Fast, Low Pressure Analysis of Food and Beverage Additives Using a Superficially Porous Agilent Poroshell 120 EC-C18 Column

Application Note

Food and Beverage

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Abstract

Eleven non-nutritive food and beverage additives are analyzed in less than three minutes using an Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column with an ammonium acetate/acetonitrile gradient. The maximum pressure of the analysis is under 400 bar, making this method suitable for use with standard HPLC instruments. Selectivity and efficiency of this Agilent Poroshell 120 EC-C18 column is similar to that of Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 1.8 µm and 4.6 mm × 250 mm, 5 µm columns. While the 1.8-µm Agilent ZORBAX Eclipse Plus column can achieve the desired separation in the same time as the Agilent Poroshell 120, the maximum pressure exceeds 400 bar making a high pressure HPLC necessary. Conversely, the 5-µm Agilent ZORBAX Eclipse Plus column can perform this analysis at less than 400 bar, but requires substantially more time to accomplish the separation. This application note demonstrates the transfer of older methods using Agilent ZORBAX Eclipse Plus C18 columns to new Agilent Poroshell 120 EC-C18 columns on an HPLC with similar efficiency and selectivity, while saving substantial time.

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Introduction

Conventional 400-bar HPLC systems are found in many laboratories. With the introduction of UHPLC, there is a desire to move towards sub-2-µm particles. HPLC columns packed with sub-2-µm particles provide superior resolution and decreased analysis time over traditional 5-µm particles. However this also increases system back pressure. For analysts that wish to translate their current methods to a sub-2-µm method but cannot afford a high pressure LC, columns packed with superficially porous particles may be the answer. Columns like the Agilent Poroshell 120 column offer resolution and speed similar to columns packed with 1.8-µm particles, without generating high back pressure.

The high efficiency of Agilent Poroshell 120 particles is similar to sub-2-µm totally porous particles. This is due to short mass transfer distance and substantially narrower particle size distribution. The larger 2.7-µm Agilent Poroshell 120 particles generate very low back pressure, about 40% to 60% of the back pressure generated by sub-2 µm totally porous particles. This allows the columns to run faster flow rates without exceeding HPLC pressures. Agilent Poroshell 120 columns with 2-µm frits are more forgiving with dirty samples than 1.8-µm columns, providing a more seamless method transfer from traditional 5-µm columns [1-3].

This application note demonstrates the transfer of established methods using longer columns packed with 5-µm particles to a UHPLC-like method using superficially porous particles packed in shorter columns, while keeping the system back pressure below 400 bar. The benefits are a decrease in sample and mobile phase consumption, significant time savings, and the selectivity and efficiency of a sub-2-µm analysis on virtually any HPLC system.

A group of 11 non-nutritive food and beverage additives is used to demonstrate this method translation. These compounds include preservatives, artificial sweeteners, an energy supplement and a flavoring agent. While these compounds are not harmful in appropriate amounts, they can cause sensitization and allergic reactions after excessive exposure [4]. Therefore, detection and quantification of these additive compounds are important.

Experimental

An Agilent 1200 Rapid Resolution LC (RRLC) system was used for this work:

- G1312B Binary Pump SL with mobile phase A: 20 mM ammonium acetate (pH 4.80) and B: acetonitrile. Gradient was 14% B at t_0, ramp to 52% B. Gradient times vary depending on column dimensions and flow rate, see Table 1.
- G1367C Automatic Liquid Sampler (ALS) SL. Injection volume was 11.8 and 2.0 µL for the 4.6 mm × 250 mm and 3.0 mm × 100 mm columns respectively.
- G1316B Thermostatted Column Compartment (TCC) SL with temperature set to 30 °C.
- G1315C Diode Array Detector (DAD) SL with the signal set to 230, 4 nm and reference set to 360, 100 nm, using a G1315-60024 micro flow cell (3-mm path, 2-µL volume).
- ChemStation version B.04.01 (491) was used to control the HPLC and process the data.

