APPLICATION NOTE

UHPLC

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A Robust Method for the Analysis of Commonly Used Sunscreen Compounds for Compliance with New FDA Regulations

Introduction

Over time, exposure to ultra violet (UV) radiation from the sun or tanning beds can damage the skin's cellular DNA, resulting in mutations that cause 3.5 million cases of skin cancers and about 11,500 deaths in the U.S. each year, for a total cost of nearly $2 billion. There are three types of UV: UVC, UVB, and UVA. Of the three, UVC has the shortest wavelengths and is the most dangerous; fortunately it is completely absorbed by the ozone layer in the atmosphere. UVB has short wavelengths that penetrate the outer layer of the skin causing sunburn. UVA has longer wavelengths that penetrate deeper into the skin, causing wrinkling and premature aging.

There are two types of skin cancers: one type develops in melanocytes and is called melanoma (melanocytes are the cells producing the skin coloring pigments called melanin); the other type develops elsewhere in the skin cells and is called non-melanoma. Though it is the least common form of skin cancer, melanoma is the most deadly. Melanoma caused the death of about 9000 people every year in the U.S., which is about 75% of the deaths attributed to skin cancers, although it accounts for only 2% of all the cases diagnosed.

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Among the non-melanoma type, basal cell carcinoma is the most common with an estimated 2.8 million diagnoses every year in the US; this form of cancer rarely results in death. The second most common is the squamous cell carcinoma with an estimated 700,000 cases diagnosed each year resulting in approximately 2,500 deaths.

As pernicious as skin cancers are, they can still be prevented by avoiding prolonged exposure to UV sources, and by shielding the skin from UV with generous applications of sunscreen lotion before and during exposure to the sun or tanning beds. These lotions provide protection because their formulations include compounds that chemically absorb UVA and UVB or physically block them (Table 1 and Figure 1).

While sunscreens are crucial for UV protection, their usage carries some risks. Several studies suggest that some benzophenones used as sunscreen agents are potential estrogenic disruptors and can interfere with thyroid hormone function. Also, because they reduce exposure to UV, sunscreens can cause vitamin D deficiency, as Vitamin D synthesis in the body is initiated by UVB rays from sunlight that reach the skin.

In the summer of 2012, new U.S. regulations went into effect requiring specific testing of sunscreen products before making claims that they offer a broad spectrum protection. Prior usage of the term broad spectrum on sunscreen labels was at the discretion of manufacturers, whose casual use of the term often mislead customers into believing that they were protected against the full range of UV rays. The new rules state that only sunscreens providing broad UVA/UVB protection with a Sun Protection Factor (SPF) value of a least 15, can claim in their labeling to be broad spectrum, to help reduce the risk of skin cancer and early skin aging. In more prosaic terms, an SPF value of 15 is UVB protection for a time period 15-fold longer than the time it takes a person under sun exposure without sunscreen to get burned, assuming the intensity level is constant.

This application note presents a method for the simultaneous analysis of five commonly used sunscreen agents (Figure 2) to help ensure that their levels are sufficient, safe and comply with new regulations. Method conditions and performance data including precision, linearity and accuracy are presented. Three sunscreen lotions with SPF claims of 30, 50 and 100 are analyzed and the levels of protective agents are determined.

Table 1. **US-FDA approved sunscreen agents.**

Most common US-FDA Approved sunscreens	
Chemical Absorbers	**Inorganic filters**
Aminobenzoic acid (PABA) UVB	Zinc Oxide UVB, UVA
Avobenzone UVA	Titanium Dioxide UVB, UVA
Cinoxate UVB	
Dioxybenzone UVB, UVA	
Ecamsule UVA	
Ensulizole UVB	
Homosalate UVB	
Meradimate UVA	
Octocrylene UVB	
Octinoxate UVB	
Octisalate UVB	
Oxybenzone UVB, UVA	
Padimate O UVB	
Sulisobenzone UVB, UVA	

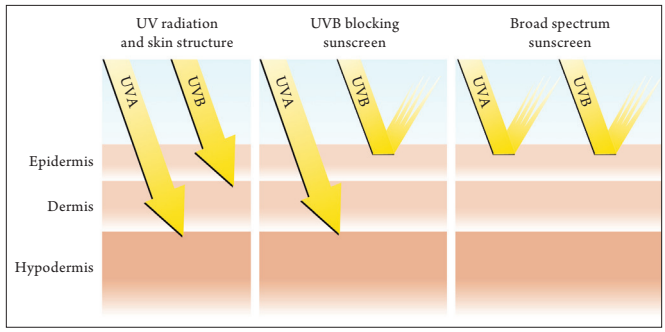

Figure 1. Type of UV rays.

