Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **32** (5): (2013)

Regular article
Received: February 13, 2013
Revised version: April 12, 2013
Accepted: April 14, 2013

Evaluation of Thermal Stability and Sun Protector Factor values *In Vitro* in O/W Emulsions Containing Benzophenone-3 and Avobenzone

Rita CERESOLE, Marta D. ASERO, Yong K. HAN, María A. ROSASCO & Adriana I. SEGALL*

Cátedra de Calidad de Medicamentos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina

***SUMMARY*. The purpose of this work is to study the thermal stability of benzophenone-3 and avobenzone in the presence and in the absence of TiO_2, in O/W emulsions for sunscreens products. The emulsions were stored in glass containers at 40 °C and 75 % RH and at room temperature. Concomitantly the Sun Protector Factor (SPF) value was evaluated *in vitro*. These formulations seem to be more stable in the presence of diethylhexyl syringylidene malonate and titanium dioxide and silica. Avobenzone and benzophenone-3 seem to be less stable in the presence of caprylic capric triglyceride though this excipient increases the SPF *in vitro* value. Thus, the aim of this study was to evaluate the thermal stability by HPLC and the efficacy of formulations containing chemical filters, a physical filter and an antioxidant measuring the SPF *in vitro* value.**

INTRODUCTION

Ultraviolet radiation (UVR) from the sun is divided into UVC (short wave UV, 270-290 nm), UVB (Middle wave UV 290-320 nm), and UVA (long wave UV) which is subdivided into UVA2 (320-340 nm) and UVA1 (340-400 nm). UVC emitted by the sun is filtered by ozone in the stratosphere; therefore, it does not reach the earth´s surface. The amount of solar UVB and UVA reaching the earth's surface is affected by latitude, altitude, season, time of day, cloudiness, and ozone layer. Acute response of human skin to UVB irradiation includes erythema, edema, and pigment darkening followed by delayed tanning, thickening of the epidermis and dermis, and synthesis of vitamin D; chronic UVB effects are photoaging, immunosupression, and photocarcinogenesis. UVA, compared with UVB, can penetrate deeper through the skin, and is not filtered by window glass. It has been estimated that approximately 50% of exposure to UVA occurs in the shade [1-8].

To minimize the deleterious effects of UVR, appropriate clothing, wide-brimmed hat, sunglasses and broad-spectrum sunscreen are recommended. Sunscreens typically contain "chemical filters", that are organic compounds that absorb strongly in the UV (most often, UVB) and physical filters, such as TiO_2 and ZnO that block UVB and UVA sunlight through reflection and scattering; however, they also absorb significant UV Radiation [9]. To remain fully efficient during the exposure period, a UV filter must be resistant to heat and sunlight. For a high UVB and UVA protector factor, sunscreens often contain a physical filter and at least 2 organic filters, one with optimal screening for UVB wavelength and the other for UVA photons [2].

Oxybenzone, benzophenone-3 (Fig. 1A) is the most commonly used benzophenone. It absorbs most efficiently in UVB and UVA2 range with two absorption peaks (λ maximum, 288 and 325 nm). Although it is a broad-spectrum UVA filter, it is photolabile and can oxidize rapidly; its oxidation will inactivate the antioxidant systems 6. Butyl methoxydibenzoylmethane, avobenzone, (Figure 1B) has strong absorption in the UVA1 range (λ maximum to

KEY WORDS: Avobenzone, Benzophenone-3, Stability, FPS

* Author to whom correspondence be addressed. *E-mail:* asegall@ffyb.uba.ar

ISSN 0326-2383

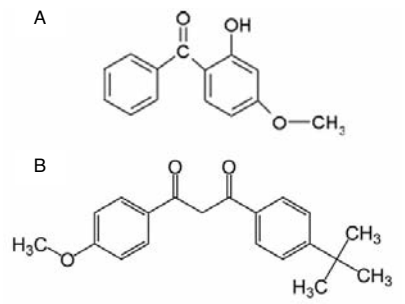

Figure 1. Chemical structure of benzophenone-3 (**A**) and avobenzone (**B**).

380 nm). Unfortunately, it has been shown that its photoprotective capacity decreased by 50% to 60% after 1 h of exposure to sunlight.

