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SOLID-PHASE EXTRACTION AND HPLC DETERMINATION OF PARABENS FROM PHARMACEUTICAL HYDROGELS, MULTIVITAMIN SYRUPS AND HAND CREAMS

Slavica SUNARIĆ,[a*] Miljan JEREMIĆ [b] and Miroslava SPASIĆ [c]

[a] University of Niš, Faculty of Medicine, Department of Chemistry, Bulevar Zorana Đinđića 81, Niš, Serbia
[b] University Clinical Center Niš, Bulevar Zorana Đinđića 48, Niš, Serbia
[c] Pharmacy Biofarm, Kosmajska 60a, Belgrade, Serbia

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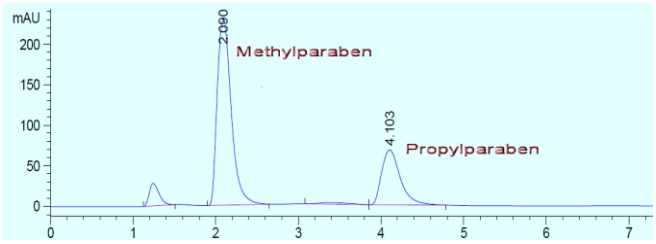
A rapid and reproducible solid-phase extraction (SPE) followed by the HPLC procedure has been developed for the routine analysis of methylparaben and propylparaben in pharmaceutical products and hand creams. Parabens were determined with LOD of 0.001%, which were significantly below the concentrations allowed by regulations. Also, the proposed SPE-HPLC procedure showed particularly good selectivity ensuring the determination of parabens in complex matrices containing various possible interfering substances (plant extracts, water-soluble and liposoluble vitamins, paracetamol, diclofenac, and hand cream´s excipients). With this procedure, the determination of active compounds in pharmaceuticals (diclofenac and paracetamol) could also be possible. This work showed that sorbent styrene-divinylbenzene was very suitable for paraben extraction from hand creams, herbal and multivitamin syrups. The methylparaben and propylparaben contents found in the analyzed products were in the expected ranges.



INTRODUCTION

Parabens are a family of related substances which have been used for decades as antimicrobial preservatives in cosmetics, food products and pharmaceutical formulations. Parabens are alkyl esters of *p*-hydroxybenzoic acid with a broad spectrum of antimicrobial activity and are also effective against yeasts and molds. Their advantage over other preservatives is that they are effective in a wide pH range (in both acidic and basic environments). Antimicrobial activity increases with increasing alkyl chain length for the commonly used methyl, ethyl, propyl, and butyl parabens, and synergy between parabens has been reported.[1] The parabens most used in cosmetics are methyl-, propyl-, butyl-, and ethylparaben. Product ingredient labels typically list more than one paraben in a product, and parabens are often used in combination with other types of preservatives to better protect against a broad range of microorganisms.[2] In oral pharmaceutical formulations, combinations of methylparaben and propylparaben are usually applied. Other parabens, such as ethylparaben and butylparaben, are also used in pharmaceuticals but to a lesser extent.

* Corresponding author: slavica.sunaric@medfak.ni.ac.rs

Thus, butylparaben is predominantly used in pharmaceutical formulations for the cutaneous route.[3]

Parabens are currently listed in the guideline on excipients in the label and package leaflet of medicinal products for human use (CPMP/463/00 Rev.1) for their allergenic potential. Further safety concerns have been raised during the last decade by the scientific community, regulatory agencies, and the public because of possible endocrine-disrupting effects.[4] Thus, it has been demonstrated that parabens bind to oestrogen receptors with binding affinities increasing with side chain length and branching.[5] Among the twelve parabens with linear alkyl chains ranging in length from C1 to C12, the most potent oestrogen receptor agonist activity was measured for heptyl- (C7) and pentylparaben (C5). Estrogenic activity decreased in a stepwise manner as the alkyl chain was shortened to C1 or lengthened to C12.[3,6] Risk assessments on parabens have been performed by several European expert panels including the European Food Safety Authority (EFSA) and the Scientific Committee on Consumer Safety (SCCS). EFSA established a full-group acceptable daily intake (ADI) of 0-10 mg/kg body weight for the sum of methylparaben, ethylparaben and propylparaben. The EFSA opinion dated July 2004 considered that propylparaben should not be included anymore in this group ADI due to effects on the male reproductive organs observed in juvenile rats, and from the year 2006 propylparaben was no longer allowed for use as a food additive within the European Union (Directive 2006/52/EC).[3]

Determination of parabens in cosmetics and pharmaceuticals has been of interest for a long time, especially from the point of view of controlling their permissible content in these products. Paraben analytics is not so simple considering that they are compounds of similar structure and are usually present in complex samples (creams, gels etc.) together with a number of interfering substances. So far, many papers on this topic have been published and various analytical methods such as spectrophotometric, chromatographic, voltammetric,[7] ion mobility spectrometric,[8] capillary electrophoresis[9] and high-performance thin-layer chromatography with image analysis[10] have been proposed.

Spectrophotometric methods are characterized as low selective for the analysis of organic substances from complex matrices such as creams, gels, syrups. Moreover, they are low selective to determine a group of substances of similar structures such as a mixture of parabens. Nevertheless, some spectrophotometric methods in which the sample was prepared by using liquid-liquid extraction[11] or extraction with methanol followed by sonication[12] have been reported. In all these papers, the lack of a complete evaluation of selectivity as part of the method validation is noticeable. However, since only methylparaben has been determined, satisfactory selectivity of the method can be assumed if the excipients did not interfere with the analysis.

