



# Journal of Applied Sciences

ISSN 1812-5654

**science**  
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## Deterioration of Parabens in Preserved Magnesium Hydroxide Oral Suspensions

<sup>1,4</sup>Pirali Hamedani Morteza, <sup>2</sup>Fazeli Mohamad Reza, <sup>2</sup>Samadi Nasrin, <sup>3</sup>Nassireslami Ehsan,  
<sup>1</sup>Rezazadeh Shams Ali and <sup>1</sup>Mohsen Amini  
<sup>1</sup>Department of Medicinal Chemistry,  
<sup>2</sup>Department of Drug and Food Control, Faculty of Pharmacy,  
<sup>3</sup>Pharmaceutical Sciences Research Center, Medical Sciences/University of Tehran,  
<sup>4</sup>Food and Drug Laboratory Research Center, Tehran, Iran

**Abstract:** In this study the chemical stability of methyl and propyl paraben in magnesium hydroxide suspension (pH about 10) was investigated using both real time (32 months incubation at 25±2°C with humidity of 60±5% RH) and also accelerated (3 months incubation at 40±2°C with humidity of 70±5% RH) methods. Preparation with no added preservative was used as control. Concentrations of methyl and propyl paraben decreased to levels lower than the reported MIC values after the first month and fifth months of real time study, respectively. Preservative effectiveness testing against the Pharmacopeal challenging microorganisms in both suspensions with or without parabens conformed the US Pharmacopeia requirements for oral antacids. It is concluded that alkaline pH of the suspension without parabens could preserve the product against Pharmacopeial challenging microorganisms and incorporation of parabens did not add any antimicrobial activity to the test product.

**Key words:** Antacids, antimicrobial preservative, magnesium hydroxide

### INTRODUCTION

Antacids are agents that neutralize stomach acid containing at least one active acid neutralizing compound such as magnesium, aluminum or calcium salts. In general, liquid antacid suspensions are preferred to tablets or powders, since they are more rapidly acting and have a greater ability to neutralize gastric acid. Although the alkaline pH of liquid antacids is a critical factor in their acid neutralizing capacity and also controlling the microbial growth within the suspension, these formulations could still be susceptible to microbial contamination thus the need for an adequate preservative policy towards oral antacid suspensions should be recognized (Beveridge, 1998; Bloomfield, 1996). Under most circumstances, the ability to restrict microbial growth can be aided by addition of a preservative. The choice of a suitable preservative should be made as a function of pH and compatibility with other ingredients (Bloomfield and Sheppard, 1996). No approved preservative systems function optimally at pH higher than 8.5. The alkyl esters of parahydroxybenzoic acid especially methyl and propyl esters are still the most widely used preservatives in oral preparations because they offer the most efficacious options such as relative

broad spectrum of activity, low toxicity and good stability (Morris and Leech, 1996; Soni *et al.*, 2002, 2001), but they undergo hydrolysis in alkaline pH (Shija *et al.*, 1992).

To achieve adequate preservative levels throughout the shelf life of a product with an alkaline pH, we investigated the extent of methyl and propyl paraben hydrolysis in magnesium hydroxide suspension using both real time (32 months incubation at 25±2°C with humidity of 60±5% RH) and also accelerated (3 months incubation at 40±2°C with humidity of 70±5% RH) methods.

### MATERIALS AND METHODS

All chemicals and culture media were obtained from Merck Co. Methyl paraben, propyl paraben and ethyl paraben were further purified by recrystallizing from ethanol-water admixture. P-hydroxy benzoic acid was recrystallized from water.

**HPLC analysis:** Methyl, propyl and p-hydroxy benzoic acid were determined by HPLC method (Waters 600 pump, Waters 486 UV detector, Waters 746 integrator and C18, 150×3.9 mm Novapack column). The injection volume was 25 µL. Detection was performed at 254 nm. The mobile

phase was water and methanol in 50:50 ratio with the flow rate of 1 mL min<sup>-1</sup>. Ethyl paraben was used as internal standard.

**Chemical analysis:** The stability study was evaluated using both accelerated (40±2°C, 70±5% RH) and real time (25±2°C, 60±5% RH) conditions and amounts of methyl and propyl parabens and p-hydroxy benzoic acid were estimated. The slope of regression line of concentration log versus time represented the rate constant (k) of the decomposition (Gullory and Poust, 1996; Mechkovski, 1991).

**Microbiological methods**

**Sample preparation:** The test samples, magnesium hydroxide 8% suspensions, were prepared by diluting a 30% magnesium hydroxide paste with distilled water. Two kinds of samples were prepared, suspensions containing a mixture of methyl and propyl paraben (1.2 mg mL<sup>-1</sup> methyl paraben and 0.6 mg mL<sup>-1</sup> propyl paraben) and also preparations without any parabens.