As a part of the ongoing project on the development of formulations containing benzophenone-3 and avobenzone, techniques of thermal analysis (DSC) and isothermal stress testing (IST) were utilized for drug-excipient compatibility testing. In the first phase of the study DSC was used as a tool to detect any interaction. Use of DSC has been proposed as a rapid method for evaluating the physico-chemical interaction between two components. Based on the DSC results alone, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, butylated hydroxytoluene, ascorbyl palmitate, non ionic self emulsifying wax, anionic self emulsifying wax, cetostearyl alcohol, propylene glycol, isopropyl myristate and avobenzone were found to exhibit interaction with benzophenone-3. Stressed binary mixtures (stored at 40 °C for 10 days) of benzophenone-3 and excipients were evaluated by HPLC. The results obtained shown that benzophenone-3 was compatible with the tested excipients [10].

In the second phase of the study, interaction of avobenzone with excipients was studied. Based on DSC, IST and FT-IR results, avobenzone is incompatible with caprylic capric triglyceride, propylparaben and butylated hydroxytoluene [11].

Literature survey revealed investigations of the stability of organic filters in the presence of TiO_2 [9,12]. Actually, our Health Authorities indicates that SPF (Sun Protector Factor, UVB radiation) should be determinate with FDA or COLIPA methodologies [13,14]. These are *in vivo* determinations. In this work, we used an *in vitro* methodology, as evaluation technique. The SPF-290S is a recording UV spectrophotometer designed and optimized for the determination of SPF *in vitro* values on a variety of sunscreen and cosmetic products reducing the need and cost for *in vivo* testing [15].

The purpose of this work is to study the stability of benzophenone-3 and avobenzone in the presence and in the absence of TiO_2, in O/W emulsions for sunscreens products stored in glass containers at 40 °C and 75 % RH and at room temperature, and the evaluation of the SPF *in vitro* value in these conditions.

MATERIALS AND METHODS

Materials and reagents

Avobenzone (98.3) and benzophenone-3 (100.3%) was received as a gift sample from Merck Química Argentina (Merck, Germany). The rest of chemical and excipients were purchased from commercial sources: paraffinum liquidum (R.A.A.M., Argentina), acetylated lanolin (Acelan L, Fabriquímica, Argentina), cetearyl alcohol & sodium lauryl sulfate and sodium cetearyl sulphate (Flamacer SX, Flamaquímica, Argentina), imidazolidinyl urea (Bioflama 115, Flamaquímica, Argentina), disodium EDTA (Merck, Germany), caprylic capric triglyceride (Flamacer CC, Flamaquímica, Argentina), titanium dioxide and silica (Eusolex T-AVO, Merck, Germany), and diethylhexyl syringylidene malonate (Oxynex ST, Merck, Germany). All chemicals used were of analytical grade. Methanol and water were of HPLC grade. Solvents were filtered through a 0.45 µm membrane and degassed.

Preparation of the emulsions

Cetearyl alcohol & sodium lauryl sulfate & sodium cetearyl sulphate, acetylated lanolin, and paraffinum liquidum were melted in a stainless steel container (in system I) and with caprylic capric triglyceride (in system II); then, diethylhexyl syringylidene malonate was added. It was mixed by slow agitation avoiding the incorporation of air and keeping temperature between 72 and 75 °C. Avobenzone and benzophenone-3 were then added. It was stirred maintaining the temperature until a full dispersion was obtained.

Demineralized water, Disodium EDTA and imidazolidinyl urea were mixed in another stainless steel container. This mixture was heated up to 75 °C; titanium dioxide and silica was then added. The mixture 2 was incorporated into 1, and stirred at 900 rpm, for 5 min. Then, cooling was started and stirring was slowed down.

The emulsions were stored for six months at 40 °C and 75 %RH and twelve months at room temperature, and were analyzed under the same conditions in all cases.The quantitative compositions of the formulations are shown in Table 1.

Analysis of the active ingredients

The analysis of benzophenone-3 and avobenzone were made by HPLC [16].

Materials and reagents

The working standard employed for benzophenone-3 and avobenzone were the same as those used in the preparation of the creams. Solvents were HPLC grade. Water HPLC grade was obtained by distillation and passed through a 0.45 micron membrane filter.

Instrumentation

The HPLC system consisted of a dual piston reciprocating Thermo Finnigan pump (Waltham, Massachusetts, United States, Model P2000), a Rheodyne injector (Model 7125), a UV-Vis KONIK detector (Barcelona, Spain, Model KNK-027-757) with operating software WinPCC Chrom XY (Buenos Aires, Argentine) was used during the study.

