Original Paper

Hydrolysis and transesterification of parabens in an aqueous solution in the presence of glycerol and boric acid.

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ABSTRACT

In a solution containing 0.067% methylparaben, 0.033% propylparaben, 3.4% glycerol and 2.0% boric acid, concentrations of both parabens, 4-hydroxybenzoic acid and 2,3-dihydroxypropyl 4-hydroxybenzoate were monitored for up to 68 months storage. 4-hydroxybenzoic acid is the main hydrolysis product of parabens, while 2,3-dihydroxypropyl 4-hydroxybenzoate was proposed as the main product of transesterification of parabens with glycerol. Results of an HPLC evaluation of parabens, 4-hydroxybenzoic acid and 2,3-dihydroxypropyl 4-hydroxybenzoate showed that the decomposition of 68 months old samples stored at room temperature did not exceed 2.0%. The stability of both parabens in a medicinal preparation of the stated composition has thus been satisfactorily demonstrated after more than 5 years of storage under ambient conditions. The transesterification reaction was shown to influence the chemical stability of parabens to an extent comparable to hydrolysis. Moreover, the presence of 2,3-dihydroxypropyl 4-hydroxybenzoate in the solution containing glycerol and boric acid was confirmed by ^{1}H-NMR spectroscopy.

KEY WORDS: Parabens, glycerol, boric acid, transesterification, hydrolysis, 2,3-dihydroxypropyl 4-hydroxy-benzoate, ^{1}H-NMR

INTRODUCTION

Parabens, which are alkyl 4-hydroxybenzoates, have been commonly used as antimicrobial preservatives in various medicinal and cosmetic preparations, as well as in food and beverages, since they were introduced by Theodor Sabalitschka in the late 1920s (1-3). Sabalitschka also investigated the fundamentals of their detection and colorimetric determination as complexes with Hg^{2+} ions (4). Polyols or sugar alcohols, such as glycerol (GLY), sorbitol, mannitol, erythritol, xylitol, etc., are widely used as pharmaceutical excipients. They are used as sweeteners, humectants and viscosity increasing agents (5). Some are also used as drugs, namely osmotic diuretics and laxatives (6). They are often used in combination with parabens in pharmaceutical formulations. Methylparaben (methyl 4-hydroxybenzoate, MP) and propylparaben (propyl 4-hydroxybenzoate, PP) are quite stable in aqueous solutions at room temperature. Based on the results of HPLC analyses of solutions containing 0.067% MP and 0.033% PP, where concentrations of MP,

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PP and 4-hydroxybenzoic acid (4-HYB) as their hydrolytic product were determined, a 3-year expiration time was proposed (7). In the presence of GLY and boric acid (BOA), which together with MP and PP are components of the *Solutio Jarisch* (SJ) preparation (0.067% MP, 0.033% PP, 3.4% GLY and 2.0% BOA), a different situation is encountered. SJ is a traditional topical preparation which, for many years, has been popular with dermatologists in some Central European countries (8). It also has been listed in some pharmacopoeias since the 1980s (9). It is typically produced by smaller pharmaceutical manufacturers, often by drug distributors, rather than large drug companies. It is used as an anti-inflammatory and antipruriginous agent, and also possesses antiseptic, keratoplastic and hydration properties. Galls, acute eczema and intertrigo are among its main indications (10, 11). It was probably fist formulated by the Austrian dermatologist Adolf Jarisch (1850-1902) who worked at universities in Vienna, Graz and Innsbruck between 1876 and 1902. He is known as one of the discoverers of the Jarisch-Herxheimer reaction, an acute skin reaction, that occurs after the treatment of an infectious disease with a highly efficacious antimicrobial drug. It is believed to be due to the release of endotoxins from the destroyed bacteria (12).

Originally SJ only contained BOA and GLY dissolved in purified water. However, some pharmacopoeias, including the Czech Pharmacopoeia 2009 (13), and prescription books (10) accept the use of *Aqua conservans* in the preparation of SJ, instead of simple purified water due to the longer microbial stability of the modified preparation. *Aqua conservans* contains identical levels of 0.067% MP and 0.033% PP to those in SJ prepared using *Aqua conservans* (7, 13). Whereas the recommended expiration date of parabens-free SJ is only one month (14), the declared shelf life of the version containing MP and PP, which is often manufactured commercially, ranges between 24 and 36 months.(15, 16). Unfortunately, internal reference documents from commercial manufacturers of SJ which declare shelf lives or expiration dates of their products are not typically available, except for Vakos XT. It has thus been difficult to obtain data which support shelf life claims for commercial products.

