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The degradation of salbutamol in ethanolic solutions

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ABSTRACT

The degradation pathways of salbutamol in ethanolic solutions have been investigated and three potential ethyl ether degradation products have been identified. Two have been confirmed as salbutamol ethyl ethers and the third as a diethyl ether. All three degradation products have been structurally elucidated by LC–MS–MS (TOF and tandem quadrupole). The two ethyl ethers have a molecular weight of 267 Da (28 units higher than salbutamol) and are structural isomers (molecules with the same molecular weight but different structural arrangements). The molecular weight of the two ethyl ethers is consistent with the addition of one ethyl group to the salbutamol molecule and elimination of one water molecule. The molecular weight of the diethyl ether is 295 Da (56 units higher than salbutamol) and is consistent with the addition of two ethyl groups to the salbutamol molecule and elimination of two water molecules. A plausible degradation mechanism for the formation of the salbutamol ethyl ethers is the acid-catalysed dehydration of alcohols. Acidic pH is required to drive the degradation of salbutamol in ethanolic solution. Higher degradation levels of salbutamol ethyl ethers are achieved in acidic pH solutions. Levels of the two salbutamol ethyl ethers reach a maximum at an ethanol concentration of around 20%. Levels of the diethyl ether increase linearly with ethanol concentration, until it becomes the major degradation product in high concentration ethanolic solutions (≥30%).

1. Introduction

Salbutamol is a widely prescribed short-acting β_2-agonist, developed in the 1960s, and used to relieve bronchospasm in patients with asthma and Chronic Obstructive Pulmonary Disease [1,2]. It is commonly administered by inhalation as a pressurized metered dose inhaler (pMDI), a multidose dry powder inhaler (DPI) or as a nebulized aerosol.

Salbutamol degrades in aqueous solution at elevated temperatures. Many factors, such as pH, buffer species, buffer concentration and drug concentration, affect the stability [3,4].

Salbutamol was found to be most stable at around pH 3.5 [5,6]. Some of the degradation products formed in aqueous solution have been identified and structurally elucidated by LC–MS [7,8]. Salbutamol can also undergo bimolecular reactions, which result in the formation of a range of dimers [9,10].

The chemical instability of salbutamol in pharmaceutical formulations (pMDIs), containing ethanol as cosolvent, has also been reported [11]. The instability was measured only as the percentage of drug remaining. The separation or identification of the degradation products formed was not investigated.

In the synthesis of salbutamol sulphate, degradation of salbutamol by ethanol has been previously observed [9]. In this process, salbutamol was dissolved in industrial methylated spirits and water and then precipitated by rapid addition of dilute sulphuric acid. If concentrated acid was used instead of dilute sulphuric acid, and the reaction mixture left to stand until salbutamol was completely degraded, three degradation compounds were detected by TLC. Two components were isolated and identified as salbutamol ethers by ^{1}H NMR (an ethyl ether and a diethyl ether derivative). The third was not isolated in sufficient quantity or a pure enough state for unambiguous identification. In typical batches of salbutamol sulphate, only small quantities of one salbutamol ethyl ether were detected as a synthetic impurity.

In this paper, the degradation pathways of salbutamol in ethanolic solutions are described for the first time in a peer-reviewed journal. Three ethyl ether degradation products of salbutamol have been unambiguously identified and structurally elucidated by LC–MS–MS (TOF and tandem quadrupole). The effect of ethanol concentration and pH on the degradation pathway is also reported.

2. Experimental

2.1. Chemicals and reagents

Ethanol (96%, manufactured by VPR, lot L735005) was used for sample preparation.

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A stock solution of salbutamol sulphate was prepared by weighing accurately 10 g of salbutamol sulphate (manufactured by Boehringer Ingelheim; lot 1032205; expiry: June 2010) into a 50 ml volumetric flask and making to volume with deionised water.

Twenty-seven salbutamol sulphate solutions of varying ethanol/water concentrations (0, 1, 2, 5, 10, 20, 30, 40, 50% ethanol) at three different pHs (1, 2, or 3), were prepared by serial dilution from this stock standard. For example: the 30% ethanolic solution at pH 1 was prepared by pipetting 10 ml of the salbutamol sulphate stock solution into a 100 ml flask, adding 30 ml of ethanol and around 50 ml of demineralised water. The pH was adjusted to pH 1 with 1N or concentrated sulphuric acid. The final solution was then made up to 100 ml with demineralised water. The other solutions were prepared in a similar manner.

