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RP- HPLC METHOD FOR ESTIMATION OF PSEUDOEPHEDRINE HYDROCHLORIDE IN BULK AND TABLET

Rahul K. Godge*[1], Nachiket S. Dighe[1] and Rohini R. Gore[2]

[1]Department of Pharmaceutical chemistry, Pravara Rural college of Pharmacy, Pravaranagar.
[2]Department of Mathematics, Pravara Rural c ollege of Engineering, Pravaranagar.

*Correspondence for Author: Rahul K. Godge

Department of Pharmaceutical chemistry, Pravara Rural college of Pharmacy, Pravaranagar.

ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high-pressure liquid chromatographic method has been developed for the estimation of Pseudoephidrine hydrochloride from pharmaceutical formulation by using internal standard Chlorpheniramine maleate. The method was carried out on a HiQ sil C18 W (250mm x 4.6mm) column, with a mobile phase consisting of Acetonitrile: Methanol : Phosphate buffer (45:40:15 v/v) at a flow rate of 1.5 ml/min. Detection was carried out at 265 nm. The retention time of Pseudoephidrine hydrochloride and Chlorpheniramine maleate were 2.508 and 3.300 min. respectively. The developed method was validated in terms of accuracy, precision, linearity, Limit of detection, Limit of quantitation. The proposed method can be used for estimation of drug in dosage form for routine analysis.

KEYWORDS: Pseudoephedrine hydrochloride, Chlorpheniramine maleate, RP-HPLC.

INTRODUCTION

Pseudoephedrine hydrochloride is chemically 2-methylamino-1- phenyl-1-propanol hydrochloride and is official in the United States Pharmacopoeia[1], British Pharmacopoeia[2], and Indian Pharmacopoeia.[3] Pseudoephedrine hydrochloride is a white, crystalline powder and the molecular mass of Pseudoephedrine hydrochloride is 201.69 g/mol.[4] Pseudoephedrine is a decongestant that shrinks blood vessels in the nasal passages. It is used to relieve nasal congestion caused by colds, allergies, and fever. Pseudoephedrine occurs naturally as an alkaloid in certain plant species, the majority of pseudoephedrine produced for commercial use is derived from yeast fermentation of dextrose in the presence of benzaldehyde. The salts pseudoephedrine hydrochloride and pseudoephedrine sulfate are found in many over the counter preparations either as single-ingredient preparations, or more commonly in combination with antihistamines active substances including cetirizine[5] in capsule or coated tablet forms for the treatment of seasonal allergic rhinitis. Several methods such as HPLC[6-7], HPTLC[8], packed column supercritical fluid chromatography[9], and spectrophotometry[10- 13] have been reported in the literature. The present HPLC method was validated as per ICH guidelines.[14]

EXPERIMENTAL

Reagents and Chemicals

Acetonitrile (HPLC grade) and Methanol (HPLC grade) was purchased from Merck specialties pvt. Ltd. (Worli, Mumbai, India) and Water (HPLC grade) was purchased from Loba Chemie (Mumbai, India). Phosphate buffer was purchased from Sisco research Laboratories Pvt. Ltd.(Mumbai, India). All other reagents used were of HPLC grade.

Pharmaceutical formulation

Commercial tablets, each containing Pseudoephidrine hydrochloride Dose 60mg (Sudafed) was procured from the local market.

Method Development

Different mobile phases containing methanol, water, Acetonitrile, and different buffers in different proportion were tried and finally of Acetonitrile: Methanol: Phosphate buffer 45:40:15 v/v was selected as moile phase which gave good resolution and acceptable peak parameters for Pseudoephedrine hydrochloride.

System Suitability Studies

The resolution, number of theoretical plates and peak asymmetry were calculated for the standard solutions and is as shown in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combinations. The typical chromatogram of standard solution is as shown in Fig.1.

