



Simultaneous Estimation of Fluticasone propionate, Azelastine Hydrochloride, Phenylethyl alcohol and Benzalkonium chloride by RP-HPLC Method in Nasal spray preparations

K. Lakshmi Narasimha Rao^{*1,2}, K. Padmaja Reddy², K. Sudheer Babu², K. Solomon Raju²,
K. Visweswara Rao², Jafer Vali Shaik²

¹Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, India

²Department of Branded Formulations, Analytical Research & Development, Dr.Reddy's Laboratories, IPDO, Bachupally, Hyderabad- 500 072 India

ABSTRACT

A simple, reproducible and accurate efficient reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Fluticasone propionate (FLP), Azelastine Hydrochloride (AZH), Phenylethyl alcohol (PEA) and Benzalkonium chloride (BKC) in combined nasal spray preparations. Formulation containing FLP with AZH are used as antihistamine-corticosteroid combination available as a metered spray formulation for nasal or intranasal administration. Chromatography was performed on a 250 mm x 4.6 mm, 5- μ m particle size, Waters Spherisorb CN column with a 55:45 (v/v) mixture of buffer and acetonitrile as a mobile phase. The detection of the combined dosage form was carried out at 215 nm and flow rate employed was 1.0 ml/min. The retention times were 3.380, 4.855, 6.647 & 10.175, 12.179 min for Phenylethyl alcohol, Fluticasone propionate, Azelastine Hydrochloride and Benzalkonium chloride (C₁₂, C₁₄), respectively. Linearity was established in the concentration range 70.0 to 120.0 μ g/ml for Phenyl ethyl alcohol, 14.0 to 24.0 μ g/ml for Fluticasone propionate, 39.2 to 67.2 μ g/ml for Azelastine Hydrochloride and in the range 2.8 to 4.8 μ g/ml for Benzalkonium chloride, with a correlation coefficient of 0.9961, 0.9992, 0.997 and 0.9997, respectively. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.

Keywords: Fluticasone propionate; Azelastine Hydrochloride; Phenylethyl alcohol; Benzalkonium chloride; Nasal preparations; RP-HPLC; simultaneous estimation.

1. INTRODUCTION

Fluticasone propionate and Azelastine Hydrochloride Nasal Spray is an antihistamine-corticosteroid combination available as a metered spray formulation for intranasal administration. It contains azelastine hydrochloride, which is a second generation H₁ receptor-antagonist with potent topical activity. Chemically it is known as 4-[(4-Chlorophenyl)methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone, hydrochloride. Azelastine hydrochloride occurs as a white, almost odorless, crystalline powder with a bitter taste. It has a molecular weight of 418.37. [The Merck Index.3, 2006, British pharmacopoeia]. Fluticasone propionate, a synthetic corticosteroid with anti-inflammatory properties. Chemically it is known as (6 α ,11 β -16 α ,17 β)-6,9-Difluoro-11-hydroxy-16-methyl-3-oxo-17-(1-oxo-propoxy)androsta-1,4-diene-17-

carbothioic acid S-(fluoromethyl) ester. Fluticasone propionate is a white to off-white powder with a molecular weight of 500.58. [The Merck Index.3, 2006, British pharmacopoeia, United States pharmacopoeia]. Benzalkonium chloride is chemically known as alkyldimethyl (phenylmethyl) ammonium chloride. Three major homologues consist of C₁₂, C₁₄ and C₁₆ straight chain alkyls. It acts as a Pharmaceutical aid (preservative), Algeside. [The Merck Index.3, 2006, British pharmacopoeia, United states pharmacopoeia, Jadwiga Dudkiewicz-Wilczynska., *et al.*, 2004; Louis-Philippe *et al.*, 2007]. Phenylethyl alcohol is chemically known as 2 - phenyl ethanol. Its molecular weight is 122.16. It acts as a Pharmaceutical aid (antimicrobial). [The Merck Index.3, 2006, United States pharmacopoeia]. In this formulation FLP and AZH was drug substance where as PEA and BKC used as a preservatives. All four peaks to be quantified in the formulation. There are very few methods appearing in the literature for the simultaneous estimation of Fluticasone propionate with other combinational drugs [J. L. Bernal *et al.*, 1998; D. Murnane *et al.*, 2006; Nichole L. Korpi-Steiner., 2010; Sriram Krishnaswami., 2000. Since these methods were based on HPLC, Capillary electrophoresis, UV spectrophotometer and LC-MS/MS. Azelastine hydrochloride also with other com-

* Corresponding Author

Email: klnrao16@gmail.com

Contact: +91-9676486567

Received on: 19-07-2010

Revised on: 24-08-2010

Accepted on: 01-09-2010

bination drugs was appearing in the literature in human plasma [J. Pivonka *et al.*, 1987; Ute Heinemann *et al.*, 2003;]. Thus, an attempt was made to develop a simple, precise, accurate and cost effective RP-HPLC method for the simultaneous estimation of Fluticasone propionate, Azelastine Hydrochloride, Phenylethyl alcohol and Benzalkonium chloride in nasal spray preparations. The chemical structures of Phenylethyl alcohol, Benzalkonium chloride, Fluticasone propionate and Azelastine Hydrochloride are shown in Fig. 1.