2.2. Stability storage

Aliquots of the above solutions were stored for 9 weeks at accelerated stability conditions (40 °C/25% r.h.) and then for 9 months at long term stability conditions (25 °C/60% r.h.). After which, samples were analysed using gradient HPLC under the following chromatographic conditions.

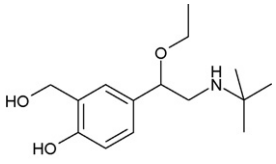
2.3. Chromatographic conditions

HPLC analysis was performed on an Agilent 1100, equipped with a diode array and a gradient pump. The separation was achieved using a Waters Symmetry C8 column, 250 mm × 4.6 mm, 5 μm stationary phase. The data was acquired and processed using Waters Empower software. Mobile phase A consisted of 0.1% TFA in water/0.1% TFA in acetonitrile (950:50) and mobile phase B was 0.1% TFA in water/0.1% TFA in acetonitrile (400:600). The eluent was maintained at 100% A for the hold time of the system and then a linear gradient to 86% A was applied for the next 22 min. The composition was maintained for a further 22 min, and then a linear gradient to 50% A was applied over the next 20 min. The composition was maintained for the next 3 min and then a linear gradient to 100% A was applied over the next minute. The composition was maintained for the next 17 min for column equilibration purposes, bringing the total run time to 85 min. The flow rate used was 1 ml/min, the column temperature was 37 °C and the injection volume was 25 μl.

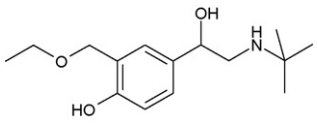
2.4. LC–MS–MS reagents and standards

Two salbutamol ethyl ethers, previously synthesized by Boehringer Ingelheim Pharma GmbH & Co. KG, and assigned internal codes, were used as standards:

BIIK 0285 (Molecular weight = 267 Da; molecular formula = $C_{15}H_{25}NO_3$).



BIIK 0277 (Molecular weight = 267 Da; molecular formula = $C_{15}H_{25}NO_3$).



2.5. LC–MS–MS Instrumentation

The LC–MS–MS analysis of the two salbutamol ethyl ethers (degradates 1 and 2) was performed using a Waters Q-Tof Premier by Boehringer Ingelheim, Biberach, Germany.

The mass spectrometer was operated in the positive ion mode. The ESI conditions were as follows: cone voltage: 35 V; source temperature: 120 °C; desolvation temperature: 300 °C; desolvation gas flow: 800 ml/min. LC analysis was performed using a Water Acquity UPLC, equipped with a diode array and a gradient pump. The separation was achieved using a Waters BEH C18 column, 50 mm × 2.1 mm, 1.7 μm stationary phase. The data was acquired and processed using MassLynx 4.1 software. Mobile phase A consisted of 0.1% Ammonium formate and mobile phase B was 100% acetonitrile. The eluent was maintained at 95% A for 1 min, followed by a linear gradient to 88% A for the next 2.5 min, and then to 87% A for the next 1.2 min. A linear gradient to 75% A was applied over the next 0.3 min, and then to 25% A over the next 1 min. The composition was maintained for the next 0.5 min and then a linear gradient to 100% A was applied over the next 0.5 min. The flow rate used was 0.5 ml/min and the column temperature was 40 °C. The chromatographic conditions were used in preference to those in Section 2.3 in order to reduce run time from 85 to 6 min.