Nowadays, chromatographic methods for parabens determination in cosmetics and pharmaceuticals have been mostly used because of good selectivity and equipment availability. Many of the chromatographic methods published in recent years employed complex analytical procedures and less available types of detectors such as amperometric detection on a boron-doped diamond electrode,[13] triple quadrupole tandem mass spectrometry QqQ or MS/MS,[14] electrospray ionization tandem mass spectrometry LC–ESI-MS-MS.[15] For paraben analysis in environmental or biological samples, LC-MS/MS is necessary,[16] however, routine quality control of pharmaceutical and cosmetic products requires simpler and less expensive analytical methods, which also meet the criteria of high selectivity and sensitivity.

For the application of simpler analytical methods, one of the limiting factors is the preparation of complex samples and the extraction of parabens. Inadequate sample preparation cannot provide a good selectivity and accuracy of the method. Due to high polarity of parabens, it is difficult to extract them selectively from hydrogels and syrups by classic liquid-liquid extraction. Also, these procedures can significantly reduce the analyte concentrations in the final solution. Ocaña-González *et al.*[17] reviewed developments in extraction methods of parabens and application of the solid phase extraction to their analysis in cosmetics and environmental samples. Modern methods performed ultrasonic-assisted extraction, supercritical fluid extraction, pressurized liquid extraction, solid-phase extraction, solid-phase microextraction, liquid phase microextraction, dispersive liquid-liquid microextraction, stir bar sorptive extraction and matrix solid-phase dispersion.[17,18]

Although most methods of paraben`s analysis required some forms of sample preparation, approaches with only dilution prior to chromatographic analysis were also published. Nevertheless,

these procedures may not be conventional in all situations, and they are valid only for simple samples. Thus, parabens and levetiracetam were analyzed in stability-indicating studies of pharmaceutical syrup by applying gradient elution.[19] Grześkowiak *et al.* applied only a dilution step in the analysis of cosmetic tonics, micellar waters, and Eau de toilettes.[20] Since they were aqueous cosmetics, this type of sample preparation was acceptable. Alagić-Džambić *et al*. determined parabens in simple pharmaceutical cream by using the HPLC method after ultrasonication and extraction with ethanol.[21] Mincea *et al*. analyzed methylparaben in semi-solid cosmetics which were only diluted and sonicated.[22] Emulsions were chromatographed by using gradient elution, but low resolution and co-elution with unidentified compound have occurred. A micellar liquid chromatographic method without previous extraction was also proposed.[23] A key limitation of this research is that only spiked cosmetic samples were analyzed. Even so, peaks of low symmetry were obtained on the chromatogram of the hand cream. All this indicates the need for adequate sample preparation and extraction prior to HPLC analysis of parabens in complex viscous or semi-solid samples.

Parabens are still widely used as preservatives in some pharmaceutical and cosmetic products. Manufacturers are required to declare their presence in the products but do not have to state the exact concentration. Therefore, product quality control and monitoring of paraben´s content in pharmaceutical syrups, creams and gels are important. Ph.Eur. 8.0 provides a procedure for the individual determination of parabens in raw materials. Due to very similar physicochemical properties, their simultaneous analysis can be difficult and resolution of chromatographic peaks can be reduced when it comes to analyzing real commercial samples. In addition, chromatographic analysis of emulsions (gels, creams etc.) cannot be done without prior sample preparation as the column would become clogged. If the gel or emulsion samples were only to be diluted, many excipients of higher molecular weights can damage the column or lead to increased column pressure. We consider that it is necessary to apply extraction which will both separate parabens and purify the sample from other molecules of excipients. Our choice was SPE because it is more selective and easier to perform than the classic liquid-liquid extraction. Also, by applying this extraction method it is possible to use small amounts of samples and solvents, which provides a high concentration of analyte in the final sample. In this paper, solid-phase extraction procedure followed by HPLC-DAD analysis was validated and applied to methylparaben and propylparaben determination in pharmaceutical hydrogels, syrups, and hand creams.

RESULTS

Optimization of HPLC conditions

Chromatographic parameters were firstly set for the mixture of MP and PP standards. However, given the complexity of the samples, it was necessary to adjust the final chromatographic conditions to the real samples obtained after extraction. Although SPE is a selective extraction method based on the principles of chromatography, there is always the possibility to extract more substances with about the same physicochemical properties. The analyzed samples contained diclofenac, paracetamol, and water-soluble vitamins, the compounds that may have similar retention like parabens on SPE sorbents and chromatographic column. Therefore, the most optimal HPLC conditions were set and corrected depending on the presence of co-eluting substances in the extracts of the individual real samples.

Since methylparaben and propylparaben are moderate polar substances, the optimal HPLC conditions were examined on C8 and C18 columns. Isocratic elution by mixtures of methanol/phosphate buffer (pH 2.5) and methanol/water in different volume ratios from 50:50 to 100:0 was tested. Gradient elution was also examined. In all cases, the selected temperature of the column was 35°C and UV detection was set at 254 nm. The flow rate was changed in the range of 0.3 to 0.8 mL/min. The peak symmetry, peak width, resolution, retention time, selectivity and the number of theoretical plates were compared for two chromatographic columns and elution modes, and for different mobile phases.

By using a mobile phase containing methanol and a phosphate buffer higher than 50% (v/v) retention time of propylparaben was longer than 12 minutes on the C8 column. On the other hand, lower buffer concentrations gave low peak`s resolution and shorter retention times of parabens under 2 minutes. Slightly better values of peak parameters and the number of theoretical plates were obtained on the C18 column. A mixture of methanol and water was also assessed as a mobile

phase by isocratic elution, but the chromatographic parameters of the analyte's peaks were poor compared to the mobile phase with phosphate buffer. To find the best separation conditions and retention times of the analytes, a gradient mode was also evaluated. However, the gradient mode was efficient only for the analysis of standards and did not achieve good peak's symmetry especially in the case of diclofenac and propylparaben in the hydrogel.