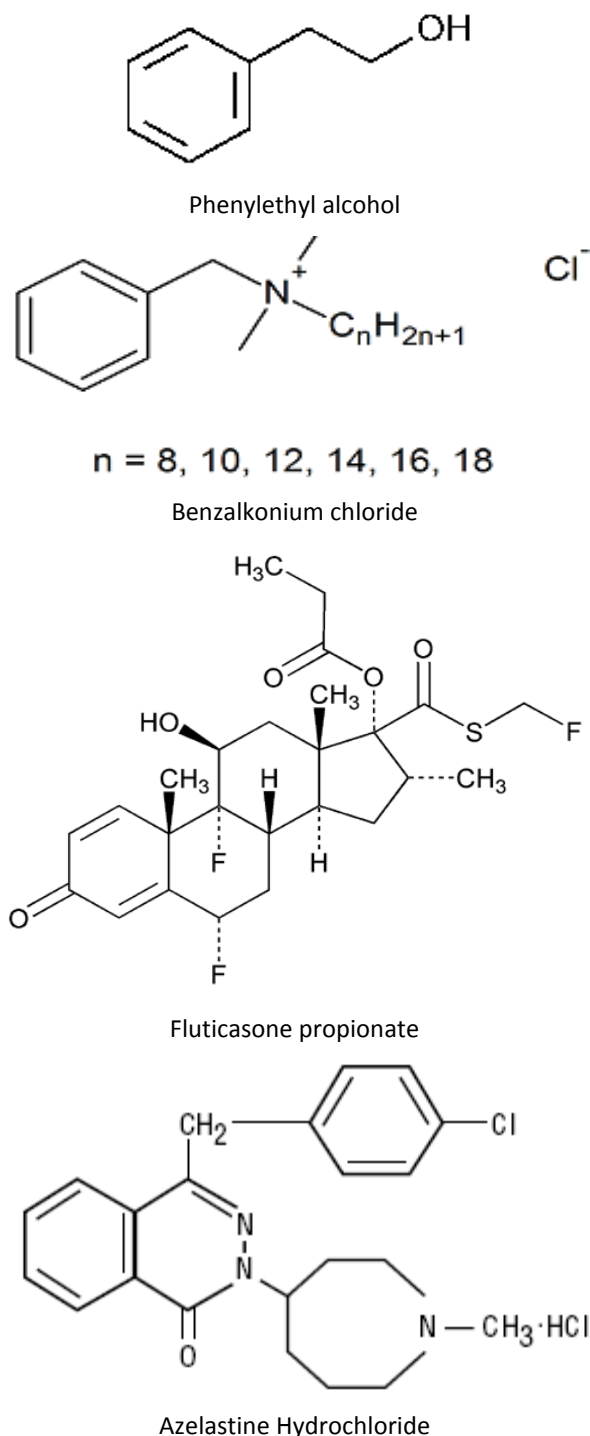


Figure 1: The chemical structures of Phenylethyl alcohol, Benzalkonium chloride, Fluticasone propionate and Azelastine Hydrochloride

2. MATERIALS & METHODS

2.1. Reagents and chemicals

HPLC-grade acetonitrile and potassium dihydrogen ortho phosphate analytical grade were procured from Rankem (Mumbai, India) and pure standards of Phenylethyl alcohol (99.9%), Benzalkonium chloride (95.76%), Fluticasone propionate (98.95%) and Azelastine Hydrochloride (99.46%) was obtained from Dr. Reddy's Laboratories Ltd. HPLC grade water was prepared by using Millipore Milli Q plus purification system. The 0.45 μ m-nylon filter was obtained from Advanced Micro Devices Pvt. Ambala Cantt, India. Waters spherisorb CN column was procured from Waters Associates, Inc.

