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Professional paper

Determination of methylparaben and propylparaben in cosmetic products using HPLC

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Abstract

This study presents the method performance verification and application of a HPLC method for determination of methylparaben (MP) and propylparaben (PP) in cosmetic products. The selected parabens were separated on a Lichrospher 100 RP 18 chromatographic column (250 x 4.6 mm I.D.; particle size of 5 µm), in an isocratic mode with a mobile phase consisting of methanol: THF:ACN:water (10:5:25:60, %, *v/v*). The flow rate was 1.0 mL/min, and the injection volume was 10 µL. All analyses were performed at room temperature and the compounds of interest were monitored at 280 nm. The levels of MP and the levels of PP in 16 analyzed products ranged from 0.06 – 0.42% (*w/w*) and from 0.1 – 0.26% (*w/w*), respectively. Highest levels were found in skin and hair care products followed by products intended for facial care. Four investigated products contained both MP as well as PP. The MP and PP levels determined in the examined products were all in agreement with requirements of the European Union Cosmetic Directive (EU Cosmetics Directive 76/768/EEC).

Key words: Preservatives, methylparaben, propylparaben, HPLC, cosmetic products

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Introduction

Cosmetic products are complex products that contain a large portion of water and other organic/inorganic compounds, and they represent a nutrient-rich medium that favors microorganism's growth (Halla et al., 2018). The protection against potentially pathogenic microorganisms that can develop in the cosmetic products, together with the product's preservation resulting from biological or physicochemical deterioration (stability of the product formulation) can be performed by addition of different antimicrobial agents (Barabasz et al., 2019; Halla et al., 2018). Parabens, alkyl esters of para-hydroxybenzoic acid esters such as methylparaben (MP) and propylparaben (PP) are widely present in cosmetic products, pharmaceuticals and the food industry because of their relatively low toxicity, their effective antimicrobial activity, and good stability (Barabasz et al., 2019; Baranowska et al., 2014; Cabaleiro et al., 2014; Halla et al., 2018; Ocana-Gonzalez et al., 2015).Generally, MP and PP are among the most commonly used parabens and combinations of two or more of these preservatives are often used to increase the ability of the system to withstand microbial contaminations (Kaur et al., 2020; Msagati et al., 2008; Zhang et al., 2005).

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Scientific research concerning the impact of the parabens on human health, especially their endocrine disrupting potential has raised much controversy about their application. Many studies suggest that parabens show different adverse effects to biological tissues ranging from allergic reactions to triggering development of cancerous tissues (e.g., breast cancer), however, the results are contradictory (Matwiejczuk et al., 2020; Pan et al., 2016; Petric et al., 2021). Nowadays, parabens are used in different categories of cosmetic products. The European Union legislation allows the use of parabens and their salts up to 0.4% (*w/w*) for MP, up to 0.4% (*w/w*) for ethylparaben, up to 0.14% (*w/w*) for the sum of PPand butylparaben and up to 0.8% (*w/w*) for the total paraben concentration in a cosmetic product (Commission regulation (EU) No.1004/2014; Grześkoviak et al., 2016).

Due to the general use of parabens and their potential risks to human health, the development and application of quantitative analytical methods play important role in measurement and monitoring of paraben levels in different cosmetic products (Kaur et al., 2020). Among different analytical techniques, high performance liquid chromatography (HPLC) coupled with diode-array (DAD), fluorescence or mass detection is most commonly used for paraben analysis in different matrices (Grześkoviak et al., 2016; Lucas- Sanchez et al., 2022). It possesses the required sensitivity, and it is available in the most quality control laboratories to be used as a technique for identification, detection or quantification of parabens in different types of cosmetic products (Gao et al., 2021).

Well-characterized and standardized methods represent the basis upon which the determination and monitoring regulated as well as unregulated substances/contaminants in different samples should be performed (Shoemaker, 2016). Laboratories could choose methods published in international, regional or national standards (standard methods) or develop their own methods. Further, if a standard method is chosen, then it must be verified prior use in the laboratory to prove method suitability for the intended use and method transferability i.e., easy implementation by many laboratories (Kotsiuba, 2022; Shoemaker, 2016).

This study presents the method performance verification of the HPLC test method established by the Seventh Commission Directive 96/45/EC (Section B) and its application for determination of parabens, MP and PP in cosmetic products samples available on the market in Republic of North Macedonia.

