

## DETERMINATION OF THREE ULTRAVIOLET FILTERS IN SUNSCREEN FORMULATIONS AND FROM SKIN PENETRATION STUDIES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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An analytical procedure to quantify 3-benzophenone, octylmethoxycinnamate and octylsalicylate was validated and employed to assess these ultraviolet filters in sunscreen formulations and from skin penetration studies. The effect of the vehicle on the skin retention of these filters was investigated. HPLC and extraction procedure were found to be reliable when obtaining data for the sunscreen formulations and for evaluation skin penetration. The results demonstrated that a cream gel generated higher epidermal concentrations of these filters than a lotion or cream-based formulation. Additionally, when comparing the skin retentions of each filter using the same formulation, 3-benzophenone showed the highest skin retention.

Keywords: 3-benzophenone; octylmethoxycinnamate; octylsalicylate.

### INTRODUCTION

Oxidative stress and inflammatory responses induced by ultraviolet (UV) radiation can cause a variety of harmful effects in skin, including premature photoaging and the induction of immunosuppression and skin carcinogenesis.<sup>1-3</sup>

To limit sun exposure, the government advises one to wear loose fitting, tightly woven clothing, to stay in the shade between 11 a.m. and 3 p.m. and to use a sunscreen with a sun protection factor (SPF) of 15 or higher liberally reapplying every 2 h or after working, swimming, playing or exercising outdoors.<sup>4,5</sup>

The necessity to provide high SPF and screening efficiency against both ultraviolet A (UVA) and ultraviolet B (UVB) wavelengths has led to the development of sunscreen formulations with multiple added sunscreen chemicals.<sup>6</sup>

Of the approved sunscreen chemicals, 3-benzophenone (3-BZ), octylmethoxycinnamate (OMC), and octylsalicylate (OS) are some of the most common active ingredients used in sunscreen formulations.<sup>7</sup> Benzophenones efficiently absorb both UVA and UVB rays, and 3-BZ is the most commonly used in sunscreen formulations. Among the cinnamates, OMC is the most widely used UVB filter in the world. It is often used in combination with other filters to achieve a high SPF in the final product. Salicylates, such as OS, absorb UVB radiation, are very stable, and are insoluble in water. They are commonly used to improve the substantivity of the formulation and reduce the photodegradation of some sunscreens.<sup>8</sup>

When formulating sunscreens, the sunscreen agents should remain on the surface of the skin, accumulate in the stratum corneum, and create a barrier against UV radiation without transdermally penetrating systemic circulation.<sup>9</sup> Unfortunately, several studies have demonstrated that some UV filters, such as 3-BZ, OMC, and OS, can penetrate the epidermis.<sup>7,10-13</sup> Additionally, recent reports

have shown that when the filters, 3-BZ, OMC and octocrylene, penetrate into these nucleated layers, the levels of reactive oxygen species produced naturally by the epidermal chromophores under UV illumination increase.<sup>14</sup>

It is important to consider that sunscreen formulations are applied to a large area of the body and for long periods of time, producing a constant and high input of the chemical into the viable skin layer and into systemic circulation.<sup>3</sup> The development of formulations and the synthesis of new sunscreen molecules with good substantivity have been of great interest to decrease absorption and to improve the safety and effectiveness of the topical sunscreen formulations.<sup>15-17</sup>

In this context, it is of great importance to develop adequate, reliable and sensitive new techniques to extract and quantify the sunscreens in the formulations and from the skin samples. These techniques can then aid in the safety of sunscreen formulations because studies have also demonstrated that UV filters can be found in the deeper layers of the skin, in urine, in plasma, and even in breastmilk.<sup>7,10-13,18</sup>

First in this study, a reverse phase-high-performance liquid chromatography (RP-HPLC) technique and an extraction procedure for simultaneous determination of the three UV filters in the sunscreen formulations, and from skin samples were developed and validated. Second, the applicability of this method for determining skin penetration was investigated. Last, the effects of the three different sunscreen formulations were investigated by testing the skin penetration of 3-BZ, OMC, and OS *in vitro*.