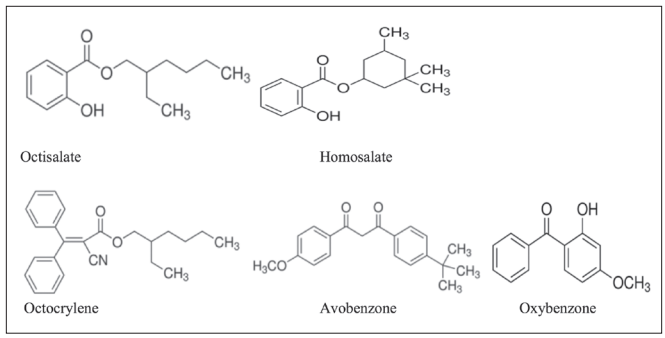

Figure 2. Sunscreens agents analyzed.

Experimental

A stock solution of 8 mg/mL of octocrylene, octisalate and homosalate, and 2 mg/mL of avobenzone and oxybenzone was prepared by transferring each appropriate net weight into the same 10 mL volumetric flask. 5 mL of isopropanol was added, the preparation was vortexed for one minute, and then sonicated for 10 minutes. The solution was allowed to return to room temperature and brought to volume with acetonitrile.

From the stock standard, five calibration levels were prepared by dilution with acetonitrile (see Table 2). The calibration curve and the repeatability were evaluated with three injections per level and six injections of level 1 standard.

Lotion samples were prepared by transferring 0.5 g of the 30 SPF, 50 SPF and 100 SPF lotions into an individual 10 mL vol. flask; 5 mL of isopropanol was added and each preparation was vortexed for one minute followed by a 10 minutes sonication. Solutions were allowed to return to room temperature and brought to volume with acetonitrile. The preparations were centrifuged for 10 min at 5000 RPM. 3 mL, 2 mL, and 1.5 mL of the aliquot from the 30 SPF, 50 SPF and 100 SPF preparations were transferred into individual 50 mL vol flask and brought to volume with acetonitrile.

For the recovery study, about 40 mg of corn oil was transferred into a 10 mL vol. flask, to which 0.3 mL of stock was added. 3 mL of isopropanol was added and the solution was vortexed for one minute and then sonicated for 10 minutes. After returning to room temperature the preparation was brought to volume with acetonitrile and centrifuged at 5000 RPM for 10 minutes.

The standard solutions and all the sample aliquots were filtered with a 0.2 µm nylon membrane prior to testing. Linearity was evaluated by serial dilutions starting with the level 1 standard covering a wide range of 0.6 to 100 µg/mL for oxybenzone and avobenzone and from 1.3 to 400 µg/mL for the remaining samples.

Table 2. **Calibration preparation.**

	Level 1	Level 2	Level 3	Level 4	[3]Level 5
Stock std. (ml)	0.5	0.3	0.2	0.1	–
Total vol. (mL)	10	10	10	10	–
[1]Conc. (µg/mL)	400	240	160	80	40
[2]Conc. (µg/mL)	100	60	40	20	10

[1]Octocrylene, octisalate, homosalate

[2]Oxybenzone, avobenzone

[3]Level 5 is a direct 1:1 dilution from level 4

Table 3. **Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar™ FX UHPLC Setting: 50 µL Loop, partial loop injection mode Injection 2 µL; flush solvent: 75:25 methanol/water
	Flush before and after injection.
UV/VIS Detector:	Analytical wavelength program: 325 nm (2.2 min) 304 nm (1.3 min)
HPLC Column:	Brownlee™ SPP C-18, 100 x 3 mm, 2.7 µm at 25 °C (Cat # N9308410)
Isocratic:	10 % Mobile phase A (1.25% acetic acid in water) 90% Mobile phase B: Acetonitrile: Two minutes wash with acetonitrile and 2 min. re-equilibration after injection
Software:	Chromera® Version 3.0
Sampling Rate:	5 pts/sec

Result and discussion

The separation was achieved using a PerkinElmer® SPP C-18, 2.7 µm, 100 x 3 mm column. The optimal flow rate was 0.6 mL/min and the run time was about 3.5 minutes with a modest back pressure of approximately 2000 psi (138 bar). All five components were well resolved. The repeatability of six injections of level 1 standard was excellent, with % RSD values ranging from 0.7 to 1.0; in addition, the average % RSDs across the fifteen injections of the five levels calibration were less than the cutoff of 2.0, with values ranging from 1.3 to 1.6. The excellent linearity of the calibration curve is demonstrated by a coefficient of determination not less than the cutoff of 0.999. Recoveries of the three sunscreen lotions analyzed match label claims. Representative chromatograms of the standard solutions are shown in Figures 3 and 4; and representative chromatograms of a spiked corn oil solution and a 100 SPF sunscreen are presented in Figure 5 and 6. The standard calibration curve, regression equations and intercepts are presented in Figure 7. Performance of the method and results of the lotions analyzed are presented in Table 4 and 5. The maximum allowable amount of different sunscreen agents is shown in Table 6.

