Research Article

SIMULTANEOUS ESTIMATION OF
P-HYDROXYBENZOIC ACID AND ITS ESTERS IN WASH-OFF / LEAVE-ON COSMETIC PRODUCTS BY HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Parabens, alkyl esters of p-hydroxybenzoic acid, including methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP), have been widely used as antimicrobial agents. Recently, parabens in cosmetic products have received keen attention, because the elevated amounts of parabens in topical products have been shown to induce allergic contact dermatitis to cancer. The potential harm of parabens to consumers increases especially when consume in large quantities. This demonstrates that any of the parabens from the long-term, low-dose levels to which humans are exposed can be absorbed and retained in human body tissues without hydrolysis by tissue esterases to the common metabolite p-hydroxybenzoic acid. So the objective of our study is to estimate simultaneously the p-hydroxybenzoic acid and its esters in commercial wash-off (Shampoo)/leave-on (Lipsticks) cosmetic products by high-performance thin layer chromatographic method. Eleven brand of shampoos analysed for the estimation. All the products are contains the parabens above the limit. In none of the labels of the products the name and quantity of the preservatives were indicated except Garnier Fructis brand in which use of methyl paraben was indicated on the label. In cosmetics methyl and propyl parabens are used as a preservative at a concentration of 0.4% and PHB at 0.8% w/w respectively. Regulatory authorities should regulate the limits and implement strict regulations on these products before it reaches the consumers.

KEY WORDS: Parabens, cosmetics, HPTLC

INTRODUCTION

Parabens, alkyl esters of p-hydroxybenzoic acid, including methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP), have been widely used as antimicrobial agents because they have no perceptible odor or taste, they do not produce discoloration, they are practically pH neutral and they do not cause hardening or “muddying” (Neidig and Burrell, 1944). The popular use of paraben preservatives in cosmetics and toiletries arises from their low toxicity, broad spectrum of activity, inertness, worldwide regulatory acceptance, biodegradability and low cost. An additional advantage is their excellent chemical stability in relation to pH (effective between pH 4.5–7.5) and temperature. Because of their stability at high temperature, products containing parabens can be safely autoclaved without significant loss of antimicrobial activity, as a result of hydrolysis (Maddox, 1982). The antimicrobial activity of parabens increases with increasing length of the alkyl chain of ester group, but in practice shorter esters are commonly used because of their high solubility in water. Combinations of two or more parabens are often used to increase the ability of the system to withstand microbial contaminations. Propyl and methyl paraben being the most commonly used (Berke et al., 1982; Jackson, 1992; Parker, 1984). The maximum permissible concentration for
each one of 0.4% (w/w) and total maximum concentration of 0.8% (w/w), expressed as p-hydroxybenzoic acid (Rastogi et al., 1995). Parabens can have multiple biological actions, but it is generally believed that their inhibitory effects on membrane transport and mitochondrial function processes are key for their actions. Parabens are approved as additives in topical products by the United States Food and Drug Administration (FDA, 1979). In the survey of 215 cosmetic products, it was found that one or more parabens were detected in 77% of rinse-off products and in 99% of leave-on products (Rastogi et al., 1995). Recently, parabens in cosmetic products have received keen attention, because the elevated amounts of parabens in topical products have been shown to induce allergic contact dermatitis (Reynolds, 1996). As estrogen is a major etiological factor in the growth and development of the majority of cases of human breast cancer, it has been proposed that the use of parabens in cosmetics, particularly underarm deodorants and antiperspirants, may contribute to the rising incidence of breast cancer. Clinical observations show a disproportionately high incidence of breast cancer in the upper outer quadrant of the breast, just the local area to which these cosmetics are applied. Recent results of the presence of paraben in tumor tissue (Darbre et al., 2004) along with results of the earlier described studies on the estrogenic potential of parabens lead to the hypothesis that parabens may be involved or contribute to the incidence of breast cancer. In a series of recent studies and reports, this hypothesis has been extensively discussed (Harvey and Darbre, 2004; Harvey, 2003; Harvey and Everett, 2004; Darbre, 2006). In a recent study, parabens were detected in a small number (n = 20) of tissue samples from human breast tumors (Darbre et al., 2004). The potential harm of parabens to consumers increases especially when consume in large quantities. This demonstrates that any of the parabens from the long-term, low-dose levels to which humans are exposed can be absorbed and retained in human body tissues without hydrolysis by tissue esterases to the common metabolite p-hydroxybenzoic acid. So the objective of our study is to estimate simultaneously the p-hydroxybenzoic acid and its esters in commercial wash-off (Shampoo)/leave-on (Lipsticks) cosmetic products by high-performance thin layer chromatographic method.

