

## Simultaneous Determination of Nine Sunscreen Agents by HPLC and Chemometric Analysis

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### Synopsis

A worldwide outbreak of skin cancer, related to ultraviolet (UV) radiations, was reported. Therefore, primary prevention programs were initiated. Application of sunscreens is one of the most efficient ways of protection; however, their efficiency and safety have remained a challenging issue. So, it seems necessary to consider the potential side effects for limiting the use and amount of sunscreens. In this study, an high performance liquid chromatography (HPLC) system equipped with a UV–visible detector has been used. For separation, an Agilent C<sub>18</sub> column was used (Agilent Technologies, Santa Clara, CA). This method was applied for quantitative determination of nine UV filters in commercial sunscreen products which were widely used in Iran. Fifty samples of Iranian and imported sunscreen products were analyzed. The detection limit was determined to be 0.439–1.481 µg/ml, and the quantization limit was determined to be 1.330–4.490 µg/ml. Also, in this study, chemometric methods were used to investigate the differences between Iranian and other countries' sunscreen brands. It was observed that despite the amount of UV filters in Iranian sunscreens, which was in the allowed range, there were some differences between Iranian and other countries' sunscreens. The proposed HPLC method allows efficient and simultaneous analysis of UV filters and is suitable as a quality control assay for commercial sunscreen products.

### INTRODUCTION

Because of the recent changes in lifestyle, people are being exposed to sunlight more and more. The harmful ultraviolet (UV) radiation, which consists of three parts (UVA, UVB, and UVC), is our main concern (1). The ozone layer screens UVC and does not let it reach the human body. UVA is the main cause of skin aging and UVB is the most hazardous part. It affects the skin quickly, causing skin burns (i.e., sunburn) and an increase in the risk of future skin cancer (2).

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Use of sunscreens is one of the helpful recommendations to prevent the deleterious effects of UV rays. The active ingredients of sunscreens consist of physical (UV-scattering agents, such as zinc oxide and titanium dioxide) and chemical filters: UV absorbers such as avobenzone (BMDBM), oxybenzone, para-aminobenzoic acid (PABA), homosalate (HMS), octocrylene, 3-(4-methylbenzylidene) camphor (MBC), ethylhexyl salicylate (EHS), ethylhexyl methoxycinnamate (EHMC), and ethylhexyl triazone (EHT) (3). These organic compounds (chemical sunscreens) can penetrate the skin (4), where they act as a photosensitizer (5,6). They can cause an increase in the production of free radicals under illumination, which may contribute to enhanced malignant melanoma incidences (7) among consumers. Furthermore, some of the organic UV filters such as HMS and oxybenzone have been identified as hormonal disruptors (8–10). As a result, types and amount of sunscreen agents need to be evaluated.

Although acid–base titration method is the current way to determine the type and amount of UV filters in British pharmacopeia (BP) and U.S. pharmacopeia (USP), manufacturers cannot apply this simple method in their analysis because the substances and impurities used in their products interfere with each other.

Many high performance liquid chromatography (HPLC) methods have been developed by researchers over the years for the determination and photostability of UV filters in sunscreen and cosmetic products. Comparison of the photostability of five UV filters in sunscreen agents was carried out by Vanquerp et al. (11) using a C<sub>8</sub> column and a mobile phase consisting of methanol and water.

Chisvert et al. (12) determined seven UV filters simultaneously, using a C<sub>18</sub> stationary phase. This was combined with a mobile phase of ethanol, water, and acetic acid, with cyclodextrins as a mobile phase modifier.

Chisvert and Salvador (13) analyzed three most common water-soluble UV filters in sunscreen sprays. They used a C<sub>18</sub> column and an isocratic mobile phase of ethanol and water with a 20 mM sodium acetate buffer. The analytical run time of this method was five and a half minutes.

Schakel et al. (14) determined 16 UV filters, including benzophenone-3 (Benz-3), 4-tert-butyl-4'-methoxydibenzoylmethane (BDM), octyl methoxycinnamate (OMC), and octocrylene (OCT), in sun care formulations. They used a C<sub>18</sub> stationary phase and a gradient ethanol–aqueous acetate buffer mobile phase, containing 0.2 mM EDTA. EDTA is an agent added to the mobile phase to reduce tailing in the determination of BDM; the analysis took 32 min.