Table 1. HPLC Method Parameters for Various Columns

Column	Flow rate (mL/min)	Gradient time (min)	Stop time (min)	Post run time (min)
Agilent ZORBAX Eclipse Plus C18 4.6 mm × 250 mm, 5.0 µm	1.000	12.00	13.10	7.00
Agilent ZORBAX Eclipse Plus C18 3.0 mm × 100 mm, 3.5 µm	0.638	3.00	3.50	2.00
Agilent ZORBAX Eclipse Plus C18 3.0 mm × 100 mm, 1.8 µm	0.851	2.10	2.60	1.80
Agilent Poroshell 120 EC-C18 3.0 mm × 100 mm, 2.7 µm	0.851	2.10	2.60	1.80

Four Agilent columns were used in this work:

- Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm

  (p/n 695975-302)

- Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 1.8 µm

  (p/n 959964-302)

- Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 3.5 µm

  (p/n 959961-302)

- Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm

  (p/n 959990-902)

The compounds of interest are shown in Figure 1, with their respective structure, pKa value and additive function. Compounds were dissolved in water at 1 mg/mL. Equal aliquots were combined to produce a mixed sample. Compounds were purchased from Sigma Aldrich (Bellefonte, PA). Additionally, acetonitrile and ammonium acetate were purchased from Sigma Aldrich. Water used was 18 M-Ω Milli-Q water (Bedford, MA).

Results and Discussion

Figure 2 shows the separation of 11 food and beverage additives on a traditional Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm column in just over 13 minutes. Figure 3 shows the same separation scaled to an Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 3.5 µm with a

