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Structural characterization of the decomposition products of salbutamol by liquid chromatography–ionspray mass spectrometry

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Abstract: Liquid chromatography–ionspray mass spectrometry was used to elucidate the structures of the decomposition products of salbutamol. The best sensitivity in mass spectrometry was achieved by using a mixture of acetonitrile and ammonium formate (10 mM, pH 3.3) as the mobile phase in liquid chromatography. Fragmentation of the compounds was obtained by increasing the nozzle voltage in the first vacuum stage of the mass spectrometer. Tentative structure elucidation showed that both acidic and basic decomposition products are formed from salbutamol.

Keywords: *Salbutamol (sulphate); liquid chromatography–mass spectrometry; structural characterization; decomposition products.*

Introduction

Salbutamol is a synthetic adrenergic amine, which has long been available to the medical profession. In aqueous solutions at elevated temperatures it decomposes [1, 2], forming polymers and several other products. There is no detailed information in the literature regarding the identity of these. In a previous study, in which an HPLC method with diode array detection was developed for stability studies on salbutamol, the retention behaviour of the compounds under different conditions indicated that some of the decomposition products had lost their basic character [3].

Because the isolation of the decomposition products of salbutamol before identification was laborious due to the small amount and polar character of the compounds, an on-line technique was sought for the structure elucidation. Gas chromatography–mass spectrometry (GC–MS) has been widely used for the characterization of unknown compounds, but derivatization is required for polar compounds. A further disadvantage of GC–MS is that thermally unstable substances tend to decompose in the column. By contrast, on-line liquid chromatography–mass spectrometry (LC–MS) techniques offer a powerful tool for the analysis of nonvolatile and polar compounds.

In this study we characterize the decomposition products of salbutamol by liquid chromatography–atmospheric pressure ionization mass spectrometry.

Experimental

Materials

Salbutamol sulphate was kindly supplied by Leiras (Turku, Finland). All organic solvents and other chemicals were of gradient or analytical grade. Acetonitrile (LiChrosolv®), ammonium acetate, acetic acid, formic acid and other buffer components were purchased from Merck (Darmstadt, Germany). Ammonium formate was from Fluka (Buchs, Switzerland). Water was purified in a Milli-Q Water Purification System (Millipore, Milford, MA, USA).

Samples

Salbutamol sulphate (0.018 or 0.036 M with respect to salbutamol) was decomposed in several different media: aqueous acetic acid (pH 2.2), sodium citrate buffer solutions (0.1 M, pH 2.1 and 4.5) [4] and a Britton–

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Robinson buffer solution (pH 2.2) [5]. Solutions were kept in an oven at 65 or 85°C for variable periods of time. A 20 μl sample of the filtered solution (Schleicher & Schuell RC 55, 0.45 μm) was injected into the liquid chromatograph. For a more concentrated sample the acetic acid sample solution was freeze-dried and the residue was dissolved in a small amount of water before injection into the LC.

LC instrumentation and conditions

The chromatographic equipment consisted of two Spectroflow 400 HPLC solvent delivery pumps (ABI Analytical Kratos Division, Ramsey, New Jersey), coupled to an ABI Analytical Kratos Division Spectroflow 450 solvent programmer with a Rheodyne 7125 manual injector (20 μl loop) and a Spectroflow 757 UV absorbance detector (ABI Analytical Kratos Division). The analytical wavelength was 265 nm. The analytical column was a LiChrospher 60 RP Select B (Merck, 125 × 4 mm i.d., 5 μm particles). A LiChrosorb RP-18 column (Merck, 125 × 4 mm i.d., 5 μm particles) was used in preliminary studies.

The final mobile phase consisted of a mixture of acetonitrile and 10 mM ammonium formate (pH 3.3 with formic acid). A linear gradient from 5 to 15% (or 4 to 15%) acetonitrile in 30 min was used. The flow rate was 1.5 ml min^{-1} with a post-column split ratio of 1:300 (pressure 10 psi) providing a flow of 5 μl min^{-1} to the mass spectrometer. The mobile phase was filtered (Schleicher & Schuell RC 55, 0.45 μm) before use and degassed with helium during the run.

