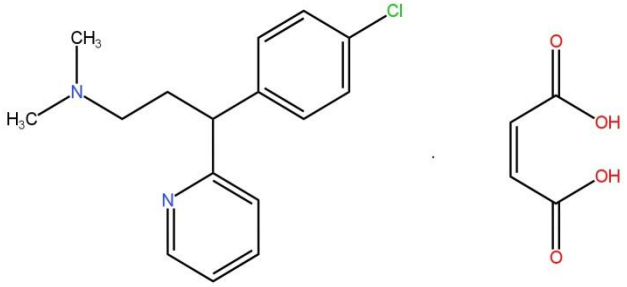

Fig.1: Chlorpheneramine maleate

Trithioparamethoxy phenylpropene is one of the drugs categorized as hepatoprotective agents. Trithioparamethoxy phenylpropene keeps the liver safe from the effects of alcohol, fatty acids, and other hepatotoxic chemicals.[3,8]Trithioparamethoxy phenylpropene lowers blood cholesterol, resulting in smoother circulation. Furthermore, trithioparamethoxy phenylpropene is prescribed to treat constipation and dry mouth caused by the usage of sleeping pills.[3,10]

The previously Mumbai, India-based company Franco Indian Remedies manufactures and distributes Hepasulfol AA pills, a fixed dose combination drug. This tablet has 3 mg of chlorpheniramine maleate and 12.5 mg of trithioparamethoxyphenylpropene.[4,11] Analytical methods like UV spectrophotometry, chemometry, fluorescence spectrophotometry, and HPLC are recommended for quantifying chlorpheniramine maleate alone in pharmaceutical samples. any method that doesn't recommend measuring trithioparamethoxy phenylpropene by itself.[5,12]

METHODOLOGY

Instruments And Glass wares used
UPLC, pH meter, Weighing machine, Volumetric flask, Pipettes and Burettes, Beakers, Digital ultra sonicator.

UPLC METHOD DEVELOPMENT:
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Chlorpheniramine and Trithioparamethoxy Phenylpropene working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.[23]
Further pipette 0.1ml of the above Chlorpheniramine and 0.375ml of the Trithioparamethoxy Phenylpropene stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.[13,25]

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACNin proportion 50:25:25 v/v respectively.[14]
Fig No.2. Optimised chromatogram
Fig No.3 Standard Chromatogram of Sample Solution
Fig No.4 Standard Chromatogram of Standard solution
Table No.1 Optimised Chromatogram conditions

METHOD VALIDATION PARAMETERS

System Suitability: The parameters of the system suitability were ascertained using a standard solution containing 1000 parts per million. Six injections were used, and metrics like resolution, peak tailing, and USP plate count were calculated. The percentage RSD was kept to a maximum of 2%.[15,24]

Specificity: The capacity to separate a certain analyte from other substances. Usually, they consist of degradants, matrix, and impurities.[16,27]

Linearity: The ability of an analytical process to demonstrate the rationale that the observed absorbance is proportionate to the analyte concentration in the sample is known as linearity. It was discovered to be linear for the drug concentration that was provided, as illustrated in figures 5, and 6.[17,28]

Accuracy: An analytical method accuracy is determined by how closely the values agree with one another, which is what constitutes an acceptable true or recognized reference value. According to ICH criteria, the percentage recovery for analytes at every level must not be greater than 2.0%. 50%, 100%, and 150% concentrations of the standard solution were spiked, and the percentage recovery was 98–102%. For the medication concentration indicated in Table 2,3 it was determined to be Accurate.[18,26]

Precision: The degree to which a set of values agree with each other The evaluation of system, method, and intermediate precision was conducted in accordance with ICH guidelines. According to the analytical methods instructions, the standard solution was injected into six replicates, and the percentage RSD was calculated. Precision for the specified drug concentration was determined, as indicated in Table 4. The percent RSD for the peak area of both analytes for six injections of standard solutions was established at no more than 2.0%.[19,30]

Reproducibility: Six samples were prepared in a single batch to test the accuracy of the technique. It was calculated what the six samples' assay percentage was. By figuring out the results' percentage RSD, the method's accuracy was assessed.[20.31]

Selectivity: The preparation of the diluent, distinct standard solutions for every analyte, placebo, and sample was indicated in the analytical procedure.[21,29]

Limit of detection (LOD): The lowest concentration of analyte in a sample that can be found.

LOD: 3.3x standard deviation /slope

Limit of quantitation (LOQ): The lowest concentration of analyte present in the sample that can be quantitatively quantified with an appropriate level of precision and accuracy. The limit of quantification was determined by applying the following equation to the response's standard deviation and the matching curve's slope.

