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Full Length Research Paper

Determination of phenylethyl alcohol by reversed-phase high-performance liquid chromatography (RP-HPLC) in Budesonide nasal spray

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Phenylethyl alcohol is used as an antimicrobial preservative in many pharmaceutical products, especially nasal sprays. A simple and accurate reverse phase high performance liquid chromatographic method was developed to assay of phenylethyl alcohol in budesonide nasal spray preparations. A waters C18 symmetry column chromatographic system (150 × 4.6 mm, 5 µm particle size) was performed with a 50:50 (%V/V) mixture of water and acetonitrile as a mobile phase. The detection of the phenylethyl alcohol was carried out at 220 nm and flow rate was employed 1.0 ml/min. The retention time of phenylethyl alcohol was about 2.8 min. Linearity was established in the concentration range of 173.28 to 259.92 mg/ml (80 to 120% of the target concentration), with a regression coefficient of 0.9991. Specificity was tested in the presence of placebo; no interference was detected at the retention time of phenylethyl alcohol. The results of the analysis were validated statistically and recovery percentage studies confirmed the accuracy and precision of the proposed method.

Key words: Phenylethyl alcohol, budesonide, nasal spray, reversed-phase high-performance liquid chromatography (RP-HPLC), preservative.

INTRODUCTION

Allergic rhinitis is a common disease and refers to inflammation of the nasal passages including sneezing, itching, nasal congestion and runny nose. Intranasal corticosteroids are among the most effective treatments for permanent allergic rhinitis. Some individuals unable to tolerate aerosols may prefer an aqueous nasal spray (Mygind, 1993).

Budesonide, the active component of budesonide nasal spray is a corticosteroid designated chemically as (RS)-11β, 16α, 17, 21-Tetrahydroxypregna-1, 4-diene-3, 20-

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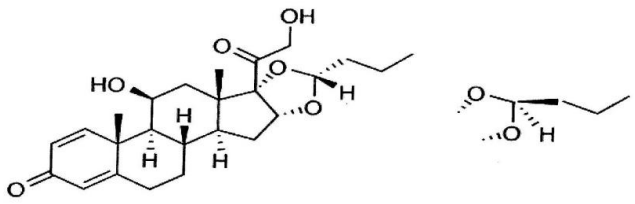

Figure 1. The structural formula of budesonide.

dione cyclic 16, 17-acetal with butyraldehyde. Budesonide is provided as a mixture of two epimers (22R and 22S). The empirical formula of budesonide is $C_{25}H_{34}O_6$ and its molecular weight is 430.5. Its structural formula was shown in Figure 1.

Budesonide is a white to off-white, tasteless, odorless powder that is practically insoluble in water and in heptanes, sparingly soluble in ethanol, and freely soluble in chloroform. Its partition coefficient between octanol and water at pH 7.4 is 1.6×10^3. Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity and weak mineralocorticoid activity. In standard *in vitro* and animal models, budesonide has approximately a 200-fold higher affinity to the glucocorticoid receptor and a 1000-fold higher topical anti-inflammatory potency than cortisol. As a measure of systemic activity, budesonide is 40 times more potent than cortisol when administered subcutaneously and 25 times more potent when administered orally in the rat thymus involution assay (Rice-Thomas et al., 2009).

Antimicrobial preservatives are included in preparations to kill or inhibit the growth of microorganisms inadvertently introduced during manufacture or use. They are used in sterile preparations such as eye-drops and multidose injections to maintain sterility during use and in cosmetics, foods, and non-sterile pharmaceutical products such as oral liquids, creams, inhalations and nasal sprays to prevent microbial spoilage. The choice of a suitable preservative for a preparation depends on pH, compatibility with other ingredients, the route, dose and frequency of administration, partition coefficients with ingredients and containers or closures, degree and type of contamination, concentration required, and rate of antimicrobial effect (Thomas et al., 1989).