HPLC conditions

The analytical column was a reversed phase C18 column (Inerstil ODS-3, GL Sciences Inc.) 250 x 4.6 mm, 5 µm. The separation was carried out under isocratic elution with methanol:water (95:5) pH 3.2 adjusted with 85% of phosphoric acid. The flow rate was 1.0 mL/min. The wavelength was monitored at 315 nm, and the injection volume was 20 µL. The HPLC was operated at ambient temperature. In these conditions benzofenone-3 retention time (tR) was roughly 5.9 min and avobenzone retention time was 9.2 min.

Procedure

Solutions of the filters were prepared on a weight basis with volumetric flasks to minimize solvent evaporation.Prior to injecting solutions, the column was stabilized for at least 30 min. with the mobile phase flowing through the system. Quantification was accomplished using an external standard method. Each solution was prepared in duplicate and was injected in triplicate and the relative standard deviation (RSD) was below 2.0%.

Working standard solutions

A standard stock solution of benzophenone-3 (1.0 mg/mL) was prepared by dissolving appropriate amount in mobile phase. The standard solution was obtained by diluting the standard stock solution with mobile phase to yield a solution containing 0.04 mg/mL. A standard stock solution of avobenzone (0.5 mg/mL) was prepared by dissolving appropriate amount in mobile phase. The standard solution was obtained by diluting the standard stock solution with mobile phase to yield a solution containing 0.02 mg/mL.

Preparation of O/W samples

Approximately 0.5 g of cream were exactly weighed, placed into a 50 mL volumetric flask, taken to volume with mobile phase and shaken for about 10 min. A 2 mL aliquot of the solution was transferred to a 50 mL volumetric flask. The solutions were passed through a 0.45 µm membrane filter before injection.

Physical stability of the systems

The centrifuge model was performed to study the physical stability of the systems. The centrifuge technique, based on theoretical principles reflected in the Stokes formula, was used

INCI names	System I					System II				
	1	2	3	4	5	A	B	C	D	E
Butyl methoxydibenzoylmethane	–	–	2.5	2.5	2.5	–	–	2.5	2.5	2.5
Benzofenone-3	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Caprylic/capric triglyceride	–	–	–	–	–	5.0	5.0	5.0	5.0	5.0
Acetylated lanolin	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Paraffinum liquidum	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Cetearyl alcohol, sodium lauryl sulfate and sodium cetearyl sulfate	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Titanium dioxide	–	1.0	–	1.0	–	–	1.0	–	1.0	–
Imidazolidinyl Urea	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Diethylhexyl syringylidene malonate	–	–	–	–	0.5	–	–	–	–	0.5
Water	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 1. Quantitative composition of formulations (g %)

as one of the ways for predicting the vulnerability of the emulsion-to-oil coalescence [17]. Centrifugation was performed for 30 min at 3500 rpm at room temperature on a Rolco (Argentina) centrifuge. Ten mL samples in graduated centrifuge tubes were used. The classification adopted was: a) good, no creaming or phase separation was observed; b) poor, a considerable creaming and/or phase separation was observed.

pH determinations

The pH data for all the systems were obtained with model Altronix TPX I (Saen S.R.L., Buenos Aires, Argentina). The pH was measured as directed in USP 32 <791>, using an indicator glass electrode. The buffer solutions for standardization were from Merck (Darmstadt, Germany) at pH 4.00 and 7.01. The emulsions were stored in glass containers at 40 °C and 75 %RH and at room temperature. Control emulsions were stored at 2-8 °C.

SPF in vitro determinations

The emulsions were applied in small spots to the Transpore Tape® with a 1 mL syringe. The substrate is placed on an open metal frame. The sample is spread lightly and evenly over 70.7 cm^2 area at 2 µl/cm2, equivalent to *in vivo* testing, the applied product was carried out by weight difference, according to amount administered. The SPF *in vitro* value is the average of 12 sampling locations. The operator can specify the positions to be read or the computer can generate them randomly. Once set, the operation, data collection and reporting are performed automatically. Covering both the UVB and UVA spectral regions, the system automatically scans from 290 to 400 nm, accumulating and storing data at intervals of 1, 2 or 5 nm. The software calculates the area under the curve.

RESULTS AND DISCUSSION

pH variations in the period are indicated in Table 2. After twelve months of storage at ambient temperature, pHs were well retained as the control. After six months of storage at 40 °C and 75 % RH generally, pH decreased. This would indicate the presence of acid degradation products in the systems.

All the O/W formulations were stable under centrifugation.

