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Original Research Article

DETERMINATION OF ALBUTEROL IN BULK AND DOSAGE FORM BY HPLC USING BUFFER-ETHANOL SYSTEM

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Abstract: A novel, simple, accurate and precise HPLC method for determination albuterol in buffer-ethanol (1:1) system has been developed and validated. The linearity is obeyed over a concentration range of 0.5-150 µg/ml with correlation coefficient of 0.999 for both the drugs. The proposed method was validated by determining accuracy, precision and stability parameters. The method was found to be robust. Specificity of the method was determined by subjecting the drugs to various stress conditions like acid, alkali, oxidation, thermal and photolytic degradation. The method was used successfully for the simultaneous determination of albuterol in aerosol dosage form.

Keywords: Albuterol, stress, accuracy, oxidation, degradation.

Introduction: Asthma is a common condition due to chronic inflammation of the lower respiratory tract. Chronic lower airway inflammation is known to be more common in individuals that also have inflammatory disorders of the upper airway. The scientific understanding of asthma continues to improve and it is important for providers who treat upper or lower airway inflammation to be familiar with asthma's definition and pathophysiology. Asthma is a serious health and socioeconomic issue all over the world, affecting more than 300 million individuals. The disease is considered as an inflammatory disease in the airway, leading to airway hyper responsiveness, obstruction, mucus hyper-production and airway wall remodeling. Asthma is extremely common, especially in poor, urban environments. Asthma is the third most common reason for pediatric hospitalizations[1].The presence of airway inflammation in asthmatic patients has been found in the nineteenth century. The diagnosis of asthma requires these symptoms and demonstration of reversible airway obstruction using spirometry. Identifying clinically important allergen sensitivities is useful. Inhaled short-acting β2-agonists provide rapid relief of acute symptoms, but maintenance with daily inhaled corticosteroids is the standard of care for

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persistent asthma. Combination therapy, including inhaled corticosteroids and long-acting β2-agonists, is effective in patients for whom inhaled corticosteroids alone are insufficient. The use of inhaled long-acting β2-agonists alone is not appropriate. Other controller approaches include long-acting muscarinic antagonists (eg, tiotropium), and biological agents directed against proteins involved in the pathogenesis of asthma (eg, omalizumab, mepolizumab, reslizumab)[2], [3].Albuterol is used for the treatment and prevention of bronchospasm (acute or severe) in patients with reversible obstructive airway disease[4]–[6]. It is also indicated for the prevention of exercise-induced bronchospasm. Albuterol acts on beta2-receptors to relax the bronchial smooth muscle[7]–[9]. It also inhibits the release of immediate hypersensitivity mediators from cells, especially mast cells. Although albuterol affects beta1-receptors, this is minimal and has little effect on the heart rate[10]–[12]. Albuterol comes in a variety of dosing forms and strengths. An aerosol metered-dose inhaler gives 90 mcg (base)/actuation, which is equivalent to 108 mcg of albuterolsulfate. The powder metered-dose inhaler form gives the same values as the aerosol metered-dose inhaler. Albuterol also is offered in 2 mg and 4 mg tablets [13]–[15]. There are several methods for estimation of albuterol. The present study deals with estimation of albuterol in ethanol-buffer system.

Materials and Methods

Chemicals: Albuterol was purchased from Sigma Eldrich, Germany. The formulation of albuterol Asthalin, Cipla) was purchased from local market. All other used in the present study were of analytical grade. Triple distilled water was used in the present study.

Preparations and chromatography

Buffer: A 25 mM KH_2PO_4 buffer was prepared by transferring 3.4 g of KH_2PO_4 to a 1000 mL volumetric flask and dissolving in 990 ml of water (purified, USP or HPLC grade). The pH was adjusted to 3.0 with 1 M hydrochloric acid and the resulting solution was diluted to 1000 mL with water and mixed.

Mobile phase: A 950 mL aliquot of buffer solution was mixed with 50 mL of methanol and filtered using a 0.2 m filter under vacuum to degas.

Standard solutions (equivalent to 0.3 mg/mL of albuterol base): Standard solutions of albuterolsulfate were prepared by dissolving approximately 90 mg, accurately weighed, of qualified albuterolsulfate reference material in 250 mL of water.

Resolution solution: About 1 mg each of albuterone hydrochloride and methoxymethylalbuterol hydrochloride, accurately weighed, were transferred to a 25 mL volumetric flask dissolved, and diluted to volume with the standard solution.

Sensitivity solution (about 0.1% of the active concentration): A 2.0 mL aliquot of the standard solution was transferred to a 200 mL volumetric flask, diluted to volume with water, and mixed. A 5.0 mL aliquot of the resulting solution was transferred to a 50 mL volumetric flask, diluted to volume with water, and mixed.