Phenylethyl alcohol is an excipient of budesonide nasal spray. It is an antimicrobial preservative designated chemically as 2-Phenylethanol. The empirical formula of phenylethyl alcohol is $C_8H_{10}O$ (Figure 2) and its molecular weight is 122.17. Phenylethyl alcohol is a clear, colorless liquid with an odor of rose oil. It has a burning taste that irritates and then anesthetizes mucous membranes (Rowe et al., 2009; O'Neil et al., 2001). Phenylethyl alcohol is very soluble in alcohol, in fixed oils, in glycerin, and in propylene glycol, and sparingly soluble in water and slightly soluble in mineral oil Franson et al., 2012).

Phenylethyl alcohol in relatively low concentrations

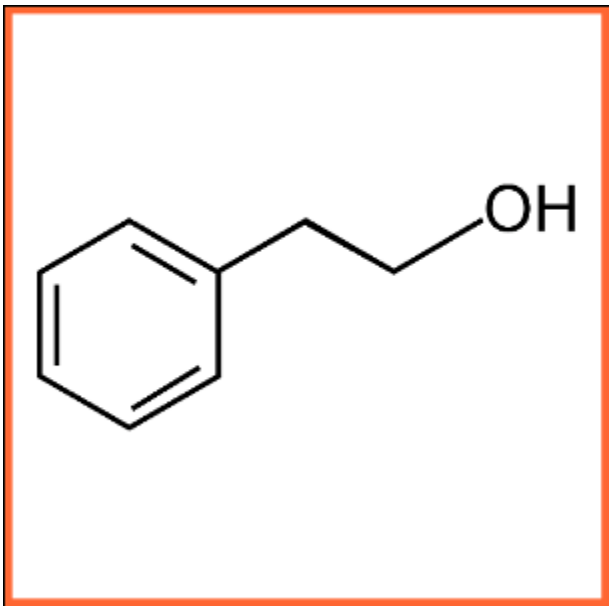

Figure 2. The structural formula of phenylethyl alcohol.

(1:400) exerts an effective inhibitory action on Gram-negative bacteria and may thus be used for differential inhibition (Lilley and Brewer, 1953; Hodges et al., 1996). Assay and detection of phenylethyl alcohol in nasal sprays is one of the important experiments during manufacturing process in quality control laboratory. Therefore the aim of this study was finding a fast and valid measurement method to assess phenylethyl alcohol in budesonide nasal sprays.

High performance liquid chromatography (HPLC) is one of the most powerful analysis methods. In recent years the use of reversed-phase high-performance liquid chromatography (RP-HPLC) method for determination of drug substances is very common and HPLC instruments and RP-HPLC solvents are available in most pharmaceutical laboratories. For this reasons, we developed a RP-HPLC method for determination of phenylethyl alcohol in Budesonide nasal spray and similar formulations which this analysis method is simple, fast, short response time, cheap price, with high accuracy and high precision. This analysis method was fully validated and can be done easily in any laboratories.

MATERIALS AND METHODS

HPLC grade acetonitrile was procured from Merck Company (Germany), pure standard of phenylethyl alcohol (99.9 % w/w) was obtained from LGC Company (England) and HPLC grade water was prepared by using Millipore Milli Q plus purification system (USA). The 0.45 µm nylon filter was obtained from Millipore Company (USA) and L1 columns were procured from both Waters Company (USA) and Agilent Company (USA). All other chemicals were analytical grade and commercially available.

