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Concerning the stability of benzyl alcohol: formation of benzaldehyde dibenzyl acetal under aerobic conditions

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Abstract

An impurity in benzyl alcohol was identified as benzaldehyde dibenzyl acetal (BDBA). The component BDBA is reversibly formed by the reaction of benzyl alcohol with benzaldehyde, an oxidative degradation product of benzyl alcohol. Whereas, BDBA is a known chemical entity, it is not typically controlled in commercial benzyl alcohol since it cannot be formed in the absence of benzaldehyde, which is itself generally controlled. However, once commercial benzyl alcohol is exposed to the atmosphere, formation of BDBA is possible. The synthesis and characterization of BDBA is reported. The ability of BDBA to react with alcohols to form other types of acetals, and the impact of low levels of BDBA on the quantitative analysis of pharmaceutical products, are considered.

Keywords: Excipients; Benzyl alcohol; Benzaldehyde dibenzyl acetal; Reversed-phase chromatography; Degradation product levels

1. Introduction

Benzyl alcohol **2** is commonly used in pharmaceutical products as an antimicrobial preservative at levels of 3–5% [1]. It is well established that when exposed to air, benzyl alcohol **2** oxidizes slowly to benzaldehyde **1** and subsequently to benzoic acid. The chemical oxidation of benzyl alcohol to benzaldehyde has been described in several papers [2–4]. The monograph for benzyl alcohol **2** in the United States Pharmacopoeia (USP) [5] includes a method for assay of benzaldehyde **1**, and USP grade benzyl alcohol **2** may contain no more than 0.20% benzaldehyde **1**.

We have identified an additional impurity in commercial benzyl alcohol **2**, benzaldehyde dibenzyl acetal **3** (BDBA). We have observed that this component grows on exposure of benzyl alcohol **2** to the atmosphere for extended periods. Whereas, this compound has been reported in synthetic literature previously [6] it has not, to our knowledge, been considered as a potential impurity in a raw material used in product manufacture. In this communication, we report the

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physical characterization of BDBA **3**, along with a short HPLC method, which achieves the resolution of BDBA **3** from benzyl alcohol **2** and benzaldehyde **1**. The potential impact of low levels of BDBA **3** on the quantitative analysis of pharmaceutical formulations with various potencies will be discussed.

2. Experimental

2.1. Apparatus and materials

^{1}H NMR and ^{13}C NMR spectra were collected on a Bruker 300 NMR Spectrometer. Preparative HPLC was performed using a Waters 600 pump and samples were injected onto a Luna C8(2) column from Phenomenex (25 cm × 2 cm, 5 μm). Analytical HPLC was performed on an Agilent 1100 system equipped with a quaternary pump, Diode Array Detector, and Autosampler. A Waters Symmetry C8 column (150 mm × 3.9 mm, 5 μm) was used to separate the compounds of interest. Standard laboratory equipment and chemicals were used in the synthesis and purification of BDBA **3**.

2.2. Synthesis of benzaldehyde dibenzyl acetal *3* (BDBA) and benzaldehyde benzylmethyl acetal *4* (BBMA)

To a mixture of benzyl alcohol **2** (20 g, 188 mmol) and benzaldehyde **1** (5 g, 46 mmol), was added sulfuric acid (0.5 ml, 9 mmol). Molecular sieves (5 g, 3 Å) were added, and the resultant mixture was stirred for 24 h at ambient temperature. The reaction mixture was chilled on an ice bath, and ice-cold water together with $NaHCO_3$ (5 g, 60 mmol) and MeOH (25 ml) were added under vigorous stirring and cooling on ice. The solution was filtered and transferred into a 500 ml separation funnel. The sample was extracted twice with 100 ml of *n*-heptane. The *n*-heptane extracts were combined, washed with saturated $NaHCO_3$, and solvents were evaporated under reduced pressure. A sample of the crude mixture was analyzed by analytical HPLC (see Section 2.3 for details). Two major components were observed in addition to benzyl alcohol **2** and benzaldehyde **1**. The sample was dissolved in 80:20 acetonitrile:H_2O and the solution was filtered. The components BDBA **3** and BBMA **4** were isolated from the reaction mixture by preparative HPLC (see Section 2.4 for details).

