The Separation of Seven Synthetic/Artificial Food Colors on Agilent HC(2)/TC(2) Reversed Phase Columns

Application

Food and Flavors

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Abstract

Many synthetic or artificial colorants (Red #33, Sunset yellow, etc.) are used in food and beverages to improve product appearance. These compounds can be easily separated by reversed-phase liquid chromatography. [1] A new C18 column was used to separate seven food colorants, using a gradient method with a phosphate buffer-acetonitrile mobile phase. This method is suitable for many samples and is applied here to the analysis of these colorants in beverages.

Introduction

Synthetic or artificial food colorings are almost all water soluble, making them ideal for analysis by HPLC with reversed phase columns. These compounds are generally safe, but there are some possible harmful effects of these compounds, including allergic reactions and hyperactivity in children. Artificial food colorings are restricted in some countries because of these possible effects. The quantity of these compounds in food quality control is becoming more important, as is the need to prove that they meet international food quality control standards. The structures of seven colorants, mostly azo dyes, are shown in Figure 1. These compounds are used in beverages to give them a colorful, attractive appearance.

In this application, we focused on developing a method for separating seven colorants on the Agilent TC-C18(2) column and then applying this method to the analysis of these food colors in beverage samples. This new column is packed with 5-µm high-purity (B type) silica with a surface area of 290 m^2/g and bonded with C18, achieving a carbon load of 12%. This is a moderate carbon load for a column with this surface area and is therefore more hydrophilic than columns with a higher carbon load. The special treatment of the surface of the silica and improved end-capping give this column excellent performance, especially for the separation of polar and basic compounds.

