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HPLC Determination of Some frequently used Parabens in Sunscreens

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Research Article

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Abstract

Parabens are esters of p-hydroxybenzoic acid and belong to group of effective preservatives commonly used in cosmetic products, drugs and food. Their antimicrobial activity increases with increasing carbon number of the ester group. A number of cosmetic products and skin-care products are preserved with parabens, as well in Europe as in the United States. Methyl, ethyl and propyl paraben are preservatives commonly used in cosmetic products. Usage of parabens should be under great attention, because some studies mentioned that the increased concentration can cause skin irritation and contact dermatitis.. This paper shows optimization of HPLC method for determination of methyl, ethyl and propyl paraben in sunscreen products. The advantage of this analytic method is that the same stationary phase with different mobile phases is used for determination UV filters and parabens as well in sunscreen products. Determination was performed using reversed stationary phase C_8 with wavelength 254 nm. Separation was performed using mobile phase methanol: water (60:40 w/w). Analytic method was validated through specificity, linearity, limit of detection (LOD) and limit of quantification (LOQ). Determined limit of detection and limit of quantification for methyl, ethyl and propyl paraben are respectively: LOD-0.035 µg/ml LOQ-0.116 µg/ml; LOD-0.061 µg/ml LOQ-0.203 µg/ml and LOD-0.009 µg/ml LOQ-0.031 µg/ml. Coefficient of quantification for methyl, ethyl and propyl paraben are respectively: R^2-0.9996; R^2-0.9988 and R^2-1. A content of parabens was examined on commercial samples available on market in Bosnia and Herzegovina. Concentration of ethyl, methyl and propyl paraben does not exceed maximal allowed concentrations (0.4% for single ester and 0.8% for mixture of esters) in tested samples.

Keywords: *parabens, HPLC, sunscreen preparation, methanol, water*

Introduction

Cosmetic composition and stability are of particular relevance in our daily life, since the average adult is estimated to use at least seven different cosmetic products a day. Therefore, antimicrobial chemicals that prevent microorganisms from growing play a crucial role. We should bear in mind that not only can a cosmetic product be damaged by microorganisms but also by other external agents, such as air and sunlight. Thus, using compounds with antioxidant and light absorbent properties can help lengthen the life of cosmetic [1].

Parabens have been used as preservatives in food and drugs about 70 years. They are class of chemicals widely used as preservatives in the cosmetic and pharmaceuticals industries. These compounds, and their salts, are used primarily for their bactericidal and fungicidal properties. They can be found in shampoo, commercial moisturizers, shaving gels, personal lubricants, topical/parenteral pharmaceuticals, spray tanning solution and toothpaste.. The antimicrobial activity of the parabens increases with increasing carbon number of the ester group. The individual esters differentiate in their relative antimicrobial activities and therefore optimal effectiveness is generally obtained by combinations of parabens [2]. A lot of cosmetics and skin care products are preserved with parabens, as well in Europe as in the United States [3].

Usage of parabens should be under great attention, because some studies mentioned that the use of preservatives can also produce other undesirable effects, which can appear either after first application or after years of cosmetic use. These effects range from mild skin irritation to estrogenic activity, and recently the possibility that they could potentially induce human breast tumors has been discussed [4]. The European Cosmetic Toiletry and Perfumery Association, COLIPA emphasizes that parabens are hydrolysed in the skin and that none are entering the blood stream [2]. Given their application in a wide range of consumables the use of parabens is regulated. Parabens are

not included in Annex 1 of the dangerous substances list (Council Directive 67/548/EC). The Council Directive 76/768/EC of the European Community restricts the preservation of cosmetic products with methyl, ethyl, propyl and butyl parabens to a maximal allowed concentration of 0.4% for one single ester or 0.8% for esters mixture [5,6]. Thus, it is important to develop analytic method for monitoring maximum allowed concentrations of parabens in sunscreen products that applies directly to skin.

