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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 864–874
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of albuterol sulfate and its related substances in albuterol sulfate inhalation solution, 0.5% by RP-HPLC

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Received 20 June 2005; received in revised form 9 August 2005; accepted 9 August 2005
Available online 21 October 2005

Abstract

An isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination of albuterol sulfate and six of its related substances in albuterol sulfate inhalation solution, 0.5% (w/v). The separation was achieved using a YMC phenyl column (250 mm × 4.6 mm ID, 5 μm fitted with a direct connect YMC phenyl guard column (20 mm × 4 mm ID) maintained at ambient conditions, and a mobile phase of 25 mM monobasic potassium phosphate (pH 3.0) and methanol (95:5, v/v). The mobile phase flow rate was 1.5 mL/min and the detection wavelength was 225 nm. Albuterol is quantitated versus an external standard. The method was capable of resolving six of the seven known albuterol-related substances. Bis-ether albuterol, a drug substance process related impurity, is retained on the column due to its different hydrophilic character. The related substances are determined by area percent. However, a correction factor of 1.6 is applied for the determination of albuterol aldehyde, a potential impurity and a degradation product, since its molar absorptivity is about 1.6 times that of albuterol. The limits of detection and quantitation for albuterol and six of its related substances ranged between 0.01 and 0.21% of the assay concentration of 0.3 mg/mL as albuterol base. The method was found to be linear for albuterol over the range of 50–150% of the active label claim. The method was also found to be linear for the six related substances over the range 0.05–0.5%. No interferences from the blank, placebo (formulation matrix), related substances or force-degraded placebo samples were observed for the determination of the active or the individual related substances. The method was found to be accurate, precise, linear, specific, sensitive, rugged, robust, and stability-indicating.

Keywords: Albuterol sulfate; Related substances; RP-HPLC; HPLC; Assay; Validation

1. Introduction

Albuterol sulfate (synonym: salbutamol sulfate) is a relatively selective β_2-adrenergic agonist and is used as a bronchodilator. The chemical name of albuterol sulfate is 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino) ethanol sulfate (2:1) (salt). Albuterol sulfate is indicated for the prevention and relief of bronchospasm with reversible obstructive airway disease (asthma), and for the prevention of exercise induced bronchospasm. It is also indicated for the management of acute attacks of bronchospasm. Albuterol sulfate acts by stimulating the adenyl cyclase enzyme, which catalyzes the formation of cyclic-3′, 5′-adenosine monophosphate (cyclic AMP) from adenosine triphosphate (ATP). The cyclic AMP thus formed, mediates the cellular responses. The increased cyclic AMP levels are associated with relaxation of bronchial smooth muscles. Albuterol sulfate is effective by oral and inhalation routes of administration. Albuterol sulfate has been used in tablets, syrups, metered dose inhalers, and nebulized inhalation solutions. Albuterol sulfate inhalation solution, 0.5% (w/v) contains 5 mg/mL of albuterol as base (about 6 mg/mL of albuterol sulfate) in the aqueous formulation matrix. The formulation pH is maintained between 3 and 4.

There are several process impurities/related substances associated with the manufacture of albuterol sulfate drug substance. Different process related impurities are observed with various synthetic routes and/or manufacturing processes. Seven of the known albuterol related substances studied here are albuterone, chloroalbuterone, chloroalbuterol, methyl albuterol, albuterol aldehyde, methoxymethyl albuterol, and bis-ether albuterol. Structures of these related substances and their chemical names

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doi:10.1016/j.jpba.2005.08.021

are provided in Table 1. These related substances are monitored during the release of drug substance raw material and finished drug products. The United States [1] and British [2] Pharmacopoeias describe a titrimetric assay method for albuterol sulfate (salbutamol sulfate) drug substance. The TLC procedures described in the United States [1] and British [2] Pharmacopoeias for the related substances do not resolve all available related substances and are not sensitive enough for the detection and quantitation of unknown impurities. A literature search revealed that very few methods are published for the determination of albuterol and its related substances. However, an isocratic HPLC method [3] and a gradient method [4] are available for the determination of albuterol and its related substances either in raw material, tablets, syrups and/or inhalers. The related substances presently studied are different from those in these research articles.

Various analytical methods utilizing glycopeptide stationary phases such as teicoplanin [5] and vancomycin [6]; diol normal

