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Decomposition of salbutamol in aqueous solutions. II. The effect of buffer species, pH, buffer concentration and antioxidants

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Abstract

The decomposition of salbutamol sulphate proceeded faster in citrate buffer than in acetate and phosphate buffers, and an increase in the concentration of citrate accelerated the decomposition. Investigated in citrate buffer over the pH range 2–6 at 85°C, salbutamol was found to be maximally stable at pH about 3. Reversed-phase high-performance liquid chromatography with diode array detection was used to follow the decomposition, which obeyed apparent first-order kinetics under all conditions studied. In all buffers (pH 5), the main decomposition product observed in chromatograms was 4-hydroxy-3-hydroxymethylbenzoic acid, but the relative amount of this product was greater in citrate than in the other buffers studied. Of the antioxidants studied, sodium metabisulphite and sodium bisulphite showed a slight stabilizing effect. Virtually no decomposition was observed when the salbutamol solutions (pH 3) were autoclaved for 20 min at 120°C.

Keywords: Salbutamol sulfate; Decomposition kinetics; HPLC; Buffer species; pH; Antioxidant

1. Introduction

Salbutamol sulphate (albuterol sulphate), the hemisulphate of 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino)ethanol, is extensively used by patients with bronchial asthma for its β_2-receptor-agonist bronchodilating effects. Commercial pharmaceutical formulations include aerosols, injection fluids, mixtures and tablets.

Salbutamol decomposes in aqueous solutions at elevated temperatures (Wall and Sunderland, 1976; Hakes et al., 1979; Roberts, 1982; Bhalla et al., 1986; Mälkki and Tammilehto, 1990). The accelerating effect of sugars on the decomposition rate depends on the pH of the solution (Hakes et al., 1980; Sequiera and Zupon, 1985). Thiourea, an antioxidant, has been shown to stabilize salbutamol solutions (Valdés Santurio and Vega Equino, 1986; Valdés Santurio et al., 1989).

In an earlier study (Mälkki and Tammilehto, 1990), decomposition kinetics were studied in Britton-Robinson buffers, in which the reaction rate increased with drug concentration and temperature. Salbutamol was most stable at a pH of about 3.5. Because the pH of commercial fluids is about 3–4, a detailed study was undertaken to

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investigate the decomposition of salbutamol in various acidic buffer solutions stressed by heat. In addition, the effects of antioxidants and autoclaving were studied.

2. Materials and methods

2.1. Materials

Salbutamol sulphate was a generous gift from 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 spectra. All chemicals used were of analytical or chromatographic grade. Water was purified on an Alpha-Q Water Purification System (Millipore, Molsheim, France).

2.2. Chromatography

The chromatographic equipment consisted of two Waters 501 HPLC pumps, coupled to a Waters automated gradient controller and a Rheodyne 7125 manual injector. A Waters 991 photodiode array system (Waters Associates, Milford, MA, USA) provided the detector. The computer was a NEC PowerMate 386/25 (Waters Associates) connected with a Waters 5200 printer/plotter. The loop volume was 20 μl. A LiChrosorb RP-18 column (125 × 4 mm i.d., particle size 5 μm) was used as the analytical column. The mobile phase consisted of a mixture of acetonitrile, sodium dihydrogen phosphate (40 mM) and triethylamine (5.74 mM), the flow rate being 1.5 ml/min. The pH of the aqueous phase was adjusted to 3.0 with concentrated phosphoric acid. After 6 min the acetonitrile content was increased from 4 to 9%. The mobile phase was filtered and degassed with helium before use.