2.2. Instrumentation and Chromatographic Conditions

Chromatography was performed with a Agilent technologies 1100 LC (Germany), gradient pump with in-built auto injector, variable wavelength detector and Waters Alliance HPLC system (Milford, USA) equipped with a 2695 separation module with inbuilt auto injector and 2996 photodiode array detector. Waters Spherisorb CN column (250 x 4.6nm, 5- μ m particles) was used for chromatographic separation under suitable condition. Detection was carried out using a UV spectrophotometric detector at 215 nm and the software used was waters Empower2. The mobile phase was a 55:45 (v/v) mixture of freshly prepared buffer (50mM of potassium dihydrogen ortho phosphate) and acetonitrile. The mobile phase was sonicated and degassed before use. The diluent was mixture of water and acetonitrile in the ratio of 40:60 (v/v). The flow rate of mobile phase was maintained at 1.0 ml/min. The column temperature was maintained at ambient conditions. The injection volume was 20 μ L and total run time was 20 min. The peaks were identified by retention time; a typical chromatogram is shown in Fig. 2.

2.3. Mixed standard Preparation

Standard stock solution of AZH (1400 μ g/ml), FLP (500 μ g/ml) PEA (2500 μ g/ml) and BKC (100 μ g/ml) was prepared individually in diluent. 2.0ml of the each stock solution was pipetted out into 50ml volumetric flask and diluted to volume with diluent to achieve a final concentration of AZH (56.6 μ g/ml), FLP (19.8 μ g/ml) PEA (100 μ g/ml) and BKC (4 μ g/ml). A system suitability test was performed for five replicate standard injections.

2.4. Sample preparation

Shaken and mixed the contents of 3 bottles (7ml each) in 100ml volumetric flask. Weighed accurately a quantity of nasal spray solution equivalent to 2ml into a 50ml volumetric flask. Added about 30ml diluent, sonicated for about 20minutes and then diluted to volume with diluent to achieve the sample concentration of AZH (56.6 μ g/ml), FLP (19.8 μ g/ml) PEA (100 μ g/ml) and BKC (4 μ g/ml). Filter through a 0.45 μ m nylon filter.