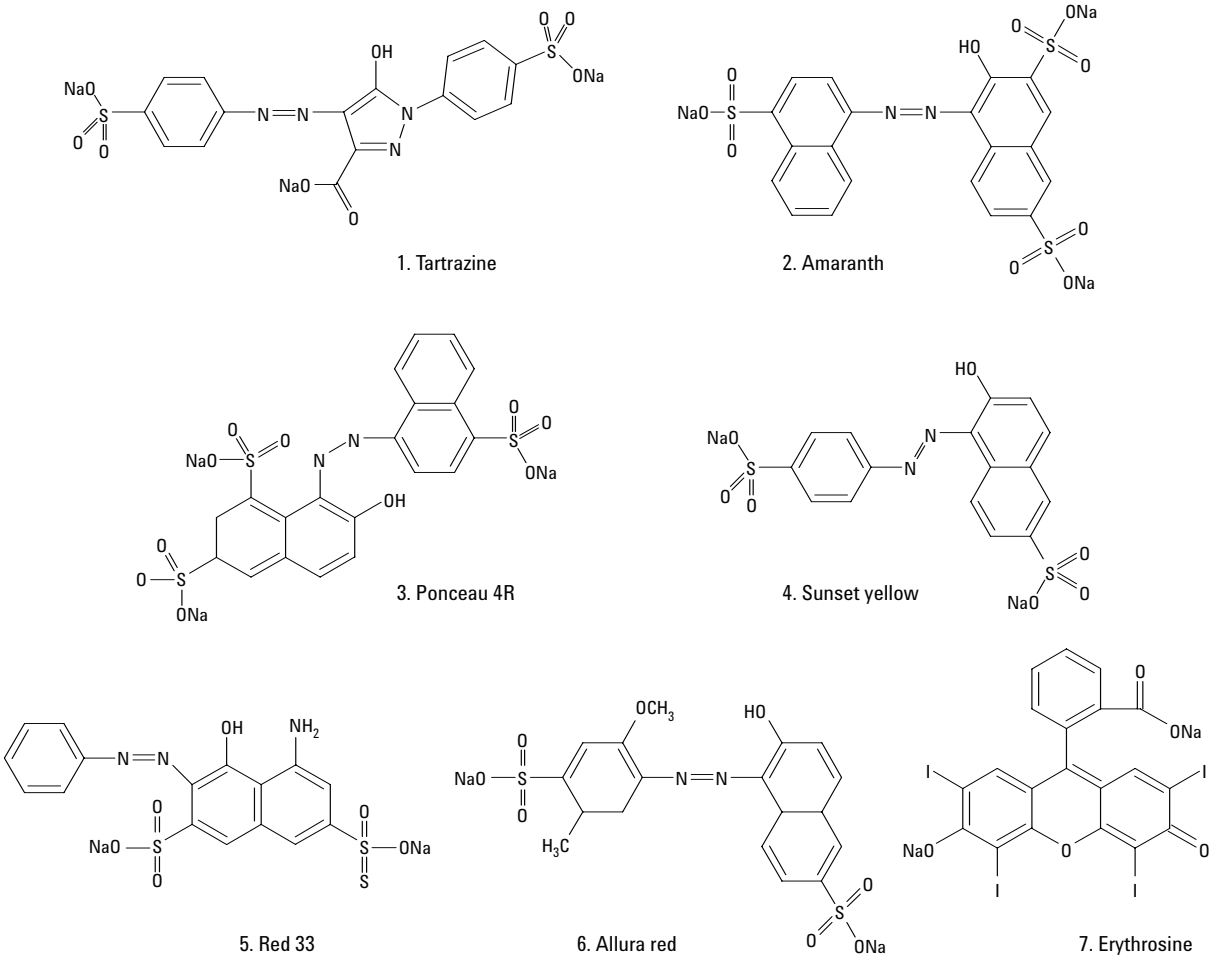

Figure 1. Structures of colorants.

HPLC conditions

Instrument	Agilent 1200SL with DAD
Column	Agilent TC-C18(2), 4.6 mm × 150 mm, 5 µm
Mobile phase	A: 20 mM phosphate buffer, pH 7.0 B: Methanol
Gradient	0–15 min, 10% B – 90% B
Flow rate	1 mL/min
Detector wavelength	254 nm
Temperature	30 °C
Injection volume	5 µL

Seven Food Color Standards Separated on Agilent TC-C18(2)

The compounds shown in Figure 1 are polar, water-soluble compounds with sulfonic acid groups on them, increasing their solubility in water. In fact, many of these compounds are used in the salt form in food. A C18 column was selected to separate these compounds. Two new C18 columns are available from Agilent for routine or QC/QA-type applications. These columns are the Agilent HC-C18(2) column with a carbon load of 17% and the TC-C18(2) column with a carbon load of 12%. The difference in carbon load was designed to provide column choices optimized for the analyses of very polar and very nonpolar analytes. Polar analytes typically require high aqueous mobile phases to be effectively retained. For the artificial food colors, a gradient method starting at low organic in the mobile phase was developed. Starting at very high aqueous mobile phases, the TC-C18(2) column with a carbon load of 12% was the better column for this method development, and can be used for mixtures of polar compounds or for samples of polar and nonpolar compounds.

Figure 2 shows all seven colorants separated on the 4.6 mm × 150 mm, 5 µm Agilent TC-C18(2) column. Using pH 7.0 phosphate buffer-methanol mobile phase, all the compounds are symmetrical with a U.S. Pharmacopeia (USP) tailing factor close to 1.0 and baseline resolution with good reproducibility. Because of their strong acidity, the peak shape and resolution of the compounds are dramatically influenced by the pH selected for the mobile phase. The separation was repeated with mobile phases at three different pH values. The chromatograms for this are shown in Figure 3. The chromatograms clearly show that the peak shape and resolution are influenced by pH; especially the first three components, which are the most acidic and polar. At low pH, pH 2.2, poor resolution was seen between peaks 1 and 2 and peaks 4 and 5; however, pH had less influence on resolution for the later eluting compounds, such as erythrosine (peak 7). Erythrosine is the most hydrophobic of these compounds. The overall elution order of these compounds was tied to their hydrophobic properties, as expected in reversed phase LC.