2.3. Kinetic studies

Kinetic studies were performed with salbutamol sulphate solutions (18 mM with respect to salbutamol) prepared in acetate (0.1 M, Walpole), citrate (0.025–0.5 M, Sörensen), phosphate (0.067 M, Sörensen) (Diem and Lentner, 1971) and Britton-Robinson buffers (0.04 M) (Brezina and Zuman, 1958). The ionic strength of the solutions was adjusted with sodium chloride (μ = 0.4 in all kinetic studies, except in the study of buffer concentration). Antioxidant concentrations were 0.1% for cysteine, ascorbic acid and sodium metabisulphite and 0.05–0.5% for sodium bisulphite. The ionic strength was not adjusted in antioxidant studies. Aliquots (3 ml) of the solutions were dispensed into 10-ml glass vials (quality of Ph.Eur.), which were tightly sealed with silicone and metal stoppers and covered with aluminium foil before being placed in a preheated oven at 85 ± 2°C (Memmert, Schwabach, Germany). At appropriate time intervals, at least duplicate samples were removed from the oven and the pH of each sample was measured at room temperature (PHM83 Autocal pH meter, Radiometer, Copenhagen, Denmark). Before injection onto the column, 0.8 ml of the sample solution was diluted with water to exactly 10 ml and filtered through a 0.45 μm disposable filter. All samples were injected in duplicate. For the quantitation of salbutamol, a calibration graph was constructed in the range 0.2–2.0 mM ($n = 5$) by plotting the peak area of salbutamol vs salbutamol concentration. At the lowest and highest concentrations, six replicate injections were performed; at other concentrations they were two. Several calibration graphs ($n = 14$) were constructed after appropriate time intervals and each day some standard solutions were co-chromatographed to check that calibration worked.

2.4. Autoclaving

Vials of salbutamol sulphate solutions (3 ml of 18 mM solution in 10-ml vials) in Britton-Robinson pH 3.5, 5.0 and 7.0 (Brezina and Zuman, 1958), Sörensen citrate pH 3.0, 5.0 and 6.0 and McIlvaine pH 3.5, 5.0 and 7.0 (Diem and Lentner, 1971) buffers and in a solution of sodium chloride and sulphuric acid (pH 3.5 and 5.0) made to resemble a commercial injection fluid were autoclaved for 20 min at 120°C. After cooling to room temperature, 0.8 ml of the solution was diluted with water to 10 ml before analysis by HPLC. After storage for about 4 months at ambi-

ent temperature the samples in citrate buffer were again checked by HPLC.

3. Results and discussion

The decomposition of salbutamol has earlier been investigated in Britton-Robinson buffers over the pH range 1.9–8.8 (Mälkki and Tammilehto, 1990). In addition to coloured polymer precipitate, several decomposition products were detected by TLC and HPLC. Upon longer heat stress their amount tended to decrease.

In this work, in addition to Britton-Robinson buffer citrate, acetate and phosphate buffers were studied to determine whether the buffer species had any effect on the decomposition pattern of salbutamol. The same decomposition products (compounds **I–VI**) as earlier (Mälkki and Tammilehto, 1990, 1993) were detected in all solutions, however, the stability of some products was considerably improved in citrate, acetate and phosphate buffers compared to Britton-Robinson buffer. Compounds **I**, **II** and **IV** were recently identified by liquid chromatography-mass spectrometry (LC/MS) as α-4-dihydroxy-3-hydroxy-

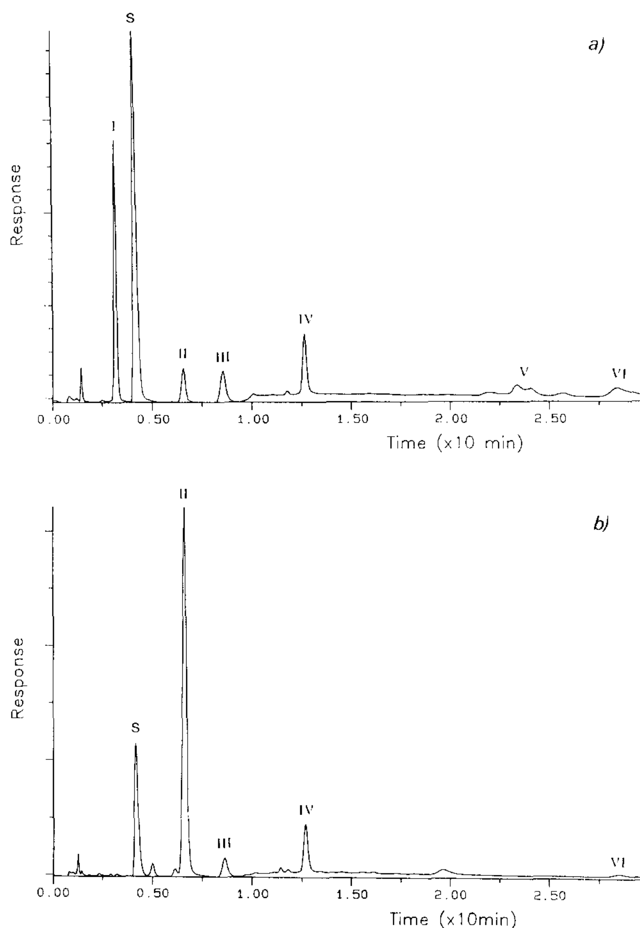


Fig. 1. Chromatograms of partly decomposed salbutamol sulphate solutions (85°C) in citrate buffer at (A) pH 2.1 (67% salbutamol left) and (B) pH 3.8 (53% salbutamol left). S, salbutamol; **I–VI**, decomposition products.

