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# Determination of methylparaben from cosmetic products by ultra performance liquid chromatography

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*Abstract*: A new method for the determination of methylparaben by ultra-performance liquid chromatography (UPLC) was developed. Methylparaben is often used as preservative, alone or in combination with other parabens, being added to cosmetic products, pharmaceutical products and foods to avoid microbial contamination. Due to its widespread use and potential risk to human health, assessing human exposure to this compound is of interest. A good determination and quantification of methylparaben was developed with a gradient elution using a mixture of methanol and water (60:40, v/v) within 1.455 min. Under optimized conditions, the linear working range extends over two orders of magnitude with relative standard deviations of intra- and inter-day precision below 2.3 %, and a detection limit of 0.02 ng $\mu L^{-1}$ for methylparaben. The proposed method was successfully applied to the assay of methylparaben in cosmetic products with minimal sample preparation.

*Keywords*: UPLC; methylparaben; preservative; cosmetic products.

## INTRODUCTION

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.

Hydroxybenzoates (parabens) are alkyl esters of *p*-hydroxybenzoic acid with antibacterial and antifungal properties. While the antimicrobial activity increases with increasing alkyl chain length of the ester group, the aqueous solubility decreases, making the use of shorter chain esters more common because of their high solubility in water.[1] The activity may also be improved by combining two

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hydroxybenzoates with short alkyl chains. Methylparaben ($C_8H_8O_3$, molecular mass 152.14 g $mol^{-1}$) is used alone or in combination with other parabens in some preparations, as they act as synergists.

Due to their broad antimicrobial spectra with relatively low toxicity, good stability and non-volatility,[2] parabens are commonly used as preservatives to prevent alteration and degradation of cosmetics, pharmaceuticals and foods from microbial and fungal contamination[3] and to protect the consumers.

Nearly all types of cosmetics products contain parabens, individually or in combination, which may come in touch with the skin, hair, scalp, lips, *mucosae*, *axillae* and nails, being used daily or occasionally,[1] in over 13200 formulations.[4]

The European Economic Community (EEC) Directive stipulates that parabens are permitted in a concentration of up to 0.8 % in cosmetics, with a maximum concentration for each individual one of 0.4 % (w/w), expressed as *p*-hydroxybenzoic acid.[5]

The super scale use of preservatives in cosmetics can result in potential health risks. Most of the preservatives may be harmful to the consumers due to their potency to induce allergic contact dermatitis. Some studies have reported that all commonly used parabens possess estrogenic activity in several *in vitro* assays and in animal models *in vivo*.[6–10]

Humans are exposed to low-dose but long-term levels of parabens and this type of preservative can be absorbed and retained in human body tissues without hydrolysis by tissue esterases to the common metabolite *p*-hydroxybenzoic acid. Consequently, a highly selective and sensitive method is required for the detection of methylparaben in cosmetic products.

The aim of this study was to develop a new, very fast and rapid method for the detection and quantification of methylparaben, the paraben most frequently used in cosmetic products.

## EXPERIMENTAL

### *Reagents and chemicals*

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 (from Waters, Mildford, USA, *via* Hemtek Co., Belgrade, Serbia and Chromaktiv, Bucharest, Romania), 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 $C_{18}$ (2.1×150) mm, 1.7 μm, column, also from Waters. Empower™ 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 5 s. 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). 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 amounts 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 sonicated for 10 min, diluted to 10 mL and filtered through 0.22 μm Millipore membrane filters.

## RESULTS AND DISCUSSION

### *Optimization of the UPLC system*

Using various liquid chromatography methods, several mobile phases have been reported for the separation of parabens, such as methanol–phosphate–water,[11–13] methanol–acetate buffer,[14] methanol–water,[1,15–18] acetonitrile–water,[19] acetonitrile–phosphate buffer,[20] acetonitrile–ammonium acetate,[21] methanol–acetic acid–water,[22] and acetonitrile–methanol–water.[23] In this work, the mobile phase of methanol–water–phosphoric acid was found to be suitable for the detection and quantification of methylparaben. Using a gradient mobile phase composed of methanol and water (60:40, v/v), the retention time of methylparaben was 1.455 min. A chromatogram of methylparaben, with PDA detection at the max-plot obtained under these conditions is shown in Fig. 1. The compounds were monitored by measurement of the peak area of methylparaben and the standard, and the ratio of peak area was calculated.

### *Linearity*

Under the above-described optimum conditions, the calibration curve obtained with standard MP showed a good linear relationship in the interval 0.1–10 μg·mL$^{-1}$. A regression curve was constructed: $y = 2.07\times10^5x + 1.71\times10^3$, with $R > 0.9987$, where $x$ represents concentration in μg mL$^{-1}$ and $y$ represents the UPLC peak area, which was automatically measured by the UPLC instrument, and $R$ is the correlation coefficient. The detection limit for methylparaben, at a signal-to-noise ratio of three, was 0.02 ng μL$^{-1}$ and the limit of quantification was 0.06 ng μL$^{-1}$. The calculations were performed by the Empower$^{TM}$ program.

