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A NOVEL RP-HPLC METHOD DEVELOPMENT AND ITS VALIDATION FOR ASSAY OF CHLORPHENIRAMINE MALEATE INJECTION

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ABSTRACT

Chlorpheniramine Maleate injection (CPM) is a first-generation H_1 antihistamine widely utilized for the management of allergic disorders. Thevarious pharmacopoeias currently employ UV spectroscopy method to determine the interference of preservative with API. This method lacks in sensitivity, specificity and robustness, particularly in the formulations as they contain various preservatives such as Chlorbutol, phenol, phenyl mercuric nitrate and benzyl alcohol, which interferes with the analysis. In order to overcome the limitation of the existing UV spectroscopic method, the present studies aim to design and develop a novel RP-HPLC method for the assay of chlorpheniramine maleate injection. Chromatographic separation of chlorpheniramine maleate and preservative was obtained by using C18 column (250mm× 4.6, 5µm) as stationary phase. The mobile phase consists of phosphate buffer (pH 3 adjusted with Ortho Phosphoric Acid) and acetonitrile in the ratio of 60:40 (v/v) at flowrate 1.0mL/min. The wavelength of the photo diode array detector was set at 265nm. The retention time was found to be 2.44min. The developed method was validated according to the ICH guidelines. i.e., linearity (R^2=0.999 between 50-120%), precision (%RSD < 2%), system suitability,accuracy (recovery 98-102%), robustness and specificity. The developed method exhibited very good resolution, reproducibility and regulatory compliance. This method effectively exhibited no interferences with main peak. The research addresses a vital void in analytical technique for injectable chlorpheniramine maleate. Hence, the novel developed RP-HPLC method can be applied for the routine analysis of chlorpheniramine maleate injection.

INTRODUCTION

Chlorpheniramine maleate (CPM) it is commonly known as chlorpheniramine, Chlorprophenpyridamine maleate and (2Z)-but-2-enedioic acid; [3-(4-chlorophrnyl)-3-(pyridine-2-yl) propyl] dimethylamine (Fig:1)The molecular formula of CPM is $C_{20}H_{23}CIN_2O_4$(PubChem), and the molecular weight is 390.9g/mol.

The four different formulations of chlorpheniramine maleate injectionare available in the market. The label claim for those different formulations were found to be same, but all the four different formulation contain different preservatives namely,

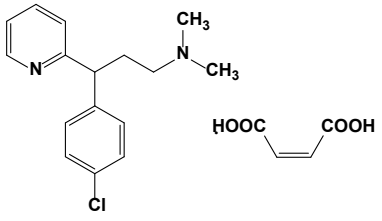

Fig.1. Structure of Chlorpheniramine maleate

benzyl alcohol, phenol, phenyl mercuric nitrate and chlorbutol. The available market samples of chlorpheniramine maleate injection are shown in below Figures 2, 3, 4 and 5.

Chlorpheniramine maleate injection is widely used in veterinary medicine for allergic conditions. Since CPM

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Fig. 2. Marketed sample contain Benzyl alcohol as preservative