BDBA **3** was also prepared in a non-catalyzed reaction monitoring the formation of the acetal from benzyl alcohol **2** in the presence of 0.2% benzaldehyde **1**. To ∼5 ml of benzyl alcohol **2** in a 10 ml volumetric flask, 20 μl of benzaldehyde **1** were added and the flask was then filled with benzyl alcohol **2** and mixed. The reaction mixture was protected from oxygen and kept in the dark for 27 days. Aliquots of 200 μl were taken at several time points and dissolved in 10 ml of acetonitrile:H_2O (80:20). Samples were subjected to analytical HPLC analysis.

2.2.1. BDBA *3*

^{1}H NMR (300 MHz, CD_3CN): δ = 4.6 (s, 4H), 5.7 (s, 1H), 7.2–7.8 (m, 15H). ^{13}C NMR (300 MHz, CD_3CN): δ = 67, 105, 123, 125, 127, 130, 131, 138, 139. IR (neat): ν = 3000 cm^{-1} (acetal CH stretch, medium), 2900 cm^{-1} (benzyl CH stretch, medium), 1300–1500 cm^{-1} (aromatic CH stretch, medium), 1000–1200 cm^{-1} (four bands, C–O–C, strong). UV: λ = 205 nm (ε = 28250 l mol^{-1} cm^{-1}), 252 nm (sh, ε = 500 l mol^{-1} cm^{-1}), 258 nm (ε = 630 l mol^{-1} cm^{-1}), 263 nm (sh, ε = 500 l mol^{-1} cm^{-1}).

2.2.2. BBMA *4*

^{1}H NMR (300 MHz, CD_3CN): δ = 3.3 (s, 3H), 4.6 (s, 2H), 5.6 (s, 1H), 7.3–7.9 (m, 10H). ^{13}C NMR (300 MHz, CD_3CN): δ = 54, 69, 104, 128, 129, 130, 134, 140, 141. UV: λ = 205 nm (ε = 19000 l mol^{-1} cm^{-1}), 252 nm (sh, ε = 325 l mol^{-1} cm^{-1}), 258 nm (ε = 405 l mol^{-1} cm^{-1}), 263 nm (sh, ε = 325 l mol^{-1} cm^{-1}).

2.3. Separation of BDBA *3*, BBMA *4*, benzaldehyde *1* and benzyl alcohol *2* by analytical HPLC

Separation of benzyl alcohol **2** (1.2 min), benzaldehyde **1** (1.3 min), BBMA **4** (2.1 min), and BDBA **3** (2.6 min) was accomplished on a Symmetry C8 column (150 mm × 3.9 mm, 5 μm). HPLC conditions were: flow rate = 1.5 ml min^{-1}, injection volume = 25 μl, detector wavelength = 210 nm. Mobile phase was 80:20 acetonitrile:H_2O. Samples were prepared by dissolving 20 μl of benzyl alcohol **2** in 980 μ1

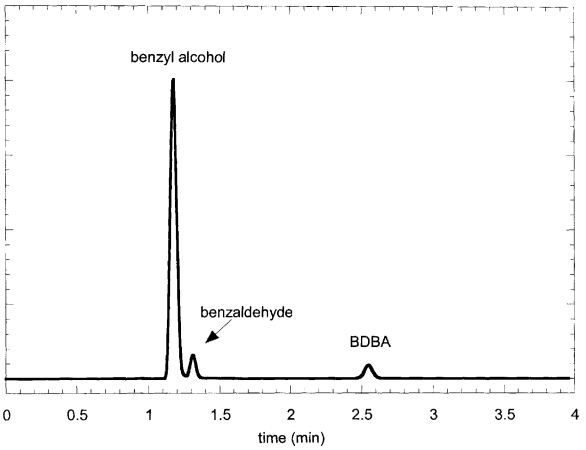


Fig. 1. A typical chromatogram of benzyl alcohol **2** by the HPLC method described in Section 2.3.

of mobile phase. A representative chromatogram is shown in Fig. 1.

2.4. Isolation of BDBA *3* and BBMA *4* by preparative scale chromatography

1 l of acetonitrile:H_2O (80:20) was degassed by purging He through the solution for 5 min. A semi-preparative HPLC C8 column (25 cm × 2 cm, 5 μm) was equilibrated at a flow rate of 10 ml per minute. The crude reaction mixture was dissolved in 10 ml of acetonitrile and a 2 ml sample was injected onto the column. Fractions of 10 ml were collected and analyzed by analytical HPLC. Fractions eluting from 10 to 11.5 min contained BBMA **4** and fractions eluting from 18 to 20 min contained BDBA **3**.