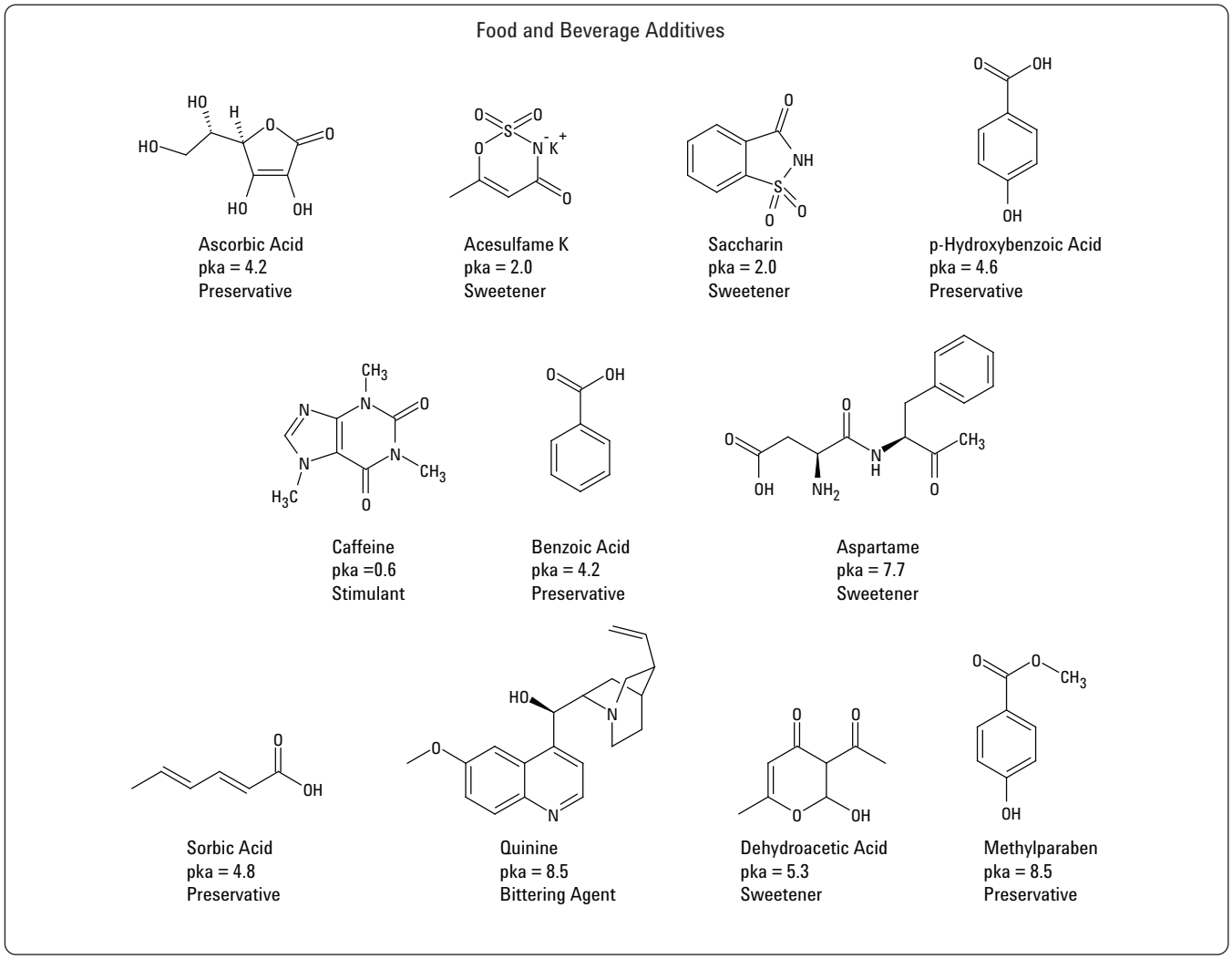

Figure 1. Compounds of interest.

significantly shorter analysis time of 3.5 minutes. The flow rate for this analysis was adjusted to compensate for the smaller internal diameter and for the smaller particle size. In this case, the 3.5-µm column does not resolve two peak pairs: benzoic acid/aspartame and dehydroacetic acid/methylparaben. Figure 4 shows the same separation, scaled to an Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 1.8 µm column. Flow rate for the 1.8 µm column was adjusted to optimize the smaller sub-2-µm particles. All compounds were well resolved in just over 2.5 minutes. This faster analysis can enhance laboratory productivity, and lower the mobile phase and solvent consumption. A smaller sample volume is required for the smaller 3.0 mm × 100 mm column, thus yielding sample preservation. The benefits of this sub-2-µm separation, however are at the cost of higher back pressure (483 bar), resulting in the need for a high pressure LC system. Figure 5 shows the same separation on a superficially porous Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7-µm with the same shortened analysis time as the 1.8-µm column. This separation has the added benefit of lower back pressure (356 bar). The more than 100-bar difference in pressure is very significant, because it determines if a high pressure HPLC system is needed or if a traditional system (400 bar maximum) is sufficient for this analysis.

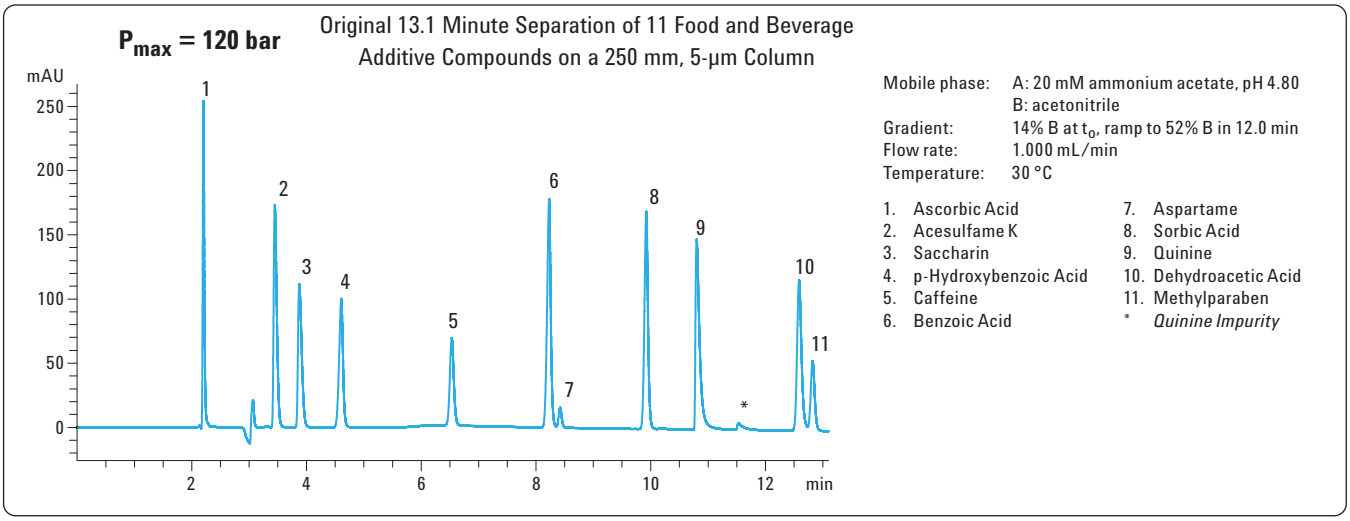

Figure 2. Separation of 11 food and beverage additives on a Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5-µm column using an ammonium acetate/acetonitrile gradient.

