A review on various analytical method developments for the identification of methyl paraben present in cosmetics

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ABSTRACT
Methyl paraben is methyl ester of P-hydroxy benzoic acid. Its use is limited in European countries to concentration range is up to 0.4%, as per USFDA guidelines concentration ranging from 0.01 to 0.3%. So the present review is aimed to study the importance and amount of methyl paraben present in cosmetic preparations. Various analytical methods are employed to determine the methyl paraben and its concentration. The methods like spectrophotometric method and further determination of paraben in bulk material by HPLC is clearly the most suitable technique if complex samples are involved. Electrokinetic capillary electrophoresis is reported for determination of methyl paraben in cosmetics using fused silica capillary column and UV detection at 220nm. A spectrofluorimetric method for determination of methyl paraben based on derivatization made with labeling reagent danzyl chloride. UPLC and flow injection analysis are other analytical methods for determination of methyl paraben concentration in cosmetics.

Keywords capillary column; electrophoresis; HPLC; methyl paraben; spectrofluorimetry; UV.

INTRODUCTION
Preservatives are substances that commonly added to various foods and pharmaceutical products in order to prolong their shelf life. The addition of preservatives to such products, especially to those that have higher water content, is essential for avoiding alteration and degradation by microorganisms during storage (K. Parfitt et al., 1999).

Other ingredients are also utilized in preparing the desired dosage form of a drug substance. Some of these agents may be used to achieve the desired physical and chemical characteristics of the product or to improve its appearance, odour and taste. In each instant, the added ingredient must be harmless in the amount used; does not exceed the minimum quantity required to provide its intended effect; its presence does not impair the bioavailability, the therapeutic efficacy or safety of the official preparation, and does not interfere with analysis and tests prescribed for determining compliance with the pharmacopeias standards (M. C. Boyce et al., 2001).

Classification of preservatives
Preservatives are classified into two main classes: antimicrobial preservatives and antioxidants.

A. Antimicrobial preservatives
Antimicrobial preservatives are included in the preparations to kill or to inhibit the growth of microorganisms inadvertently introduced during manufacture or use. They are used in sterile preparations such as eye drops and multidose injections to maintain sterility during use. They may be also added to aqueous injections that cannot be sterilized in their final containers and have to be prepared using aseptic precautions. Preservatives are also used in cosmetics, foods, and non sterile pharmaceutical products such as oral liquids and creams to prevent microbial spoilage. They are not used indiscriminately, and preparations that should not contain preservatives include; injection into cerebrospinal fluids, eye or heart (H. Y. Hang et al., 2003).

The British pharmacopoeia (the National Formulary XIX) stated that the addition of antimicrobial preservatives to radio-pharmaceutical preparations in multidose containers is not obligatory unless their addition is prescribed in the monograph.

Antimicrobial preservatives are classified into two main sub-groups: anti-fungal preservatives and anti-bacterial preservatives. Anti-fungal preservatives include compounds such as benzoic and ascorbic acids and their salts, and phenolic compounds such as methyl, ethyl, propyl and butyl p-hydroxybenzoate (parabens). Anti-bacterial preservatives include compounds such as quaternary ammonium salts, alcohols, phenols, mercury salts and biguanidines.

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B. Antioxidants

Antioxidants are included in the pharmaceutical products to prevent deterioration from oxidation. Antioxidants are classified into 3 groups. The first group is known as true antioxidants, or anti-oxygen, probably inhibit oxidation by reacting with free radicals blocking the chain reaction. Examples are alkylgallates butylated hydroxyanisol, butylated hydroxytoluene, nordihydroguaiaretic acid and the tocopherols. The second group consists of reducing agents; these substances have lower redox potentials than the drug or adjuvant which they are intended to protect, and are therefore, more readily oxidized. Reducing agents may act also by reacting with free radicals. Examples are ascorbic acid, the potassium and sodium salts of sulphurous acid. The third group consists of antioxidant synergists which usually have little antioxidant effect themselves but probably enhance the action of antioxidants in the first group by reacting with heavy metal ions which catalyze oxidation. Example of antioxidant synergists are citric acid, edetic acid and its salts, lecithin and tartaric acid (W. J. Reilly et al., 2000)

Parabens, which are commonly found in skin creams and other beauty products, glues and some foods, have been around since the 1930s. They’re currently the most widely-used preservatives in cosmetic, pharmaceutical and industrial products. Methyl and ethyl parabens are the most frequently used parabens and, with the exception of water, the most commonly used ingredients in cosmetic preparations. Parabens are popular because they are inexpensive, colourless, odourless and non-toxic, with a wide spectrum of antimicrobial activity – they’ve been used as preservatives for over 80 years.

Antimicrobial preservatives are used in cosmetics, foods, beverages and non-sterile pharmaceutical products (such as oral liquids and creams) to inhibit the growth of micro-organisms involuntarily introduced during manufacture or use.

