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Analytical method development and validation for simultaneous estimation of loratadine and pseudoephedrine in bulk and tablet dosage form by RP HPLC method

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Pseudoephedrine and Loratadine , in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Luna C18 (4.6×150mm, 5µ) column using a mixture of Acetonitrile: Water (10:90% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 240nm. The retention time of the Pseudoephedrine and Loratadine was 1.933, 3.396±0.02min respectively. The method produce linear responses in the concentration range of 16.5-82.5mg/ml of Pseudoephedrine and 5-25mg/ml of 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, Loratadine , RP-HPLC, validation.

INTRODUCTION

Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the

collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (QC)

In many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants

The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies

The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays

The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and applied research

The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

MATERIALS AND METHODS

Pseudoephedrine from Sura labs, Loratadine from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Pseudoephedrine and Loratadine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of Loratadine and 0.49ml the above Pseudoephedrine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile: water and Phosphate buffer pH 4.0: Methanol with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 10:90v/v respectively.

Optimization of Column

The method was performed with various columns like C18 and C8 columns, Symmetry and Xterra column. Luna C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 0.9ml/min flow.

Optimized chromatographic conditions

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	35ºc
Column	:	Phenomenex Luna C18 (4.6×150mm, 5µ)
Mobile phase	:	Acetonitrile: Water (10:90v/v)
Flow rate	:	0.9ml/min
Wavelength	:	240 nm
Injection volume	:	10 µl
Run time	:	6min

Validation

Preparation of buffer and mobile phase

Preparation of mobile phase

Accurately measured 100 ml (10%) of Acetonitrile and 900 ml of Water (90%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the Diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: Water (10:90%v/v)
Column : Phenomenex Luna C18 (4.6×150mm, 5µ)
Column temperature : 35ºC
Wavelength : 240nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 6minutes

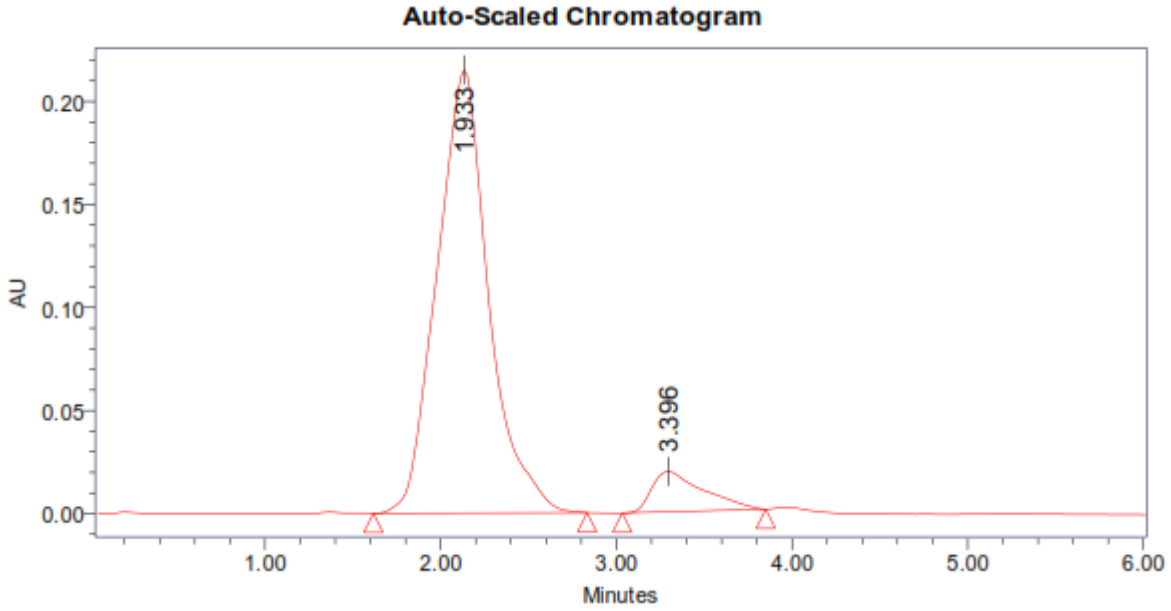


Fig 1: Optimized Chromatogram (Standard)

Table1: Optimized Chromatogram (Standard)

S.No	Name	Rt (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP Plate count
1	Pseudoephedrine	1.933	409905	214828		1.15	4242
2	Loratadine	3.396	392596	19612	4.9	1.78	6515

By the above chromatogram it shows that separation of two peaks is well, it shows proper plate count, tailing and shows proper resolution. So it was optimized chromatogram.