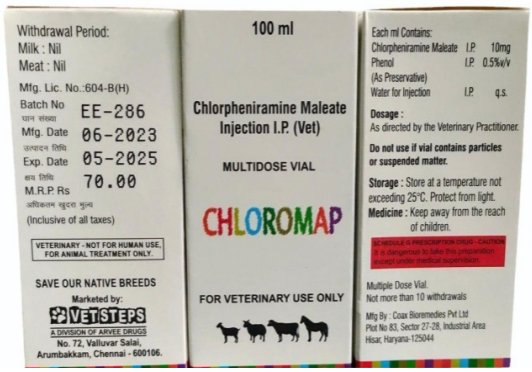

Fig. 3. Marketed sample contain phenol as preservative

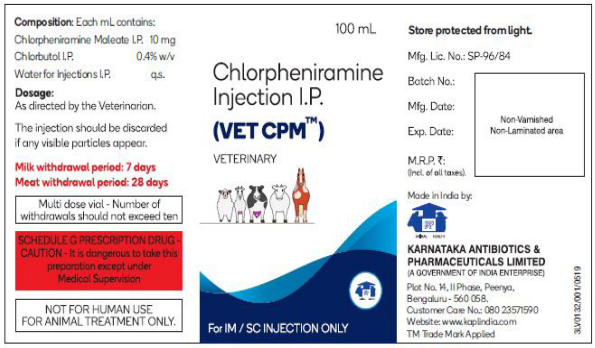

Fig. 4. Marketed sample contain Chlorbutol as preservative

Fig. 5. Marketed sample contain Phenyl Mercuric Nitrate as preservative

belongs to the class of first-generation alkyl amines, which is a H_1receptor antagonist. There is documentation on CPM injection for different routes of administration such as subcutaneous, intramuscular and intravenous routes (Rizvi *et al.,* 2022). Literature review indicates that there are no reportson HPLC analytical method for assay of CPM injection. This formulation is recognized in all the pharmacopoeias including the Indian Pharmacopeia (IP), British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP), and the United State Pharmacopoeia 29 (USP 29). However, all the Pharmacopoeias currently implements theUV spectroscopic method with different parametric conditions to analyse and determine the interferences of the preservative with active ingredient (CPM).

Lately, regulatory authorities arefavouring the RP-HPLC analytical method over UV spectroscopic method as to its more precise, accurate, specific, robust and reproducible. Majorly injectable product demand rigorous quality control and a robust analytical method is essential for precise and reliable quantification of the active ingredient. The injectable nature of the formulation makes it susceptible to environmental factors like temperature, light, and pH. The RP-HPLC approach offers better resolution, faster analysis, and adaptability to modern pharmaceutical requirements.

UV spectroscopic method was used to analyse four different formulations of CPM injection. 10mg of CPM injection is accurately weighed and diluted to 500ml with 0.25M sulphuric

Table 1. Different parameters involved in pharmacopeias'(IP, BP, USP and JP) for the assay of chlorpheniramine maleate injection

Parameters	Indian pharmacopeia (IP)	British pharmacopeia (BP)	United states pharmacopeia (USP)	Japanese pharmacopeia (JP)
Sample concentration	20ppm	20ppm	24ppm	24ppm
Standard concentration	20ppm	20ppm	24ppm	24ppm
Solvent	0.25M sulphuric acid	0.25M sulphuric acid	Diluted sulphuric acid	Water, sodium hydroxide, diethyl ether and 0.25mol/L sulfuric acid
Wavelength of absorbance	265nm	265nm	264nm	265nm
Method	UV spectroscopy	UV spectroscopy	UV spectroscopy	UV spectroscopy
Acceptance limit	90-110% label claim	90-110% label claim	90-110% label claim	95-105% label claim

The parametric conditions implemented in pharmacopoeias are mentioned in Table No.1.

acid. Absorbance of the resulting solution is measured at 265nm and the percentage purity of CPM was calculated using given formula.

Table 2. Assay of Placebo

Formulation	Absorbance (Placebo)	Assay value (%)
Blank	0.000	0
CPM(Chlorbutol)	0.000	0
CPM (Phenyl Mercuric Nitrate)	0.000	0
CPM(Phenol)	0.141	33.25
CPM (Benzyl Alcohol)	0.042	9.905

Table 3. Assay of Sample

Formulation	Absorbance	Assay value (%)
Blank	0.000	0
CPM(Chlorbutol)	0.429	100.94
CPM (Phenyl Mercuric Nitrate)	0.425	100.23
CPM(Phenol)	0.564	133.01
CPM (Benzyl Alcohol)	0.468	110.37

Table No. 2 and 3 shows the results for the analysis of four different samples of CPM injection by UV spectroscopic method. The formulation containing phenol and benzyl alcohol as preservative shows interference with the API. Hence, UV spectroscopic method is lackingin specificity, sensitivity, accuracy, precision and robustness. Overall, this study aims to address the existing gaps in analytical methodologies and enhance the reliability and efficiency of quality control measures for Chlorpheniramine Maleate Injection.

In this context, there is a need to develop a novel RP-HPLC method for the assay of Chlorpheniramine Maleate Injection and validate it according to ICH guidelines.

