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Original Research

The Determination of Preservatives in Cosmetics and Environmental Waters by HPLC

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Abstract

A method for the simultaneous determination of six preservatives in cosmetic and pharmaceutical products, as well as in surface waters, using high performance liquid chromatography (HPLC) has been developed.

Methylisothiazolinone (MI), chloromethylisothiazolinone (CMI), benzyl alcohol (BA), potassium sorbate (PS), sodium benzoate (SB), and methylparaben (MP) were separated on a Develosil RP Aqueous AR-5 RP-30 (250 x 4.6 mm, 5.0 μm) column, using a gradient elution with acetonitrile and 0.1% aqueous solution of formic acid. Acetonitrile content is increased during the analysis from 15% to 25%. The analysis time was 22 minutes. The linearity ranges of calibration curves for the standards dissolved in methanol are: 1.83-18.33 μg/mL for MI, 2.50-25.00 μg/mL for CMI, 10.00-100.00 μg/mL for BA, 0.50-10.00 μg/mL for PS, and 1.00-10.00 μg/mL for SB and MP. The linearity ranges of analytes for the water matrix are as follows: 3.17-18.33 μg/mL for MI, 3.50-25.00 μg/mL for CMI, 25.00-100.00 μg/mL for BA, 2.50-15.00 μg/mL for PS, and 2.50-10.00 μg/mL for SB and MP. The limits of detection and quantification for the determined compounds are within the following ranges: LOD – 0.15-5.3 μg/mL and LOQ – 0.45-16 μg/mL.

The quantitative extraction of analytes from solid samples and liquid samples with a high density and viscosity (cosmetics, pharmaceuticals) of the recoveries order 69-119% was performed using an ultrasound-assisted extraction with methanol. For the purpose of the analysis of environmental water samples, a method for extracting the analytes using solid-phase extraction technique (SPE) also was developed, allowing for the quantitative isolation of the analytes from water samples (recoveries of 65.4-105.6%) and for the 600-fold concentration. As a sorbent in SPE, cartridges with HLB filling were used (Oasis HLB, 6 mL, 500 mg).

The method developed was applied to the analysis of the following samples: face tonics, creams, lotions, shower gels, face masks, and syrups for the content of determined preservatives, as well as for the detection and identification of its residues in surface waters. Four of the six chosen analytes were identified in the waters. The most detected and determined compound was SB (up to 3.12 μg/L). There are also detected and determined CMI (up to 11.57 μg/L), BA (up to 35.1 μg/L), and MP (in amounts higher than method detection limit but smaller than method quantification limit).

The long-term stability of the compounds in surface waters also was determined. The stability of the determined compounds in environmental water varies, the most stable compounds are MI and BA, while the least stable are CMI and MP.

Keywords: HPLC, SPE, surface waters, cosmetics

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Introduction

Cosmetics, detergents, and pharmaceutical preparations require protection against microbial growth to ensure the safety of their use, and to extend the length of their shelf life. The use of different types of preservatives ensures the quality of these products. The most commonly used preservatives (for the European market, but also in the USA) are methylisothiazolinone (MI), chlorometyloizotiazolinon (CMI), benzyl alcohol (BA), potassium sorbate (PS), sodium benzoate (SB), and parabens, particularly methylparaben (MP) (the structural formulas of these preservatives are shown in Table 1).

The determination of preservatives in cosmetic and pharmaceutical industry products is important, not only when considering the quality control of these preparations, but also because of the numerous reports of allergic reactions that they can cause. Therefore, the relevant regulations limiting the contents of the individual ingredients in cosmetics and pharmaceuticals are introduced to increase the safety of their application. On the other hand, increased use of preservatives is a potential threat to the environment. Nowadays, wastewater treatment plants are not equipped with the tools to effectively eliminate residues of such compounds, usually organic, that after the cleaning process can get into natural water and affect aquatic ecosystems. Due to their properties, (bactericidal, stability, lack of biodegradability), these compounds become a real threat to the environment. Therefore, it is necessary to develop analytical methods and procedures to allow for the determination of preservatives, not only in the samples of drugs and cosmetics, but also their residues in surface waters. These methods should allow for quantitative extraction, concentration (especially in the water samples in which the analytes will be present at low concentrations), separation, and the simultaneous determination of all chosen preservatives in different cosmetic products, or in water. The methods should also eliminate interference from the matrix (cosmetics, pharmaceuticals, environmental waters).

Of the compounds tested in this paper, the most widely used preservative is MP. It is used mainly because of the wide spectrum of antibacterial activity, but also due to the fact that this compound does not modify the physical properties of the final products, such as taste, odor, color or texture. MP is used in the food, cosmetic, and pharmaceutical industries. In recent years, more attention has been paid to the need for qualitative and quantitative determination of parabens because of the allergies that they can cause [1]. Previous studies have shown that parabens also have estrogenic activity (these have an effect on secretion of testosterone and on the function of the reproductive system in females) [2]. Furthermore, long-term exposure to parabens, even at low concentrations, results in their absorption into biological tissues, which may affect the growth and development of cancerous tissue (such as breast cancer) [1]. A European Economic Community Directive (EEC) provides the maximum level of parabens in cosmetics to 0.4% (w/w) for a single compound or the total content of all parabens in the formulation to 0.8% (w/w). The acceptable content of parabens in food is 0.1%. The total exposure to parabens is 76 mg per day or 1.3 mg per kilogram of body weight. These doses include the following distribution of impacts: 1 mg per day may be derived from food, 50 mg from cosmetics, and 25 mg from drugs [3]. MP occurs almost in all categories of cosmetics (tonics, creams, gels, lotions, etc.), and also in many pharmaceuticals (ointments, gels, syrups, creams, and aerosols).

BA is used as a bacteriostatic agent – more often in pharmaceuticals than in cosmetics. The monitoring of its contents in medicines is important because of its potential to oxidize to toxic benzaldehyde [4]. BA is also used as an ingredient in perfumed products. The Commission of the European Union has issued directive 2003/15/EC, whereby it is necessary that the packaging of ended cosmetic products carry lists with declared ingredients present in the product in concentrations greater than 0.01% for rinse-off products and 0.001% for products remaining in contact with the skin (so-called leave-on products) [5].

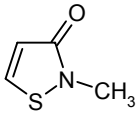
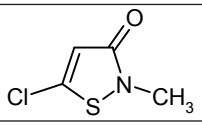
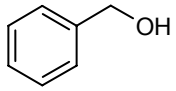
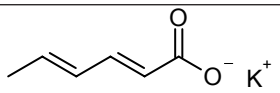
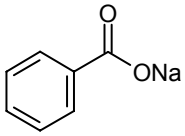
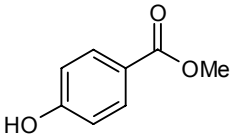
The compounds from the isothiazolinone group are widely used as preservatives, especially in industrial products based on water, such as detergents, cosmetics, paints, resins, emulsions, plasticizers, fibers, and products for polishing. These compounds are also used in the textile and paper industries. Particularly important compounds in this group are MI and CMI, which are, on one hand, strong skin irritants and sensitizers, and on the other hand, the most commonly used (among isothiazolinones) in the cosmetic industry. Limits to the possibility of their use are as follows: for a mixture of CMI:MI (3:1) the maximum concentration in cosmetics is 0.0015%, and 0.01% for MI [6].

SB is a preservative most commonly used to maintain freshness and to inhibit the growth of yeast, mold, and bacteria in food products, but also in cosmetics and pharmaceuticals. SB shows the desired effect in an acidic medium. Because of the reported activities of sensitizing, the U.S. Food and Drug Administration (FDA) limits its content as follows: the maximum allowable content in cosmetics is dependent on the category of product – rinse-off products: maximum 2.5%, products for oral care: maximum 1.7%, leave-on products: maximum 0.5%, food: maximum 0.1% [7].

PS is the other compound commonly used as a preservative in food, pharmaceutical, and cosmetic industries. It is mainly used as a food additive (mainly in fruit juice), which prevents the growth of mold. From the reports on the activities of sensitizing, and side effects it produces, its content in commercial products is limited (FDA, EU) and is permitted to a maximum 0.2% for food and 0.6% for pharmaceuticals and cosmetics [8, 9].

