J. Soc. Cosmet. Scientists Korea
Vol. 33, No. 4, December 2007, 263-267

UPLC™를 이용한 화장품 중 보존제 8종(파라벤 6종, 페녹시에탄올, 클로페네신)의 동시분석

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(2007년 8월 23일 접수, 2007년 9월 28일 채택)

Simultaneous Determination of 8 Preservatives (6 Parabens, 2-Phenoxyethanol, and Chlorphenesin) in Cosmetics by UPLC™

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(Received August 23, 2007; Accepted September 28, 2007)

요 약: 미생물의 오염을 통해 화장품이 변질되거나 분해되는 것을 방지하여 소비자를 보호하기 위해 화장품에 보존제가 사용된다. 파라벤류는 제형화하기 쉽고, 활성 범위가 넓으며, pH에 화학적으로 안정하면서 저렴하여 거의 모든 종류의 화장품에 널리 사용된다. 페녹시에탄올과 클로페네신 역시 화장품에 일반적으로 사용되는 보존제로 보통 파라벤과 함께 사용된다. 파라벤의 독성은 일반적으로 낮지만, 손상된 피부에는 자극을 유발할 수 있으며, estrogenic 잠재성, 마취 효과 및 생식 독성의 가능성에 대한 논란이 있어 왔다. 따라서 파라벤은 배합한도 원료로 지정 관리되고 있으며, 페녹시에탄올과 클로페네신도 마찬가지로 최대 허용량이 지정되어 있다. 그러므로 제품 중 보존제의 함량을 관리하는 것은 중요하다. 그러나 일반적으로 사용되는 역상 액체크로마토그래프법으로는 이성질체를 포함한 6종의 파라벤과 페녹시에탄올 및 클로페네신을 동시에 분리 분석하기 어려웠다. 용출 시간이 길어져, 피크 모양이 나쁘고 분리능이 좋지 않아 정확한 정량이 불가능하였다. 본 연구에서는 ultra performance liquid chromatography™ (UPLC™)를 이용하여 8종의 보존제를 10 min 이내에 동시분석을 시도하였다. 또한, International conference on harmonisation (ICH) 가이드라인의 밸리데이션 방법에 근거하여 본 시험법의 적합성을 검증하고, 로션, 팩트, 파운데이션 및 립글로스 등 다양한 제형에 적용이 가능함을 보였다. 본 시험법은 파라벤류를 포함한 다양한 보존제를 함유한 화장품 중 보존제의 함량을 단시간에 간편하고 정확하게 정량하는데 활용될 수 있을 것이다.

Abstract: Parabens are used in nearly all types of cosmetics and toiletries because they are formulated well and have broad spectrum of activity, inertness, low costs and excellent chemical stability in relation to pH. 2-phenoxyethanol and chlorphenesin are common preservatives which are usually used in combination with parabens in cosmetics. Toxicity of parabens is generally low but application of parabens to damaged or broken skin has resulted in sensitization. Moreover, the possibility of their estrogenic potential, anesthetic effects and reproductive toxicity has been reported. Consequently there are some regulations in use of parabens. And the maximum permitted concentrations of chlorphenesin and 2-phenoxyethanol in cosmetic products are authorized by the same reasons. So it is important to control and estimate the amount of parabens in products. In this article, we proposed a valid method for the simultaneous determination of 8 preservatives including parabens in a short time using ultra performance liquid chromatography™ (UPLC™). Separation of eight components was achieved in less than 10 min and resolutions were reasonable (USP resolution ≧ 2). And limit of detection and quantification were evaluated. The method was suitably validated for specificity, linearity, precision (repeatability, intermediate precision) and accuracy for assay (recovery) based on International conference on harmonisation (ICH) guideline. The method was applicable to analysis of preservatives in cosmetic products.

