Spectrophotometric method development and validation for determination of chlorpheniramine maleate in bulk and controlled release tablets

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Abstract: Spectrophotometric technique is considered to be the simplest and operator friendly among other available analytical methods for pharmaceutical analysis. The objective of the study was to develop a precise, accurate and rapid UV-spectrophotometric method for the estimation of chlorpheniramine maleate (CPM) in pure and solid pharmaceutical formulation. Drug absorption was measured in various solvent systems including 0.1N HCl (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8) and distil water (pH 7.0). Method validation was performed as per official guidelines of ICH, 2005. High drug absorption was observed in 0.1N HCl medium with λ_{max} of 261nm. The drug showed the good linearity from 20 to 60µg/mL solution concentration with the correlation coefficient linear regression equation Y= 0.1853 X + 0.1098 presenting R^2 value of 0.9998. The method accuracy was evaluated by the percent drug recovery, presents more than 99% drug recovery at three different levels assessed. The % RSD value <1 was computed for inter and intraday analysis indicating the high accuracy and precision of the developed technique. The developed method is robust because it shows no any significant variation in with minute changes. The LOD and LOQ values were assessed to be 2.2µg/mL and 6.6µg/mL respectively. The investigated method proved its sensitivity, precision and accuracy hence could be successfully used to estimate the CPM content in bulk and pharmaceutical matrix tablets.

Keywords: Analytical method, validation, spectrophotometeric detection, Chlorpheniramine maleate, Spectrophotometer, Pharmaceutical assay

INTRODUCTION

Chlorpheniramine maleate (CPM) is chemically2-[p-Chloro-α-[2-(dimethylamino) ethyl]benzyl] pyridine maleate, with a molecular formula of $C_{16}H_{19}ClN_2.C_4H_4O_4$. is a white , odorless, crystalline and bitter taste powder that is freely soluble in water, soluble in alcohol and chloroform and slightly soluble in ether and benzene (USP, 2007). It is a potent first generation H-1 receptor antagonist belongs to the class of alkyl amines. CPM is effectively used to treat common cold, conjunctivitis and acute allergic symptoms like rhinitis and urticaria (Schatz *et al*., 2010). It also relieves the symptoms of sneezing, rhinorrhea and itching of eyes, nose, throat and some others conditions including pruritus, atopic dermatitis, contact dermatitis insect bites and poison ivy (Sweetman, 2009). The most observed adverse effect of the drug is CNS depression with minor effect of drowsiness to deep sleep, lassitude, dizziness, and in-coordination (Paradoxical stimulation may occur occasionally, especially at higher doses). However; these sedative effects may diminished after few days of treatment (Spratto and Woods, 2009).

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CPM is commercially available in various pharmaceutical dosage forms as single medicament or in combination with other therapeutic agent(s). Several analytical techniques have been reported for the estimation of CPM including second-derivative absorption spectrophotometry (Leung and Law, 1989), HPLC (Miyamoto, 1987), polarography (Jacobsen and Hogberg, 1974) and colorimetric (Hudanick, 1964) determination. This comprehensive literature research reveals the lack of a simple spectrophotometric analytical method for detection of CPM in bulk and controlled release matrix tablet formulations. All these methods are time consuming and based on complex solvent system procedures, which are less economical. The chances of trouble shooting also increased with the complexity and also lead to the need of efficient technical person. This successful attempt of a simple, accurate, and precise analytical procedure via spectrophotometer was made to quantify drug content in pure and dosage form as well.

MATERIAL AND METHODS

Materials

CPM with the potency of 99.17%, manufactured by Supriya life science ltd and all other excipients (Hydroxyl propyl methyl cellulose, Micro-crystalline cellulose,

povidone, colloidal silicon dioxide and magnesium stearate) were kindly provided by "Karachi Pharmaceutical Laboratories Pvt. Ltd.," (Karachi Pakistan). All chemicals and reagents like hydrochloric acid (37%), sodium acetate, potassium dihydrogen phosphate, potassium hydroxide and sodium hydroxide were of analytical grade procured from the Merck (KGaA 64271 Darmstadt Germany).