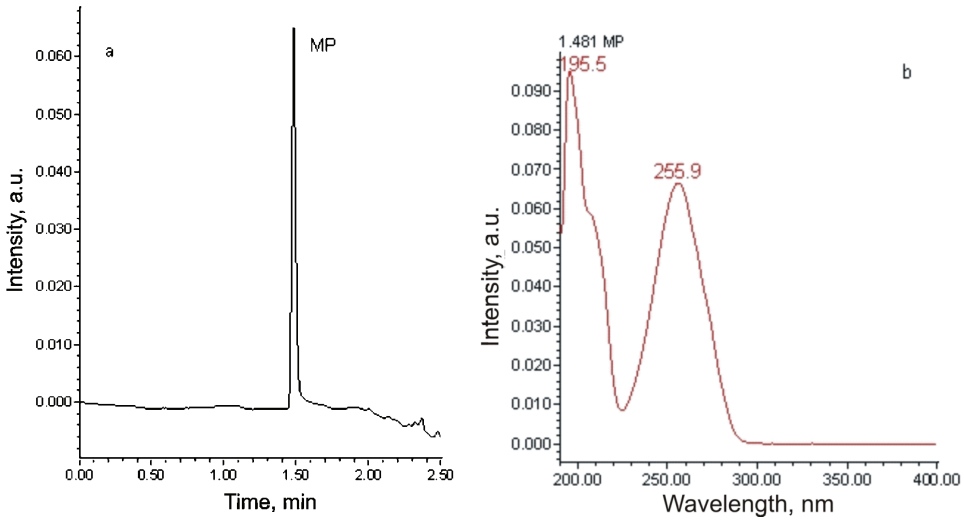


Fig. 1. A typical chromatogram of standard methylparaben using PDA detection at max-plot (a); for gradient elution, see the text; spectrum of methylparaben (b).

*Precision*

The intraday precision was tested with 5 repeated injections of methylparaben standard solutions at three concentration levels of 0.50, 1.0 and 5.0 μg·mL$^{-1}$. The relative standard deviations (*RSD*) were below 2.3 %. The reproducibility of the chromatographic separation was very good as shown by the very narrow window of the retention time (Table I).

TABLE I. Precision and accuracy of the UPLC validation parameters of methylparaben

| Characteristic | Type | Recovery, % | Peak area *RSD*, % | Retention time *RSD*, % |
|---|---|---|---|---|
| Precision | Intra-day | 99–101 | 0.4–0.9 | $10^{-6}$ |
| | Inter-day | 98.2–101.7 | 0.3–1.3 | $10^{-4}$ |
| Accuracy | Intra-day | 97.7–102.1 | 0.9–2.1 | $10^{-4}$ |
| | Inter-day | 96.5–103.1 | 1.4–2.8 | $10^{-4}$ |

*Accuracy*

Five placebo samples of products without methylparaben were spiked with reference standard solutions. These samples were treated as described in the sample preparation procedure. The data obtained were compared with the theoretical concentrations. Under these conditions, the accuracy was expressed as percentage recovery. The relative standard deviations of the set results were determined. The extraction efficiency was determined by comparing the analysis of the standards solutions, and un-spiked and spiked samples.

*Recovery*

The recovery of spiked methylparaben in some cosmetic samples is shown in Table II. A comparison of an un-fortified and fortified shower gel sample is presented in Fig. 2.

TABLE II. The recovery of spiked methylparaben in cosmetic samples[a]

| Spiked level, ng $g^{-1}$ | Recovery, % | | |
|---|---|---|---|
| | Balsam | Shower gel | Make-up removal (cleaning milk) |
| 20 | 97.6±3.2 | 102.6±1.3 | 101.1±4.1 |
| 50 | 98.0±2.1 | 97.4±1.9 | 104.2±2.3 |
| 200 | 101.4±1.9 | 102.6±2.1 | 101.8±3.8 |

[a]Values are the means of three determinations ± standard deviation

It was found that the contents of methylparaben in the tested cosmetic products all satisfied the permitted concentration of the EEC Directive.

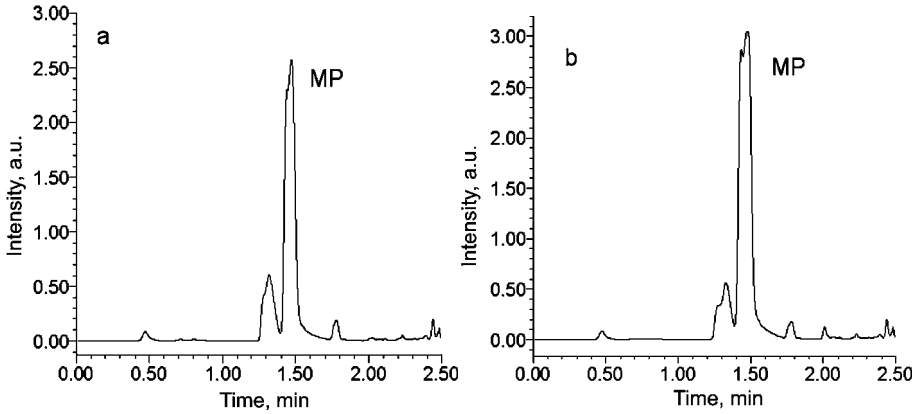


Fig. 2. Chromatogram of shower gel extract (a) and the same sample fortified with 90 ng $g^{-1}$ MP (b).