MS instrumentation and conditions

The mass spectrometer was a Nermag R 30-10 (Delsi-Nermag, Argenteuil, France) equipped with a custom-built atmospheric pressure ionization (API) source. During the LC–MS operation the quadrupole was typically scanned from *m/z* 50 to *m/z* 600 at 2 s $scan^{-1}$. The multiplier was 600 V in positive mode and 700 V in negative mode. Nozzle voltage was increased from 70 to 180 V to obtain the fragmentation. Nitrogen (99.9%) was used as a curtain gas (75–100 l h^{-1}).

The fused silica capillary was connected to an ionspray interface. A voltage of 3 kV was applied to a stainless steel tube. Nitrogen (99.9%) was used as a nebulizing gas and the pressure was 3 bar.

Tuning was done by a continuous mobile phase flow via the silica capillary to the mass spectrometer.

Results and Discussion

Liquid chromatography–mass spectrometry

The use of an atmospheric pressure ion source was described as early as in 1974 by Horning's group [6]. Both ionspray and electrospray exploit the principle of ion emission from charged droplets at atmospheric pressure, with the difference that, in the ionspray method, the generation of charged droplets is assisted by pneumatic nebulization [7]. The ionspray used in this study operates without heat in the spray-ionization step ensuring that labile and polar samples do not undergo thermal degradation. The interface and ion source of the ionspray LC–MS is shown in Fig. 1 [8].

The effluent from the liquid chromatograph is fed through a fused silica capillary, which is connected to a stainless steel capillary floating at several kilovolts, and dispersed into charged droplets in dry nitrogen at atmospheric pressure. The best sensitivity is obtained with flow rates of about 5 μl min^{-1}, which means that microbore columns or a split of the effluent from the liquid chromatograph are needed [8].

After the formation of an electrically charged aerosol and evaporation of solvents, the sample ions are transported into the mass spectrometer. Ions are drawn into the first stage through a nozzle orifice in a stainless steel disc and then into the second stage through a skimmer orifice. The nozzle orifice is protected from solvent molecules by a curtain of dry nitrogen gas [8].

At low nozzle voltage, e.g. 70 V, only $[M + H]^+$ in the positive mode and $[M - H]^-$ in the negative mode are observed. When the nozzle voltage is increased, e.g. from 70 to 120 V, fragment ions are obtained by collision induced dissociation in the region between the nozzle

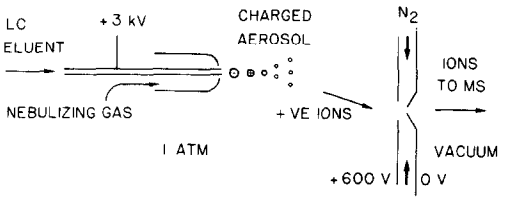


Figure 1
Ionspray LC–MS interface in an atmospheric pressure ion source [8].

and the skimmer. The degree of fragmentation greatly increases when the nozzle voltage is raised to still higher values. At the same time the signal-to-noise ratio improves since background ions are fragmented into small ions outside the mass range of interest, and the background ion spectrum becomes less abundant. At higher values of the nozzle voltage, the $[M + H]^+$ and $[M - H]^-$ peaks almost disappear.

Salbutamol and its decomposition products require an acidic mobile phase for good separation by liquid chromatography [3]. For mass spectrometry a volatile mobile phase is necessary because solvents and buffer components have to evaporate before the sample ions are introduced into the vacuum system of the mass spectrometer. Commonly used volatile buffers are ammonium acetate and ammonium formate and, of these, formate gave the better sensitivity for the samples of salbutamol.

