Simultaneous Determination of Six Parabens in Cosmetics by a New High Performance Liquid Chromatography Method with Fluorescence Detection

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Recieved: November 05, 2019; Accepted: January 31, 2020
DOI: 10.17721/moca.2020.27-32

In this study, a new high performance liquid chromatography method with fluorescence detection was developed and validated for the simultaneous determination of methyl paraben, ethyl paraben, propyl paraben, isopropyl paraben, butyl paraben and benzyl paraben in cosmetics. Separations were achieved using a C18 guard column (2.1 × 10 mm, 3 µm) and a C18 analytical column (2.1 × 150 mm, 3 µm). Isocratic elution was applied with a mobile phase consisting of 45% aqueous o-phosphoric acid solution (0.08%) and 55% methanol/water mixture (90 : 10 v/v). The excitation and the emission wavelengths were 254 and 310 nm, respectively. Column temperature was fixed at 40°C. The linear range was 0.50-10.00 µg/mL for all of the parabens. Limits of detection and quantification were in the range of 0.29-0.32 µg/mL and 0.88-0.97 µg/mL, respectively. Precision and accuracy values were calculated by analysis results of standard solutions at 0.50, 2.50 and 10.00 µg/mL. The developed and validated method was applied for simultaneous quantitative determination of six paraben species in cosmetic tonic and micellar water samples successfully.

Keywords: cosmetics, paraben, HPLC, fluorescence

Parabens are esters of p-hydroxybenzoic acid. They are used as preservatives in a wide variety of food products, pharmaceuticals and cosmetics due to their low cost, antimicrobial activity over a wide pH range, high stability and water solubility. Methyl paraben, ethyl paraben, propyl paraben and butyl paraben are the mostly utilized types individually or as mixtures to provide a wide antimicrobial spectrum [1, 2].

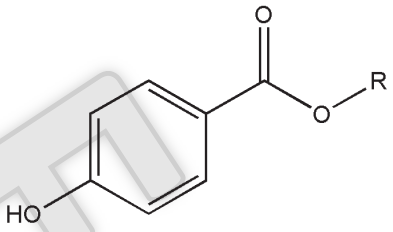
Researches have shown that parabens possessed weak estrogenic activity with an affinity for binding to estrogen reseptors [3, 4]. Because of their estrogenic activity, it was assumed that parabens would be able to promote breast cancer [5, 6]. Also they might effect the male reproductive system negatively [7]. Considering these studies, parabens are currently classified as suspected endocrine disruptors and carcinogens.

European Union recommended a maximum permitted concentration of 0.14% for propyl paraben and butyl paraben when used individually or together with other esters in cosmetics as a consequence of their toxicological properties. They are banned from leave-on products for the nappy area of young children below the age of three. The use of isopropyl, isobutyl, phenyl, benzyl and pentyl parabens in cosmetics were restricted due to the lack of data necessary to evaluate the human risk [8]. Also parabens with branched or long linear chains are known to possess higher toxicity.

Reliable determination of parabens in different matrices as biological fluids [9-11], environmental samples [12, 13], pharmaceuticals [14, 15] and cosmetics [16, 17] became more of an issue considering the widespread utilization and the effects on human health. The detection of parabens is generally performed by high performance liquid chromatography (HPLC) [10, 11] or gas chromatography (GC) [18, 19]. GC methods for the determination of parabens may require preconcentration or derivatization [20, 21]. HPLC coupled with ultraviolet or diode array detection [22, 23] is one of the commonly used techniques which has drawbacks of interfering of other ingredients in the sample with parabens and high detection limits causing the requirement of preconcentration. Liquid chromatography with mass spectrometry (LC-MS) or with tandem mass spectrometry (LC-MS/MS) detection may overcome these problems, but on the other hand these systems are more expensive and unavailable in many laboratories [24-26]. HPLC with fluorescence detection (FD) may be an alternative for analysis of parabens with considerably higher selectivity than UV detection and being an available system unlike MS. In a study an HPLC-FD method was developed, validated and applied for the determination of methyl paraben, ethyl paraben, propyl paraben and butyl paraben in cosmetic products successfully [27].