The goal of this paper was to develop analytical method for identification and determination of methyl, ethyl and propyl paraben in sunscreen products, using the same stationary phase with different mobile phase that have been used for identification and determination UV filters from same preparations. [7]. This kind of analytic method hastens and facilitates work of an analyst in quality control laboratory.

Developed method has been previously optimized. Optimization was performed on C_8 stationary phase and two different mobile phases. After optimization, analytical method validation was performed according to International Commission of Harmonization (ICH) [7] directives through specificity, linearity, repeatability, limit of detection (LOD) and limit of quantification (LOQ). After that, concentration of methyl, ethyl and propyl parabens were determined in treated sunscreen products. Samples were taken from manufacturers, most widely present in Bosnia and Herzegovina.

Materials and Methods

Standards

The standards included methyl p-hydroxybenzoate (Sigma- Aldrich), ethyl p-hydroxybenzoate (Sigma-Aldrich) and propyl p-hydroxybenzoate (Sigma- Aldrich).

Samples

Chemicals and reagents

All solvents were of analytical grade. The chemicals and reagents used were absolute ethanol, methanol, acetonitrile (UV-IR-HPLC; Panreac Quimica, Barcelona, Spain), sulphuric acid (Kemika-Zagreb) and purified water for HPLC. Sunscreens were used as samples specified in Table I.

Table I Sunscreens used as samples

Samples used for analysis	Declared parabens in samples
Sample 1: Sunscreen milk SPF 8 (UVA and UVB protection)	Methyl, ethyl and propyl p-hydroxybenzoate
Sample 2: Sunscreen cream SPF 12 (UVA and UVB protection)	Methyl and propyl p-hydroxybenzoate
Sample 3: Sunscreen milk SPF 20 (UVA and UVB protection)	Methyl, ethyl and propyl p-hydroxybenzoate
Sample 4: Sunscreen cream SPF 25 (UVA and UVB protection)	Methyl and propyl p-hydroxybenzoate

Chromatographing condition

Separation was performed using HPLC pump Shimadzu 10 Avp with autosampler and Shimadzu SPD-M10A DAD detector (Shimadzu Europe GmbH, Dulsburg, Germany) using column and mobile phases specified in Table II. Ten µL of each solution was injected. Separation was performed on temperature 42^{0}C with flow speed of 1 ml min^{-1} and with detection on wavelength maximal absorption at 245 nm. Record time was 30 min.

Table II Column and mobile phases used in HPLC

Column	Mobile phase
Beckman C_8 5µm 250 x 4.6	**(1)** Acetonitrile:water-70:30 **(2)** Methanol:water-60:40

Stock solution

Stock solutions parabens was prepared by accurate weight of 50.0 mg methyl, 40.0 mg ethyl and 50.0 propyl parabens into 100 ml volumetric flasks. Forty ml mixture ethanol/water (90/10) was added in volumetric flasks and the mixture was shaken approximately 2 minutes. Thereafter mixture ethanol/water was added in flasks up to the mark.

Working standards

From stock solutions of methyl, ethyl and propyl parabens, series of working standards were made with following concentrations: 10, 20, 50, 100, 200 µg ml^{-1}.

Sample preparation

Samples for analysis were prepared by accurate weight of 1 g of sample into 50 ml volumetric flask. In each flask 1 ml 2 M sulphuric acid was added and filled up to the mark with mixture ethanol/water. Solutions were shaken approximately one minute. Thereafter solutions were transfer into water bath and kept five minute on 60^{0}C. Solutions were rapidly cool and kept into refrigerator for one hour. Ten µl of each sample was injected.

Results and Discussion

Separating conditions optimization

The objectives of this study were to determine optimal separating conditions for methyl, ethyl and propyl parabens in sunscreen products. Choice for analytic method for determination and quantification of methyl, ethyl and propyl parabens depends of analytic method for determination and quantification UV filters presented in tested samples. The goal was to determine examined parabens using the same stationery phase that have been used for examined UV filters, with appropriate mobile phase [5]. The first choice was mobile phase **1** (Table II). Separation was performed on mixture of methyl, ethyl and propyl parabens. Mobile phase 1 retention times are given in Table III. It can be seen from chromatogram of

mixture of paraben standards (fig.1) that resolution between peaks is weak.