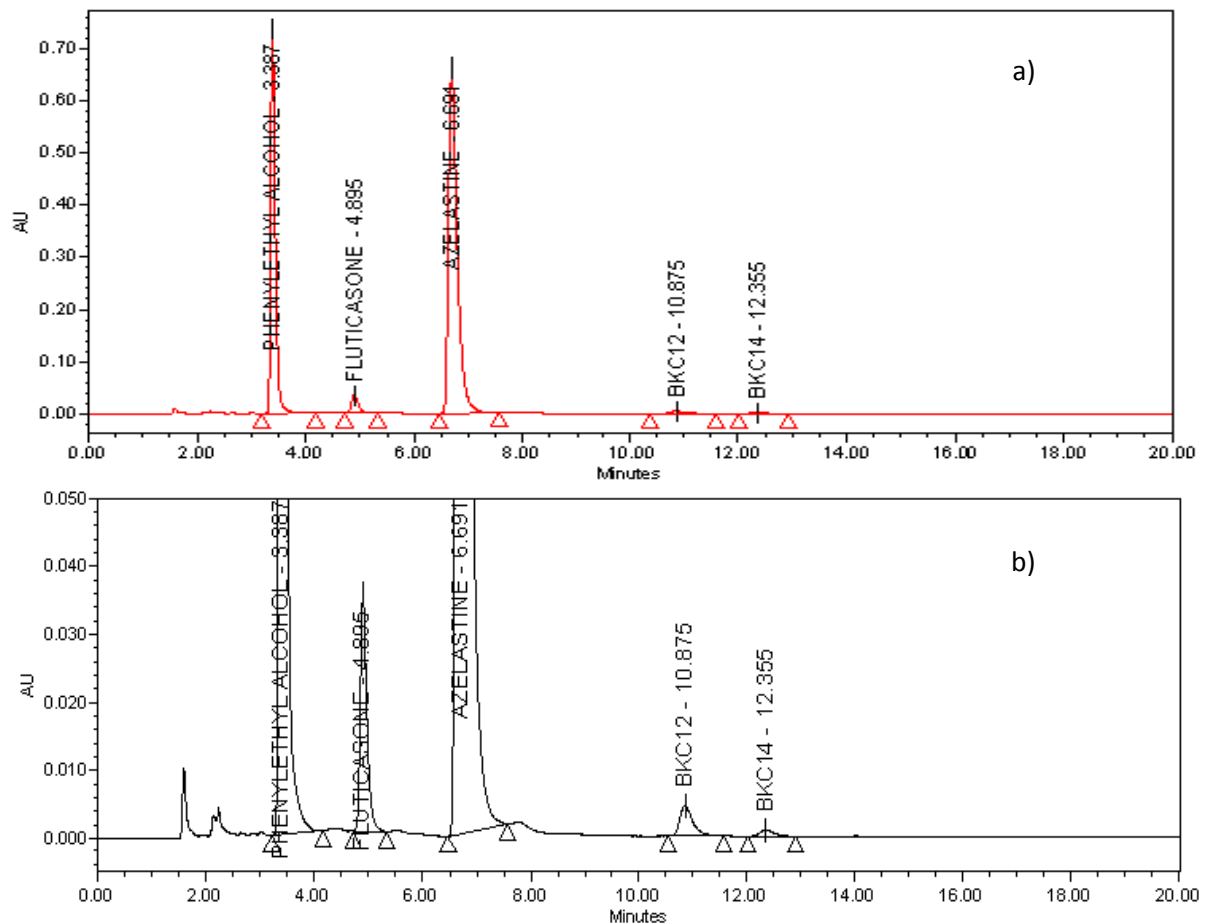


Figure 2: Typical chromatogram obtained from assay sample a) Full scale chromatogram b) Expanded chromatogram

A typical chromatogram obtained from a sample solution of assay is shown in Fig. 2.

3. RESULTS AND DISCUSSION

3.1. Method development

The objective of this study was to develop a method for simultaneous estimation of four components AZH, FLP, PEA and BKC under isocratic conditions. The mobile phase used was the mixture of acetonitrile with phosphate buffer in different ratios. Finally a mixture of acetonitrile-phosphate buffer in the ratio of 45:55 (v/v), proved to be effective mixture than the other mixture used for the separation. Then the flow rate tested includes 0.8, 1.0, 1.2 and 1.5 ml/min. Among the flow rates 1.0 ml/min was selected for the assay because of better visibility and resolution of the peaks. The final chromatographic conditions revealed to provide better resolution among Phenylethyl alcohol, Fluticasone propionate, Azelastine Hydrochloride and Benzalkonium chloride peaks ie greater than 2.0. Number of theoretical plates and tailing factor for each individual peak was more than 2000 and less than 2.0, respectively. The optimum wavelength for detection was 215 nm, no indigenous interfering compounds eluted at the retention times of the drugs. Labeled

amount of the drugs present in nasal spray preparations are given in Table.1.

3.2. Validation of the Method

The method was validated, in accordance with ICH guidelines (ICH Q2B), for precision, accuracy, linearity, specificity, ruggedness and robustness.

3.2.1. Linearity

Linearity was obtained in the concentration range 70.0 to 120.0 $\mu\text{g/mL}$ for Phenylethyl alcohol, 14.0 to 24.0 $\mu\text{g/ml}$ for Fluticasone propionate, 39.2 to 67.2 $\mu\text{g/ml}$ for Azelastine Hydrochloride and in the range 2.8 to 4.8 $\mu\text{g/ml}$ for Benzalkonium chloride. Linearity was performed with the aid of serially diluted calibration solutions from 70% to 120% of the target concentration. Calibration graphs were plotted on the basis of analysis of each calibration solutions. The coefficient of regression obtained was 0.9961, 0.9992, 0.997 and 0.9997, respectively for PEA, FLP, AZH and BKC. The slope obtained was 50988, 3883, 79373 and 854, respectively for PEA, FLP, AZH and BKC.

3.2.2. Precision and intermediate precision

The method was found to be precise with six sample preparations for the quantification of PEA, FLP, AZH

Table 1: Results from validation and system suitability studies of the Method

Method characteristic	Phenyl ethyl alcohol	Fluticasone Propionate	Azelastine HCL	Benza - lkonium chloride (C12)	Benza-lkonium chloride (C14)
Theoretical plates	7810	7292	9342	12050	12598
Resolution	---	7.4	6.9	11.7	3.4
Tailing factor	1.6	1.5	1.7	1.5	1.3
% RSD for six injections	0.6	0.9	0.3	0.6	1.4
Accuracy (RSD(%))*	1.4	1.9	1.5	1.4***	
Precision (RSD(%)) **	0.11	0.10	0.07	0.61***	
Intermediate Precision (RSD(%))**	0.04	0.05	0.06	1.36***	

* Results are mean of three sample preparations with six different drug concentration levels.

