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Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1380–1385
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Time-dependent conversion of benzyl alcohol to benzaldehyde and benzoic acid in aqueous solutions

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Received 1 November 2005; received in revised form 10 February 2006; accepted 15 February 2006
Available online 24 March 2006

Abstract

The oxidative reaction: benzyl alcohol–benzaldehyde–benzoic acid was investigated in time in aqueous solutions of benzyl alcohol widely used as a preservative in medicine and cosmetology. The solutions of benzyl alcohol were stored at concentrations from 0.005 to 2.09 mg/ml for a long time under different conditions. The presence of benzaldehyde and benzoic acid in these solutions was controlled by liquid chromatography on silica sorbent in water. The content of benzoic acid and potentially toxic benzaldehyde in solutions depending on the initial concentration of benzyl alcohol, on time, and on storage conditions was evaluated quantitatively.

Keywords: Benzyl alcohol; Oxidation in aqueous solutions; Benzaldehyde; Benzoic acid

1. Introduction

Benzyl alcohol (BALC) solutions are very popular in cosmetology [1] and pharmaceutical industry. In medicine, BALC is commonly used as preservative in many injection drugs and solutions [2], sometimes it is used as local anesthetic [3].

There is much information in the literature about complications appearing in patients who received medications preserved with BALC. Adult patients rarely demonstrate hypersensitivity reactions [4–6]. The most significant adverse reactions were observed in pediatric practice [7–10] up to death of premature infants from asphyxia, which is thought to be due to BALC toxicity [7,11].

Biodegradation of BALC in the organism takes place in liver and kidneys in two stages. First, the enzymes: alcohol dehydrogenase and cytochrome P-450 catalyze BALC oxidation to benzaldehyde (BALD), then BALD is oxidized with the aid of aldehyde dehydrogenase to benzoic acid (BAC) which is bound to glycine and isolated with urine in the form of hippuric acid [11–13]. The authors of papers [11,12] explain tragic consequences of BALC poisoning in the neonatal practice by immaturity of the detoxification process in premature newborns. Up to now no unique opinion exists about the possibility of using BALC-containing drugs and solutions for newborns [2].

The process of BALC biodegradation assumes the investigation of BALC toxicity as well as the toxicity of its oxidation products BALD and BAC. Benzyl alcohol is known to destabilize interferon γ [14] and exhibits hepatotoxicity [15]. Nephro-, neuro-, and hepatotoxicity are also exhibited by BALD [15–18]. The risk of convulsions and lethal complications associated with the therapeutic use of aminophylline increase in the presence of BALD [19]. The possible toxicity mechanism of BALC and BALD is the ability to inhibit antioxidative enzymes [15,16,18] and thus to increase the content of reactive oxygen species in the organism. Benzoic acid used as food and medical creams preservative also leads to slight allergic reactions, especially in children [20].

So far there is no single opinion in the literature about the reason for serious adverse reactions appearing when medications preserved with BALC are used: either it is BALC itself or its metabolites [15–21]. In any case, the British Pharmacopoeia and the United States Pharmacopoeia limit the presence of BALD in BALC, used as preservative in the manufacture of parenteral forms, to levels of 0.05% and 0.2%, respectively [22,23].

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doi:10.1016/j.jpba.2006.02.028

However, the authors of Ref. [24] determined BALD quantity in different commercial injection formulations containing BALC preservative and found the excess of BALD permitted level in some formulations. The authors of Ref. [25] found that the presence of 0.2% BALD impurity in BALC under aerobic conditions leads to the formation of a slight amount of benzaldehyde dibenzyl acetal the content of which increases with time. In the presence of water this additional impurity is separated into the initial compounds increasing BALD content. Hence, BALD content may depend on BALC storage time.

It is known that BALC kept in the air and visible light is oxidized to BALD and then to BAC [26,27]. Benzyl alcohol oxidation proceeds more intensively in the presence of various catalysts, e.g. reduced forms of metals [27–30]. Thus, when BALC is used as a preservative of injection drugs and solutions, it is necessary to take into account the possibility of its oxidation in water to BALD and BAC.

The aim of this work was to elucidate the possibility of BALC oxidation upon prolonged storage of its dilute aqueous solutions at different conditions.