**Test organisms:** Cultures of following microorganisms were used for the challenge test according to US Pharmacopeia 27 (10):

- Staphylococcus aureus* PTCC 1112 (ATCC 6538)
- Pseudomonas aeruginosa* PTCC 1074 (ATCC 9027)
- Escherichia coli* PTCC 1338 (ATCC 8739)
- Aspergillus niger* PTCC 5011 (ATCC 16404)
- Candida albicans* PTCC 5027 (ATCC 10231)

**Antimicrobial preservative effectiveness test:** The preservative effectiveness testing was carried out according to the US Pharmacopeia 27 guidelines (10).

The magnesium hydroxide suspensions with or without parabens were inoculated separately with 10<sup>5</sup>-10<sup>6</sup> cfu mL<sup>-1</sup> of each challenge microorganism having cultured under the conditions described in the US Pharmacopeia (10). Recovery of viable microorganisms was performed on 7, 14 and 28 days of experiment by diluting serially 1 mL of the test product into 0.9% sterile NaCl. Samples of 1 mL from each dilution were plated onto Tryptone Soy Agar (TSA) media for bacteria and

incubated at 30-35°C for 3 days. In term of the test fungi, Sabouraud Dextrose Agar (SDA) media was used and the resulting plates were incubated at 20-25°C for 3-5 days. The number of viable challenging organisms was determined and the results were interpreted according to the US Pharmacopeia 27.

**In use test:** Six samples from each type of magnesium hydroxide suspension (with or without parabens) were consumed by 6 individuals for a period of two weeks. The test samples were maintained at room temperature and about 10 mL suspension was drunk off directly from magnesium hydroxide bottle, three times a day before each meal. The bioburden of magnesium hydroxide suspensions were determined immediately after preparation and at 7 and 14 days during consumption.

**RESULTS**

**Chemical analysis:** The rate of methyl and propyl paraben hydrolysis in accelerated and real time studies showed a pseudo first orders kinetic (Fig. 1 and 2). The rate of methyl paraben decomposition was higher than propyl paraben in both accelerated and real time studies. By the end of 12 weeks of accelerated study about 1 log decrease in methyl paraben concentration was observed, while that for propyl paraben was about 0.3 log. The hydrolysis rate of parabens in real time study was slower than accelerated conditions, showing about 1 log decrease in methyl paraben and propyl paraben concentrations after 48 weeks and more than 128 weeks, respectively. The pH of suspensions reduced from 9.8 to 9.5 during 32 months of real time study.

**Microbiological tests**

**Antimicrobial preservative effectiveness test:** Both of magnesium hydroxide formulations containing parabens and without parabens eradicated the inoculated bacteria more than 4 log at 7 days. In both formulations the number of *A. niger* and *C. albicans* at 7 days were at least 2 and 3 log lower than the initial counts, respectively. The bacterial and fungal counts at 14 and 28 days did not show any increase compared to those at 7 days counts (Table 1).

**Table 1: Antimicrobial effectiveness testing of magnesium hydroxide suspensions with or without parabens**

Organisms	Product with parabens							Product without parabens						
	Count (cfu mL <sup>-1</sup> )				Log reduction			Count (cfu mL <sup>-1</sup> )				Log reduction		
	Starting day	7th day	14th day	28th day	7th day	14th day	28th day	Starting day	7th day	14th day	28th day	7th day	14th day	28th day
<i>S. aureus</i>	2×10 <sup>6</sup>	<100	<100	<100	>4	>4	>4	6×10 <sup>6</sup>	<100	<100	<100	>4	>4	>4
<i>E. coli</i>	3×10 <sup>6</sup>	<100	<100	<100	>4	>4	>4	2×10 <sup>7</sup>	<100	<100	<100	>5	>5	>5
<i>P. aeruginosa</i>	8×10 <sup>6</sup>	<100	<100	<100	>4	>4	>4	9×10 <sup>6</sup>	<100	<100	<100	>4	>4	>4
<i>A. niger</i>	5×10 <sup>4</sup>	<100	<100	<100	>2	>2	>2	5×10 <sup>4</sup>	<100	<100	<100	>2	>2	>2
<i>C. albicans</i>	6×10 <sup>5</sup>	<100	<100	<100	>3	>3	>3	7×10 <sup>5</sup>	<100	<100	<100	>3	>3	>3

Table 2: Microbial quality of magnesium hydroxide suspensions with parabens (6 samples) or without parabens (6 samples) during in-use test