Table 1
Chemical names and structures for albuterol and its related substances

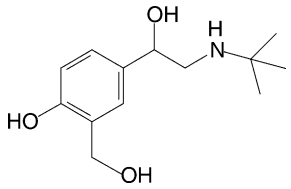
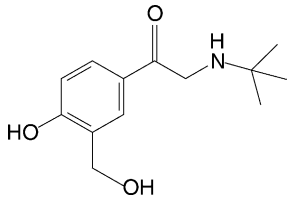
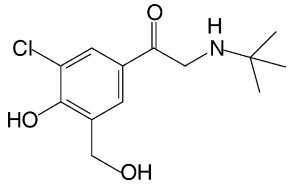
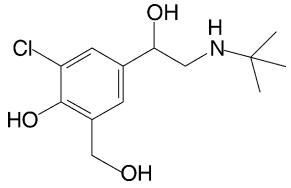
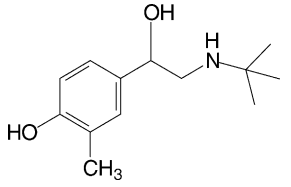
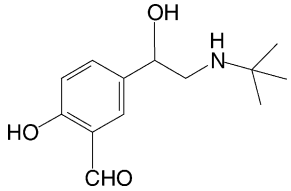
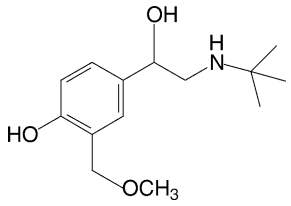
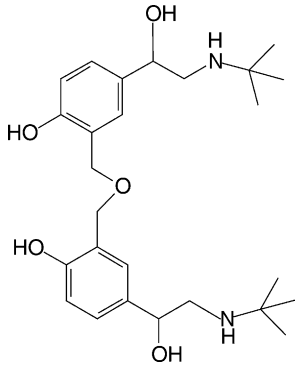
Related substance	Structure
1-(4-Hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino) ethanol (albuterol), active ingredient	
Tert-butylamino-4-hydroxy-5-hydroxymethylacetophenone (albuterone), process impurity	
Tert-butylamino-3-chloro-4-hydroxy-5-hydroxymethylacetophenone (chloroalbuterone), process impurity	
1-(5-Chloro-4-hydroxy-3-hydroxymethyphenyl)-2-(*tert*-butylamino)ethanol (5-chloroalbuterol), process impurity	
1-(4-Hydroxy-3-methylphenyl)-2-(*tert*-butylamino)ethanol (methyl albuterol), process impurity	
5-(2-((1,1-Dimethylethyl)amino)-1-hydroxyethyl)-2-hydroxybenzaldehyde (albuterol aldehyde), process impurity, degradant	
2-*Tert*-butylamino-1-(4-hydroxy-3-methoxymethylphenyl)-ethanol (methoxymethyl albuterol), process impurity	

Table 1 (*Continued*)

Related substance	Structure
Bis-(2-hydroxy)-5-(2-*tert*-butylamino-1-hydroxyethyl)phenylmethyl)ether (bis-ether albuterol), process impurity	

phase column [7]; and techniques such as isotachophoresis and capillary zone electrophoresis [8]; flow injection spectrophotometry [9] and potentiometry [10] for the determination of albuterol are available in the literature. The present analytical method discussed in this article is a simple isocratic high performance liquid chromatography with ultraviolet detection for the determination of albuterol sulfate and its related substances in inhalation solution.

2. Experimental

2.1. Materials

Albuterol sulfate and its related substances (albuterone hydrochloride, chloroalbuterone, chloroalbuterol, methyl albuterol, albuterol aldehyde sulfate, methoxymethyl albuterol hydrochloride, and bis-ether albuterol diacetate) were provided by Profarmaco S.r.I., Milan, Italy. Potassium phosphate, monobasic (KH_2PO_4), 1 M hydrochloric acid, and water (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ). Methanol (HPLC grade) was purchased from Fisher Scientific (Fairlawn, NJ). In-house purified water (USP) was used.

The HPLC systems used were ThermoSeparations Products (Waltham, MA) liquid chromatographs (SCM 1000 degasser, P4000 pump, AS3000 autosampler, UV3000 detector or UV6000LP photodiode array detector, and ChromQuest Chromatography Data System – Version 2.51), and Hitachi (San Jose, CA) liquid chromatography system (L-7100 pump, L-7200 autosampler, L-7450 detector, D-7000 HPLC System Manager – Version 3.1). The YMC (Kyoto, Japan) phenyl columns (120 Å, 250 mm × 4.6 mm ID, 5 μm) with a direct connect YMC phenyl guard columns (120 Å, 20 mm × 4 mm ID, 5 μm) were obtained from Waters Corporation (Milford, MA).

2.2. Preparations and chromatography

2.2.1. 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.

2.2.2. Mobile phase

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

2.2.3. Standard solutions (equivalent to 0.3 mg/mL of albuterol base)

Standard solutions of albuterol sulfate were prepared by dissolving approximately 90 mg, accurately weighed, of qualified albuterol sulfate reference material in 250 mL of water.

2.2.4. Resolution solution

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

2.2.5. 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.

2.2.6. 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.

2.2.7. Placebo

A placebo (formulation matrix) was prepared consisting of purified water, sodium citrate, edetate disodium at 0.01% (w/v), and adjusted to pH between 3 and 4 with hydrochloric acid.

2.2.8. Chromatographic conditions

Mobile phase flow rate: 1.5 mL/min; column temperature: ambient; detection: ultraviolet, 225 nm; injection volume: 20 μL; run time: about 40 min.

