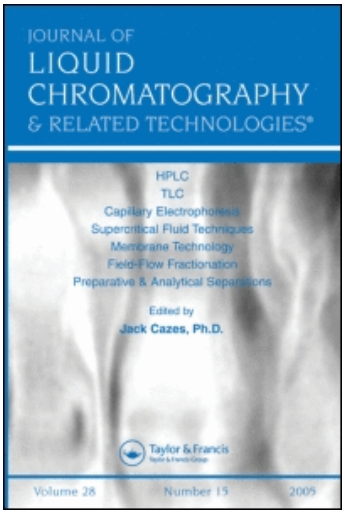

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High Performance Liquid Chromatographic Assay of Chlorpheniramine Maleate in Tablet Formulations

O. A. Al-Deeb[a]; N. H. Foda[b]; F. El Shafie[a]; A. Al-Afifi[a]

[a] Department of Pharmaceutical Chemistry, College of Pharmacy King Saud University, Riyadh, Saudi Arabia [b] Department of Pharmaceutics, College of Pharmacy King Saud University, Riyadh, Saudi Arabia

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF CHLORPHENIRAMINE MALEATE IN TABLET FORMULATIONS

Omar A. Al-Deeb,[1] Nagwa H. Foda,[2,*]
Faiza El Shafie,[1] Ahmed Al-Afifi[1]

[1]Department of Pharmaceutical Chemistry
[2]Pharmaceutics
College of Pharmacy
King Saud University
P.O. Box 22452
Riyadh 11495, Saudi Arabia

ABSTRACT

A simple, rapid, specific, and reliable high performance liquid chromatographic assay of chlorpheniramine maleate in tablets has been developed. Reverse phase chromatography was conducted using a mobile phase of 0.05 M ammonium acetate and acetonitrile, (60%, v/v) pH 3.5 with UV detection at 265 nm. The % recovery and coefficient of variation from six placebo tablets containing 4 mg of chlorpheniramine maleate were 100.2, 98.25, and 0.4, 2.2 by the HPLC and B.P. 93 methods, respectively. Replicate regression analyses of three standard plots in the concentration range 0.5 - 20 mcg/mL obtained on three different days gave a correlation coefficient >0.9998 and a coefficient of variation of the slopes <1.54%.

The assay was precise within day and between days as indicated by ANOVA test. The average percentage recoveries from 10 replicate tablets of chlorpheniramine maleate was 101.35 and 100.464 of the label amount, and their coefficients of variation were 0.8 and 4.6 by the HPLC and B.P. 93. methods, respectively.

A comparison of the proposed HPLC and the B.P. 93. method, indicated that the HPLC method is more rapid, simple and reproducible. It is suggested that the proposed HPLC procedure could be used for routine quality control and dosage form assay of chlorpheniramine maleate.

INTRODUCTION

Chlorpheniramine maleate, is an antihistaminic agent which is effective in allergic and vasomotor rhinitis, allergic conjunctivitis, mild urticaria, angioedema and as adjunct therapy in anaphylactic shock.[1] It is widely used as an ingredient in proprietary antitussive formulations.[1]

Several methods for the quantitation of chlorpheniramine maleate in pharmaceutical dosage forms have been described, such as Proton NMR Spectroscopy,[2] second derivative Photoiodide-array spectroscopy,[3] near-infrared reflectance spectroscopy,[4] and various HPLC methods employing ion pairing and buffering.[5-12]

Most of the described HPLC procedures focused on the improvement of the separation of chlorpheniramine from other drug substances, rather than on the drug resolution from additives in various formulations containing the drug.

The B.P. 93 for the assay of chlorpheniramine maleate in tablets entails an ether-based extraction, followed by a UV spectrophotometric determination. All of these approaches require either lengthy sample preparation steps and/or non-specific quantitation.

The purpose of the present work was to develop a simple and direct HPLC assay for the quantitation of chlorpreniramine maleate in tablet formulations. The developed HPLC and the B.P. 93 procedures were compared with respect to their accuracy, simplicity and reproducibility.

