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RP-HPLC FOR THE SIMULTANEOUS DETERMINATION OF CHLORPHENIRAMINE MALEATE AND DEXTROMETHORPHAN PHARMACEUTICAL PREPARATION

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ABSTRACT

Objective: The present study aimed to develop and validate a novel, simple, rapid, and precise RP-HPLC (Reverse-Phase High-Performance Liquid Chromatography) method for the simultaneous estimation of Chlorpheniramine Maleate (CPM) and Dextromethorphan Hydrobromide (DXM) in combined pharmaceutical dosage forms. Methods: Using an isocratic mobile phase composed of Phosphate Buffer (pH 6.2): Acetonitrile: Methanol in the ratio of 35:34:31 (v/v), a Spherisorb 5µ CN column (250 mm x 4.6 mm) was used to optimize chromatographic separation. The flow rate was 1.5 mL/min. The column temperature throughout runs was held at 40°C and detection used 228 nm with an injection volume of 20 µL. The method was validated comprehensively according to general ICH Q2 (R1) guidelines for the outtake of specificity, linearity, accuracy and precision. Results: The method was capable of providing sharp, well-resolved peaks for CPM and DXM at retention times of 6.53 min and 8.18 min, respectively. The method showed excellent linearity in the range of 80-120% of the test concentration for both drugs. Correlation coefficients (r^2) were 0.9995 for CPM and 0.9998 for DXM. The accuracy, determined by recovery studies, was mean recoveries of 98.96-101.16% for CPM and 95.87-98.06% for DXM. The method was precise with %RSD for repeatability and intermediate precision both being less than 1% of that measured (1.9-0.39% respectively) for both analytes. Robustness studies confirmed that the method remained unaffected by minor, intentional variations in chromatographic parameters. Conclusion: The present RP-HPLC method is simple, accurate, precise, robust, and specific. It provides a means for simple simultaneous determination of CPM and DXM in combined pharmaceutical formulations. It is an ideal method for routine quality control and KNP analysis of all pharmaceutical formulas containing Chlorpheniramine Maleate and Dextromethorphan Hydrobromide.

Keywords: *RP-HPLC, Method Validation, Chlorpheniramine Maleate, Dextromethorphan Hydrobromide, Simultaneous Estimation, ICH Guidelines, Quality Control.*

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1. INTRODUCTION

Based on critical data for drug substances and products, it is evident that analytical chemistry is the cornerstone of pharmaceutical development and quality assurance. It provides essential information regarding identity, purity, and potency that is used to maintain quality for both trial batches as well as commercially available items manufactured by manufacturers under contract. This is really all about ensuring consistency in finished product production -through quantifiable methods like those found in analytical chemistry rather than leaving things to chance at every stage of the fabrication process [1]. When it comes to the various analytical techniques, High-Performance Liquid Chromatography (HPLC) and, in particular, Reverse-Phase (RP) have stood out as preeminent tools. Above all else, this is because their resolving power is superior. The separation of closely-related species which cannot be resolved under any other conditions will be carried out far easier with this technique than any of those that we saw mentioned before [2, 3]. It is vital to develop validated analytical methods in order to guarantee consistent product quality throughout its shelf life. With the chemical name of (RS) -3-(4-chlorophenyl)-3-(pyrid-2-yl) propyl dimethylamine hydrogen maleate, Chlorpheniramine Maleate (CPM) is a first-generation alkylamine antihistamine used mainly by eldery persons. It is widely used for the symptomatic relief of allergic conditions such as allergic rhinitis, urticaria and conjunctivitis by

competitively antagonizing histamine at the H1-receptor [4]. An ent-3-methoxy-9-methylmorphinan hydrobromide monohydrate is called Dextromethorphan Hydrobromide Ch,the base of DXM.It is a non-opioid antitussivity agent for cough, suppression centrally acting centrally on the cough center in the medulla oblongata to cause an inhibitory effect comparable to that of codeine but with significantly less potential for addiction [5].The combination of these two drugs is rational clinically, and also popular for formulation to control the cough orewhere with irritation of the throat or nasopharynx; indeed it does provide both allergic harbinger and cure. A comprehensive review of international Sigma literature reveals a number of analytical procedures for CPM and DXM, individually or together with other drugs and using methods such as picric acid spectrophotometry or HPLC [6-10].However, many of these current methods employ complicated mobile phases, gradient elution or long chromatographic procedure times that may not be suitable for use in high throughput quality control laboratories. Others refuse to supply a thorough validation data set as required by contemporary standards of assertion. Consequently, the aim of this study was to develop an innovative, economic and readily performed RP-HPLC method with complete validation data in accordance with ICH Q2(R1) guidelines [11], which can be used to determine CPM and DXM simultaneously in such pharmaceutical preparations.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

