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Liquid chromatographic methods for the determination of albuterol (salbutamol), albuterol sulphate and related compounds in drug raw materials, tablets and inhalers

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Abstract: Liquid chromatographic methods for the determination of albuterol (salbutamol), albuterol sulphate and related compounds in drug raw materials, tablets and inhalers are described. The methods resolve five known related compounds from the drug and, in the case of inhalers, several compounds not related to the drug. Two of these were identified as 2,6-di-t-butyl-4-methylphenol, a common antioxidant, and 2,2'-methylene bis(6-t-butyl-4-methylphenol). Related compounds are detectable at levels of about 0.03%. Eleven albuterol and 12 albuterol sulphate raw materials and eight tablet formulations were found to contain related compounds ranging from 0.03 to 0.54%, 0.09 to 0.50% and 0.32 to 0.95%, respectively. Non-drug compounds in three inhaler samples ranged from 4.6 to 12% of the drug delivered through the valve. Some of the non-drug compounds may be excipients.

Keywords: *Salbutamol; albuterol; reversed-phase liquid chromatography.*

Introduction

Albuterol (**1**) is the United States Adopted Name for 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(t-butylamino) ethanol [1] and is used throughout this report. The International Nonproprietary Name is salbutamol.

Physical and spectral properties of the drug raw material, a route of synthesis and methods of analysis were published in a 1980 review article on albuterol [2]. More recently, LC methods have appeared for the determination of albuterol in inhalers [3], plasma [4] and hydro-alcoholic solutions with theophylline and saccharin [5]. However, none of these papers address the determination of related compounds. The United States [6] and British [7] pharmacopoeial TLC procedures for related compounds do not resolve all available compounds and are insufficiently sensitive for detection and quantitation of unknown impurities.

The purpose of this paper is to describe procedures for the separation and quantitation of albuterol and albuterol related compounds in drug raw material and in tablet and inhaler formulations. Some of these compounds, which originate during synthesis or upon degradation, are listed in Table 1 and their structures are given in Fig. 1.

Experimental

Chemicals and related compounds

Albuterol related compounds (Table 1) were obtained as follows: **2**, **3** and **5**, Huhtamaki Oy (Turku, Finland); **4**, ICFI (Milan, Italy); **6**, Cipla Ltd (Bombay, India). Albuterol and albuterol sulphate raw materials were obtained from Huhtamaki Oy, ICFI, Cipla and other sources. Acetonitrile (J.T. Baker Co., Phillipsburg, NJ, USA) and phosphoric acid (85%, Fisher Scientific) were HPLC grade. Deionized water was used.

Apparatus

The LC system (Varian 5560) consisted of an autosampler fitted with a 10-μl loop (Varian Model 8085), a detector set at 229 nm (Varian Model UV-200) and a data station (Varian model 402). A 3-μm hexyl bonded phase column (Spherisorb, 150 × 4.6 mm, Phase Separations Ltd, No. N0125), was used at ambient temperature with a mobile phase flow rate of 1.0 ml min^{-1}. Other equipment used was a UV–vis spectrophotometer (Varian

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Table 1
Albuterol related compounds

Compound	Name
1	Albuterol: 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(t-butylamino) ethanol [salbutamol]
2	1-(4-hydroxy-3-methylphenyl)-2-(t-butylamino) ethanol
3	1-(4-hydroxy-3-methylphenyl)-2-(t-butylamino) ethane hydrochloride
4	1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*N*-benzyl-*N*-t-butylamino) ethanol [*N*-benzyl albuterol]
5	1-(4-benzyloxy-3-hydroxymethylphenyl)-2-(t-butylamino) ethanol
6	2-(*N*-benzyl-*N*-t-butylamino)-4-hydroxy-3-hydroxymethyl acetophenone HCl

