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Application News

No. L546

High Performance Liquid Chromatography

Qualitative Analysis of UV-Absorbents in Cosmetics Based on UV-Vis Spectrum

Many cosmetic products contain ultraviolet absorbers (UV-absorbents) which protect the skin from ultraviolet rays. In Japan, ingredients of UV-absorbents and their allowable quantities are regulated under Standards for Cosmetics (2000, Ministry of Health, Labour and Welfare Notification No. 331) based on the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (PMD Act). Because these standards differ depending on the country and region, compliance with the applicable regulations is verified by high performance liquid chromatography (HPLC) when importing/exporting cosmetics.

Against this background, Application News No. L541 introduced an example of high-speed analysis of 23 UV-absorbents. This article introduces a method for qualitative analysis of six UV-absorbents based on the UV-Vis spectrum.

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Analysis of Standard Substances

In this article, six UV-absorbents shown in Fig. 1 were focused on, and Fig. 1 shows the chromatogram of a mixed standard solution of six UV-absorbents (100 mg/L each). Table 1 shows the analytical conditions.

Fig. 2 on the following page shows the UV-Vis spectra of each UV-absorbent obtained by analyzing the mixed standard solution (100 mg/L of each UV-absorbent). A qualitative analysis of the UV-absorbents contained in cosmetics was conducted based on these spectra.

Table 1 Analytical Conditions

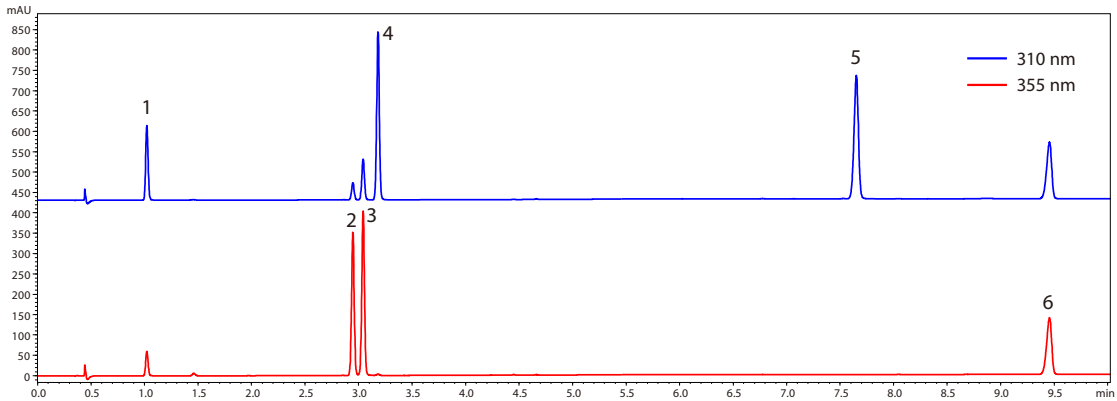
Column	: Shim-pack Velox™ C18 (100 mm L. × 3.0 mm I.D., 2.7 µm)
Mode	: High pressure gradient
Mobile phase	: A) 0.085% Phosphoric acid in water
	: B) Acetonitrile
Time program	: 60%B (0 min) → 60%B (0.5 min) → 90%B (4 min) → 100%B (10 min) → 60%B (10.01 min) → 60%B (15 min)
Flow rate	: 1 mL/min
Column temp.	: 60 °C
Injection volume	: 1 µL
Detection	: SPD-M40 (190-800 nm)

Linearity and Repeatability

Calibration curves for each UV-absorbent were prepared from the mixed standard solution of six UV-absorbents. Calibration curves for the calibration points of 1, 5, 25, 50, and 100 mg/L were prepared for each absorbent, and their linearity was evaluated. Repeatability of retention time and area was evaluated by a repeated analysis (n = 6) at 100 mg/L. Table 2 shows these results. Satisfactory linearity with a contribution ratio (R^2) of 0.9999 or more was obtained for all UV-absorbents. Repeatability was also satisfactory in terms of both retention time and peak area.

Table 2 Linearity and Repeatability of Six UV-Absorbents

No.	Compound	Linearity (R^2)	Retention time (%RSD)	Area (%RSD)
1	2-hydroxy-4-methoxybenzophenone	0.9999	0.24	0.35
2	4-*tert*-butyl-4'-methoxydibenzoylmethane	0.9999	0.10	0.34
3	2-ethylhexyl-4-methoxycinnamate	0.9999	0.09	0.29
4	2-[4-(diethylamino)-2-hydroxybenzoyl]benzoic acid hexyl ester	0.9999	0.08	0.33
5	2,4,6-tris[4-(2-ethylhexyloxycarbonyl)-anilino]-1,3,5-triazine	0.9999	0.03	0.30
6	bis-ethylhexyloxyphenol methoxyphenyl triazine	0.9999	0.02	0.33



1. 2-hydroxy-4-methoxybenzophenone/2. 4-*tert*-butyl-4'-methoxydibenzoylmethane/3. 2-ethylhexyl-4-methoxycinnamate/4. 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoic acid hexyl ester/5. 2,4,6-tris[4-(2-ethylhexyloxycarbonyl)-anilino]-1,3,5-triazine/6. bis-ethylhexyloxyphenol methoxyphenyl triazine

Fig. 1 Chromatogram of Mixed Standard Solution of Six UV-Absorbents (100 mg/L Each)