Table III Retention times with mobile phase **1**

Substance	Methyl paraben	Ethyl paraben	Propyl paraben
Retention time (min.)	3.00	3.55	3.97

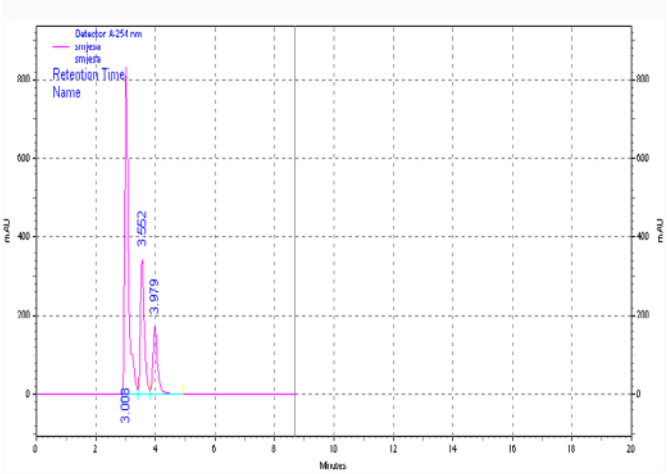

Figure 1. Chromatogram of mixture of ethyl, methyl and propyl paraben with mobile phase**1**

Separation using mobile phase **2** gave much better results. Retention times with mobile phase **2** are given in Table IV. It can be seen from chromatogram of mixture that all three parabens were successfully separated and that resolution is acceptable (see Fig.2).

Table IV. Retention times with mobile phase **2**

Substance	Methyl paraben	Ethyl paraben	Propyl paraben
Retention time (min.)	4.33	5.49	7.68

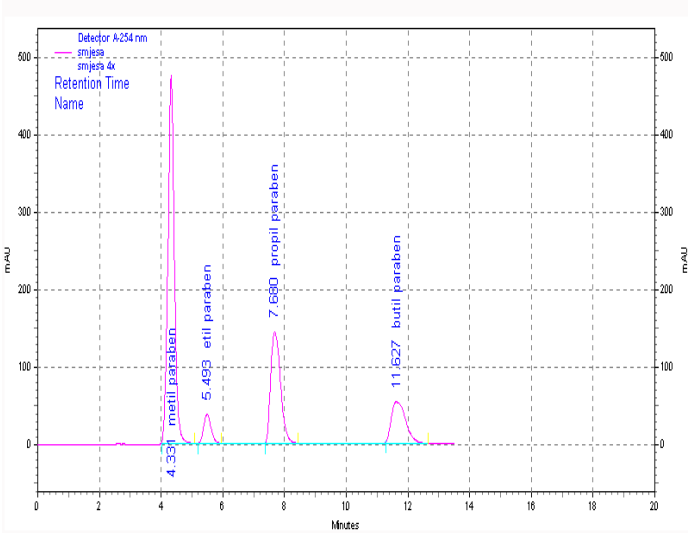

Figure 2. Chromatogram of mixture ethyl, methyl and propyl paraben with mobile phase **2**

Quantitative analyses of methyl, ethyl and propyl paraben in sunscreen products was performed using stationary phase C_8 and mobile phase *methanol:water-60:40*. Separation has been performed on wavelength with maximum absorption 254 nm.