The reaction of GLY with BOA causes a shift in pH of the solution whereby it becomes more acidic due to the formation of hydrogen bis[1,2,3-propanetriolato(2-)-$\varkappa O^1$,$\varkappa O^2$] borate(1-) (shown in Figure 1).This is a particular example of a common reaction of hydroxylated aliphatic compounds with at least two vicinal hydroxyls that has been known for many years (17, 18).

This type of reaction is used *inter alia* to increase the acidity of BOA for the purpose of titrimetric determination in many pharmacopoeias. For example, the Czech Pharmacopoeia uses the addition of mannitol for this purpose (13) while the United States Pharmacopeia uses GLY (19). Less well known is that, similarly to other polyols, GLY can also interact with parabens through a reaction in which the alkyl moiety of a particular paraben is replaced with a polyhydroxylated chain or ring originating from the initial sugar alcohol, thus forming 2,3-dihydroxypropyl-2-yl (2,3-diOHPP) or 1,3-dihydroxypropan-2-yl ester in our case. Such a reaction is often simply called transesterification (20-22). 2,3-diOHPP and its isomer 1,3-dihydroxypropan-2-yl 4-hydroxybenzoate (1,3-diOHPP) are the most probable products of such a reaction due to the large molar excess of GLY over MP and PP. However, 2,3-diOHPP is likely to be preferred due to reduced steric hindrance (shown in Figure 2). Such transesterification products could also be hydrolyzed into GLY and 4-HYB (dotted line pathway in Figure 2).

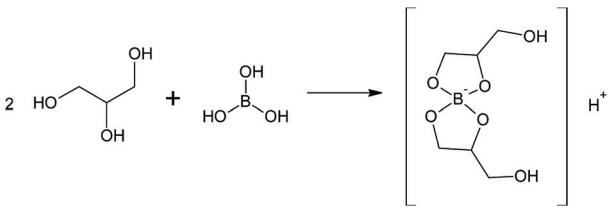

Figure 1 Reaction of GLY with BOA

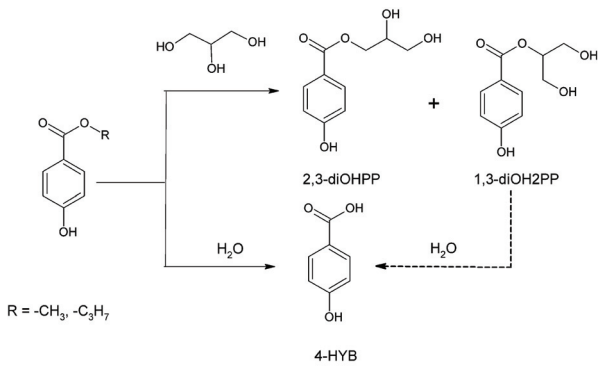

Figure 2 Possible decomposition reactions of MP and PP in aqueous solution in the presence of GLY

Although this type of reaction could be considered to be unlikely in aqueous media from the point of view of the reaction mechanism, it has nevertheless been reported several times in the literature (20-23). Evidence given in the cited papers is usually based on chromatographic data without use of any standard of the proposed polyol-paraben ester. However, they are usually supported by mass spectroscopic measurements, either by a simple identity of the m/z of a molecular ion of a compound present in the sample having a molecular weight identical with that of a polyol monoester (21), or by a complete spectra of such products (20, 22). Evidence of the presence of polyol-parabens esters acquired by means of NMR spectroscopy are also mentioned, but they often lack exact values of chemical shifts of polyol parabens signals or detailed diagrams of appropriate parts of the spectra (21, 22). Moreover, these studies often use reaction conditions which are unlikely to occur during actual storage of preparations containing polyols and parabens, such as increased temperature (up to 90°C) (21, 22), and more extreme pH values, e.g. pH 3 and pH 8 (23) or 11 (22). These pH values together with a "physiological" value of 7.3 (21) differ markedly from natural pH of SJ, which ranges between 4.7 and 5.6 due to the presence of BOA and GLY. Finally, hydrolysis of MP and PP to 4-hydroxybenzoic acid (4-HYB) can also be expected (Figure 2).