Optimized Chromatogram (Sample)

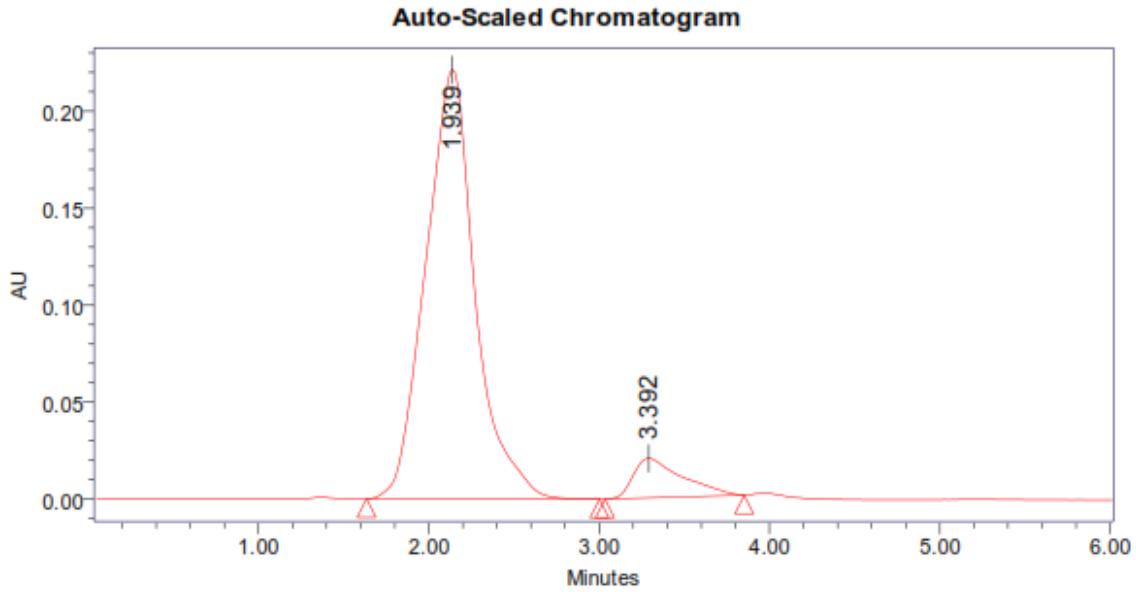


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	Rt (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP Plate count
1	Pseudoephedrine	1.939	409003	221408		1.13	5253
2	Loratadine	3.392	323731	20385	4.9	1.79	7569

Assay (Standard)

Table 3: Peak results for assay standard of Pseudoephedrine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pseudoephedrine	1.939	407105	219674	5249	1.14
2	Pseudoephedrine	1.943	407333	218266	5248	1.14
3	Pseudoephedrine	1.949	409824	221080	5254	1.13
4	Pseudoephedrine	1.949	403182	221866	5255	1.12
5	Pseudoephedrine	1.953	407276	221578	5253	1.13
Mean			406944			
Std.Dev.			2384.036			
%RSD			0.585839			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Peak results for assay standard of Loratadine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Loratadine	3.390	390942	20057	6569	1.23	4.9
2	Loratadine	3.397	392296	20602	6613	1.29	4.9
3	Loratadine	3.395	398056	21296	6672	1.29	4.9
4	Loratadine	3.391	393286	21242	6619	1.29	4.9
5	Loratadine	3.388	392284	21592	6672	1.22	4.9
Mean			393372.8				
Std.Dev.			2747.438				
%RSD			0.698431				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Sample)

Table 5: Peak results for Assay sample of Pseudoephedrine

S.No	Name	Rt	Area	Height	USPTailing	USPPlateCount
1	Pseudoephedrine	1.955	409895	218842	1.16	5218
2	Pseudoephedrine	1.956	409411	221359	1.14	5216
3	Pseudoephedrine	1.956	409066	219684	1.14	5427

Table 6: Peak results for Assay sample of Loratadine

S.No	Name	Rt	Area	Height	USPTailing	USP	Resolution
1	Loratadine	3.395	387469	21283	1.20	4612	4.9
2	Loratadine	3.388	387471	22171	1.25	4690	4.9
3	Loratadine	3.392	386604	21731	1.20	4640	4.9

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{1\ 00} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=409457.3 / 406944 ×10/49.5×49.5/0.0315×99.7/100×0.3151/100×100
= 100.3%
The % purity of Pseudoephedrine and Loratadine in pharmaceutical dosage form was found to be 100.3%.