In general, the addition of a buffer salt at a concentration of 10 mM or more will strongly reduce the sensitivity in MS for ionic compounds [7]. When ammonium formate was used at a concentration of 1 mM, HPLC separation of the decomposed salbutamol solution was inadequate, but when the concentration was increased to 10 mM, separation was good and, the peak shapes of the basic compounds in the chromatogram were better.

Since salbutamol and some of its decomposition products are basic, a column packing material specially designed for basic analytes was tested in place of the normal reversed-phase packing. A LiChrospher 60 RP Select B column improved the peak shapes of salbutamol and decomposition product IV compared with a LiChrosorb RP-18 column.

The UV chromatogram obtained with the LiChrospher column and the total ion chromatograms (TIC) obtained from MS are shown in Fig. 2.

Decomposition products

Britton–Robinson and sodium citrate buffer solutions were chosen because of their use in previous kinetic studies of salbutamol [1]. Decomposition products II–VI (Fig. 2) were produced in all the studied solutions, while compound I was formed only in solutions of very low pH (pH $\approx$2). The aqueous acetic acid solution was used because it contained adequate amounts of all the decomposition products formed in other solutions. Furthermore, compound VII existed only in this solution.

Compounds I–III were only observed with the negative mode of the MS, indicating loss of the basic character. Salbutamol and compounds IV–VII were observed with both positive and negative modes, but the sensitivity was better in the positive mode. The fragment ions for salbutamol and its decomposition products are listed in Table 1 and the proposed structures are presented in Scheme 1.

The main decomposition product in citrate buffer pH 4.5 was compound II. With a nozzle voltage of 70 V only a $[M - H]^-$ ion at *m/z* 167 was observed, corresponding to the molecular weight of 168. At increased nozzle voltage the $[M - H]^-$ peak decreased and fragment ions appeared. The most important fragment ions were at *m/z* 137, 123, 121 and 93 corresponding to $[M - H - CH_2O]^-$, $[M - H - CO_2]^-$, $[M - H - CO, - H_2O]^-$ and $[M - H - CH_2O, - CO_2]^-$, respectively (Fig. 3). The spectrum suggested that the side-chain of salbutamol had been partly split off, with the subsequent oxidation of the secondary alcohol to a carboxylic acid. For a carboxylic acid, the loss of CO_2 from the $[M - H]^-$ ion is characteristic. A small amount of compound II could be extracted with ethyl acetate and analysed with the high resolution MS (Jeol JMS-SX 102 HR-MS). The elemental formula and the unit of unsaturation obtained by HR-MS for the mass value of 168.0417 were $C_8H_8O_4$ and 5.0, respectively. The extracted compound was also silylated using bis(trimethylsilyl)trifluoroacetamide and 1% trimethylchlorosilane (Pierce, Rockford, IL, USA) and the product that formed was analysed by GC–MS (HP 5890 GC and HP 5970A MS) with a Nordion bonded 54 column. The TMS derivative of II showed a molecular ion at *m/z* 384 indicating that three functional groups had been derivatized. Spectra recorded with high resolution MS and GC–MS confirmed the results obtained by LC–MS. Further evidence of compound II being a derivative of 4-hydroxybenzoic acid was given by UV. The UV spectrum obtained from II [3] closely resembled that of 4-hydroxybenzoic acid. All these data pointed to the identification of compound II as 4-hydroxy-3-hydroxymethylbenzoic acid.

Another acidic compound which was observed only with the negative mode of the MS was compound I — the main decomposition product in strongly acidic solutions.