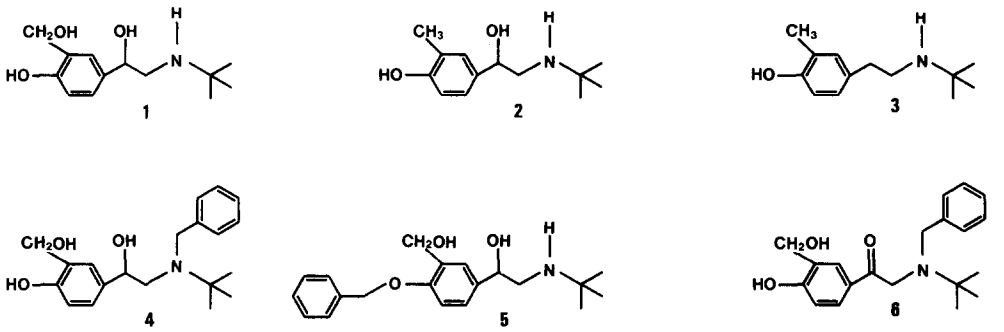

Figure 1
The structures of albuterol and five related compounds.

DMS 90) connected to a computer (Hewlett–Packard Model HP85) and a plotter (Hewlett–Packard Model 7470A), an autotitrator (Mettler DL40RC Memotitrator) equipped with a 5-ml burette and a glass calomel electrode, and a Finnigan Mat Model H610B mass spectrometer.

Mobile phase

The mobile phase consisted of a solution of 600 ml acetonitrile, 400 ml water and 1 ml phosphoric acid that was mixed, filtered and degassed under vacuum.

Solutions

Note — albuterol sulphate standards should be used for the analysis of albuterol sulphate raw materials and formulations.

Standard solutions. The dissolution medium consisted of acetonitrile–water (60:40, v/v). It was used to prepare the resolution solution (0.01 mg ml^{-1} each of albuterol and **2**, the related compounds standard solution (0.002 mg ml^{-1} albuterol standard) and the assay standard solution (0.1 mg ml^{-1} albuterol standard).

Test solutions. Drug raw material related compounds and assay solutions contained 1.0 and 0.1 mg ml^{-1} albuterol, respectively, in the dissolution medium.

The test solution for related compounds in tablets was prepared by weighing, crushing and powdering 20 tablets, transferring the equivalent of 20 mg of albuterol, accurately weighed, to a 70-ml centrifuge tube fitted with a Teflon-lined screw-cap and adding 20.0 ml of dissolution medium. The tube was shaken for 30 min and centrifuged at 2000 rpm for 10 min to obtain a final albuterol concentration of about 1.0 mg ml^{-1}. This solution was diluted to 0.1 mg ml^{-1} with dissolution medium to obtain the tablet assay test solution.

The test solution for related compounds in inhalers was made in two ways.

Method (A). The entire contents of one inhaler canister were expelled into a 500-ml separatory funnel containing 5 ml methanol by repeated depression of the valve (about 200 times). The contents of the funnel were dissolved, transferred quantitatively to a 25-ml volumetric flask and made up to volume, all with methanol, to a final albuterol concentration of about 0.8 mg ml^{-1}.

Method (B). The inhaler canister was frozen by immersion in liquid nitrogen. When frozen, the can was opened with metal cutters and the contents were allowed to melt into a beaker at room temperature with the propellant and

other volatiles evaporating into a fume hood. The residue was dissolved, transferred to a 25-ml volumetric flask and made up to volume, all in methanol. The final albuterol concentration was about 0.8 mg ml^{-1}. It was diluted to 0.1 mg ml^{-1} with dissolution medium to obtain the inhaler assay test solution.

Method (C). Inhaler test solutions prepared by methods A and B were evaporated to dryness. The residue was dissolved in 0.1 M HCl and extracted into dichloromethane. A portion of the dichloromethane solution was evaporated to dryness and the residue was redissolved in methanol for analysis.

System suitability

Six aliquots of the resolution solution were injected daily. The system was deemed to be suitable if, using USP procedures, the number of theoretical plates for the albuterol peak was not less than 30,000 plates, the tailing (peak asymmetry) factor was <1.5, the relative standard deviation (RSD) of the albuterol area response was not more than 3% and the resolution between albuterol and **2** was >6. The retention times of albuterol and **2** were about 6 and 10 min, respectively. The system was considered to be suitable for assay if five replicate injections of the assay standard solution gave a RSD <1%.

