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Research Article

Method Development and Validation for Triprolidine and Phenylepherine in Bulk and Its Pharmaceutical Dosage Forms by Using RP-HPLC as per ICH Guidelines

Angara Ganesh[1]*, Gope Edward Raju[2], Vaddi Lakshmi Priya[3], Doonaboyina Raghava[4], Kavala Nageswara rao[5]

Department of Pharmaceutical Analysis, K.G.R.L College of Pharmacy, Bhimavaram-534201, Andhra Pradesh, India

Abstract

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Triprolidine and Phenylephrine was done by RP-HPLC. The Phosphate buffer was p^H4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of P^H 4.5(20:80 v/v). Kromosil C_{18} column (250mm x 4.6mm) 5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min^{-1}. The linearity range of Triprolidine and Phenylephrine were found to be from 100-500 µg/ml of Triprolidine and 1-5µg/ml of Phenylephrine. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. Triprolidine % RSD 0.2 and Phenylephrine % RSD 0.6.Intermediate precision for Triprolidine %RSD 0.2 and Phenylephrine%RSD0.1The percentage recovery varies from 98-102% of Triprolidine and Phenylephrine. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Kromosil $C_{18,}$ Triprolidine and Phenylephrine, RP-HPLC

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Corresponding Author:
Angara Ganesh
Department of Pharmaceutical Analysis,
K.G.R.L College of Pharmacy,
Bhimavaram-534201, Andhra Pradesh, India

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Contents

1. Introduction

Triprolidine is a sedating antihistamine combined with pseudoephedrine and guaifenesin in various types of cold and allergy medications to relieve allergy symptoms, hay fever and common cold symptoms, and to aid in sleep.

Phenylephrine is an alpha-1 adrenergic agonist used in the management of hypotension, generally in the surgical setting associated with the use of anaesthetics.

2. Materials and Methds

Instrumentation

The instrument used was HPLC waters 2690 separation module with photo diode array detector, Software-empower. The stationary phase used was Inertsil (250×4.6mm, 5µ) ODS C-18 RP-column Digital weighing balance-Model number BSA224SCW (Ascoset), Sonicator (Enertech)-SE60US, pH meter Model number AD102U

Materials and reagents

Roflumilast and Montelukast were gift samples provided by Hetero Laboratories, Hyderabad, Ortho phosphoric acid , Potassium dihydrogen, Tri ethyl amine, Methanol and Water for HPLC were supplied by Merck India Ltd, Mumbai

Method development

Six trials were made by changing the mobile phase ratios and solvents Buffer: Methanol P^H 2.5 (30:70 v/v) Buffer: Methanol P^H 2.5 (30:70 v/v) Buffer: Methanol P^H 2.5 (60:40 v/v) Phosphate buffer: Methanol P^H 2.5 (20:80 v/v) Phosphate buffer: Methanol P^H 2.5 (55:45 v/v) Phosphate buffer: Methanol P^H 2.5 (25:75 v/v). Finally, the mobile phase was optimized to Methanol: Phosphate buffer P^H 2.5 (25:75 v/v).

Chromatographic conditions: From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Methanol 25:75 were set (Buffer P^H 2.45 adjusted with Triethylamine), Inertsil C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm.

3. Results and Discussion

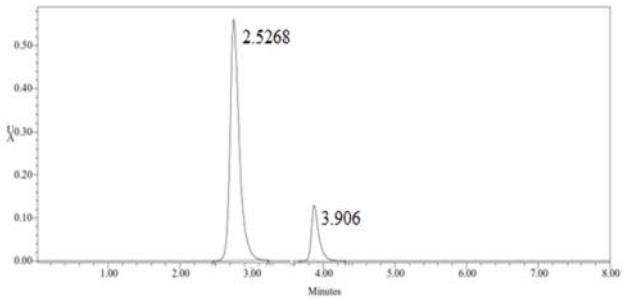

Figure 1: chromatogram for system suitability

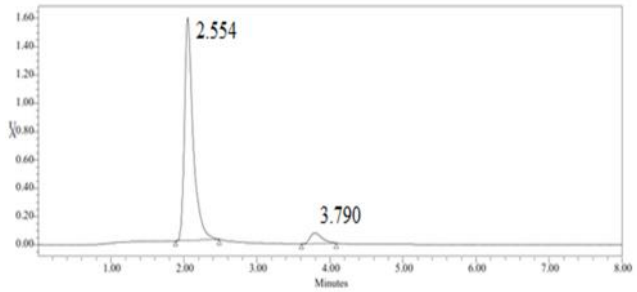

Figure 2: chromatogram for standard injection

Table 1 Results of system suitability parameters for Triprolidine and Phenylephrine

