Simultaneous Determination of Nine UV Filters and Four Preservatives in Suncare Products by High-Performance Liquid Chromatography

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Abstract

HPLC method for quantitative determination of four preservatives and nine UV filters worldwide authorized in commercial suncare product was developed and validated, and then 101 samples of commercial suncare products were analyzed for the UV filters and preservatives using the proposed method. The mobile phase was acetonitrile-water containing 0.5% acetic acid using a gradient elution at a flow rate of 0.9 mL/min and UV measurements were carried out at 320 nm for UV filters and 254 nm for preservatives. The correlation coefficients of each calibration curves were mostly higher than 0.999. The percent relative standard deviations (%RSD) ranged from 0.97% to 6.1% for five sample aliquots. The recoveries from the spiked solutions were 98–102%. 2-ethylhexyl-*p*-methoxycinnamate (EHMC) was detected in 96 of 101 commercial suncare products and the concentration was in the range of 3.08–8.16% and 18 samples were found to exceed the 7.5% which has been defined as the maximum allowed concentration in Korea. Methyl paraben was detected in 81 of 101 samples and the next-most often detected preservatives were propyl paraben (25), ethyl paraben (18), and butyl paraben (4). Three samples of 101 suncare products exceeded the maximum allowed concentration (i.e., 0.58–0.79%). The proposed HPLC method allows efficient and simultaneous analysis of preservatives and UV filters suitable for quality control assays of commercial suncare products.

Introduction

Public concern about skin damage by sunlight has increased due to an increase in exposure to harmful UV, which at least in part may be related to a depletion of the ozone layer. Accordingly the use of suncare product containing UV filters has become increasingly widespread to protect human skin against sunlight-induced damages such as photo-aging, skin cancer and damage to the skin's immunological system (1–3). UV filters are chemical compounds that mitigate the deleterious effects of sunlight and they are used in a variety of pharmaceutics and cosmetics such as sunscreen creams, lotions and spray and other products. The screening efficiency of suncare products against both UV-B (290–320 nm) and UV-A (320–400 nm) has resulted in the development of cosmetic preparation and sunscreen chemicals. A list of approved UV filters and their maximum allowed concentrations in commercial products have been set by the regulatory authority in Korea (Table I) as well as Europe, USA and Japan (4–6). Most of the organic UV filters that are available are lipophilic and can be expected to accumulate in humans (7–10) and environmental media (11,12).

Alternatively these chemicals may be subject to photochemical transformation including isomerization which may result in the formation of potentially toxic compounds. Many analytical

Table I. Levels of UV-filters and Preservatives in Commercial Suncare Products (101 items)

Compound	Frequency in use	Conc. (%)	Average (%)	Authorized conc.* (g/100 g %)
2-ethylhexyl p-methoxycinnamate	96(18)‡	3.08~8.16	6.77	7.5%
Isoamyl p-methoxycinnamate	41	0.33~7.79	2.91	10%
Ethylhexylsalicilate	37(2)‡	1.78~5.33	4.20	5%
3-(4-Methyl benzylidene) camphor	20	2.01~4.96	3.42	5%
Benzophenone-3	10(2)‡	3.04~5.37	4.25	5%
Ethylhexyldimethyl-p-aminobenzoate	3	2.23~5.71	4.46	8%
Octocrylene	6	1.13~6.75	3.53	10%
Butyl-methoxydibenzoilmethane	23	0.49~3.41	2.01	5%
Methyl paraben	81	0.018~0.419	0.232	< 0.5
(by Sum of all parabens)				
Ethyl paraben	18	0.040~0.251	0.110	
Propyl paraben	25	0.052~0.250	0.106	
Butyl paraben	4	0.081~0.186	0.105	

* Maximum allowed concentrations stated in Korea.
‡ The number of items which were over the authorized concentration.

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techniques for determining UV filters in sunscreen products have been reported. These include for example analytical methods based on separation and/or quantification using UV–vis spectroscopy (13), gas chromatography (14), and high-performance liquid chromatography (HPLC) (15–20). In particular, reversed-phase HPLC with C18 column is the most common method for the simultaneous analysis of several UV filters in pharmaceutics and cosmetics. Chisvert et al. (18) separated seven UV filters using hydroxypropyl-β-cyclodextrin as modifier of the mobile phase ethanol–water–acetic acid (70:29.5:0.5).