LOQ: 10 x standard deviation /slope

For the drug's specified concentration, it was determined to be LOD and LOQ, as indicated in Table 5. [22,33]

Robustness: Although minor, intentional adjustments were made to the temperature, mobile phase ratio, and flow rate, the results remained unchanged and fell within the acceptable range as per ICH recommendations. As indicated by Table 6, it was determined to be Robustness for the specified medication concentration.[23,32]

RESULTS AND DISCUSSIONS:

Parameters		**Chlorpheniramine**	**Trithioparamethoxy Phenylpropene**	**Limit**
Linearity(µg/ml)		0-100 µg/ml	0-50 µg/ml	R2<1
Regression coefficient		0.9993	0.9991	
Slope (m)		1292.3	58802	
Intercept (c)		1868.6	43918	
Regression equation (Y=mx+c)		y=1292.3x + 1868.6	y=58802x + 43918	
Assay (% mean assay)		99.89%	99.75%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.177123	0.595695	NMT 2.0%
Method precision %RSD		0.480593	0.197436	NMT 2.0%
Accuracy % recovery		100.28%	100.56%	98-102%
LOD		0.62µg/ml	2.97 µg/ml	NMT 3
LOQ		1.89µg/ml	3.3 µg/ml	NMT 10
Robustness	Actual flow	0.97	1.26	%RSD NMT 2.0
	Less flow	0.96	1.25	
	More flow	0.94	1.24	
	Less organic	0.92	1.23	
	More organic	0.95	1.22	

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-UPLC method wasdeveloped for the quantitative estimation ofChlorpheniramine and TrithioparamethoxyPhenylpropene in bulk drug and pharmaceutical dosage forms.Chlorpheniramine was found to be soluble in organic solvents such as which should bepurged with an inert gas. Chlorpheniramine is also slightly soluble in ethanol. Trithioparamethoxy Phenylpropene was found to be is easily soluble in water, soluble inpropylene glycol, and slightly soluble in ethanol and isopropyl alcohol, Soluble in Methanol.Methanol: TEA buffer: ACN (50:25:25 v/v) was chosen as the mobile phase. The solvent system used in this method was economical.The %RSD values were within 2 and the method was found to be precise.The results expressedin Tables for RP-HPLC method was promising. The RP-HPLCmethod is more sensitive, accurate and precise compared to the Spectrophotometricmethods.This method can be used for the routine determination of Chlorpheniramine andTrithioparamethoxyPhenylpropene in bulk drug and in pharmaceutical dosage forms.

