Volume 2 (6), 2014, Page-463-469
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Method Development and Validation for the Simultaneous Estimation of Pseudoephedrine and Loratidine in Bulk and Pharmaceutical Dosage Form Using RP-HPLC

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ARTICLE INFO

Received: 22 Nov 2014
Accepted: 12 Dec 2014

ABSTRACT

A simple specific, accurate and precise RP-HPLC method has been developed for the simultaneous estimation of Pseudoephedrine and Loratidine in pharmaceutical dosage form. The Isosbestic point for Pseudoephedrine and Loratidine was found to be 283nm using mixture of Acetonitrile: 0.02M Phosphate Buffer (33:67) as the mobile phase system. The retention times of Pseudoephedrine and Loratidine were found to be 3.90 and 2.60 minutes respectively. Different analytical performance parameters such as Specificity, Linearity, Precision, Limit of detection (LOD) and Limit of quantification (LOQ) were determined according to ICH guidelines. Calibration graphs were potted using standard peak areas vs. concentration of standard solutions. The slope, intercept and correlation coefficient values were found to be 13801, 10378 and 0.997 and 44623, 10569 and 0.999 for Pseudoephedrine and Loratidine, respectively. Pseudoephedrine and Loratidine were found to be linear in the range of 0 to 50µg/ml. The LOD of Pseudoephedrine and Loratidine were found to be 0.06µg/ml and 0.02µg/ml respectively. The LOQ of Pseudoephedrine and Loratidine were found to be 1.2µg/ml and 0.04/ml respectively. The recovery values were found within the limits indicating that the method is accurate. The developed method was found to be robust. System suitability parameters like number of theoretical plates (N), Tailing factor (T) and Resolution (R_s) were studied. The validated liquid chromatographic method was applied to simultaneous determination of Pseudoephedrine and Loratidine for routine studies.

Keywords: RP-HPLC, Method Development, Validation, Pseudoephedrine, Loratidine.

1. INTRODUCTION

Pseudoephedrine (PSE) chemically known as (1S,2S)-2-methylamino-1-phenylpropan-1-ol (Figure 1), is a sympathomimetic and Nasal decongestant. The

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vasoconstriction that pseudoephedrine produces is believed to be principally an α-adrenergic response and the β2-receptor response is responsible for the relaxation of smooth muscle in the bronchi. [1, 2]

Loratidine chemically known as Ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)-1-piperidinecarboxylate (Figure 2), is a second generation H_1 histamine antagonist drug used to treat allergies. It is a tricyclic antihistamine, which acts as a selective inverse agonist of peripheral histamine H_1-receptors.

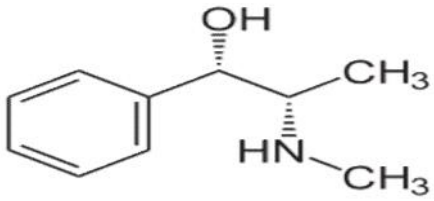

Fig 1: Pseudoephedrine

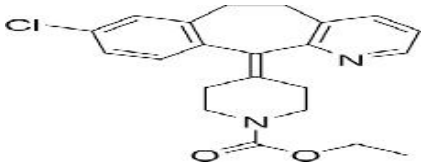

Fig 2: Loratidine

The combination of Pseudoephedrine and Loratidine is prescribed by the physician for the treatment of Sinusitis and Asthmatic allergy. Literature survey reveals several methods that have been used for the quantitative determination of these two drugs individually and in combination with other drugs. The objective of the present work is to develop a new RP-HPLC method for the simultaneous estimation of Pseudoephedrine and Loratidine in bulk and pharmaceutical dosage form.

2. MATERIALS AND METHOD

Materials and Instrument:

The pharmaceutical grade Pseudoephedrine and Loratidine were obtained as gift samples from Aurobindo Laboratories. HPLC grade Acetonitrile, Water, Orthophosphoric acid of AR grade and Potassium phosphate was procured from S.D. Fine Chemicals, Mumbai, India.

