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METHOD DEVELOPMENT AND VALIDATION OF PSEUDOEPHEDRINE HCL AND LORATADINE BY RP-HPLC IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Pseudoephedrine Hcl and Loratadine in bulk and tablet dosage form. Chromatography was carried out on a Symmetry C_{18} (4.6×250mm)5µ column using a mixture of Methanol and water(60:40) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 224nm. The retention time of the Pseudoephedrine Hcl and Loratadine was 2.4, 4.7 ±0.02min respectively. The method produce linear responses in the concentration range of 24-120µg/ml of Pseudoephedrine Hcl and Loratadine 10-50 µg /ml of Pseudoephedrine Hcl and Loratadine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Pseudoephedrine Hcl and Loratadine**,** RP-HPLC, ICH validation. PDA detection; Tablet dosage form.

INTRODUCTION

Pseudoephedrine hydrochloride is chemically 2-methylamino-1- phenyl-1-propanol hydrochloride (Fig**.** 1) 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.

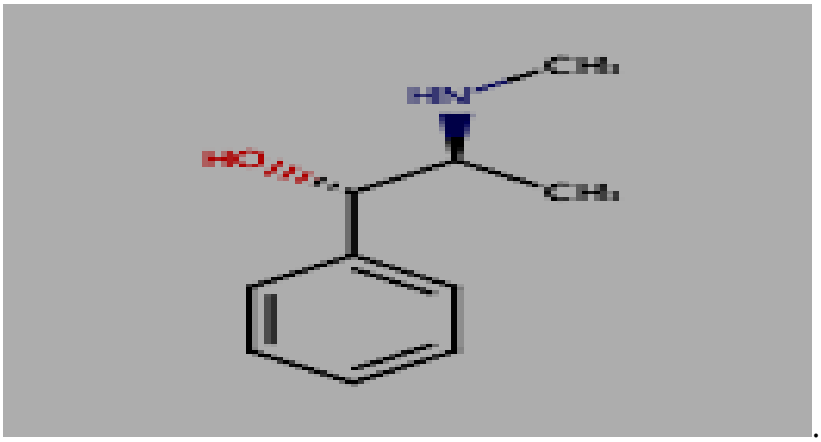

Fig.No.1.Chemical structure of Pseudoephedrine hydrochloride

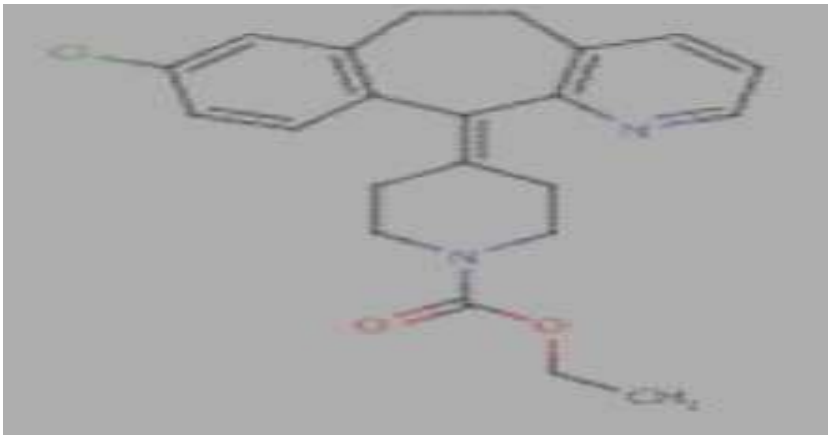

Fig.No.2.Chemical structure of Lortadine

The objective of the present study was to develop simple, precise, accurate and validated, economic and analytical methods for estimation of Pseudoephedrine Hcl and Loratadine in tablet dosage forms.

MATERIALS AND METHODS

Chemicals and solvents

Pure samples of PSD and LTD were obtained respectively from sura labs, Hyderabad, India. The commercial pharmaceutical preparation containing 60mg and 5mg PSD and LTD

respectively (Marketed by Piramal Health care Pvt. Ltd) were procured from local pharmacy. Methanol and water used are of HPLC grade.

Instrumentation: The chromatographic separations were performed using HPLC-Waters alliance (Model-2695) consisting of an inbuilt auto sampler, a column oven and 2996 PDA detector. The data was acquired through Empower-2- software. The A symmetry C18 4.6 x 250mm, 5µm particle size. Enertech sonicator was used for enhancing dissolution of the compounds. Lab India pH meter was used for adjusting the pH of buffer solution. All weighing was done on sartorious balance (model AE-160).

