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RP-HPLC-UV DETERMINATION OF PARABENS IN COSMETIC PRODUCTS

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Abstract: In this research, a simple reverse phase high performance liquid chromatographic (RP-HPLC) method with ultraviolet (UV) detection was developed for the simultaneous determination of parabens such as methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP). The system consisted of Phenomenex C₁₈, (125 mm length, 4.6 mm i.d. and 3 µm) as an analytical column. The separations were affected by using mobile phase acetonitrile: water (32:68) with a flow rate 1.0 mL/min. The UV detector was set at wavelength 254 nm, and 20 µL of the sample were injected. Under these conditions, separation of the four paraben compounds was achieved in less than 10 minutes. The limits of detection (LOD) of MP, EP, PP and BP standards were 0.02, 0.01, 0.02 and 0.02 µg/mL, respectively. This proposed RP-HPLC method was successfully applied to determine parabens in cosmetic cream and lotion samples and the linearity of each paraben was studied in the concentration range of 0.04-80 µg/mL.

1. Introduction

Parabens are esters of *p*-hydroxybenzoic acid; they are a group of effective preservatives for cosmetics, skin care products, drugs, beverages and food. Parabens show significant effects on the antimicrobial activity. The biological activity of the parabens increases with increasing carbon number of the ester group [1]. In contrast, their water solubility decreases correspondingly. The individual esters exhibit differences in their relative antimicrobial activities and, therefore, optimal effectiveness is generally obtained by combinations of parabens. Many cosmetics and skin care products are preserved with parabens [2, 3]. Since paraben combinations exhibit powerful antimicrobial activity against an extremely broad spectrum of microorganisms, many manufacturers market paraben mixtures in different compositions under different trade names. Methylparaben and propylparaben are often used together due to showed synergetic effects [4]. 2-Phenoxyethanol and 1-phenoxypropan-2-ol show some structural similarities with parabens and are also used as antimicrobial preservatives for cosmetic, toiletry and pharmaceutical applications. Unlike the parabens they are suitable for the preservation of liquid protein concentrates, such as those based on collagen [5]. Initially, parabens were considered among the preservatives with low systemic toxicity, merely caused allergic reactions. In 1940, Bonnevie already

observed a case of contact dermatitis from ethyl paraben [6]. In 1973, Fischer reported that parabens in topical therapeutic agents occasionally sensitize, whereas parabens in cosmetics do not [7, 8].

This apparent contradiction was termed the "paraben paradox"; the application of paraben containing cosmetics is generally well tolerated provided the product is applied to the normal and not to the damaged skin, which is more readily sensitized. Recently, J. Sumpter's group at Brunel University, UK, suggested that the safety of the parabens should be reassessed because some alkyl hydroxybenzoate preservatives are estrogenic [9]. The European Cosmetic Toiletry and Perfumery Association, COLIPA, emphasizes that parabens are hydrolysed in the skin and that none are entering the blood stream [10]. Whether parabens at the concentrations used in cosmetics and other consumable goods exhibit endocrine disrupting effects in humans is still unknown. There are, however, indications that skin hydrolysis of parabens is incomplete [11]. Given their application in a wide range of consumables the use of parabens and paraben mixtures is regulated. Parabens are not included in Annex 1 of the dangerous substances' list (Council Directive 67/548/EC). The Council Directive 76/768/EC of the European Community [12] restricts the preservation of cosmetic products with methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP) and benzyl paraben (BeP) to a maximal authorized concentration of 0.4% for one single ester or 0.8 % for ester mixtures (w/w, calculated as acid). The reported methods for the determination of parabens in foods, cosmetics, and pharmaceuticals are based on gas chromatography (GC) [13,14], capillary electrophoresis (CE) [15] chemiluminescence (CL) [16] and high-performance liquid chromatography (HPLC) [17-20]. This paper proposed and validated an HPLC method using Phenomenex C₁₈ analytical column and an eluent based on acetonitrile: water (32:68 v/v) with UV-detector at wavelength 254 nm. The method has been successfully developed for simultaneous determination of MP, EP, PP and BP in cosmetic products with a very simple sample pretreatment prior to analysis. This proposed RP-HPLC method can also be successfully applied to cosmetics analysis.