Chromatography (Waters HPLC system, USA) was performed

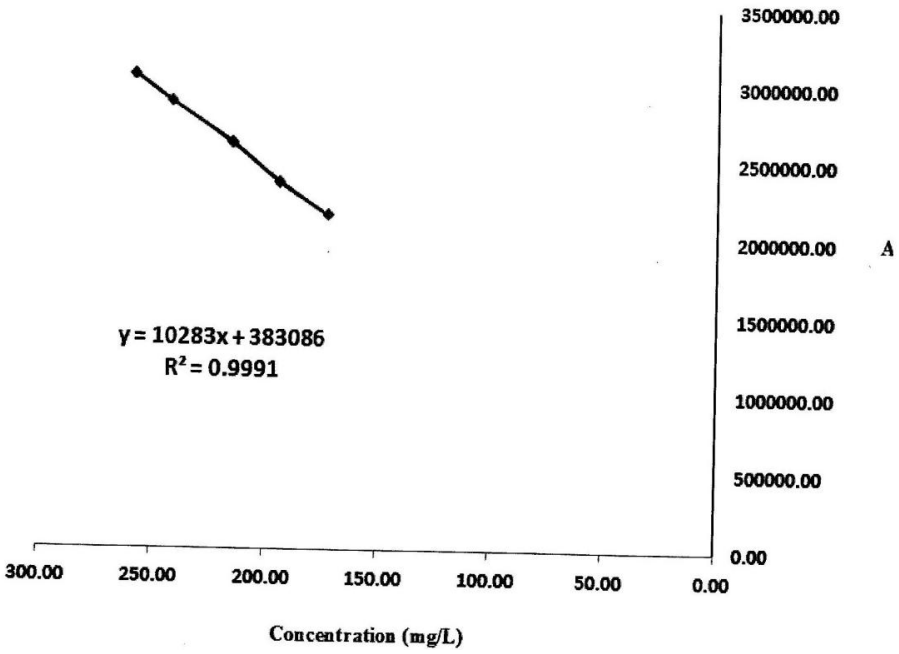

Figure 3. Linearity curve of phenylethyl alcohol in budesonide nasal spray preparation.

with a 1525 separation module through inherent manual injector jointed to 2487 UV detector and also a 2695 separation module with inbuilt auto injector and 2996 photodiode array detector. Waters C18 symmetry column (150×4.6 mm, 5 µm particle size) and Agilent C18 column were used for chromatographic separation under isocratic elution. Detection was carried out using an UVspectrophotometric detector at 220 nm and Waters Breez and Empower software was used. The mobile phase was a 50:50 (% v/v) mixture of prepared water and acetonitrile. Mobile phase was sonicated and degassed before use. The flow rate of mobile phase was adjusted at 1.0 ml/min. The column temperature was maintained at ambient conditions. The injection volume was 20 µl and total run time was 5 min. The phenylethyl alcohol was identified by retention time of the standard phenylethyl alcohol peak. Also in specificity test, phenylethyl alcohol peak was identified against standard compound peak in the presence of placebo.

Validation of the method

Based on previous and similar chromatographic methods, the best system for determining of phenylethyl alcohol was selected (Harris, 1991; Skoog et al., 1991; Moffat et al., 2011). The method is intended to assay phenylethyl alcohol in budesonide nasal spray during analytical method validation. The method was validated, in accordance with ICH guidelines (Authors Group, 2005) and other similar works (Rao et al., 2010; Blanco et al., 1999). All validation factors such as linearity, specificity, accuracy, precision, repeatability, reproducibility and robustness were assessed.

RESULTS

Linearity

Linearity was obtained with the concentration range of 173.28 to 259.92 mg/L for phenylethyl alcohol. Linearity was performed with different dilutions. Calibration solutions were 80 to 120% of the target concentration. Calibration graph was plotted on the basis of analysis of calibration solutions. The coefficient of regression was obtained 0.9991 and the slop of 10283 was achieved (Figure 3). Standard stock solution of phenylethyl alcohol (2.166 g/L) was prepared by dissolving it in water. From this stock, concentrations of 173.28, 194.94, 216.60, 243.67, 259.92 mg/L were prepared in acetonitrile. Each solution was injected three times except that the target solution (216.60 mg/L) was injected six times. The results are shown in Table 1 and Figure 3.

