

EVALUATION OF THE INGESTION OF PARABENS DURING THE USE OF DENTAL CARE PRODUCTS BY HEALTHY HUMAN VOLUNTEERS

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Abstract

Parabens are effective antibacterial agents for the cosmetic and food industries. Methyl and ethyl parabens have been considered safe by regulatory authorities and an acceptable daily intake (ADI) of 10 mg/kg bw was established. Recently, animal studies linked negative health effects with the use of heavier chained parabens. Our study focused on the safe use of methyl and propyl parabens in dental care products, such as mouthwash and toothpaste. A HPLC-UV method was developed for quantifying methyl and propyl parabens in dental care products and oral fluids. The results of the study, conducted on volunteers, showed that the ingestion of methyl paraben, using mouthwash and toothpaste, was very low (only 1-2% of the ADI). The ingested amount of propyl paraben is about the same, since these compounds are coexisting ingredients of dental care products in similar concentrations. This could be considered toxicologically significant, if further studies would confirm the presumption that propyl paraben can be hundred times more toxic than methyl paraben.

Rezumat

Parabenii sunt agenți antimicrobieni eficienți pentru industria cosmetică și alimentară. Metil și etil parabenii au fost considerați siguri de către autoritățile de reglementare, având stabilită o doză zilnică acceptată (DZA) de 10 mg/kg corp. Recent, studiile pe animale au evidențiat efectele negative asupra sănătății în urma administrării parabenilor cu radical alchil lung și o DZA nu a fost stabilită încă. Studiul nostru s-a concentrat pe siguranța utilizării metil și propil parabenilor în produsele de îngrijire dentară. A fost dezvoltată o metodă HPLC-UV pentru determinarea metil și propil parabenilor din produse de îngrijire dentară și fluide din cavitatea bucală. Rezultatele studiului, realizat pe voluntari, au demonstrat că ingestia de metil paraben, folosind apă de gură și pastă de dinți, este foarte scăzută (doar 1 - 2% din doza zilnică). Cantitatea ingerată de propil paraben este asemănătoare, deoarece acești compuși coexistă în concentrații similare, ca și ingredientele ale produselor stomatologice. Acest lucru ar putea fi considerat semnificativ din punct de vedere toxicologic, dacă studii suplimentare ar confirma prezumția că propil parabenul poate fi de sute de ori mai toxic decât metil parabenul.

Keywords: paraben, mouthwash, toothpaste, HPLC, acceptable daily intake (ADI)

Introduction

Parabens, alkyl esters of p-hydroxybenzoic acid, are widely used as preservatives in the food, cosmetic and pharmaceutical industries in order to inhibit microbial growth and extend product's shelf life [9, 13, 14].

Methyl and propyl parabens are currently used in mouthwash products recommended to persons with sensitive teeth. Since parabens are not included in the strategies of dentine hypersensitivity treatment [15], they are probably included in the formulation of mouthwash products due to their antibacterial properties. Nevertheless, their presence in these products may also have therapeutic effects, since there are studies that prove their efficacy against strains of bacteria with deleterious effects in the oral cavity. A synergistic antibacterial effect was

found in the case of several combinations of parabens on immobilized and planktonic *Streptococcus sobrinus* [4].

Parabens were shown to inhibit glycolysis in the cariogenic dental plaque bacterium *Streptococcus mutans*. In this way, the ability of the bacterium to lower the pH will be reduced. The lethal action was attributed, at least in part, to the irreversible damage to key enzymes, such as those of the phosphotransferase system [11]. Less pronounced beneficial effects were found in other studies [18].

A possible deleterious effect of parabens is their weak estrogenic property, which was proven in recently published *in vitro* studies [19]. Concerning propyl paraben, reduced testosterone levels, reduced numbers of sperm cells and impaired spermatogenesis were observed *in vivo* at doses of 10 mg/kg bw/day, which is considered far lower

than the no-observed-adverse-effect level (NOAEL) in the case of methyl and ethyl parabens [2, 20]. However, not every animal study succeeded to prove that parabens possess estrogenic properties, so the debate is still open regarding this issue [1, 6]. Human studies provided conflicting results regarding the estrogenic activity of parabens [1, 17].