2. Experimental

2.1. Materials and preparation of solutions

Benzyl alcohol (analytical reagent grade, Reachim, Russia) was purified by a standard procedure of distillation before solutions preparation. Benzaldehyde (synthesis grade, Ferak, Germany) and benzoic acid (analytical reagent grade, Reachim, Russia) were used without further purification as chromatographic standards.

Water for preparing BALC solutions was additionally purified in two different ways. Distilled water (DW): it is filtered through a Millipore 0.22 GS membrane filter and vacuum degassed before experiment. Purified water (PW): apyretic deionized water filtered through a carbon filter and Milli Q-purified. The mean conductivity of PW water was 0.5×10^{-7} $\Omega^{-1}\,cm^{-1}$.

Benzyl alcohol solutions prevented from air oxygen and from trace amounts of metal ions were prepared on PW previously saturated with Ar. After adding BALC the solution was saturated with Ar during 20 min. Then it was placed into ampoules, Ar was bubbled through again for 2 min, and the ampoules were sealed off. They were stored in darkness.

2.2. Instrumentation

Liquid chromatography was performed on the chromatographic installation consisting of standard units. Two successively connected photometric detectors at wavelengths $\lambda = 254$ nm and $\lambda = 214$ nm were used. Silica sorbent (Si 300) packed in a standard stainless steel column (30.0 cm × 0.4 cm i.d.) was used as a porous media. The analyzes time was 25 min at the eluent (water DW) flow rate 15 ml/h. The injector volume was 10 μl. Spectrophotometry of solutions was performed on a UV–vis Specord M-40 spectrophotometer.

2.3. Testing technique of BALC oxidation products determination

It may be suggested that the main reason for exceeding the allowed BALD level in injection drugs and solutions is gradual BALC oxidation. In the literature there are many precise and reliable chromatographic techniques of determination of BALC and its oxidation products [31] including in various pharmaceutical formulations [24,32], in plasma [33] or in cell extracts [34,35]. All of these techniques include either previous solution treatment, for example, dilution with chloroform [31], extraction by chloroform and vacuum drying [24] in the case of gas–liquid chromatography or the elution by solvent mixtures [25,32–35].

In this work, a simple system was used: BALC solution in water, and a simple method of diagnosing its oxidation products: liquid chromatography on a silica sorbent in water.

2.3.1. Identification of peaks

The possibility of BALC oxidation products' separation on the chromatographic system used was examined. Fig. 1 shows individual chromatogram of BALC solution in 22 h after preparation (curve 1) and individual chromatograms of standard solutions: BAC (curve 2) and BALD (curve 3). The elution volumes of standards differ greatly. Due to electroexclusion, BAC was eluted as the unretained component with an elution volume corresponding to dead volume, $V_0 = 1.9$ ml, BALC was eluted with complete column volume, $V_T = 4.1$ ml, and BALD was eluted in a weakly adsorbing regime with $V_A = 5.1$ ml. The typical BALC chromatogram given as an example in Fig. 1 (curve 1) reveals the presence of oxidation products in the BALC solution already in 22 h after preparation. It should be noted that the presence of a small BAC peak in the BALD chromatogram (curve 3), was probably, caused by high spontaneous oxidation rate of BALD to BAC [27].

In 56 days after preparation, the BALC solution was fractionated on this chromatographic system with collection of fraction

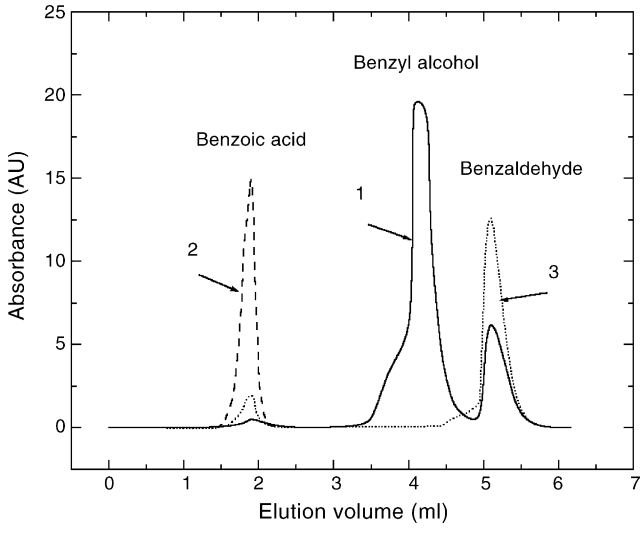

Fig. 1. Chromatograms of: [1] benzyl alcohol solution in distilled water (DW), 22 h after dissolution; [2] benzoic acid standard; [3] benzaldehyde standard. The conditions of chromatography in water: sorbent Si 300, flow rate 15 ml/h.