Smyrniotakis and Archontaki (15) used a 5- $\mu$ m Hypersil BDS column for the determination of OCT, OMC, Tinosorb M, and octyl salicylate using a mobile phase of methanol–acetonitrile (90:10, v/v).

Salvador and Chisvert developed an environmentally friendly method for the determination of 18 UV filters in cosmetics. Their method used a C<sub>18</sub> stationary phase and a mobile phase of ethanol and acetic acid, which was used for fat-soluble compounds. A mixture of ethanol and sodium acetate buffer was used for water-soluble compounds. The analysis run time was less than 30 min for the 12 fat-soluble filters, whereas the water-soluble filters took <10 min. They validated their method by the analysis of 27 samples with different cosmetic forms, e.g., creams, lipsticks, makeup, and sunscreens (16).

Kedor-Hackmann et al. (17) operated two C<sub>18</sub> columns connected in series and a mobile phase containing acetonitrile and water to determine five sunscreens in synthetic formulations.

One of the most recent studies has developed and validated a HPLC method for the simultaneous determination of 12 sunscreens in 30 minutes using a C<sub>18</sub> column and a gradient mobile phase consisting of ethanol and acidified water (18).

Table I  
Gradient Timetable Used for the Mobile Phase

Time (min)	Flow (ml/min)	Solvent A (%)	Solvent B (%)
0	1	30	70
18	1	25	75
22	1	0	100
30	1	0	100
35	1	30	70

Temperature of the column was set at 40°C. The injected volume was 20  $\mu$ l. The wavelength was set at 312 nm. Because of the overlap of the BMDBM chromatogram with the EHMC chromatogram, another wavelength (390 nm) on which only BMDBM had absorbance was chosen. To compute the exact amount of EHMC, the area seen at 390 nm should be subtracted from the area seen at 312 nm (which is related to both substances).

In another recent study, Wharton et al. (19) used a 3- $\mu$ m Hypersil BDS C<sub>18</sub> column for the simultaneous determination of seven UV filters. The mobile phase was used in a gradient method consisting of ethanol and 1% acetic acid.

In general, most of the reported methods dealing with the determination of a large number of sunscreen compounds by HPLC recommend the use of binary, ternary, or quaternary solvent mixtures as the mobile phase. Gradient elutions have been used to obtain adequate resolution in the separation of a large number of UV filters as they allow a wide range of solvent polarity. However, this type of elution has the disadvantage of requiring longer analysis time and higher costs.

Although the high boiling points of UV filters made them less suited for gas chromatography (GC) analysis, several successful examples of identification and quantitation (20-24) by this technique have been described.

A method was developed by Haunschmidt et al. (25) based on direct analysis in real-time mass spectrometry for the qualitative and semiquantitative analysis of eight organic UV filters and four parabens in 12 cosmetic products with substantially different formulations (such as cream, milk, lotion, oil, and lipstick).

The aim of this study was to develop a fast, simple, and practical HPLC method using phosphoric acid with distilled water and ethanol to determine the most widely used UV filters in cosmetics commercialized in Iran. The method was validated and applied for the determination of nine sunscreens in formulations (lotions) commercially available in Iran to verify if they are in conformity with the current legislation. An established extraction technique using ethanol was applied to eliminate the possible interaction effects between various ingredients used in sunscreens. The stability of UV filters was assessed before method development. All the agents were kept away from light and heat.

## METHOD AND MATERIAL

### INSTRUMENTATION

A Knauer<sup>®</sup> K-1000 liquid chromatograph, which was equipped with a mixing chamber, 20- $\mu$ l loop, degasser, and Knauer<sup>®</sup> K-2500 UV detector (Knauer, Berlin, Germany), was applied. To separate substances, an Agilent C<sub>18</sub> (4.6  $\times$  150 mm, 5  $\mu$ m) column was used.