LITERATURE REVIEW

The vital voids in the research are illustrated in Table No.4

METHODS AND METHODOLOGY

Instrumentation

Shimadzu HPLC instrument, with an auto injector was used for the analysis. It employs deuterium lamp as the light source, Photo diode array detector to measure the emerging signals and a four-line filterquaternary pump(www.ssi.shimadzu.com,n.d.). Chromatography outcomes and output data study is processed by lab solution software. pH of the phosphate buffer solutionwas measured using Eutech pH meter. Ultra sonicator

Table 4. Review of literature

Sl. No	Authors	Tittle of the paper	Journal name	Volume and issue	Research gap
1.	-	Chlorpheniramine maleate injection	Indian pharmacopeia	IP 2022, pp. 1857-58	UV Spectroscopy method
2.	-	Chlorpheniramine maleate injection	British pharmacopeia	BP 2024, pp. 1-2(electronic copy)	UV Spectroscopy method
3.	-	Chlorpheniramine maleate injection	Japanese pharmacopeia	JP XIV, pp. 357-58	UV Spectroscopy method
4.	-	Chlorpheniramine maleate injection	United states pharmacopeia 29	USP 29, (Electronic Copy)	UV Spectroscopy method
5.	-	Chlorpheniramine maleate injection	United states pharmacopeia 2024	USP 2024 (Electronic Copy)	UV Spectroscopy method
6.	Blessy Sirigiri*et al.,*	A Novel HPLC Method for the Simultaneous Determination of Chlorpheniramine Maleate and Dextromethorphan in Bulk and Pharmaceutical Formulation	International Journal of Pharmaceutical Sciences and Research	IJPSR (2018), Volume 9, Issue No. 3	Analysis of combination drug in tablet formulation
7.	Amir Ali *et al.,*	Stability – Indicating RP-HPLC Assay for Simultaneous Determination of Chlorpheniramine Maleate and Prednisolone in Veterinary Injection	Acta Chromatographica	Volume 32, Issue No. 2	Analysis and determination of combination drugs
7.	Hasan Aldewachi and Thamer A. Omar	Development Of HPLC Method for Simultaneous Determination of Ibuprofen and Chlorpheniramine Maleate	Scientia Pharmaceutica	Volume 90, Issue No. 3	No RP-HPLC method reported for simultaneous estimation

8.	Akwasi Acheampong *et al.,*	Validated RP-HPLC method for simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation	Springer Plus	Volume 5, Issue No. 1	RP-HPLC method for simultaneous estimation in tablet formulation

was used for solvent degassing. The buffer solution was passed through a nylon membrane of 0.45microns.ShimackC18 column (4.6mm×250mm, 5µm) used as stationary phase.

Chemicals and reagents

Karnataka Antibiotics and Pharmaceuticals Ltd. (A Govt of India Enterprise), Bengaluru, India, has provided CPMAPI and CPM injection. Merk Ltd. supplied the acetonitrile HPLC-grade, while SD Fine Chem Ltd. India supplied the orthophosphoric acid and the 99.9% pure potassium dihydrogen phosphate. Shimadzu Ltd. supplied the Milli-Q purification apparatus with HPLC-grade water, and a stationary phase (Shimack C18 column (4.6mm×250mm, 5µm).

Preparation of mobile phase and diluent

Preparation of the standard solution and the mobile phase are essential and critical in the development and validation of chromatographic techniques. In this method, the mobile phase was preparedusing potassium di-hydrogen phosphate buffer of pH 3.0, adjusted with 10M orthophosphoric acid and acetonitrile in the ratio of 60:40 v/v for one liter. This mobile phase is used as a diluent in sample and standard preparation.

Preparation of standard and sample solutions

20mg of chlorpheniramine maleate was accurately weighed and transferred to a 100ml volumetric flask and made up to mark using mobile phase to prepare the standard solution.From the above stock solution 5ml was pipetted and transferred into 50ml volumetric flask and made up to the mark.Similar procedure is fallowed for sample preparation.