Instrumentation

Digital analytical balance (Sortorious, Japan), Ultrasonic cleaner (Elma; America), pH meter (Mettler Toledo, Switzerland) and UV-visible spectrophotometer, model UV 1800 (Shimadzu, Japan) with 1cm matched open top UV quartz cells (Germany) were used.

A. Method development

Optimization and selection of wavelength

For solvent selection four aliquots of 40µg/mL CPM was transferred to the 100mL volumetric flask separately and dissolved by adding each solvent system including 0.1N HCl (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8) and distil water (pH 7.0). All buffers were prepared as per USP recommendations (USP, 2007). Drug scans were run in all mediums between 400nm to 200 nm wave length to obtain maxima of absorption (λ_{max}).The wavelength of 261 nm was found to be optimized for the quantification of CPM using 0.1N HCl acid as a blank.

Preparation of standard stock solution

100mg exactly weighed quantity of CPM was transferred to100mL volumetric flask and dissolved by adding small amount of 0.1N HCl then volume was make up using the same solvent.

Preparation of sample stock solution

For sample stock a multinational brand was purchased from an international market, with a label claim of 12mg. 20 tablets were crushed and the sample weight was taken equivalent to 100mg CPM (1500 mg of the crushed powder). This quantity was dissolved in 0.1N HCl then filtered through filter paper.

B. validation parameters performance

Method validation was performed according to the ICH guidelines (ICH, 2005) including following parameters;

Specificity

Standard CPM solution (120µg/mL) was prepared in 100 mL volumetric flask using 0.1N HCl medium. Sample was obtained by crushing CPM controlled release tablets (20 units) in mortar then powder weight equivalent to one tablet i.e. 180mg (label claim=12mg) was dissolved in 20mL of 0.1N HCl solution with volume making up-to 100mL. Placebo solution was prepared by dissolving the formulation ingredient except the active pharmaceutical ingredient (CPM). These excipients included microcrystalline cellulose 99.6 mg/tablet, Hydroxypropyl methyl cellulose 54mg/tablet, magnesium stearate 3.6 mg/tablet, colloidal silicon dioxide 1.8mg/tablet and povidone 9 mg/tablet. Overlay spectrum of standard, sample and placebo were observed to assess the impurities, degradation or any hindrance due to the presence of excipients.

Linearity

Five dilutions were made from standard stock solution at 50% (20µg/mL), 75% (30µg/mL), 100% (40µg/mL), 125% (50µg/mL) and 150% (60µg/mL) strengths of working range. Linearity and correlation was determined by plotting the absorption against various strengths of solutions.

Accuracy and recovery

Three standard CPM solutions having 50%, 100% and 150% levels were prepared in triplicate. The acceptable limit of active response is range of 98% - 102%.

Precision and reproducibility

The Precision of an analytical method expresses the closeness of agreement (degree of scatter) between a series of observations obtained from multiple sampling of same homogenous sample under the prescribed conditions. Precision is considered to be a marker of random error. It was measured by following methods;

a) Repeatability / intra-day precision

Repeatability is a precision under the same operating conditions over a short interval of time. It was determined by analyzing six samples of 40µg/mL CPM solutions for two times in the same day.

b) Inter-day precision

It is the precision within-laboratories variations, either by different analyst, different equipment or different day. Inter-day precision was determined by analyzing six aliquots 0f 40µg/mL drug solutions for consecutively two days by two analysts.

Robustness and ruggedness

Wave length was varied to 261±2nm (259 nm and 263 nm) and the difference in drug content was determined at each level of wavelength.

Detection limit (LOD)

The detection limit can be determined using formula following expression;

LOD=3.3 δ/ slope (equation 1)

Where δ is the root mean square error.

Quantification limit (LOQ)

The quantitation limit can be determined by using formula LOQ = 10 δ/ slope (equation 2).

