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Optimization of the separation of salbutamol and its decomposition products by liquid chromatography with diode-array detection

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Abstract: A high-performance liquid chromatographic (HPLC) method based on diode-array detection is developed and optimized for stability studies on salbutamol. In establishing the method, the effects of mobile phase constituents (buffers, buffer concentration, pH, modifiers, organic solvents, ion-pair reagents) upon the resolution of salbutamol and its degradation products are studied. The optimum method involves the use of a LiChrosorb RP-18 column with a one-step gradient elution with acetonitrile–sodium dihydrogen phosphate (40 mM)–triethylamine (5.74 mM) (pH 3.0). The acetonitrile content is increased from 4 to 9% after 6 min.

Keywords: *Salbutamol (sulphate); LC separation; method optimization; diode-array detection.*

Introduction

Salbutamol is one of the most widely used drugs in the treatment of asthma. As a salicyl alcohol derivative it is relatively stable compared with adrenergic catecholamines. In earlier work the effects of pH, temperature and drug concentration on the decomposition of salbutamol in Britton–Robinson buffer solutions were studied [1]. The decomposition of salbutamol obeyed apparent first-order kinetics with respect to salbutamol sulphate. The rate of the decomposition was followed by reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection, which is one of the conventional analytical techniques for stability studies on salbutamol [2–5]. Other techniques include spectrofluorometry [6] and colorimetry [6–11]. The spectrofluorometric and colorimetric methods are not necessarily specific, because it is possible that unidentified decomposition products react with the reagents used.

The aim of the present study was to develop and optimize an HPLC method with diode-array detection for stability studies on salbutamol in buffer solutions, and to obtain further information on the decomposition products of salbutamol by comparing their retention behaviour with that of salbutamol.

Experimental

Materials

Salbutamol sulphate, the hemisulphate of 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino)ethanol, was kindly supplied by Leiras (Turku, Finland). The identity and purity of the substance were verified by TLC and HPLC and by UV, IR and ^{1}H-NMR and ^{13}C-NMR spectroscopy. All organic solvents and other chemicals were of chromatographic or analytical grade. Acetonitrile (AcN), methanol (MeOH) and tetrahydrofuran (THF) were from Rathburn (Walkerburn, UK). Phosphoric acid (H_3PO_4), sodium dihydrogen phosphate (NaH_2PO_4), ammonium acetate (CH_3COONH_4) and acetic acid (CH_3COOH) were supplied by Merck (Darmstadt, Germany). Triethylamine (TEA) was from Fluka (Buchs, Switzerland) and dimethyloctylamine (DMOA) was obtained from Aldrich-Chemie (Steinheim, Germany). Tetrabutylammonium phosphate (TBAP) and 1-heptane sulphonic acid sodium salt (Na-HSA) were supplied by Sigma (St Louis, MO, USA). Water was purified in an Alpha-Q Water Purification System (Millipore, Molsheim, France).

Sample

Salbutamol sulphate in Britton–Robinson

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buffer solutions (0.036 M) was heated in an oven at 75°C (pH 8.8) or 85°C (pH 2.2) long enough to ensure optimum decomposition of salbutamol (maximum number and amount of decomposition products). A 10-μl aliquot of the filtered solution (Spartan 30/B) was injected into the liquid chromatograph.

Instrumentation

The chromatographic equipment consisted of two Waters 501 HPLC pumps, coupled to a Waters automated gradient controller with a Rheodyne 7125 manual injector and a Waters photodiode array (PDA) system with a Waters 5200 printer/plotter. The PDA system consisted of a Waters 991 diode-array detector, an NEC PowerMate 386/25 computer and PDA software (all from Waters Assoc., Milford, MA, USA). The loop volume was 20 μl.

The UV absorption spectral data were obtained and the spectral analyses performed with the PDA technique in the range 210–350 nm.