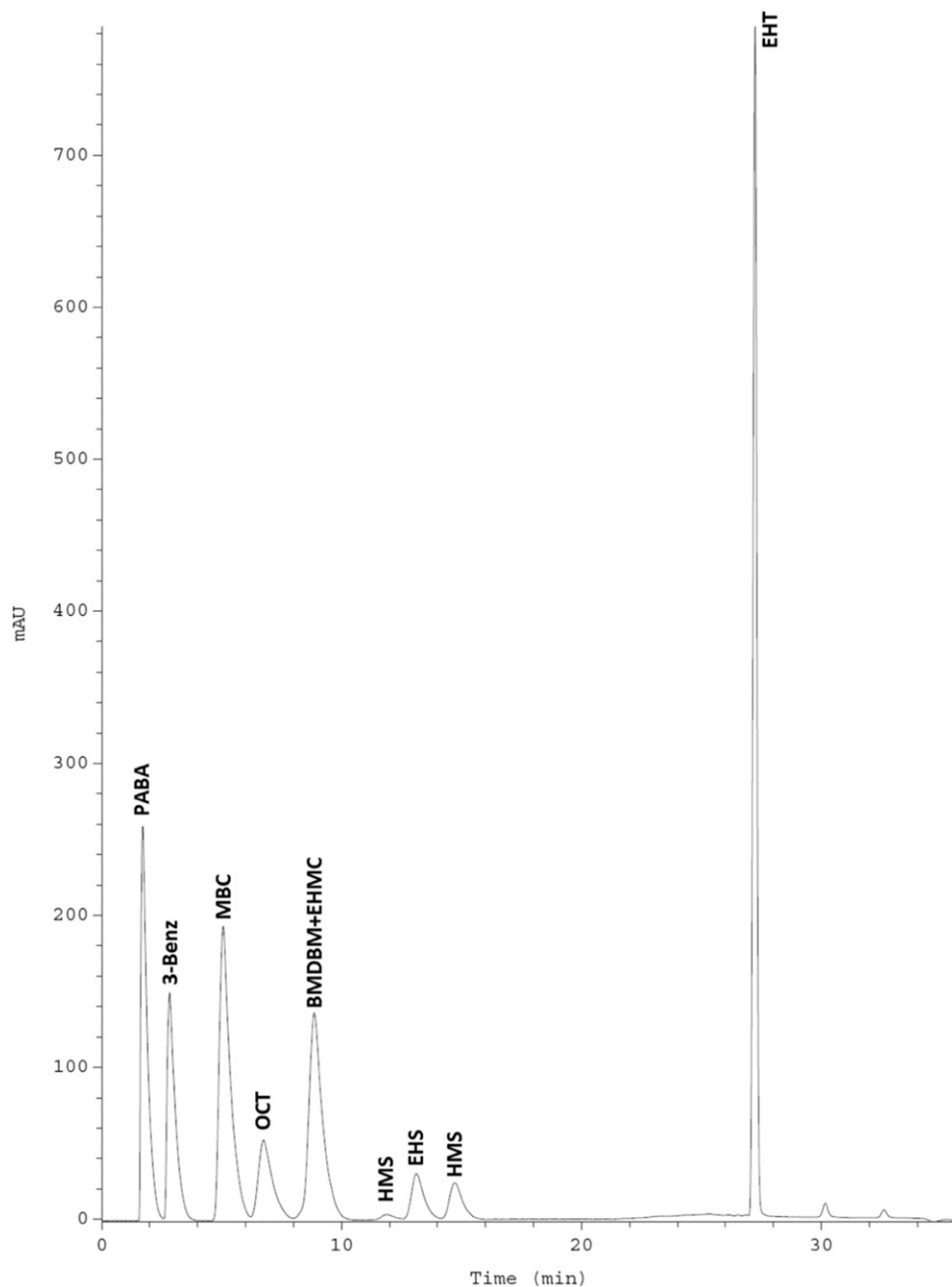


Figure 1. Separation of nine sunscreen agents at 312 nm (3-Benz: 3-benzophenone, OCT: octocrylen).

#### STANDARDS AND REAGENTS

The chemical standards include BMDMB, 3-benzophenone (3-Benz), MBC, octocrylene (OCT), PABA, EHMC, HMS, EHS, and EHT. All were purchased from Sigma-Aldrich (St. Louis, MO). The solvents were prepared by Merck (HPLC-grade high-purity product of Merck, Darmstadt, Germany).