In the literature data, the technique which is the most commonly used in the analysis of preservatives is high-performance liquid chromatography (HPLC) [3, 5, 7-22], and less ultra high performance liquid chromatography (UHPLC) [20-23]. These methods use a mainly reversed-phase system – a column packed with octadecylsilane filling, and a gradient elution of mobile phase. The organic solvents used as the mobile phase were methanol and acetonitrile. The other components of the mobile phase were acetate

Table 1. Abbreviations, structural formulas, CAS numbers, names, and synonyms for the names and values of $logP_{ow}$ for determined compounds.

Preservatives (abbreviations)	Structural formulas	CAS numbers	IUPAC names/ synonyms for the names	$logP_{ow}$
MI		2682-20-4	2-methyl-2H-isothiazol-3-one/ methylisothiazolinone	0.486
CMI		26172-55-4	5-chloro-2-methyl-2H-isothiazol-3-one/ chloromethylisothiazolinone, methylchloroisothiazolinone	0.75
BA		100-51-6	phenylmethanol/ benzyl alcohol	1.12
PS		24634-61-5	potassium 2,4-hexadienoate/ potassium sorbate, sorbic acid potassium salt	1.72
SB		532-32-1	sodium benzoate/ benzoic acid sodium salt	1.87
MP		99-76-3	methyl 4-hydroxybenzoate/ methylparaben	1.96

buffer, citrate buffer, or water solutions of acetic acid, sodium acetate, ammonium acetate, triethylamine, and hexane-1-sulfonic acid sodium salt. These solvents were used in different combinations [3, 5-12, 14-19, 21, 23-30].

Gas chromatography and capillary electrophoresis are much less frequently used techniques [28, 29, 31-35]. Individual methods occur with the use of spectrophotometric [36] and voltammetric methods [37]. The most commonly used detector in chromatographs during the determination of the aforementioned compounds are the spectrophotometric detector [5-7, 10-19, 21, 23], and mass spectrometer [3, 24-26, 28, 29, 33], less a chemiluminescence detector [1, 20].

Preservatives are usually determined in the samples of pharmaceuticals [8-11, 12-19, 21-23, 35-37], cosmetics [1, 3, 5, 34], and food [7, 30, 31], but, there are also publications that describe the determination of its residues in waste waters and surface waters [6, 24, 25]. The methods available for the preparation of samples for analysis generally use extraction techniques. Solid-phase extraction (SPE) was used for preparation of water samples [24, 25]. Ultrasound-assisted extraction (UAE) [1, 12, 15, 21, 34] and subcritical fluid extraction (SFE) [3] were used for preparation of cosmetics. In other cases [5, 7-11, 13-14, 16-19, 22, 30, 35-37] samples were diluted, stirred and centrifuged before analyses. Solvents used for sample preparation were methanol, ethanol, acetonitrile, ether (acidified with glacial acetic acid), dichloromethane, water, 0.1 M solution of sodium hydroxide, and 0.1 M solution of hydrochloric acid, or mixtures of acetonitrile:acetate buffer:water, actonitrile:phosphate buffer, acetonitrile:citrate buffer, acetonitrile:water, and methanol:5% solution of ammonium hydroxide in methanol.

The methods previously described in the literature allow for the determination of a single preservative, or in mixtures – with drugs, other preservatives, or other ingredients of cosmetics and cleaning agents. However, these methods do not allow for the simultaneous determination of all six preservatives in a single analysis. In addition, the previously described methods for preparing cosmetic and pharmaceutical samples for analysis are dedicated to a specific category of products. However, there is no universal approach to this type of analysis, both in developed chromatographic methods, and also the procedures for sample preparation (different matrices: liquids, pastes, ointments, creams, gels).

The alternative method for the determination of preservatives in cosmetics and medicines is the method with the application of fast liquid chromatography for the simultaneous determination of five preservatives (MI, CMI, BA, SB, MP) developed by our team [38]. This method does not allow for the separation of SB and PS, and is suitable for analysis only in cases where the product does not contain both preservatives simultaneously. This method is useless in environmental analyses, for which we are not able to predict the qualitative composition (as opposed to commercial products, for which the composition is usually indicated on the package). The method is dedicated to the analysis of pharmaceuticals and cosmetics, and does not cover the study of extraction of analytes from environmental water.

Our previous studies also included analyses of surface water samples from Poland for the content of pharmaceuticals [39] and disinfectants [40].

Other research centers in Poland also tend to research less conventional environmental contaminants, including their presence in different types of Polish waters. These tests relate to the residues of pharmaceuticals, pesticides, detergents, surfactants, and phenols [41-48]. Concerning the methods presented above for determining the residues of water pollutants, determining the preservatives is entirely justifiable, especially if the bactericidal and bacteriostatic properties of these compounds are take into account, which may have an impact on aquatic ecosystems.

Rather than determining the preservatives in industrial products (drugs, cosmetics), the presented publication focuses on the study of the detection of preservative residues in surface waters. For this purpose, the solid-phase extraction method has been developed, allowing for quantitative isolation (R: 65-106%), and a 600-fold concentration of the determined compounds. This is also innovative research. So far only some of the compounds (MI, CMI, MP) were tested in waters and wastewaters [6, 21, 22, 27, 28].

The analytical method proposed in this article allows for its wide potential use – the simultaneous determination in a single chromatographic analysis of all six selected compounds, and also the possible extraction procedures that could be used for analyses of different types of samples (for cosmetics and pharmaceuticals with different matrices, as well as surface water). Laboratories using this method can analyze any number of tested compounds (from the six chosen preservatives), present both individually as well as some side by side, without the necessity of changing the stationary or mobile phase.

Materials and Methods

Chemicals

Methylisothiazolinone (MI, 2-methyl-2H-isothiazol-3-one, CAS: 2682-20-4; min. 98.0%), chloromethylisothiazolinone (CMI, 5-chloro-2-methyl-2H-isothiazol-3-one, CAS: 26172-55-4), benzyl alcohol (BA, CAS: 100-51-6; min. 99.0%), potassium sorbate (PS, CAS: 24634-61-5; min. 99.0%), sodium benzoate (SB, CAS: 532-32-1; min. 99.0%), and methylparaben (MP, metyl 4-hydroxybenzoate, CAS: 99-76-3; min. 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloromethylisothiazolinone was purchased as a mixture containing 1.14% of CMI, 0.38% of MI, 21.9% of magnesium nitrate and water (to 100%), available under the trade name 'ProClin 150' (Sigma-Aldrich, St. Louis, MO, USA).

Both the acetonitrile and water (HPLC grade) and formic acid (analytical grade, 99%) used to prepare the mobile phase were purchased from Merck (Darmstadt, Germany). 0.1% formic acid was prepared by the appropriate dilution of the 99% acid with HPLC water.

The methanol (pure for analysis) used for the preparation of standard solutions and extraction was purchased from CHEMPUR COMPANY (Piekary Śląskie, Poland).

The sulfuric acid (95%, pure for analysis) used for preparing 2M solution (used for acidification of water) was purchased from PPH "STANLAB" Sp.J. (Gliwice, Poland).

The standard solutions with concentrations of 1 mg/mL were prepared by dissolving (MI, PS, SB, MP) or diluting (CMI, BA) the appropriate amount of standard in methanol. All the solutions were stored in dark glass containers at 4ºC. The stability of the standard solutions is at least three months. The working standard solutions were prepared by diluting the standard solution with methanol, immediately before their being used in chromatographic analysis.

Instrumentation

The chromatographic analyses were carried out using a chromatograph La Chrom ELITE Hitachi (Merck, Germany) equipped with a pump (Hitachi La Chrom ELITE, type L-2130, Merck, Germany) and DAD detector (Hitachi La Chrom ELITE, L-type 2455, Merck, Germany). Analyses were carried out at room temperature using a reversed-phase system.

During the development of the chromatographic system for simultaneous determination of six preservatives, the following chromatographic columns were tested: LiChrosorb RP 8 (250×4 mm, 7 μm) (Merck, Germany), LiChrosorb RP18 (250×4 mm, 7 μm) (Merck, Germany), LiChrosorb RP18 (125×4 mm, 7 μm) (Merck, Germany), Acclaim 120 C8 (150×4.6 mm, 3 μm) (Dionex Corporation, USA), Acclaim C18 PA II (150×2.1 mm, 3 μm) (Dionex Corporation, USA), Chromolith Performance RP-18 (100×4.6 mm, -) (Merck, Germany), Chromolith Performance RP-18 (2×100×4.6 mm, -) (Merck, Germany), Wide Pore RP 18 (250×4.6, 5 μm) (J. T. Baker, USA), and Develosil RP Aqueous AR-5 RP-30 (250×4.6 mm, 5.0 μm) (Nomura Chemical, Japan).

