APPLICATION NOTE

Ultra High Performance Liquid Chromatography

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Analysis of Sunscreen Agents with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Azobenzene and benzophenone are chemical compounds that can absorb ultraviolet light. They are used in sunscreen cream to prevent cutaneous disorders, including skin cancer and premature aging caused by sunlight. However, they are not a panacea for all cutaneous ailments caused by the sun. These chemical compounds do not protect against the full spectrum of UV light, especially when the exposure time is increased. While sunscreen can enhance the protection from UV light, its usage is not without risks. Some studies suggest that some benzophenones are potential estrogenic disruptors that can also interfere with the thyroid hormone function by inhibiting the thyroid peroxidase enzyme. Moreover, because they reduce the exposure to UV, sunscreen agents can cause vitamin D deficiency since vitamin D synthesis in the body is initiated by ultraviolet rays from sunlight that reach the skin.

The usage of sunscreen agents comes with health concerns that in many countries are addressed in a regulatory framework that limit the concentration at which they can be used safely. In the U.S., the FDA and Cosmetic Act (FD&C Act) states that it is against the law to market products with ingredients that may cause injuries under label conditions. It is therefore important for the cosmetic industry to develop robust analytical methods to monitor the type and amount of sunscreen agents in cosmetics to ensure that their levels are safe and comply with regulations.

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This application note presents a method for the simultaneous analysis of five sunscreen agents from the benzophenone and azobenzene groups (Figure 1) using a superficially porous particle column. Method conditions and performance data including precision, linearity and accuracy are presented.

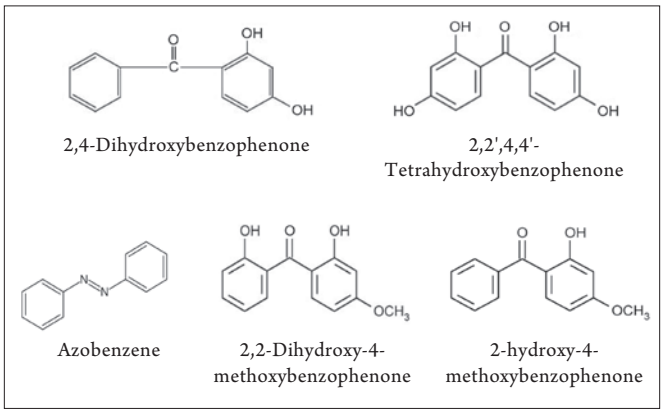

Figure 1. Molecular structures of the sunscreen compounds analyzed.

Experimental

A stock solution of 1 mg/mL of each compound was prepared by transferring the appropriate net weight into a 10 mL volumetric flask, to which some diluent (70:30 methanol/water) was added, the solution was mixed well and sonicated for five min. The solution was left to return to room temperature and brought to volume with diluent. Similarly, an internal standard of 1 mg/mL of benzoic acid was prepared. The working standard was prepared by transferring 1.0 mL of each stock solution and 1.0 mL of the internal standard into 10 mL vol. flask, the solution was brought to volume with diluent and mixed well. Precision was evaluated with five injections of the working standard. Linearity was determined across the concentration range of 2.5 µg/mL to 100 µg/mL. Accuracy was evaluated by spiking one gram of corn oil in a 10 mL vol. flask with 2.0 mL of working standard solution as to obtain (when brought to vol. with diluent) a final solution of 20 µg/mL of sunscreen agent or 0.02% sunscreen in the oil. Samples were thoroughly mixed and centrifuge for 10 min. at 5000 RPM; the aliquot was filtered through a 0.2 µm nylon membrane prior to testing. Because Azobenzene consists in two photoisomers, the cis isomer and the trans isomer, the standard was exposed to light overnight prior to testing to convert as much of the cis form as possible to the more stable trans form.

A PerkinElmer® Flexar™ FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector was the platform for this experiment. The separation was achieved using a PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm column with 2.7 µm superficially porous particles.