Figure 2. Separation of BMDDBM at 390 nm.

#### STANDARD SOLUTION

The stock solution with 1,000 ppm concentration level of each sunscreen agent was prepared in ethanol daily by dissolving 250 mg of each material in 250 ml ethanol. To draw the calibration curve, seven different concentration levels (1, 2.5, 5, 10, 25, 50, and 100 ppm) were made by diluting the stock solution with ethanol.

**Table II**  
Linear Equation, LOD (ppm), LOQ (ppm), and  $r^2$  for Nine UV Filters

Analyte	Linear range (ppm)	Linear equation	$r^2$	LOD (ppm)	LOQ (ppm)
PABA	1–100	$Y = 1.448X + 0.828$	0.999	1.325	4.016
3-Benz	1–100	$Y = 1.245X + 0.527$	0.999	0.946	2.923
MBC	1–100	$Y = 1.968X + 2.05$	0.999	0.677	2.053
OCT	1–100	$Y = 0.855X + 0.41$	0.999	1.088	3.297
EHMC	1–100	$Y = 1.797X + 0.237$	1	0.439	1.330
HMS	1–100	$Y = 0.307X + 0.291$	0.999	1.481	4.490
EHS	1–100	$Y = 0.353X - 0.007$	0.999	1.322	4.008
EHT	1–100	$Y = 3.201X + 3.115$	0.999	0.813	2.464
BMDBM	1–100	$Y = 1.072X + 0.111$	0.999	0.572	1.733

#### METHOD VALIDATION

To validate the method, the following parameters were evaluated: selectivity, linearity, linear range, inter-assay and intra-assay precision, and recovery. Linearity and linear range were calculated through the calibration curve for which seven concentration levels ranging 1–100 ppm were injected into the device in triplicate analyses.

Intra-assay precision was measured by injecting each of the seven concentration levels into the device three times. Next, the relative standard deviations (RSDs) were computed.

For inter-assay precision, the selected concentration level was injected on three different days.

To obtain the recovery of the method, six various sunscreens with different SPFs were chosen, and 10, 25, and 50 ppm of each standard were added to them. The recovery percentage was reported after data analysis.

#### SAMPLES

Fifty sunscreens with different SPFs were purchased from various manufacturers (32 of them were Iranian and the rest were made in other countries). These creams were chosen from the widely used sunscreens in Iran.

For analysis, 0.1 g of each cream was weighed, mixed, dissolved, and extracted with 100 ml ethanol; then filtered using a 0.22- $\mu$ m syringe filter; and injected into a HPLC system (26).

**Table III**  
Intra-day and Inter-day Precision (RSD, %) of the Developed Method

Analyte	RSD (%) (intra-assay precision)	RSD (%) (inter-assay precision)
PABA	0.39–1.88	0.791
3-Benz	0.34–1.46	0.387
MBC	0.81–2.05	0.425
OCT	0.52–3.86	0.553
EHMC	0.25–2.14	0.935
HMS	0.61–1.73	0.333
EHS	0.27–1.9	0.420
EHT	0.8–2.03	0.224
BMDBM	0.61–1.35	0.683

3-Benz: 3-benzophenone, OCT: octocrylen.

Table IV  
Accuracy Results Obtained through the Recovery Test

Analyte	% Recovery		
	10 (ppm)	25 (ppm)	50 (ppm)
PABA	98.591	100.234	102.560
3-Benz	100.145	99.169	99.672
MBC	97.681	99.783	101.528
OCT	99.966	100.333	100.997
EHMC	102.343	98.925	103.140
HMS	101.531	98.867	103.778
EHS	98.026	100.185	102.485
EHT	101.555	100.413	100.050
BMDBM	100.490	99.467	102.769

#### HPLC-UV ANALYSIS

In the current study, the mobile phase was prepared by adding 0.1 M phosphoric acid to distilled water (solvent A) and ethanol (solvent B) to obtain 1% concentration in each of them. The gradient condition shown in Table I was applied.

## RESULT AND DISCUSSION

#### CHROMATOGRAPHY

The reversed-phase HPLC method was developed for the detection and measurement of the amount of nine different UV filters in creams. Fifty-two creams from diverse brands were screened. Figures 1 and 2 show the chromatographic separation of these materials.