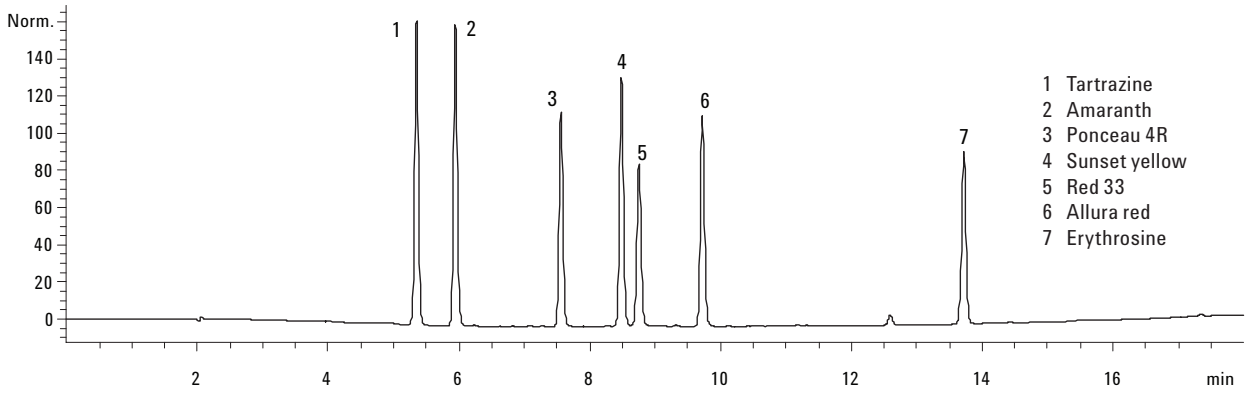

Figure 2. Chromatogram of colorants standards on the Agilent TC-C18(2) column.