Linearity

Table 7: Chromatographic Data For Linearity Studyof Pseudoephedrine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	16.5	154449
66	33	280463
100	49.5	449653
133	66	590193
166	82.5	755619

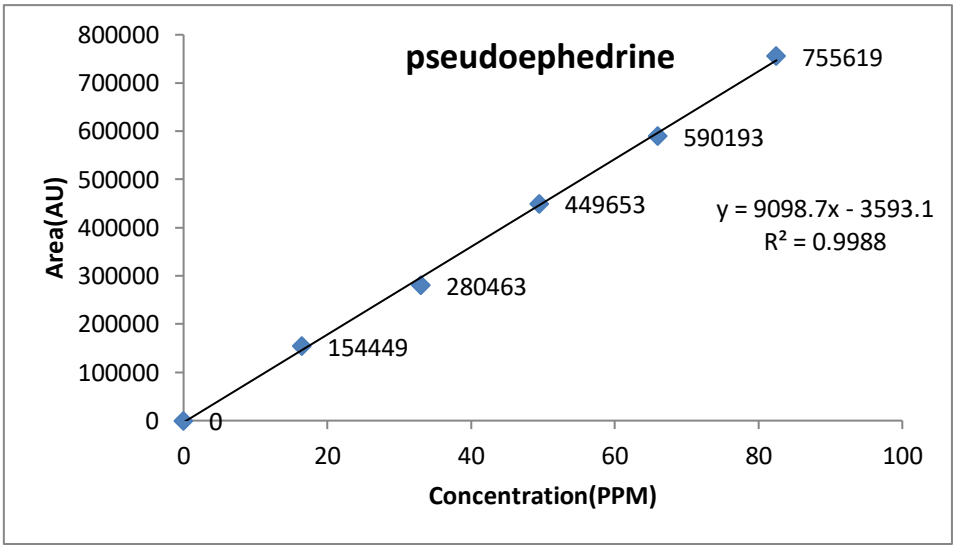
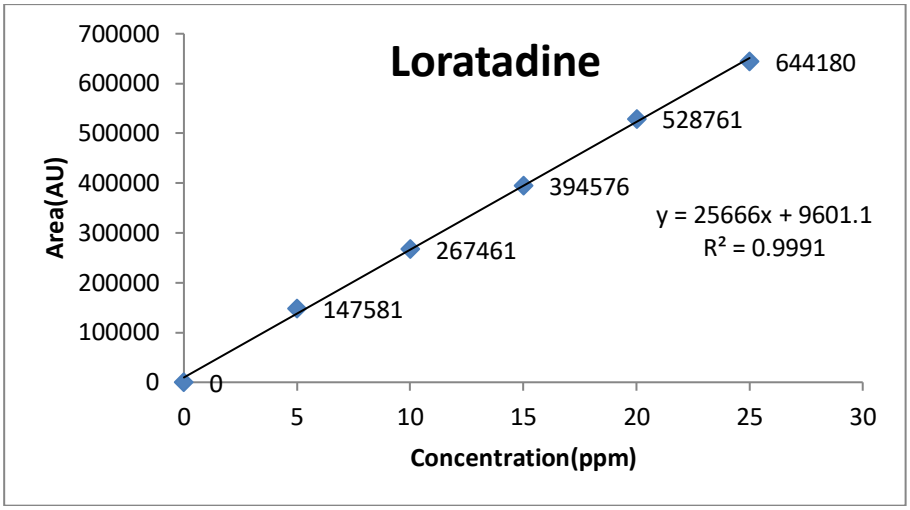


Fig 3:

Table 8: Chromatographic Data For Linearity Studyof Loratadine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	5	147581
66	10	267461
100	15	394576
133	20	528761
166	25	644180



Repeatability

Table 9: Results of repeatability for Pseudoephedrine

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pseudoephedrine	1.961	409349	208879	5200	1.18
2	Pseudoephedrine	1.966	409980	214656	5213	1.16
3	Pseudoephedrine	1.966	407839	214544	5208	1.17
4	Pseudoephedrine	1.968	409731	212354	5202	1.18
5	Pseudoephedrine	1.966	408042	218482	5193	1.16
Mean			408988.2			
Std.dev			985.0826			
%RSD			0.240858			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 10: Results of repeatability for Loratadine