***Table 1.* Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC Setting: 50 µL Loop and 15 µL needle volume, partial loop mode 350 µL mixer volume Injection 2 µL; injector wash and carrier: water
PDA Detector:	Scanned from 190 – 700 nm, recording setting 240 nm Reference 400 nm, bandwidth 10 nm
UHPLC Column:	PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm at 45 °C Cat # N9308402
Mobile Phase:	A: 0.1% Phosphoric acid in water B: 0.1% Phosphoric acid in 60:40 (v/v) Acetonitrile/water

Time (min)	Flow rate (mL/min)	B %	Curve
4	0.6	10-100	1
2	0.6	100	1

	Three minutes equilibration after injection.
Software:	Chromera® Version 3.0
Sampling Rate:	5 pts/sec

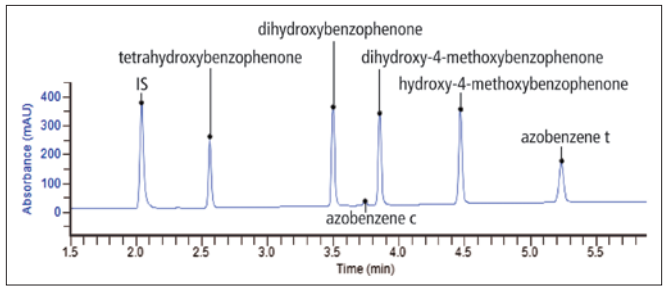

Figure 2. Chromatogram from the analysis of the standard solution.

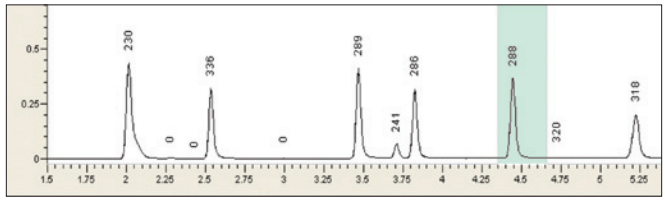

Figure 3. Chromatogram from the analysis of the standard solution showing the maximum absorbance for each peak.