EXPERIMENTAL

Chemicals

Samples of SJ with the declared content of 0.067% MP, 0.033% PP, 3.4% GLY and 2.0% BOA were obtained from a commercial producer in the Czech Republic (C.S.C., Prague, Herbacos-Bofarma, Pardubice, RNDr. Jan Kulich, Hradec Králové and Vakos XT, Prague). A sample of SJ prepared in a pharmacy was also obtained (SJ No. 4). All the samples were marked with the date of their preparation in accordance with the Pharmacopoeia (13), and were of various ages at the time of their acquisition. The samples were collected over time, not immediately after their manufacturing. A more detailed characterization of the samples is given in Table 1. Their pH values were measured 12 and 6 months respectively before the determination of the degradation products of the parabens. These are summarized in Table 4.

Table 1 Origin and characterization of analyzed samples of SJ

Sample number	Date of production	Expiration	Manufacturer	Lot number
1	10/2001	10/2002	Herbacos-bofarma, Pardubice, Czech Republic	12301001-2
2	10/2001	10/2002	C.S.C., Prague, Czech Republic	1101001
3	7/2002	7/2003	Vakos XT, manufactory C.S.C, Prague, Czech Republic	360702
4	5/2003	5/2003	Pharmacy of J. G. Mendel, Brno, Czech Republic	---
5	4/2006	3/2007	RNDr. Jan Kulich, Hradec Králové, Czech Republic	280306
6	9/2005	9/2007	Herbacos-bofarma, Pardubice, Czech Republic	51098
7	2/2006	2/2009	Vakos XT Co., Prague, Czech Republic	100206

Methanol, acetic acid and ethyl acetate (Lach-Ner, Neratovice, Czech Republic) were of analytical grade, MP and PP were Czech Pharmacopoeia grade, 4-HYB (Fluka, Buchs, Switzerland) was of puriss. grade. Sodium hydroxide (Lach-Ner, Neratovice, Czech Republic), anhydrous sodium sulfate (Lachema, Neratovice, Czech Republic), sodium tetraborate decahydrate and potassium hydrogenphthalate (Merck, Darmstadt, Germany) were of analytical grade, 3-chloro-1,2-propandiol (Merck, Darmstadt, Germany) was of 98% purity (synthetic grade). The deute-

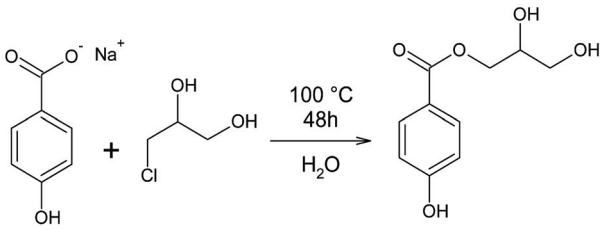

Figure 3 Reaction scheme of preparation of 2,3-dihydroxypropyl 4-hydroxybenzoate (2,3-diOHPP)

rated solvents, deuterium oxide, deuterated acetone and deuterated dimethyl sulfoxide, were of NMR grade with 99% deuteration (Aldrich, Milwaukee, USA). Solid phase extraction (SPE) tubes Discovery® DPA-6S with bed weight of 500 mg and overall volume of 6 ml were purchased from Supelco (Bellefonte, USA).