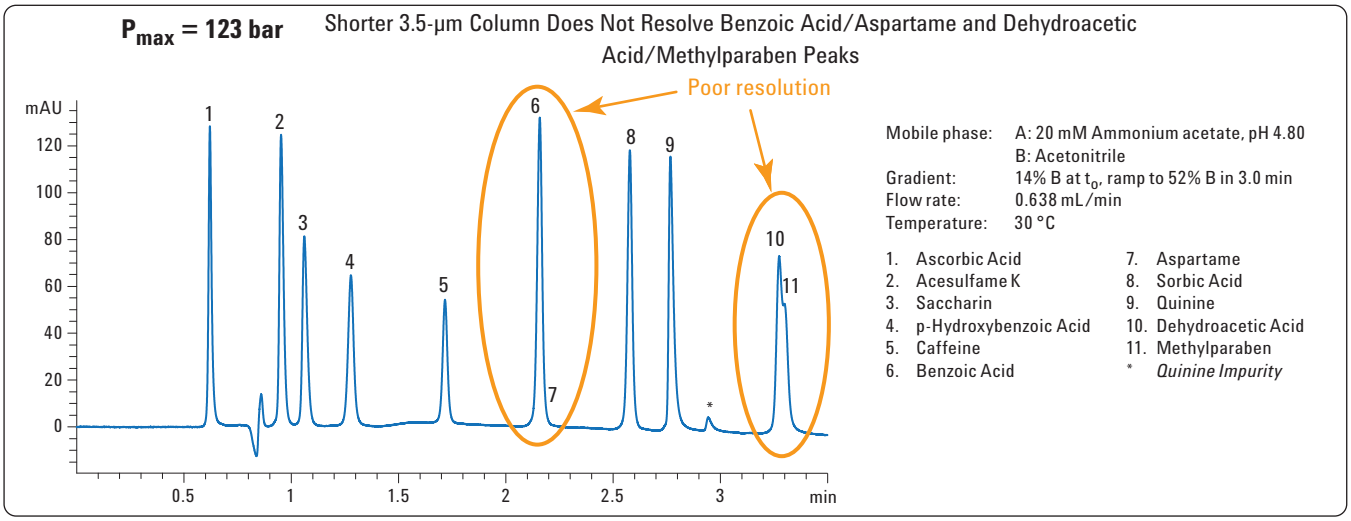

Figure 3. Separation of 11 food and beverage additives on a Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 3.5-µm column using an ammonium acetate/acetonitrile gradient.