Different LC–MS–MS conditions were used for the structural elucidation of the salbutamol diethyl ether (degradate 3), because it was performed at a later time point than degradates 1 and 2 and another group was responsible for the analysis (Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany). The mass spectrometer used was a Micromass Quattro Premier, which was operated in the positive ion mode. The ESI conditions were as follows: cone voltage: 35 V; source temperature: 120 °C; desolvation temperature: 270 °C; desolvation gas flow: 594 L/h; cone gas flow: 18 L/h. The HPLC conditions used were modified slightly from Section 2.3. The separation was achieved using a Waters Symmetry C8 column, 150 mm × 4.6 mm, 5 μm stationary phase. Mobile phase A consisted of 0.1% TFA in water and mobile phase B was 0.1% TFA in acetonitrile. The eluent was maintained at 95% A for 5 min and then a linear gradient to 82% A was applied for the next 17 min. The composition was maintained for a further 22 min, and then a linear gradient to 30% A was applied over the next 20 min. The composition was maintained for the next 3 min and then a linear gradient to 95% A was applied over the next 1 min. The composition was maintained for the next 17 min for column equilibration purposes, bringing the total run time to 85 min. The flow rate used was 1 ml/min and the column temperature was 35 °C.

3. Results and discussion

3.1. Initial observations

Using the HPLC conditions described in Section 2.3, three major degradation products were observed in the stability samples. An HPLC chromatogram of the salbutamol stability sample in 10% ethanolic solution at pH 1, is given in Fig. 1. Salbutamol sulphate elutes after 16 min, degradate 1 elutes after 33 min, degradate 2 after 40 min and degradate 3 after 68 min, using the above gradient HPLC method.

3.2. Structural elucidation of the salbutamol ether degradation products by LC–MS–MS

Initial investigations by Boehringer Ingelheim Ltd., Bracknell, UK, using a single quad LC–MS (Waters ZQ 2000), indicated that degradates 1 and 2 had the same *m*/*z* ratios (268) and were

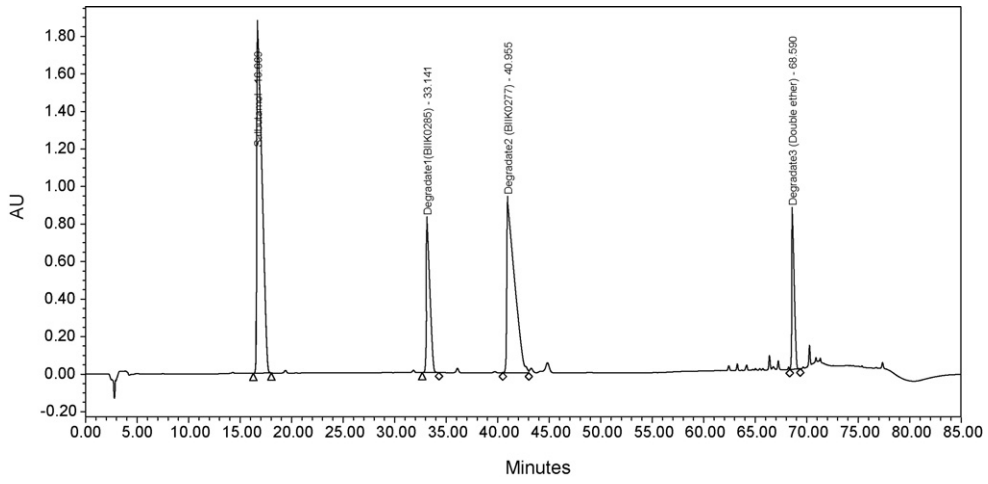


Fig. 1. HPLC separation of salbutamol and its three ethyl ether degradation products in 10% ethanolic solution at pH 1.

likely to be structural isomers (molecules with the same molecular weight but different structural arrangements). Since the molecular weight of degradates 1 and 2 were 28 units higher than salbutamol, the most likely hypothesis was that a single ethyl group had been added to the molecule (via nucleophilic substitution) at two different positions. The most likely positions for nucleophilic substitution, being the two –COH groupings adjacent to the benzene ring. The molecular weight of degradate 3 was 56 units higher than salbutamol, which suggested that two ethyl groups had been added to the molecule (via nucleophilic substitution), at two different positions. To confirm this hypothesis, more detailed structural elucidation work was undertaken.

Degradates 1 and 2 were elucidated at Boehringer Ingelheim, Biberach, Germany using LC–MS–MS (TOF). Degradate 3 was elucidated, at a later time point, by Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany using LC–MS–MS (tandem quadrupole).

The collision-induced dissociation (CID) mass spectra of degradate 1 (upper spectrum) and BIIK 0285 (lower spectrum) are compared in Fig. 2.

