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Application News

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High Performance Liquid Chromatography

# Analysis of Formaldehyde Using HPLC and Post-Column Derivatization with Acetylacetone

Formaldehyde is a useful ingredient for wood preservatives and resin products, but because it is a causative agent of sick house syndrome, it has attracted attention.

Shampoos, skin lotion, and foundation products used in everyday life are generally called cosmetics, and the ingredients used in them are subject to particularly strict regulation because they are applied to the human body. Japan's Standards for Cosmetics (Ministry of Health and Welfare Notification No. 331, 2000) list formaldehyde as one material which is prohibited to include 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 article, formaldehyde in cosmetics was detected using the HPLC method and the 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 examples of analysis of formaldehyde 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 achieves strong retention of high-polarity compounds such as formaldehyde, compared to general ODS column, 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, and Fig. 2 shows the appearance of the system. Formaldehyde is detected by a process of separation with an ODS reversed-phase column, followed by online reaction with acetylacetone at 90 °C, and selective detection of the reaction product (3,5-diacetyl-1,4-dihydrolutidine) by using a PDA detector (414 nm). Table 1 shows the analysis conditions. Because temperature control up to 100 °C is possible with the CTO-40C column oven of the Nexera XR used in the analysis, the oven could be converted to use as a chemical reactor under the conditions of this analysis. Although the conventional chemical reactor (CRB-6A) can not be controlled from a workstation, the CTO-40C can. Integrated management of the CTO-40C such as setting the reaction temperature, logging the oven temperature, and the use time of consumables is performed by the workstation.

**Table 1 Formaldehyde Analysis Conditions**

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

*1 Using phosphoric acid, adjust to pH = 2.1.

*2 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.

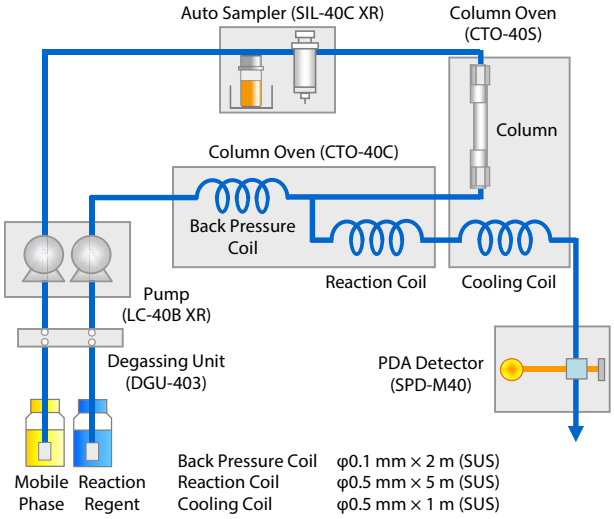


**Fig. 1 Flow Path Diagram of Analytical System**


**Fig. 2 Image of Appearance of Analytical System**

## Derivatization of Formaldehyde by Acetylacetone

Formaldehyde was reacted with two molecules of acetylacetone in the presence of ammonium acetate and formed one molecule of 3,5-diacetyl-1,4-dihydrolutidine, as shown in Fig. 3. The analysis was conducted using this reaction product (derivative).

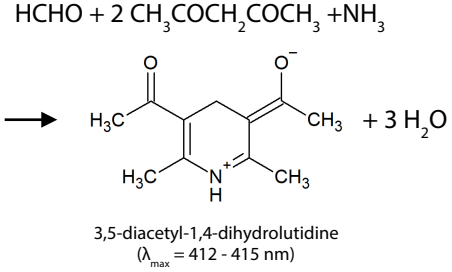


**Fig. 3 Reaction of Formaldehyde and Acetylacetone**

## Analysis of Formaldehyde Standard Solution

Fig. 4 shows the chromatogram obtained by analysis of the standard solution of formaldehyde. A peak corresponding to 3,5-diacetyl-1,4-dihydrolutidine, which was derivatized from formaldehyde, was detected at around 2.8 min. The formaldehyde standard solution was adjusted by diluting 100 mg/L (water medium) of the standard with ultrapure water. Because the mobile phase contains no organic solvents, the peak shape may be degraded when methanol, acetonitrile, or the other organic solvents were added to the standard solution.