Table 1: Accuracy and Percent Recovery in 50%, 100% & 150% Drug Solution

% Drug Solution	mg Obtained	% Recovery	Mean	SD	% RSD
50%	50.150	100.30	99.71	0.597	0.599
	49.255	99.10			
	49.956	99.71			
100%	100.136	99.94	99.65	0.352	0.353
	99.256	99.26			
	99.957	99.76			
150%	150.375	100.05	99.46	0.509	0.512
	148.242	99.16			
	148.764	99.18			

Table 2: Intra & Inter day precision of the newly developed method

Add conc. (%)	Intra-day precision		Inter-day precision	
	% Assay At Time t1	% Assay At Time t2	% Assay At Day 1 (Analyst 1)	% Assay At Day 2 (Analyst 2)
100%	100.474	100.462	100.362	100.474
100%	99.822	99.195	99.970	99.822
100%	100.944	100.720	99.732	100.944
100%	99.336	100.039	99.027	99.336
100%	100.203	98.725	99.680	99.028
100%	98.533	99.315	99.161	99.194
Mean	99.88	99.74	99.74	99.80
SD	0.86	0.78	0.78	0.77
RSD	0.861	0.786	0.786	0.76

Solution stability

The solution stability of test sample was obtained by the analysis of the same samples under minor modifications with respect to the standard test conditions. For this Standard and sample were prepared and analyzed at time interval of 0hr, 12hr, 24hr and 48 hr.

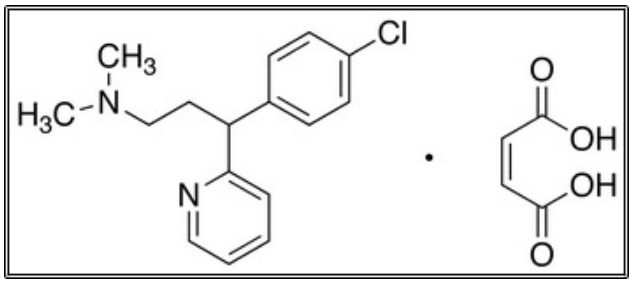

Fig. 1: Structure of chlorpheniramine maleate.

RESULTS

Optimization of analytical procedures

In present study the assay of CPM alone (bulk & dosage form) was carried out in different solvent systems of varying pH environment. These solvents were 0.1N HCl (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8) and distil water (pH 7.0) with absorbance of 0.8192A, 0.6239A, 0.5410A and 0.5001A respectively for each solvent. Solvent selection was made on the basis of maximum absorption (Beer Lambert Law) by standard CPM solution in all mediums. Among all 0.1N HCl solution exhibited the higher absorbance while the maxima were observed at wavelength of 261 nm.

Validation of developed method

The proposed assay method of CPM in bulk and pharmaceutical dosage form has been validated in accordance to the guidelines given by the "International conference on the harmonization of technical Requirements for the Registration of Pharmaceuticals for Human Use" (ICH). Different test procedures were followed to validate the sensitivity and accuracy of the method. CPM was measured specifically without the inference of formulation ingredients and shown in fig. 4. The developed method was found to be linear at these drug concentrations (50% (20µg/mL), 75% (30µg/mL), 100% (40µg/mL), 125% (50µg/mL) and 150% (60µg/mL). The coefficient of regression (r^2) value was computed to be Y=0.1853 X + 0.1098 presenting R^2 value of 0.9998 and slope of the plot was 0.745 with standard error of ±0.08268 (fig. 5). The method was accurate with the mean recovery values of 99.71, 99.65 and 99.46% at 50%, 100% and 150% respectively. Furthermore, the %RSD was also found to be less than 1 for both intra and inter-day precision. The details of the results for accuracy and precision are given in table 1 and 2.The present analytical method was tested for robustness and even by making deliberate changes in wave lengths (±1nm) as presented in fig. 6. The label claim of multinational brand

is 12 mg and the result of assay was measured within the acceptable limits i.e. 99.74%. The limit of detection (LOD) of CPM was found to be 2.2µg/mL while the limit of quantification (LOQ) was found as 6.6µg/mL. The solutions were stable as well for longer duration in medium. The result of solution stability is graphically shown in fig. 7 at various time points.

