Determination of Preservatives in Cosmetics, Cleaning Agents and Pharmaceuticals Using Fast Liquid Chromatography

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This paper reports the development of a method for simultaneously determining five preservatives in cosmetics, cleaning agents and pharmaceuticals by fast liquid chromatography. Methylisothiazolinone, methylchloroisothiazolinone, benzyl alcohol, sodium benzoate and methylparaben were separated on a Chromolith Fast Gradient reversed-phase 18ε column using gradient elution with acetonitrile and a 0.1% aqueous solution of formic acid, with a run time of 3 min. The preparation of solid and liquid samples included ultrasonic extraction with methanol with recoveries ranging from 69 to 119%.

The developed method was used to analyze samples of cosmetics (66 samples), cleaning agents (five samples) and pharmaceutical industry products (17 samples).

Introduction

Cosmetics, cleaning agents and pharmaceuticals that contain water require protection against microorganism growth to ensure product quality. Preservatives ensure product quality. The most frequently used preservatives include the following compounds: methylisothiazolinone (MI), methylchloroisothiazolinone (MCI), benzyl alcohol (BA), sodium benzoate (SB) and parabens, particularly methylparaben (MP). Structural formulas of these compounds are presented in Figure 1. The determination of these substances in cosmetics and pharmaceuticals is important in quality control, especially considering the numerous reports of allergic reactions caused by preservatives. Detection methods should simultaneously extract, separate and determine multiple preservatives in a variety of samples, while also eliminating interferences.

Of the preservatives examined in this study, the most frequently used is MP due to its broad antimicrobial spectrum and the fact that it does not modify the physical properties of the final products (i.e., taste, smell, color or consistency). It is used in food, cosmetics and pharmaceuticals. Recently, more attention has been paid to the qualitative and quantitative analysis of parabens because of the allergies they cause (1). Previous studies using in vitro animal models have also shown that parabens exhibit estrogenic activity. They influence testosterone production and the function of the female reproductive system (2). Moreover, long-term exposure to parabens, even at low concentrations, causes biological tissues to absorb up to 20.6 ± 4.2 ng/g, which may influence the growth and development of cancerous tissues (e.g., breast cancer) (1). The European Economic Community Directive (EEC) lists an allowable content of parabens in cosmetics of up to 0.4% for a single compound or up to 0.8% for the total content of all parabens used in a preparation. In food, however, the allowable content of parabens is 0.1%. Total exposure to parabens is 76 mg per day or 1.3 mg per kg of body weight. These doses take into account the following distribution: 1 mg daily from food, 50 mg from cosmetics and 25 mg from pharmaceuticals (3). MP is found in nearly all categories of cosmetics (e.g., lotions, creams, gels and fluids) and in many pharmaceuticals (i.e., ointments, gels, syrups, creams and aerosols).

BA is commonly used as a bacteriostatic agent or as one of the components in various liquid pharmaceuticals. Monitoring of its content in medicines is significant due to its potential oxidation to toxic benzaldehyde (4). BA is also used as one of the fragrance ingredients in perfume products. The European Union has issued a directive (2003/15/EC) that requires the packages of final cosmetic products to include a list of ingredients with concentrations greater than 0.01% for rinse-off products and 0.001% for leave-on products (5).

Isothiazolinone group compounds are commonly used as preservatives in industrial water-based products (e.g., detergents, cosmetics, paints, resins, emulsions, textile softeners and products for polishing) in the cosmetic, textile and paper industries. MI and MCI are especially relevant because they are strong skin irritants and sensitizers. These preservatives are particularly common in cosmetics, warranting specific limitations on their maximum concentrations at 0.0015% for a 3:1 mixture of MCI:MI and 0.01% for MI (6).

SB is a preservative most commonly used to protect food, but is also found in cosmetics and pharmaceuticals to keep them fresh and inhibit the growth of yeast, mold and bacteria. It exhibits positive activity in an acidic medium. In response to reports on sensitization to this preservative, the American Food and Drug Administration (FDA) limits its concentration in products to different levels, depending on the category of the product: 2.5% in rinse-off products, 1.7% in oral care products and 0.5% in leave-on products (7).