Sample preparation: The contents of at least 15 vials (0.5 mL each) were composited. A 3.0 mL aliquot of the composite was transferred to a 50 mL volumetric flask and diluted to volume with water.

Chromatographic conditions: Mobile phase flow rate: 1.5 mL/min; column temperature: ambient; detection: ultraviolet, 225 nm; injection volume: 20L; run time: about 40 min. Post analysis column wash was performed with methanol: water (25:75, v/v) before column storage.

Limit of detection (LOD) and limit of quantitation (LOQ): Solutions of albuterol and six of its related substances were prepared in duplicate (from independently prepared stock solutions) at concentrations equivalent to 0.042, 0.025, 0.017, and 0.0083% of the 0.3 mg/mL albuterol base assay concentration. Each of the prepared solutions was chromatographed[16]. The signal-to-noise ratios for albuterol and formulation were calculated. The LOD was evaluated as the concentration, which produced a peak with a signal-to-noise ratio of about 3. The

LOQ was evaluated as the concentration that produced a peak with a signal-to-noise ratio of about 10.

Specificity

Chromatographic profiles : Solutions of albuterol formulation containing about 75g/mL were individually prepared and chromatographed[17]. Retention times and relative retention times were determined to evaluate the potential co-elution or interference to the determination of albuterol and/or the related substances.

Force-degradation studies : Solutions of albuterolsulfate drug substance, formulation, and formulation placebo (without the active) were stressed with acidic, basic, oxidative, thermal, and photolytic conditions[18]. Details are presented in Table 2. Prior to analysis, the acid stressed samples were neutralized with base, and the base stressed samples were neutralized with acid. The force degraded samples were analyzed. This detector was equipped with a long path length flow cell and a reduced injection volume of 5 µl was required in order to achieve detector responses for the albuterol peak that were below 1 V. A "marker solution" containing albuterol and the formulation was injected within the HPLC run to aid in identification of the degradation products[19], [20].

Stability of standard and sample solutions: The stability of albuterol in prepared standard and sample solutions was evaluated under refrigerated condition. The assay values obtained at the end of the storage period were compared to the initial concentrations to evaluate the stability of solutions[17], [21], [22].

Results and Discussion

System suitability: The results of system suitability are shown in Table 1. The resolution was found to be 2.5 where as tailing factor was 1.6. The retention time was found to be 32.3 min. The method was fairly precise (2.9 %).

Table 1: Results of System Suitability

Parameter	Acceptance criteria	Observations	
		Drug	Drug Product
%RSD	Not more than 2.0%	0.1 %	0.1 %
Resolution	Not less than 1.5	2.5	2.4
Tailing factor	Not more than 3.5	1.6	1.8
Retention time	Elutes within the chromatogram	32.3 min	32.3 min
Precision	Not more than 10%	2.9 %	3.0 %

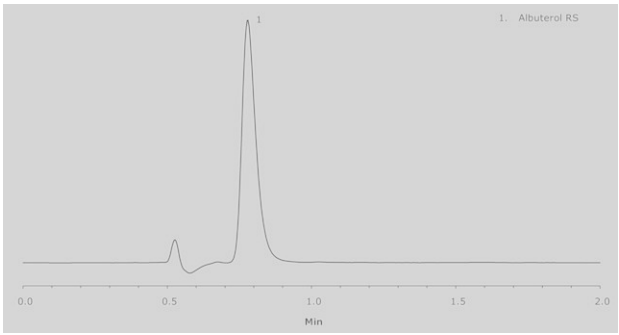

Figure 1: Typical HPLC Chromatogram of Albuterol

Results of validation parameters: The results of validation (Table 2) revealed that drug substances and drug product met typically similar level of results.

Table 2: Results of LOD, LOQ, Response Factors, System Suitability And Retention Time

Parameter	Observations	
	Drug	Drug Product
Retention Time	0.7 min	0.8 min
Response Factor	1.3	1.5
LOD	0.02 %	0.02 %
LOD	0.04 %	0.04 %

Forced degradation studies: The results of forced degradation study are summarized in Table 3. The drug in bulk as well as in formulation was stable in base. However, exposure to heat as well as UV light did not caused any harm to the drug in bulk as well as in drug product.

Table 3: Results of Forced Degradation Studies

Parameter	Observations	
	% Drug	%Drug in Drug Product
Control	100.20	101.32
Base	94.25	93.57
Acid	83.47	84.52
Peroxide	82.78	82.47
Heat	99.32	99.54
UV light	99.51	99.45

Conclusion: The method developed in this study was found to be specific, accurate, and followed the parameters for detection and quantisation of minimal amount of the drug. The method also helped to determine the stability under various stress conditions. Further the developed method may be applied for bioavailability and bioequivalence study of both the drugs in different biological samples.

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