Original Research Article **Pharmaceutical Chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

STABILITTY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF CHLORPHENAMINE MALEATE RELATED SUBTANCES USING HPLC TECHNIQUE

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ABSTRACT

Chlorpheniramine Maleate (CPM) is an antihistamine used for the treatment of allergic disorders and common cold since, the method for determination of CPM related substances in pharmaceutical products is not described in current pharmacopoeias; the aim of this work was to develop and validate a precise, accurate and robust method. Separation was achieve on a RP C-18 column (250 x 4.6 mm, 5 µm particle size), column temperature kept at 40°C. The mobile phase content A: 80 volumes buffer solution (pH 3.00) and Mobile phase B 20 volumes of Acetonitrile, and UV detection wavelength at 225nm. The method was validated by ICH guidelines and validation studies proved that the method is stability indicating and used for determination of related substances of CPM tablets.

KEYWORDS: **Chlorpheniramine maleate, HPLC, Validation, Method development**

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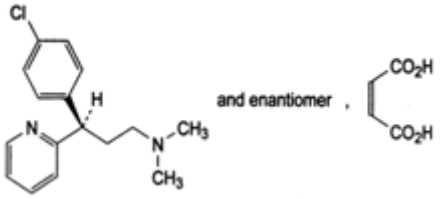
INTRODUCTION

Use of High performance liquid chromatography (HPLC) technique[1] for the quantification of the drugs and their impurities is nowadays most widely used by the pharmaceutical industries and also most of the regulatory agencies rely and believe the data generated on these techniques. Chlorpheniramine Maleate (CPM) is an antihistamine used for the treatment of allergic disorders and common cold.[2] Chemically CPM is [(3RS')-3-(4-chloophenyl)-N, N-dimethyl-3-(pyridin-2-yl) propan-1-amine hydrogen (Z)-butenedioate].[3] In the past recent years many of the analytical methods has been developed for the quantification of CPM in pure form and in finished pharmaceutical products. However, the efficient, rapid and robust analytical methods for the quantification of CPM impurities have not been developed so far. There are no simple, rapid and economic studies, however, of such studies for HPLC method development of CPM impurities. The aim of this work was, therefore, to develop economic, simple, precise, accurate and robust HPLC method and to validate the developed method for the quantification of CPM impurities as per ICH guidelines.[4]



MATERIAL AND METHODS

CPM, Impurity A, Impurity B and Impurity C were procured from LGC promochem. Maleic acid was procured from Merck. Ammonium dihydrogen phosphate, Acetonitrile, Methanol and Orthophosphoric acid used were of HPLC or Analytical grades. Milli-Q water was used for the preparation of mobile phase and diluent. Filters used are 0.45 µm PTFE of PALL Life Sciences.

Instrumentation and optimized chromatographic conditions

Waters alliance HPLC instrument equipped with UV or PDA detector and Empower 2 software.
Coloumn: Inertsil ODS -3v, 250 x 4.6 mm, 5µm
Column temperature: 40°C.
Mobile phase A (MPA): Buffer solution of pH 3.00- 80 parts
Mobile phase B (MPB): Acetonitrile-20 parts.
The buffer solution (MPA) was prepared by dissolving 8.57g of ammonium dihydrogen phosphate in 1000 ml of water. pH was then adjusted to 3.00 with dilute ortho phosphoric acid, filtered and degassed.Run time for test solution was 70 minutes, for standard and resolution solution run time used as 35 minutes.UV detection at a wavelength of 225 nm was used. Injection volume used was 20 µl. Diluent used was pH 3.00 ammonium acetate buffer : acetonitrile, 80:20 (v:v). Retention time for CPM was 22 minutes and it was well separated from the closely eluting Impurity C peak (Figure 1).

Standards and sample preparation

Standard stock solution (1000ppm)

Weighed accurately and transfered 25 mg of CPM reference standard into a 25 ml volumetric flask. Dissolved and diluted to volume with mobile phase and mixed well.

Standard solution (5ppm)

Transferred 1ml of standard stock solution into a 200 ml volumetric flask and diluted to volume with mobile phase and mixed well.