The chromatographic separation was performed on a Waters 2690/5, integrated with Auto Sampler and PDA detector and Empower2 software. The analytical Develosil ODS HG-5 RP C_{18} column (5µm, 15cmx4.6mm i.d.) was used for the separation. The mobile phase consisted of 0.2M Potassium dihydrogen phosphate buffer (pH 2.5):Acetonitrile (67:33 v/v).The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1.0ml/min and the detector wavelength was set at 283 nm. The injection volume was 20 µl. [3-6]

Preparation of Mobile Phase

A potassium dihydrogen phosphate buffer and acetonitrile was used as mobile phase. Buffer was prepared by adding potassium dihydrogen phosphate in to a 1000mLvolumetric flask, dissolved by adding 500mL of distilled water to it and sonicated. The pH was adjusted to 2.5 with dilute orthophosphoric acid and diluted to volume with distilled water. The mobile phase with a mixture of potassium dihydrogen phosphate buffer and acetonitrile in the ratio of 67:33 v/v was prepared. This mobile phase was filtered through 0.45µ membrane filter and degassed in ultrasonic water bath. The mobile phase was delivered at a flow rate of 1.0mL per min. [7-9]

Preparation of standard stock solutions

In a 100mL volumetric flask10mg of Pseudoephedrine was added and mixed with 10mL of mobile phase. This solution was sonicated to dissolve completely and diluted to the mark. From this, 3mL of the solution was taken in a 10mL volumetric flask and the volume was made up to the mark with the mobile phase. The final concentration obtained was 30µg/ml. Similarly, 30µg/ml solution of Loratidine was prepared. [10-11]

Chromatographic conditions

The selected and optimized mobile phase was 0.2M Potassium dihydrogen phosphate buffer pH 2.5 (67:33 v/v) and conditions optimized were: flow rate (1.0 mL/min), wavelength (283nm), run time (10min) at which better resolution was observed. All the experiments were performed in the isocratic mode. Detection of the analytes was done at a wavelength of 283nm. Injection volume of the analytes was set at a constant volume of 20µl by using a fixed sample loop. Twenty micro litres of the standard solution of Pseudoephedrine and Loratidine was injected each time in to the stream of mobile phase system at a flow rate of 1.0 ml/min and the corresponding chromatograms were obtained. [12, 13]

Sample preparations

Analysis of marketed formulation

A commercial brand, Claritin-D tablet was procured for testing suitability of the proposed analytical method to estimate Pseudoephedrine and Loratidine in tablet formulation. The label claim was 120 mg and 5mg for Pseudoephedrine and Loratidine respectively. Twenty tablets were weighed and average weight was determined. These tablets were crushed to a fine powder and weighed quantity of powder equal to the average weight was transferred in the 100 ml volumetric flasks. Then 50 ml of mobile phase is added in this volumetric flask. The contents of the flask were allowed to dissolve with intermittent sonication to ensure complete solubility of the drug. The mixture was diluted to 100 ml with mobile phase, thoroughly mixed and then filtered through 0.45µ membrane filter. 20µl of each of this solution was injected into the HPLC system. The drug content in the test preparation was quantified by comparing with the known amount of standard injected. The related amounts of Pseudoephedrine and Loratidine in binary mixtures or dosage forms were individually calculated using the related linear regression equations. [14-16]

3. RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

During the optimization of this method for better separation two different columns were tried (Waters C_{18}, 5µm, 25cmx4.6mm & Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm), different organic solvents viz, acetonitrile, water, methanol, acetate and phosphate buffers were tried. Acetonitrile and potassium phosphate buffer solvent system which proved to be useful for better resolution was tried at different pH values from 2 to 5. As a result of pH screening, the optimum mobile phase was chosen as 0.02M Potassium phosphate buffer (pH 2.5) & Acetonitrile in the ratio of 67:33. The flow rate was set to 1.0ml per min for all experiments. Detector wavelength of 283nm was chosen at which both the drugs absorbed appreciably with the respective retention times of 3.85 min and 2.59 min for Pseudoephedrine and Loratidine, respectively as shown in the Figure 1. [17]

Validation of the method

The aim of the method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2A and Q2B. The method was validated as per the recommendation of ICH for the parameters like specificity, linearity, precision, and accuracy, Limit of detection and limit of quantification. [18, 19]

Specificity

Specificity can be described as the capability of the method to accurately measure the response of the analysed compound with no interferences originating from sample matrix. For the specificity of the method the marketed formulation has been taken & the solution was injected into the HPLC system. The chromatogram obtained is shown in the Figure 2.No peaks were found at the retention of Pseudoephedrine and Loratidine

which indicated that the excipients did not interfere with the analysis.