Material and methods

Chemicals, reagents and materials

Reference standards of methylparaben (methyl-4-hydroxybenzoate) (MP) and propylparaben (propyl-4-hydroxybenzoate) (PP), (99.9%) were purchased from CPAchem Ltd., Bulgaria. Ethanol, absolute (99%) was purchased from Merck (Darmstadt, Germany). Tetrahydrofuran (THF, HPLC grade) as well as acetonitrile (ACN, HPLC grade) were purchased from Sharlau (Turkey) and methanol HPLC grade was purchased from Fisher Chemical (Belgium). Sulfuric acid (95-97%, p.a) was obtained from Carlo Erba, France. Double-distilled water (HPLC-grade, Direct – Q 5UV system) was used throughout the entire analysis.

Stock standard solutions of MP (2 mg/mL) and PP (2 mg/mL) were prepared using a solvent mixture of ethanol and water (90:10, %, *v/v*). The solutions for HPLC analysis were filtered through a 0.45 µm nylon syringe filters before use. The stock solutions were stable for one week, when kept in the refrigerator in amber glass flasks, at 4 °C. Working standard solutions were prepared by diluting the stock standard solutions. Appropriate quantity of the stock standard solutions was transferred in 50 mL volumetric flasks, 1 mL sulfuric acid (2 mol/L) was added, and the flask was filled up to the mark with the solvent. The concentration range for MP was 0.011-0.23 mg/mL and 0.01-0.218 mg/mL for PP.

Chromatographic analysis

Chromatographic analysis was performed on a Shimadzu LC-2010 chromatographic system (Shimadzu, Kyoto, Japan) consisting of a LC-20AT Prominence liquid chromatograph pump with DGU-20A5 Prominence degasser, a SPD-M20A Prominence Diode Array Detector and a SIL-20 AC Prominence auto sampler. Data analyses were done using Class VP 7.3 Software. Chromatographic separation was performed on a Lichrospher 100 RP 18 reversed-phase column (250 x 4.6 mm I.D.; particle size 5 µm), in an isocratic mode with a mobile phase consisting of methanol:THF:ACN:water (10:5:25:60, %, *v/v*). The flow rate was 1.0 mL/min and the injection volume was 10 µL All analyses were performed at ambient temperature and the compounds of interest were monitored at 280 nm.

Sample preparation

16 cosmetic products from different companies were investigated in this study. They included facial creams, body milks, body lotions, shampoos, makeup powders as well as hand creams. All products were obtained during an inspection procedure of the State Sanitary and Health Inspectorate aiming to control the declared amount of preservatives and heavy metals in cosmetic products available on the market in Republic of North Macedonia.

For the sample preparation, 0.5 g of sample was accurately weighed into a 50 mL volumetric flask and 1 mL of sulfuric acid (2 mol/L) and 30 mL solvent was added. The flask was closed and shaken vigorously for about 5 min until a homogeneous suspension was obtained. Then, the same solvent was added up to the mark. To facilitate the extraction of the preservative into the ethanol phase, the suspension was heated for about 5 min in a water bath, at 60 °C. Then, the flask was cooled immediately in a stream of cold water and kept at 5 °C for one hour and then stored at 5 °C until analysis. Samples were filtered through a 0.45 µm nylon syringe filters before use. The HPLC determination was performed within 24 hours of sample preparation.

Method verification

A method performance verification study of the EEC method was performed in accordance with the requirements of the MKS ISO 17025:2018 standard. Specificity, linearity, precision and accuracy were investigated.

Results and discussion

Method verification is the process of assessment of the analytical test procedure suitability under actual experimental conditions, for a specific substance or product, environment, personnel or equipment. During the analytical transfer, the laboratories must verify already validated methods before their application in routine analysis thereby confirming that the proposed method can be applied in their laboratory (ALLAC, 2007). The quantitative determination of parabens in the cosmetic products in our study was based on the standard EEC method (Seventh Commission Directive 96/45/EC (Section B)).

Specificity – The specificity of the method in our study was investigated in order to prove that there are no matrix components unique to the lab's samples which would interfere the quantitative determination (ALACC, 2007). The representative chromatograms of solvent and paraben-free products (having similar composition to the investigated products, but no MP or PP present) are presented in Fig. 1. No interfering peaks from the matrix were observed at the retention times of MP and PP.