The most frequently used technique in preservative analysis is high-performance liquid chromatography (HPLC) (3, 5, 7–21), with fewer reports on ultrahigh-performance liquid chromatography (UHPLC) (22–25). A reversed-phase system employing octadecylsilane stationary phases is typically used, with gradient elution by a mixture of water and organic solvents (3, 5–12, 14–19, 21–28). Gas chromatography and capillary electrophoresis have been reported much less frequently (27–31), and single determinations have been made using spectrophotometric (32) or voltammetric methods (33). Spectrophotometric detection is most common in chromatography (5–19, 21–22) and some
mass spectrometry (3, 23–25, 27–29) or chemiluminescent detection (1, 20).

Preservatives are most commonly determined in samples of pharmaceuticals (8–9, 11–19, 21–22, 31–33), cosmetics (1, 3, 5, 10, 30) and food (7). There are, however, also publications describing determination of their residues in wastewater and surface water (6, 23, 24).

Previous methods have determined single preservatives or preservatives in a mixture with drugs or other preservatives, but never the simultaneous determination of the five mentioned preservatives. Moreover, the described methods of sample preparation for chromatographic analysis have only focused on specific product categories. There is currently no universal approach to this type of analysis, either in developed chromatographic methods or sample preparation procedures (i.e., for different sample matrices, such as liquids, pastes, ointments, creams and gels). This procedure can simultaneously determine MI, MCI, BA, SB and MP in a single chromatographic analysis in various kinds of sample matrices.

### Experimental Procedure

#### Reagents and chemicals

MI (2-methyl-2H-isothiazol-3-one; CAS: 2682-20-4), MCI (5-chloro-2-methyl-2H-isothiazol-3-one; CAS: 26172-55-4), BA (CAS: 100-51-6), SB (CAS: 532-32-1) and MP (methyl 4-hydroxybenzoate; CAS: 99-76-3) were purchased from Sigma Aldrich (St. Louis, MO). MCI was purchased as a mixture consisting of 1.14% MCI, 0.38% MI, 21.9% magnesium nitrate (V) and water (to 100%) under the trade name ProClin 150 (Sigma Aldrich).

Acetonitrile (HPLC grade), water (HPLC grade) and formic acid (99%) used as the mobile phase were purchased from Merck (Darmstadt, Germany). Formic acid (0.1%) was obtained by appropriate dilution of the concentrated reagent with water for HPLC. Methanol (pure for analysis) used in standard solution preparation and extraction was purchased from POCh (Gliwice, Poland).

Standard solutions were prepared at 1 mg/mL by dissolving MI, SB and MP or diluting MCI and BA in methanol. All standard solutions were stored in amber glass containers at 4 °C. Standard solutions were stable for at least three months. Working solutions were prepared by diluting the standard solutions with methanol immediately before use in chromatographic analyses.

#### Apparatus

Chromatographic analyses were conducted on a La Chrom ULTRA chromatograph (Hitachi, Merck) equipped with two pumps (L-2160U), an autosampler (L-2200U), an ultraviolet (UV) detector with variable wavelength (L-2400U) and a thermostat (L-2350). Analyses were conducted at 12 °C on a reversed phase Chromolith Fast Gradient RP 18e column (50 x 2 mm i.d.; Merck) with gradient elution in acetonitrile and a 0.1% aqueous solution of formic acid. The elution program is shown in Table I. The flow rate of the mobile phase was 0.7 mL/min. The injection volume was 2 μL. Compounds were detected at the following wavelengths: 237 nm (SB), 257 nm (BA, MP) and 274 nm (MI, MCI). Data were acquired on a DAD ELITE HSM control program (Merck). A Sonic-1 ultrasonic bath (Polsonic, Poland) was used to extract compounds from sample matrices, and nylon filters (NY 0.20 μm; JT Baker, Phillipsburg, NJ) were used for sample filtration.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Gradient Elution Program</th>
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<tbody>
<tr>
<td>t (%)</td>
<td>ACN (%)</td>
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<tr>
<td>0.0</td>
<td>5</td>
</tr>
<tr>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td>2.0</td>
<td>20</td>
</tr>
<tr>
<td>3.0</td>
<td>5</td>
</tr>
</tbody>
</table>