Table 1
Within-day and day-to-day reproducibility of retention times of salbutamol and four of its decomposition products ($n = 8$)

Compound	Retention time (min), RSD (%)			
	Day 1	Day 2	Day 3	Mean
I	3.48(1.04)	3.54(1.40)	3.51(0.20)	3.51(0.85)
Salbutamol	4.61(1.20)	4.75(1.96)	4.76(0.59)	4.71(1.78)
II	7.12(1.65)	7.43(1.73)	7.51(1.60)	7.35(2.80)
III	9.22(0.26)	9.72(1.95)	9.44(1.32)	9.46(2.65)
IV	12.70(0.88)	12.21(1.45)	12.20(1.16)	12.37(2.31)

methylbenzene acetic acid, 4-hydroxy-3-hydroxymethylbenzoic acid and 2-hydroxy-5-(2-*tert*-butylamino-1-hydroxyethyl)benzaldehyde, respectively (Mälkki-Laine and Bruins, 1994).

3.1. Analytical procedure

The decomposition of salbutamol was followed using a one-step gradient RP-HPLC method (Mälkki and Tammilehto, 1993), which allowed all decomposition products to be detected within a reasonable time (Fig. 1).

The mean retention times and their precision for salbutamol and four major decomposition products are listed in Table 1. For all compounds the within-day precision of the retention time was better than 2%. The method resolution was $R_s >$ 2.2, with poorest separation between salbutamol and compound **I**. The calibration graph of salbutamol sulphate showed excellent linearity. The mean slope was 1.61×10^{-2} (SD = 6.53×10^{-4}, $n = 14$) and the mean intercept was 3.97×10^{-4} (SD = 2.19×10^{-4}, $n = 14$). In all separate equations the correlation coefficients (r) were greater than 0.999. The repeatability of the chromatography expressed as relative standard deviations ($n = 6$) was 0.53% for 2.0 mM solution and 0.66% for 0.2 mM solution. The mean recovery of salbutamol obtained from the initial solution was 101.19% (RSD = 2.74%; $n = 25$). The relative standard deviations for parallel decomposed salbutamol sulphate solutions ($n = 6$) varied between 0.45 and 5.19%.

3.2. Effect of buffer species and of citrate pH and concentration

Semilogarithmic plots of the concentration of residual salbutamol vs time were linear and indicated that the decomposition of salbutamol followed apparent first-order kinetics under all conditions studied.

The apparent decomposition rate constants (k_{obs}) were extracted from the slopes of the first-order plots; shelf-lives were calculated from the equation $t_{10} = 0.105/k_{obs}$.

The effect of different buffers was investigated at constant pH (pH 5.2) and ionic strength (μ = 0.4). The decomposition proceeded faster in citrate than in acetate and Britton-Robinson buffers, where the k_{obs} values were 3.9×10^{-7}, 2.1×10^{-7} and 2.3×10^{-7} s^{-1}, respectively. Decomposition was slowest in phosphate buffer, but the results were not comparable with those for other buffers because of a strong decrease in pH of the solution during the decomposition, perhaps due to the low buffering capacity of phosphate at pH 5.2.

The pH profile studies were carried out with citrate buffer (0.1 M, $\mu = 0.4$). The log k_{obs}-pH profile (Fig. 2) has approximately the same shape as with Britton-Robinson buffers (Mälkki and Tammilehto, 1990), with salbutamol proving to be

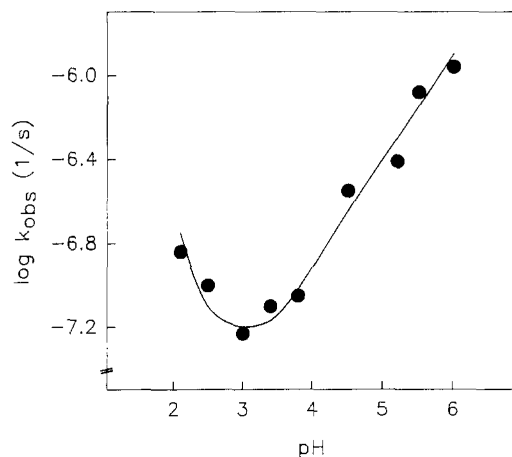


Fig. 2. pH-rate profile for the decomposition of salbutamol sulphate (18 mM) in citrate buffer ($\mu = 0.4$) at 85°C.