The reference standards of chlorpheniramine maleate(CPM, 99.8%) and dextromethorphan hydrobromide(DXM, 99.7%) were given to us as free samples. HPLC-grade methanol and acetonitrile were both purchased from Qualigens Fine Chemicals(Mumbai, India). Sodium dihydrogenorthophosphate and triethylamine(AR grade) were also acquiredfrom this supplier.Using a Millipore purification system, high-purity osmotic reverse (RO) water was produced. A syrup medicament that is commercially available is proved to contain 2mg of chlorpheniraminemaleate and 10mg of dextromethorphanhydrobromide in every 5mL.

2.2. Instrumentation and Chromatographic Conditions

Electrospray ionization mass spectrometry (ESI-MS) is a technique used to identify the chemical composition of substances based on the differences in their mass/charge ratio. Detailed procedures and results are reported in the literature1 and are omitted here. The chromatographic analysis was performed using a Waters Alliance HPLC System (Waters Corporation, Milford, USA, Model: W-2695) equipped with a quaternary solvent manager, an auto-sampler, a column heater and a 2998 Photodiode Array (PDA) detector. Data acquisition and processing were managed by Empower 3 software. The separation was achieved on a Spherisorb(r) 5[mu] C n column (250 mm x 4.6 mm i.d.). The optimized mobile phase consisted of a mixture of Phosphate Buffer (pH 6.2), Acetonitrile and Methanol (35:34:31, v/v/v). The buffer was prepared by dissolving 7.8 g Sodium dihydrogen orthophosphate in 1000 mL R.O. water, and the pH was adjusted to 6.2 using Triethylamine. The mobile phase was filtered through a 0.45[mu] nylon membrane filter (Millipore), and sonicated for 15 min to remove bubbles. Isocratic elution was employed with a constant flow rate of 1.5 mL/min. The column temperature was maintained at 40[degree]C, and the detection wavelength set at 228-10*->(h) % s. The injection volume was 20 [mu]L, and the total run time 11 min.

2.3. Preparation of Standard and Sample Solutions

Preparation of Standard Stock Solutions: CP M and DXM stock solutions (1 mg/mL) were obtained separately by weighing accurately, transferring 40 mg CP M into one 50 mL volumetric flask and into another 100 mg DXM. Each drug was dissolved in 50 ml of water, then volume made up to with water.

Working Standard Solution: A mixed working standard solution was prepared by pipetting 5 ml from each stock solution into a 50 ml volumetric flask, diluting to 50 ml with water, and sonicating for 10 minutes to mix. The solution was then diluted to the mark with water. The final concentrations were about 4 ug/ml for CPM and 10 ug/ml for DXM.

Sample Solution (Syrup): A quantity equivalent to 4 mg of CPM and 10 mg of DXM syrup was quantitatively weighed and transferred to a 50 mL volumetric flask. 30 ml of water was added, and the flask was sonicated 20 minutes with periodic stirring to assure complete recovery of active ingredients. Next, the solution was allowed to cool to room temperature and made up to volume with water. The solution was thoroughly mixed and then filtered through a 0.45 µ syringe filter. 5 ml of the resultant filtrate was transferred to another 100 mL volumetric flask and volume made up to with water to provide a solution with nominal concentrations similar to those in the mixed standard.

2.4. Method Validation

The developed approach meets strict guidelines following ICH Q2 (R1) [Contribution 11] and deviates from the situation in which an analytic method should be validated for following environments:

System Suitability: A standard solution was injected six times to obtain the results on such parameters as theoretical plates, tailing factor, and % RSD of peak areas and retention times

Specificity: The method's specificity was assessed by injecting the mobile phase (blank), a placebo solution (containing all excipients without any active drugs), and a standard solution to confirm the absence of interference at the retention times of CPM and DXM.