### EXPERIMENTAL

#### Reagents

3-benzophenone (99.9%, Eusolex® 4360), octylmethoxycinnamate (99.9%, Eusolex® 2292), and octylsalicylate (99.8%, Eusolex® OS) were purchased from Merck (Darmstadt, Germany). Acetic acid (chromatographic grade) was supplied by Merck (Darmstadt, Germany) and methyl

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alcohol (MeOH) for use in the chromatograph was purchased from J. T. Baker (USA). The water used to prepare the solutions and the mobile phase was purified by a Milli-Q-plus System (Millipore®, Bedford, MA, USA). Ethyl alcohol was supplied from Synth (Sao Paulo, Brazil). All of the raw materials used for the formulations were purchased from Galena (Campinas, SP, Brazil) or Clariant (Sao Paulo, SP, Brazil). All other chemicals were of reagent grade and were used without further purification.

### Sunscreen formulations

The study was performed using 3 model formulations of sunscreen: cream, lotion and cream gel, each containing a mixture of the 3 organic UV filters.

3-BZ (4.0%, w/w), OMC (7.5%, w/w), and OS (5.0%, w/w) were incorporated into the formulations at room temperature 24 h after their preparation, and the pH was adjusted to 6.0 using aminomethylpropanol. 3-BZ was first solubilized in propylene glycol and was then incorporated into the formulations. Blank formulations were prepared containing all of the components without the UV filters. The percent composition of each formulation is described in Table 1.

**Table 1.** Percent composition (w/w) of the formulations

Component	Cream	Lotion	Cream gel
Polawax <sup>a</sup>	3.0	1.0	1.5
Carbopol <sup>®</sup> 940 <sup>b</sup>	-	-	0.5
Cetyl alcohol	3.5	1.5	1.75
Stearic acid	4.0	1.5	2.0
Glyceril monostearate	5.5	3.5	2.75
Propylparaben	0.05	0.025	0.025
Methylparaben	0.05	0.025	0.175
EDTA	0.15	0.15	0.075
Propylene glycol	15.0	10.0	10.0
Distilled water	68.75	82.3	81.23

<sup>a</sup>Self-emulsifying wax (cetostearyl alcohol and polyoxyethylene derived from fatty acid ester sorbitan 2OE). <sup>b</sup>Anionic hydrophilic colloid (carboxy-polymethylene).

### Determination of 3-benzophenone, octylmethoxycinnamate and octylsalicylate by HPLC

#### Chromatographic conditions

The presence of the UV filters 3-BZ, OMC, and OS, was determined using a Shimadzu (Kyoto, Japan) liquid chromatograph system equipped with an LC-10 AT VP solvent pump unit and an SPD-10A VP UV-Visible detector operating at 305 nm. Separation was performed on a Supelcosil<sup>™</sup> LC-18 (25 cm x 4.6 mm, 5 µm) column equipped with a C-18 (4 x 4 mm, 5 µm, Merck) precolumn. This procedure was adapted from previously described techniques.<sup>19,20</sup>

The mobile phase was a MeOH/water (84:16, v/v) mixture containing 0.1% (v/v) acetic acid. The flow rate was 1 mLmin<sup>-1</sup>, and the system was run at room temperature. Data were collected using a Chromatopac C-R6A integrator (Shimadzu, Kyoto, Japan). All solutions and solvents were filtered through a Millipore<sup>®</sup> filter membrane (pore size = 0.45 µm) and were vacuum degassed by sonication before use.

#### Standard solution

Standard solutions of the UV filters were prepared daily. The individual solutions of all of the UV filters were prepared by dis-

solving 0.025 g of samples in 25 mL of mobile phase followed by ultrasonication at room temperature for 15 min. This solution was further diluted (10x) in a mobile phase. Standard solutions with concentrations of at 4 µg/mL for 3-BZ, 7.5 µg/mL for OMC, and 5 µg/mL for OS were prepared. All samples were quantified by comparing the peak areas of the samples with the peak areas of the reference substances present in the standard solution.