Specificity

Specificity was tested against standard compound and against potential interferences in the presence of placebo. As Figures 4, 5 and 6 depict, no interference was detected at the retention time of phenylethyl alcohol in placebo solution.

Accuracy

Accuracy was determined by the two methods including: 1) Spiking the phenylethyl alcohol standard in placebo. 2) Using linearity curve. Standard stock solution of phenylethyl alcohol (2.1508 g/L) was prepared in water. 1.0 ml of this solution was transferred into 10 ml volumetric flask and diluted with acetonitrile to achieve a

Table 1. Linearity results of phenylethyl alcohol in budesonide nasal spray preparation.

Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
173.28	2.810 2.811 2.811	2168590 2165472 2166079	2166713.67	0.02	0.08
194.94	2.811 2.810 2.811	2372223 2376421 2376063	2374902.33	0.02	0.10
216.60	2.811 2.812 2.812 2.811 2.813 2.812	2628468 2629975 2625099 2626837 2626590 2626687	2627276	0.03	0.06
243.67	2.812 2.812 2.812	2876793 2891142 2889632	2885855.67	0.00	0.27
259.92	2.811 2.811 2.810	3052790 3055870 3050643	3053101	0.02	0.09

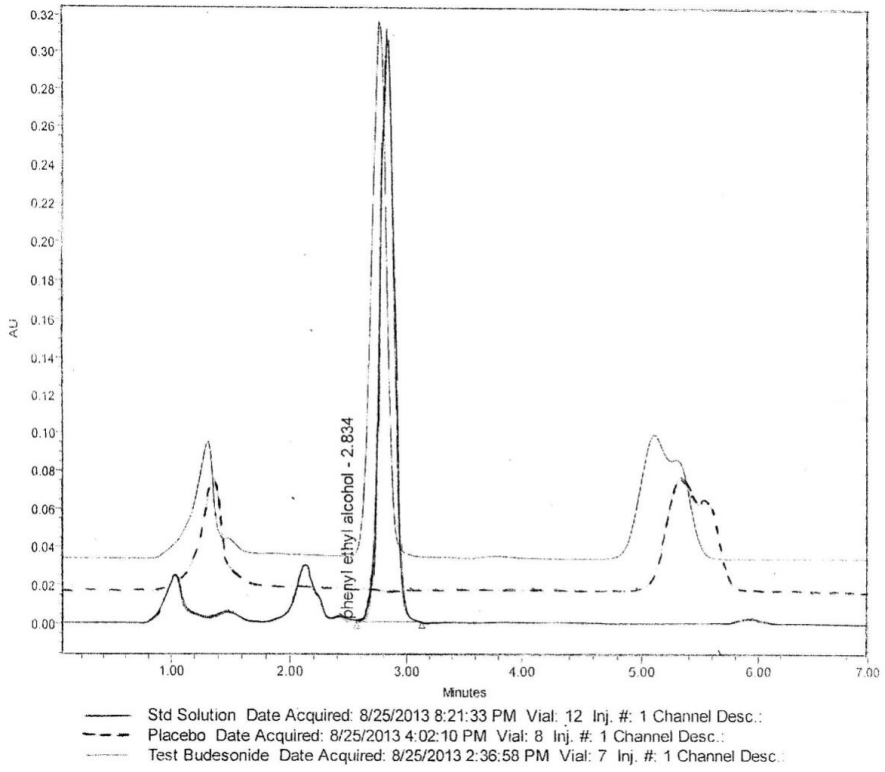

Figure 4. Chromatograms of Standard solution, Test Solution and Placebo solution (with PDA detector).