Chisvert et al. (21) also studied a HPLC method for quantitative determination of three UV filters, namely benzophenon-4, 2-phenylbenzimidazole-5-sulphonic acid (PBS) and terephthalylidene dicamphor sulfonic acid (TDS), using ethanol–20mM sodium acetate buffer of pH 4.6 (30:70, v/v) in sunscreen sprays. Simeoni et al. (15) studied the separation of eight of the most common sunscreen agents (2-ethylhexyl-4-methoxycinnamate, oxybenzone, butyl-methoxydibenzoilmethane, octylsalicilate, methybenzylidene camphor, octyldimethylamminobenzoate, phenylbenzimidazole sulphonic acid and octoctylene) in sun protection product, using a cyanopropyl silica column eluted with methanol-acetonitrile-tetrahydroguran-aqueous acetic acid. Sixteen UV filters were determined by Schakel et al. (22) using gradient of ethanol-aqueous acetate buffer containing 0.2 mM of EDTA; however, isoamyl-p-methoxycinnamate and 3-(4-methylbenzyliden) camphor peaks were partially unresolved , and terephthalidene dicamphor sulfonic acid (TDSA) and benzophenone-4(B-4) were mostly unresolved in this study. Smyrniotakis et al. (20) developed and validated a HPLC method for the determination of four UV filters including tinosorb M, which is a very hydrophobic compound.

Most of the studies have focused on the analysis of only UV filters, whereas the presences of other compounds in samples were not discussed. On the other hand there is a need for example to establish methods that allows simultaneous analysis of UV filters as well as other chemicals such as parabens in a given suncare product. Parabens are ester compounds of *p*-hydroxybenzoic acid, widely used as antimicrobial preservatives in cosmetic products, pharmaceuticals and beverage because of their relatively low toxicity and their effective antimicrobial activity (23). However, recent in vivo (24–26) and in vitro (27–29) studies have revealed weak estrogenic activity of some parabens and have raised concern about the safety of widespread paraben use. In particular, it has been suggested that propyl paraben (*n*-propyl-p-hydroxybenzoate) has the highest estrogenic activity among paraben esters (30).

Accordingly analysis of preservatives in commercial suncare products is equally important as UV filters for quality control and for carrying on the observance of the existing legislation. HPLC assays have been reported for methyl paraben, ethyl paraben, propyl paraben and butyl paraben in creams and ointments (31), gels (32), and lotions (33).

The aim of this study was to develop and evaluate a HPLC method for the simultaneous analysis of nine common UV filters and four antimicrobial preservatives that are common in sunscreen products. The outcome aims to provide a rapid and accurate assay, with a basic HPLC configuration using solvents that are considered to be of relatively low toxicity. Importantly the aim is to show that the method is sufficiently robust and simple to provide a means for allowing assessment of an evaluation of the composition of suncare products based on existing legislation that regulates the composition of commercial products. The method was validated for nine UV filters and four preservatives and 101 samples of commercial suncare products were analyzed for the UV filters and preservatives.

Experimental

Reagents

Four esters of p-hydroxybenzoic acid (methyl, ethyl, propyl, and butyl parabens), 2-ethylhexyl-*p*-methoxycinnamate (EHMC), benzophenone-8 (B-8) and benzophenone-3 (B-3) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Isoamyl-*p*-methoxycinnamate, 3-(4-methylbenzylidene) camphor, 4-tert-butyl-4'-methoxy-dibenzylmethane, 2-ethylhexyl-4-dimethyl aminobenzoate and 2-ethylhexylsalicilate (EHS) were purchased from Sigma-Aldrich (Tokyo, Japan). All other chemicals were of analytical reagents grade. One hundred and one suncare products containing UV filters and preservatives were purchased on the local market in Korea and were analyzed using the developed method.