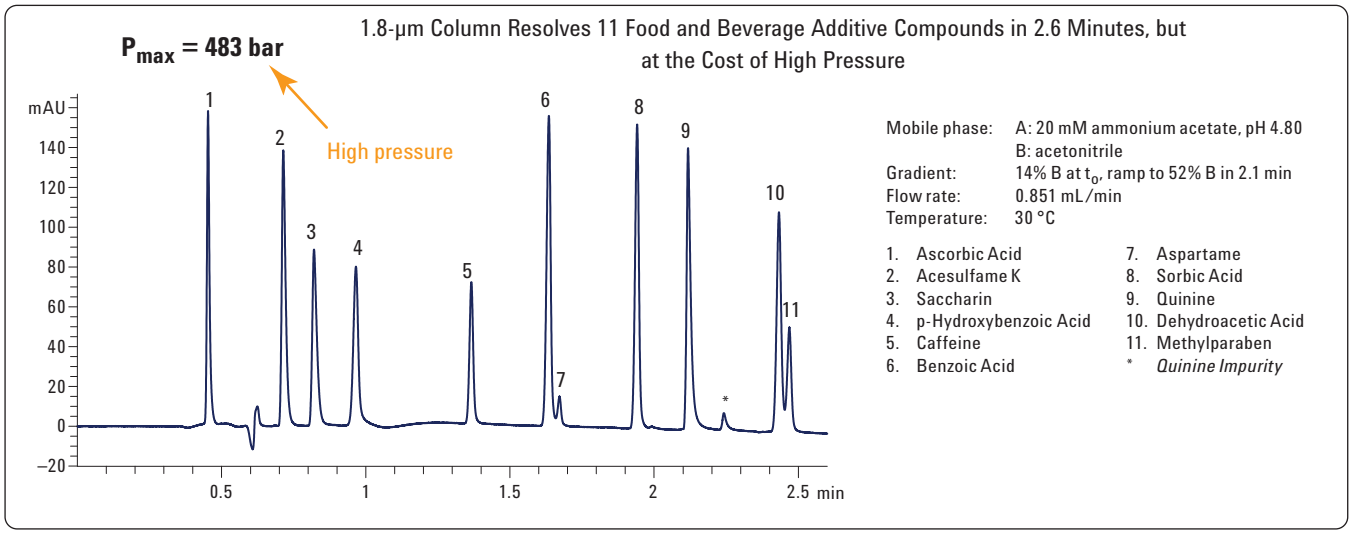

Figure 4. Separation of 11 food and beverage additives on a Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 1.8 µm column using an ammonium acetate/acetonitrile gradient.

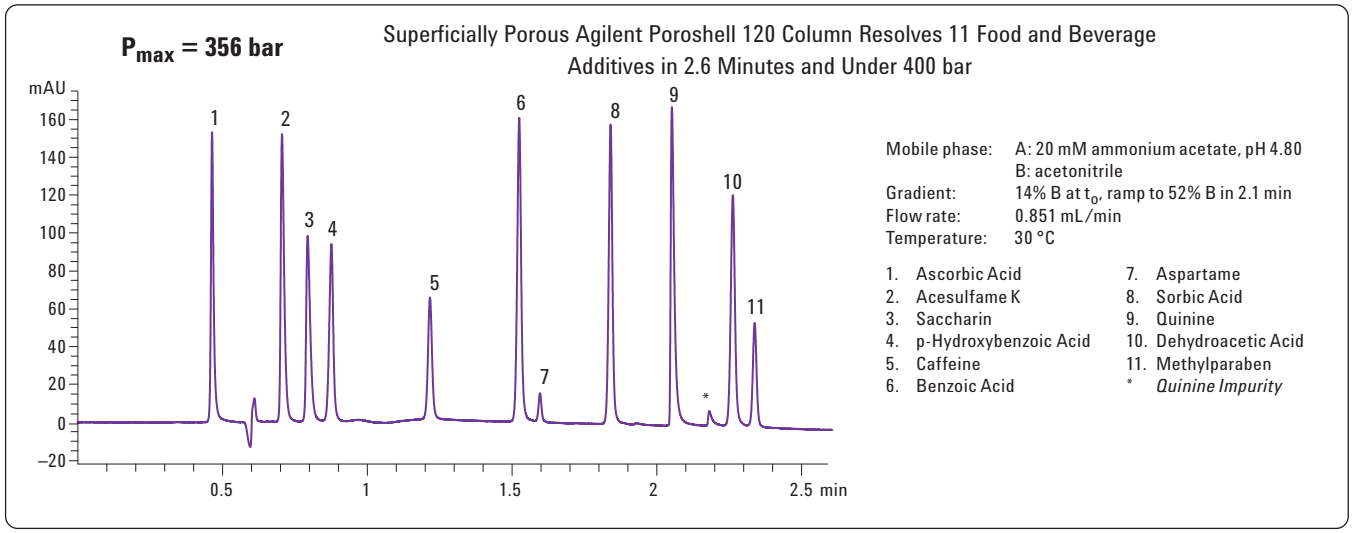

Figure 5. Separation of 11 food and beverage additives on a Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm superficially porous column using an ammonium acetate/acetonitrile gradient.

Figure 6 shows an overlay of the original 5-µm method compared to the new Agilent Poroshell 120 method. Analysis time is reduced from 13.1 to 2.6 min, with the post run time reduced from 7 to 1.8 min. Solvent and mobile phase consumption are reduced by more than 80%. Resolution of the critical pair (dehydroacetic acid and methylparaben) improved from 1.79 to 3.01 on the Agilent Poroshell 120 method, compared to the longer 5-µm Agilent ZORBAX Eclipse Plus method. Note in Figure 6, that the last peak on Agilent Poroshell 120 elutes at approximately the same time as the first peak on the 5-µm Agilent ZORBAX Eclipse Plus.