System suitability – The system suitability testing represents a significant component of any analytical procedure and is used as part of the qualification (Zhang et al., 2020). The results from the system suitability testing are presented in Table 1. The results imply that the chromatographic criteria were met, and the method can be further used for quantitative analysis of the investigated samples.

Table 1. System suitability criteria

Chromatographic parameter	Criterion	MP	PP
t_R (min)		3.456	6.11
Repeatability (% RSD)	≤ 2.0	0.65	0.70
Peak symmetry	0.8 – 1.8	1.05	1.0
Number of theoretical plates (N)	≥ 2000	2941	2323

Table 2. Results from testing the linearity and precision of the method

Verification parameter	MP	PP
Linearity		
Concentration range (mcg/ml)	11.5 –229.8 µg/mL	10.9 –217.9 µg/mL
Slope	12922.15	11575.5
Intercept	8532.67	11821.4
Correlation coefficient (R^2)	0.9999	0.9998
Precision (RSD %)		
Repeatability(*n* = 6)	0.9	0.9
Intermediate precision (*n* = 6)		
Day 1	0.8	1.1
Day 2	1.3	1.5

Table 3. Accuracy of the method

MP			PP		
Concentration level (%)	Recovery (%)	RSD (%)	Concentration level (%)	Recovery (%)	RSD (%)
50%	98.24±0.86	1.70	50%	98.04±0.48	0.98
100%	97.36±0.57	0.59	100%	100.95±0.56	0.55
150%	100.33±0.46	0.30	150%	99.62±0.25	0.17

Linearity – Linear calibration curves for MP and PP were obtained using solutions of five different levels of concentration ranging from 11.5–229.8 µg/mL for MP, and five different levels of concentration ranging from 10.9-217.9 µg/mL for PP. The obtained values for the correlation coefficients were 0.9999 and 0.9998, for MP and PP, respectively. The results are shown in Table 2.

Accuracy and Precision – To verify the accuracy of the method, recovery tests were performed on products containing none of the examined parabens (paraben-free products). Different known amounts of MP and PP (corresponding to a concentration of 50, 100 and 150% of the working concentration) were added, and the results were expressed as % recovery (Table 3).

The recovery values ranged 97.24-100.37% for MP and 97.69-100.90% for PP were within the acceptance criteria (95-100%) verifying appropriate method accuracy (EMA/CHMP/ICH/82072/2006, 2023).

For the verification of method precision, three different products were used (body lotion B1, hydrating cream C4, hair and body shampoo S2). Each sample was divided into five aliquots (0.15-0.2g) and then analyzed separately. The RSD values (%) which represented the method's precision, were evaluated by repeated analyses (*n*=6) of the same sample solution and the results ranged from 1.3-1.5% for the sample (Table 2). The RSD values for intermediate precision ranged from 0.8-1.1% for MP, and from 1.3-1.5% for PP, respectively. The obtained values from precision testing indicated that the method is precise (Little, 2016).

Method application

The verified method was further applied on cosmetic products obtained from the Macedonian market. The levels (%, *w/w*) of MP and PP as well as the sum of the investigated parabens in each cosmetic product are presented in Table 4. Representative chromatograms of blank samples, standard solutions containing MP and PP, as well samples containing MP and PP, are presented in Fig. 1.

The results show that in all of the cosmetic products analyzed, the sum of the parabens was not greater that 0.8% (*w/w*) which is in accordance with the national and European Union legislation (Official Gazette No. 94/2010 of the Republic of Macedonia; Commission regulation (EU) No.1004/2014). In all samples the levels of MP were higher than the levels of PP.

The sum of MP and PP (%, *w/w*) in the investigated types of products ranged 0.20-0.60% (*w/w)* for creams, 0.33-0.56% (*w/w)* for body milks and 0.12-0.32% (*w/w)* for face powders. The levels of the investigated parabens in individual products ranged from 0.06-0.42% *(w/w)* for MP and 0.1-0.26% *(w/w)* for PP, respectively. In some of the examined products the declared preservatives (MP and PP) were not identified (products HG1, C5 and C4) and in some products they were identified but their levels were below LOQ. There are studies that have examined the levels of methylparaben (MP) and propylparaben (PP) in cosmetic products which reported discrepancies between declared and detected concentrations (Baranovska et al., 2014; Kaur et al., 2020). In the study by Baranowska et al. (2014) some samples of cosmetic and personal care products contained parabens at levels below those specified by manufacturers. These findings suggest that factors such as formulation inconsistencies, degradation over time, or errors in manufacturing could lead to lower-than-declared paraben levels. Similarly, Kaur et al. (2020) detected inconsistencies in paraben concentrations in various commercial cosmetics, though their primary concern was the potential for exceeding regulatory limits.

