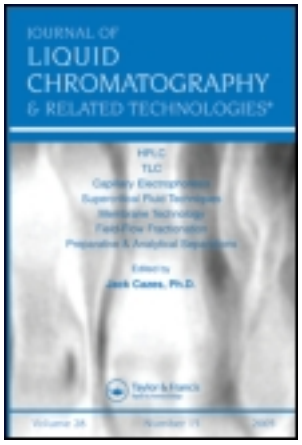

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ljlc20

STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF BENZOPHENONE-3 AND AVOBENZONE IN COSMETIC FORMULATIONS

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Accepted author version posted online: 08 Jan 2013.Published online: 02 Sep 2013.

To cite this article: Rita Ceresole, Yong K. Han, Laura D. Simionato & Adriana I. Segall (2013) STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF BENZOPHENONE-3 AND AVOBENZONE IN COSMETIC FORMULATIONS, Journal of Liquid Chromatography & Related Technologies, 36:20, 2882-2894

To link to this article: http://dx.doi.org/10.1080/10826076.2012.731667

Journal of Liquid Chromatography & Related Technologies, 36:2882–2894, 2013

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2012.731667

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□ *An accurate, simple, and reproducible liquid chromatographic method was developed and validated for the determination of benzophenone-3 and avobenzone in a cosmetic formulation. The analyses were performed at room temperature on a reverse-phase C18 column (Inerstil ODS-3) (250 × 4.6 mm, 5 μm). The mobile phase, which consisted of methanol:water (95:5) and pH 3.2 adjusted with 85% of phosphoric acid, was pumped at a constant flow rate of 1 mL/min. Detection was performed on a UV detector at 315 nm. The method was validated in terms of linearity, precision, accuracy, and specificity by forced decomposition of benzophenone-3 and avobenzone using acid, base, water, hydrogen peroxide, heat, and light. The response of was linear in the range 0.08 to 0.24 mg/mL and 0.04 to 0.12 mg/mL for benzophenone-3 ($r^2 = 0.9984$), and avobenzone ($r^2 = 0.9925$), respectively. The relative standard deviation values for intra- and inter-day precision studies were 0.81 and 0.91 for benzophenone-3 and 1.57 and 1.13 for avobenzone. Recoveries ranged between 99.58 and 101.39 for benzophenone-3 and 98.63 and 102.05 for avobenzone.*

Keywords avobenzone, benzophenone-3, cosmetic formulation, HPLC, stability indicating, validation

INTRODUCTION

Ultraviolet radiation (UVR) from the sun is divided into UVC (270–290 nm), UVB (290–320 nm), and UVA which is subdivided into UVA2 (320–340 nm) and UVA1 (340–400 nm). UVC is filtered by ozone in the stratosphere and does not reach the earth's surface. The amount of UVB and UVA 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,

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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] UVA penetrates deeper into the skin than UVB. It particularly affects connective tissue where it produces detrimental reactive oxygen species. UVA is a stronger inducer of immune suppression and is suspected to contribute to photocarcinogenesis. A great majority of exogenous photosensitization has an action spectrum in the UVA range and is also involved in idiopathic photodermatosis such as most cases of polymorphous light eruption.[1]

Oxybenzone, Benzophenone-3 (Figure 1), is the most commonly used benzophenone. It absorbs most efficiently in the UVB and UVA2 range with two absorption peaks (λ maximum, 288 and 325 nm). It is photolabile, can oxidized rapidly, and its oxidation will inactivate the antioxidant systems.[6]

Butyl methoxydibenzoylmethane, Avobenzone (Figure 2), has strong absorption in the UVA1 range (λ maximum to 380 nm). Unfortunately, its photoprotective capacity was decreased by 50% to 60% after 1 hour of exposure to sunlight. Because of both benzophenone-3 and avobenzone are photolabile, they are frequently combined with octocrylene, salicylates, methylbenzylidene camphor, micronized ZnO, and/or TiO_2 to enhance their photostability.[6]

Most of the analytical techniques for benzophenone-3 and avobenzone described in the literature are based on the determination of these drugs in cosmetic formulations,[9–21] in aqueous solutions,[22,23] in environmental samples,[24] in pigskin,[23,25] and in biological fluids.[26–29] Between these, some methods use mass spectrometry detector[12,24,26,29] and gradient elution.[9–12,14,22]. Literature survey revealed a

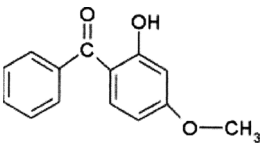

FIGURE 1 Benzophenone-3.