The chromatographic column used in the analyses was Develosil Aqueous AR RP-5 RP-30 (250×4.6 mm, 5.0 μm) (Nomura Chemical, Japan) equipped with a precolumn Develosil RP RP Aqueous AR-30 (10×4.0 mm, 5.0 μm) (Nomura Chemical, Japan). The developed method uses a gradient elution of the mobile phase, which consisted of a mixture of acetonitrile and 0.1% aqueous solution of formic acid. The gradient program is shown in Table 2. The flow rate of the mobile phase was 1.0 mL/min. The injection volume was 20 μL. The chromatograms were registered using a DAD detector; quantitative analyses were performed for the wavelengths λ = 237 nm (SB), λ = 257 nm (BA, MP), λ = 261 nm (PS), and λ = 274 nm (MI, CMI) . Data was obtained through the DAD ELITE HSM program (Merck, Germany).

To isolate the determined compounds from samples of cosmetics an ultrasonic bath, Sonic-1 (Polsonic, Poland), was used. The filtration of samples or obtained extracts were made by using NY 0.20 μm nylon filters (J. T. Baker, USA).

Table 2. Gradient elution program and chromatographic parameters determined using system suitability test (SST) for Develosil RP Aqueous AR-5 RP-30 column.

Program of gradient elution			SST parameters for Develosil RP Aqueous AR-5 RP-30 (250×4.6 mm, 5 μm) (Nomura Chemical)						
t[a] [min]	ACN [%]	0.1% HCOOH [%]	Analytes	t_r[b]	N (USP)[c]	k'[d]	A_s[e]	R_s (USP)[f]	N/m (USP)[g]
0	15	85	MI	3.90	9236	0.55	1.26	0	36945
5	15	85	CMI	8.83	5566	2.49	1.34	15.41	22263
7	25	75	BA	12.98	22472	4.15	1.31	10.25	89887
17	25	75	PS	17.07	20143	5.83	1.17	10.22	80570
19	15	85	SB	17.51	19652	5.21	1.28	1.29	78606
22	15	85	MP	20.77	16226	6.99	1.26	4.58	64905

[a]t – time, min., [b]t_r – retention time, min., [c]N (USP) – number of theoretical plates, [d]k' – capacity factor, [e]A_s – peak assymetry, [f]R_s (USP) – peak resolution, [g]N/m (USP) – theoretical plates/meter.

In order to isolate and concentrate the determined compounds from water samples, the Bakerbond solid phase extraction system was used (Baker type spe-12 G+, J. T. Baker, USA). Oasis HLB cartridges (500 mg, 6 mL) (Waters, USA) were used to SPE. For the filtration of water samples, hard paper filters were used.

Samples and Their Origin

Samples of cosmetics subjected to analyses for the content of preservatives were purchased from the local market (Poland). Cosmetic products for the analyses were face tonics, creams, lotions, shower gels, and face masks. These products came from different manufacturers. The analyses also are conducted on syrups containing potassium sorbate purchased on the local market.

The environmental water samples analyzed in this study were from Polish rivers and lakes. The samples were collected in the province of Silesia in accordance with the Polish standard for water sampling (PN-74/C-04620.00). The samples were collected in September 2011 and in June 2012. Water samples subjected to analysis were derived from the following polish rivers: Krzywa River (Bielsko-Biała), Wisła River (Skoczów), Brennica River (Brenna), Jesionka River (Jaworze Nałęże), Potok Szeroki River (Jaworze Górne), Drama River (Kamieniec), Drama River (Dzierżno), Kłodnica River (Gliwice), and the Bytomka River (Zabrze), and Polish lakes Dzierżno Duże (Dzierżno), Dzierżno Małe (Dzierżno), Żywieckie (Zarzecze), and Pławniowice Lake (Pławniowice).

Preparation of Cosmetics

Clear liquid formulations were analyzed directly or, if necessary, after the appropriate dilution with distilled water.

Solid samples and liquid formulations with a high density and viscosity required ultrasonic extraction with methanol prior to analysis. Approximately 1 g of the samples (toothpaste, cream, gel, etc.) were weighed and 6 mL of methanol was added, after which the samples were sonicated in an ultrasonic bath for 15 minutes, and later transferred quantitatively into a 10.0 mL volumetric flask filled to the mark with methanol and mixed. Then the solutions were filtered through a nylon filter with a pore size of 0.20 μm (J.T. Baker, USA) and the resulting filtrates were analyzed by the HPLC method (where necessary, samples were diluted with methanol or water).

Preparation of Water Samples

In developing the procedure for extracting the analytes from water samples the following extraction cartridges were used: C18 (500 mg, 6 mL, J. T. Baker), Polar Plus C18 (1000 mg, 6 mL, J. T. Baker), and Oasis HLB (500 mg, 6 mL, Waters).

The preliminary stage of the water preparation consisted of: acidification with 2M sulfuric acid to pH 2 and the filtration of the samples by hard paper filter.

The procedure of SPE (Oasis HLB, 500 mg, 6 mL; Waters) involved successively: conditioning with methanol (5 mL) and distilled water acidified with 2M sulfuric acid to pH 2 (3 mL); then passing the previously prepared water samples (3 L); and drying the cartridge (approximately 2 min) and eluting the analytes with a mixture of acetonitrile and 0.1% aqueous solution of formic acid (25:75, v:v) (2.5 mL) and acetonitrile (2.5 mL). The eluates were analyzed by the chromatography method developed.

Method Validation

For the developed chromatographic method using a liquid chromatography technique in reversed-phase system, the calibration curves for the standards dissolved in methanol and in the water matrix (Wapienica River, Bielsko-Biała, Poland) were designated. The analytes in each mixture of the standards were determined six times (n=6). Mixtures of standards dissolved in methanol contained, respectively:

Table 3. Analytical wavelengths (λ, nm), retention times (t_r, min), and their standard deviations for determined compounds in methanol solutions and matrices of samples.

Analytes	λ [nm]	Matrix	t_r [min]	SD [min]
MI	274	methanol	3.90	0.15
		water matrix	3.92	0.01
		cosmetic matrix	4.03	0.06
CMI	274	methanol	8.83	0.02
		water matrix	9.00	0.21
		cosmetic matrix	9.03	0.08
BA	257	methanol	12.98	0.01
		water matrix	13.23	0.05
		cosmetic matrix	13.04	0.38
PS	261	methanol	17.07	0.02
		water matrix	17.65	0.10
		cosmetic matrix	16.87	0.12
SB	237	methanol	17.51	0.03
		water matrix	18.30	0.14
		cosmetic matrix	17.43	0.12
MP	257	methanol	20.77	0.06
		water matrix	19.60	0.21
		cosmetic matrix	20.25	0.24

- mixture I: MI – 18.33 μg/mL, CMI – 25.00 μg/mL, BA – 100.00 μg/mL, PS – 10.00 μg/mL, SB – 10.00 μg/mL, MP – 10.00 μg/mL
- mixture II: MI – 13.75 μg/mL, CMI – 18.75 μg/mL, BA – 80.00 μg/mL, PS – 8.00 μg/mL, SB – 8.00 μg/mL, MP – 8.00 μg/mL
- mixture III: MI – 9.17 μg/mL, CMI – 12.50 μg/mL, BA – 60.00 μg/mL, PS – 6.00 μg/mL, SB – 6.00 μg/mL, MP – 6.00 μg/mL
- mixture IV: MI – 4.58 μg/mL, CMI – 6.25 μg/mL, BA – 40.00 μg/mL, PS – 4.00 μg/mL, SB – 4.00 μg/mL, MP – 4.00 μg/mL
- mixture V: MI – 3.17 μg/mL, CMI – 3.50 μg/mL, BA – 25.00 μg/mL, PS – 2.50 μg/mL, SB – 2.50 μg/mL, MP – 2.50 μg/mL

Standard additions to the extract obtained from the river water after SPE were as follows:

- mixture I: MI – 18.33 μg/mL, CMI – 25.00 μg/mL, BA – 100.00 μg/mL, PS – 10.00 μg/mL, SB – 10.00 μg/mL, MP – 10.00 μg/mL
- mixture II: MI – 13.75 μg/mL, CMI – 18.75 μg/mL, BA – 75.00 μg/mL, PS – 7.50 μg/mL, SB – 7.50 μg/mL, MP – 7.50 μg/mL
- mixture III: MI – 9.17 μg/mL, CMI – 12.50 μg/mL, BA – 50.00 μg/mL, PS – 5.00 μg/mL, SB – 5.00 μg/mL, MP – 5.00 μg/mL
- mixture IV: MI – 4.58 μg/mL, CMI – 6.25 μg/mL, BA – 40.00 μg/mL, PS – 2.50 μg/mL, SB – 2.50 μg/mL, MP – 2.50 μg/mL
- mixture V: MI – 1.83 μg/mL, CMI – 2.50 μg/mL, BA – 10.00 μg/mL, PS – 0.50 μg/mL, SB – 1.00 μg/mL, MP – 1.00 μg/mL.