Methylparaben is a Antimicrobial preservatives with the chemical formula CH₃C₆H₄(OH)COO). It is the methyl ester of p-hydroxybenzoic acid. Methylparaben is found in several fruits, in particular blueberries (The Japanese Standards of Cosmetic Ingredients-with Commentary, 1984) where it acts as an antimicrobial agent.

Methylparaben is produced naturally and found in several fruits, primarily blueberries, along with other parabens. There is controversy about whether methylparaben or propylparabens are harmful at concentrations typically used in body care or cosmetics. Some studies of breast tumors show a buildup of methylparabens in the breast tissue. Methyl paraben is also estrogenic (Miebs et al., 1986). Methylparaben and propylparaben are considered generally recognized as safe (GRAS) for food and cosmetic antibacterial preservation. Methylparaben is readily absorbed from the gastrointestinal tract or through the skin (G. Popovic et al., 2003). It is hydrolyzed to p-hydroxybenzoic acid and rapidly excreted in urine without accumulating in the body. Acute toxicity studies have shown that methylparaben is practically non-toxic by both oral and parenteral administration in animals. In a population with normal skin, methylparaben is practically non-irritating and non-sensitizing; however, allergic reactions to ingested parabens have been reported. Methylparaben is not carcinogenic, mutagenic, teratogenic or embryo-toxic; in addition, it is negative in the uterotrophic assay.

Methyl paraben uses in cosmetics (M. Blanco et al., 1997)

- Parabens have been successfully used in cosmetics for more than 80 years.
- The use of Paraben, alone or in combination with other compounds, is well suited for the preservation of cosmetics.
- In most cosmetics, Parabens are used at very low levels ranging from 0.01 to 0.3%.
- The product containing Parabens may be used on an occasional or a consistent basis and their use may extend product life.

SAFETY MEASURES (S. Ouanes et al., 1998)

The safety has been proven by various studies (in vivo tests) as stated below:

- skin stimulating test
- reproduction toxicity studies
- carcinogenesis studies
- photo-contactsensitization and phototoxicity studies
- absorbing, metabolizing, and excreting studies
- acute chronic and subchronic toxicity studies

Table 1: Toxicity nature of methyl paraben

<table>
<thead>
<tr>
<th>TOXICITY</th>
<th>METHYL PARABEN</th>
</tr>
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<tbody>
<tr>
<td>Acute toxicity</td>
<td>&lt;2-8g/kg</td>
</tr>
<tr>
<td>Mutagenicity(Bacterium)</td>
<td>Negative</td>
</tr>
<tr>
<td>ADI</td>
<td>0-10mg/kg</td>
</tr>
</tbody>
</table>

The CIR again looked at the safety of parbens in cosmetics in 2003 and 2005 and again determined that parbens are safe as used in cosmetics (K. Shimizu et al., 1992). In July 2010 the CIR updated its safety assessment of parabens and determined the following are safe: Methylparaben: Up to 0.4% if used alone

According to the U.S. Food and Drug Association, the average amount of parabens in cosmetics is 0.01% to
0.3% (E. H. Girgis et al., 1984). They are in shaving creams, skin care, hair care, and personal care products. They are not used in major brands of deodorants or antiperspirants.

ANALYTICAL METHOD DEVELOPMENT USING VARIOUS METHODS

Spectrophotometric Method

UV-visible spectrophotometry is still considered to be a convenient and low cost method for the determination of preservatives. Several spectrophotometric and colorimetric methods have been reported for the determination of parabens in bulk material. While in pharmaceutical products, the preservatives and excipients prevent a direct conventional spectrophotometric analysis, due to severe spectral overlap. To overcome such an overlap, a second order derivative spectrophotometric method was developed by Popovic et al. for the determination of methyl parabens, propyl parabens and bifonazole in cream (Shabir et al., 2004). The method is based on measurements of acidic extract at 241.5 nm.

Partial least-squares calibration method for the UV-spectrophotometric determination of parabens and ketoprofen in a gel preparation (R. Hajkova et al., 2003) In that method, the sample was extracted into methanol and sodium hydroxide solution was added before measurement at 240-330 nm.

UV-derivative spectroscopic method has been described for the analysis of parabens and haloperidol in preparations (R. Hajkova et al., 2002). First-derivative spectroscopic method was also applied for the determination of parabens, benzyl alcohol and phenol in different pharmaceutical products. The measurements were made at 240-286 nm.

High Performance Liquid Chromatographic Method

High performance liquid chromatographic methods (HPLC) are clearly the most suitable technique if complex samples are involved. So, several HPLC methods were reported for the determination of parabens in pharmaceutical products. Regular or silanol-deactivated C18 or C8 columns coupled with UV detection are the most common configuration for the analysis of parabens in pharmaceutical products and cosmetics. This review refers only to the most recent HPLC methods for the determination of parabens in various pharmaceutical products.