Post analysis column wash was performed with methanol: water (25:75, v/v) before column storage.

2.3. System suitability

The system was deemed suitable if the following acceptance criteria were satisfied. The relative standard deviation (RSD) of the peak area responses for albuterol from five standard solution injections was not more than 2.0%. The RSD for the peak area responses for albuterol from three sensitivity solution injections was not more than 10%. The tailing factor for the albuterol peak in the resolution solution was not more than 3.5. The resolution between the albuterone and albuterol peaks was not less than 1.5 and the methoxymethyl albuterol peak eluted within the chromatogram.

2.4. Response factor determination

Two separate solutions containing about 4.5 μg/mL (corrected for base fractions and purity) of each of the six related substances were prepared in the aqueous matrix and chromatographed. The response factors for each of the six related substances were calculated by dividing their individual peak area responses by their respective concentrations. The response factor for albuterol (as base) from the standard solution was similarly calculated. The relative response factors for each of the six related substances were then calculated by dividing their determined response factors by the albuterol response factor. An average from the two sets was used to report.

2.5. 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. The signal-to-noise ratios for albuterol and the six related substances 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.

2.6. Specificity

2.6.1. Chromatographic profiles

Solutions of albuterol and the six related substances (see Table 4) containing about 75 μg/mL were individually prepared and chromatographed. 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.

2.6.2. Force-degradation studies

Solutions of albuterol sulfate drug substance, formulation, and formulation placebo (without the active) were stressed with acidic, basic, oxidative, thermal, and photolytic conditions [11]. 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 using a ThermoSeparations HPLC system equipped with a UV6000LP photodiode array detector. 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 six related substances was injected within the HPLC run to aid in identification of the degradation products.

2.7. Validation studies

2.7.1. Accuracy/recovery/linearity for albuterol

Samples of product placebo were spiked with albuterol drug substance at 50, 75, 100, 125, and 150% of the product label claim. Each level was prepared in triplicate using three different lots of product placebo. Each sample was individually prepared by weighing albuterol sulfate drug substance and dissolving in the placebo solution. The spiked samples were assayed for albuterol content (% label claim, LC) versus albuterol standard preparations. The percent recovery for albuterol was calculated for each sample. The determined concentrations (%LC) were plotted versus the spiked concentrations (%LC) and a linear regression analysis was performed.

Table 2
Conditions for the forced degradation studies

Parameter	Drug substance condition	Drug product condition
Control (undegraded)	Ambient room temperature, protected from light	Ambient room temperature, protected from light
Base	1 M sodium hydroxide stored at 50 °C for 3 days; neutralized with 1 M hydrochloric acid prior to analysis	2.5 M sodium hydroxide stored at 60 °C for 16 h; neutralized with 1 M hydrochloric acid prior to analysis
Acid	1 M hydrochloric acid stored at 70 °C for 4.5 h	1 M hydrochloric acid stored at 60 °C for 12 h
Peroxide	2.1% (w/v) hydrogen peroxide stored at 50 °C for 3 days	10% (w/v) hydrogen peroxide stored at 60 °C for 16 h
Heat	Aqueous solution stored at 50 °C for 3 days	Aqueous solution stored at 60 °C for 16 h
Photolytic	UV-A light for 72 h (GE black light, 20 W)	UV-A light for 312 h (GE black light, 20 W)
Photolytic	CWF light for 168 h (Philips, 20 W)	CWF light for 312 h (Philips, 20 W)

2.7.2. Accuracy/recovery/linearity for related substances

Drug product solution was spiked with various aliquots of a stock solution containing about 7.5 μg/mL of each of the six related substances. After dilution to volume with water, the resulting solutions contained about 0.05, 0.1, 0.2, 0.3, and 0.5% of the normal albuterol assay concentration. These solutions were prepared and analyzed in triplicate. A composite standard solution containing 1.5% of albuterol base concentration (about 4.5 μg/mL) of each of the six related substances was prepared and injected in triplicate. The amount of each of the related substances was determined as the % label claim (% area corrected for relative response factors) and versus their respective external standards from the composite standard solution. The % label claim for each related substance was calculated by dividing individual peak area by the total area (sum of individual related substances and albuterol peak areas) and their determined relative response factors and multiplying by 100. The determined % label claims versus the theoretical % spiked were plotted and a linear regression analysis was performed. In addition, agreement of recoveries from the % area approach and values determined versus the respective external standard calculations were determined to demonstrate that the % area calculations were accurate and can be used in routine analyses.

2.7.3. Robustness

The robustness of the HPLC method was demonstrated by studying the effects of changes in the HPLC system parameters using a resolution solution. The HPLC parameters varied were column temperature (ambient, 27 °C, 30 °C), flow rate (±0.2 mL/min), wavelength (±2 nm), pH (±0.2 units) of the aqueous portion of the mobile phase, and organic/aqueous (±1%) ratios of the mobile phase.