** Results are mean of six sample preparations.

***Results are mean of total area of benzalkonium chloride (C12, C14).

Table 2: Accuracy results of Phenylethyl alcohol and Fluticasone propionate in nasal spray preparations

% Spike level	Phenylethyl alcohol			Fluticasone propionate		
	µg/ml added	µg/ml found	% Avg recovery	µg/ml added	µg/ml found	% Avg recovery
70	70.0	70.9	101.3	13.9	13.9	99.8
80	80.0	82.8	103.6	15.8	16.1	102.2
90	90.0	93.1	103.5	17.8	18.4	103.2
100	100.0	102.8	102.8	19.8	20.7	104.4
110	110.0	111.9	101.7	21.8	23.0	105.4
120	120.0	120.1	100.1	23.8	24.9	104.6

Table 3: Accuracy results of Azelastine Hydrochloride and Benzalkonium chloride (C12,C14) in nasal spray preparations

% Spike level	Azelastine Hydrochloride			Benzalkonium chloride (C12,C14)		
	µg/ml added	µg/ml found	% Avg recovery	µg/ml added	µg/ml found	% Avg recovery
70	39.6	39.1	98.7	2.8	2.8	99.0
80	45.3	45.7	101.0	3.2	3.2	101.3
90	50.9	52.1	102.4	3.6	3.6	101.4
100	56.6	58.3	102.9	4.0	4.1	102.3
110	62.3	63.6	102.0	4.4	4.5	102.7
120	67.9	68.6	101.1	4.8	4.9	102.7

and BKC. The precision was also studied on different day. The precision and intermediate precision variation was calculated in terms of percentage relative standard deviation and the results of PEA, FLP, AZH and BKC were found to be less than 2.0% and the results are given in Table. 1.

3.2.3. Accuracy

Accuracy was determined by the method of spiking the standard stock solution in placebo at six different levels, by multiple level recovery studies. Solution containing 2500 µg/ml PEA, 500 µg /ml FLP, 1400 µg/ml AZH and 100 µg/ml BKC standard stock solutions was prepared and spiked with amounts of the standard drugs equivalent to 70, 80, 90, 100, 110 and 120% of the amounts present in the original solution. These

solutions were then analyzed for recovery studies and the average recoveries ranged from 97% to 106%. Results for determination of accuracy are presented in Table. 2 and 3.

3.2.4. Specificity

3.2.4.1. Placebo interference

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interference was detected at the retention time of PEA, FLP, AZH and BKC (C12, C14) in placebo solution.

3.2.4.2. Forced degradation

The Forced degradation of placebo and formulation was carried out as per ICH guidelines (ICH, Q2B) in acid,