After twelve months of storage at room temperature for systems 1 to 5 and systems A to E, benzophenone-3 and avobenzone seem to be stable; all the results are above 90.0%. The SPF *in vitro* values are well maintained after twelve months at room temperature. After six months of storage at 40 °C and 75 %RH for all the Systems the content of avobenzone and benzophenone-3 decreased between 15% and 20% or more. The presence of caprylic capric triglyceride increase SPF *in vitro* values (Table 3)

Fig. 2 shows the ultraviolet absorption spectra for benzophenone-3 alone (Systems I. 1 and II. A) and for benzophenone-3 and avobenzone (Systems I. 3 and II. C) obtained with SPF-290S, Optometrics.

Room temperature					40 °C, 75% RH				
	Time (months)					Time (months)			
System	0	1	3	6	System	0	1	3	6
1	5.59	5.47	5.42	5.38	1	5.59	5.37	5.27	4.74
2	5.70	5.78	5.75	5.78	2	5.70	5.70	5.61	5.18
3	5.51	5.59	5.48	5.40	3	5.51	5.45	5.00	4.64
4	5.70	5.90	5.70	5.75	4	5.70	5.70	5.47	4.99
5	5.59	5.64	5.53	5.56	5	5.59	5.47	5.18	4.80
control	5.26	5.36	5.39	5.37	control	5.26	5.33	5.11	4.67
A	5.18	5.39	5.37	5.34	A	5.18	5.36	5.17	5.01
B	5.45	5.68	5.60	5.61	B	5.45	5.58	5.31	4.99
C	5.20	5.34	5.33	5.37	C	5.20	5.35	5.16	4.93
D	5.46	5.62	5.58	5.62	D	5.46	5.61	5.43	5.26
E	5.20	5.42	5.38	5.42	E	5.20	5.35	5.12	4.82
control	5.27	5.30	5.24	5.29	control	5.27	5.29	5.10	4.89

Table 2. pH variation in systems 1-5 and A-E at room temperature and 45 °C, 75% RH.

Room temperature				
System	**inicial SPF** ***in vitro***	**12 months SPF** ***in vitro***	**Final concentration of Benzophenone-3 (12 months)**	**Final concentration of Avobenzone (12 months)**
System I Control	1.2	1.2		
1	2.2	2.1	95.49	
2	3.8	3.8	100.84	
3	6.6	4.7	97.72	95.18
4	9.4	9.2	100.00	96.81
5	4.9	5.1	100.40	98.83
System II Control	1.3	1.3		
A	2.9	2.9	95.45	
B	4.9	4.8	99.21	
C	12.3	10.9	94.38	93.39
D	13.9	14.1	98.13	94.36
E	10.4	10.6	99.41	96.24
40 °C and 75 %RH				
System	**inicial SPF** ***in vitro***	**6 months SPF** ***in vitro***	**Final concentration of Benzophenone-3 (6 months)**	**Final concentration of Avobenzone (6 months)**
System I Control	1.2	1.3		
1	2.2	2.6	79.22	
2	3.8	3.7	87.95	
3	6.6	5.2	79.70	79.92
4	9.4	9.9	84.35	84.46
5	4.9	5.9	85.35	85.55
System II Control	1.3	1.2		
A	2.9	3.0	85.61	
B	4.9	4.2	89.13	
C	12.3	7.3	83.73	74.71
D	13.9	9.4	84.33	81.95
E	10.4	8.2	88.71	81.95

Table 3.