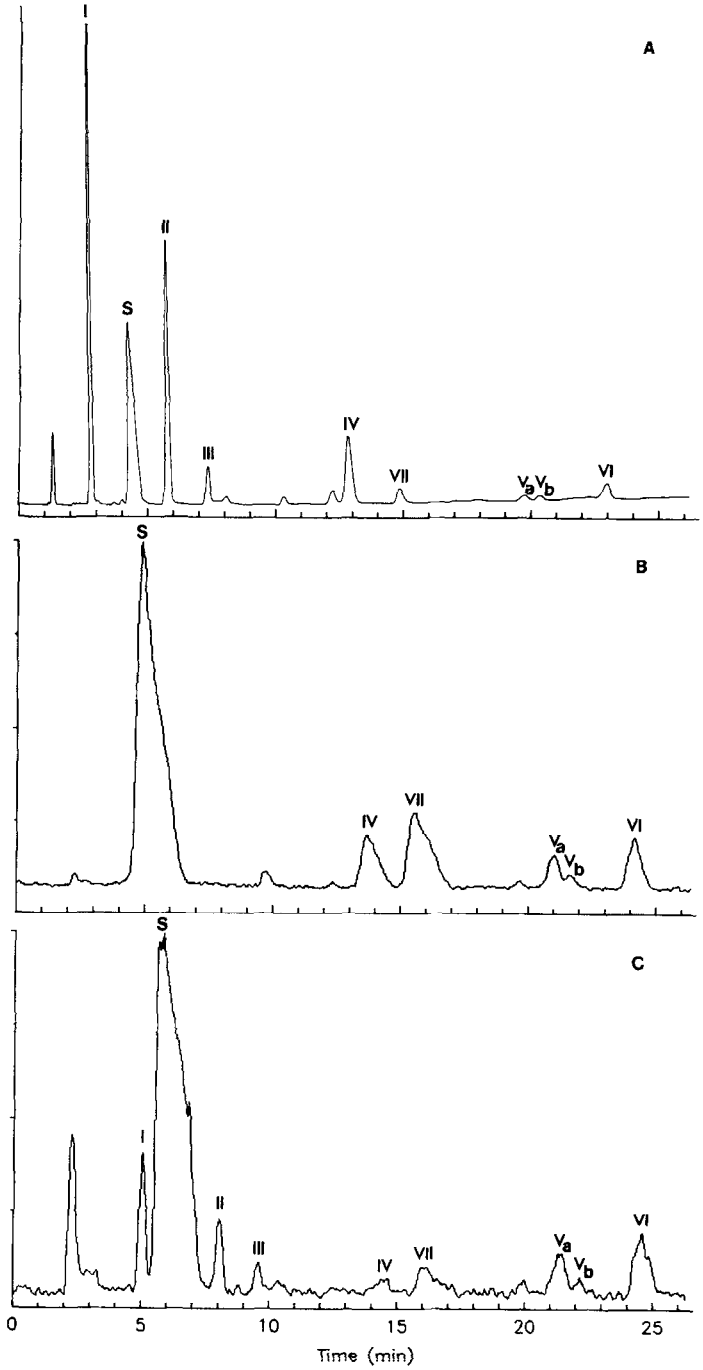
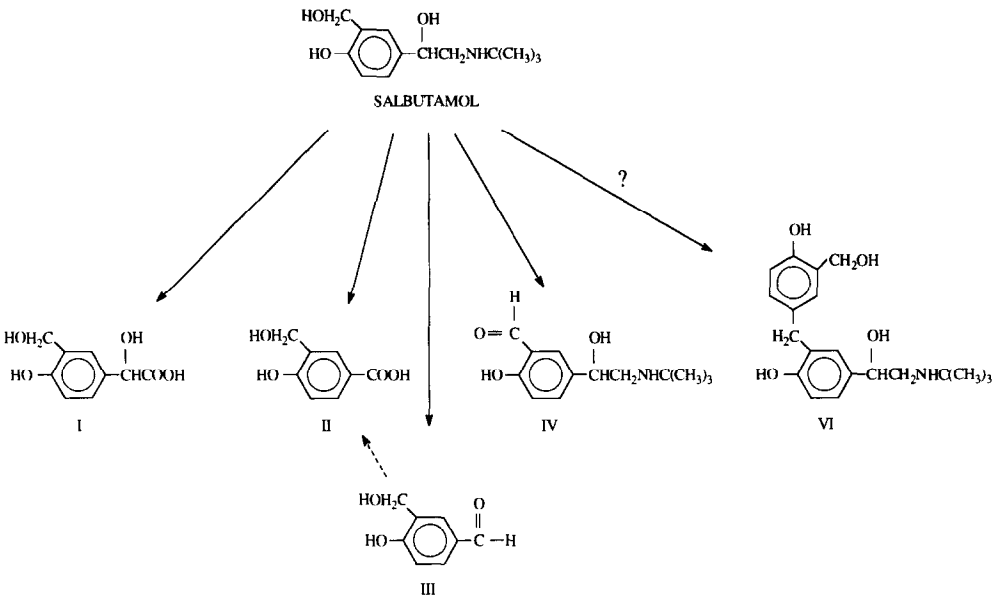