### Method validation

Validation was performed following the ICH guidelines.<sup>21</sup> The method was validated considering the parameters of linearity, accuracy, precision, specificity and the limits of detection and quantitation.

Linearity was tested using the standard solutions of 3-BZ, OMC, and OS. Calibration curves were prepared using 5 different concentrations of each of the UV filters (2.5-15.0 µg/mL) and were analyzed by the linear regression of the peak areas versus the UV filter concentrations.

Precision was expressed as a relative standard deviation (RSD%) and was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by the analysis of six replicates of each experiment during the same day and under the same experimental conditions. The intermediate precision was tested by assaying six replicates on three different days. The concentrations used were 4.0 µg/mL for 3-BZ, 7.5 µg/mL for OMC, and 5.0 µg/mL for OS.

Accuracy was determined based on recovery tests performed by adding known amounts of the UV filters to blank formulations. Samples of 0.05 g of the formulations containing all three UV filters were dissolved into 50 mL of ethanol. For the preparation of samples of the low, medium and high concentration, 0.8, 1.0, and 1.2 mL, respectively, of these solutions were diluted with mobile phase in a 10-mL volumetric flask. Each of the solutions were filtered and injected into the HPLC system. Accuracy was expressed as a percent of recovery, which was estimated from the relationship between the experimental and theoretical concentrations ( $(C_p/C_t) \times 100$ ).

The detection limit (LD) and quantitation limit (LQ) were determined from the standard deviation of the response and from the slope of the constructed calibration curve. The LD was expressed as  $(3.3 \times \sigma)/S$  and, the LQ was expressed as  $(10 \times \sigma)/S$ , where  $\sigma$  is the standard deviation of the response and  $S$  is the slope of calibration curve.

The specificity was determined by comparing the results obtained by the analyses of the standard solution, of the blank formulation and of the formulations containing the three UV filters.

### Applicability of the technique

In this study, the applicability of the HPLC technique for the analysis of the *in vitro* retention studies of the topical formulations spiked with 3-BZ, OMC, and OS was investigated by treating the pig ear skin sections (area of 1.77 cm<sup>2</sup>) with methanolic solutions of the UV filters, prepared as described below. These methanolic solutions consisted of 0.5, 0.1 or 0.02% of the UV filters contained in 300 mg of the formulation in every 100 µL of sample. The treated skin sections were allowed to rest for 30 min before they were submitted to the extraction process (procedure described below). The UV filters extracted from these skin sections were quantified by HPLC, and the pig skin sections without addition of any solution were also analyzed.

### In vitro skin penetration

To conduct this study the skin from the outer surface of an excised porcine ear was used. Pig ears were obtained within 2 h after the

slaughter of the animals. The whole skin membrane was carefully removed from the underlying cartilage with a scalpel. Additionally, the subcutaneous tissues were removed, and the skin was stored at  $-20\text{ }^{\circ}\text{C}$  for a maximum period of 30 d before use.<sup>22,23</sup>

A Franz diffusion cell (diffusion areas of  $1.77\text{ cm}^2$ ) was used to perform the study, with the stratum corneum facing the donor compartment (where the formulation was applied) and the dermis facing the receptor compartment. The receptor chamber contained 150 mM of phosphate buffer (pH 7.2) in polyoxyethylene (20) sorbitan monolaurate (Tween 20<sup>®</sup>) (0.5%, v/v), and the fluid was maintained under constant stirring and temperature ( $37 \pm 0.5\text{ }^{\circ}\text{C}$ ).<sup>24</sup>

The formulations cream, lotion, and cream gel, containing a UV filters and unloaded formulations (300 mg of each) were applied to the surface of the stratum corneum, while avoiding light exposure. Six hours post-application of the formulations, the skin surfaces were wiped with a cotton swab to remove any excess formulation. To separate the stratum corneum from the remaining epidermis and dermis, the skin sections were subjected to tape stripping with 15 pieces of adhesive tape.