Parabens in liquid pharmaceutical products were determined by RP-HPLC using C18 column. The mobile phase was a mixture of methanol-and phosphate buffer of pH 7.05. Detection was carried out at 254 nm (E. Koundourellis et al., 2000). RP-HPLC with C18 column was also reported for the determination of methyl and propyl parabens besides hydrocortisone and its degradation products in a topical cream (L. Labat et al., 2000). The mobile phase was a mixture of methanol-acetonitrile and water. Detection was carried out at 238 nm. A similar configuration, with a different pH mobile phases (pH 2.5), was used for the analysis of parabens with diclofenac sodium and its degradation products (H. Y. Huang et al., 2003). Another RP-HPLC configuration, with a different mobile phase (at pH 3.45 with glacial acetic acid), was used for the analysis of parabens with ambroxol expectorant. Detection was carried out using at 247 nm (R. Driouich et al., 2000).

Ultra Performance Liquid Chromatography

Methylparaben was obtained from Fluka, Switzerland. All the employed solvents were of HPLC grade and were obtained from Merck (Darmstadt, Germany). All other chemicals were analytical-reagent grade and deionized water was used to prepare all solutions.

UPLC instrument and conditions

The employed UPLC system was a Waters Acquity UPLC, consisting of a binary solvent manager, a sample manager with an integral column heater module, a solvent tray module and a photo-diode array (PDA) detector. The analyte was determined using a BEH C18 (2.1×150) mm, 1.7 μm, column, also from Waters. EmpowerTM software was used. The column temperature was maintained at 30 °C. The autosampler temperature was set to 6 °C. The mobile phase A was 100 % methanol and mobile phase B was 0.05 % phosphoric acid in 60 % methanol. The flow rate was 0.250 ml/min. A gradient program was used starting with 100 % mobile phase B, followed by a linear increase in phase A until 30 % in 1 min and then the percentage of mobile phase A was increased to 100 in the next 30 s. The column was eluted isocratically for 40 s and re-equilibrated for the next injection in 5s. The injection volumes were varied between 1 and 7 μl (partial loop method). The UV signal was detected as the max plot in the range 190–400 nm (sampling rate: 20 pts/s)[(B. Baalbki et al., 2002)]. The advantages of the sub-routines of the Empower software, such as purity check and library match, were used throughout the sample analysis.

The UPLC mobile phases were freshly prepared daily and filtered through a 0.22 μm membrane filter (Millipore).

Stock solutions

The initial stock solutions of methylparaben (= 1 mg·ml⁻¹) were prepared by dissolving measured amount of the analyte (approx. 0.01 g) in methanol (10 ml). Standard solutions were prepared by further dilution of the stock solutions with mobile phase B.

Sample preparation

The tested cosmetic products, including shampoo, shower gels, body lotions, balsams, body creams, sun creams, make-up removals, were obtained at local markets. A 5.0 ml volume of methanol was added to the cosmetic samples (0.50 g). The emulsions were
Capillary Electrophoresis Method

Electrokinetic capillary electrophoresis (CE) was reported for the determination of methyl, ethyl, propyl and butyl parabens in cosmetic preparations using fused silica capillary column and applied voltages of 22, 25, 27 and 30 kV respectively. The UV detection was carried out at 220 nm (P. E. Mahuzier et al., 2001). Micellar CE for the determination of 4-hydroxybenzoic acid and its methyl, ethyl and propyl esters (parabens) in liquid preparations using fused silica capillary column and an applied voltage of 30 kV with UV detection at 205 nm. Q. Zhang et al., 2005 reported the use of CE and HPLC methods for the determination of parabens in cosmetic products. CE was carried out using fused silica gel column and an applied voltage of 30 kV with UV detector at 290 nm.

Micellar Electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) were reported as analytical methods for the simultaneous separation and determination of preservatives in commercial drug products. The determined preservatives were parabens, benzoic acid, sorbic acid, imidurea, triclosan and dehydroacetic acid. Fused silica capillary column was used with an applied voltage of 25 kV. Detection was carried out at 200 nm (F. F. Cantwell et al., 1854) MEKC method was reported, for the determination of haloperidol tranquilizer, parabens and benzoic acid using fused silica capillary column and an applied voltage of 30 kV with UV detection at 200 nm. Imidurea and parabens, in ointments were determined by MEKC. Fused silica capillary column was used with an applied voltage of 30 kV. Detection was carried out at 200 nm (M. G. Soni et al., 2005).

MEEKC method was reported (R. L. Elder et al., 1984), for the determination of 4-hydroxybenzoic acid and its derivatives parabens using fused silica capillary column and an applied voltage of 11kV with UV detection at 200 nm (Anon et al., 1976).

Flow Injection Analysis

Recently, a flow injection–chemiluminescence (FIA-CL) method has been reported for the determination of methyl, ethyl, propyl and butyl parabens in food, pharmaceutical products and cosmetics (E. J. Routledge et al., 1998). The method is based on the fact that parabens greatly enhance the chemiluminescence reaction between the cerium (IV)-rhodamine system in strong sulphuric acid. The method has very low detection limits and wide dynamic range.

Spectrofluorimetric Method

A spectrofluorimetric method for the determination of methyl paraben based on derivatization with the labeling reagent dansyl chloride (DNS-Cl), is presented.
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