Figure 2
LC–UV and LC–MS chromatograms (four times smoothing) obtained from a sample of decomposed salbutamol in aqueous acetic acid. (A) UV chromatogram at 265 nm, (B) TIC from positive mode and (C) TIC from negative mode. Mobile phase: acetonitrile–ammonium formate (10 mM, pH 3.3) 4:96 to 15:85 in 30 min.

The molecular weight of compound I was 30 amu higher than that of compound II. Evidently salbutamol had lost the *tert*-butylamino group, and the α-carbon had oxidized to a carboxylic group. The base peak in the spectrum (Fig. 4A) at *m/z* 179 corresponds to the loss of H_2O from the $[M - H]^-$ ion, confirming the presence of the side-chain hydroxyl group. The favourable cleavage of H_2O from the molecular ion has been reported in the literature for β-agonists containing the secondary alcohol group in their side-chain [9]. Vanillin



Scheme 1
Proposed structures for the decomposition products formed from salbutamol in aqueous solutions.

Table 1
m/z Values for salbutamol and its decomposition products obtained by LC–API-MS

Product	MW	Positive mode*	Negative mode*	Fragments (*m/z*, %) at 120 V	150 V	180 V
Salbutamol	239	240		480 (3) 479 (8) 241 (13) 240 (83) 223 (8) 222 (59) 167 (6) 166 (61) 149 (8) 148 (100) 130 (4) 121 (3)		480 (6) 479 (23) 241 (7) 240 (47) 222 (6) 166 (11) 149 (8) 148 (89) 130 (29) 121 (52) 103 (31) 91 (100) 77 (55)
I	198		197	395 (10) 197 (38) 180 (8) 179 (100) 177 (8) 151 (6) 149 (10) 133 (4) 121 (9) 105 (3)	197 (46) 180 (19) 179 (100) 177 (38) 150 (10) 151 (5) 149 (44) 133 (13) 122 (43) 121 (88) 120 (38) 105 (14) 92 (18)	
II	168		167	335 (7) 168 (6) 167 (100) 166 (4) 165 (9) 137 (4) 124 (3)	167 (36) 165 (8) 123 (9) 121 (28) 105 (10) 94 (13) 93 (100)	

(Table 1 continues overleaf)

Table 1
Continued

Product	MW	Positive mode*	Negative mode*	Fragments (*m/z*, %) at 120 V	150 V	180 V
				123 (18) 122 (6) 121 (40) 120 (5) 105 (5) 93 (28)		
III	152		151	151 (59) 149 (43) 121 (100) 105 (19)		
IV	237	238		239 (9) 238 (64) 220 (16) 165 (10) 164 (100) 136 (5) 119 (5) 91 (4)		238 (11) 164 (21) 136 (14) 135 (21) 119 (21) 118 (12) 117 (17) 107 (16) 92 (9) 91 (100)
VI	345	346		347 (22) 346 (100) 329 (7) 328 (29) 255 (4) 254 (27)		346 (8) 328 (4) 255 (20) 254 (100) 237 (7) 209 (7) 148 (11) 135 (25) 119 (10) 91 (13)
VII	281	282		283 (16) 282 (100) 265 (4) 264 (26) 223 (3) 222 (21) 208 (6) 205 (3) 204 (23) 166 (4) 148 (73)		282 (27) 148 (100) 133 (29) 130 (32) 121 (64) 120 (26) 103 (26) 93 (23) 79 (12) 77 (32)