MATERIALS AND METHODS

Standard solutions:
Five milli grams of para hydroxy benzoic acid (PHB), 50 mg of methyl paraben (MP) and 25 mg of propyl paraben (PP) were dissolved in a mixture of 50 ml acetone - ethyl acetate (2:1) and then it was diluted in the ratio of 1:10. From this further dilutions were made to get the concentration of 0.05 to 0.35% of PHB, 0.5 to 3.5% of MP and 0.25 to 1.75% of PP and its area was measured to plot a calibration curve.

Sample preparation:
One gram of the each cosmetic preparation was dissolved or suspended in 40 ml acetone - ethyl acetate (2:1) mixture at 40°C. Let it be cooled to room temperature. It was filtered and rinsed with 7 ml acetone - ethyl acetate (2:1) mixture and the final volume was made up to 50 ml. These samples were analyzed by HPTLC instrument.

Chromatographic conditions:
Precoated HPTLC plates silica gel MERCK 60 F 254, 20 x 10 cm, Bandwise with CAMAG Linomat, 8 mm band length,
distance between bands 4 mm, distance from side edge 22 mm, distance from bottom edge 8 mm. Plate was development in CAMAG Twin Trough Chamber 20 x 10 cm, without pre-equilibration. Developing solvent: pentane - dichloromethane - acetic acid (25:25:3). Solvent migration distance about 40 mm (³10 min); after chromatography, dry plate 3 min with hair dryer. Densitometric scanning in CAMAG Scanner with CATS software, deuterium lamp, scanning by absorbance at 255 nm; monochromator bandwidth 10 nm, slit dimensions 0.3 x 5 mm (Thomassin et al., 1997; Zimmermann, 1989; Camag, 1998). Several investigators reported methods for determination of parabens in cosmetics, food products and pharmaceuticals (Mahuzier et al., 1927; Wang and Chang, 1998; Ali et al., 1999; Kreuz et al., 1999; Driouich et al., 2000; Lin Yu et al., 2000; Labat et al., 2000).

RESULTS
Out of eleven shampoos analyzed, paraben was found in 3 brands such as Head & Shoulders, Clinic All Clear and Garnier Fructis. In Head & Shoulders 11.8 % w/w of methyl paraben was found, Clinic All Clear was found to contain 11.8 % w/w of PHB and in Garnier shampoo 0.2% w/w of methyl paraben was found be to present. Except Garnier brand the other 2 brands namely Head & Shoulders (11.8 % w/w) and Clinic All Clear (11.8 % w/w) contained parabens much higher than the permissible limit (0.8% w/w). Out of 14 brand lipsticks identified with paraben content, Aviva was found with highest quantity of 8.58% w/w PHB, which was very high than the permissible quantity of 0.8% w/w. The other brand Revlon contain 1.08 % w/w of PHB, 1.94 % w/w of MP and 0.71 % w/w of PP; all these were above the permissible limit (0.4%w/w) individually and the total amount of 3.73 % w/w as a preservative. Lakme and Elle-18 has 1.89 and 1.06 % w/w of PP, which was above the permissible limit of 0.4% individually. Lakme also had 0.82 % of PHB and Elle-18 has 0.38 % of MP. Tips & Toes had 1.29 and 1.32% w/w of PHB and PP respectively, total of 2.61% which was also very high individually and in combined form as a preservative in cosmetic formulations. In figure 1 & 2 HPTLC peaks of parabens in Wedding Bells and Aviva brand are given.
DISCUSSIONS