The parameters of the calibration curves and the parameters from method validation are shown in Tables 4 and 5.

The limit of detection (LOD) was calculated based on the parameters of calibration curves, using the formula:

$$LOD = (3.3 \times S_{xy})/a$$

...where S_{xy} is the residual standard deviation of the calibration curve and a is the slope. The limit of quantification (LOQ) was determined as a multiple of the limit of detection LOQ = 3×LOD.

Method validation also included determining the intra- and inter-day precision of the method, both for the standard

Table 4. The parameters of calibration curves and values of LOD and LOQ determined for standards of preservatives in methanol solution (n=6).

Analytes	Linearity range[a] [μg·mL^{-1}]	a[b]	S_a[c]	b[d]	S_b[e]	R^2[f]	S_{xy}[g]	LOD[h] [μg·mL^{-1}]	LOQ[i] [μg·mL^{-1}]
MI	18.33-1.83	68131.59	869.51	-14318.81	9786.99	0.9995	11638.74	0.56	1.7
CMI	25.00-2.50	242979.98	2305.22	27766.28	35385.31	0.9997	42074.21	0.57	1.7
BA	100.00-10.00	89364.04	1141.42	66682.74	70083.69	0.9995	83331.64	3.1	9.2
PS	10.00-0.50	1013109.26	5448.11	-55155.38	30557.19	0.9999	46134.99	0.15	0.45
SB	10.00-1.00	314945.33	3680.14	-20727.52	22596.15	0.9996	26867.51	0.28	0.84
MP	10.00-1.00	642756.30	7733.53	21907.12	47484.10	0.9996	56460.04	0.29	0.87

[a]Equation of the calibration curve: y = a x + b, where: y – peak area, mAU and x – concentration, μg·mL^{-1}; [b]a – slope; [c]S_a – standard deviation of slope; [d]b – intercept; [e]S_b – standard deviation of intercept; [f]R^2 – correlation coefficient; [g]S_{xy} – residual standard deviation; [h]LOD – limit of detection assigned for chromatographic method, μg·mL^{-1}; [i]LOQ – limit of quantification assigned for chromatographic method, μg·mL^{-1}.

Table 5. The parameters of calibration curves and values of LOD and LOQ determined for preservatives in a water matrix (n=6).

Analytes	Linearity range[a] [μg·mL^{-1}]	a[b]	S_a[c]	b[d]	S_b[e]	R^2[f]	S_{xy}[g]	LOD[h] [μg·mL^{-1}]	LOQ[i] [μg·mL^{-1}]	MDL[j] [μg·L^{-1}]	MQL[k] [μg·L^{-1}]
MI	18.33-3.17	228793.66	3592.49	92868.01	40648.85	0.9993	45444.47	0.66	2.0	1.1	3.3
CMI	25.00-3.50	186857.53	3431.96	9995.93	52814.81	0.9990	60710.26	1.1	3.2	1.8	5.4
BA	100.00-25.00	9461.21	273.77	19911.50	18743.95	0.9983	15304.37	5.3	16	8.9	27
PS	15.00-2.50	765331.34	20149.23	33968.67	137952.36	0.9986	112637.63	0.49	1.5	0.81	2.4
SB	10.00-2.50	248786.21	6570.27	-4022.17	44983.59	0.9986	36728.95	0.49	1.5	0.81	2.4
MP	10.00-2.50	463395.27	14573.03	37537.50	99774.72	0.9980	81645.72	0.58	1.7	0.97	2.9

[a]Equation of the calibration curve: y = a x + b, where: y – peak area, mAU and x – concentration, μg·mL^{-1}; [b]a – slope; [c]S_a – standard deviation of slope; [d]b – intercept; [e]S_b – standard deviation of intercept; [f]R^2 – correlation coefficient; [g]S_{xy} – residual standard deviation; [h]LOD – limit of detection assigned for chromatographic method, μg·mL^{-1}; [i]LOQ – limit of quantification assigned for chromatographic method, μg·mL^{-1}; [j]MDL – limit of detection assigned for all analytical procedure (SPE and HPLC), ng·mL^{-1}; [k]MQL – limit of quantification assigned for all analytical procedure (SPE and HPLC), ng·mL^{-1}.

solutions in methanol (Table 6), as well as for the matrix of river water (Table 7). In order to determine the intra-day precision, six mixtures of standards for three concentration levels were prepared. These concentrations correspond to the working ranges of the calibration curves' limit values (about 95 and 5%) and intermediate (50%). These solutions were analyzed by the developed chromatography method. To determine the inter-day precision, analogous operations were performed within five days. The precision of the developed methods are based on the calculated values of the coefficient of variation (CV).

The accuracy of the method is based on the calculated relative errors (RE).

Stability Test

The stability study for all six compounds were determined in river water over a long period of time (24 months). For the stability tests of analytes to the river water sample (not containing the determined compounds), the appropriate amounts of standards were added. Samples containing analytes on two concentration levels, approximately equal to 90% and 10% of the working range of the calibration curves, were tested. These tests were carried out in different conditions – part of the samples were stored in a refrigerator (4ºC) and the other part was stored at room temperature (20-35ºC, depending on the season) in lit conditions. The results of long-term stability for the determined compounds are presented in Table 12 and as the graphs in Figs. 5-7. The measurements were performed successively in the following intervals: first day, 1 week, 2 weeks, 1 month, 1.5 months, 2.5 months, 3.5 months, 7 months, 9 months, 11 months, 13.5 months, 17 months, 20.5 months, and 24 months. For each of the concentration levels tested, and for each of the temperatures tested (4ºC and 20-35ºC), six parallel chromatographic analyses were performed. The result is the average of these analyses. The content [%] was calculated in relation to the amount of standards introduced to water on the first day of analysis. The river water matrix on which the study was conducted was the water from the Wapienica River (Bielsko-Biała, Poland). This water was a blank sample; it was confirmed before testing that the water did not contain any analytes.

Statistical Analysis

All reported results are the average of six or three independent chromatographic analysis. Analysis were performed six times during determination of retention times (t_r) in different matrices and their SD (Table 3), preparation of the calibration curves (Tables 4 and 5), designation of intra- and inter-day precision and also accuracy (Tables 6 and 7), assignation of the recoveries for different cosmetic matrices (Table 8), during sample analyses of cosmetics (Table 9) and surface waters (Table 11), and during stability tests (Table 12).

Analysis were performed three times during determination of chromatographic parameters (Table 2) and recoveries after SPE procedure (for water samples – Table 10).

Results and Discussion

Chromatographic System

As a part of the study, a chromatography method allowing for the simultaneous determination of six preservatives was developed. Nine chromatographic columns from different manufacturers, with different dimensions and filling, were tested. These columns are listed in the '*Materials and Methods*' section. Different compositions of the mobile phase, and different flow rates and elution methods (isocratic, gradient) were tested. Different solvents were tested as the components of the mobile phase e.g.: water, methanol, acetonitrile, 0.05% aqueous solution of trifluoroacetic acid, 0.1% aqueous solution of formic acid, and mixtures thereof. Analyses were carried out at room temperature (20-22ºC). For the nine chromatography columns

Table 6. Parameters characterizing the precision and accuracy of the developed chromatographic method derived from the analyses of standard solutions in methanol (n=6).