The remaining epidermis and dermis were cut in small pieces, bath sonicated for 30 min in 2.5 mL of MeOH, vortex mixed for 1 min and centrifuged for 15 min at 15000 rpm. The supernatant was transferred to a 5 mL volumetric flask. To the remaining precipitate, 2.5 mL of methanol was added, and the extraction procedure was repeated. The supernatant of the second extraction was added to the previous sample in the volumetric flask, and these samples were diluted with methanol to fill the flask. The resulting mixture was filtered using a  $0.45\text{-}\mu\text{m}$  membrane, and the sunscreens were assayed by HPLC, as described previously.

### Statistical analysis

Data were statistically analyzed by one-way ANOVA, followed by Bonferroni's multiple comparison t-tests. The level of significance was set to a p-value  $< 0.05$ .

## RESULTS

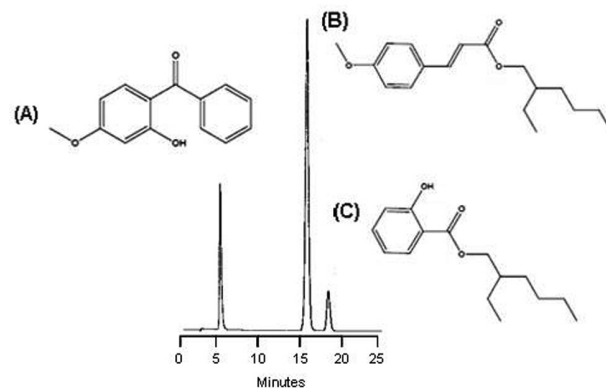
### Determination of 3-benzophenone, octylmethoxycinnamate and octylsalicylate by HPLC

The chromatographic analysis for the quantitative determination of the UV filters was validated according to ICH guidelines<sup>21</sup> to obtain reproducible analyses with high accuracy and precision in the range of the concentrations investigated.

The proposed HPLC technique enabled the separation and quantitative determination of the three UV filters present in the sunscreen formulations and from the porcine skin samples. Isocratic elution with MeOH/water (84:16, v/v) containing 0.1% (v/v) acetic acid was found to be simple in comparison to other studies that used gradient elution techniques.<sup>7</sup> As demonstrated in Figure 1, 3-BZ, OMC, and OS had retention times of approximately 6, 17, and 19 min, respectively, allowing for the rapid determination of these UV filters, which is essential for routine analysis.

Furthermore, the specificity study showed that the blank formulations did not display interference with regard to the chromatographic characteristics. Additionally, in the presence of the different formulation components, the chromatographic profiles of 3-BZ, OMC, and OS (retention times and areas) were not affected (data not shown).

The calibration curves were linear within the concentration range of 2.5-15  $\mu\text{g/mL}$  for each of the UV filters, and the regression analyses gave the following representative linear equations:  $y = 41490x - 13080$  for 3-BZ,  $y = 60800x + 11150$  for OMC, and  $y = 15350x - 3130$  for OS. The correlation coefficients (r) were all above 0.999.



**Figure 1.** Chromatogram of the standard solution for (A) 3-benzophenone ( $4.0\text{ }\mu\text{g/mL}$ ), (B) octylmethoxycinnamate ( $7.5\text{ }\mu\text{g/mL}$ ), and (C) octylsalicylate ( $5.0\text{ }\mu\text{g/mL}$ )

The detection limits of 3-BZ, OMC, and OS were 0.36, 0.38, and  $0.27\text{ }\mu\text{g/mL}$ , respectively, and the quantitation limits were 1.12, 1.15, and  $0.81\text{ }\mu\text{g/mL}$ , respectively.

The precision of the method was determined by repeatability (intra-assay) and intermediate precision (inter-assay), and is shown as the relative standard deviation (R.S.D%). The repeatability and intermediate precision for the three sunscreens were all less than 5% which can be considered satisfactory for the purpose of this analysis (Table 2).