2. Materials and Methods

2.1. Materials

All reagents used were analytical grade or, guaranteed reagent tested according to International Standardization Organization. Methanol and acetonitrile were obtained from Merck. Standards were purchased from Aldrich. Standard solutions were prepared by dissolving reference standards of MP, EP, PP and BP in mobile phase system acetonitrile: water (32:68 v/v). High quality water was prepared with a Milli-Q purification system (Millipore).

2.2. HPLC method

The HPLC method was performed on a Shimadzu HPLC system equipped with LC-10 ATPV pump, SPD-10ADVP UV detector, and Rheodyne injector system fitted with 20 μ l loop. The HPLC analysis was performed on reversed phase high-performance liquid chromatographic (RP-HPLC) system with isocratic elution mode using a mobile phase of acetonitrile:water (32:68% v/v) with 1.0 mL/min flow rate, and C-18 column (Phenomenex, 125 $\times$ 4.6 mm, 3 μ m particle size) was used as an

analytical column. Detection was performed at 254 nm. The running time for each analysis was 10 min. All separations were carried out at ambient temperature.

2.3 Preparation of Standard Solutions

The different concentrations of standard solutions were prepared to contain 0.02 to 100.0 μ g/mL of MP, EP, PP and BP, respectively (Table 1). All solutions were filtered through a 0.45 μ m membrane filter prior to use. Five standard solutions of each paraben compounds were prepared for each product by dissolving accurately weighed amounts of paraben compounds in a water: methanol mixture in order to obtain the ranges of concentration as reported in Table 1. Calibration curves were constructed by plotting the peak areas of paraben compounds (y) against their concentrations (x); regression analysis of the data (n=5) for each component gave the values for slope, along with the intercept and correlation coefficient for each calibration curve (Table 1). The calibration curves were used for the quantification of MP, EP, PP and BP in commercial cosmetic cream samples.

Table 1 Method validation results (n=5)

Std parabens	Concentration range (μ g/ml)	Calibration curves	Corr. Coeff.	Recovery average %	Recovery RSD %	LOD (μ g/ml)	LOQ (μ g/ml)
MP	0.04-80.0	$y = 44338 x - 283918$	0.9999	99.4	1.32	0.02	0.05
EP	0.04-80.0	$y = 42281 x - 425729$	0.9989	100.5	1.15	0.01	0.05
PP	0.04-80.0	$y = 93354 x - 157332$	0.9986	101.2	1.52	0.02	0.07
BP	0.04-80.0	$y = 35998 x - 375051$	0.9997	99.8	1.46	0.02	0.07

2.5. Sample preparation

Approximately 1 g of commercial cream sample, accurately weighed, was diluted 1:10 with tetrahydrofurane-phosphate buffer (pH 3.5; 0.025 M) (3:7, v/v) in a screw-capped tube. It was stirred in a vortex mixer and slightly warmed until a homogeneous suspension was obtained. This suspension was injected into the chromatograph.

3. Results and Discussion

3.1. Observed retention times

The aim of the present chromatographic method was to separate a mixture containing four components of parabens. The observed retention times of the reference compounds were found to be 2.8, 3.7, 5.4 and 8.4 min for MP, EP, PP, and BP respectively. The variation in retention time was found to be $\pm$ 5% of the observed retention time. Fig. 1 shows the separation of a standard mixture of parabens.

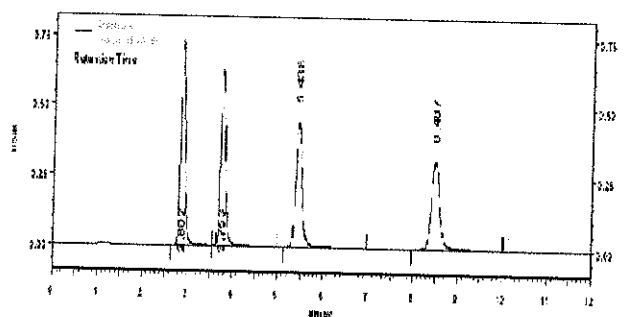


Fig.1. Chromatograms of a standard mixture of parabens at 254 nm.