EXPERIMENTAL

Chemicals and Reagents

Chlorpheniramine maleate[13] and propyl paraben[14] were used without further purifications. Acetonitrile,[15] methanol[15] and water were of HPLC grade. All other chemicals and reagents were of U.S.P. or A.C.S. quality and were used as received.

Instrumentation

A Waters HPLC system[16] was utilized consisting of the following components: Model 45 pump, the WISP model 710 B autosampler, the model 481 UV detector set at 265 nm at 0.02 AUFS, the model 730 data system. Chromatographic separation was accomplished using C_{18} column, 8 mm i.d. x 10cm μ Bondapack C_{18} column with 10μm packing.

Chromatographic Conditions

The eluting medium consisting of 0.05 M ammonium acetate and acetonitrile (60% v/v) adjusted to pH 3.5 with glacial acetic acid, was prepared and degassed by bubbling helium gas for 5 min. prior to use. Column equilibrium with the eluting solvent was established by pumping the mobile phase at a rate of 0.2 mL/min. overnight. The flow rate was set at 1.8 mL/min. during analysis. The chromatogram was recorded and integrated at a speed of 0.3 cm/min.

Internal Standard

A stock solution of propyl paraben containing 10 mg in 100 mL methanol was prepared weekly and stored at 4°C.

Standard Solution of Chlorpheniramine Maleate

A stock solution of chlorpheniramine maleate was prepared by dissolving 10 mg of chlorpheniramine maleate in 10 mL water. Ten aliquots equivalent to 0.5, 1, 2, 4, 6, 8, 10, 12, 15 and 20 mcg of chlorpheniramine maleate were added to 1 mL volumetric flask. After an aliquot of the internal standard

equivalent to 2 mcg were added, the flasks were brought to volume by water and thoroughly mixed. Twenty μL of the standard solutions were injected onto the column for analysis. The peak area ratio of the drug internal standard was plotted against the standard chlorpheniramine maleate concentrations. Least square linear regression analysis was performed to determine the slope, y-intercept, and the correlation coefficients of the standard plots.

Sample Preparation

Individual tablets were pulverized using a mortar and pestle, and completely transferred to 100 mL volumetric flask. The volume was adjusted with water and the flask was mechanically shaken for five min. Five mL of the solution were centrifuged at 3000 r.p.m. in a centrifuge tube for 5 min. Three hundred μL were transferred to a one mL volumetric flask containing 20 μL of propyl paraben stock solution, and diluted to the volume with acetonitrile. Twenty μL were loaded into the sample loop for chromatography. Ten replicate commercial tablets of chlorpheniramine maleate were analyzed for statistical evaluation of the assay.

Quantitation

The amount of chlorpheniramine maleate per tablet was determined from the following equation:

$$Q = [R/A + B] \text{ X dilution factor}$$

were Q is the mg chlorpheniramine maleate per tablet, R is the peak area ratio (drug/internal standard), A is the slope of the calibration curve and B is the y-intercept.

Recovery of Chlorpheniramine Maleate from the Fabricated Placebo Tablets

Placebo samples containing 4 mg of chlorpheniramine maleate and 50 mg each of starch and lactose were prepared and subjected to the described HPLC assay and the B.P 93 method to compare the accuracy and precision of the methods.

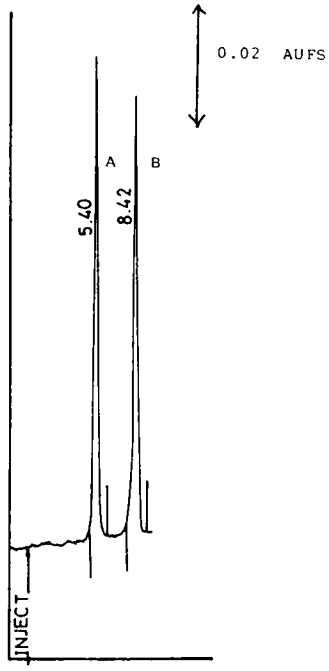

Figure 1. Chromatogram of chlorpheniramine maleate tablet.
Key: A- Chlorpheniramine maleate, B- Propyl paraben