Separations were performed on LiChrosorb RP-18 (250 × 4 mm i.d., 10 μm; 125 × 4 mm i.d., 5 μm) columns. The LiChrosorb RP-18 (250 × 4 mm i.d., 10 μm) column was equipped with a LiChrosorb RP-18 (10 μm) precolumn.

Chromatographic conditions

The mobile phases (see Table 1) were filtered (Fluoropore for organic solvents and Durapore for aqueous solutions) and degassed with helium before use. The flow rate was 1.5 ml min^{-1} and the column operated at room temperature. The column void volume was estimated by an injection of sodium nitrate when this was possible.

Results and Discussion

Salbutamol decomposes in aqueous solutions at elevated temperatures [1]. Besides polymer formation, several decomposition products in small amounts were detected by HPLC. The decomposition pattern depended on the pH of the solution: compounds **II**, **III**, **V** and **VI** were the major constituents in basic solutions (pH 8.8), whilst two additional products (**I** and **IV**) were formed in acidic solutions (pH 2.2). As yet it has not been possible to isolate the pure decomposition products in sufficient quantity for NMR characterization. However, liquid chromatography and photodiode-array (PDA) detection enabled the compounds to be characterized on the basis of their UV spectra. The UV spectra of salbutamol and its decomposition products are shown in Fig. 1. The absorption patterns of **II** and **IV** differed substantially from those of the other compounds, indicating major changes in the chromophoric system of these products relative to salbutamol. In all cases the spectra allowed an unequivocal differentiation of the compounds, so that their elution order

Table 1
Mobile phases used in the development of a LC method for stability studies on salbutamol

Number	Components	Organic modifier percentage (v/v)
1	AcN-H_2O	2–20
2	MeOH-H_2O	20–27
3	AcN-H_3PO_4 (0.05 M, pH 3.5)	5–20
4	AcN-NaH_2PO_4 (a, 0.02 or b, 0.04 M, pH 3.0 with H_3PO_4)	3–20
5	AcN-NaH_2PO_4 (0.04 M, pH 6.0 with NaOH)	12
6	AcN-CH_3COONH_4 (0.1 M, pH 4.4 with CH_3COOH)	5–20
7	AcN-NaH_2PO_4 (0.04 M) + TEA (5.74–50.2 mM) (pH 3.0 with H_3PO_4)	4–12
8	AcN-NaH_2PO_4 (0.04 M) + DMOA (2.43–14.6 mM) (pH 3.0)	4–8
9	AcN-NaH_2PO_4 (0.04 M) + TEA (43 mM) + CH_3COOH (0.4%) (pH 3.0)	8
10	THF-NaH_2PO_4 (0.04 M) + TEA (43 mM) (pH 3.0)	4–6
11	MeOH-NaH_2PO_4 (0.04 M) + TEA (43 mM) (pH 3.0)	12
12	THF-MeOH-NaH_2PO_4 (0.04 M) + TEA (43 mM) (pH 3.0)	2–4.5 4.5–7
13	THF-AcN-NaH_2PO_4 (0.04 M) + TEA (43 mM) (pH 3.0)	0.5–2 6–7.5
14	MeOH-NaH_2PO_4 (0.04 M) + Na-HSA (0.005 M) (pH 3.0 with H_3PO_4)	30–50
15	MeOH-NaH_2PO_4 (0.04 M) + TBAP (a, 0.005 or b, 0.002 M) (pH 3.0 with H_3PO_4)	0–35
16	MeOH-H_3PO_4 (0.05 M) + CH_3COOH (0.05 M) + TBAP (0.005 M) (pH 5.0 with NaOH)	5–10
17	MeOH-H_3PO_4 (0.05 M) + CH_3 COOH (0.05 M) + TBAP (0.005 M) (pH 7.0 with NaOH)	10–20