Related compounds procedure

A 10 μl aliquot of the related compounds standard solution and the related compounds test solution were injected separately into the column and eluted for ≈25 min. The percentage of each impurity was calculated from $[100(A_u/A_s)(C_s/C_u)]$, where A_u is the peak area of each impurity in the related compounds test solution, A_s is the peak area of albuterol in the related compounds standard solution and C_u and C_s are the concentrations of albuterol in the related compounds test and standard solutions, respectively.

Assay procedure

A 10 μl aliquot of the assay standard solution and the assay test solution were injected separately into the column. The percentage of albuterol was calculated from $[100(A_u/A_s)(C_s/C_u)]$, where A_u is the peak area of albuterol in the assay test solution, A_s is the peak area of albuterol in the assay standard solution and C_u and C_s are the concentrations of albuterol in the assay test and standard solutions, respectively.

Ultraviolet spectra

The spectra of albuterol and the available related compounds were measured in methanol.

Infrared spectra

The spectra in KBr of all available samples of albuterol and albuterol sulphate were virtually identical.

Mass spectra

The following conditions were used: ion source 150°C; emission current 300 μA; interface 250°C; electron energy 40 eV; DB-5 column, 15 m by 0.25 mm.

Non-aqueous titration

About 75 mg albuterol, accurately weighed, was dissolved in 50 ml glacial acetic acid and titrated with 0.1 N perchloric acid in glacial acetic acid to a potentiometric end point. The perchloric acid solution was standardized against potassium biphthalate using Crystal Violet as an indicator.

Results and Discussion

A chromatogram showing the resolution of the five available related substances from an albuterol inhaler formulation is given in Fig. 2. Retention times of the drug and related compounds are given in Table 2 and UV absorption maxima, absorbance at 229 nm and LC response are given in Table 3. The response of the LC system to the drug was linear over the range 5 ng to 2 μg on-column (0.5 to 200 μg ml^{-1}), and related compounds were shown to be linear from 5 to 200 ng on column (0.5 to 20 μg ml^{-1}) (Table 2). Minimum quantifiable amounts of the related compounds were about 0.03%.

The method provides for quantitation of the impurities in albuterol against an albuterol standard. The relative LC responses in Table 3 show that each related compound will be over or underestimated due to a different detector response as compared to the drug. From the point of view of evaluating albuterol samples, this loss of accuracy is acceptable because there is no indication that a more accurate quantitation of related compounds is needed and

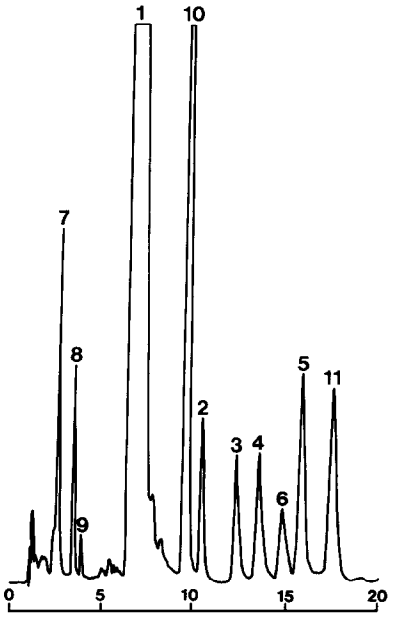

Figure 2
Chromatogram of inhaler product JJ to which has been added about 1% of related compounds **2**, **3**, **4**, **5** and **6**, virtually none of which was originally present. Peaks 10 and 11 have been identified as BHT and 2,2′-methylene bis(6-t-butyl-4-methylphenol), respectively. The abscissa scale is in minutes.