Apparatus and chromatographic Conditions

Chromatographic separation was performed on a Jasco chromatographic system equipped with a Jasco PU-2080 plus HPLC pump, Jasco UV-2075 plus UV / VIS detector and Rheodyne injector with 20 ml loop volume. HiQ SiL C18 (250mm x 4.6 mm i.d) was used for the

separation; mobile phase of a mixture of Acetonitrile: Methanol: phosphate buffer was delivered at a flow rate of 1.5 ml/min with detection at 265nm. The mobile phase was filtered through a 0.2 m membrane filter and degassed. The injection volume was 20 ml; analysis was performed at ambient temperature.

Preparation of Standard Solutions
Standard stock solutions of strength 0.5 mg/ml of Pseudoephedrine hydrochloride and 0.1 mg/ml of chlorpheniramine maleate were prepared separately using acetonitrile. From Standard stock solution of each drug, mixed standard solution was prepared in mobile phase to contain 50mg/ml of Pseudoephedrine hydrochloride and 5mg/ml chlorpheniramine maleate as an internal standard.

Calibration Curve
Linearity of the system was investigated by serially diluting the stock solutions to give concentrations in the range of 10 mg/ml to 60 mg/ml for Pseudoephedrine hydrochloride. An aliquot (20 ml) was injected using mixture Acetonitrile: Methanol: Phosphate buffer (45:40:15) v/v, as mobile phase. Calibration curves were obtained by plotting the response factor vs. concentration. The response factor is calculated as area of the drug peak divided by area of peak for internal standard. The calibration curves are as shown in Fig.2. The equations of the regression lines is
$$y = 0.117X - 1.127 \ (R^2 = 0.985)$$

Assay
Preparation of Sample Solutions
Twenty Tablets, containing 60 mg for Pseudoephedrine hydrochloride was weighed and finely powdered. A quantity of powder equivalent to 50mg was weighed and transferred to 25 ml volumetric flask. To this, acetonitrile was added and sonicated for 10 min; the volume was made up to 25 ml with acetonitrile to get solution of 500 mg/ml. The solution was filtered using whatmann filter paper. From the filtrate appropriate dilutions were made to obtain concentration in the range of 10 to 60mg/ml. Chlorpheniramine maleate was added to each sample dilution at 5mg/ml as internal standard. With the optimized chromatographic conditions, a steady baseline was recorded, the standard solution was injected and the chromatogram was recorded. The retention time of Pseudoephidrine hydrochloride and chlorpheniramine were found to be 2.508 and 3.300 min respectively. This procedure was repeated for the sample solution obtained from the formulation. The proposed method was found to be specific and no interference from common tablet excipients like lactose, starch etc was observed. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated. The assay was calculated from the equation of regression line for each drug. The percentage of individual drugs found in formulations was calculated and presented in table 2. The results of analysis shows that the amounts of drugs were

in good agreement with the label claim of the formulations.

Method Validation
As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantization and robustness.

Linearity and Range
The linearity of the method was determined for the formulation at five concentration levels ranging from 10 to 60 mg/ml. The equation for regression line was y = 0.0073X + 0.0089. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

Accuracy and Precision
The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery was calculated and presented in Table 2. Recovery was within the range of $100 \pm 2\%$ which indicates accuracy of the method. The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, 3 repeated injections of standard and sample solutions were made in a day and the response factor of drug peaks and percentage RSD were calculated and presented in Table 3. In the inter day variation studies, 3 repeated injections of standard and sample solutions were made on 3 consecutive days and response factor of drugs peaks and percentage RSD were calculated and presented in Table 3. The data obtained, %RSD not more than 1.5%, indicates that the developed RP-HPLC method is precise.

Limit of Detection and Limit of Quantification
The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula
LOD = (3.3 x standard deviation)/ Slope of calibration curve
The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula
LOQ = (10 x standard deviation) / Slope of calibration curve.
The LOD and LOQ for Pseudoephedrine hydrochloride were found to be 0.23mg/ml and LOQ 0.71mg/ml.

Robustness
Robustness is checked by making slight deliberate change in the experimental procedures. In the present method a deliberate change of room temperature and pH was made and the effects were noted. The method was found to be robust with respect to change in room temperatures.