2.7.4. Ruggedness

Six replicate samples of the inhalation solution from the same bulk formulation were prepared and assayed per the test method using two different laboratories, analysts, instruments, and on different days. The individual assay results for albuterol as %LC and as % area for related substances were calculated. The RSD for the six assays were determined for each laboratory and agreement between the mean results was calculated.

2.7.5. Stability of standard and sample solutions

The stability of albuterol in prepared standard and sample solutions was evaluated for 1 and 2 week intervals 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.

3. Results and discussion

Various mobile phases and columns were used to arrive at a method that achieved an optimal separation for all the components. The chromatographic method described here separates six of the seven related substances of albuterol. The bis-ether albuterol is retained on the column due to its different hydrophilic character. Since, bis-ether albuterol is an impurity originating from the drug substance manufacturing process and is not a potential degradation product, it is monitored only in the drug substance using a different method.

3.1. System suitability

The typical system suitability requirements were met and the results obtained are presented in Table 3. The tailing factor for the albuterol peak was 1.7 (criteria of NMT 3.5). The proposed method does not use a competing base in the mobile phase resulting in some tailing of the albuterol peak. However, the data shows that the tailing was consistently below 2.0 throughout the study.

3.2. Response factors

The determined response factors for the six related substances relative to that of albuterol ranged between 0.7 and 1.6 and are presented in Table 4. These results demonstrate that the UV responses for most of the related substances are comparable to those of the active (albuterol). However, albuterol aldehyde, a potential impurity and degradation product, exhibited a response that was 1.6 times that for the albuterol peak. Therefore, a correction factor of 1.6 is applied for its determination in routine use.

3.3. Limits of detection (LOD) and quantitation (LOQ)

Solutions of albuterol and six of its related substances containing each at 0.042, 0.025, 0.017, and 0.0083% of target concentration (corrected for base fractions and purity) were prepared in duplicate from each of two stock solutions and chromatographed. The signal-to-noise ratios for each component were determined. The LOD concentration was that concentration yielding a signal-to-noise ratio of at least 3. The LOQ

Table 3
Typical system suitability results

Parameter	Acceptance criteria	Laboratory 1	Laboratory 2
%RSD for albuterol sulfate peak areas from five standard injections	Not more than 2.0%	0.1%	0.1%
Resolution between albuterone and albuterol peaks	Not less than 1.5	2.9	3.1
Tailing factor for albuterol peak in resolution solution	Not more than 3.5	1.8	1.7
Retention time of methoxymethyl albuterol	Elutes within the chromatogram	Conforms (33.7 min)	Conforms (37.0 min)
Precision for three sensitivity solution injections	Not more than 10%	3.8%	0.9%

Table 4
Typical relative retention time and response factors, LOD and LOQ concentrations for components

Peak ID	Component	Typical retention time (min)	Relative retention time (RRT)	Relative response factor (RRF)	LOD[a] concentration (%)	LOQ[a] concentration (%)
1	Albuterone	8.1	0.8	1.1	0.01	0.03
2	Albuterol	9.6	1.0	1.0	0.01	0.02
3	Chloroalbuterone	13.7	1.4	1.3	0.02	0.05
4	Chloroalbuterol	22.1	2.3	0.7	0.02	0.08
5	Methyl albuterol	25.0	2.6	0.8	0.03	0.11
6	Albuterol aldehyde	27.1	2.8	1.6	0.02	0.07
7	Methoxymethyl albuterol	32.7	3.4	0.8	0.06	0.21

[a] Percentage of albuterol assay concentration, 0.3 mg/mL.

concentration was that concentration yielding a signal-to-noise ratio of at least 10. For albuterol (as a model compound for potential unknown related substances) these LOD and LOQ limits were about 0.01 and 0.02% of the normal assay concentration. Table 4 provides the determined LOD and LOQ values for the related substances.

3.4. Specificity

3.4.1. Chromatographic profiles

The specificity of the method was determined by individually chromatographing albuterol and six of its related substances (see Table 4), formulation, placebo, and a blank (purified water). The chromatograms show that the method is specific and no interferences from the placebo, blank, or related substances was observed for the determination of albuterol or the individual related substances.

The typical retention times and relative retention times for each component are presented in Table 4. Fig. 1 shows typical overlaid chromatograms of the blank, the placebo, and a 0.1% sensitivity solution of albuterol. Overlaid chromatograms of the drug product and drug product solution spiked with 0.5% of each of the six related substances is presented in Fig. 2.

3.4.2. Force-degradation studies

Initially, similar stress conditions were applied to both the drug substance and to the drug product solutions. However, stressed samples of drug substance showed up to 17% degradation, while the drug product solutions showed little or no degradation. Thus, stronger stress conditions, as presented in Table 2, were applied to the drug product solutions. The application of stress conditions to drug substance and drug product solutions did not generate any degradation products that interfered with the detection/determination of albuterol. Further, the peak purity results for the albuterol peak in all the stressed samples was unity, indicative of single pure peak. Undegraded samples of drug substance and drug product were analyzed as "controls". The data from the stressed samples was compared to that of the respective controls to determine that no unknown peak(s) was formed that co-eluted with the known related substance(s). This was determined by evaluating peak(s), if any, corresponding to synthetic related substance(s) that was formed or increased under the stress condition. The albuterol assay results, mass balance, and the peak purity indices for each of the stressed samples are presented in Table 5.