Table 2
Linearity data for albuterol and related compounds

Compound	Slope*	Intercept†	(R^2)‡	RRT§	RT‖
1	507	1	0.999	1.0	6.4
2	492	8	0.999	1.5	10.0
3	349	5	0.999	1.8	12.0
4	361	12	0.999	2.1	13.5
5	738	30	0.999	2.5	16.3
6	377	−34	0.999	2.4	15.9

* Area count/ng.
† Area counts.
‡ The coefficient of determination.
§ Retention time relative to albuterol.
‖ Retention time in min.

samples of the related compounds are not generally available for direct area comparison.

Precision

The precision of the system was determined by making six replicate injections of a solution containing 0.1 mg ml^{-1} albuterol in acetonitrile–water (60:40, v/v). The RSD of the peak area was found to be <1.0%. Six replicate injections of albuterol and related compounds gave, at concentrations of about 5 μg ml^{-1} RSDs of 1.32 (albuterol), 2.53 (**2**), 1.76 (**3**), 3.59 (**4**), 1.40 (**5**) and 2.15% (**6**).

Raw material assay

Eleven samples of albuterol raw material from seven manufacturers and 12 samples of albuterol sulphate from five manufacturers were analysed in duplicate for impurities. Results are given in Tables 4 and 5, respectively. Raw material assay results for selected samples by LC and non-aqueous titration are compared in Table 6. Impurity levels in eight samples of albuterol sulphate tablets from four manufacturers are given in Table 7, and albuterol assay results of selected tablets are given in Table 8.

Impurity levels in three samples of inhalers from two manufacturers are given in Table 9. Compared with other types of formulations, the levels are high and some of the impurities may be due to excipients or leachates from the metered dose delivery system. The level of the compound eluting at a relative retention time (RRT), referenced to albuterol, of 0.69 was several times higher in samples obtained by delivery through the valve (method A) as compared with that obtained by opening a frozen can (method B), a strong indication that this impurity is associated with the valve system.

Table 3
UV absorbance of albuterol related compounds

Compound	Concentration* (μg ml^{-1})	UV maxima (nm)	Absorbance at 229 nm	Relative absorbance†	LC† response
1	5.00	205, 230, 280	0.128	1.00	1.00
2	4.89	205, 230, 280	0.114	0.91	0.97
3	5.03	206, 229, 282	0.09	0.70	0.69
4	5.13	205, 230, 281	0.092	0.70	0.71
5	5.16	206, 231, 278	0.162	1.22	1.46
6	5.02	202, 278	0.137	1.06	0.78

* In methanol.
† Relative to albuterol.

Table 4
Percent related compounds in albuterol raw material

RRT* of related compounds	Albuterol raw material samples										
	A	B	C	D	E	F	G	H	I	J	AA
0.23	0.02			0.15						0.03	
0.25	0.03	0.03	0.05	0.06	0.03	0.05	0.06	0.03	0.03	0.08	
0.90				0.10		0.05	0.07		0.20		
0.92				0.02		0.03	0.08				0.01
1.32							0.07				tr†
1.43			ni‡	tr			0.02	ni			
1.71				0.06		0.25			tr		
1.82§				0.13		0.06					
2.84	ni									0.07	
Total	0.05	0.03	0.05	0.52	0.03	0.44	0.30	0.03	0.23	0.18	0.02

*Retention time relative to albuterol at 6.4 min.
†Trace is <0.005%.
‡Peak not integrated.
§RRT of **3** is 1.8.

Table 5
Percent related compounds in albuterol sulphate

RRT* of related compounds	Albuterol sulphate raw material samples											
	K	L	M	N	O	P	Q	R	S	T	U	V
0.23	0.03	0.01	0.05					0.03		0.04		
0.25	0.04	0.06	0.06		0.03	0.03	0.02	0.02	0.04	0.03		0.02
0.90	0.03	0.01		0.02			0.01			ni†	0.01	
1.22				tr‡			tr	tr			0.08	
1.32	ni	ni		ni	ni	0.04	0.02				0.06	0.04
1.43				0.05	ni	0.03		ni			0.11	ni
1.58			0.03									
1.71	0.06	0.08	0.04	0.02	0.06	0.05	0.08				0.16	0.02
1.82§	0.05	0.06	ni	ni	0.06	0.05	0.21	ni				0.06
2.19								0.04				
2.50											0.08	
2.76			0.05					ni				0.06
Total	0.21	0.22	0.23	0.09	0.15	0.20	0.34	0.09	0.04	0.07	0.50	0.20

*Retention time relative to albuterol at 6.4 min.
†The peak was not integrated.
‡Trace is <0.005%.
§RRT of **3** is 1.8.