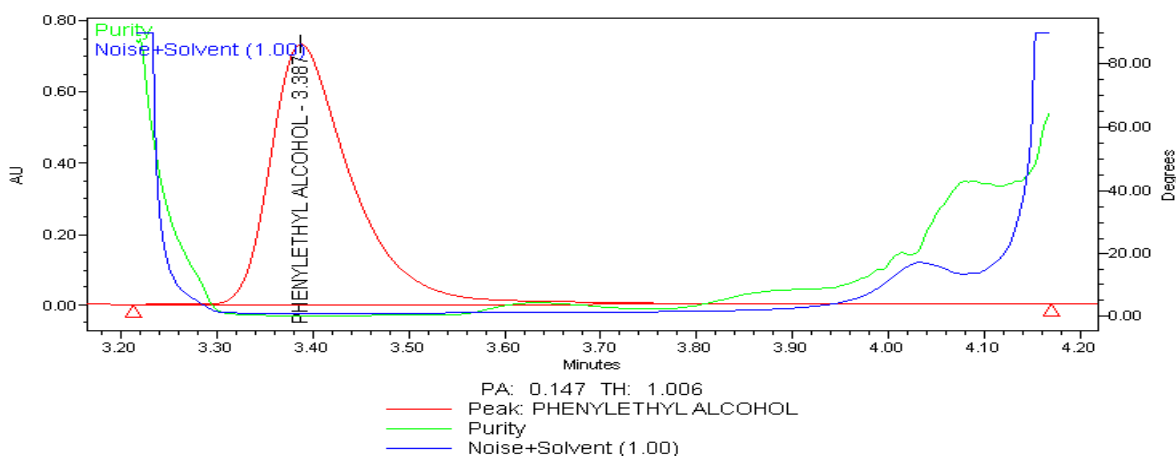


Figure 3: Peak purity of Phenylethyl alcohol

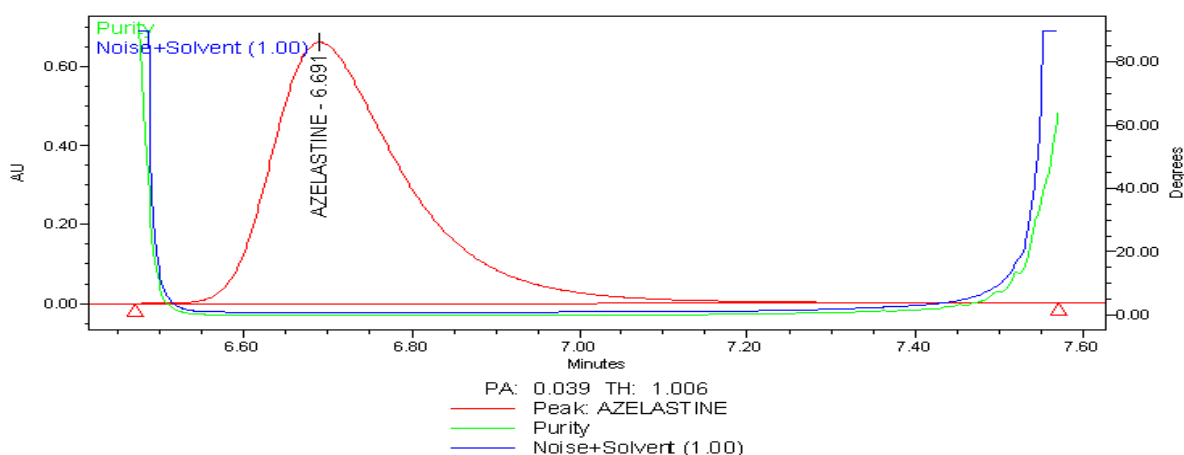


Figure 4: Peak purity of Azelastine hydrochloride

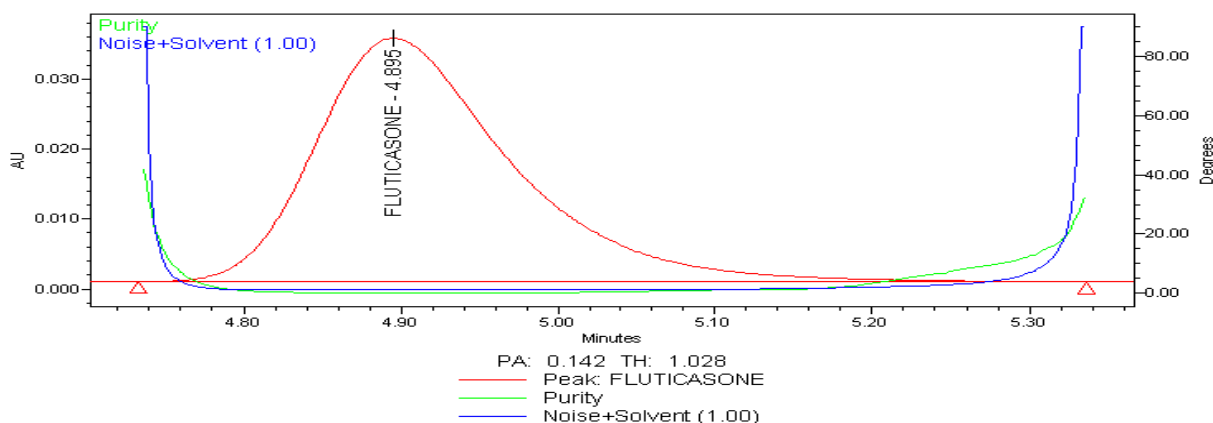


Figure 5: Peak purity of Fluticasone Propionate

base, oxidation and water. The acid, base, oxidation and water stress studies were carried out by refluxing sample & placebo into individual flasks at 60°C for half an hour with 10ml 0.1N HCl, 0.1N NaOH, 1% hydrogen peroxide and water respectively. The drug and the formulation were found to be stable under all the stress conditions. All the stress conditions with purity angle, purity threshold and purity flag results are reported in Table. 4 to Table. 8. Purity plots are shown in Fig. 3 to Fig. 7.

3.2.5. Solution stability

To demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results showed that for all the solutions, the retention times and peak areas of PEA, FLP, AZH and BKC remained almost unchanged (RSD<2.0%) indicating that no significant degradation occurred within this period, i.e. both solutions were stable for at least 24 h,