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Triprolidine	2.669	124505	223532	**1.2**	1.2	4523.3
2	Triprolidine	2.5264	123442	134544	1.2	1.2	5020.2
3	Triprolidine	2.5265	123431	124386	1.2	1.2	4061.2
4	Triprolidine	2.5266	125432	134568	1.2	1.2	5032.4
5	Triprolidine	2.5267	122434	146852	1.2	1.2	5076.4
6	Triprolidine	2.5268	124438	145782	1.2	1.2	6024.8
7	Phenylephrine	3.855	1308495	154566	1.3	1.3	6090.3
8	Phenylephrine	3.902	1309496	156428	1.3	1.3	5023.2
9	Phenylephrine	3.903	1306498	152634	1.3	1.3	8060.7
10	Phenylephrine	3.904	1342499	158426	1.3	1.3	7080.1
11	Phenylephrine	3.905	1343451	158484	1.3	1.3	6054.4
12	Phenylephrine	3.906	1346455	158423	1.3	1.3	7080.6

Table 2 Showing %RSD results method precession for Triprolidine

Injection	Peak Name	Rt	Area	Height
1	Triprolidine	3.699	1302729	341432.2
2	Triprolidine	3.790	1302947	523341.4
3	Triprolidine	3.663	1303236	374642.4
4	Triprolidine	3.658	1303977	327514.3
5	Triprolidine	3.647	1309759	374028.1
6.	Triprolidine	3.645	1309789	346280.2
mean			1304529.8	

Std.dev			2961.1	
%RSD			0.2	

Table 3 Showing % RSD results method precession for Phenylephrine

Injection	Peak Name	Rt	Area	Height
1	Phenylephrine	3.616	123149	248742.3
2	Phenylephrine	3.634	123766	281441.2
3	Phenylephrine	3.460	124271	271721.2
4	Phenylephrine	3.446	124691	284393.8
5	Phenylephrine	3.437	124956	256318.0
6	Phenylephrine	3.438	125845	226813.0
mean			124162.7	
Std.dev			725.6	
%RSD			0.6	

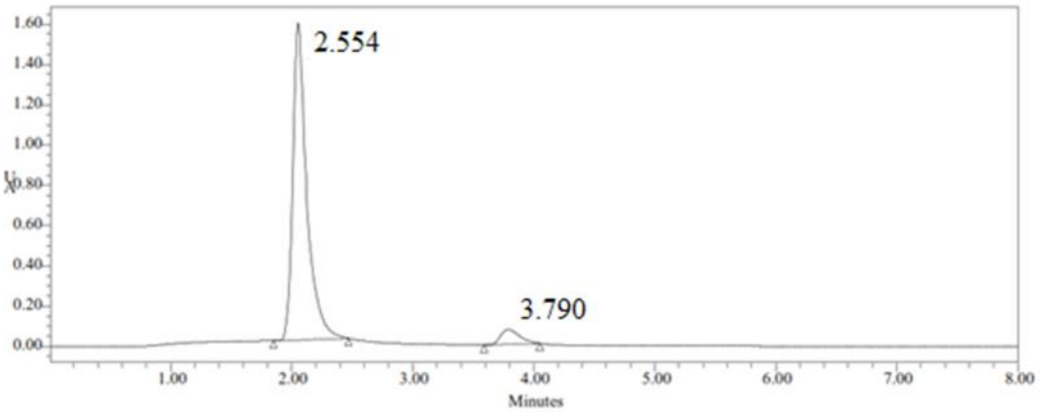

Figure 3 Chromatogram for standard injection

Table 4 Showing results for intermediate precision of Triprolidine

Injection	Peak name	Rt	Area	Height
1	Triprolidine	2.554	1300148	438467.1
2	Triprolidine	2.557	1304520	436873.3
3	Triprolidine	2.563	1305937	438572.1
4	Triprolidine	2.562	1306476	435587.5
5	Triprolidine	2.561	130871	432826.4
6	Triprolidine	2.561	130872	432838.3
mean			1305070.2	
Std.dev			3061.8	
%RSD			0.2	