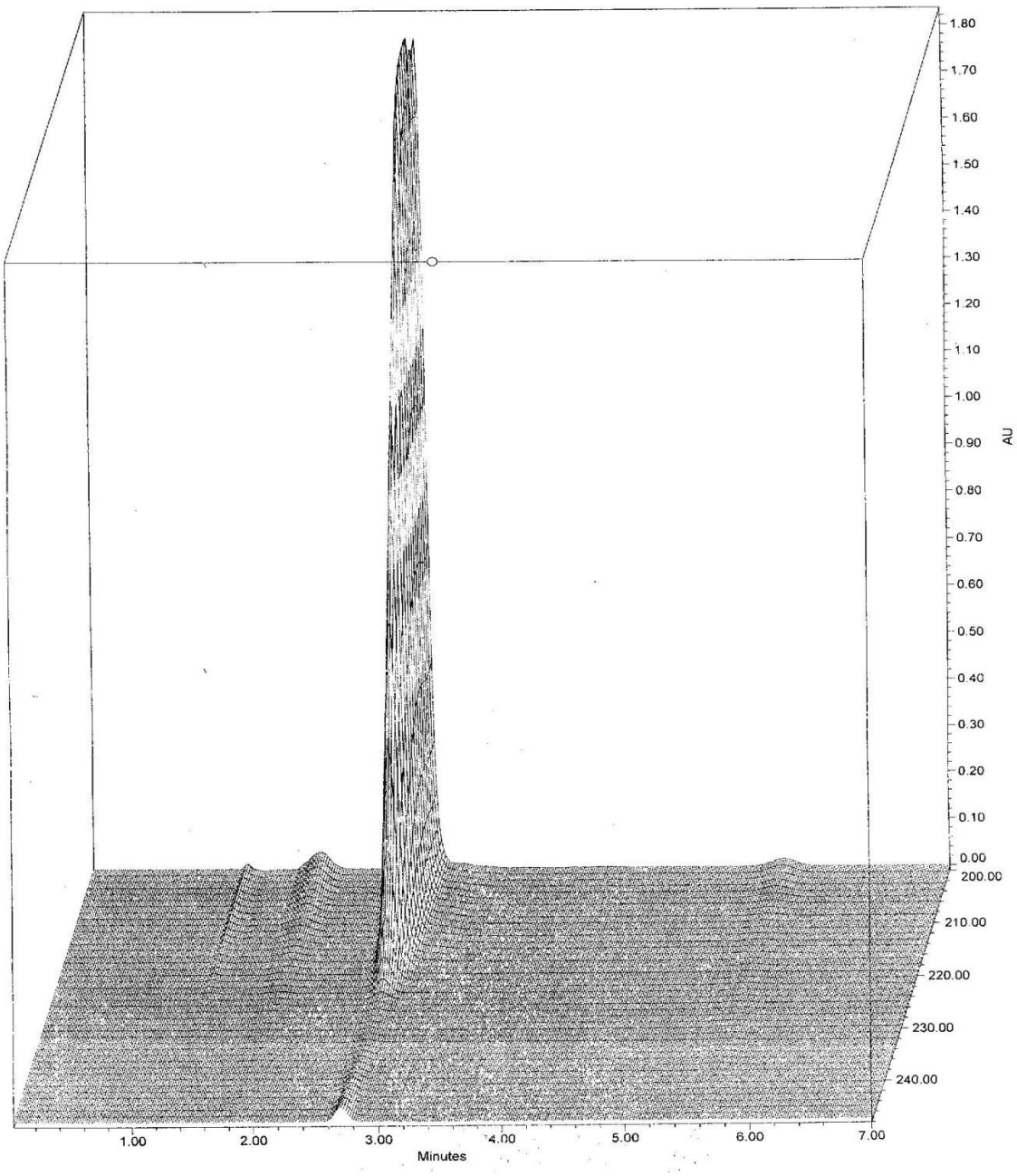

Figure 5. Three-dimensional chromatogram of phenylethyl alcohol peak in Standard solution (with PDA detector).

final concentration of 215.08 mg/L. Concentrations of 187.5, 206.25, 176.25 mg/L with acetonitrile were prepared from budesonide nasal spray.