The isocratic elution on a C18 column by using methanol/phosphate buffer (pH=2.5) was chosen for all samples so that there would not be significant differences between chromatographic procedures for different samples. However, to obtain chromatograms without interfering peaks, a small difference in chromatographic conditions for different types of samples were necessary. The difference in the chromatographic conditions is only in the percentage of solvents, while the type of solvents and the column are the same. Thus, the final chromatographic conditions were selected after the estimation of chromatographic parameters for all sample extracts. Thus, for the syrup samples, the optimal ratio of MeOH vs. buffer was found to be 60:40 (v/v), while for gels optimal ratio was 70:30 (v/v). Gel samples can also be analyzed using a ratio of 60:40, but in this case, a long retention time of diclofenac is obtained (longer than 14 minutes). Therefore, to shorten the analysis duration and save time for washing the column between runs, as well as to register the peak of diclofenac, a small change in the percentage ratio of the two solvents of the mobile phase is recommended. For the hand creams, the optimal ratio of methanol vs. buffer was slightly modified to 55:45 (v/v) to achieve good resolution between the propylparaben peak and the unknown co-eluting compound from the cream.

Under these conditions, successfully resolved, and well-shaped peaks with run time below 6 min were obtained. The low retention times enabled a fast chromatographic analysis maintaining a good selectivity of the method. The chromatographic system suitability parameters (number of theoretical plates, peak width and symmetry, capacity factor k', resolution and selectivity) are calculated by the Agilent ChemStation program and are summarized in Table 1. It can be concluded that a good peak symmetry, resolution and selectivity were achieved which indicates satisfactory overall column efficiency and appropriate selection of chromatographic conditions.

Optimization of sample preparation procedures

To optimize the sample preparation process, it was necessary to select the solvent for the sample dissolution. Our previous tests of extraction recovery showed that the extraction yield on all cartridges was significantly higher when methanol was used to dissolve the samples instead of water. Since parabens have high solubility in methanol, all the examined products were dissolved in this solvent. In this way, water-methanol mixtures were obtained for all analyzed samples. As syrups are already liquids, only the dilution step prior to SPE was applied. For gels and creams, the preparation process was more complex. After dissolution in methanol, the samples of gels and creams remained opalescent and viscous, which indicated that gelling agent carbomer should be removed. Polymeric chains of carbomer kept their structure that would have clogged the pores of the SPE cartridge. Literature data showed that carbomer could be successfully precipitated by the addition of ammonium hydroxide[24]. The pH value of the gels and creams solutions was increased to seven by adding 0.1M ammonium hydroxide. The change of pH value caused the net-like structure of the carbomer to destabilize leading to its precipitation in the form of white grains. Afterwards, the carbomer was filtered out by centrifugation and the clear solution was subjected to an SPE cartridge. The final adopted procedure is described in detail in the Sample preparation section.

Optimization of solid-phase extraction

To optimize solid-phase extraction, C4, C18 and HR-P cartridges were evaluated. While C18 and C4 were silica-based sorbents, HR-P cartridges were of hydrophobic polystyrene-divinylbenzene copolymer structure. The cartridge was selected based on the extraction yields obtained for standard solutions of parabens and also for the real samples on each type of the tested sorbents. The known amount of MP and PP standard mixtures, at three different concentration levels (0.08 mg/mL, 0.3 mg/mL, and 0.8 mg/mL of each), which corresponded to MP and PP content of 0.008%, 0.03% and 0.08% in products, were extracted on different cartridges and the extraction yield was calculated as the ratio of the loaded and eluted parabens. The percentage extraction yield was found to be 85-94% on HR-P, 60-75% on C_{18} and 80-85% on C_4 cartridges. However, the final cartridge selection depended on the sample matrix, therefore, an extraction yield test was also made for all real samples on three cartridges.

Table 1

Analytical and HPLC system suitability parameters of the method

	Methylparaben (MP)	**Propylparaben (PP)**
Linearity range	10-100 µg/mL	3-30 µg/mL
Equation of the calibration line	y = -14.7 + 120.9x	y = -18.7 + 107.2x
R^2	0.998	0.996
LOD (µg/mL)	0.5	0.5
LOQ (µg/mL)	1.5	1.5
Number of theoretical plates	2865	3720
Peak width	0.156	0.187
Peak symmetry	0.814	0.806
k'	0.64	2.20
Resolution	0.88	5.72
Selectivity	1.74	3.47

For the gel samples, it was found that HR-P cartridges were low selective for paraben extraction. An interfering substance from the sample has remained in the extract and methylparaben peak was poorly resolved from the peak of this compound. Thus, after extraction of heparin gel on the HR-P cartridge, the chromatogram showed an interference of some substance from the sample with the methylparaben peak which disabled its accurate UV determination. Additionally, when Diclofenac gel was extracted on this cartridge, the active substance diclofenac was removed from the sample (Figure 1). This is not favourable if the goal is simultaneous determination of parabens and diclofenac. Therefore, HR-P cartridges were found to be unsuitable for the extraction of parabens from gels. On the other hand, chromatograms of gels extracted on C4 and C18 cartridges showed successful and selective extraction of methylparaben and propylparaben without any interference from other compounds from the sample. Further analysis of peak areas showed that extraction on the C4 cartridge resulted in a higher yield, therefore, the C4 cartridge was selected as optimal for SPE of gels.