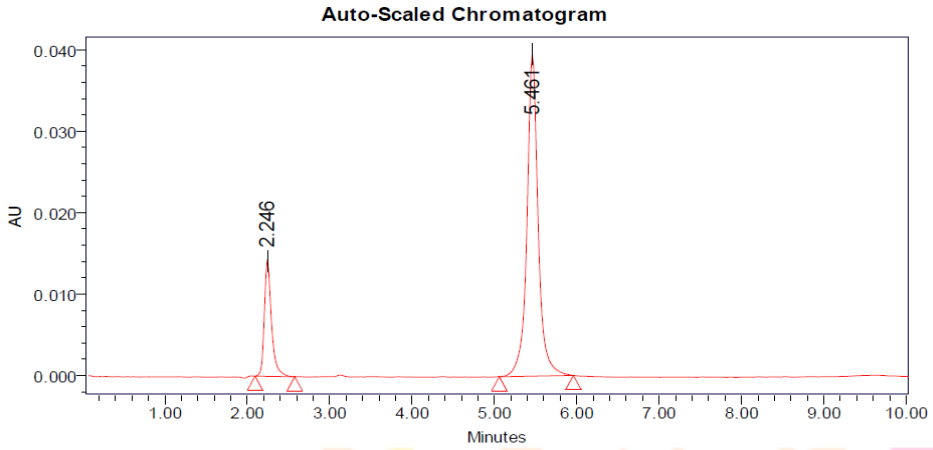

Fig. 2: Optimized Chromatogram

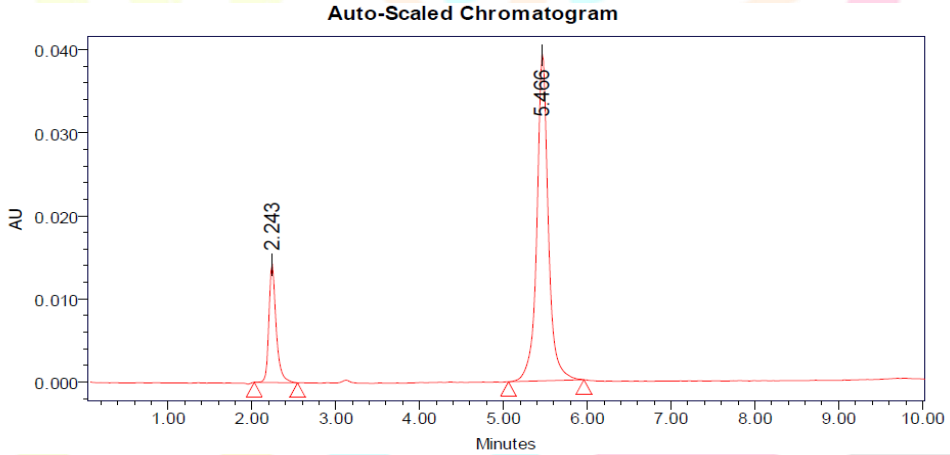

Fig.3: Standard chromatogram of Sample solution

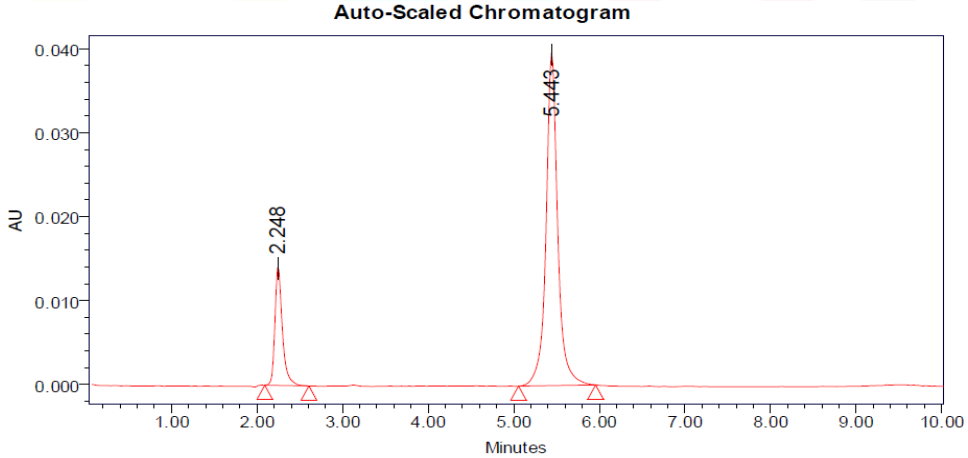

Fig.4: Standard chromatogram of Standard solution

Table.1: Optimised chromatographic conditions:

Parameters	Conditions
Instrument used	Waters ACQUITY, Software: Empower 2, PDA detector.
Mobile phase	Methanol: TEA buffer: ACN (50:25:25 v/v)
Flow rate	1ml/min

Temperature	50^0C
Column	BEH C18 (2.1 × 50 mm, 1.7 µm)
Buffer	Dissolve 1.5ml of Triethyl amine in 250 ml HPLC water and adjust the pH 4.5.
Wavelength	210 nm
Injection volume	10 µl
Run time	10 min