Synthesis of 2,3-dihydroxypropyl 4-hydroxybenzoate as a standard

4-hydroxybenzoic acid (5.0 g, 36 mmol) was added to a solution of sodium hydroxide (1.5 g, 37.5 mmol) in 110 ml of water while stirring. After its dissolution, 3-chloro-1,2-propandiol (3.1 ml, 32 mmol) was added. The reaction mixture was refluxed while stirring for 2 days using a vent tube containing potassium hydroxide. It was then cooled and the contents extracted with ethyl acetate (5x20 ml). The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residuum was recrystallized from ethanol and yielded crystals which were dried *in vacuo* at 45°C. The reaction scheme is shown in Figure 3. The yield was 35%, and the final product had an m.p. 157-162°C (lit. 154°C (24), 155-157°C (25). The ^{1}H-NMR (200 MHz, DMSO-D_6, δ[ppm], J[Hz]) was characterized as follows: 10,28 1H bs (*p*-OH); 7,84 2H dd J= 8,4 1,6 (*o*-H_{ar}); 6,85 2H dd J=8,4 1,6 (*m*-H_{ar}); 5,00 1H s (OH); 4,70 1H s (OH), 4,25-4,05 2H m ($COOCH_2$); 3,90-3,60 1H m (C*H*OH); 3,55-3,15 2H m (CH_2OH); ^{1}H-NMR (200 MHz, D_2O, δ[ppm], J[Hz]):7.96 2H dd J = 9.2 2.0 (*o*-H_{ar}); 6.94 2H dd J = 8.8 2.2 (*m*-H_{ar}); 5.08-4.79 3H bs (3 OH); 4.46-4.24 2H m ($COOCH_2$); 4.16-4.03 1H m (C*H*OH); 3.76-3.68 2H m (CH_2OH); ^{13}C-NMR (50 MHz, DMSO-D_6, δ[ppm]): 165,8; 162,0; 131,6; 120,7; 115,4; 69,6; 66,0; 62,8; IR (ATC, ν[cm^{-1}]): 3324 ν(NH), 2925 ν(CH_x), 2850 ν(CH_x), 1627 ν(C=O), 1570 δ(NH), 1439 δ(CH_2).

Storage of samples

The samples of SJ were stored in the original 50 – 1000 ml brown screw-cap glass bottles. The screw caps were fitted with plastic gaskets. The temperature range during their storage was between 19 – 26 °C.

pH measurement

A digital 691 Metrohm pH meter (Metrohm, Herisau, Switzerland) with a combined Metrohm 6.0218.010 glass electrode was used for pH measurement. Standard solutions for pH meter scale calibration were prepared in accordance with the requirements of the European Pharmacopoeia (26). Sodium tetraborate decahydrate and potassium hydrogen phthalate were used as standards. The first measurement, 12 months before the determination of the decomposition products of the parabens, was performed in duplicate on samples 1, 3, 5, 6 and 7 and the second determination six months later was performed on all the samples in trplicate. Arithmetic means were calculated for all measurements for all samples.

HPLC

The HPLC system consisted of a P 2000 gradient pump (Spectra Physics, San Jose, USA), a Rheodyne®(Rheodyne, Rohnert Park, USA) manual sample injector equipped with 20 μl loop, a Kromasil® 100-7-C18 column 150 × 4.6 mm, particle size 7.6 μm (Prochrome, Mumbai, India), maintained at ambient temperature, and an LCD 2083 spectrophotometric detector (Ecom, Prague, Czech Republic). A suitable flow rate for the mobile phase was 0.7 ml min^{-1}. A wavelength of

257 nm was used for spectrophotometric detection conforming to the absorption maximum of 2,3-diOHPP in the mobile phase. For the determination of MP and PP, the samples were diluted 10-fold with a mixture of methanol and water 70:30 volume %. The injection volume was 20 µl. The chromatographic analyses were evaluated using Clarity Lite 2.1 software (Data Apex, Prague, Czech Republic). Results of the analyses of the samples were statistically processed by first testing for outliers, and then calculating arithmetic mean and estimating relative standard deviation (RSD). Linear regression analyses were performed using QC Expert 2.5 statistical software (Trilobyte, Pardubice, Czech Republic). The separation of 2,3-diOHPP, 4-HYB, MP and PP by HPLC was found to be satisfactory using a linear gradient elution program 0 – 7 minutes 30 → 70% A (methanol + acetic acid 99:1 volume), 70 → 30% B (water + acetic acid 99:1 volume), 7 – 33 minutes 70% A, 30% B, 33 – 43 minutes 70 → 30% A, 30 → 70% B.

NMR

^{1}H- and ^{13}C-NMR spectra were measured at 200 MHz (FT-NMR spectrometer Varian Gemini 2000, Varian, Palo Alto, USA) and evaluated using VNMR 6.1c (Varian, Palo Alto, USA) and MestReNova Lite 5.2.5-4119 software (Mestrelab Research S. L., Santiago de Compostela, Spain).

50 ml of a sample of SJ was saturated with sodium chloride and extracted with 4x15 ml of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The temperature of the evaporator bath did not exceed 35°C. The weights of residues after evaporation were about 200 mg.

