APPLICATION NOTE

UHPLC

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Analysis of Sunscreen Compounds for Compliance with New FDA Regulations Using PerkinElmer FX-15 Platform and Third Party Software

Introduction

Prolonged exposure to ultraviolet (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 cancer and about 11,500 deaths in the U.S. each year. There are three types of UV rays: 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, and UVA has longer wavelengths that penetrate deeper into the skin. In addition to causing sunburn and premature skin aging, both types of UV can cause mutations responsible for skin cancer.

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Skin cancers can be prevented by avoiding prolonged exposure to UV sources and by shielding the skin with generous applications of sunscreen lotion before and during exposure. These lotions provide protection because their formulations include compounds that chemically absorb or physically block UVA and UVB. In the summer of 2012, new regulations set forth in the U.S clarified the use of the term "broad spectrum" by manufacturers of sunscreen lotions. The regulation stipulates that only manufacturers of sunscreen lotions that provide UVA/UVB protection with a Sun Protection Factor (SPF) value of at least 15 can claim in their labeling to be "broad spectrum". A SPF value of 15 provides 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 compounds in order to help ensure that their levels are sufficient, safe and comply with new regulations. Three sunscreen lotions with SPF claims of 30, 50 and 100 are analyzed and the levels of protective agents are determined.

Experimental

A stock solution containing 6 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 20 mL volumetric flask. 10 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, a working standard was prepared by spiking 0.5 mL of the stock into a 10 mL vol. brought to volume with acetonitrile. Repeatability was evaluated with six injections of the working standard.

Table 1. Detailed UHPLC system and chromatographic conditions.

Platform:	Flexar FX-15 UHPLC
Autosampler:	Flexar™ FX UHPLC Setting: 20 µL Loop, variable loop injection mode Injection 2 µL; flush solvent: 75:25 methanol/water
	Flush before and after injection.
UV/VIS Detector:	Analytical wavelength program: 320 nm
HPLC Column:	Brownlee™ Analytical C-18, 150 x 4.6 mm, 5 µm at 35°C (Cat # N9303513)
Isocratic:	10 % Mobile Phase A (1.25% acetic acid in water) 90% Mobile Phase B (1:1 acetonitrile methanol)
Flow Rate:	1.2 mL/min
Software:	Agilent® EZChrom Elite™ Version 3.3.2
Sampling Rate:	5 pts/sec

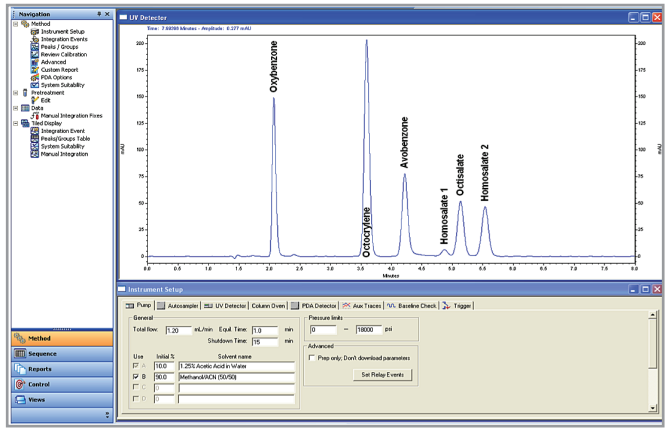

Figure 1. Chromatogram from the analysis of a working standard solution.

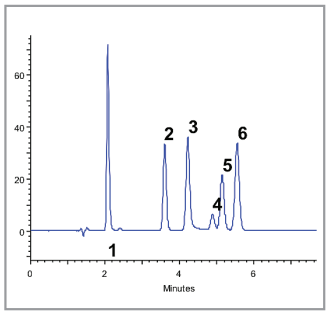

Figure 2. Chromatogram from the analysis of a solution of a 30 SPF lotion.

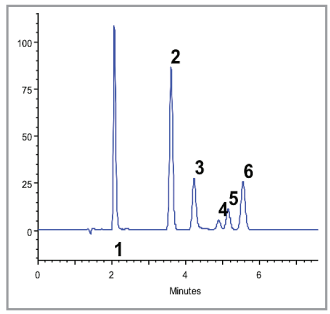

Figure 3. Chromatogram from the analysis of a solution of a 100 SPF lotion.

1.Oxybenzone, 2 Octocrylene, 3 Avobenzone, 4 Homosalate 1, 5 Octisalate, 6 Homosalate 2

Lotion samples were prepared by transferring 0.5 g of the 30 SPF, 50 SPF and 100 SPF lotions into individual 10 mL volumetric flasks, 5 mL of isopropanol was added and each preparation was vortexed for one minute followed by a 10 minute sonication. Solutions were allowed to return to room temperature and the flasks were 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 volumetric flasks and the flasks brought to volume with acetonitrile. Samples were filtered through a 0.2 µm nylon membrane prior to testing.

Result and Discussion

The separation was achieved using a PerkinElmer Brownlee™ Analytical C-18, 150 x 4.6 mm, 5 µm. The run time was about eight min., the optimal flow rate was 1.2 mL/min with a modest back pressure of 2,000 psi (138 bars). All five components were well resolved. The repeatability was good, with %RSD values ranging from 0.7 to 1.2. Recoveries of the three sunscreen lotions analyzed were 83%, 78%, and 85% of the label claims, for 30 SPF, 50 SPF and 100 SPF, respectively. Representative chromatograms of the working standard solution are shown in Figure 1; and representative chromatograms of the 30 SPF and 100 SPF sunscreen samples are presented in Figure 2, 3. Performance of the method and results of the lotions analyzed are presented in Table 2. The maximum allowable amount of different sunscreen agents are shown in Table 3.

Table 2. Repeatability and % weight/weight analysis results.

Compounds	Repeatability %RSD (n=6)	SPF 30			SPF 50			SPF 100		
		Claim	Result	%	Claim	Result	%	Claim	Result	%
Octocrylene	1.03%	2.0	1.7	85.0	7.0	5.5	78.6	10.0	8.6	86.0
Octisalate	0.77%	5.0	4.3	86.0	5.0	4.1	82.0	5.0	4.5	90.0
Homosalate	1.21%	10.0	7.9	79.0	13.0	9.7	74.6	15.0	12.2	81.3
Oxybenzone	1.09%	2.0	1.7	85.0	4.0	3.2	80.0	6.0	5.2	86.7
Avobenzone	0.70%	2.0	1.6	80.0	3.0	2.2	73.3	3.0	2.4	80.0
Average	0.96%	---	---	83.0	---	---	77.7	---	---	84.8

Table 3. % (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, the incidence of skin cancers is ever increasing, reaching epidemic proportions. The growing usage of sunscreen lotions to prevent the disease is eliciting more scrutiny on their efficacy and safety. The analytical method presented in this study resolved five major sunscreen agents within six minutes. Method performance was outstanding with precisions ranging from 0.7% to 1.2% RSD. The levels of the five sunscreen compounds in the sunscreen lotions analyzed were within the maximum quantities allowed in the U.S., Europe, Japan and Brazil. This analysis was done with PerkinElmer's FX -15 system reliably under the control of Agilent's® EZChrom Elite™ chromatography data system.

References

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

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   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

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

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