Fig. 5 shows the calibration curve prepared with the formaldehyde standard solution for 5 points in the concentration range from 0.01 to 1.0 mg/L. Although preparation of a calibration curve for the concentration range of 1 to 4 mg/L is specified in the cosmetic test method, in this experiment the calibration curve was prepared for a lower concentration range than in that test method in order to quantify formaldehyde at the trace level.

Good linearity was obtained, as the coefficient of determination $r^2$ = 0.9999 or higher.

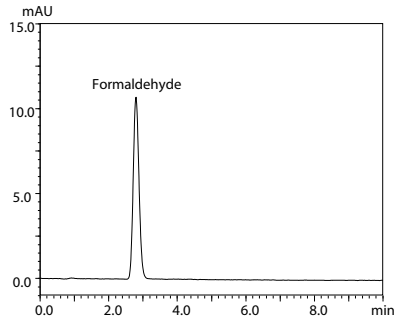


**Fig. 4 Chromatogram of Formaldehyde Standard Solution (1.0 mg/L)**

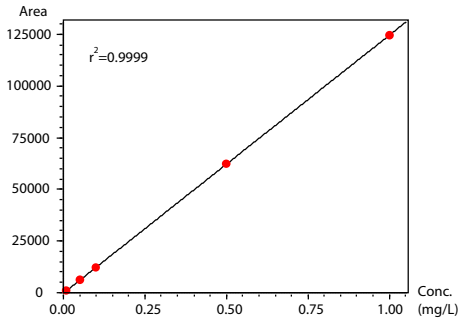


**Fig. 5 Calibration Curve of Formaldehyde Standard Solution**

## Analysis of Formaldehyde in Shampoo, Conditioner, and Skin lotion

A shampoo, conditioner, and skin lotion which are sold commercially in Japan were used as cosmetics. The analysis was carried out after extracting samples from these 3 products with water. As mentioned above, use of formalin in cosmetics that are manufactured, sold, or distributed domestically in Japan is prohibited.

Fig. 6 to Fig. 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 2 shows the results of the spike and recovery tests. In all the samples of shampoo, conditioner, and skin lotion, the formaldehyde concentration was below the minimum concentration (1 mg/L) of the calibration point specified in the cosmetic test method.

In the pretreatment, 100 mL of ultrapure water was added to 1g of each sample, and after stirring, the solutions were filtered with a membrane filter (0.45 µm).

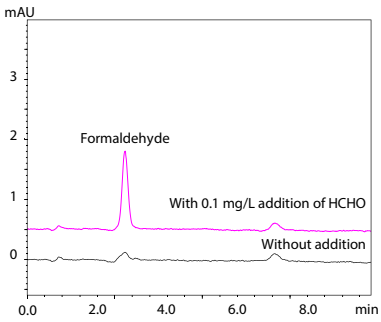


**Fig. 6 Chromatogram of Commercially Available Shampoo**

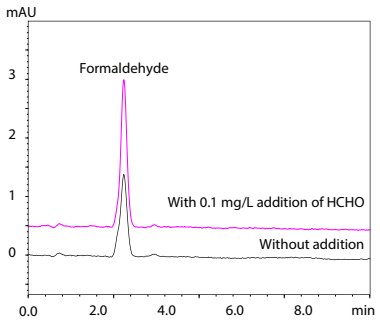


**Fig. 7 Chromatogram of Commercially Available Conditioner**

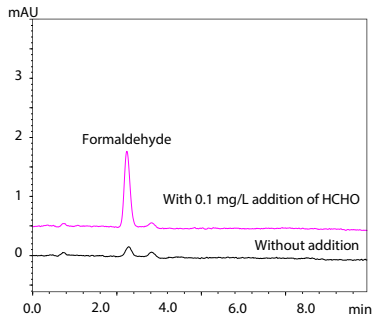


**Fig. 8 Chromatogram of Commercially Available Skin lotion**

**Table 2 Concentrations of Formaldehyde in Shampoo, Conditioner and Skin lotion, and Recovery Rates of Each Pretreatment**

| Product | Formaldehyde (mg/L) | Recovery Rate (%) |
|---|---|---|
| Shampoo | 0.025 | 101 |
| Conditioner | 0.167 | 104 |
| Skin lotion | 0.018 | 109 |

## Conclusion

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



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