RESULT AND DISCUSSION

The proposed method was found to be simple and linear in the concentration range of 10 to 60 µg/ml. The method was found to be accurate and precise as indicated by recovery studies and % RSD not more than 1.5. Moreover LOD and LOQ were found to be 0.23 mg/ml and 0.71 mg/ml, respectively. Thus the method is specific and sensitive.
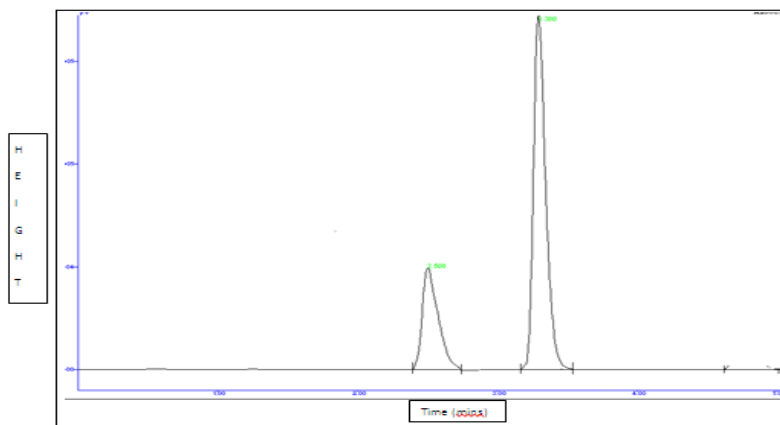

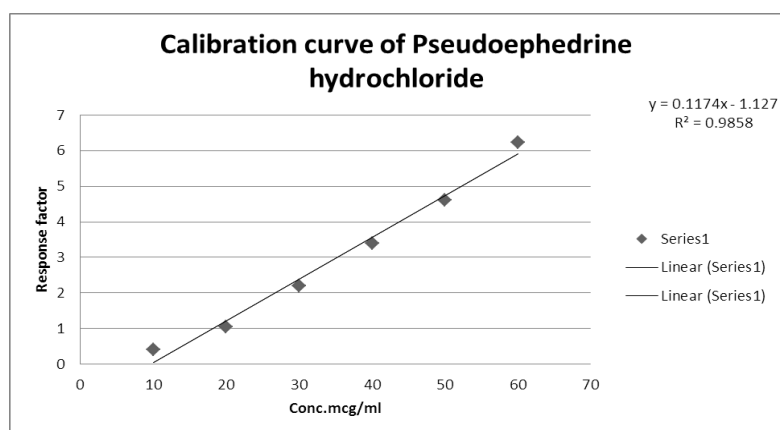**Figure:1 Chromatogram of Pseudoephidrine hydrochloride (2.508 min), Chlorpheniramine maleate 3.300 min.**

Fig.2 Calibration curve of Pseudoephedrine hydrochloride

Table 1: System Suitability Studies

Sr. No.	Parameters	Pseudoephedrine hydrochloride
1	Theoretical plate/ meter	6681.8
2	Resolution Factor	2.81
3	Asymmetry	1.10
4	LOD (µg/ml)	0.23
5	LOQ (µg/ml)	0.71

Table 2: Recovery Studies of Pseudoephedrine hydrochloride

Level of % Recovery	% Mean Recovery*	Standard Deviation	% R.S.D.†
80	100.37	0.137	0.07
100	98.87	0.075	0.052
120	100.82	0.1514	0.075

Table 3: Intraday and Inter day Precision Studies (System precision)

Conc. (mg/ml)	% RSD	
	Intra Day	Interday
10	0.12	0.29
20	0.27	0.38
30	0.2	0.9
40	0.37	0.28
50	0.25	0.37
60	0.36	0.42

CONCLUSION
The proposed RP-HPLC method for the estimation of for Pseudoephedrine hydrochloride in dosage form was found to be sensitive, accurate, precise, simple and rapid. Hence the present RP –HPLC method may be used for routine analysis of the raw materials and formulations.

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