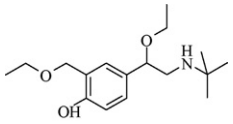
Based on evidence from the experimental mass, the CID mass spectra and retention time, it can be confirmed that degradate 1 corresponds to the structure of BIIK 0285.

The collision-induced dissociation (CID) mass spectra of degradate 2 (upper spectrum) and BIIK 0277 (lower spectrum) are compared in Fig. 3.

Based on evidence from the experimental mass, the CID mass spectra and retention time, it can be confirmed that degradate 2 corresponds to the structure of BIIK 0277.

Finally, the collision-induced dissociation (CID) mass spectra of degradate 3 is given in Fig. 4.

The experimental mass and the CID mass spectra of degradate 3 corresponds to the structure of the salbutamol diethyl ether, given below:



Salbutamol diethyl ether (Molecular weight = 295 Da; molecular formula = $C_{17}H_{29}NO_3$).

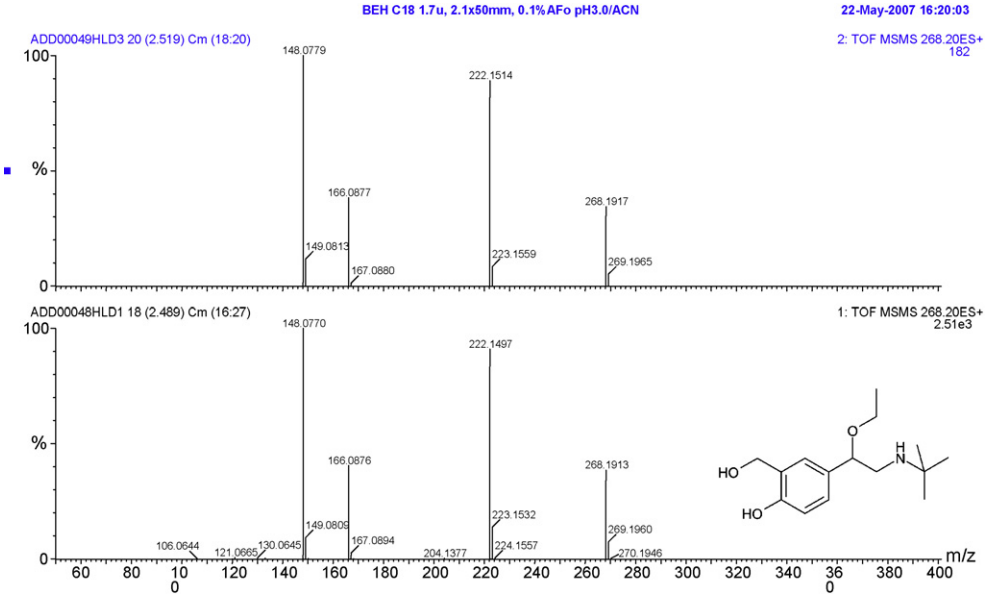


Fig. 2. CID mass spectra of degradate 1 (upper spectrum) and BIIK 0285 (lower spectrum).