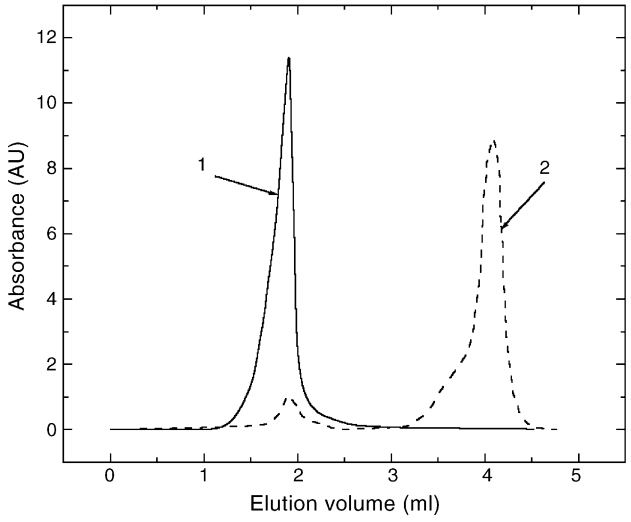

Fig. 2. Rechromatography of fractions obtained at the chromatography of a benzyl alcohol solution stored in distilled water (DW) for 56 days: [1] fraction 1; [2] fraction 2. Chromatography conditions were as in Fig. 1.

1 corresponding to the position of peak BAC ($V_0 = 1.9$ ml) and of fraction 2 corresponding to BALC ($V_T = 4.1$ ml) on a typical chromatogram of BALC solution (Fig. 1). Fig. 2 shows chromatograms of these fractions. It is evident that the elution volume of fraction 1 (Fig. 2, curve 1) conforms to BAC elution volume (Fig. 1, curve 2). Fractionation and rechromatography of fractions 1 and 2 were carried out during about 3 h. Nevertheless, the component with BAC elution volume is present in the chromatogram of fraction 2 (Fig. 2, curve 2). This means that solution dilution during fractionation stimulates further irreversible transition of BALC into BAC.

To perform final identification of peaks, additional UV spectroscopic investigation were carried out. Comparing the spectrum of fraction 1 and that of BAC reference solution confirms that fraction 1 is really benzoic acid (Fig. 3, curves 1 and 2).

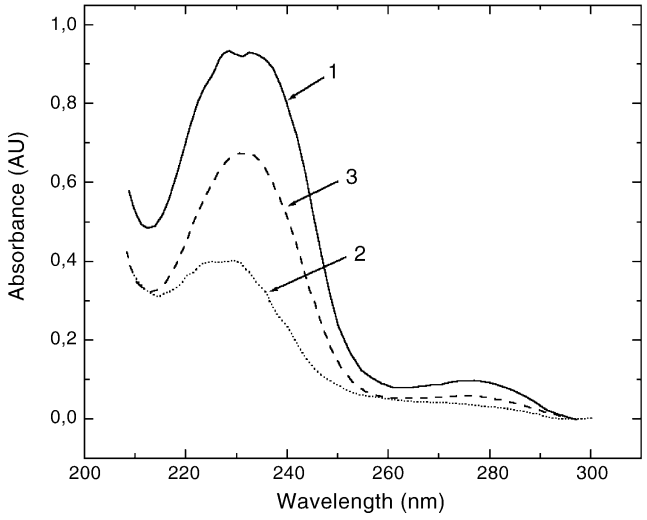

Fig. 3. Spectra of solutions: [1] benzoic acid; [2] fraction 1 of benzyl alcohol, 56 days after dissolution; [3] benzyl alcohol, 70 days after dissolution, $C_0 = 0.005$ mg/ml.