The standard was prepared at five different concentrations for linearity study. Exactly weighed 20mg of CPM and transferred to 100ml volumetric flask made up to the markusing diluent. From the stock solution 2.5ml was pipetted and transferred to another 100ml of volumetric flask. Similarly,50%, 80%, 100%, 120% and 150% was prepared to obtain a calibration curve. The concentration of the standard solution ranges from 50 to 150%

Chromatographic conditions

In the proposed methodology, an isocratic mode was employed. The flow rate of mobile phase wasset at 1 mL/min, with a fixed wavelength of 265nm to identifythe CPM. The column temperature was maintained at 25°C. Auto sample injector was utilized. Phosphate buffer at pH 3.0 and an acetonitrile in a ratio of 60:40 (v/v)(Phosphate buffer: acetonitrile) employed as a mobile phase.A C18 column (4.6mm×250mm, 5µm) used asstationary phase. 10 µL is the sample injection volume. The retention time (RT) of CPM was found to be 2.44 minutes. For the proposed method, all parameters met the same requirements, and these conditions gave a clear and sharp CPM peak shown in Fig:6.

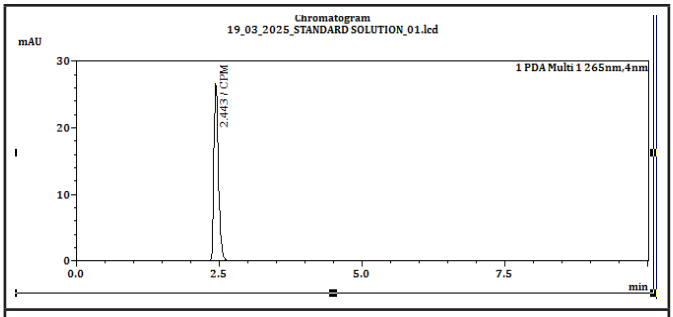

Fig. 6. Standard CPM by liquid chromatography

RESULTS AND DISCUSSION

Method development

Trials were carried out with variations in mobile phase, stationary phase and other chromatographic conditions like flow rate, wavelength and column temperature. The major consideration in choosing the mobile phase is polarity of the solvent mixture.Followed by a modification in the ratio of mobile phases.

Methanol and water in a ratio of 70:30 (v/v) was used as a mobile phase in trial 1. No peaks were eluted. Acetonitrile and methanol in a ratio of 80:20 v/v was utilized in trial 2; nevertheless, this trial›s peak eluted and was board showing less resolution. The mobile phase was adjusted by using a 70:30 (v/v) ratio of methanol and phosphate buffer; in this trial 3 the obtained peak is not symmetrical and had a tailing factor. In trial 4,Phosphate buffer and Acetonitrilewere used in a ratio of 60:40 v/v.The resultant peak was narrow and symmetrical with good resolution.Hence, this ratio of solvents was utilized as mobile phase for further analysis.

During the initial trial, the stationary phase ODS, C18 (5 µm, 150mm x 4.6)was employed. In this trial peak eluted but inadequately. For the next trial, C18 (5 µm, 250mm x 4.6) was utilized which gave aprominent result with significant theoretical plates. Tailing factor found to be within the limit according to the ICH recommendations.Thus, the fallowing stationary phase was employed throughout the analysis.

A number of trials were conducted to determine the flow rate, such as 2.0mL/min, 1.5 mL/min and1.0mL/min. Among all these trials, flow rate of 1.0 mL/min provided the optimum retention time (RT). The proposed method was utilized with the flowrate of 1.0ml/min and the column temperature was set at 25°C,30°C and 35°C. At 25°Coptimum outcomes were observed.

Therefore, for the proposed method the stationary phaseC18 (25 x 4.6 mm, 5 µm) was employed. The mobile phase comprisesof phosphate buffer and acetonitrile in the ratio of (60:40v/v) with the flowrate of 1.0mL/min was utilized. While the wavelength was set at 265nm with 15min of run time. The column temperature was maintained at 25°C by injecting 10µL of sample, an 8°C constant temperature was maintainedto cool

the samples. This enabled stability of the solution and ensured that the test and standard samples prevents deteriorating over a prolonged period of examination.The chromatogram of finalized method along with blank, standard, placebo and samples are shownin figures 7 to 15.