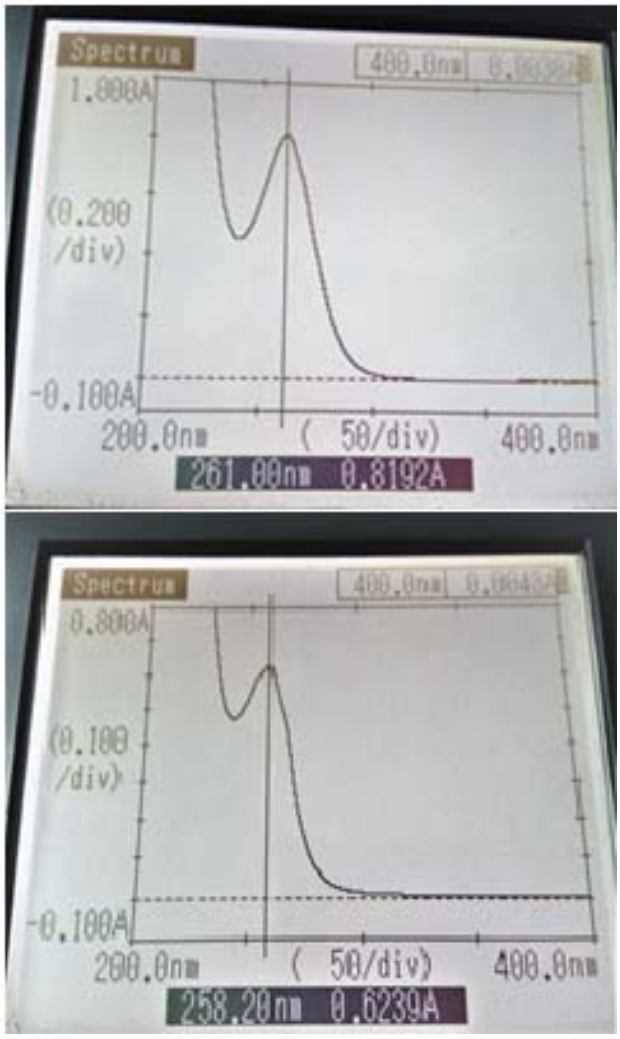

Fig. 2: Spectrum of 0.1N HCl and acetate buffer (pH: 4.5)

DISCUSSION

Chlorpheniraminemelaete formulations (plain & extended release) have been prescribed worldwide to overcome many allergic complains especially common cough-cold by blocking the permeability of histamine (Kaura *et al.,* 2013). Pharmaceutically drug content determination is considered to be significant to assure the drug's therapeutic efficacy. In addition to the official methods various other analytical methods were also reported to assess chlorpheniraminemeleate alone or in combination with one or more active pharmaceutical ingredients (Heneedak*et al*., 2015; Hadad *et al.,* 2007; Baray and wahbi, 1991). Literature survey revealed that spectrophotometric technique of estimation is found to be the simplest, cheap, easiest and economical way among all other analytical procedures (Souri*et al*., 2017; Parmar *et al*., 2013; shah *et al*., 2011). Extensive data has been documented for determination of CPM combinations using spectrophotometric analysis but none is available for sole drug quantification by spectrophotometer. It is a well-known fact that solvent selection exhibits immense influence on the quality and shape of the peak obtained. Hence in the present study the assay of CPM alone (bulk & dosage form) was carried out in different solvent systems as shown in fig. 2 and fig. 3. 0.1N HCl solution showed the maximum absorbance satisfying all the conditions related to peak quality and non-interference at the specified wavelength.