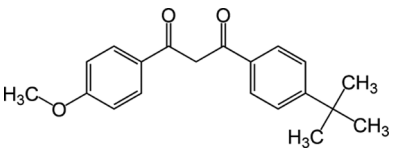

FIGURE 2 Avobenzone.

determination of benzophenone-3 alone[24,31] or with other active drug substances.[9,12,14,19,21,24,30–34]

Other analytical techniques such as capillary electrophoresis[35] have also been described.

We have developed and validated a new chromatographic method for determining these two ingredients in cosmetic formulations. This method consists of a single operation, saving time and materials. Additionally, as both drugs are quantified in the same chromatographic system, the risk of contamination with solvents is lower, protecting human health and the environment. The method was validated following the analytical performance parameters suggested by International Conference on Harmonization (ICH).[36]

EXPERIMENTAL

Chemicals and Reagents

Benzophenone-3 (100.3%) and avobenzone (98.3%) were obtained from Merck Química Argentina (Merck, Darmstadt, Germany).

The method was developed using a formulation containing: benzophenone-3 and avobenzone in a matrix of paraffinum liquidum, acetylated lanolin, cetearyl alcohol & sodium lauryl sulfate & sodium cetearyl sulphate, imidazolidinyl urea, disodium EDTA, and caprylic capric triglyceride.

Methanol used was HPLC grade, J. T. Baker, (Estado de Mexico, México), Phosphoric acid 85%, Mallinckrodt Baker Inc., (Phillipsburg, New Jersey, USA). Distilled water was passed through a 0.45-μm membrane filter.

Equipment

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.

Chromatographic Conditions

The analytical column was a reversed phase C18 column (Inerstil ODS-3, GL Sciences Inc.) 250 × 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

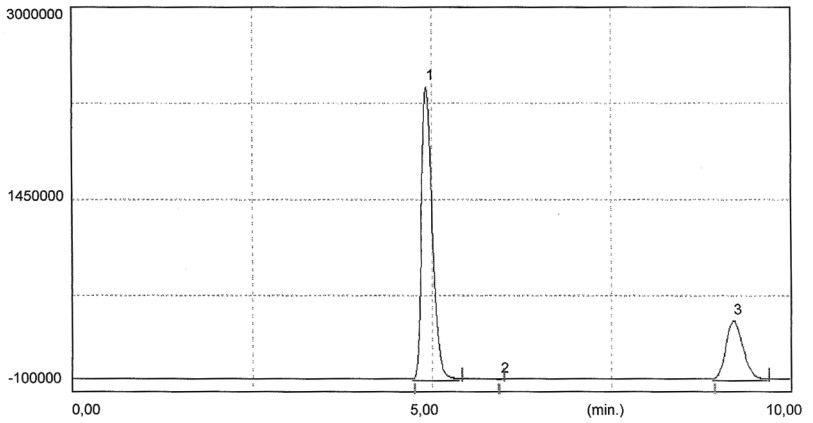

FIGURE 3 Typical chromatogram.

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 (t_R) was roughly 4.9 min and avobenzone retention time was 9.2 min (Figure 3).

Standard Solutions

A standard stock solution of benzophenone-3 was prepared at a concentration of 1.0 mg/mL in diluents which was a mixture of methanol and water (95:5, *v*/*v*). The standard solution was obtained by diluting the standard stock solution with diluent to obtain a solution containing 0.2 mg/mL of benzophenone-3

A standard stock solution of avobenzone was prepared (0.5 mg/mL) and the working standard solution was obtained by diluting the stock standard solution to obtain a solution containing 0.1 mg/mL of avobenzone.

Sample Preparation

Approximately 0.5 g of cream were exactly weighed, placed into a 100-mL volumetric flask, taken to volume with diluent and shaken for about 5 min.

The solutions were passed through a 0.22-µm nylon membrane filter before injection (25 mm disposable filter; Cat. N° Y02025WPH µicroclar, Buenos Aires, Argentina).

Method Validation

System Suitability

Relative standard deviations (RSD) values of the peak area, tailing factor, retention time, and resolution were the chromatographic parameters selected for the system suitability test.[37]

Specificity

Forced degradation studies were performed to evaluate the specificity of the method. Degraded samples were prepared by refluxing for at least 30 min 1.0 mg/mL benzophenone-3 and 0.5 mg/mL avobenzone working standard with acid (1 N hydrochloric acid), base (1 N NaOH), water, and hydrogen peroxide 100 vol. Drugs were subjected to thermal degradation (either in the solid state or in solution in an open container in an oven at 110°C for 24 hr) and photochemical degradation (a solution was transferred to a container and exposed to daylight for 24 hr). After degradation treatment, samples were allowed to cool at room temperature and diluted, if necessary, to the same concentration as that of the standard solution, after being neutralized. After degradation, samples were analyzed using the methodology and the chromatographic conditions described.