For the drug substance and the drug product samples that were stressed with thermal and photolytic conditions, no appreciable degradation was observed. However, for the base stressed condition, an unknown peak at a retention time relative to albuterol (RRT) of 1.57 was the major degradant, being detected at 2.6 and 0.44% (peak labeled as U2 of Fig. 3) in the drug substance and drug product, respectively. Albuterol aldehyde peak (peak ID 7)

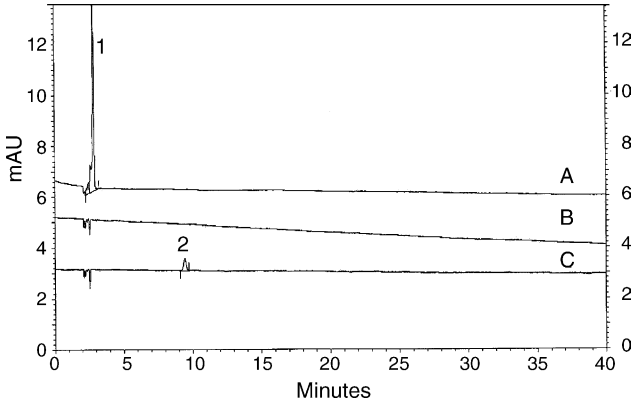


Fig. 1. Overlaid chromatograms (enhanced scale) of (A) placebo (B), diluent (purified water), and (C) sensitivity solution; 2: albuterol.

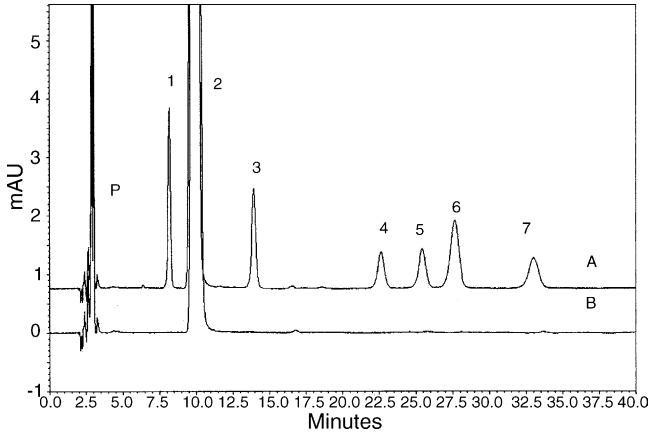


Fig. 2. Overlaid chromatograms (enhanced scale) of (A) drug product spiked with 0.5% of each of the six related substances and (B) drug product. P: placebo and solvent peaks. See Table 4 for peak identification.

Table 5
Mass balance and purity indices for albuterol for stressed samples

Condition	Drug substance				Drug product			
	Albuterol %LC	Total degradants and impurities	Mass balance	Peak purity	Albuterol %LC	Total degradants and impurities	Mass balance	Peak purity
Control	100.2	0.04	100.2	1.0	97.3	0.00	97.3	1.0
Base	93.9	3.50	97.4	1.0	95.6	0.62	96.2	1.0
Acid	85.1	0.19	85.3	1.0	82.2	0.10	82.3	1.0
Peroxide	83.4	2.99	86.4	1.0	96.1	1.07	97.2	1.0
Heat	99.5	0.06	99.6	1.0	98.4	0.00	98.4	1.0
UV light	101.4	0.08	101.5	1.0	97.2	0.00	97.2	1.0
CWF light	102.7	0.05	102.8	1.0	97.8	0.07	97.9	1.0

was detected at levels 0.08% or less. Chromatogram A of Fig. 3 was obtained for sample stressed with base (2.5 M) for 16 h at 60 °C per conditions in Table 2. In addition, a chromatogram for a sample stressed with base (1.75 M) for 16 h at 60 °C was obtained for comparison and is shown as chromatogram B of Fig. 3. No change in the profile of degradation products was observed by changing the concentration of sodium hydroxide.

Unknown peak U2 was not observed at or above the threshold level of 0.1% for the actual product storage conditions in the stability studies. Since the drug product is controlled in the acidic range (pH 3.0–4.0), no further characterization of these unknown peaks was performed.

For the sample stressed with peroxide (Fig. 4) small amounts (less than or equal to 0.1%) of two unknown peaks (RRT = 0.83 and 1.32; peaks U1 and U2 of Fig. 4) and albuterol aldehyde (peak ID 7) were observed. Chromatogram A of Fig. 4 was obtained for sample stressed with 10% peroxide for 16 h at 60 °C per conditions in Table 2. In addition, a chromatogram for a sample stressed with 4% peroxide for 12 h at 60 °C was obtained for comparison and is shown as chromatogram B of Fig. 4. No change in the profile of the formed degradation products was observed. However, the rate of formation of the two unknown peaks and albuterol aldehyde were different. Fig. 5 shows the chromatographic profile of samples of drug product, placebo and blank that were stressed with acid (1 M) for 12 h at 60 °C.