In the present work, it was aimed to develop a sensitive and simple method for the determination of methyl (MP), ethyl (EP), propyl (PP), isopropyl (IPP), butyl (BP) and benzyl (BzP) parabens (Fig. 1) by HPLC-FD without a derivatization reaction. The developed method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. To our best knowledge the present study could be the first report on detection

of the selected six parabens simultaneously by HPLC-FD. The developed and validated method was applied for the paraben analysis of cosmetic tonic and micellar water samples.



R=CH_3 methyl paraben
R= C_2H_5 ethyl paraben
R= C_3H_7 propyl and isopropyl paraben
R= C_4H_9 butyl paraben
R= C_6H_5 benzyl paraben

Fig. 1. Chemical structures of the determined parabens.

Materials and methods

Chemicals and solutions. The standards of MP, EP, PP, IPP, BP and BzP were purchased from Sigma (Darmstadt, Germany). The HPLC grade MeOH was purchased from Isolab (Eschau, Germany) and o-phosphoric acid was from Merck (Darmstadt, Germany). The stock solution containing each of the parabens at a concentration of 100.00 µg/mL was prepared with HPLC grade methanol (MeOH). The standard solutions were prepared daily by dilution of the stock solution with the mobile phase to desired concentrations.

Three cosmetic tonic and two micellar water samples (Istanbul, Turkey, 2018) were analyzed by the developed method. The sample codes and the ingredients indicated on the labels were given in Table 1. The original and the spiked samples were diluted 1000 times with the mobile phase considering the maximum permitted concentration values in the EU regulation [8, 27]. The final concentrations of the spiked samples were 0.50, 2.50 and 10.00 µg/mL for each of the parabens. All of the sample solutions were filtered (0.45 µm) prior to injection to the HPLC-FD system.

Instruments and analytical conditions. A Shimadzu (Shimadzu, Kyoto, Japan) LC20AT high performance liquid chromatography system with fluorescence detection was used. The separation of parabens was performed using a GL Sciences (GL Sciences Inc., Tokyo, Japan) Intersil ODS-3 guard column (C18, 2.1 × 10 mm, 3.0 µm) and an Intersil ODS-3 analytical column (C18, 2.1 × 150 mm, 3.0 µm). The data were analysed by the LabSolutions software (version 1.25).

Isocratic elution was applied with a mobile phase system consisting of 45 % aqueous o-phosphoric acid solution (0.08 %) and 55 % methanol/water mixture (90:10 v/v) by volume. The flow rate was set to 0.3 mL/min and the injection volume was 5 µL. The column temperature was adjusted to 40 °C.

Quantification. Parabens were identified by comparing their retention times with those of the ones in the standard solutions. The quantification was performed by the external standard method. The linear range was 0.50-10.00 µg/mL for each of the parabens. The calibration curves were plotted as the analytes' peak areas versus the concentrations with the data of triplicate analyses.

Results and discussion

Several mobile phase systems consisting of water, methanol, acetonitrile, o-phosphoric acid and formic acid at different proportions with gradient and isocratic elutions were tested for the appropriate separation of the parabens with resolution values higher than 2.

Table 1. Sample codes and ingredients indicated on the labels.

Sample code	Ingredients
TS1	Aqua, denatured alcohol (alcohol denat.), PPG-5-ceteth-20, glycerin, salicyclic acid, menthyl lactate and parfum
TS2	Aqua, glycerin, PEG-8, PEG-40 hydrogenated castor oil, glyceryl glucoside, prunus amygdalus dulcis oil, tocopheryl acetate, panthenol, polyquaternium-10, citric acid, sodium chloride, sodium acetate, 1,2-hexanediol, trisodium edta, phenoxyethanol, butylphenyl methylpropional, geraniol, limonene, alpha-isomethyl ionone, parfum
TS3	Aqua, alcohol denat., PEG-8, glycerin, PEG-40 hydrogenated castor oil, magnolia officinalis bark extract, glyceryl glucoside, polyquaternium-10, citric acid, trisodium edta, phenoxyethanol, alpha-isomethyl ionone, benzyl salicylate, benzyl alcohol, butylphenyl methyl-propional, citronellol, hexyl cinnamal, limonene, linalool, parfum
MW1	Aqua, PEG-40 hydrogenated castor oil, glycerin, *Prunus amygdalus dulcis* oil, panthenol, sorbitol, decyl glucoside, glyceryl glucoside, poloxamer 124, propylene glycol, disodium cocoyl glutamate, sodium chloride, trisodium EDTA, polyquaternium-10, 1,2-hexanediol, citric acid, sodium acetate, phenoxyethanol
MW2	Aqua, poloxamer 124, *Camellia sinensis* leaf extract, glycerin, decyl glucoside, sodium cocoamphoacetate, 1,2-hexanediol, sodium chloride, citric acid, Trisodium EDTA, phenoxy-ethanol

The mobile phase system consisting of 0.08% aqueous o-phosphoric acid solution and methanol/water (90:10, v/v) (45:55, v/v) was selected which provided the highest resolution. The analyte peaks were detected at the excitation wavelength of 254 nm and the emission wavelength of 310 nm with the highest sensitivity.