Analytical method validation

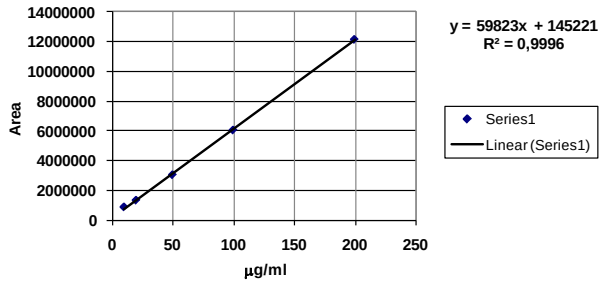

Figure 3. Calibration curve for methyl paraben

Analytic method validation was performed according ICH directives [8] over linearity, repeatability, LOD and LOQ.

Linearity, repeatability, detection limit and quantification limit

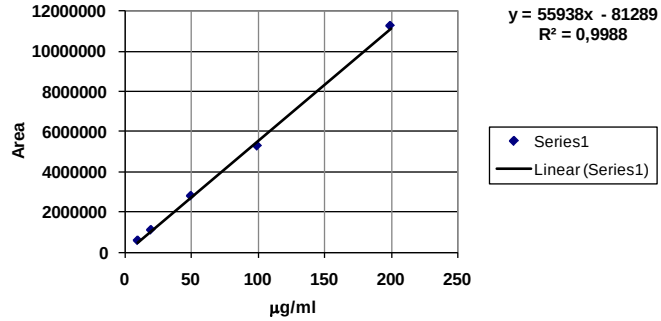

Figure 4. Calibration curve for ethyl paraben

Chromatograms of different methyl, ethyl and propyl parabens standards concentrations were recorded at wavelength of 254 nm. Areas under the signal were scanned and arithmetic mean was calculated for three repetitions. For each arithmetic mean, SD and linearity correlation coefficient were calculated (Table V, VI and VII). Low coefficient of variation indicates good repeatability of this method.

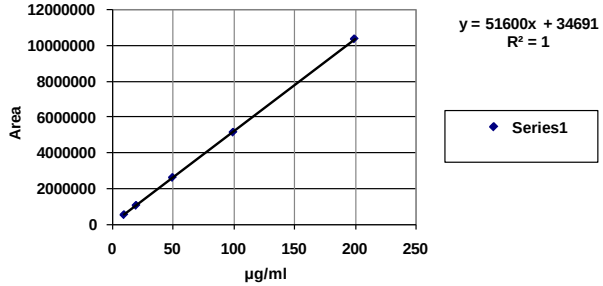

Figure 5. Calibration curve for propyl paraben

Calibrations curve were constructed based on five concentrations in range of 10-200 µg ml^{-1} (Fig. 3, 4 and 5). Linearity coefficient of determination (R^2), LOD and LOQ were calculated. From calibrations curves it could be seen that linearity coefficient of determination (R^2) were 0.9996 for methyl paraben, 0.9986 for ethyl paraben and 1 for propyl paraben (Fig. 3, 4 and 5). LOD and LOQ were determined, according ICH [8] directives, from SD of detector response and calibration curve slope.

Table V. Areas scanned from methyl paraben standard chromatogram and calculate values: arithmetic mean (AM), SD and coefficient of variation (CV).

Concentration µg/ml	RT(min)	P1	P2	P3	AM	SD	CV%
10	4.46	870308	876301	873787	873465.33	3009.42	0.34
20	4.46.	1309327	1332360	1335005	1325564.00	14123.71	1.07
50	4.46.	2983602	3044067	3090036	3039235.00	53381.27	1.76
100	4.46	5924090	6116980	6124655	6055241.67	113645.46	1.88
200	4.46	11981060	12290514	12224606	12165393.33	163003.22	1.34

Table VI. Areas scanned from ethyl paraben standard chromatogram and calculate values: arithmetic mean (AM), SD and coefficient of variation (CV).

Concentration µg/ml	RT(min)	P1	P2	P3	AM	SD	CV %
10	5.43	546213	546883	550578	547891.33	2350.71	0.43
20	5.43	1049980	1082285	1080597	1070954.00	18183.61	1.70
50	5.43	2703423	2792851	2799283	2765185.67	53584.63	1.94
100	5.43	5164217	5287161	5284556	5245311.33	70241.83	1.34
200	5.43	11004733	11306733	11350750	11220738.67	188356.60	1.68

Table VII. Areas scanned from propyl paraben standard chromatogram and calculate values: arithmetic mean (AM), SD and coefficient of variation (CV).