#### LINEARITY AND LINEAR RANGE

To calculate the linearity, nine UV filters that were prepared in seven concentration levels (1, 2.5, 5, 10, 25, 50, and 100 ppm) were injected into the HPLC system. The areas were considered and the calibration curves were drawn. Limit of detection (LOD) and limit of qualification (LOQ) were estimated based on the standard deviation of the response and the slope of the curves. The results are shown in Table II.

#### INTRA-ASSAY AND INTER-ASSAY PRECISION (REPEATABILITY AND REPRODUCIBILITY)

Repeatability of the method was estimated by applying three frequent injections of the mixture of nine UV filters at the different concentration levels (1, 2.5, 5, 10, 25, 50, and 100 ppm) in 1 d. All of the RSDs were less than 4%, which seemed to be appropriate.

Reproducibility was estimated by injecting 50 ppm concentration level in three different days. The upper range of RSD for each of the components was more than 1. The RSD for each component is shown in Table III.

#### RECOVERY

Six different creams were selected from common Iranian sunscreens. For analysis, 0.1 g of each cream was weighed and extracted by ethanol. Then, 10, 25, and 50 ppm of UV-filter

**Table V**  
Results of the Percentage of Each UV Filter in Samples That Was Detected by HPLC

Sample number	PABA (%)	3-Benz (%)	MBC (%)	OCT (%)	EHMC (%)	HMS (%)	EHS (%)	EHT (%)	BMDMB (%)
1	—	—	—	—	0.253	—	0.214	0.124	—
2	—	—	0.293	0.438	0.140	—	0.100	0.806	0.306
3	—	—	—	0.743	0.695	—	0.942	0.199	0.226
4	0.221	—	0.166	—	0.716	—	—	—	0.143
5	—	—	—	—	0.775	—	—	—	0.564
6	—	—	—	—	—	—	—	—	0.231
7	—	—	0.300	—	0.885	—	—	—	0.176
8	—	0.316	0.292	0.170	0.918	—	—	—	0.100
9	—	—	0.264	0.330	0.739	—	—	—	0.114
10	—	—	0.186	—	0.112	—	—	—	0.122
11	—	—	0.244	—	0.113	—	—	—	0.323
12	—	0.343	—	—	0.597	—	—	—	0.164
13	—	0.903	0.412	—	0.954	—	0.526	0.900	—
14	0.819	0.242	—	—	0.986	—	0.519	—	—
15	—	—	—	—	0.356	—	—	—	0.541
16	—	—	—	—	0.452	—	—	4.440	0.541
17	—	—	—	0.594	0.240	—	—	—	—
18	—	—	0.01	0.453	0.147	—	0.223	0.001	0.316
19	—	—	0.005	0.739	0.702	—	0.102	0.008	0.223
20	0.210	—	0.159	—	0.722	—	0.009	0.210	0.137
21	—	—	—	—	0.769	—	—	—	0.563
22	—	—	—	—	0.783	—	—	—	0.245
23	—	—	0.315	—	0.879	—	—	—	0.164
24	—	0.322	0.302	0.001	0.900	—	—	—	0.001
25	—	—	0.259	0.003	0.726	—	—	—	0.010
26	—	—	0.179	—	1.134	—	—	—	0.108
27	—	—	0.243	—	1.129	—	—	—	0.317
28	—	0.373	—	—	0.601	—	—	—	0.158
29	—	0.009	0.150	—	0.523	—	—	—	—
30	0.083	0.237	—	—	1.001	—	0.522	—	—
31	—	—	—	—	0.348	—	—	—	—
32	—	—	—	0.601	0.040	—	—	4.390	0.539
									0.538



