

## Application News

Software for Efficient Method Development “LabSolutions™ MD”  
Organic Silica Hybrid Core-Shell Column “Shim-pack™ NovaCore C18-HB”

### Simultaneous Analysis of Synthetic Colorants, Including Red No. 3, Using an Organic Silica Hybrid Core-Shell Column

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#### User Benefits

- ◆ 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.
- ◆ A reduction in analysis time compared to fully porous columns can be achieved because separation on the Shim-pack NovaCore C18-HB column occurs only in the porous outer layer of the particle (core-shell column).
- ◆ 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 the optimization of separation conditions for the simultaneous analysis of these twelve synthetic colorants using the organic silica hybrid core-shell column, Shim-pack NovaCore C18-HB, in combination with LabSolutions MD, a dedicated software for supporting method development. Furthermore, the applicability of the optimized method is demonstrated through the analysis of a real sample, Konpeito sugar candy.

#### 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 Konpeito sugar candy.

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)

Table 2 Analytical Conditions

System	: Nexera™ X3 (Method Scouting System)
Column	: Shim-pack NovaCore C18-HB (100 mm × 3.0 mm I.D., 2.6 μm)*1
Temperature	: 40 °C
Injection volume	: 5 μL (STD), 10 μL (sample)
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% (5-7 min) → 5% (7-15 min)
Sample Conc.	: 50 mg/L
Sample solvent	: Water
Detection	: 254 nm (SPD-M40, 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. 1) 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. 1, 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.

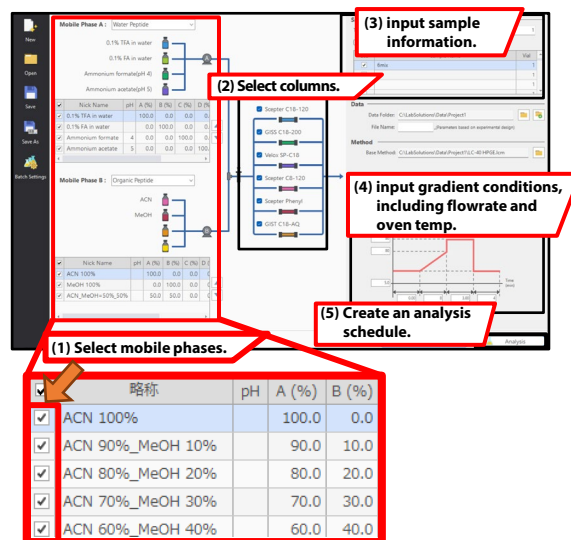


Fig. 1 Steps for Creating Analysis Schedule

## ■ Optimization of Organic Solvent Composition in Mobile Phase

Fig. 2 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. 2), R104 and R106 were not completely separated. 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. 2) 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.

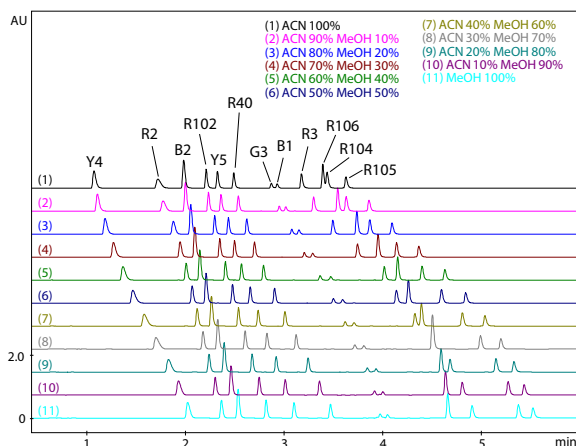


Fig. 2 Chromatograms Obtained at Different Organic Solvent Composition

## ■ Analysis of Konpeito Sugar Candy

The chromatogram obtained by applying the optimized method (analytical conditions : Table 2) to the analysis of Konpeito sugar candy is shown in Fig. 3. For sample preparation, Konpeito was dissolved in ultrapure water, followed by filtration through a 0.22  $\mu$ m membrane filter. The analysis resulted in the detection of Y4, Y5, and B1 in the sample.

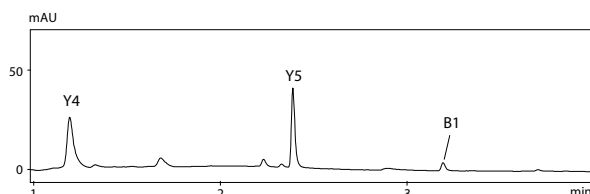


Fig. 3 Chromatogram of Konpeito Sugar Candy  
(Pump B: Acetonitrile/Methanol = 80:20)

## ■ Comparison of Analysis Time with Fully Porous Column

Fig. 4 presents a comparison of chromatograms obtained from the mixed standard solution using a core-shell column (Shim-pack NovaCore C18-HB) and a fully porous column (Shim-pack Scepter C18-120), both of the same dimensions (length : 150 mm, inner diameter : 4.6 mm; analytical conditions : Table 3). The gradient profile in Table 3 was automatically generated by the method transfer software (a standard feature of LabSolutions), based on the gradient in Table 2, with adjustments made to account for differences in column dimensions. The core-shell column demonstrated a shorter analysis time compared to the fully porous column, as separation occurs only in the thin porous outer layer of the particle. This allows for a reduction in analysis time simply by switching from a fully porous column to a core-shell column, without the need for time-consuming and tedious modifications to the analytical conditions. The reduction in analysis time generally leads to decreased mobile phase consumption, resulting in cost savings and a reduction in environmental impact.

Table 3 Analytical Conditions

System	: Nexera lite
Column	: Shim-pack NovaCore C18-HB (150 mm $\times$ 4.6 mm I.D., 5 $\mu$ m) <sup>*1</sup> Shim-pack Scepter™ C18-120 (150 mm $\times$ 4.6 mm I.D., 5 $\mu$ m) <sup>*2</sup>
Temperature	: 40 °C
Injection volume	: 10 $\mu$ L
Mobile phases	
Pump A	: 20 mmol/L ammonium acetate
Pump B	: Acetonitrile/Methanol = 80:20
Flow rate	: 1 mL/min
Time program (%B)	: 5% (0-0.6 min) $\rightarrow$ 95% (11.2-15.4 min) $\rightarrow$ 5% (15.4-30 min)
Sample Conc.	: 50 mg/L
Sample solvent	: Water
Detection	: 254 nm (SPD-M40, STD cell)

\*1 P/N : 227-32903-11

\*2 P/N : 227-31020-05

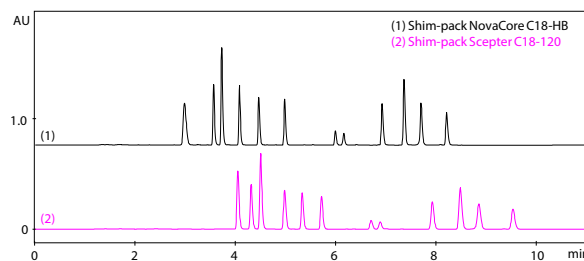


Fig. 4 Comparison of Core-Shell (above) and Fully Porous Column (below)

## ■ Conclusion

A case study was presented on the optimization of separation conditions for the simultaneous analysis of twelve synthetic tar colorants using Shim-pack NovaCore C18-HB column and LabSolutions MD, followed by the analysis of a real sample (Konpeito sugar candy). 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 the mobile phase and sample solvents. Additionally, it offers the advantage of reduced analysis times compared to fully porous columns.

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