Concentration µg/ml	RT(min)	P1	P2	P3	AM	SD	CV%
10	7.2	546448	547823	560985	551752	8025.51	1.45
20	7.2	1077120	1072397	1068184	1072567	4470.42	0.42
50	7.2	2605534	2641483	2645974	2630997	22165.64	0.84
100	7.2	5101514	5179719	5191944	5157725.67	49062.98	0.95
200	7.2	10265832	10373158	10466684	10368558	100504.98	0.97

Limit of detection and limit of quantification for methyl paraben were: LOD-0,035 µg/ml, LOQ-0,116 µg/ml; for ethyl paraben LOD-0,061 µg/ml, LOQ-0,203 µg/ml; and for propyl paraben LOD-0,009 µg/ml, LOQ-0,031 µg/ml.

Quantitative analysis

Chromatograms of samples were recorded (Figs. 6-9), and then repeatability was tested. Repeatability was obtained by repeating every sample six times. Arithmetic mean, SD and coefficient variation (CV) were calculated from scanned areas (Table VIII-X). It can be seen from tables that concentrations in the samples do not exceed maximum allowed concentration (0.4% for single ester and 0.8% for ester mixture

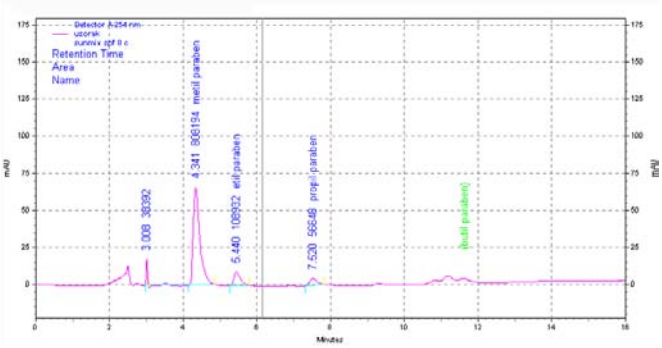

Figure 6. Chromatogram of sample 1

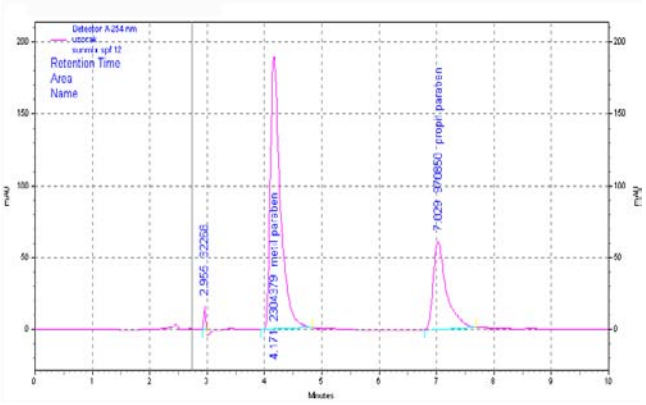

Figure 7. Chromatogram of sample 2

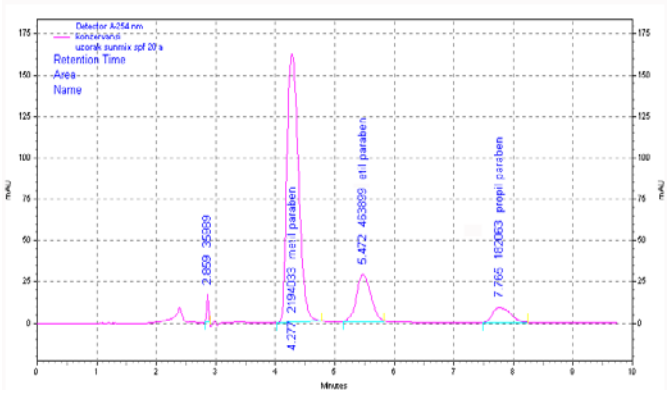

Figure 8. Chromatogram of sample 3

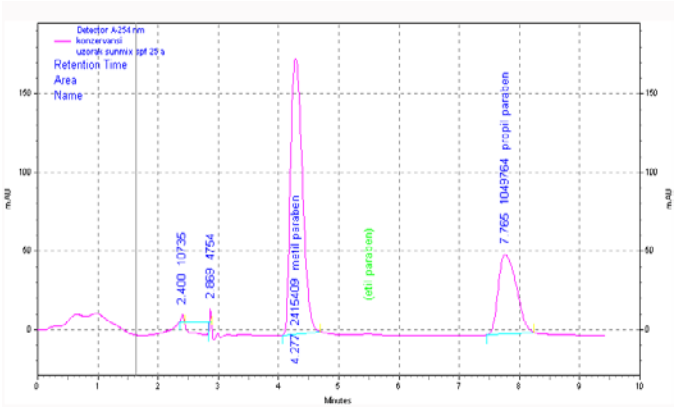

Figure 9. Chromatogram of sample 4

Conclusion

Presented results show that developed analytic method enables identification and quantification of methyl, ethyl and propyl paraben using the same stationary phase and different mobile phase that has been used for identification and determination of UV filters in same samples. This fact gave great advantage to this analytic method compared to others because it facilitates work of analyst in an analytic laboratory. This method also satisfy validation criterion through parameters: specificity, linearity, repeatability, LOD and LOQ.