Linearity

The linearity curve was made using standard solutions containing Pseudoephedrine and Loratidine at different concentrations. The area under the peaks for Pseudoephedrine and Loratidine were found to be linear over the concentration range of 0-50 µg/ml as shown in the Figure 3 & 4. The data of regression analysis of the calibration curves is shown in Table 1. The correlation coefficient, slope and intercept were found to be 0.997, 13801, 10378 & 0.999, 44623, 10569 for Pseudoephedrine and Loratidine, respectively.

Accuracy (Recovery study)

Accuracy of the proposed method was established by recovery experiments using standard addition method. This study was employed by addition of known amounts (80%, 100% &120%) of pure drugs of Pseudoephedrine and Loratidine to the pre-analysed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The mean recovery was found to be 100.10% for Pseudoephedrine and 99.45% for Loratidine. The % recovery is between 98-102% which indicates specificity and accuracy of the method. Results obtained from recovery studies are as shown in Table 2.

Precision

Repeatability

The precision of the method were assessed by repeatability in which five replicates of a fixed amount of drug were injected and analysed. The relative standard deviation for Pseudoephedrine and Loratidine were calculated and are shown in the Table 3.The % R.S.D. values of the measurements ranged between 0.35 and 0.61%, confirming good precision of the proposed method.

Intermediate precision

For intraday studies, drug solutions of three concentrations were injected six times each into the HPLC system and for inter day studies, the solutions of three concentrations were injected into the HPLC on different days. Data obtained {shown in Table 4(a) &4(b)} were analysed. The % R.S.D. of results obtained in intermediate precision study was not greater than 2% confirming good precision.

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

The limit of detection and limit of quantification were determined using standard deviation of the response and the slope of the calibration curve. The LOD and LOQ values obtained for Pseudoephedrine and Loratidine were calculated and are shown in the Table 5.

Robustness

For testing the robustness of method a few parameters like flow rate, percentage of composition of acetonitrile in the mobile phase were deliberately changed. One parameter was changed at one time to evaluate the effect in results. The % R.S.D. of results of samples obtained for robustness with respect to change in flow & change in composition were within 2% of method precision & thus ensures that the method is Robust.

System suitability

System suitability testing is an integral part of analytical method validation. The system suitability was determined by injecting six replicate injections from freshly prepared standard solutions and analysing each solute for resolution (Rs),tailing factor (T) and number of theoretical plates (N). The results are shown in Table 6.

Table 1: Method validation-Linearity (Standard curve for PSE &LOR)

Concentration (µg/ml)	Peak Area (PSE)	Peak Area (LOR)
0	0	0
10	1228747	4224838
20	2638031	904737
30	3983572	1302869
40	5249436	1746831
50	6979310	2250813

Table 2: Method validation-Accuracy studies (Recovery data)

PSE		LOR	
Level-%	**% Recovery**	**Level-%**	**%Recovery**
80	100.03	80	100.03
80	100.25	80	99.72
80	99.48	80	99.28
100	99.98	100	99.29
100	99.86	100	99.15
100	102.34	100	99.13
120	99.59	120	99.21
120	99.64	120	99.55
120	101.99	120	99.69
Mean	100.35	**Mean**	99.45
Std. dev	1.146	**Std. dev**	0.167
%R.S.D.	1.140	**%R.S.D.**	0.1684

Table 3: Method validation-Method Precision (Repeatability)

PSE		LOR	
Peak Area		**Peak Area**	
Replicate 1	3983572	Replicate 1	1302869
Replicate 2	3985214	Replicate 2	1302586
Replicate 3	3990228	Replicate 3	1318521
Replicate 4	3985261	Replicate 4	1302569
Replicate 5	3996512	Replicate 5	1302896
Average	3988157	Average	1305888
Std. Dev	5295.407	Std. dev	7063.605
%R.S.D.	0.132778	%R.S.D.	0.540904

Table4(a):Methodvalidation-Method Precision(Intermediate Precision) for Pseudoephedrine

Conc. of PSE (µg/ml)	Intra day		Inter day	
	Observedconc.	%R.S.D.	Observedconc.	%R.S.D.
	(Mean n=6)		(Mean n=6)	
20	19.94	0.96	19.43	0.97
30	30.04	0.40	29.93	0.96
40	39.91	0.93	40.15	0.19

Table4(b):Methodvalidation-Method Precision(Intermediate Precision) for Loratidine.

