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Application News

No. L574A

High Performance Liquid Chromatography

Analysis of Formaldehyde Using Post-Column Derivatization with Acetylacetone

Formaldehyde, which is used as an ingredient of wood preservatives and resin products, has attracted considerable attention because it is a causative agent of the sick house syndrome.

Ingredients contained in shampoos, skin lotions, foundation products, and other cosmetics are subject to particularly strict regulations because they are applied to the human body. Japan's Standards for Cosmetics (Ministry of Health and Welfare Notification No. 331, 2000) prohibit the inclusion of formaldehyde in cosmetics. Moreover, in the EU, the content of formaldehyde in nail polish and other nail products is limited to no more than 5% under Regulation (EC) No. 1223/2009, Annex III.

In this study, formaldehyde was detected in cosmetics using the HPLC method and post-column derivatization with acetylacetone, which is an established test method under the Methods of Analysis in Health Science (The Pharmaceutical Society of Japan, 2015).

This article introduces formaldehyde analysis using a Nexera™ Series Nexera XR ultra high performance liquid chromatograph.

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System and Analysis Conditions

The Shim-pack™ GIST C18-AQ column used in this experiment can achieve strong retention of high-polar compounds such as formaldehyde, compared to general ODS columns, and thus can maintain good retention and a superior peak shape in highly or 100% aqueous mobile phases.

Fig. 1 shows the flow path diagram of the system used in this analysis. Fig. 2 shows the appearance of the system. Table 1 lists the analysis conditions.

Table 1 Analysis Conditions

	System	: Nexera XR
■	Separation	
	Column	: Shim-pack GIST C18-AQ *1 (150 mm × 4.6 mm I.D., 5 µm)
	Mobile Phase	: 6 mmol/L Na_2HPO_4 (pH=2.1) *2
	Flow Rate	: 1.0 mL/min
	Column Temp.	: 30 °C
	Injection Vol.	: 10 µL
	Vial	: LabTotal Vial*3
■	Post Column Derivatization and Detection	
	Reaction Reagent	: Solution of acetyl acetone *4
	Flow Rate	: 0.5 mL/min
	Reaction Temp.	: 90 °C
	Detection	: SPD-M40 at 414 nm

*1 P/N: 227-30742-07
*2 Using phosphoric acid, adjust to pH 2.1.
*3 P/N: 227-34001-01
*4 Adjust to a constant volume of 1,000 mL while dissolving 150 g of ammonium acetate, 3 mL of acetic acid, and 2 mL of acetylacetone in ultrapure water.

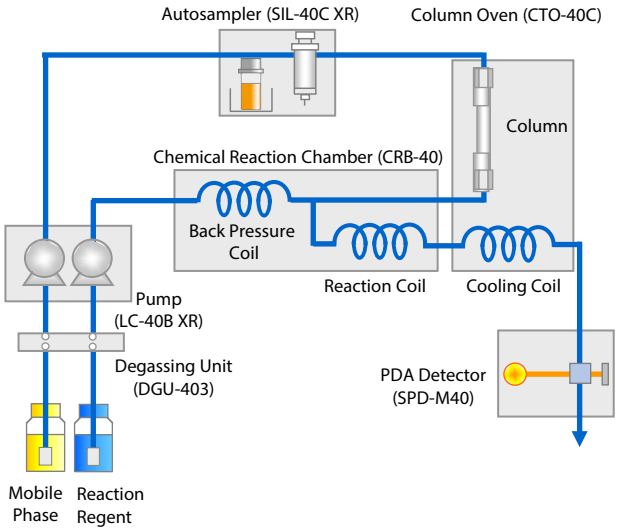

Fig. 1 Flow Path Diagram of Analytical System

Fig. 2 Analytical System

Derivatization of Formaldehyde by Acetylacetone

Formaldehyde was reacted with two molecules of acetylacetone in the presence of ammonium acetate, and one molecule of 3,5-diacetyl-1,4-dihydrolutidine was formed, as shown in Fig. 3. This reaction product (derivative) is selectively detected using a PDA detector (414 nm).

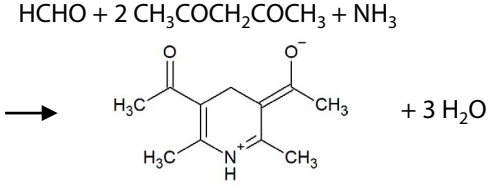

Fig. 3 Reaction of Formaldehyde and Acetylacetone

Analysis of Formaldehyde Standard Solution

Table 2 shows the sample solvent established by Japan's Standards for Cosmetics. Two types of the calibration curve were obtained for water-soluble or water-insoluble samples. Fig. 4 shows the chromatogram obtained by analyzing the standard solution of formaldehyde.