The validation of the developed HPLC-FD method was performed in terms of linearity, LOD, LOQ, precision and accuracy. A representative chromatogram of the analyzed parabens at 5.00 µg/mL was shown in Fig. 2. The linearity was determined by 5 point calibration curves for each of the parabens. The calibration equations and correlation coefficients (r) were calculated by linear regression analysis based on least squares method. A good linearity with r values higher than 0.99 was obtained (Table 2).

LOD and LOQ values were calculated as 3.3 and 10 times of the ratio of the standard deviation of the calibration curve to the slope of the calibration curve, respectively. The LODs and LOQs of six parabens were in the range of 0.29-0.32 µg/mL and 0.88-0.97 µg/mL, respectively. The linear ranges, calibration equations, correlation coefficients, LODs, LOQs, resolutions and tailing factors were given in Table 2.

The precision and the accuracy of the developed method were determined at low, middle and high concentrations (0.50, 2.50 and 10.00 µg/mL) in triplicate analysis (Table 3). The precision was expressed as standard deviation of triplicate analyses in one day and in three separate days and the accuracy was determined in terms of recovery percent.

Non of the analyzed parabens were detected in the paraben-free cosmetic tonic sample TS1 above LOD values of the validated method. To check the recovery of the parabens TS1 was spiked to final concentrations of 0.50 (Fig. 3a), 2.50 (Fig. 3b.) and 10.00 µg/mL (Fig. 3c) and analyzed in triplicate. Appropriate standard deviation values and recoveries in the range of 90.50-118.96% were obtained (Table 3). Two other tonic samples, TS2 and TS3, and two micellar water samples, MW1 and MW2 were analyzed. Since all of the samples were labeled to be paraben-free, all analyzed parabens were spiked to provide a final concentration of 2.5 µg/mL (Table 4). The results indicated that different types of aqueous cosmetic product matrices could be analyzed by the proposed method.

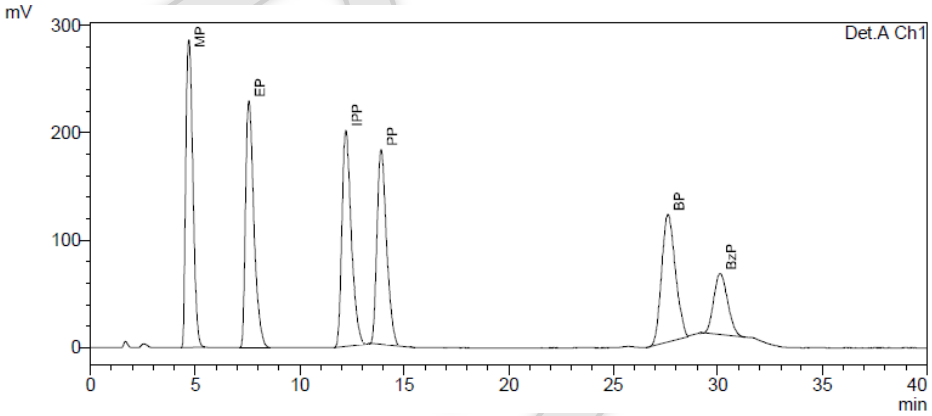


Fig. 2. A representative chromatogram of the determined parabens (standard solutions at 5 µg/L).

Table 2. Analytical figures of merit for parabens.