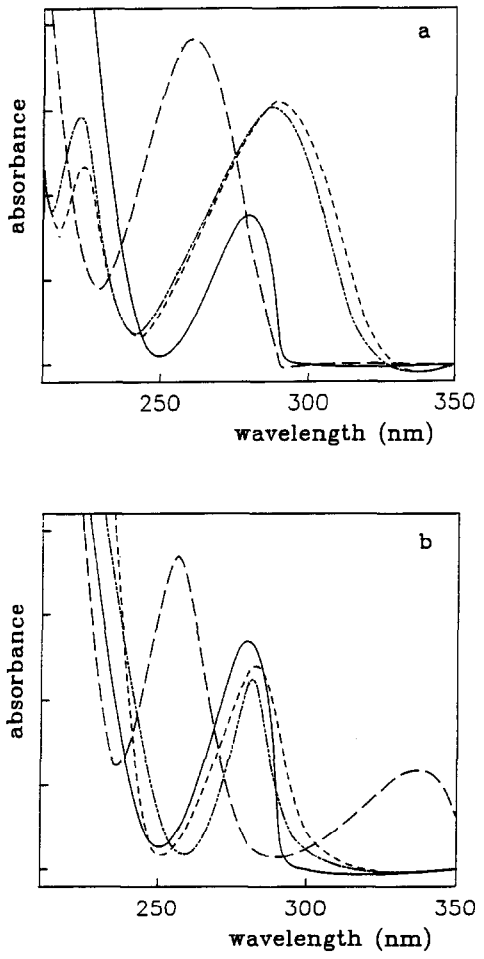

Figure 1
UV spectra obtained for salbutamol (——) and its decomposition products (a) **I** (– –), **II** (— —), **III** (–·–) and (b) **IV** (— —), **V** (– –), **VI** (–·–). Experimental conditions as described in the text.

could be followed during the method development. The homogeneity of the peaks was checked with the PDA software, mainly by using the overlaid normalized spectra taken from different regions of a peak and the absorbance ratio at two pre-selected wavelengths.

The HPLC method used in an earlier study was accurate and precise for the determination of salbutamol in the degradation solutions, but it was not optimum for the separation of the decomposition products [1]. Traces of strongly retained compounds frequently escaped detection because they eluted as broad bands and were lost in the noise or drift of the baseline. The method required some improvements to resolve all the decomposition products from one another and from salbutamol within a reasonable time. In establishing the method, mobile phase constituents (buffers, buffer concentration, pH, modifiers, organic solvents, ion-pair reagents) were optimized with respect to capacity factor ($1 < k' < 20$), resolution ($R_s > 1.0$) and asymmetry factor ($0.9 < A_s < 1.2$).

The chromatographic experiments with mobile phases 1–13 (Table 1) were performed on a LiChrosorb RP-18 (250 × 4 mm i.d., 10 μm) column. The composition of the aqueous phase (mobile phases 1, 3, 4 and 6) particularly affected the retention of salbutamol and compounds **V** and **VI** (Fig. 2). When an unbuffered aqueous phase was used, with acetonitrile providing the organic component, salbutamol was strongly retained on the column and the separation of the decomposition products was inadequate. With phosphoric acid [Fig. 2(C)] the capacity factors of compounds **V** and **VI** were $k' > 17$ even with 20% acetonitrile and the peak shape of salbutamol was very broad ($A_s > 4$). A change from phosphoric acid to sodium dihydrogen phosphate in the aqueous phase [Fig. 2(B)] decreased sharply the retention of salbutamol and compounds **V** and **VI**. With ammonium acetate [Fig. 2(A)] the resolution was good except for compound **II**, which separated from salbutamol only when the acetonitrile content was less than 10%. In this case, however, **V** and **VI** were strongly retained ($k' > 17$). An increase in the buffer concentration from 0.02 to 0.04 M sodium dihydrogen phosphate resulted in better resolution and higher