Analytes	Added [μg·mL^{-1}]	Intra-day precision				Inter-day precision			
		Measured [μg·mL^{-1}]	SD [μg·mL^{-1}]	Relative error [%]	Recovery [%]	Measured [μg·mL^{-1}]	SD [μg·mL^{-1}]	Relative error [%]	Recovery [%]
MI	17.33	17.07	0.10	-1.50	98	17.80	0.97	2.71	103
	10.33	10.61	0.58	2.71	103	10.58	0.76	2.42	102
	3.83	3.74	0.19	-2.35	98	3.91	0.23	2.09	102
CMI	22.00	22.34	0.66	1.55	102	22.85	1.10	3.86	104
	13.00	13.22	0.37	1.69	102	13.51	0.64	3.92	104
	4.00	4.00	0.11	0.12	100	4.12	0.14	3.00	103
BA	90.00	91.40	0.88	1.56	102	90.93	2.40	1.03	101
	60.00	61.42	1.60	2.37	102	60.07	2.50	0.12	100
	30.00	30.53	0.87	1.77	102	30.08	1.10	0.27	100
PS	9.00	9.00	0.11	0.08	100	9.34	0.32	3.78	104
	6.00	6.01	0.22	0.17	100	6.12	0.29	2.00	102
	3.00	3.01	0.11	0.33	100	3.13	0.14	4.33	104
SB	9.00	9.26	0.21	2.89	103	8.90	0.36	-1.11	99
	6.00	6.01	0.25	0.17	100	6.21	0.24	3.50	104
	3.00	2.94	0.074	-2.00	98	2.88	0.13	-4.00	96
MP	9.00	9.10	0.055	1.11	101	9.25	0.45	2.78	103
	6.00	6.23	0.22	3.83	104	6.04	0.32	0.67	101
	3.00	3.02	0.12	0.67	101	2.91	0.13	-3.00	97

Table 7. Parameters characterizing precision and accuracy of the developed method for river water matrix (n=6).

Analytes	Added [μg·mL^{-1}]	Intra-day precision				Inter-day precision			
		Measured [μg·mL^{-1}]	SD [μg·mL^{-1}]	Relative error [%]	Recovery [%]	Measured [μg·mL^{-1}]	SD [μg·mL^{-1}]	Relative error [%]	Recovery [%]
MI	17.87	17.09	0.94	-4.36	96	18.03	1.20	0.90	101
	9.17	8.97	0.73	-2.18	98	9.51	0.62	3.71	104
	3.67	3.47	0.37	-5.45	95	3.83	0.27	4.36	104
CMI	23.75	23.73	1.40	-0.08	100	24.55	1.40	3.37	103
	12.50	12.19	0.89	-2.48	98	12.89	0.87	3.12	103
	4.75	4.74	0.29	-0.21	100	4.99	0.48	5.05	105
BA	95.00	93.96	5.39	-1.09	99	91.13	7.30	-4.07	96
	50.00	49.77	3.60	-0.46	100	49.56	3.10	-0.88	99
	30.00	29.56	2.10	-1.47	99	29.60	2.20	-1.33	99
PS	14.25	14.61	0.80	2.53	103	15.21	0.86	6.74	107
	7.50	7.49	0.55	-0.13	100	7.81	0.45	4.13	104
	3.25	3.06	0.24	-5.85	94	3.21	0.24	-1.23	99
SB	9.50	9.93	0.50	4.52	105	9.15	0.71	-3.68	96
	5.00	5.17	0.40	3.40	10	4.65	0.39	-7.00	93
	3.00	2.99	0.16	-0.33	100	2.88	0.17	-4.00	96
MP	9.50	9.33	0.55	-1.79	98	9.64	0.57	1.47	101
	5.00	5.01	0.35	0.20	100	5.27	0.32	5.40	105
	3.00	2.99	0.22	-0.33	100	3.12	0.26	4.00	104

Table 8. Average recovery (R, %) (n=6) and its standard deviatons (SD, %) for the ultrasound-assisted extraction procedure, designated for blank sample (cosmetics – cream and gel) with standards addition.

Samples	Analytes	R [%]	SD [%]
Cream	MI	97.3	4.6
	CMI	79.5	3.4
	BA	114.2	3.5
	PS	84.40	0.71
	SB	70.1	3.3
	MP	111.1	5.0
Gel	MI	92.8	3.5
	CMI	84.3	3.5
	BA	118.7	3.7
	PS	81.8	2.9
	SB	69.6	3.2
	MP	81.4	2.5

tested in this study, and elution solvents with a flow rate equal to 1.0 mL/min, and according to the gradient elution program shown in Table 2, the following chromatographic parameters were determined: retention time (t_r), number of theoretical plates (N), capacity factor (k'), peak asymmetry (A_s), resolution (R_s), and number of theoretical plates/meter (N/m). The best results were obtained with columns Acclaim C18 PA II and Develosil RP Aqueous AR-5 RP-30. For Acclaim C18 PA II (150 x 2.1 mm, 3 µm) (Dionex Corporation, USA) column, all six analytes were separated in less than 13 minutes and the desired values (>1.7) of peak resolution (R_s) were obtained. However, for further study the Develosil RP Aqueous column AR-5 RP-30 (250×4.6 mm, 5.0 um) (Nomura Chemical, Japan) was selected, since it showed better parameters for the number of theoretical plates (N/m), capacity factor (k'), and peak asymmetry (A_s). The assigned values are shown in Table 2. The application of these conditions for analysis allow for the efficient separation of all six compounds in 22 minutes. The chromatogram obtained from the analysis of the mixture of the standard solutions is shown in Fig. 1.

In order to achieve suitably strong analytical signals, and to avoid interference from matrices, the registering of chromatograms and quantitative analysis were carried out at different wavelengths, suitable for the individual compounds. Table 3 shows the analytical wavelengths for each analyte, the average retention times of the standards, and its standard deviations. The average retention times of analytes were registered for standard solutions prepared in methanol, and the standards added to both water and cosmetic matrices.

Chromatographic Method Validation

The high values of the correlation coefficients (0.9980-0.9999) confirm the linearity of the calibration curves in the concentration ranges tested, and the close relationship between the variables.

The high values of slope for the determined calibration curves indicate the sensitivity of this method.

Values of CV are less than 6% for standards in methanol (Table 6), and less than 11% for standards in a water matrix (Table 7), which confirms the good precision of the presented method. The higher values for the environmental water matrix are due to matrix effects and interferences from the matrix.

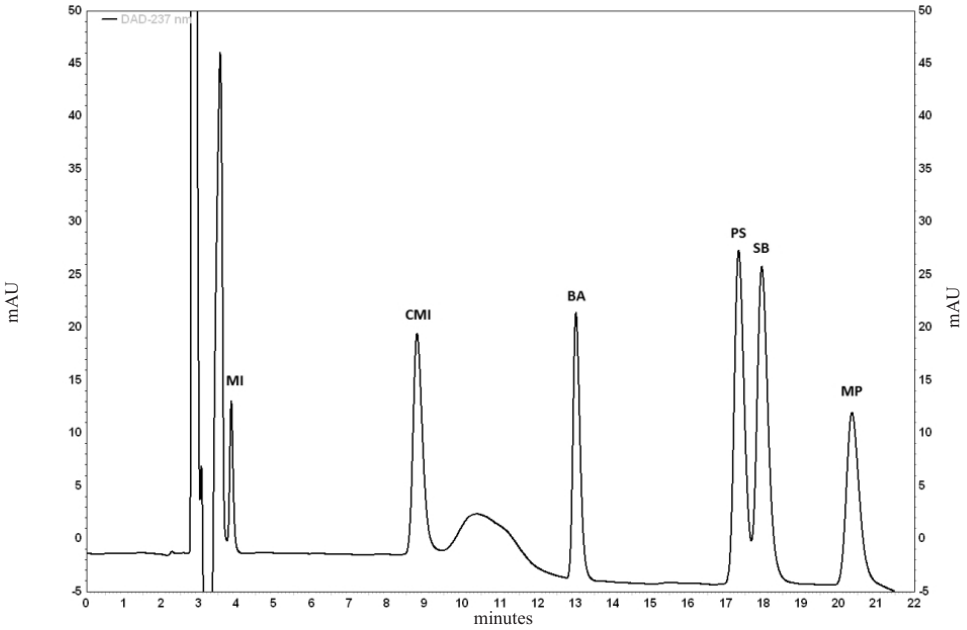


Fig. 1. Chromatogram of a mixture of standards with concentrations: 18.33 µg·mL^{-1} (MI), 25.00 µg·mL^{-1} (MCI), 600.00 µg·mL^{-1} (BA), 10.00 µg·mL^{-1} (PS, MP), 40.00 µg·mL^{-1} (SB), registered for monitoring wavelength λ=237 nm.