Table V  
Continued

Sample number	PABA (%)	3-Benz (%)	MBC (%)	OCT (%)	EHMC (%)	HMS (%)	EHS (%)	EHT (%)	BMDBM (%)
33	—	—	0.452	0.334	—	—	—	0.218	0.611
34	—	—	—	0.449	0.250	—	—	0.296	0.656
35	—	—	—	0.439	0.626	—	—	0.09	0.532
36	—	0.989	—	—	0.07	—	—	0.498	0.524
37	—	—	—	—	0.543	—	—	—	—
38	—	—	0.335	0.524	0.698	—	—	—	0.436
39	—	—	—	0.480	—	—	—	8.3	0.685
40	—	—	—	2.37	—	—	—	—	—
41	—	0.720	0.290	—	0.900	—	—	0.140	0.574
42	—	—	0.449	0.331	—	—	—	0.202	0.624
43	—	—	—	0.453	0.251	—	—	0.302	0.664
44	—	—	—	0.441	0.618	—	—	0.091	0.528
45	—	1.001	—	—	0.081	—	—	0.501	0.517
46	—	—	—	—	0.527	—	—	—	—
47	—	—	0.333	0.517	0.701	—	—	—	0.441
48	—	—	—	0.501	—	—	—	8.26	0.669
49	—	—	—	2.290	—	—	—	—	—
50	—	0.731	0.301	—	0.090	—	—	0.151	0.571
FDA standards [30]	Up to 15%	Up to 6%	Forbidden	Up to 10%	Up to 7.5%	Up to 15%	Up to 5%	Up to 5%	Up to 3%
European standard [31]	Forbidden	Up to 10%	Up to 4%	Up to 10%	Up to 10%	Up to 10%	Up to 5%	Up to 5%	Up to 5%

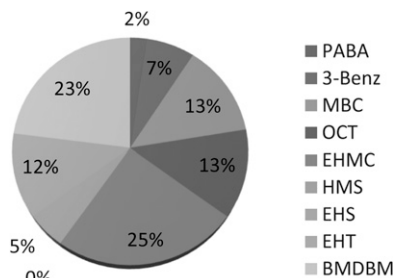


Figure 3. Pie chart displaying the contribution of each value to the total.

standards were spiked to the selected samples. The following formula determines the recovery percentage:

$$\% \text{ recovery} = \left[ \frac{\text{concentration of UV filter in spiked cream} - \text{concentration of UV filter in non-spiked cream}}{\text{concentration of UV filter in spiked cream}} \right] \times 100.$$

The recovery was determined to be 97.681–103.778% (Table IV).

#### SAMPLE ANALYSIS

Method development for the determination of sunscreen agents in sun care products has been a controversial issue. Peruchi and Rath (27) worked to separate and determine the concentration of eight agents in 2011 in Brazil. Chang et al. (26) determined 14 widely

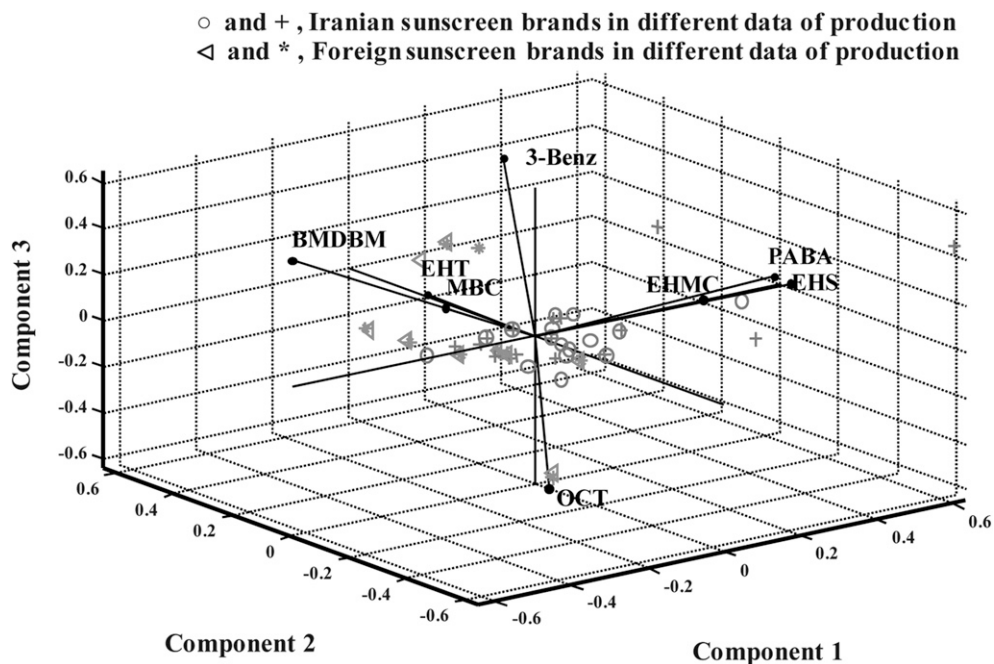


Figure 4. Illustration of the objects (sunscreen brand) and variables (UV-filter components) in biplot. Circle and plus symbol show two different production dates for the Iranian brand. Triangle and star are used for demonstration of different production dates for the foreign brands.