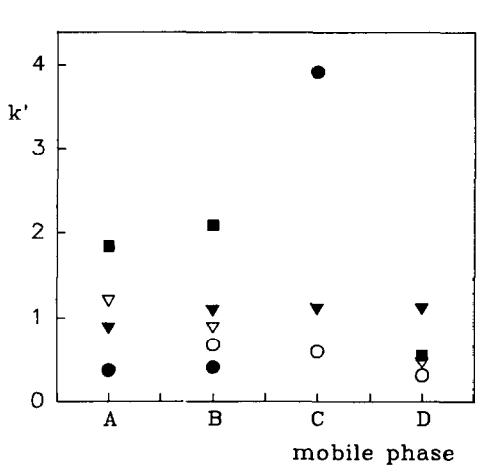

Figure 2
Effect of aqueous phase composition on the capacity factors of salbutamol (●) and its decomposition products **II** (○), **III** (▼), **V** (▽) and **VI** (■). Mobile phase: 20% acetonitrile with (A) CH_3COONH_4 (0.1 M, pH 4.4), (B) NaH_2PO_4 (0.02 M, pH 3.0), (C) H_3PO_4 (0.05 M, pH 3.5) and (D) H_2O.

sensitivity. The change of pH from 3.0 to 6.0 did not affect the retention of salbutamol and compound **III**, indicating that there was no change in their ionic state. On the other hand, a marked increase in the retentions of **V** and **VI** was observed. Perhaps these compounds are weaker bases than salbutamol and exist partly in non-protonated form at pH 6.0. The opposite behaviour was noticed for compound **II** suggesting a strongly acidic character. Sodium dihydrogen phosphate (0.04 M) at pH 3.0 gave the most promising results and was used as the aqueous phase in further studies.

The effect of modifiers (amines and acetic acid) was studied with acetonitrile–sodium dihydrogen phosphate (0.04 M) as the mobile phase (eluents 7–9 in Table 1). Amine modifiers usually improve the peak shape for basic compounds. It is also possible to adjust the retention of the compound of interest by adjusting the concentration of amine, rather than adding an organic solvent to the mobile phase [12]. Both amines studied, triethylamine (TEA) and dimethyloctylamine (DMOA), improved peak symmetry. An addition of amine, as well as an increase in the amine concentration, strongly reduced the capacity factors of compounds **V** and **VI** (Fig. 3), perhaps because amines facilitate the elution of basic compounds by blocking the acidic silanol groups of the stationary phase. The retentions of **II** and **III** remained essentially unchanged, suggesting the loss of their basic character. This was supported by their ready extraction into ethyl acetate from acidic solutions. Acetic acid had no effect on the retention of the compounds or the peak shapes.

Table 2 shows the capacity factors for salbutamol and some of its decomposition products when different organic solvents of equal solvent strength were used in the mobile phase (eluents 7, 10 and 11). The solvent strength was calculated using the nomograph of Snyder *et al.* [13], which is an approximation. The less hydrophilic solvent, tetrahydrofuran, caused a marked decrease in the retentions of compounds **V** and **VI**, which usually were strongly retained on the stationary phase in the presence of acetonitrile or methanol. Their differing chromatographic behaviour from salbutamol might be due to their larger molecular size. Ternary solvent systems (mobile phases 12 and 13) gave no satisfactory resolution of the compounds investigated.