Beside their estrogenic properties, inhibition of mitochondrial functions was also observed [3].

International regulatory agencies consider methyl and ethyl parabens as safe cosmetic ingredients [8]. European Food Safety Agency (2013) established an ADI of 10 mg/kg bw for methyl and ethyl parabens, while in the case of propyl paraben there is insufficient data to establish an adequate ADI [20].

There are many papers focusing on skin exposure and absorption of parabens from cosmetics and their fate in organism after the absorption [1, 5, 12]. Even if there is a strong interest in studying the skin exposure as a consequence of using cosmetics, to our knowledge, there have been no papers dealing with the fate of parabens ingested *via* dental care products. The purpose of this study was to assess, with the help of healthy human volunteers, the amounts of ingested methyl and ethyl parabens using paraben containing dental care products and to compare these amounts with the respective ADI values.

Many simple and effective HPLC-UV methods were published for the detection of parabens in cosmetic products, but none of them focused on simultaneous detection of methyl and propyl parabens from dental care products or saliva [5, 10, 16]. Therefore, we decided to develop an HPLC method suitable for the detection of methyl and propyl paraben from toothpaste, mouthwash and saliva.

Materials and Methods

Materials

Merck D-7000 HPLC system with diode array detector (DAD), LichroCART 250-4, Lichrospher 100, RP-18 (5 µm) column (Merck KgaA), disodium phosphate (Merck KgaA), phosphoric acid (Merck KgaA), HPLC quality water, methyl paraben reagent. Ph. Eur. (Merck), propyl paraben reagent. Ph. Eur. (Merck), internationally commercialised (paraben containing) mouthwash and tooth paste.

Methods

HPLC conditions: injected volume 100 µL (loop method); monitoring wavelength 254 nm; DAD peak purity limit 95%; retention time (RT) methyl paraben 6.08 min, RT propyl paraben 9.07 min. The gradient program is shown in Table I.

Table I
Gradient parameters

Time (minutes)	% acetonitrile	% 20 mM phosphate buffer pH = 2.5	Flow (mL/min)
0	40	60	1.0
7.5	70	30	1.0
12	70	30	1.0
12.1	40	60	1.5
15	40	60	1.5

Calibration curve: consisted of solutions of methyl and propyl paraben in the following concentrations: 0.1, 1.0, 5.0, 10.0, 15.0 µg/mL.

Parabens' detection in the products used by the volunteers: mouthwash was diluted in a 1:50 ratio with water, then filtered through an HPLC nylon syringe filter and 100 µL of the solution was injected in the HPLC system. In the case of toothpaste, 1 g toothpaste was carefully dispersed in 50 mL water, the solution was filtered and 100 µL of the solution were injected into the HPLC system.

Volunteer samples: twenty five healthy individuals were enrolled in this study, made with respect to international regulations (Helsinki Declaration of 1975) and with the approval of our University's Committee on Ethics. Written consent was also obtained from each volunteer and they were informed, prior to the experiment, about the purpose of the study and the products that will be used. All volunteers were requested to rinse their oral cavity with 10 mL of mouthwash for 30

seconds (as recommended by the manufacturer). The expelled residual fluids (mouthwash and saliva) were collected in a sterile 50 mL collection cup. On a second occasion (24 hours later) nine volunteers were requested to use the same mouthwash in the same amount but for only 10 seconds. At the same time, five volunteers were requested to brush their teeth with 1.3 - 1.5 g (exactly weighted) of tooth paste. The eliminated oral fluids were collected as mentioned above. The samples were stored at -20°C until analysis. Next, after being defrosted, the samples were diluted to 500 mL with HPLC quality purified water, filtered and 100 µL of the solutions were injected into the HPLC system.

Results and Discussion

Performance of the analytical method

Specificity: injection of blanks from dental care products (similar products without parabens) did

not yield peaks with similar retention times to those obtained with parabens. No interferences were observed in the case of blanks made with human saliva obtained from five volunteers who rinsed their mouth with 10 mL water. Peak purities were in all cases higher than 99%. A typical chromatogram for a biological sample is shown in Figure 1.