Keywords: *paraben, 2-phenoxyethanol, chlorphenesin, UPLC™, preservatives*

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1. Introduction

Parabens are used in nearly all types of cosmetics and toiletries because they were formulated well and have a broad spectrum of activity. They are also inert, inexpensive, and have an excellent chemical stability in relation to pH. 2-phenoxyethanol and chlorphenesin are common preservatives that are usually used in combination with parabens in cosmetics. The toxicity of parabens is generally low, but applying parabens to damaged or broken skin can makes result in a sensitization[1]. Moreover, there have been reports that parabens may have estrogenic potential, anesthetic effects, and reproductive toxicity[2]. Consequently, parabens have been regulated. For example, a European Economic Community (EEC) directive only permits the use of parabens with a maximum concentration of 0.4 wt% and a total maximum concentration of 0.8 wt%, expressed as *p*-hydroxybenzoic acid. And the maximum concentrations of chlorphenesin and 2-phenoxyethanol are authorized as 0.3 wt% and 1.0 wt% respectively[3]. Therefore, it is important to control and estimate the amount of parabens in products. With traditional RP-HPLC method, separation of paraben and its isomer took so long time, and the simultaneous determination of parabens, paraben isomers, 2-phenoxyethanol and chlorphenesin was hard to get good resolution and sharpness of peaks. This article shall propose a valid, quickly executed method to make a simultaneous determination of eight preservatives, including parabens, using ultra performance liquid chromatography™ (UPLC™).

2. Materials and Methods

2.1. Chemicals and Reagents

Working standards of 6 parabens, 2-phenoxyethanol and chlorphenesin were used. The reference standards of 6 parabens and 2-phenoxyethanol were bought from Sigma-Aldrich (USA). Chlorphenesin was obtained from Cognis/Serobiologiques (France). Methanol, acetonitrile and tetrahydrofuran HPLC grade were provided by Fischer Scientific (Germany), and phosphoric acid was obtained from Shinyo pure chemicals co., ltd. (Japan). HPLC grade water was prepared by Milli-Q Gradient (Milipore, USA).

2.2. Equipments

UPLC™ analysis was performed on a Waters Acquity™ ultra performance LC (UPLC™, USA) equipped with 2996 photodiode array (PDA) detector, a gradient elution pump with degassing device and mixer, a cooling auto-sampler and a column oven. Waters Acquity™ UPLC systems use small particles, very low system volumes and stand high pressure so enable to detect more peaks with faster run times. The data was acquired via Empower waters data system software. A Mettler Toledo AT200 balance (Mettler Toledo, USA) was used for weighing standards. In addition, a Millipore filter was used in the study.

2.3. Chromatographic Conditions

Chromatographic separation of the 8 preservatives was performed using a Waters C18 BHC column, 210 × 50 mm, 2 μm particle size. The mobile phase consisted of acetonitrile and buffer solution of phosphoric acid (pH 2.5) that were carried as a gradient program (Table 1). The wavelength was chosen for UV detection to provide acceptable absorbance for 8 components. The PDA detector was operated at 230 nm and 270 nm each. The absorbance of chlorphenesin at 270 nm was relatively good but it is eluted early (1.2 min) so there can be interference with sample solution. Because of this, chlorphenesin was detected at 230 nm and the others were detected at 270 nm. The injection volume was 2 μL. During analysis, the column was equilibrated at 25 ℃. Each determination of 8 preservatives required 10 minutes (Figure 1).