**Table 2.** Precision of the HPLC technique for the quantification of the UV filters in the sunscreen formulations

	Cream gel	Cream	Lotion
Inter-day variation <sup>a</sup>	RSD (%)	RSD (%)	RSD%
3-BZ ( $4\text{ }\mu\text{g/mL}$ )	2.92	3.15	4.23
OMC ( $7.5\text{ }\mu\text{g/mL}$ )	3.53	2.39	3.51
OS ( $5\text{ }\mu\text{g/mL}$ )	3.03	2.16	3.28
Intra-day variation <sup>b</sup>			
3-BZ ( $4\text{ }\mu\text{g/mL}$ )	2.44	2.55	2.31
OMC ( $7.5\text{ }\mu\text{g/mL}$ )	1.88	2.31	2.61
OS ( $5\text{ }\mu\text{g/mL}$ )	2.47	1.69	2.66

The results are presented as the mean  $\pm$  the standard deviation (SD). <sup>a</sup>Six replicates were assayed on three different days. <sup>b</sup>Six replicates were assayed on the same day.

The accuracy for the detection of the UV filters in the formulations varied from 88.3-101.1% for the cream, 87.1-106.2% for the lotion, and 87.3-100.1% for the cream gel (Table 3). These results can be accepted due to the complexity of the cosmetic formulation determination.<sup>25</sup>

### Applicability of the technique

The recoveries of 3-BZ, OMC, and OS from the skin samples were all above 70% (Table 4). Therefore, the combined procedures for the extraction and HPLC assaying of these sunscreen agents in the skin samples are efficient in terms of recovery and fast manipulation.

### In vitro skin penetration

As demonstrated by Figure 2 and Table 5, 3-BZ, OMC, and OS showed a potential for skin penetration in the cream, lotion, and cream gel formulations.

**Table 3.** Accuracy of the HPLC technique for the quantification of the UV filters in the sunscreen formulations

Theoretical concentration ( $\mu\text{g/mL}$ )	Cream gel		Cream		Lotion	
	Experimental concentration ( $\mu\text{g/mL}$ )	Recovery (%)	Experimental concentration ( $\mu\text{g/mL}$ )	Recovery (%)	Experimental concentration ( $\mu\text{g/mL}$ )	Recovery (%)
3-BZ						
3.2	$3.03 \pm 0.11$	94.05	$3.09 \pm 0.03$	95.37	$3.06 \pm 0.01$	94.43
4.0	$3.79 \pm 0.01$	94.13	$3.94 \pm 0.03$	97.17	$3.75 \pm 0.03$	92.55
4.8	$4.51 \pm 0.05$	93.44	$4.73 \pm 0.06$	97.18	$4.53 \pm 0.04$	93.37
OMC						
6.0	$6.04 \pm 0.21$	100.11	$6.11 \pm 0.11$	100.33	$6.46 \pm 0.09$	106.23
7.6	$7.48 \pm 0.08$	99.16	$7.66 \pm 0.02$	100.66	$7.81 \pm 0.05$	102.75
9.1	$8.86 \pm 0.04$	99.00	$9.23 \pm 0.20$	101.06	$9.44 \pm 0.11$	103.43
OS						
4.1	$3.82 \pm 0.15$	92.84	$3.64 \pm 0.04$	88.29	$3.62 \pm 0.01$	87.93
5.1	$4.52 \pm 0.01$	87.89	$4.64 \pm 0.04$	90.07	$4.45 \pm 0.01$	87.14
6.1	$5.39 \pm 0.06$	87.26	$5.59 \pm 0.07$	90.4	$5.39 \pm 0.05$	87.93

The results are the mean  $\pm$  SD of three experiments.