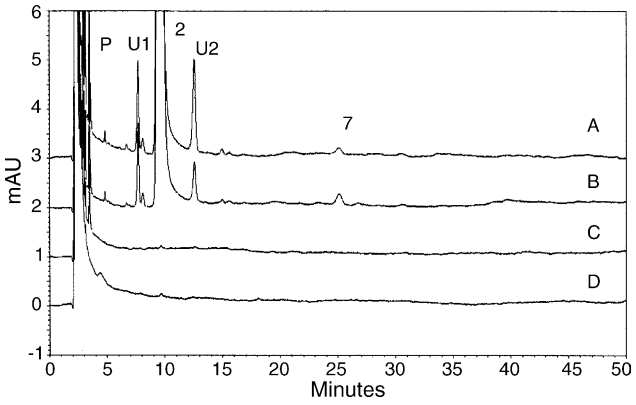


Fig. 4. Overlaid chromatograms (enhanced scale) of peroxide stressed (A and B) drug product; (C) placebo; (D) blank. P: placebo and solvent peaks; 2: albuterol; U1: unknown (RRT 0.78); U2: unknown (RRT 1.32).

The chromatographic profiles of the force-degraded samples were compared to the actual drug product that was exposed to 40 °C/75%RH condition for 6 months (conventional stability) and to 70 °C/ambient humidity for 1 month. The results indicate that albuterol aldehyde was the only degradation product that was observed in the drug product. No other peaks were observed in the drug product that corresponded to degradation peaks observed in the samples stressed with acidic, basic, and or oxidative conditions.

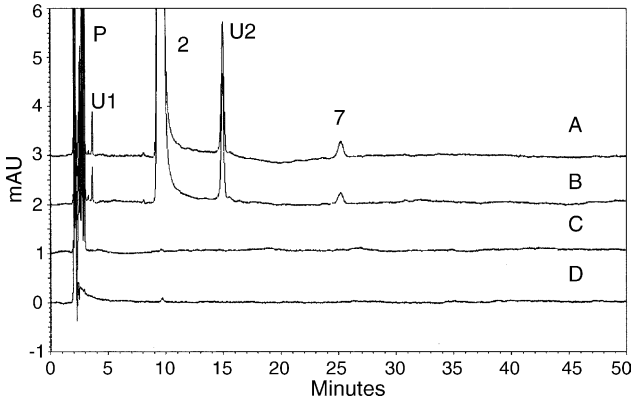


Fig. 3. Overlaid chromatograms (enhanced scale) of base stressed (A and B) drug product; (C) placebo; (D) blank. P: placebo and solvent peaks; 2: albuterol; U1: unknown (RRT 0.38); U2: unknown (RRT 1.57).

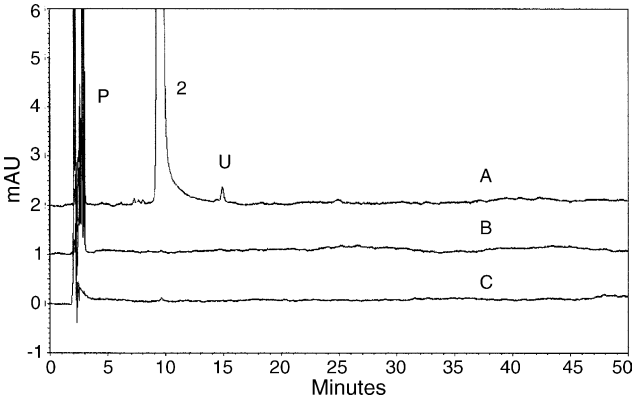


Fig. 5. Overlaid chromatograms (enhanced scale) of acid stressed (A) drug product; (B) placebo; (C) blank. P: placebo and solvent peaks; 2: albuterol; U: unknown (RRT 1.57).

Table 6
Accuracy and recovery for albuterol

Level (%)	Spiked albuterol concentration (% label claim)	Determined albuterol concentration (% label claim)	Percent recovery	Mean	RSD
50	51.1	51.6	101.0	100.9	0.5
	52.4	52.6	100.4		
	51.9	52.6	101.3		
75	75.5	76.2	100.9	101.2	0.5
	74.8	76.2	101.9		
	75.4	76.1	100.9		
100	100.3	99.9	99.6	100.4	0.7
	101.5	102.4	100.9		
	101.6	102.4	100.8		
125	125.5	126.2	100.6	100.9	0.3
	126.4	127.8	101.1		
	125.4	126.6	101.0		
150	151.5	151.0	99.7	99.8	0.2
	150.5	150.5	100.0		
	151.7	151.2	99.7		

Overall mean: 100.6; overall RSD: 0.7.