CPM impurity C stock solution (100 PPM)

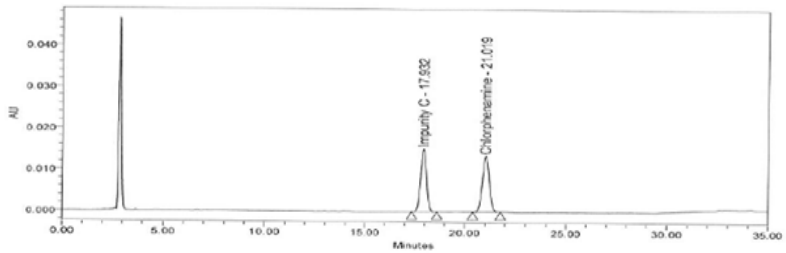
Accurately weighed 2.5 mg of CPM impurity C was transferred into a 25 ml volumetric flask. Dissolved and diluted to volume with methanol.

Resolution solution (10ppm of CPM and impurity C)

Transferred 1ml of CPM impurity C stock solution into a 10 ml volumetric flask. To this added 100 µl of standard stock solution and diluted to volume with the mobile phase and mixed well.

Test solution (1000ppm)

Weighed 20 tablets and determined the average weight of the tablet. Weighed the tablet powder equivalent to 25 mg of Chlorpheniramine maleate into a 25 ml volumetric flask and added about 18 ml of mobile phase, shook to dispersed and sonicated for 30 minutes with occasional shaking. Then diluted to volume with mobile phase, mixed well and filtered through 0.45µm PTFE filter after discarding the first 2 ml of the filtrate.



	Name	RT	Area	% Area	USP Resolution
1	Impurity C	17.932	316147	49.47	
2	Chlorphenamine	21.019	322920	50.53	5.1

Figure 1
Separation between impurity C and CPM(Chlorpheniramine)

Validation of HPLC method

The developed method was validated in terms of system suitability test, specificity, linearity and range, accuracy, precision, Detection Limit (DL), Quantitation Limit (QL), and solution stability as per ICH guidelines.

RESULTS

Specificity

Specificity[5] of the method was determined by spiked sample, individual impurity of A, B and C, impurity mixture, placebo solution, sample solution were prepared and injected into a HPLC system equipped with PDA Detector (HPLC-PDA). No peaks detected at the retention time of CPM in the chromatograms of diluent and placebo solution. The related substances peaks also well separated from the CPM peak and each other. The purity angle (0.150) is less than the purity threshold (0.524) for CPM peak and there is no tick mark in the purity flag column for CPM peak in spiked test solution (Figure 4).

Forced degradation

A forced degradation study was conducted to demonstrate that the method is stability indicating[6]. Separate portions of drug product, drug substance and placebo were exposed to the following stress condition to induce degradation.Acid Stress-Samples were stressed with 4M HCl at 80°C for 4 hours. Base Stress - Samples were stressed with 4M NaOH at 80°C for 4 hours. Oxidative degradation was induced for the samples with 3% hydrogen peroxide (H_2O_2) at 70°C for 1 hour. Thermal degradation was induced for the samples with the exposure of heat at 105°C for about 72h.Photolytic stress was induced for the samples with the exposure of UV light for 14days.Stressed samples were then injected into the HPLC-PDA.The chromatograms of the stressed test samples were evaluated for peak purity of CPM peak using Waters Empower-2 networking software. For all forced degradation test solutions, the purity angle is less than the purity threshold for the CPM peak. In placebo solution, no peak was observed at the retention time of CPM in all stress conditions.Degradation of CPM was observed in Thermal and Oxidative stress conditions at 3% and 15%, respectively.

Linearity

Linearity for peak area of CPM in relation to their respective concentration was determined. The best-fit line through an unweighed least square linear regression was generated. The result is shown in Figure 2 for CPM impurity C and Figure 3 for CPM.