Spiking the phenylethyl alcohol standard in placebo

Accuracy was evaluated by spiking the phenylethyl alcohol standard in placebo at three different concentrations level and were calculated the recovery percentages with external standard method. Results are presented in Tables 2 and 3. Recovery percentages were in the range of 98.0 to 102.0% that show this method has suitable accuracy.

Using linearity curve

Accuracy was estimated by this method at three different concentration levels and recovery percentages were

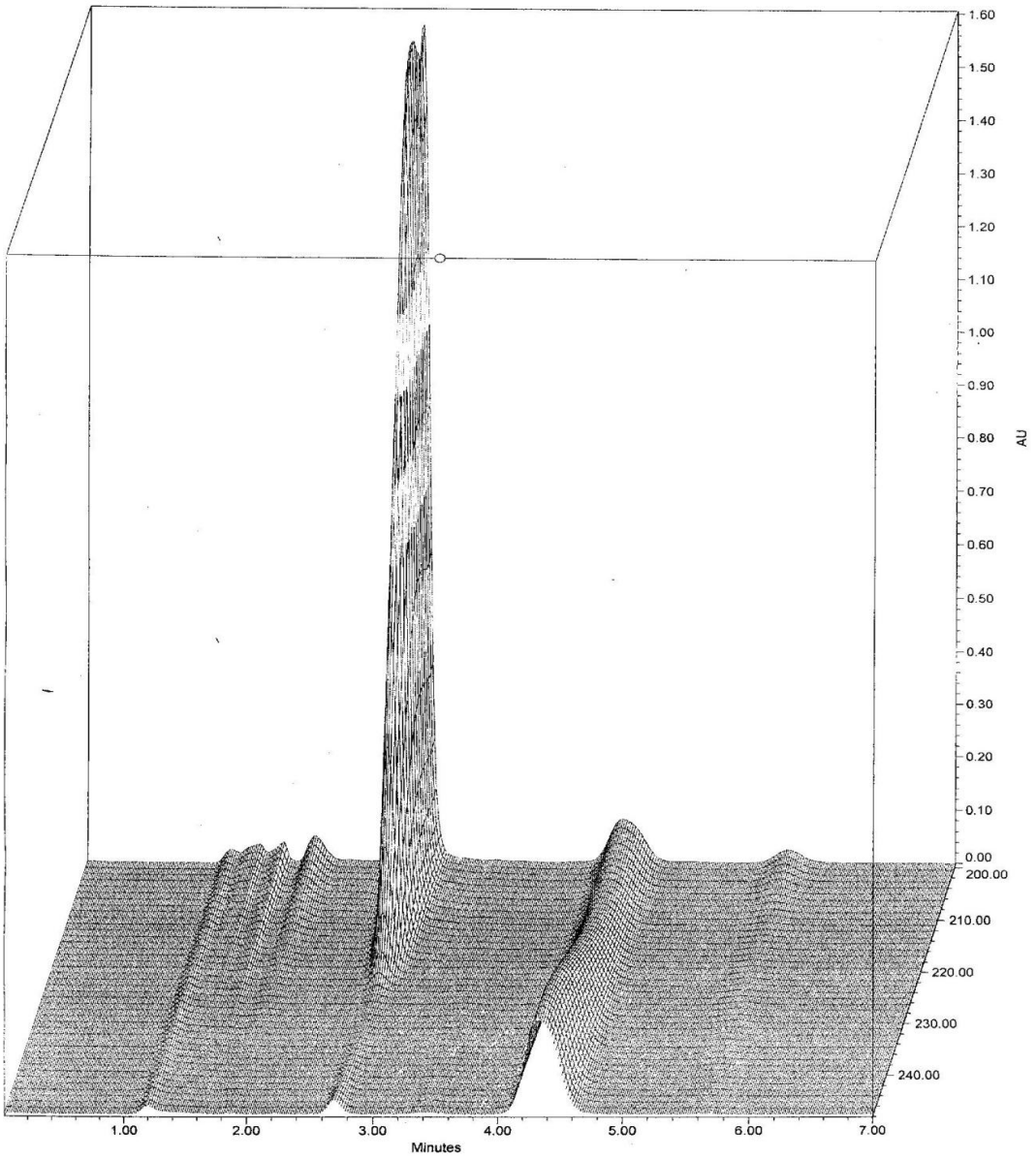

Figure 6. Three-dimensional chromatogram of phenylethyl alcohol peak in Sample solution of budesonide nasal spray preparation (with PDA detector).

calculated with use linearity curve. In this method concentrations were obtained by a linear equation and recovery percentages were in the range of 98.0 to 102.0% that demonstrate this method has suitable accuracy. These results are represented in Table 4. Spiking in placebo and using linearity curve overall confirm which accuracy of this method is in the series of very high-quality.

Precision

Precision was studied in the three levels including: Repeatability (Intra-assay precision), ruggedness and solution stability (Intermediate precision) and reproducibility. Standard stock solution of phenylethyl alcohol (2.1508 g/L) was prepared in water. 1.0 ml of this solution was moved to 10 ml volumetric flask and diluted with acetonitrile to achieve a final concentration of 215.08 mg/L. Concentrations of 200, 250 and 220 mg/L with acetonitrile were prepared from budesonide nasal spray.

Repeatability

Repeatability was studied at three different concentration

Table 2. Chromatographic results of accuracy test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
		2.834	2300932			
	187.5	2.910	2300298	2300642.00	1.38	0.01
		2.892	2300696			
		2.854	2497974			
Sample	206.25	2.834	2502115	2500475.00	0.35	0.09
		2.843	2501336			
		2.835	2177020			