From a safety and quality perspective, lower-than-declared levels of preservatives could impact product stability and microbial resistance. Parabens serve as antimicrobial agents, preventing bacterial and fungal contamination in water-rich cosmetic products. If their concentrations are insufficient, the product may be more susceptible to microbial growth, leading to potential risks such as skin irritation or infections, particularly for consumers with sensitive skin or compromised immune systems. Additionally, such discrepancies raise concerns about quality control and regulatory compliance within the cosmetic industry. Inadequate preservative levels could shorten shelf life and increase contamination risks, particularly in high-water-content cosmetics. Therefore, continuous monitoring and verification of preservative concentrations in cosmetic products remains essential to ensure both efficacy and consumer safety.

Table 4. Levels of the investigated parabens (% *w/w*) in cosmetic products from the Macedonian market.

Sample code	Product characteristics	MP (%, *w/w*)	PP (%, *w/w*)	Sum of parabens (%, *w/w*)
Creams				
C1	Face cream	0.37±0.02*	0.19±0.01	0.56
C2	Daily Face cream	0.40±0.02	ND	0.40
C3	Night Face cream	0.34±0.02	0.26±0.01	0.60
C4	Hydrating Face cream	ND	ND	ND
C5	Hand cream	ND	ND	ND
C6	Hand cream	0.2±0.01	ND	0.20
Body lotion				
BL1	Hydrating	ND	ND	ND
Body milk				
BM1	Soothing	0.39±0.01	0.17±0.02	0.56
BM2	Face cleaning	0.33±0.03	ND	0.33
Hair shampoos				
S1	For Dry hair	0.42±0.01	ND	0.42
S2	Hair and body	ND	ND	ND
Hair gel				
HG1	Wet looking	ND	ND	ND
Liquid soap				
LS1	Soap	0.16±0.01	ND	0.16
Face powder				
FP1	Liquid	0.22±0.01	0.10±0.02	0.32
FP2	Powder	0.12±0.01	ND	0.12

*ND-not detected; results expressed as mean±SD

Conclusion

In our study, method performance verification and the application of a standard RP-HPLC method for determination of MP and PP content in cosmetic samples (creams, body lotions, shampoo sample and similar cosmetic products) is presented. The laboratory verified the requirements for analytical method suitability and this method can be used for routine monitoring of MP and PP in different cosmetic products available on the market. All the tested samples contained parabens at the levels within the current law regulations. However, some of the samples contained the investigated parabens below the declared values on their label. The method can be used for monitoring paraben concentrations in order to assure adequate quality of different cosmetic products available to the consumers.