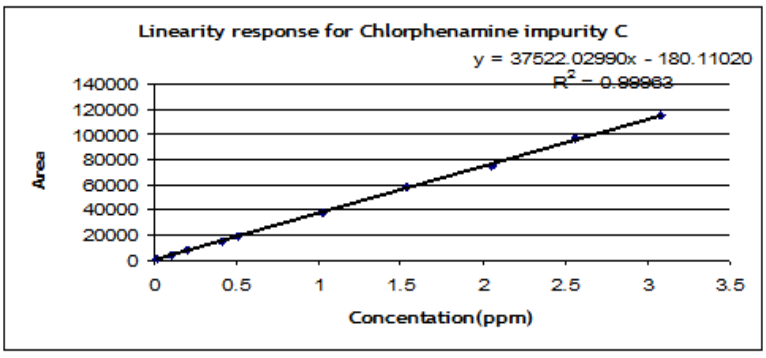


Figure 2
Linearity response for CPM Impurity C

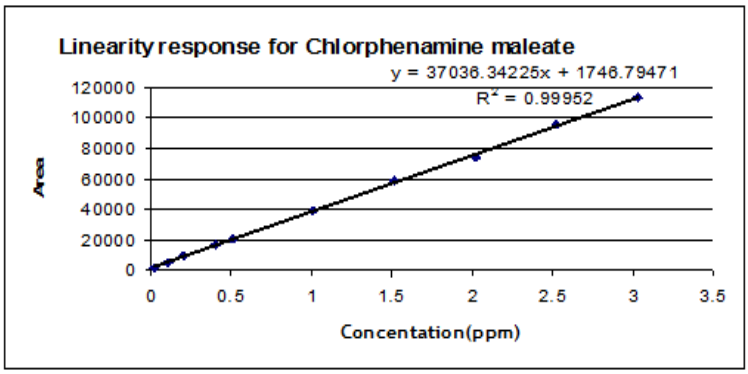


Figure 3
Linearity response for CPM
Slope(S):37036.3422, Intercept (I): 1746.7947, % I: 2.3 The regression coefficient, r^2 for CPM: 0.999, CPM impurity C: 0.999.The percent deviation of the y-intercept from the origin for CPM: 2.3%, CPM impurity C: 0.2%.The results for Linearity experiment fulfils predefined acceptance criteria, r^2 (NLT 0.990) and deviation from y-intercept (NMT 5.0%) was met. No apparent non-linearity is observed.

Limit of Quantitation (QL) and limit of Detection (DL)
To determine the Quantitation limit and detection limit for CPM and CPM impurity C obtained from linearity curve, samples were prepared and injected into HPLC to get the signal to noise ratio of about 10 and 3. The results obtained are given in Table 1.

Table 1
QL and DL results

Compound name	Detection limit (DL)		Quantification limit(QL)	
	Concentration (ppm)	S/N ratio	Concentration (ppm)	S/N ratio
Chlorpheniramine maleate	0.02	7	0.06	13
Chlorpheniramine impurity - C	0.02	6	0.06	15

Precision
Six test solutions of CPM 4mg tablets were prepared by spiking the Impurity C for determining the precision of the method,. The acceptance criteria % RSD should be not more than 20.0 for impurities greater than Quantitation Limit) was met. Hence, the method is acceptable with respect to method precision.

Accuracy
To determine the accuracy of the method[7-10], three test solutions of 4mg strength in each spike level (QL level, 50%, 75%, 100%, 125% and 150% for CPM impurity C) were prepared. The acceptance criteria (each individual and average recovery should be from 80% to 120% of theoretical value) were met. This shows that the method is acceptable with respect to the accuracy.