3.5. Validation studies

3.5.1. Accuracy/recovery/linearity for albuterol

The results of the recovery studies show that the method is accurate for the determination of albuterol. The individual albuterol recoveries for placebo samples spiked at 50–150% of label claim ranged from 99.6 to 101.9%. The overall mean recovery was 100.7%. All recovery results are presented in Table 6. The method was found to be linear for albuterol in the 50–150% of label claim. The correlation coefficient (R^2) was 1.000 and the linear regression equation is presented in Table 6.

3.5.2. Accuracy/recovery/linearity for related substances

The recovery results obtained indicate that the method is also accurate for the determination of the six related substances in the range of 0.05–0.5% of product label claim. The method was found to be linear with correlation coefficients (R^2) of 0.987–0.995 for the six related substances, with slopes near unity and *y*-intercepts near zero for these low-level determinations. The linear regression data for the six related substances is presented in Table 7. The mean of three determinations was calculated at each of the five levels studied. The mean recoveries for the related substances at the low levels ($n = 5$, 0.05–0.5%) ranged between 96.3 and 118.4%. The relative standard deviations for these determinations ranged between 3.8 and 24.6%. The recoveries determined by the % area approach and corrected for the relative response factors was in good agreement for the recoveries determined versus the respective related substance external standard. The agreements ranged between 99.7 and 103.9%. The results of accuracy and recovery for the related substances spiked at low levels are presented in Table 8.

Table 7
Linear range, coefficient of correlation, slope and intercepts for albuterol and six of its related substances

Component	Linear range (%LC)	R^2	Intercept	Slope
Albuterol	50–150	0.9997	1.1107	0.9942
Albuterone	0.062–0.624	0.9999	0.0096	0.9497
Chloroalbuterone	0.052–0.516	0.9998	0.0080	0.9621
Chloroalbuterol	0.052–0.524	0.9991	0.0113	1.0089
Methyl albuterol	0.053–0.534	0.9980	0.0260	1.0023
Albuterol aldehyde	0.059–0.595	0.9998	0.0069	0.9112
Methoxymethyl albuterol	0.062–0.624	0.9966	0.0314	0.8801

3.5.3. Robustness

The data obtained from the deliberate variations of the HPLC parameters shows that the variations did not significantly affect the system suitability requirements. The observed responses to the parameter changes were as expected. Decreasing the flow rate and increasing the aqueous portion of the mobile phase slightly increased the retention of components. Increasing the flow rate, column temperature, and increasing the organic portion of the mobile phase slightly decreased the retention of components. Variations of detection wavelength did not affect separation. However, it had a slight effect on the absorption intensities (peak responses) for the components. The results of variation of HPLC parameters on system suitability are presented in Table 9.

3.5.4. Ruggedness

The method was shown to be rugged. The mean results from six replicate samples from a homogeneous sample prepared and assayed using two different laboratories, analysts, instruments, and on different days yielded results with an agreement of 100.2%. The mean assay value obtained by laboratory 1 was 97.9% (RSD = 0.2%) and laboratory 2 obtained 98.1% (RSD = 0.5%). The individual results from both laboratories are presented in Table 10. Results obtained for the related substances

Table 8
Recovery and accuracy for related substances

Level (%)	Spiked amount (%)	Amount determined area (%)	Recovery (%)	Assay vs. standard (%)	Recovery (%)	Agreement between recoveries
Albuterone						
0.05	0.062	0.067	108.1	0.066	106.5	98.5
0.1	0.125	0.128	102.4	0.126	100.8	98.4
0.2	0.249	0.250	100.4	0.249	100.0	99.6
0.3	0.374	0.363	97.1	0.366	97.9	100.8
0.5	0.624	0.602	96.5	0.609	97.6	101.2
Mean			100.9		100.5	99.7
%RSD			4.7		3.6	1.3
Chloroalbuterol						
0.05	0.052	0.066	126.9	0.068	130.8	103.0
0.1	0.105	0.114	108.6	0.117	111.4	102.6
0.2	0.209	0.217	103.8	0.225	107.7	103.7
0.3	0.314	0.337	107.3	0.351	111.8	104.2
0.5	0.524	0.537	102.5	0.564	107.6	105.0
Mean			109.8		113.9	103.7
%RSD			9.0		8.5	0.9
Albuterol aldehyde						
0.05	0.059	0.064	108.5	0.065	110.2	101.5
0.1	0.119	0.112	94.1	0.114	95.8	101.8
0.2	0.238	0.222	93.3	0.228	95.8	102.7
0.3	0.357	0.334	93.6	0.347	97.2	103.9
0.5	0.595	0.549	92.3	0.573	96.3	104.4
Mean			96.3		99.1	102.9
%RSD			7.1		6.3	1.2
Chloroalbuterone						
0.05	0.052	0.055	105.8	0.056	107.7	101.8
0.1	0.103	0.109	105.8	0.112	108.7	102.8
0.2	0.206	0.209	101.5	0.215	104.4	102.9
0.3	0.310	0.305	98.4	0.316	101.9	103.6
0.5	0.516	0.504	97.7	0.525	101.7	104.2
Mean			101.8		104.9	103.0
%RSD			3.8		3.1	0.9
Methyl albuterol						
0.05	0.053	0.069	130.2	0.07	132.1	101.4
0.1	0.107	0.146	136.4	0.148	138.3	101.4
0.2	0.214	0.242	113.1	0.248	115.9	102.5
0.3	0.320	0.342	106.9	0.353	110.3	103.2
0.5	0.534	0.562	105.2	0.582	109.0	103.6
Mean			118.4		121.1	102.4
%RSD			11.9		11.0	1.0
Methoxymethyl albuterol						
0.05	0.062	0.098	158.1	0.100	161.3	102.0
0.1	0.125	0.127	101.6	0.131	104.8	103.1
0.2	0.249	0.244	98.0	0.255	102.4	104.5
0.3	0.374	0.372	99.5	0.389	104.0	104.6
0.5	0.624	0.578	92.6	0.609	97.6	105.4
Mean			109.9		114.0	103.9
%RSD			24.6		23.3	1.3