Table 5 Showing results for intermediate precision of Phenylephrine

INJECTION	Peak name	Rt	Area	Height
1	Phenylephrine	3.790	122487	241421.6
2	Phenylephrine	3.657	122626	233417.3
3	Phenylephrine	3.663	122632	281751.1
4	Phenylephrine	3.646	122702	241843.6
5	Phenylephrine	3.662	122962	281564.1
6	Phenylephrine	3.663	122972	284917.2
mean			122681.8	
Std.dev			174.8	
%RSD			0.1	

Table 6 Details of Accuracy 50%

INJECTION	Peak Name	RT	Area	Height
1	Triprolidine	2.572	132457	86026
2	Triprolidine	2.573	132458	85549
3	Triprolidine	2.576	134242	84196
4	Phenylephrine	3.881	122487	21744
5	Phenylephrine	3.882	122489	21909
6	Phenylephrine	3.792	122392	21382
Mean			371513.5	
Std.Dev			253899.3	
% RSD			0.532	

Table 9 accuracy (recovery) data for Triprolidine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table 10 accuracy (recovery) data for Phenylephrine

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

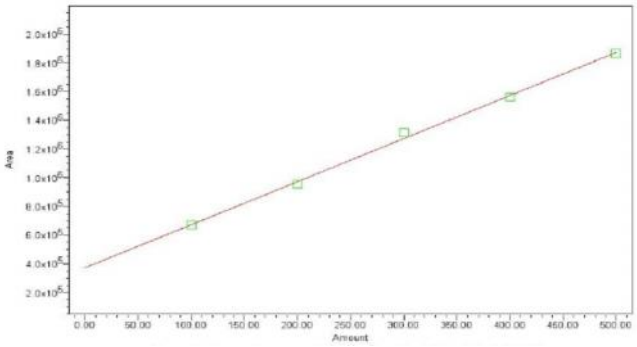

Figure 8 calibration graph for Triprolidine

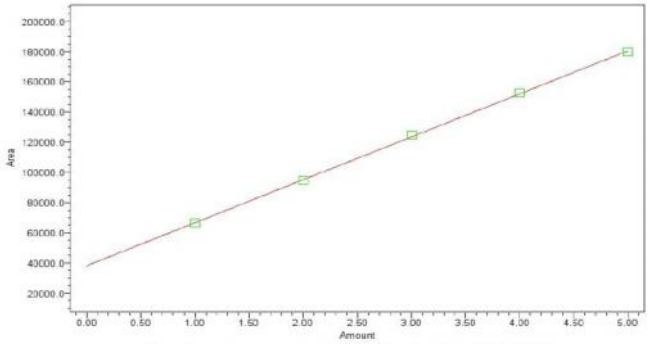

Figure 9 calibration graph for Phenylephrine

Table 13 Analytical performance parameters of Triprolidine and Phenylephrine

Parameters	Triprolidine	Phenylephrine
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 14 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Triprolidine	52	152	2.9
Phenylephrine	52	156	3

Table 15 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Triprolidine	52	522	10.03
Phenylephrine	52	524	10.1

Table 16 Flow Rate (ml/min) data for Triprolidine

		System Suitability Results	
S.No	**Flow Rate (ml/min)**	**USP Plate Count**	**USP Tailing**
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 17 flow rate (ml/min) data for Phenylephrine

		System Suitability Results	
S.No	**Flow Rate (ml/min)**	**USP Plate Count**	**USP Tailing**
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Triprolidine and Phenylephrine was done by RP-HPLC. The Phosphate buffer was pH4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of P^H 4.5(20:80 v/v). KromosilC_{18} Column (250mm x 4.6mm) 5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min^{-1}. The linearity range of Triprolidine and Phenylephrine were found to be from 100-500 µg/ml of Triprolidine and 1-5µg/ml of Phenylephrine. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. Triprolidine %RSD 0.2 and Phenylephrine % RSD0.6. Intermediate precision for Triprolidine %RSD 0.2 and Phenylephrine %RSD0. 1. The percentage recovery varies from 98-102% of Triprolidine and Phenylephrine. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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