	176.25	2.843	2164883	2169686.67	0.15	0.30
		2.837	2167157			
		2.832	2609245			
Standard	215.08	2.832	2612129	2609373.33	0.02	0.10
		2.831	2606746			

Table 3. Accuracy results of phenylethyl alcohol in budesonide nasal spray preparation by external standard method.

True concentration (mg/L)	Found concentration (mg/L)	% Recovery
187.5	189.63	101.14
206.25	206.10	99.93
176.25	178.84	101.47

Table 4. Accuracy results of phenylethyl alcohol in budesonide nasal spray preparation by linearity curve.

True concentration (mg/L)	Found concentration (mg/L)	% Recovery
187.5	186.48	99.46
206.25	205.91	99.84
176.25	173.74	98.58

levels and relative standard deviations of results were calculated. Results in Tables 5 and 6 were found less than 2.0%.

Ruggedness and solution stability

The ruggedness of the method was studied on three different days with different analysts. The relative standard deviations of results were found less than 2.0%. To demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 48 h at room temperature. The results showed that for all solutions, the retention times and peak areas of phenylethyl alcohol remained almost unchanged (RSD<2.0%) which indicating that no significant degradation occurred within this period. Both solutions were stable for at least 48 h. These results are presented in Tables 7 and 8.

Reproducibility

The reproducibility of method was studied in the two

Table 5. Chromatographic results of repeatability test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
Sample	200	2.779	2316501	2318291	0.05	0.09
		2.780	2317825			
		2.777	2320547			
	250	2.776	2940676	2963738	0.04	0.68
		2.775	2971893			
		2.777	2978645			
	220	2.775	2620006	2622414.67	0.06	0.08
		2.772	2624101			
		2.773	2623137			
Standard	215.08	2.843	2632891	2632455.67	0.23	0.14
		2.832	2628565			
		2.831	2635911			

Table 6. Repeatability results of phenylethyl alcohol in budesonide nasal spray preparation by external standard method.