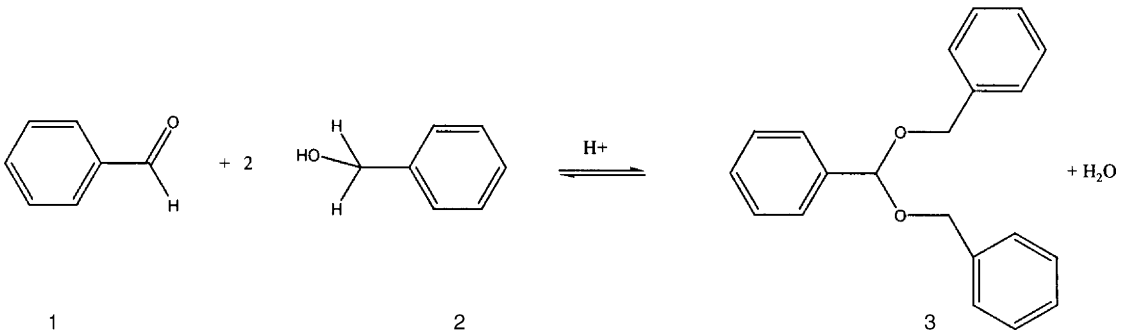
3. Results and discussion

3.1. Synthesis

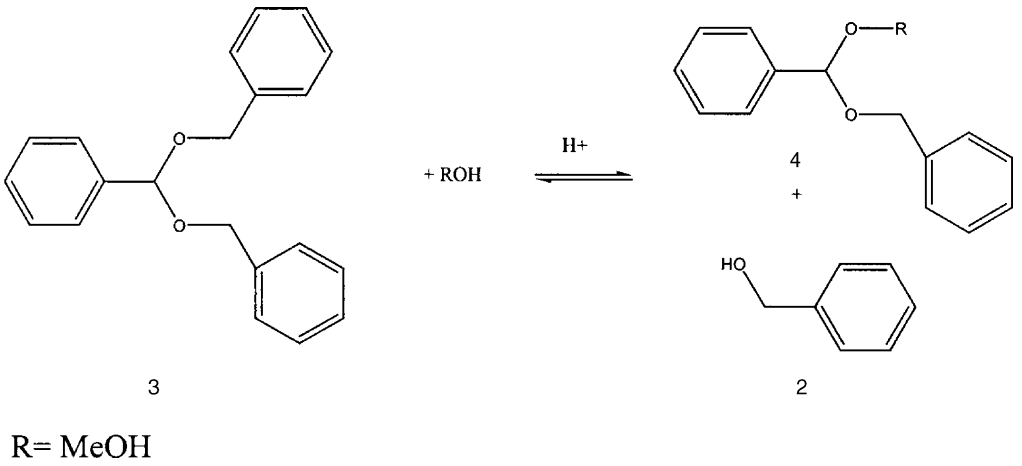
Benzaldehyde dibenzyl acetal **3** (BDBA) is formed by reaction between benzaldehyde **1** and benzyl alcohol **2**, as shown in Scheme 1.

BDBA **3** was synthesized by condensation of benzyl alcohol **2** with benzaldehyde **1** in the presence of catalytic amounts of H_2SO_4 at ambient temperature. On completion of the reaction, the reaction mixture was chilled on ice and the acid was neutralized with sodium bicarbonate in water. Addition of *n*-heptane caused a phase separation in the water-benzyl alcohol-benzaldehyde mixture; MeOH was added so that two phases were obtained. BDBA **3** showed increased solubility in *n*-heptane. The *n*-heptane layer was washed with saturated $NaHCO_3$ to remove some of the benzyl alcohol **2**, which was also extracted. Upon HPLC separation of the partially purified reaction mixture, it was observed that a significant portion of the BDBA **3** so formed was converted to a new compound, which was identified as benzaldehyde benzylmethyl acetal **4** [7] (BBMA). It therefore appears that before neutralization of the acid was complete, partial exchange of a benzyloxy group for a methoxy took place, as depicted in Scheme 2. Additional losses due to hydrolysis may have occurred.