Chromatographic conditions

A Symmetry C18 (4.6 x 150mm, 5□m, Make: Waters) and 5µm particle size was used.at ambient temperature. 7 g of Potassium dihydrogen orthophosphate was weighed and 1000ml of Milli-Q water was added to it. The mobile phase was considered buffer: acetonitrile. pH was adjusted to 5 with ortho phosphoric acid and was filtered through 0.45µm PVDF membrane filter disc and was degassed. Flow rate was maintained at 1ml/min. The elution was observed at 245nm. Some trials were carried out with respect to change in the ratio of constituents of the mobile phase like 50: 60,50:50, 60:40 (Water: Metanol). Injection volume and run time were 20 µl l and 10 mins respectively.In the ratio 60:40 retention time for PSD and LTD were observed to be 2.25 and 7.58 min respectively. The two peaks were well resolved with good peak shape and symmetry was obtained.

Optimized Chromatographic Condition

Mobile phase ratio	: Methaol: water (60:40)
Column	: Phenomenex Gemini C18 (4.6×250mm)5µ
Column temperature	: 30°C
Wavelength	: 224nm
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 8mins

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of pseudoephedrine Hcl and Loratadine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to

dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

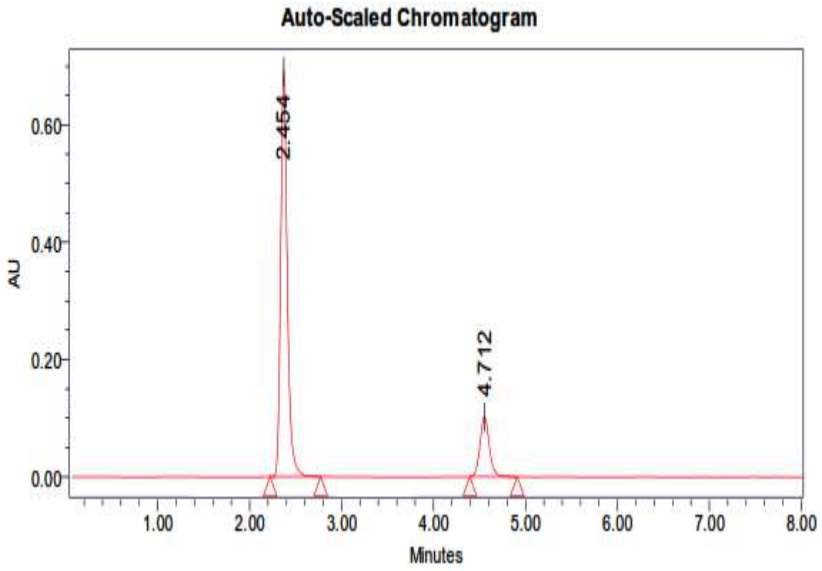

Figure: 3.Typical Optimized Chromatogram for PSD and LRTD.

Further pipette 0.72ml of pseudoephedrine Hcl and Loratadine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution: Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of pseudoephedrine Hcl and Loratadine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

METHOD VALIDATION

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precission, Specificity, Linearity, Accuracy, Limit of detection and Limit of quantification. Further pipette 0.72ml of pseudoephedrine Hcl and Loratadine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Linearity

Aliquots of 24, 48, 72, 96, and 120 ml were taken from stock solution of concentration 20-140µg/ml for PSD and LRDT, and then diluted up to mark with methanol. Such that the final concentrations were in the range 60-140µg/ml for PSD and LRDT, Volume of 10µl of each

sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. The observations and calibration curve were shown in Table 1 and Fig. 2, 3.

Table No.1.Linearity Results of pseudoephedrine Hcl and Lortadine

Concentration Level (%)	Concentration (µg/ml)	(PSD) Peak Area	LRTD Peak Area
60	24	865128	205948
80	48	1515612	368615
100	72	2312647	554821
120	96	3082541	728159
140	120	3818412	901481

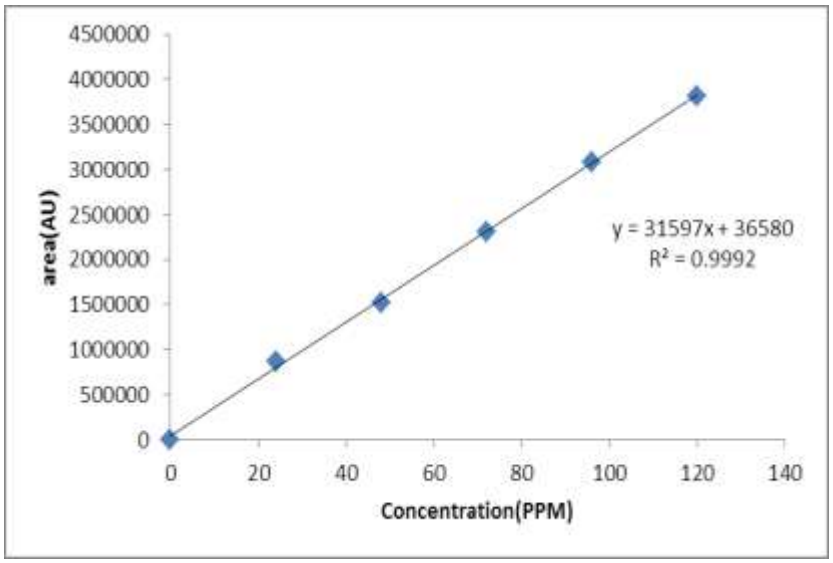

Fig No.4 Calibration graph of Pseudoephedrine Hcl.