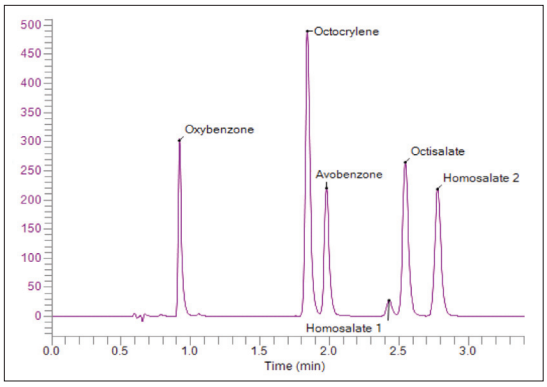

Figure 3. Chromatogram from the analysis of the standard solution (100 µg/mL, 400 µg/mL).

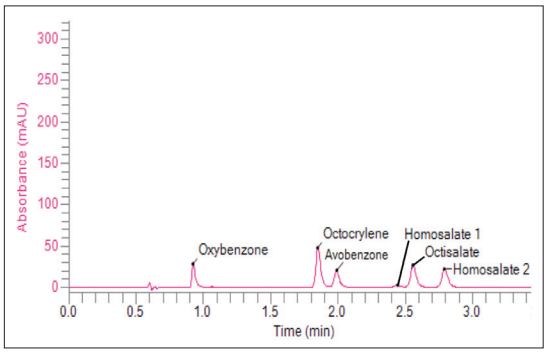

Figure 4. Chromatogram from the analysis of the standard solution (10 µg/mL, 40 µg/mL).

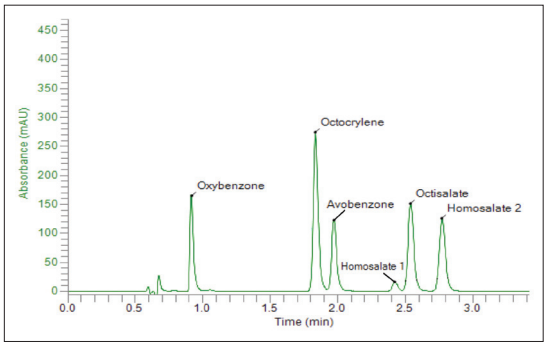

Figure 5. Chromatogram from the analysis of spiked corn oil solution (60 µg/mL, 250 µg/mL).

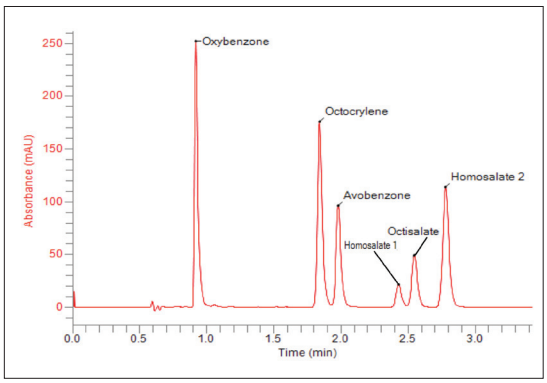

Figure 6. Chromatogram from the analysis of a solution of 100 SPF lotion.

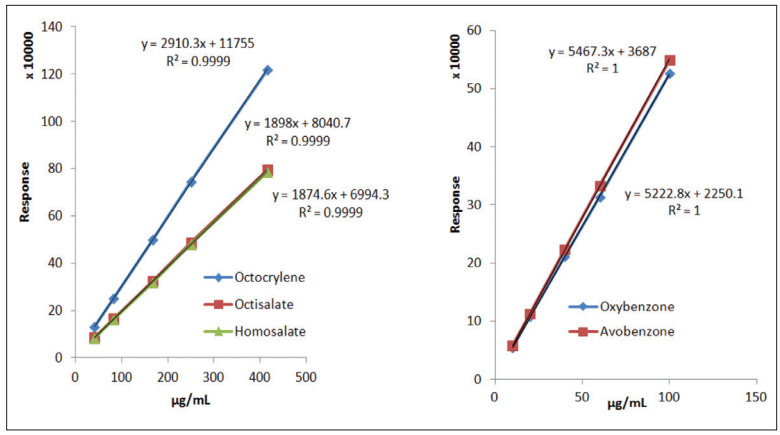

Figure 7. Calibration curves, regression and intercepts.