Table 9. Results of analyses for cosmetics and pharmaceuticals (n=6).

Sample type	Name[a]	Analytes	Dilution	Content [μg·g^{-1}]	SD [μg·g^{-1}]	Acceptable content [μg·g^{-1}]
Hand cream	1a	BA	5	2451	119	-
		PS	0	5.70	0.26	6000
		MP	20	1112	48	4000
	2	PS	0	14.96	0.60	6000
		SB	0	54.43	0.95	5000
		MP	20	973	27	4000
Face cream	1b	PS	2	149.9	3.1	6000
		MP	25	1463	17	4000
Eye cream	1c	PS	0	71.64	0.79	6000
		MP	0	9.68	0.31	4000
Body cream	1d	BA	10	6849	138	-
		PS	0	22.00	0.65	6000
		SB	0	80.0	3.2	5000
		MP	25	1046	30	4000
Body lotion	1e	PS	0	2.201	0.057	6000
		MP	50	2268	26	4000
Shower gel	1f	MI	0	<LOQ	-	15
		CMI	0	8.59	0.30	15
		PS	12.5	1.503	0.062	6000
	1g	MI	0	<LOQ	-	15
		CMI	0	7.94	0.40	15
		PS	0	0.491	0.014	6000
Face gel	1h	PS	0	1242	18	6000
		SB	0	28.63	0.38	5000
		MP	0	38.75	0.49	4000
Face mask	1i	BA	10	1317.53	53.55	-
		PS	0	<LOQ	-	6000
		SB	0	8.76	0.40	25000
		MP	20	497	16	4000
Shampoo	1j	MI	0	<LOQ	-	15
		CMI	0	8.54	0.19	15
		PS	0	1.204	0.047	6000
		SB	10	317.5	8.7	25000
	3	MI	0	<LOQ	-	15
		CMI	0	3.40	0.12	15
		PS	0	2.493	0.014	6000
		SB	0	7.20	0.29	25000
	4	PS	0	5.85	0.13	6000
		SB	100	7947	384	25000

Table 9. Continued.

Kind of sample	Name[a]	Analytes	Dilution	Content [μg·g^{-1}]	SD [μg·g^{-1}]	Acceptable content [μg·g^{-1}]
Lifting	1k	PS	0	20.01	0.17	6000
		SB	0	141.9	2.2	5000
		MP	25	1510	66	4000
Syrups	5	PS	25	1737	80	6000
	6	PS	500	769.1	7.5	6000
Face tonic[b]	7	PS	500	1021	17	6000
		SB	500	2167	41	5000
		MP	500	2298	26	4000
	8	PS	250	786.0	7.0	6000
		SB	250	1698	17	5000

[a]numbers 1-8 indicate manufacturers, letters a-k replace the trade name of the sample;
[b]concentrations of preservatives in face tonics are given in [μg·mL^{-1}].

Table 10. Average recovery for the SPE procedure (R, %) and its standard deviatons (SD, %) for the samples of river water (3 L) with standards addition (n=3).

Analytes	C18		C18 PP		Oasis HLB									
	Distilled water		Distilled water		Distilled water		Tap water		River water					
									95%[a]		50%[a]		5%[a]	
	R [%]	SD [%]	R [%]	SD [%]	R [%]	SD [%]	R [%]	SD [%]	R [%]	SD [%]	R [%]	SD [%]	R [%]	SD [%]
MI	17.64	0.74	61.5	2.6	89.7	3.4	86.0	3.2	80.7	1.9	72.5	2.2	65.4	2.0
CMI	91.7	1.0	101.4	1.1	102.7	2.5	102.8	5.7	103.2	2.0	102.9	2.6	103.2	4.6
BA	67.1	1.5	78.63	0.43	82.4	2.2	79.7	4.9	79.6	3.6	78.6	4.1	77.2	5.6
PS	18.56	0.29	83.5	1.3	102.5	2.2	101.5	6.3	103.3	3.8	102.2	5.0	105.6	7.6
SB	8.5	0.65	9.18	0.70	86.6	5.3	78.3	5.3	75.6	3.2	74.5	2.9	72.3	3.0
MP	103.9	3.5	105.5	3.1	82.3	1.5	76.5	4.2	74.3	1.8	74.4	1.1	73.6	4.5

[a]addition of the analytes corresponding to about 95, 50, and 5% of the linearity range for calibration curves in real water matrix.

The accuracy of the method is based on the calculated relative errors (RE), whose values are also given in Tables 6 and 7 and do not exceed 4.4% for standards in the methanol solution and 7.0% for standards in the water matrix.

The recovery values presented in Tables 6 and 7 are the quantitative expression of the bias and allow for the detection and elimination of some systematic errors that occurred during the measurements.

The Preparation of Cosmetics

In the case of liquid samples (face tonics), water was used as a solvent for dilution; three parallel dilutions were made, and each of them was subjected to chromatographic analysis twice.

In the case of solid samples and liquid samples with a high density and viscosity (creams, gels, lotions, masks, syrups), it was necessary that preparation procedures should allow for the quantitative isolation of the analytes from the samples. Based on a review concerning previous methods tested by other researchers, the number of methods for analyzing cosmetics and pharmaceuticals, such as shaking the sample with a solvent (acetonitrile, methanol), followed by their centrifugation, were tested in this study. However, the most effective method proved to be ultrasound-assisted extraction. For this purpose, the number of solvents (methanol, ethanol, ethyl acetate, acetone, acetonitrile, methylene chloride), various pH (pH 1, pH 5, pH 7, and pH 10), and the different extraction times (5, 15, 45, 90 minutes) were tested to optimize the extraction conditions. The most effective in terms of recoveries received turned out to be the procedure mentioned in "*Experimental Procedures – the Preparation of Cosmetics*" above. The values of recoveries obtained for two different matrices (cream and gel – with a composition similar to the composition of the tested samples, but not containing the ana-

Table 11. Results of the analyses of environmental water samples from Poland (the Silesian region).

Samples (location)	Analytes	Concentration [μg·L^{-1}]	SD [μg·L^{-1}]	CV [%]
Krzywa River (Bielsko-Biała)	SB	2.68	0.13	5
	MP	< MQL	-	-
Wisła River (Skoczów)	SB	< MQL	-	-
Brennica River (Brenna)	SB	< MQL	-	-
	MP	< MQL	-	-
Jesionka River (Jaworze Nałęże)	SB	2.87	0.14	5
Potok Szeroki River (Jaworze Górne)	SB	< MQL	-	-
Drama River (Kamieniec)	SB	2.713	0.092	3.4
Drama River (Dzierżno)	SB	3.06	0.20	7
Kłodnica River (Gliwice)	CMI	7.89	0,32	4
	SB	3.12	0.18	6
Bytomka River (Zabrze)	CMI	11.57	0.61	5
	BA	35.1	1.9	5
	SB	2.98	0.19	6
Lake Dzierżno Duże (Dzierżno)	CMI	5.72	0.29	5

Table 12. Stability of preservatives in river water – the content of the analytes in the environmental water samples after 24 months storage at room temperature conditions (T: 20-35ºC) and in the refrigerator (T=4ºC).