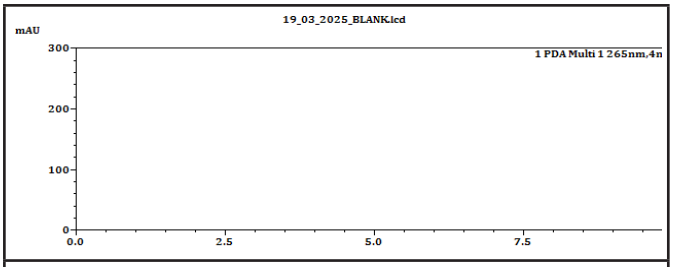

Fig .7. Blank

System suitability

At 2.44 minutes, the CPM retention time for the estimated method was constant **Table 5.** presents the system suitability results at normal concentrations,andFig:6 illustrates the standard chromatogram. The %RSD value was found to be below 2.0%. Based on the results, other parameter like theoretical plates and tailing factors were found to be within the acceptable limits of ICH recommendations.

Table 5. Results of system suitability

Parameter	Observed	Limit
Theoretical plates	6008	2000
Tailing factor	1.28	< 2
RSD%	0.91	2%
Retention time	2.44 min	-

Linearity

The linearity graph that was achieved for the area and concentration of CPM revealed a straight line (Fig: 16) with various concentration of the solution relating across the areas of the peaks (Fig: 17), the regression coefficient found within the limit of ICH recommendation (R^2- 0.9999). These findings revealed that CPM can be measured and quantified at lower to higher levels, that confirmsthe proposed method is sensitive. The value of R^2 was calculated based on the equation Y = MX+ C. Results are given in **Table 6.**