The observation of this reaction product demonstrates, as expected, that not only the impurity of BDBA **3** be unstable in aqueous formulations, in accordance with Scheme 1, but also in alcohol-based formulations due to exchange with the matrix.



Scheme 1. Acid catalyzed formation of BDBA **3** from benzaldehyde **1** and benzyl alcohol **2**.



Scheme 2. Formation of BMBA **4** from BDBA **3**.

Pure samples of BDBA **3** and BBMA **4** for characterization were isolated by preparative chromatography, as described in the Section 2.4.

3.2. Characterization of BDBA *3* and BBMA *4*

As described in the Section 2.4, the components BDBA **3** and BBMA **4** were isolated from benzyl alcohol **2** by preparative scale chromatography. The NMR spectra of each compound showed proton signals between 5 and 6 ppm, and carbon signals near 100 ppm, regions characteristic of acetals. BDBA **3** showed only high-field signal for each nucleus (^{1}H 4.6 ppm, ^{13}C 67 ppm) for the benzyl ether centers, whereas BBMA **4** showed an additional high field signal for each nucleus (^{1}H 3.3 ppm, ^{13}C 54 ppm), indicative of the additional methoxy.

The electronic spectra of BDBA **3** and BBMA **4** are very similar to that of benzyl alcohol **2**. A strong band was observed near 205 nm, and a weaker band with some fine structure was observed near 260 nm in each compound. These absorbances appear to be derived from transitions associated with the phenyl groups [8]. The strong absorbances characteristic of the conjugated aldehyde group are absent as expected.

3.3. BDBA *3* contamination in benzyl alcohol *2*

Several lots of benzyl alcohol **2** were tested for BDBA **3** using the method described in Section 2.3. A chromatogram of a typical lot of benzyl alcohol **2** is shown in Fig. 1. Note that this method is very similar to the method to control benzaldehyde **1**, which is described in the USP monograph for benzyl alcohol **2**. Further, note that the short method (4 min) is also selective for the formation of other acetals, including BBMA **4** (RRT 0.69 versus BDBA **3**) and benzaldehyde dimethyl acetal (RRT 0.50 versus BDBA **3**).

Fresh lots of benzyl alcohol **2** are usually "free" from BDBA **3** (<0.05%), however, they contain varying levels of benzaldehyde **1**. The formation of BDBA **3** in benzyl alcohol **2** spiked with approximately 0.2% of benzaldehyde **1** (the limit specified in USP) was monitored as a function of time. Fig. 2 depicts the formation of BDBA, which rises to 0.01% after 27 days. Similar levels were observed in aged lots of commercial benzyl alcohol **2**. These low levels of BDBA **3** are typically of little concern. However, BDBA **3** has a strong absorbance at wavelengths below 220 nm, and liquid formulations of potent actives containing 3–5% benzyl alcohol **2** may show significant interference.

For example, consider a typical formulation comprised of an active ingredient delivered at 1 mg ml^{-1}, in a total volume containing 3% benzyl alcohol **2** (30 mg ml^{-1}). At a level of 0.05% in benzyl alcohol **2** raw material, BDBA **3** would be delivered at 0.015 mg ml^{-1} in the finished product. From a safety perspective, this level is relatively insignificant considering that Material Safety Data Sheets for

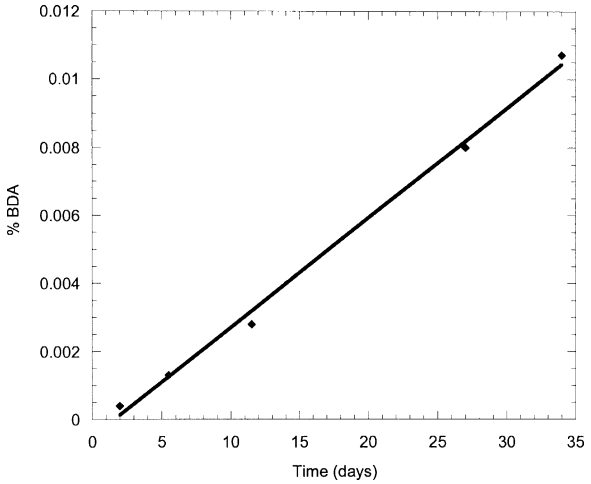


Fig. 2. A plot of BDBA **3** concentration vs. time for a mixture of 99.8% benzyl alcohol **2** and 0.2% benzaldehyde **1**.

benzaldehyde **1** and benzyl alcohol **2** indicate that most biological systems can tolerate several hundred milligrams per milliliter of these components. However, from an analytical perspective, 0.015 mg ml^{-1} of BDBA **3** in a 1 mg ml^{-1} active formulation represents an impurity level of 1.5% with respect to the active ingredient.