Chromatography

The HPLC apparatus comprised dual-gradient analytical pumps, injector and autosampler and diode array and multiple wavelength detector (Model: UltiMate 3000, Dionex, Sunnyvale, CA). The above system was controlled using chromeleon 6.8 software. Separations were performed on a SunFire C18 column (5 µm, 4.6 × 250 mm: Waters, Milford, MA). Separation using

Table II. Recovery Studies of E ight UV Filters and Four Preservatives in Suncare Products

Samples*	Compounds†	Spiked amount (mg)	Recovery (%)	RSD (%, *n* = 3)
A	Methyl paraben	0.10	102.1	2.80
B	Ethyl paraben	0.10	100.0	1.23
B	Propyl paraben	0.10	98.5	0.64
A	Butyl paraben	0.10	98.3	2.47
B	Benzophenone-3	0.92	99.1	0.51
C	Isoamyl–p-methoxycinnamate	1.54	100.4	1.76
A	3-(4-Methyl benzylidene) camphor	1.24	99.8	2.31
B	Octocrylene	0.97	99.9	2.09
B	Butyl-methoxy-dibenzoilmethane	1.06	99.3	0.65
A	2-ethylhexyl methoxycinnamate	1.30	97.3	2.68
C	Ethylhexyl dimethyl-p-aminobenzoate	1.31	98.0	2.71
B	Ethylhexylsalicilate	0.90	101.4	3.43

* A, B, C: samples used for recovery studies.
† benzophenon-8 is excluded from recovery studies because it wasn't detected in 101 samples.

HPLC was performed at 30°C with a gradient elution of acetonitrile (containing 0.5% acetic acid)–water (containing 0.5% acetic acid) at a flow rate of 0.9 mL/min as described in Table II. UV absorption was quantified at 254 nm for preservatives and 320 nm for UV filters. The isomerization of 2-ethylhexyl-p-methoxycinnamate was confirmed by LC-ES–MS–MS on a XTerra MS C18 column (3.5 µm, 2.1 × 150 mm, Waters) and a ion trap LC–MS (LCQ, Finnigan, Hercules, CA) with electrospray (ESI) using the same HPLC system described above, and the chromatography was performed under isocratic condition of methanol–water (89:11, v/v) at a flow rate of 0.2 mL/min.

Preparation of solutions

Standard solutions

The multicomponent stock solution of UV filters and preservatives were made as follows. The mixed stock solution of four preservatives with concentrations of 1–1.5 g/L was prepared in methanol. The nine UV filters were accurately weighed (50–100 mg) into a 50 mL volumetric flasks filled up with 5mL of the solution containing the preservatives and then made up to the mark with ethyl acetate-methanol (50:50, v/v) containing 0.5% acetic acid. Working standard solutions for the calibration curve were prepared in the concentration range of 25–200 mg/L for UV filters, 2.5–20 mg/L for preservatives. All the working standard solution of UV filters and preservatives were prepared daily.

Sample preparation

Commercial suncare products were purchased in local markets in Korea. An aliquot from each sample (~ 500 mg) was accurately weighed into a 50 mL volumetric flask and dissolved into 40 mL of methanol-ethyl acetate (50:50, v/v). Samples were extracted using a sonifier (Branson, Teltow, Germany) for 10 min and then made up to the mark with ethyl acetate–methanol (50:50, v/v) containing 0.5% acetic acid. A 5 mL of stock sample solutions was diluted to 50–100 mL with methanol containing 0.5% acetic acid so that the final expected concentrations were in the range of 25.0–100 mg/L for UV filters and 2.5–20 mg/L for preservatives. Prepared solutions were then injected into the HPLC system after filtering with 0.45 µm membrane filters (Whatman, Piscataway, NJ). In this study, brown colored glasses were used to minimize the degradation of chemicals by sunlight.

Validation of Analytical method

The linearity of the proposed method was determined using six and four point calibration. Regression equations were obtained through unweighed least squares linear regression analysis, using peak area as a function of concentration. The accuracy of the developed method was examined by recovery test—standard addition method and the precision was expressed as the percent relative standard deviation or standard deviation for the results of replicate measurements.

Results and Discussion

Chromatography

In the work presented here the most optimized mobile phase was acetonitrile-water containing 0.5% acetic acid using a gradient elution as described in Table III. Acetic acid, which was added to the mobile phase, was used for preventing the ionization of alkyl parabens because these compounds might exist in molecular and ionic forms and ultimately cause the split or distortion of peaks in the chromatogram. The chromatography and detection of UV filters was not influenced by acetic acid. A typical chromatogram for nine UV filters and four preservatives obtained under these conditions shows that resolution factors for all components is > 1.8 and the analytical conditions can apply for quantitative analysis (Figure 1).