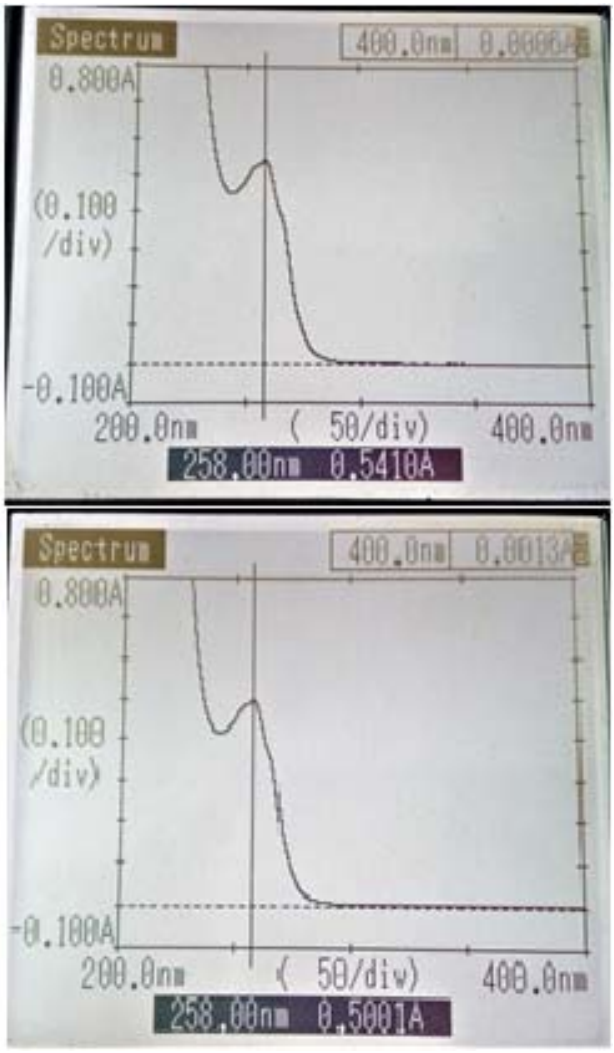

Fig. 3: Spectrum of phosphate buffer (pH: 6.8) and distil water (pH: 7.0)

Specificity is the ability to estimate the analyte in the presence of components that may expect to be present including formulation ingredients and impurities as well (Kalra, 2011). Spectrum (fig. 4) showed the absence of interference between spectrum of standard drug, sample

drug and placebo, since none of the peaks appeared at the retention time of CPM.

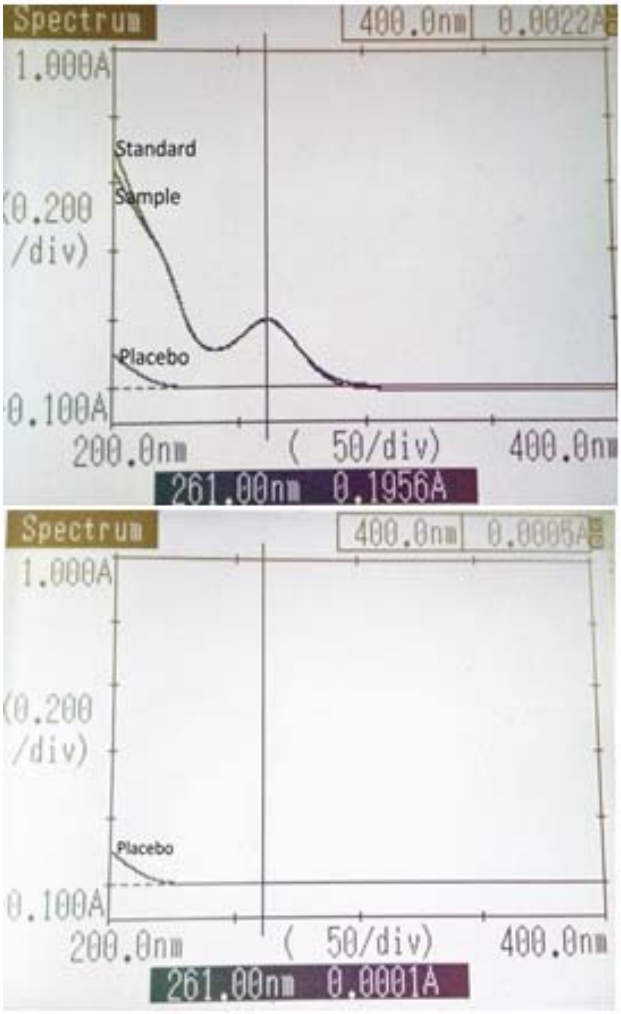

Fig. 4: Scan of Standard, Sample and Placebo

The Linearity of an analytical method is the ability to obtain test result (within a given range) that is directly proportional to the concentration (amount) of analyte in the sample (ICH, 2005). In the present study linearity of the assay method was observed at 50%, 75%, 100%, 125% and 150% strength of CPM standard solution. fig. 5 shows the plot between drug concentration and their corresponding absorption as per Beer Lambert Law**.** The correlation coefficient (r^2) was found to be 0.9998 indicating excellent linearity (> 0.999).