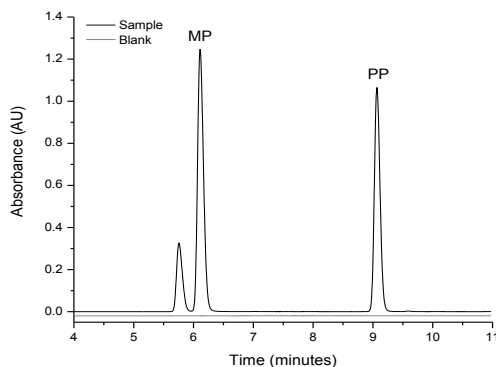


Figure 1.

Chromatograms of a sample obtained from a volunteer and a corresponding blank (MP-methyl paraben, PP-propyl paraben)

Linearity: intraday linearity testing yielded the following results (concentration is given in $\mu\text{g/mL}$, peak area in $\mu\text{V}\cdot\text{s}$): slope: 247 855 and 216 914 for methyl and propyl paraben, respectively; intercept: -8144 and -8136 for methyl and propyl paraben, respectively; coefficient of correlations: 0.9998 and 0.9997 for methyl and propyl paraben, respectively; the residuals showed no correlation with the theoretical concentration.

Recovery and precision: biological material (obtained identically as in the case of specificity testing) was spiked with methyl and propyl paraben in the concentration of 20 $\mu\text{g/mL}$ for each compound (five replicates were made). Recovery ranged between 89.8 - 105.7% and 89.7 - 106.3% for methyl and propyl paraben, respectively. The coefficients of variation were 7.3% and 10.7% for methyl and propyl paraben, respectively.

Limits of detection and quantification: limits of quantification for undiluted samples were 0.08 and 0.09 $\mu\text{g/mL}$ for methyl and propyl paraben, respectively. The limit of detection for undiluted samples was 0.2 $\mu\text{g/mL}$ for both compounds.

The HPLC-UV method used in this study, proved a fast and effective way to quantify methyl and propyl paraben from dental care products and from oral fluids. The achieved limits of detection and quantification, allow the measurements of persistence of these products in the oral cavity.

Ingestion of parabens via mouthwash and toothpaste

The amount of ingested parabens was calculated as the difference between the amount of parabens

introduced in the oral cavity with the mouthwash and the amount recovered in the fluids eliminated after rinsing. The determination of parabens from the mouthwash showed a content of 9.94 mg methyl paraben and 9.07 mg propyl paraben in 10 mL of mouthwash used by the volunteers.

The average amount of ingested parabens consecutive to the use of the mouthwash is shown in Figure 2.

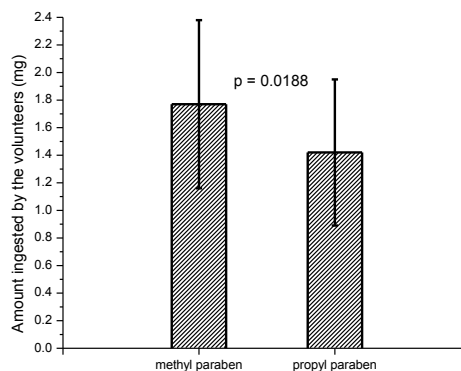


Figure 2.

Amounts of parabens ingested after using paraben containing mouthwash by the volunteers

The used toothpaste contained 0.49 mg/g methyl paraben and 0.43 mg/g propyl paraben. The ingestion of parabens consecutive to the use of toothpaste is 0.30 mg methyl paraben and 0.24 mg propyl paraben.

Influence of the rinsing time on the amounts of parabens ingested via mouthwash

Statistically significant difference was observed between the amounts of parabens ingested by the volunteers during the 30 seconds (as recommended by the manufacturer) and the 10 seconds of rinsing time. Interestingly, the simultaneous ingestion of methyl and propyl paraben was higher when the volunteers rinsed with the mouthwash for a shorter period of time (Figure 3).