Table 6
Percent albuterol in raw material by LC and non-aqueous titration

Sample	Non-aqueous titration	LC*
H	99.8†	101.3, 100.5
J	100.0,† 99.5, 99.5, 99.2	100.8, 99.7
Q	98.7,† 98.4,† 99.2, 99.2, 99.0	99.8, 99.9
T	98,7,† 98.9†	100.5, 100.6
F	99.9, 99.4, 99.5, 99.4, 98.8	
L	102.3, 99.2, 99.6	

*Lot F was used as the LC standard for albuterol base assay and lot L was used for albuterol sulphate.
†Sample not dried before assay.

Ruggedness

A solution of albuterol, 1.0 mg ml^{-1}, in acetonitrile–water (60:40, v/v) and kept at room temperature, was injected at intervals over a period of 16 h. No evidence of albuterol decomposition was observed in the chromatograms.

Most of the development work upon which this method was based was done on a 15 cm, 3-μm Spherisorb hexyl column (Phase Separations Ltd) and the results were checked on a similar column from Chromatography Science Co. (No. 048821). A 15 cm, 3-μm Spherisorb octyl column from Chromatography Sciences Co. gave long retention times and tailing for some impurities. Decreasing the amount of phosphoric acid in the mobile phase from 0.1 to 0.05% increased the retention time and peak tailing. Increasing the amount to 0.2% gave shorter retention times. Increasing or decreasing the amount of acetonitrile in the mobile phase had little effect on the retention time of the known impurities.

Table 7
Percent related compounds in albuterol tablets

RRT* of related compounds	Albuterol sulphate tablet samples XX	BB	CC	DD†	EE	FF	GG	HH†
0.48	—	—	—	—	—	—	0.01	—
0.52	—	—	—	—	—	—	0.02	—
0.53	—	—	—	—	—	—	0.01	—
0.60	—	—	—	—	—	—	0.01	—
0.73	—	—	—	—	0.02	0.02	0.01	0.01
0.92	—	—	—	0.01	—	—	—	—
0.96	0.02	0.03	0.02	0.03	0.44	0.45	0.55	0.44
1.30	0.01	0.09	0.02	0.02	—	—	—	—
1.60	0.19	0.13	0.13	0.15	—	—	—	0.01
1.68	0.13	0.11	0.15	0.17	0.31	0.31	0.04	0.06
2.35‡	—	—	—	—	0.18	0.16	—	—
Total	0.35	0.36	0.32	0.38	0.95	0.94	0.65	0.52

*Relative to albuterol at retention time of 6.4 min.
†Results are the average of duplicate determinations.
‡RRT relative to related compound **6** (RRT = 2.4).

Table 8
Percentage albuterol in 2 and 4 mg tablets by LC

XX (2 mg)	DD (4 mg)	GG (2 mg)	HH (4 mg)
104.0	99.4	100.2	95.5
101.0	101.2	98.9	95.6

Table 9
Percent impurities in albuterol inhalers

RRT of impurties	Albuterol inhaler samples JJ Valve*	JJ Frozen	KK Valve	KK Frozen	MM Valve	MM Frozen
0.48	—	1.0	0.4	—	1.7	1.4
0.54	1.4	2.1	2.3	2.2	0.3	0.4
0.69†	1.7	0.2	1.5	0.2	1.9	0.1
1.70‡	6.5	2.3	2.4	2.4	—	—
2.80§	—	3.4	3.4	3.7	0.4	0.4
3.10	2.4	0.1	0.1	—	0.1	—
7.30	—	—	—	—	0.2	1.0
Total	12.0‖	9.1	10.1	8.5	4.6	3.3

*Samples were obtained through the metered dose valve or by opening a frozen can.
†This peak is related to valve delivery.
‡This peak has been identified as butylated hydroxy toluene [2,6-bis-(1,1-dimethylethyl)-4-methyl-phenol].
§This peak tentatively has been identified as 2,2′-methylene bis(6-t-butyl-4-methylphenol).
‖Some samples from this lot have a significant amount of impurity at RRT = 3.1, and none at 2.8 (two cans), while others exhibit a major peak at RRT = 2.8, and none at 3.1 (two cans).