The alkyl esters of p-hydroxybenzoic acid (parabens) up to 0.8% are widely used as preservatives in many thousands of pharmaceutical, food and cosmetic products. Paraben(s) are used either singly or in combination to exert the intended antimicrobial effect. Parabens are particularly useful against molds and yeasts. These substances can have multiple biological effects, but it is generally considered that their inhibitory effects on membrane transport and mitochondrial function process are key for their action. The parabens meet several criteria of an ideal preservative, as they have a broad spectrum of antimicrobial activity and are relatively non-irritating, non-sensitizing, and biodegradable with no perceptible odor and taste. Moreover parabens have excellent chemical stability in relation to pH. Antimicrobial activity of parabens increase as the chain length of the ester group increases, but since solubility decreases with increasing chain length, the lower esters (methyl and propyl) are of practical choices as preservatives. Products containing parabens may contact the skin, hair and scalp, lips, mucosae (oral, ocular and vagina), axillae and nails. Products containing parabens may be used on an occasional or daily basis and their use may extend over a period of years (Soni et al., 2002). A survey of 215 cosmetic products found that parabens were used in 99% of leave-on cosmetics products and in 77% of rinse-off cosmetics. Total paraben content in paraben-positive cosmetic products was 0.01–0.87% (Rastogi et al., 1995). Animal studies have shown that parabens are quickly absorbed from the gastrointestinal tract and from the blood, hydrolyzed to p-hydroxybenzoic acid, conjugated and the conjugate excreted in urine. Parabens can also be absorbed rapidly through intact skin. Human skin has been shown to possess isozymes of carboxylesterase and it has been suggested that this enzyme activity would result in hydrolysis of dermally applied paraben esters to p-hydroxybenzoic acid in the skin and this can also influence the absorption. These paraben preservatives are
Endocrine disruptors and are found to possess oestrogenic activity which was proved by *in vitro* and *in vivo* studies (Lobemeier, 1996; Bando *et al*., 1997). **Eleven brand of shampoo** were used for analysis. In none of the labels of the products the name and quantity of the preservatives were indicated except Garnier Fructis brand in which use of methyl paraben was indicated on the label. In cosmetics methyl and propyl parabens are used as a preservative at a concentration of 0.4% and PHB at 0.8% w/w respectively (Oishi, 2002; EFSA, 2004; Zhang *et al*., 2005). As estrogen is a major etiological factor in the growth and development of the majority of cases of human breast cancer, it has been proposed that the use of parabens in cosmetics, particularly underarm deodorants and antiperspirants, may contribute to the rising incidence of breast cancer. Clinical observations showed a disproportionately high incidence of breast cancer in the upper outer quadrant of the breast, just the local area to which these cosmetics are applied. In a recent study, parabens were detected in a small number (n = 20) of tissue samples from human breast tumors (Darbre *et al*., 2004). Out of the parabens detected, methyl paraben represented approximately 60% of the total parabens. Results of the presence of paraben in tumor tissue along with results of the earlier described studies on the estrogenic potential of parabens lead to the hypothesis that parabens may be involved or contribute to the incidence of breast cancer. In a series of recent studies and reports, this hypothesis has been extensively discussed (Harvey and Darbre, 2004; Harvey, 2003; Harvey and Everett, 2004; Darbre *et al*., 2004; Darbre, 2006). In young boys who are exposed to these parabens before puberty, they may cause a statistically marked increased risk for birth defects and reproductive abnormalities. Meanwhile, among young women, one disturbing trend is premature sexual development (Oishi, 2002). From these we could understand the problems with paraben preservatives in cosmetics especially even within the permissible limit. Recently, a study in China has reported paraben content in cosmetics, which states that, Avon brand contain, MP as preservative at a concentration of 0.1033 %, Adidas brand has PP at 0.033% and Clean & Clear brand contains mixture of MP, EP, PP and BP as preservatives at a concentration of 0.0317%, 0.0172%, 0.0114% and 0.0226% respectively (Zhang *et al*., 2005). As it mentioned earlier, continuous usage of paraben containing cosmetics are suspected to cause mild skin reaction to breast cancer even if they are present in a permissible limit; our results has shown much higher amount of parabens in 2 brand shampoos. Even though shampoos are coming under the category of “rinse off” cosmetics, entry of these parabens in to the blood circulation through scalp and skin are possible. This demonstrates that any of the parabens even at low-dose concentration for a long term exposure could lead to undesirable effects.

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