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Application News

# Simultaneous Analysis of Synthetic Colorants, Including Red No. 3, Using Integrated LC System

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## User Benefits

- The integrated LC system i-Series enables simultaneous analysis of 12 synthetic colorants, including Red No. 3.
- The organic silica hybrid-based Shim-pack™ NovaCore C18-HB provides a wide pH tolerance range from 1 to 12 for both the mobile phase and sample solvent.
- LabSolutions™ MD, dedicated software for supporting method development, enables efficient exploration of separation conditions.

## ■ Introduction

Food colorants can be categorized into natural and synthetic types. The availability of synthetic colorants varies across countries, depending on regulatory frameworks. In Japan, twelve synthetic colorants are currently approved as food additives, and their safety can be assessed through qualitative and quantitative analysis using liquid chromatography. Recently, the U.S. Food and Drug Administration announced a prohibition on the use of Red No. 3, one of these twelve colorants, in food products, further increasing interests regarding the safety of synthetic colorants. This study presents an example of simultaneous analysis of twelve synthetic colorants using the integrated LC system i-Series (Fig. 1) in combination with the organic silica hybrid core-shell column, Shim-pack NovaCore C18-HB. The optimization of separation conditions was performed efficiently using LabSolutions MD, a dedicated software for supporting method development. Furthermore, the applicability of the optimized method is demonstrated through the analysis of real samples.

## ■ About Shim-pack NovaCore C18-HB

Shim-pack NovaCore C18-HB is a core-shell column incorporating an organic silica hybrid material. Conventional silica gel is known for its low chemical stability under basic conditions, with a pH tolerance range of 2 to 7.5. In contrast, organic silica hybrid materials exhibit a broader pH tolerance, ranging from 1 to 12. As a result, the Shim-pack NovaCore C18-HB is applicable not only under acidic conditions but also in basic pH environments, making it suitable for method development processes. In core-shell particles, the inner core is non-porous, while the outer layer is porous. In contrast, fully porous particles allow analytes to diffuse deeply into the inner structure, where separation and diffusion occur. In core-shell particles, however, molecular diffusion is restricted to the thin outer porous layer, leading to more efficient separation due to reduced band broadening. This structural difference enables core-shell columns to achieve sharper peak shapes and shorter analysis times.

## ■ Target Compounds and Analytical Conditions

The twelve synthetic colorants classified as tar colorants and the corresponding analytical conditions are presented in Table 1 and 2, respectively. Initially, separation conditions were optimized using a mixed standard solution of the twelve colorants with the aid of LabSolutions MD. The optimized method was subsequently applied to the analysis of real samples.

Table 1 Target Compounds

| Compound name | |
|---|---|
| R2 (Amaranth) | R106 (Acid Red) |
| R3 (Erythrosine) | Y4 (Tartrazine) |
| R40 (Allura Red AC) | Y5 (Sunset Yellow FCF) |
| R102 (New Coccine) | G3 (Fast Green FCF) |
| R104 (Phloxine B) | B1 (Brilliant Blue FCF) |
| R105 (Rose Bengal) | B2 (Indigo Carmine) |

Fig. 1 Integrated LC System "i-Series" (LC-2070C 3D)

Table 2 Analytical Conditions

| | |
|---|---|
| System | : LC-2070C 3D |
| Column | : Shim-pack NovaCore C18-HB (100 mm × 3.0 mm I.D., 2.6 µm) *1 |
| Temperature | : 40 °C |
| Injection volume | : 3 µL |
| Mobile phases | |
| Pump A | : 20 mmol/L ammonium acetate |
| Pump B – Line A | : Acetonitrile |
| – Line B | : Methanol |
| Flow rate | : 0.6 mL/min |
| Time program (%B) | : 5% (0 min) →95% (10-12 min)→5% (12-17 min) |
| Sample Conc. | : 50 mg/L |
| Sample solvent | : Water |
| Detection | : 254 nm (STD cell) |

*1 P/N : 227-32902-08

## ■ Optimization of Separation Conditions for the Twelve Tar Colorants

Optimal separation conditions for the mixed standard solution of twelve synthetic tar colorants were investigated by varying the mixing ratio of acetonitrile and methanol in the organic solvent of the mobile phase. Specifically, an analytical schedule was designed in which the methanol ratio in acetonitrile was incrementally adjusted from 0% to 100% in 10% steps (a total of eleven levels) to determine the optimal composition. LabSolutions MD facilitates the automatic creation of analytical schedules (steps (1) to (5) in Fig. 2) under various conditions, including different mobile phase compositions and column selections, minimizing the risk of human error. Additionally, the organic solvent composition was adjusted automatically using the mobile phase blending function. By simply selecting the desired solvent compositions from a predefined list (Fig. 2, step (1)), the mobile phase is prepared with the specified organic solvent ratio, significantly reducing manual preparation efforts and preventing errors associated with solvent preparation.