Analytes	Content [%] after 24 months			
	T: 20-35ºC		T=4ºC	
	A[a]	B[a]	A[a]	B[a]
MI	94.4	87.6	93.8	92.7
CMI	18.7	18.6	91.2	90.5
BA	94.2	90.4	95.3	92.4
PS	50.5	51.3	94.9	87.4
SB	73.0	70.2	81.2	81.7
MP	34.7	34.3	33.2	28.7

[a]the addition of the analytes corresponding to about 90 (level A) and 10% (level B) of the linearity range for calibration curves determined in real water matrix.

lytes), are shown in Table 8. In order to determine the recoveries for each tested matrix, the appropriate amount of standards were added to three portions of preparation weighed to approximately 1 g, thoroughly mixed, after which 6 mL of methanol was added. The sample was mixed again and then sonicated for 15 minutes. The samples were then quantitatively transferred to a 10.0 mL volumetric flask, filled with methanol to volume, and mixed thoroughly. The obtained sample was filtered through a nylon filter (0.20 μm), and then each of them were analyzed three times by the chromatography method. This method is similar to the procedure described by Q. Zhang et al. for the determination of MP only [49]; however, the ultrasound-assisted extraction time was extended to 15 minutes and extraction was used for the quantitative isolation of all six analytes. The ultrasound-assisted extraction method applied in this study is similar to our previously described methods [38], but this one also includes additional recoveries determined in the cream and gel matrix for PS.

In the analysis of cosmetic samples – the ultrasound-assisted extraction method was used for three weighed portions (approximately 1 g) of each of the commercial samples (the procedure was performed according to the one described in "*Experimental Procedures – the Preparation of Cosmetics*" above). If it was necessary to dilute samples before the chromatographic analysis – water (for gels and syrups) or methanol (for creams and lotions) were used as solvents. Samples that were not diluted were analyzed twice; for samples requiring dilution – two parallel dilutions were made, and each of them was subjected to chromatographic analysis.

Results of the Analyses of Cosmetics

The analytical procedure developed was used for the analysis of samples of cosmetics and pharmaceuticals. The identification of the determined compounds in the samples was based on the comparison of their retention times with the retention times of the standards, and the comparison of their absorption spectra, as well as the method of standard addition, was used. The results of the analyses are shown in Table 9.

Chromatograms of cosmetic samples or their extracts are shown in Figs. 2-3.

The determined contents of individual analytes in commercial samples are within the aforementioned limits.

Preparation of Water Samples

In order to concentrate the analytes and to separate interferences from the water matrix, the solid-phase extraction technique (SPE) was selected. During the study extraction cartridges with various fillings were tested. Tested cartridges are listed in the "*Materials and Methods*" section. Various extraction procedures were also tested (using various solvents for conditioning the cartridges, different methods for water preparation, and different solvents for the elution of the analytes). These studies were based on the methods described before in the literature, and allowing for the quantitative extraction of the individual substances from water or sewage samples. However, none of the methods described before was suitable for the simultaneous, quantitative isolation of all six compounds. Due to the best results obtained for the polymer packed cartridge to further test – involving a comparison of the recoveries for distilled water, tap water and river water – Oasis HLB (500 mg, 6 mL)

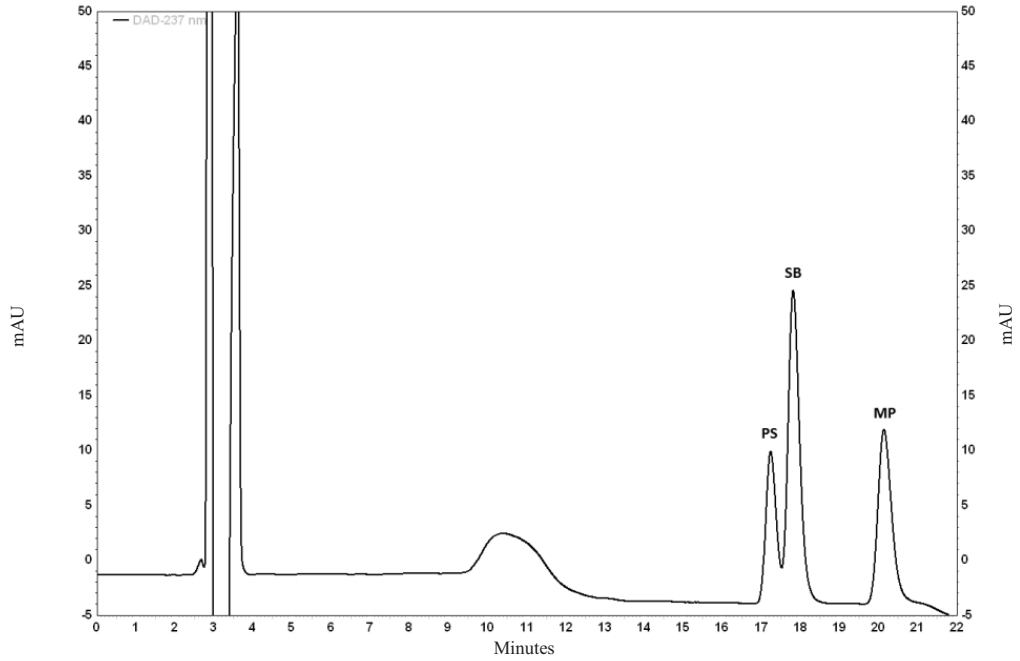


Fig. 2. Chromatogram of facial tonic registered for monitoring wavelength λ=237 nm.

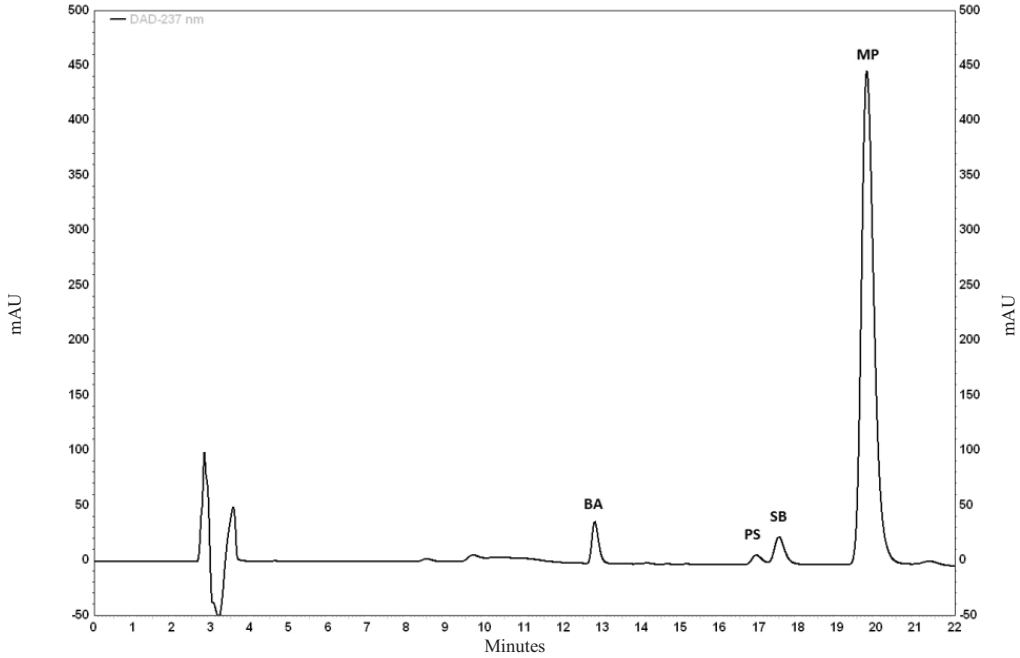


Fig. 3. Chromatogram of the extract obtained from body lotion registered for monitoring wavelength: λ=237 nm.

(Waters) cartridges were used. The solid-phase extraction method described in the experimental section allows for the extraction of analytes with satisfactory recovery values (R: 65.4-105.6%, but for most of the analyzed compounds these are higher than 72.3%). The mean values of recoveries and their standard deviations for the tested cartridges and the tested water samples are shown in Table 10. In order to determine recoveries, three water samples (3 L) of each type of water were extracted in parallel (respectively, these were: distilled water, tap water, and river water. These waters do not contain analytes; the river water was from the Wapienica River, Bielsko-Biała, Poland). To each water sample, the appropriate amount of standards (corresponding to approximately 95% of the linearity range of the calibration curves) was added. The obtained eluates were analyzed twice by chromatographic method (the final number of samples: n=6). Then the recovery for Oasis HLB cartridges and for river water samples (3 L) with standard additions of about 95, 50, and 5% of the linearity range of calibration curves were determined.