*Obtained by using 70 V.
Only the checked fragment ions are shown.

mandelic acid (VMA) was used as a model compound for compound I. The MS spectrum of VMA showed only a minor contribution from loss of CO_2 from the $[M - H]^-$ ion; so the absence of an $[M - H - CO_2]^-$ ion in the spectrum of compound I does not imply the absence of a COOH group in the molecule. The UV absorption maxima of both VMA and compound I were around 280 nm. The MS and UV data led to the conclusion that compound I was α,4-dihydroxy-3-hydroxymethylbenzene acetic acid.

The HPLC behaviour of compound III suggested that it has a neutral character [3]. The molecular weight of 152 obtained with negative mode MS is consistent with the structure of 4-hydroxy-3-hydroxymethylbenzaldehyde. The presence of an aldehyde in decomposed salbutamol solutions is supported by the easy discolouring (yellow to brown) of the solutions, perhaps due to a reaction between compound III and other decomposition products, e.g. *tert*-butylamine. Since the amount of III was consistently small it may be

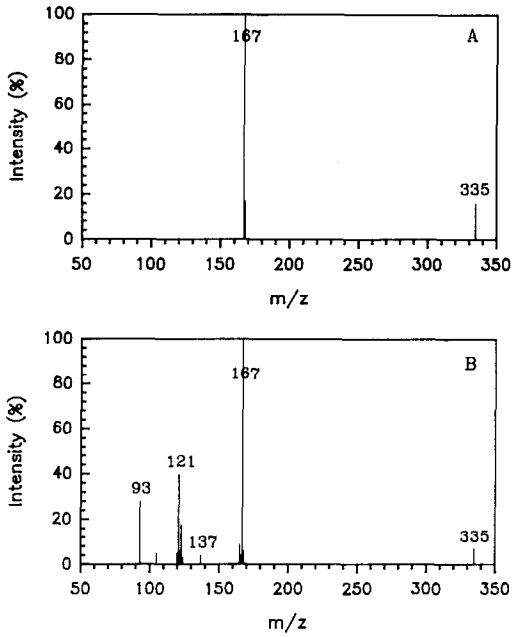


Figure 3
Ionspray mass spectra of decomposition product II recorded at nozzle voltages (A) 70 V and (B) 120 V.

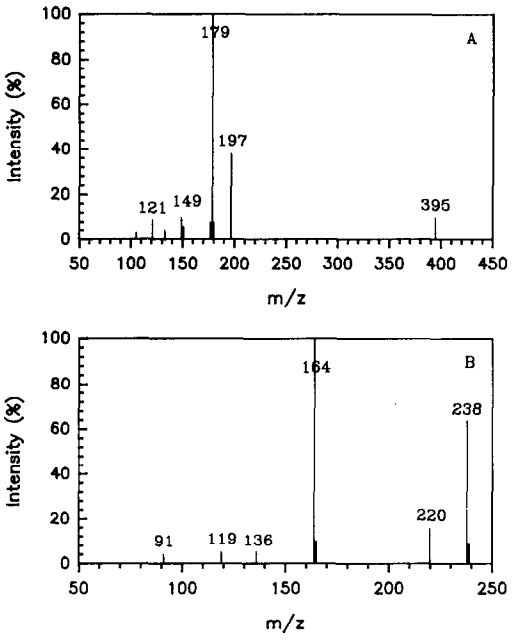


Figure 4
Ionspray mass spectra of decomposition products (A) I and (B) IV recorded at nozzle voltage of 120 V.

that it further oxidized to the carboxylic acid, namely to compound II. Oxidation of an aldehyde-type decomposition product to the corresponding carboxylic acid has been reported for another β-agonist, terbutaline [10].