were also satisfactory. The means for six results from each laboratory are presented in Table 11.

3.5.5. *Stability of standard and sample solutions*

Prepared samples and standards have been shown to be stable for at least 2 weeks when stored refrigerated. Additionally, the standard solutions have been shown to be stable while in use for assays for at least 40 h. The stability of albuterol in standard and sample solutions was evaluated after 1 and 2 weeks under refrigerated condition. The results obtained for refrigerated standard solution were 100.2 and 100.9% of the initial concentration for 1 and 2 week time points, respectively. The result for the

Table 9
Robustness study

Parameter	Variation	Albuterol peak			Methoxymethyl albuterol, tR (min)
		R^a	T	tR (min)	
Flow rate	1.3 mL/min	3.7	1.4	11.9	40.6
	1.5 mL/min (normal)	**3.6**	**1.5**	**10.6**	**36.2**
	1.7 mL/min	3.5	1.5	9.4	32.3
Column temperature	25 °C (ambient) (normal)	**3.6**	**1.5**	**10.6**	**36.2**
	27 °C	3.5	1.4	10.3	34.8
	30 °C	3.3	1.3	10.1	33.9
Detection wavelength	223 nm	3.6	1.6	10.3	35.0
	225 nm (normal)	**3.6**	**1.5**	**10.6**	**36.2**
	227 nm	3.5	1.4	10.4	35.1
Mobile phase pH	2.8	3.7	1.4	10.2	34.3
	3.0 (normal)	**3.6**	**1.5**	**10.6**	**36.2**
	3.2	3.5	1.6	10.1	33.9
Mobile phase methanol content	4% Methanol	3.5	1.4	11.1	39.0
	5% Methanol (normal)	**3.6**	**1.5**	**10.6**	**36.2**
	6% Methanol	3.4	1.3	9.31	30.0
Column lots	042556114 (W)	2.9	1.8	9.9	33.7
	042547769 (W) (normal)	3.6	1.5	10.6	36.2
	042554814 (W)	3.5	1.6	9.6	32.6

Values in bold are for method conditions. T: tailing factor.
[a] Resolution between albuterone and albuterol.

Table 10
Method ruggedness for albuterol assay

Sample	Albuterol (% label claim)	
	Laboratory 1	Laboratory 2
1	97.9	98.2
2	98.1	97.4
3	97.6	98.5
4	97.9	98.7
5	98.1	98.2
6	97.9	97.8
Average	97.9	98.1
RSD	0.2	0.5

Agreement: 100.2.

Table 11
Method ruggedness for related substances

Component	Mean related substance (% label claim) ($n = 6$)		Difference (% area)
	Laboratory 1	Laboratory 2	
Albuterone	0.01	0.01	0.00
Chloroalbuterone	ND	ND	0.00
Chloroalbuterol	ND	ND	0.00
Methyl albuterol	ND	0.01	0.01
Albuterol aldehyde	0.03	0.03	0.00
Methoxymethyl albuterol	0.02	0.02	0.00
Other (RRT = 0.82)	0.02	0.02	0.00
Other (RRT = 2.02)	ND	0.01	0.01
Total	0.08	0.09	0.01

ND: not detected.

refrigerated sample solution were 101.2 and 99.9% of its initial concentration after 1and 2 week's storage, respectively. No degradation products were observed for any of the solutions tested. A standard solution that had been held at ambient conditions for 41.5 h was stable with a response that was 101% of its initial value.

4. Conclusion

The proposed method was found to be accurate, precise, specific, sensitive, linear, rugged, robust, and stability-indicating for the determination of albuterol and six of its related substances, in the inhalation solution, over the entire range investigated. The method is therefore suitable for the determination of albuterol and its related substances in albuterol sulfate inhalation solution, 0.5%.

Acknowledgements

The authors would like to thank Miriam Swanson, Jill Brians and Gorm Yoder for the successful completion of the study.

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