Linearity: Linearity was verified by preparing standard solutions at five concentration levels (80%, 90%, 100%, and 110% of the target concentration). Calibration curves were drafted based on peak area versus concentration for each drug

Accuracy: The accuracy of a standard addition (recovery) assay at three levels (80%, 100 %, and 120%) was assessed in triplicate. A known quantity of the standard drug was added to a pre-analyzed sample, and the percent recovery obtained

Precision: The method's precision was assessed using intra-day reproducibility (intra-day precision) and inter-day reproducibility (inter-day precision and different analysts). To test reproducibility, six independent preparations of 100 % content sample were analyzed on the same day. In the case of intermediate precision, the study was carried out on separate days or by another analyst exactly as before

Robustness: The robustness was tested by making minor alterations in the chromatographic method environment, including flow rate (±0.3 mL/min), detection wavelength (±5 nm), injection volume (±4 µL), and column temperature(±5°C). The standard solution was found proper for investigation in each altered condition, its system suitability parameters adaptation.

3. RESULTS AND DISCUSSION

3.1. Method Development and Optimization

The main goal of method development is to obtain baseline separation of CPM and DXM with symmetric peak shapes and a short analysis time. However, the first trials achieved poor peak shape and insufficient resolution using Acetonitrile: Methanol. Thus, it appears that a mobile phase comprising Acetonitrile: Methanol: Water (50:40:10, v/v/v) on a C18 column is unsatisfactory. In contrast, significant improvement ensued upon switching to a cyanopropyl [CN] column, and CN offers a different selectivity than C18.A phosphate buffer in the mobile phase is necessary to control analyte ionization and improve peak symmetry. The pH of the buffer was varied systematically. At both pH 4.2 and 5.2, the separation was incomplete. Optimal separation, with a well-defined baseline and symmetrical peak shapes for both drugs, was achieved at a buffer pH of 6.2. Finally, the mobile phase comprised Buffer (pH 6.2): Acetonitrile: Methanol (35:34:31, v/v/v), which provided the best compromise between resolution, peak shapes, and analysis time. After a PDA scan showed that both compounds were more sensitive and less noisy at the lower wavelength, we changed the detection wavelength from 254nm to 228 nm. Figure 1 shows are presentative chromatogram of the standard solution. It clearly illustrates this excellent separation.

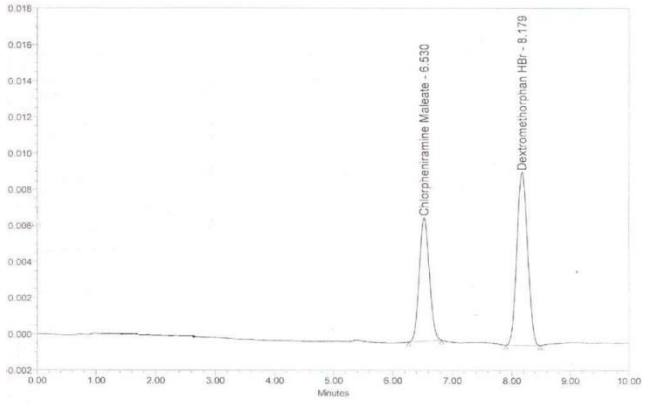

Figure 1. Typical Chromatogram of Standard Solution showing CPM (Rt = 6.53 min) and DXM (Rt = 8.18 min).

3.2. Method Validation

3.2.1. System calibration

Both peak all system suitability parameters were within acceptable limits. The theoretical plates exceeded 4000 for both peaks, and the tailing factor was less than 1.5. The % RSD for six replicate injections of the standard solution was less than 1% for both retention time and peak area, showing the system was suitable for intended analysis.

3.2.2. Selectivity

No interfering substances from the blank mobile phase or placebo solution were detected at the retention times of CPM and DXM (Figure 2A &2B). This demonstrates that the method is both selective and specific, and that ingredients in the formulation other than those intended will not affect the analysis.