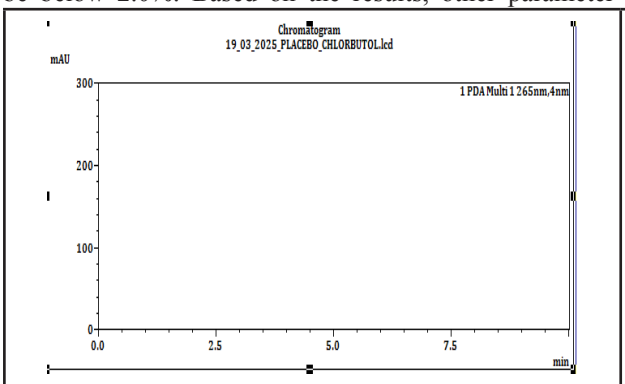

Fig. 8. Placebo chlorbutol

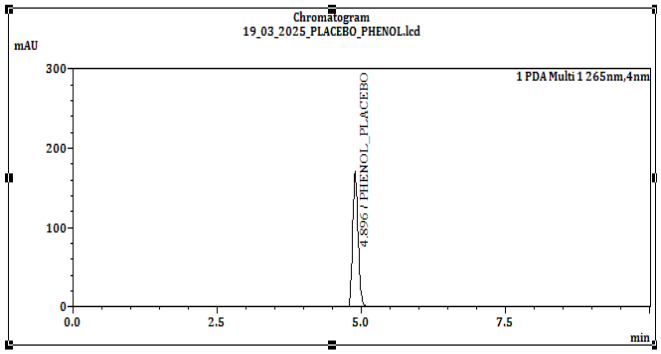

Fig. 9. Placebo phenol

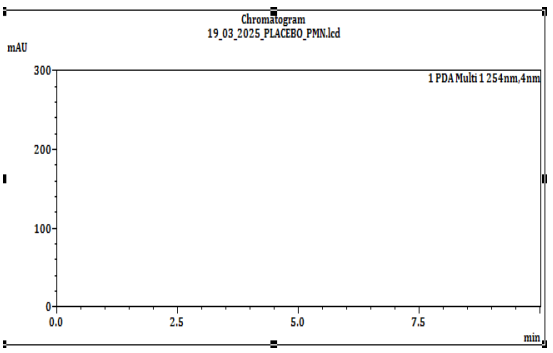

Fig. 10. Placebo phenyl mercuric nitrate

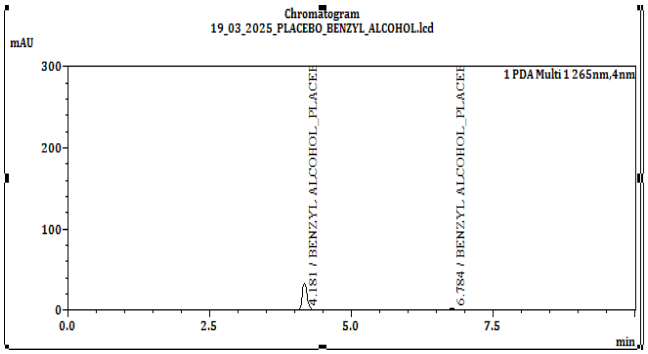

Fig. 11. Placebo benzyl alcohol

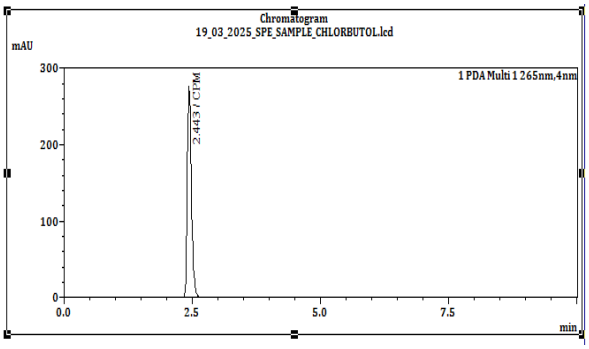

Fig. 12. Sample chlorbutol

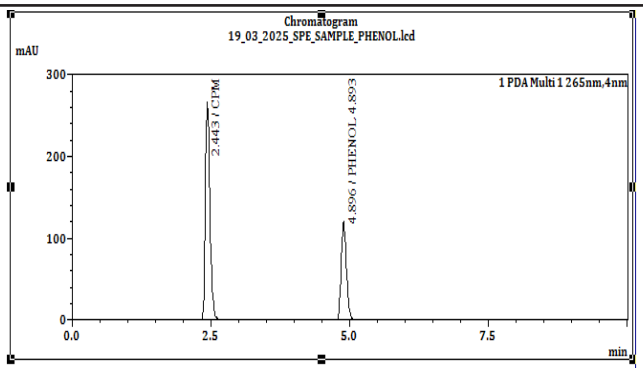

Fig. 13. Sample phenol

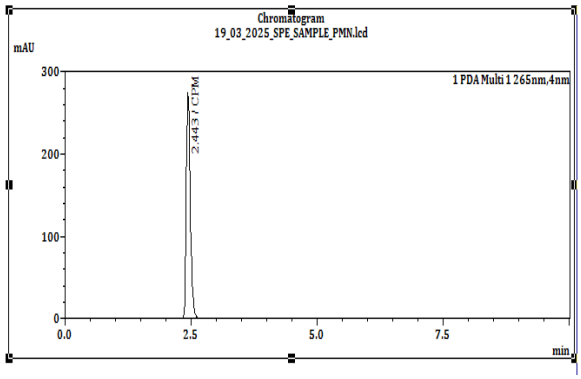

Fig. 14. sample phenyl mercuric nitrate

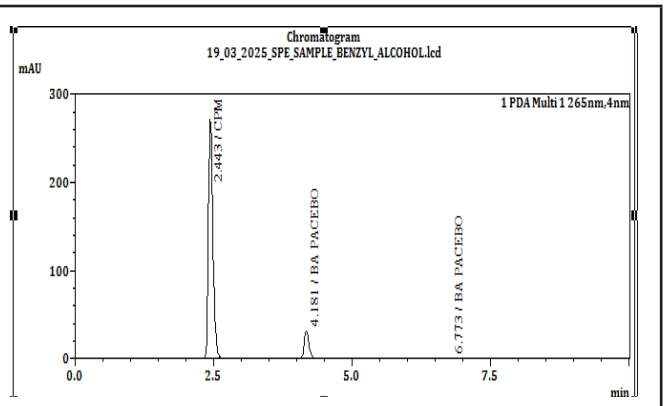

Fig. 15. sample benzyl alcohol

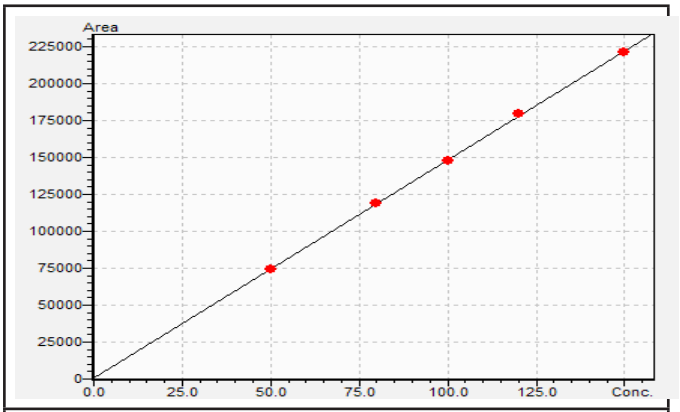

Fig.16. Linearity graph of CPM

Table 6. Results of linearity for CPM		
Parameter	**Results**	**Limit**
Slope (b)	1660.0	
Intercept(c)	7500.0	
Correlation coefficient (R^2)	0.9999	$R^2 > 0.995$

Precision and Intermediate precision

This parameter was conducted on a different day, by a different analyst with a different manufacturer's column and different instruments with the same specifications. Initially the precision for each of the four test samples was compared against the standard. The %RSD of four test samples were found below 2%.The intermediate precision was performed and the assay data results were below 2%RSD after spiking the four individual test samples against the standard solution. Therefore, the proposed method is reliable, precise, and reproducible. The ICH regulations specify that the precision, intra and intermediateresults are good and acceptable. The obtained data are given in **Table 7.**

Table 7. Data of precision and intermediate precision		
Parameter	**Results**	**Limit**
Intra day	0.9	2%
Inter day	0.92	2%

Robustness studies