0.04 g of each evaporation residue was suspended in 0.7 ml of deuterium oxide and stirred at ambient temperature for 20 minutes. The mixture was then filtered using a Pasteur pipette equipped with a cotton wool filter plug at its end. The filtrate was used for the measurement of ^{1}H-NMR spectra under standard conditions, with the exception of performing 2048 scans which was used for signal accumulation for a better signal-noise ratio instead of the usual 16. The chemical shifts axis was referenced to D*H*O signal (4.8 ppm – residual hydrogen in deuterium oxide). The part of the region of aliphatic hydrogens between 4.7 – 3.9 ppm was observed and the strongest maxima of 2,3-diOHPP signals, namely those at about 4.3 ppm (COOCH_2) and 4.05 ppm (C*H*OH), were monitored.

Measurements in deuterated acetone and deuterated dimethyl sulfoxide were also attempted but no signals corresponding to 2,3-diOHPP hydrogens were observed probably due to the almost complete overlapping with signals of MP, PP and GLY. 2,3-diOHPP is practically insoluble in deuterated chloroform which is the most commonly used solvent in NMR spectroscopy. Solid-phase extraction (SPE) using Discovery® DPA-6S tube (polyamide resin containing multiple -OH and -COOH groups recommended for adsorbing of polar compounds from aqueous solutions) followed by elution with methanol was also investigated but no 2,3-diOHPP hydrogens signals were found for evaporation residues of methanolic extracts of SJ samples re-extracted to deuterium oxide.

RESULTS AND DISCUSSION

The stability of MP and PP in an aqueous solution in the presence of GLY and BOA in SJ stored under ambient conditions encountered in pharmacies without any means of acceleration of proposed decomposition reactions was determined. Reverse-phase HPLC, a common analytical method in pharmaceutical applications, was used. An analytical procedure, that had been originally developed for the determination of the decomposition of parabens in the aqueous solution *Aqua conservans* (7), was adapted and improved, to enable resolution of analytes of both different (e.g. 4-HYB and PP) and similar

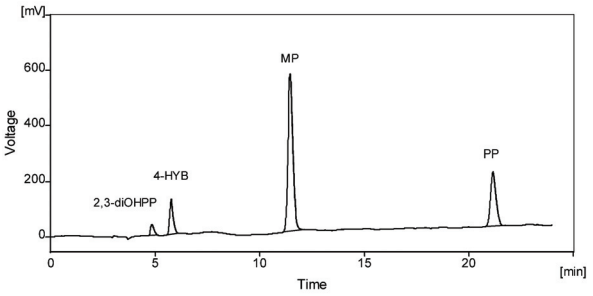

Figure 4 A chromatogram of the mixture initially containing 1.2 μg mL^{-1} 2,3-diOHPP, 3.5 μg mL^{-1} 4-HYB, 6.3 μg x mL^{-1} MP and 2.5 μg x mL^{-1} PP (see Experimental for detailed conditions)

(eg. 4-HYB and 2,3-diOHPP) lipophilicities, and thus retention times, by means of gradient elution.

From the possible products of the reactions of MP and PP with GLY in solutions containing 0.067% MP, 0.033% PP, 3.4% GLY and 2.0% BOA stored at room temperature, formation of 2,3-diOHPP was proposed as the most likely degradation product due to reduced steric hindrance compared to 1,3-diOHPP. The separation of 2,3-diOHPP, 4-HYB, MP and PP by HPLC was satisfactory with the use of a linear gradient elution program. The retention time of PP as the last eluted analyte was 21 minutes (Figure 4).

The dependence of the analyte signal on the analyte concentration was verified: MP and PP 0.13 – 16 μg ml^{-1}, n = 14, linear correlation coefficient r ≥ 0.9996 (7), 2,3-diOHPP 1.9 – 38 μg ml^{-1}, 4-HYB 2.0 – 40 μg ml^{-1}, n = 6, linear correlation coefficient r ≥ 0.999. The limit of detection for 2,3-diOHPP was found to be 0.31 μg $ml^{-1}$and the limit of quantification for the same compound at 1.03 μg ml^{-1} (n = 8) were determined in accordance with the requirements of the European Pharmacopoeia (26). The respective values for 4-HYB were 0.052 μg ml^{-1} and 0.175 μg ml^{-1}(n = 5) respectively. Quantitative analysis of samples which had initially contained 0.067% MP, 0.033% PP, 3.4% GLY and 2.0% BOA of various ages was made by comparison with a standard solution which contained all the analytes. The arithmetic mean of the RSD of HPLC results was 6.3%. The maximum RSD obtained in the analyses of any of the samples was 9.9%. More exact results (lower mean RSD) for 2,3-diOHPP, 4-HYB and MP were obtained from peak areas, and from peak heights for PP. For detailed values of analyte concentrations see Table 2.