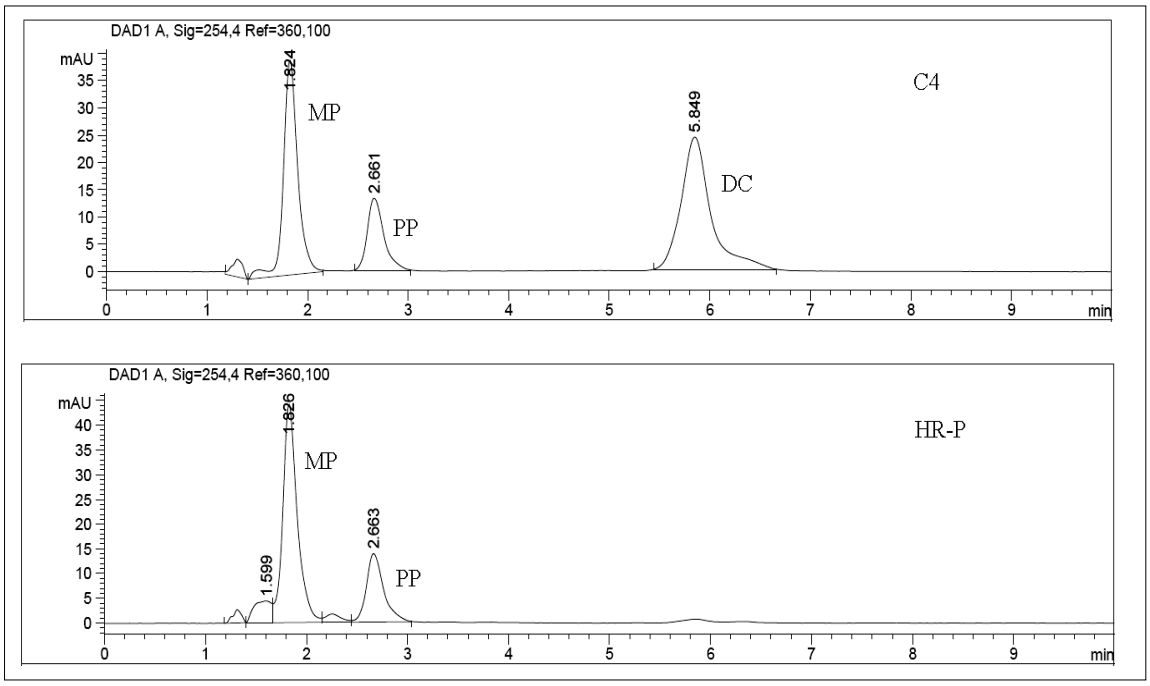


Fig. 1 – Chromatograms of Diclofenac gel obtained by solid-phase extraction on C4 and HR-P cartridges; DC-diclofenac.

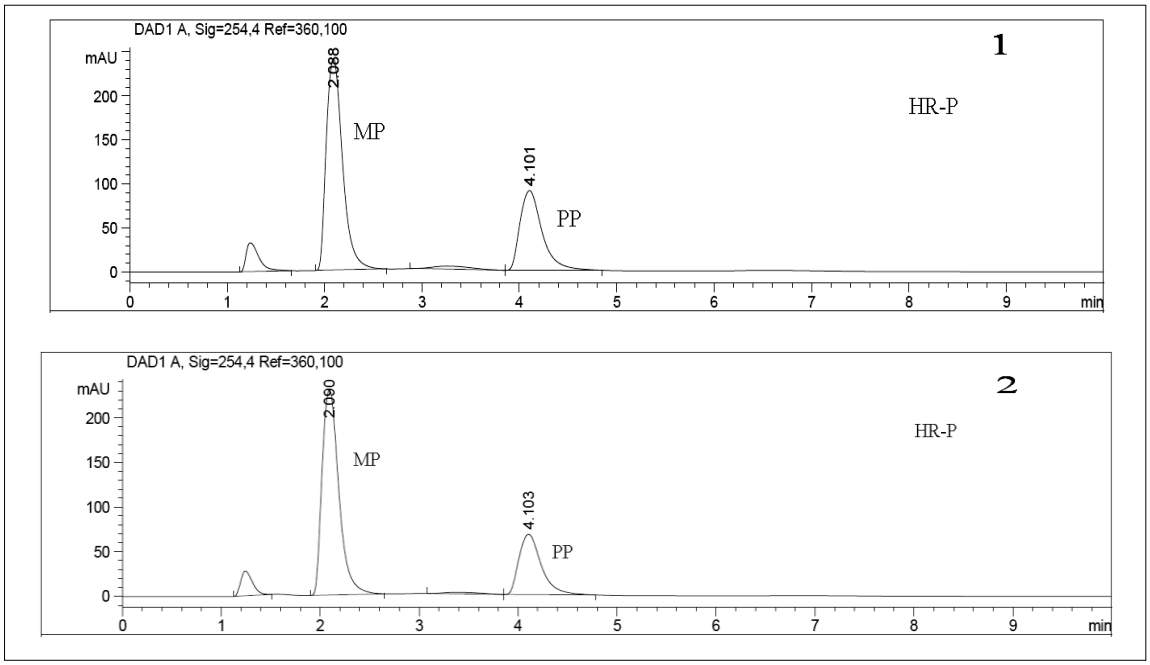


Fig. 2 – Chromatograms obtained by solid-phase extraction on HR-P cartridge:
1) Marshmallow cough syrup; 2) Ribwort plantain cough syrup.