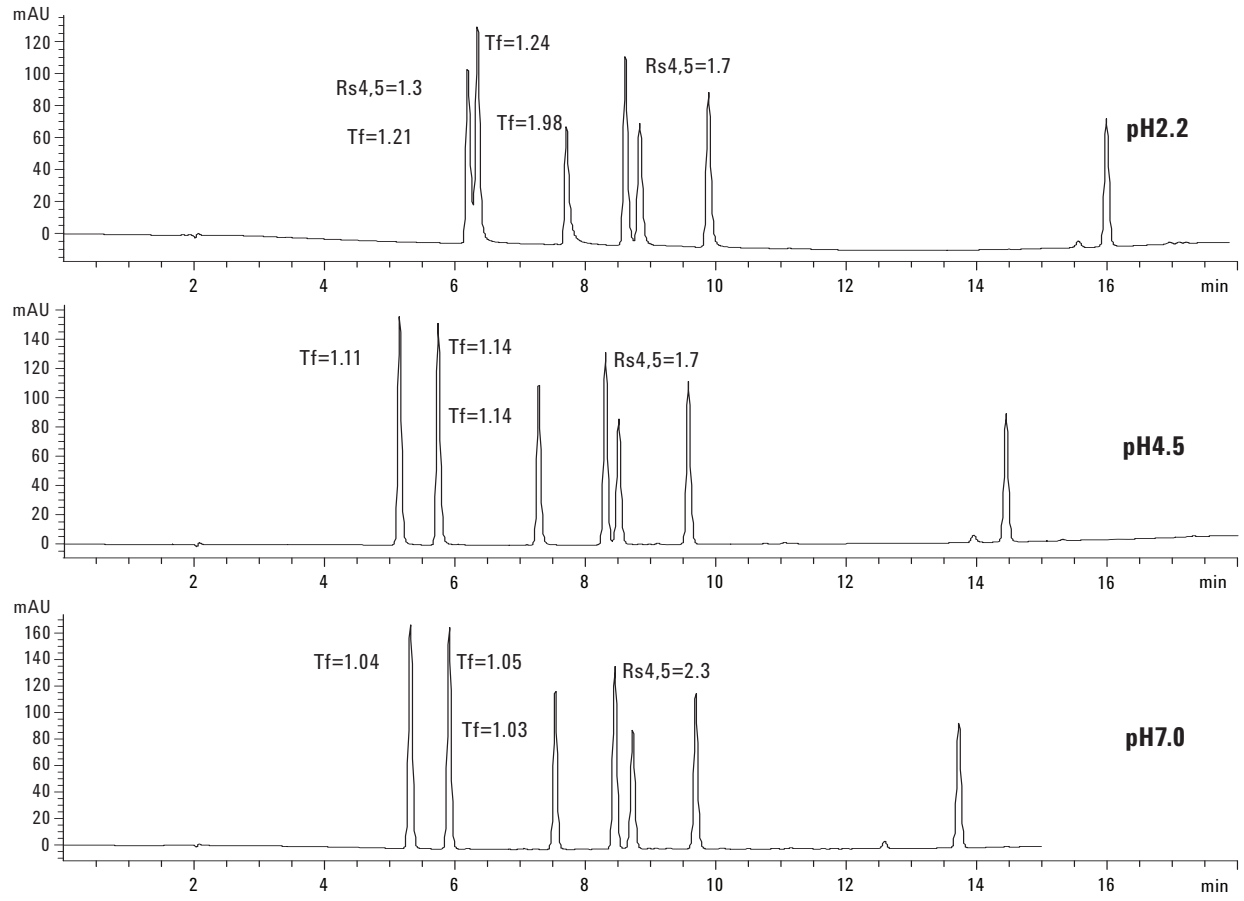

Figure 3. Chromatogram of colorants standards in different pH mobile phase on the Agilent TC-C18(2) column.

Lot-to-Lot Reproducibility

Lot-to-lot reproducibility of columns is important for rugged HPLC methods and routine monitoring. Figure 4 shows the reproducibility test used to evaluate the TC-C18(2) column in this method. Three different batches of columns were used in the test under the same HPLC conditions. The chromatograms show almost no retention time drift and no peak shape variations occurring from column lot-to-lot, which demonstrates the high-quality manufacturing and packing techniques used for these columns.

Analysis of Artificial Food Colors in Beverages

Synthetic or artificial food colorants exist in many common beverages and food, such as fruit-flavored drinks and sodas, cakes, and candy. Some of the colorants in two different beverages were separated in this application. Two popular beverages were selected, an orange flavored soda and a drink powder. For the sample preparation, orange flavored soda was degassed, filtered through a 0.45-µm filter, and then analyzed. The drink powder was dissolved in water, filtered through a 0.45-µm filter, and injected for analysis. The chromatograms from these samples are shown in Figure 5. Three colorants were found and completely separated in the orange flavored soda sample. Based on the standard, the first known peak could be resolved from the unknown peak before it. Two target colorants were found and adequately resolved in the beverage powder sample for effective quantitative analysis.