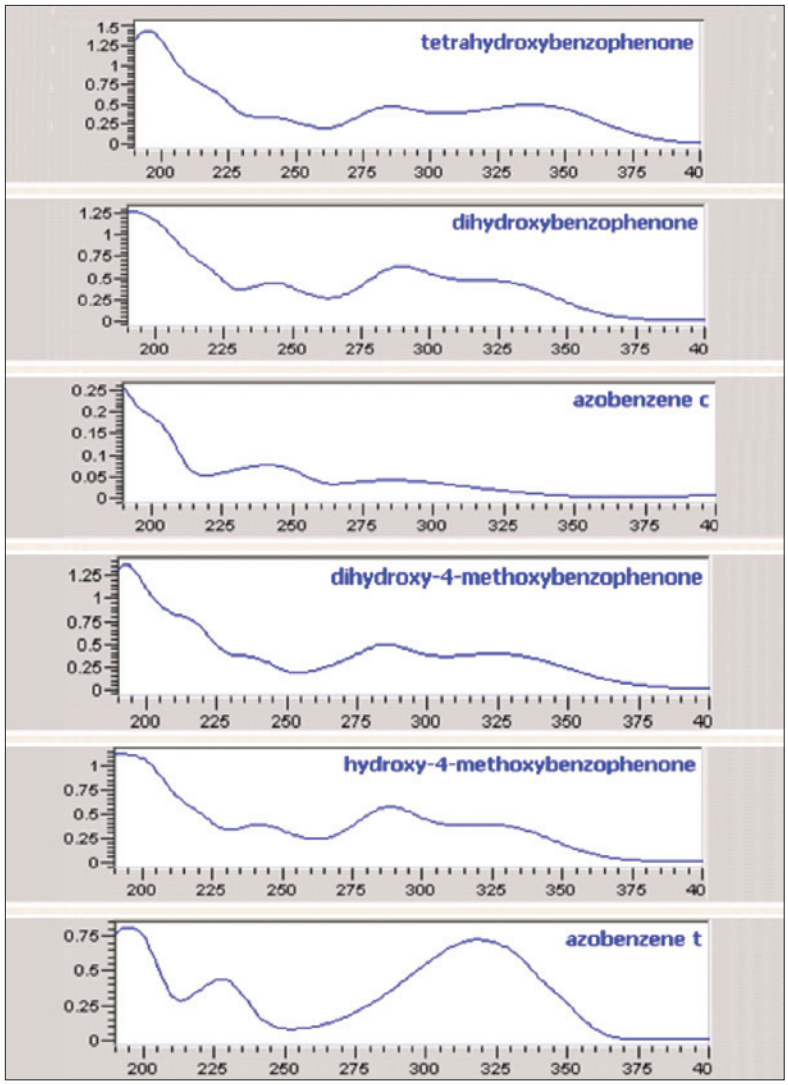

Figure 4. UV spectra from the standard solution run.

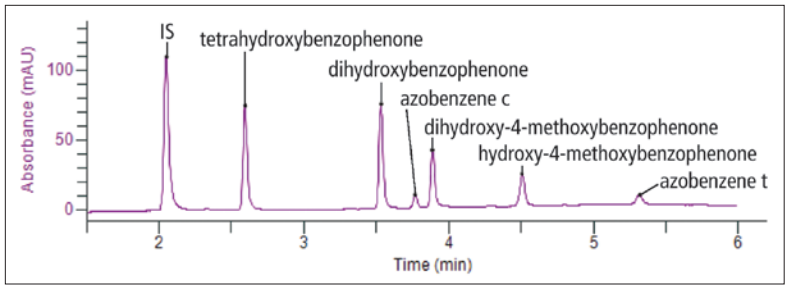

Figure 5. Chromatogram from the analysis a 0.02% spiked corn oil.

***Table 2.* Precision and amount in samples.**

Compound	Precision	r^2	Accuracy
2,2',4,4'-Tetrahydroxybenzophenone	0.66	0.999	105.2
2,4-Dihydroxybenzophenone	0.46	0.999	93.5
2,2-Dihydroxy-4-methoxybenzophenone	0.55	0.999	97.0
2-hydroxy-4-methoxybenzophenone	0.55	0.999	96.7
Azobenzene	0.49	0.999	90.2
Average	**0.54**	**0.999**	**96.5**

Results and Discussion

The column temperature was set at 45 °C, and the optimal flow rate was determined to be 0.6 mL/min. Pressure stabilized at about 3000 PSI and the five peaks eluted within six min. All the peaks were well resolved and the method performance was excellent. From 2.5 µg/mL to 100 µg/mL, the method was linear with r-squared not less than 0.999 for all five components. Precision values ranged from 0.5% to 0.7% RSD, and the average recovery was 97%. Details of the method performance are in Table 2.

Chromatograms from the standard solution preparations are shown in Figures 2, and the chromatogram showing the maximum absorbance for each sunscreen agent is presented in Figure 3. The spectral library created from the standard solution is presented in Figure 4 and a chromatogram of a corn oil spiked with the sunscreen agents is shown in Figure 5.

Conclusion

PerkinElmer's Flexar FX-15 UHPLC system and the PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm column with a 2.7 µm superficially porous particle resolved all six sunscreen compounds within six min. The method was shown to be precise with a %RSD average of 0.5, linear with r-squared of no less than 0.999; the average accuracy was 97%, ranging from 90% to 105%.

References

1. National Institutes of Health http://ods.od.nih.gov/factsheets/vitamind, retrieved 11/23/11

2. Simultaneous Identification of Eight Sunscreen Compounds in Cosmetic Product, Journal of Food and Drug Analysis, Vol 16, No. 6, 2008, pages 22-28.

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