Analyte	t_R (min)	Calibration range (µg/mL)	Linear equation	r^2	LOD (µg/mL)	LOQ (µg/mL)	Tailing factor (t)	Resolution (R_s)
MP	4.684	0.50-10.00	y = 1189764.2x - 345888.6	0.9955	0.32	0.97	1.343	3.784
EP	7.533	0.50-10.00	y = 1192070.2x - 342612.8	0.9963	0.30	0.90	1.562	4.375
PP	12.125	0.50-10.00	y = 1166196.7x - 255872.3	0.9961	0.30	0.30	1.681	6.167
IPP	13.787	0.50-10.00	y = 342612.8x - 1192070.2	0.9961	0.30	0.90	1.842	2.057
BP	27.272	0.50-10.00	y = 276355.0x - 1031110.5	0.9957	0.31	0.95	1.476	13.356
BzP	29.664	0.50-10.00	y = 345888.6x - 1189764.2	0.9965	0.29	0.88	1.178	2.041

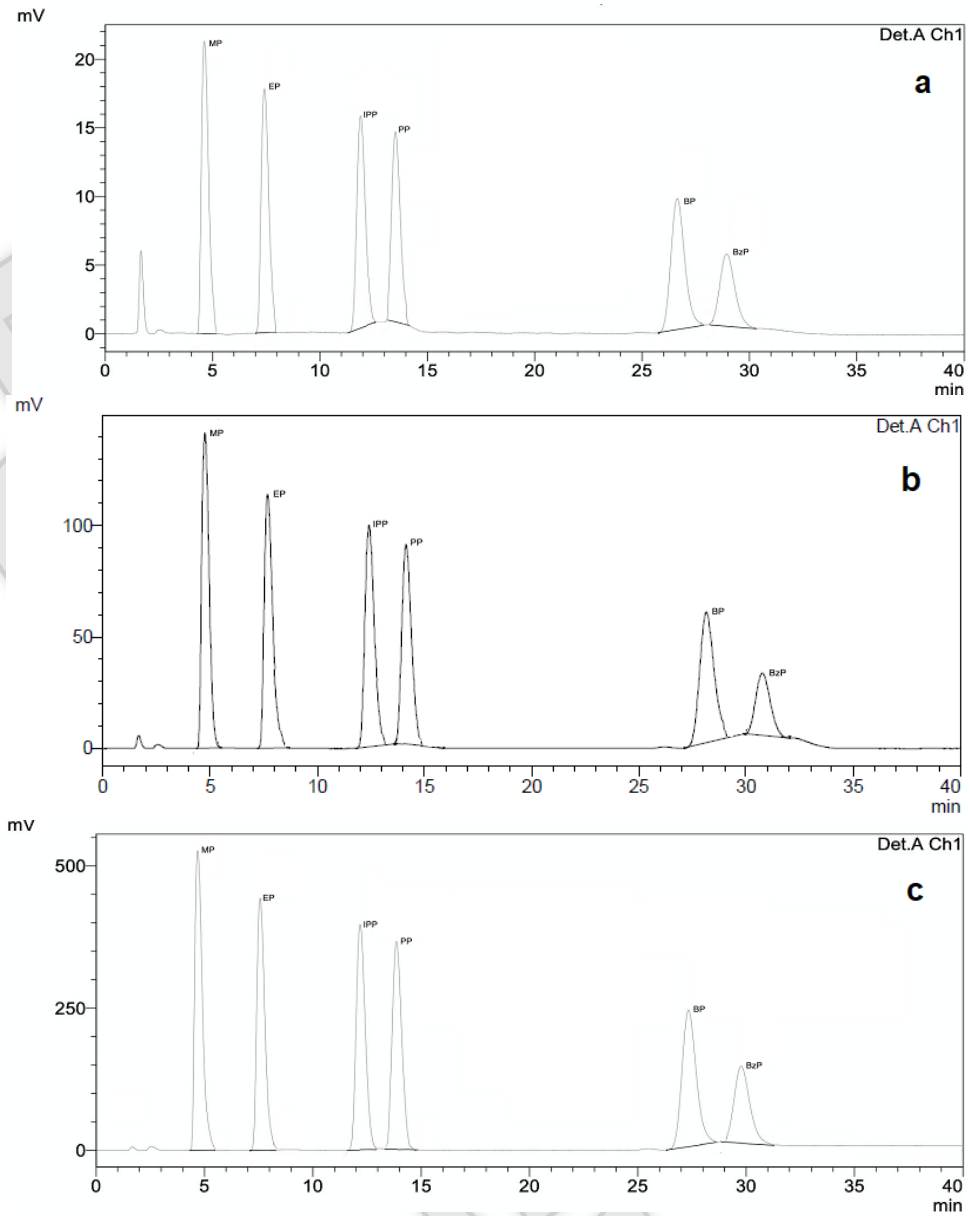


Fig. 3. Chromatograms of the spiked tonic sample, TS1: a) 0.50 μg/mL, b) 2.50 μg/mL, c) 10.00 μg/mL.