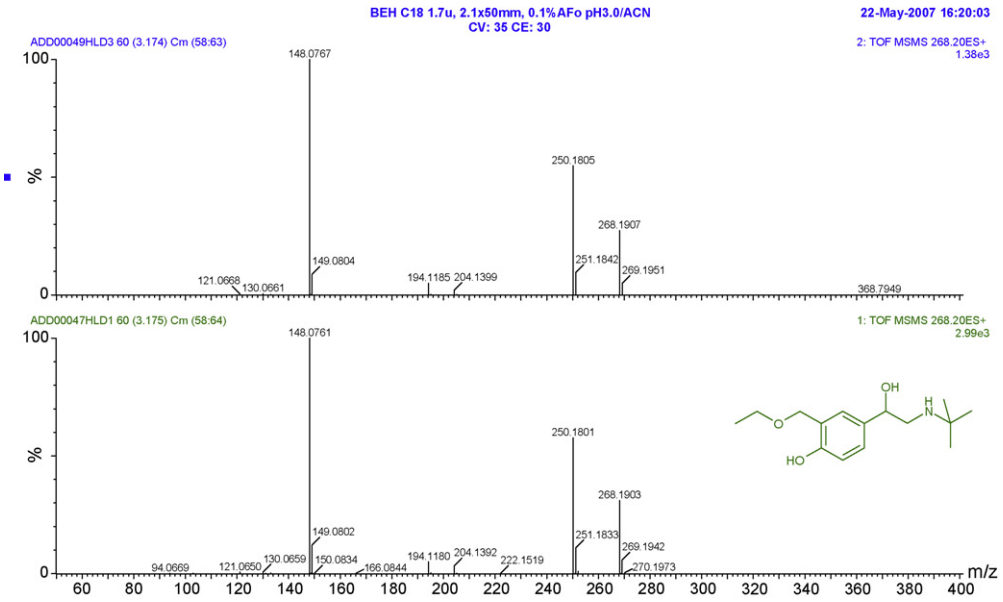


Fig. 3. CID mass spectra of degradate 2 (upper spectrum) and BIIK 0277 (lower spectrum).

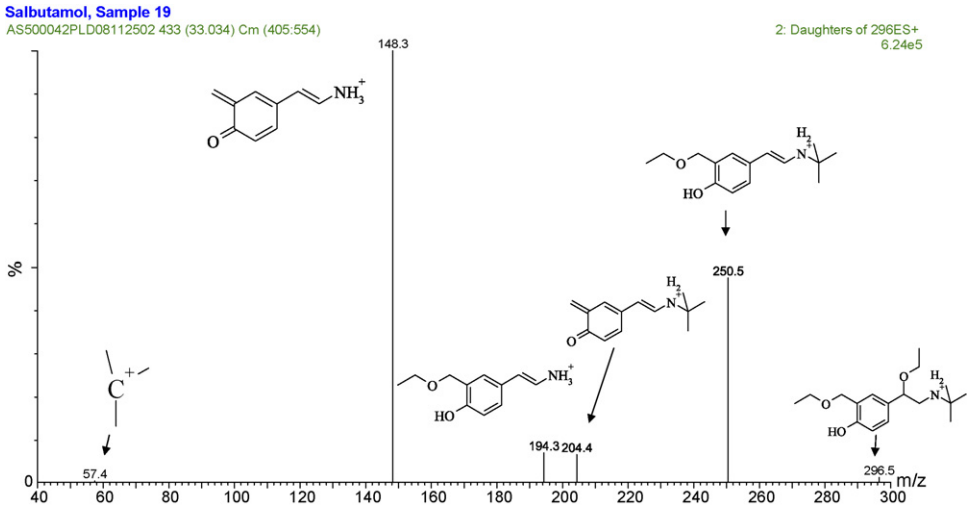


Fig. 4. CID mass spectra of degradate 3.

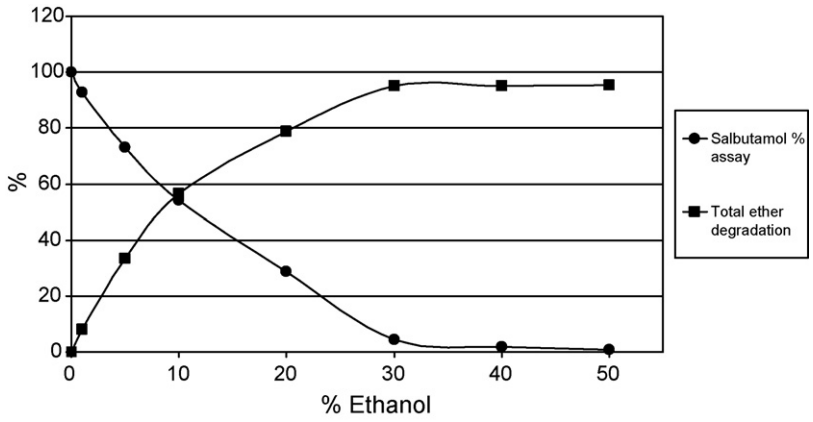
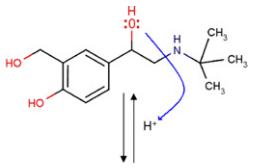


Fig. 5. The effect of ethanol concentration (at pH 1) on salbutamol assay and total ether degradation levels.