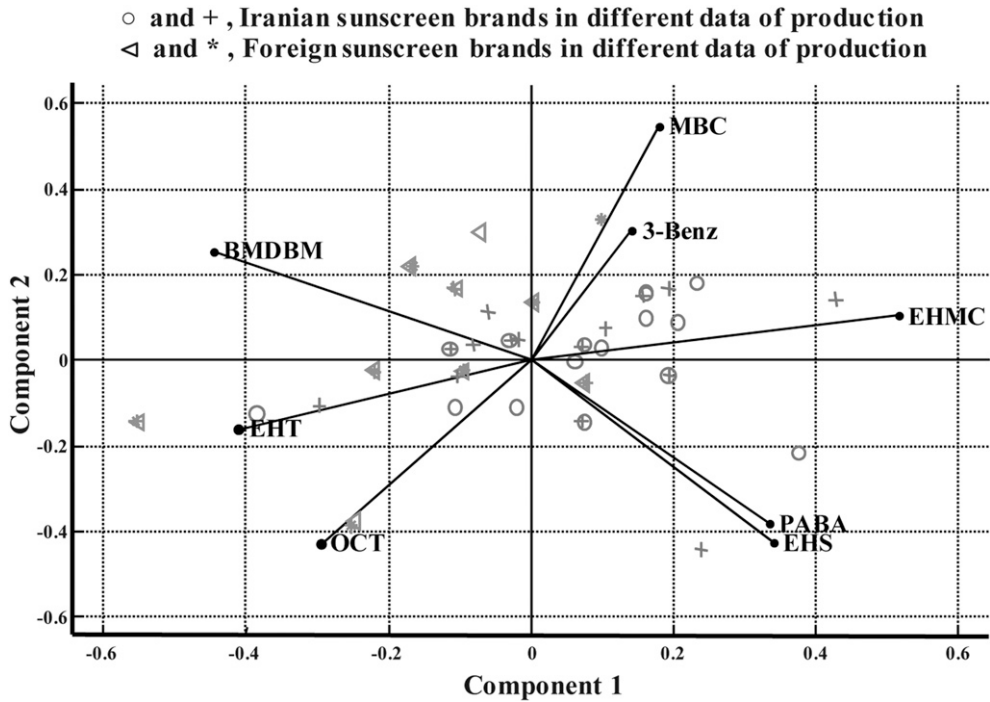


Figure 5. Illustration of the biplot in two-dimensional space. The UV filters EHC and BMDDBM are the mostly used components and make separate groups because of their frequency and total amount. EHS and PABA, MBC and 3-Benz, and OCT and EHT formed different groups.

used sunscreen agents in Korean sun care products using the RP-HPLC method in 2014. Six active agents were studied by Chisvert et al. (28) and Salvador in Spain in 2000.

Twenty-five different brands with various SPFs and series production (50 samples) were purchased from Iranian markets. Samples were prepared by the method mentioned earlier. Results are shown in Table V. The first 32 samples are Iranian and the others are made in other countries.

#### STATISTICAL DATA ANALYSIS

Data analysis was performed by using Matlab (MathWorks, Natick, MA) and Excel (Microsoft Redmond, WA). The results are mentioned in the next paragraph.

According to Table V, all of the UV filters used in sun care products followed the international standards. Only Iranian brands used PABA and EHS. The percentage of MBC in the sunscreens from other countries was more than that in the Iranian ones. The number of creams that had OCT was greater in sunscreens made in countries other than Iran. Figure 3 demonstrates the percentage of samples that used each UV-filter component.

HMS was observed in none of the sunscreens, and most of our products used BMDDBM because of its efficacy. EHC was the most widely used agent.