Linearity

A stock solution of 1.0 mg/mL of benzophenone-3 was prepared in a 100 ml volumetric flask by dissolving 100 mg in diluent. Appropriate amounts of the stock solutions were diluted with diluent, yielding concentrations 80.0, 120.0, 160.0, 200.0, and 240.0 µg/mL. A stock solution of 0.5 mg/mL of avobenzone was prepared and appropriate volumes of the stock standards were diluted, yielding concentrations of 40.0, 60.0, 80.0, 110.0, and 120.0 µg/mL. Triplicate injections of each were made.

Precision

Method precision (repeatability) and intermediate precision: six replicates ($n = 6$) of sample solutions were analyzed in the same day to determine method precision and in two different days by two different analyst to evaluate intermediate precision.

Accuracy

The recovery method was studied at concentration levels of 80%, 100%, and 120% (three samples each). It was calculated the amount of benzophenone-3 and avobenzone recovered in relation with the results obtained in the intermediate precision study.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The organic strength was varied by ±5.0%, while pH was varied by ±0.2 units. Robustness was established by changing the mobile phase proportion. Retention time, tailing, and resolution were evaluated.

RESULTS AND DISCUSSION

The described reversed-phase liquid chromatography method was developed to provide a rapid quality control determination of benzophenone-3 and avobenzone in cosmetic formulations. Validation of the method was performed according to ICH. This method uses a simple mobile phase. All samples were analyzed using the assay chromatographic conditions described.

The analytical column was equilibrated with the eluting solvent system used. After an acceptable stable baseline was achieved, the standards and then the samples were analyzed.

System Suitability

System suitability results were calculated according to the USP 32 <621> from typical chromatograms. Instrument precision as determined by six successive injections of the Standard preparation provided a relative standard deviation (RSD) below 1.5%. Peak asymmetry or tailing factor, T, was calculated as $T = W_{0.05}/2f$, where $W_{0.05}$ is the distance from the leading edge to the tailing edge of the peak, measured at 5% of the peak height from the baseline and f is the distance from the peak maximum to the leading edge of the peak. The tailing factor did not exceed 1.55. The resolution between benzophenone-3 and avobenzone should be greater than 2.5

Stability of the standard solution and sample preparation was studied by injecting the prepared solution at periodic intervals into the chromatographic system up to about 24 hr stored at room temperature and refrigerated. The solutions maintained at least 99.2% (benzophenone-3) and 98.5% (avobenzone) of their initial concentration under the test conditions.

Selectivity

Selectivity was demonstrated showing that benzophenone-3 and avobenzone were free of interference from degradation products and no interference from the sample excipients could be observed at this detection

TABLE 1 Selectivity: Degradation Conditions of Benzophenone-3

Condition	Time (h)	% of Benzophenone-3	RRT of Degradation Products
Acid (1 N HCl, reflux)	0.5	93.9	0.43
Base (1 N NaOH, reflux)	0.5	89.0	0.43
Hydrogen peroxide 100 vol (reflux)	0.5	96.3	0.66
Water (reflux)	0.5	100.4	None detected
Dry heat, 50°C (solution)	24.0	96.2	None detected
Dry heat, 50°C (solid)	24.0	91.4	None detected
Daylight exposure	24.0	99.8	None detected

Note. RRT, relative retention time.

TABLE 2 Selectivity: Degradation Conditions of Avobenzone

Condition	Time (h)	% of Avobenzone	RRT of Degradation Products
Acid (1 N HCl, reflux)	0.5	81.7	0.28, 0.37, 0.49
Base (1 N NaOH reflux)	0.1	42.1	0.28, 0.49, 0.55
Hydrogen peroxide 100 vol (reflux)	0.5	89.9	0.47, 0.53
Water (reflux)	0.5	61.1	0.48, 0.66
Dry heat, 50°C (solution)	24.0	96.4	0.53
Dry heat, 50°C (solid)	24.0	89.8	0.53
Daylight exposure	24.0	99.2	0.54, 0.67

Note. RRT, relative retention time.

wavelength, indicating that the proposed method can be used in a stability assay (Tables 1 and 2; Figures 4 and 5).