2.4. Preparation of the Standard Solutions

Stock standard solutions were prepared in methanol at a concentration of 1,000 g/mL (methylparaben and 2-phenoxyethanol) and 500 g/mL (ethylparaben, propylparaben, iso-propylparaben, butylparaben, iso-butylparaben and chlorphenesin) approximately. 6 stock standard solutions were prepared as a mixture of 8 preservatives. Linearity experiments were performed using 6 standard solutions which were made by serial dilution from 6 stock solutions to 1/5, 1/10, 1/25, 1/50, 1/100 and 1/200 each. These solutions were found to be stable for several weeks at 4 ℃. A portion of the solutions was taken and filtered through a PTFE membrane filter (0.45 μm), and each of the filtered

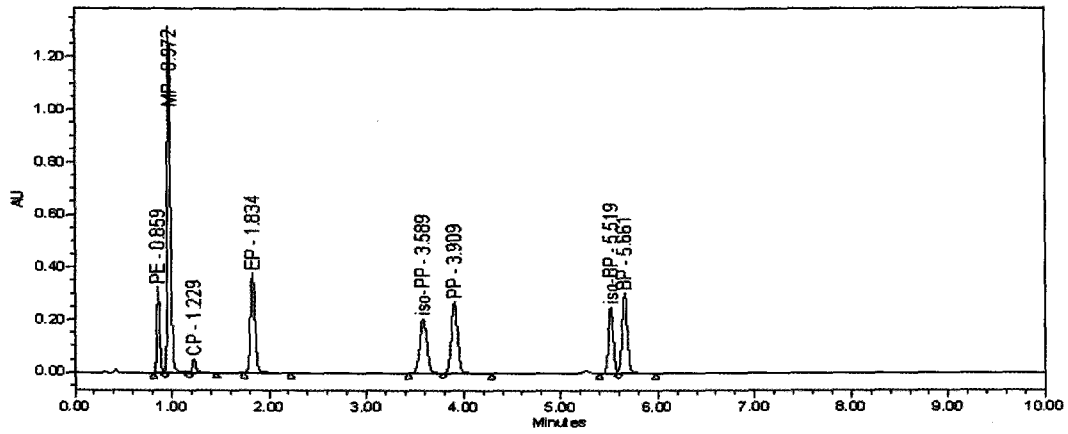

Figure 1. UPLC™ chromatogram of eight preservatives; 2-phenoxyethanol (PE); methylparaben (methyl 4-hydroxybenzoate, MP); chlorphenesin (CP); ethylparaben (ethyl 4-hydroxybenzoate, EP); isopropylparaben (isopropyl 4-hydroxybenzoate; iso-PP); propylparaben (propyl 4-hydroxybenzoate; PP); isobutylparaben (isobutl 4-hydroxybenzoate; iso-BP).

Table 1. Gradient Profile Program to Carry out This Chromatographic Method

Time (min)	Acetonitrile (%)	Phosphate buffer solution (%)	Flow rate (mL/min)
0	75	25	0.45
3	75	25	0.45
5	65	35	0.50
6.5	65	35	0.50
6.7	75	25	0.45
10	75	25	0.45

solutions were analysed in triplicate.

2.5. Preparation of the Sample Solutions

Sample solutions were prepared with four commercial cosmetic products; O/W emulsion, foundation, twin-cake and lip gloss. 1 g of sample was weighed into a 100 mL volumetric flask and 30 mL of methanol was added. The solution was stirred and sonicated to be homogenized, and then mess up to 100 mL with methanol. The sample solutions were filtered through a PTFE membrane filter (0.45 μm) before injection. But in case of lip gloss, approximately 10 mL of tetra-hydrofuran (THF) was added at first after weighing the sample to disperse polymers in it.

3. Results

3.1. Linearity

Linearity was studied using six concentration points

Table 2. Results of Linearity Test

Compounds	Range (μg/mL)	Equation for regression line	Correlation coefficient
PE	5.6 - 266.8	y = 2476x - 3377	0.9997
MP	5.4 - 213.6	y = 13686x - 5201	0.9999
CP	2.4 - 102.6	y = 12536x - 7817	0.9996
EP	2.5 - 95.2	y = 13523x - 7727	0.9995
Iso-PP	2.3 - 90.4	y = 12097x - 4901	0.9991
PP	2.6 - 105.8	y = 12029x - 6501	0.9999
Iso-BP	2.2 - 88.0	y = 10244x - 4260	0.9992
BP	2.6 - 109.8	y = 10760x - 6667	0.9996

in the range 2.2 to 266.8 g/mL (n = 3). The correlation coefficient obtained in each case was not less than 0.999. This result showed strong linear relationship beween peak area and concentration of 8 preservatives respectively (Table 2).