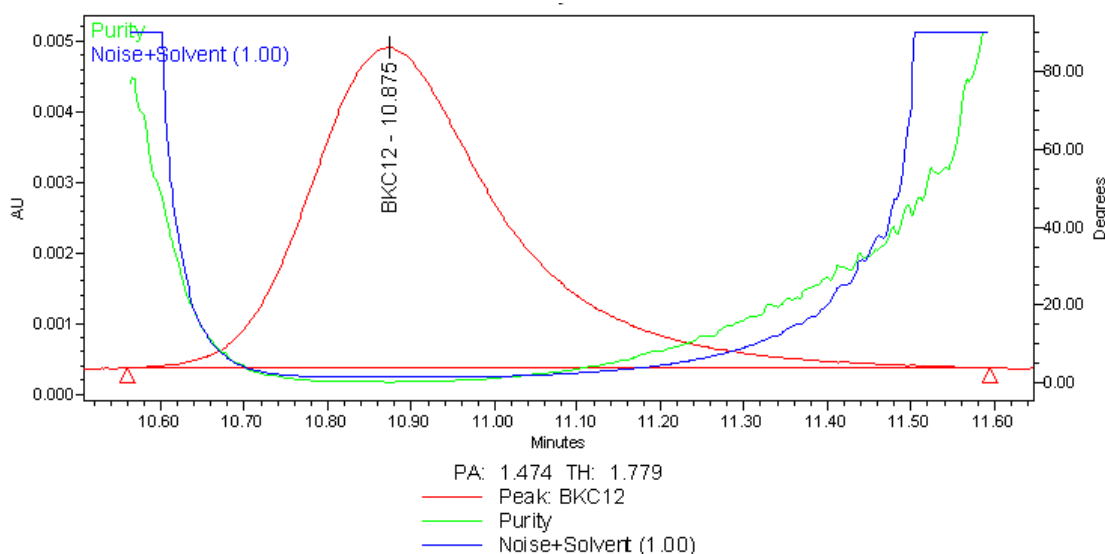


Figure 6: Peak purity of Benzalkonium Chloride(C12)

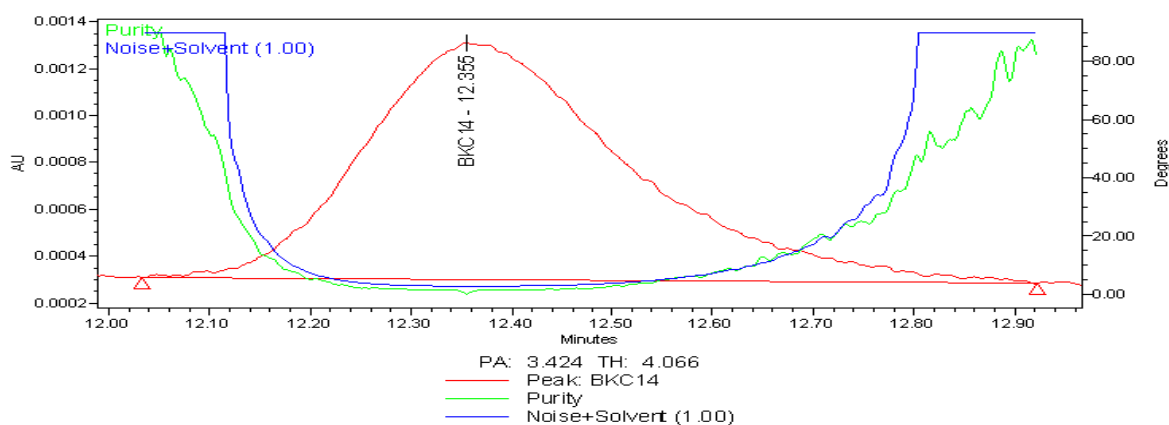


Figure 7: Peak purity of Benzalkonium Chloride(C14)

Table 4: Peak purity of as such Sample preparation (undegraded)

S.No	Stress Condition	Purity Angle	Purity Threshold	Purity Flag	% Degradation
1.	Azelastine Hydrochloride	0.039	1.006	No	NA
2.	Fluticasone Propionate	0.142	1.028	No	NA
3.	Phenylethyl Alcohol	0.147	1.006	No	NA
4.	Benzalkonium chloride	1.474 & 3.424	1.779 & 4.066	No	NA

Table 5: Peak purity of acid degraded sample

S.No	Stress Condition	Purity Angle	Purity Threshold	Purity Flag	% Degradation
1.	Azelastine Hydrochloride	0.037	1.005	No	6.5
2.	Fluticasone Propionate	0.354	1.025	No	5.4
3.	Phenylethyl Alcohol	0.137	1.005	No	6.5
4.	Benzalkonium chloride	0.888 & 2.936	1.739 & 3.948	No	10.3

which was sufficient to complete the whole analytical process.

3.2.6. Ruggedness and Robustness

The ruggedness of the method was determined by using different instruments (Waters 2489). The robustness of the method was determined by making slight changes in the chromatographic conditions ie organic phase composition $\pm 10\%$, flow rate ± 0.2 ml/min and

column oven temperature $\pm 5^\circ\text{C}$. These results indicated that the method was rugged and robust with regard to these conditions. However, proper resolution also been achieved; separation of the drugs was very robust to the mobile phase ratio, flow rate and column oven temperature.