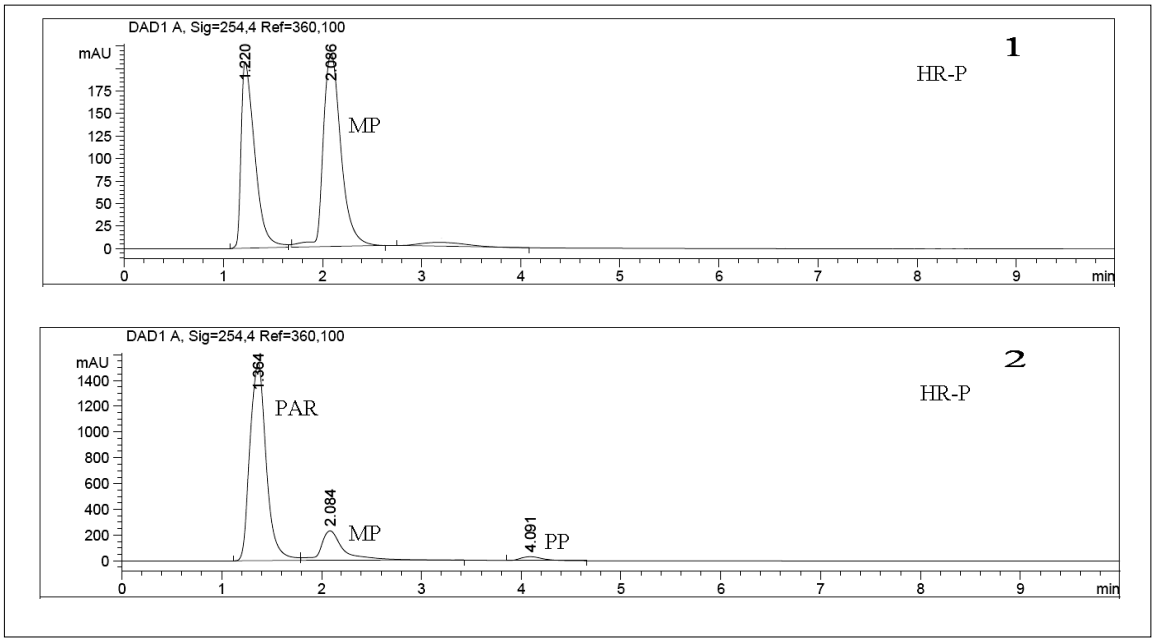


Fig. 3 – Chromatograms obtained by solid-phase extraction on HR-P cartridge:
1) Multivitamin syrup; 2) Paracetamol syrup; PAR-paracetamol.

For syrups and hand creams HR-P cartridges were an excellent choice providing high extraction yields and good selectivity. For all samples, the obtained paraben's peaks were successfully resolved with high peak purity (Figures 2-4). Also, paracetamol peak was identified on the chromatogram of syrup (Figure 3(2)) which enabled simultaneous paracetamol determination.

Since parabens are highly soluble in methanol, it was chosen as a solvent for elution from the SPE cartridges. Twice the volume of methanol was used for elution to achieve quantitative removal. Although the interaction of parabens with SPE sorbent is stronger than their interaction with the water-methanol sample matrix, the elution with double volume of pure methanol successfully removes

parabens from the sorbent. The obtained eluates were clear, so there was no need for further filtering through membrane HPLC filters.

Analytical parameters

The calibration curves for methylparaben and propylparaben were constructed by plotting the peak areas versus the concentrations of the standards. A least-squares linear regression analysis of six-point calibration curves was used to calculate analytical parameters such as linearity, regression coefficient R^2, LOD, and LOQ (Table 1). The linearity was obtained for a wide range of methylparaben and propylparaben concentrations (0.1-100 µg/mL), but only two calibration lines (10-100 µg/mL for MP and 3-30 µg/mL for PP) were used for analysis. These concentration ranges covered paraben`s amounts in products given in Table 2. The regression coefficients R^2 indicated a good linear relationship (Table 1).

The real LOD and LOQ were estimated by spiking paraben-free cough syrup and paraben-free hand cream (from different producers) to obtain the content of 0.005% for methylparaben and 0.005% for propylparaben. After solid-phase extraction, different volumes of syrup and cream extracts were injected into the column until the signal-to-noise ratio (S/N) reached three for LOD and ten for LOQ. In this way, it was found that the proposed method can detect and quantify parabens in concentrations that are far less than those commonly used in products (Table 1).

The accuracy and precision of the method

The accuracy and precision of the developed HPLC-SPE procedure were estimated for HR-P cartridges by the standard addition method. Paraben-free cough syrup and hand cream were used as a blank. The blank syrup and cream samples were weighed and dissolved as described in the Sample preparation section. The dissolved samples were spiked with standard methylparaben and propylparaben solutions at three concentration levels (0.08 mg/mL, 0.3 mg/mL, and 0.8 mg/mL of each), which corresponded to MP and PP content of 0.008%, 0.03% and 0.08% in products and three replicate extractions on HR-P cartridges were conducted. The mean recoveries of the extraction, calculated as the ratio of found and added MP and PP, were 83%, 87% and 91% respectively for three spiked concentration levels, while relative standard deviations were from 4% to 9%.