Linearity

Linearity of the detector responses was determined by preparing calibration graphs. The linearity of the peak responses versus concentration was studied from 0.08 to 0.24 mg/mL and 0.04 to 0.12 mg/mL for

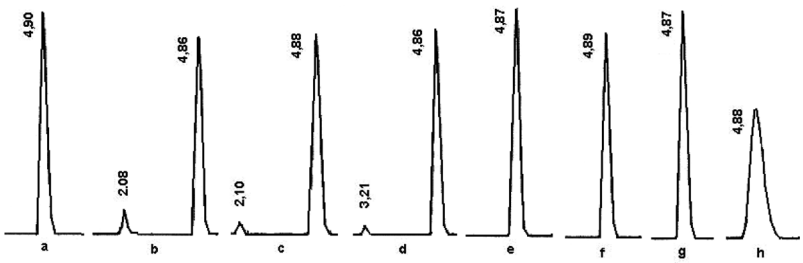

FIGURE 4 Chromatograms of benzophenone-3: a) Standard; b) Acid hydrolysis; c) Alkaline hydrolysis); d) Oxidation; e) Hydrolysis; f) Heat dry, (solution); g) Heat dry, (solid); h) Daylight exposure.

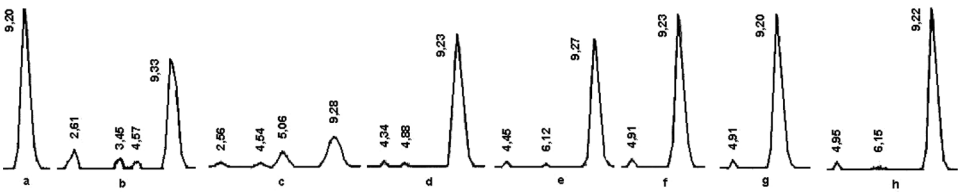

FIGURE 5 Chromatograms of avobenzone: a) Standard; b) Acid hydrolysis; c) Alkaline hydrolysis); d) Oxidation; e) Hydrolysis; f) Heat dry, (solution); g) Heat dry, (solid); h) Daylight exposure.

benzophenone-3 and avobenzone, respectively. The representative linear equation for benzophenone was 5809892.3x +16274777.3 with a standard error ($S_{x,y}$) of 12947261.6 and a correlation coefficient (r) of 0.99922 whereas intercept was not significantly different from zero ($p = 0.05$) (Table 3). The representative linear equation for avobenzone was 3420345.2x + 10031588.6 with a standard error ($S_{x,y}$) of 3990272.8 and a correlation coefficient (r) of 0.99625, whereas intercept was not significantly different from zero ($p = 0.05$) (Table 4).

TABLE 3 Linearity Data of Benzophenona-3

% of Nominal Value	Injected (μg)	Average Peak Area Response	RSD
40	1.6	10009582.7	0.57
60	2.4	14588647.0	0.61
80	3.2	19664754.3	0.27
100	4.0	24383395.0	0.15
120	4.8	28351777.3	0.36
Slope[a]	5809892.3 ± 16274777.3		
Intercept[b]	807975.7 ± 55238425.9		

[a]Confidence limits of the slope ($p = 0.05$).
[b]Confidence limits of the intercept ($p = 0.05$).

TABLE 4 Linearity Data of Avobenzone

% of Nominal Value	Injected (μg)	Average Peak Area Response	RSD
40	0.8	3620147.7	0.74
60	1.2	4562498.3	0.71
80	1.6	6107405.0	0.98
100	2.0	7748993.3	0.77
120	2.4	8867590.7	0.30
Slope[a]	3420345.2 ± 10031588.6		
Intercept[b]	708774.6 ± 17024170.3		

[a]Confidence limits of the slope ($p = 0.05$).
[b]Confidence limits of the intercept ($p = 0.05$).

Precision

The relative standard deviation (RSD) obtained was 0.15% and 0.77% for benzophenone-3 and avobenzone, respectively. In all these cases, the RSD obtained was below 1.5%; the limit percentage was set for the precision study of the instrumental system and showed that the equipment used for the study worked correctly for the developed method and is highly repetitive.

The inter-day precision of the assay was performed by assaying 6 samples and showed a RSD with a maximum 1.6% for both drugs. The intra-day precision was performed by assaying the samples on 2 different days by 2 different analysts. The results were given both individually and as a whole. For each precision assay the results were as follows: mean values 5.05 and 5.06 g%, RSD 0.81% and 0.91% for benzophenone-3 and mean values 2.93 and 2.92 g%, and RSD 1.57% and 1.13% for avobenzone. Test "*t*" comparing two sample with 95% confidence for 10 degrees of freedom disclosed that both results were not significantly different *inter se* ($t_{n-2,\ \alpha:0.05}$) = 2.23 (Tables 5 and 6).