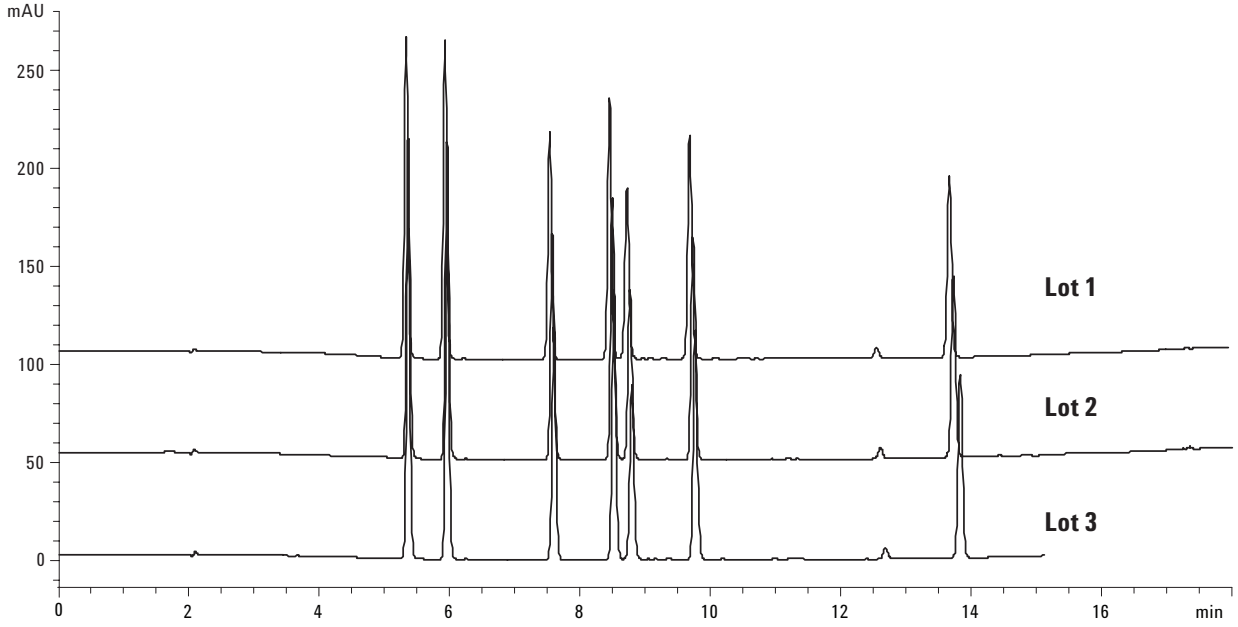

Figure 4. Lot-to-lot reproducibility(TC-C18[2], 4.6 mm × 150 mm, 5 µm).