Conc. of LOR (µg/ml)	Intra day		Inter day	
	Observed conc.	%R.S.D.	Observedconc.	%R.S.D.
	(Mean n=6)		(Mean n=6)	
20	20.09	1.54	20.13	0.46
30	30.03	0.75	29.84	0.82
40	39.94	0.48	39.37	0.91

Table 5: Method validation-Limit of detection & Limit of quantification

	Conc. (µg/ml)	Conc. (µg/ml)
Pseudoephedrine	0.06	1.2
Loratidine	0.02	0.04

Table 6: Method validation-System suitability parameters

S.No.	Parameter	Limit	Result
1	Resolution	Rs>2	9.15
2	Tailing factor	T≤2	PSE=0.5
			LOR=0.12
3	Theoretical plates	N>2000	PSE=4693
			LOR=3246

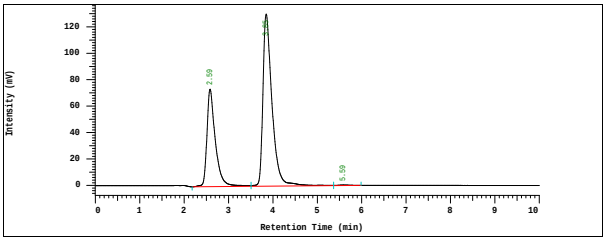

Fig 1: Chromatogram of synthetic mixture

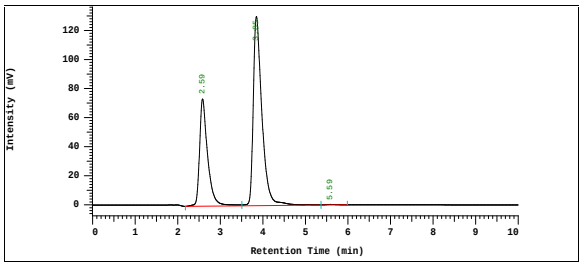

Fig 2: Method validation-Chromatogram for Specificity

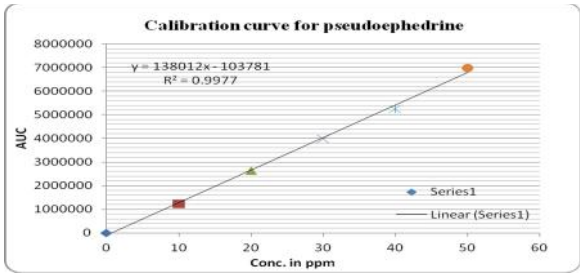

Fig 3: Method validation-Linearity (Standard curve for Pseudoephedrine)

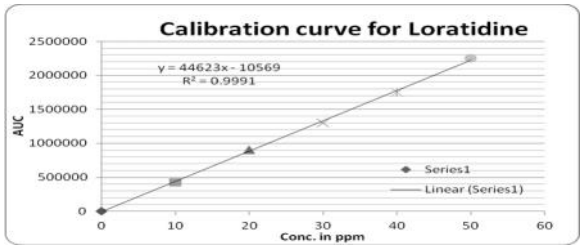

Fig 4: Method validation-Linearity (Standard curve for Loratidine)

4. CONCLUSION

The developed method was validated HPLC method has been proved to be simple, precise, accurate, rapid and reliable. To achieve sharp peaks with good resolution under isocratic conditions, mixture of 0.2M Potassium buffer and Acetonitrile in different proportion were tested as mobile phase on a C_{18} stationary phase. The mixture of 0.2M Phosphate buffer and Acetonitrile in the proportion of 67:33 v/v was proved to be the most suitable for estimation. Both the drugs absorbed appreciably at 283nm & hence this wavelength was selected for detection of both the compounds. Since the chromatographic peaks were better defined, resolved with this system, under the above mentioned chromatographic conditions the retention time obtained for Pseudoephedrine and Loratidine were 3.85 and 2.59 min respectively. The calibration curve for Pseudoephedrine and Loratidine was found to be linear over the range of 0-50µg/ml. The data of regression analysis of the calibration curves is shown in Table 1.

The proposed method was validated for the test parameters like specificity, linearity, accuracy, precision, LOD, LOQ and system suitability. The developed method was found to be simple, sensitive and selective for analysis of Pseudoephedrine and Loratidine in combination without any interference from excipients. Thus the method was successfully used for the determination of Pseudoephedrine and Loratidine in pharmaceutical formulation.

The developed method can be used for the simultaneous estimation of Pseudoephedrine and Loratidine. It can be used for routine quality control analysis of tablet dosage forms containing Pseudoephedrine and Loratidine.

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