Table 2 Concentrations of MP and PP and their decomposition products in samples of SJ of various age and origin. (Arithmetic means of results of three analyses)

Sample No.	Sample age (months)	Content of 2,3-diOHPP (μg/ml) calculated from peak areas	Content of 4-HYB (μg/ml) calculated from peak areas	Content of MP (μg/ml) calculated from peak areas	Content of PP (μg/ml) calculated from peak heights
1	68	[b]	2.49[a]	562	305
2	68	4.80	10.2	632	337
3	59	3.96	7.24	654	328
4	49	[b]	2.18	589[a]	323
5	14	[b]	[b]	601	301
6	21	[b]	[b]	624[a]	341
7	16	0.782[a]	5.29	573	283

Notes:
[a] Arithmetic mean from n = 2 after excluding of an outlier value
[b] Under detection limit

These samples were used for investigation of the decomposition of parabens to 2,3-diOHPP and 4-HYB in a similar manner to a previous report (7) shown in Table 3.

Table 3 Decomposition of parabens in solutions initially containing 0.067% MP 0.033% PP, 3.4% GLY and 2.0% BOA (mean from n = 3) expressed in% of original total concentration of parabens

Sample No.	Sample age (months)	Parabens decomposition (%)		
		to 2,3-diOHPP	to 4-HYB	Total
1	68	0.047	0.36	0.407
2	68	0.52	1.5	2.02
3	59	0.38	1.1	1.48
4	49	0.024	0.26	0.284
5	14	[a]	0.12	0.12
6	21	[a]	0.15	0.15
7	16	0.098	0.87	0.968

Note: [a] Under detection limit

From the results shown in Table 2 it was evident that the decomposition of parabens in a solution containing 0.067% MP and 0.033% PP in presence of 3.4% GLY and 2.0% BOA in 68 months old samples did not significantly exceed 2%. The stability of MP and PP in the SJ

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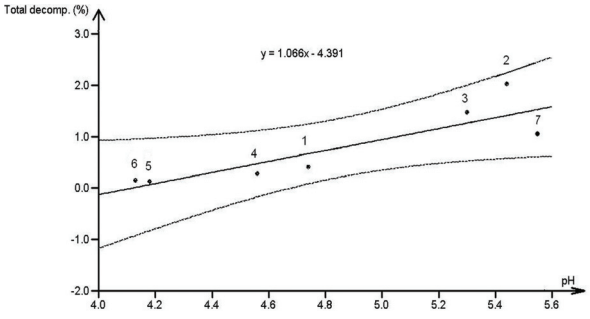

Figure 5 A plot of dependence of total decomposition of MP and PP, i.e. overall concentration of 4-HYB and 2,3-diOHPP, on the pH of SJ samples

preparation is thus satisfactory for a period greater than 5 years. Surprisingly, in the presence of BOA and GLY, the total decomposition of parabens did not correlate significantly with the period of storage of solutions. The linear correlation coefficient r was 0.532 while its critical value for n = 7 in one-sided test for p = 0.95 was 0.669 (27). The percentage of total decomposition of parabens correlated better with the pH measured 6 months before the HPLC analyses, the pH of the SJ samples ranged between 4.13 – 5.56 in samples 1 – 7 (n = 3 – see Table 4), the r value was 0.858, and r_{crit} (n = 7, p = 0.99) was 0.833 (Figure 5). It should be emphasized that the pH values of the samples did not change significantly during 6 months storage although they tended to decrease slightly (see Table 4).

Table 4 Values of pH of SJ samples 12 and 6 months before parabens degradation products assay

Sample number	Sample age [months]	pH value	Sample age [months]	pH value
1	56	4.71[a]	62	4.74[b]
2	-	-[c]	62	5.44[b]
3	47	5.51[a]	53	5.30[b]
4	-	-[c]	43	4.56[b]
5	2	4.25[a]	8	4.18[b]
6	9	4.28[a]	15	4.13[b]
7	4	5.61[a]	10	5.56[b]

[a] Arithmetic mean from n = 2
[b] Arithmetic mean from n = 3
[c] Not available at the time of the first pH measurement

Thus, in solutions containing 0.067% MP, 0.033% PP, 3.4% GLY and 2.0% BOA, the decomposition of parabens depended on differences in pH of the solutions rather than on the period of their storage. The decomposition was lower than in pure aqueous solutions stored and analyzed in the same manner as the solutions investigated in this study. The pH values of these aqueous solutions ranged between 6.2 – 6.8 (7) and were thus higher. It was not clear from the results of this study if 2,3-diOHPP was hydrolyzed into 4-HYB and GLY.