Validation of analytical method

Linearity

The analytical parameters of representative calibration curves were summarized as follows. Linear calibration curves were

Table III. Gradient Time Table of Mobile Phase

Time (min)	Solvent A (%)*	Solvent B (%)*	UV-vis Detector wavelength (nm)
0	50	50	
10	50	50	254 nm
15	100	0	(preservatives, 0~14.5 min)
23	100	0	
25	50	50	313 nm
28	50	50	(UV filters, 14.5~28.0 min)

* A: acetonitrile (containing 0.5% acetic acid); B: water (containing 0.5% acetic acid).

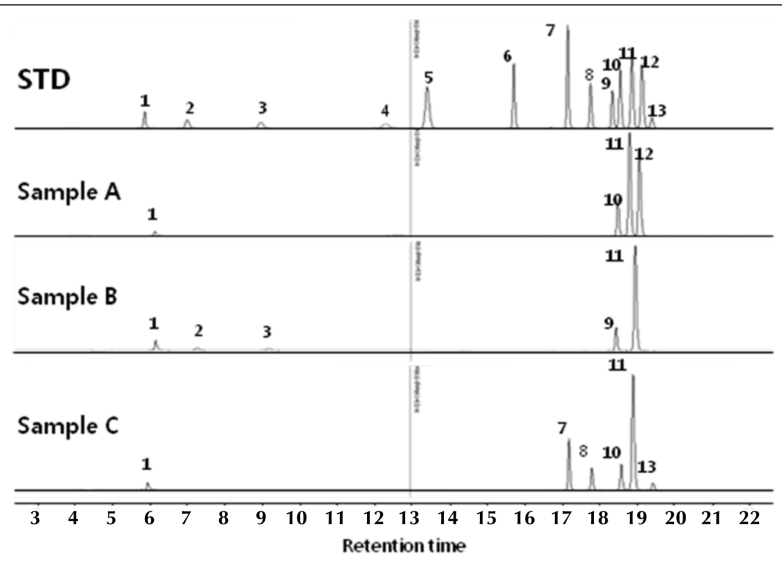

Figure 1. Typical HPLC chromatogram of a standard mixture and sample solutions of UV-filters and preservatives: 1. Methyl paraben, 2. ethyl paraben, 3. propyl paraben, 4. butyl paraben, 5. benzophenone-8, 6. benzophenone-3, 7. isoamyl p-methoxy cinnamate, 8. 3-(4-methylbenzylidene) camphor, 9. octocrylene, 10. butyl-methoxydibenzoilmethane, and 11. 2-ethylhexyl-*p*-methoxycinnamate.

obtained using solutions of four and six different levels of concentration ranging from 2.5 to 20 mg/L for four preservatives and from 25 to 200 mg/L for nine UV filters. The correlation coefficients of each calibration curves were mostly higher than 0.999 except for isoamyl-*p*-methoxycinnamate. The slopes of calibration curves for preservatives were similar for all ranges from 2.5 to 20 mg/L, on the other hand slopes for UV filters a trend towards decreasing slopes (i.e., non-linearity) was observable toward higher concentrations (i.e., 200 mg/L). Accordingly when analyzing the UV filters, the intervals between standard solutions and calibration ranges need to be considered thoroughly.

Inter-day comparison of calibration curves

To evaluate the stability of standard solutions as time goes by, linear calibration curves were measured at 24 h intervals for three days. The slopes of the calibration curves, which expressed the sensitivity of standard chemicals, were very slightly decreased for three days. It shows that standard solutions of nine UV filters and four preservatives could be available for at least 3 days.

Limit of detection (LOD) and limit of quantification (LOQ)

In this study, LOD and LOQ were calculated based on the slope (S) of the calibration curves and the standard deviation (SD) of y-intercepts of regression lines according to the formula: LOD = 3.3(SD/S), LOQ = 10(SD/S) (34). Based on the analytical parameters of calibration curves, the calculated LOD values for methyl paraben, ethyl paraben, propyl paraben, butyl paraben, benzophenone-8, benzophenone-3, isoamyl-*p*-methoxycinnamate, 3-(4-methylbenzylidene) camphor, octocrylene, butyl-methoxydibenzoilmethane, 2-ethylhexyl-*p*-methoxycinnamate, ethylhexyl dimethyl-*p*-aminobenzoate and ethylhexyl salicilate were 0.03, 0.14, 0.07, 0.23, 1.5, 0.87, 1.2, 0.88, 1.4, 1.5, 0.89, 0.61, and 0.5 mg/L, respectively. The calculated LOQ values were 0.08, 0.43, 0.21, 0.68, 4.6, 2.6, 3.5, 2.7, 4.4, 4.5, 2.7, 1.8, and 1.7 mg/L, respectively.