The formaldehyde standard solution was adjusted by diluting 100 mg/L (water medium) of the standard with water or water/THF=20:80. In analyses where the mobile phase contains no organic solvents, the peak shape may be degraded when methanol, acetonitrile, or the other organic solvents are added to the standard solution.

Fig. 5 shows the calibration curve prepared with the formaldehyde standard solution for five points in the concentration range of 0.05–1.0 mg/L. Although preparation of a calibration curve for the concentration range of 1–4 mg/L is specified in the cosmetic test method, in this experiment the calibration curve was prepared for a lower concentration range to quantify formaldehyde at the trace level.

Good linearity was obtained with the coefficient of determination r^2 greater than 0.9999.

Table 2 Solvent for Sample and Standard Established by Japan's Standards for Cosmetics

	Solvent for Sample	Solvent for Standard
Water-soluble	Water	Water
Water-insoluble	Water/THF = 50:50	Water/THF = 20:80

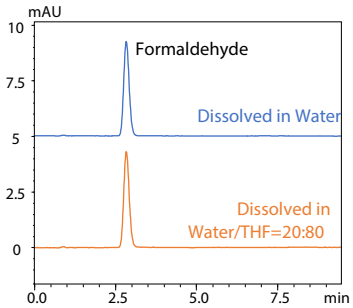

Fig. 4 Chromatogram of Formaldehyde Standard Solution (1.0 mg/L)

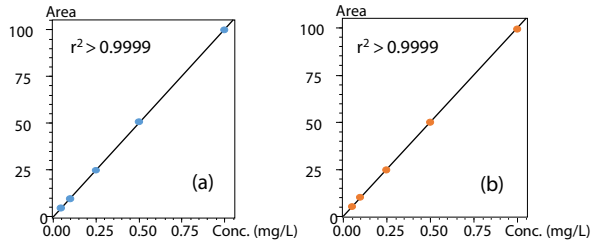

Fig. 5 Calibration Curve of Formaldehyde Standard Solution (a) Dissolved in Water, (b) Dissolved in Water/THF=20:80

Table 3 Concentrations of Formaldehyde in the Sample, and Recovery Rates of Each Pretreatment

	Formaldehyde (mg/L)	Recovery Rate (%)
Skin Lotion	<0.05	102
Shampoo	n.d.*	102
Hair Conditioner	<0.05	97

*n.d.: not detected

Analysis of Formaldehyde in Shampoo, Conditioner, and Skin lotion

Figs. 6–8 show the chromatograms of the solutions extracted by pretreatment of the shampoo, conditioner, and skin lotion, together with the chromatograms of samples obtained by spiking those solutions with formaldehyde to a concentration of 0.1 mg/L. Table 3 shows the results of the spike and recovery tests. In all the samples, the formaldehyde concentration was below the minimum concentration (0.05 mg/L) of the calibration.

In the pretreatment step, 20 mL of water was added to 0.2 g of the sample, or 10 mL of THF was added to 0.2 g of the sample and diluted with 10 mL of water. After stirring, the solutions were filtered with a membrane filter (0.45 µm).

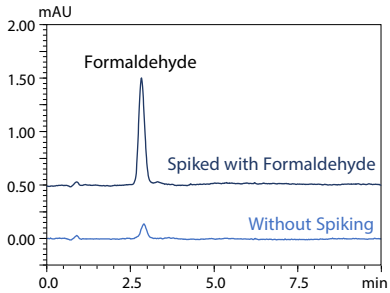

Fig. 6 Chromatogram of Skin Lotion (Dissolved in Water)

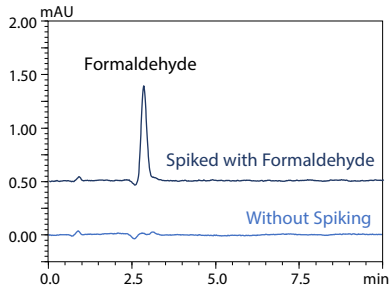

Fig. 7 Chromatogram of Shampoo (Dissolved in Water)

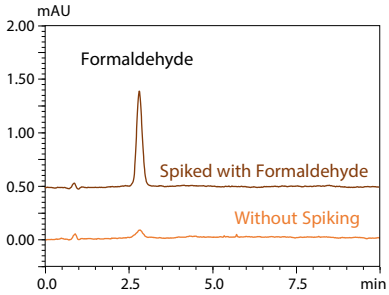

Fig. 8 Chromatogram of Hair Conditioner (Dissolved in Water/THF=50:50)

Conclusion

Formaldehyde was measured by the HPLC post-column derivatization with acetylacetone using a Nexera XR ultra high performance liquid chromatograph. As detection at the wavelength with high selectivity was possible by derivatizing formaldehyde, the analysis was substantially unaffected by impurities in the sample.

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