Two different situations arise from this study. The first is the case of methyl paraben where an ADI value was established by international authorities, based on well-conducted animal studies. Compared with this ADI (10 mg/kg bw), which means an allowed amount of 600 mg for a 60 kg person, the ingestion brought by the use of the mouthwash (an average of 1.76 mg for single use and an expected 5.28 mg for a three times daily use) is less than 1%. Even if combined with other products containing methyl parabens in high amounts, the 1% extra amount could not be expected to bring an observable increase in toxic effect. In the case of toothpaste, almost half the amount of methyl paraben was not recovered after the volunteers brushed their teeth. In this case also, the amount ingested (0.3 mg for single use and 0.6 mg for

twice a day use) is insignificant compared with the accepted non-toxic dose. The ingestion of methyl paraben *via* the combined use of methyl paraben containing toothpaste and mouthwash can also be considered toxicologically insignificant compared with the ADI.

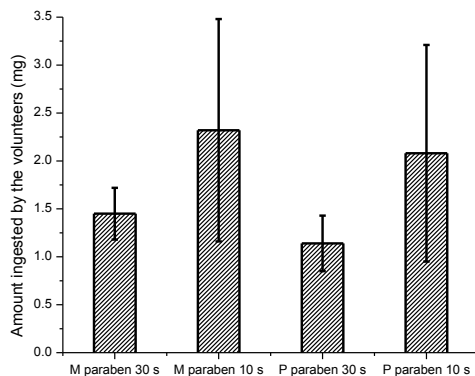


Figure 3.

Amounts of parabens ingested by eight healthy volunteers after two different rinsing times: 30 and 10 seconds

In the case of propyl paraben, even if the ingested amounts were comparable with the ones measured for methyl paraben, a safety evaluation is not possible because there is no currently ADI for this compound. Animal studies showed unwanted effects of propyl paraben on male experimental animals at doses of 10 mg/kg bw, which is 100 times lower than the NOAEL dose for methyl and ethyl parabens [2]. If further experiments will confirm this value, the ADI will be of a same order of magnitude with the doses ingested by the volunteers who used the dental care products that are commercially available. In this case using propyl paraben containing dental care products could become a health hazard.

Regarding the concentrations of parabens in the tested products, one can see that in the case of the mouthwash used by the volunteers, both compounds are present in concentrations that represent about 25% of the maximum allowed concentrations [2]. In the case of the toothpaste, the concentrations of parabens are half of those recorded for the mouthwash. This leads to the conclusion that in the case of methyl paraben, using dental care products that contain the maximum permitted amounts does not lead to an ingestion that exceeds or even approaches the ADI. In some cases, where an ADI is stated, it is accepted that a dose increase, even if significant, will not lead to unwanted effects if remains under the accepted value. This is not the case for propyl paraben for which an internationally accepted ADI is not approved yet and therefore, using more concentrated products could significantly increase the potential unwanted effects.

It is interesting to observe that reducing the rinsing time increases the ingestion of parabens in a statistically significant way. This could be explained by the diluting effect brought by the increased salivary secretion induced by the use of an intensely flavoured mouthwash. When rinsing time is the 30 seconds recommended by the manufacturer, saliva dilutes the mouthwash, and the fluid remained in the oral cavity will have a paraben concentration lower than the mouthwash itself. By reducing the rinsing time, the diluting effect will not be as intense, and as a consequence, the fluids retained in the mouth (in the same amounts as previously) will contain more parabens. However, the effect might be statistically significant but has no toxicological significance in the case of methyl paraben and impossible to evaluate in the case of propyl paraben.

Conclusions

Our results show that the use of dental care products containing methyl parabens are safe even if two such products (mouthwash and toothpaste) are used in the same day for more than one occasion. In the case of propyl paraben, a safety evaluation is difficult to make because an ADI has not been stated so far. If further animal studies will confirm the high potency of propyl paraben to cause deleterious health effects, the amounts of propyl paraben ingested during the use of the dental care products might be of toxicological significance. Unfortunately, all paraben containing products used for oral hygiene that are available in hypermarkets contain propyl paraben associated with methyl paraben. Products with safer parabens as ethyl paraben are not available, therefore it is advisable for manufacturers to modify their formulas in order to offer safer products to consumers. On the other hand, media campaigns against parabens should differentiate between safe (methyl and ethyl parabens) and unsafe parabens (propyl and butyl parabens). We consider that avoidance of all parabens is inadequate since efficient and safe products could be replaced with more unsafe ones.

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