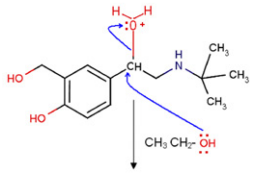
3.3. Plausible degradation mechanism for the formation of salbutamol ethyl ethers

It is known that alcohols can undergo an acid-catalysed dehydration reaction to form ethers [12–14]. This reaction is also responsible for the formation of diethyl ether from ethanol [15,16]. It is possible that the formation of salbutamol ethyl ethers from ethanol and salbutamol follows a similar reaction pathway: A plausible degradation mechanism for the formation of BIIK 0285 is given below.

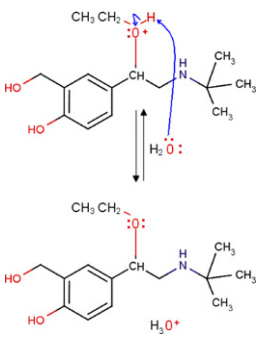
Step 1: An alcoholic oxygen in Salbutamol is protonated in acidic solution to form a good leaving group. This step is fast and reversible.



Step 2: The oxygen of the ethanol molecule functions as a nucleophile and attacks the asymmetric carbon next to the benzene ring to displace a neutral water molecule, in an S_N2 reaction, by cleaving the C–O bond. This creates an oxonium ion intermediate.



Step 3: A proton is removed by a suitable base (here a water molecule), to yield the salbutamol ethyl ether product.



In order to explain the behaviour of the three salbutamol ether degradates with increasing ethanol concentration, it is postulated that up to four separate degradation reactions occur when salbutamol is present in ethanolic solutions:

Primary degradation process:

Reaction 1: Salbutamol + Ethanol → BIIK 0285.
Reaction 2: Salbutamol + Ethanol → BIIK 0277.

Secondary degradation process:

Reaction 3: BIIK 0285 + Ethanol → Salbutamol diethyl ether.
Reaction 4: BIIK 0277 + Ethanol → Salbutamol diethyl ether.

At low ethanol concentrations (<20%), reactions 1 and 2 are likely to predominate over reactions 3 and 4: BIIK 0277 being the major degradation product in low concentration ethanolic solutions (≤20%). When the concentration of ethanol reaches 30%, reactions 1 and 2 cannot occur because there is virtually no salbutamol present in solution, since it has all been degraded (see Fig. 5). As the level of ethanol increases further, a secondary degradation process must occur (reactions 3 and 4) between ethanol and the salbutamol ethers (BIIK 0285 and BIIK 0277). This results in a decrease in the BIIK 0285 and BIIK 0277 concentrations and a corresponding increase in the diethyl ether concentration over time (see Fig. 6). From reactions 3 and 4 it is clear that the amount of diethyl ether will increase at the expense of BIIK 0285 and BIIK 0277. For each additional molecule of diethyl ether formed, there will be one molecule less of BIIK 0285 or BIIK 0277. The level of the diethyl ether increases linearly with ethanol concentration, until it becomes the principal degradation product in high concentration ethanolic solutions (>30%).

3.4. Effect of ethanol concentration on salbutamol assay value

The effect of increasing ethanol concentration (at pH 1) on the salbutamol assay is given in Fig. 5. The salbutamol assay value decreases with ethanol concentration up to about 30% ethanol and thereafter it tends to zero as ethanol concentration increases.

3.5. Effect of ethanol concentration on total salbutamol ether degradation levels

The total salbutamol ether degradation is defined as the sum of the % degradation of BIIK 0285, BIIK 0277 and the diethyl ether.

The effect of increasing ethanol concentration on the total salbutamol ether degradation is also given in Fig. 5. It can be seen that the total degradation increases with ethanol concentration up to about 30% of ethanol and thereafter it reaches a plateau, as the total degradation approaches 100%. This correlates well with the fall in assay and confirms that the three ether degradation products are the major degradates of salbutamol in ethanolic solutions and that no significant mass balance gap exists.