RESULTS AND DISCUSSION

Figure 1, shows a typical chromatogram obtained following analysis of chlorpheniramine maleate in tablets. Using the chromatographic conditions described, chlorpheniramine maleate and the internal standard, propyl paraben were well separated and their retention times were 5.42 and 8.45 min., respectively. For both compounds, sharp and symmetrical peaks were obtained with good baseline resolution and minimal tailing, thus facilitating the accurate measurement of the peak area ratio. No interfering peaks were found in the chromatogram due to tablet excipients. Figure 2, shows a calibration plot for the peak area ratio of varying amounts of chlorpheniramine maleate (0.5-20 mcg/mL) to a constant amount of propyl paraben (2 mcg/mL). The plots were linear (r = 0.99985) and the regression analysis of the data gave the slope and intercept as:

$$Y = 0.0677 x - 0.0033$$

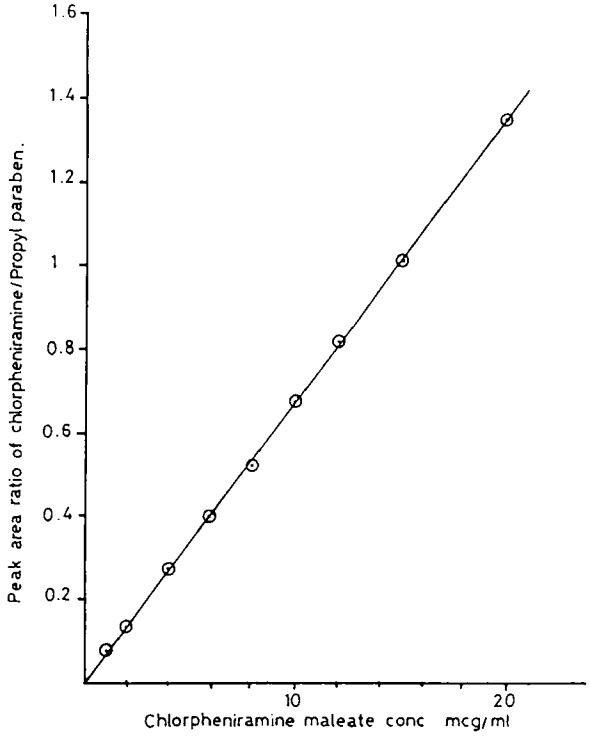

Figure 2. Standard calibration plot of chlorpheniramine maleate.

where Y and X are the peak area ratio and chlorpheniramine maleate concentration, respectively. Three replicate analyses of chlorpheniramine maleate at a concentration of 0.5 - 20 mcg/mL were performed at three different days over one week period. The results of this evaluation are summarized in Table 1.

The average correlation was higher than 0.9998 and the coefficient of variation of the slopes of the three lines was <1.54%. Analysis of variance of the data showed no detectable difference in the slopes of the three standard plots ($F=3.2$, $P > 0.01$). The similarities in the slopes and the high correlation coefficients indicate that the assay possesses excellent reproducibility and linearity. Thus, the method should be accurate and precise within the assay day as well as between assay days.

Table 1

Regression Analysis of the Three Standard Plots of Chloropheniramine Maleate

Standard [a]	Slope [b]		Intercept[b]	Correlation Coefficient[b]
1	0.06777	-	0.00330	0.99985
2	0.07024	-	0.00450	0.99977
3	0.06976	-	0.00399	0.99989

[a] Obtained in 3 different days

[b] The mean of 3 determinations at each drug concentration.

Precision and Accuracy

Six placebo tablets containing 50 mg each of lactose and starch and 4 mg chlorpheniramine maleate were assayed for four consecutive days for intra- and interday precision studies. The average recovery shown in Table 2 was (4.008 mg) with the coefficient of variation 1.3156%. Estimation of day to day and within day precision were calculated by ANOVA test. The calculated F values, $F_{0.05}$ (5, 15) = 0.1873 and $F_{0.05}$ (3, 15) = 2.003 were smaller than the table values $F_{0.05}$ (5, 15) = 2.44 and $F_{0.05}$ (3, 15) = 2.24 respectively.