System suitability tests are an integral part of chromatographic method. They were used to verify that the

Table 6: Peak purity of base degraded sample

S.No	Stress Condition	Purity Angle	Purity Threshold	Purity Flag	% Degradation
1.	Azelastine Hydrochloride	0.075	1.028	No	4.6
2.	Fluticasone Propionate	0.945	1.099	No	18.2
3.	Phenylethyl Alcohol	0.373	1.132	No	5.4
4.	Benzalkonium chloride	14.917 & 21.584	22.175 & 32.912	No	11.4

Table 7: Peak purity of peroxide degraded sample

S.No	Stress Condition	Purity Angle	Purity Threshold	Purity Flag	% Degradation
1.	Azelastine Hydrochloride	0.041	1.005	No	7.4
2.	Fluticasone Propionate	0.088	1.025	No	7.4
3.	Phenylethyl Alcohol	0.228	1.003	No	6.2
4.	Benzalkonium chloride	0.781 & 2.821	1.724 & 3.898	No	8.9

Table 8: Peak purity of water degraded sample

S.No	Stress Condition	Purity Angle	Purity Threshold	Purity Flag	% Degradation
1.	Azelastine Hydrochloride	0.042	1.005	No	6.3
2.	Fluticasone Propionate	0.131	1.026	No	5.3
3.	Phenylethyl Alcohol	0.121	1.004	No	6.0
4.	Benzalkonium chloride	0.962 & 2.769	1.692 & 3.740	No	9.7

Table 9: Robustness- Flow rate variation

Robustness Parameter	Flow rate variation (ml/min)									
	PEA		FLP		AZH		BKC- C12		BKC- C14	
Name of the peak										
Changes in parameter	0.8	1.2	0.8	1.2	0.8	1.2	0.8	1.2	0.8	1.2
% RSD of Standard injections	0.3	0.1	1.8	0.3	0.1	0.3	0.5	0.3	1.1	1.7
Resolution	--	--	8.1	6.6	6.2	5.6	12.0	10.4	3.3	2.9
The tailing factor	1.2	1.2	1.2	1.1	1.4	1.3	1.3	1.3	1.3	1.2

Table 10: Robustness- Organic phase composition variation

Robustness Parameter	Organic phase composition variation (%)									
	PEA		FLP		AZH		BKC - C12		BKC - C14	
Name of the peak										
Changes in parameter	90	110	90	110	90	110	90	110	90	110
% RSD of Standard injections	0.2	0.2	0.3	1.0	1.0	0.2	0.4	0.6	1.3	1.1
Resolution	--	--	9.6	6.8	4.9	6.7	13.6	11.0	4.1	2.8
The tailing factor	1.2	1.2	1.2	1.4	1.4	1.3	1.2	1.2	1.2	1.3

Table 11: Robustness- Column oven temperature variation

Robustness Parameter	Column oven temperature variation (°C)									
	PEA		FLP		AZH		BKC - C12		BKC - C14	
Name of the peak										
Changes in parameter	20	30	20	30	20	30	20	30	20	30
% RSD of Standard injections	0.1	0.2	0.4	0.6	0.9	0.4	0.3	1.0	1.2	1.4
Resolution	--	--	7.3	7.0	7.1	6.7	11.4	10.9	3.3	3.1
The tailing factor	1.2	1.1	1.2	1.3	1.3	1.3	1.1	1.3	1.2	1.2

reproducibility of the chromatographic system is adequate for the analysis. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solution of Phenylethyl alcohol, Fluticasone propionate, Azelastine Hydrochloride and Benzalkonium chloride. In addition, relative standard deviation of PEA, FLP, AZH and BKC peaks were evaluated by injecting mixed standard of the PEA, FLP,

AZH and BKC (100, 20, 56.6 and 4.0 µg/ml). All the parameters are shown in Table. 1 & Table. 9 to Table.11.

CONCLUSION

The proposed RP-HPLC method for simultaneous assay of Phenylethyl alcohol, Fluticasone propionate, Azelastine Hydrochloride and Benzalkonium chloride in combined nasal spray preparations is simple, precise, specific, highly accurate and less time consumption for

analysis could be recorded. So, it can definitely be employed for the routine and stability study analysis. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations.

ACKNOWLEDGEMENTS

The authors are grateful to the Dr.Reddy's Laboratories Ltd. (Hyderabad, India) for gift samples (Phenylethyl alcohol, Fluticasone propionate, Azelastine Hydrochloride and Benzalkonium chloride) for providing laboratory facility for this research work.

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