Table 10
UV and GC/MS characteristics of inhaler impurities

RRT of impurity	UV maxima (nm)	MS ions (*m/z*)
0.54	225, 235	
0.69	211, 270	
1.00	225, 275	
1.70*	211, 275	220(28%), 205(100%), 177(10%), 145(12%), 105(8%), 57(38%), 41(13%)
2.80	211, 268	340(38%), 284(11%), 177(100%), 164(50%), 161(71%), 149(48%), 121(22%), 57(28%)
3.10	229, 278	
BHT (1.70)	211, 277	Identical to impurity at RRT 1.70

*The absorptivity of BHT relative to albuterol at 229 nm is 0.5.

Identification of inhaler impurities

Liquid chromatographic analysis of the inhaler test solution from method (C) showed that the impurities at RRTs of 1.7, 2.8 and 3.1 had been isolated from the drug. GC/MS analysis of the methanol solution (Table 10) led to identification of the impurity at RRT 1.7 as 2,6-di-t-butyl-4-methylphenol (BHT), a common anti-oxidant, and that at RRT 2.8 as 2,2′-methylene bis(6-t-butyl-4-methylphenol), of which the corresponding 4-ethyl compound is a known rubber additive. An injected BHT standard co-eluted with the peak at RRT 1.7, and gave a mass spectral fragmentation pattern identical to that of the peak in the inhaler at RRT 1.7. The mass spectral pattern of the compound eluting at RRT 2.8 conformed to that which would be expected from 2,2′-methylene bis(6-t-butyl-4-methylphenol). The peak at RRT 3.10 exhibited a UV spectrum similar to that given by the compound eluting at RRT 2.80 and may be structurally similar to it; two possible structures are the plasticizers 2,2′-methylene bis(4-ethyl-6-t-butylphenol) and 4,4′-methylene bis(2,6-di-t-butylphenol). A portion of the dichloromethane solution was extracted into 0.1 M NaOH solution, which was injected into the liquid chromatograph. There did not appear to be a measurable difference in the amount of the three major impurities relative to the extraction of the acidic solution, suggesting that the compounds are neutral or sterically hindered acids or bases. The UV maxima of the impurities are given in Table 10.

Conclusions

The detection of impurities, both known and unknown, in albuterol and albuterol formulations from several manufacturers indicates that the method for related compounds described here is suitable for purity evaluation of albuterol raw materials, tablets and inhalers. The assay procedure can be used to determine total drug content in raw materials and in formulations. The impurities found in the inhaler products are indicative of the chemical complexity of this formulation.

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References

[1] *USAN and the USP Dictionary of Drug Names*, p. 26. United States Pharmacopeial Convention, Rockville, MD (1988).
[2] H.Y. Aboul-Enein, A.A. Al-Badir and S.E. Ibrahim, in *Analytical Profiles* (K. Flory, Ed.), Vol. 10, pp. 665–689. Academic Press, New York (1980).
[3] K. Kawakatsu and H. Kuwata, *Byoin Yakusaku* **9**, 40–44 (1983).
[4] M.J. Hutchings, *J. Chromatogr.* **277**, 423–426 (1983).
[5] O. Agostini, A. Chiari and D.C. Baffoni, *Boll. Chim. Farm.* **121**, 612–618 (1982).
[6] *The United States Pharmacopeia XXI and the National Formulary XVI*, p. 2717. United States Pharmacopeial Convention, Rockville, MD (1985).
[7] *British Pharmacopoeia*, Vol. I, pp. 496–497. Her Majesty's Stationery Office, London (1988).

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