***Table 4.* Method performance.**

Compound	Repeatability % RSD (n =6)	Accuracy %	Linear range (µg/mL)	r^2	Detection Limit (µg/mL)
Octocrylene	1.03	97.9	1.3 - 400	0.9999	< 0.26
Octisalate	0.98	98.2	1.3 - 400	0.9999	< 0.26
Homosalate	0.93	98.0	1.3 -400	0.9998	< 0.26
Oxybenzone	0.65	95.7	0.6 - 100	1	< 0.06
Avobenzone	0.81	101.3	0.6 - 100	1	< 0.06
Average	**0.88**	**98.2**	–	**1**	–

***Table 5.* % weight/weight label claim and method % recovery.**

	SPF 30			SPF 50			SPF 100		
Compounds	**Claim**	**Result**	**%**	**Claim**	**Result**	**%**	**Claim**	**Result**	**%**
Octocrylene	2	1.8	90	7	6.5	93	10	9.6	96
Octisalate	5	4.9	98	5	4.7	94	5	4.8	96
Homosalate	10	9.7	97	13	12.3	95	15	14.8	99
Oxybenzone	2	1.7	85	4	3.4	93	6	5.4	90
Avobenzone	2	1.9	95	3	2.8	93	3	2.9	97
Average	–	–	93	–	–	93	–	–	95

***Table 6.* % (weight/weight) maximum allowed of the sunscreen analyzed.**

Sunscreen	USA	EU	Brazil	Japan
Octocrylene	10	10	10	10
Octisalate	5	5	5	10
Homosalate	15	10	15	10
Oxybenzone	6	10	10	5
Avobenzone	3	5	5	10

Source: International Journal of Cosmetic Science 2012, 34, 228.

Conclusion

Worldwide, skin cancer is reaching epidemic proportions, eliciting a renewed interest in the efficiency and safety of sunscreens. Over the last decade, serious efforts have been made to improve the quality of sunscreen products and their labeling requirements. However, since the levels of sunscreen agents allowed in sunscreen products vary from country to country, there is a need to harmonize regulations worldwide and to adapt them to the latest scientific knowledge

The isocratic method presented in this study resolves all the five sunscreen agents within three minutes. Method performance was outstanding with precisions ranging from 0.7 to 1.0% RSD. In the range 0.6 to 100 µg/mL (oxybenzone and avobenzone) and 1.3 to 400 µg/mL (Homosalate, Octocrylene, Octisalate), all five compounds were linear with correlation coefficient not less than the cutoff of 0.999. The detection limits were below 0.26 µg/mL and 0.06 µg/mL for the two groups and the accuracy was well within the 90 -110% limits. In the three sunscreens analyzed, the five compounds levels were within 93 to 95% of the label claim, and within the maximum allowed in the U.S., Europe, Japan and Brazil.

During this analysis, PerkinElmer's FX 15 pump fitted with durable pistons delivered reproducible pressure injection after injection as evidenced by the identical pressure graphs. The PerkinElmer superficially porous column (SPP) with its particles designed for high efficiency (porous silica layer surrounding a solid silica core) delivered sharp peaks with a modest back pressure of less than 2000 PSI.

References

1. American Cancer Society. Cancer Facts & Figures 2011. http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-facts-figures-2011. Retrieved June 5, 2012.

2. Centers for Diseases Control and Prevention. http://www.cdc.gov/cancer/skin/pdf/toolkit/GuidelineToolsResources.pdf, retrieved June 5, 2012.

3. Skin Cancer Foundation. Skin cancer facts: http://www.skincancer.org/skin-cancer-information/skin-cancer-facts#general, retrieved June 5, 2012.

4. FDA Regulatory Action on Sunscreen: www.FDA.gov, retrieved June 5, 2012. Federal Register / Vol. 77, No. 92 / Friday, May 11, 2012 / Rules and Regulations Federal Register / Vol. 76, No. 117 / Friday, June 17, 2011 / Proposed Rules.

5. National Institute of Health, http://ods.od.nih.gov/factsheets/vitamind, retrieved June 5, 2012.

6. Schmutzler, C., Bacinski, A., Gotthardt, I., June 2007. The ultraviolet filter benzophenone 2 interferes with the thyroid hormone axis in rats and is a potent in vitro inhibitor of human recombinant thyroid peroxidase. Endocrinology; 148(6): 2835-44.

7. Jarry, H., Christoffel, J., Rimoldi, G., Koch, L., Wuttke, W., Dec. 2004. Multi-organic endocrine disrupting activity of the UV screen benzophenone 2 (BP2) in ovariectomized adult rats after 5 days treatment. Toxicology 205(1-2): 87-93.

Note: this application note is subject to change without prior notice.

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