This rough calculation does not take into account the extinction coefficient of BDBA **3**, which is 98.6 ml (mg cm)$^{-1}$ at 210 nm and 2.046 ml (mg cm)$^{-1}$ at 257 nm. Actives with one or more phenyl ring systems will have similar extinction coefficients at these wavelengths, however, actives lacking conjugation may exhibit much lower UV-absorption in these regions. Therefore, BDBA **3** may appear as a significant peak in related substance methods for the active ingredient in these cases. The effects of 0.015 mg ml^{-1} BDBA **3** contamination on hypothetical formulations (3% benzyl alcohol **2**) with varying potencies and different extinction coefficients relative to BDBA **3** are summarized in Table 1. The table shows that for potent drugs (10 mg ml^{-1} or less) with comparable extinction coefficients to BDBA **3**, the apparent level of BDBA **3** (relative to the active) will exceed 0.1%. For less potent drugs with higher dosages, traces of BDBA **3** only become significant when the ratio of the extinction coefficients is more pronounced (e.g. $\varepsilon_{BDBA}/\varepsilon_{Active} > 10$). In this case, high BDBA **3** levels (relative to the active) will be observed even if the BDBA **3** delivery is much lower than 0.015 mg ml^{-1}.

Table 1
Theoretical levels of BDBA **3** contaminations (0.015 mg ml^{-1}) in hypothetical formulations containing 3% benzyl alcohol **2** with varying potencies and different extinction coefficients relative to BDBA **3**

Active (mg ml^{-1})	Degradation product levels		
	A[a] (%)	B[b] (%)	C[c] (%)
0.5	3	6	30
1	1.5	3	15
2	0.75	1.5	7.5
5	0.3	0.6	3
10	0.15	0.3	1.5
25	0.06	0.12	0.6
50	0.03	0.06	0.3
100	0.015	0.03	0.15

[a] Numbers (%) are figured based on a 1:1 ratio of the extinction coefficient of BDBA **3** and active.
[b] Numbers (%) are figured based on a 2:1 ratio of the extinction coefficient of BDBA **3** and active.
[c] Numbers (%) are figured based on a 10:1 ratio of the extinction coefficient of BDBA **3** and active.

4. Conclusions

It is evident from Scheme 1 that formation of BDBA **3** is contingent on the presence of benzaldehyde **1**. In USP grade benzyl alcohol **2**, benzaldehyde **1** is controlled at levels of 0.20%; the data presented in Fig. 2 demonstrate that BDBA **3** forms readily at this threshold. Indeed the likelihood is that if benzaldehyde **1** is present, so also is BDBA **3**. Since the formation of BDBA **3** is reversible in the presence of water, neglecting its contribution may effectively increase the allowable level of benzaldehyde **1** in USP benzyl alcohol **2**.

Furthermore, as exemplified by the ready formation of BBMA **4** in the presence of methanol, the impurity BDBA **3** will clearly degrade to form other benzaldehyde acetal impurities in alcohol- or glycol-based formulations. Indeed, in additional experiments not reported herein, we have observed rapid exchange of BDBA **3** with both ethylene glycol and propylene glycol to produce cyclic dioxolanes of benzaldehyde **1**. Thus, in the presence of alcohol-bearing matrix components, it is possible to form a wide range of

acetal impurities which have not been considered as contaminants in pharmaceutical products.

Finally, the presence of even trace levels of BDBA **3** (0.05% or lower) in benzyl alcohol **2** can present considerable analytical challenges in method development of formulations containing potent actives. These low levels of BDBA **3** can be manifested as peaks considerably higher than 0.1% relative to the active, a level above which an innovator company is typically bound to identify (at a minimum) impurity peaks.

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