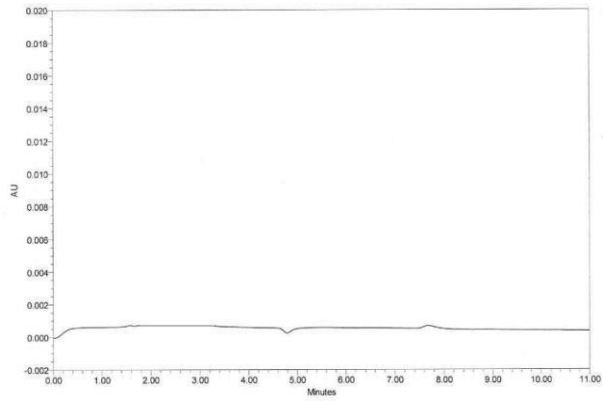

Figure 2. (A) Chromatogram of Mobile Phase (Blank), (B) Chromatogram of Placebo Solution.

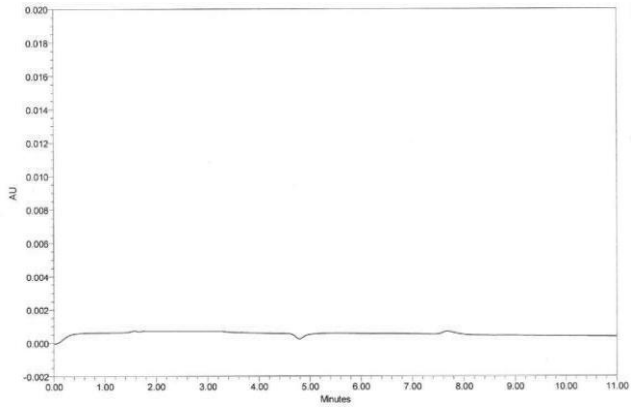

Figure2.B): Placebo for Specificity

3.2.3. Linearity and Range

This approach has an outstanding linear range for both CPM and DXM, from 80% to 120%. The concentration range for these two drugs is well-fitted within three portions of the straight line defined by people's data. The correlation coefficients (r^2) are 0.9995 and 0.9998 for CPM and DXM respectively, and the linearity is therefore obvious. The linear regression data are listed in Table 1: it is in the form of Y=0.0748X+0.0011 for CPM and Y=0. 0287X-0.0005 for DXM, where Y is peak area and X concentration.

Table 1. Linearity Data for CPM and DXM

Analytic	Linearity Range (µg/mL)	Regression Equation (y = mx + c)	Correlation Coefficient (r^2)
CPM	3.2 - 4.8	y = 27945x - 1258.4	0.9995
DXM	8.0 - 12.0	y = 19982x - 1856.7	0.9998

3.2.4. Accuracy

Accuracy of the method, as shown by percentage recovery results, was very high. The mean percentage recoveries from CPM were 98.96%101.16% and from DXM were 95.87%98.06% at three different levels of concentration. The

very low %RSD values (all below 1%) were evident proof for its high accuracy & reliability. The results are summarized in Table 2 and Figure 3.

Table 2. Accuracy (Recovery) Data

Spike Level	Mean % Recovery ± %RSD (n=3)	
	CPM	DXM
80%	101.16 ± 0.64	98.06 ± 0.58
100%	98.96 ± 0.88	97.01 ± 0.41
120%	98.99 ± 0.45	95.87 ± 0.37

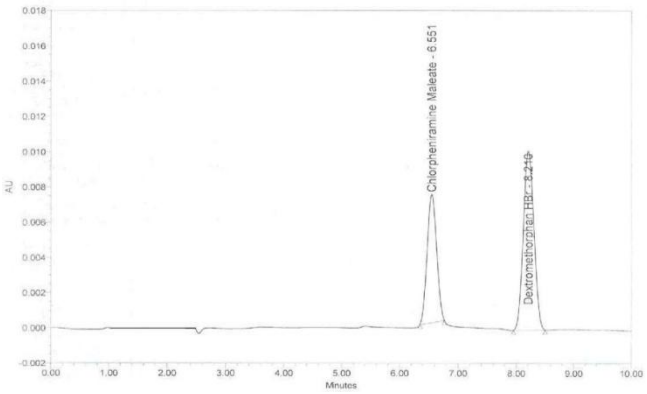

Figure 3.A): Recovery for Chlorpheniramine maleate and dextromethorphan HBr at80%:

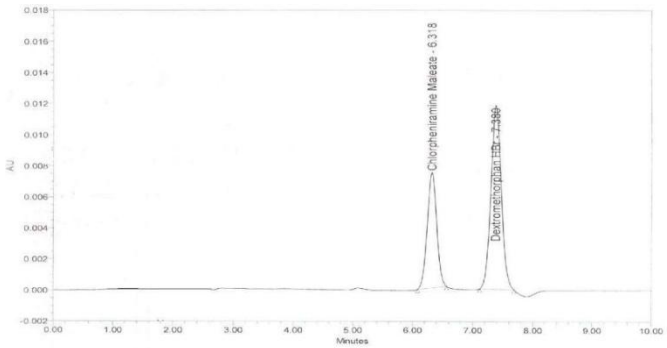

Figure3.B): Recovery for Chlorpheniramine maleate and dextromethorphan HBr at100%

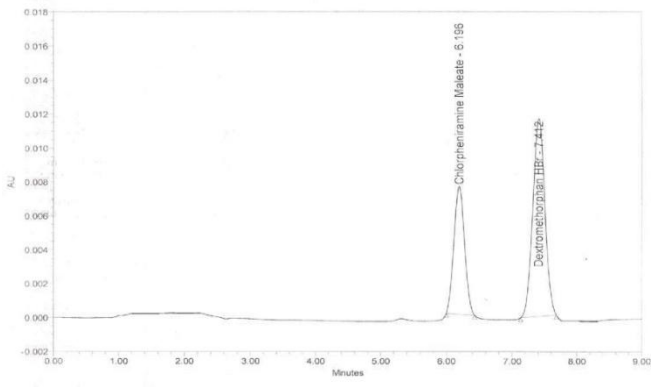

Figure 3(C): Recovery for Chlorpheniramine Maleate and Dextromethorphan HBr at 120%

3.2.5. Precision

The precision study results (Table 3) show that the method has high reproducibility. %RSDs for intra-day repeatability was 0.42% for CPM and 0.45% for DXM. The precision at different days （inter-day & different analyists） was shown as % RSD values less than 1 %, which demonstrates that the method performs consistently under different conditions.

Table 3. Precision Data

Precision Type	Conditions	Mean % Assay ± %RSD	
		CPM	DXM
Repeatability	n=6, Same Day	98.95 ± 0.42	95.82 ± 0.45
Intermediate	Day 2, Analyst 2	97.50 ± 0.36	96.14 ± 0.38
Intermediate	Different Analyst	98.68 ± 0.62	96.69 ± 0.27

3.2.6. Robustness

Upon checking even, the robustness of this method under all sorts of adverse conditions–flow rate, wavelength, column temperature, injection volume–system suitability parameters remained within their limits, and no peak RSD reached 2 % for either one of our drugs over any set period. This indicates that the method is reliable and can tolerate small intentional variations in a routine laboratory setting.

3.3. Application to Commercial Formulation

The validated method was successfully applied to determine the content of CPM and DXM in a commercially available cough syrup. The results were 99.2% for CPM and 98.5% for DXM of the labeled amounts indicated on each bottle, in line with acceptable limits of 90%-110%. This demonstrates that the method is applicable to routine quality-control testing of commercial products.

4. CONCLUSION

This Rapid RP-HPLC method, developed for the simultaneous determination of Chlorpheniramine Maleate and Dextromethorphan Hydrobromide, is simple, rapid, robust, and novel. Its mobile phase is low-cost and easy to prepare; in addition, it achieves good separation in 11 minutes without being unduly long for high-throughput laboratories. All parameters for method validation, as defined by ICH guidelines, were found to be acceptable, demonstrating that the method is specific, accurate, precise, linear, and robust. Therefore, this method may be readily used at any time for routine quality control testing of these substances in combination pharmaceutical dosage forms.

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6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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