The fragmentation of salbutamol in the positive mode (Table 1) was in agreement with the data reported in the literature [9]. The main basic decomposition product was compound IV. The $[M + H]^+$ ion of IV was only 2 amu lower than the protonated molecule of salbutamol, indicating the loss of two hydrogens. Although the fragmentation pattern of IV was similar to that of salbutamol, there were two distinct differences in the spectra. In the case of salbutamol there was an elimination of two water molecules: *m/z* 222 ($MH^+ - 18$) and *m/z* 148 (166-18). Only loss of water from the protonated molecule was seen in the spectrum of IV (Fig. 4B), indicating an intact secondary alcohol group in the side-chain. In addition, the peak at *m/z* 121, which was typical for all decomposition products with the salicyl alcohol moiety, was lacking in IV. These results allowed the conclusion that the oxidation had taken place at the primary alcohol group attached to the ring and giving rise to the salicyl aldehyde derivative. The salicyl aldehyde structure was further supported by the UV spectrum of IV, which was similar to that of salicyl aldehyde with maxima at about 220, 255 and 330 nm (Fig. 5). On the basis of the data above it was concluded that compound IV was 2-hydroxy-5-(2-*tert*-butylamino-1-hydroxyethyl)benzaldehyde.

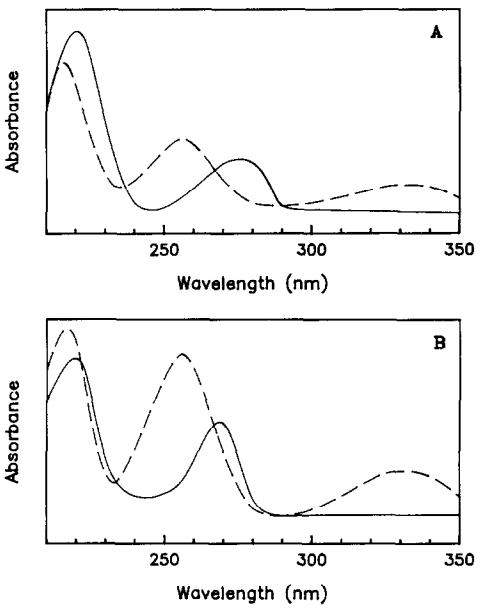


Figure 5
UV spectra of (A) salbutamol (—) and decomposition product IV (--), (B) salicyl alcohol (—) and salicyl aldehyde (--) recorded in mobile phase used in LC (see Experimental section).

In a previous study [3], the strong retention of compounds V_a, V_b and VI on the stationary phase in liquid chromatography was attributed to their large molecular size. This was confirmed by LC–MS, which gave molecular weights of 460, 419 and 345 for V_a, V_b and VI, respectively. The amount of compounds V_a and V_b was too low to obtain meaningful fragmentation. At the nozzle voltage of 120 V the most important fragment ions of compound VI were *m/z* 328 and 254 corresponding to $[M + H - H_2O]^+$ and $[M + H - H_2O, - C(OH)(CH_3)_3]^+$, respectively. One possible structure of VI is 1-(4-hydroxy-3-(4-hydroxy-3-hydroxymethylphenyl) methylphenyl)-2-(*tert*-butylamino)ethanol.

Compound VII was formed only in decomposed solutions prepared in aqueous acetic acid. The molecular weight was 42 amu higher than that of salbutamol, indicating compound VII to be an acetylated derivative of the parent drug. The loss of two water molecules in the spectrum of VII indicated that it was the phenolic hydroxyl group that had been acetylated.

The side-chains cleaved from the molecule of salbutamol (*tert*-butylamine with MW of 73 and *tert*-butylaminoacetic acid with MW of 131) eluted at t_0 in LC and were observed in the positive ion mode in MS.

Conclusions

On-line LC–ionspray MS is a very useful technique for the structural characterization of small amounts of polar compounds. Fragment ions can be obtained by increasing the nozzle voltage above 70 V. With a mixture of acetonitrile and ammonium formate in the mobile phase of LC, the decomposition products of salbutamol were easily characterized with this technique.

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