#### Sample preparation

Samples were purchased at a local market in Poland. The cosmetic products included facial tonics, mouthwashes, toothpastes, creams, body lotions, shaving gels, shower gels, body oils, body sprays and face masks. These products came from various companies, including Avon Cosmetics, Ziaja Ltd., Nivea Beisdorf-Lechia, Adidas International Marketing, Unilever Poland, Laboratorium Kosmetyczne Dr. Irena Eris, L’Oreal Poland, Oceanic, Colgate-Palmolive and Johnson & Johnson Poland.

Pharmaceutical products included injection solutions, syrups, aerosols, gels and ointments from various companies:

Household cleaning and disinfecting agents from Colgate-Palmolive and Reckitt-Benckiser (Poland) were also analyzed.

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

Solid samples and liquids with high density or viscosity required ultrasonic extraction with methanol prior to analysis. Approximately 1 g of sample was weighed out (e.g., toothpaste, cream, gel or ointment), and 6.0 mL of methanol was added. These solutions were mixed and placed in an ultrasonic bath for 15 min. Solutions were then quantitatively transferred to a 10 mL volumetric flask and completed to the final volume with methanol. Next, solutions were filtered through a nylon filter with a pore diameter of 0.20 μm, and the obtained filtrates were analyzed by the developed method. If necessary, samples were diluted further.

Results and Discussion

Chromatographic method development

A chromatographic method was developed for the rapid, simultaneous determination of five preservatives in various consumer products. Two chromatographic columns, the Hypersil GOLD (50 × 2.1 mm i.d., 1.9 μm; Thermo Scientific, Palo Alto, CA) and Chromolith Fast Gradient RP 18e (50 × 2 mm i.d.; Merck) were examined. The Chromolith column was selected for further study because of its better chromatographic parameters. Different mobile phase compositions, flow rates and elution methods (i.e., isocratic, gradient) were examined. Components of the mobile phase included water, methanol, acetonitrile, a 0.05% aqueous solution of trifluoroacetic acid, a 0.1% aqueous solution of formic acid and mixtures thereof. The best results were obtained using gradient elution with acetonitrile and 0.1% formic acid with flow rate of 0.7 mL/min.

The gradient elution program is shown in Table I. The column temperature was also optimized. Tested temperatures were: 30, 25, 20, 15 and 12°C. A temperature of 12°C (this temperature allows for very good separation of SB and MP, which have similar retention times). These conditions effectively separated all five compounds in less than 3 min with good repeatability, resolution and peak symmetry.

Standard chromatograms are shown in Figure 2. To acquire sufficiently strong signals, various detection wavelengths were evaluated. For quantitative analyses, analytical wavelengths were chosen at the point of maximum absorption for each compound: 237 nm for SB, 257 nm for BA and MP and 274 nm for MI and MCI.

Analytical method validation

Calibration curves were generated based on the analysis of a series of mixtures prepared by the appropriate dilution of the standard solutions with methanol. Analytes in each of the mixtures were determined six times. The determined compounds were examined at the following concentrations: MI at 13.33, 10.00, 6.67, 3.33 and 0.33 μg/mL; MCI at 10.00, 7.50, 5.00, 2.50 and 0.25 μg/mL; BA at 100.00, 75.0, 50.00, 25.00 and 15.00 μg/mL; SB at 10.00, 7.50, 5.00, 2.50 and 1.00 μg/mL; MP at 10.00, 7.50, 5.00, 2.50 and 0.25 μg/mL. Concentration ranges from minimal to maximal concentration are shown additionally in Table II.

Retention times, analytical wavelengths, limits of detection (LOD) and quantitation (LOQ), calibration curve and validation parameters of the developed method are shown in Table II. High correlation coefficients (0.9989–0.9999) confirm the linearity of determined calibration curves over the tested range of concentrations.