Precision and accuracy

The precision of the proposed analytical method was expressed as the percentage of relative standard deviation (RSD). Three of the suncare products were used to estimate the precision of the methods. Each sample was divided into five aliquots of 0.15–0.2 g each and then analyzed separately. The percent relative standard deviations (%RSD), which represented the precision of the proposed method, ranged from 0.97% to 6.1% for five aliquots. The chromatographic precision was evaluated by repeated analyses (n = 6) of the same sample solution and the results (%RSD) ranged from 0.56% to 1.15%.

To verify the accuracy of the method, the recovery tests were studied for eight UV filters and four preservatives. Three suncare products containing known concentrations of eight UV filters and four preservatives were prepared and divided into a set of three aliquots of 0.15–0.20 g each. Known amounts of twelve standard solutions were directly added to the sample aliquots, which were submitted the overall analytical method. The recoveries from the spiked solutions were 98–102% (Table II).

Photo-instability of 2- ethylhexyl-p-methoxycinnamate (trans-EHMC)

2-Ethylhexyl-*p*-methoxycinnamate (EHMC), one of the most widely used UV filters over the world, has been known as an unstable compound under sunlight exposure (15). In particular, EHMC can be easily isomerized to cis-EHMC by irradiation as

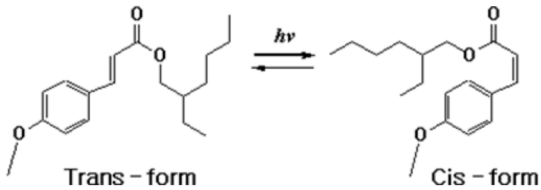

Figure 2. Photo-isomerization of 2-ethylhexyl-p-methoxycinnamate by irradiation of sunlight.