3.2. Precision

The precision of instrumental system (repeatability) was estimated by calculating relative standard solution (RSD) value (%) for repeated (n = 6) standard injections at 100 % concentration level. In all these cases, the RSD was obtained below 1 %. Intermediate precision was demonstrated by the same analyst working on different days. Standard solutions at three concentration levels (90, 100 and 110 %) were injected three times (n = 3). The RSD values were less than 2 %, and demonstrated the good precision of the an-

Table 3. Results of Method Validation

Compounds	Repeatability (n = 6) RSD values (%)	Intermediate precision (3 samples × 3 injections) RSD values (%)	LOD (μg/mL)	LOQ (μg/mL)
PE	1.27	0.48	0.31	0.94
MP	1.38	0.38	0.32	0.98
CP	1.38	0.28	0.17	0.51
EP	1.29	0.63	0.34	1.04
Iso-PP	1.23	0.68	0.49	1.49
PP	1.24	0.09	0.07	0.20
Iso-BP	1.28	0.96	0.55	1.67
BP	1.24	0.91	0.28	0.84

Table 4. Results of Recovery and Application Studies with Four Cosmetic Products

Added (μg/mL)	Recovery (%)			Quantitative results (w/w%)
	5	10	20	
O/W emulsion	95.1 - 101.8	95.7 - 102.5	95.3 - 99.1	MP: 0.2, PP: 0.1
Foundation	96.1 - 100.2	97.9 - 102.8	95.7 - 101.8	MP: 0.2, PP: 0.05
Twin-cake	98.4 - 101.3	98.5 - 103.7	98.1 - 103.3	MP: 0.1
Lip gloss	96.8 - 102.5	96.0 - 100.5	95.6 - 101.0	iso-PP: 0.13, BP: 0.1 iso-BP: 0.1

alytical method (Table 3).

3.3. Limit of Detection and Quantification

Limits of detection (LOD) and quantification (LOQ) were based on the standard deviation of response and the slope of calibration curve according to the ICH validation guideline[4].

$LOD = 3.3\sigma \times S^{-1}$

$LOQ = 10\sigma \times S^{-1}$

where, σ =standard deviation (n = 3) of the response of the stardard solution prepared in 2.4 with the lowest concentration.

S = the slope of the calibration curve

The LOD for 8 preservatives was found in the range 0.07 ~ 0.55 g/mL and LOQ was 0.20 ~ 1.67 g/mL (Table 3). Usually cosmetics contain preservatives at the level of 0.01 ~ 0.3 wt% and the limit of total parabens in cosmetics is 0.8 wt%. So that this method will be useful to quantify each preservative and total paraben in cosmetics.

3.4. Accuracy and Application

The analytical method was applied for cosmetic products; O/W emulsion, foundation, twin-cake and lip gloss. The results of quantification were listed in Table 4. The accuracy was verified by recovery studies with 3 different concentrations of test solutions for each five samples prepared with a known added amount (5, 10 and 20 g/mL and 10, 20 and 40 g/mL for PE) of each preservative and injected in triplicate. The results of standard addition reported as percent recoveries are presented in Table 4 for eight compounds and four types of cosmetic products were quantified specifically with this UPLC™ method. The recoveries obtained were between 95 ~ 105 % and they satisfied the conditions for accuracy for accuracy of Association of Analytical Chemists (AOAC)[5].

Therefore, the UPLC™ method is accurate within the regulation range for various formulas of cosmetics.

4. Conclusion

We developed a new analytical method for 8 preservatives simultaneously within ten minutes. This method is simple and quick so it will be usefully applied in routine determination of preservatives.

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