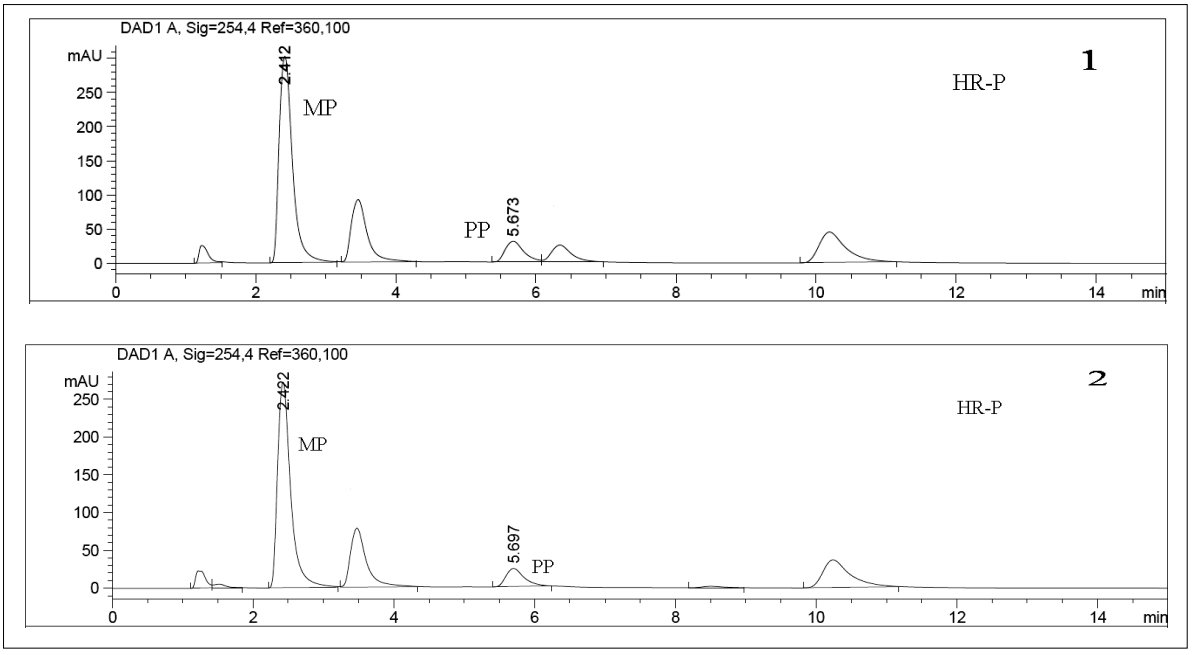


Fig. 4 – Chromatograms obtained by solid-phase extraction on HR-P cartridge: 1) Hand cream 1; 2) Hand cream 2.

Table 2

The average MP and PP content obtained for three measurements with standard deviation

Product		**Average concentration**			
		$\bar{X} \pm SD^*$ in the extract (μg/mL)	$\bar{X} \pm SD^*$ in the product (mg/g; mg/mL)	RSD (%)	Content in the product (%)
Diclofenac gel (mg/g)	MP	3.44±0.25	1.45±0.052	3.6	0.145
	PP	1.47±0.13	0.62±0.05	8.1	0.062
Heparin gel (mg/g)	MP	1.42±0.07	0.60±0.03	5.0	0.06
	PP	0.80±0.07	0.34±0.03	8.8	0.034
Multivitamin syrup (mg/mL)	MP	28.6±0.53	0.57±0.03	5.3	0.057
	PP	/	/	/	/
Marshmallow syrup (mg/mL)	MP	32.8±0.68	0.66±0.014	2.2	0.066
	PP	20.3±0.62	0.41±0.013	3.2	0.041
Ribwort plantain syrup (mg/mL)	MP	29.6±0.76	0.59±0.015	2.5	0.059
	PP	14.15±0.85	0.28±0.02	7.1	0.028
Paracetamol syrup (mg/mL)	MP	24.5±0.5	0.49±0.010	2.1	0.049
	PP	4.1±0.28	0.08±0.006	7.5	0.008
Hand cream 1 (mg/g)	MP	41.5±0.25	0.84± 0.009	1.1	0.084
	PP	6.7±0.46	0.14±0.009	6.4	0.014
Hand cream 2 (mg/g)	MP	36.7±0.78	0.74±0.016	2.1	0.074
	PP	5.35±0.54	0.11±0.008	7.3	0.011

* The standard deviation of three measurements

Method application

After choosing optimal analytic conditions, parabens were determined in commercially available products. The solid-phase extraction of gels, syrups and hand creams was applied, and clear non-viscous extracts were subjected to HPLC analysis. As can be seen in Figures 1-4, well-resolved peaks of parabens and other active substances in formulations containing paracetamol, diclofenac and vitamins were achieved indicating a good selectivity of the method. The identification of methylparaben and propylparaben was achieved based on retention times as well as by UV spectrum of the chromatographic peaks obtained by the DAD detector. The average retention times of MP and PP in syrup samples were 2.084± 0.0033 min and 4.09±0.013 min respectively, while in gel samples they were 1.82±0.014 min and 2.61±0.076 min respectively. The average retention times of MP and PP in hand cream samples were 2.42±0.008 min and 5.71±0.029 min respectively. The methylparaben and propylparaben contents in the examined samples are presented in Table 2. Paraben's content is shown as an average value of three measurements in milligrams of substance per gram or milliliter of the product and as the average percent content.

DISCUSSION

According to current regulations, in oral pharmaceutical formulations combinations of methylparaben and propylparaben are applied with concentrations up to 0.2% for MP and 0.06% for PP which correspond to maximal intakes of approximately 140 mg/day and 50 mg/day, respectively.[3] On the other hand, methylparaben and ethylparaben are allowed in cosmetics at a maximum concentration of 0.4% for a single and 0.8% for a mixture of parabens, respectively, while propylparaben and butylparaben are approved if the sum of their individual concentrations does not exceed 0.14%.[3] As can be seen in Table 2, the methylparaben and propylparaben contents found in the analyzed products were in the expected ranges.

As we reviewed in the Introduction, most of the recently published chromatographic methods for paraben's analysis in cosmetics and pharmaceuticals are complex. The question is whether it is necessary to use such demanding and complex techniques for the determination in commercial samples in which paraben`s concentration (from the analytical point of view) is not small. On the other hand, applications of simpler analytical methods require sample preparation and extraction steps which provide good selectivity and sensitivity. A particular challenge in the analysis and extraction of parabens is their high polarity, which makes them difficult to separate from aqueous mixtures containing many other polar compounds. Additionally, active substances (such as paracetamol and diclofenac) may have absorption at the same wavelength as parabens and may be also quantified if the extraction and chromatographic conditions are set correctly. We developed SPE-HPLC procedure for selective and sensitive determination of parabens in complex matrices containing hydrophilic and hydrophobic components, including vitamins, pharmaceutical active substances (paracetamol and diclofenac), plant extracts, excipients, and additives. It has been published in previous works that Florisil, HLB, C18, C8, multi-walled carbon nanotubes, polyaniline coated Fe_3O_4 nanoparticles, graphene and molecularly imprinted polymer have been used as sorbents for SPE of parabens from different matrices.[18] In our work, it was shown that sorbent styrene-divinylbenzene (HR-P) can also be used for paraben extraction with the recovery of 83-91%. This type of sorbent has been shown to be very suitable for paraben extraction from herbal and multivitamin syrups. After extraction, chromatograms with very few peaks were obtained, although some of the products contained number of possible interfering substances.