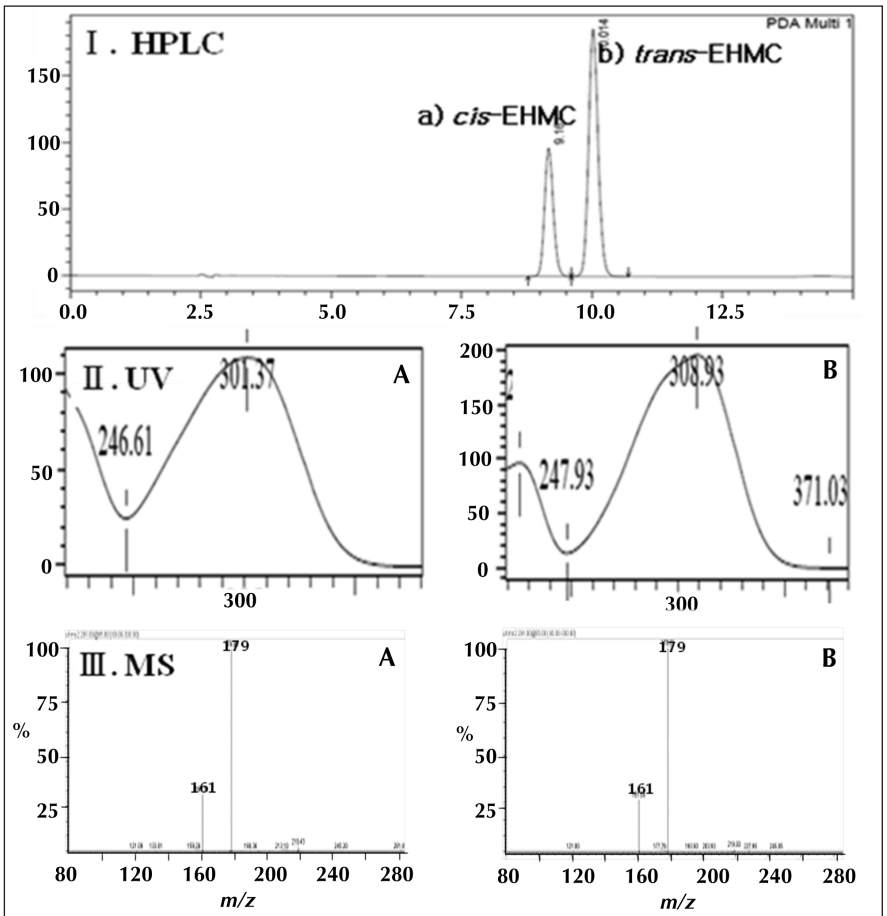

Figure 3. HPLC (I), UV (II), and MS (III) spectra of EHMC isomers: (a) cis-isomer and (b) trans-isomer.

showed in Figure 2, and the successive degradation might be induced by decomposition of cis-EHMC because it has stereometrically unstable molecular structure. Also cis-EHMC could be reversely isomerized to trans-EHMC. The HPLC chromatogram, UV-spectrum and MS spectrum for the standard solution of EHMC measured after irradiating to the sunlight for 1 h are presented in Figure 3. In HPLC chromatogram, two isomers were separated at the mobile phase of methanol–water (89:11, v/v) and the λmax of cis-EHMC was slightly shifted to the direction of short wavelength compare to trans-EHMC in UV spectrum. However MS spectra of two isomers were very similar and major fragment ion formed by fragmentation of molecular ion (M+1, m/z = 291) in LC–MS was *p*-methoxycinnamic acid (m/z = 179). Accordingly, when analyzing the sample containing EHMC, it is very important to cut off the light, in particular UV-ray, if possible, through whole analytical step.

Analysis of UV filters and preservatives in commercial suncare products

The levels of UV filters and preservatives in 101 commercial suncare products were presented in Table I. For all products at least one organic UV filter could be quantified with some products containing up to five different UV filters. In this study, the most frequently detected UV filter was 2-ethylhexyl-p-methoxycinnamate (EHMC), which is known as a sunscreen ingredient with a strong absorbance in the UV-B region (290–320 nm). EHMC was detected in 96 of 101 commercial suncare products and the concentration of EHMC was in the range of 3.08–8.16%. Other filters that were often detected (number of detection in brackets) were isoamyl-p-methoxycinnamate (41), ethylhexyl salicilate (37) and butyl methoxy dibenzoilmethane (23). The most frequently used preservative was methyl paraben which is detected in 81 of 101 samples. The next-most often detected preservatives were propyl paraben (25), ethyl paraben (18) and butyl paraben (4). Propyl paraben and butyl paraben were mostly used with methyl paraben in suncare product. In case of EHMC, 18 samples were found to exceed the 7.5% which has been defined as the maximum allowed concentration in Korea though it is noteworthy that the concentrations in these samples were in the range of 7.69–8.16%. For both ethylhexyl salisilate and benzophenone-3, two samples exceeded the maximum allowed concentration (5.0%) though only marginally (i.e., 5.19–5.33% and 5.34–5.37%, respectively). In case of preservatives, three samples of 101 suncare products exceeded the maximum allowed concentration (i.e., 0.58–0.79%). Methyl paraben, the most highly detected paraben, were in the range of 0.30–0.34%.

Conclusion

In this study, the HPLC method for quantitative determination of four preservatives and nine UV filters in commercial suncare product were developed and validated. The good merit of this method is that it is possible to determine the mostly used preservatives and UV filters, simultaneously, in a single analysis. Using 0.5% of acetic acid as mobile phase modifier makes it possible to create a good chromatographic peak for four alkyl parabens (pKa > 8.0). The method was employed to determine these preservatives and UV filters in 101 commercial suncare products. The result showed that trans-EHMC, one of the mostly used UV filters, were determined over 7.5%, the maximum allowed concentration in Korea, in 18 of 101 samples. It is assumed that some manufactories used an excess of trans-EHMC intentionally because of its photo-instability. Cis-EHMC, one of the materials formed by photochemical reaction of trans-EHMC, is unstable and its toxicities onto the body haven't been known yet. Therefore further study will be considered for the potential toxicities of cis-EHMC.

In the end, the proposed HPLC method allows efficient and simultaneous analysis of preservatives and UV filters suitable for quality control assays of commercial suncare products.

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