Table2.Calibration Results Loratadine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	1	205948
80	2	368615
100	3	554821
120	4	728159
140	5	901481

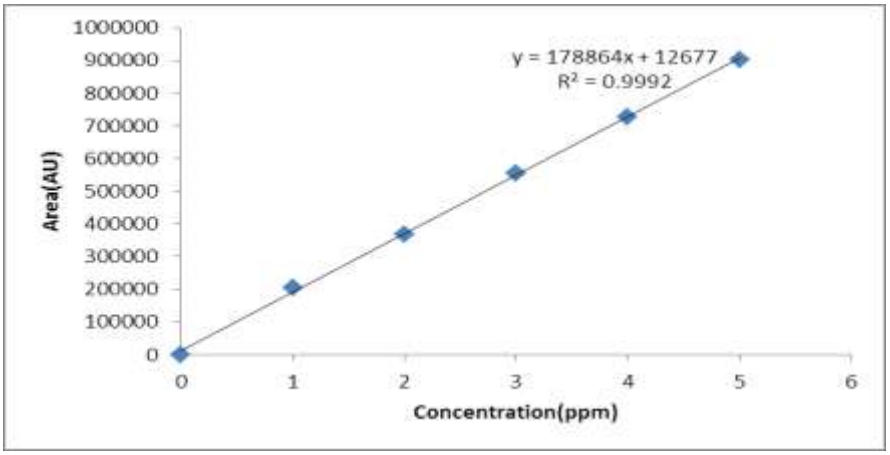

Fig.No.5.Calibration graph of Lortadine.

Accuracy

Accuracy of the method was done by recovery study. Sample solutions were prepared by spiking at about 50%, 100%, and 150% of specification limit to placebo and analyzed by the proposed HPLC method. Results are shown in Table-3.

Table No.3 Accuracy studies

%Concentration (at specification Level)	Amount Added (ppm)		Amount Found (ppm)		% Recovery	
	PSD	LRD	PSD		PSD	LRTD
50%	36	1.5	1.5	35.33895	98%	100%
100%	72	3.0	3.0	71.57961	99.3%	100%
150%	108	4.5	4.5	108.8065	100.7%	101%

Specificity

The specificity of the method was performed by injecting blank solution(without any sample) and then a drug solution of 10µl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both PSD and LRTD from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be Specific.

Limit of detection (LOD) and Limit of quantification (LOQ)

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The linearity for PSD and LRTD was performed from 40-140µg/ml and 4.3 and 7.6 for LOD and 1.3-23.12µg/ml for LOQ respectively.

System precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Standard solution of 40- 140µg/ml for PSD and 2.5-15.µg/ml for LRDT were prepared as per test method and injected for 3 times. Results are shown in Table-4.

Method precision

Three samples were prepared and analyzed as per the test method on same day and three different days and calculated the % RSD for assay of five preparations. Results were shown in Table- 5.

Robustness

Robustness studies were carried out by variations in flow rate, mobile phase compositions and temperature. It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak interest. The degree of reproducibility of the results proven that the method is robust.

RESULTS AND DISCUSSION

The nature of sample, its molecular weight and solubility decides the proper selection of stationary phase. The drugs PSD and LRTD were preferably analyzed by reverse phase chromatography and accordingly C18 column was selected. The elution of the compounds from column was influenced by polar mobile phase. The ratio of phosphate buffer to Acetonitrile was optimized to (60:40) to give well resolved and good symmetrical peaks with short run time. The retention time of PSD and LRTD were found to be 2.25 & 7.59 min respectively. The calibration curve was linear over the concentration range of 20-140µg/ml for PSD and LRTD. The linearity of the method was statistically confirmed. RSD values for accuracy and precission studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters were given in table-5. The analytical recovery at five different concentrations of PSD and LRTD was determined and the recovery results were in the range of 100-300µg/ml. Therefore proposed validated method was successfully applied to determine PSD and LRTD in Bulk and Pharmaceutical dosage form.

CONCLUSION

The developed method is accurate, simple, rapid and selective for the simultaneous estimation of PSD and LRTD in tablet dosage form. The sample preparation is simple, the

analysis time is short and the elution is by gradient method. The retention time of PSD and LRTD were found to be 2.25 & 7.59 min respectively. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. Hence the proposed method can be conveniently adopted for the routine quality control analysis in the combined formulation.

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