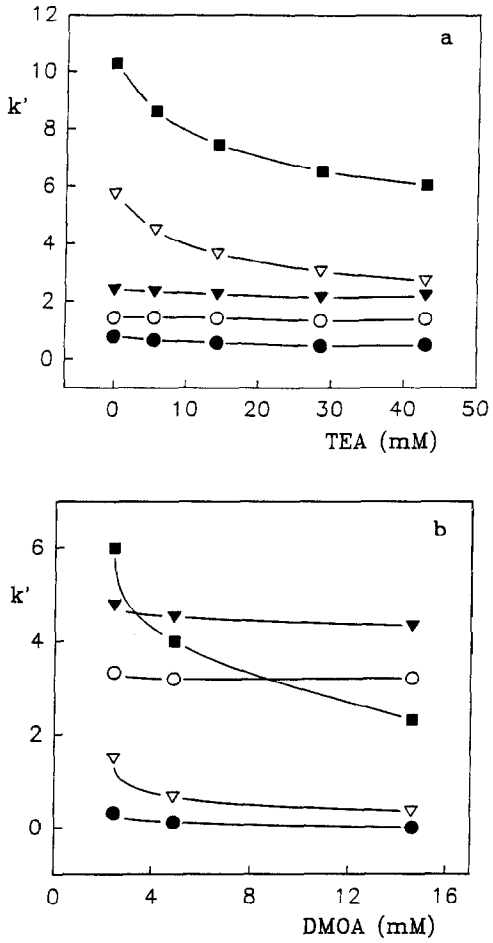

Figure 3
Effect of amine modifiers on the capacity factors of salbutamol and its decomposition products. Mobile phase: (a) AcN – NaH_2PO_4 + TEA (0–43.0 mM) (pH 3.0) 12:88, (b) AcN – NaH_2PO_4 + DMOA (2.43–14.6 mM) (pH 3.0) 8:92. Symbols as in Fig. 2.

Table 2
Effect of organic modifiers on the capacity factors of salbutamol and its decomposition products

Compound	Capacity factor		
	8% AcN*	12% MeOH*	6% THF*
Salbutamol	0.90	1.52	0.18
II	2.68	5.11	3.60
III	4.05	6.67	3.31
V	13.06	>25.16	0.96
VI	20.33	>25.16	5.65

Mobile phase: organic solvent–0.04 M sodium dihydrogen phosphate–43 mM triethylamine (pH 3.0).
*In order to achieve the same solvent strength in all phases [13].

Salbutamol and its decomposition products were well resolved with acetonitrile–sodium dihydrogen phosphate and 5.74 mM TEA

(4:96) as the mobile phase, but the k'-values of compounds **V** and **VI** were >35. Therefore a gradient elution regime was introduced in which the acetonitrile gradient was increased after 6 min from 4 to 9% in one step. The column was LiChrosorb RP-18 (125 × 4 mm i.d., 5 μm). A complete separation of all the compounds was achieved in about 30 min and the resolutions (R_s) were greater than 1.15 (Fig. 4).

Since salbutamol and presumably also its decomposition products contain basic or acidic groups or both, effect of both anionic (heptanesulphonic acid) and cationic (tetrabutylammonium phosphate) ion-pair reagents (mobile phases 14–17) was studied. Methanol was used as organic solvent instead of acetonitrile to avoid the precipitation that would occur with a mixture of a large percentage of acetonitrile and phosphate buffer. Heptanesulphonic acid gave too wide a k'-range for the compounds; the retention of compounds **V** and **VI** sharply increased, suggesting possible ion-pair formation. With tetrabutylammonium

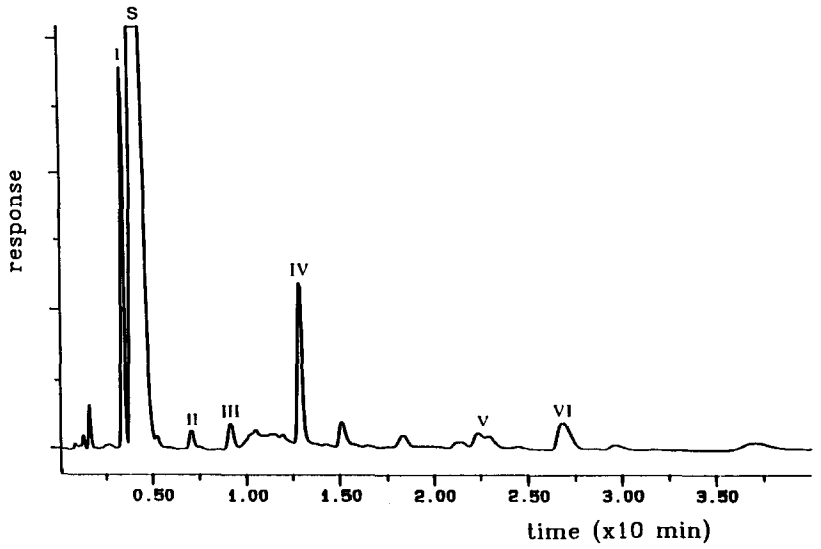

Figure 4
Chromatogram of partly decomposed salbutamol sulphate solution obtained with gradient elution and UV detection at 265 nm. Mobile phase: 4–9% AcN – NaH_2PO_4 (0.04 M) + TEA (5.74 mM) (pH 3.0).