Four consumer product samples are successfully analyzed in less than 2.6 minutes and under 400 bar (Figure 7). The energy drink contains caffeine, benzoic acid and sorbic acid. The diet soda has saccharin, caffeine, benzoic acid and aspartame. Mouthwash includes saccharin and benzoic acid. The sugar-free chewing gum contains acesulfame k and aspartame.

When high throughput is important and HPLC system limits allow, the flow rate can be increased with little loss in chromatographic quality, as shown in Figure 8. The flow rate on the Agilent Poroshell 120, 3.0 mm × 100 mm column can be increased from 0.851 to 1.489 mL/min to further reduce run time by 40% in under 600 bar. This achieves little loss in resolution of the critical pair and has minimal effects on conditional peak capacity (n_c) [5]. The result is a baseline separation of 11 compounds in under 600 bar back pressure with an analysis time of 1.5 minutes.

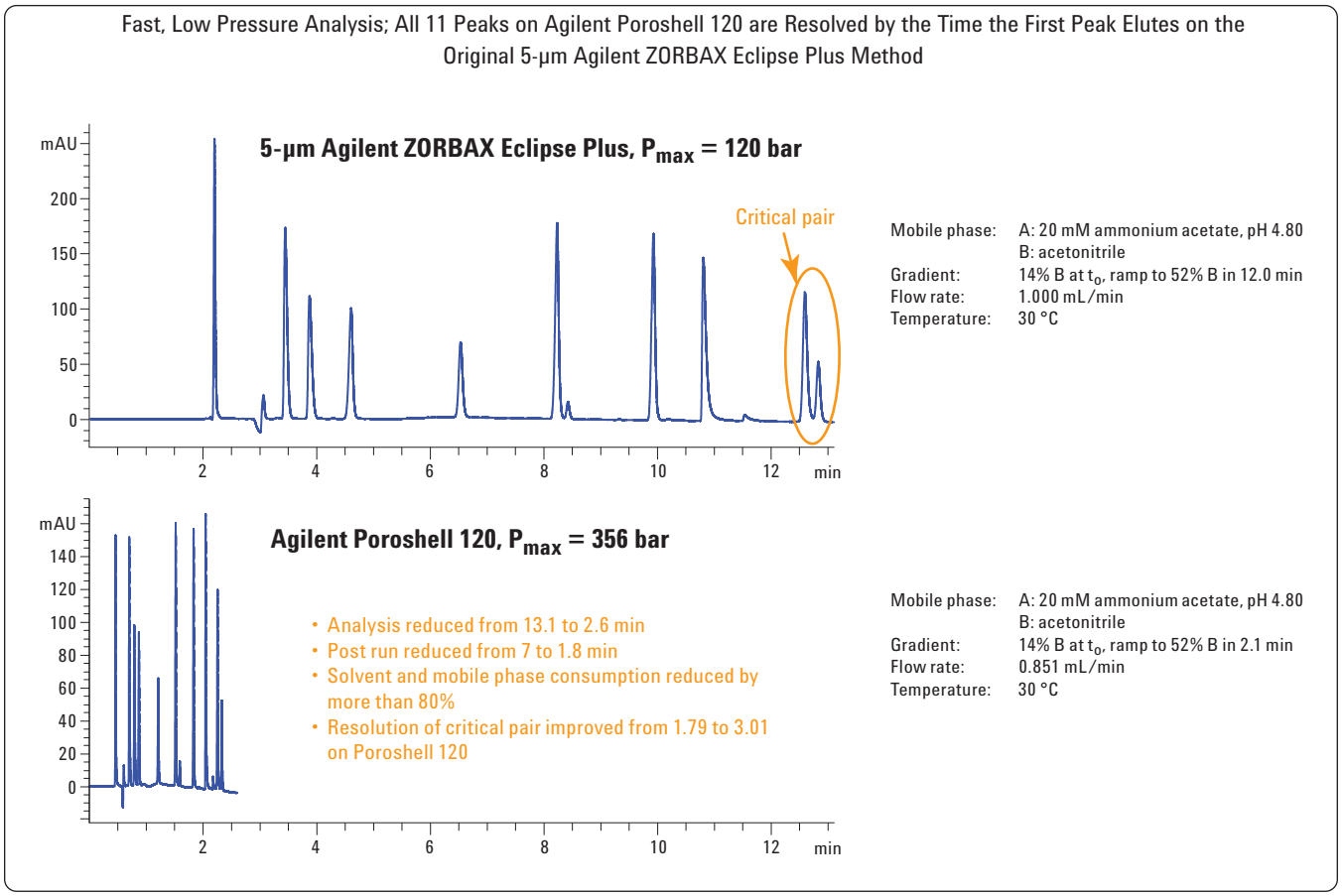

Figure 6. An overlay of the original Agilent Eclipse Plus 5-µm method and new Agilent Poroshell 120 method.