Table 2
Decomposition rates (k_{obs}) and shelf-lives (t_{10}) of salbutamol sulphate in citrate buffer of various pH values (85°C, μ = 0.4)

pH	k_{obs} (s^{-1})	t_{10} (days)	r (n = 6)
2.1	1.43×10^{-7}	8.5	0.9997
2.5	9.89×10^{-8}	12.3	0.9985
3.0	5.89×10^{-8}	20.6	0.9720
3.4	7.97×10^{-8}	15.2	0.9657
3.8	8.94×10^{-8}	13.6	0.9791
4.5	2.81×10^{-7}	4.3	0.9965
5.2	3.92×10^{-7}	3.1	0.9971
5.2 [a]	2.26×10^{-7}	5.4	0.9844
5.5	8.39×10^{-7}	1.4	0.9984
6.0	1.09×10^{-6}	1.1	0.9898

[a] Britton-Robinson buffer pH 5.2

most stable at a pH of about 3 (Table 2). Within the pH range 2.1–6.0, at 85 ± 2°C, the shelf-lives varied from 1.1 to 20.6 days.

The effect of buffer concentration (0.025–0.5 M) was studied in citrate buffer pH 5.5 at constant ionic strength. An increase in the buffer concentration accelerated the decomposition of salbutamol, but at very high buffer concentrations the relation between k_{obs} and the concentration deviated from linearity and began to level off (Table 3). The leveling effect in high buffer concentrations has also been noted in studies of doxorubicin (Beijnen et al., 1986).

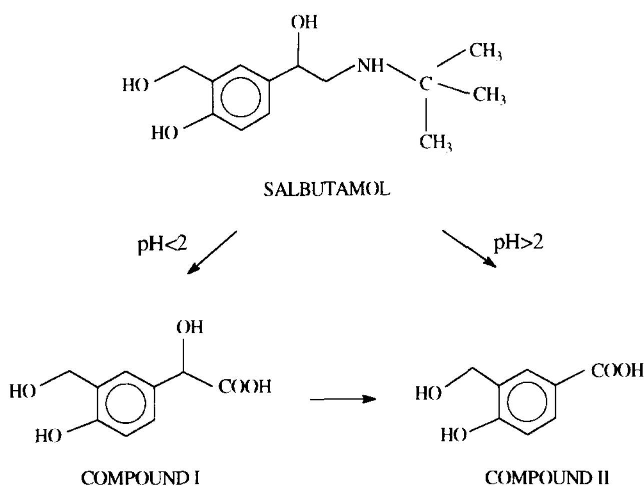
Table 3
Decomposition rates (k_{obs}) and shelf-lives (t_{10}) for the decomposition of salbutamol sulphate in various concentrations of citrate buffer of pH 5.5 (85°C, μ = 1.43)

Buffer (M)	k_{obs} (s^{-1})	t_{10} (days)	r (n = 6)
0.025	7.72×10^{-7}	1.6	0.9963
0.05	8.72×10^{-7}	1.4	0.9980
0.1	1.12×10^{-6}	1.1	0.9997
0.25	1.33×10^{-6}	0.92	0.9996
0.5	1.37×10^{-6}	0.89	0.9988

The pH of the solutions also influenced product formation. Compound **I** was the primary product in strongly acidic solution (Fig. 1a), however, as the pH of the sample solution increased, the formation of **II** became dominating (Fig. 1b). Probably compound **I** is an intermediate in the oxidation of salbutamol and at higher pH values the reaction proceeds directly to compound **II** (Scheme 1). However, at pH 6.0 the formation of **II** decreased as the decomposition proceeded further. At the same time the solutions discoloured red instead of yellow. Fig. 3 shows the formation of the decomposition products at pH 2.5.

3.3. Effect of antioxidants

Oxidative pathways must be involved in the decomposition of salbutamol, since the decompo-



Scheme 1. Proposed oxidation of salbutamol to α-4-dihydroxy-3-hydroxymethylbenzeneacetic acid (compound **I**) and 4-hydroxy-3-hydroxymethylbenzoic acid (compound **II**).

sition products identified were oxidation products. Accordingly, to study their ability to stabilize the solutions, several antioxidants acting with different mechanisms were added to solutions of salbutamol (citrate buffer pH 5.2).