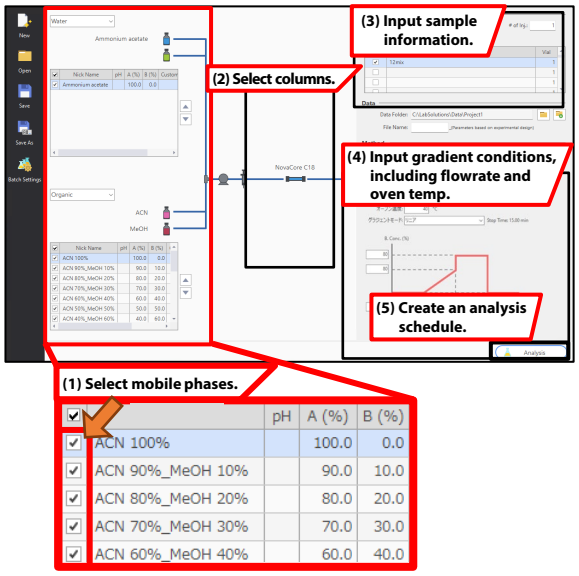


Fig. 2 Steps for Creating Analysis Schedule

## ■ Optimization of Organic Solvent Composition in Mobile Phase

Fig. 3 displays the chromatograms obtained when the methanol ratio in acetonitrile was varied from 0% to 100% in 10% steps. Under the condition of 0% methanol (chromatogram (1) in Fig. 3), R104 and R106 were not completely separated (Rs < 1.5). As the separation of R104 and R106 improved with the increase in the methanol ratio in acetonitrile, a methanol ratio of 20% (chromatogram (3) in Fig. 3) was selected as the optimal condition, considering both the improvement in separation and the need to shorten the analysis time. In this study, an aqueous ammonium acetate solution, which exhibits approximately neutral pH (pH around 7), was used as the aqueous mobile phase. Despite these conditions, the Shim-pack NovaCore C18-HB column demonstrated high durability due to the organic silica hybrid material used in its construction.

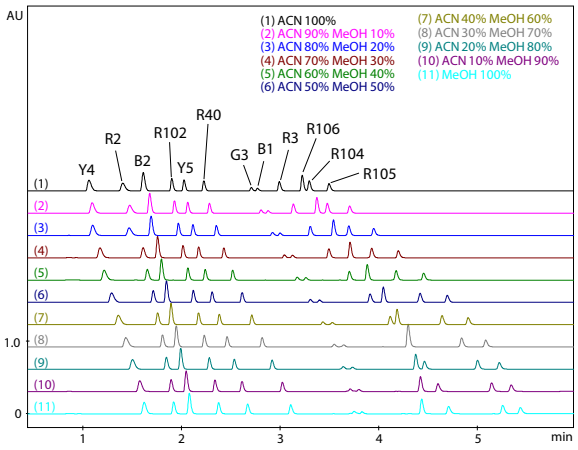


Fig. 3 Chromatograms Obtained at Different Organic Solvent Composition

## ■ Analysis of Real Samples

Chromatograms obtained by applying the optimized method (Table 2, Pump B: Acetonitrile / Methanol = 80 : 20) to the analysis of four real samples are shown in Fig. 4 to 7. As a result of the analysis, synthetic colorants were successfully detected in each sample.

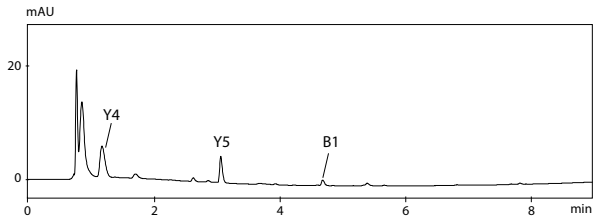


Fig. 4 Chromatogram of Konpeito Sugar Candy
(Dissolved in ultrapure water and 0.2 µm filtration)

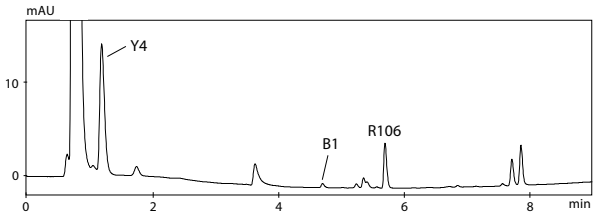


Fig. 5 Chromatogram of Tablet-type Candy (ramune)
(Dissolved in ultrapure water and 0.2 µm filtration)

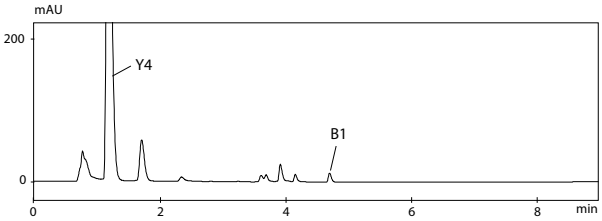


Fig. 6 Chromatogram of Carbonated Soft Drink (melon soda)
(0.2 µm filtration)

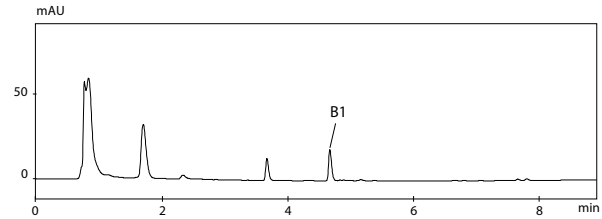


Fig. 7 Chromatogram of Shaved Ice Syrup (hawaiian blue)
(0.2 µm filtration)

## ■ Conclusion

A case study was presented on the simultaneous analysis of twelve synthetic tar colorants using i-Series, Shim-pack NovaCore C18-HB column, and LabSolutions MD for optimizing separation conditions. The core-shell column is constructed with an organic silica hybrid material, allowing the use of a wide pH range (from 1 to 12) for both mobile phases and sample solvents. LabSolutions MD also contributed to streamlining the process of separation condition optimization.



01-00935A-EN First Edition: Sep. 2025
Revision A: Oct. 2025

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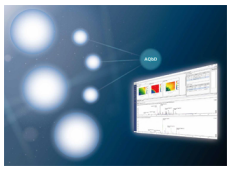

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