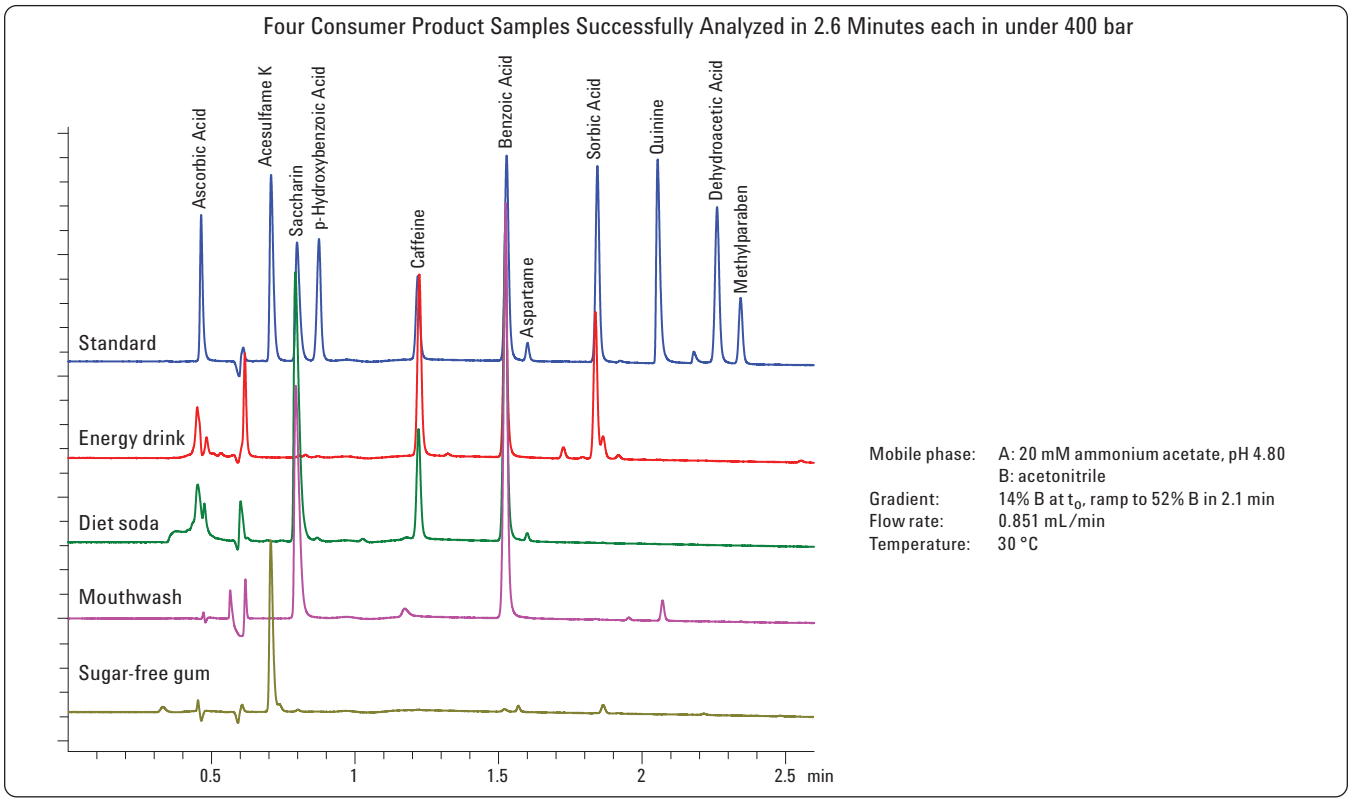

Figure 7. Four consumer product samples analyzed on a Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm superficially porous column.