LODs were calculated based on the parameters of the calibration curves using the following formula: LOD = (3.3 × S_y/x) / S_x, where S_y/x is the residual standard deviation of the calibration curve, and S_x is the slope. LOQ were determined as LOQ = 3 × LOD.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** RP-UHPLC–UV chromatograms of standards with concentrations of: 5 μg/mL (MP), 10 μg/mL (MI, SB), 25 μg/mL (MCI), 600 μg/mL (BA); and registered with wavelengths of: 274 nm (0.0–1.1 min) and 237 nm (1.1–3.0 min) (designated by a solid line), and 274 nm (0.0–1.1 min) and 257 nm (1.1–3.0 min) (designated by a dotted line).
Intra-day precision was determined by preparing six mixtures of the standard solutions at three levels of concentration: at the lower limit, upper limit and intermediate values of the calibration curves. To determine inter-day precision, similar solutions were prepared and analyzed over five days (each day, the new set of standard solutions at three levels of concentration were prepared). Precision was based on the standard deviation (SD) and coefficient of variation (CV). These values were less than 6% (Table III), confirming the precision of the developed method. The accuracy of the method was determined based on the calculated relative error (Table III).

**Preparation of samples for analyses**

Water was used to dilute the liquid samples (e.g., facial tonics, mouthwashes, body tonics and sprays, injection solutions, clear syrups, aerosols and cleaning agents) as needed. For samples requiring dilution, three parallel dilutions were made, and each was analyzed twice by chromatography. Undiluted samples were analyzed six times.

Solid samples (e.g., toothpastes, creams, masks and ointments) and liquid samples with high density and viscosity (e.g., gels, dense syrups and oils) had to be quantitatively extracted from their matrices. Many methods were tested, including shaking samples with different solvents and centrifugation. However, ultrasonic extraction was the most effective. Many solvents were tested with this method (methanol, ethanol, ethyl acetate, acetonitrile and methylene chloride), in addition to different pH levels of the matrix (pH 1, 5, 7 and 10) and different extraction times (5, 15, 45 and 90 min).

The procedure yielding the highest recoveries was previously outlined. Table IV provides the recoveries from various matrices (toothpaste, cream and gel) with compositions similar to those of the examined samples, but not containing the analytes. To determine the recoveries for each of the tested matrices, three amounts of each matrix were weighed to approximately 1 g and standards were added. These samples were thoroughly mixed and 6.0 mL of methanol was added. This solution was mixed again and subjected to sonication for 15 min. Next, the samples were quantitatively transferred to 10 mL volumetric flasks, filled with methanol to the mark and thoroughly mixed. Samples treated with this procedure were then filtered through a nylon filter (0.20 μm) before chromatographic analysis, which was repeated two times. This method is similar to that described by Zhang et al. (1) for the determination of MP, however, the sonication time was extended to 15 min, and all five analytes were extracted together, instead of only MP.

In the analysis of commercial samples, three aliquots (1.0 g) of each sample were subjected to the optimized method. Samples with high analyte concentrations were diluted with either water (gels and syrups) or methanol (creams and body lotions). Undiluted samples were analyzed twice, and two parallel dilutions were analyzed separately for diluted samples.

**Results of sample analyses**

The optimized analytical procedure was used to analyze all commercial samples of cosmetics, pharmaceuticals and cleaning agents. Compound identification was based on the standard
addition method and on the comparison of analyte retention times in the samples with those of the standards. Figures 3 and 4 present the chromatograms obtained from the analyses of appropriate samples or their extracts. Table V shows the results of analyses for selected samples. The results of all samples tested are presented as supplementary data, available online. Data for cosmetics and personal hygiene products are shown in Supplementary Table I, pharmaceuticals in Supplementary Table II and cleaning agents in Supplementary Table III.

Concentrations of individual analytes in commercial samples did not exceed the regulatory limits. Preservative concentrations were the highest in aqueous samples by as much as four times more than samples with alcohol as the primary ingredient; for example.