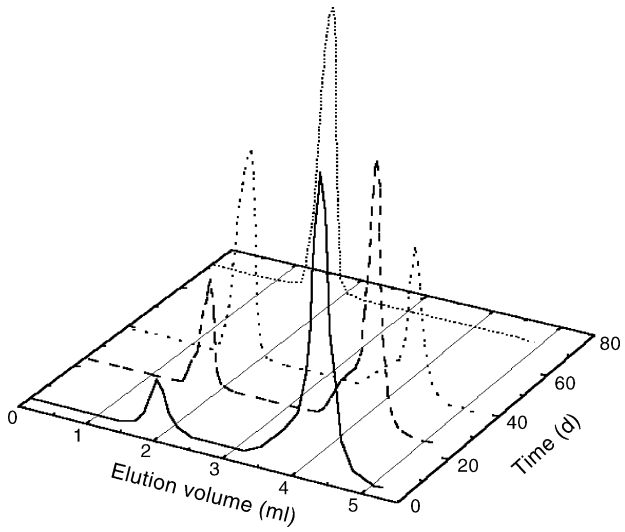

Fig. 4. Chromatograms of benzyl alcohol in distilled water (DW) at different times of solution storage, $C_0 = 0.005$ mg/ml, $\lambda = 214$ nm.

Thus, the proposed technique makes it possible to determine in a single experiment the presence in solution of both BALC itself and the products of its oxidation without introducing any change during testing.

In the general case during chromatography on silica sorbent, the BALC sample was eluted by water with two (BALC and BAC) or three (BALC, BAC, and BALD) peaks. The ratios of peak areas were varied in time and depended on the initial concentration and storage conditions of BALC solutions. Experimental results revealed gradual transformation of BALC into BAC with time. As an example, the total conversion of BALC into BAC during 70 days is shown in Fig. 4. This fact was confirmed by the spectroscopic data (Fig. 3, curves 1 and 3).

2.3.2. *Determination of component concentrations*

The quantitative evaluation of components' content was made by simple peak area method. First, the calibration dependence of peak area on BAC reference concentration $S_{BAC} = F_1$ (C_{BAC}) was obtained. Then BALC concentration was evaluated for chromatograms of BALC solutions containing only two components (BALC and BAC) from the difference between the known initial sample concentration and the concentration of BAC determined from its peak area by using F_1 calibration. In this way the dependence $S_{BALC} = F_2$ (C_{BALC}) was obtained. The concentration calibration dependence for benzaldehyde $S_{BALD} = F_3$ (C_{BALD}) was found by processing in a similar way the chromatograms of BALC solutions containing all three components. All calibration dependences were linear with the correlation coefficient not less than 0.995. Two-beam-detection ($\lambda = 214$ nm and 254 nm) makes it possible to determine more reliably the concentration of components having characteristic absorbance bands at different wavelengths [36]. Thus, BAC is detected more distinctly at $\lambda = 214$ nm and BALD at $\lambda = 254$ nm. Calibration dependences for BALC and BAC were plotted on the basis of chromatograms obtained in detection at both wavelengths and for BALD only at $\lambda = 254$ nm. The coincidence of concentrations calculated by using calibrations at different λ was within 5%.

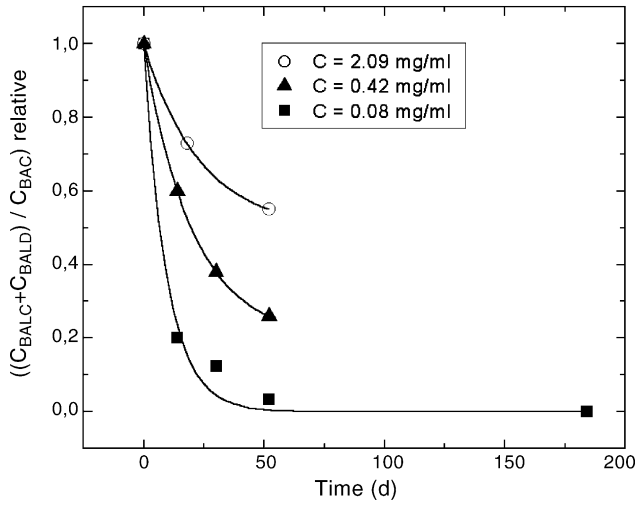

Fig. 5. Long-term composition changes of benzyl alcohol solutions prepared at different initial concentrations: dependences of reduced concentrations' ratio of components (($C_{BALC} + C_{BALD}$)/C_{BAC}, relative) on storage time (days). Distilled water (DW), room conditions.

3. Results and discussion

The effect of initial BALC concentration on the dynamics of its oxidation was studied in the solutions prepared in water with different degrees of purification and stored under different conditions.