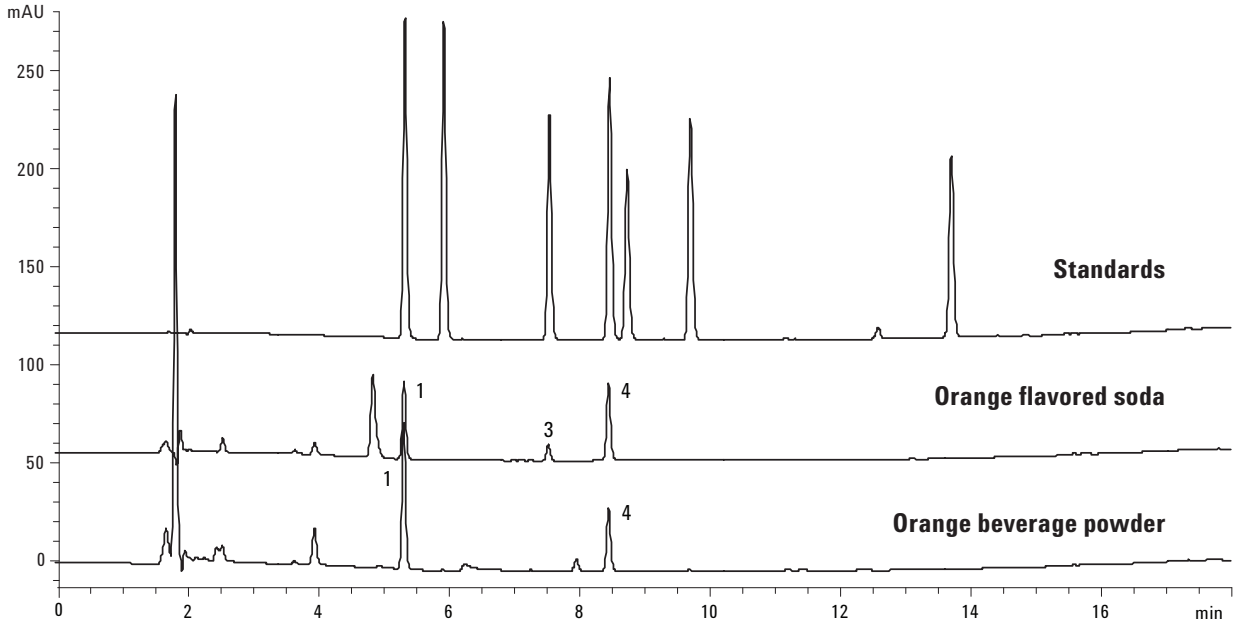

Figure 5. Chromatogram of two samples.

In the beverage powder, there were lower levels of these food colors added. For the low-concentration samples, a large-volume injection is needed to meet the detection and quantitation requirements. Two chromatograms of large-volume 50-µL injections are shown in Figure 6. The separation was still quite good, with symmetrical peaks and little band broadening. For the sample orange flavored soda, peak 1 was completely separated from the unknown compound. All these demonstrate the high capacity of the high surface area TC-C18(2) column.

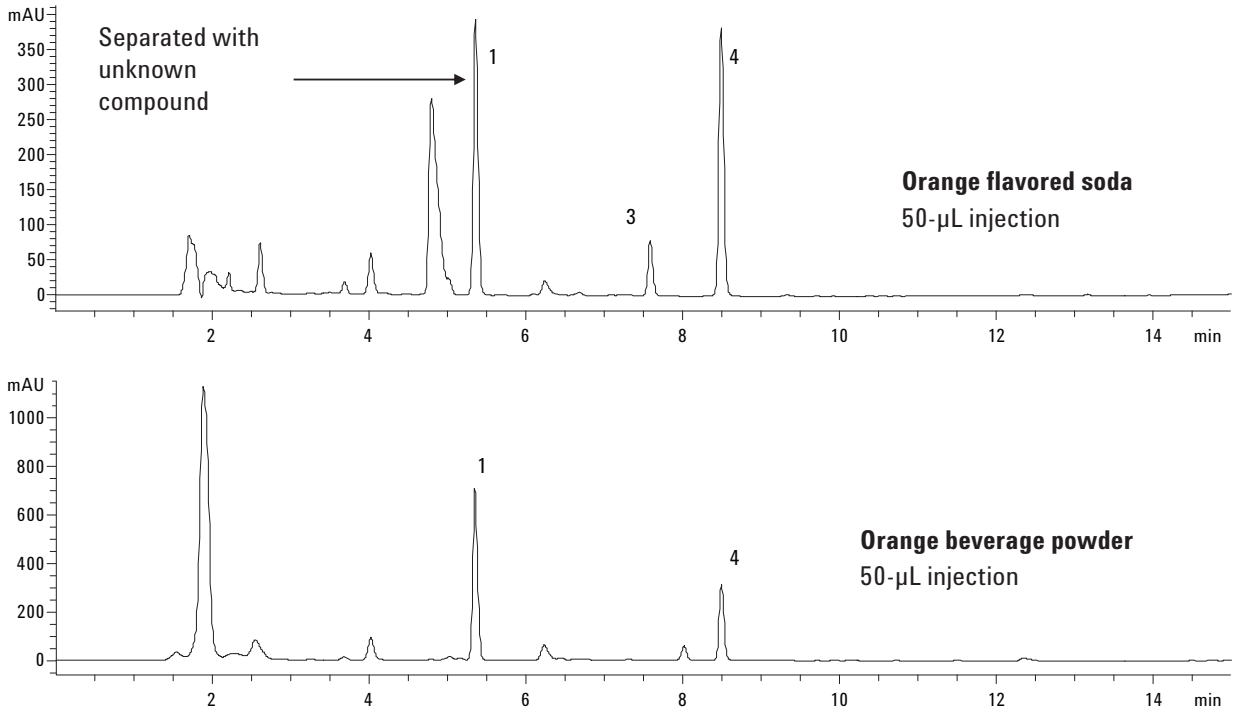

Figure 6. Chromatogram of 50-µL injection.