Time	With parabens		Without parabens	
	Total aerobic microbial count (cfu mL <sup>-1</sup> )	<i>E. coli</i> (10 mL <sup>-1</sup> )	Total aerobic microbial count (cfu mL <sup>-1</sup> )	<i>E. coli</i> (10 mL <sup>-1</sup> )
Starting day	<100	Absent	<100	Absent
7th day	<100	Absent	<100	Absent
14th day	<100	Absent	<100	Absent

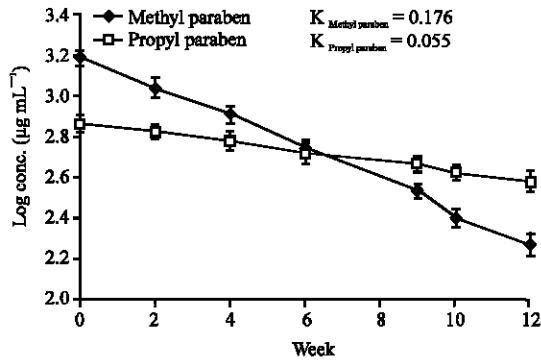


Fig. 1: Hydrolysis of methyl paraben and propyl paraben in accelerated conditions of 40±2°C, 70±5% RH

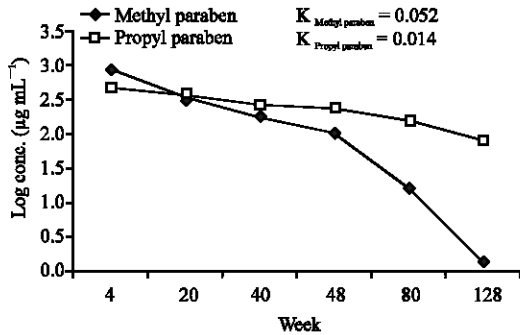


Fig. 2: Hydrolysis of methyl paraben and propyl paraben in real time conditions of 25±2°C, 60±5% RH

**In use test:** The results of in-use microbiological testing of magnesium hydroxide suspensions containing parabens and without parabens at 7 and 14 days were similar and total aerobic viable counts of lower than 100 cfu mL<sup>-1</sup> were obtained for all 12 suspensions. *Escherichia coli* was not detected in any samples (Table 2).

**DISCUSSION**

Sufficient preservation of liquid and semi liquid formulations in pharmaceuticals and cosmetics is essential for the efficacy and safety of these products. In this study the chemical stability of paraben esters in magnesium hydroxide suspension with initial pH of 9.8 was evaluated.

The methyl and propyl paraben hydrolysis showed a pseudo first order kinetic in both accelerated and real time conditions. While decomposition rate constant (k) in accelerated conditions was higher than real time, the rate of methyl paraben hydrolysis was about 3 times faster than propyl paraben in both conditions.

The methyl and propyl paraben concentrations were lower than the reported MIC values of parabens after the first and the fifth months of real time study, respectively (Table 3). It was supposed that suspensions containing parabens will not be further preserved against microbial contamination after first month of preparation. A reduction in pH values during 32 months of study was related to benzoic acid production following parabens hydrolysis.

Preservative effectiveness testing results were similar in both suspensions with or without parabens and conforms the US Pharmacopeias requirements for oral antacids which requires no increase from the initial calculated counts at 14 and 28 days with lower initial inoculation of 10<sup>3</sup>-10<sup>4</sup> cfu mL<sup>-1</sup> of the test product at starting date (10). It was concluded that alkaline pH of magnesium hydroxide suspension without parabens could preserve the product against Pharmacopeias challenging microorganisms and incorporation of parabens did not add any antimicrobial activity to the product.

Although the results of in-use testing was in accordance with the challenge test results, indicating good microbiological stability of antacid suspensions with high pH and without any parabens, there are some reports of their microbial contamination (Vanhaecke *et al.*, 1987). To achieve adequate preservative levels throughout the shelf life of a product with an alkaline pH, higher initial levels of parabens, due to their poor solubility in water, is practically impossible and usefulness. Lowering the pH of the antacid suspensions to inhibit degradation of the parabens, by addition of large amounts of buffers such as organic acids, will adversely affect the acid neutralizing capacity of the antacid. Accordingly, there is still a need for an effective preservative system in pH levels above 7 which inhibits microbial contamination over the shelf life of liquid antacid preparations.

Table 3: Methyl and propyl paraben concentrations and MIC values

Preservative	Conc. real time study ( $\mu\text{g mL}^{-1}$ )			Conc. accelerated study ( $\mu\text{g mL}^{-1}$ )	MIC ( $\mu\text{g mL}^{-1}$ )
	1 month	5 months	32 months	3 months	
Methyl paraben	724.43	302.00	1.32	186.21	1000-2000
Propyl paraben	524.81	371.53	85.11	380.19	500-1000

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