None of the antioxidants prevented the decomposition of salbutamol. At the beginning of the process, there was a rapid decrease in salbutamol content in solutions (Fig. 4), but after several days, sodium metabisulphite, sodium bisulphite and cysteine exhibited a slight stabilizing effect on the drug. Similar behaviour has been observed in adrenalin solutions to which cysteine and sodium metabisulphite were added as antioxidants (Thoma and Struve, 1986). Ascorbic acid had no effect because it itself degraded in aqueous solutions at elevated temperatures. In an attempt to enhance the effectiveness of the antioxidants, salbutamol solutions were purged with carbon dioxide, however, this had no favourable influence on the stability of the drug. In autooxidation reactions even trace amounts of oxygen may be sufficient to trigger the reaction.

To study the effect of concentration on the stabilizing effect of antioxidants, sodium bisulphite was added in three different concentrations, 0.05, 0.1 and 0.5%. The best stabilizing effect was obtained with 0.1% sodium bisulphite. The concentration of 0.05% was too low to stabilize the solution and that of 0.5% accelerated the decomposition of salbutamol.

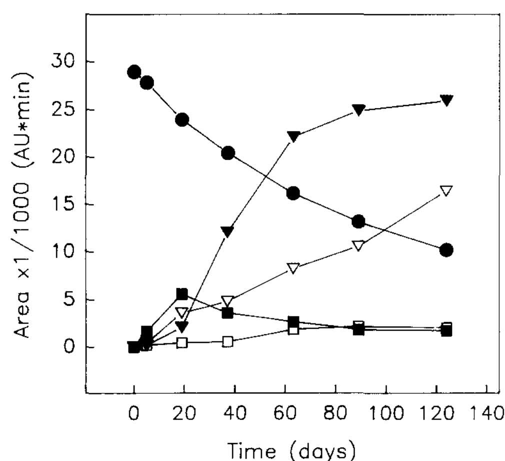


Fig. 3. Time courses for salbutamol sulphate (18 mM) and its decomposition products in citrate buffer pH 2.5 (85°C). (●) salbutamol, (■) **I**, (▼) **II**, (□) **III**, (▽) **IV**.

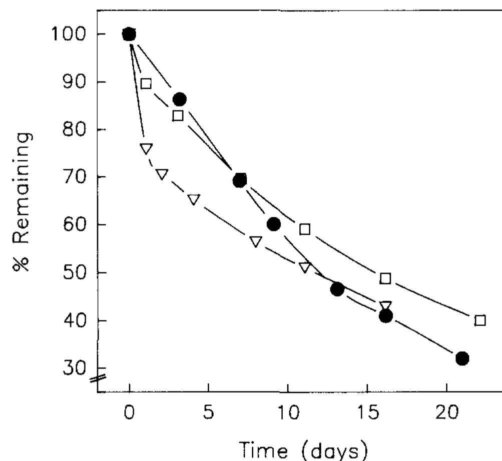


Fig. 4. Stability of salbutamol sulphate (18 mM) in citrate buffer pH 5.2 at 85°C with added sodium bisulphite and cysteine (0.1%). (●) Salbutamol without antioxidant; (□) salbutamol with sodium bisulphite; (▽) salbutamol with cysteine.

The high sodium bisulphite concentration also seemed to change the degradation pattern, since no precipitate was observed in the solution and the yellow colour appeared only after 1 week. Three unknown decomposition products were formed, while the amount of compound **II**, the main degradation product in all other solutions, was small. One of the unknown compounds might be the derivative of the sulphonic acid of the parent drug as has been shown in the case of adrenalin (Schroeter et al., 1958). The same substance was also detected in solutions containing sodium metabisulphite.

3.4. Autoclaving

To obtain information about the effect of autoclaving on the stability of salbutamol solutions, salbutamol in three common buffers – McIlvaine, Britton-Robinson and Sörensen citrate – and in a solution imitating commercial injection fluid was studied. The decomposition of salbutamol was accelerated with increasing pH of the solutions. At pH 3–5 virtually no decomposition was observed in the buffer solutions. In the solution

containing sulphuric acid and sodium chloride the loss of salbutamol was about 3% at pH 5. After autoclaving, storage at room temperature for 4 months had no effect on the stability of salbutamol.

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