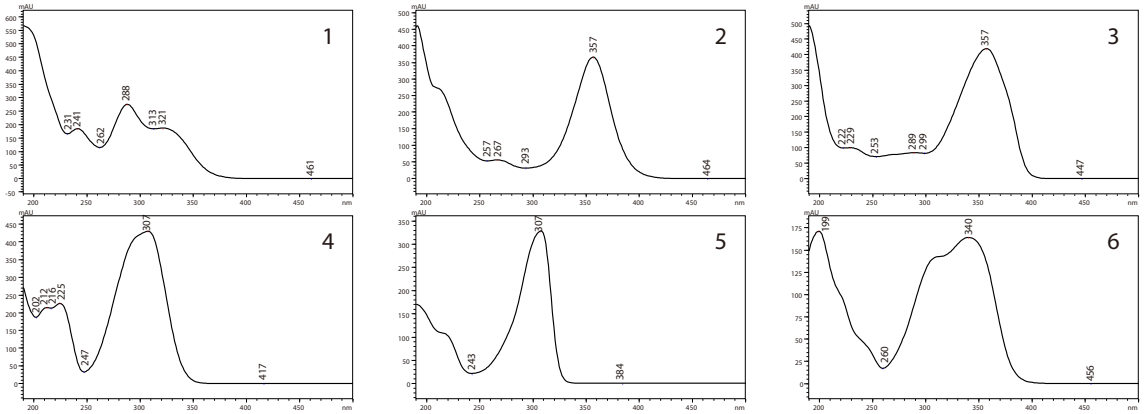


Fig. 2 UV-Vis Spectra of Six UV-Absorbents

Analysis of Cosmetic Product (Foundation)

100 mg of a commercial foundation was weighed, 5 mL of tetrahydrofuran (THF) was added, and ultrasonic extraction was conducted, followed by centrifugal separation, and 0.5 mL of the supernatant was adjusted to a constant volume of 10 mL with THF. The sample for analysis was then prepared by filtering that solution with a 0.22 μm pore size membrane filter.

Fig. 3 shows the chromatogram of the foundation. It was clarified that three types of UV-absorbents are contained in the foundation, and their contents satisfied the regulation values.

Fig. 4 shows the superimposed UV-Vis spectra of compound 4 in the analysis of the mixed standard solution and analysis of the cosmetic product. Similar spectra were obtained for the standard sample and the actual sample, demonstrating that qualification based on UV-Vis spectra is possible.

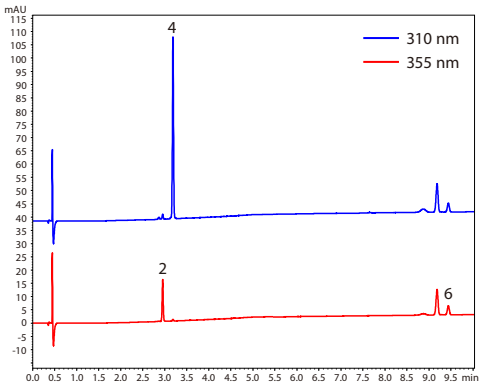


Fig. 3 Chromatogram of Foundation

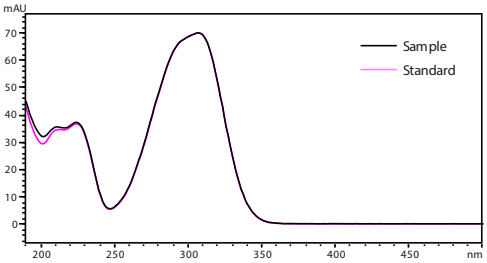


Fig. 4 Comparison of UV-Vis Spectra of Compound 4

Qualitative Analysis Based on UV-Vis Spectrum

In HPLC, retention time is used to qualify compounds, and for this reason, qualitative analysis is not a strong point of HPLC in comparison with NMR, LC-MS and similar techniques that can acquire information on the chemical structure. However, use of a photo diode array (PDA) detector makes it possible to obtain spectral information, and as a result, more reliable qualitative analysis also becomes possible with HPLC.

When the UV-Vis spectra of compounds are registered in the library file of the LabSolutions™ workstation manufactured by Shimadzu, it is possible to compare unknown spectra with the spectra in the library file. Moreover, quantitative evaluation using numerical values, and not simple visual comparison of the similarity between the spectra, is also possible.

The spectra of six UV-absorbents with concentrations of 100 mg/L were registered in the library, and were then compared with the spectra of 1 mg/L mixed standard solutions of each UV-absorbent and the foundation in the library. Table 3 shows the results. The spectra of each UV-absorbent at 1 mg/L shows high similarity with the 100 mg/L mixed solution, demonstrating that similar spectra can be obtained over a wide range of concentrations. Furthermore, because the spectra of the UV-absorbents contained in the foundation also displayed high similarity with the standard sample, it can be understood that using similarity is an effective technique for qualitative analysis.

Table 3 Comparison of Similarity of Spectra

No.	Similarity	
	Standard (1 mg/L)	Sample
1	0.999979	-
2	0.991281	0.998648
3	0.999114	-
4	0.999913	0.999988
5	0.999938	-
6	0.999957	0.999991

Similarities are calculated from the spectra from 230 nm to 500 nm.

Conclusion

This article introduced a qualitative analysis method based on the UV-Vis spectrum. Although this introduction is only a simple comparison of similarity, setting for automatic peak identification based on similarity and retention time is also possible. As demonstrated in this experiment, it is possible to enhance qualitative analysis capabilities by using a PDA detector.

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