Table 3. Repeatability (intraday), intermediate precision (interday) and recovery of the developed method.

	Conc. (μg/mL)	MP	EP	IPP	PP	BP	BzP
Intraday[a)] (n=3)	0.50	0.47±0.00	0.51±0.00	0.55±0.00	0.46±0.00	0.44±0.00	0.46±1.25
	2.50	3.06±0.02	2.91±0.03	3.02±0.02	2.99±0.02	2.63±0.01	2.54±0.02
	10.00	11.65±0.08	11.21±0.05	11.70±0.06	11.43±0.06	10.10±0.04	9.82±0.09
Interday (n=3)	0.50	0.46±0.00	0.53±0.00	0.52±0.00	0.43±0.00	0.42±0.00	0.41±0.01
	2.50	3.00±0.02	2.85±0.02	2.92±0.03	2.85±0.03	2.55±0.02	2.48±0.02
	10.00	11.05±0.09	11.11±0.05	11.00±0.06	11.23±0.06	10.50±0.04	9.72±0.08
Recovery (%)[b)] (n=3)	0.50	94.00±0.00	102.00±0.00	110.00±0.00	92.00±0.00	88.00±0.00	92.67±1.15
	2.50	122.27±0.61	116.27±1.01	120.93±0.61	119.69±0.60	105.20±0.40	101.60±0.80
	10.00	116.47±0.75	112.07±0.49	116.97±0.57	114.33±0.60	101.03±0.40	98.23±0.87
Recovery (%) from the spiked TS1 (n=3)	0.50	90.50±0.00	104.00±0.09	112.10±0.32	98.98±0.56	91.00±0.78	96.87±1.20
	2.50	118.96±0.61	114.23±1.94	117.05±0.76	118.09±0.70	104.94±0.67	106.68±0.90
	10.00	111.07±0.75	109.07±1.09	112.98±0.97	112.47±0.18	104.30±0.80	99.97±0.93

Notes. a) mean ± SD, b) recovery ± SD.

Table 4. Analysis results of the cosmetic samples.

	Concentration (μg/mL)[a)]					
Sample	**MP**	**EP**	**IPP**	**PP**	**BP**	**BzP**
TS1	2.97±0.01	2.86±0.05	2.93±0.02	2.95±0.02	2.62±0.02	2.67±0.02
TS2	2.50±0.04	2.76±0.08	2.63±0.09	2.65±0.05	2.52±0.04	2.59±0.08
TS3	2.62±0.03	2.55±0.04	2.77±0.08	2.61±0.04	2.65±0.06	2.67±0.06
MWS1	2.77±0.05	2.67±0.07	2.66±0.05	2.70±0.07	2.55±0.01	2.64±0.07
MWS2	2.65±0.04	2.70±0.08	2.66±0.03	2.65±0.05	2.54±0.04	2.53±0.02

a) Samples spiked to final concentration of 2.50 μg/mL.

In literature there is only one HPLC-FD method for the quantification of parabens as MP, EP, PP, BP in cosmetics [27]. Comparatively, the proposed HPLC-FD method had the advantage of determination of IPP and BzP together with MP, EP, PP, BP with acceptable resolution values, sensitivity, precision and accuracy. The LOD and LOQ values were lower in the referred study [27] in which the S/N = 3 was used for the calculation of the LOD and the S/N = 10 for the calculation of the LOQ. In the present work 3.3 and 10 times of the ratio of the standard deviation of the calibration curve to the slope of the calibration curve were calculated as LOQ and LOQ, respectively, by taking account the significance of the calibration equations on these values.

Conclusions

To our best knowledge, this study could be considered as the first report on simultaneous determination of MP, EP, PP, IPP, BP and BzP by HPLC-FD. The procedure was easy to perform and enabled the quantification of six parabens with good precision and accuracy in cosmetic tonic and micellar water samples containing different ingredients. Further extraction studies may be performed for the analysis of more complex matrices (e.g creams, lotions, etc.) and lower concentrations using the developed and validated HPLC-FD method.

Acknowledgements

This study was funded by Scientific Research Projects Coordination Unit of Istanbul University (Project number: 27374).

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