A comparison between Iranian and other countries' products was performed using a two-sample *t*-test. The results demonstrated significant difference in three of the UV filters (OCT, BMDDBM, and EHC) ( $p < 0.05$ ), but others showed no notable variation.

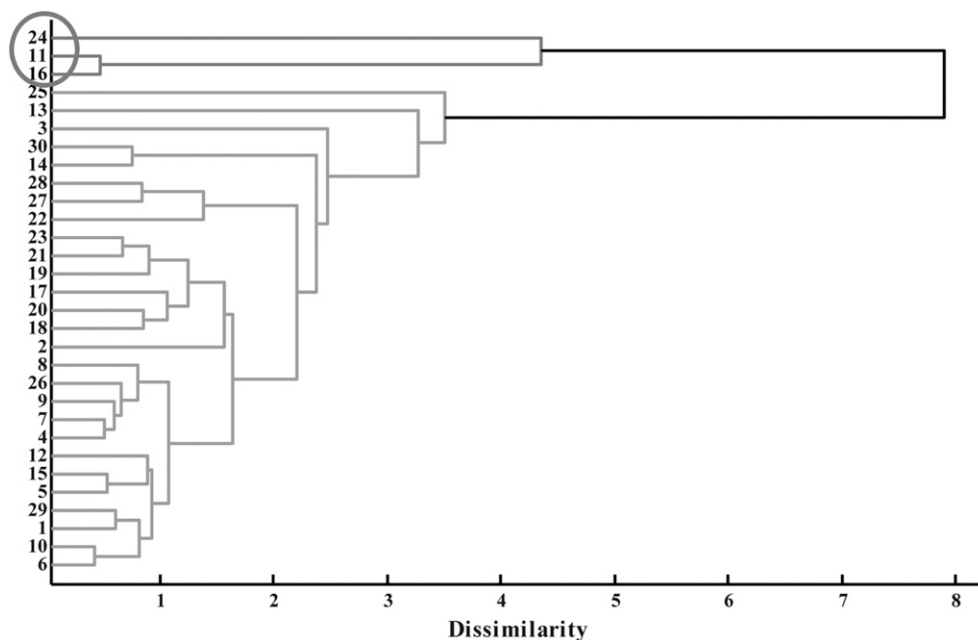


Figure 6. Dendrograms of HCA. Class numbers 11, 16, and 24 belong to sample numbers 16, 32, 39, and 48. In this group, the amount of EHT is more than that in the other group.

#### PRINCIPAL COMPONENT ANALYSIS (PCA)

Also, the PCA was performed with this data set, and the results are shown in Figure 4.

In the original data, the variable “HMS” was omitted because it was not used in any of the samples. As is illustrated in this figure, Iranian and imported brands had different amounts of the mentioned UV filters. As opposed to imported products, Iranian ones showed a diverse amount of UV-filter components in different production dates.

The 2D biplot of the analyzed data is illustrated in Figure 5.

As clearly shown in this figure, UV filters can be divided into five different groups. The division was based on the algebraic sum of each component in all the samples. EHMC and BMDBM are the mostly used components, and they make separate groups because of their frequency and total amount. EHS and PABA, MBC and 3-Benz, and OCT and EHT formed different groups.

#### HIERARCHICAL CLUSTER ANALYSIS (HCA)

HCA is a multivariate analysis technique that is used to sort samples into groups. HCA provides a visual representation of complex data. A method called average linkage was applied between groups, and cityblock was selected as a measurement. The results of the HCA are shown in Figure 6.

The results of the HCA showed that the samples could be divided into two quality clusters. Samples 16, 32, 39, and 48 were categorized into Cluster I and the rest into Cluster II. It was determined that Cluster I contains more of EHT than Cluster II.

On the other hand, samples with the same total amount of UV filters can be seen in the same groups.

## CONCLUSION

A RP-HPLC method was developed to determine nine UV-filter agents commonly used in Iran. The method was simple, practical, reliable, and sensitive due to validation parameters. Iranian sun care products were compared with those of other countries, and with FDA and European standards. The method seems to be an appropriate one that can be used in laboratories to determine and evaluate organic sunscreen materials.

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