3.2 Analytical parameters and validation

The optimised method was validated by a standard procedure. Accuracy of the method was determined by using spiked placebo solutions, three preparations each, two injections of each preparation. Relative standard deviation (RSD) values were calculated for repeated standard injections (system precision) as well as repeated injections of multiple sample preparations (method precision). Linearity was determined in the 20% to 150% range of the prescribed amount in the cream at six different concentrations. The method validation results obtained under the final conditions are shown in Table 1.

The recovery data (Table 1) clearly showed that other constituents of the formulation in cosmetic cream did not interfere with the assay. Regression analysis of data ($n = 5$) for each component gave the values for slope, intercept and correlation coefficient for each calibration curve are summarized in Table 1. The validity of listed regression data was tested by the assay of an authentic mixture containing known quantities of MP, EP, PP, and BP. The result showed good accuracy (as revealed by the percentage recovery). The recovery data were generated by the assay of solutions containing 80, 100 and 120% of the theoretical assay concentration. The external standard method is routinely used as a quality control procedure for the analysis of the formulation containing parabens. The parameters LOD and LOQ were determined using signal to noise ratio. The results of the linearity, LOD ($S/N = 3$), and LOQ ($S/N = 10$) are shown in Table 1.

3.3 Analytical applications

Parabens including MP, EP, PP and BP are used as preservatives in cosmetic products. The proposed RP-HPLC method was applied to several cosmetic samples (cream, face wash foam, and cleaning towels) commonly available in different supermarkets in the town of Phitsanulok, Thailand. The results indicated that cream and face wash for men contained two parabens, MP and PP, and four parabens, MP, EP, PP and BP, were found in cream for men product. Thus, we tried recuperation assays with all the samples in order to test that the extraction methods were valid, adding the paraben to spike the sample and the quantitative recoveries for the different concentration levels varied from 99.9 to 100.5 %. This RP-HPLC method was successfully applied to determine parabens in the selected cosmetic products and also able to reduce analysis cost and time. The results are shown in table 2, 3 and table 4.

Table 2 Analytical applications of the proposed RP-HPLC-UV for qualitative analysis of cosmetics

Sample	Paraben found in sample			
	MP	EP	PP	BP
Cream	✓	-	-	-
Cream for men	✓	✓	✓	✓
Face wash for men	✓	-	✓	-
Cream	✓	-	✓	-

Table 3 Paraben contents (MP, EP, PP, BP) in some cosmetics products obtained by RP-HPLC-UV method

Sample	Paraben Contents (% w/w) ^a			
	MP	EP	PP	BP
Cream	0.25 ± 0.40	-	-	-
Cream for men	0.28 ± 0.24	0.01 ± 0.30	0.06 ± 0.20	0.01 ± 0.25
Face wash for men	0.15 ± 0.18	-	0.01 ± 0.16	-
Cream	0.12 ± 0.20	-	0.05 ± 0.30	-

Note: ^a Mean of three determinations ± CV, %

Table 4 Simultaneous determination of methyl paraben (MP), ethyl paraben (EP), *n*-propyl paraben (PP), and *n*-butyl paraben (BP) under the proposed HPLC method

Compound	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)
MP	16.05	16.10	101.02
	20.36	20.40	100.46
	24.54	24.46	99.64
Mean (±RSD)			100.36 (± 0.65)
EP	4.05	4.07	100.82
	5.04	5.04	100.00
	5.98	5.97	99.88
Mean (±RSD)			100.46 (± 0.84)
PP	10.20	10.18	99.80
	20.16	20.14	99.88
	20.20	20.26	100.30
Mean (±RSD)			99.99 (± 0.75)
BP	4.02	4.03	100.39
	5.04	5.02	99.70
	6.06	6.04	99.66
Mean (±RSD)			99.92 (± 0.42)

4. Conclusions

In this work, a simple RP-HPLC method was developed for simultaneous determination of MP, EP, PP and BP. Under the optimum conditions, separation of the four parabens was achieved in less than 10 minutes. The limit of detection (LOD) of standard MP, EP, PP and BP was 0.02, 0.01, 0.02 and 0.02 µg/mL, respectively, and the limit of quantification (LOQ) was 0.05, 0.05, 0.07 and 0.07 µg/mL, respectively. This proposed method was successfully applied to determine parabens in cosmetic samples. The developed method offers several advantages such as a short analysis time, a low LOD, LOQ, and reagent consumption. It can be concluded that the proposed method provides an alternative procedure for the quality control of parabens in cosmetic products.

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