**Table 4.** Recoveries of 3-benzophenone, octylmethoxycinnamate, and octylsalicylate extracted from the viable epidermis and dermis

	Theoretical concentration ( $\mu\text{g}$ ) <sup>a</sup>	Experimental concentration ( $\mu\text{g}$ )	Recovery (%)
3-BZ	2.4	$1.76 \pm 0.03$	73.32
	12.0	$9.51 \pm 0.39$	79.19
	60.0	$44.26 \pm 2.00$	73.72
OMC	4.5	$4.08 \pm 0.11$	90.68
	22.5	$19.60 \pm 0.66$	87.03
	112.5	$111.82 \pm 5.82$	99.33
OS	3.0	$2.95 \pm 0.24$	98.41
	15.0	$11.22 \pm 0.55$	74.77
	75.0	$53.15 \pm 1.62$	70.81

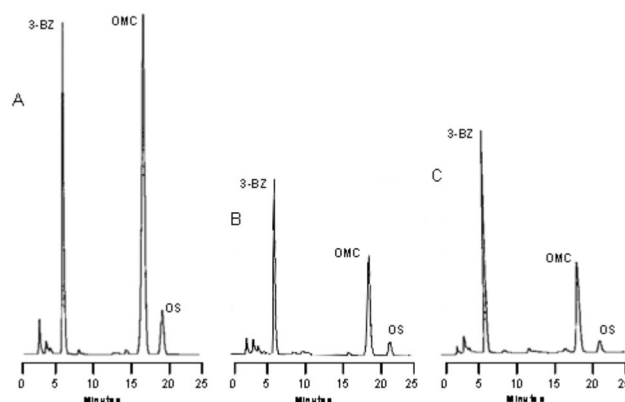
<sup>a</sup>3-BZ, OMC, and OS, each in a 100  $\mu\text{L}$  methanolic solution added to the skin samples. The results are the mean  $\pm$  SD of three experiments.

The cream gel formulation provided the highest retention of the sunscreens in the epidermis and dermis, while the skin retentions of the cream and lotion emulsions were not significantly different. The skin retention of 3-BZ in the cream gel was 1.9- and 1.8-fold higher than in the cream and lotion, respectively. The skin retention of OMC when the carrier was a cream gel was 2.86 and 3.66 higher than when the cream and lotion, respectively, were used. Finally, the skin retention of OS in the cream gel was 2.53 and 3.22 higher than in the cream and lotion formulations, respectively.

## DISCUSSION

Considering the great necessity for the development of adequate, reliable and sensitive techniques to extract and quantify the sunscreens in the formulations and from the skin samples as well as to ensure the safety of the sunscreen formulations, the present study aimed to develop and validate a RP-HPLC technique for the analysis of three UV filters found in sunscreen formulations and skin samples. In addition, the *in vitro* skin penetration effects of three different sunscreen formulations containing UV filters were investigated.

The values obtained for linearity, precision, accuracy, detection limit and quantitation limit were in accordance with the ICH guidelines,<sup>21</sup> which indicate that the chromatographic conditions used are reliable to quantify 3-BZ, OMC, and OS in the range evaluated.

**Figure 2.** HPLC chromatograms of the skin retentions for the three sunscreen formulations cream gel (A), cream (B), and lotion (C) containing the UV filters

Moreover, no interference from the formulations and the extracted skin endogenous compounds were observed, indicating the applicability of this technique with the *in vitro* retention studies of the topical formulations containing the UV filters.

Considering the significant difference between the retention values of the sunscreen incorporated into the cream gel in comparison with the other formulations, it might be suggested that the affinities of the sunscreens to the vehicles differed. The cream and lotion formulations have higher oil contents than the cream gel; therefore, the UV filters, which have high lipophilic characteristics, most likely remain in the cream and lotion formulations. Thus, the UV filter in these formulations would not as readily penetrate the skin.