MP and SB were the most commonly used preservatives in cosmetics, whereas the other preservatives were determined in

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Analytes concentration (µg/mL)</th>
<th>R (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toothpaste</td>
<td>MI 13.33</td>
<td>93.96</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td>MCI 10.00</td>
<td>83.98</td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td>BA 100.00</td>
<td>74.36</td>
<td>4.98</td>
</tr>
<tr>
<td></td>
<td>SB 10.00</td>
<td>93.32</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>MP 10.00</td>
<td>89.25</td>
<td>2.52</td>
</tr>
<tr>
<td>Cream</td>
<td>MI 13.33</td>
<td>97.34</td>
<td>4.68</td>
</tr>
<tr>
<td></td>
<td>MCI 10.00</td>
<td>79.49</td>
<td>4.21</td>
</tr>
<tr>
<td></td>
<td>BA 100.00</td>
<td>114.17</td>
<td>3.06</td>
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<tr>
<td></td>
<td>SB 10.00</td>
<td>70.12</td>
<td>4.72</td>
</tr>
<tr>
<td></td>
<td>MP 10.00</td>
<td>111.14</td>
<td>4.50</td>
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<tr>
<td>Gel</td>
<td>MI 13.33</td>
<td>92.78</td>
<td>3.82</td>
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<tr>
<td></td>
<td>MCI 10.00</td>
<td>84.28</td>
<td>4.18</td>
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<tr>
<td></td>
<td>BA 100.00</td>
<td>118.66</td>
<td>3.13</td>
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<tr>
<td></td>
<td>SB 10.00</td>
<td>69.58</td>
<td>4.56</td>
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<tr>
<td></td>
<td>MP 10.00</td>
<td>81.37</td>
<td>3.07</td>
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</table>

Figure 3. RP-UHPLC–UV chromatograms registered with wavelengths of: 274 nm (0.0–1.1 min) and 237 nm (1.1–3.0 nm). The solid line delineates the chromatogram of the extract from toothpaste, diluted twice; the dotted line delineates the chromatogram of the extract with standard addition.

Figure 4. RP-UHPLC–UV chromatograms registered with wavelengths of: 274 nm (0.0–1.1 min) and 257 nm (1.1–3.0 nm). The solid line delineates the chromatogram of facial tonic, diluted four times; the dotted line delineates the chromatogram of tonic with standard addition.
only a few products. In contrast, pharmaceuticals primarily contained BA as a preservative and rarely contained MP. All of the five preservatives were detected in the cleaning products.

The previously described method could be considered competitive with the method described by Wu et al. (34) because it allows the simultaneous determination of SB and MP. Wu et al. simultaneously determined four preservatives examined in this study, but did not determine SB. An analytical signal of SB partly overlaps with the signal for MP, but the current method allows for its separation and simultaneous determination; moreover, the current method also allows for determination of MI, MCI and BA.

Additionally, an extraction procedure is verified for more matrices like gels and toothpastes; it does not only concern creams and lotions. Many samples were analyzed to confirm a wide application of the elaborated method.

For samples with a quantitative list of ingredients (Papaverinum, Heparinum WZF, Cavinton and Biseptol), the differences were calculated between the concentration declared on the manufacturer’s package and that determined by the optimized method. The differences between the declared amount of BA and the labeled amount is a few percent (from -8 to 4).

**Conclusions**

This analytical procedure uses fast liquid chromatography to simultaneously determine MI, MCI, BA, SB and MP in cosmetics and pharmaceuticals. These compounds are the most common preservatives used in products at the market. The optimized method reduces costs and increases efficiency with its 3 min analysis. Additionally, the sample preparation procedure is not time-consuming and utilizes the application of standard instrumentation.

The optimized procedure can be used to control the quality of cosmetics, pharmaceuticals and cleaning agents and to estimate both short-term and long-term product stability.

**Acknowledgments**

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**References**


a bioadhesive gel by HPLC; *Journal of Pharmaceutical and Biomedical Analysis*, (2005); 39: 920–927.