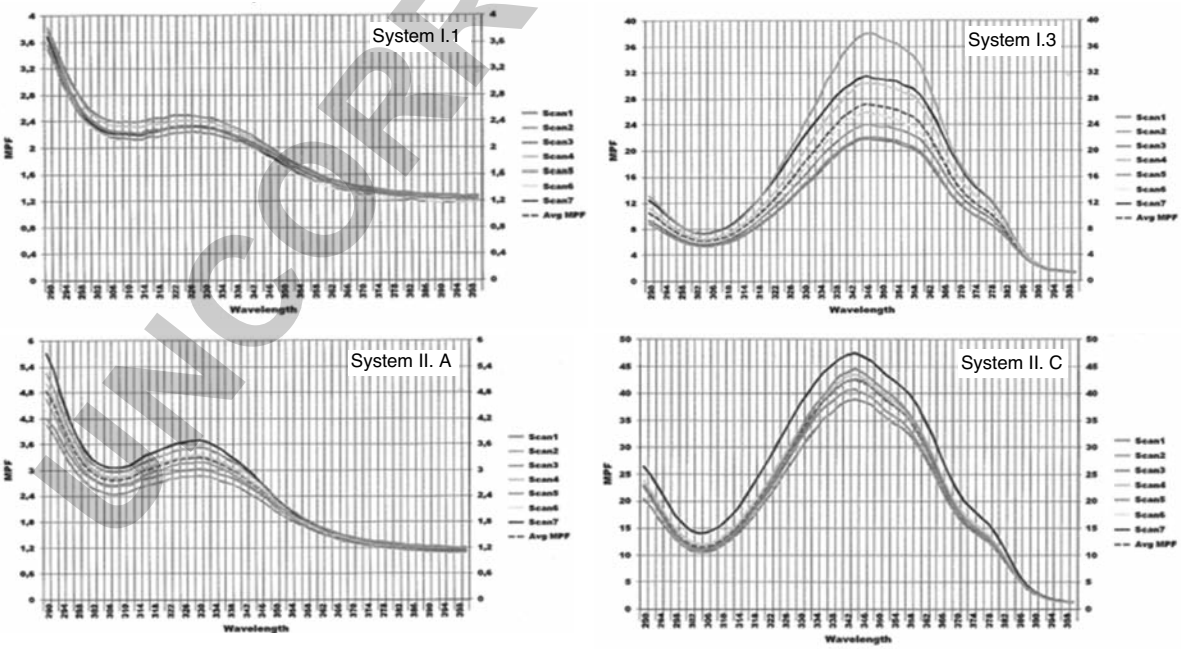

Figure 2. Ultraviolet absorption spectra.

CONCLUSIONS

The content of caprylic capric triglyceride increases SPF values. At room temperature stability studies, the SPF *in vitro* values were well maintained, but decreases in the accelerated conditions. Avobenzone and benzophenone-3 were less stable in the accelerated stability studies.

Acknowledgements. This work was supported by grant B072 and 20020100100816 given to A. I. Segall from UBA. The authors thank Glaxo Smith Klein (Argentina) for assistance in the use of the spectrophotometer SPF-290S and Merck Quimica Argentina S.A.I.C. and Flamaquímica S.R.L. (Argentina) for the donation of UV filters and reagents.

REFERENCES

1. Forestier, S. (2008) *J. Am. Acad. Dermatol.* **58**: S133-8.
2. González, S., M. Fernández-Lorente & Y. Gilaberte-Calzada (2008) *Clin. Dermatol.* **26**: 614-26.
3. Lautenschlager, S., H.C. Wulf & M.R. Pittelkow (2007) *Lancet* **370**: 528-37.
4. Serpone, N., D. Dondi & A. Albini (2007) *Inorg. Chim. Acta* **360**: 794-802.
5. Nash, J.F. (2006) *Dermatol. Clin.* **24**: 35-51.
6. Kullavanijaya, P. & H.W. Lim, (2005) *J. Am. Acad. Dermatol.* **52**: 937-58.
7. Diffey, B.L., P.R. Tanner, P.J. Matts & J.F. Nash (2000) *J. Am. Acad. Dermatol.* **43**: 1024-35.
8. Fernandez, C., G. Marti Mestres, J. Ramos & H. Maillols (2000) *J. Pharm. Biomed. Anal.* **24**: 155-65.
9. Dondi, D., A. Albini & N. Serpone (2006) *Photochem. Photobiol. Sci.* **5**: 835-43.
10. Ceresole, R., M.A. Rosasco, L.D. Simionato & A.I. Segall (2009) *Industria Farmacéutica* **146**: 96-100.
11. Ceresole, R., Y.K. Han, M.A. Rosasco, L.R. Orelli & A. Segall, *J. Cosm. Sci.* in evaluation.
12. Tyner, K.M., A.M. Wokovich, D.E. Godar, W.H. Doub & N. Sadrieh (2011) *Int. J. Cosmet. Sci.* **33**: 234-244.
13. Food and Drug Administration (2012) *Labeling and Effectiveness Testing: Sunscreen Drug Products for Over-The-Counter Human Use - Small Entity Compliance Guide.* U.S. Department of Health and Human Services Center for Drug Evaluation and Research (CDER).
14. COLIPA/JCIA/CTFA-SA (2006) *International Sun Protection Factor (SPF) Test Method.*
15. Optometrics Corporation. SPF-290S *Analyzer System,* rev 5-5-11, pag 5. Available at <http://www.es-france.com/pdf/SPF-Brochure.pdf>.
16. Ceresole, R., Y.K. Han, L.D. Simionato & A. I. Segall (2013) *J. Liq. Chromatogr. R.T.* On line: 01-08-2013.
17. Agencia Nacional de Vigilancia Sanitaria (2005) *Guía de Estabilidad de Productos Cosméticos,* Editora ANVISA 1, 17.