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Loratadine	3.389	317876	20821	7639	1.28
2	Loratadine	3.388	320133	21502	6718	1.22
3	Loratadine	3.386	323930	22054	6762	1.21
4	Loratadine	3.387	324517	22022	6748	1.23
5	Loratadine	3.386	323107	21455	6878	1.21
Mean			321912.6			
Std.dev			2816.936			
%RSD			0.875062			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 11: Results of Intermediate precision for Pseudoephedrine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pseudoephedrine	1.968	409600	200415	5192	1.1
2	Pseudoephedrine	1.972	409792	204737	5202	1.1
3	Pseudoephedrine	1.971	409710	202315	5198	1.1
4	Pseudoephedrine	1.978	408131	210538	5213	1.1

5	Pseudoephedrine	1.978	409596	208031	5213	1.1
6	Pseudoephedrine	1.976	409932	206543	5217	1.1
Mean			409460.2			
Std.Dev.			663.3016			
%RSD			0.161994			

Table 12: Results of Intermediate precision for Loratadine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Loratadine	3.386	323199	20851	6281	1.2	4.9
2	Loratadine	3.388	324588	21266	6392	1.2	4.9
3	Loratadine	3.386	321726	21070	6293	1.2	4.9
4	Loratadine	3.387	326955	21217	6039	1.2	4.9
5	Loratadine	3.389	323546	21257	6153	1.2	4.9
6	Loratadine	3.385	327755	20978	6293	1.2	4.9
Mean			324628.2				
Std.Dev.			2316.421				
%RSD			0.713561				

- *%RSD of five different sample solutions should not more than 2*

Table 13: Results of Intermediate precision Day 2 for Pseudoephedrine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pseudoephedrine	1.980	409042	209754	5237	1.1
2	Pseudoephedrine	1.982	409920	210411	5023	1.1
3	Pseudoephedrine	1.979	407912	208055	5983	1.1
4	Pseudoephedrine	1.979	409213	207720	5294	1.1
5	Pseudoephedrine	1.963	406475	206740	5819	1.1
6	Pseudoephedrine	1.965	409079	209516	5183	1.1
Mean			408606.8			
Std.Dev.			1227.327			
%RSD			0.300369			

Table 14: Results of Intermediate precision Day 2 for Loratadine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Loratadine	3.379	323744	21401	6173	1.2	4.9
2	Loratadine	3.379	325554	21446	6183	1.2	4.9
3	Loratadine	3.376	323154	21266	6103	1.2	4.9
4	Loratadine	3.377	331213	21312	6482	1.2	4.9
5	Loratadine	3.323	323263	21750	6831	1.2	4.9
6	Loratadine	3.317	328951	21602	6153	1.2	4.9
Mean			325979.8				
Std.Dev.			3369.293				
%RSD			1.033589				

%RSD of five different sample solutions should not be more than 2

Accuracy

Table 15: The accuracy results for Pseudoephedrine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	222026.7	24.75	24.75	100	99.8%
100%	443552.3	49.5	49.2	99.2	
150%	674558	74.25	74.25	100	

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 16: The accuracy results for Loratadine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	202430	7.5	7.3	98.6	99.8%
100%	394993.7	15	14.9	99.8	
150%	593559	22.5	22.6	101.1	

Robustness
Pseudoephedrine

Table 17: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	409905	1.933	4242	1.1
Less Flow rate of 0.9 mL/min	407262	2.451	5405	1.6
More Flow rate of 1.1 mL/min	409250	1.630	5365	1.5
Less organic phase	407722	2.064	4393	1.6
More Organic phase	406458	1.960	4358	1.5

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 18: Loratadine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	392596	3.396	6515	1.7
Less Flow rate of 0.9 mL/min	322247	4.178	4698	1.1
More Flow rate of 1.1 mL/min	321244	2.754	7934	1.7
Less organic phase	317397	3.455	4368	1.4
More Organic phase	318735	3.287	5371	1.3

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Pseudoephedrine and Loratadine in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Pseudoephedrine and Loratadine was freely soluble in ethanol, methanol and sparingly soluble in water.

Acetonitrile: Water (10:90% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed inTablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Pseudoephedrine and Loratadine in bulk drug and in Pharmaceutical dosage forms.

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