Thus, it was concluded that, there was no significant difference for the assay which was tested within day and between days.

Recovery from Placebo Samples

Table 3 compares the average recoveries of chlorpheniramine maleate from placebo samples containing 4 mg chlorpheniramine maleate and 50 mg each of lactose and starch, using the HPLC and the B.P. 93 methods. The average recoveries were 4.008 and 3.93 for the HPLC and the B.P. 93 methods, respectively, and their respective relative standard deviations were 0.4 and 2.2.

The values obtained by the HPLC method compared favorably with those obtained by the B.P. 93 procedure. The difference may have been caused by a loss of sampling during several extraction steps in the B. P method.

Table 2

Analysis of Variance for Intra- and Inter day Precision

Day/Assay	1	2	3	4	5	6
1	3.97	4.02	4.03	4.08	3.94	4.08
2	3.96	4.03	4.06	4.01	3.92	4.02
3	3.91	4.02	4.03	4.05	4.03	4.07
4	4.012	3.94	4.05	3.96	4.08	3.93

Mean = 4.008 mg
SD = 0.052735
C.V.% = 1.3156

ANOVA Test

Source of Variation	DF	Sum of Squares	Mean of Squares	F Ratio	P
Within day	5	0.0028471	0.000569	0.1873	0.05
Between day	3	0.0182758	0.00609	2.003	0.05
Error	15	0.0456209	0.00304		
Total:	23				

Table 3

Average Recoveries of Chlorpheniramine Malete from Spiked Placebo Samples by the HPLC and B.P. 93 Procedures

Method	n	Amount Added mg	Amount Recovered mg	CV%
B.P. 93	6	4	3.930	2.2
HPLC	6	4	4.008	0.4

Table 4

Recovery of Chlorpheniramine Maleate from Commercial Tablets by HPLC and B.P. 93 Procedures

Method	n[a]	Mean % Recovery	SD	%CV
B.P. 93	10	100.464	4.6200	4.6
HPLC	10	101.350	0.8108	0.5

[a] Number of replicates

The B.P. 93 assay for chlorpheniramine maleate is very time consuming, as it requires shaking with 0.05 M sulphuric acid for 5 min, then extraction with ether; the etherial layer is extracted twice with sulphuric acid, then the acidic extract is rendered alkaline and re-extracted twice with ether. The etherial layer is washed and re-extracted 3 times with sulphuric acid, followed by spectrophotometric determination at 265 nm. As such, the method requires many hours of analytical time to analyze 6 tablets, compared to <1 hr by the proposed HPLC procedure.

Analysis of Chlorpheniramine Maleate Tablets

Table 4 presents the results comparing mean % recoveries, % CV, and SD of chlorpheniramine maleate tablets by the HPLC and the B.P. 93 assay procedure.

The average % recovery and coefficients of variation were 101.350, 100.464, and 0.8, 4.6 for the HPLC and B.P 93 procedures, respectively. The requirements for content uniformity of chlorpheniramine maleate tablets in the B.P. 93, specify that the potency must fall within 92.5 - 107.5% of the label claim. Thus, the tablets selected randomly in this determination met the B.P. 93 requirement for the content uniformity.

The stability indicating nature of the assay has not been demonstrated in this study, since no sign of degradation was observed by TLC after subjecting the drug solution (pH 3 and 9) at 70°C for 2 hr, which was also evident from the absence of any additional peaks in the chromatogram.

CONCLUSION

The HPLC method developed in this study has the advantage of simplicity, precision, and reliability. It allows for the direct determination of chlorpheniramine maleate bypassing several tedious steps involved in the B.P. 93 method. It should be useful for routine analytical and quality control assay of chlorpheniramine maleate in dosage forms.

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