Sample concentration (mg/L)	Result (%)	RSD (%)
200	94.71	
250	96.86	1.47
220	97.39	

Table 7. Chromatographic results of ruggedness test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
Sample	200	2.761	2395008	2396151.67	0.00	0.06
		2.761	2397899			
		2.761	2395548			
	250	2.761	2967927	2978031	0.04	0.30
		2.763	2983895			
		2.763	2982271			
	220	2.813	2617292	2614569	0.02	0.09
		2.813	2612827			
		2.814	2613588			
Standard	215.08	2.834	2626307	2627129	0.05	0.14
		2.832	2623848			
		2.831	2631232			

Table 8. Ruggedness results of phenylethyl alcohol in budesonide nasal spray preparation by external standard method.

Sample concentration (mg/L)	Result (%)	RSD (%)
200	98.09	
250	97.52	0.42
220	97.30	

Table 9. Chromatographic results of reproducibility test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
Sample	200	2.760	2377528	2382569.33	1.02	0.19
		2.762	2384364			
		2.810	2385816			
	250	2.766	2989100	2987751.33	0.39	0.10
		2.763	2989812			
		2.783	2984342			
	220	2.813	2618586	2618884.67	0.00	0.01
		2.813	2618922			
		2.813	2619146			
Standard	215.08	2.834	2627913	2626403	0.05	0.06
		2.832	2626417			
		2.831	2624879			

Table 10. Reproducibility results of phenylethyl alcohol in budesonide nasal spray preparation (in other laboratory with other Waters HPLC instrument).

Sample concentration (mg/L)	Result (%)	RSD (%)
200	97.56	
250	97.87	0.21
220	97.48	

different laboratories. The percentage relative standard deviations of results with other Waters HPLC Instrument and in other laboratory were found less than 2.0%. The results are given in Tables 9 and 10.

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions that is, mobile phase ±5%, flow rate ±0.1 ml/min (Woolfson et al., 2014). Also this method was done with another C18 column (150×4.6 mm, 5 µm, Agilent Company), and finally similar results were obtained.

DISCUSSION

The purpose of this study was development a method to determination of phenylethyl alcohol in budesonide nasal spray and other similar nasal anti allergic formulations. The mixture of water and acetonitrile in different ratios was examined as a mobile phase and lastly a mixture of water and acetonitrile in the ratio of 50:50 (V/V) and flow

rate of 1.0 ml/min was selected. The optimum wavelength for detection was considered at 220 nm (because of no interfering and suitable shape). After obtaining these final conditions of the chromatographic system, validation of the method was performed.

(i) Linearity was recognized in the concentration range of 173.28 to 259.92 mg/ml (80 to 120% of the target concentration) with a regression coefficient of 0.9991.
(ii) Specificity was experienced in the presence of placebo; no interference was detected at the retention time of phenylethyl alcohol.
(iii) Accuracy was determined by the two technique of spiking and linearity curve. Recovery percentages were calculated and results were in the range of 98.0 to 102.0%.
(iv) Precision was studied in the three levels including; repeatability, ruggedness and reproducibility.

Percentage relative standard deviations of the results were calculated that were less than 2.0%. The results of the analysis were validated statistically and confirmed the accuracy and precision of the proposed method.
We concluded that proposed RP-HPLC method for determination of phenylethyl alcohol in budesonide nasal spray is simple, precise, specific, and highly accurate and this method is very less time consuming in quality control laboratories. So, this method can definitely be used in phenylethyl alcohol drug substance analysis and determination of phenylethyl alcohol in budesonide nasal spray and other similar nasal anti-allergic formulations such as Fluticasone nasal spray, Beclometasone nasal spray, Mometasone nasal spray and etc. The advantages of this method over other old methods are short retention time for determination of phenylethyl alcohol (about 2.7 min), simple mobile phase, economical and practical procedure to assay phenylethyl alcohol in other similar pharmaceutical products.

Conflict of Interest

The authors of this paper declared no conflict of interest.

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