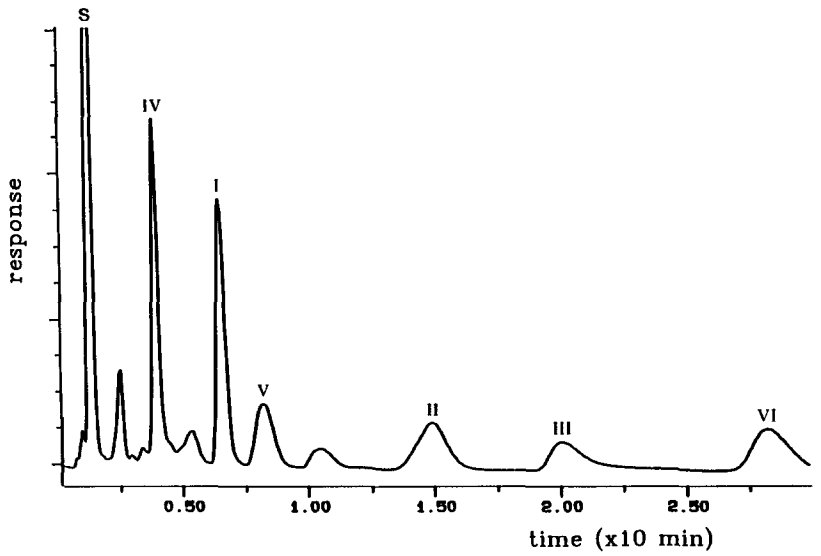

Figure 5
Chromatogram of partly decomposed salbutamol sulphate solution obtained with isocratic elution and UV detection at 265 nm. Mobile phase: NaH_2PO_4 (0.04 M) + TBAP (0.005 M) (pH 3.0).

phosphate (TBAP) the retention of salbutamol and compounds **IV–VI** decreased. In decreasing the retention of basic compounds, TBAP may be behaving as a silanol-blocking agent. At the same time with TBAP, the affinity of compounds **I–III** for the stationary phase was increased, suggesting an ion-pair formation and confirming the acidic character of these compounds. Salbutamol and the decomposition products **I–VI** were resolved from each other within 30 min when the mobile phase was 0.04 M sodium dihydrogen phosphate with 0.005 M TBAP without an organic solvent (Fig. 5). With this system, however, the capacity factor of salbutamol ($k' = 0.56$) was too low for accurate quantitative analysis. The retention times with the two HPLC methods are shown in Table 3.

Conclusions

Adjustments to the pH, buffer species and organic modifiers enabled the best resolution of salbutamol and its decomposition products on a LiChrosorb RP-18 column to be obtained with a mobile phase of acetonitrile, sodium dihydrogen phosphate and triethylamine (pH 3.0), along with one-step gradient (4–9%) elution.

Table 3
Retention times of salbutamol and its decomposition products obtained by gradient and isocratic elution

Compound	Retention time (min)	
	RP-HPLC	Ion-pair
I	3.37	6.49
Salbutamol	4.07	1.23
II	7.05	14.95
III	9.13	20.05
IV	12.85	3.92
V	22.66	8.23
VI	26.83	28.22

Mobile phase: RP-HPLC: 4–9% AcN–NaH_2PO_4 (0.04 M) + TEA (5.74 mM) pH 3.0. Ion-pair: NaH_2PO_4 (0.04 M) + TBAP (0.005 M) pH 3.0.

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