The robustness of the method was testedby introducing slight modification in the chromatographic parameters (e.g., flow rate, column temperature, and wavelength variation). The known concentration was injected with the standard solution. A shift in the column temperature (30°C, 25°C and 35°C), mobile phase flow rate (0.9mL/min, 1.0mL/min. and 1.1mL/min), and a wavelength(265 nm to 264 nm and 265 nm) all parameters were related to different conditions.The results fell within the predetermined limits of the ICH requirements and are displayed in **Table 8.**

Table 8. Results of robustness studies			
Parameters	**low**	**High**	**Actual**
Flow rate	0.9 mL/ min	1.1 mL/ min	1 mL/ min.
Column temperature	30°C	35°C	25°C
RSD	1.0%	1.2%	0.9%

Accuracy

Three levels of upper, middle and lower concentrations at 80%, 100%, and 120%, were employed in the calculation of accuracyor recovery parameter against the sample concentration of 100%. The standard procedure was applied to assess the collected data and the accuracy results were found to be adequate. Allthe experiment results were found to be within the range of the accuracy test of 98.0% to 102.0%. Consequently, the developed method is accurate.

Specificity

Specificity studies confirmed that the excipient and solvent peaks does not interfere with the CPM main peak. The sample solutions were examined following spiking of the blank, placebo and standard. The sample peak showed a peak purity of 1. The chromatogram illustrated that the main peakof CPM was free from interference. These finding confirm the specificity of the method and ensure that excipient does not interference or impact on the main peak (Fig:17 to 20).

Assay

The assay test spiked the sample solution against the standard solution at a 100% concentration to accurately measure the CPM's content. The results are presented in Table 9. The obtained data was calculated by a standard formula

$$\textbf{Formula}: Assay = \frac{Test\ area}{Standard\ area} \times \frac{Standard\ weight}{Test\ weight} \times \frac{Standard\ purity}{100} \times 100$$