3.6. Effect of ethanol concentration on levels of BIIK 0285 and BIIK 0277

The effect of increasing ethanol concentration on the level of BIIK 0285 and BIIK 0277 (at pH 1) is given in Fig. 6. Both degradation products increase with increasing ethanolic concentration, reach a maximum (around 20% ethanol), and then start

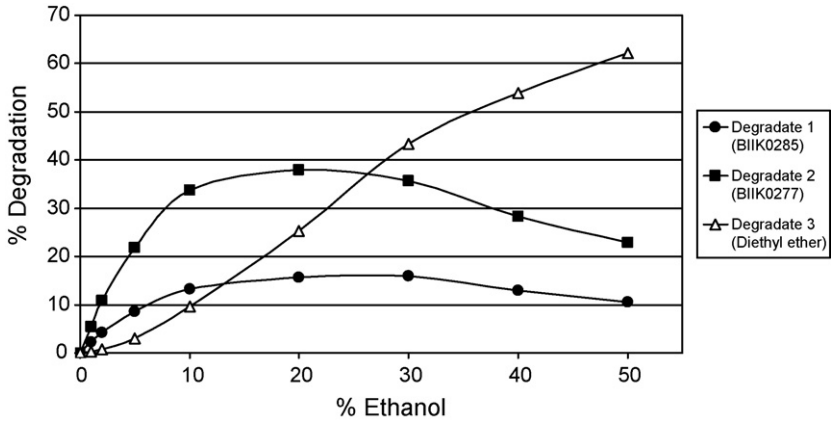


Fig. 6. The degradation of salbutamol in ethanolic solution (at pH 1) as a function of ethanol concentration.

decreasing at higher ethanolic levels. From Fig. 5 it is clear than the salbutamol assay value tends to zero at ethanol concentrations of 30% and above. The concentrations of BIIK 0285 and BIIK 0277 would therefore be expected to reach a plateau at higher ethanol concentrations. The fact that they decrease with increasing ethanol concentration suggests that a secondary degradation process is occurring. This is further explained in Section 3.7.

3.7. Effect of ethanol concentration on levels of the salbutamol diethyl ether

The effect of increasing ethanol concentration on levels of salbutamol diethyl ether (at pH 1) is given in Fig. 6. The levels of the diethyl ether increase linearly with ethanol concentration. At ethanol concentrations of 30% or above, there is virtually no salbutamol left in the sample (see Fig. 5), and therefore the diethyl ether cannot be formed by degradation of salbutamol. The only other compounds present in solution are the two salbutamol ethyl ether degradation products, BIIK 0285 and BIIK 0277, at levels of 15% and 35%, respectively. Since the levels of the diethyl ether continue to increase with increasing ethanol concentration, it follows that BIIK 0285 and BIIK 0277 must interact with ethanol, in a secondary degradation process, to form the diethyl ether. As the ethanol concentration increases further, the diethyl ether becomes the principal degradation product.

3.8. Effect of pH on levels of BIIK 0285, BIIK 0277 and salbutamol diethyl ether

Higher degradation levels of BIIK 0285, BIIK 0277 and the diethyl ether are achieved under acidic pH conditions. In 20% ethanolic solutions, the % degradation of BIIK 0285 at pH 1, 2 and 3 was 16%, 8% and 2%, respectively. Similar results were observed for BIIK 0277 (38%, 26% and 14%) and for the salbutamol diethyl ether (25%, 3% and <0.5%). If the dehydration of salbutamol is acid-catalysed, then the amount of degradation would be expected to vary inversely with pH.

4. Conclusion

The degradation pathways of salbutamol in ethanolic solutions have been investigated. Three salbutamol ethyl ether degradation products have been structurally elucidated by LC–MS–MS (TOF and tandem quadrupole). A plausible mechanism for the formation of the salbutamol ethyl ethers is the acid-catalysed dehydration of alcohols. Higher degradation levels of salbutamol ethyl ethers are achieved in acidic pH solutions. Levels of the two salbutamol ethyl ethers reach a maximum at an ethanol concentration of around 20%: BIIK 0277 being the major degradation product in low concentration ethanolic solutions ($\leq$20%). The diethyl ether is thought to be produced in a secondary degradation reaction between ethanol and the single ethyl ethers (BIIK 0285 and BIIK 0277).

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