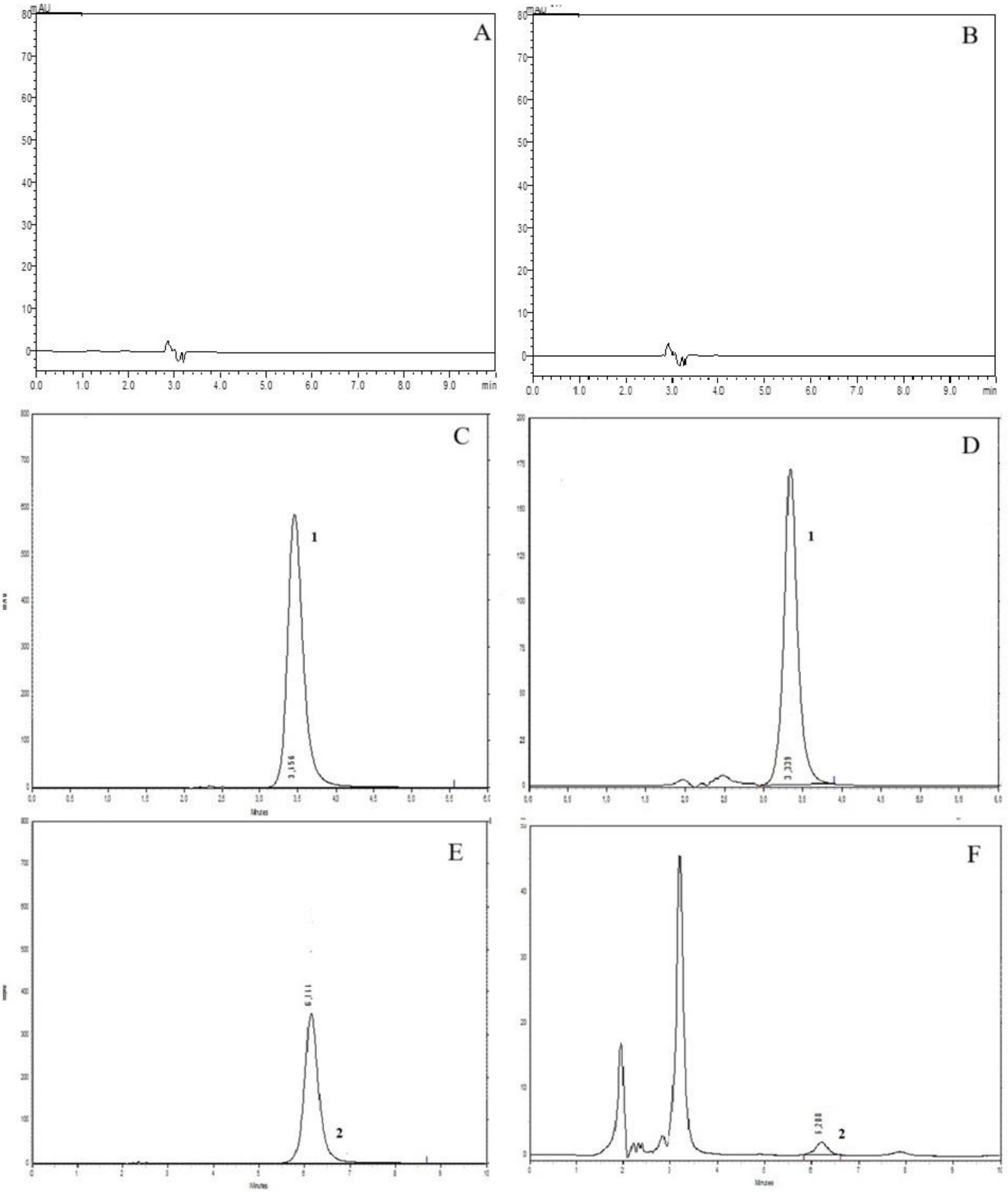

Fig. 1. Representative chromatograms of: A) Blank solution (Solvent), B) Paraben free sample, C) Standard solution containing MP (1) (35.50 μg/mL), D) Sample solution containing MP (1) (37.00 μg/mL), E) Standard solution containing PP (2) (21.80 μg/mL), F) Sample solution containing PP (2) (12.00 μg/mL). Unidentified peaks are derived from the matrix.

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Резиме

Определување на метилпарабен и пропилпарабен во козметички производи со примена на HPLC

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Клучни зборови: Конзерванси, метилпарабен, пропилпарабен, HPLC, козметички производи

Ова истражување ја претставува верификацијата на перформансите и примената на HPLCметод за определување насодржината на метилпарабен (MP) и пропилпарабен (PP) во козметички производи. Испитуваните парабени беа разделени на Lichrospher 100 RP 18 реверзно-фазна колона (250 x 4.6 mm; големина на честички од 5 µm), изократски, со мобилна фаза составена од метанол:THF:ACN: вода (10:5:25:60, %, *v/v*). Протокот на мобилната фаза беше 1,0 mL/min, а волуменот на инјектирање изнесуваше 10 µL. Анализите беа изведени на собна температура, а соединенијата од интерес беа одредеувани на бранова должина од 280 nm. Содржината на метилпарабен се движеше од 0,06-0,42% (*w/w*), додека содржината на пропилпарабен се движеше од 0,1-0,26% (*w/w*). Највисоки вредности за содржината беше определени во козметички производи за нега на кожата и косата, а потоа и во производи за нега на лицето. Четири од испитуваните производи содржеа и метилпарабен и пропилпарабен. Определената содржина на испитуваните парабени во анализираните производи е во согласност со барањата на националната и Европската козметичка регулатива (EU Cosmetics Directive 76/768/EEC).