3.1. Effect of initial concentration

The dynamics of BALC transformation was investigated at three initial concentrations (C_0) in the range from 0.08 to 2.09 mg/ml. The high sensitivity of detectors did not make it possible to use higher concentrations. Figs. 5 and 6 show the reduced time dependences obtained as the ratio of the total concentration of initial (BALC) and intermediate (BALD) substances to the concentration of final (BAC) oxidation product ($C_{BALC} + C_{BALD}$)/C_{BAC}, in solution stored during time t, divided by the same ratio obtained during the first hour after BALC dissolution. These dependences illustrate the rates of conversion BALC through BALD to BAC. The lower the initial BALC concentration, the more rapidly its transformation into BAC proceeded. The relatively short time period (70 days) of the complete BALC transformation into BAC was observed at the lowest concentration ($C_0 = 0.005$ mg/ml) from those investigated in this work (Fig. 4). Only two peaks (BALC and BAC) were present on the chromatograms up to initial concentration of BALC solution 0.08 mg/ml.

3.2. Effect of the quality of water purification and the storage conditions of solutions

To evaluate the effect of the quality of water purification and the storage conditions of solutions, the dynamics of BALC conversion dissolved in distilled (DW) and purified (PW) water and stored under different conditions were compared (Figs. 5 and 6). From the comparison of the time dependences corresponding to the same concentrations in Figs. 5 and 6 it was clear that oxidation rate of BALC to BAC was higher under room conditions. Moreover, the differences in oxidation rates concerned with storage conditions were greater at higher initial BALC concentrations. The water purification quality as well as the storage conditions of solution, virtually did not affect the process of BALC transition into BAC under the investigated time period at the lowest concentration of BALC used ($C_0 = 0.08$ mg/ml) (Fig. 7).

As mentioned above, at relatively low concentrations of BALC solutions (in this case at $C_0 = 0.08$ mg/ml) the intermediate oxidation product (BALD) was not detected. With increasing BALC concentration (in this case at $C_0 = 0.42$ mg/ml and $C_0 = 2.09$ mg/ml) BALD was present in solutions. Decrease of oxidation rate with increasing C_0 in Figs. 5 and 6 indicated the

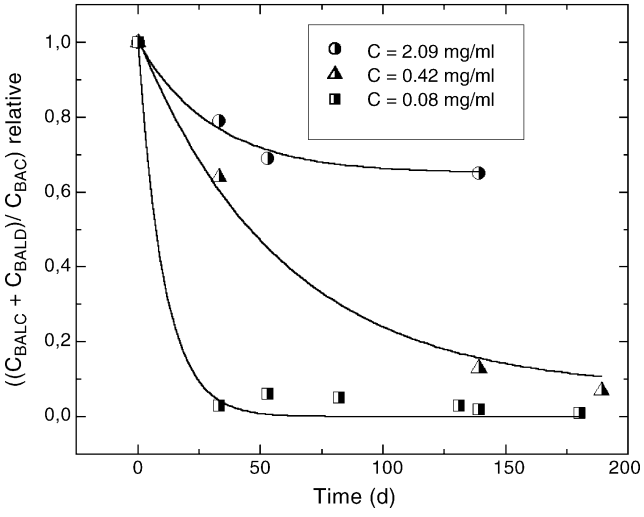

Fig. 6. Long-term composition changes of benzyl alcohol solutions prepared at different initial concentrations: dependences of reduced concentrations' ratio of components (($C_{BALC} + C_{BALD}$)/C_{BAC}, relative) on storage time (days). Purified water (PW), Ar, ampoules, darkness.

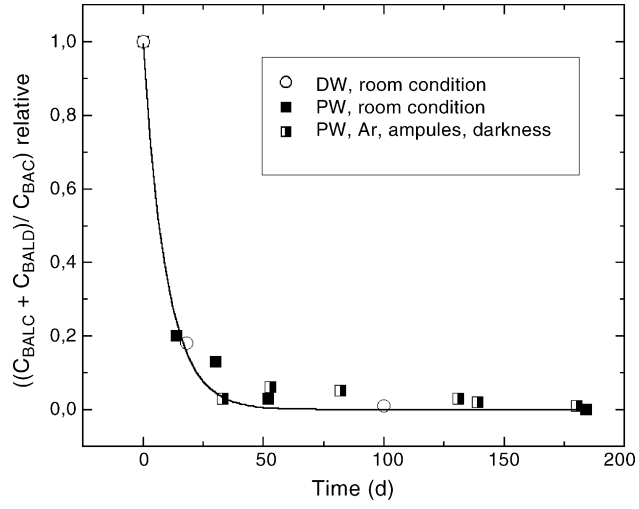

Fig. 7. Long-term composition changes of benzyl alcohol solutions stored under different conditions: dependences of reduced concentrations' ratio of components (($C_{BALC} + C_{BALD}$)/C_{BAC}, relative) on storage time (days). $C_0 = 0.08$ mg/ml.