*Application of the method to cosmetic samples*

The UPLC method was used for the quantification of methylparaben in various types of cosmetic products. The fact that in some sample methylparaben co-eluted with unidentified compounds was solved using the spectral analysis routine. The chromatogram of a hair balsam extract, as an example where methylparaben co-eluted with an unknown compound, is presented in Fig. 3. This drawback was, however, solved with the help of the purity check and library match routines of the Empower software.

Various types of cosmetic samples, including balsams, shower gels, sun creams, body lotions, anti-cellulite, feminine hygiene products and make-up removal (cleaning milk), were tested in this study. All the tested sample solutions

were found to contain between 16 and 680 mg·kg$^{-1}$ methylparaben. In all the analyzed samples, the level of methylparaben was under 0.4 %, the high limit imposed by European regulations.

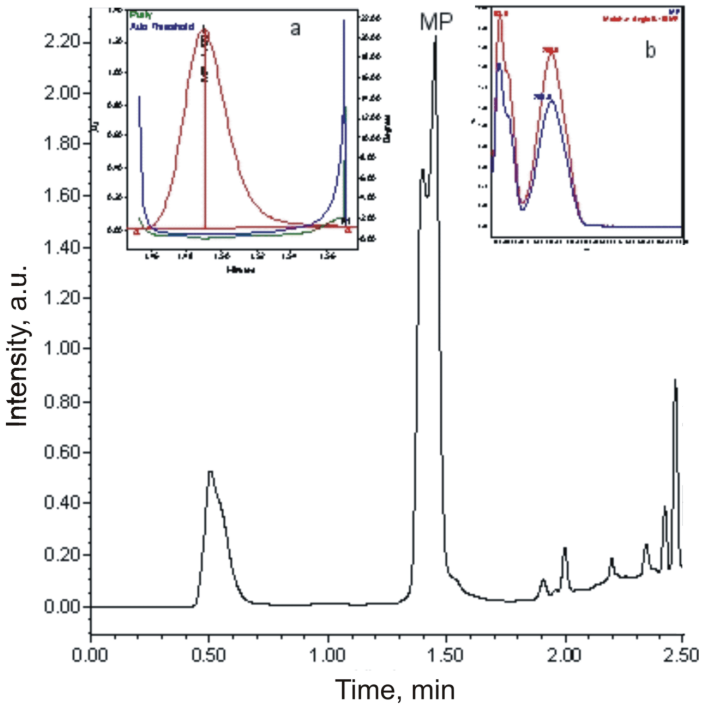


Fig. 3. Chromatogram of a hair balsam extract. The methylparaben co-eluted with an unknown compound. The peak purity check and library match routines helped in the identification and quantification of the analyte.

## CONCLUSIONS

In conclusion, the proposed method allows a rapid and sound quantification of methylparaben in cosmetic samples, being based on a simple and rapid sample preparation procedure and a very fast and reliable chromatographic separation. The method can be used to monitor the occurrence at trace level of methylparaben preservative in cosmetic samples found on the market.

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ИЗВОД

## ОДРЕЂИВАЊЕ МЕТИЛПАРАБЕНА У КОЗМЕТИЧКИМ ПРОИЗВОДИМА ПРИМЕНОМ UPLC МЕТОДЕ

MANUELA M. MINCEA[1,3], IOANA R. LUPŞA[3], DAN F. CINGHIŢĂ[1], CIPRIAN V. RADOVAN[1], IOAN TALPOS[2] и VASILE OSTAFE[1,2]

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Развијена је метода за одређивање метилпарабена у козметичким производима применом UPLC методе. Из класе парабена, метилпарабени су најчешће коришћена једињења као конзерванси у козметичким и фармацеутским производима и храни. Анализа поменутог једињења је значајна имајући у виду широку употребу и потенцијални здравствени ризик због изложености. Идентификација и одређивање метилпарабена су изведени веома брзом процедуром, за око 1,5 min, са минималном припремом узорка, UPLC системом уз коришћење PDA детектора (BEH $C_{18}$ (2,1×50) mm, 1,7 μm колона, градијентно елуирање смешом метанол–вода (60:40, v/v). Под оптималним условима радни линерани опсег одређивања метилпарабена обухвата два реда величине са релативном стандардном девијацијом до 2,4 %, и границама детекције од 2,45 ng $ml^{-1}$.

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