Selectivity Comparison with HC-C18(2)

As mentioned above, the carbon load of HC-C18(2) is higher than TC-C18(2), which leads to strong retention of nonpolar compounds but not quite so much retention of polar compounds. Figure 7 shows that the HC-C18(2) column retains the more polar food colors slightly less than the TC-C18(2) column does. The change in carbon load also causes a change in selectivity of peaks 4 and 5 between the two columns, which is also shown in the chromatograms. Figure 8 shows chromatograms of orange flavored soda both on TC-C18(2) and HC-C18(2) columns. Peak 1 and the potential interfering compound on the TC-C18(2) column reverse order on the HC-C18(2) column. The possible interfering compound elutes before the target compound 1 on the TC-C18(2) column and has excellent resolution. When the large-volume injection was made, baseline separation could still be achieved; when the elution order was reversed eluted, the two compounds were not baseline separated until the mobile phase composition was adjusted. Because the mobile phase is a low organic (only 10%) starting gradient for this method, we chose the TC-C18(2) column with a lower carbon load for this application. It's important to note that the different selectivity of these two columns provides more column selection opportunities for method development.

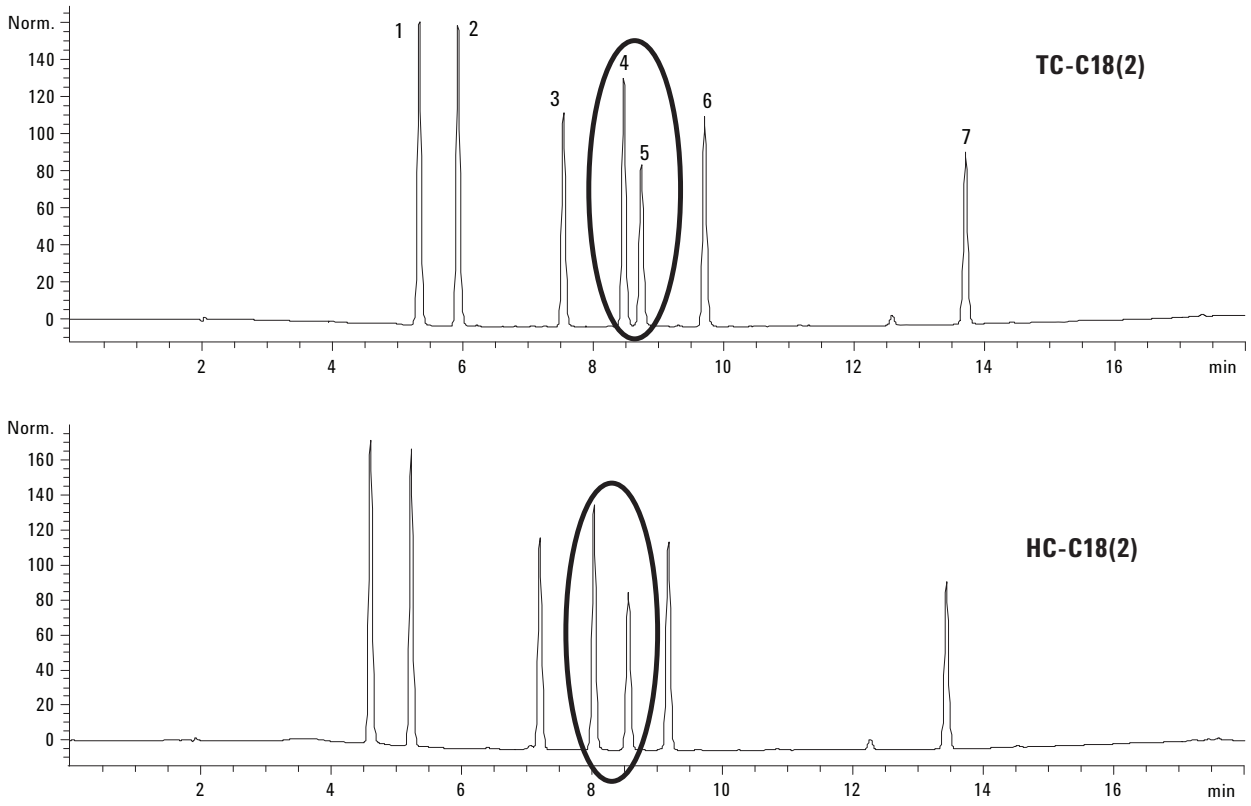

Figure 7. Chromatogram of colorants standards on TC-C18(2) and HC-C18(2).

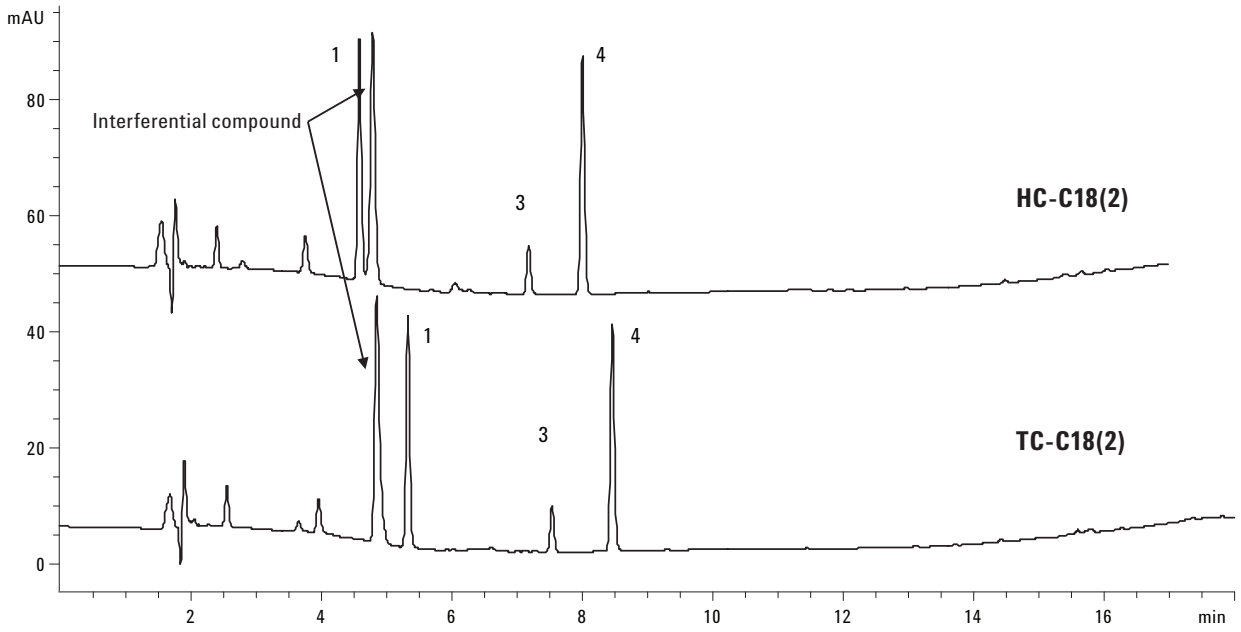

Figure 8. Different selectivity of TC-C18(2) and HC-C18(2).

Conclusions

Using a simple gradient method, many common synthetic colorants can be separated on the Agilent TC-C18(2) column. This method allows baseline separation for many artificial food colors and provides symmetrical peaks and good reproducibility. The Agilent TC-C18(2) column can be used for daily QC analysis of synthetic colorants in foods and beverages with reliable results.

Reference

1. Fang Yanyan, Agilent Technologies, publication 5989-3639CHCN. www.agilent.com/chem

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