Table 1
Composition of benzyl alcohol solutions stored under different conditions

Experiment number	Time (d)	$C_0 = 0.42$ (mg/ml)		$C_0 = 2.09$ (mg/ml)		Experimental conditions
		$\frac{C_{BALD}}{C_{BALC}}$ (%)	$\frac{C_{BAC}}{C_{BALC}}$ (%)	$\frac{C_{BALD}}{C_{BALC}}$ (%)	$\frac{C_{BAC}}{C_{BALC}}$ (%)	
1	33	0.3	2.7	5.5	1.4	Ampoule, water PW, Ar, darkness
2	53	1.2	2.1	7.3	1.6	
3	139	0.0	11.0	4.1	1.9	
4	189	14.6	22.8			
5	18	0.0	1.2	9.1	0.8	Flask, water PW, without Ar, light
6	30	1.0	6.9	18.9	1.3	
7	52	2.1	9.8	9.6	1.6	
8	14	8.9	3.7			Flask, water DW, without Ar, light
9	30	22.1	6.2			
10	52	16.9	8.3			

presence of toxic impurity (BALD) during a long time in prepared BALC solution. It is essential, that the higher was initial BALC concentration and the more thoroughly the solutions were stored, the longer BALD was present in solutions.

3.3. Benzaldehyde content in the benzyl alcohol solutions

The quantitative evaluation of oxidation products' content was carried out in different storage times of BALC solutions. It is interesting to analyze the data from the viewpoint of the correspondence to pharmacopoeia requirements [22,23].

Table 1 gives the results of calculating BALD and BAC contents in BALC solutions at two initial concentrations and stored at different conditions. Virtually, in all samples, BALD content in BALC solutions exceeded the critical level of 0.2%. Unfortunately, in this stage of the work, it was not possible to give quantitative relations making it possible to predict solution composition change with time. It was not the aim of this work to investigate the kinetics of a two-stage reaction of BALC transition into BALD and BALD into BAC. It is clear from general considerations that reaction rates in the first and the second stages may be different and may depend on concentrations of oxidation products in solution at this time. However, general relationships can be easily seen. The higher the initial BALC concentration, the greater the amount of BALD presents in solution all other conditions being equal. Visible light and oxygen dissolved in water increase oxidation rate of BALC to BALD. It may be shown by comparing solution content in experiments 1 and 6, as well as 2 and 7. A more important difference was observed in solution compositions in experiments 6 and 9 as well as 7 and 10. This can be explained by the effect of trace amounts of metal ions in distilled water on BALC oxidation rates. With time the content of toxic BALD impurity exceeded permissible level even under "ideal" conditions of BALC storage: in the dark, in PW, and without oxygen (Table 1, experiments 1–4). After six months of storage, an ampoule with initial BALC concentration 0.42 mg/ml contained ~15% BALD and ~23% BAC (Table 1, experiment 4).

Real medical solutions have complicated compositions, and the concentration of BALC used in them as preservative can attain 5% [37]. It is clear that the process of BALC oxidation in such solutions can take place at rates different from those in model experiments, the results of which are given in this work. Nevertheless, it is necessary to take into account the time and conditions of storage of injection drugs and solutions containing BALC for two reasons. First, because the content of potentially toxic BALD increases and second, because the content of BALC fulfilling the functions of a preservative decreases and it is gradually transformed into a food preservative, BAC.

4. Conclusion

It was first shown that even when aqueous BALC solutions are stored extremely carefully BALC is oxidized and it is difficult to predict the content of its reaction product (BALD and BAC) with time. This oxidation can result in a considerable excess of the allowed level of potentially toxic BALD content.

Acknowledgement

The authors thank Koroleva E.M. for the technical assistance.

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