Studies have shown that emulsion-gel provides greater retention of 3-BZ, OMC, and OS in the epidermis compared to the petroleum jelly.<sup>13</sup> Additionally, the penetrations and stratum corneum retentions of the UV filters are formulation dependent.<sup>10</sup> Therefore, corroborating with Treffel and Gabard<sup>13</sup> and Gupta *et al.*,<sup>10</sup> the formulation-dependent skin penetrations of the investigated UV filters were detected in the present study. Also for these reasons, the vehicle should be carefully chosen to prevent the skin penetration of the cosmetic formulations containing the sunscreen agents.

In comparing the skin retentions of each UV filter in the same formulation, expressed as percentages of the applied doses, 3-BZ showed higher skin retention than the other two filters. This difference in the 3-BZ retention, when compared to those of OMC

**Table 5.** Amount of UV filters retained in the epidermis and dermis for the three formulations after 6

	Cream gel		Cream		Lotion	
	Retention <sup>a</sup>	Retention <sup>b</sup>	Retention <sup>a</sup>	Retention <sup>b</sup>	Retention <sup>a</sup>	Retention <sup>b</sup>
3-BZ	27.75 ± 5.97*	0.41 ± 0.09*	14.84 ± 1.76	0.22 ± 0.03	15.83 ± 1.73	0.23 ± 0.02
OMC	32.70 ± 9.91*	0.26 ± 0.07*	11.40 ± 3.36	0.09 ± 0.02	8.93 ± 3.96	0.07 ± 0.03
OS	18.33 ± 5.85*	0.22 ± 0.06*	7.24 ± 1.86	0.08 ± 0.02	5.70 ± 1.95	0.07 ± 0.02

<sup>a</sup>The UV filter retentions are expressed as µg/cm<sup>2</sup>. <sup>b</sup>The UV filter retentions are expressed as percentages of the applied doses. Results are represented as means ± SD (n = 6). Statistical analysis was performed by a one-way ANOVA followed by a Bonferroni's test of the multiple comparisons. \*Significant statistical difference compared to the cream and lotion formulations (p < 0.05).

and OS, was approximately 1.7-, 2.6-, and 3.3-fold higher in the cream gel, cream, and lotion formulations, respectively. These results may be attributed to the different physical characteristics and chemical properties of each UV filter; these properties affect the penetrability of the compounds into the stratum corneum and into the deeper layers of skin.

3-BZ is characterized by a relatively low molecular weight (228.25) and a log P of 3.58<sup>13</sup> which suggest that 3-BZ has a good ability to penetrate the skin.<sup>26</sup> Comparatively, the oil-water partition coefficients (octanol/water partition) for OMC and OS are 5.96 and 6.02, respectively, indicating they are both highly lipophilic. Due to these high lipophilicities, it is likely that the compounds are capable of accumulating and forming reservoirs within the lipid phases of the stratum corneum. Additionally, these agents would have difficulty penetrating the viable epidermis because of the hydrophilic nature of this layer.<sup>27</sup> In other words, these characteristics explain the superior penetrating capability of 3-BZ into the skin when compared to OMC and OS. Furthermore, Gupta *et al.*<sup>10</sup> showed that 3-BZ presented a higher penetration into the viable epidermis and dermis, whereas the OMC showed better retention in the stratum corneum.

## CONCLUSION

The results demonstrate that the proposed chromatographic technique is a useful and reliable tool for the analysis of sunscreens in formulations and from skin samples. It not only provided the detection and quantification of the microquantities in the samples, but it also eliminated most of the interference problems caused by the formulations and the skin in the samples. In other words, this technique should enable the development of studies and quality controls of the sunscreen formulations.

Furthermore, cream gel formulation generated the highest epidermal concentration of the studied UV filters, and by comparing the skin retention amounts of each sunscreen in the same formulation, 3-BZ showed the highest skin retention ability. Therefore, formulations must be developed to minimize the penetration of these UV filters into the deeper layers of skin, and they must take into account the physical and chemical properties of the sunscreen formulation components and of the UV filters. Additionally, it is indispensable that penetration studies of the sunscreens into the skin be developed to ensure the safety of the sunscreen formulations.

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