One of the more significant conclusions is that the choice of cartridge sorbent is particularly important, which can be seen from the example of diclofenac gel. Diclofenac was eliminated after extraction on the styrene-divinylbenzene, while it was retained on the C4 sorbent. Therefore, if the goal is the simultaneous determination of diclofenac and parabens, it is necessary to use a C4 cartridge for extraction. All of this shows that the appropriate cartridge must be selected not only depending on the properties of the analyte but also depending on the sample matrix. For that reason, method must be adapted to the nature of sample to achieve the best results of the analysis. Consequently, optimization of the SPE procedure is an important and mandatory part of the HPLC method for determining parabens in different types of samples. Also, individual optimization of chromatographic conditions and adaptation to the analyzed matrices is important to prevent the overlap of the peaks of parabens, paracetamol, diclofenac, vitamins, and other compounds. Our method was developed for the eight products of three basic types of sample matrices in which parabens are used (gel, cream, syrup), so similar behavior can be expected for other commercial products of the same type. Nevertheless, prior partial validation is necessary for all new samples to exclude the possible interferences.

EXPERIMENTAL

Apparatus and reagents

Methylparaben (MP) and propylparaben (PP) of extra pure quality were purchased from Centrohem (Serbia). Paracetamol (PAR) of Ph. Eur. quality was from Fagron Hellas (Trikala, Greece). Diclofenac sodium (DC) active substance was granted by Jugoremedija (Zrenjanin, Serbia). Methanol (MeOH) was of HPLC grade (Sigma-Aldrich). Phosphate buffer pH=2.5 was made of sodium dihydrogen phosphate.

Solid-phase extraction

Visiprep™ SPE vacuum manifold Supelco 57030-U (Sigma-Aldrich Chemie GmbH, Eschenstrasse, Germany) was used for the solid-phase extraction. Three different types of SPE sorbents (C4, C18 and HR-P) of the capacity 1 mL/ 100 mg (CHROMABOND® RP development kit II, Macherey-Nagel, GmbH, Düren, Germany) were used.

HPLC equipment

HPLC analyses were performed on Agilent Technologies 1200 Series apparatus with diode array detector (DAD) and fluorescence detector (FL). The separation was carried out on Zorbax Eclipse Plus C8 (150 mm L x 3.0 mm i.d., 3.5 µm d.p.) and Zorbax Eclipse Plus C18 (150 mm L x 3.0 mm i.d., 3.5 µm d.p.) columns.

Pharmaceutical products

Nowadays, a small number of pharmaceuticals contain parabens, so the choice of the analyzed products was limited. Thus, our study included the following commercial products: marshmallow cough syrup containing 2.3% of *Althaea officinalis* aqueous extract, ribwort plantain cough syrup containing 52 mg/mL *Plantago lanceolata* extract, one multivitamin immunity syrup containing vitamins A, D, E, C, B1, B2, B6, dexpanthenol and niacin, one analgesic-antipyretic syrup containing paracetamol (120 mg/5 mL), one

vasoprotective gel containing heparin-sodium (300 IU/g), allantoin and dexpanthenol, one topical pain relief gel containing 1% of diclofenac diethylamine and two hand creams. According to the manufacturer's declaration multivitamin immunity syrup contained only methylparaben. All these products contained a number of excipients and additives such as flavours, natural and artificial sweeteners, preservatives, acidity regulators, humectants etc. The gels additionally contained emulsion stabilizers and a polymer carbomer 940.

Analytical procedures

Stock solutions and calibration curve

The stock solutions of methylparaben (MP) and propylparaben (PP) concentrations of 1.0 mg/mL were prepared in methanol. Aliquots of 0.5 mL of both stock solutions were mixed and diluted with methanol to obtain a working standard solution containing each of the parabens in the concentration of 20 μg/mL. Further dilution of this working solution produced the solutions of concentrations 1 μg/mL, 2 μg/mL, 4 μg/mL, 6 μg/mL, 8 μg/mL and 10 μg/mL, which were used to construct the calibration curves.

HPLC conditions

Standard substances and prepared samples were subjected to HPLC analysis on Zorbax Eclipse Plus C8 (150x3.0 mm, 3.5 μm) and Zorbax Eclipse Plus C18 (150x3.0 mm, 3.5 μm) columns. After optimization, the final adopted HPLC conditions were: Zorbax Eclipse Plus C18 column, the mobile phase consisting of a MeOH/phosphate buffer (pH=2.5) and a flow rate of 0.5 mL/min. The optimal volume ratio methanol vs. buffer was found to be different depending on the analyzed product. Thus, for syrup samples, the ratio MeOH vs. buffer was 60:40 (v/v), for gels 70:30 (v/v), while for hand creams the ratio of methanol vs. buffer was 55:45 (v/v). The temperature was kept at 35°C and the detection was carried out at 254 nm. For the monitoring of chromatographic system and data acquisition, the Agilent ChemStation program was used.