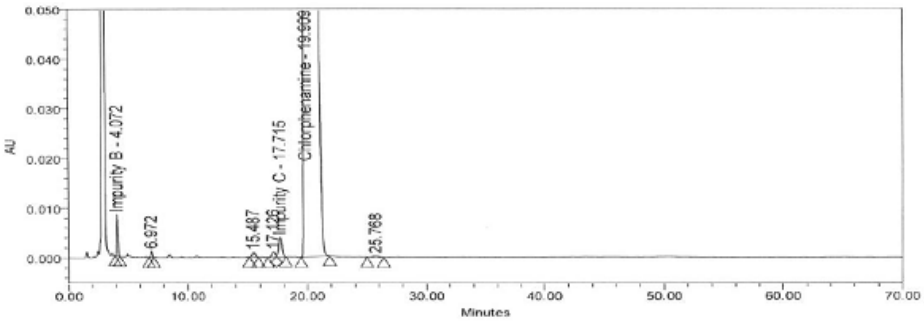
Stability of analytical solutions
The stability of standard solution and CPM related substances in the test solution were determined. Two test solutions by spiking the CPM impurity C at specification level and two standard solutions were prepared and kept at ambient temperature (25°C-30°C) on bench top for bench top stability study and in refrigerator (2-8°C) for refrigerator stability study.The Solution stability data were collected up to 48 hours for standard and test solution and evaluated against freshly prepared standard solution. From the obtained results it revealed that, the test solution and standard solution is stable for up to 48 hours at bench top (25°C-30°C) and refrigerator (2°C-8°C) condition..

Robustness
Effect of variation in flow rate from 0.9 ml/min and 1.1 ml/min, Effect of variation in mobile phase composition ± 5% variation of organic, change in the column temperature of 38°C and 42°C and change in the pH of 2.8 and 3.2 were performed with a test solution of 4mg strength and standard solutions were prepared by spiking CPM impurity C. The results revealed that the resolution between Impurity C and CPM was more than 2.0, %RSD of standard is less than 2.0 and Difference in % impurities from the unchanged chromatographic condition for unknown impurities and specified impurities were less than 20.0%.

DISCUSSION

The present study describes the development of a sensitive, precise and accurate HPLC method for the analysis of related substances of CPM Tablets. The most suitable mobile phase for separating the CPM and Impurities was optimized as pH 3.0 buffer solution (MPA) and Acetonitrile (MPB). The peaks obtained with this system were better defined and well separated for the related substances of CPM tablets. Retention time obtained for CPM was about 22 minutes, Impurity A, B and C were 3.8, 4.1 and 17.7 minutes, respectively. The impurity peak areas were reproducible as indicated by low coefficient of variation were found. A good linear relationship for impurity C (r^2 = 0.999) and CPM (r^2 = 0.999) were observed between the concentrations and respective peak areas. This reveals that the method is quite precise. The absence of additional peaks in the

chromatogram indicates non-interference of the common excipients used in the tablets. No peaks detected at the retention time of CPM in the chromatograms of diluent and placebo solution. The related substances peaks also well separated from the CPM peak and this was authenticated using purity angle method in that the purity angle is less than the purity threshold for CPM peak in spiked test solution. Therefore, the developed method is acceptable with respect to specificity. The method was duly validated by evaluation of the required parameters such as accuracy, precision and DL and QL. The method is robust with respect to flow rate from 0.9 mL to 1.1 mL/min, Column temperature variation from 38°C to 42°C, while change in mobile phase composition at ± 5% and change in buffer pH in mobile phase from 2.9 to 3.1.does not reveal any change in chromatographic behaviour.



	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag
1	Impurity B	4.072	63967	0.19	0.168	0.201	No
2		6.972	12637	0.04	0.853	0.462	Yes
3		15.487	17071	0.05	1.585	0.968	Yes
4		17.126	22083	0.07	0.755	0.813	No
5	Impurity C	17.715	78526	0.24	0.288	0.375	No
6	Chlorphenamine	19.909	32710702	99.38	0.150	0.524	No
7		25.768	9371	0.03	12.688	2.932	Yes

Figure 4
Spiked test solution Chromatogram

CONCLUSION

The proposed method is sensitive, precise, accurate, robust, rugged and economic. Validation studies have proved that developed analytical method is stability indicating. Therefore, it can be concluded that the proposed HPLC method is reproducible, accurate and precise for related substances of CPM in CPM 4mg tablets.

CONFLICT OF INTEREST

Conflict of interest declared none.

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