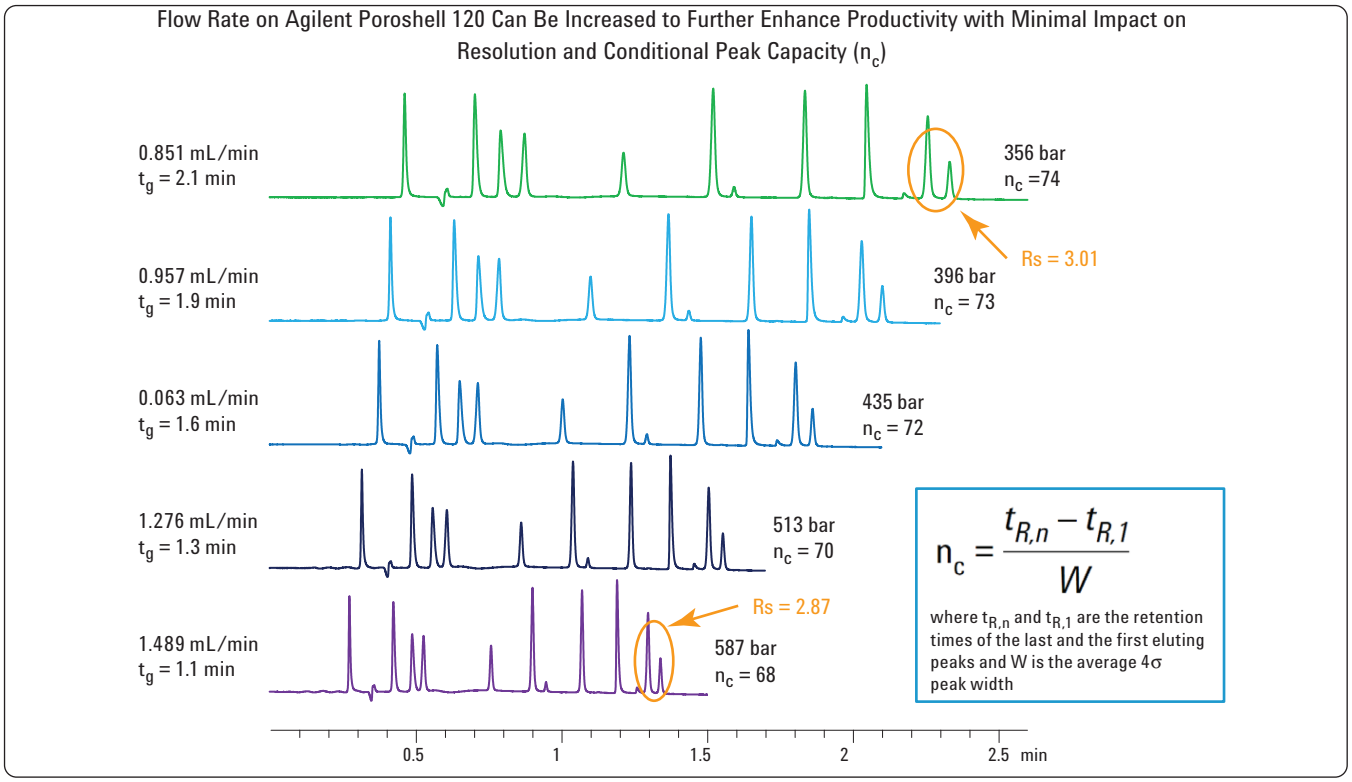

Figure 8. Overlay showing effects on chromatographic quality with increased flow rate on Agilent Poroshell 120.

Conclusion

HPLC columns packed with superficially porous particles offer many advantages over columns packed with conventional, fully porous particles. The superficially porous 2.7-µm Agilent Poroshell 120 EC-C18 column offers similar efficiency and selectivity to the 1.8-µm Agilent ZORBAX Eclipse Plus C18 column, without the high back pressure. While larger 5-µm particles packed in longer columns can yield similar efficiency without high back pressure, they result in a significantly longer analysis time. Due to the similar selectivity between Poroshell 120 EC-C18 and Eclipse Plus C18, methods can easily be transferred from older Eclipse Plus C18 columns to new Poroshell 120 EC-C18 columns. This achieves lower back pressure for older 400 bar HPLC systems, shorter run times, and solvent savings.

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