Accuracy

The results obtained for the accuracy study (recovery method) from 9 samples studied ($n = 3$ for 80%, 100%, and 120%) indicated that the mean

TABLE 5 Precision of the Assay Method for Benzophenona-3

Analyst 1 Sample N°	cc. g%	RSD (%)	Analyst 2 Sample N°	cc g%	RSD (%)
1	4.97	0.06	1	5.07	0.59
2	5.06	0.06	2	5.01	0.59
3	5.06	0.06	3	5.03	0.59
4	5.08	0.06	4	5.07	0.59
5	5.07	0.06	5	5.05	0.59
6	5.07	0.06	6	5.14	0.59
Mean	5.05	0.81	Mean	5.06	0.91

TABLE 6 Precision of the Assay Method for Avobenzone

Analyst 1 Sample N°	cc g%	RSD (%)	Analyst 2 Sample N°	cc g%	RSD (%)
1	2.88	0.03	1	2.95	0.48
2	2.90	0.03	2	2.91	0.48
3	2.89	0.03	3	2.90	0.48
4	2.93	0.03	4	2.88	0.48
5	2.96	0.03	5	2.96	0.48
6	3.00	0.03	6	2.90	0.48
Mean	2.93	1.57	Mean	2.92	1.13

recovery was 100.59% for benzophenone-3 and 99.73% for avobenzone and RSD were 0.56 and 1.35%, respectively.

Method accuracy was also demonstrated by plotting the amount (expressed in mg) of benzophenone-3 and avobenzone found against the amount present in mg. Linear regression analysis rendered slopes not significantly different from 1 (t test $p = 0.05$), intercepts not significantly different from zero (t test $p = 0.05$) and r = 0.99976 and r = 0.99690, respectively (Tables 7 and 8).

Robustness

The effect of mobile phase proportion and pH on resolution, retention time, and tailing for both drugs could be seen in Table 9.

A decrease of methanol content resulted in longer retention time for avobenzone and benzophenone-3. Tailing factor was increased for both drugs.

TABLE 7 Recovery Analysis of Benzophenone-3

% of Nominal Value	Added Amount (mg)	Found Amount (mg)	Recovery (%)	Average Recovery ($n = 3$)	RSD (%)
80	20.20	20.48	101.38	101.18	0.34
	20.23	20.39	100.79		
	20.21	20.49	101.39		
100	25.26	25.41	100.59	100.40	0.20
	25.26	25.36	100.40		
	25.24	25.29	100.20		
120	30.28	30.16	99.58	100.19	0.54
	30.28	30.40	100.40		
	30.30	30.48	100.59		
Mean ($n = 9$)				100.59	0.56

TABLE 8 Recovery Analysis of Avobenzone

% of Nominal Value	Added Amount (mg)	Found Amount (mg)	Recovery (%)	Average Recovery ($n = 3$)	RSD (%)
80	11.68	11.60	99.32	98.96	0.53
	11.70	11.58	98.97		
	11.69	11.49	98.29		
100	14.60	14.50	99.32	100.46	1.20
	14.61	14.86	101.71		
	14.59	14.64	100.34		
120	17.51	17.33	98.97	99.89	1.89
	17.51	17.87	102.05		
	17.52	17.28	98.63		
Mean ($n = 9$)				99.73	1.35

TABLE 9 Robustness of Benzophenone-3 and Avobenzone Method

Mobile Phase	RT Benzophenone-3 (min)	Tailing	RT Avobenzone (min)	Tailing	RS
Methanol: Water pH 3.2 (85:15)	5.29	1.50	10.93	1.51	14.58
Methanol: Water pH 3.2 (90:10)	5.29	1.51	10.85	1.36	14.16
Methanol: Water pH 3.2 (95:5)	4.90	1.30	9.20	1.07	12.47
Methanol: Water pH 3.0 (95:5)	5.18	1.27	10.32	1.03	13.01
Methanol: Water pH 3.4 (95:5)	4.90	1.30	8.95	1.08	12.27
Methanol: Water pH 3.6 (95:5)	4.92	1.28	9.08	1.08	12.24

An increase of pH reduces retention time for both drugs. The tailing factor was not affected.

The resolution was good in all cases.

CONCLUSIONS

The liquid chromatographic method developed in this study has the advantage of simplicity, precision, accuracy, and convenience. The method uses simple reagents, with minimal sample preparation procedures. The results demonstrated that this method is useful for the routine quality control for benzphenone-3 and avobenzone in cosmetic formulations.

The method is very simple and specific. The peaks are well-separated from its impurities and with total runtime of 15 min, which makes it especially suitable for routine quality control analysis work.

ACKNOWLEDGEMENT

This work was supported by grant B072/2008-2010 to A. I. Segall from UBA.

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