Accuracy is the best indication of systematic errors. To ensure the accuracy of the newly developed method, three samples having three different drug concentrations were assessed. The mean percent recovery at various working strengths was found to be 99.61 ± 0.446 (mean ± SD). The detail observations of various drug concentration solutions are given in table 1.

Precision was evaluated by repetitive intra-day determination of drug solution at two different time intervals. While inter- day precision was determined by conducting the same procedure by two analyst in two different days. The observations of intra and inter day precision is shown in table 2.

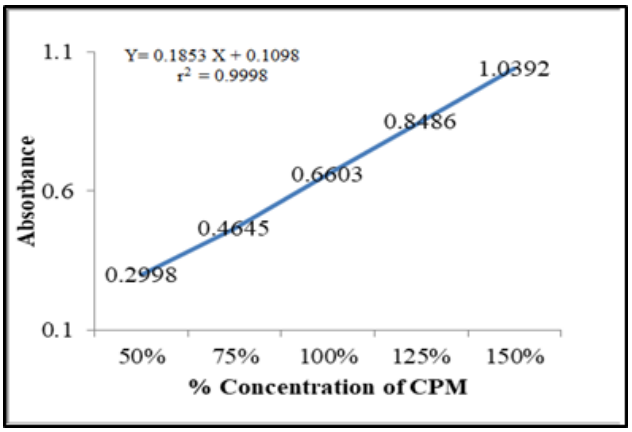

Fig. 5: Linearity Plot of Chlorpheniramine Meleate at various concentrations

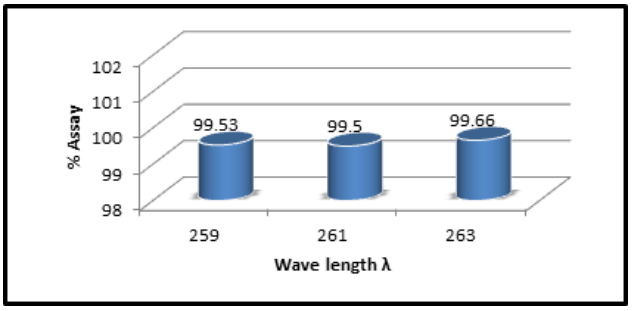

Fig. 6: Robustness and the ruggedness of the spectrophotometeric analysis

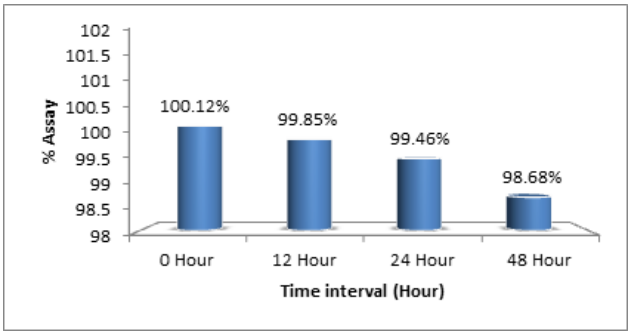

Fig. 7: Solution stability

Robustness is basically the capability of an assay technique to remain uninfluenced at deliberate changes to various parameters. Robustness and ruggedness was observed by making deliberate changes in wave length**.** All results were found within the official acceptable limits. The robustness of the procedure is shown in fig. 6.

The detection limit indicates the lowest amount of analyte in a sample that could be detected but not necessarily

exactly quantified. The quantitation limit indicates the lowest amount of analyte in a sample which can be determined with suitable precision and accuracy and express the quantitation of parameters for quantitative assay for low levels of compounds in sample matrices, and it also useful for the determination of impurities and/or degradation of products.

Solution stability was ascertained at various time intervals using 100% CPM solution. Samples were found to be stable from 0 hour to 48 hours with minor change of drug concentration fig. 7 shows the tested strengths (In percent %) of the drug at different timings.

CONCLUSION

The developed UV-Spectrophotometric method for estimation of CPM was validated in accordance with the ICH guideline and this method was appear to be a suitable technique for the reliable analysis of commercial formulations containing CPM. The most striking features of this method are its simplicity, specificity, linearity, accuracy, precision, and robustness. It is also an easier, rapid and cost effective method then HPLC and does not require the use of any expensive or toxic reagent. Hence the present UV-Spctrophotometric method is suitable for routine analysis of CPM tablet dosage form.

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