The presence of 2,3-diOHPP in the solutions under investigation was confirmed by ^{1}H-NMR spectroscopy of evaporation residues of ethyl acetate extracts of samples. In ^{1}H-NMR spectra of deuterium oxide extracts of such residues from samples No. 2 and 3, signals of chemical shift about 4.3 ppm which were assigned to hydrogens on the ester methylene (COOCH_2) of 2,3-diOHPP and those at about 4.05 ppm which were assigned to methine hydrogen of 2,3-dihydroxypropyl moiety (C*H*OH) were found. All other signals from 2,3-diOHPP were unfortunately either too weak or overlapped with much stronger signals of MP, PP or GLY (see Figure 6. For the corresponding fragment

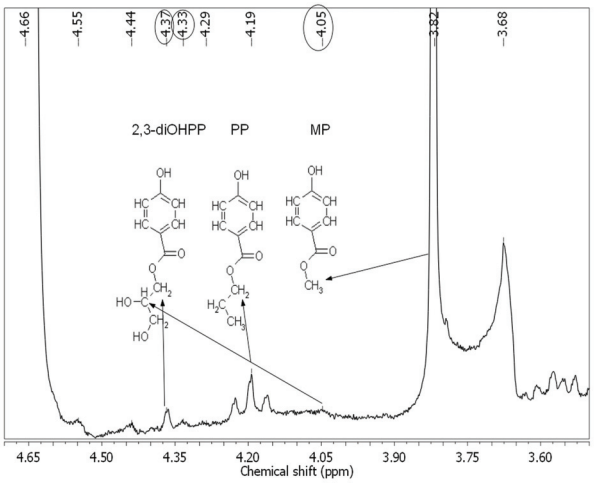

Figure 6 A detail of the ^{1}H-NMR spectrum of the evaporation residue of the ethyl acetate extract of the sample of SJ No. 3 measured in deuterium oxide – the region of aliphatic hydrogens. Chemical shifts of ester methylene and methine groups of 2,3-diOHPP are indicated

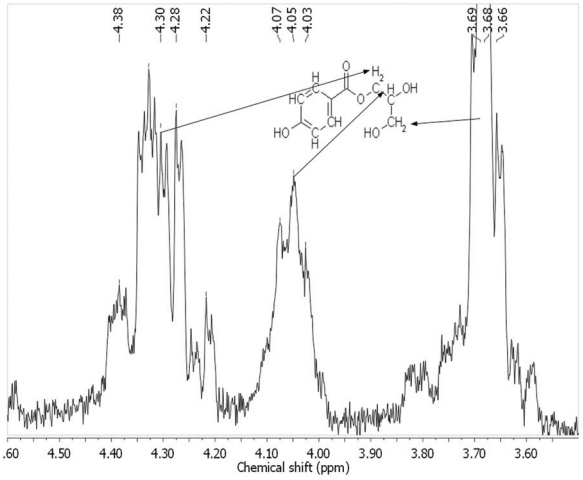

Figure 7 The "aliphatic region" of ^{1}H-NMR spectra of a saturated solution of 2,3-diOHPP in deuterium oxide. Particular multiplets are assigned to corresponding hydrogens by arrows

of ^{1}H-NMR spectrum of pure 2,3-diOHPP in deuterium oxide see Figure 7). No signals which could be assigned to 2,3-diOHPP were found in extracts from any other samples of SJ.

CONCLUSION

This study shows that alkyl 4-hydroxybenzoates in weakly acid aqueous solutions in presence of boric acid and glycerol are more stable than in pure water. Surprisingly it was also shown that the overall concentration of decomposition products correlates better with the pH value of a particular solution than with the period of its storage. In most samples, 2,3-diOHPP was determined as a product of transesterification of parabens with glycerol and, in two samples, its presence was also confirmed using ^{1}H-NMR spectroscopy.

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