Sample preparation

After optimization of the sample preparation procedure, the following different protocols were adopted: 1) A 0.5 g of the pharmaceutical gel was dissolved in 5 mL of methanol. In this solution, 0.1M NH_4OH was added to obtain pH 7. Insoluble excipients were separated by centrifugation for 10 min at 4000 rpm at 20°C. Then, 1.0 mL of the supernatant was applied to the cartridge and the SPE procedure was performed; 2) A 1.0 mL of pharmaceutical syrup was dissolved in 9.0 mL of methanol. After vortex mixing 1 mL of the sample solution was loaded on SPE cartridge; 3) A 1.0 g of the hand care cream was dissolved in 9.0 mL of methanol. The solution was vigorously mixed to complete the dissolution of the cream and to obtain an emulsion. After that, 0.1M NH_4OH was added to pH 7. A large amount of the precipitate was separated by centrifugation for 5 min (4000 rpm at 20°C), and 1 mL of the supernatant was inserted on the SPE cartridge.

Solid-phase extraction

Before extraction SPE cartridges were conditioned with 3 mL of methanol and 3 mL of deionized water. One milliliter of the prepared sample was loaded on the cartridge at the flow rate of 1 mL/min. The elution of parabens was achieved with 2 x 1 mL of methanol.

CONCLUSIONS

The development of analytical methods for selective determination of parabens is important in modern pharmaceutical analysis. In this paper, the SPE procedure with styrene-divinylbenzene and C4 cartridges was developed. It has been shown that the selection of the optimal cartridge depends not only on the analyte but also on the sample matrix. SPE is a simple, inexpensive, and environmentally friendly extraction method which in combination with the appropriately selected column and the mobile phase achieves excellent results in the selectivity and yield of paraben extraction and determination from complex pharmaceutical and cosmetic samples. Also, it is an effective way to improve the purification of the complex cream and syrup samples to protect the chromatographic column. The proposed method can be readily used in practice. In addition, it is possible simultaneous determination of parabens, paracetamol, and diclofenac after complete validation of the methods for these two active substances.

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REFERENCES

1. C. Charnock and T. Finsrud, *J. Clin. Pharm.Ther.*, **2007**, *32*, 567-572.
2. USFDA, https://www.fda.gov/cosmetics/cosmetic-ingredients/parabens-cosmetics; 2020 (accessed 18 March 2020).
3. EMA/CHMP/SWP/272921/2012, Committee for Medicinal Products for Human Use (CHMP), Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use, 22 October 2015.
4. P. D. Darbre, A. Aljarrah, W. R. Miller, N. G. Coldham, M. J. Saue and G. S. Pope, *J. Appl.Toxicol.*, **2004**, *24*, 5-13.
5. R. M. Blair, H. Fang, W. S. Branham, B. S. Hass, S. L. Dial, C. L. Moland, W. Tong, L. Shi, R. Perkins and D. M. Sheehan, *Toxicol. Sci.*, **2000**, *54*, 138-153.
6. Y. Watanabe, H. Kojima, S. Takeuchi, N. Uramaru, S. Ohta and S. Kitamura, *Food. Chem.Toxicol.*, **2013**, *57*, 227-234.
7. S. Michalkiewicz, M. Jakubczyk and A. Skorupa, *Int. J. Electrochem. Sci.*, **2016**, *11*, 1661-1675.

8. J. K. Lokhnauth and N. H. Snow, *Anal. Chem.,* **2005**, *77*, 5938-5946.
9. U. D. Uysal and T. J. Guray, *Anal. Chem.,* **2008**, *63*, 982-986.
10. D. Casoni, I. A. Tuhutiu and C. J. Sarbu, *Liq. Chromatogr. Relat. Technol.*, **2011**, *34*, 805-816.
11. M. F. Ambarak, *Asian. J. Green Chem*., **2020**, *4*, 192-201.
12. T. Bhandari, A. Patel, K. Dhodi, Z. Desai and S. Desai, *Int. J. Pharm. Sci. Rev. Res.*, **2019**, *59*, 17-21.
13. I. Martins, F.C. Carreira, L.S. Canaes, F.A. de Souza Campos Junior, L.M. da Silva Cruz and S. Rath, *Talanta*, **2011**, *85*, 1-7.
14. C. Moreta, M. T. Tena and K. Kannan, *Environ. Res.*, **2015**, *142*, 452-460.
15. T. H. Pritchett, E. A. Myers and T. A. Brettell, *LC GC N. Am.*, **2015**, *33*, 16-22.
16. S. Yakkundi, H. Mulla, H. Pandya, M. A. Turner and J. Mc Elnay, *Bioanalysis*, **2016**, *8*, 1173-1182.
17. J. A. Ocana-Gonzalez, M. Villar-Navarro, M. Ramos-Payan, R. Fernandez-Torres and M. A. Bello-Lopez, *Anal. Chim. Acta.,* **2015**, *858*, 1-15.
18. C. Piao, L. Chen and Y. J. Wang, *J. Chromatogr. B.*, **2014**, *969*, 139-148.
19. S. S. Abd El-Hay and M. S. Mohram, *Chromatogr. Res. Int.*, **2016**, *Article ID 4909547*, 5 pages.
20. A. Zgola-Grzeskowiak, J. Werner, M. Jeszka-Skowron and B. Czarczynska-Goslinska, *Anal. Methods*, **2016**, *8*, 3903-3909.
21. L. Alagić-Džambić, M. Vehabović, N. Avdić, S. Kaljun, N. Kokorović and M. Džambić, *Int. J. Pharma. Teaching & Practices (IJPTP)*, **2014**, *5*, 1571-1573.
22. M. M. Mincea, I. R. Lupsa, D. F. Cinghita, C. V. Radovan, I. Talpos and V. J. Ostafe, *Serb. Chem. Soc.*, **2009**, *74*, 669-676.
23. J. F. Noguera-Orti, R. M. Villanueva-Camanas and G. J. Ramis-Ramos, *Chromatogr. Sci.*, **1999**, *37*, 83-87.
24. Separation of Carbopol® polymers from complex formulations for quantitive analysis, https://www.yumpu.com/en/document/view/12345746/tds-231-separation-of-carbopolr-polymers-from-lubrizol; 2016 (accessed 21 July 2016).