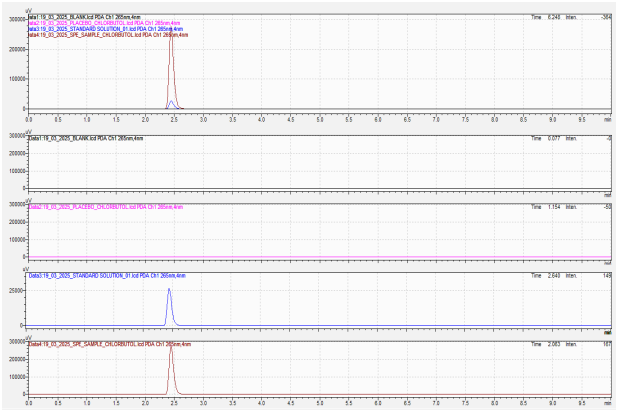

Fig. 17. Specificity of chlorbutol, blank and standard

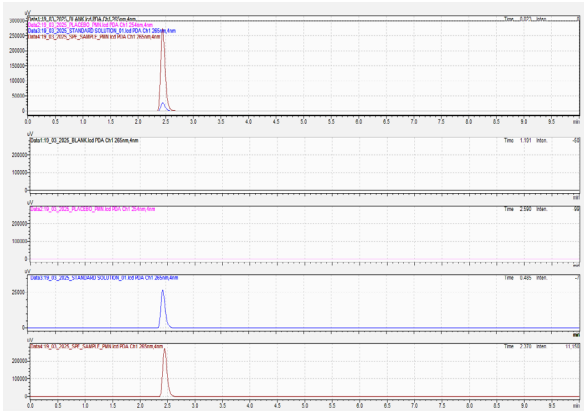

Fig. 18. Specificity of phenyl mercuric nitrate, blank and standard

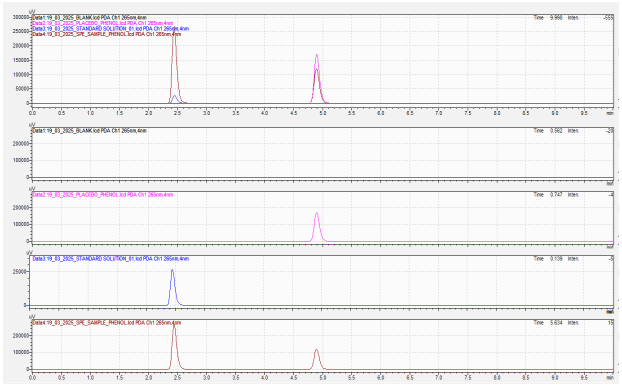

Fig. 19. Specificity of phenol, blank and standard

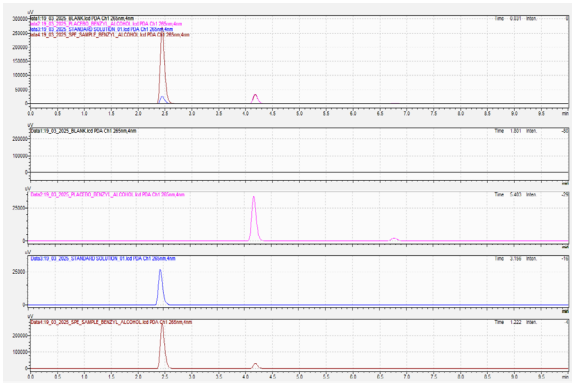

Fig. 20. Specificity of benzyl alcohol, blank and standard

Table 9. Results of recovery studies and assay outcome

Parameter	Label claim	%RSD	Concentration %	Found mg	Assay in %	Value recovery %	Limit
Assay	10mg	0.9%	100%	10mg	99.6	-	98.0-102%
Recovery		0.92%	80% 100% 120%			99.2% 99.6% 100.2%	

CONCLUSION

A major void in the analytical methods for this widely used antihistamine is addressed by the development approach to the evaluation of Chlorpheniramine Maleate (CPM) injection. The analysis of CPM injection in pharmacopoeias has traditionally been dominated by UV spectroscopic methods. Though this method often lacksin sensitivity and specificity required for injectable drugs. This study provides RP-HPLC protocol that has been validated according to ICH guidelines. The developed method displayed high sensitivity and accuracy allow the drug to be identified and quantified accurately, even in low to higherconcentrations. The developed method shows good linearity over a broad concentrationrange, ensuring stableperformanceoveraseriesofsamplestrengths.Asignificant advantage is the low retention time, which minimizes solvent usage and acceleratesanalysis time and laboratory productivity. Additionally, this method provided sharp, symmetrical peaks with high resolution, enabling precise identification and quantitation without interference from excipient. The proposed RP-HPLC method was found to be easy, quick economical and validated as per regulatory requirements, rendering it suitable for routine assay of CPM injection. Further stability studies need to be performed on CPM injection.

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Conflict of Interest: The authors declares that there is no conflict of interest

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