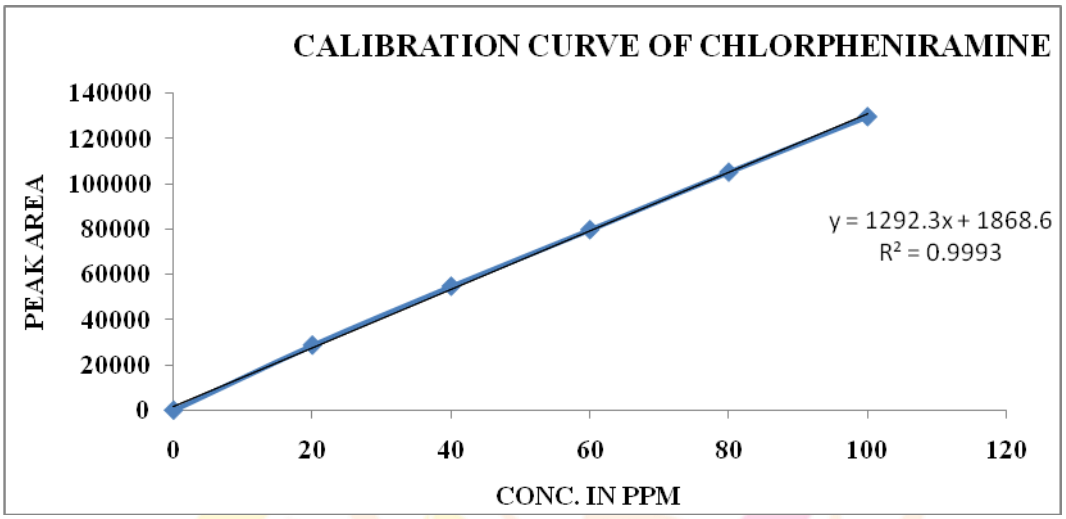

Fig 5: Linearity Curve of Chlorpheniramine

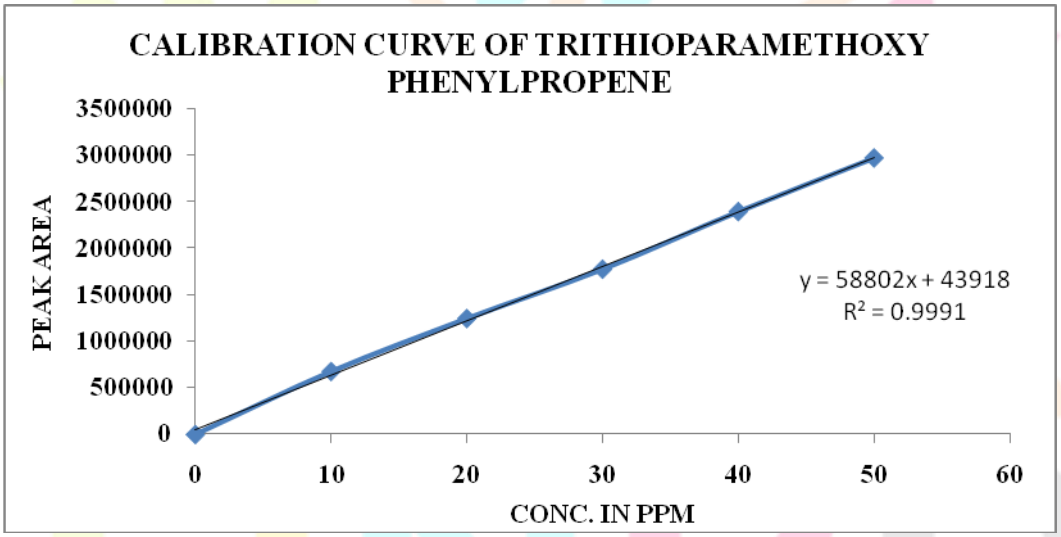

Fig 6: Linearity Curve of Trithioparamethoxy Phenylpropene

Table-2: The accuracy results for Chlorpheniramine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	42594.67	25	25.070	100.280%	100.28%
100%	84867	50	49.965	99.930%	
150%	127654	75	75.164	100.218%	

Table-3: The accuracy results for Trithioparamethoxy Phenylpropene

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2079124	50	50.445	100.890%	100.56%
100%	4082412	100	100.571	100.571%	
150%	6070195	150	150.309	100.206%	

Table-4:Precision

S.NO	Repeatability		Intermediate Precision	
	Area of chlorpheniramine	Area of Trithioparamethoxy Phenylpropene	Area of chlorpheniramine	Area of Trithioparamethoxy Phenylpropene
1.	766854	2569865	758955	2659852
2.	765884	2578474	759869	2648574
3.	765842	2568985	758985	2659865
4.	768985	2586845	756894	2658547
5.	765845	2545898	759854	2648981
Mean	766682	2570013	758590.3	2655079
Std. Dev.	1357.973	15309.45	1339.793	5242.086
%RSD	0.177123	0.595695	0.480593	0.197436

Table-5: LOD and LOQ

Molecule	**LOD**	**LOQ**
Chlorpheniramine	0.625	2.97
Trithioparamethoxy Phenylpropene	1.895	3.3

Table-6: Robustness

Sr.no.	**Parameter**	Chlorpheniramine	Trithioparamethoxy Phenylpropene
1.	Actual Flow rate of 1.0 mL/min	0.97	1.26
2.	Less Flow rate of 0.9 mL/min	0.96	1.25
3.	More Flow rate of 1.1	0.94	1.24
4.	Less organic phase	0.92	1.23
5.	More Organic phase	0.95	1.22

Table-7: Stability studies of Chlorpheniramine and Trithioparamethoxy Phenylpropene

S. No	**Type of degradation**	**Weight of sample (µg/ml)**	**Area of sample**		**Assay content (% w/w)**	
			Chlorpheniramin	**Trithioparamethoxy**	**Chlorpheniramin**	**Trithioparamethoxy n**

1	Acid (0.5N HCl)	50µg/ml of Chlorpheniramine and 75µg/ml of Trithioparamethoxy Phenylpropene	21074471	2971811	90% (10%)	92% (8%)
2	Base (0.5N NaOH)	50µg/ml of Chlorpheniramine and 75µg/ml of Trithioparamethoxy Phenylpropene	21074674	3078484	92% (8%)	91% (9%)
3	Peroxide (3% H_20_2)	50µg/ml of Chlorpheniramine and 75µg/ml of Trithioparamethoxy Phenylpropene	20011633	3071191	95% (5%)	95% (5%)
4	Thermal (at 60^0 c)	50µg/ml of Chlorpheniramine and 75µg/ml of Trithioparamethoxy Phenylpropene	21001918	3071919	93% (7%)	93% (7%)
5	Photolytic (Sunlight)	50µg/ml of Chlorpheniramine and 75µg/ml of Trithioparamethoxy Phenylpropene	21016363	3072992	91% (9%)	92% (8%)

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