Advantage of this analytical method is in high sensitivity and low coefficient of variation that show good repeatability of this method. Small CV, low detection and LOQ enable accurate and reliable quantification of parabens in cosmetic sunscreen products. In addition to that is the fact that sample preparation is simple and easy feasible despite complex formulation of cosmetic products. Because of its easy sample preparation, rate and efficiency, this method is also applicable for parabens identification and determination in other cosmetic products.

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Table VIII Areas scanned from methyl paraben sample chromatogram and calculate values: arithmetic mean (AM), SD and coefficient of variation (CV).

Sample	P1	P2	P3	P4	P5	P6	AM	SD	CV %	Amount in sample %
1	801523	803169	808194	803162	807732	802453	804372.17	2849.841	0.35	0.110
2	2304379	2297934	2301134	2326180	2289734	2335661	2309170.33	17791.29	0.77	0.362
3	2194033	2174383	2169235	2170443	2163542	2180134	2175295	10712.22	0.49	0.339
4	2415409	2420109	2400422	2421254	2408581	2413273	2413174.67	7772.963	0.32	0.379

Table IX Areas scanned from ethyl paraben sample chromatogram and calculate values: arithmetic mean (AM), SD and coefficient of variation (CV).

Sample	P1	P2	P3	P4	P5	P6	AM	SD	CV %	Amount in sample %
1	109872	109484	103048	109102	109372	108932	108301.67	2594.16	2.39	0.034
3	490978	474754	463899	463274	471025	468501	472071.83	10224.29	2.17	0.099

Table X Areas scanned from propyl paraben sample chromatogram and calculate values: arithmetic mean (AM), SD and coefficient of variation (CV).

Sample	P1	P2	P3	P4	P5	P6	AM	SD	CV %	Amount in sample %
1	57783	57344	56648	57329	56621	57021	57124.33	450.53	0.797	0.004
2	970850	961182	968873	970940	971003	980010	970476.33	6007.96	0.621	0.181
3	182063	188953	179409	179354	180723	186003	182750.83	3907.30	2.14	0.029
4	1049764	1048446	1034368	1043210	1047210	1049721	1045453.17	5945.65	0.57	0.196

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed

CONFLICTS OF INTEREST

The authors declare that they have no competing interests