Results of Analyses of Water Samples

The method of solid-phase extraction developed in this study allows for a 600-fold concentration of the analytes. In this study, 13 samples of surface waters were tested; their results are presented in Table 11. The most frequently identified and determined compound is SB (7 samples), then CMI (3 samples), and BA (1 sample). Three of the examined waters (Dzierżno Małe Lake, Żywieckie Lake, Pławniowice Lake) did not identify any of the determined compounds. Additionally, in the case of three samples, it was possible to detect small amounts of MP and SB (c>LOD), but because of their too low concentrations (c<LOQ) it was impossible to quantitatively analyze them.

The chromatogram registered for the analysis of the eluate obtained after SPE of the water sample is shown in Fig. 4.

The presence of preservatives in the surface waters tested may be caused by their use in many branches of human activity, not only in the cosmetic and pharmaceutical industries, but also during food production (SB is the most frequently detected compound, probably due to its wide use, especially in the food industry).

The Stability Test of Preservatives in Surface Water

The results obtained after 24 months affirm the grounds of the study, that compounds are stable in water, as indicated. The results show that the stability of analytes at room temperature is less than in the case of storage in the refrigerator. The largest decline (to about 18.6% of the introduced amount) was observed for CMI. The most stable compound was BA (in which was measured greater than 90.4% of the initial content after 24 months). In the cases of MI and BA the changes are small, and the decline oscillates above the limits of 5.6-12.4%. In the case of SB a decrease at about 18.3-29.8% was observed. In other cases (CMI, PS, MP), the differences in degradation are significant, which is illustrated in Figs. 5-7.

Conclusions

Our paper presents the new chromatography method and sample preparation procedures (for cosmetics and pharmaceuticals with different matrix composition and physical form, and also for environmental water samples) for the simultaneous isolation, concentration, and determination of

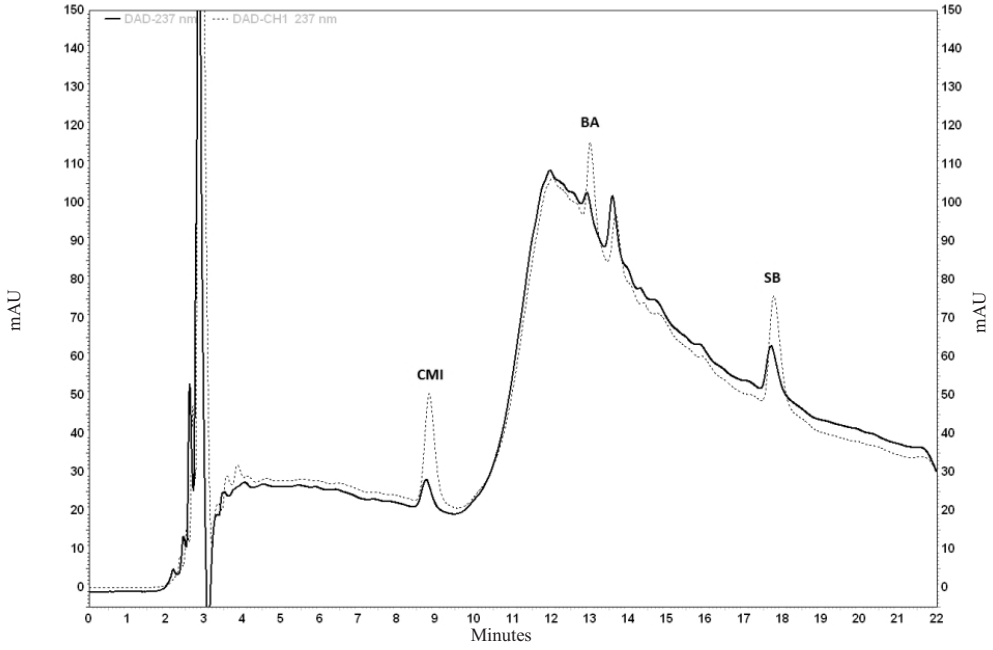


Fig. 4. Chromatogram of the extract obtained after SPE of water (3 L) from Bytomka River (Zabrze, Poland) – solid line; chromatogram of the extract with the addition of standards (CMI, BA, SB) – dotted line, both registered with the wavelength λ=237 nm.

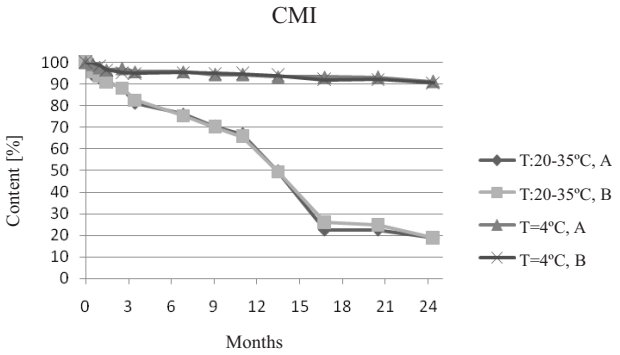


Fig. 5. Graph showing the long-term stability (24 months) of methylchloroisothiazolinone in environmental water, for samples stored at room temperature (T: 20-35ºC) and in a refrigerator (T=4ºC), containing the analyte at two concentration levels: level A – about 90% of the working range of the calibration curve and level B – about 10% of the working range of the calibration curve.

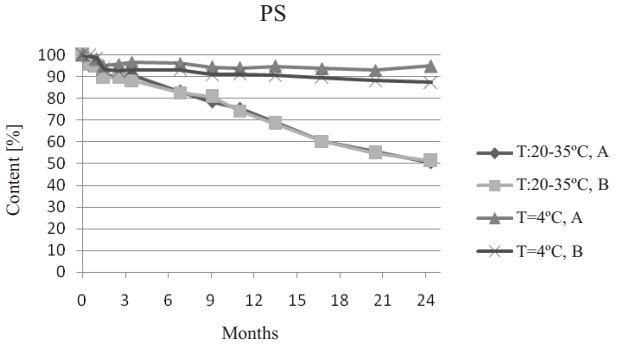


Fig. 6. Graph showing the long-term stability (24 months) of potassium sorbate in environmental water, for samples stored at room temperature (T: 20-35ºC) and in a refrigerator (T=4ºC), containing the analyte at two concentration levels: level A – about 90% of the working range of the calibration curve and level B – about 10% of the working range of the calibration curve.

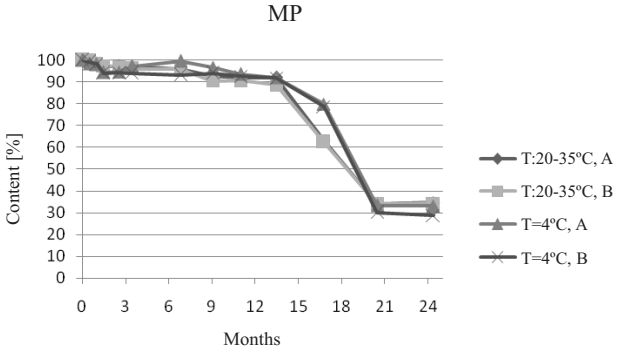


Fig. 7. Graph showing the long-term stability (24 months) of methylparaben in environmental water, for samples stored at room temperature (T: 20-35°C) and in a refrigerator (T=4ºC), containing the analyte at two concentration levels: level A – about 90% of the working range of the calibration curve and level B – about 10% of the working range of the calibration curve.

six preservatives: MI, CMI, BA, PS, SB, and MP. These methods can be used in the cosmetics and pharmaceutical industries for the quality control of products and for the evaluation of their stability. This method may also be useful in environmental monitoring and assessment of the degree of environmental pollution by residues of preservatives. The methods and procedures developed in this study have been validated.

The results obtained for the samples of cosmetics and medicines indicate the possibility of the practical application of the developed methods; both the ultrasound-assisted extraction procedure, and chromatographic methods for the qualitative and quantitative analysis of select preservatives.

In addition, the analyses of environmental water samples also confirmed the potential application of this method. The solid-phase extraction, coupled with the chromatographic method developed in this study, are suitable for the analysis of residues of preservatives in aquatic ecosystems. The results for surface water samples indicate the presence of this type of pollution in the environment, which is of significance to living organisms. This study is innovative, but their results should draw the researchers' attention to the problem of the presence of the non-conventional organic pollutants in the environment. Currently, the presence of various chemicals (usually drugs), is testing in the environment, but the scale of that study should be expanded and include other substances that potentially threaten living organisms, interfering with their habitat. The designation of the stability of preservatives in water confirms the validity of the study.

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